
NEW ENGLAND
BioLabs® Inc.
be INSPIRED
drive DISCOVERY
stay GENUINE
2019•20

Learn about NEB

NEB EMPLOYEE PHOTO © Kim Indresano
COVER PHOTO – Pygmy Seahorse, Philippines, Southeast Asia, Pacific Ocean © Giordano Cipriani/Getty Images
COVER VIDEO – Pink Pygmy seahorse on gorgonian coral, Puerto Galera, Philippines © Sabangvideo

Stay genuine.

Since our establishment in 1974, New England Biolabs has been different. From our founding principles — placing the advancement of science and stewardship of the environment as our highest priorities — to our unique corporate culture, NEB's philosophy can be distilled down to three core values: passion, humility and being genuine.

As part of our ongoing commitment to these values, in 2014, we established the Passion in Science Awards® to recognize the unsung heroes in the scientific community who contribute to making the world a better place through their inspirational and innovative work in artistic expression, humanitarian service, environmental stewardship and scientific mentorship. We have always believed that science is more than just a vocation — it embodies an ethos that inspires acts of compassion, brilliance and originality.

Thank you for your ongoing trust and support.
If there is anything you believe we should be doing differently, please share your thoughts with us.
We wish you continued success in your research.

Want to experience the NEB catalog in augmented reality?

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the NEB Augmented Reality (AR) app for iPhone® or iPad® at the Apple® App Store or for Android™ on Google Play™.

Explore
the NEB Catalog & Technical Reference and keep an eye out for the augmented reality icon.

Discover
videos, tutorials and immersive experiences that can be accessed by scanning the icon using the AR app.

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At New England Biolabs, we are motivated by a set of core values that are still as true today as they were when the company was founded 45 years ago. These principles continue to guide us both as a company and as individuals.

Advancement of Science

We believe that basic research and the cultivation of scientific knowledge is critical for us to stay connected with our customers and to drive scientific breakthroughs. At NEB, over 30 labs participate in research projects, which are aided by post-doctoral fellows and students in Masters and Ph.D. programs. NEB researchers have authored or co-authored over 1,200 publications to date, most of which are in peer-reviewed journals.

Environmental Stewardship

We continuously strive to promote ecologically sound practices and environmental sustainability in order to protect our natural resources, both locally and globally. Further, it is our goal to continuously improve our business processes to minimize our impact on the environment.

Humanitarianism

We see opportunities where science can be used to improve lives, and are inspired by researchers who help push the social barometer to a kinder, more compassionate, and healthier future. This philosophy lies behind NEB's longstanding commitment to its parasitology research program, which contributes to the understanding and treatment of poorly-funded and understudied tropical diseases. NEB has also helped to establish several foundations devoted to humanitarian efforts.

Delivering the Highest Quality Product

In order to accelerate your research, it is our goal to deliver best-in-class product quality and technical support. With a reliance on recombinant technologies, our products are designed and manufactured in our ISO 13485:2016 and ISO 9001:2015 certified facility in Ipswich, MA, USA. We are constantly improving the stringency and range of our quality controls to ensure that our products will perform to your expectations, every time. In addition, we recently expanded our manufacturing footprint by opening a facility in Rowley, MA, for the production of GMP-grade materials for customers requiring an enhanced level of quality documentation and support.

nqa ISO 13485 Registered Medical Devices

nqa ISO 9001 Registered Quality Management

nqa ISO 14001 Registered Environmental Management

Learn more about our commitment to the environment

A Unique Approach to Wastewater Treatment

Our state-of-the-art Solar Aquatics System™ utilizes and accelerates the process found in streams and wetlands to treat the campus' wastewater, making it clean enough for groundwater recharge.

Solar Aquatics System™ is a trademark of Ecological Engineering Associates.

The NEB Facility

Our research and production facility, located in Ipswich, MA, USA, is LEED® certified, which is awarded based on environmentally-focused standards that include:

- Water efficiency
- Energy conservation
- Atmospheric protection
- Sustainable building materials and resources
- Indoor environmental quality
- Innovation and building design

LEED® is a registered trademark of the U.S. Green Building Council Corporation.

Recycling at NEB

NEB established the first shipping box recycling program over 40 years ago, in order to divert polystyrene from landfills.

Our extensive in-house recycling program includes:

- Paper
- Plastics
- Glass
- Aluminum
- Batteries
- Electronics

NEB also composts its waste, diverting as much as 75% of the waste from landfills.

Partnering with NEB

With over 40 years of leadership in the life science industry, our scientific expertise, global reach, and proven track record of turning innovative ideas into successful products positions NEB as a compelling partner. With experience in fields as diverse as next generation sequencing, RNA biology, qPCR and protein engineering, NEB is ready to work with you to develop custom solutions specific to your needs, and to help bring your technologies to market. Further, our global distribution network can help to ensure that your products will have worldwide reach.

Custom Solutions

From development to commercialization, NEB provides the technical expertise, consistent scalable manufacturing, quality systems and a global distribution network to enable a successful long-term partnership. Our dedicated team is ready to work with you to develop novel, high performance enzymes tailored to your application, optimize these enzymes in your workflow, enable small to large scale production, generate quality controls and customize packaging. With our ISO 13485 and ISO 9001 certified manufacturing processes, as well as the ability to manufacture GMP-grade products, you can be confident in our robust process, documentation and risk mitigation for the product you need. For more information, contact **custom@neb.com**.

Global Business Development

The business development team at NEB operates on a global basis to enable innovation in molecular technologies through strategic partnerships, licensing and new ventures. We do so by leveraging the talents and assets of NEB, including our scientific, commercial and international resources. Co-development collaborations benefit from access to our expertise and proprietary technologies, ensuring commercial outcomes with shorter innovation cycles. Further success is then achieved due to our privileged market position as products enter a well-managed global distribution network. For more information, contact **busdev@neb.com**.

International Network

NEB has extensive worldwide distribution capabilities through a network of exclusive distributors and agents, plus wholly-owned subsidiaries located in Australia, Canada, China, France, Germany, Japan, Singapore and the United Kingdom. For more information, please see the back inside cover.

NEBnow Freezer Program Network

Your research doesn't have to be put on hold waiting for that critical reagent to be delivered. With NEBnow® on-site freezers, enjoy convenient and affordable access to NEB's high quality reagents, anytime. Our NEBnow Freezer Program Team works closely with your institution to customize inventory best suited to your research program. Save time and avoid shipping fees with consolidated shipments. For more information, contact **freezers@neb.com**.

Enzymes for Innovation

The NEB catalog highlights a wide variety of enzyme functionalities found in nature or engineered for specific purposes. However, in molecular biology, new tools can often lead to new discoveries. Taking advantage of the enzymology expertise at NEB, we now offer a growing selection of novel enzymes with interesting and unique activities for manipulating DNA, RNA, proteins and glycans, even if specific applications for them have yet to be discovered. Our hope is that by engaging researchers' imaginations, our "Enzymes for Innovation" initiative will enable the discovery of new molecular tools and workflows. If you are looking for an enzyme functionality that it is not currently available, visit www.enzymesforinnovation.com or contact **enzymesforinnovation@neb.com**.

What are Enzymes for Innovation?

"We see ourselves as an extension of your manufacturing and operations team, dedicated to enabling you to develop new technologies, products and services, and delivering them with exceptional service and support."

– Director, OEM & Custom Solutions, New England Biolabs

"Trust, transparency and timely communications are key to achieving a mutually beneficial outcome. Our goal is to exceed your expectations as we work together to carry your innovations forward."

– Executive Director, Global Business Development, New England Biolabs

"The NEBnow freezer program is an amazing addition; having 24/7 access to the NGS reagents has been extremely helpful. NEB allows you to customize the freezer to hold the items you use most, saving us valuable time by cutting out the ordering process!"

– Assistant Director, Genomic Sequencing and Analysis Facility, University of Texas, Austin

Non-Profits and Research Foundations

New England Biolabs has played a role in the establishment of several organizations that are advancing humanitarian efforts and environmental stewardship.

New England Biolabs Foundation

The New England Biolabs Foundation is a private independent foundation whose mission is to foster community-based conservation of landscapes and seascapes, and the bio-cultural diversity found in these places. The foundation supports projects in selected countries of Central America, Andean South America, West Africa, and in coastal communities on the north shore of Massachusetts. Learn more at **NEBF.org**.

creative action INSTITUTE home of ArtCorps

Creative Action Institute transforms the way people communicate and collaborate to catalyze community-driven solutions that advance gender equality and build a sustainable planet. Through our experiential trainings, convenings and coaching, we develop leadership, build networks and support grassroots advocacy. Visit **CreativeActionInstitute.org** to learn more.

Northeastern University
The Ocean Genome Legacy Center of New England Biolabs
dedicated to preserving the biological diversity of the sea
www.northeastern.edu/ogl

The Ocean Genome Legacy Center of New England Biolabs is a non-profit research center dedicated to the conservation of marine genome diversity and maintaining a repository of genomic DNA from marine organisms around the world. Learn more at **northeastern.edu/ogl**.

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Featured Products from NEB

NEBNext® Reagents for NGS Sample Prep

- Streamline workflows, minimize inputs, and improve library yields & quality with our growing portfolio.
- Avoid DNA damage generated with bisulfite sequencing! Our NEBNext Enzymatic Methyl-seq Kit (EM-seq™)generates high quality libraries for superior detection of 5-mC and 5-hmC from fewer sequencing reads.
- Generate high quality, full length transcript sequencing libraries from single cells or as little as 2 pg of total RNA with our NEBNext Single Cell/Low Input RNA Library Prep Kit.
- The NEBNext Ultra™ II FS DNA Library Prep Kit incorporates our novel fragmentation reagent and offers a fast and reliable solution for library construction.

See page 134 for details

Monarch® Nucleic Acid Purification Kits

- Maximize performance and minimize your environmental impact.
- Choose from kits for genomic DNA & total RNA extraction, DNA & RNA cleanup, plasmid miniprep and gel extraction. Buffers and columns are also available separately.
- Obtain highly-pure DNA & RNA from a wide variety of sample types.
- Elute in small volumes and prevent buffer carryover with our unique column designs.
- Save time with fast, user-friendly protocols.

See page 122 for details

Cloning & Synthetic Biology

- Assemble multiple DNA fragments and transform in under two hours, regardless of fragment length or compatibility, with the NEBuilder HiFi DNA Assembly Kit. This versatile kit can be used for a variety of DNA assembly methods.
- Achieve 20+ fragment assembly with high efficiency and accuracy with our NEB Golden Gate Assembly Kit (BsaI-HF®v2). Couple with our Ligase Fidelity Viewer online tool to ensure the highest fidelity ligation.

See pages 88–90 for details

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AbaSI	BcgI	BspCNI	DraIII-HF	HpyAV	NruI	ScaI-HF
AccI	BciVI	BspDI	DrdI	HpyCH4III	NruI-HF	ScrFI
Acc65I	BclI	BspEI	EaeI	HpyCH4IV	NsiI	SexAI
AciI	BclI-HF	BspHI	EagI	HpyCH4V	NsiI-HF	SfaNI
AclI	BcoDI	BspMI	EagI-HF	KasI	NspI	SfcI
AcuI	BfaI	BspQI	EarI	KpnI	PacI	SfiI
AfeI	BfuAI	BsrI	EciI	KpnI-HF	PaeR7I	SfoI
AflII	BglI	BsrBI	Eco53kI	LpnPI	PciI	SgrAI
AflIII	BglII	BsrDI	EcoNI	MboI	PflFI	SmaI
AgeI	BlpI	BsrFI-v2	EcoO109I	MboII	PflMI	SmlI
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AhdI	BmrI	BsrGI-HF	EcoRI	MfeI-HF	PluTI	SpeI
AleI-v2	BmtI	BssHII	EcoRI-HF	MluI	PmeI	SpeI-HF
AluI	BmtI-HF	BssSI-v2	EcoRV	MluI-HF	PmlI	SphI
AlwI	BpmI	BstAPI	EcoRV-HF	MluCI	PpuMI	SphI-HF
AlwNI	Bpu10I	BstBI	Esp3I	MlyI	PshAI	SrfI
ApaI	BpuEI	BstEII	FatI	MmeI	PsiI	SspI
ApaLI	BsaI	BstEII-HF	FauI	MnlI	PspGI	SspI-HF
ApeKI	BsaI-HFv2	BstNI	Fnu4HI	MscI	PspOMI	StuI
ApoI	BsaAI	BstUI	FokI	MseI	PspXI	StyI
ApoI-HF	BsaBI	BstXI	FseI	MslI	PstI	StyI-HF
AscI	BsaHI	BstYI	FspI	MspI	PstI-HF	StyD4I
AseI	BsaJI	BstZ17I-HF	FspEI	MspA1I	PvuI	SwaI
AsiSI	BsaWI	Bsu36I	HaeII	MspJI	PvuI-HF	Taq$^{\alpha}$I
AvaI	BsaXI	BtgI	HaeIII	MwoI	PvuII	TfiI
AvaII	BseRI	BtgZI	HgaI	NaeI	PvuII-HF	TseI
AvrII	BseYI	BtsI-v2	HhaI	NarI	RsaI	Tsp45I
BaeI	BsgI	BtsIMutI	HincII	NciI	RsrII	TspMI
BaeGI	BsiEI	BtsCI	HindIII	NcoI	SacI	TspRI
BamHI	BsiHKAI	Cac8I	HindIII-HF	NcoI-HF	SacI-HF	Tth111I
BamHI-HF	BsiWI	ClaI	HinfI	NdeI	SacII	XbaI
BanI	BsiWI-HF	CspCI	HinP1I	NgoMIV	SalI	XcmI
BanII	BslI	CviAII	HpaI	NheI	SalI-HF	XhoI
BbsI	BsmI	CviKI-1	HpaII	NheI-HF	SapI	XmaI
BbsI-HF	BsmAI	CviQ1	HphI	NlaIII	Sau3AI	XmnI
BbvI	BsmBI	DdeI	Hpy99I	NlaIV	Sau96I	ZraI
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Cellular Analysis

Orbiculate cardinalfish (*Sphaeramia orbicularis*) sheltering amongst roots of mangroves (*Rhizophora sp.*), Mangrove Ridge, Yanggefo Island, Raja Ampat, West Papua, Indonesia. Credit: Linda Pitkin, Minden Pictures

Conservation of Biodiversity

Each edition of the New England Biolabs Catalog contains a collection of mini-reviews that addresses various scientific, environmental and/or humanitarian topics. The theme of the 2019-20 Catalog is “Conservation of Biodiversity”.

Mini-reviews were authored by Joanne Gibson, a Science Writer at New England Biolabs, Inc. Joanne received her Ph.D. in Molecular Biology from the Unversity of Sydney, Faculty of Medicine, Sydney, Australia. She then became a postdoctoral associate at the Massachusetts Institute of Technology, Cambridge, MA. Prior to joining NEB, Joanne served as an Editor for American Journal Experts. When she is not working, Joanne enjoys spending time with her family, traveling & camping, and volunteering for local educational outreach opportunities.

Orbiculate cardinalfish (*Sphaeramia orbicularis*) sheltering amongst roots of mangroves (*Rhizophora sp.*), Mangrove Ridge, Yanggefo Island, Raja Ampat, West Papua, Indonesia.
Credit: Linda Pitkin, Minden Pictures

What is Biodiversity and Why Conserve it?

Biodiversity is a broad concept that refers to the “web of life”. It describes all of the organisms on Earth, the variety of organisms in an ecosystem and the genetic make-up of those organisms. Biodiversity is responsible for sustaining life as we know it — from producing oxygen, to pollinating our food sources and cleaning our water supplies.

Relationships between organisms have evolved over millions of years into a complex network that functions in harmony with the Earth. All species within an ecosystem have a role to play, and the productivity and stability of any given ecosystem depends on the ability of all of the organisms to work together to maintain balance and ensure each other’s survival.

Since humans have inhabited the Earth, there has been a reduction in biodiversity and genetic diversity, yet our health and well-being are intricately tied to its survival. For example, our food sources require a vast range of native pollinators. Diverse ecosystems are responsible for purifying water and recycling nutrients. Our economic well-being relies on the availability of natural resources, such as timber and crude oil. Rainforests help manage the reduction of CO_2 to regulate our climate. Fifty percent of modern pharmaceutical products used in developed countries are derived from plants, animals and microorganisms, and this number increases to 80% if you take into consideration traditional medicines used across the globe. Biodiversity enriches our cultural and recreational experiences: it pleases the senses and gives us the opportunity to exercise, whether it be hiking, bird watching or boating on a lake.

Ecosystems are vulnerable to collapse, and a loss of biodiversity makes them more susceptible to disease and sudden environmental changes. It renders the ecosystem less adaptable.

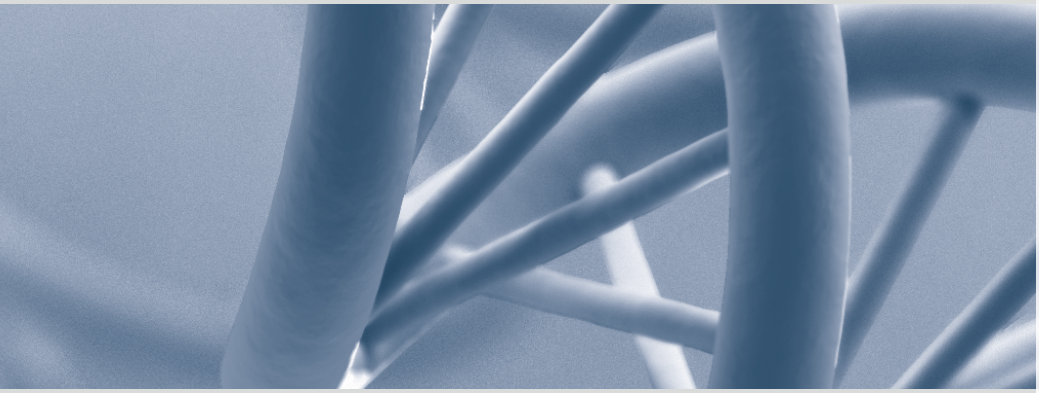
Humans have caused rapid changes to ecosystems and a decline in biodiversity. Habitat destruction, particularly in biodiverse-rich tropical regions, is the primary cause of an accelerated level of extinction of many species. Upstream of habitat destruction is overpopulation, which has led to an unsustainable level of resource consumption. Downstream of habitat destruction is climate change — to which the burning of fossil fuels consequently warming the atmosphere, the destruction of forests that play a critical role in regulating climate, ocean acidification and changes in plant morphology all contribute. Other factors, such as the accidental and deliberate introduction of non-native species to an ecosystem and overfishing of our lakes and oceans, are also significant threats to biodiversity.

One key challenge we face is to better understand the complexity of all biodiversity and the network of interactions that occur within ecosystems — this knowledge will help us to better evaluate the threats. Some of the most significant disruptions to ecosystems have been the result of a lack of awareness of the complexity within that ecosystem. Our attempts to control an ecosystem for human benefit has, in many cases, been disastrous.

We also need to expand protected areas of land and ocean, with a desire to both preserve ecosystems and benefit from what they have to offer. Precious resources must be carefully utilized, while fully considering the implications.

Biodiversity is declining, and human population is growing exponentially; however, we are now gaining tools and the necessary knowledge to protect our planet for future generations. Scientific and community involvement is continually expanding, which has the potential to lead to policy changes designed to conserve biodiversity.

Restriction Endonucleases



The leader in the discovery & production of restriction enzymes.

Having supplied restriction enzymes to the research community for over 40 years, NEB has earned the reputation of being a leader in enzyme technologies. Working continuously to be worthy of that distinction, NEB strives to develop enzymes of the highest purity and unparalleled quality.

NEB scientists continue to improve our existing portfolio, as well as explore the utility of NEB reagents in new technologies. As a result, NEB scientists continue to publish scientific papers and be awarded grants in this area. With the industry's largest research and development group dedicated to restriction enzymes, we are proud to have been there first: the first to commercialize a recombinant enzyme, the first to introduce a nicking enzyme, and the first to supply a true restriction enzyme master mix. In addition, NEB has a continuing history of innovation by engineering restriction enzymes with altered specificities and improved performance. Through ongoing research in these areas, we are committed to driving the innovations that allow us to offer maximum performance and convenience.

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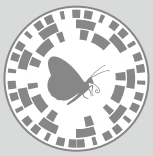
Visit NEBrestrictionenzymes.com to find additional online tools, video tech tips and tutorials to help you in setting up restriction enzyme reactions.



What are restriction enzymes?

Icon Descriptions

The gene encoding this enzyme was cloned at NEB.

RR This enzyme is purified from a recombinant source.

e This enzyme has been engineered for maximum performance.

Time-Saver qualified enzymes will digest 1 µg of substrate DNA in 5–15 minutes using 1 µl of enzyme under recommended reaction conditions. These enzymes can also be used overnight with no loss of sample. For more information, see pages 302–303.

2*site Indicates that the restriction enzyme requires two or more sites for cleavage.

NEB 1.1 NEB 2.1 NEB 3.1 CutSmart NEB U
Indicates which reaction buffer is supplied with the enzyme for optimal activity. Enzymes with buffer requirements not met by one of the four standard NEBuffers (1.1, 2.1, 3.1 or CutSmart®) are supplied with their own unique NEBuffer (NEB U). NEBuffers are color-coded (NEB 1.1-yellow, NEB 2.1-blue, NEB 3.1-red, CutSmart-green) and supplied as 10X stocks with each enzyme. For more information, consult the Performance Chart on pages 293–298.

Epi This enzyme is EpiMark validated for epigenetics studies.

SAM This enzyme is supplied with a separate tube of S-adenosylmethionine (SAM). To obtain 100% activity, SAM should be added to the 1X reaction mix as indicated. When required, a concentrated stock of SAM is supplied with the enzyme.

dam dcm CpG This restriction enzyme is sensitive to dam, dcm, or CpG methylation. (Note that CpG methylation is applicable to eukaryotic genomic DNA only.) For more information, see pages 334–336.

65° 80° No Indicates whether or not the enzyme can be heat inactivated. Enzymes are first tested by incubation at 65°C for 20 minutes; any enzyme not inactivated at 65°C is then tested by incubation at 80°C for 20 minutes. If an enzyme can be heat inactivated, the temperature is indicated in the icon.

25° 37° 50° 55° 60° 65° 75°
Indicates the enzyme's optimal incubation temperature.

dilA dilB dilC Indicates which diluent buffer (A, B or C) is recommended for making dilutions of restriction enzymes. For more information see pages 293–298.

Enzymes for Innovation.

- AatII
- AbaSI
- AccI
- Acc65I
- AciI
- AclI
- AcuI
- AfeI
- AflII
- AflIII
- AgeI
- AgeI-HF
- AhdI
- AleI-v2
- AluI
- AlwI
- AlwNI
- ApaI
- ApaLI
- ApeKI
- ApoI
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- BslI
- BsmI
- BsmAI
- BsmBI
- BsmFI
- BsoBI
- Bsp1286I
- BspCNI
- BspDI
- BspEI
- BspHI
- BspMI
- BspQI
- BsrI
- BsrBI
- BsrDI
- BsrFI-v2
- BsrGI
- BsrGI-HF
- BssHII
- BssSI-v2
- BstAPI
- BstBI
- BstEII
- BstEII-HF
- BstNI
- BstUI
- BstXI
- BstYI
- BstZ17I-HF
- Bsu36I
- BtgI
- BtgZI
- BtsI-v2
- BtsIMutI
- BtsCI
- Cac8I
- ClaI
- CspCI
- CviAII
- CviKI-1
- CviQ1
- DdeI
- DpnI
- DpnII
- DraI
- DraIII-HF
- DrdI
- EaeI
- EagI
- EagI-HF
- EarI
- EciI
- Eco53kI
- EcoNI
- EcoO109I
- EcoP15I
- EcoRI
- EcoRI-HF
- EcoRV
- EcoRV-HF
- Esp3I
- FatI
- FauI
- Fnu4HI
- FokI
- FseI
- FspI
- FspEI
- HaeII
- HaeIII
- HgaI
- HhaI
- HincII
- HindIII
- HindIII-HF
- HinfI
- HinP1I
- HpaI
- HpaII
- HphI
- Hpy99I
- Hpy166II
- Hpy188I
- Hpy188III
- HpyAV
- HpyCH4III
- HpyCH4IV
- HpyCH4V
- KasI
- KpnI
- KpnI-HF
- LpnPI
- MboI
- MboII
- MfeI
- MfeI-HF
- MluI
- MluI-HF
- MluCI
- MlyI
- MmeI
- MnlI
- MscI
- MseI
- MslI
- MspI
- MspA1I
- MspJI
- MwoI
- NaeI
- NarI
- NciI
- NcoI
- NcoI-HF
- NdeI
- NgoMIV
- NheI
- NheI-HF
- NlaIII
- NlaIV
- NmeAIII
- NotI
- NotI-HF
- NruI
- NruI-HF
- NsiI
- NsiI-HF
- NspI
- PacI
- PaeR7I
- PciI
- PflFI
- PflMI
- PleI
- PluTI
- PmeI
- PmlI
- PpuMI
- PshAI
- PsiI
- PspGI
- PspOMI
- PspXI
- PstI
- PstI-HF
- PvuI
- PvuI-HF
- PvuII
- PvuII-HF
- RsaI
- RsrII
- SacI
- SacI-HF
- SacII
- SalI
- SalI-HF
- SapI
- Sau3AI
- Sau96I
- SbfI
- SbfI-HF
- ScaI-HF
- ScrFI
- SexAI
- SfaNI
- SfcI
- SfiI
- SfoI
- SgrAI
- SmaI
- SmlI
- SnaBI
- SpeI
- SpeI-HF
- SphI
- SphI-HF
- SrfI
- SspI
- SspI-HF
- StuI
- StyI
- StyI-HF
- StyD4I
- SwaI
- Taq$^{\alpha}$I
- TfiI
- TseI
- Tsp45I
- TspMI
- TspRI
- Tth111I
- XbaI
- XcmI
- XhoI
- XmaI
- XmnI
- ZraI

High-Fidelity Restriction Enzymes

AgeI-HF, ApoI-HF, BamHI-HF, BbsI-HF, BclI-HF, BmtI-HF, BsaI-HFv2, BsiWI-HF, BsrGI-HF, BstEII-HF, BstZ17I-HF, DraIII-HF, EagI-HF, EcoRI-HF, EcoRV-HF, HindIII-HF, KpnI-HF, MfeI-HF, NcoI-HF, NheI-HF, NotI-HF, NruI-HF, NsiI-HF, PstI-HF, PvuI-HF, PvuII-HF, SacI-HF, SalI-HF, SbfI-HF, ScaI-HF, SpeI-HF, SphI-HF, SspI-HF, StyI-HF
19, 301

Methylation Sensitive Restriction Enzymes for Epigenetics Studies

AbaSI, DpnI, DpnII, FspEI, HpaII, LpnPI, McrBC, MspI, MspJI
264–265

Nicking Endonucleases

Nb.BbvCI, Nb.BsmI, Nb.BsrDI, Nb.BssSI, Nb.BtsI, Nt.AlwI, Nt.BbvCI, Nt.BsmAI, Nt.BspQI, Nt.BstNBI, Nt.CviPII
53–55

Homing Endonucleases

I-CeuI, I-SceI, PI-PspI, PI-SceI
55–56

NEBuffers, Diluents, Gel Loading Dyes, BSA & Recombinant Albumin, Molecular Biology Grade, NEB Tube Opener
56–57

Enzymes in green are all 100% active in CutSmart Buffer (see page 293–298).

Looking to bring convenience to your workflow?

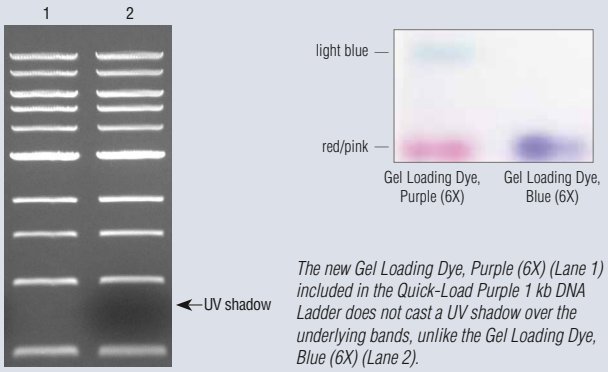
Speed up digestions with Time-Saver™ Qualified Restriction Enzymes

There are 195 NEB restriction enzymes that can digest DNA in 5–15 minutes; many of which are CutSmart or High-Fidelity (HF®) Restriction Enzymes. If you prefer, you can also digest overnight with no unwanted star activity. All of our enzymes are rigorously tested for nuclease contamination. Only NEB can offer enzymes with the power to digest in 5–15 minutes, and the flexibility to withstand overnight digestions with no loss of sample (see page 302–303).

For more information, visit **www.neb.com/timesaver**

Improve your analysis with our Purple Gel Loading Dye

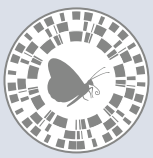
Our Gel Loading Dye, Purple (6X), which is supplied with most restriction enzymes and all HF enzymes, sharpens bands and eliminates the UV shadow seen with other dyes. This solution contains SDS, which often results in sharper bands, as some restriction enzymes are known to remain bound to DNA following cleavage.



The new Gel Loading Dye, Purple (6X) (Lane 1) included in the Quick-Load Purple 1 kb DNA Ladder does not cast a UV shadow over the underlying bands, unlike the Gel Loading Dye, Blue (6X) (Lane 2).

Bring flexibility to your workflow

NEB offers the largest selection of restriction enzymes commercially available. With an evergrowing list to choose from, currently at 286 restriction enzymes – including traditional restriction enzymes, nicking endonucleases, homing endonucleases and methylation-sensitive enzymes for epigenetics studies – there is no need to look anywhere else.



Learn about the benefits of CutSmart.

Simplify reaction setup and double digestion with CutSmart® Buffer

Over > 215 enzymes are 100% active in a single buffer, CutSmart Buffer, making it significantly easier to set up double digest reactions. Since CutSmart Buffer includes BSA, there are fewer tubes and pipetting steps to worry about. Additionally, many DNA modifying enzymes are 100% functionally active in CutSmart Buffer, eliminating the need for subsequent purification (see page 299).

For more information, visit **www.NEBCutSmart.com**

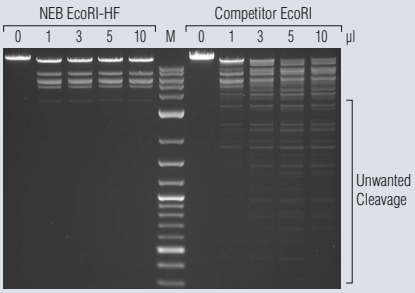
Looking to optimize performance in your reaction?

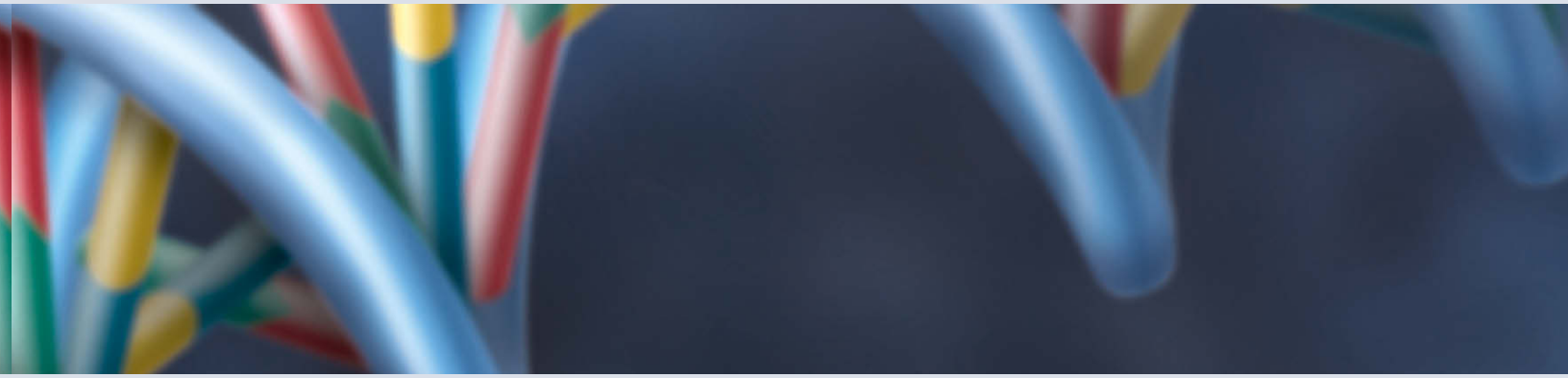
Choose a High-Fidelity (HF) Restriction Enzyme

NEB High-Fidelity (HF) restriction enzymes have the same specificity as native enzymes, with the added benefits of reduced star activity, rapid digestion (5–15 minutes), and 100% activity in CutSmart Buffer. Enjoy the improved performance of our engineered enzymes at the same price as the native enzymes!

For more information, visit **www.neb.com/HF**

EcoRI-HF (NEB #R3101) shows no star activity in overnight digests, even when used at higher concentrations. 50 µl rxns were set up using 1 µg of Lambda DNA, the indicated amount of enzyme and the recommended reaction buffer. Rxns were incubated overnight at 37°C. Marker M is the 1 kb DNA Ladder (NEB #N3232).



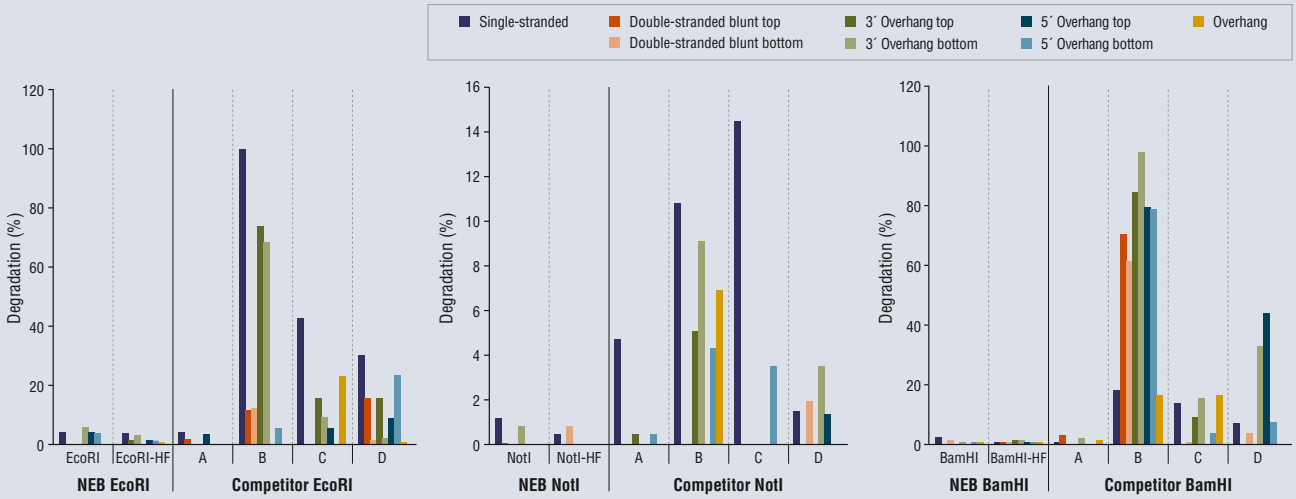


Benefit from industry-leading quality controls

NEB's reputation as a leader in enzyme technologies stems from the quality and reliability of our restriction enzymes. All of our restriction enzymes undergo stringent quality control testing, ensuring the highest levels of purity and lot-to-lot consistency.

For more information on quality at NEB, visit **www.neb.com/quality**

Restriction Enzyme Competitor Study: Nuclease Contamination



EcoRI, NotI, and BamHI from multiple suppliers were tested in reactions containing a fluorescent labeled single stranded, double stranded blunt, 3´ overhang or 5´ overhang containing oligonucleotides. The percent degradation is determined by capillary electrophoresis and peak analysis. The resolution is at the single nucleotide level.

Learn about the benefits of HF enzymes.

AatII

CutSmart · RR · dilB · 37° · 80° · CpG

#R0117S 500 units
#R0117L 2,500 units

5′... G A C G T C ...3′
3′... C T G C A G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50*	50	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: *May exhibit star activity in this buffer.

AbaSI

CutSmart · RR · Epi · dilC · 25° · 65°

#R0665S 1,000 units

See page 264 for more information.

AccI

CutSmart · RR · dilA · 37° · 80° · CpG

#R0161S 1,000 units
#R0161L 5,000 units

5′... G T M K A C ...3′
3′... C A K M T G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	50	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

Acc65I

RR · NEB3.1 · dilA · 37° · 65° · dcm · CpG

#R0599S 2,000 units
#R0599L 10,000 units

5′... G G T A C C ...3′
3′... C C A T G G ...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	75*	100	25

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: *May exhibit star activity in this buffer.

AciI

CutSmart · RR · dilA · 37° · 65° · CpG

#R0551S 200 units
#R0551L 1,000 units

5′... C C G C ...3′
3′... G G C G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	25	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

AclI

CutSmart · RR · dilB · 37° · No · CpG

#R0598S 300 units
#R0598L 1,500 units

5′... A A C G T T ...3′
3′... T T G C A A ...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	<10	<10	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

AcuI

CutSmart · RR · SAM · dilB · 37° · 65°

#R0641S 300 units
#R0641L 1,500 units

5′... C T G A A G $(N)_{16}$ ...3′
3′... G A C T T C $(N)_{14}$ ...5′

Reaction Conditions: CutSmart Buffer + SAM, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	50	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

AfeI

CutSmart · RR · dilB · 37° · 65° · CpG

#R0652S 200 units
#R0652L 1,000 units

5′... A G C G C T ...3′
3′... T C G C G A ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	25	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

AflII

CutSmart · RR · dilA · 37° · 65°

#R0520S 2,000 units
#R0520L 10,000 units

5′... C T T A A G ...3′
3′... G A A T T C ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

AflIII

#R0541S 250 units
#R0541L 1,250 units

5′... A▼CRYGT...3′
3′... TGYRC▲A...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50	100	50

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

AgeI

#R0552S 300 units
#R0552L 1,500 units

5′... A▼CCGGT...3′
3′... TGGCC▲A...5′

Reaction Conditions: NEBuffer 1.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	75	25	75

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from extended digestion.

AgeI-HF®

High-Fidelity

#R3552S 300 units
#R3552L 1,500 units

5′... A▼CCGGT...3′
3′... TGGCC▲A...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

AhdI

#R0584S 1,000 units
#R0584L 5,000 units

5′...GACNNNNNGTC...3′
3′...CTGNNNNNCAG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	25	10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 334).

NEW

AleI-v2

#R0685S 500 units
#R0685L 2,500 units

5′...CACNNNNGTG...3′
3′...GTGNNNNCAC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	10	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Impaired by overlapping CpG methylation (see p. 334).

AluI

#R0137S 1,000 units
#R0137L 5,000 units

5′... AG▼CT...3′
3′... TC▲GA...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	50	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

AlwI

#R0513S 500 units
#R0513L 2,500 units

5′... GGATC(N)$_4$▼...3′
3′... CCTAG(N)$_5$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	50	10	100

Methylation Sensitivity: Blocked by *dam* methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or glycerol concentration of > 5%.

AlwNI

#R0514S 500 units
#R0514L 2,500 units

5′...CAGNNN▼CTG...3′
3′...GTC▲NNNGAC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	50	100

Methylation Sensitivity: Blocked by overlapping *dcm* methylation (see p. 334).

Note: Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5%, or pH > 8.0 may result in star activity (see p. 300).

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ApaI

CutSmart · RR · e · dilA · 25° · 65° · dcm · CpG

#R0114S 5,000 units
#R0114L 25,000 units

5′... G G G C C▼C ...3′
3′... C▲C C G G G ...5′

Reaction Conditions: CutSmart Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 50,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	25	<10	100

Activity at 37°C: 100% However, the half-life of ApaI at 37°C is only 30 minutes.

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

ApaLI

CutSmart · RR · e · dilA · 37° · No · CpG

#R0507S 2,500 units
#R0507L 12,500 units
for high (5X) concentration
#R0507M 12,500 units

5′... G▼T G C A C ...3′
3′... C A C G T▲G ...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

ApeKI

RR · e · NEB3.1 · dilB · 75° · No · CpG

#R0643S 250 units
#R0643L 1,250 units

5′... G▼C W G C ...3′
3′... C G W C▲G ...5′

Reaction Conditions: NEBuffer 3.1, 75°C

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	100	10

Activity at 37°C: 10%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

ApoI

RR · e · NEB3.1 · dilA · 50° · 80°

#R0566S 1,000 units
#R0566L 5,000 units

5′... R▼A A T T Y ...3′
3′... Y T T A A▲R ...5′

Reaction Conditions: NEBuffer 3.1, 50°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	75	100	75

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

High-Fidelity

ApoI-HF®

CutSmart · RR · e · e · dilB · 37° · 80°

#R3566S 1,000 units
#R3566L 5,000 units

5′... R▼A A T T Y ...3′
3′... Y T T A A▲R ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

AscI

CutSmart · RR · e · dilA · 37° · 80° · CpG

#R0558S 500 units
#R0558L 2,500 units

5′... G G▼C G C G C C ...3′
3′... C C G C G C▲G G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	10	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

AseI

RR · e · NEB3.1 · dilB · 37° · 65°

#R0526S 2,000 units
#R0526L 10,000 units
for high (5X) concentration
#R0526M 10,000 units

5′... AT▼T A A T ...3′
3′... T A A T▲T A ...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	100	10

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

AsiSI

CutSmart · RR · dilB · 37° · 80° · CpG

#R0630S 500 units
#R0630L 2,500 units

5′... G C G A T▼C G C ...3′
3′... C G C▲T A G C G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	25	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from extended digestion.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

AvaI

CutSmart · dinA · 37° · 80° · CpG

#R0152S 2,000 units
#R0152L 10,000 units
for high (5X) concentration
#R0152T 2,000 units
#R0152M 10,000 units

5′... C▼Y C G R G ...3′
3′... G R G C Y▲C ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	100	25	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

AvaII

CutSmart · dinA · 37° · 80° · dcm · CpG

#R0153S 2,000 units
#R0153L 10,000 units
for high (5X) concentration
#R0153M 10,000 units

5′... G▼G W C C ...3′
3′... C C W G▲G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	75	10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

AvrII

CutSmart · dinB · 37° · No

#R0174S 100 units
#R0174L 500 units

5′... C▼C T A G G ...3′
3′... G G A T C▲C ...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	50	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BaeI

CutSmart · SAM · dinA · 25° · 65° · CpG

#R0613S 250 units
#R0613L 1,250 units

5′..▼10(N) AC (N)4 GTAY C (N)12▼.3′
3′..▲15(N) TG (N)4 CATR G (N)7▲.5′

Reaction Conditions: CutSmart Buffer + SAM, 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	50	100

Activity at 37°C: 20%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

BaeGI

NEB3.1 · dinA · 37° · 80°

#R0708S 500 units

5′... G K G C M▼C ...3′
3′... C▲M C G K G ...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	75	100	25

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BamHI

NEB3.1 · dinA · 37° · No

#R0136S 10,000 units
#R0136L 50,000 units
for high (5X) concentration
#R0136T 10,000 units
#R0136M 50,000 units

5′... G▼G A T C C ...3′
3′... C C T A G▲G ...5′

Reaction Conditions: NEBuffer 3.1, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

High-Fidelity

BamHI-HF®

CutSmart · e · dinA · 37° · No

#R3136S 10,000 units
#R3136L 50,000 units
for high (5X) concentration
#R3136T 10,000 units
#R3136M 50,000 units

5′... G▼G A T C C ...3′
3′... C C T A G▲G ...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BanI

CutSmart · dinA · 37° · 65° · dcm · CpG

#R0118S 5,000 units

5′... G▼G Y R C C ...3′
3′... C C R Y G▲G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	25	<10	100

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BanII

#R0119S 2,000 units

5′... G R G C Y▼C ...3′
3′... C▲Y C G R G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	50	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

BbsI

#R0539S 300 units
#R0539L 1,500 units

5′... G A A G A C (N)$_2$▼...3′
3′... C T T C T G (N)$_6$▲...5′

Reaction Conditions: NEBuffer 2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	25	75

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Store at -80°C.

BbsI-HF®

High-Fidelity

#R3539S 300 units
#R3539L 1,500 units

5′... G A A G A C (N)$_2$▼...3′
3′... C T T C T G (N)$_6$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	10	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BbvI

#R0173S 300 units

5′... G C A G C (N)$_8$▼...3′
3′... C G T C G (N)$_{12}$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	25	100

Concentration: 2,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

BbvCI

#R0601S 100 units
#R0601L 500 units

5′... C C▼T C A G C ...3′
3′... G G A G T▲C G ...5′

Reaction Conditions: CutSmart Buffer, 37°C.

Concentration: 2,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	50	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

BccI

#R0704S 1,000 units
#R0704L 5,000 units

5′... C C A T C (N)$_4$▼...3′
3′... G G T A G (N)$_5$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	10	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

BceAI

#R0623S 50 units
#R0623L 250 units

5′... A C G G C (N)$_{12}$▼...3′
3′... T G C C G (N)$_{14}$▲...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	100	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

BcgI

#R0545S 250 units
#R0545L 1,250 units

5′...▼$_{10}$(N) C G A (N)$_6$ T G C (N)$_{12}$▼...3′
3′...▲$_{12}$(N) G C T (N)$_6$ A C G (N)$_{10}$▲...5′

Reaction Conditions: NEBuffer 3.1 + SAM, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	75*	100	50*

Methylation Sensitivity: Impaired by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: *May exhibit star activity in this buffer.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

BciVI

CutSmart RR e dinC 37° 80°

#R0596S 200 units
#R0596L 1,000 units

5′... G T A T C C $(N)_6$▼... 3′
3′... C A T A G G $(N)_5$▲... 5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	25	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BclI

RR e NEB3.1 dinA 50° No dam

#R0160S 3,000 units
#R0160L 15,000 units

5′... T▼G A T C A ... 3′
3′... A C T A G▲T ... 5′

Reaction Conditions: NEBuffer 3.1, 50°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	75

Activity at 37°C: 50%

Methylation Sensitivity: Blocked by *dam* methylation (see p. 334).

NEW

BclI-HF®

High-Fidelity

CutSmart RR e dinB 37° 65° dam

#R3160S 3,000 units
#R3160L 15,000 units

5′... T▼G A T C A ... 3′
3′... A C T A G▲T ... 5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Blocked by *dam* methylation (see p. 334).

BcoDI

CutSmart RR e dinB 37° No CpG

#R0542S 1,000 units

5′... G T C T C $(N)_1$▼... 3′
3′... C A G A G $(N)_5$▲... 5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	75	75	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 334).

BfaI

CutSmart RR dinB 37° 80°

#R0568S 500 units
#R0568L 2,500 units

5′... C▼T A G ... 3′
3′... G A T▲C ... 5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	10	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

BfuAI

RR 2*site e NEB3.1 dinB 50° 65° CpG

#R0701S 250 units
#R0701L 1,250 units

5′... A C C T G C $(N)_4$▼... 3′
3′... T G G A C G $(N)_8$▲... 5′

Reaction Conditions: NEBuffer 3.1, 50°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	25	100	10

Activity at 37°C: 50%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

BfuCI

BfuCI has been replaced by its isoschizomer Sau3AI.

BglI

RR e NEB3.1 dinB 37° 65° CpG

#R0143S 2,000 units
#R0143L 10,000 units

5′... G C C N N N N▼N G G C ... 3′
3′... C G G N▲N N N N C C G ... 5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	25	100	10

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

BglII

RR e NEB3.1 dinA 37° No

#R0144S 2,000 units
#R0144L 10,000 units
for high (5X) concentration
#R0144M 10,000 units

5′... A▼G A T C T ... 3′
3′... T C T A G▲A ... 5′

Reaction Conditions: NEBuffer 3.1, 37°C.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	10	100	<10

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BlpI

#R0585S 500 units
#R0585L 2,500 units

5′... G C▼T N A G C ...3′
3′... C G A N T▲C G ...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BmgBI

#R0628S 500 units
#R0628L 2,500 units

5′... C A C▼G T C ...3′
3′... G T G▲C A G ...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	10	100	10

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

BmrI

#R0600S 100 units

5′... A C T G G G(N)$_5$▼...3′
3′... T G A C C C(N)$_4$▲...5′

Reaction Conditions: NEBuffer 2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	75	100*

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

BmtI

#R0658S 300 units

5′... G C T A G▼C ...3′
3′... C▲G A T C G ...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	100	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

High-Fidelity

BmtI-HF®

#R3658S 300 units
#R3658L 1,500 units

5′... G C T A G▼C ...3′
3′... C▲G A T C G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	10	100

Concentration: 20,000 units/ml.

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BpmI

#R0565S 100 units
#R0565L 500 units

5′... C T G G A G(N)$_{16}$▼...3′
3′... G A C C T C(N)$_{14}$▲...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	100	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

Bpu10I

#R0649S 200 units
#R0649L 1,000 units

5′... C C▼T N A G C ...3′
3′... G G A N T▲C G ...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	25	100	25

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

BpuEI

#R0633S 500 units

5′... C T T G A G(N)$_{16}$▼...3′
3′... G A A C T C(N)$_{14}$▲...5′

Reaction Conditions: CutSmart Buffer + SAM, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50*	100	50*	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

BsaI

CutSmart · rr · dinB · 37° · 65° · dcm · CpG

#R0535S 1,000 units
#R0535L 5,000 units

5′... G G T C T C (N)$_1$▼... 3′
3′... C C A G A G (N)$_5$▲... 5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	75	100	100

Methylation Sensitivity: Impaired by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

BsaHI

CutSmart · rr · e · dinC · 37° · 80° · dcm · CpG

#R0556S 2,000 units

5′... G R▼C G Y C ... 3′
3′... C Y G C▲R G ... 5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

NEW

High-Fidelity

BsaI-HF®v2

CutSmart · rr · e · e · dinB · 37° · 80° · dcm · CpG

#R3733S 1,000 units
#R3733L 5,000 units

5′... G G T C T C (N)$_1$▼... 3′
3′... C C A G A G (N)$_5$▲... 5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	100	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Impaired by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

BsaJI

CutSmart · rr · dinA · 60° · 80°

#R0536S 1,000 units
#R0536L 5,000 units

5′... C▼C N N G G ... 3′
3′... G G N N C▲C ... 5′

Reaction Conditions: CutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100

Concentration: 10,000 units/ml

Activity at 37°C: 20%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsaAI

CutSmart · rr · e · dinC · 37° · No · CpG

#R0531S 500 units
#R0531L 2,500 units

5′... Y A C▼G T R ... 3′
3′... R T G▲C A Y ... 5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	100	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

BsaWI

CutSmart · rr · e · dinA · 60° · 80°

#R0567S 250 units

5′... W▼C C G G W ... 3′
3′... W G G C C▲W ... 5′

Reaction Conditions: CutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	50	100

Concentration: 10,000 units/ml

Activity at 37°C: 20%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsaBI

CutSmart · rr · dinB · 60° · 80° · dam · CpG

#R0537S 2,000 units
#R0537L 10,000 units

5′... G A T N N▼N N A T C ... 3′
3′... C T A N N▲N N T A G ... 5′

Reaction Conditions: CutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 20%

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	75	100

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: Star activity may result from extended digestion.

BsaXI

CutSmart · e · dinC · 37° · No

#R0609S 100 units
#R0609L 500 units

5′... ▼$_9$(N) A C (N)$_8$ C T C C (N)$_{10}$▼... 3′
3′... ▲$_{12}$(N) T G (N)$_7$ G A G G (N)$_7$▲... 5′

Reaction Conditions: CutSmart Buffer, 37°C

Concentration: 2,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50*	100*	10	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BseRI

#R0581S 200 units
#R0581L 1,000 units

5′...GAGGAG(N)$_{10}$▼...3′
3′...CTCCTC(N)$_{8}$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	75	100

Concentration: 5,000 units/ml.

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BseYI

#R0635S 100 units
#R0635L 500 units

5′...C▼CAGC...3′
3′...GGGTC▲G...5′

Reaction Conditions: NEBuffer 3 1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50	100	50

Concentration: 5,000 units/ml.

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

BsgI

#R0559S 50 units
#R0559L 250 units

5′...GTGCAG(N)$_{16}$▼...3′
3′...CACGTC(N)$_{14}$▲...5′

Reaction Conditions: CutSmart Buffer + SAM, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	25	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsiEI

#R0554S 1,000 units

5′...CGRY▼CG...3′
3′...GC▲YRGC...5′

Reaction Conditions: CutSmart Buffer, 60°C.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	<10	100

Activity at 37°C: 30%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

BsiHKAI

#R0570S 1,000 units

5′...GWGCW▼C...3′
3′...C▲WCGWG...5′

Reaction Conditions: CutSmart Buffer, 65°C.

Concentration: 10,000 and 50,000 units/ml.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Activity at 37°C: 5%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsiWI

#R0553S 300 units
#R0553L 1,500 units

5′...C▼GTACG...3′
3′...GCATG▲C...5′

Reaction Conditions: NEBuffer 3.1, 55°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50*	100	25

Activity at 37°C: 50%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: *May exhibit star activity in this buffer.

High-Fidelity

BsiWI-HF®

#R3553S 300 units
#R3553L 1,500 units

5′...C▼GTACG...3′
3′...GCATG▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

BsmI

#R0555S 1,000 units
#R0555L 5,000 units

5′...CCNNNNN▼NNGG...3′
3′...GGNN▲NNNNNCC...5′

Reaction Conditions: CutSmart Buffer, 55°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	75	100	100

Activity at 37°C: 30%

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

BsmI

CutSmart | dirA | 65° | 80°

#R0134S 500 units
#R0134L 2,500 units

5′...G A A T G C N▼...3′
3′...C T T A C▲G N...5′

Reaction Conditions: CutSmart Buffer, 65°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	<10	100

Concentration: 10,000 units/ml

Activity at 37°C: 20%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsmAI

CutSmart | dirB | 55° | No | CpG

#R0529S 1,000 units
#R0529L 5,000 units

5′...G T C T C (N)$_1$▼...3′
3′...C A G A G (N)$_5$▲...5′

Reaction Conditions: CutSmart Buffer, 55°C

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100

Activity at 37°C: 50%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

BsmBI

NEB3.1 | dirB | 55° | 80° | CpG

#R0580S 200 units
#R0580L 1,000 units

5′...C G T C T C (N)$_1$▼...3′
3′...G C A G A G (N)$_5$▲...5′

Reaction Conditions: NEBuffer 3.1, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50*	100	25

Activity at 37°C: 20%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: *May exhibit star activity in this buffer.

BsmFI

CutSmart | dirA | 65° | 80° | dcm | CpG

#R0572S 100 units
#R0572L 500 units

5′...G G G A C (N)$_{10}$▼...3′
3′...C C C T G (N)$_{14}$▲...5′

Reaction Conditions: CutSmart Buffer, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 2,000 units/ml

Activity at 37°C: 50%

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	50	100

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or glycerol concentration of > 5%.

BsoBI

CutSmart | dirA | 37° | 80°

#R0586S 10,000 units

5′...C▼Y C G R G...3′
3′...G R G C Y▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Bsp1286I

CutSmart | dirA | 37° | 65°

#R0120S 500 units

5′...G D G C H▼C...3′
3′...C▲H C G D G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	25	25	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

BspCNI

CutSmart | SAM | dirA | 25° | 80°

#R0624S 100 units

5′...C T C A G (N)$_{10}$▼...3′
3′...G A G T C (N)$_8$▲...5′
and
5′...C T C A G (N)$_9$▼...3′
3′...G A G T C (N)$_7$▲...5′

Note: The cleavage site of BspCNI varies. Two equally represented species of fragments are produced from BspCNI cleavage.

Reaction Conditions: CutSmart Buffer + SAM, 25°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 2,000 units/ml

Activity at 37°C: 75%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	75	10	100

BspDI

CutSmart | dirA | 37° | 80° | dam | CpG

#R0557S 2,000 units

5′...A T▼C G A T...3′
3′...T A G C▲T A...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	75	50	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BspEI

#R0540S 1,000 units
#R0540L 5,000 units

5′... T▼C C G G A ...3′
3′... A G G C C▲T ... 5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	10	100	<10

Concentration: 10,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is impaired by CpG methylation (see p. 334).

BspHI

#R0517S 500 units
#R0517L 2,500 units

5′... T▼C A T G A ...3′
3′... A G T A C▲T ... 5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	25	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Impaired by overlapping *dam* methylation (see p. 334).

BspMI

#R0502S 100 units

5′... A C C T G C (N)$_4$▼... 3′
3′... T G G A C G (N)$_8$▲... 5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50*	100	10

Concentration: 2,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

BspQI

#R0712S 500 units .....
#R0712L 2,500 units .....

5′... G C T C T T C (N)$_1$▼... 3′
3′... C G A G A A G (N)$_4$▲... 5′

Reaction Conditions: NEBuffer 3.1, 50°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100*	100*	100	100*

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

BsrI

#R0527S 1,000 units
#R0527L 5,000 units

5′... A C T G G N▼... 3′
3′... T G A C▲C N ... 5′

Reaction Conditions: NEBuffer 3.1, 65°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	100	10

Concentration: 10,000 units/ml

Activity at 37°C: 20%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsrBI

#R0102S 1,000 units
#R0102L 5,000 units

5′... C C G▼C T C ...3′
3′... G G C▲G A G ... 5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

BsrDI

#R0574S 200 units
#R0574L 1,000 units

5′... G C A A T G N N▼... 3′
3′... C G T T A C▲N N ... 5′

Reaction Conditions: NEBuffer 2.1, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	75	25

Activity at 37°C: 30%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

BsrFI-v2

#R0682S 1,000 units

5′... R▼C C G G Y ...3′
3′... Y G G C C▲R ... 5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	25	0	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

BsrGI

#R0575S 1,000 units
#R0575L 5,000 units

5′... T▼G T A C A ...3′
3′... A C A T G▲T ...5′

Reaction Conditions: NEBuffer 2.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	**2.1**	3.1	CutSmart
% Activity	25	**100**	100	25

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsrGI-HF®

High-Fidelity

#R3575S 1,000 units
#R3575L 5,000 units

5′... T▼G T A C A ...3′
3′... A C A T G▲T ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	**CutSmart**
% Activity	10	100	100	**100**

Concentration: 20,000 units/ml.

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BssHII

#R0199S 500 units
#R0199L 2,500 units
for high (5X) concentration
#R0199M 2,500 units

5′... G▼C G C G C ...3′
3′... C G C G C▲G ...5′

Reaction Conditions: CutSmart Buffer, 50°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	**CutSmart**
% Activity	100	100	100	**100**

Concentration: 5,000 and 25,000 units/ml

Activity at 37°C: 75%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

BssSI-v2

#R0680S 200 units
#R0680L 1,000 units

5′... C▼A C G A G ...3′
3′... G T G C T▲C ...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	**CutSmart**
% Activity	10	25	<10	**100**

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BstAPI

#R0654S 200 units
#R0654L 1,000 units

5′... G C A N N N N▼N T G C ...3′
3′... C G T N N N N N▲A C G ...5′

Reaction Conditions: CutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	**CutSmart**
% Activity	50	100	25	**100**

Activity at 37°C: 10%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

BstBI

#R0519S 2,500 units
#R0519L 12,500 units

5′... T T▼C G A A ...3′
3′... A A G C▲T T ...5′

Reaction Conditions: CutSmart Buffer, 65°C

Concentration: 20,000 units/ml

NEBuffer	1.1	2.1	3.1	**CutSmart**
% Activity	75	100	10	**100**

Activity at 37°C: 10%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

BstEII

#R0162S 2,000 units
#R0162L 10,000 units
for high (5X) concentration
#R0162M 10,000 units

5′... G▼G T N A C C ...3′
3′... C C A N T G▲G ...5′

Reaction Conditions: NEBuffer 3.1, 60°C

Concentration: 10,000 and 50,000 units/ml

NEBuffer	1.1	2.1	**3.1**	CutSmart
% Activity	10	75	**100**	75

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

BstEII-HF®

High-Fidelity

#R3162S 2,000 units
#R3162L 10,000 units
for high (5X) concentration
#R3162M 10,000 units

5′... G▼G T N A C C ...3′
3′... C C A N T G▲G ...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	**CutSmart**
% Activity	<10	10	<10	**100**

Concentration: 20,000 and 100,000 units/ml.

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BstNI

#R0168S 3,000 units
#R0168L 15,000 units

5′... C C^W G G ...3′
3′... G G W C^ C ...5′

Reaction Conditions: NEBuffer 3.1, 60°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	100	75

Activity at 37°C: 30%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

High-Fidelity

BstZ17I-HF®

#R3594S 1,000 units
#R3594L 5,000 units

5′... G T A^ T A C ...3′
3′... C A T^ A T G ...5′

Reaction Conditions: CutSmart Buffer, 37°C.

Concentration: 20,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

BstUI

#R0518S 1,000 units
#R0518L 5,000 units

5′... C G^ C G ...3′
3′... G C^ G C ...5′

Reaction Conditions: CutSmart Buffer, 60°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	25	100

Activity at 37°C: 20%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Bsu36I

#R0524S 1,000 units
#R0524L 5,000 units

5′... C C^ T N A G G ...3′
3′... G G A N T^ C C ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BstXI

#R0113S 1,000 units
#R0113L 5,000 units

5′... C C A N N N N N N^ N T G G ...3′
3′... G G T N^ N N N N N N A C C ...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	100	25

Concentration: 10,000 units/ml

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

BtgI

#R0608S 1,000 units

5′... C^ C R Y G G ...3′
3′... G G Y R C^ C ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BstYI

#R0523S 2,000 units
#R0523L 10,000 units

5′... R^ G A T C Y ...3′
3′... Y C T A G^ R ...5′

Reaction Conditions: NEBuffer 2.1, 60°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	75	100

Activity at 37°C: 30%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BtgZI

#R0703S 100 units
#R0703L 500 units

5′... G C G A T G (N)$_{10}$^ ...3′
3′... C G C T A C (N)$_{14}$^ ...5′

Reaction Conditions: CutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	25	<10	100

Activity at 37°C: 75%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

BtsI-v2

CutSmart RR e dA 55° No

#R0667S 500 units
#R0667L 2,500 units

5′...GCAGTGNN▼...3′
3′...CGTCAC▲NN...5′

Reaction Conditions: CutSmart Buffer, 55°C.

Concentration: 10,000 units/ml

Activity at 37°C: 75%

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	25	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

BtsIMutI

CutSmart RR e dA 55° 80°

#R0664S 100 units

5′...CAGTGNN▼...3′
3′...GTCAC▲NN...5′

Reaction Conditions: CutSmart Buffer, 55°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	10	100

Concentration: 1,000 units/ml

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BtsCI

CutSmart RR dB 50° 80°

#R0647S 2,000 units

5′...GGATGNN▼...3′
3′...CCTAC▲NN...5′

Reaction Conditions: CutSmart Buffer, 50°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	25	100

Concentration: 20,000 units/ml

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Cac8I

CutSmart dB 37° 65° CpG

#R0579S 100 units
#R0579L 500 units

5′...GCN▼NGC...3′
3′...CGNNC▲G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	75	100	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

ClaI

CutSmart RR dA 37° 65° dam CpG

#R0197S 1,000 units
#R0197L 5,000 units

5′...AT▼CGAT...3′
3′...TAGC▲TA...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50	50	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

CspCI

CutSmart RR 2*site SAM dA 37° 65°

#R0645S 500 units

5′...▼$_{10\text{-}11}$(N)CAA(N)$_5$GTGG(N)$_{12\text{-}13}$▼...3′
3′...$_{12\text{-}13}$▲(N)GTT(N)$_5$CACC(N)$_{10\text{-}11}$▲...5′

Note: The cleavage point may shift one base pair depending on the DNA sequence context before and after the recognition site. For a given sequence, one site will predominate. For details, see www.neb.com.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	10	100

Reaction Conditions: CutSmart Buffer + SAM, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

CviAII

CutSmart RR dC 25° 65°

#R0640S 200 units
#R0640L 1,000 units

5′...C▼ATG...3′
3′...GTA▲C...5′

Reaction Conditions: CutSmart Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	50	10	100

Concentration: 10,000 units/ml

Activity at 37°C: 20%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

CviKI-1

CutSmart RR dA 37° No

#R0710S 250 units

5′...RG▼CY...3′
3′...YC▲GR...5′

Reaction Conditions: CutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

CviQI

#R0639S 2,000 units
#R0639L 10,000 units

5′...GTAC...3′
3′...CATG...5′

Reaction Conditions: NEBuffer 3.1, 25°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100*	100	75*

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

DdeI

#R0175S 1,000 units
#R0175L 5,000 units

5′...CTNAG...3′
3′...GANTC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

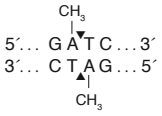
NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

DpnI

#R0176S 1,000 units
#R0176L 5,000 units



Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	75	100

Concentration: 20,000 units/ml

Methylation Sensitivity: DpnI cleaves only when its recognition site is methylated. DNA purified from a *dam*+ strain will be a substrate for DpnI. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

DpnII

#R0543S 1,000 units
#R0543L 5,000 units

for high (5X) concentration
#R0543T 1,000 units
#R0543M 5,000 units

5′...GATC...3′
3′...CTAG...5′

Reaction Conditions: NEBuffer DpnII, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	25	100*	25

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Blocked by *dam* methylation (see p. 334).

Note: *May exhibit star activity in this buffer.

DraI

#R0129S 2,000 units
#R0129L 10,000 units

5′...TTTAAA...3′
3′...AAATTT...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	75	50	100

Concentration: 20,000 units/ml.

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

High-Fidelity

DraIII-HF®

#R3510S 1,000 units
#R3510L 5,000 units

5′...CACNNNGTG...3′
3′...GTGNNNCAC...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 334).

DrdI

#R0530S 300 units
#R0530L 1,500 units

5′...GACNNNNNNGTC...3′
3′...CTGNNNNNNCAG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

EaeI

#R0508S 200 units
#R0508L 1,000 units

5′...YGGCCR...3′
3′...RCCGGY...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50	<10	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · diluent A Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

EagI

#R0505S 500 units
#R0505L 2,500 units
for high (5X) concentration
#R0505M 2,500 units

5′...C▼GGCCG...3′
3′...GCCGG▲C...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	25	100	10

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Eco53kI

#R0116S 1,000 units

5′...GAG▼CTC...3′
3′...CTC▲GAG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	<10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

High-Fidelity

EagI-HF®

#R3505S 500 units
#R3505L 2,500 units
for high (5X) concentration
#R3505M 2,500 units

5′...C▼GGCCG...3′
3′...GCCGG▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

EcoNI

#R0521S 1,000 units
#R0521L 5,000 units

5′...CCTNN▼NNNAGG...3′
3′...GGANNN▲NNTCC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	75	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

EarI

#R0528S 500 units
#R0528L 2,500 units

5′...CTCTTC(N)$_1$▼...3′
3′...GAGAAG(N)$_4$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	10	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 334).

EcoO109I

#R0503S 2,000 units

5′...RG▼GNCCY...3′
3′...YCCNG▲GR...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	50	100

Methylation Sensitivity: Blocked by overlapping *dcm* methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

EciI

#R0590S 100 units
#R0590L 500 units

5′...GGCGGA(N)$_{11}$▼...3′
3′...CCGCCT(N)$_9$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	50	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: Star activity may result from extended digestion.

EcoP15I

#R0646S 500 units
#R0646L 2,500 units

5′...CAGCAG(N)$_{25}$▼...3′
3′...GTCGTC(N)$_{27}$▲...5′

Reaction Conditions: NEBuffer 3.1 + ATP, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

EcoRI

#R0101S	10,000 units
#R0101L	50,000 units
for high (5X) concentration	
#R0101T	10,000 units
#R0101M	50,000 units

5′...G▼AATTC...3′
3′...CTTAA▲G...5′

Reaction Conditions: NEBuffer EcoRI, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100*	50	50*

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combination of overlapping CpG methylation (see p. 334).

Note: *May exhibit star activity in this buffer.

NEW

Esp3I

#R0734S	300 units
#R0734L	1,500 units

5′...CGTCTC(N)$_1$▼...3′
3′...GCAGAG(N)$_5$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

High-Fidelity

EcoRI-HF®

#R3101S	10,000 units
#R3101L	50,000 units
for high (5X) concentration	
#R3101T	10,000 units
#R3101M	50,000 units

5′...G▼AATTC...3′
3′...CTTAA▲G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	<10	100

Concentration: 20,000 and 100,00 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

FatI

#R0650S	50 units
#R0650L	250 units

5′...▼CATG...3′
3′...GTAC▲...5′

Reaction Conditions: NEBuffer 2.1, 55°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	50	50

Concentration: 2,000 units/ml

Activity at 37°C: 20%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

EcoRV

#R0195S	4,000 units
#R0195L	20,000 units
for high (5X) concentration	
#R0195T	4,000 units
#R0195M	20,000 units

5′...GAT▼ATC...3′
3′...CTA▲TAG...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50	100	10

Concentration: 20,000 and 100,000 units/ml.

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 334).

FauI

#R0651S	200 units
#R0651L	1,000 units

5′...CCCGC(N)$_4$▼...3′
3′...GGGCG(N)$_6$▲...5′

Reaction Conditions: CutSmart Buffer, 55°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	10	100

Activity at 37°C: 20%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

High-Fidelity

EcoRV-HF®

#R3195S	4,000 units
#R3195L	20,000 units
for high (5X) concentration	
#R3195T	4,000 units
#R3195M	20,000 units

5′...GAT▼ATC...3′
3′...CTA▲TAG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 334).

Fnu4HI

#R0178S	200 units
#R0178L	1,000 units

5′...GC▼NGC...3′
3′...CGN▲CG...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	<10	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

FokI

CutSmart · RR · 2°site · diiA · 37° · 65° · dcm · CpG

#R0109S 1,000 units
#R0109L 5,000 units

5′...G G A T G $(N)_9$▼...3′
3′...C C T A C $(N)_{13}$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	75	100

Methylation Sensitivity: Impaired by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

FseI

CutSmart · RR · diiB · 37° · 65° · dcm · CpG

#R0588S 100 units
#R0588L 500 units

5′...G G C C G G▼C C...3′
3′...C C▲G G C C G G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	75	<10	100

Concentration: 2,000 units/ml

Methylation Sensitivity: Impaired by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

FspI

CutSmart · RR · diiC · 37° · NO · CpG

#R0135S 500 units
#R0135L 2,500 units

5′...T G C▼G C A...3′
3′...A C G▲C G T...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	10	100

Concentration: 10,000 units/ml.

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

FspEI

CutSmart · RR · Epi · diiB · 37° · 80°

#R0662S 200 units

See page 264 for more information.

HaeII

CutSmart · RR · diiA · 37° · 80° · CpG

#R0107S 2,000 units
#R0107L 10,000 units

5′...R G C G C▼Y...3′
3′...Y▲C G C G R...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

HaeIII

CutSmart · RR · diiA · 37° · 80°

#R0108S 3,000 units
#R0108L 15,000 units
for high (5X) concentration
#R0108T 3,000 units
#R0108M 15,000 units

5′...G G▼C C...3′
3′...C C▲G G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	25	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

HgaI

RR · NEB1.1 · diiA · 37° · 65° · CpG

#R0154S 100 units
#R0154L 500 units

5′...G A C G C $(N)_5$▼...3′
3′...C T G C G $(N)_{10}$▲...5′

Reaction Conditions: NEBuffer 1.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	25	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

HhaI

CutSmart · RR · diiA · 37° · 65° · CpG

#R0139S 2,000 units
#R0139L 10,000 units

5′...G C G▼C...3′
3′...C▲G C G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

HincII

RR · NEB3.1 · diiB · 37° · 65° · CpG

#R0103S 1,000 units
#R0103L 5,000 units

5′...G T Y▼R A C...3′
3′...C A R▲Y T G...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

HindIII

#R0104S 10,000 units
#R0104L 50,000 units
for high (5X) concentration
#R0104T 10,000 units
#R0104M 50,000 units

5′...A▼AGCTT...3′
3′...TTCGA▲A...5′

Reaction Conditions: NEBuffer 2.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	50	50

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

HpaI

#R0105S 500 units
#R0105L 2,500 units

5′...GTT▼AAC...3′
3′...CAA▲TTG...5′

Reaction Conditions: CutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	75	25	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

High-Fidelity

HindIII-HF®

#R3104S 10,000 units
#R3104L 50,000 units
for high (5X) concentration
#R3104T 10,000 units
#R3104M 50,000 units

5′...A▼AGCTT...3′
3′...TTCGA▲A...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

HpaII

#R0171S 2,000 units
#R0171L 10,000 units
for high (5X) concentration
#R0171M 10,000 units

5′...C▼CGG...3′
3′...GGC▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	<10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

HinfI

#R0155S 5,000 units
#R0155L 25,000 units
for high (5X) concentration
#R0155T 5,000 units
#R0155M 25,000 units

5′...G▼ANTC...3′
3′...CTNA▲G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

HphI

#R0158S 1,000 units
#R0158L 5,000 units

5′...GGTGA $(N)_8$▼...3′
3′...CCACT $(N)_7$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	50	<10	100

Methylation Sensitivity: This enzyme is blocked by overlapping *dam* and *dcm* methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

HinP1I

#R0124S 2,000 units

5′...G▼CGC...3′
3′...CGC▲G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Hpy99I

#R0615S 100 units
#R0615L 500 units

5′...CGWCG▼...3′
3′...▲GCWGC...5′

Reaction Conditions: CutSmart Buffer Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	10	<10	100

Concentration: 2,000 units/ml.

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

Hpy166II

CutSmart · RR · dil C · 37° · 65° · CpG

#R0616S 1,000 units

5′... G T N▼N A C ...3′
3′... C A N N T G ▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	50	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

HpyCH4III

CutSmart · RR · dil A · 37° · 65°

#R0618S 250 units
#R0618L 1,250 units

5′... A C N▼G T ...3′
3′... T G▲N C A ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	25	<10	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Hpy188I

CutSmart · RR · dil A · 37° · 65° · dam

#R0617S 1,000 units
#R0617L 5,000 units

5′... T C N▼G A ...3′
3′... A G▲N C T ...5′

Reaction Conditions: CutSmart Buffer, 37°C.Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	50	100

Methylation Sensitivity: Blocked by overlapping *dam* methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

HpyCH4IV

CutSmart · RR · dil A · 37° · 65° · CpG

#R0619S 500 units
#R0619L 2,500 units

5′... A▼C G T ...3′
3′... T G C▲A ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	25	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Hpy188III

CutSmart · RR · dil B · 37° · 65° · dam · CpG

#R0622S 500 units
#R0622L 2,500 units

5′... T C▼N N G A ...3′
3′... A G N N▲C T ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	10	100

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

HpyCH4V

CutSmart · RR · dil A · 37° · 65°

#R0620S 100 units
#R0620L 500 units

5′... T G▼C A ...3′
3′... A C▲G T ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	50	25	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

HpyAV

CutSmart · RR · 37° · 65° · CpG

#R0621S 100 units
#R0621L 500 units

5′... C C T T C (N)$_6$▼...3′
3′... G G A A G (N)$_5$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	25	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation. (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

KasI

CutSmart · RR · dil B · 37° · 65° · CpG

#R0544S 250 units
#R0544L 1,250 units

5′... G▼G C G C C ...3′
3′... C C G C G▲G ...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	50	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

KpnI

#R0142S 4,000 units
#R0142L 20,000 units
for high (5X) concentration
#R0142M 20,000 units

5′...GGTAC▼C...3′
3′...C▲CATGG...5′

Reaction Conditions: NEBuffer 1.1, 37°C

Concentration: 10,000 and 50,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	75	<10	50

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

KpnI-HF®

High-Fidelity

#R3142S 4,000 units
#R3142L 20,000 units
for high (5X) concentration
#R3142M 20,000 units

5′...GGTAC▼C...3′
3′...C▲CATGG...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	25	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

LpnPI

#R0663S 200 units

See page 264 for more information.

MboI

#R0147S 500 units
#R0147L 2,500 units
for high (5X) concentration
#R0147M 2,500 units

5′...▼GATC...3′
3′...CTAG▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	100	100

Concentration: 5,000 and 25,000 units/ml

Methylation Sensitivity: Blocked by *dam* methylation. Its isoschizomer Sau3AI is not. Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 334).

MboII

#R0148S 300 units
#R0148L 1,500 units

5′...GAAGA(N)$_8$▼...3′
3′...CTTCT(N)$_7$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100*	100	50	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation (see p. 334).

Note: *May exhibit star activity in this buffer.

MfeI

#R0589S 500 units
#R0589L 2,500 units

5′...C▼AATTG...3′
3′...GTTAA▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	50	10	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

MfeI-HF®

High-Fidelity

#R3589S 500 units
#R3589L 2,500 units

5′...C▼AATTG...3′
3′...GTTAA▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	25	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MluI

#R0198S 1,000 units
#R0198L 5,000 units

5′...A▼CGCGT...3′
3′...TGCGC▲A...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50	100	25

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

MluI-HF®

High-Fidelity

#R3198S 1,000 units
#R3198L 5,000 units

5′...A▼CGCGT...3′
3′...TGCGC▲A...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

MluCI

CutSmart · RR · dif A · 37° · No

#R0538S 1,000 units
#R0538L 5,000 units

5′...▼A A T T...3′
3′...T T A A▲...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	10	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MlyI

CutSmart · RR · dif A · 37° · 65°

#R0610S 1,000 units
#R0610L 5,000 units

5′...G A G T C (N)$_5$▼...3′
3′...C T C A G (N)$_5$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	50	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MmeI

CutSmart · RR · 2*site · SAM · dif B · 37° · 65° · CpG

#R0637S 100 units
#R0637L 500 units

5′...T C C R A C (N)$_{20}$▼...3′
3′...A G G Y T G (N)$_{18}$▲...5′

Reaction Conditions: CutSmart Buffer + SAM, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	50	100

Concentration: 2,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

MnlI

CutSmart · RR · dif B · 37° · 65°

#R0163S 500 units
#R0163L 2,500 units

5′...C C T C (N)$_7$▼...3′
3′...G G A G (N)$_6$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	50	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MscI

CutSmart · RR · dif C · 37° · 80° · dcm

#R0534S 250 units
#R0534L 1,250 units
for high (5X) concentration
#R0534M 1,250 units

5′...T G G▼C C A...3′
3′...A C C▲G G T...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 5,000 and 25,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dcm* methylation (see p. 334). The single MscI site in pBR322 overlaps a *dcm* methylation site; consequently, pBR322 which has been grown in a *dcm*$^-$ host should be used for cloning.

MseI

CutSmart · RR · dif A · 37° · 65°

#R0525S 500 units
#R0525L 2,500 units
for high (5X) concentration
#R0525M 2,500 units

5′...T▼T A A...3′
3′...A A T▲T...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	75	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MslI

CutSmart · RR · dif A · 37° · 80°

#R0571S 500 units
#R0571L 2,500 units

5′...C A Y N N▼N N R T G...3′
3′...G T R N N▲N N Y A C...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	50	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MspI

CutSmart · RR · Epi · dif A · 37° · No

#R0106S 5,000 units
#R0106L 25,000 units
for high (5X) concentration
#R0106T 5,000 units
#R0106M 25,000 units

5′...C▼C G G...3′
3′...G G C▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	50	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MspA1I

CutSmart · RR · dilB · 37° · 65° · CpG

#R0577S 500 units
#R0577L 2,500 units

5′...CMG▼CKG...3′
3′...GK▲CGMC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

MspJI

CutSmart · RR · Epi · dilB · 37° · 65°

#R0661S 200 units
#R0661L 1,000 units

See page 264 for more information.

MwoI

CutSmart · RR · dilB · 60° · No · CpG

#R0573S 500 units
#R0573L 2,500 units

5′...GCNNNNN▼NNGC...3′
3′...CGNN▲NNNNNCG...5′

Reaction Conditions: CutSmart Buffer, 60°C

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	100	100	100

Activity at 37°C: 10%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

NaeI

CutSmart · RR · 2*site · dilA · 37° · No · CpG

#R0190S 500 units
#R0190L 2,500 units

5′...GCC▼GGC...3′
3′...CGG▲CCG...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	25	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

NarI

CutSmart · RR · 2*site · dilA · 37° · 65° · CpG

#R0191S 500 units
#R0191L 2,500 units

5′...GG▼CGCC...3′
3′...CCGC▲GG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	10	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

NciI

CutSmart · RR · dilA · 37° · No · CpG

#R0196S 2,000 units
#R0196L 10,000 units

5′...CC▼SGG...3′
3′...GGS▲CC...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	25	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 334).

NcoI

RR · NEB3.1 · dilA · 37° · 80°

#R0193S 1,000 units
#R0193L 5,000 units
for high (5X) concentration
#R0193T 1,000 units
#R0193M 5,000 units

5′...C▼CATGG...3′
3′...GGTAC▲C...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	100	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

High-Fidelity

NcoI-HF®

CutSmart · RR · e · dilB · 37° · 80°

#R3193S 1,000 units
#R3193L 5,000 units
for high (5X) concentration
#R3193M 5,000 units

5′...C▼CATGG...3′
3′...GGTAC▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NdeI

CutSmart · RR · dilA · 37° · 65°

#R0111S 4,000 units
#R0111L 20,000 units

5′...CA▼TATG...3′
3′...GTAT▲AC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	100	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

NgoMIV

CutSmart · RR · 2*site · dam · dcmA · 37° · 80° · CpG

#R0564S 1,000 units
#R0564L 5,000 units

5′...G▼CCGGC...3′
3′...CGGCC▲G...5′

Reaction Conditions: Cutsmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

NheI

RR · dam · NEB2.1 · dcmC · 37° · 65° · CpG

#R0131S 1,000 units
#R0131L 5,000 units
for high (5X) concentration
#R0131M 5,000 units

5′...G▼CTAGC...3′
3′...CGATC▲G...5′

Reaction Conditions: NEBuffer 2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

NheI-HF®

High-Fidelity

CutSmart · RR · exact · dam · dcmC · 37° · 80° · CpG

#R3131S 1,000 units
#R3131L 5,000 units
for high (5X) concentration
#R3131M 5,000 units

5′...G▼CTAGC...3′
3′...CGATC▲G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	25	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

NlaIII

CutSmart · RR · dam · dcmB · 37° · 65°

#R0125S 500 units
#R0125L 2,500 units

5′...CATG▼...3′
3′...▲GTAC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	<10	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NlaIV

CutSmart · RR · dcmB · 37° · 65° · dcm · CpG

#R0126S 200 units

5′...GGN▼NCC...3′
3′...CCN▲NGG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	10	10	100

Concentration: 2,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

NmeAIII

CutSmart · RR · 2*site · SAM · dcmB · 37° · 65°

#R0711S 250 units

5′...GCCGAG(N)$_{20\text{-}21}$▼...3′
3′...CGGCTC(N)$_{18\text{-}19}$▲...5′

Note: The cleavage point may shift one base pair depending on the DNA sequence context between the recognition site and the position of cleavage. For a given sequence, generally one site will predominate. For details, see www.neb.com.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	10	<10	100

Reaction Conditions: CutSmart Buffer + SAM, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NotI

RR · NEB3.1 · dcmC · 37° · 65° · CpG

#R0189S 500 units
#R0189L 2,500 units
for high (5X) concentration
#R0189M 2,500 units

5′...GC▼GGCCGC...3′
3′...CGCCGG▲CG...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	100	25

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

NotI-HF®

High-Fidelity

CutSmart · RR · exact · dam · dcmA · 37° · 65° · CpG

#R3189S 500 units
#R3189L 2,500 units
for high (5X) concentration
#R3189M 2,500 units

5′...GC▼GGCCGC...3′
3′...CGCCGG▲CG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	25	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

NruI

#R0192S 1,000 units
#R0192L 5,000 units
for high (5X) concentration
#R0192T 1,000 units
#R0192M 5,000 units

5′...TCG▼CGA...3′
3′...AGC▲GCT...5′

Reaction Conditions: NEBuffer 3.1, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	10	100	10

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

NspI

#R0602S 250 units
#R0602L 1,250 units

5′...RCATG▼Y...3′
3′...Y▲GTACR...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

High-Fidelity

NruI-HF®

#R3192S 1,000 units
#R3192L 5,000 units

5′...TCG▼CGA...3′
3′...AGC▲GCT...5′

Reaction Conditions: CutSmart Buffer, 37°C.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	0	25	50	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

PacI

#R0547S 250 units
#R0547L 1,250 units

5′...TTAAT▼TAA...3′
3′...AAT▲TAATT...5′

Reaction Conditions: CutSmart Buffer, 37°C.Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	75	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NsiI

#R0127S 1,000 units
#R0127L 5,000 units

5′...ATGCA▼T...3′
3′...T▲ACGTA...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	75	100	25

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

PaeR7I

#R0177S 2,000 units

5′...C▼TCGAG...3′
3′...GAGCT▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

High-Fidelity

NsiI-HF®

#R3127S 1,000 units
#R3127L 5,000 units

5′...ATGCA▼T...3′
3′...T▲ACGTA...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	20	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

PciI

#R0655S 200 units
#R0655L 1,000 units

5′...A▼CATGT...3′
3′...TGTAC▲A...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	75	100	50*

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

PflFI

CutSmart · RR · dinA · 37° · 65°

#R0595S 2,000 units

5′...GACNNNGTC...3′
3′...CTGNNNCAG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	25	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

PflMI

RR · NEB3.1 · dinA · 37° · 65° · dcm

#R0509S 1,000 units
#R0509L 5,000 units

5′...CCANNNNNTGG...3′
3′...GGTNNNNNNACC...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	0	100	100	50

Methylation Sensitivity: Blocked by overlapping *dcm* methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

PleI

CutSmart · RR · 2°site · dinA · 37° · 65° · CpG

#R0515S 1,000 units

5′...GAGTC(N)$_4$...3′
3′...CTCAG(N)$_5$...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	25	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

PluTI

CutSmart · RR · 2°site · dinA · 37° · 65° · CpG

#R0713S 500 units

5′...GGCGCC...3′
3′...CCGCGG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	25	<10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

PmeI

CutSmart · RR · dinA · 37° · 65° · CpG

#R0560S 500 units
#R0560L 2,500 units

5′...GTTTAAAC...3′
3′...CAAATTTG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

PmlI

CutSmart · RR · dinA · 37° · 65° · CpG

#R0532S 2,000 units
#R0532L 10,000 units

5′...CACGTG...3′
3′...GTGCAC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	<10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

PpuMI

CutSmart · RR · dinB · 37° · N6 · dcm

#R0506S 500 units
#R0506L 2,500 units

5′...RGGWCCY...3′
3′...YCCWGGR...5′

Reaction Conditions: CutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity: Blocked by overlapping *dcm* methylation (see p. 334).

PshAI

CutSmart · RR · dinA · 37° · 65° · CpG

#R0593S 1,000 units
#R0593L 5,000 units

5′...GACNNNNGTC...3′
3′...CTGNNNNCAG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

PsiI

CutSmart · RR · dilB · 37° · 65°

#R0657S 200 units
#R0657L 1,000 units

5′...T T A▼T A A...3′
3′...A A T▲A T T...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	10	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

PspGI

CutSmart · RR · dilA · 75° · NO · dcm

#R0611S 1,000 units

5′...▼C C W G G...3′
3′...G G W C C▲...5′

Reaction Conditions: CutSmart Buffer, 75°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	50	100

Activity at 37°C: 10%

Methylation Sensitivity: Blocked by *dcm* methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

PspOMI

CutSmart · RR · dilB · 37° · 65° · dcm · CpG

#R0653S 1,500 units
#R0653L 7,500 units

5′...G▼G G C C C...3′
3′...C C C G G▲G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	10	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Impaired by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

PspXI

CutSmart · RR · dilB · 37° · NO · CpG

#R0656S 200 units
#R0656L 1,000 units

5′...V C▼T C G A G B...3′
3′...B G A G C T▲C V...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	100	25	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by CpG methylation (see page 334).

PstI

RR · e · NEB3.1 · dilC · 37° · 80°

#R0140S 10,000 units
#R0140L 50,000 units
for high (5X) concentration
#R0140T 10,000 units
#R0140M 50,000 units

5′...C T G C A▼G...3′
3′...G▲A C G T C...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	75	100	50*

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

High-Fidelity

PstI-HF®

CutSmart · RR · e · dilC · 37° · NO

#R3140S 10,000 units
#R3140L 50,000 units
for high (5X) concentration
#R3140T 10,000 units
#R3140M 50,000 units

5′...C T G C A▼G...3′
3′...G▲A C G T C...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	75	50	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

PvuI

RR · e · NEB3.1 · dilB · 37° · NO · CpG

#R0150S 500 units
#R0150L 2,500 units

5′...C G A T▼C G...3′
3′...G C▲T A G C...5′

Reaction Conditions: NEBuffer 3.1, 37°C.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	25	100	<10

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

High-Fidelity

PvuI-HF®

CutSmart · RR · e · dilB · 37° · NO · CpG

#R3150S 500 units
#R3150L 2,500 units

5′...C G A T▼C G...3′
3′...G C▲T A G C...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

PvuII

#R0151S 5,000 units
#R0151L 25,000 units
for high (5X) concentration
#R0151T 5,000 units
#R0151M 25,000 units

5′...CAG▼CTG...3′
3′...GTC▲GAC...5′

Reaction Conditions: NEBuffer 3.1, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100*

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

*May exhibit star activity in this buffer.

PvuII-HF®

High-Fidelity

#R3151S 5,000 units
#R3151L 25,000 units
for high (5X) concentration
#R3151M 25,000 units

5′...CAG▼CTG...3′
3′...GTC▲GAC...5′

Reaction Conditions: CutSmart Buffer, 37°C.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	<10	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

RsaI

#R0167S 1,000 units
#R0167L 5,000 units

5′...GT▼AC...3′
3′...CA▲TG...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

RsrII

#R0501S 500 units
#R0501L 2,500 units

5′...CG▼GWCCG...3′
3′...GCCWG▲GC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	75	10	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

SacI

#R0156S 2,000 units
#R0156L 10,000 units
for high (5X) concentration
#R0156M 10,000 units

5′...GAGCT▼C...3′
3′...C▲TCGAG...5′

Reaction Conditions: NEBuffer 1.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

SacI-HF®

High-Fidelity

#R3156S 2,000 units
#R3156L 10,000 units
for high (5X) concentration
#R3156M 10,000 units

5′...GAGCT▼C...3′
3′...C▲TCGAG...5′

Reaction Conditions: Cutsmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	50	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam* or *dcm* methylation. Blocked by some combinations of overlapping CpG methylation.

SacII

#R0157S 2,000 units
#R0157L 10,000 units

5′...CCGC▼GG...3′
3′...GG▲CGCC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

SalI

#R0138S 2,000 units
#R0138L 10,000 units
for high (5X) concentration
#R0138T 2,000 units
#R0138M 10,000 units

5′...G▼TCGAC...3′
3′...CAGCT▲G...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	<10	100	<10

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

SalI-HF®

High-Fidelity

CutSmart · RR · e · ◐ · diluA · 37° · 65° · CpG

#R3138S 2,000 units
#R3138L 10,000 units
for high (5X) concentration
#R3138T 2,000 units
#R3138M 10,000 units

5′...G▼TCGAC...3′
3′...CAGCT▲G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

SapI

CutSmart · RR · ◐ · diluB · 37° · 65°

#R0569S 250 units
#R0569L 1,250 units

5′...GCTCTTC(N)$_1$▼...3′
3′...CGAGAAG(N)$_4$▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	50	>10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam, dcm* or mammalian CpG methylation.

Sau3AI

RR · NEB1.1 · diluA · 37° · 65° · CpG

#R0169S 200 units
#R0169L 1,000 units

5′...▼GATC...3′
3′...CTAG▲...5′

Reaction Conditions: NEBuffer 1.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	10	100

Methylation Sensitivity: Unlike DpnII and MboI, Sau3AI is not blocked by *dam* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

Sau96I

CutSmart · RR · diluA · 37° · 65° · dcm · CpG

#R0165S 1,000 units

5′...G▼GNCC...3′
3′...CCNG▲G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

SbfI

CutSmart · RR · ◐ · diluA · 37° · 80°

#R0642S 500 units
#R0642L 2,500 units

5′...CCTGCA▼GG...3′
3′...GG▲ACGTCC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	25	<10	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

SbfI-HF®

High-Fidelity

CutSmart · RR · e · ◐ · diluB · 37° · 80°

#R3642S 500 units
#R3642L 2,500 units

5′...CCTGCA▼GG...3′
3′...GG▲ACGTCC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	25	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

ScaI-HF®

High-Fidelity

CutSmart · RR · e · ◐ · diluB · 37° · 80°

#R3122S 1,000 units
#R3122L 5,000 units
for high (5X) concentration
#R3122M 5,000 units

5′...AGT▼ACT...3′
3′...TCA▲TGA...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

ScrFI

CutSmart · RR · diluC · 37° · 65° · dcm · CpG

#R0110S 1,000 units

5′...CC▼NGG...3′
3′...GGN▲CC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	100	100

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 334).

Note: Star activity may result from extended digestion.

CutSmart or NEB1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · ◐ Time-Saver Qualified · diluA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

SexAI

CutSmart · dinA · 37° · 65° · dcm

#R0605S 200 units
#R0605L 1,000 units

5′...A▼CCWGGT...3′
3′...TGGWCC▲A...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	75	50	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Blocked by *dcm* methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

SfaNI

NEB3.1 · dinB · 37° · 65° · CpG

#R0172S 300 units
#R0172L 1,500 units

5′...GCATC(N)$_5$▼...3′
3′...CGTAG(N)$_9$▲...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	75	100	25

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

SfcI

CutSmart · dinB · 37° · 65°

#R0561S 200 units
#R0561L 1,000 units

5′...C▼TRYAG...3′
3′...GAYRT▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	50	25	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%.

SfiI

CutSmart · 2*site · dinC · 50° · dcm · CpG

#R0123S 3,000 units
#R0123L 15,000 units

5′...GGCCNNNN▼NGGCC...3′
3′...CCGGN▲NNNNCCGG...5′

Reaction Conditions: CutSmart Buffer, 50°C

Concentration: 20,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	50	100

Activity at 37°C: 10%

Methylation Sensitivity: Impaired by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

SfoI

CutSmart · dinB · 37° · dcm · CpG

#R0606S 500 units
#R0606L 2,500 units

5′...GGC▼GCC...3′
3′...CCG▲CGG...5′

Reaction Conditions: CutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

SgrAI

CutSmart · 2*site · dinA · 37° · 65° · CpG

#R0603S 1,000 units
#R0603L 5,000 units

5′...CR▼CCGGYG...3′
3′...GYGGCC▲RC...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

SmaI

CutSmart · dinB · 25° · 65° · CpG

#R0141S 2,000 units
#R0141L 10,000 units

5′...CCC▼GGG...3′
3′...GGG▲CCC...5′

Reaction Conditions: CutSmart Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	<10	<10	100

Concentration: 20,000 units/ml

Activity at 37°C: 50% (15 minute half-life)

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

SmlI

CutSmart · dinA · 55°

#R0597S 500 units
#R0597L 2,500 units

5′...C▼TYRAG...3′
3′...GARYT▲C...5′

Reaction Conditions: CutSmart Buffer, 55°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	75	25	100

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

SnaBI

CutSmart · RR · dilA · 37° · 80° · CpG

#R0130S 500 units
#R0130L 2,500 units
for high (5X) concentration
#R0130M 2,500 units

5′...TAC▼GTA...3′
3′...ATG▲CAT...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 and 25,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	50	10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.

SpeI

CutSmart · RR · e · dilC · 37° · 80°

#R0133S 500 units
#R0133L 2,500 units
for high (5X) concentration
#R0133M 2,500 units

5′...A▼CTAGT...3′
3′...TGAT▲CA...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	25	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

High-Fidelity

SpeI-HF®

CutSmart · RR · e · dilC · 37° · 80°

#R3133S 500 units
#R3133L 2,500 units
for high (5X) concentration
#R3133M 2,500 units

5′...A▼CTAGT...3′
3′...TGAT▲CA...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 and 100,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	<10	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

SphI

RR · NEB2.1 · dilB · 37° · 65°

#R0182S 500 units
#R0182L 2,500 units
for high (8X) concentration
#R0182M 2,500 units

5′...GCATG▼C...3′
3′...C▲GTACG...5′

Reaction Conditions: NEBuffer 2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	100	50	100

Concentration: 10,000 and 80,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

High-Fidelity

SphI-HF®

CutSmart · RR · e · dilB · 37° · 65°

#R3182S 500 units
#R3182L 2,500 units
for high (5X) concentration
#R3182M 2,500 units

5′...GCATG▼C...3′
3′...C▲GTACG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	25	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

SrfI

CutSmart · RR · e · dilB · 37° · 65° · CpG

#R0629S 500 units
#R0629L 2,500 units

5′...GCCC▼GGGC...3′
3′...CGGG▲CCCG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	<10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p 334).

SspI

RR · e · NEBU · dilC · 37° · 65°

#R0132S 1,000 units
#R0132L 5,000 units
for high (5X) concentration
#R0132M 5,000 units

5′...AAT▼ATT...3′
3′...TTA▲TAA...5′

Reaction Conditions: NEBuffer SspI, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	50	50

Concentration: 5,000 and 25,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

High-Fidelity

SspI-HF®

CutSmart · RR · e · dilB · 37° · 65°

#R3132S 1,000 units
#R3132L 5,000 units
for high (5X) concentration
#R3132M 5,000 units

5′...AAT▼ATT...3′
3′...TTA▲TAA...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

StuI

#R0187S 1,000 units
#R0187L 5,000 units
for high (10X) concentration
#R0187M 5,000 units

5′...AGG▼CCT...3′
3′...TCC▲GGA...5′

Reaction Conditions: CutSmart Buffer, 37°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	50	100

Concentration: 10,000 and 100,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dcm* methylation (see p. 334).

StyI

#R0500S 3,000 units

5′...C▼CWWGG...3′
3′...GGWWC▲C...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	25	100	10

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

StyI-HF®

High-Fidelity

#R3500S 3,000 units
#R3500L 15,000 units

5′...C▼CWWGG...3′
3′...GGWWC▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	25	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

StyD4I

#R0638S 200 units

5′...▼CCNGG...3′
3′...GGNCC▲...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	100	100

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation. (see p. 334).

SwaI

#R0604S 2,000 units
#R0604L 10,000 units

5′...ATTT▼AAAT...3′
3′...TAAA▲TTTA...5′

Reaction Conditions: NEBuffer 3.1, 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	10	100	10

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Taq$^{\alpha}$I

#R0149S 4,000 units
#R0149L 20,000 units
for high (5X) concentration
#R0149T 4,000 units
#R0149M 20,000 units

5′...T▼CGA...3′
3′...AGC▲T...5′

Reaction Conditions: CutSmart Buffer, 65°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	75	100	100

Concentration: 20,000 and 100,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity: Blocked by overlapping *dam* methylation (see p. 334).

TfiI

#R0546S 500 units

5′...G▼AWTC...3′
3′...CTWA▲G...5′

Reaction Conditions: CutSmart Buffer, 65°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	100	100

Activity at 37°C: 10%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

TseI

#R0591S 75 units
#R0591L 375 units

5′...G▼CWGC...3′
3′...CGWC▲G...5′

Reaction Conditions: CutSmart Buffer, 65°C

Concentration: 5,000 units/ml.

Activity at 37°C: 20%

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	100	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Tsp45I

CutSmart · dilA · 65° · No

#R0583S 200 units
#R0583L 1,000 units

5′...▼GTSAC...3′
3′...CAST▲G...5′

Reaction Conditions: CutSmart Buffer, 65°C

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	<10	100

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

TspMI

CutSmart · dilB · 75° · No · CpG

#R0709S 200 units

5′...C▼CGGG...3′
3′...GGGC▲C...5′

Reaction Conditions: CutSmart Buffer, 75°C

Concentration: 5,000 units/ml

Activity at 37°C: 20%

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50*	75*	50*	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Note: *May exhibit star activity in this buffer.

TspRI

CutSmart · dilB · 65° · No

#R0582S 1,000 units
#R0582L 5,000 units

5′...NNCASTGNN▼...3′
3′...▲NNGTSACNN...5′

Reaction Conditions: CutSmart Buffer, 65°C

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	25	100

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Tth111I

CutSmart · dilB · 65° · No

#R0185S 400 units

5′...GACNNNGTC...3′
3′...CTGNN▲NCAG...5′

Reaction Conditions: CutSmart Buffer, 65°C

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	25	100

Concentration: 5,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

XbaI

CutSmart · RR · dilA · 37° · 65° · dam

#R0145S 3,000 units
#R0145L 15,000 units
for high (5X) concentration
#R0145T 3,000 units
#R0145M 15,000 units

5′...T▼CTAGA...3′
3′...AGATC▲T...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	100	75	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation (see p. 334).

XcmI

RR · NEB21 · dilC · 37° · 65°

#R0533S 1,000 units
#R0533L 5,000 units

5′...CCANNNNN▼NNNNTGG...3′
3′...GGTNNNN▲NNNNNACC...5′

Reaction Conditions: NEBuffer 2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	25	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

XhoI

CutSmart · RR · dilA · 37° · 65° · CpG

#R0146S 5,000 units
#R0146L 25,000 units
for high (5X) concentration
#R0146M 25,000 units

5′...C▼TCGAG...3′
3′...GAGCT▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by CpG methylation (see p. 334).

XmaI

CutSmart · RR · dilA · 37° · 65° · CpG

#R0180S 500 units
#R0180L 2,500 units
for high (5X) concentration
#R0180M 2,500 units

5′...C▼CCGGG...3′
3′...GGGCC▲C...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	50	<10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by CpG methylation (see p. 334).

Note: Star activity may result from a glycerol concentration of > 5%.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

XmnI

CutSmart dA 37° 65°

#R0194S 1,000 units
#R0194L 5,000 units

5′...G A A N N ▼ N N T T C...3′
3′...C T T N N N N A A ▲ G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	75	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

ZraI

CutSmart dB 37° 80° CpG

#R0659S 200 units
#R0659L 1,000 units

5′...G A C ▼ G T C...3′
3′...C T G ▲ C A G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	25	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Nicking Endonucleases

As a rule, when restriction endonucleases bind to their recognition sequences in DNA, they cleave both strands of the duplex at the same time. Two independent hydrolytic reactions proceed in parallel, most often driven by the presence of two catalytic sites within each enzyme, one for hydrolyzing each strand. One of the focuses of our research program is to engineer restriction enzymes so that they hydrolyze only one strand of the duplex, generating DNA molecules that are "nicked", rather than cleaved. These conventional nicks (3′-hydroxyl, 5′-phosphate) can serve as initiation points for a variety of further enzymatic reactions such as replacement DNA synthesis, strand-displacement amplification (1), exonucleolytic degradation or the creation of small gaps (2).

Nicking endonucleases (NEases) are as simple to use as restriction endonucleases. Since the nicks generated by 6- or 7-base nicking endonucleases do not fragment DNA, their activities are monitored by conversion of supercoiled plasmids to open circles. Alternatively, substrates with nicking sites close enough on opposite strands to create a double-stranded cut can be used instead.

The uses of nicking endonucleases are still being explored. NEases can generate nicked or gapped duplex DNA for DNA mismatch repair studies and for diagnostic applications. The long overhangs that nicking enzymes make can be used in DNA fragment assembly. Nt.BbvCI has been used to generate long and non-complementary overhangs when used with XbaI in the USER® cloning protocol from NEB. Nicking endonucleases are also useful for isothermal DNA amplification, which relies on the production of site-specific nicks. For example, isothermal DNA amplification using Nt.BstNBI in concert with Vent® (exo⁻) DNA Polymerase (NEB #M0257) (EXPAR) has been reported for detection of a specific DNA sequence in a sample (3). Another isothermal DNA amplification technique [Nicking Endonuclease Mediated- DNA Amplification (NEMDA)] (4) has been described using the 3-base cutter Nt.CviPII and *Bst* DNA Polymerase I. Frequent cutting NEases can generate short partial duplex DNA fragments from genomic DNA. These fragments can be used for cloning or used as probes for hybridization-based applications. Nicking enzymes have also been used for genome mapping.

NEB continues to engineer more nicking enzymes, particularly in response to specific customer needs and applications.

References:

(1) Walker, G.T. et al. (1992) *Proc. Natl. Acad. Sci. USA*, 89, 392–396.
(2) Wang, H. and Hays, J.B. (2000) *Mol. Biotechnol.*, 15, 97–104.
(3) Van Ness, J. et al. (2003) *Proc. Natl. Acad. Sci. USA*, 89, 4504–4509.
(2) Chan, S.H. et al. (2004) *Nucl. Acids Res.*, 32, 6187–6199.

Nb.BbvCI

CutSmart dA 37° 80°

#R0631S 1,000 units
#R0631L 5,000 units

5′...C C T C A G C...3′
3′...G G A G T ▲ C G...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nb.BsmI

NEB3.1 dA 65° 80°

#R0706S 1,000 units

5′...G A A T G C N...3′
3′...C T T A C ▲ G N...5′

Reaction Conditions: NEBuffer 3.1, 65°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	100	10

Concentration: 10,000 units/ml

Activity at 37°C: 25%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nb.BsrDI

CutSmart · RR · dilA · 65° · 80°

#R0648S 1,000 units
#R0648L 5,000 units

5′... GCAATGNN...3′
3′... CGTTAC▲NN...5′

Reaction Conditions: CutSmart Buffer, 65°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	25	100	100	100

Concentration: 10,000 units/ml

Activity at 37°C: 75%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nt.BbvCI

CutSmart · RR · dilA · 37° · 80° · CpG

#R0632S 1,000 units
#R0632L 5,000 units

5′... CCTCAGC...3′
3′... GGAGTCG...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	100	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 334).

Nb.BssSI

RR · NEB3.1 · dilB · 37° · NG

#R0681S 1,000 units
for high (5X) concentration
#R0681T 5,000 units

5′... CACGAG...3′
3′... GTGCT▲C...5′

Reaction Conditions: NEBuffer 3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	100	100	25

Concentration: 20,000 and 100,000 units/ml.

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nt.BsmAI

CutSmart · RR · dilA · 37° · 65° · CpG

#R0121S 500 units

5′... GTCTCN▼N...3′
3′... CAGAGNN...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	100	50	10	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Nb.BtsI

CutSmart · RR · dilA · 37° · 80°

#R0707S 1,000 units

5′... GCAGTGNN...3′
3′... CGTCAC▲NN...5′

Reaction Conditions: CutSmart Buffer, 37°C.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	75	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nt.BspQI

RR · NEB3.1 · dilB · 50° · 80°

#R0644S 1,000 units

5′...GCTCTTCN▼...3′
3′...CGAGAAGN...5′

Reaction Conditions: NEBuffer 3.1, 50°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	25	100	10

Concentration: 10,000 units/ml

Activity at 37°C: 80%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nt.AlwI

CutSmart · RR · dilA · 37° · 80° · dam

#R0627S 500 units

5′...GGATCNNNNN▼N...3′
3′...CCTAGNNNNN...5′

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage is blocked by *dam* methylation (see p. 334).

Nt.BstNBI

RR · NEB3.1 · dilA · 55° · 80°

#R0607S 1,000 units
#R0607L 5,000 units

5′...GAGTCNNNNN▼N...3′
3′...CTCAGNNNNN...5′

Reaction Conditions: NEBuffer 3.1, 55°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	0	10	100	10

Concentration: 10,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

NEW

Nt.CviPII

CutSmart RR dilA 37° 65° CpG

#R0626S 40 units

5´...▼CCD...3´
3´...GGH...5´

D = A or G or T (not C)
H = A or C or T (not G)

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	100	25	100

Concentration: 2,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

Homing Endonucleases

Homing endonucleases are double stranded DNases that have large, asymmetric recognition sites (12–40 base pairs) and coding sequences that are usually embedded in either introns or inteins (1). Introns are spliced out of precursor RNAs, while inteins are spliced out of precursor proteins (2,3). Homing endonucleases are named using conventions similar to those of restriction endonucleases with intron-encoded endonucleases containing the prefix, "I-" and intein endonucleases containing the prefix, "PI-"(1,7).

Homing endonuclease recognition sites are extremely rare. For example, an 18 base pair recognition sequence will occur only once in every 7 x 10^{10} base pairs of random sequence. This is equivalent to only one site in 20 mammalian-sized genomes (4). However, unlike standard restriction endonucleases, homing endonucleases tolerate some sequence degeneracy within their recognition sequence (5,6). As a result, their observed sequence specificity is typically in the range of 10–12 base pairs.

Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved.

References:

(1) Belfort, M. and Roberts, R.J. (1997) *Nucleic Acids Res.*, 25, 3379–3388.
(2) Dujon, B. et al. (1989) *Gene*, 82, 115–118.
(3) Perler, F.B. et al. (1994) *Nucleic Acids Res.*, 22, 1125–1127.
(4) Jasin, M. (1996) *Trends in Genetics*, 12, 224–228.
(5) Gimble, F.S. and Wang, J. (1996) *J. Mol. Biol.*, 263, 163–180.
(6) Argast, M.G. et al. (1998) *J. Mol. Biol.*, 280, 345–353.
(7) Roberts, R.J. et al. (2003) *Nucleic Acids Res.*, 31, 1805–1812.

I-CeuI

CutSmart RR dilB 37° 65°

#R0699S 500 units
#R0699L 2,500 units

(Supplied with 5 µg of plasmid DNA)

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Specificity: The homing or recognition site for this endonuclease is shown below:

5´...TAACTATAACGGTCCTAA▼GGTAGCGAA...3´
3´...ATTGATATTGCCAGGA▲TTCCATCGCTT...5´

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	10	10	<10	100

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences.

I-SceI

CutSmart RR dilB 37° 65°

#R0694S 500 units
#R0694L 2,500 units

(Supplied with 5 µg of plasmid DNA)

Reaction Conditions: CutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Specificity: The homing or recognition site for this endonuclease is shown below:

5´...TAGGGATAA▼CAGGGTAATA...3´
3´...ATCCCTATT▲GTCCCATTAT...5´

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	50	25	100

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences.

PI-PspI

#R0695S 500 units

(Supplied with 5 µg of plasmid DNA)

Reaction Conditions: NEBuffer PI-PspI + BSA, 65°C

Specificity: The homing or recognition site for this endonuclease is shown below:

```
5´...TGGCAAACAGCTATTATGGGTATTATGGGT...3´
3´...ACCGTTTGTCGATAATACCCATAATACCCA...5´
```

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	10	10	10

Concentration: 5,000 units/ml

Activity at 37°C: 5%

Note: Homing endonucleases do not have stringently-defined recognition sequences.

PI-SceI

#R0696S 250 units
#R0696L 1,250 units

(Supplied with 5 µg of plasmid DNA)

Reaction Conditions: NEBuffer PI-SceI + BSA, 37°C. Heat inactivation: 65°C for 20 minutes.

Specificity: The homing or recognition site for this endonuclease is shown below:

```
5´...ATCTATGTCGGGTGCGGAGAAAGAGGTAATGAAATGG...3´
3´...TAGATACAGCCCACGCCTCTTTCTCCATTACTTTACC...5´
```

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	<10	<10	<10	<10

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences.

NEW

Recombinant Albumin, Molecular Biology Grade

#B9200S 12 mg

Companion Product:

BSA, Molecular Biology Grade
#B9000S 12 mg

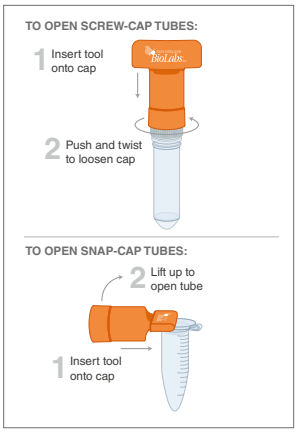
Reaction Conditions: Recombinant Albumin, Molecular Biology Grade, is a non-bovine derived albumin that can serve as an alternative to Bovine Serum Albumin (BSA). Like BSA, it has been shown to prevent adhesion of enzymes to reaction tubes and pipette surfaces. It also stabilizes some proteins during incubation. Choose Recombinant Albumin, when there is a need to avoid BSA sequences.

NEW

NEB Tube Opener

#C1008S 2 units

Description: Use to open a variety of microcentrifuge tubes. Can be used for snap-on caps or screw-on caps.



Reaction Buffers

NEBuffer™ 1.1 (10X)
#B7201S 5.0 ml

NEBuffer 2.1 (10X)
#B7202S 5.0 ml

NEBuffer 3.1 (10X)
#B7203S 5.0 ml

CutSmart Buffer (10X)
#B7204S 5.0 ml

NEW
NEBuffer Set (EcoRI, DpnII)
#B7006S 5.0 ml

S-adenosylmethionine (SAM)
#B9003S (32 mM) 0.5 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

Description: New England Biolabs provides a color-coded 10X NEBuffer with each restriction endonuclease to ensure optimal (100%) activity. Most of our enzymes are supplied with one of four standard NEBuffers. Occasionally, an enzyme has specific buffer requirements not met by one of the four standard NEBuffers, in which case the enzyme is supplied with its own unique NEBuffer.

Nuclease-free Water is ideal for the preparation of reagents and for use in enzymatic reactions. No toxic agents, such as DEPC, are used in the production of this water, so as to avoid inhibition in enzymatic reactions.

For more information about NEBuffer compatibility, please turn to page 293.

Reaction Buffer Compositions: Visit www.neb.com for details.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

Diluent Buffers

Diluent A
#B8001S 5.0 ml

Diluent B
#B8002S 5.0 ml

Diluent C
#B8003S 5.0 ml

Description: Diluent Buffers are recommended for making dilutions of restriction endonucleases. When necessary, we recommend diluting enzymes just prior to use and suggest that the final concentration of diluted enzymes be at least 1,000 units/ml. Diluent preference for each restriction endonuclease is listed with its catalog entry; a complete listing of all restriction endonucleases and their diluents can be found on page 293.

Storage Conditions:
Store at –20°C.

Diluent Buffer Compositions: Visit www.neb.com for details.

Gel Loading Dyes

Gel Loading Dye, Blue (6X)
#B7021S 4 ml

Gel Loading Dye, Orange (6X)
#B7022S 4 ml

Gel Loading Dye, Purple (6X)
#B7024S 4 ml

Gel Loading Dye, Purple (6X), no SDS
#B7025S 4 ml

Description: Pre-mixed loading dye solutions are available with a choice of tracking dyes, for agarose and non-denaturing polyacrylamide gel electrophoresis. Three of the solutions contain SDS, which often results in sharper bands, as some restriction enzymes remain bound to their DNA substrates following cleavage. Some interference may be observed when using SYBR® or GelRed® as precast dyes in the presence of increased concentrations of SDS. When using these dyes as precast dyes, NEB recommends using our Gel Loading Dye, Purple (6X), no SDS (NEB #B7025). Gel Loading Dyes contain EDTA to chelate up to 10 mM magnesium, thereby stopping the reaction. Bromophenol blue is the standard tracking dye for electrophoresis. It migrates at approximately 300 bp on a standard 1% TBE agarose gel. Orange G will not appear in gel photographs, and runs ahead of all but the smallest restriction fragments, migrating at approximately 50 bp on a standard 1% TBE agarose gel. NEB also offers a unique purple dye which migrates similarly to Bromophenol blue. Specifically chosen, this dye does not leave a shadow under UV light.

Gel Loading Dye Compositions: Visit www.neb.com for details.

Note: Use 5 µl of Gel Loading Dye per 25 µl reaction, or 10 µl per 50 µl reaction. Mix well before loading gel. Store at room temperature.

Twice a year, we bring together NEB team members from around the world to learn about the latest NEB innovations. U.S. Sales Representatives Corina, Faraz, Lynn, and the General Manager of NEB France, Eric Beguec, join NEB's CEO Jim Ellard (center), as he welcomes them to NEB's Ipswich, Massachusetts headquarters.

Magdalena River at Sunset, Colombia.
Credit: John Quintero, Getty Images

Habitat Fragmentation

Habitat fragmentation occurs when a continuous habitat is disrupted and divided into smaller habitats that are physically isolated from each other. It carves the environment into patches and divides plant and animal populations. Fragmentation can occur when a forest is disrupted for urban development or agricultural purposes, following a forest fire, or by the addition of roadways through a previously continuous habitat. The result is that the number of plants and animals in the fragments decreases, and the further apart the fragments are, the greater the loss in species diversity and genetic diversity. This increases the likelihood of species becoming extinct, as they are less able to deal with environmental changes or disturbances.

Furthermore, “edge effects” are observed in fragmented habitats. Edge effects refer to the contrast between adjacent habitats that can hinder the safe travel of species. When the contrast is great, for example, with suburban development, abiotic factors, such as an increase in sunlight, wind and temperature are observed. These factors influence which plants and animals live near the edge. A sharp contrast between habitats affects the safety of animals as they move around to forage for food and water. It also makes seasonal migration more dangerous. If an animal has to leave its core habitat and enter a more hostile environment, they become more vulnerable to attack by a predator.

In a fragmented forest, such as the Middle Magdalena Valley in Colombia, the Brown Spider Monkey no longer has a continuous canopy to move around — 80% of its habitat has been cleared for farmland and cattle ranches. Spider monkeys have a prehensile tail that acts like a fifth limb and gives them the appearance of a spider; they require large tracts of undisturbed forest due to their large body size.

While the monkeys rely on the forest, the forest also relies on the monkeys to regenerate. Spider monkeys disperse the seeds of approximately 100 tropical plants through ingestion of the fruit in one location, and then elimination of the seeds in a different part of the forest. Spider monkeys defecate 13 to 17 times a day, and this represents the dispersal of hundreds to thousands of seeds. Many of the seeds that they disperse belong to the dense, hardwood trees that are the most effective in eliminating carbon dioxide from the atmosphere.

Habitat fragmentation limits the distance that can be traveled safely by the spider monkey, and consequently, the area of seed dispersal. Fragmentation is causing a decline in the spider monkey population, which causes concern regarding the future of the forest. In an effort to prevent this, researchers observe the monkeys and are directed to their feces by dung beetles. They collect, wash and categorize the seeds in order to predict which species of plants will be dispersed, the quantity of each species, and to see if there is a pattern between the type of seeds dispersed and the monkey’s gender or age. They then plant the seeds in degraded areas that could potentially provide continuity, a nature corridor, between fragmented areas.

It is hoped that efforts to restore the continuity of the Middle Magdalena Valley will allow spider monkeys to again move freely around the canopy and carry out their crucial role in preserving the biodiversity of the forest. The tale of the spider monkey demonstrates how habitat fragmentation effects not just a single species, but an entire ecosystem.

DNA Polymerases & Amplification Technologies

NEB has pursued the discovery & development of DNA polymerases for over 25 years.

As the first company to sell *Taq* DNA polymerase and the first to discover a PCR-stable high-fidelity DNA polymerase, NEB established a foundation in developing innovative, high quality tools for the research and diagnostic community.

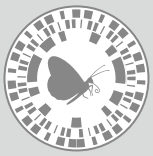
PCR & qPCR

NEB's product portfolio features a large selection of polymerases for PCR. Q5 High-Fidelity DNA Polymerase offers fidelity 280 times higher than *Taq*, along with superior performance with minimal optimization. One*Taq* is ideal for robust amplification in routine PCR applications. Both are available in hot start formulations, utilizing a novel aptamer-based approach that does not require an activation step.

Fluorescence-based quantitative real-time PCR (qPCR) is the gold standard for detection and quantitation of nucleic acids due to its specificity and sensitivity. Luna qPCR & RT-qPCR products feature an inert blue tracking dye for easy reaction setup and are available for intercalating dye or fluorescent probe-based detection methods.

Isothermal Amplification

Sequence specific isothermal amplification approaches eliminate the need for temperature cycling, providing advantages for certain point-of-care diagnostic needs. NEB's broad suite of reagents continue to enable advancement in isothermal amplification. Nicking enzymes, WarmStart enzymes, strand-displacing DNA polymerases, and RNA polymerases offer flexibility to assemble and design an isothermal amplification platform.



Find an overview of PCR.

Featured Products

Featured Tools & Resources

Visit www.neb.com/PCR to find additional online tools, video tech tips and tutorials to help you in setting up your PCR experiments.

RI Recombinant Enzyme

PCR Polymerase Selection Chart

For over 40 years, New England Biolabs, Inc. has been a world leader in the discovery and production of reagents for the life science industry. NEB offers a wide range of DNA polymerases, and through our commitment to research, ensures the development of innovative and high quality tools for PCR and related applications. The following table simplifies the selection of a polymerase that best suits your experiment.

High-fidelity polymerases benefit from a Tm+3 annealing temp. Use the **NEB Tm Calculator** to ensure successful PCR at **TmCalculator.neb.com**

★ **indicates recommended choice for application**

	STANDARD PCR		HIGH-FIDELITY PCR			SPECIALTY PCR			
			HIGHEST FIDELITY		MODERATE FIDELITY	LONG AMPLICONS	dU TOLERANCE		BLOOD DIRECT PCR
	One *Taq*®/ One *Taq* Hot Start	*Taq* / Hot Start *Taq*	Q5®/Q5 Hot Start	Phusion®(4)/ Phusion(4) Flex	Vent®/ Deep Vent™	LongAmp®/ LongAmp Hot Start *Taq*	Epimark® Hot Start *Taq*	Q5U™	Hemo KlenTaq®
PROPERTIES									
Fidelity vs. *Taq*	2X	1X	~280X(2)	> 39X	5–6X	2X	1X	ND	ND
Amplicon Size	< 6 kb	≤ 5 kb	≤ 20 kb	≤ 20 kb	≤ 6 kb	≤ 30 kb	≤ 1 kb	app-specific	≤ 2 kb
Extension Time	1 kb/min	1 kb/min	6 kb/min	4 kb/min	1 kb/min	1.2 kb/min	1 kb/min	2 kb/min	0.5 kb/min
Resulting Ends	3´ A/Blunt	3´ A	Blunt	Blunt	Blunt	3´ A/Blunt	3´ A	Blunt	3´ A
3´→5´ exo	Yes	No	Yes	Yes	Yes	Yes	No	Yes	No
5´→3´ exo	Yes	Yes	No	No	No	Yes	Yes	No	No
Units/50 µl Reaction	1.25	1.25	1.0	1.0	0.5–1.0	5.0	1.25	1.0	N/A
Annealing Temperature	Tm-5	Tm-5	Tm+3	Tm+3	Tm-5	Tm-5	Tm-5	Tm-3	Tm-5
APPLICATIONS									
Routine PCR	★	•	•	•	•	•			
Colony PCR	★	•							
Enhanced Fidelity	•		★	•	•	•			
High Fidelity			★	•					
High Yield	★	•	★	•					
Fast			★	•					
Long Amplicon			★	•		★			
GC-rich Targets	★		★		•	•			
AT-rich Targets	★	•	★	•		•	•	★	
High Throughput	•	•	•	•			•	★	
Multiplex PCR	•	★(1)	•	•					
Extraction-free PCR									★
DNA Labeling		★							
Site-directed Mutagenesis			★	•					
Carryover Prevention							•	★	
USER® Cloning							•	★	
NGS APPLICATIONS									
NGS Library Amplification			★(3)	•			★(5)		
FORMATS									
Hot Start Available	•	•	•	•		•	•		
Kit		•	•	•		•			
Master Mix Available	•	•	•	•		•			
Direct Gel Loading	•	•							

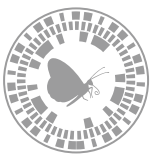
(1) Use Multiplex PCR 5X Master Mix.

(2) Due to the very low frequency of misincorporation events being measured, the error rate of high-fidelity enzymes like Q5 is challenging to measure in a statistically significant manner. We continue to investigate improved assays to characterize Q5's very low error rate to ensure that we present the most robust accurate fidelity data possible (Popatov, V. and Ong, J.L. (2017) PLoS One, 12(1):e0169774. doi 10.1371/journal. pone. 0169774).

(3) Use NEBNext High-Fidelity 2X PCR Master Mix.

(4) Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific.

(5) Use NEBNext Enzymatic Methyl-seq Kit (EM-seq™) for Illumina.



Why choose Q5 for your PCR?

Cloned at NEB | Recombinant Enzyme | High-Fidelity | PCR Enzyme | Hot Start/ WarmStart | Requires BSA | Heat Inactivation | Annealing Temperature

Q5® High-Fidelity DNA Polymerase

Q5 Hot Start High-Fidelity DNA Polymerase

Q5 High-Fidelity DNA Polymerase
#M0491S 100 units
#M0491L 500 units

Q5 High-Fidelity 2X Master Mix
#M0492S 100 rxns (50 µl vol)
#M0492L 500 rxns (50 µl vol)

Q5 Hot Start High-Fidelity DNA Polymerase
#M0493S 100 units
#M0493L 500 units

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 rxns (2 x 1.25 ml)
#M0494L 500 rxns (10 x 1.25 ml)
#M0494X 500 rxns (1 x 12.5 ml)

NOW AVAILABLE: NEBNext Ultra II Q5 Master Mix – see page 155.

Q5 POLYMERASE DETAILS	
Extension Rate	6 kb/min
Amplicon Size	≤ 20 kb
Fidelity	~ 280X *Taq*
Units / 50 µl rxn	1 unit
Resulting Ends	Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	No
Supplied Buffer	Q5 Reaction Buffer
Supplied Enhancer	Q5 High GC Enhancer
PRODUCT FORMATS	
Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
PCR Kit Available	Yes
NGS Version Available	Yes
APPLICATIONS	
High-Fidelity PCR	Yes
Difficult PCR	Yes
High GC PCR	Yes
T/A, U/A Cloning	No
Colony PCR	No
Blunt Cloning	Yes
Multiplex PCR	Yes
USER Cloning	Yes (Q5U)
Carryover Prevention	Yes (Q5U)

Tm Calculator

High-Fidelity polymerases benefit from a Tm+3 annealing temp. Use the **NEB Tm Calculator** to ensure successful PCR at **TmCalculator.neb.com**

NEW

Q5U™ Hot Start High-Fidelity DNA Polymerase
#M0515S 100 units
#M0515L 500 units

Q5 High-Fidelity PCR Kit
#E0555S 50 rxns (50 µl vol)
#E0555L 200 rxns (50 µl vol)

Q5 Site-Directed Mutagenesis Kit
#E0554S 10 rxns

Q5 Site-Directed Mutagenesis Kit (without competent cells)
#E0552S 10 rxns

For a complete listing of Deoxynucleotide Solutions, see page 79.

For a complete listing of Reaction Buffers, see pages 79.

Description: Q5 High-Fidelity DNA Polymerase sets a new standard for both fidelity and performance. With the highest fidelity amplification available (~ 280 times higher than *Taq*), Q5 DNA Polymerase results in ultra-low error rates. Q5 DNA Polymerase is composed of a novel polymerase that is fused to the processivity-enhancing Sso7d DNA binding domain, improving speed, fidelity and reliability of performance.

The Q5 buffer system is designed to provide superior performance with minimal optimization across a broad range of amplicons, regardless of GC content. For routine or complex amplicons up to ~65% GC content, Q5 Reaction Buffer provides reliable and robust amplification. For amplicons with high GC content (>65% GC), addition of the Q5 High GC Enhancer ensures continued maximum performance.

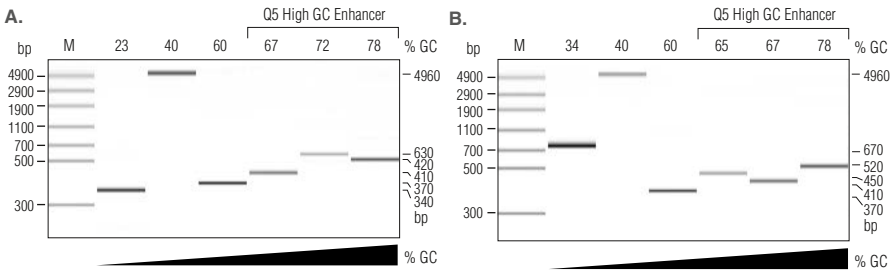
Q5 Hot Start DNA Polymerase: In contrast to chemically-modified or antibody-based hot start polymerases, NEB's Q5 Hot Start utilizes a unique synthetic aptamer. This structure binds to the polymerase through non-covalent interactions, blocking activity during the reaction setup. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. Q5 Hot Start does not require a separate high temperature activation step, reducing the potential for sample degredation, shortening reaction times and increasing ease-of-use. Q5 Hot Start is an ideal choice for high specificity amplification and provides robust amplification of a wide variety of amplicons, regardless of GC content.

Q5U Hot Start High-Fidelity DNA Polymerase: A modified version of Q5 High-Fidelity DNA Polymerase capable of incorporating dUTP for carryover prevention. Q5U is also compatible with USER cloning methods and enables the amplification of bisulfite treated/deaminated DNA.

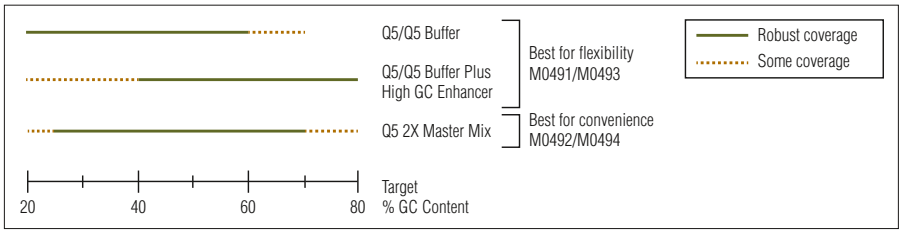
Additional Formats: For added convenience, Q5 DNA Polymerase is available in master mix format or as a kit. Master mix formulations include dNTPs, Mg^{++} and all necessary buffer components. The Q5 High-Fidelity PCR Kit contains the Q5 High-Fidelity 2X Master Mix, nuclease-free water and the Quick-Load Purple 1 kb Plus DNA Ladder. For information on the Q5 Site-Directed Mutagenesis Kit, with or without competent cells, see page 92.

Concentration: 2,000 units/ml

Visit **www.Q5PCR.com** for more information.



Robust amplification with Q5 (A) and Q5 Hot Start (B) High-Fidelity DNA Polymerases, regardless of GC content.
Amplification of a variety of human genomic amplicons from low-to-high GC content using either Q5 or Q5 Hot Start High-Fidelity DNA Polymerase. Reactions using Q5 Hot Start were set up at room temperature. All reactions were conducted using 30 cycles of amplification. and visualized by microfluidic LabChip® analysis.



The stand-alone enzyme comes with a reaction buffer that supports robust amplification of high AT to routine targets. Addition of the High GC Enhancer allows amplification of GC rich and difficult targets. For added convenience, the master mix formats allow robust amplification of a broad range of targets with a single formulation.

Epi EpiMark Extraction Free

Phusion® High-Fidelity DNA Polymerase

RR HiFi PCR No Tm+3

Phusion Hot Start Flex DNA Polymerase

RR HiFi PCR Hot No Tm+3

Phusion High-Fidelity DNA Polymerase
#M0530S 100 units
#M0530L 500 units

Phusion High-Fidelity PCR Master Mix with HF Buffer
#M0531S 100 rxns (50 µl vol)
#M0531L 500 rxns (50 µl vol)

Phusion High-Fidelity PCR Master Mix with GC Buffer
#M0532S 100 rxns (50 µl vol)
#M0532L 500 rxns (50 µl vol)

Phusion Hot Start Flex DNA Polymerase
#M0535S 100 units
#M0535L 500 units

Phusion Hot Start Flex 2X Master Mix
#M0536S 100 rxns (50 µl vol)
#M0536L 500 rxns (50 µl vol)

Phusion High-Fidelity PCR Kit
#E0553S 50 rxns (50 µl vol)
#E0553L 200 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 79.

For a complete listing of Reaction Buffers, see pages 79.

PHUSION POLYMERASE DETAILS	
Extension Rate	4 kb/min
Amplicon Size	≤ 20 kb
Fidelity	> 50X *Taq*
Units / 50 µl rxn	1 unit
Resulting Ends	Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	No
Supplied Buffers	- 5X Phusion HF Buffer - 5X Phusion GC Buffer
Supplied Enhancer	100% DMSO
PRODUCT FORMATS	
Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
PCR Kit Available	Yes
APPLICATIONS	
High-Fidelity PCR	Yes
T/A, U/A Cloning	No
Colony PCR	No
Blunt Cloning	Yes

Description: DNA polymerases with high fidelity are important for applications in which the DNA sequence needs to be correct after amplification. Manufactured and quality controlled at New England Biolabs, Thermo Scientific® Phusion High-Fidelity DNA Polymerase offers both high fidelity and robust performance, and thus can be used for all PCR applications. Its unique structure, a novel *Pyrococcus*-like enzyme fused with a processivity-enhancing domain, increases fidelity and speed. Phusion Hot Start Flex DNA Polymerase is available as a standalone enzyme or in a master mix format, and enables high specificity amplification. Phusion DNA Polymerase is an ideal choice for cloning and can be used for long amplicons.

Additional Formats: Phusion and Phusion Hot Start Flex DNA Polymerases are also available in master mix format. Phusion master mixes are available with HF or GC Buffer.

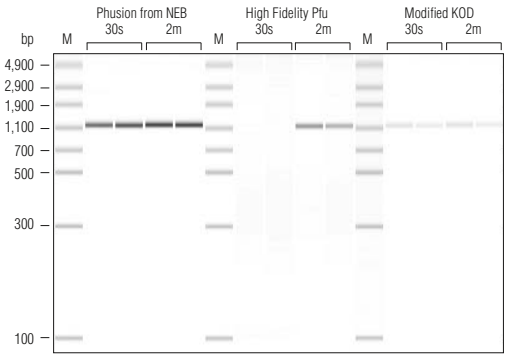
The Phusion PCR Kit contains Phusion Polymerase, Phusion HF and GC buffers, deoxynucleotides, $MgCl_2$, DMSO and DNA size standards.

Concentration: 2,000 units/ml

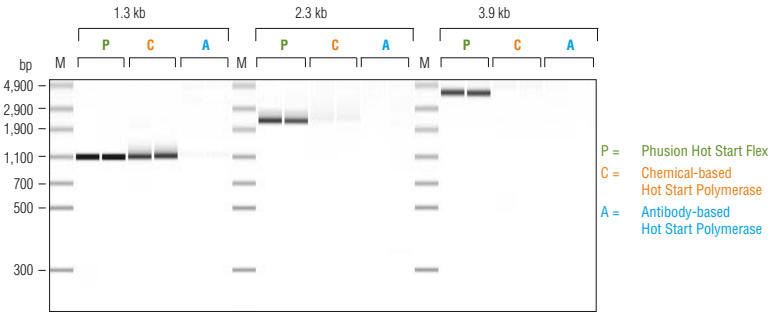
* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

Tm Calculator

High-Fidelity polymerases benefit from a Tm+3 annealing temp. Use the **NEB Tm Calculator** to ensure successful PCR at **TmCalculator.neb.com**



Phusion DNA Polymerase generates robust amplification even with short extension times. *A 1.2 kb C. elegans genomic amplicon was analyzed using different polymerases. Reactions were set up in duplicate and visualized by microfluidic LabChip analysis. All reactions were conducted according to manufacturer's instructions using 30 cycles and extension times of either 30 seconds or 2 minutes as indicated.*



Phusion Hot Start Flex DNA Polymerase delivers robust amplification. *All amplicons are from human Jurkat template except for the 1.3 kb C. elegans amplicon. Amplicon sizes are indicated above gel. All reactions were conducted in duplicate according to manufacturer's instructions using 30 cycles and visualized after microfluidic LabChip analysis.*

Cloned at NEB · RR Recombinant Enzyme · HiFi High-Fidelity · PCR PCR Enzyme · Hot Start/ WarmStart · BSA Requires BSA · 65° Heat Inactivation · Tm-5 Annealing Temperature · Epi EpiMark · Extraction Free

One*Taq*® DNA Polymerase

One*Taq* Hot Start DNA Polymerase

One*Taq* Products

One*Taq* DNA Polymerase
#M0480S 200 units
#M0480L 1,000 units
#M0480X 5,000 units

One*Taq* 2X Master Mix with Standard Buffer
#M0482S 100 rxns (50 µl vol)
#M0482L 500 rxns (50 µl vol)

NEW
One*Taq* Quick-Load® DNA Polymerase
#M0509S 100 units
#M0509L 500 units
#M0509X 2,500 units

One*Taq* Quick-Load 2X Master Mix with Standard Buffer
#M0486S 100 rxns (50 µl vol)
#M0486L 500 rxns (50 µl vol)

One*Taq* RT-PCR Kit
#E5310S 30 rxns

One*Taq* One-step RT-PCR Kit
#E5315S 30 rxns

ONE*Taq* POLYMERASE DETAILS	
Extension Rate	1 kb/min
Amplicon Size	≤ 6 kb
Fidelity	> 2X *Taq*
Units / 50 µl rxn	1.25 units
Resulting Ends	3´ A/Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	Yes
Supplied Buffers	- One*Taq* Std Rxn Buffer - One*Taq* GC Rxn Buffer
Supplied Enhancer	One*Taq* High GC Enhancer
PRODUCT FORMATS	
Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
Direct Gel-loading Available	Yes
PCR Kit Available	Yes
APPLICATIONS	
Routine PCR	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes

One*Taq* Hot Start Products

One*Taq* Hot Start DNA Polymerase
#M0481S 200 units
#M0481L 1,000 units
#M0481X 5,000 units

One*Taq* Hot Start 2X Master Mix with Standard Buffer
#M0484S 100 rxns (50 µl vol)
#M0484L 500 rxns (50 µl vol)

One*Taq* Hot Start 2X Master Mix with GC Buffer
#M0485S 100 rxns (50 µl vol)
#M0485L 500 rxns (50 µl vol)

One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer
#M0488S 100 rxns (50 µl vol)
#M0488L 500 rxns (50 µl vol)

One*Taq* Hot Start Quick-Load 2X Master Mix with GC Buffer
#M0489S 100 rxns (50 µl vol)
#M0489L 500 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 79.

For a complete listing of Reaction Buffers, see pages 79.

Description: One*Taq* DNA Polymerase is an optimized blend of *Taq* and Deep Vent® DNA polymerases for use with routine and difficult PCR experiments. The 3´→5´ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robust amplification of *Taq* DNA Polymerase. The One*Taq* reaction buffers and High GC Enhancer have been formulated for robust yields with minimal optimization, regardless of a template's GC content.

One*Taq* Hot Start DNA Polymerase: One*Taq* Hot Start DNA Polymerase utilizes an aptamer-based inhibitor. The hot start formulation combines convenience with decreased interference from primer-dimers and secondary products.

The aptamer-based inhibitor binds reversibly, blocking polymerase activity at temperatures below 45°C, allowing reactions to be set up at room temperature. One*Taq* Hot Start DNA Polymerase is activated during normal cycling conditions, eliminating the need for a separate high temperature incubation step to activate the enzyme. One*Taq* Hot Start DNA Polymerase can therefore be substituted into typical or existing *Taq*-based protocols.

One*Taq* and One*Taq* Hot Start are provided with Standard Reaction Buffer, GC Reaction Buffer and High GC Enhancer. Recommendations for buffer selection, based on % GC content, are shown below.

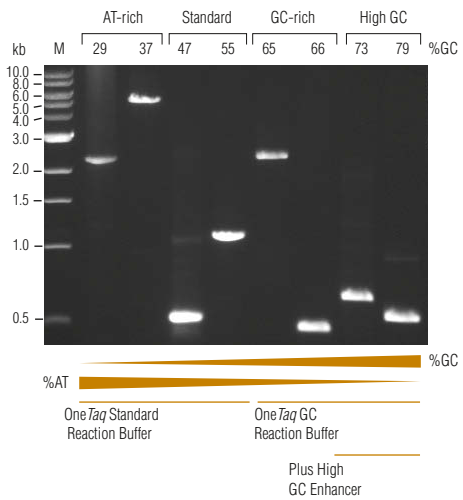
Quick-Load formats offer direct loading of PCR products onto gels, eliminating the need for a PCR clean-up step.

Additional Formats: For added convenience, One*Taq* and One*Taq* Hot Start DNA Polymerases are available in master mix format. Master mixes are available with Standard or GC Buffer. High GC Enhancer is also provided with master mixes containing GC Buffer. For direct gel loading, Quick-Load versions of master mixes are also available. For information on the One*Taq* RT-PCR Kit or One*Taq* One-step RT-PCR Kit, see page 72.

Concentration: 5,000 units/ml

One*Taq* Buffer Recommendations

AMPLICON % GC CONTENT	RECOMMENDED DEFAULT BUFFER	OPTIMIZATION NOTES
< 50% GC	One*Taq* Standard Reaction Buffer	Adjust annealing temperature, primer/template concentration, etc., if needed.
50–65% GC	One*Taq* Standard Reaction Buffer	One*Taq* GC Reaction Buffer can be used to enhance performance of difficult amplicons.
> 65% GC	One*Taq* GC Reaction Buffer	One*Taq* GC Reaction Buffer with 10-20% One*Taq* High GC Enhancer can be used to enhance performance of difficult amplicons.



Amplification of a selection of sequences with varying GC content from human and C. elegans genomic DNA using OneTaq DNA Polymerase. *GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).*

Taq DNA Polymerase

Hot Start *Taq* DNA Polymerase

Taq Polymerases

Taq DNA Polymerase with ThermoPol® Buffer
#M0267S 400 units
#M0267L 2,000 units
#M0267X 4,000 units
#M0267E 20,000 units

Taq DNA Polymerase with Std *Taq* Buffer
#M0273S 400 units
#M0273L 2,000 units
#M0273X 4,000 units
#M0273E 20,000 units

Taq DNA Polymerase with Std *Taq* (Mg-free) Buffer
#M0320S 400 units
#M0320L 2,000 units

Taq PCR Kit
#E5000S 200 rxns

Taq 2X Master Mix
#M0270L 500 rxns (50 µl vol)

Quick-Load *Taq* 2X Master Mix
#M0271L 500 rxns (50 µl vol)

Taq 5X Master Mix
#M0285L 500 rxns (50 µl vol)

Multiplex PCR 5X Master Mix
#M0284S 100 rxns (50 µl vol)

Hot Start *Taq* Products

Hot Start *Taq* DNA Polymerase
#M0495S 200 units
#M0495L 1,000 units

Hot Start *Taq* 2X Master Mix
#M0496S 100 rxns (50 µl vol)
#M0496L 500 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 79.

For a complete listing of Reaction Buffers, see pages 79.

Taq DNA POLYMERASE DETAILS	
Extension Rate	1 kb/min
Amplicon Size	≤ 5 kb
Units / 50 µl rxn	1.25 units
Resulting Ends	3´ A
3´→5´ Exonuclease Activity	No
5´→3´ Exonuclease Activity	Yes
Supplied Buffer (product dependent)	- Std *Taq* Rxn Buffer or - ThermoPol Rxn Buffer
PRODUCT FORMATS	
Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
Direct Gel-loading Available	Yes
PCR Kit Available	Yes
APPLICATIONS	
Routine PCR	Yes
SNP Detection	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes

Description: *Taq* DNA Polymerase is a thermostable DNA polymerase that possesses a 5´→ 3´ polymerase activity and a 5´ flap endonuclease activity. It is the most widely used enzyme for PCR. To accomodate a variety of PCR applications, *Taq* is available with different reaction buffers. Standard *Taq* Reaction Buffer is designed to support existing PCR platforms, and is an ideal choice for DHPLC and high-throughput applications. ThermoPol Reaction Buffer was designed at NEB, and is formulated to promote high product yields, even under demanding conditions. For additional convenience, *Taq* DNA Polymerase is also available in kit and master mix formats. For direct gel loading, a Quick-Load version of the *Taq* 2X Master Mix is also available.

Hot Start *Taq* DNA Polymerase: With value pricing and attractive commercial terms, Hot Start *Taq* from NEB is an ideal choice for molecular diagnostics and other applications. In contrast to chemically modified or antibody-based hot start polymerases, NEB's Hot Start *Taq* utilizes an aptamer-based technology. The unique aptamer binds to the polymerase through non-covalent interactions, inhibiting polymerization at non-permissive temperatures. This novel method eliminates the need for an activation step, reducing the potential for sample degradation and decreasing overall reaction time.

Additional Formats: For added convenience, *Taq* and Hot Start *Taq* DNA Polymerase are available in master mix format. For direct gel loading, a Quick-Load version of the *Taq* 2X Master Mix is also available. The *Taq* PCR Kit contains *Taq* DNA Polymerase, dNTP Mix, Buffer, $MgCl_2$ and the Quick-Load Purple 1 kb Plus DNA Ladder.

The Multiplex PCR 5X Master Mix formulation has been specifically optimized for enhanced performance in multiplex PCR reactions.

Concentration: 5,000 units/ml

Taq Buffer Selection Chart

CHOICE OF BUFFER	AVAILABLE PRODUCTS	NEB #
ThermoPol Reaction Buffer: Designed for optimal yield and specificity	*Taq* DNA Polymerase with ThermoPol Buffer	M0267
Standard *Taq* Reaction Buffer: Detergent-free and designed to be compatible with existing assay systems	*Taq* DNA Polymerase with Standard *Taq* Buffer	M0273
	Taq DNA Polymerase with Standard *Taq* (Mg-free) Buffer	M0320

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Requires BSA · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

LongAmp® *Taq* DNA Polymerase

LongAmp Hot Start *Taq* DNA Polymerase

LongAmp *Taq* DNA Polymerase
#M0323S 500 units
#M0323L 2,500 units

LongAmp Hot Start *Taq* DNA Polymerase
#M0534S 500 units
#M0534L 2,500 units

LongAmp *Taq* 2X Master Mix
#M0287S 100 rxns (50 µl vol)
#M0287L 500 rxns (50 µl vol)

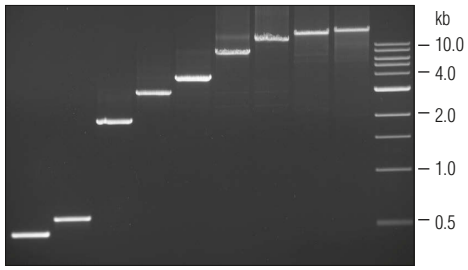
LongAmp Hot Start *Taq* 2X Master Mix
#M0533S 100 rxns (50 µl vol)
#M0533L 500 rxns (50 µl vol)

LongAmp *Taq* PCR Kit
#E5200S 100 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 79.

For a complete listing of Reaction Buffers, see pages 79.

LONGAMP *Taq* POLYMERASE DETAILS	
Extension Rate	1.2 kb/min
Amplicon Size	≤ 30 kb
Fidelity	2X *Taq* DNA Polymerase
Units / 50 µl rxn	5 units
Resulting Ends	3´ A/Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	Yes
Supplied Buffer (product dependent)	LongAmp *Taq* Rxn Buffer
PRODUCT FORMATS	
Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
Direct Gel-loading Available	No
PCR Kit Available	Yes
APPLICATIONS	
Long Amplicons	Yes
Routine PCR	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes



Amplification of longer templates with LongAmp Taq. *Amplification of specific sequences from human genomic DNA using LongAmp Taq DNA Polymerase. Amplicon sizes are indicated below gel. Ladder (M) is NEB 1 kb DNA Ladder (NEB #N3232).*

Description: An optimized blend of *Taq* and Deep Vent DNA Polymerases, LongAmp *Taq* DNA Polymerase enables amplification of longer PCR products with higher fidelity than *Taq* DNA Polymerase alone.

LongAmp Hot Start *Taq* DNA Polymerase: LongAmp Hot Start *Taq* DNA Polymerase utilizes a unique synthetic aptamer. This structure binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions.

Additional Formats: For added convenience, LongAmp *Taq* and LongAmp Hot Start *Taq* are available in master mix format. The LongAmp *Taq* PCR Kit includes LongAmp *Taq* DNA Polymerase (2,500 units/ml), dNTP Mix (10 mM), LongAmp *Taq* Reaction Buffer Pack (5X), $MgSO_4$ (100 mM) and nuclease-free water.

Concentration: 2,500 units/ml

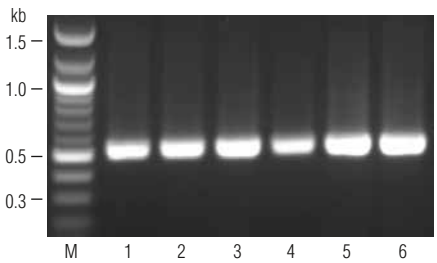
Hemo KlenTaq®

#M0332S 200 rxns (25 µl vol)
#M0332L 1,000 rxns (25 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 79.

HEMO KLENTaq DETAILS	
Extension Rate	0.5 kb/min
Amplicon Size	≤ 2 kb
Units / 50 µl rxn	4 units
Resulting Ends	3´ A
3´→5´ Exonuclease Activity	No
5´→3´ Exonuclease Activity	No
Supplied Buffer	Hemo KlenTaq Rxn Buffer
APPLICATIONS	
Extraction-free PCR	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes

Description: Hemo KlenTaq is a truncated version of *Taq* DNA Polymerase, lacking the first 280 amino acids. Hemo KlenTaq also contains mutations that make it resistant to inhibitors present in whole blood. It can amplify DNA from whole blood samples from humans and mice, without the need for DNA extraction. Hemo KlenTaq tolerates up to 20% whole blood in a 25 µl reaction (30% in a 50 µl reaction). Hemo KlenTaq works well with most common anticoagulants, including heparin, citrate and EDTA.



Amplification of human whole blood with Hemo KlenTaq. *Lane 1: 5% blood + Na-EDTA; Lane 2: 5% blood + K-EDTA; Lane 3: 5% blood + Na-Heparin; Lane 4: 5% blood + Na-Citrate; Lane 5: 1.2 mm² FTA Guthrie Card containing dried human blood + Na-Heparin; Lane 6: 1.2 mm² PTA Guthrie Card containing dried human blood + Na-Heparin (washed with 50 µl H_2O at 50°C for 5 min.). Ladder (M) is the 1 kb Plus DNA Ladder (NEB #N3200).*

Source: An *E. coli* strain that carries a mutant *Taq* DNA polymerase gene. The protein lacks the N-terminal 5´ flap endonuclease domain and the gene has three internal point mutations.

Reaction Buffer: 1X Hemo KlenTaq Reaction Buffer

EpiMark® Hot Start *Taq* DNA Polymerase

#M0490S 100 rxns (50 µl vol)
#M0490L 500 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 79.

EPIMARK POLYMERASE DETAILS	
Extension Rate	1 kb/min
Amplicon Size	≤ 1 kb
Units / 50 µl rxn	1.25 units
Resulting Ends	3´A
3´→5´ Exonuclease Activity	No
5´→3´ Exonuclease Activity	Yes
Supplied Buffer	EpiMark Hot Start *Taq* Rxn Buffer
APPLICATIONS	
AT-rich Targets	Yes
Bisulfite-converted DNA	Yes
Routine PCR	Yes
T/A, U/A Cloning	Yes

Description: EpiMark Hot Start *Taq* DNA Polymerase is an excellent choice for use on bisulfite-converted DNA. It is a mixture of *Taq* DNA Polymerase and a temperature-sensitive, aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits room temperature reaction assembly with no separate high-temperature incubation step to activate the enzyme.

Companion Product:

EpiMark Bisulfite Conversion Kit
#E3318S 48 reactions

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from Thermus aquaticus YT-1.

Concentration: 5,000 units/ml

Vent® & Deep Vent® DNA Polymerases

Vent DNA Polymerase
#M0254S 200 units
#M0254L 1,000 units

Vent (exo⁻) DNA Polymerase
#M0257S 200 units
#M0257L 1,000 units

Deep Vent DNA Polymerase
#M0258S 200 units
#M0258L 1,000 units

Deep Vent (exo⁻) DNA Polymerase
#M0259S 200 units
#M0259L 1,000 units

For a complete listing of Deoxynucleotide Solutions, see page 79.

VENT/DEEP VENT POLYMERASES DETAILS	
Extension Rate	1 kb/min
Amplicon Size	≤ 6 kb
Fidelity	5-6X *Taq*
Resulting Ends	Blunt
3´→5´ Exonuclease Activity	Yes (M0254, M0258)
5´→3´ Exonuclease Activity	No
Supplied Buffer	ThermoPol Rxn Buffer

Description: Vent DNA Polymerase was the first high-fidelity thermophilic DNA polymerase available for PCR. The fidelity is 5-fold higher than that observed for *Taq* DNA Polymerase, and is derived in part from an integral 3´→ 5´ proofreading exonuclease activity. Greater than 90% activity remains following a 1 hour incubation at 95°C.

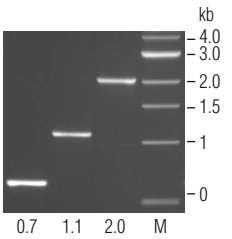
Deep Vent DNA Polymerase is a modified version of Vent DNA Polymerase. Deep Vent has similar fidelity with even more stability than Vent at temperatures of 95 to 100°C, with a half-life of 8 hours at 100°C.

Vent (exo⁻) DNA Polymerase has been genetically engineered to eliminate the 3´→ 5´ proofreading exonuclease activity associated with Vent DNA Polymerase. The fidelity of polymerization by this form is reduced to a level about 2-fold higher than that of *Taq* DNA Polymerase. Likewise, Deep Vent (exo⁻) DNA Polymerase has been genetically engineered to eliminate the 3´→ 5´ proofreading exonuclease activity associated with Deep Vent DNA Polymerase.

Source: Vent DNA Polymerase is purified from a strain of *E. coli* that carries the Vent DNA Polymerase gene from the archaea *Thermococcus litoralis*. Vent (exo⁻) is purified from an *E. coli* strain that carries the Vent (D141A/E143A) DNA Polymerase gene, a genetically engineered form of the native DNA polymerase.

Deep Vent DNA Polymerase is purified from a strain of *E. coli* that carries the Deep Vent DNA Polymerase gene from *Pyrococcus* species GB-D. Deep Vent (exo⁻) is purified from an *E. coli* strain that carries the Deep Vent (D141A/E143A) DNA Polymerase gene, a genetically engineered form of the native polymerase.

Concentration: 2,000 units/ml



Amplification of Jurkat genomic DNA with Vent DNA Polymerase. *Amplicon Sizes are indicated below gel. Marker (M) is the 1 kb DNA Ladder (NEB #N3232).*

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Requires BSA · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Luna qPCR and RT-qPCR

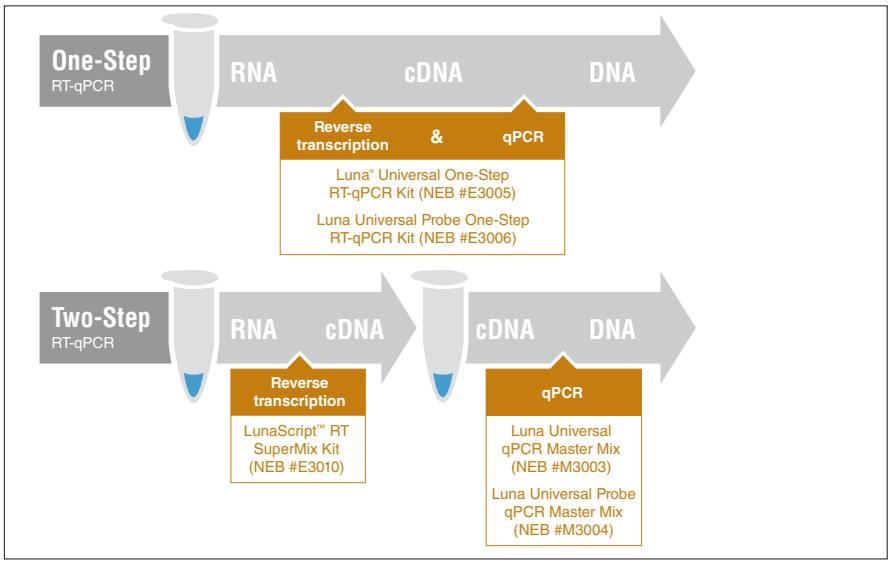
- *Convenient master mix and supermix formats with user-friendly protocols simplify reaction setup*
- *Non-interfering, visible tracking dye helps to eliminate pipetting errors*
- *Novel, thermostable reverse transcriptase (RT) improves performance*
- *One product per application streamlines selection process*

Fluorescence-based quantitative real-time PCR (qPCR) is the gold standard for the detection and quantification of nucleic acids due to its sensitivity and specificity. Luna products from NEB are optimized for qPCR or RT-qPCR, and are available for either intercalating dye or probe-based detection methods. Each Hot Start *Taq*-based Luna qPCR master mix has been formulated with a unique passive reference dye that is compatible across a wide variety of thermal cyclers, regardless of ROX requirements. No additional components are required to ensure compatibility. For two-step RT-qPCR, the LunaScript® RT SuperMix Kit offers a fast (less than 15 minute), robust, and sensitive option for cDNA synthesis upstream of your Luna qPCR experiment.

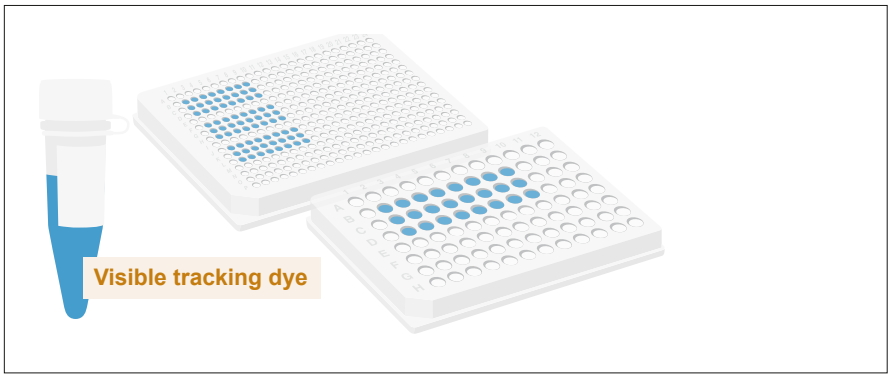
What is the Difference Between One-Step and Two-Step RT-PCR?

In one-step RT-PCR, cDNA synthesis and PCR amplification are performed in a single reaction. This offers the benefit of a streamlined workflow with fewer chances of contamination. In two-step RT-PCR, cDNA synthesis and PCR amplification are done separately to allow for more flexibility and customization.

Find the right Luna product for your application



Avoiding pipetting errors with Luna



Find an overview of qPCR.

Luna® Universal qPCR & Probe qPCR Master Mixes

Luna Universal qPCR Master Mix

#M3003S	200 reactions
#M3003L	500 reactions
#M3003X	1,000 reactions
#M3003E	2,500 reactions

Luna Universal Probe qPCR Master Mix

#M3004S	200 reactions
#M3004L	500 reactions
#M3004X	1,000 reactions
#M3004E	2,500 reactions

Description: The NEB Luna Universal qPCR Master Mix is an optimized 2X reaction mix for real-time qPCR detection and quantitation of target DNA sequences using the SYBR®/FAM channel of most real-time qPCR instruments.

The NEB Luna Universal Probe qPCR Master Mix is a 2X reaction mix optimized for real-time qPCR detection and quantitation of target DNA sequences using hydrolysis probes.

Each Hot Start *Taq*-based Luna qPCR master mix has been formulated with a unique passive reference dye that is compatible across a wide variety of instrument platforms, including those that require a ROX reference signal. This means that no additional components are required to ensure machine compatibility. The mixes also contain dUTP, enabling carryover prevention when reactions are treated with NEB's Antarctic Thermolabile UDG. A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates. These features, combined with rapid, sensitive and precise real-time qPCR performance, make Luna the universal choice for all your qPCR experiments.

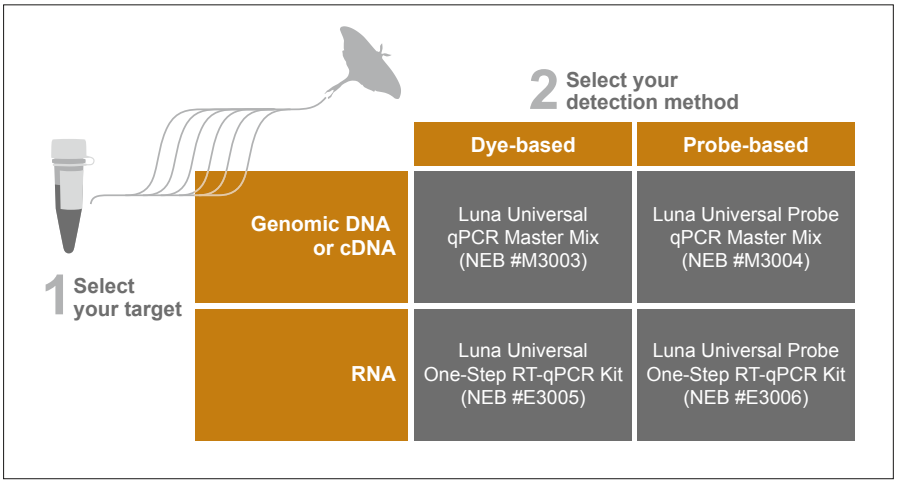
Companion Product:

Antarctic Thermolabile UDG

#M0372S	100 units
#M0372L	500 units

- *One product per application*
- *Convenient master mix formats and user-friendly protocols simplify reaction setup*
- *Non-interfering, visible tracking dye helps to eliminate pipetting errors*
- *Rigorously tested to optimize specificity, sensitivity, accuracy and reproducibility*
- *Unique passive reference dye for compatibility across wide range of instruments*

Find the right Luna product for your application



NEW

LunaScript® RT SuperMix Kit

#E3010S	25 reactions
#E3010L	100 reactions

Description: LunaScript RT SuperMix Kit is an optimized master mix containing all the necessary components for first strand cDNA synthesis in the context of a two-step RT-qPCR workflow. It features the thermostable Luna Reverse Transcriptase, which supports cDNA synthesis at elevated temperatures. Murine RNase Inhibitor is also included to protect template RNA from degradation. The LunaScript RT SuperMix Kit contains random hexamer and poly-dT primers, allowing for even coverage across the length of the RNA targets.

In addition, the LunaScript RT SuperMix Kit contains an inert blue tracking dye, providing a visual indicator that can be followed throughout the two-step RT-qPCR process. The LunaScript RT SuperMix offers robust, linear, and sensitive detection using total RNA inputs as high as 1 µg and as low as single copies of RNA.

The Luna Universal One-Step RT-qPCR Kit Includes:

- LunaScript RT SuperMix
- No-RT Control Mix
- Nuclease-free Water

Companion Products:

Luna Universal qPCR Master Mix

#M3003S	200 reactions
#M3003L	500 reactions
#M3003X	1,000 reactions
#M3003E	2,500 reactions

Luna Universal Probe qPCR Master Mix

#M3004S	200 reactions
#M3004L	500 reactions
#M3004X	1,000 reactions
#M3004E	2,500 reactions

- *Less than 15 minute first-strand cDNA synthesis protocol*
- *Combine with Luna qPCR master mixes for robust RT-qPCR results*

How can we ensure best in class performance with Luna?

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/ WarmStart · Requires BSA · Heat Inactivation · Annealing Temperature

Luna Universal One-Step RT-qPCR & Probe One-Step RT-qPCR Kits

Luna Universal One-Step RT-qPCR Kit

#E3005S	200 reactions
#E3005L	500 reactions
#E3005X	1,000 reactions
#E3005E	2,500 reactions

Luna Universal Probe One-Step RT-qPCR Kit

#E3006S	200 reactions
#E3006L	500 reactions
#E3006X	1,000 reactions
#E3006E	2,500 reactions

Companion Product:

Antarctic Thermolabile UDG

#M0372S	100 units
#M0372L	500 units

- *Novel, thermostable reverse transcriptase (RT) improves performance*
- *WarmStart RT paired with Hot Start Taq increases reaction specificity and robustness*
- *Non-interfering, visible tracking dye helps to eliminate pipetting errors*
- *Products perform consistently across a wide variety of sample sources*
- *Unique passive reference dye for compatibility across wide range of instruments*

Description: One-Step RT-qPCR provides a convenient and powerful method for RNA detection and quantitation. In a single tube, RNA is first converted to cDNA by a reverse transcriptase, and then a DNA-dependent DNA polymerase amplifies the cDNA, enabling quantitation via qPCR.

The Luna RT-qPCR kits contain a novel, *in silico*-designed reverse transcriptase (RT) engineered for improved performance. Both the Luna WarmStart Reverse Transcriptase and Hot Start *Taq* DNA Polymerase, included in these kits, utilize a temperature-sensitive, reversible aptamer, which inhibits activity below 45°C. This enables room temperature reaction setup and prevents undesired non-specific activity. Furthermore, the WarmStart RT has increased thermostability, improving performance at higher reaction temperatures.

The NEB Luna Universal One-Step RT-qPCR Kit is optimized for dye-based real-time quantitation of target RNA sequences via the SYBR/FAM fluorescence channel of most real-time instruments.

The NEB Luna Universal Probe One-Step RT-qPCR Kit is optimized for real-time quantitation of target RNA sequences using hydrolysis probes.

Each Hot Start *Taq*-based Luna RT-qPCR kit has been formulated with a unique passive reference dye that is compatible across a wide variety of instrument platforms, including those that require a ROX reference signal. This means that no additional components are required to ensure machine compatibility. The mixes also contain dUTP, enabling carryover prevention when reactions are treated with NEB's Antarctic Thermolabile UDG. A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates. These features, combined with rapid, sensitive and precise real-time qPCR performance, make Luna the universal choice for all your qPCR experiments.

The Luna Universal One-Step RT-qPCR Kit Includes:

– Luna Universal One-Step Reaction Mix

– Luna WarmStart RT Enzyme Mix

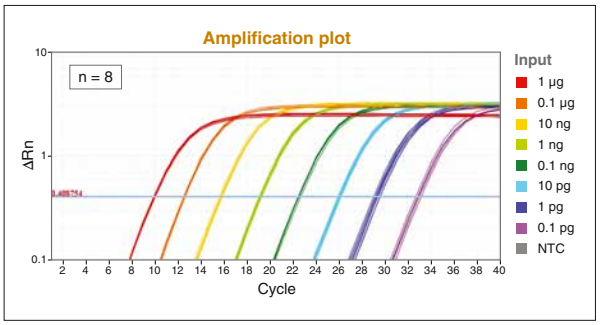
– Nuclease-free Water

The Luna Universal Probe One-Step RT-qPCR Kit Includes:

– Luna WarmStart® RT Enzyme Mix

– Luna Universal Probe One-Step Reaction Mix

– Nuclease-free Water



Luna RT-qPCR products offer exceptional sensitivity, reproducibility and performance. *RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit over an 8-log range of input template concentrations (1 µg – 0.1 pg Jurkat total RNA) with 8 replicates at each concentration. Reaction setup and cycling conditions followed recommended protocols, including a 10-minute RT step at 55°C for the thermostable Luna WarmStart Reverse Transcriptase. NTC = non-template control.*

NEB's OEM & Custom Solutions team works closely with our customers to ensure they receive the innovative solutions they need to accelerate their own research. Denisa, Julie and Beth support the operations of this growing branch within the company.

Epi EpiMark

Extraction Free

One*Taq* One-Step RT-PCR Kit

#E5315S 30 reactions

Companion Products:

ProtoScript II First Strand cDNA Synthesis Kit
#E6560S 30 reactions
#E6560L 150 reactions

ProtoScript II Reverse Transcriptase
#M0368S 4,000 units
#M0368L 10,000 units
#M0368X 40,000 units

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

For a complete listing of One*Taq* products, see page 65.

- *Combine cDNA synthesis and PCR in a single reaction*
- *Detect at little as 0.1 pg of a GAPH target*
- *Robust amplification from 100 bp to 9 kb*
- *Faster protocols with less hands-on time*
- *Quick-Load Reaction Mix allows instant gel loading*

Description: The One*Taq* One-Step RT-PCR Kit offers sensitive and robust end-point detection of RNA templates. cDNA synthesis and PCR amplification steps are performed in a single reaction using gene-specific primers, resulting in a streamlined RT-PCR protocol.

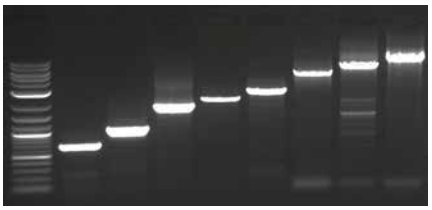
The kit combines three optimized mixes: One*Taq* One-Step Enzyme Mix, One*Taq* One-Step Reaction Mix and One*Taq* One-Step Quick-Load Reaction Mix. The Enzyme Mix combines ProtoScript II Reverse Transcriptase, Murine RNase Inhibitor and One*Taq* Hot Start DNA Polymerase. ProtoScript II Reverse Transcriptase is a mutant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. One*Taq* Hot Start DNA Polymerase is mixture of a Hot Start *Taq* DNA Polymerase combined with a proof-reading DNA polymerase, resulting in high-yield amplification with minimal optimization. The One*Taq* One-Step RT-PCR Kit is capable of amplifying long transcripts up to 9 kb in length.

Two optimized reaction mixes are included, One*Taq* One-Step Reaction Mix and Quick-Load One*Taq* One-Step Reaction Mix. The reaction mixes offer robust conditions for both cDNA synthesis and PCR amplification. The unique Quick-Load One*Taq* One-Step Reaction Mix contains additional dyes, offering color indication for reaction setup as well as direct gel loading.

Both total RNA and mRNA can be used as template. The kit can detect a GAPDH target as low as 0.1 pg per reaction. It can routinely detect RNA targets up to 9 kb. The One*Taq* One-Step RT-PCR Kit is capable of multiplex detection of two or three targets.

The One*Taq* One-Step RT-PCR Kit Includes:

– One*Taq* One-Step Enzyme Mix
– One*Taq* One-Step Reaction Mix
– Quick-Load One*Taq* One-Step Reaction Mix
– Nuclease-free Water



Detection of RNA templates of different length. *About 100 ng of Jurkat total RNA was used in 50 µl reactions following the standard protocol. The target sizes were Lane 1: 0.7 kb, Lane 2: 1.1 kb, Lane 3: 1.9 kb, Lane 4: 2.3 kb, Lane 5: 2.5 kb, Lane 6: 5.5 kb, Lane 7: 7.6 kb and Lane 8: 9.3 kb. The marker lane (M) contains Quick-Load 1 kb Plus DNA Ladder (NEB #N0469).*

One*Taq* RT-PCR Kit

#E5310S 30 reactions

Companion Products:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

M-MuLV Reverse Transcriptase
#M0253S 10,000 units
#M0253L 50,000 units

One*Taq* Hot Start 2X Master Mix with Standard Buffer
#M0484S 100 rxns (50 µl vol)
#M0484L 500 rxns (50 µl vol)

PolyA Spin mRNA Isolation Kit
#S1560S 8 isolations

For a complete listing of One*Taq* products, see page 65.

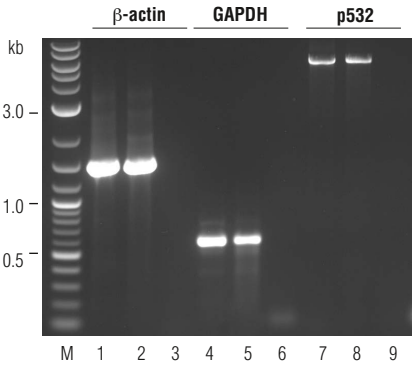
- *Robust RT-PCR reactions*
- *Convenient master mix formats*
- *OneTaq DNA Polymerase offers robust amplification for a wide range of templates*

Description: One*Taq* RT-PCR Kit combines two powerful mixes, M-MuLV Enzyme Mix and One*Taq* Hot Start 2X Master Mix with Standard Buffer for 2-step RT-PCR applications. The two mixes require minimal handling during reaction setup and yet offer consistent and robust RT-PCR reactions.

The first strand cDNA synthesis is achieved by using two optimized mixes, M-MuLV Enzyme Mix and M-MuLV Reaction Mix. M-MuLV Enzyme Mix combines M-MuLV Reverse Transcriptase and RNase Inhibitor, Murine while M-MuLV Reaction Mix contains dNTPs and an optimized buffer. The kit also contains two optimized primers for reverse transcription and nuclease-free water. An anchored Oligo-dT primer [$d(T)_{23}$VN] forces the primer to anneal to the beginning of the polyA tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs.

The One*Taq* RT-PCR Kit Includes:

– 10X M-MuLV Enzyme Mix
– 2X M-MuLV Reaction Mix
– One*Taq* Hot Start 2X Master Mix with Standard Buffer
– Random Primer Mix (60 µM)
– Oligo $d(T)_{23}$VN Primer (50 µM)**
– Nuclease-free Water



First strand cDNA synthesis. *Synthesis was carried out in the presence of 1X M-MuLV Enzyme Mix at 42°C using 0.5 µg of human spleen total RNA in the presence of dT_{23}VN (lanes 1, 4 and 7) or Random Hexamer Mix (lanes 2, 5 and 8). No-RT controls were lanes 3, 6 and 9. OneTaq Hot Start Master Mix was used to amplify a 1.5 kb fragment of beta-actin gene, a 0.6 kb fragment of GAPDH gene, and a 5.5 kb fragment from p532 gene in 35 cycles. The marker lane (M) contains 1 kb Plus DNA Ladder (NEB #N3200).*

** Oligo $d(T)_{23}$ VN and Random Primer Mix contain 1 mM dNTP

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/ WarmStart · Requires BSA · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Bst DNA Polymerase-based Products for Isothermal DNA Amplification

	5´→ 3´ EXO ACTIVITY	AMPLIFICATION SPEED	ROOM TEMPERATURE SETUP	REVERSE TRANSCRIPTASE ACTVITY	INHIBITOR TOLERANCE	APPLICATIONS
***Bst* DNA Polymerase, Full Length**	★★	N/A	N/A	N/A	★	• Nick translation reactions at elevated temperatures • Primer extension
***Bst* DNA Polymerase, Large Fragment**	N/A	★	N/A	★	★	• General strand-displacement reactions
***Bst* 2.0 DNA Polymerase**	N/A	★★	N/A	★★	★	• Improved LAMP, SDA, and other amplification reactions • Minimal effect of substitution of dTTP with dUTP
***Bst* 2.0 WarmStart DNA Polymerase**	N/A	★★	★★★	★★	★★	• Consistent, room-temperature, and high-throughput amplification assays • Minimal effect of substitution of dTTP with dUTP
***Bst* 3.0 DNA Polymerase**	N/A	★★★	★★	★★★	★★★	• Fastest, most robust LAMP and RT-LAMP reactions • High reverse transcriptase activity up to 72°C • Strand displacement DNA synthesis

★★★ Optimal, recommended product for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
N/A Not applicable to this application

Bst DNA Polymerases

Bst DNA Polymerase, Large Fragment
#M0275S 1,600 units
#M0275L 8,000 units
for high (15X) concentration
#M0275M 8,000 units

Bst DNA Polymerase, Full Length
#M0328S 500 units

Bst 2.0 DNA Polymerase
#M0537S 1,600 units
#M0537L 8,000 units
for high (15X) concentration
#M0537M 8,000 units

Bst 2.0 WarmStart DNA Polymerase
#M0538S 1,600 units
#M0538L 8,000 units
for high (15X) concentration
#M0538M 8,000 units

Bst 3.0 DNA Polymerase
#M0374S 1,600 units
#M0374L 8,000 units
for high (15X) concentration
#M0374M 8,000 units

Companion Products:

WarmStart RTx Reverse Transcriptase
#M0380S 50 reactions
#M0380L 250 reactions

Tte UvrD Helicase
#M1202S 0.5 µg

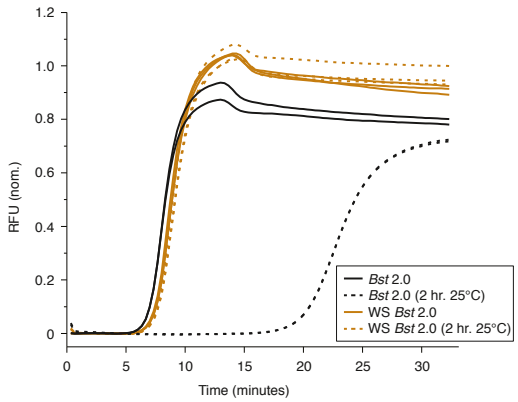
For a complete listing of Deoxynucleotide Solutions, see page 79.

Description: *Bst* DNA Polymerase, Large Fragment, is the portion of the *Bacillus stearothermophilus* DNA Polymerase protein that contains the 5´→ 3´ polymerase activity, but lacks 5´→ 3´ exonuclease activity.

Bst DNA Polymerase, Full Length is the full length polymerase from *Bacillus stearothermophilus*. It has 5´→ 3´ polymerase and double-strand specific 5´→ 3´ exonuclease activities, but lacks 3´→ 5´ exonuclease activity.

Bst 2.0 DNA Polymerase is an *in silico* designed homologue of *Bst* DNA Polymerase I, Large Fragment. It contains 5´→ 3´ DNA polymerase activity and strong strand displacement activity but lacks 5´→ 3´ exonuclease activity. It has improved amplification speed, yield, salt tolerance and thermostability compared to wild-type *Bst* DNA Polymerase, Large Fragment.

Bst 2.0 WarmStart DNA Polymerase utilizes aptamer technology to inhibit activity at non-permissive temperatures (< 50°C). Like "Hot Start" PCR polymerases, the WarmStart feature enables room temperature set up and prevents non-templated addition of nucleotides, increasing reaction efficiencies. Additionally, no separate activation step is required to release the aptamer from the enzyme. *Bst* 2.0 WarmStart DNA Polymerase permits reaction temperatures from 60–72°C.



Benefits of Bst 2.0 WarmStart: *Identical LAMP reactions were run either immediately after setup (solid line) or after a 2 hour incubation at 25°C. Without the protection from Bst 2.0 WarmStart, this room temperature incubation results in variable LAMP performance. Bst 2.0 WarmStart provides more consistent amplification reaction and enables room-temperature and high-throughput setup.*

Bst 3.0 DNA Polymerase is a similarly designed *in silico* homologue engineered for improved performance and increased reverse transcriptase activity. *Bst* 3.0 DNA Polymerase contains 5´→ 3´ DNA polymerase activity with either DNA or RNA templates but lacks 5´→ 3´ and 3´→ 5´ exonuclease activity. It demonstrates robust performance in the presence of inhibitors and significantly increase reverse transcriptase activity compared to *Bst* DNA Polymerase.

Concentration: *Bst* DNA Polymerase, Full Length: 5,000 units/ml. All others: 8,000 and 120,000 units/ml

Heat Inactivation: 80°C for 20 minutes

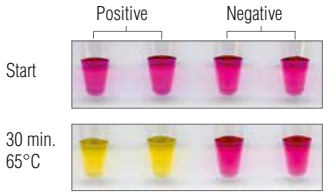
Usage Notes: No *Bst* DNA Polymerase-based products can be used for thermal cycle sequencing or PCR. *Bst* 2.0 WarmStart DNA Polymerase permits reaction temperatures from 60–72°C. Generally, reaction temperatures above 72°C are not recommended for any *Bst* DNA Polymerase-based product.

WarmStart® Colorimetric LAMP Master Mix (DNA & RNA)

#M1800S	100 reactions
#M1800L	500 reactions

- *LAMP amplification of RNA and DNA targets*
- *One-step field and point-of-need LAMP reactions*
- *Set up reactions at room temperature with our unique dual WarmStart formulation*
- *Simple visual indication of amplification for easy detection and analysis*

Description: The WarmStart Colorimetric LAMP 2X Master Mix is an optimized formulation of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx in a special low-buffer reaction solution containing a visible pH indicator for rapid and easy detection of Loop-Mediated Isothermal Amplification (LAMP) and RT-LAMP reactions. This system is designed to provide a fast, clear visual detection of amplification based on the production of protons and subsequent drop in pH that occurs from the extensive DNA polymerase activity in a LAMP reaction, producing a change in solution color from pink to yellow (an overview of LAMP and primer design can be found in our video library, https://www.neb.com/tools-and-resources/video-library). This mix can be used for any LAMP or RT-LAMP reaction and requires only a heated chamber and samples, with readout of positive amplification judged by eye in 15–40 minutes.



WarmStart LAMP Kit (DNA & RNA)

#E1700S	100 reactions
#E1700L	500 reactions

Companion Product:

Tte UvrD Helicase
#M1202S 0.5 µg

- *LAMP amplification of RNA and DNA targets*
- *Improve LAMP specificity and sensitivity with optimized master mixes*
- *Set up reactions at room temperature with our unique dual WarmStart formulation*
- *Use with a variety of detection methods including fluorescence, turbidity, visual detection and electrophoresis*

Description: The WarmStart LAMP Kit (DNA & RNA) is designed to provide a simple, one-step solution for Loop-Mediated Isothermal Amplification (LAMP) of DNA or RNA (RT-LAMP) targets. LAMP and RT-LAMP are commonly used isothermal amplification techniques that provides rapid detection of a target nucleic acid using LAMP-specific primers (supplied by the user) and a strand-displacing DNA polymerase. This kit is supplied with the WarmStart LAMP 2X Master Mix, which contains a blend of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase in an optimized LAMP buffer solution. Both *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase have been engineered for improved performance in LAMP and RT-LAMP reactions. A fluorescent dye is also supplied to enable real-time fluorescence measurement of LAMP. The WarmStart LAMP Kit is compatible with multiple detection methods, including turbidity detection, real-time fluorescence detection (when used with LAMP fluorescent dye) and end-point visualization.

The WarmStart LAMP Kit Includes:

– WarmStart LAMP 2X Master Mix
– LAMP Fluorescent Dye (50X)

How is colorimetric LAMP used in point of care?

Cloned at NEB | Recombinant Enzyme | High-Fidelity | PCR Enzyme | Hot Start/ WarmStart | Requires BSA | Heat Inactivation | Annealing Temperature

IsoAmp® II Universal tHDA Kit

#H0110S 50 reactions

- *Easy-to-use for assay development*
- *Helicase eliminates need for thermocycler*
- *Reactions performed at constant temp*
- *Amplify & detect short DNA sequences (70–120 bp)*
- *Use with a variety of templates (microbial genomic DNA, viral DNA, plasmid DNA and cDNA)*
- *Amplify a single copy of target DNA by tHDA when optimized primers and buffer are used*

Description: Thermophilic Helicase-Dependent Amplification (tHDA) is a novel method for isothermal amplification of nucleic acids. Like PCR, the tHDA reaction selectively amplifies a target sequence defined by two primers. However, unlike PCR, tHDA uses an enzyme called a helicase to separate DNA, rather than heat. This allows DNA amplification without the need for thermocycling. The tHDA reaction can also be coupled with reverse transcription for RNA analysis.

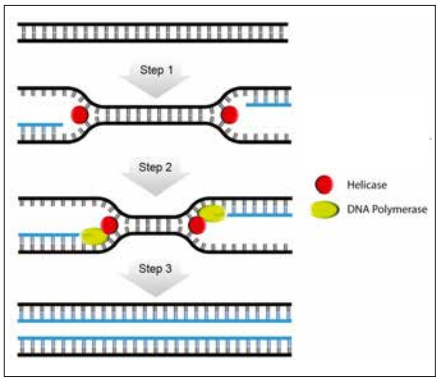
IsoAmp II Universal tHDA Kit is based on a second-generation thermophilic Helicase-Dependent Amplification platform. The reactions supported by IsoAmp II Universal tHDA Kit include tHDA, reverse transcription HDA (RT-HDA), real-time quantitative HDA (qHDA) and real-time quantitative RT-HDA (qRT-HDA), from a single reaction buffer.

The IsoAmp II Universal tHDA Kit Includes:

– IsoAmp dNTP solution and IsoAmp Enzyme Mix

– 10X Annealing Buffer II, 100 mM $MgSO_4$ and 500 mM NaCl

– Control template and amplification primers



HDA technology. Helicase Dependent Amplification: Step 1: Helicase unwinding and primer binding. Step 2: DNA polymerization. Step 3: DNA amplification.

Developed by BioHelix Corporation a NEB-affiliated company, now part of Quidel Corporation. ISOAMP® is a registered trademark of BioHelix Corporation

phi29 DNA Polymerase

#M0269S 250 units
#M0269L 1,250 units

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Extreme processivity*
- *Extreme strand displacement*
- *Replication requiring a high degree of strand displacement and/or processive synthesis*
- *High-fidelity replication at moderate temperatures*

Description: phi29 DNA Polymerase is the replicative polymerase from the *Bacillus subtilis* phage phi29 (ϕ29). This polymerase has exceptional strand displacement and processive synthesis properties. The polymerase has an inherent 3´→ 5´ proofreading exonuclease activity.

Concentration: 10,000 units/ml

Heat Inactivation: 65°C for 10 minutes

Steve is a Product Marketing Manager and has been with NEB for 2 years. In his role, Steve works with our scientists specializing in amplification technologies to bring the most useful products to market.

PreCR® Repair Mix

#M0309S 30 reactions
#M0309L 150 reactions

Companion Product:

beta-Nicotinamide adenine dinucleotide (NAD+)
#B9007S 0.2 ml

- *Repair DNA prior to its use in DNA-related technologies*
- *Easy-to-use protocols*
- *Does not harm template*

Need to repair FFPE-treated DNA prior to next gen sequencing?
See page 153 for more information.

Description: The PreCR Repair Mix is an enzyme cocktail formulated to repair damaged template DNA prior to its use in the polymerase chain reaction (PCR), microarrays, or other DNA technologies. The PreCR Repair Mix is active on a broad range of DNA damage, including those that block PCR (e.g., apurinic/apyrimidinic sites, thymidine dimers, nicks and gaps) and those that are mutagenic (e.g., deaminated cytosine and 8-oxo-guanine). In addition, it will remove a variety of moieties from the 3´ end of DNA leaving a hydroxyl group. The PreCR Repair Mix will not repair all damage that inhibits/interferes with PCR. It can be used in conjunction with any thermophilic polymerase.

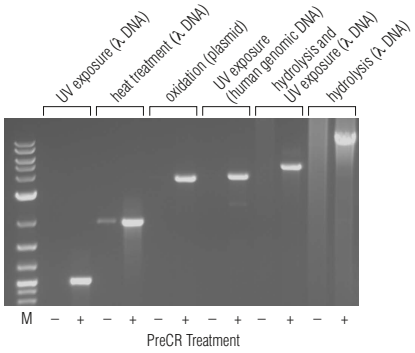
Applications:

- Repair DNA prior to its use as a template in PCR or other DNA technologies

Reagents Supplied:
1X PreCR Repair Mix
10X ThermoPol Reaction Buffer
100X NAD^+ solution
Control Template (UV damaged λ DNA)
PCR primers for control template
Purified BSA

Types of DNA Damage

DNA DAMAGE	CAUSE	REPAIRED BY PRECR REPAIR MIX?
abasic sites	hydrolysis	yes
nicks	hydrolysis nucleases shearing	yes
thymidine dimers	UV radiation	yes
blocked 3´-ends	multiple	yes
oxidized guanine	oxidation	yes
oxidized pyrimidines	oxidation	yes
deaminated cytosine	hydrolysis	yes
fragmentation	hydrolysis nucleases shearing	no
protein-DNA crosslinks	formaldehyde	no



Repair of different types of DNA damage with the PreCR Repair Mix. *The gel shows amplification of damaged DNA that was either not treated (–) or treated (+) with the PreCR Repair Mix. Type of DNA damage is shown. Note: heat treated DNA is incubated at 99°C for 3 minutes. Marker (M) is the 1 kb Plus DNA Ladder (NEB #N3200).*

Sulfolobus DNA Polymerase IV

#M0327S 100 units

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Synthesis of DNA through DNA lesions (lesion bypass)*
- *DNA Repair*

Description: *Sulfolobus* DNA Polymerase IV is a thermostable Y-family lesion-bypass DNA Polymerase that efficiently synthesizes DNA across a variety of DNA template lesions.

Concentration: 2,000 units/ml

Therminator™ DNA Polymerase

#M0261S 200 units
#M0261L 1,000 units

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Incorporation of modified nucleotides*
- *DNA sequencing by partial ribosubstitution*
- *DNA sequencing or SNP analysis using dideoxy or acyclo chain terminators*

Description: Therminator DNA Polymerase is a 9°N™ DNA Polymerase variant with an enhanced ability to incorporate modified substrates such as dideoxynucleotides, ribonucleotides and acyclonucleotides.

Source: An *E. coli* strain that carries the 9°N (D141A / E143A / A485L) DNA Polymerase gene, a genetically engineered form of the native DNA polymerase from *Thermococcus* species 9°N-7.

Concentration: 2,000 units/ml

Usage Notes: Amplification of extended regions may require optimization of reaction conditions.

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DNA Polymerase I (*E. coli*)

#M0209S 500 units
#M0209L 2,500 units

- *Nick translation of DNA*
- *Second strand cDNA synthesis*

Description: DNA Polymerase I (*E. coli*) is a DNA-dependent DNA polymerase with inherent 3´→ 5´ and 5´→ 3´ exonuclease activities. The 5´→ 3´ exonuclease activity removes nucleotides ahead of the growing DNA chain, allowing nick translation.

Concentration: 10,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Usage Notes: DNase I is not included with this enzyme and must be added for nick translation reactions.

DNA Polymerase I, Large (Klenow) Fragment

#M0210S 200 units
#M0210L 1,000 units
for high (10X) concentration
#M0210M 1,000 units

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Generates probes using random primers*
- *Removal of 3´ overhangs or fill-in of 5´ overhangs to form blunt ends*
- *Second strand cDNA synthesis*

Description: DNA Polymerase I, Large (Klenow) Fragment was originally derived as a proteolytic product of *E. coli* DNA Polymerase I that retains polymerase and 3´→ 5´ exonuclease activity, but lacks 5´→ 3´ exonuclease activity. Klenow retains the polymerization fidelity of the holoenzyme without degrading 5´ termini.

Source: An *E. coli* strain that contains the *E. coli polA* gene that has had its 5´→ 3´ exonuclease domain removed.

Concentration: 5,000 and 50,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Usage Notes: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.

Klenow Fragment (3´→ 5´ exo⁻)

#M0212S 200 units
#M0212L 1,000 units
for high (10X) concentration
#M0212M 1,000 units

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Generates probes using random primers*
- *Random priming labeling*
- *Second strand cDNA synthesis*

Description: Klenow Fragment (3´→ 5´ exo⁻) is an N-terminal truncation of DNA Polymerase I that retains polymerase activity, but has lost the 5´→ 3´ exonuclease activity, and has mutations (D355A, E357A) that abolish the 3´→ 5´ exonuclease activity.

Source: An *E. coli* strain containing a plasmid with a fragment of the *E. coli polA* (D355A, E357A) gene starting at codon 324.

Concentration: 5,000 and 50,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Usage Notes: Klenow Fragment (3´→ 5´ exo⁻) is not suitable for generating blunt ends because it lacks the 3´→ 5´ exonuclease activity necessary to remove non-templated 3´ additions.

T4 DNA Polymerase

#M0203S 150 units
#M0203L 750 units

Companion Product:

Quick Blunting™ Kit
#E1201S 20 reactions
#E1201L 100 reactions

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Gap filling (no strand displacement activity)*
- *Removal of 3´ overhangs or fill-in of 5´ overhangs to form blunt ends*
- *Probe labeling using replacement synthesis*
- *Single-strand deletion subcloning*

Description: T4 DNA Polymerase catalyzes the synthesis of DNA in the 5´→ 3´ direction and requires the presence of template and primer. This enzyme has a 3´→ 5´ exonuclease activity which is much more active than that found in *E. coli* DNA Polymerase I. Unlike DNA Polymerase I, T4 DNA Polymerase does not have a 5´→ 3´ exonuclease function.

Concentration: 3,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Usage Notes: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.

T7 DNA Polymerase (unmodified)

#M0274S 300 units
#M0274L 1,500 units

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Gap-filling reaction (no strand displacement)*

Description: T7 DNA Polymerase catalyzes the replication of T7 phage DNA during infection. The protein dimer has two catalytic activities: DNA polymerase activity and strong 3´→ 5´ exonuclease. The high fidelity and rapid extension rate of the enzyme make it particularly useful in copying long stretches of DNA template.

Source: T7 DNA Polymerase consists of two subunits: T7 gene 5 protein (84 kDa) and *E. coli* thioredoxin (12 kDa). Each protein is cloned and overexpressed in a T7 expression system in *E. coli*.

Reaction Conditions: 1X T7 DNA Polymerase Reaction Buffer. Supplement with BSA and dNTPs (not included). Incubate at 37°C. Heat inactivation: 75°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C.

Concentration: 10,000 units/ml

Usage Notes: The high polymerization rate of the enzyme makes long incubations unnecessary. T7 DNA Polymerase is not suitable for DNA sequencing.

Bsu DNA Polymerase, Large Fragment

#M0330S 200 units
#M0330L 1,000 units

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Random primer labeling*
- *Second strand cDNA synthesis*
- *Single dA tailing*
- *Strand displacement DNA synthesis*

Description: *Bsu* DNA Polymerase I, Large Fragment retains the 5´→ 3´ polymerase activity of the *Bacillus subtilis* DNA polymerase I, but lacks the 5´→ 3´ exonuclease domain. This large fragment naturally lacks 3´→ 5´ exonuclease activity.

Source: An *E. coli* strain that contains the *Bacillus subtilis* DNA polymerase I gene (starting from codon 297 thus lacking the 5´→ 3´ exonuclease domain).

Concentration: 5,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Usage Notes: *Bsu* DNA Polymerase, Large Fragment is not suitable for generating blunt ends because it lacks the 3´→ 5´ exonuclease activity necessary to remove non-templated 3´ additions.

Bsu DNA Polymerase, Large Fragment retains 50% activity at 25°C and is twice as active as Klenow Fragment (3´→ 5´ exo⁻) at this temperature.

Terminal Transferase

#M0315S 500 units
#M0315L 2,500 units

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Addition of homopolymer tails to the 3´ ends of DNA*
- *Labeling the 3´ ends of DNA with modified nucleotides (e.g., ddNTP, DIG-dUTP)*
- *TUNEL assay (in situ localization of apoptosis)*
- *TdT dependent PCR*

Description: Terminal Transferase (TdT) is a template independent polymerase that catalyzes the addition of deoxynucleotides to the 3´ hydroxyl terminus of DNA molecules. Protruding, recessed or blunt-ended double or single-stranded DNA molecules serve as a substrate for TdT. The 58.3 kDa enzyme does not have 5´ or 3´ exonuclease activity. The addition of Co^{2+} in the reaction makes tailing more efficient.

Concentration: 20,000 units/ml

Heat Inactivation: 75°C for 20 minutes

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Polymerase Reaction Buffers

Q5

Q5 Reaction Buffer Pack [Q5 Reaction Buffer (5X), Q5 High GC Enhancer (5X)]
#B9027S 6.0 ml

Phusion*

Phusion HF Buffer Pack [Phusion HF Reaction Buffer (5X), $MgCl_2$ (50 mM), DMSO]
#B0518S 6.0 ml

Phusion GC Buffer Pack [Phusion GC Reaction Buffer (5X), $MgCl_2$ (50 mM), DMSO]
#B0519S 6.0 ml

Taq

Standard *Taq* Reaction Buffer [Standard *Taq* Reaction Buffer (10X), $MgCl_2$ (25 mM)]
#B9014S 6.0 ml

Standard *Taq* (Mg-free) Reaction Buffer Pack [Standard *Taq* (Mg-free) Reaction Buffer (10X), $MgCl_2$ (25 mM)]
#B9015S 6.0 ml

Other

ThermoPol Reaction Buffer Pack [ThermoPol Reaction Buffer (10X), $MgSO_4$ (100 mM)]
#B9004S 6.0 ml

Isothermal Amplification Buffer Pack [Isothermal Amplification Buffer (10X)]
#B0537S 6.0 ml

Isothermal Amplification Buffer II Pack [Isothermal Amplification Buffer II (10X)]
#B0374S 6.0 ml

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

Description: Q5 Reaction Buffer and High GC Enhancer are provided with both Q5 and Q5 Hot Start High-Fidelity DNA Polymerases.

Phusion High-Fidelity DNA Polymerase is supplied with 5X Phusion HF Buffer, 5X Phusion GC Buffer, DMSO, and 50 mM $MgCl_2$.

Standard *Taq* Reaction Buffer is provided with *Taq* DNA Polymerase as an alternative to the ThermoPol Reaction Buffer.

ThermoPol Reaction Buffer is provided with *Taq*, Vent, Deep Vent, *Bst* Full Length and *Bst* Large Fragment, Sulfolobus IV and Terminator DNA Polymerases; this buffer contains 2 mM $MgSO_4$ when the buffer is diluted to its final 1X concentration.

Isothermal Amplification Buffer is supplied with *Bst* 2.0 and *Bst* 2.0 WarmStart DNA Polymerases.

Isothermal Amplification Buffer II is supplied with *Bst* 3.0 DNA Polymerase.

Nucleotides

Acyclonucleotide Set
#N0460S 0.5 µmol of each

Deoxynucleotide (dNTP) Solution Set
#N0446S 25 µmol of each

Deoxynucleotide (dNTP) Solution Mix
#N0447S 8 µmol of each
#N0447L 40 µmol of each

Ribonucleotide Solution Set
#N0450S 10 µmol of each
#N0450L 50 µmol of each

Ribonucleotide Solution Mix
#N0466S 10 µmol of each
#N0466L 50 µmol of each

7-deaza-dGTP
#N0445S 0.3 µmol of each
#N0445L 1.5 µmol of each

Adenosine 5´-Triphosphate (ATP)
#P0756S 1.0 ml
#P0756S 5.0 ml

5-methyl-dCTP
#N0356S 1 µmol

dATP Solution
#N0440S 25 µmol

Description:
Deoxynucleotide Solution Set:
Four separate solutions of ultrapure deoxynucleotide (dATP, dCTP, dGTP and dTTP). Each deoxynucleotide is supplied at a concentration of 100 mM.

Deoxynucleotide Solution Mix:
An equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP. Each deoxynucleotide is supplied at a concentration of 10 mM.

Ribonucleotide Solution Set:
Four separate solutions of ATP, CTP, GTP and UTP, pH 7.5, as sodium salts.

Ribonucleotide Solution Mix:
A buffered equimolar solution of ribonucleotide triphosphates: rATP, rCTP, rGTP and rUTP. Each nucleotide is supplied at a concentration of 25 mM (total rNTP concentration equals 100 mM).

7-deaza-dGTP:
7-deaza contains a 5 mM solution of 7-deaza-dGTP as a dilithium salt.

5-methyl-dCTP:
dm5CTP supplied as 10 mM solution at pH 7.0.

dATP Solution:
dATP Solution contains a 100 mM solution of dATP as a sodium salt at pH 7.4.

Acyclonucleotide Set:
Four separate tubes of acyNTPs (acyATP, acyCTP, acyGTP and acyTTP). Acyclonucleotides are supplied as a dry powder. Addition of 50 µl of distilled or de-ionized (Milli-Q®) water will result in a final concentration of 10 mM acyNTP.

Acyclonucleotides (acyNTPs) act as chain terminators and are thus useful in applications that normally employ dideoxynucleotides such as DNA sequencing and SNP detection. AcyNTPs are especially useful in applications with archaeal DNA Polymerases, more specifically with Therminator DNA Polymerase. Therminator DNA Polymerase is an engineered enzyme with an increased capacity to incorporate analogs with altered sugars, such as ribonucleotides, dideoxynucleotides, 2´ deoxynucleotides and especially acyclo-base analogs.

For RNA Cap Analogs, see page 190.

MILLI-Q® is a registered trademark of Millipore, Inc.

Products for cDNA Synthesis

cDNA SYNTHESIS	FEATURES	SIZE
KITS		
NEW **LunaScript® RT SuperMix Kit** (NEB #E3010)	• Ideal for cDNA synthesis in a two-step RT-qPCR workflow • Single tube supermix contains random hexamer and oligo-dT primers, dNTPs, Murine RNase Inhibitor, and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol	25/100 rxns
ProtoScript® II First Strand cDNA Synthesis Kit (NEB #E6560)	• Generates cDNA at least 10 kb in length • Contains ProtoScript II Reverse Transcriptase, an enzyme with increased thermostability and reduced RNase H activity • Convenient 2-tube kit includes dNTPs, Oligo-dT primer and Random Primer Mix	30/150 rxns
ProtoScript First Strand cDNA Synthesis Kit (NEB #E6300)	• Generates cDNA at least 5 kb in length • Contains M-MuLV Reverse Transcriptase • Convenient 2-tube kit includes dNTPs, Oligo-dT primer and Random Primer Mix	30/150 rxns
NEW **Template Switching RT Enzyme Mix** (NEB #M0466)	• Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction • Enzyme mix and buffer are optimzed for efficient template switching • RT enzyme mix includes RNase Inhibitor • High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA • Robust and simple workflow for 5´ Rapid Amplification of cDNA Ends (RACE) • Retains the complete 5´ end of transcripts for 2nd Strand cDNA Synthesis	20/100 rxns
STANDALONE REAGENTS		
ProtoScript II Reverse Transcriptase (NEB #M0368) An alternative to SuperScript® II	• RNase H⁻ mutant of M-MuLV Reverse Transcriptase with increased thermostability and reduced RNase H activity • Increased reaction temperatures (37–50°C)	4,000/10,000/40,000 units
M-MuLV Reverse Transcriptase (NEB #M0253)	• Robust reverse transcriptase for a variety of templates • Standard reaction temperatures (37–45°C)	10,000/50,000 units
AMV Reverse Transcriptase (NEB #M0277)	• Robust reverse transcriptase for a broad temperature range (37–52°C) • Can be used for templates requiring higher reaction temperatures	200/1,000 units
WarmStart RTx Reverse Transcription (NEB #M0380)	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection	50/250 rxns

More information about our reverse transcriptases and cDNA synthesis kits can be found in the RNA analysis chapter, pages 192–194.

Ellen has been with NEB for 29 years, and will retire this spring with much fanfare. NEB's Research and Development teams have been lucky to have a Principal Development Scientist with Ellen's dedication and expertise. She is also an advocate for science education and has brought joy to many children at the holiday party as Mrs. Claus. Ellen is looking forward to spending more time gardening, knitting and traveling in the coming years.

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Requires BSA · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Monarch PCR & DNA Cleanup Kit (5 µg)

#T1030S 50 preps
#T1030L 250 preps

Companion Products:

Monarch DNA Cleanup Columns (5 µg)
#T1034L 100 columns

Monarch DNA Cleanup Binding Buffer
#T1031L 235 ml

Monarch DNA Wash Buffer
#T1032L 25 ml

Monarch DNA Elution Buffer
#T1016L 25 ml

Monarch Plasmid Miniprep Kit
#T1010S 50 preps
#T1010L 250 preps

Monarch DNA Gel Extraction Kit
#T1020S 50 preps
#T1020L 250 preps

- *Elute in as little as 6 µl*
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Purify small DNA and oligos with a slight protocol modification*
- *Save time with fast, user-friendly protocol*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*

Description: The Monarch PCR & DNA Cleanup Kit (5 µg) is a rapid and reliable method for the purification and concentration of up to 5 µg of high-quality, double-stranded DNA from enzymatic reactions such as PCR, restriction digestion, ligation and reverse transcription. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 5 minutes. DNA Cleanup Binding Buffer is used to dilute the samples and ensure they are compatible for loading onto the proprietary silica matrix under high salt conditions. The DNA Wash Buffer ensures enzymes, short primers (≤ 40 nt), detergents and other low-molecular weight reaction components (e.g., nucleotides, DMSO, betaine) are removed, thereby allowing low-volume elution of concentrated, high-purity DNA. Eluted DNA is ready for use in restriction digests, DNA sequencing, ligation and other enzymatic manipulations. The unique column design ensures no buffer retention and no carryover of contaminants, allowing elution of sample in volumes as low as 6 µl. A slight protocol modification enables purification of small DNA and oligonucleotides.

Applications:

- PCR cleanup
- Enzymatic reaction cleanup
- cDNA cleanup
- Labeling cleanup
- Plasmid cleanup
- Oligo cleanup

The Monarch PCR & DNA Cleanup Kit Includes:

– Monarch DNA Cleanup Columns (5 µg)
– Monarch DNA Cleanup Binding Buffer
– Monarch DNA Wash Buffer
– Monarch DNA Elution Buffer
– Monarch Collection Tubes

With Monarch PCR & DNA Cleanup Kit, you can purify your DNA in as little as 5 minutes.

NEW

Exo-CIP™ Rapid PCR Cleanup Kit

RR

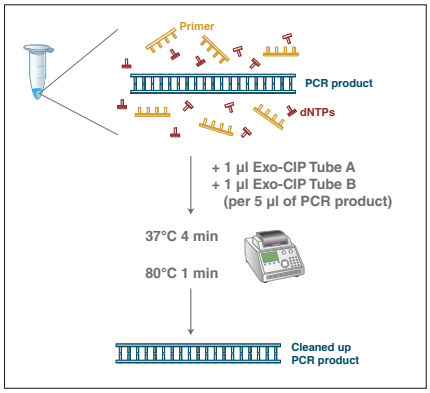
#E1050S 100 reactions
#E1050L 400 reactions

- *5 minute protocol for enzymatic cleanup of primers and dNTPs*
- *Improves sequencing results, allowing longer reads*

Description: The Exo-CIP Rapid PCR Cleanup Kit contains optimized formulations of thermolabile Exonuclease I and thermolabile Calf Intestinal Phosphatase and is used to rapidly degrade residual PCR primers and dephosphorylate excess dNTPs after amplification. Degradation occurs in only 4 minutes at 37°C, and is immediately followed by rapid inactivation of the enzymes by heating for 1 minute at at 80°C. In just 5 minutes, the PCR product is ready for downstream analysis such as Sanger sequencing, SNP detection, or library preparation for NGS. The Exo-CIP Rapid PCR Cleanup Kit is compatible with all commonly-used reaction buffers.

The Exo-CIP Rapid PCR Cleanup Kit Includes:

– Exo-CIP Tube A (thermolabile Exo I)
– Exo-CIP Tube B (thermolabile CIP)



Exo-CIP Rapid PCR Cleanup Kit workflow. *1 µl of Exo-CIP Tube A (thermolabile Exo I) and 1 µl of Exo-CIP Tube B (thermolabile CIP) are added to the PCR product to degrade excess primers and dNTPs. The mixture is incubated at 37°C for 4 minutes, followed by a 1 minute incubation at 80°C to irreversibly inactivate both enzymes. The cleaned PCR product is ready for downstream applications or analysis.*

A fisherman returns oyster shells to the sea to provide a surface for new oysters to grow.
Credit: Jeff J Mitchell, Getty Images

Whisky and Oysters

Oysters are essential members of a diverse marine ecosystem because of their specific roles in contributing to biodiversity. They purify water by pumping it through their gills — obtaining food for themselves and filtering plankton and chemical contaminants from the water. Additionally, generations of oysters settle on top of each other, forming a reef of sorts, that provides habitats for other marine organisms, such as crabs.

While oyster reefs all over the world are threatened due to overfishing, in one protected location in northeast Scotland, there is a university, a marine conservation society, and a whisky distillery all striving to restore a long-lost oyster habitat.

The Dornoch Firth is a large and complex estuary that has been designated both a Special Protection Area (SPA) and Special Area of Conservation (SAC), because it is one of the northern-most estuaries for migrating and wintering birds. White sandy beaches at the mouth of the estuary lead to salt marshes, sandflats and mudflats that support an incredibly diverse plant and animal environment, including breeding osprey, waders and wildfowl, seal, dolphins, otters and mussel reefs.

The Native European Oyster thrived in the waters of the Dornoch Firth for over 10,000 years, until it was overfished and disappeared completely just over 100 years ago.

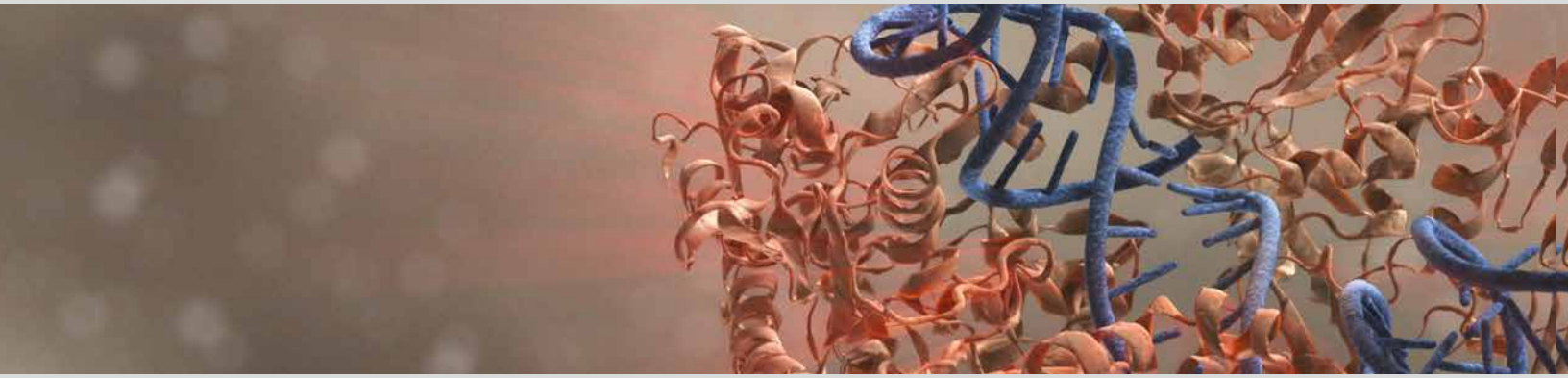
Glenmorangie Distillery is picturesquely situated on the banks of Dornoch Firth, producing single malt whisky for the past 175 years. In 2014, Glenmorangie pioneered a project to reduce their environmental footprint. The whisky distillery teamed up with Heriot-Watt University and the Marine Conservation Society in a partnership called DEEP (Dornoch Environmental Enhancement Project) to make some drastic changes to the water quality, re-introduce the Native European Oyster, and set a precedent for the re-establishment of disappearing reefs worldwide.

The DEEP project had two major initiatives. First was the introduction of an anaerobic digestion plant that purifies 95% of the by-products of whisky distillation that were previously released into the Firth. The remaining 5% consists of mostly organic compounds, such as barley, which is used as food by the oysters that then go on to further improve the water quality in the firth.

Next was the re-establishment of the Native European Oyster reef. Following an initial small scale exploratory step, twenty tons of waste mussel and scallop shells were laid down to stabilize the sediment and give the oysters a surface on which to grow. Divers then laid down 20,000 Native European Oysters in an area that covers 40 hectares of the firth. Their hope is that the oyster population will grow to 200,000 in three years, and then four million after five years, generating a fully sustainable oyster reef.

Ultimately, the introduction of an anaerobic digestion plant and the re-introduction of the Native European Oyster reef will drastically improve the water quality in Dornoch Firth. In turn, this supports all of the other organisms that form the diverse marine ecosystem there. A picturesque landscape for a renowned distillery on the edge of an ecosystem teaming with bird, plant and marine life, is a victory for both whisky connoisseurs and for the conservation of biodiversity.

DNA Modifying Enzymes & Cloning Technologies



The trusted source for DNA-modifying enzymes & cloning technologies.

Molecular biology, which also includes synthetic biology, is a fundamental area of research and development. Central to these advances has been the use of DNA modifying enzymes and novel cloning technologies. Some common examples of DNA-modifying enzymes include kinases, ligases and methylases, while newer cloning technologies include NEBuilder® HiFi DNA Assembly and Golden Gate Assembly.

NEB's 40+ year history as a leader in enzyme technologies gives you confidence in the products and support you'll receive. NEB continues to serve the scientific community by providing the tools to carry out the most innovative research, from start to finish. All NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

NEB offers several online tools to aid in your cloning experiments, including:

- **NEBcloner®** – find the right products and protocols for each step of your traditional cloning experiment, including double digests
- **NEBioCalculator®** – use this tool for your scientific calculations and conversions
- **NEBuilder Assembly Tool** – use this tool for help with your DNA assembly primer design
- **Thermostable Ligase Reaction Temperature Calculator** – estimate incubation temperature when using thermostable ligases
- **NEB Golden Gate Assembly Tool** – use this tool for help with construct design for Golden Gate Assembly
- **Ligase Fidelity Viewer** – visualize overhang ligation preferences for Golden Gate Assembly design

To view the full list of online tools available, see page 288.

Featured Products

88 NEBuilder® HiFi DNA Assembly Master Mix & Cloning Kit

91 NEB® PCR Cloning Kit (with & without competent cells)

90 NEB Golden Gate Assembly Kit (BsaI-HF®v2)

97 HiFi *Taq* DNA Ligase

99 Quick Dephosphorylation Kit

Featured Tools & Resources

86 Cloning Overview

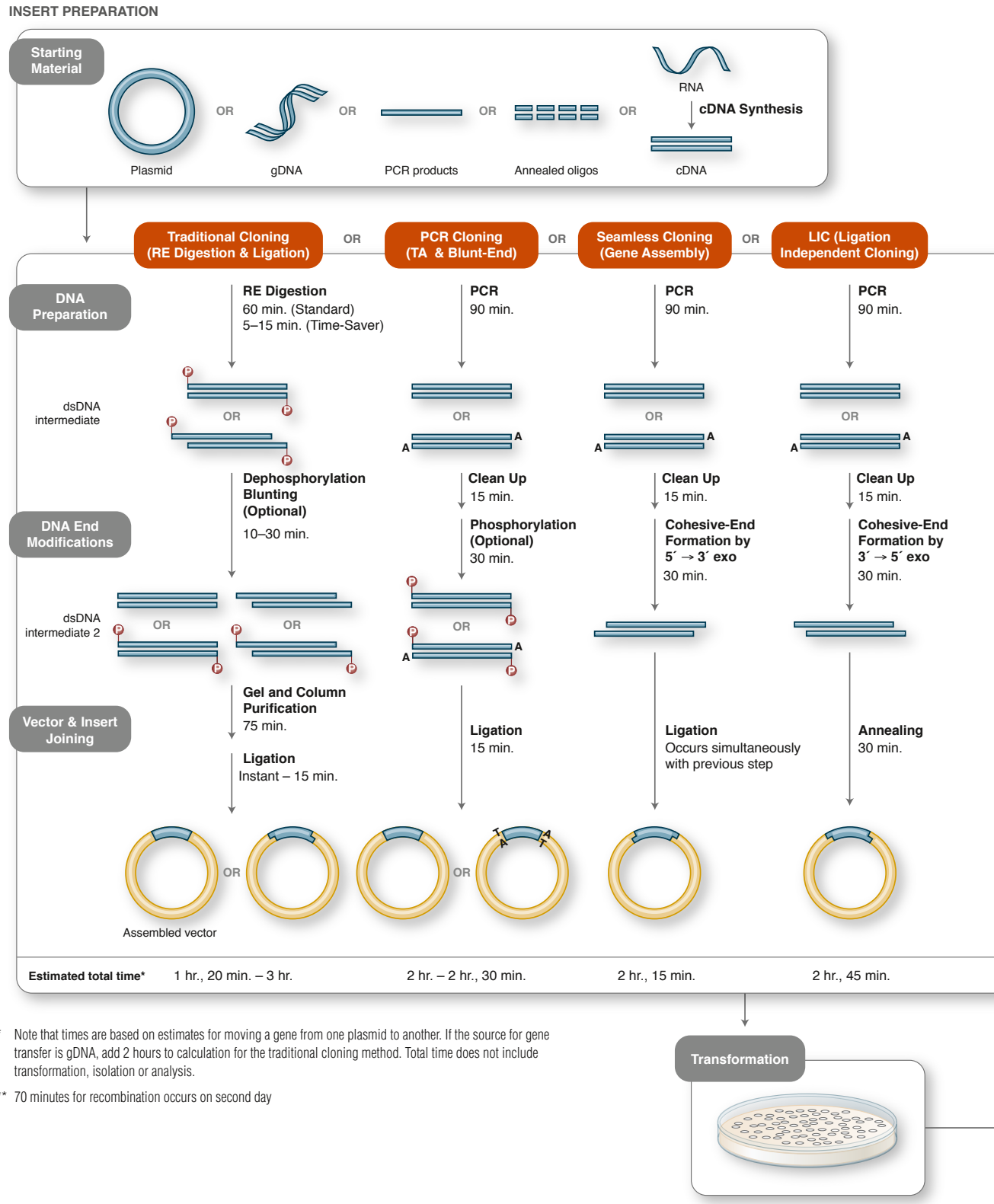
349 Troubleshooting Guide for Cloning

Visit ClonewithNEB.com to view our online tutorials explaining each of the steps in the cloning workflow.

RI Recombinant Enzyme

Cloning Workflow Comparison

The figure below compares the steps for the various cloning methodologies, along with the time needed for each step in the workflows.

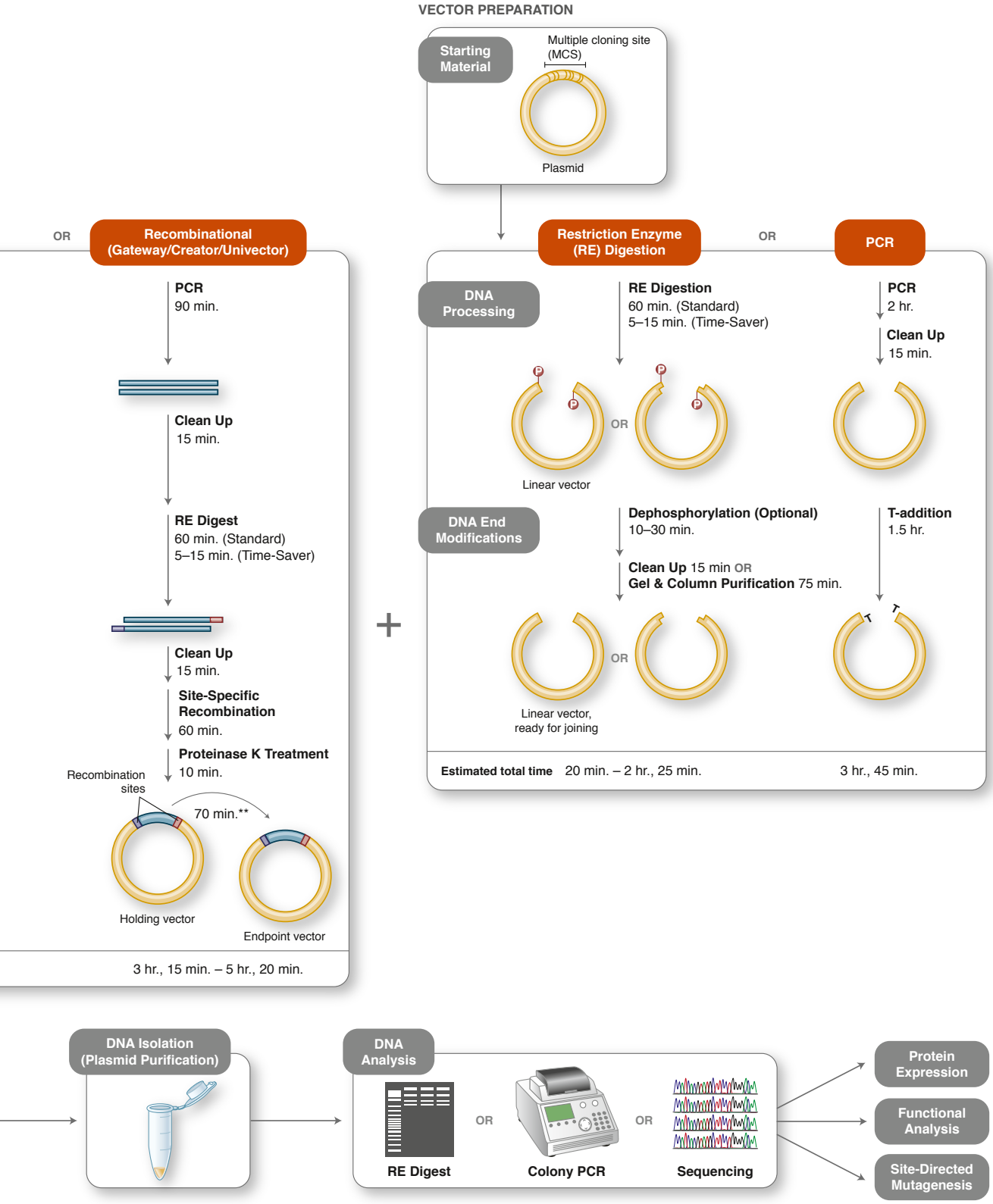


* Note that times are based on estimates for moving a gene from one plasmid to another. If the source for gene transfer is gDNA, add 2 hours to calculation for the traditional cloning method. Total time does not include transformation, isolation or analysis.

** 70 minutes for recombination occurs on second day

NEBcloner®

For help with choosing the right product for each step in the cloning workflow, try NEBcloner at **NEBcloner.neb.com**



NEBuilder® HiFi DNA Assembly Master Mix & Cloning Kit RR

NEBuilder HiFi DNA Assembly Master Mix
#E2621S 10 reactions
#E2621L 50 reactions
#E2621X 250 reactions

NEBuilder HiFi DNA Assembly Cloning Kit
#E5520S 10 reactions

NEBuilder HiFi DNA Assembly Bundle for Large Fragments
#E2623S 20 reactions

- *Simple and fast seamless cloning*
- *Increased number of successful assembly products, particularly for longer or greater numbers of fragments*
- *Flexible sequence design, with no need to engineer cloning site*
- *Complex assembly achieved in an hour*
- *Less screening/re-sequencing of constructs, virtually error-free, high-fidelity assembly*
- *Use in successive rounds of assembly; removes 5´ and 3´ restriction enzyme mismatches*
- *Bridge two ds-fragments with a synthetic ss-DNA oligo*
- *DNA can be used immediately for transformation or as template for PCR or RCA*
- *Switch from other systems easily; compatible with Gibson Assembly-, In-Fusion- designed fragments*
- *Adapts for multiple DNA manipulations, including site-directed mutagenesis.*
- *Assemble multiple DNA fragments and transform in just under 2 hours*
- *Clone into any vector with no additional sequence added (scarless)*

To learn how simple NEBuilder HiFi is, visit **NEBuilderHiFi.com**

Description: NEBuilder HiFi DNA Assembly Master Mix was developed to improve the efficiency and accuracy of DNA assembly. This method allows for seamless assembly of multiple DNA fragments, regardless of fragment length or end compatibility. This method has been used to assemble either single-stranded oligonucleotides or different sizes of DNA fragments with varied overlaps (15–80 bp). It has utility for the synthetic biology community, as well as those interested in one-step cloning of multiple fragments due to its ease of use, flexibility and simple master-mix format. The reaction features different enzymes that perform in the same buffer:

- Exonuclease creates single-stranded 3´ overhangs that facilitate the annealing of fragments that share complementarity at one end (the overlap region)
- The polymerase fills in gaps within each annealed fragment
- The DNA ligase seals nicks in the assembled DNA

The end result is a double-stranded, fully sealed DNA molecule that can serve as template for PCR, RCA or a variety of other molecular biology applications, including direct transformation of *E. coli*.

The NEBuilder HiFi DNA Assembly Cloning Kit combines the power of the NEBuilder HiFi DNA Assembly Master Mix with NEB 5-alpha Competent *E. coli*, enabling fragment assembly and transformation in just under 2 hours.

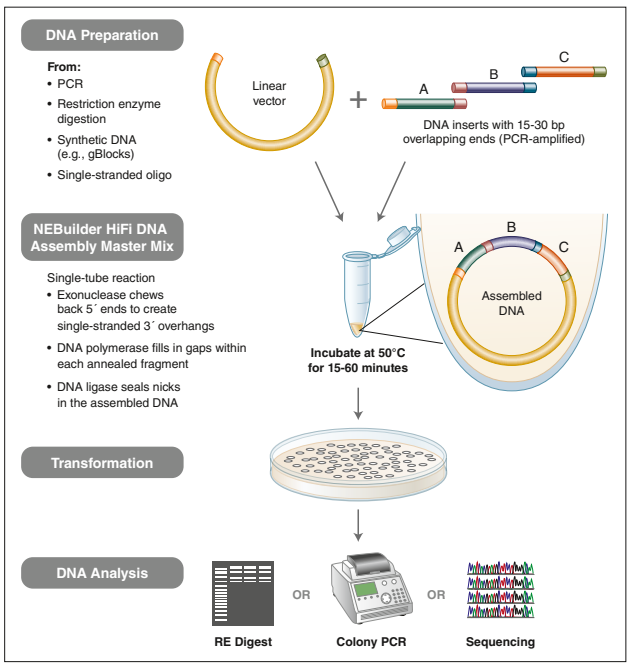
NEBuilder HiFi Kits can be purchased with NEB 5-alpha Competent *E. coli* (Cloning Kit, NEB #E5520) or as a bundle with NEB 10-beta Competent *E. coli* (Bundle for Large Fragments, NEB #E2623). NEB 5-alpha competent cells are excellent for routine assemblies of 15 kb or less. NEB recommends NEB 10-beta competent cells for assemblies larger than 15 kb.

The NEBuilder HiFi DNA Assembly Master Mix Includes:
– NEBuilder HiFi DNA Assembly Master Mix
– NEBuilder Positive Control

The NEBuilder HiFi DNA Assembly Cloning Kit Includes:
– NEBuilder HiFi DNA Assembly Master Mix
– NEBuilder Positive Control
– NEB 5-alpha Competent *E. coli* (High Efficiency)
– SOC Outgrowth Medium
– pUC19 Control DNA

The NEBuilder HiFi DNA Assembly Bundle for Large Fragments Includes:
– NEBuilder HiFi DNA Assembly Master Mix
– NEBuilder Positive Control
– NEB 10-beta Competent *E. coli* (High Efficiency)
– NEB 10-beta/Stable Outgrowth Medium
– pUC19 Control DNA

NEBuilder®
Assembly Tool
Speed up your experimental design with our primer design tool at **NEBuilder.neb.com**



Overview of the NEBuilder HiFi DNA Assembly Cloning Method.

How does NEBuilder HiFi DNA Assembly work?

CutSmart or NEB 1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | BSA Requires BSA | SAM Requires SAM

Gibson Assembly® Master Mix & Cloning Kit

Gibson Assembly Master Mix
#E2611S 10 reactions
#E2611L 50 reactions

Gibson Assembly Cloning Kit
#E5510S 10 reactions

- *Increased number of successful assembly products, particularly for longer or greater numbers of fragments*
- *Flexible sequence design with no need to engineer cloning sites*
- *Assemble multiple DNA fragments and transform in just under 2 hours*
- *Clone into any vector with no additional sequence added (scarless)*
- *No PCR clean-up step required*

Description: Gibson Assembly Master Mix was developed by Dr. Daniel Gibson and his colleagues at the J. Craig Venter Institute and licensed to NEB by Synthetic Genomics, Inc. It allows for successful assembly of multiple DNA fragments, regardless of fragment length or end compatibility.

Gibson Assembly efficiently joins multiple overlapping DNA fragments in a single-tube isothermal reaction. The Gibson Assembly Master Mix includes three different enzymatic activities that perform in a single buffer:

- The exonuclease creates a single-stranded 3´ overhang that facilitates the annealing of fragments that share complementarity at one end
- The polymerase fills in gaps within each annealed fragment
- The DNA ligase seals nicks in the assembled DNA

The Gibson Assembly Cloning Kit combines the power of the Gibson Assembly Master Mix with NEB 5-alpha Competent *E. coli*, enabling fragment assembly and transformation in just under 2 hours.

The Gibson Assembly Cloning Kit has been optimized for the assembly and cloning of up to 6 fragments.

The Gibson Assembly Master Mix Includes:
- Gibson Assembly Master Mix
- NEBuilder Positive Control

The Gibson Assembly Cloning Kit Includes:
- Gibson Assembly Master Mix
- NEBuilder Positive Control
- NEB 5-alpha Competent *E. coli* (High Efficiency)
- SOC Outgrowth Medium
- pUC19 Control DNA

NEBuilder®
Assembly Tool

Speed up your experimental design with our primer design tool at **NEBuilder.neb.com**

To learn how simple Gibson Assembly is, view our online tutorials at **NEBGibson.com**

Synthetic Biology/DNA Assembly Selection Chart

	NEBuilder® HiFi DNA Assembly (NEB #E2621) (NEB #E5520) (NEB #E2623)	NEB Gibson® Assembly (NEB #E5510) (NEB #E2611)	NEB® Golden Gate Assembly Kit (BsaI-HFv2) (NEB #E1601)	USER® Enzyme (NEB #M5505)
PROPERTIES				
Removes 5´ or 3´ End Mismatches	★★★	★	N/A	N/A
Assembles with High Fidelity at Junctions	★★★	★★	★★★	★★★
Tolerates Repetitive Sequences at Ends	★	★	★★★	★★★
Generates Fully Ligated Product	★★★	★★★	★★★	NR
Joins dsDNA with Single-stranded Oligo	★★★	★★	NR	NR
Assembles with High Efficiency with Low Amounts of DNA	★★★	★★	★★	★★
Accommodates Flexible Overlap Lengths	★★★	★★★	★	★★
APPLICATIONS				
Simple Cloning (1-2 Fragments)	★★★	★★★	★★★	★★★
4-6 Fragment Assembly (one pot)	★★★	★★★	★★★	★★★
7-11 Fragment Assembly (one pot)	★★★	★★	★★★	★★★
12-24 Fragment Assembly (one pot) (1)	★	★	★★★	NR
Template Construction for In vitro Transcription	★★★	★★★	★★★	★★★
Synthetic Whole Genome Assembly	★★★	★	★	★
Multiple Site-directed Mutagenesis	★★★	★★	★★	★★
Library Generation	★★★	★★★	★★★	★★
Metabolic Pathway Engineering	★★★	★★	★★★	★★★
TALENs	★★	★★	★★★	★★
Short Hairpin RNA Cloning (shRNA)	★★★	★★	★	★
gRNA Library Generation	★★★	★★	★	★
Large Fragment (> 10 kb) Assembly	★★★	★★★	★★★	★★
Small Fragment (< 100 bp) Assembly	★★★	★	★★★	★★★
Use in Successive Rounds of Restriction Enzyme Assembly	★★★	★	NR	★

★★★ Optimal, recommended product for selected application
★★ Works well for selected application
★ Will perform for selected application, but is not recommended
N/A Not applicable to this application
NR Not recommended

(1) Please visit www.neb.com/GoldenGate for more information.

NEW

NEB Golden Gate Assembly Kit (BsaI-HF®v2)

RR

#E1601S	20 reactions
#E1601L	100 reactions

Companion Products:

NEB 5-alpha Competent *E. coli* (High Efficiency)

#C2987H	20 x 0.05 ml
#C2987I	6 x 0.2 ml
#C2987P	1 x 96 well plate
#C2987R	1 x 384 well plate
#C2987U	96 x 50 µl/tube

NEB 10-beta Competent *E. coli* (High Efficiency)

#C3019H	20 x 0.05 ml
#C3019I	6 x 0.2 ml

NEB Cloning Competent *E. coli* Sampler

#C1010S	8 tubes

Q5 Hot Start High-Fidelity 2X Master Mix

#M0494S	100 rxns (50 µl vol)
#M0494L	500 rxns (50 µl vol)

- *Updated to include BsaI-HFv2 (optimized for Golden Gate)*
- *Seamless cloning – no scar remains following assembly*
- *Includes destination plasmid with T7/SP6 promoters*
- *Ordered assembly of multiple fragments (2-20+) in a single reaction*
- *Can also be used for cloning of single inserts and library preparations*
- *Efficient with regions of high GC content and areas of repeats*
- *Compatible with a broad range of fragment sizes (< 100 bps to > 15 kb)*

Type IIS Enzymes used in Golden Gate:

- BsaI (NEB #R0535)
- BsaI-HFv2 (NEB #R3733)
- BbsI (NEB #R0539)
- BbsI-HF (NEB #R3539)
- BsmBI (NEB #R0580)
- Esp3I (NEB #R0734)

Description: The NEB Golden Gate Assembly Kit (BsaI-HFv2) contains an optimized mix of BsaI-HFv2 and T4 DNA Ligase. Together these enzymes can direct the assembly of multiple inserts/modules using the Golden Gate approach. Also included is the pGGA destination plasmid, which provides a backbone for your assembly, features convenient restriction enzyme sites for subcloning, and has T7/SP6 promoter sequences to enable *in vitro* transcription.

The efficient and seamless assembly of DNA fragments, commonly referred to as Golden Gate assembly, has its origins in 1996, when for the first time it was shown that multiple inserts could be assembled into a vector backbone using only the sequential or simultaneous activities of a single Type IIS restriction enzyme and T4 DNA Ligase.

Type IIS restriction enzymes bind to their recognition sites but cut the DNA downstream from that site at a positional, not sequence-specific, cut site. Thus, a single Type IIS restriction enzyme can be used to generate DNA fragments with unique overhangs. As an example, BsaI has a recognition site of GGTCTC(N1/ N5), where the GGTCTC represents the recognition/binding site, and the N1/ N5 indicates the cut site is one base downstream on the top strand, and five bases downstream on the bottom strand. Assembly of digested fragments proceeds through annealing of complementary four base overhangs on adjacent fragments. The digested fragments and the final assembly no longer contain Type IIS restriction enzyme recognition sites, so no further cutting is possible. The assembly product accumulates with time.

While particularly useful for multi-fragment assemblies such as Transcription Activator Like Effectors (TALEs) and TALEs fused to a FokI nuclease catalytic domain (TALENs), the Golden Gate method can also be used for cloning of single inserts and inserts from diverse populations that enable library creation.

Advances in Ligase Fidelity: Research at NEB has led to increased understanding of ligase fidelity, including the development of a comprehensive method for profiling end-joining ligation fidelity in order to predict which overhangs have improved fidelity. This research allows careful choice of overhang sets, which is especially important for achieving complex Golden Gate Assemblies. To learn more, visit www.neb.com/goldengate.

The NEB Golden Gate Assembly Kit (BsaI-HFv2) Includes:

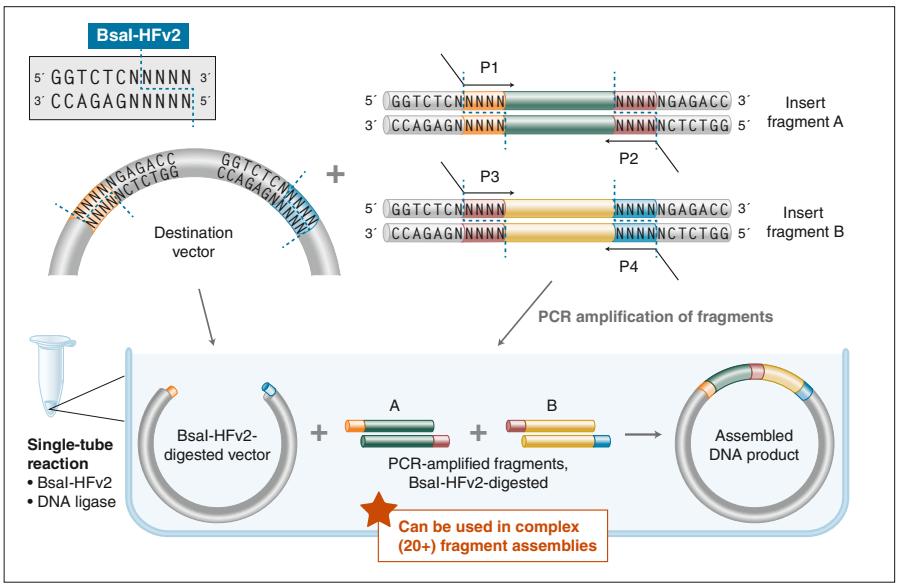
- NEB Golden Gate Assembly Mix
- T4 DNA Ligase Reaction Buffer (10X)
- pGGA Destination Plasmid

NEB Golden Gate Assembly Tool

Speed up your experimental design with our assembly tool at **GoldenGate.neb.com**

Ligase Fidelity Viewer*

Visualize overhang ligation preferences for Golden Gate Assembly design. *This is a NEBeta™ Tool which is not yet optimized for design and usability. Please share your feedback with us. Access this tool at **www.neb.com/research/nebeta-tools**.



In its simplest form, Golden Gate Assembly requires a Type IIS recognition site, in this case, BsaI (GGTCTC), added to both ends of a dsDNA fragment. After digestion, these sites are left behind, with each fragment bearing the designed 4-base overhangs that direct the assembly.

How does Golden Gate Assembly work?

BioBrick® Assembly Kit

#E0546S 50 reactions

The BioBrick Assembly Kit was developed in partnership with Ginkgo BioWorks. For more details and for technical questions, please see: **ginkgobioworks.com/support**

Description: The BioBrick Assembly Kit provides a streamlined method for assembly of BioBrick parts into multi-component genetic systems. BioBrick parts are DNA sequences that encode a defined biological function and can be readily assembled with any other BioBrick part. The process for assembling any two BioBrick parts is identical and results in a new composite BioBrick part.

Please refer to the individual datacards for each reagent's recommended use and storage conditions.

BIOBRICK® is a registered trademark of The BioBricks Foundation. (http://www.biobricks.org)

The BioBrick Assembly Kit Includes:

- EcoRI-HF
- XbaI
- SpeI
- PstI
- 10X NEBuffer 2.1
- T4 DNA Ligase
- 10X T4 DNA Ligase Buffer

NEB® PCR Cloning Kit (with or without competent cells)

NEB PCR Cloning Kit
#E1202S 20 reactions

NEB PCR Cloning Kit
(without competent cells)
#E1203S 20 reactions

- *In vitro transcription with both SP6 & T7 promoters*
- *Easy cloning of all PCR products, including blunt and TA ends*
- *Fast cloning with 5-minute ligation step*
- *Simplified screening with low/no colony background and no blue/white selection*
- *Save time by eliminating purification steps*
- *More flanking restriction sites available for easy subcloning, including choice of two single digest options*
- *BsaI site removed to allow cloning of Golden Gate modules*

Description: The NEB PCR Cloning Kit contains an optimized 2X Cloning Master Mix with a proprietary ligation enhancer and a linearized vector that uses a novel mechanism for background colony suppression to give a low background. It allows simple and quick cloning of any PCR amplicon, whether the amplification reactions are performed with proofreading DNA polymerases, such as Q5® or Phusion® which produce blunt ends; or nonproofreading DNA polymerases, such as *Taq* or *Taq* mixes (One*Taq*, LongAmp *Taq*) which produce single-base overhangs. This is possible due to "invisible" end polishing components in the master mix that are active during the ligation step. The kit also allows direct cloning from amplification reactions without purification, and works well whether or not the primers used in the PCR possess 5´-phosphate groups.

- Provided analysis primers allow for downstream colony PCR screening or sequencing
- Ready-to-use kit components include 1 kb control amplicon, linearized cloning vector and single-use competent *E. coli* (NEB #E1202 only)
- Longer shelf life (12 months), as compared to some commercially available products

The PCR Cloning Kit Includes:

- Linearized pMiniT™ 2.0 Vector
- Cloning Mix 1
- Cloning Mix 2
- Amplicon Cloning Control (1 kb)
- Cloning Analysis Forward Primer
- Cloning Analysis Reverse Primer
- NEB 10-beta Competent *E. coli* (Cloning Efficiency) (NEB #E1202 only)
- NEB 10-beta/Stable Outgrowth Medium (NEB #E1202 only)
- pUC19 Control DNA

PHUSION® is a registered trademark of Thermo Fisher Scientific.

USER® Enzyme

CutSmart 37° 65°

Thermolabile USER II Enzyme

CutSmart 37° 65°

USER Enzyme
#M5505S 50 units
#M5505L 250 units

NEW
Thermolabile USER II Enzyme
#M5508S 50 units
#M5508L 250 units

- *USER Cloning*
- *Directional RNA-Seq*
- *NEBNext adaptor cleavage*

Description: USER (Uracil-Specific Excision Reagent) Enzyme generates a single nucleotide gap at the location of a uracil. USER Enzyme is a mixture of Uracil DNA glycosylase (UDG) and the DNA glycosylase-lyase Endonuclease VIII. UDG catalyses the excision of a uracil base, forming an abasic (apyrimidinic) site while leaving the phosphodiester backbone intact. The lyase activity of Endonuclease VIII breaks the phosphodiester backbone at the 3´ and 5´ sides of the abasic site so that base-free deoxyribose is released.

Thermolabile USER (Uracil-Specific Excision Reagent) II Enzyme generates a single nucleotide gap at the location of a uracil residue. It can be 100% inactivated at temperatures > 65°C

Reaction Conditions: CutSmart Reaction Buffer. Incubate at 37°C. Heat Inactivation of Thermolabile USER II Enzyme: 65°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to nick 10 pmol of a 34-mer oligonucleotide duplex containing a single uracil base, in 15 minutes at 37°C in a total reaction volume of 10 µl. Unit assay conditions can be found at www.neb.com.

Concentration: 1,000 units/ml

How does the NEB PCR Cloning Kit work?

Q5® Site Directed Mutagenesis Kit (with or without competent cells)

Q5 Site Directed Mutagenesis Kit
#E0554S 10 reactions

Q5 Site Directed Mutagenesis Kit
(without competent cells)
#E0552S 10 reactions

Companion Products:

NEB PCR Cloning Kit
#E1202S 20 reactions

NEB PCR Cloning Kit
(without competent cells)
#E1203S 20 reactions

KLD Enzyme Mix
#M0554S 25 reactions

- *Robust exponential amplification generates high yields of desired mutations from a wide range of templates.*
- *Low error rate of Q5 High-Fidelity DNA Polymerase reduces screening time.*
- *Room temperature reaction setup*
- *Use of standard primers eliminates need for phosphorylated or purified oligos*
- *Easy-to-use master mix format*

For help with primer design, try
NEBaseChanger at **NEBaseChanger.neb.com**

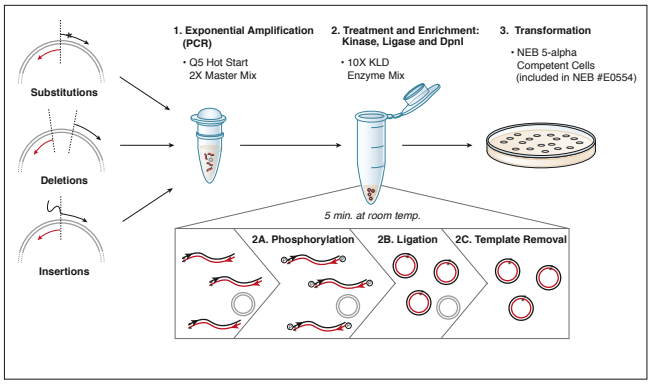
Description: The Q5 Site-Directed Mutagenesis Kit allows rapid site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours. The kit utilizes the robust Q5 Hot Start High-Fidelity DNA Polymerase along with custom mutagenic primers to create substitutions, deletions and insertions in a wide variety of plasmids. Transformation into high-efficiency NEB 5-alpha Competent *E. coli*, provided with (NEB #E0554), ensures robust results with plasmids up to 14.3 kb in length.

Applications:

- Generation of mutations, insertions or deletions in plasmid DNA

The Q5 Site Directed Mutagenesis Kit Includes:

– Q5 Hot Start High-Fidelity Master Mix (2X)
– KLD Enzyme Mix (10X) and Reaction Buffer (2X)
– Control Primer Mix (10 µM each) and Template DNA (5 ng/µl)
– NEB 5-alpha Competent *E. coli* (High Efficiency) (NEB #E0554 only)
– pUC19 Transformation Control Plasmid (5 pg/µl)
– SOC Outgrowth Medium



Q5 Site-Directed Mutagenesis Kit Overview. The first step is an exponential amplification using Q5 Hot Start High-Fidelity DNA Polymerase. The second step is a unique enzyme mix containing a kinase, ligase and DpnI. Together, these enzymes allow for rapid circularization of the PCR product and removal of the template DNA. The last step is a high-efficiency transformation into chemically competent cells.

Quick Blunting™ Kit

#E1201S 20 reactions
#E1201L 100 reactions

Special Offer:

Quick Blunting and Quick Ligation Kits
#E0542S 20 reactions
#E0542L 100 reactions

See page 95 for details on the Quick Ligation Kit.

- *Restriction enzyme-digested DNA is blunted in less than 30 minutes*
- *Reactions are performed at room temperature in a ready-to-use mix*
- *Suitable for restriction enzyme-digested DNA, sheared or nebulized DNA or PCR product*

Description: The Quick Blunting Kit is used to convert DNA with incompatible 5´or 3´overhangs to 5´phosphorylated, blunt-ended DNA for efficient blunt-end ligation into DNA cloning vectors. DNA is blunted using T4 DNA Polymerase (NEB #M0203) which has both 3´→ 5´ exonuclease activity and 5´→ 3´ polymerase activity. T4 Polynucleotide Kinase (NEB #M0201) is included in the enzyme mix for phosphorylation of the 5´ends of blunt-ended DNA for subsequent ligation into a cloning vector. This kit is optimized for blunting up to 5 µg of DNA in a single reaction.

Applications:

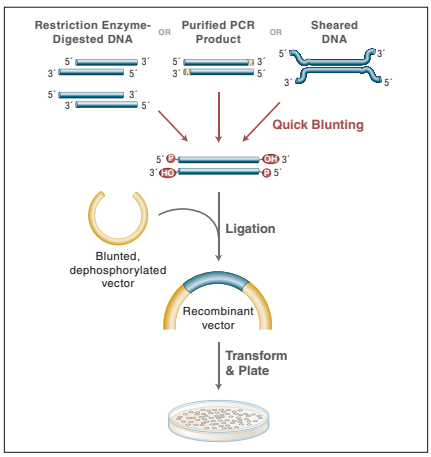
- Prepare sheared, nebulized or restriction enzyme-digested DNA for blunt-end ligation into a plasmid, cosmid, fosmid or BAC vector
- Prepare PCR products for efficient blunt-end cloning

The Quick Blunting Kit Includes:

– Blunting Enzyme Mix
– 10X Blunting Buffer
– 1 mM Deoxynucleotide (dNTP) Solution Mix

Heat Inactivation: 70°C for 10 minutes.

Notes: PCR generated DNA must be purified before blunting by using a commercial purification kit, such as Monarch PCR & DNA Cleanup Kit (NEB #T1030) phenol extraction/ethanol precipitation or gel electrophoresis. Restriction enzyme digested DNA can be blunted directly without purification.



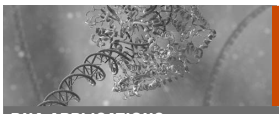
Learn more about the benefits of the Q5 SDM Kit.

CutSmart or NEB 1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | BSA Requires BSA | SAM Requires SAM

DNA Ligase Selection Chart

NEB offers a variety of ligases for DNA research. Many of these enzymes are recombinant, and all offer the quality and value you have come to expect from our products. While more than one ligase may work for your application, the following selection chart presents our recommendations for optimal performance.

Visit **NEBStickTogether.com** for more information on DNA Ligases.



	Blunt/TA Ligase Master Mix	Instant Sticky-end Ligase Master Mix	Electro Ligase™	T4 DNA Ligase	Quick Ligation™ Kit	T3 DNA Ligase	T7 DNA Ligase	HiFi *Taq* DNA Ligase	*E. coli* DNA Ligase	*Taq* DNA Ligase	9°N™ DNA Ligase	NEBNext Ultra II Ligation Module	SplintR® Ligase
DNA APPLICATIONS													
Ligation of sticky ends	★★	★★★	★★	★★	★★★	★★	★★	★	★	★	★		
Ligation of blunt ends	★★★	★	★★	★★	★★★	★★							
T/A cloning	★★★	★	★★	★★	★★	★	★						
Electroporation			★★★	★★									
Ligation of sticky ends only							★★★						
Repair of nicks in dsDNA	★★	★★	★★	★★★	★★	★★	★★	★★	★★	★★	★★		★★
High complexity library cloning	★★	★★	★★	★★★	★★								
Adaptor Ligation	★★★	★★	★★	★	★★	★						▲	
Ligation-Dependent DNA Sequence & SNP Detection (LCR, LDR & related methods)								★★★		★★	★★		
Ligation-Dependent RNA Sequence & SNP Detection				★									★★★
Ligation of adjacent ssDNAs on an RNA Splint													★★★
NGS APPLICATIONS													
NGS Library Prep dsDNA-dsDNA (ligation)	▲			▲		▲						▲	
FEATURES													
Salt tolerance (> 2X that of T4 DNA Ligase)						✔							
Ligation in 15 min. or less	✔	✔		✔	✔	✔	✔	✔		✔	✔	✔	✔
Master Mix Formulation	✔	✔										✔	
Thermostable								✔		✔	✔		
Recombinant	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔

★★★ Optimal, recommended ligase for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
▲ Please consult the specific NGS protocol to determine the optimal enzyme for your needs

Helpful Online Tools:

For help with choosing the best ligase for your experiment, try **NEBcloner** at **NEBcloner.neb.com**

For help with estimating incubation temperature when using thermostable ligases, try the **Thermostable Ligase Reaction Temperature Calculator** at **LigaseCalc.neb.com**

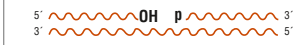
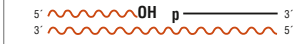
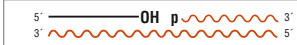
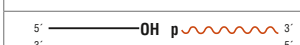
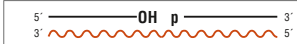
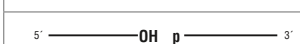
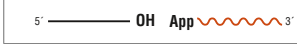
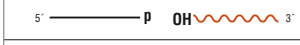
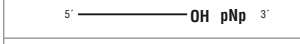
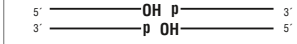
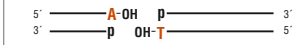
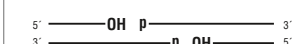
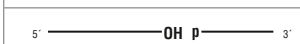
For help with scientific calculations and conversions, try **NEBioCalculator** at **NEBioCalculator.neb.com**

Find an overview of ligation.

Substrate-based Ligase Selection Chart

DNA
RNA

This chart provides our recommendation for a choice of ligase to use in a reaction, based upon the type of substrate present. DNA, RNA and hybrid substrates are represented and require specific enzymes to achieve the highest-efficiency ligation.

	RECOMMENDED LIGASE	COMMENTS
NICKED DNA/RNA		
	T4 RNA Ligase 2	
	T4 RNA Ligase 2	
	T4 RNA Ligase 2	
	T4 DNA Ligase	
	N/A	No ligase optimized for this activity
	N/A	No ligase optimized for this activity
	SplintR Ligase	100 – 1,000-fold higher efficiency than T4 DNA Ligase
	T4 DNA Ligase	For high temperatures, we recommend *Taq* DNA Ligase. For highest fidelity, we recommend HiFi *Taq* DNA Ligase.
ssDNA/RNA		
	N/A	See CircLigase™
	N/A	No ligase optimized for this activity
	T4 RNA Ligase 1	Supplement with ATP
	T4 RNA Ligase 1	
	T4 RNA Ligase 2 Truncated KQ	
	T4 RNA Ligase 2 Truncated KQ	
	Thermostable 5´ App DNA/RNA Ligase	We recommend a Proteinase K cleanup
	Thermostable 5´ App DNA/RNA Ligase	We recommend a Proteinase K cleanup
	RtcB Ligase	Supplement with GTP and Mn^{2+}
	RtcB Ligase	Supplement with GTP and Mn^{2+}
	T4 RNA Ligase 1	
	T4 RNA Ligase 1	Reported to work, but ligates inefficiently. Consider pdCp.
	T4 RNA Ligase 1	
	T4 RNA Ligase 1	
dsDNA/RNA		
	Blunt T/A Ligase Master Mix	
	Blunt T/A Ligase Master Mix	
	Quick Ligation Kit or Instant Sticky-end Ligase Master Mix	For ligating ends under high salt conditions, we recommend T3 DNA Ligase. For ligation of cohesive ends ONLY, we recommend T7 DNA Ligase.
	Quick Ligation Kit or Instant Sticky-end Ligase Master Mix	For ligating ends under high salt conditions, we recommend T3 DNA Ligase. For ligation of cohesive ends ONLY, we recommend T7 DNA Ligase.
	T4 RNA Ligase 2	
	T4 RNA Ligase 2	

CIRCLIGASE™ is a trademark of EpiCentre Technologies Corp.

CutSmart or NEB 1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · BSA Requires BSA · SAM Requires SAM · Enzymes for Innovation · 37° Incubation Temperature · 65° Heat Inactivation

T4 DNA Ligase

Regular Concentration
(400,000 cohesive end units/ml)
#M0202S 20,000 units
#M0202L 100,000 units

High Concentration
(2,000,000 cohesive end units/ml)
#M0202T 20,000 units
#M0202M 100,000 units

- *Efficient ligation of sticky or blunt ends*
- *T/A Cloning*
- *Repair of nicks in dsDNA*
- *High complexity library cloning*
- *Ligation of RNA to DNA*

For help with molar ratio calculations, try
NEBioCalculator at **NEBioCalculator.neb.com**

Competitive Nuclease Contamination Study.
T4 DNA Ligase from multiple suppliers was tested in reactions containing a fluorescent labeled single stranded, double stranded blunt, 3´overhang or 5´overhang containing oligonucleotides. The percent degradation by contaminating nucleases is determined by capillary electrophoresis and peak analysis. The resolution is at the single nucleotide level.

Description: Catalyzes the formation of a phosphodiester bond between juxtaposed 5´phosphate and 3´ hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt end and cohesive end termini, as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

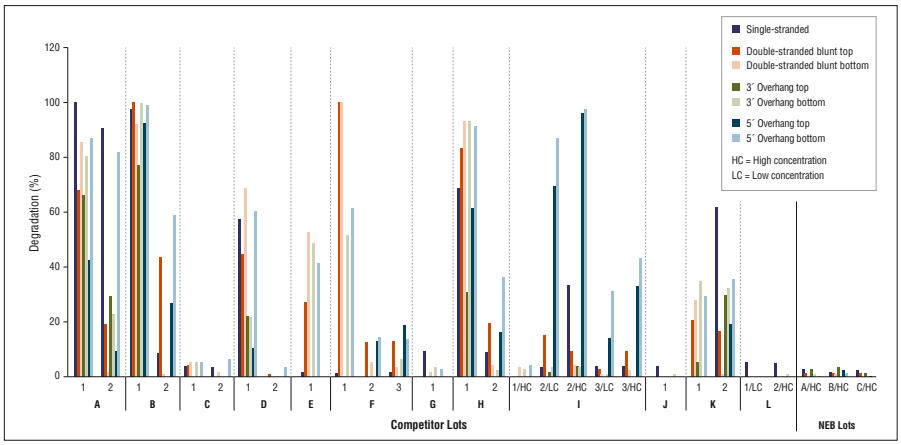
Reaction Conditions: 1X T4 DNA Ligase Reaction Buffer. Recommended DNA concentration (0.1 to 1 µM of 5´ termini). Incubate at 16°C. Heat inactivation: 65°C for 10 minutes.

Unit Definition (Cohesive End Unit):
One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λ DNA (5´ DNA termini concentration of 0.12 µM [300 µg/ml]) in 20 µl of 1X T4 DNA Ligase Reaction Buffer in 30 minutes at 16°C.

Concentration: 400,000 and 2,000,000 cohesive end units/ml

Usage Notes: ATP is an essential cofactor for the reaction. This contrasts with *E. coli* DNA Ligase which requires NAD^+.

Ligation can also be performed in any of the four restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer if they are supplemented with 1 mM ATP.



Quick Ligation™ Kit

#M2200S 30 reactions
#M2200L 150 reactions

Special Offer:

Quick Blunting and Quick Ligation Kits
#E0542S 20 reactions
#E0542L 100 reactions

See page 106 for details on the Quick Blunting Kit.

- *5 minute ligation reactions for sticky or blunt ends*
- *Reactions performed at room temperature*
- *T/A Cloning*
- *Repair of nicks in dsDNA*

Description: The Quick Ligation Kit enables ligation of cohesive or blunt end DNA fragments in 5 minutes at room temperature (25°C).

The Quick Ligation Kit Includes:
– Quick T4 DNA Ligase (recombinant)
– 2X Quick Ligation Reaction Buffer

Reaction Conditions: 1X Quick Ligation Reaction Buffer. Incubate at room temperature (25°C).

Notes:
Most ligations performed using the Quick Ligation Kit reach an end point at 5 minutes or less at 25°C. Incubation beyond this time provides no additional benefit. Overnight incubations can result in lower transformation efficiencies.

For electroporation applications we recommend the use of ElectroLigase (NEB #M0369).

Instant Sticky-end Ligase Master Mix

#M0370S 50 reactions
#M0370L 250 reactions

- *Instant ligation of sticky ends*
- *Repair of nicks in dsDNA*
- *High-complexity library cloning*

Description: Instant Sticky-end Ligase Master Mix is a ready-to-use 2X solution of T4 DNA ligase and a proprietary ligation enhancer in an optimized reaction buffer. It is specifically formulated to rapidly ligate cohesive-end (2–4 bp) substrates. No thawing of the master mix is required, as it maintains a liquid state during storage at –20°C, and no incubation time is necessary to achieve ligation efficiencies sufficient for successful cloning of sticky-end substrates.

Reaction Conditions: 1X Instant Sticky-end Ligase Master Mix with DNA substrates in a 10 µl reaction volume. A 10 µl reaction contains 1,800 cohesive end units of T4 DNA Ligase.

Usage Note: Product maintains a liquid state at –20°C. Freeze-thaw testing at –70°C has confirmed that performance after 20 freeze/thaw cycles is close to that of the original mix.

Blunt/TA Ligase Master Mix

#M0367S 50 reactions
#M0367L 250 reactions

- *Ligation of blunt ends*
- *T/A Cloning*
- *Repair of nicks in dsDNA*
- *High-complexity library cloning*
- *Ligation of sticky ends*

Description: Blunt/TA Ligase Master Mix is a ready-to-use 2X solution of T4 DNA Ligase, proprietary ligation enhancer and optimized reaction buffer. This master mix is specifically formulated to improve ligation and transformation of both blunt-end and single-base overhang substrates. No thawing is necessary as it remains liquid during storage at –20°C.

Reaction Conditions: 1X Blunt/TA Ligase Master Mix with DNA substrates in a 10 µl reaction volume incubated at 25°C. A 10 µl reaction contains 1,800 cohesive end units of T4 DNA Ligase.

Usage Note: Product maintains a liquid state at –20°C. Freeze-thaw testing at –70°C has confirmed that the performance is unchanged after 20 freeze/thaw cycles.

ElectroLigase®

#M0369S 50 reactions

- *Ideal for transformation by electroporation*
- *Vector or library construction*
- *Linker ligation*
- *Fragment assembly*
- *T/A Cloning*

Description: ElectroLigase combines T4 DNA ligase and an optimized, ready-to-use 2X reaction buffer containing a proprietary ligation enhancer and no PEG. This combination is specifically formulated to promote robust ligation of all types of DNA ends (blunt, sticky, TA). It is directly compatible, without desalting or purification, with electrocompetent cells used for transformation by electroporation.

Reaction Conditions: 1X ElectroLigase Reaction Buffer with DNA substrates and 1 µl ElectroLigase in an 11 µl reaction volume incubated at 25°C. Heat inactivation: 65°C for 15 minutes.

Usage Note: Product maintains a liquid state at –20°C.

T3 DNA Ligase

#M0317S 100,000 units
#M0317L 750,000 units

- *Ligation of sticky or blunt ends*
- *Increased salt tolerance*
- *Repair of nicks in dsDNA*

Description: T3 DNA Ligase is an ATP-dependent ds-DNA ligase from bacteriophage T3. Cohesive ends, blunt ends, and nick sealing can all be efficiently catalyzed by T3 DNA Ligase. Blunt end ligation is enhanced by the addition of PEG 6000 to the reaction. T3 DNA Ligase exhibits a higher tolerance (2-fold) for NaCl in the reaction compared to T4 DNA Ligase, making the enzyme a versatile choice for *in vitro* molecular biology protocols requiring DNA ligase activity.

Reaction Conditions: 1X T3 DNA Ligase Reaction Buffer. Incubate at 25°C.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of 100 ng HindIII fragments of λ DNA in a total reaction volume of 20 µl in 1 minute at 25°C in 1X T3 DNA Ligase Reaction Buffer.

Concentration: 3,000,000 units/ml

Notes: ATP is an essential cofactor for the reaction.

T3 DNA Ligase is also active in buffers without PEG 6000, including T4 DNA Ligase Buffer for applications in which PEG 6000 is detrimental. Supplement with 1 mM ATP (final concentration). In these buffers T3 DNA Ligase exhibits ~10-fold reduction in activity. In applications where a high concentration of NaCl needs to be maintained, we suggest using a reaction buffer without PEG 6000.

T7 DNA Ligase

#M0318S 100,000 units
#M0318L 750,000 units

- *Ligation of sticky ends only*
- *Repair of nicks in dsDNA*

Description: T7 DNA Ligase is an ATP-dependent ds-DNA ligase from bacteriophage T7. It will catalyze the formation of a phosphodiester bond between adjacent 5´ phosphate and 3´ hydroxyl groups of duplex DNA. Cohesive end ligation and nick sealing can be efficiently catalyzed by T7 DNA Ligase. However, unlike T4 and T3 DNA Ligases, blunt end ligation is not efficiently catalyzed by T7 DNA Ligase, making it a good choice for applications in which blunt and sticky ends of DNA are present but only the sticky ends are to be joined.

Reaction Conditions: 1X T7 DNA Ligase Reaction Buffer. Incubate at 25°C.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of 100 ng HindIII fragments of λ DNA in a total reaction volume of 20 µl in 30 minutes at 25°C in 1X T7 DNA Ligase Reaction Buffer.

Concentration: 3,000,000 units/ml

Notes: ATP is an essential cofactor for the reaction.

T7 DNA Ligase is also active in buffers without PEG 6000, including T4 DNA Ligase Buffer for applications in which PEG 6000 is detrimental. Supplement the reaction with 1 mM ATP (final concentration). Using these buffers, the activity of T7 DNA Ligase is reduced ~10-fold.

HiFi *Taq* DNA Ligase

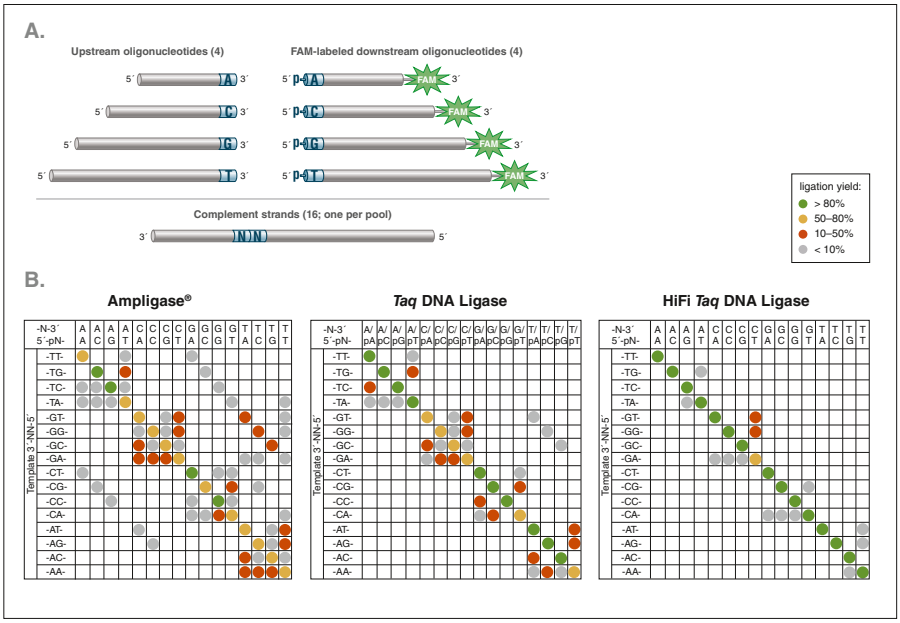
#M0647S 50 reactions

- *High fidelity, thermostable*
- *Repair of nicks in dsDNA*
- *Allele-specific gene detection using ligase-dependent methods, including the Ligase Chain Reaction (LCR) and Ligase Detection Reaction (LDR)*
- *Ligation of padlock probes*

Description: An optimized blend of a thermostable DNA Ligase and a proprietary additive, HiFi *Taq* DNA Ligase efficiently seals nicks in DNA with unmatched high fidelity. The formation of a phosphodiester bond between juxtaposed 5´ phosphate and 3´ hydroxyl termini of two adjacent oligonucleotides that are hybridized to a complementary target DNA is enhanced in the improved reaction buffer and mismatch ligation is dramatically reduced. The improved formulation allows higher resolution discrimination between ligation donors and acceptors, enabling precise detection of SNPs and other allele variants. HiFi *Taq* DNA Ligase is active at elevated temperatures (37–75°C).

Reaction Conditions: 1X HiFi *Taq* DNA Ligase Reaction Buffer. Incubate at 25°C.

For help with calculating ligation temp, try our **Thermostable Ligase Reaction Temperature Calculator** at **LigaseCalc.neb.com**



HiFi Taq DNA Ligase displays increased fidelity. *(A) Schematic of multiplexed substrate pools. Each substrate pool contained a single splint with a defined NN at the ligation junction (e.g., AA, AC, AG…) along with all four upstream probes and all four FAM-labeled downstream probes. Each probe that encodes the base at the ligation junction is of unique length allowing for separation and analysis by capillary electrophoresis. A total of 16 substrate pools were prepared, one for each unique splint. (B) Comparison of the ligation fidelity of Ampligase (Epicentre), Taq DNA Ligase and HiFi Taq DNA Ligase. Fidelity measurements were performed using 1 µl of ligase in a 50 µl reaction mixture in the supplied buffers at 1X concentration. Reactions were incubated 30 min at 55°C, using multiplexed substrate pools as outlined in (A). Rows represent a single template sequence, while columns indicate a particular ligation product resulting from a specific pair of probes ligating with the indicated bases at the ligation junction. A dot indicates detection of a product (see legend above). The diagonal from the top left to the bottom right represents Watson-Crick ligation products; all other spaces indicate mismatch ligation products. While Taq DNA Ligase and Ampligase perform similarly under these conditions, with a range of mismatch products detectable, HiFi Taq DNA Ligase shows dramatically fewer mismatch products while maintaining high yields.*

Thermus aquaticus (Taq) DNA Ligase

#M0208S 2,000 units
#M0208L 10,000 units

- *Thermostable*
- *Repair of nicks in dsDNA*
- *Used in Gibson Assembly method*
- *Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction*

Description: *Taq* DNA Ligase is a thermostable ligase that catalyzes the formation of a phosphodiester bond between the 5´-phosphate and 3´ -hydroxyl termini of two adjacent DNA strands. The strands to be ligated need to be hybridized and accurately paired, with no gap, to a complementary DNA strand; allowing resolution of single nucleotide variants. *Taq* DNA Ligase uses NAD as a cofactor and is active at elevated temperatures (37–75°C).

Reaction Conditions: 1X *Taq* DNA Ligase Reaction Buffer. Incubate at 45°C.

Requires NAD^+ as a cofactor. NAD^+ is supplied in the 10X *Taq* DNA Ligase Reaction Buffer; *the buffer should be stored at –70°C to extend the half life of the NAD^+ cofactor.*

Unit Definition: (Cohesive End Unit)
One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of BstEII-digested λ DNA in a total reaction volume of 50 µl in 15 minutes at 45°C. Unit assay conditions can be found at www.neb.com.

Concentration: 40,000 units/ml

Notes: Will not ligate short 4-base overlaps (typical of restriction enzyme digests), while it efficiently ligates 12 base pair overlaps.

E. coli DNA Ligase

RR NEBU 16° 65°

#M0205S 200 units
#M0205L 1,000 units

- *Repair of nicks in dsDNA*
- *Okayama and Berg cDNA cloning*

Description: *E. coli* DNA Ligase catalyzes the formation of a phosphodiester bond between the 5´-phosphate and 3´-hydroxyl of two adjacent DNA strands in duplex DNA with cohesive ends. It is not appreciably active on blunt-ended substrates. *E. coli* DNA Ligase uses NAD as a cofactor and can be heat-inactivated. *E. coli* DNA Ligase is active at a range of temperatures (4–37°C).

Reaction Conditions: 1X *E. coli* DNA Ligase Reaction Buffer. Optimal ligation occurs at 16°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λ DNA (5´ DNA termini concentration of 0.12 µM, 300 µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Usage Notes:
Requires NAD^+ (nicotinamide adenine dinucleotide) as a cofactor.

Ligation of blunt-ended fragments is extremely inefficient. For ligation of blunt-ended fragments we recommend the Blunt/TA Ligase Master Mix (NEB #M0367) or the Quick Ligation Kit (NEB #M2200).

9°N™ DNA Ligase

RR NEBU 45° NO

#M0238S 2,500 units

- *Repair of nicks in DNA while incubating at high temperatures*
- *Thermostable*
- *Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction*

Description: 9°N DNA Ligase is a thermostable ligase that catalyzes the formation of a phosphodiester bond between the 5´-phosphate and 3´-hydroxyl of two adjacent DNA strands that are hybridized and accurately paired, with no gap, to a complementary DNA strand. 9°N DNA Ligase uses ATP as a cofactor and it is active at elevated temperatures (45–70°C).

Reaction Conditions: 1X 9°N DNA Ligase Reaction Buffer. Incubate at 45°C.

Unit Definition: (Cohesive End Unit)
One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of BstEII-digested λ DNA in a total reaction volume of 50 µl in 15 minutes at 45°C. Unit assay conditions can be found at www.neb.com.

Concentration: 40,000 units/ml

Notes: Will not ligate short 4-base overlaps (typical of restriction enzyme digests), while it efficiently ligates 12 base pair overlaps.

SplintR® Ligase

RR 25° 65°

See page 199 for more information.

#M0375S 1,250 units
#M0375L 6,250 units

The SplintR Ligase, also known as PBCV-1 DNA Ligase or *Chlorella* virus DNA Ligase, efficiently catalyzes the ligation of adjacent, single-stranded DNA oligonucleotides splinted by a complementary RNA strand.

T4 Polynucleotide Kinase & T4 Polynucleotide Kinase (3´ phosphatase minus)

RR NEBU 37° 65°

T4 Polynucleotide Kinase
#M0201S 500 units
#M0201L 2,500 units

T4 Polynucleotide Kinase
(3´ phosphatase minus)
#M0236S 200 units
#M0236L 1,000 units

- *5´ phosphorylation of DNA/RNA for subsequent ligation*
- *End labeling DNA or RNA for probes and DNA sequencing*
- *Removal of 3´phosphoryl groups with T4 Polynucleotide Kinase (NEB #M0201)*
- *T4 PNK (3´ phosphatase minus) (NEB #M0236) can be used for the 5´ phosphorylation of 3´ phosphorylated mononucleotide to generate a substrate (pNp) that can be added to the 3´ end of DNA or RNA*
- *5´ end labeling of 3´ phosphorylated oligos with T4 PNK (3´ phosphatase minus) (NEB #M0236)*

Description: T4 Polynucleotide Kinase catalyzes the transfer and exchange of P_i from the γ position of ATP to the 5´ hydroxyl terminus of polynucleotides (double- and single-stranded DNA and RNA), as well as nucleoside 3´ monophosphates. The T4 Polynucleotide Kinase (NEB #M0201) also catalyzes the removal of 3´ phosphoryl groups from 3´ phosphoryl polynucleotides, deoxynucleoside 3´ monophosphates and deoxynucleoside 3´ diphosphates. The modified version (NEB #M0236) exhibits full kinase activity with no 3´ phosphatase activity.

Reaction Conditions: 1X T4 Polynucleotide Kinase Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Usage Notes:
Fresh buffer is required for optimal activity (in older buffers, loss of DTT due to oxidation lowers activity).

CTP, GTP, TTP, UTP, dATP or dTTP can be substituted for ATP as a phosphate donor.

Protocols for phosphorylation (radioactive and non-radioactive) of DNA & RNA can be found at www.neb.com.

The efficiencies of blunt and recessed 5´ end phosphorylation can be improved by heating to 70°C for 5 minutes, then chilling on ice prior to kinase addition and by adding PEG-8,000 to 5% (w/v).

T4 Polynucleotide Kinase requires ATP for activity, but the supplied reaction buffer does not contain ATP to allow for high specific activity radiolabeling reactions.

Often, a kinase reaction is followed by a ligation reaction. In such cases, the T4 PNK reaction is performed in ligase buffer at 37°C for 30 minutes. The product of this reaction can be used directly in the ligation reaction without a buffer change or heat inactivation UNLESS there is a need to keep other DNA fragments dephosphorylated during ligation. When this is desirable, PNK should be heat inactivated prior to ligation.

Unit Definition: One Richardson unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of acid-insoluble [^{32}P] in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

NEW

5-hydroxymethyluridine DNA Kinase

#M0659S 1,000 units

This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Description: 5-hydroxymethyluridine DNA Kinase (5-HMUDK) transfers the gamma phosphate from ATP to the hydroxymethyl moiety of 5-hydroxymethyluridine in polymeric DNA.

Reaction Conditions: 1X T4 DNA Ligase Reaction Buffer. Incubate at 37°C. Heat inactivation: 80°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of Bacillus subtilis bacteriophage SP8 genomic DNA in 30 minutes at 37°C in a total reaction volume of 20 µl against subsequent cleavage by NcoI-HF restriction endonuclease.

Concentration: 20,000 units/ml

Phosphatase Selection Chart

	Quick Dephosphorylation Kit	Recombinant Shrimp Alkaline Phosphatase (rSAP)	Antarctic Phosphatase	Alkaline Phosphatase Calf Intestinal (CIP)
FEATURES				
100% heat inactivation	2 minutes/80°C	5 minutes/65°C	2 minutes/80°C	No
High specific activity	●	●		●
Improved stability	●	●		
Works directly in NEBuffers	●	●	●	●
Requires additive			● (Zn^{2+})	
Quick Protocol	●			

Quick Dephosphorylation Kit

#M0508S 100 reactions
#M0508L 500 reactions

- *Dephosphorylation of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs and pyrophosphate from PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to 5´ end-labeling using T4 Polynucleotide Kinase*

Description: The Quick Dephosphorylation Kit contains Quick CIP, a heat-labile recombinant version of calf intestinal alkaline phosphatase (CIP). Quick CIP nonspecifically catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. It also hydrolyses ribo- and deoxyribonucleoside triphosphates (NTPs and dNTPs). Quick CIP is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents re-ligation of linearized plasmid DNA. The enzyme can quickly dephosphorylate 5´ protruding, 5´ recessed, and blunt ends in just 10 minutes. Quick CIP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis.

Quick CIP is completely and irreversibly inactivated by heating it at 80°C for 2 minutes, unlike wild type CIP, which is not inactivated by heat. This makes removal of Quick CIP prior to ligation or end-labeling unnecessary.

The Quick Dephosphorylation Kit Includes:

– CutSmart Buffer

– Quick CIP

Find an overview of dephosphorylation.

Shrimp Alkaline Phosphatase (rSAP)

CutSmart RR 37° 65°

#M0371S 500 units
#M0371L 2,500 units

- *Dephosphorylation of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs and pyrophosphate from PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to 5´ end-labeling using T4 Polynucleotide Kinase*

Description: Shrimp Alkaline Phosphatase (rSAP) is a heat labile alkaline phosphatase purified from a recombinant source. rSAP is identical to the native enzyme, and contains no affinity tags or other modifications. rSAP nonspecifically catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. Also, rSAP hydrolyzes ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). rSAP is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents re-ligation of linearized plasmid DNA. rSAP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis. rSAP is completely and irreversibly inactivated by heating at 65°C for 5 minutes, thereby making removal of rSAP prior to ligation or end-labeling unnecessary.

Reaction Conditions: 1X CutSmart Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of *p*-Nitrophenyl Phosphate, PNPP (NEB #P0757) in a total reaction volume of 1 ml in 1 minute at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 1,000 units/ml

Antarctic Phosphatase

RR NEB 1.1 37° 80°

#M0289S 1,000 units
#M0289L 5,000 units

- *Dephosphorylation of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs and pyrophosphate from PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to 5´ end-labeling using T4 Polynucleotide Kinase*

Description: Antarctic Phosphatase catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. Antarctic Phosphatase also hydrolyzes ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). Antarctic Phosphatase is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents religation of linearized plasmid DNA. The enzyme acts on 5´ protruding, 5´ recessed and blunt ends. Antarctic Phosphatase may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing. The enzyme is completely and irreversibly inactivated by heating at 70°C for 5 minutes, thereby making removal of Antarctic Phosphatase prior to ligation or end-labeling unnecessary.

Reaction Conditions: 1X Antarctic Phosphatase Reaction Buffer. Incubate at 37°C. Heat inactivation: 80°C for 2 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will dephosphorylate 1 µg of pUC19 vector DNA cut with a restriction enzyme generating 5´ recessed ends in 30 minutes at 37°C. Dephosphorylation is defined as > 95% inhibition of recirculation in a self-ligation reaction and is measured by transformation into *E. coli*. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Alkaline Phosphatase, Calf Intestinal (CIP)

CutSmart 37° No

#M0290S 1,000 units
#M0290L 5,000 units

- *Dephosphorylation of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs and pyrophosphate from PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to 5´ end-labeling using T4 Polynucleotide Kinase*

Description: Alkaline Phosphatase, Calf Intestinal (CIP) nonspecifically catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. CIP also hydrolyses ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). CIP is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents religation of linearized plasmid DNA. In cloning, dephosphorylation prevents religation of linearized plasmid DNA. The enzyme acts on 5´ protruding, 5´ recessed and blunt ends. CIP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis.

Source: Calf intestinal mucosa

Reaction Conditions: 1X CutSmart Reaction Buffer. Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of *p*-Nitrophenyl Phosphate, PNPP (NEB #P0757) in a total reaction volume of 1 ml in 1 minute at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Inorganic & Thermostable Inorganic Pyrophosphatases

RR

See page 189 for more information.

Pyrophosphatase, Inorganic (*E. coli*)
#M0361S 10 units
#M0361L 50 units

Pyrophosphatase Inorganic (Yeast)
#M2403S 10 units
#M2403L 50 units

Thermostable Inorganic Pyrophosphatase
#M0296S 250 units
#M0296L 1,250 units

Apyrase

RR NEBU 30° 65°

#M0398S 10 units
#M0398L 50 units

- *Highly efficient degradation of ATP to AMP*
- *Removal of deoxynucleotides in DNA pyrosequencing between cycles*
- *Conversion of 5´ triphosphorylated RNA to ligatable monophosphorylated*
- *Conversion of 5´ triphosphorylated RNA to 5´ exonuclease XRN-1 (NEB #M0338) sensitive monophosphorylated RNA*
- *Supplied at 10-fold higher concentration*

Description: Apyrase (recombinant, *E. coli*) is a highly active ATP-diphosphohydrolase that catalyses the sequential hydrolysis of ATP to ADP and ADP to AMP releasing inorganic phosphate. It is a recombinant version of one of several isoforms of apyrase. It can also hydrolyse 5´ tri- and diphosphate ribonucleosides and deoxyribonuclesides to their respective 5´ monophosphates. Apyrase can catalyse the conversion of 5´ triphosphorylated RNA to 5´ monophosphorylated RNA by sequential removal of γ and β phosphates.

Reaction Conditions: 1X Apyrase Reaction Buffer. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that catalyses the release of 1 µmol of inorganic phosphate from ATP (1 mM, NEB #P0756) in 1X Apyrase Reaction Buffer in 1 minute at 30°C in a total reaction volume of 50 µl. Unit assay conditions can be found at www.neb.com.

Concentration: 500 units/ml

Notes: Apyrase has a higher ratio of activity for ATP:ADP (14:1).

Apyrase is a calcium-activated enzyme. It is approximately 50% active when Mg^{2+} substitutes Ca^{2+} in Apyrase Reaction Buffer.

As a metal-dependent enzyme, Apyrase can be inhibited by EGTA and EDTA.

The activity of Apyrase is approximately 30% in NEBuffers 1.1, 2.1, 3.1 and CutSmart Buffer.

Apyrase does not remove 5´ caps from eukaryotic mRNA.

NEW

Tte UvrD Helicase

RR NEBU 65° 80°

#M1202S 0.5 µg

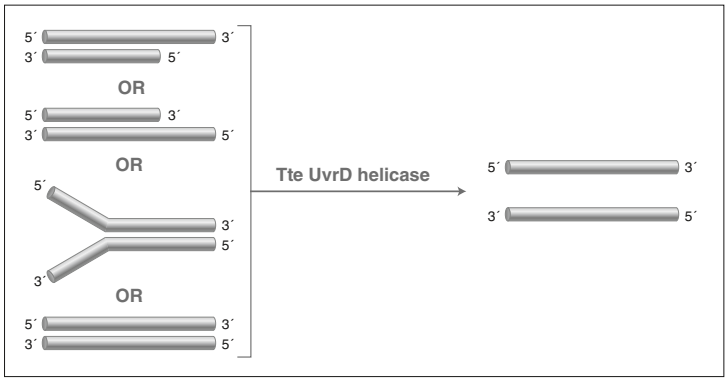
This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

- *Unwinds double-stranded DNA*
- *Thermostable to 65°C*
- *Reduces non-specific product formation in isothermal amplification (e.g. LAMP)*

Description: *Tte* UvrD Helicase is a repair helicase from the thermophilic organism *Thermoanaerobacter tengcongensis*. It is capable of unwinding double-stranded DNA without a requirement for a specific flap or overhang structure. *Tte* UvrD Helicase is active on a wide range of DNA substrates and, along with its thermostability (active to 70°C), the *Tte* UvrD Helicase has been demonstrated to be a useful additive for improving specificity of isothermal amplification reactions.

Reaction Conditions: 1X Isothermal Amplification Buffer. Supplement with 1 mM ATP. Heat inactivation: 80°C for 20 minutes.

Concentration: 20 µg/ml



Tte UvrD Helicase activity.

Properties of Exonucleases and Endonucleases

ENZYME	POLARITY	DNA SUBSTRATE		ACTIVITY WITHOUT 5´ PHOSPHATE	ABLE TO INITIATE ON DNA WITH[1]:				PARTIAL DIGESTION ss EXTENSION[2]	PRODUCTS PRODUCED[3]	END-LABELED FAM CLEAVAGE[4]	PHOSPHO-ROTHIOATE CLEAVAGE[5]	UNITS FOR 90% DIGESTION OF 2 M OLIGO[6]
		ss	ds		5´ext	3´ext	BLUNT	NICK					
Lambda Exonuclease	5´→3´	+/–	+	+/–[7]	+/–	+	+	–	3´	ssDNA, dNMP	+	–	2
$RecJ_f$	5´→3´	+	–	+	+/–[9]	–	+/–[10]	–	NA	dNMP	+/–	–	> 1500
Exonuclease III	3´→5´	+/–	+	+	+	+/–	+	+	5´	ssDNA, dNMP	+	–	10
Exonuclease I	3´→5´	+	–	+	–	+/–[9]	+/–[10]	NR	NA	dNMP, dinucleotide[11]	+	–	20
Thermolabile Exonuclease I	3´→5´	+	–	+	–	+/–[9]	+/–[10]	NR	NA	dNMP, dinucleotide[11]	+	–	20
Exonuclease T	3´→5´	+	–	+	–	+[12]	+/–[10]	NR	NA	dNMP	+	–	> 100
Exonuclease V	both	+	+	+	+	+	+	–	Both 5´, 3´	short oligos	+	NA	NA
Exonuclease VIII, truncated	5´→3´	–	+	+	+	+	+	–	3´	ssDNA, dNMP	NA	NA	NA
Exonuclease VII	both	+	–	+	+/–	+/–	–	–	5´	short oligos	NA	+	> 100
BAL-31 Nuclease	3´→5´, endo[13]	+	+	+	+	+	+	+	NA	dsDNA, dNMP	NA	NA	NR
Mung Bean Nuclease	endo	+	–	+	+	+	–	–	NA	ssDNA, dsDNA	NA	NA	10
DNase I	endo	+	+	NA	NA	NA	NA	NA	NA	ssDNA, dsDNA oligonucleotides, di- and trinucleotides	NA	NA	0.1
Micrococcal Nuclease	endo	+	+	NA	NA	NA	NA	NA	NA	ssDNA, dsDNA 3´ monophospho- and diphosphonucleotides[14]	NA	NA	NR
Nuclease P1	endo	+	–	+	+	+	–	–	NA	5´ mononucleotides	NA	NA	NR
T5 Exonuclease	5´→3´, ss endo	+	+	+	+	+	+	+	NA	dNMP to 6-mer	+	NA	2
T7 Exonuclease	5´→3´	+/–	+	+	+/–	+	+	+	3´	ssDNA, dNMP, dinucleotide[8]	+	–	10
T7 Endonuclease I[15]	endo	–	+	NA	NA	NA	NA	+/–	NA	dsDNA	NA	NA	NA
Thermostable FEN1	endo	NA	NA	+	–	–	–	–	NA	cleaved flap	?	?	?

This table is intended to be used as a guide. Not all reported activities and properties for each exonuclease or endonuclease are listed. The amount of enzyme, substrate and time of incubation can have a dramatic effect upon the desired outcome of the experiment.

Table Legend:

- **+** activity; preferred substrate
- **–** no significant activity
- **+/–** activity greatly reduced relative to preferred substrate
- **NR** not reported
- **NA** not applicable
- **ss** single-stranded
- **ds** double-stranded
- **ext** extension
- **dNMP** deoxyribonucleoside monophosphate

Footnotes:

1. The ability to act on short extensions and blunt ends distinguishes these enzymes; such ends are conveniently generated by restriction digestion. The 5´ and 3´ extensions tested were 4 nt in length.
2. Partial digestion of dsDNA by Lambda Exonuclease, T7 Exonuclease and Exonuclease III will produce dsDNA products with ss extensions. Complete digestion produces ssDNA as a product.
3. Complete hydrolysis of the preferred substrate will generate the listed products.
4. The ability of an exonuclease to initiate on the end of the preferred DNA substrate (ss or ds) containing a fluorescein group linked to either the 5´ or 3´ end. Phosphoramidite chemistry was used to synthesize oligonucleotides with FAM groups. The 5´ FAM was added to the oligonucleotide as a [(3´,6´-dipivaloylfluoresceinyl)-6-carboxamidohexyl]-1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite while the 3´ FAM oligonucleotides were synthesized using the 1-dimethoxytrityloxy-3-[O-(N-carboxy-(di-O-pivaloyl-fluorescein)-3-aminopropyl)]-propyl-2-O-succinoyl-long chain alkylamino-CPG support.
5. The S_p stereoisomer of the phosphorothioate linkage is known to greatly inhibit cleavage of many nucleases while the R_p stereoisomer shows either no or less inhibition. Synthetic oligonucleotides containing approximately an equal ratio of both isomers necessitates the use of multiple phosphorothioates to block cleavage. The presence of 6 consecutive phosphorothioates on oligonucleotides of the preferred substrate blocked all exonucleases effectively (5% or less degradation) except T7 exonuclease which had approximately 10% cleavage. Analysis of exonucleases with oligonucleotides having either one or two consecutive phosphorothioates revealed significant degradation (not shown). T5 Exonuclease is not recommened for this application since it also has ssDNA endonuclease activity.
6. The amount of enzyme in units to cleave greater than 90% of 2 µM 35-mer oligonucleotide(s) of the preferred substrate (ss or dsDNA) in a 10 µl volume using the unit digest reaction conditions. All enzymes tested, except for Exo T and $RecJ_f$, could effectively cleave the substrate to completion.
7. Lambda exonuclease has a strong preference for initiating degradation on dsDNA containing a 5´ phosphate. Thus, if linear dsDNA has a 5´ phosphate at one end and lacks a 5´ phosphate on the other end, then lambda exonuclease will preferentially degrade the DNA from the phosphorylated end.
8. It has been reported that the initial first product hydrolyzed from dsDNA by T7 Exonuclease is a dinucleotide. Subsequent hydrolytic cleavage releases dNMP.
9. $RecJ_f$ is not suitable for making 5´ extensions blunt. Exo I is not suitable for making 3´ extensions blunt. Both $RecJ_f$ and Exo I require longer length ssDNA extensions to initiate than those generated by restriction enzymes.
10. Depending upon the DNA sequence and amount of exonuclease, $RecJ_f$, Exo I and Exo T may remove a few nucleotides from flush termini.
11. Exo I releases dNMP from ssDNA, except at the last hydrolytic step where a dinucleotide is produced.
12. Exo T can be used to make 3´ extensions blunt, however, it yields 2–4 fold fewer ligatable blunt ends when compared to Klenow polymerase plus dNTP on a test substrate.
13. BAL31 has been reported as having both ss endonuclease activity as well as 3´→5´ ds exonuclease activity. Thus, any linear DNA is a substrate for BAL31.
14. Products of Micrococcal Nuclease degradation have 3´ phosphates.
15. T7 endonuclease recognizes and cleaves non-perfectly matched DNA, cruciform DNA, Holliday structures or junctions. It will act more slowly on nicked dsDNA.

Common Applications for Exonucleases and Endonucleases

APPLICATION	RECOMMENDED ENZYME(s)
Removal of 3´ overhangs	Quick Blunting Kit T4 DNA Polymerase* + dNTPs, Klenow + dNTPs
5´ overhang treatment: Fill in Cleavage	Quick Blunting Kit T4 DNA Polymerase* + dNTPs, Klenow + dNTPs Mung Bean Nuclease
Removal of oligonucleotides post PCR	Exonuclease I, Thermolabile Exonuclease I, Exonuclease VII
Removal of chromosomal DNA in plasmid preparations	Lambda Exonuclease (Exonuclease I can be added to remove ssDNA generated by Lambda Exonuclease) T5 Exonuclease (Degrades linear ss + dsDNA, nicked DNA) Exonuclease V (RecBCD) (Degrades linear ss + dsDNA)
Removal of DNA in RNA preparations	DNase I
Chromatin immunoprecipitation (ChIP) analysis	Micrococcal Nuclease
Generating ssDNA from linear dsDNA: If 5´ → 3´ polarity required If 3´ → 5´ polarity required Best general choice	 Lambda Exonuclease Exonuclease III Lambda Exonuclease

*T4 DNA Polymerase has a strong 3´→ 5´ exo activity.

DNase I (RNase-Free)

RR

See page 202 for more information.

#M0303S 1,000 units
#M0303L 5,000 units

DNase I (RNase-free) is an endonuclease that nonspecifically cleaves DNA to release di-, tri- and oligonucleotide products with 5´-phosphorylated and 3´-hydroxylated ends.

Exonuclease I (*E. coli*)

RR NEBU 37° 80°

#M0293S 3,000 units
#M0293L 15,000 units

- *3´→5´ single strand exonuclease*
- *Degradation of post-PCR primers*

Description: Catalyzes the removal of nucleotides from single-stranded DNA in the 3´ to 5´ direction.

Reaction Conditions: 1X Exonuclease I Reaction Buffer. Incubate at 37°C. Heat inactivation: 80°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 10 nmol of acid-soluble nucleotide in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 20,000 units/ml

NEW

Thermolabile Exonuclease I

RR NEB3.1 37° 80°

#M0568S 3,000 units
#M0568L 15,000 units

- *Removal of single-stranded primers in PCR reactions prior to DNA sequencing or SNP analysis*
- *Removal of single-stranded primers for nested PCR reactions*
- *Removal of single-stranded DNA from dsDNA*

Description: Catalyzes the removal of nucleotides from single-stranded DNA in the 3´ to 5´ direction.

Reaction Conditions: 1X NEBuffer 3.1. Incubate at 37°C. Heat inactivation: 80°C for 1 minute.

Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 2 nmol of acid-soluble nucleotide in a total reaction volume of 100 µl in 6 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 20,000 units/ml

Exonuclease III (*E. coli*)

RR NEB1 37° 70°

#M0206S 5,000 units
#M0206L 25,000 units

- *3´→5´ exonuclease*
- *Production of unidirectional nested deletions*
- *Site-directed mutagenesis*
- *Preparation of strand-specific probes*
- *Preparation of single-stranded substrates for dideoxy sequencing*

Description: Catalyzes the stepwise removal of mononucleotides from 3´ hydroxyl temini of duplex DNA. A limited number of nucleotides are removed during each binding event, resulting in coordinated progressive deletions within the population of DNA molecules.

The preferred substrates are blunt or recessed 3´ termini, although the enzyme also acts at nicks in duplex DNA to produce single-strand gaps. The enzyme is not active on ssDNA, and thus 3´ protruding termini are resistant to cleavage. The degree of resistance depends on the length of the extension, with 4 bases or longer being essentially resistant to cleavage. This can be exploited to produce unidirectional deletions from a linear molecule with one resistant (3´overhang) and one susceptible (blunt or 5´ overhang) terminus.

Exonuclease III has also been reported to have RNase H, 3´ phosphatase and AP-endonuclease activities.

Reaction Conditions: 1X NEBuffer 1. Incubate at 37°C. Heat inactivation: 70°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble total nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 100,000 units/ml

Usage Notes: Phosphorothioate linkages are not cleaved by Exonuclease III. Unidirectional deletions can also be created by protecting one terminus by incorporation of an α-phosphorothioate-containing nucleotide.

Exonuclease V (RecBCD)

RR NEBU 37° 70°

#M0345S 1,000 units
#M0345L 5,000 units

- *Degradation of linear ssDNA and dsDNA, while preserving nicked and supercoiled plasmid DNA*

Description: Exonuclease V, a RecBCD complex from *E. coli*, has several different enzyme activities, including an ATP-dependent single-stranded DNA endonuclease activity, ss- and ds- DNA exonuclease activity. The hydrolysis in each case is bi-directional (from both the 3´ and 5´ ends) and processive, producing oligonucleotides. All Exonuclease V activities have divalent cation requirements. Mg^{2+} is required for the exonuclease activity, while Ca^{2+} inhibits the exonuclease activity and allows double-stranded DNA unwinding (helicase activity) without hydrolysis.

Reaction Conditions: 1X NEBuffer 4. Supplement with 1 mM ATP. Incubate at 37°C. Heat inactivation: 70°C for 30 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in 30 minutes at 37°C in a total reaction volume of 50 µl. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Exonuclease VII

RR NEBU 37° 95°

#M0379S 200 units
#M0379L 1,000 units

- *Removal of ss-oligonucleotide primers*
- *Removal of terminal phosphorothioated ss-oligonucleotide primers*
- *Mapping positions of introns in genomic DNA*
- *Removal of single-stranded DNA from dsDNA*

Description: Exonuclease VII, (Exo VII) derived from *E. coli*, cleaves single-stranded DNA (ssDNA) from both 5´→3´ and 3´→5´ direction. This enzyme is not active on linear or circular dsDNA. It is useful for removal of ss-oligonucleotide primers from a completed PCR reaction when different primers are required for subsequent PCR reactions. Digestion of ssDNA by Exonuclease VII is metal-independent.

Reaction Conditions: 1X Exonuclease VII Reaction Buffer. Incubate at 37°C. Heat inactivation: 95°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 1 nmol of acid-soluble nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Exonuclease VIII, truncated

RR NEB4 37° 70°

#M0545S 1,000 units
#M0545L 5,000 units

- *Degradation of linear dsDNA while maintaining double-stranded circular DNA*

Description: Exonuclease VIII, truncated, is a genetically engineered active domain of exonuclease VIII from *E. coli*. Exonuclease VIII, truncated is able to initiate nucleotide removal from the 5´ termini of linear double-stranded DNA in the 5´ to 3´ direction. The enzyme does not degrade supercoiled dsDNA and circular ssDNA.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C. Heat inactivation: 70°C for 15 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 4 with 0.15 mM sonicated duplex [^{3}H] DNA. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Exonuclease T

#M0265S 250 units
#M0265L 1,250 units

- *Specific for single-stranded DNA or RNA*
- *Generation of blunt ends from RNA or DNA with a 3´ extension*

Description: Exonuclease T (Exo T), also known as RNase T, is a single-stranded RNA or DNA specific nuclease that requires a free 3´ terminus and removes nucleotides in the 3´→ 5´ direction. Exonuclease T can be used to generate blunt ends from RNA or DNA molecules that have 3´ extensions.

Source: Exonuclease T is overexpressed and purified as a C-terminal fusion to maltose-binding protein (MBP). Following affinity chromatography, Exo T is cleaved from MBP leaving an additional amino acid on the N-terminus and a Phe instead of a Met (i.e., Glu-Phe-Exo T instead of Met-Exo T).

Reaction Conditions: 1X NEBuffer 4. Incubate at 25°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 0.1 nmol of TCA soluble nucleotides from 1 nmol of [^{3}H]-labeled polythymidine in a total reaction volume of 100 µl in 30 minutes at 25°C in 1X NEBuffer 4 with 1 nmol [^{3}H]-labeled polythymidine DNA.

Concentration: 5,000 units/ml

Usage Note: Exo T is has different activity on RNA vs. DNA. For RNA, 1 unit of Exo T is required to completely digest 1.0 pmol of rA20 under standard reaction conditions.

Thermostable FEN1

#M0645S 1,600 units

- *Cleavage of flap DNA structure*

This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Description: Thermostable Flap Endonuclease 1, FEN1, catalyzes the cleavage of 5´ DNA flaps from branched double stranded DNA substrates, creating a 5´ phosphate terminus. FEN1 products can be ligated by DNA ligase to create double stranded DNA. *In vivo*, FEN1 is an essential component of the Okazaki fragment maturation pathway, and also plays a role in base excision repair.

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 10 pmol of 5´ flap containing oligonucleotide substrate in a total reaction volume of 10 µl in 10 minutes at 65°C. Unit assay conditions can be found at www.neb.com.

Concentration: 32,000 units/ml

Lambda Exonuclease

#M0262S 1,000 units
#M0262L 5,000 units

- *Highly processive 5´→3´ exonuclease*
- *Removal of 5´ mononucleotides from duplex DNA*

Description: A highly processive enzyme that catalyzes the removal of mononucleotides from duplex DNA in a 5´→ 3´ direction. The preferred substrate is 5´ phosphorylated double-stranded DNA, although it will also degrade single-stranded and non-phosphorylated substrates at a reduced rate. Lambda Exonuclease is unable to initiate digestion at nicks or gaps.

Source: A genetic fusion of the *E. coli* Lambda Exonuclease gene with the gene encoding maltose binding protein (MBP). Following affinity chromatography, Lambda Exonuclease is cleaved from the fusion construct and purified away from MBP.

Reaction Conditions: 1X Lambda Exonuclease Reaction Buffer. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 10 nmol of acid-soluble deoxyribonucleotide from double-stranded substrate in a total reaction volume of 50 µl in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Note: 5´-OH ends are digested 20X slower than 5´-PO_4 ends. ssDNA is digested 100X slower than dsDNA.

Micrococcal Nuclease

#M0247S 320,000 gel units

- *Degradation of nucleic acids present in protein preparations*
- *In vitro translation*
- *Reduction of the viscosity of cell lysates during non-mechanical cell lysis preparation*
- *Chromatin structure analysis*
- *Rapid RNA sequencing*
- *ChIP analysis*

Description: Micrococcal Nuclease is derived from *Staphylococcus aureus* and is a relatively non-specific endo-exonuclease. The enzyme digests double-stranded, single-stranded, circular and linear nucleic acids. Cleavage preferences have been observed at sites rich in adenylate, deoxyadenylate or thymidylate. Both DNA and RNA are degraded to 3´ phosphomononucleotides and dinucleotides.

Source: An *E. coli* strain containing a genetic fusion of the micrococcal nuclease gene (Gene ID: 3238436) and the gene coding for maltose binding protein (MBP). The micrococcal nuclease is cleaved from the fusion protein and purified away from MBP.

Reaction Conditions: 1X Micrococcal Nuclease Reaction Buffer. Supplement with 100 µg/ml BSA. Incubate at 37°C.

Unit Definition: (Agarose Gel Unit) One gel unit is defined as the amount of enzyme required to digest 1 µg of lambda genomic DNA in 15 minutes at 37°C into molecular DNA fragments (100–400 base pairs) on a 1.2% agarose gel.

Note: 10,000 Gel Units are approximately equal to 1,000 Kunitz Units.

Concentration: 2 x 10^6 gel units/ml

Notes: 1–5 mM Ca^{2+} is required for activity. The enzyme is active in the pH range 7–10, with optimal activity at pH 9.2, as long as salt concentration is less than 100 mM. Enzyme can be inactivated by addition of excess EGTA.

Mung Bean Nuclease

NEBU 30° No

#M0250S 1,500 units
#M0250L 7,500 units

- *Removal of single-stranded extensions (3´ and 5´) to leave ligatable blunt ends*
- *Transcriptional mapping*
- *Cleavage of hairpin loops*
- *Excision of gene coding sequences from genomic DNA*

Description: A single-strand specific DNA and RNA endonuclease which will degrade single-stranded extensions from the ends of DNA and RNA molecules, leaving blunt, ligatable ends.

Source: Mung bean sprouts

Reaction Conditions: 1X Mung Bean Nuclease Reaction Buffer. Incubate at 30°C.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 µg of acid-soluble total nucleotide in a total reaction volume of 50 µl in 1 minute at 37°C in 1X Mung Bean Nuclease Reaction Buffer with 0.5 mg/ml denatured Calf Thymus DNA. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Usage Note: Do not attempt to heat inactivate, DNA will "breathe" before enzyme inactivates, causing undesirable degradation.

Nuclease BAL-31

NEBU 30° 65°

#M0213S 50 units

- *Progressive shortening of duplex DNA*
- *Restriction site mapping*

Description: Nuclease BAL-31 exonuclease degrades both 3´ and 5´ termini of duplex DNA without generating internal scissions. The enzyme is also a highly-specific single-stranded endonuclease which cleaves at nicks, gaps and single-stranded regions of duplex DNA and RNA.

Source: Purified from the culture medium of *Alteromonas espejiana* BAL-31. Contains a mixture of "fast" and "slow" species of the enzyme.

Reaction Conditions: 1X Nuclease BAL-31 Reaction Buffer. Incubate at 30°C. Heat inactivation: 65°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to remove 200 base pairs from each end of linearized double-stranded ϕX174 DNA (40 µg/ml) in 50 µl of 1X Nuclease BAL-31 Reaction Buffer in 10 minutes at 30°C.

Concentration: 1,000 units/ml

Usage Notes: Duplex products of the exonuclease are a mixture of blunt and staggered ends. This mixture can be cloned directly, although maximal ligation efficiency requires repairing the staggered ends with a suitable DNA polymerase.

If necessary, the enzyme may be diluted in reaction buffer just prior to use.

NEW

Nuclease P1

NEBU 37° 75°

#M0660S 10,000 units

- *Removal of single-stranded tails from DNA molecules to create blunt ends*
- *Cleavage of hairpin loops*
- *DNA or RNA base compositional analysis*
- *Removal of nucleic acids during protein purification*

Description: Nuclease P1 (from *P. citrinum*) is a zinc-dependent single-strand specific nuclease which hydrolyzes 3´→ 5´ phosphodiester bonds in RNA and ssDNA with no base specificity. Nuclease P1 also exhibits 3´-phosphomonoesterase activity.

Although a single-strand specific nuclease, Nuclease P1 does display some activity toward double-stranded DNA (dsDNA) in Nuclease P1 Reaction Buffer. If preferentially degrading single-stranded nucleic acids (ssDNA or RNA) in the presence of dsDNA, we recommend using Nuclease P1 in 1X NEBuffer 1.1, to limit activity on dsDNA while maintaining single-strand nuclease activity.

Reaction Conditions: 1X Nuclease P1 Reaction Buffer. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to liberate 1.0 µg of acid soluble nucleotides from *Torula* Yeast total RNA per minute at 37°C in 1X Nuclease P1 Reaction Buffer.

Concentration: 100,000 units/ml

Usage Notes: Substrate specificity for Nuclease P1 is as follows: 3´ AMP > RNA > ssDNA >> dsDNA.

The rate of hydrolysis of 2´ AMP is 3,000-fold less than that of 3´ AMP.

$RecJ_f$

RR NEB2 37° 65°

#M0264S 1,000 units
#M0264L 5,000 units

- *Single-stranded DNA specific 5´→3´ exonuclease*
- *Removal of deoxynucleotide monophosphates from DNA*

Description: $RecJ_f$ is a single-stranded DNA-specific exonuclease that catalyzes the removal of deoxy-dNMPs from DNA in the 5´ → 3´ direction.

DNA substrate containing a 22 base 5´ extension results in products that are a mixture of DNA fragments that have blunt-ends, 5´ extensions (1–5 nucleotides) and recessed 5´ ends (1–8 nucleotides). $RecJ_f$ does not require a 5´ phosphate.

Source: $RecJ_f$ is overexpressed and purified as a C-terminal fusion to MBP. MBP does not affect the catalytic activity of $RecJ_f$, but does enhance $RecJ_f$ solubility.

Reaction Conditions: 1X NEBuffer 2. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 0.05 nmol TCA soluble deoxyribonucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 30,000 units/ml

T5 Exonuclease

#M0363S 1,000 units
#M0363L 5,000 units

- *Degradation of linear ssDNA, linear dsDNA and nicked plasmid DNA while preserving supercoiled plasmid DNA*
- *Used in Gibson Assembly method*

Description: T5 Exonuclease degrades DNA in the 5´ to 3´ direction. T5 Exonuclease is able to initiate nucleotide removal from the 5´ termini or at gaps and nicks of linear or circular dsDNA. However, the enzyme does not degrade supercoiled dsDNA. T5 Exonuclease also has ssDNA endonuclease activity.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in a total reaction volume of 50 µl in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

T7 Exonuclease

#M0263S 1,000 units
#M0263L 5,000 units

- *5´→3´ exonuclease*
- *Removal of 5´ mononucleotides from DNA*

Description: T7 Exonuclease acts in the 5´ to 3´ direction, catalyzing the removal of 5´ mononucleotides from duplex DNA. T7 Exonuclease is able to initiate nucleotide removal from the 5´ termini or at gaps and nicks of double-stranded DNA. It will degrade both 5´ phosphorylated or 5´ dephosphorylated DNA. It has also been reported to degrade RNA and DNA from RNA/DNA hybrids in the 5´ to 3´ direction, but it is unable to degrade either double-stranded or single-stranded RNA.

Reaction Conditions: 1X NEBuffer 4. Incubate at 25°C.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in a total reaction volume of 50 µl in 30 minutes at 25°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

NEW Nucleoside Digestion Mix

See page 202 for more information.

#M0649S 50 reactions

The Nucleoside Digestion Mix is a mixture of enzymes that provides a convenient one-step method to generate single nucleosides from DNA or RNA. Optimized for quantitative analysis by liquid chromatography-mass spectrometry (LC-MS), this reagent eliminates the need for sequential multi-step, time-consuming digestion protocols.

Properties of DNA Repair Enzymes

Substrates and Cleavage Products of DNA Repair Glycosylases

ENZYME	SUBSTRATE	CLEAVAGE SITE	TERMINI CREATED FROM CLEAVAGE	
			5´-terminus	3´-terminus
APE 1	AP sites	1st phosphodiester bond 5´ to the lesion	dR5P	OH
Endo III	Oxidized Pyrimidines, AP sites	1st phosphodiester bond 3´ to the lesion	P	PA
Endo IV	AP sites	1st phosphodiester bond 5´ to the lesion	dR5P	OH
Endo V	Deoxyinosine, mismatches, hairpin/unpaired loop, flaps, pseudo Y structures	2nd phosphodiester bond 3´ to the lesion	P	OH
Endo VIII	Oxidized Pyrimidines, AP sites	1st phosphodiester bond both 5´ and 3´ to the lesion	P	P
Fpg	Oxidized Purines, AP sites	1st phosphodiester bond both 5´ and 3´ to the lesion	P	P
hOGG1	Oxidized Purines, AP sites	1st phosphodiester bond 3´ to the lesion	P	PA
T7 Endo I	Cruciform, Holliday junctions, mismatches, herteroduplexes	1st, 2nd or 3rd phosphodiester bond 5´ to the mismatches	P	OH
T4 PDG	Pyrimidine Dimers	N-glycosidic bond of the 5´ Thymine of the dimer and the 1st phosphodiester bond 3´ to the AP site	P*	PA
UDG Afu UDG Antarctic Thermolabile UDG	Deoxyuridine	N-glycosidic bond	AP site (no break)	
hSMUG1	Deoxyuridine, 5-hyroxyuracil, 5-hydroxymethyluracil 5-formyluracil	N-glycosidic bond	AP site (no break)	
hAAG	Deoxyinosine, Alkylated purines	N-glycosidic bond	AP site (no break)	

**A pyrimidine dimer still covalently attached.*

Table Legend:

AP	apurinic/apyrimidinic sites
P	phosphate
OH	hydroxyl
dR5P	deoxyribose-5´-phosphate
PA	3´-phospho-α, β-unsaturated aldehyde

DNA Repair Glycosylases on Various Damaged Bases

		DOUBLE-STRANDED DNA OLIGOS (34-MERS)										
	ENZYME	AP:A	DHT:A	5-hmU:A	5-hmU:G	I:T	6-MeA:T	8-OG:C	8-OG:G	U:A	U:G	THYMINE GLYCOL:A
REPAIR ENZYMES	APE 1	++++	+	–	–	–	–	–	–	–	–	–
	Endo III	++++	+	–	–	–	–	–	–	–	–	+
	Tma Endo III	++++	++	–	–	–	–	+	+	–	–	++
	Endo IV	++++	+	–	–	–	–	–	–	–	–	–
	Tth Endo IV	++++	+	–	–	–	–	–	+	–	–	–
	Endo V*	+++	+	+	+	++++	+	++	+	+	++++	++
	Endo VIII	++++	++	–	–	–	–	–	–	–	–	+++
	Fpg	+	+	–	–	–	–	++++	++++	–	–	+
	hAAG	–	–	–	–	++++	–	–	–	–	–	–
	hNEIL1	++++	++	–	–	–	–	+	+	–	–	++
	hOGG1	++	–	–	–	–	–	++++	+	–	–	–
	T4 PDG	++++	–	–	–	–	–	–	–	–	–	–
	UDG	N/A	–	–	–	–	–	–	–	++++	+	–
	Afu UDG	N/A	–	–	–	–	–	–	–	++++	+	–
	hSMUG1	N/A	–	+++	+++	–	–	–	–	++++	++++	–

Standard reaction conditions were used to titer the enzymes with the alternate base.
**Nicks only, does not remove damage*

		SINGLE-STRANDED DNA OLIGOS (34-MERS)							
	ENZYME	AP	DHT	5-hmU	I	6-MeA	8-OG	U	THYMINE GLYCOL:A
REPAIR ENZYMES	APE 1	++	–	–	–	–	–	–	–
	Endo III	++	–	–	–	–	–	–	–
	Tma Endo III	++	+	–	–	–	–	–	–
	Endo IV	–	–	–	–	–	–	–	–
	Tth Endo IV	–	–	–	–	–	–	–	–
	Endo V	+	–	–	++++	–	+	–	–
	Endo VIII	+++	–	–	–	–	–	–	–
	Fpg	+	+	–	–	–	+	–	+
	hAAG	–	–	–	+	–	–	–	–
	hNEIL1	+	+	–	–	–	–	–	+
	hOGG1	++	–	–	–	–	+	–	–
	T4 PDG	–	–	–	–	–	–	–	–
	UDG	N/A	–	–	–	–	–	++++	–
	Afu UDG	N/A	–	–	–	–	–	++++	–
	hSMUG1	N/A	–	++	–	–	–	+++	–

Table Legend:

AP	apurinic/apyrimidinic site. The AP site is created by treating a uracil containing oligo with UDG.
DHT	5,6-dihydrothymine
5-hmU	5-hydroxymethyluracil
I	inosine
6-MeA	6 methyladenine
8-OG	8 oxoguanine
U	uridine
AP:A	apurinic/apyrimidinic site base paired with adenine
DHT:A	5,6 dihydrothymine base paired with an adenine
5-hmU:A	5-hydroxymethyluracil base paired with an adenine
5-hmU:G	5-hydroxymethyluracil base paired with a guanine
I:T	inosine base paired with a thymine
6-MeA:T	6-methyladenine base paired with a thymine
8-OG:C	8-oxoguanine base paired with a cytosine
8-OG:G	8-oxoguanine base paired with a guanine
U:A	uridine paired with an adenine
U:G	uridine paired with a guanine

Activity Level:

++++	100%
+++	50%
++	10% – 25%
+	< 10%
–	no detectable enzyme activity (< 0.7%)
N/A	not applicable

APE 1

#M0282S 1,000 units
#M0282L 5,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*
- *Modified nick translation*

Description: Human apurinic/apyrimidinic (AP) endonuclease, APE 1, also known as HAP 1 or Ref-1, shares homology with *E. coli* Exonuclease III. APE 1 cleaves the phosphodiester backbone immediately 5´ to an AP site via hydrolytic mechanism, generating a ssDNA break that leaves a 3´-hydroxyl and 5´-deoxyribose phosphate terminus. Besides AP endonuclease activity, APE 1 has also been reported to have weak DNA 3´-diesterase, 3´ to 5´ exonuclease and RNase H activities.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 20 pmol of a 34-mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

Recommended Dilution for the Comet Assay: $1:10^3$. A detailed protocol can be found at www.neb.com.

Concentration: 10,000 units/ml

Endonuclease IV

#M0304S 1,000 units
#M0304L 5,000 units

- Single-cell gel electrophoresis (Comet assay)
- Alkaline elution
- Alkaline unwinding

Description: Endonuclease IV can act on several types of oxidative damage in DNA. The enzyme is an apurinic/apyrimidinic (AP) endonuclease that will hydrolyze intact AP sites in DNA. AP sites are cleaved at the first phosphodiester bond that is 5´ to the lesion leaving a hydroxyl group at the 3´ terminus and a deoxyribose 5´-phosphate at the 5´ terminus. The enzyme also has a 3´-diesterase activity and can release phosphoglycolaldehyde, intact deoxyribose 5´-phosphate and phosphate from the 3´ end of DNA.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C. Heat inactivation: 85°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34-mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

Recommended Dilution for the Comet Assay: $1:10^4$ to $1:10^5$. A detailed protocol can be found at www.neb.com.

Concentration: 10,000 units/ml

Tth Endonuclease IV

#M0294S 500 units

- *Thermostable*
- *Alkaline elution*
- *Alkaline unwinding*

Description: *Tth* Endonuclease IV is a thermostable apurinic/apyrimidinic (AP) endonuclease. *Tth* Endo IV will hydrolyze an AP site at the first phosphodiester bond 5´ to the lesion leaving a 3´ hydroxyl and a deoxyribose 5´-phosphate. The enzyme also has a 3´-diesterase activity.

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 60-mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 µl in 1 hour at 65°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Endonuclease III (Nth)

#M0268S 1,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Endonuclease III (Nth) protein from *E. coli* acts both as a *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines from dsDNA generating a basic (AP) site. The AP-lyase activity of the enzyme cleaves 3´ to the AP site leaving a 5´ phosphate and a 3´-phospho -α, β unsaturated aldehyde.

Some of the damaged bases recognized and removed by Endonuclease III (Nth) include urea, 5, 6 dihydroxythymine, thymine glycol, 5-hydroxy-5 methylhydanton, uracil glycol, 6-hydroxy-5, 6-dihdrothimine and methyltartronylurea.

Source: An *E. coli* strain which carries the cloned *nth* gene.

Reaction Conditions: 1X Endonuclease III (Nth) Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34-mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

Recommended Dilution for the Comet Assay: $1:10^4$ to $1:10^5$. A detailed protocol can be found at www.neb.com.

Concentration: 10,000 units/ml

Tma Endonuclease III

#M0291S 500 units

- *Alkaline elution*
- *Alkaline unwinding*

Description: A thermostable homolog of the *E. coli* Endonuclease III (Nth). It acts as an *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines from double-stranded DNA, generating an apurinic (AP) site. The AP-lyase activity then cleaves the resulting abasic site.

Tma Endonuclease III recognizes abasic sites, 5,6 dihydroxythymine and thymine glycol in DNA.

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 60-mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 µl in 1 hour at 65°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Endonuclease V

#M0305S 250 units

- *Cleavage of oligonucleotides containing deoxyinosines*
- *Mismatch cleavage*

Description: Endonuclease V is a repair enzyme found in *E. coli* that recognizes deoxyinosine, a deamination product of deoxyadenosine in DNA. Endonuclease V, often called Deoxyinosine 3´ Endonuclease, recognizes DNA containing deoxyinosines (paired or not) on double-stranded DNA, single-stranded DNA with deoxyinosines and, to a lesser degree, DNA containing abasic sites (AP) or urea, base mismatches, insertion/deletion mismatches, hairpin or unpaired loops, flaps and pseudo-Y structures.

Endonuclease V cleaves the second phosphodiester bond 3´ to the mismatch of deoxyinosine, leaving a nick with 3´-hydroxyl and 5´-phosphate.

Source: An *E. coli* strain containing a gene fusion of the Endo V gene and the gene coding for the maltose binding protein (MBP). The fusion protein is purified to near homogeneity and is active as a fusion. The protein contains 223 amino acids and has a molecular weight of 24.9 kDa.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34-mer oligonucleotide duplex containing a single deoxyinosine site in a total reaction volume of 10 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Endonuclease VIII

#M0299S 1,000 units
#M0299L 5,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Endonuclease VIII acts as both an *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines from double-stranded DNA, generating an apurinic (AP) site. The AP-lyase activity cleaves 3´ and 5´ to the AP site leaving a 5´ phosphate and a 3´ phosphate. Damaged bases recognized and removed by Endonuclease VIII include urea, 5, 6- dihydroxythymine, thymine glycol, 5-hydroxy-5- methylhydanton, uracil glycol, 6-hydroxy-5, 6-dihydrothymine and methyltartronylurea. While Endonuclease VIII is similar to Endonuclease III, Endonuclease VIII has β and δ lyase activity while Endonuclease III has β lyase activity.

Reaction Conditions: 1X Endonuclease VIII Reaction Buffer. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34 mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

Recommended Dilution for the Comet Assay: $1:10^4$ to $1:10^5$. A detailed protocol can be found at www.neb.com.

Concentration: 10,000 units/ml

Fpg

#M0240S 500 units
#M0240L 2,500 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Fpg (formamidopyrimidine [fapy]-DNA glycosylase), also known as 8-oxoguanine DNA glycosylase, acts both as an *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged purines from double-stranded DNA, generating an apurinic (AP) site. The AP-lyase activity cleaves both 3´ and 5´ to the AP site thereby removing the AP site and leaving a 1-base gap with a 5´ and 3´ phosphate.

Some of the damaged bases recognized and removed by Fpg include 7, 8-dihydro-8-oxoguanine (8-oxoguanine), 8-oxoadenine, fapy-guanine, methy-fapy-guanine, fapy-adenine, aflatoxin B1-fapy-guanine, 5-hydroxy-cytosine and 5-hydroxy-uracil.

Reaction Conditions: 1X NEBuffer 1. Supplement with 100 µg BSA. Incubate at 37°C. Heat inactivation: 60°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 10 pmol of a 34-mer oligonucleotide duplex containing a single 8-oxoguanine base paired with a cytosine in a total reaction volume of 10 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

Recommended Dilution for the Comet Assay: $1:10^3$ to $1:10^4$. A detailed protocol can be found at www.neb.com.

Concentration: 8,000 units/ml

hAAG

#M0313S 500 units

- *Single-cell gel electrophoresis (Comet Assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Human Alkyladenine DNA Glycosylase (hAAG) excises alkylated and oxidative DNA damaged sites, including 3-methyladenine, 7-methylguanine, 1,N^6-ethenoadenine and hypoxanthine. hAAG catalyzes the hydrolysis of the *N*-glycosidic bond to release the damaged base. hAAG is also known as methylpurine DNA glycosylase (MPG) or 3-methyladenine-DNA glycosylase (ANPG).

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to create an AP site from 1 pmol of a 34-mer oligonucleotide duplex containing a single deoxyinosine site in a total reaction volume of 10 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

T4 PDG (T4 Endonuclease V)

#M0308S 2,000 units

- *DNA damage studies*
- *Single-cell gel electrophoresis (Comet assay)*

Description: T4 PDG (pyrimidine dimer glycosylase) has both DNA glycosylase and AP-lyase activity. The 16 kd protein recognizes cis-syn-cyclobutane pyrimidine dimers caused by UV irradiation. The enzyme cleaves the glycosyl bond of the 5´ end of the pyrimidine dimer and the endonucleolytic activity cleaves the phosphodiester bond at the apurinic/apyrimidinic (AP) site.

Reaction Conditions: 1X T4 PDG Reaction Buffer. Supplement with BSA. Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the conversion of 0.5 µg of UV irradiated supercoiled pUC19 DNA to > 95% nicked plasmid in a total reaction volume of 20 µl in 30 minutes at 37°C. Nicking is assessed by agarose gel electrophoresis. Irradiated plasmid contains an average of 3–5 pyrimidine dimers. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Note: Incubation time should be ≤ 30 minutes for best results.

Uracil-DNA Glycosylase (UDG)

#M0280S 1,000 units
#M0280L 5,000 units

Companion Product:

Uracil Glycosylase Inhibitor (UGI)
#M0281S 200 units
#M0281L 1,000 units

- *Eliminates PCR carry-over contamination*
- *Release of uracil from ss- or ds- DNA*

Description: *E. coli* Uracil-DNA Glycosylase (UDG) catalyzes the release of uracil from uracil-containing DNA. UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA, but not from oligomers (6 or fewer bases).

Reaction Conditions: 1X UDG Reaction Buffer Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10^4-10^5 cpm/µg) in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Usage Notes: UDG is active over a broad pH range with an optimum at pH 8.0, does not require divalent cation, and is inhibited by high ionic strength (> 200 mM).

Afu Uracil-DNA Glycosylase (UDG)

#M0279S 200 units
#M0279L 1,000 units

- *Thermostable*
- *Release of uracil from ss- or ds- DNA*

Description: A thermostable homolog of the *E. coli* Uracil-DNA Glycosylase (UDG) from *Archaeoglobus fulgidus*. *Afu* UDG catalyses the release of uracil from uracil-containing DNA. *Afu* UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA.

Reaction Conditions: 1X ThermoPol II (Mg-free) Reaction Buffer. Incubate at 65°C.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10^4–10^5 cpm/µg) in 30 minutes at 65°C. Unit assay conditions can be found at www.neb.com.

Concentration: 2,000 units/ml.

Usage Notes: *Afu* UDG retains 50% activity in the presence of 150 mM NaCl. *Afu* UDG retains less than 1% activity after boiling for 30 minutes in standard reaction conditions. Under standard reaction conditions, uracil glycosylase inhibitor (UGI) does not inhibit *Afu* UDG.

Antarctic Thermolabile UDG

RR NEBU 37° 50°

#M0372S 100 units
#M0372L 500 units

- *Eliminates PCR carry-over contamination*
- *Release of uracil from ss- or ds- DNA*

Description: Antarctic Thermolabile UDG (Uracil-DNA Glycosylase) catalyzes the release of free uracil from uracil-containing single-stranded or double-stranded DNA. The resulting abasic sites are susceptible to the hydrolytic cleavage at the elevated temperature and high pH. This enzyme is sensitive to heat and can be rapidly and completely inactivated at temperatures above 50°C.

Reaction Conditions: 1X Standard *Taq* Reaction Buffer. Incubate at 37°C. Heat inactivation: 50°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl Standard *Taq* reaction buffer containing 0.2 µg DNA (10^4-10^5 cpm/ug) in 30 minutes at 37°C.

Concentration: 1,000 units/ml

hSMUG1

RR NEB1 BSA 37° 65°

#M0336S 500 units

- *Oxidative DNA damage studies*
- *Single-cell gel electrophoresis (Comet assay)*

Description: Human single-strand-selective monofunctional uracil-DNA Glycosylase (hSMUG1) excises deoxyuracil and deoxyuracil-derivatives bearing an oxidized group at C5, such as 5-hydroxyuracil, 5-hydroxymethyluracil and 5-formyluracil in ssDNA and dsDNA.

Reaction Conditions: 1X NEBuffer 1. Supplement with 100 µg BSA. Incubate at 37°C.Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to excise 1 pmol of deoxyuracil from a 34 mer oligonucleotide duplex containing a single dU site in a total reaction volume of 10 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml.

Usage Notes: hSMUG1 has 50% activity on 5-hydroxymethyluracil when compared to uracil. hSMUG1 has 50% activity on ssDNA compared to dsDNA.

PreCR® Repair Mix

RR No

See page 76 for more information.

#M0309S 30 reactions
#M0309L 150 reactions

The PreCR Repair Mix is an enzyme cocktail formulated to repair damaged template DNA prior to its use in the polymerase chain reaction (PCR), microarrays or other DNA technologies.

β-Agarase I

RR NEBU 42° 95°

#M0392S 100 units
#M0392L 500 units

- *Extraction of DNA from agarose gels*
- *DNase and RNase free*

Description: β-Agarase cleaves the agarose subunit, unsubstituted neoagarobiose [3,6-anhydro-α-L-galactopyranosyl-1-3-D-galactose] to neoagaro-oligosaccharides. β-Agarase I digests agarose, releasing trapped DNA and producing carbohydrate molecules which can no longer gel. The remaining carbohydrate molecules and β-Agarase I will not, in general, interfere with subsequent DNA manipulations such as restriction endonuclease digestion, ligation and transformation.

Reaction Conditions: 1X β-Agarase I Reaction Buffer. Incubate at 42°C.

Unit Definition: One unit is defined as the amount of enzyme required to digest 200 µl of molten low melting point or NuSieve agarose to nonprecipitable neoagaro-oligosaccharides in 1 hour at 42°C.

Concentration: 1,000 units/ml

Heat Inactivation: Incubation at 95°C for 2 minutes or incubation at 65°C for 15 minutes inactivates 50 units of β-Agarase I. β-Agarase I retains activity for several hours at 40–45°C and is stabilized by the presence of agarose in the reaction.

Cre Recombinase

#M0298S 50 units
#M0298L 250 units
for high (15X) concentration
#M0298M 250 units

- *Excision of DNA between two loxP sites*
- *Fusion of DNA molecules containing loxP sites*
- *Inversion of DNA between loxP sites*

Description: Cre Recombinase is a Type I topoisomerase from bacteriophage P1 that catalyzes the site-specific recombination of DNA between *loxP* sites. The enzyme requires no energy cofactors, and Cre-mediated recombination quickly reaches equilibrium between substrate and reaction products. The *loxP* recognition element is a 34 base pair (bp) sequence comprised of two 13 bp inverted repeats flanking an 8 bp spacer region which confers directionality. Recombination products depend on the location and relative orientation of the *loxP* sites. Two DNA species containing single *loxP* sites will be fused. DNA between repeated *loxP* sites will be excised in circular form while DNA between opposing *loxP* sites will be inverted with respect to external sequences.

Reaction Conditions: 1X Cre Recombinase Reaction Buffer. Incubate at 37°C. Heat inactivation: 70°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme necessary to produce maximal site-specific recombination of 0.25 µg pLox2+ control DNA in a total reaction volume of 50 µl in 30 minutes at 37°C. Maximal recombination is determined by agarose gel analysis and by transformation of reactions followed by selection on ampicillin plates.

Concentration: 1,000 units/ml and 15,000 units/ml.

T7 Endonuclease I

#M0302S 250 units
#M0302L 1,250 units

- *Recognition of mismatched DNA*
- *Resolve four-way junction or branched DNA*
- *Detection or cleavage of heteroduplex and nicked DNA*
- *Random cleavage of linear DNA for shotgun cloning*

Description: T7 Endonuclease I recognizes and cleaves non-perfectly matched DNA, cruciform DNA structures, Holliday structures or junctions, heteroduplex DNA and more slowly, nicked ds- DNA. It cleaves at the first, second or third phosphodiester bond 5´ to the mismatch.

Source: An *E. coli* strain that carries a fusion of maltose binding protein (MBP) and T7 Endo I.

Reaction Conditions: 1X NEBuffer 2. Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to convert > 90% of 1 µg of supercoiled cruciform pUC(AT)* to > 90% linear form in a total reaction volume of 50 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

*pUC(AT) is derived from pUC19 with a modification of the polylinker between the EcoRI and PstI sites.

Concentration: 10,000 units/ml

Usage Note: It is important to control the amount of enzyme and the reaction time used for cleavage of a particular substrate. Temps. > 42°C increase nonspecific nuclease activity.

NEW

TelN Protelomerase

#M0651S 250 units

- *Isolated from phage N15*
- *Cuts dsDNA at a TelN recognition sequence (56 bp)*
- *Leaves covalently closed ends at the site of cleavage*

This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Description: TelN Protelomerase, from phage N15, cuts dsDNA at a TelN recognition sequence (56 bp) and leaves covalently closed ends at the site of cleavage.

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 30°C. Heat inactivation: 75°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 0.5 µg of pMiniT-TelN BsaI-linearized control plasmid (313 fmol of TelN recognition sites) in a total reaction volume of 50 µl in 30 minutes at 30°C in 1X ThermoPol Reaction Buffer.

Concentration: 5,000 units/ml

Topoisomerase I (*E. coli*)

#M0301S 100 units
#M0301L 500 units

- *Recognition of mismatched DNA*
- *Catalyzes relaxation of negatively-supercoiled DNA*

Description: Topoisomerase I (*E. coli*) catalyzes the relaxation of negatively-supercoiled DNA. Topoisomerase I has also been implicated in knotting and unknotting DNA, and in linking complementary rings of single-stranded DNA into double-stranded rings. The intact holoenzyme is a 97 kDa protein.

Reaction Conditions: 1X CutSmart Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

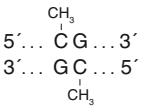
Unit Definition: One unit is defined as the amount of enzyme that catalyzes the relaxation of > 95% of 0.5 µg of negatively supercoiled pUC19 RF I DNA in a total reaction volume of 25 µl in 15 minutes at 37°C. DNA supercoiling is assessed by agarose gel electrophoresis in the absence of ethidium bromide.

Concentration: 5,000 units/ml.

CpG Methyltransferase (M.SssI)

RR NEB2 SAM 37° 65°

#M0226S	100 units
#M0226L	500 units
for high (5X) concentration	
#M0226M	500 units



- *Blocking restriction enzyme cleavage*
- *Studying of CpG methylation-dependent gene expression*
- *Probing sequence-specific contacts within the major groove of DNA*
- *Altering the physical properties of DNA*
- *Uniform [^{3}H]-labeling of DNA*
- *Decreasing the number of RE cut sites, yielding an apparent increase in specificity*

Description: The CpG Methyltransferase (M.SssI) methylates all cytosine residues (C^5) within the double-stranded dinucleotide recognition sequence 5´...CG...3´.

Reaction Conditions: 1X NEBuffer 2 + SAM. Supplement with 160 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65° for 20 minutes.

Note: $MgCl_2$ is not required as a cofactor. In the presence of Mg^{2+}, methylation becomes distributive rather than processive and exhibits topoisomerase activity.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of λ DNA in a total reaction volume of 20 µl in 1 hour at 37°C against cleavage by BstUI restriction endonuclease.

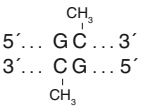
Concentration: 4,000 units/ml and 20,000 units/ml. Assayed on λ DNA.

See pages 334–336 for a complete list of restriction endonucleases blocked by CpG methylation.

GpC Methyltransferase (M.CviPI)

RR NEBU SAM 37° 65°

#M0227S	200 units
#M0227L	1,000 units



- *Blocking restriction enzyme cleavage*
- *Altering the physical properties of DNA*
- *Uniform [^{3}H]-labeling of DNA*

Description: The GpC Methyltransferase (M.CviPI) methylates all cytosine residues (C^5) within the double-stranded dinucleotide recognition sequence 5´...GC...3´.

Reaction Conditions: 1X GC Reaction Buffer. Supplement with 160 µM SAM (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Note: $MgCl_2$ is not required as a cofactor.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of λ DNA in a total reaction volume of 20 µl in 1 hour at 37°C against cleavage by HaeIII restriction endonuclease.

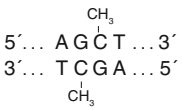
Concentration: 4,000 units/ml. Assayed on λ DNA.

For more information on products to study DNA methylation, see pages 260–273.

AluI Methyltransferase

RR NEBU SAM 37° 65°

#M0220S	100 units



Description: AluI Methyltransferase modifies the cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: 1X AluI Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

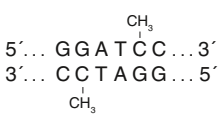
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by AluI restriction endonuclease.

Concentration: 5,000 units/ml

BamHI Methyltransferase

RR NEBU SAM 37° No

#M0223S	100 units
#M0223L	500 units



Description: BamHI Methyltransferase modifies the internal cytosine residue (N^4) in the sequence to the left.

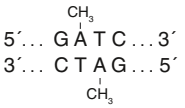
Reaction Conditions: 1X BamHI Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by BamHI restriction endonuclease.

Concentration: 4,000 units/ml

dam Methyltransferase

#M0222S 500 units
#M0222L 2,500 units



Description: *dam* Methyltransferase modifies the adenine residue (N^6) in the sequence to the left.

Reaction Conditions: 1X *dam* Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by MboI restriction endonuclease.

Concentration: 8,000 units/ml

EcoGII Methyltransferase

#M0603S 200 units

This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Description: EcoGII Methyltransferase is a non-specific methyltransferase that modifies adenine residues (N^6) in any sequence context.

Reaction Conditions: 1X CutSmart Buffer + SAM. Supplement with 160 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

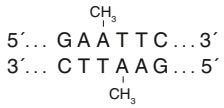
Unit Definition: One unit is defined as the amount of enzyme required to protect 100 ng FAM-labeled dsDNA in 30 minutes at 37°C in a total reaction volume of 20 µl against cleavage by MboI restriction endonuclease.

Concentration: 5,000 units/ml

Note: SAM is unstable at 37°C (pH 7.5) and should be replenished in reactions incubated > 4 hours. For use of methylation reaction SAM should be diluted 1:200 to a final concentration of 160 µM. EcoGII Methyltransferase is sensitive to salt. Make sure DNA solution is low in salt concentration or that it makes up only a small % of the final reaction volume.

EcoRI Methyltransferase

#M0211S 10,000 units



Description: EcoRI Methyltransferase modifies the internal adenine residue (N^6) in the sequence to the left.

Reaction Conditions: 1X EcoRI Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

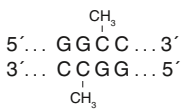
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by EcoRI restriction endonuclease.

Concentration: 40,000 units/ml

Note: EcoRI Methyltransferase is inhibited by $MgCl_2$. Only 50% activity is retained at a concentration of 4 mM $MgCl_2$.

HaeIII Methyltransferase

#M0224S 500 units



Description: HaeIII Methyltransferase modifies the internal cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: 1X HaeIII Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

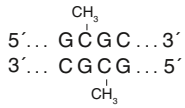
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HaeIII restriction endonuclease.

Concentration: 10,000 units/ml

Note: HaeIII Methyltransferase protects DNA against cleavage by NotI.

HhaI Methyltransferase

#M0217S 1,000 units



Description: HhaI Methyltransferase modifies the internal cytosine residue (C^5) in the sequence to the left.

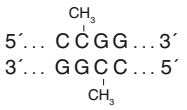
Reaction Conditions: 1X HhaI Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HhaI restriction endonuclease.

Concentration: 25,000 units/ml

HpaII Methyltransferase

#M0214S 100 units



Description: HpaII Methyltransferase recognizes the same sequence as the MspI Methyltransferase, but modifies the internal cytosine residue (C^5) in the sequence to the left.

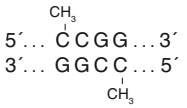
Reaction Conditions: 1X HpaII Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HpaII restriction endonuclease.

Concentration: 4,000 units/ml

MspI Methyltransferase

#M0215S 100 units



Description: MspI Methyltransferase recognizes the same sequence as the HpaII Methyltransferase, but modifies the external cytosine residue (C^5) in the sequence to the left.

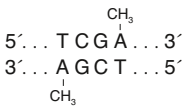
Reaction Conditions: 1X MspI Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by MspI restriction endonuclease.

Concentration: 5,000 units/ml

*Taq*I Methyltransferase

#M0219S 1,000 units



Description: *Taq*I Methyltransferase modifies the adenine residue (N^6) in the sequence to the left.

Reaction Conditions: 1X CutSmart Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 65°C.

Note: Activity at 37°C is 25%.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 65°C in a total reaction volume of 20 µl against cleavage by *Taq*I restriction endonuclease.

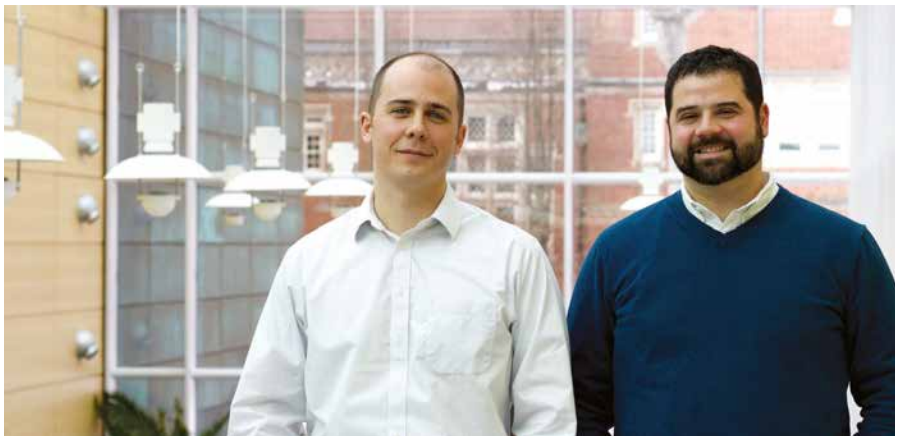
Concentration: 10,000 units/ml

Human DNA (cytosine-5) Methyltransferase (Dnmt1)

See page 270 for more information.

#M0230S 50 units
#M0230L 250 units

Dnmt1 methylates cytosine residues in hemimethylated DNA at 5´...CG...3´. Mammalian Dnmt1 is believed to be involved in carcinogenesis, embryonic development and several other biological functions. The bulk of the methylation takes place during DNA replication in the S-phase of the cell cycle.



Colby and Andrew are the newest members of our Global Business Development group. Andrew specializes in next generation sequencing, while Colby brings expertise in RNA structural biology, metabolic engineering and microbiology.

RecA & RecA$_f$

RR NEB U 37° 65°

RecA
#M0249S 200 µg
#M0249L 1,000 µg

RecA$_f$
#M0355S 200 µg
#M0355L 1,000 µg

- *Visualization of DNA structures with electron microscopy*
- *D-loop mutagenesis*
- *Screening libraries using RecA-coated probes*
- *Cleavage of DNA at a single predetermined site*
- *RecA mediated affinity capture for full length cDNA cloning*

Description: *E. coli* RecA is necessary for genetic recombination, reactions involving DNA repair and UV-induced mutagenesis. RecA promotes the autodigestion of the lexA repressor, umuD protein and lambda repressor. Cleavage of LexA derepresses more than 20 genes. *In vitro* studies indicate that in the presence of ATP, RecA promotes the strand exchange of single-strand DNA fragments with homologous duplex DNA. The reaction has three distinct steps: (i) RecA polymerizes on the single-strand DNA, (ii) the nucleoprotein filament binds the duplex DNA and searches for a homologous region, (iii) the strands are exchanged.

RecA$_f$ is a N-terminal 6X His tagged recombinant protein.

Reaction Conditions: 1X RecA Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Molecular Weight: RecA: 37,973 daltons. RecA$_f$: 38,907 daltons.

Concentration: 2 mg/ml

T4 Gene 32 Protein

RR NEB 4 37° 65°

#M0300S 100 µg
#M0300L 500 µg

- *Increase yield and processivity of reverse transcriptase during RT-PCR*
- *Increase yield and specificity of PCR products from soil samples*
- *Stabilization and marking of ssDNA structures*

Description: T4 Gene 32 Protein is a single-stranded DNA (ssDNA) binding protein required for bacteriophage T4 replication and repair. It cooperatively binds to and stabilizes transiently formed regions of ssDNA and plays an important structural role during T4 phage replication. It also has been used extensively to stabilize and mark regions of ssDNA for electron microscopic examination of intracellular DNA structures. Recently, it has been shown to improve restriction enzyme digestion, improve the yield and efficiency of reverse transcription (RT) reactions during RT-PCR, enhance T4 DNA polymerase activity, as well as increase the yield of PCR products.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: Sold by mass of pure protein as determined by OD_{280} (A_{280}=1.184 at 1 mg/ml, 1 cm)

Molecular Weight: 33,485 daltons.

Concentration: 10 mg/ml

ET SSB (Extreme Thermostable Single-Stranded Binding Protein)

RR

#M2401S 50 µg

- *Improve the processivity of DNA polymerase*
- *Stabilization and marking of ssDNA structure*
- *Increase the yield and specificity of PCR*
- *Increase the yield and processivity of RT during RT-PCR*
- *Improve DNA sequencing through regions with strong secondary structure*

Description: ET SSB (Extreme Thermostable Single-Stranded DNA Binding Protein) is a single-stranded DNA binding protein isolated from a hyperthermophilic microorganism, which remains fully active after incubation at 95°C for 60 minutes. Due to the extreme thermostability, ET SSB can be used in applications that require extremely high temperature conditions, such as nucleic acid amplification and sequencing.

Unit Definition: Sold by mass of pure protein as determined by OD_{280} (A_{280} = 0.774 at 1 mg/ml, 1 cm).

Concentration: 500 µg/ml

Molecular Weight: 16 kDa

Usage Note: ET SSB is active in any polymerase buffer. Add 200 ng of ET SSB per 50 µl reaction.

Cloning Plasmids and DNAs

CLONING PLASMID/DNA	NEB #	FEATURES	CONCENTRATION	MW/SIZE	SIZE
pBR322 Vector	N3033S/L	• Commonly used cloning vectors • Tet, Amp resistance	1,000 µg/ml	2.83 x 10^6 Da/4,361 bp	50/250 µg
pUC19 Vector	N3041S/L		1,000 µg/ml	1.75 x 10^6 Da/2,686 bp	50/250 µg
M13mp18 RF I DNA	N4018S	• Phage vectors derived from bacteriophage M13 • DNA, covalently closed circular • 13 Unique RE sites with β–gal gene • Blue/white selection	100 µg/ml	7,249 bp	10 µg
M13mp18 Single-stranded DNA	N4040S		250 µg/ml	7,249 bp	10 µg
Lambda DNA	N3011S/L	• Commonly used DNA substrate	500 µg/ml	31.5 x 10^6 Da/48,502 bp	250/1,250 µg
Lambda DNA (N^6-methyladenine-free)	N3013S/L		500 µg/ml	31.5 x 10^6 Da/48,502 bp	250/1,250 µg
φX174 RF I DNA	N3021S/L	• Commonly used DNA substrate • Covalently closed circular form of φX174	1,000 µg/ml	3.5 x 10^6 Da/5,386 bp	30/150 µg
φX174 RF II DNA	N3022L	• Commonly used DNA substrate • Double-stranded nicked circular form of φX174	1,000 µg/ml	3.5 x 10^6 Da/5,386 bp	150 µg
φX174 Virion DNA	N3023S/L	• Single-stranded viral DNA	1,000 µg/ml	1.7 x 10^6 Da/5,386 bp	50/250 µg

NEB offers a selection of common cloning plasmids and DNAs for use as substrates.
Additional information for many of these DNAs can be found in the technical reference section of this catalog or at www.neb.com.

M13KO7 Helper Phage

#N0315S 1.8 ml

- *Production of single-stranded phagemid DNA for sequencing and mutagenesis*

Description: M13KO7 is a derivative of M13 phage with the origin of replication from P15A and the kanamycin resistance gene from Tn903 both inserted within the M13 origin of replication, which is able to replicate in the absence of phagemid DNA. In the presence of phagemid bearing a wild-type M13 or f1 origin, single-stranded phagemid is packaged preferentially and secreted into the culture medium. This allows easy production of single-stranded phagemid DNA for mutagenesis or sequencing. M13KO7 carries the kanamycin resistance marker.

Source: M13KO7 phage supernatant is isolated from infected *E. coli* ER2738 by a standard procedure.

Concentration: 1.0 x 10^{11} pfu/ml

Note: NEB does not recommend the use of M13KO7 as a cloning vector. For cloning peptides displayed on M13 phage, we recommend the Ph.D.™ Peptide Display Cloning System (see page 251).

Companion Product:

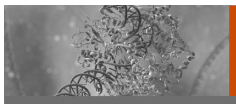
Anti-M13 pIII Monoclonal Antibody

Anti-M13 pIII Monoclonal Antibody (mouse isotype IgG2a) is derived from the A23 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with the C-terminal half of M13 coat protein III (residues 259–406).

Note: ELISA against whole phage using this antibody is not recommended since the epitope lies in a region that is not accessible on intact M13 phage virions.

#E8033S 0.1 ml

Competent Cell Selection Chart for Cloning



	NEB 5-alpha Competent *E. coli* (#C2987)	NEB Turbo Competent *E. coli* (#C2984)	NEB 5-alpha F´ I^q Competent *E. coli* (#C2992)	NEB 10-beta Competent *E. coli* (#C3019)	*dam*⁻/*dcm*⁻ Competent *E. coli* (#C2925)	NEB Stable Competent *E. coli* (#C3040)
FEATURES						
Versatile	●					●
Fast growth (< 8 hours)		●				
Toxic gene cloning		●	●			●
Large plasmid/BAC cloning				●		
Dam/Dcm-free plasmid growth					●	
Retroviral/lentiviral vector cloning						●
recA⁻	●		●	●		●
endA⁻	●	●	●	●	●	●
FORMATS						
Chemically competent	●	●	●	●	●	●
Electrocompetent	●	●		●		
Subcloning	●					
96-well format*	●					
384-well format*	●					
12 x 8-tube strips*	●					

** Other strains are available upon request. For more information, contact custom@neb.com.*

Monarch® Nucleic Acid DNA Purification Kits

Monarch kits are also available for Genomic DNA & RNA extraction, see pages 128–130.

Monarch kits provide fast and reliable purification of high quality DNA from bacterial cultures, agarose gels, and enzymatic reactions using best-in-class technology. Our unique column design offers elution in lower volumes than standard purification kits, providing concentrated, high quality DNA suitable for use in downstream applications such as DNA sequencing, PCR, restriction enzyme digests and other enzymatic manipulations.

Our column design also eliminates buffer retention and the risk of carryover contamination, providing fast, worry-free DNA purification. Designed with sustainability in mind, Monarch kits use significantly less plastic and responsibly-sourced packaging. **Learn more about Monarch Nucleic Acid Purification Products on pages 122-131.**

PRODUCT	NEB #	SIZE
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps
Monarch DNA Gel Extraction Kit	T1020S/L	50/250 preps
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030S/L	50/250 preps
COLUMNS AVAILABLE SEPARATELY		
Monarch DNA Cleanup Columns (5 µg)	T1034L	100 columns
Monarch Plasmid Miniprep Columns	T1017L	100 columns
BUFFERS AVAILABLE SEPARATELY		
Monarch DNA Cleanup Binding Buffer	T1031L	235 ml
Monarch DNA Wash Buffer	T1032L	25 ml
Monarch DNA Elution Buffer	T1016L	25 ml
Monarch Gel Dissolving Buffer	T1021L	235 ml
Monarch Plasmid Lysis Buffer (B2)	T1012L	2 x 27 ml
Monarch Plasmid Neutralization Buffer (B3)	T1013L	110 ml
Monarch Plasmid Resuspension Buffer (B1)	T1011L	55 ml
Monarch Plasmid Wash Buffer 1	T1014L	2 x 27 ml
Monarch Plasmid Wash Buffer 2	T1015L	30 ml

Fish swimming over coral reef,
Cenderawasih Bay, West Papua, Indonesia.
Credit: Pete Oxford, Minden Pictures

The Race to Save the Reefs

Coral reefs occupy less than 1% of Earth's ocean floor; however, they are home to 25% of all marine wildlife. Coral is the central infrastructure of reefs, and the trees of an underwater forest, which provides microhabitats, shelter and breeding grounds for thousands of species.

There is an incredible network of collaborative relationships between thousands of reef species that underpin all life in this rich habitat, and each of the residents have a role. First and foremost, coral is made up of millions of polyps that house microalgae (*Zooxanthellae*), which photosynthesize and provide 90% of the coral's food. There is also an abundance of unrelated animals hunting together to share a meal, cleaning and protecting each other, recycling waste and defending the reef in a reciprocally altruistic fashion.

Coral is a keystone species, meaning it has a crucial role that no other species in its ecosystem can perform. This role is essential for the survival of the ecosystem, and therefore, if it becomes threatened, it jeopardizes the entire ecosystem.

The threats to the world's reefs include pollution, infectious disease, overfishing and climate change. Rising sea temperatures cause the polyps to eject the microalgae. Without its food source, the coral becomes photobleached and subsequently leaves all of the reef species without their habitat.

In the past 30 years, more than half the world's corals have been affected by bleaching, and the intervals between bleaching events have become shorter, leaving the coral without time to recover. Scientists have concluded that if water temperatures continue to rise at the current rate, all coral reefs will die by the turn of the century.

Can these fragile ecosystems be restored? A glimmer of hope comes from the pioneering work of marine biologists who are manually growing and planting corals that can tolerate higher temperatures and ocean acidification.

Researchers break slow-growing, massive reef-building corals into small pieces, which stimulates rapid healing and growth. The corals are then outplanted back onto the damaged reefs. However, this clonal method of reproduction is not enough; genetic diversity comes from sexual reproduction, and luckily, corals reproduce both asexually and sexually.

Biologists are also collecting eggs and sperm from colonies of Brain coral during spawning events that occur typically one week after a full moon. Swarms of butterflyfish direct the divers to coral that is about to spawn. They collect the sperm and eggs in tents and fertilize them in the lab. The reproductive success rate is approximately 0.2% in nature, but in the lab, it is upwards of 90%.

Other marine biologists are breeding "super coral" by crossing the most robust species of coral in the lab and then transplanting them back onto the reef, where they can hopefully withstand the current stresses that are leading to their decline.

It remains unclear at this point whether the scale of assisted coral transplantation can match that of coral loss, and ocean warming continues to pose a threat to these recovery efforts. Regardless, the passion of these coral enthusiasts and marine biologists to save the Earth's coral reefs is genuinely inspiring.

Nucleic Acid Purification

Time for change.

Nucleic acid purification is an important step in molecular biology workflows. Further, there are many commercially-available solutions from which to choose. Our Research and Development team spent time with customers to better understand what could be done to improve upon current nucleic acid purification kits. This feedback helped us develop our new line of Monarch® Nucleic Acid Purification kits, which have been optimized for maximum performance and minimal environmental impact.

Monarch kits are available for DNA and RNA extraction and cleanup, plasmid purification, and gel extraction. They utilize unique column designs which enable the isolation of highly-pure nucleic acids, free from contaminants and often in low volumes. Monarch kits are supported by a variety of validated, user-friendly protocols to support multiple workflows and applications.

We know that it can be difficult to be environmentally friendly in the lab, where sterility and convenience are of utmost importance. At times, it may seem that sustainability and benchwork are at odds with each other. Although we can't completely solve this problem, we can make changes to our product design to help move toward the goal of a greener lab, and that's exactly what we did with the design of our Monarch kits. These kits use less plastic, as well as responsibly-sourced and recyclable packaging. The columns have thinner walls, reducing total plastic usage without affecting performance. All bottles were carefully chosen to minimize plastic usage, and the kit boxes provide a detailed explanation of how to recycle each component.

Let's work together to clean up the world of nucleic acid purification, one prep at a time.

Featured Products

125 Monarch Plasmid Miniprep Kit

128 Monarch Genomic DNA Purification Kit

130 Monarch Total RNA Miniprep Kit

Featured Tools & Resources

Visit NEBMonarch.com to view our online protocols and tips for optimization of nucleic acid purification

Make the right choice and migrate to Monarch®

Purification of DNA and RNA is an essential step in many molecular biology workflows, including enzyme digests, transformation, electrophoresis, PCR, qPCR, RT-PCR, RT-qPCR and library preparation for next gen sequencing. Monarch kits enable quick and easy purification of high-quality DNA and RNA, suitable for use in a variety of downstream applications. Recover pure, intact DNA and RNA in minutes with fast, user-friendly protocols and optimized buffer systems, and focus your time on the experiments that will drive your research forward. Monarch kits are all designed with sustainability in mind; whenever possible, kits and components are made with significantly less plastic and are packaged with responsibly-sourced, recyclable packaging.

Experience exceptional performance and streamlined workflows

- *Efficient extraction of high quality DNA and RNA from a variety of samples*
- *Simplified DNA and RNA cleanup in low elution volumes*
- *Enhanced column designs for improved performance*
- *Fast, user-friendly protocols*
- *Optimized buffer systems*

Choose Monarch Kits for pure value

- *Buffers and columns available separately*
- *No additional shipping or handling charges***
- *No hazardous materials fees***
- *Competitive pricing*

Reduce your impact on the environment

- *Less plastic used in product design*
- *Responsibly sourced and recyclable packaging*
- *Packaging and protocol cards are printed with water and soy-based inks*
- *Reusable kit boxes made from post-consumer content*

*** In the US and select subsidiary locations. Contact your local distributor for shipping policies.*

* *Visit www.NEBMonarchPackaging.com for details.*

To learn more, visit **NEBMonarch.com**

Monarch Plasmid Miniprep Kit

#T1010S 50 preps
#T1010L 250 preps

Companion Products:

Monarch Plasmid Miniprep Columns
#T1017L 100 columns + collection tubes

Monarch Plasmid Resuspension Buffer (B1)
#T1011L 55 ml

Monarch Plasmid Lysis Buffer (B2)
#T1012L 2 x 27 ml

Monarch Plasmid Neutralization Buffer (B3)
#T1013L 110 ml

Monarch Plasmid Wash Buffer 1
#T1014L 2 x 27 ml

Monarch Plasmid Wash Buffer 2
#T1015L 30 ml

Monarch DNA Elution Buffer
#T1016L 25 ml

Monarch DNA Gel Extraction Kit
#T1020S 50 preps
#T1020L 250 preps

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 preps
#T1030L 250 preps

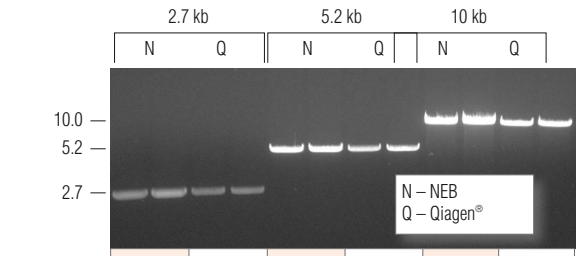
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Monitor completeness of certain steps using colored buffer system*
- *No need to add RNase before starting*
- *Elute in low volumes*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*
- *Easily label columns using tab and frosted surfaces*

Description: The Monarch Plasmid Miniprep Kit is a rapid and reliable method for the purification of high-quality plasmid DNA. This method employs standard cell resuspension, alkaline lysis and neutralization steps, with the additional benefit of color indicators at certain steps to easily monitor completion. After clarification of the lysate by centrifugation, the DNA is bound to the proprietary silica matrix under high salt conditions. Unique wash buffers ensure salts, proteins, RNA and other cellular components (endotoxins) are removed, allowing low-volume elution of concentrated, highly pure DNA, ready for use in restriction digests, DNA sequencing, PCR and other enzymatic manipulations.

The Monarch Plasmid Miniprep Kit Includes:

– Monarch Plasmid Miniprep Columns
– Monarch Plasmid Resuspension Buffer (B1)
– Monarch Plasmid Lysis Buffer (B2)
– Monarch Plasmid Neutralization Buffer (B3)
– Monarch Plasmid Wash Buffer 1
– Monarch Plasmid Wash Buffer 2 (5X)
– Monarch DNA Elution Buffer
– Monarch Collection Tubes

For your convenience, Monarch kit components, including columns and buffers, are available separately.



	2.7 kb N	2.7 kb Q	5.2 kb N	5.2 kb Q	10 kb N	10 kb Q
Conc. (ng/µl)	334.9	202.2	384.2	216.4	607.6	351.0
Total yield (µg)	10.0	10.1	11.5	10.8	18.2	17.6
$A_{260/280}$	1.9	1.9	1.9	1.9	1.9	1.9
$A_{260/230}$	2.4	2.3	2.4	2.4	2.3	2.4

Monarch Plasmid Miniprep Kits consistently yield more concentrated plasmid DNA with equivalent purity and functionality as the leading supplier. *Preps were performed according to recommended protocols using 1.5 ml aliquots of the same overnight culture. One microliter of each prep was digested with HindIII-HF (NEB #R3104) to linearize the vector and the digests were resolved on a 1% w/v agarose gel.*

Monarch DNA Gel Extraction Kit

#T1020S 50 preps
#T1020L 250 preps

Companion Products:

Monarch DNA Cleanup Columns (5 µg)
#T1034L 100 columns + collection tubes

Monarch Gel Dissolving Buffer
#T1021L 235 ml

Monarch DNA Wash Buffer
#T1032L 25 ml

Monarch DNA Elution Buffer
#T1016L 25 ml

Monarch Plasmid Miniprep Kit
#T1010S 50 preps
#T1010L 250 preps

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 preps
#T1030L 250 preps

- *Elute in as little as 6 µl*
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Save time with fast, user-friendly protocol*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*

With Monarch DNA Cleanup Columns, DNA can be eluted in as little as 6 µl.

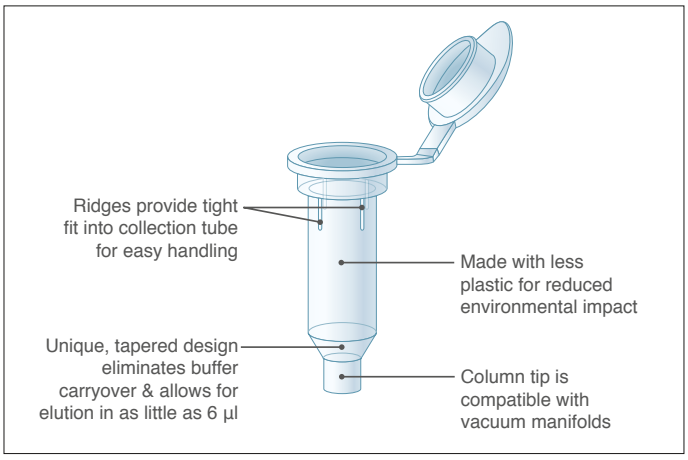
Description: The Monarch DNA Gel Extraction Kit rapidly and reliably purifies up to 5 µg of concentrated high-quality, double-stranded DNA from agarose gels. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 15 minutes. The Monarch Gel Dissolving Buffer is used to digest the agarose gel slice and ensure the sample is compatible for loading the DNA onto the proprietary silica matrix under high salt conditions. The wash buffer ensures trace amounts of DNA binding dyes, electrophoresis buffer salts and gel loading buffer components are removed. Low-volume elution produces concentrated, highly pure DNA ready for use in restriction digests, DNA sequencing, ligation, and other enzymatic manipulations. The unique column design ensures no buffer retention and no carryover of contaminants, allowing elution of sample in volumes as low as 6 µl.

The Monarch DNA Gel Extraction Kit Includes:

– Monarch DNA Cleanup Columns (5 µg)
– Monarch Gel Dissolving Buffer
– Monarch DNA Wash Buffer
– Monarch DNA Elution Buffer
– Monarch Collection Tubes

SPECIFICATIONS	
Binding Capacity	5 µg
DNA Size Range	50 bp–25 kb
Elution Volume	≥ 6 µl
Typical Recovery	DNA 50 bp–10 kb 70–90% DNA 11–25 kb 50–70%

Monarch DNA Gel Extraction Kit reproducibly recovers DNA over a broad range of molecular weights. *A mixture of 7 DNA fragments ranging from 10 kb down to 0.5 kb was prepared and one-half of the mixture was resolved on a 1% gel. Each fragment was manually excised from the agarose gel and processed using the Monarch DNA Gel Extraction Kit. The entire elution of each fragment was resolved on a new gel with the remainder of the original mixture for comparison.*



Our optimized column design supplied with the Monarch Gel Extraction and PCR & DNA Cleanup Kits enables elution in as little as 6 µl, and eliminates buffer retention

Monarch PCR & DNA Cleanup Kit (5 µg)

#T1030S 50 preps
#T1030L 250 preps

Companion Products:

Monarch DNA Cleanup Columns (5 µg)
#T1034L 100 columns + collection tubes

Monarch DNA Cleanup Binding Buffer
#T1031L 235 ml

Monarch DNA Wash Buffer
#T1032L 25 ml

Monarch DNA Elution Buffer
#T1016L 25 ml

Monarch Plasmid Miniprep Kit
#T1010S 50 preps
#T1010L 250 preps

Monarch DNA Gel Extraction Kit
#T1020S 50 preps
#T1020L 250 preps

- *Elute in as little as 6 µl*
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Purify small DNA and oligos with a slight protocol modification*
- *Save time with fast, user-friendly protocol*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*

With the Monarch PCR & DNA Cleanup Kit, you can purify your DNA in as little as 5 minutes.

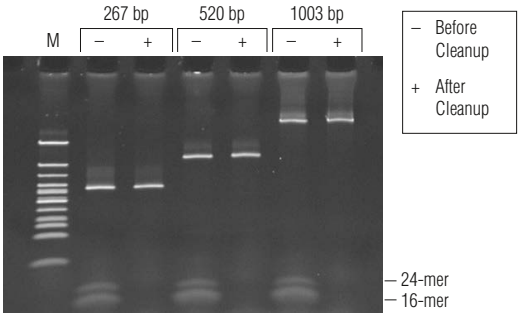
Description: The Monarch PCR & DNA Cleanup Kit (5 µg) is a rapid and reliable method for the purification and concentration of up to 5 µg of high-quality, double-stranded DNA from enzymatic reactions such as PCR, restriction digestion, ligation and reverse transcription. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 5 minutes. DNA Cleanup Binding Buffer is used to dilute the samples and ensure they are compatible for loading onto the proprietary silica matrix under high salt conditions. The DNA Wash Buffer ensures enzymes, short primers (≤ 40 nt), detergents and other low-molecular weight reaction components (e.g., nucleotides, DMSO, betaine) are removed, thereby allowing low-volume elution of concentrated, high-purity DNA. Eluted DNA is ready for use in restriction digests, DNA sequencing, ligation and other enzymatic manipulations. The unique column design ensures no buffer retention and no carryover of contaminants, allowing elution of sample in volumes as low as 6 µl. A slight protocol modification enables purification of small DNA and oligonucleotides.

Applications:

- PCR cleanup
- Enzymatic reaction cleanup
- cDNA cleanup
- Labeling cleanup
- Plasmid cleanup
- Oligo cleanup

The Monarch PCR & DNA Cleanup Kit Includes:

– Monarch DNA Cleanup Columns (5 µg)
– Monarch DNA Cleanup Binding Buffer
– Monarch DNA Wash Buffer
– Monarch DNA Elution Buffer
– Monarch Collection Tubes



Monarch PCR & DNA Cleanup Kit (5 µg) removes low molecular weight primers from dsDNA samples. *Three independent amplicons (267 bp, 520 bp, 1003 bp) were spiked with two oligonucleotides (16-mer, 24-mer) to a final concentration of 1 µM. Half of each mix was purified with the Monarch PCR & DNA Cleanup Kit (5 µg) following the included protocol. Equivalent fractions of the original mixture and the eluted material were resolved on a 20% TBE acrylamide gel at 100V for one hour and stained with SYBR® Green II.*

Monarch Microfuge Tube EcoRack

#T5020S 2 racks

Description: The Monarch Microfuge Tube EcoRack is a bench-top tube rack made from plastic recovered during the manufacture of Monarch Nucleic Acid Purification Columns. Plastic that would otherwise be discarded during the injection molding process is recovered and re-molded into this useful lab accessory that can hold up to 48 tubes each side. One side can accommodate tubes 1.5-2 mls and the other can accommodate 0.5 ml tubes.

NEW

Monarch Genomic DNA Purification Kit

#T3010S	50 preps
#T3010L	150 preps

Companion Products:

Monarch gDNA Purification Columns	
#T3017L	100 columns
Monarch Collection Tubes II	
#T2018L	100 tubes
Monarch gDNA Tissue Lysis Buffer	
#T3011L	34 ml
Monarch gDNA Cell Lysis Buffer	
#T3012L	20 ml
Monarch gDNA Blood Lysis Buffer	
#T3013L	20 ml
Monarch gDNA Binding Buffer	
#T3014L	65 ml
Monarch gDNA Wash Buffer	
#T3015L	60 ml
Monarch gDNA Elution Buffer	
#T3016L	34 ml
Monarch RNase A	
#T3018L	1 ml
Proteinase K, Molecular Biology	
#P8107S	2 ml

- *Purify high quality gDNA from a wide variety of sample types (cells, blood, tissues, and more)*
- *Experience extremely low residual RNA contamination (typically < 1%)*
- *Isolate high molecular weight gDNA (peak size typically ≥ 50 kb)*
- *Take advantage of user-friendly protocols with fast and efficient lysis steps*
- *Additionally, use the kit to clean up genomic DNA*
- *Enjoy the flexibility to purchase kit components separately*

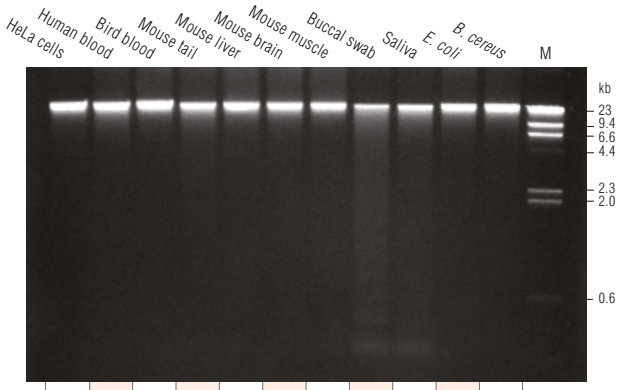
The Monarch Genomic DNA Purification Kit is an excellant complement to the NEBNext Library Preparation products for NGS. The large peak sizes of purified DNA makes it an exceptional choice for purification upstream of long read sequencing platforms.

Description: The Monarch Genomic DNA Purification Kit is a comprehensive solution for cell lysis, RNA removal, and purification of intact genomic DNA (gDNA) from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, bacteria and yeast can be processed with extra steps to enhance lysis in these tough-to-lyse samples. Protocols are also included to enable purification from clinically-relevant samples, such as saliva and cheek swabs, as well as rapid cleanup of previously extracted gDNA. Purified gDNA has high quality metrics, including $A_{260/280} > 1.8$ and $A_{260/230} > 2.0$, high DIN scores and minimal residual RNA. The purified gDNA is suitable for downstream applications, such as endpoint PCR, qPCR and library prep for next generation sequencing (NGS). Typical peak size is 50–70 kb, making this kit an excellent choice upstream of long-read sequencing platforms.

The Monarch gDNA Purification Kit Includes:

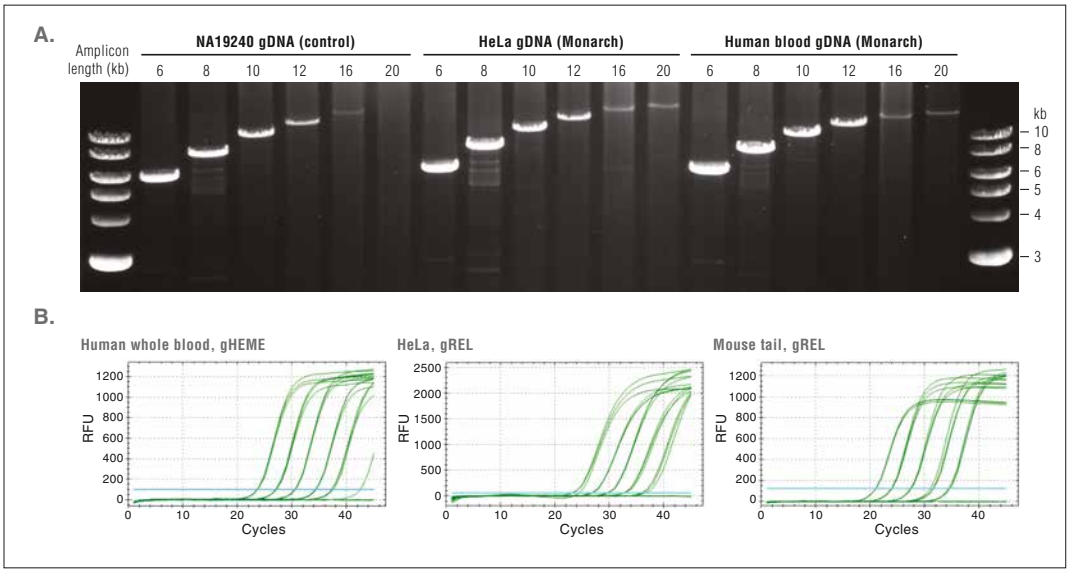
– Monarch gDNA Purification Columns
– Monarch Collection Tubes II
– Monarch gDNA Tissue Lysis Buffer
– Monarch gDNA Cell Lysis Buffer
– Monarch gDNA Blood Lysis Buffer
– Monarch gDNA Binding Buffer
– Monarch gDNA Wash Buffer
– Monarch gDNA Elution Buffer
– Monarch RNase A
– Proteinase K, Molecular Biology

SPECIFICATIONS	
Input	• Cultured mammalian cells: up to 5 x 10^6 cells • Mammalian whole blood: 100 µl • Tissue: up to 25 mg, depending on tissue type • Bacteria: up to 2 x 10^9 • Yeast: up to 5 x 10^7 • Saliva: up to 500 µl • Buccal swabs • Genomic DNA requiring cleanup
Binding Capacity	30 µg genomic DNA
Yield	Varies depending on sample type, see "Guidelines for Choosing Sample Input Amounts", (page 355)
Genomic DNA Size	Peak size > 50 kb for most sample types; may be lower for saliva and buccal swabs
RNA Content	< 1% (with included RNase A treatment)
Purity	$A_{260/280} \geq 1.8$, $A_{260/230} \geq 2.0$



Peak size (kb)	> 60	54	> 60	> 60	> 60	> 60	> 60	39	45	53	> 60
DIN	9.3	9.0	9.2	8.7	9.4	9.3	9.2	6.6	7.4	8.7	8.9
A_{260}/A_{280}	1.86	1.85	1.87	1.85	1.85	1.87	1.87	1.85	1.86	1.86	1.88
A_{260}/A_{230}	2.30	2.20	2.40	2.36	2.34	2.47	2.40	2.00	2.17	2.33	2.04

The Monarch Genomic DNA Purification Kit efficiently purifies high-quality, high molecular weight gDNA from a variety of sample types. *100 ng of genomic DNA from each sample was loaded on a 0.75% agarose gel. gDNA was isolated following the standard protocols for blood, cultured cells and tissue, and the supplemental protocols for buccal swabs, saliva, Gram- and Gram+ bacteria. Starting material used: 1 x 10^5 HeLa cells, 100 µl human blood, 10 µl bird blood, 10 mg frozen tissue powder, 1 buccal swab, 500 µl saliva and ~1 x 10^9 bacterial cells. Lambda DNA-Hind III digest (NEB #N3012) was used as a marker in the last lane (M). Purified gDNA samples were analyzed using a Genomic DNA ScreenTape® on an Agilent Technologies® 4200 TapeStation®. Samples typically yield peak sizes 50–70 kb and DINs of ~9. The cell fractions processed in the buccal swab and saliva preps contain dead cells, as expected, causing a smear like pattern with typical low molecular weight apoptotic bands.*



The Monarch Genomic DNA Purification Kit generates high quality genomic DNA suitable for sensitive applications like long range PCR and qPCR.

A: *Amplification reactions were set up with primer pairs specific for 6, 8, 10, 12, 16, 20 kb amplicons from human DNA. LongAmp® Hot Start Taq 2X Master Mix (NEB #M0533) was used and 25 ng template DNA was added to each sample. PCR reactions were carried out on an Applied Biosystems® 2720 Thermal Cycler. Monarch-purified gDNA isolated from HeLa cells and human blood were compared to commercially available reference DNA from the human cell line NA19240 F11. 10 µl was loaded on a 1.5% agarose gel, using the 1 kb DNA Ladder (NEB #N3232) as a marker. Results indicated DNA was of high-integrity and suitable for long range PCR.*

B: *Monarch-purified gDNA from human whole blood, HeLa cells and mouse tail was diluted to produce a five log range of input template concentrations. The results were generated using primers targeting gHEME (human whole blood) and gREL (HeLa, mouse tail) for qPCR assays with the Luna® Universal qPCR Master Mix (NEB #M3003) and cycled on a Bio-Rad® CFX Touch qPCR thermal cycler. Results indicated that DNA is highly pure and free from inhibitors, optimal for qPCR.*

NEW

Monarch Total RNA Miniprep Kit

#T2010S 50 preps

Companion Products:

Monarch RNA Purification Columns
#T2007L 100 columns + collection tubes

Monarch gDNA Removal Columns
#T2017L 100 columns + collection tubes

Monarch Collection Tubes II
#T2018L 100 collection tubes

Monarch DNA/RNA Protection Reagent
#T2011L 56 ml

Monarch RNA Lysis Buffer
#T2012L 100 ml

Monarch Total RNA Miniprep Enzyme Pack
#T2019L 1 pack

Monarch RNA Priming Buffer
#T2013L 56 ml

Monarch RNA Wash Buffer
#T2014L 50 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

- *Use with a wide variety of sample types*
- *Purify RNA of all sizes, including miRNA & small RNAs > 20 nucleotides*
- *Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent*
- *Efficiently remove contaminating genomic DNA*
- *Protocols available for RNA fractionation and RNA cleanup*
- *Save money with value pricing for an all-in-one kit*

See our "Guidelines for Choosing Sample Input Amounts," including expected yields on page 357.

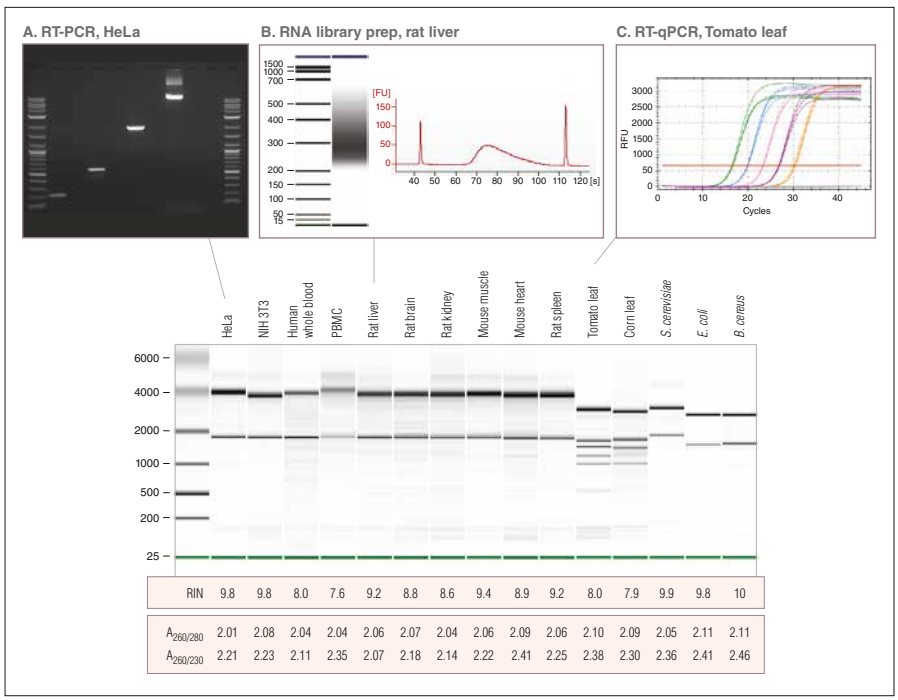
Description: The Monarch Total RNA Miniprep Kit is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, tough-to-lyse samples, such as bacteria, yeast, and plant, can be processed with additional steps that enhance lysis. Cleanup of enzymatic reactions or purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has high quality metrics, including $A_{260/280}$ and $A_{260/230}$ ratios > 1.8, high RIN scores, and minimal residual gDNA. Captured RNA ranges in size from full-length rRNAs down to intact miRNAs. Additionally, differential binding conditions allow selective capture or exclusion of the sub-200 nucleotide RNA pool that includes miRNA, 5S rRNA, and tRNA. Purified RNA is suitable for downstream applications, such as RT-qPCR, cDNA synthesis, RNA-seq, Northern blot analysis, etc.

The Monarch Total RNA Miniprep Kit Includes:

– Monarch gDNA Removal Columns
– Monarch RNA Purification Columns
– Monarch Collection Tubes II
– Monarch DNA/RNA Protection Reagent (2X)
– Monarch RNA Lysis Buffer
– Monarch Proteinase K
– Monarch Proteinase K Resuspension Buffer
– Monarch Proteinase K Reaction Buffer
– Monarch DNase I
– Monarch DNase I Reaction Buffer
– Monarch RNA Priming Buffer
– Monarch RNA Wash Buffer (5X)
– Monarch Nuclease-free Water

SPECIFICATIONS	
Binding Capacity	100 µg RNA
RNA Size	≥ 20 nt
Purity	$A_{260/280}$ and $A_{260/230}$ usually ≥ 1.8
Input Amount	up to 10^7 cells or 50 mg tissue*
Elution Volume	30–100 µl
Yield	varies depending on sample type
Compatible downsteam applications	RNA Library prep for NGS, RT-PCR, RT-qPCR, Northern blots

*See "Guidelines for Choosing Sample Input Amounts" on page 357.



Monarch-purified RNA is high-quality and compatible with a wide variety of downstream applications. *Total RNA from a broad array of sample types was purified using the Monarch Total RNA Miniprep Kit. Aliquots were run on an Agilent Bioanalyzer® 2100 using the Nano 6000 RNA chip (S. cerevisiae RNA was run using a plant Nano assay). RIN values and O.D. ratios confirm the overall integrity and purity of the RNA. To demonstrate compatibility with downstream applications, samples were subsequently used for RT-PCR (+/– RT) (A) for detection of 4 different RNA species using Protoscript® II Reverse Transcriptase/LongAmp Taq DNA Polymerase, NGS library prep (B) using NEBNext® Ultra™ II RNA Library Prep Kit and RT-qPCR (C) using Luna® One-Step RT-qPCR Reagents.*

NEW

Monarch RNA Cleanup Kits

Monarch RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Companion Products:

Monarch RNA Cleanup Columns (10 µg)
#T2037L 100 columns + collection tubes

Monarch RNA Cleanup Columns (50 µg)
#T2047L 100 columns + collection tubes

Monarch RNA Cleanup Columns (500 µg)
#T2057L 100 columns + collection tubes

Monarch Collection Tubes II
#T2018L 100 tubes

Monarch RNA Cleanup Binding Buffer
#T2041S 80 ml

Monarch RNA Cleanup Wash Buffer
#T2042S 40 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

- *Choose from 3 different binding capacities and flexible elution volumes*
- *Quickly and easily purify large quantities of high-quality RNA from in vitro transcription (IVT) reactions*
- *Efficiently remove unincorporated nucleotides from your RNA sample*

Great for RNA cleanup following *in vitro* transcription with HiScribe™ Kits, see pages 185–187.

Description: The Monarch RNA Cleanup Kits provide a fast and simple silica spin column-based solution for RNA cleanup and concentration after any enzymatic reaction (including *in vitro* transcription, DNase I treatment, capping and labeling) and after other purification methods such as phenol/chloroform extraction. The Monarch RNA Cleanup Kits are available in 3 different binding capacities: 10 µg, 50 µg and 500 µg. Each kit contains unique columns, all designed to prevent buffer retention and ensure no carryover of contaminants, enabling low-volume elution of highly-pure RNA. Following the standard protocol, RNA ≥ 25 nt is purified with this kit; however, a modified protocol is available to enable the binding of RNA as small as 15 nt (including miRNAs).

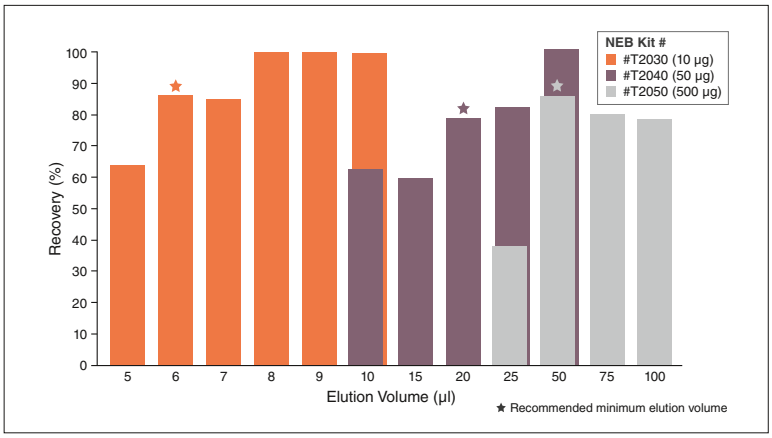
Applications:

- RNA Cleanup and Concentration (including from the TRIzol aqueous phase)
- Enzymatic Reaction Cleanup
- *In vitro* Transcription Cleanup
- RNA Gel Extraction
- RNA Fractionation

The Monarch RNA Cleanup Kits Include:

– Monarch RNA Cleanup Columns (10, 50 or 500 µg)
– Monarch RNA Cleanup Binding Buffer
– Monarch RNA Cleanup Wash Buffer
– Monarch Collection Tubes II
– Nuclease-free Water

Monarch RNA Cleanup Kit	NEB #T2030 (10 µg)	NEB #T2040 (50 µg)	NEB #T2050 (500 µg)
Binding Capacity	10 µg	50 µg	500 µg
RNA Size Range	≥ 25 nt (≥ 15 nt with modified protocol)		
Typical Recovery	70–100%		
Eluion Volume	6–20 µl	20–50 µl	50–100 µl
Purity	$A_{260/280}$ > 1.8 and $A_{260/230}$ > 1.8		
Protocol Time	5 minutes of spin and incubation time		10–15 minutes of spin and incubation time



Recovery of RNA from Monarch RNA Cleanup Kits with Varying Elution Volumes. *rRNA (10, 50 or 500 µg, respectively of 16S and 23S Ribosomal Standard from E. coli, Sigma) was purified using a Monarch RNA Cleanup Kit (10 µg, NEB #T2030) (50 µg, NEB #T2040) (500 µg, NEB #T2050). Nuclease-free water was used to elute the RNA. The percent recovery of the RNA was calculated from the resulting A_{260} as measured using a Trinean® DropSense® 16. ~80% of RNA can be efficiently recovered in 6 µl from the Monarch RNA Cleanup Kit (10 µg, NEB #T2030), 20 µl from the Monarch RNA Cleanup Kit (50 µg, NEB #T2040), and 50 µl from the Monarch RNA Cleanup Kit (500 µg, NEB #T2050).*

Choking the Oceans with Plastic

By the year 2050, it is predicted that the Earth's oceans will be home, by weight, to more plastic than fish. Eight million metric tons of plastic trash enters the sea every year. This plastic collects in all the oceans on Earth in large, circular currents, or gyres. The largest gyre has been coined The Great Pacific Garbage Patch. Many estimates are made to convey the immensity of this patch — it is 1.6 million km^2 or 618,000 miles^2, or three times the size of France. In other words, it is big!

Ocean debris consists of large and small pieces of plastic; however, the predominant contaminant is tiny confetti-sized pieces of plastic that have been photodegraded by the sun. This fog of particulate easily enters the food chain, and approximately 12,000 to 14,000 tons are ingested by fish and invertebrates each year, threatening the biodiversity of ocean life.

Much of the media coverage focuses on sea turtles, birds, seals and other marine animals entangled in, or ingesting, the trash. However, the plastic problem also extends to chemical pollution and its worrying effect on the reproductivity of various marine organisms. Previous research on plasticizers, such as bisphenol A (BPA) and phthalates, has shown that they induce endocrine toxicity and reproductive alterations. Furthermore, plastic polymers attract polychlorinate biphenyls (PCBs) and other toxic chemicals.

Pacific Oysters (*Crassostrea gigas*) that were experimentally exposed to polystyrene microparticles at concentrations estimated for the water-sediment interface (a typical oyster habitat) produced fewer and smaller egg cells, less-mobile sperm and slow-growing offspring[1]. Additionally, exposure to nanoplastics caused green algae (*Scenedesmus obliquus*) to exhibit reduced growth and chlorophyll concentrations, and a small planktonic crustacean (*Daphnia magna*) to present reduced neonate body size and increased malformations[2].

Many marine organisms, such as bryozoans and crustaceans, are known to colonize floating wood or seaweed, using them as a raft. Now, non-rafting, invasive species, such as coral pathogens, are using plastic debris to travel to, and damage, new ecosystems. Also "hitching a ride" are communities of microbes that are vastly different than those found in the surrounding seawater, for example, members of the genus *Vibrio*, which is a human pathogen. A marine insect, *Halobates sericeus*, that typically lays its eggs on natural rafts, such as shells and bird feathers, has now been observed laying eggs on plastic debris. The long-term effects of this have yet to be determined.

In the first decade of this century, more plastic was generated than ever before — and every piece produced is likely still here on the planet. Intervention at the source of this problem, specifically a reduction of single-use plastics, clearly needs to be promoted. However, we can find a glimmer of hope in ongoing efforts — massive trawling clean-ups, GPS tagging of trash to model its movement, materials scientists turning their attention to more environmentally-friendly packaging, large companies reducing their waste and consumers who are making more informed decisions every day.

(1) Sussarellu, R., et al (2016) *PNAS*, 113, 2430–2435.
(2) Besseling, E., et al (2014) *Environ Sci Technol*, 48, 12336–12343.

Trash in the ocean water.
Credit: Juan Camilo Bernal, Getty Images

NEBNext® Reagents for Next Generation Sequencing

The leading reagents for sample preparation for next generation sequencing.

Library preparation is a critical part of the next generation sequencing workflow; successful sequencing requires the generation of high quality libraries of sufficient yield and quality.

As sequencing technologies improve and capacities expand, boundaries continue to be pushed on sample preparation: high performance is required from ever-decreasing input quantities and from samples of lower quality.

To meet these growing challenges, the NEBNext suite of products continues to evolve to support next generation sequencing with sample preparation tools that streamline workflows, minimize inputs, and improve library quality and yields.

NEBNext reagents are available for sample preparation for DNA, RNA, ChIP, FFPE, Small RNA, single cell and microbiome samples, for use with Illumina®, Ion Torrent™ and other sequencing platforms. Products are in user-friendly formats including kits and modules, with bulk or customized formats also available. Adaptors and primers are available separately, for maximized flexibility. Use of NEBNext products has been cited in thousands of peer-reviewed publications.

For additional convenience and cost-effectiveness in high-throughput workflows, NEBNext reagents are also available in bulk and customized formats. For more information, contact NEBSolutions@neb.com.

Featured Products

Featured Tools & Resources

Visit **NEBNextSelector.neb.com** for help with selecting products.

Publications Related to Sample Prep

Visit **www.NEBNext.com** to keep up to date on everything NEBNext.

Find an overview of NGS library preparation.

REAGENTS FOR ILLUMINA SEQUENCING

Product Selection Charts

Ultra II DNA

METHYLATION ANALYSIS

Ultra II RNA

Single Cell RNA

Small RNA

Modules & Enzymes

Adaptors & Primers

Target Enrichment

Library Quantification

REAGENTS FOR ION TORRENT

REAGENTS FOR rRNA DEPLETION

REAGENTS SUITABLE FOR ANY PLATFORM

DNA Separation

DNA Repair

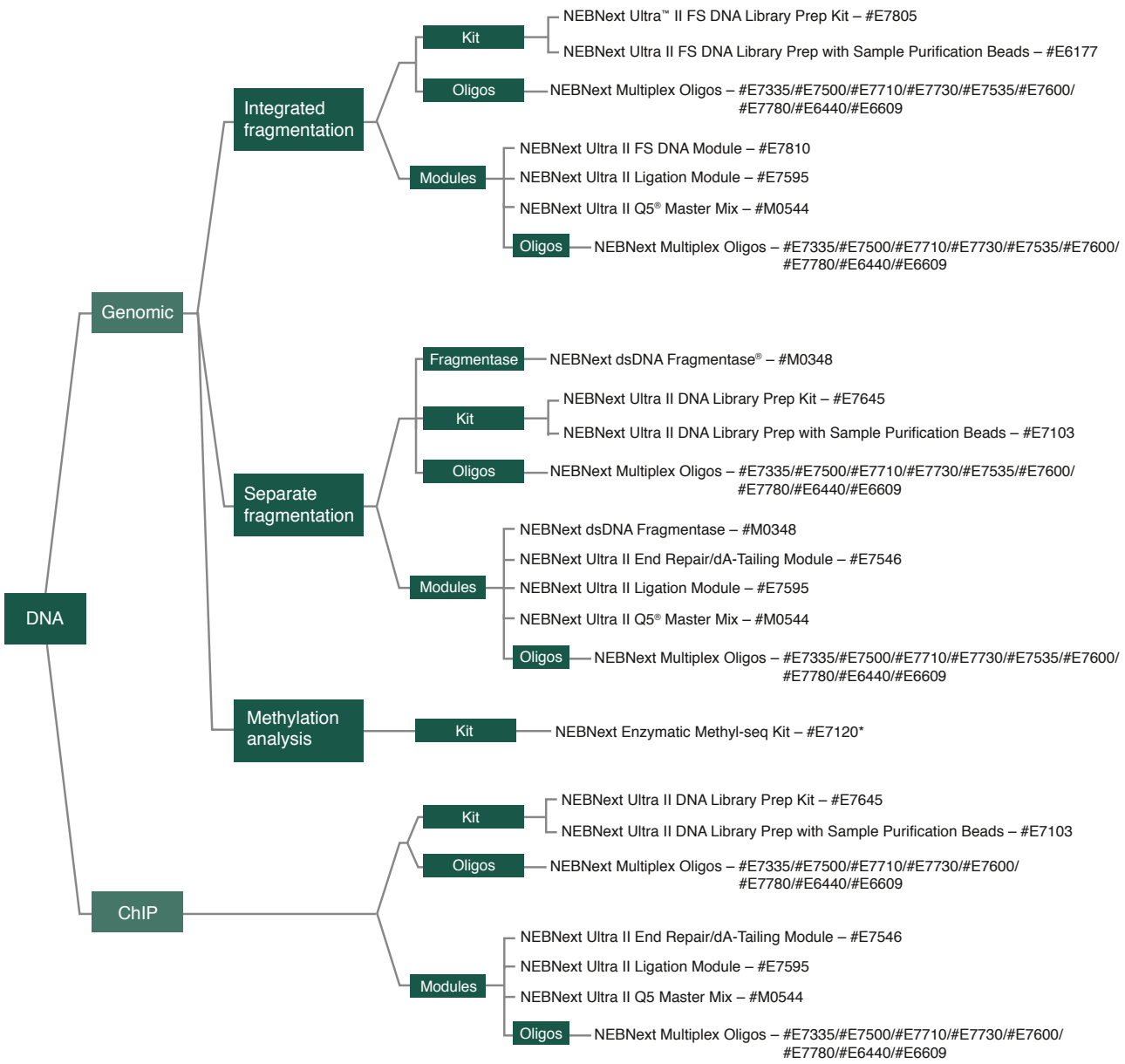
Modules & Enzymes

Magnetic Separation

Illumina® DNA Product Selection Chart

Use the following chart to determine the best NEBNext® products for your Illumina DNA library prep needs. For the most up-to-date product and pricing information, visit **NEBNext.com**.

For help selecting products, try our online product selection tool at **NEBNextSelector.neb.com**

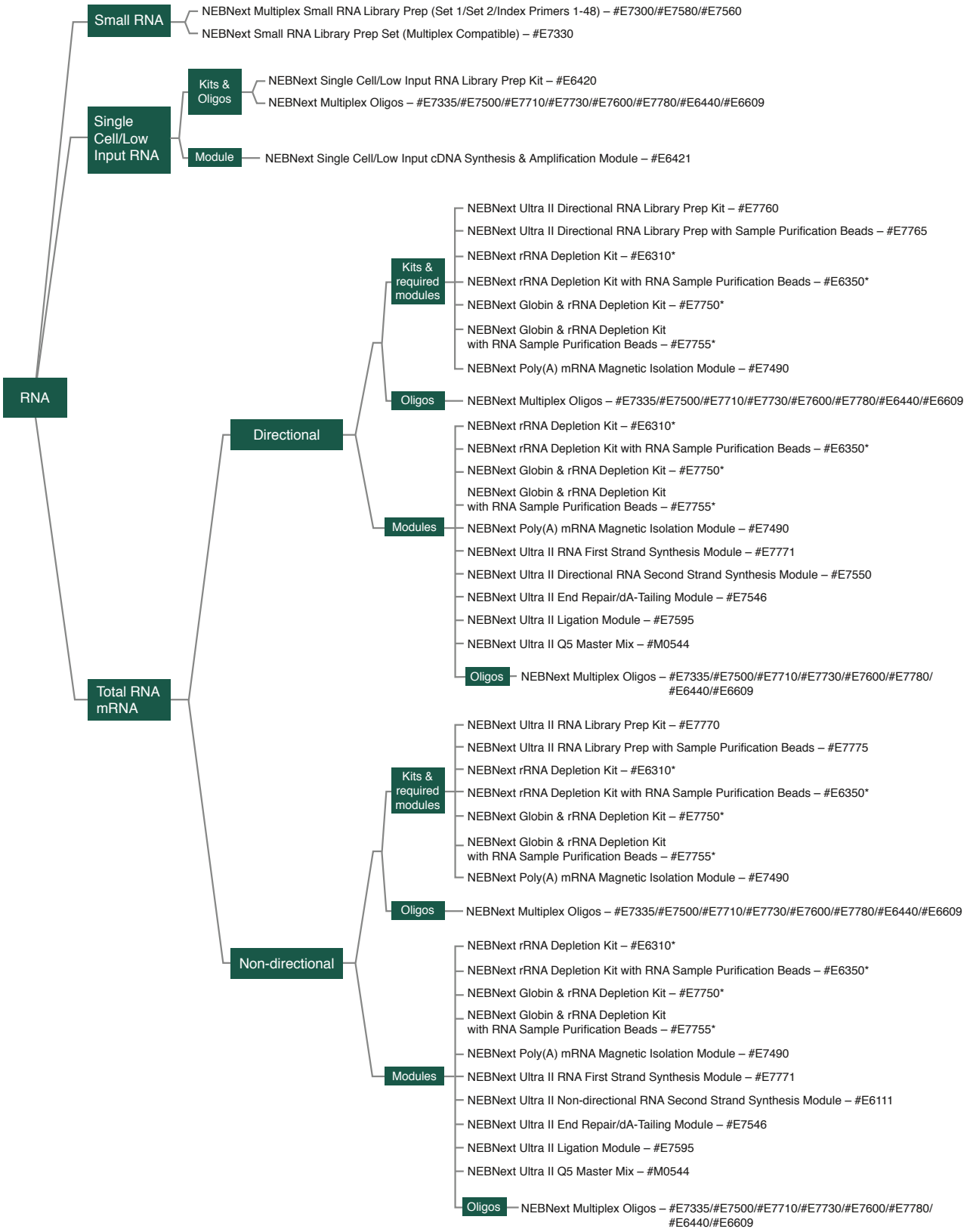


** Module and EM-seq Oligos also available.*
Reagents for original Ultra workflow and standard workflow are also available. See ordering information.

Illumina RNA Product Selection Chart

Use the following chart to determine the best NEBNext products for your Illumina RNA sequencing needs. For the most up-to-date product and pricing information, visit **NEBNext.com**.

For help selecting products, try our online product selection tool at **NEBNextSelector.neb.com**



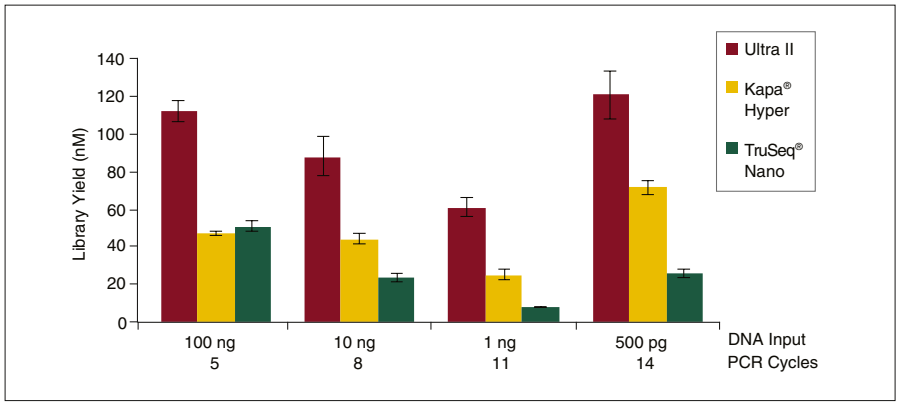
** For human/mouse/rat samples.*

Reagents for original Ultra workflow and standard workflow are also available. See ordering information.

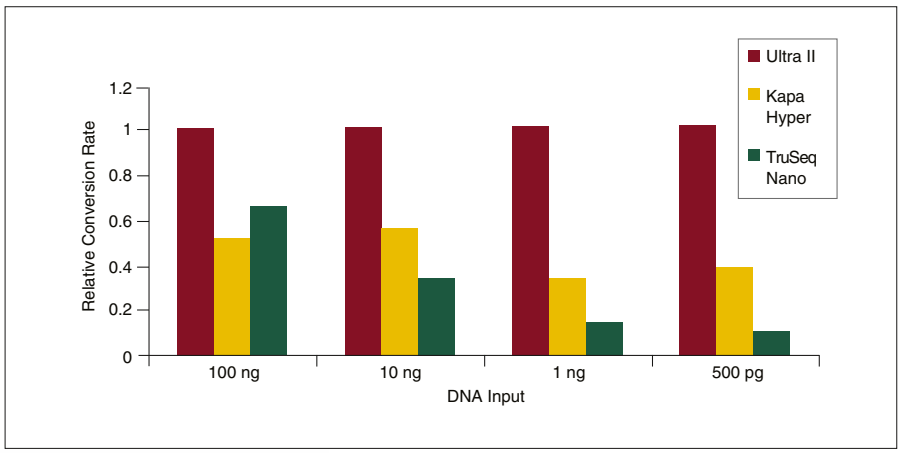
Even more *from* less – NEBNext® Ultra™ II DNA Library Prep Kits for Illumina

Are you challenged with trying to obtain higher library yields using ever-decreasing input amounts? Each component in the NEBNext Ultra II DNA Library Prep Kit from NEB has been reformulated, resulting in a several-fold increase in library yield with as little as 500 picograms of input DNA. These advances deliver unprecedented performance, while enabling lower inputs and fewer PCR cycles.

To learn more and to view performance data, visit **NEBNextUltraII.com**.



The NEBNext Ultra II DNA Library Prep Kit produces the highest yield libraries from a broad range of input amounts. *Libraries were prepared from Human NA19240 genomic DNA using the input amounts and numbers of PCR cycles shown. Manufacturers' recommended protocols were followed, with the exception that size selection was omitted.*



NEBNext Ultra II produces the highest rates of conversion to adaptor-ligated molecules from a broad range of input amounts. *Libraries were prepared from Human NA19240 genomic DNA using the input amounts and library prep kits shown without an amplification step, and following manufacturers' recommendations. qPCR was used to quantitate adaptor-ligated molecules, and quantitation values were then normalized to the conversion rate for Ultra II. The Ultra II kit produces the highest rate of conversion to adaptor-ligated molecules, for a broad range of input amounts.*

NEBNext Ultra II is available with or without sample purification beads, and/or fragmentation reagents, for added convenience.

NEBNext Ultra II DNA and FS DNA Library Prep Kits for Illumina

NEBNext Ultra II DNA Library Prep Kit for Illumina
#E7645S 24 reactions
#E7645L 96 reactions

NEBNext Ultra II DNA Library Prep with Sample Purification Beads
#E7103S 24 reactions
#E7103L 96 reactions

NEBNext Ultra II FS DNA Library Kit for Illumina
#E7805S 24 reactions
#E7805L 96 reactions

NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads
#E6177S 24 reactions
#E6177L 96 reactions

See ordering information for NEBNext Ultra II modules.

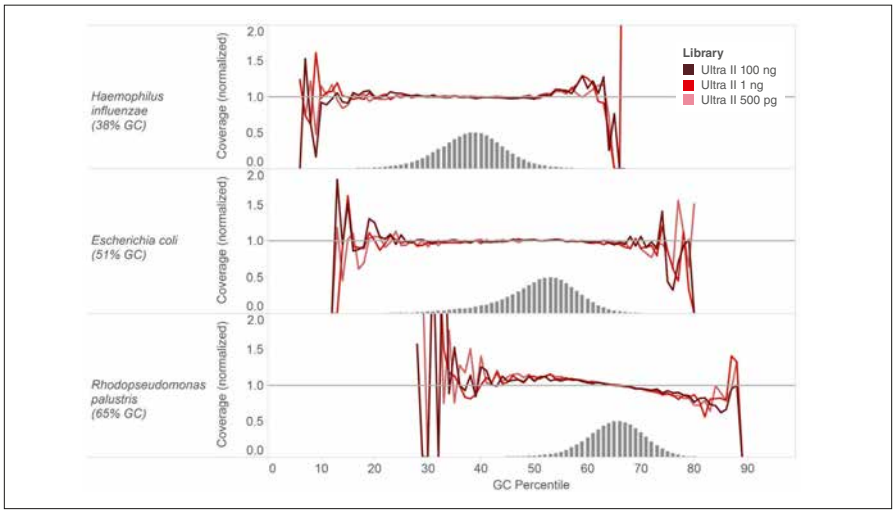
- *Get more of what you need, with the highest library yields*
- *Use to generate high quality libraries even when you have only limited amounts of DNA, with inputs as low as 500 pg*
- *Prepare libraries from ALL of your samples, including GC-rich targets and FFPE DNA samples*
- *Improve yields for target enrichment applications*
- *Save time with streamlined workflows, reduced hands-on time, and automation compatibility, and enjoy the flexibility of kit or module format products*
- *Access reliable and easy-to-use, scalable enzymatic DNA fragmentation, integrated into the Ultra II DNA workflow with the FS version of the kit*
- *Enjoy the flexibility and reliability of the gold standard SPRIselect size selection and clean-up beads, supplied in just the amounts you need*

Visit **NEBNextUltraII.com** for more information, including our technical note and protocol videos

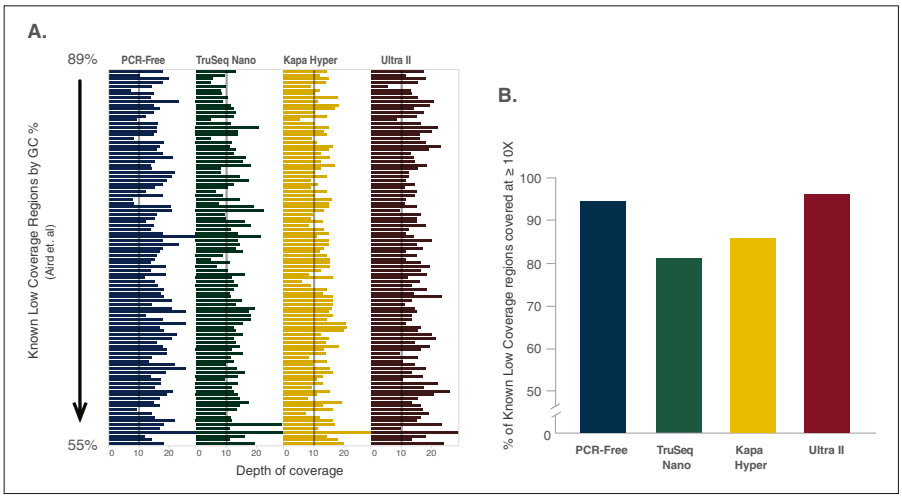
Description: The NEBNext Ultra II DNA Library Prep Kits for Illumina meet the challenge of constructing high quality libraries from ever-decreasing input quantities. The reagents for each step in the library preparation workflow have been reformulated to enable high yield preparation of high quality libraries from 500 picograms to 1 microgram of input DNA. This new generation of NEBNext reagents uses a fast, streamlined, automatable workflow and enables use of fewer PCR cycles while also improving GC coverage. The kit is also compatible with PCR-free workflows and is effective with challenging samples such as FFPE DNA.

The Ultra II FS DNA Library Prep Kit combines robust enzymatic DNA fragmentation with end repair and dA-tailing, integrated into a streamlined library prep workflow.

Both the Ultra II DNA and Ultra II FS DNA kits are available with or without SPRIselect® beads.



NEBNext Ultra II provides uniform GC coverage for microbial genomic DNA over a broad range of GC composition and input amounts. *Libraries were made using 500 pg, 1 ng and 100 ng of the genomic DNAs shown and the Ultra II DNA Library Prep Kit and sequenced on an Illumina MiSeq®. Reads were mapped using Bowtie 2.2.4 and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library.*



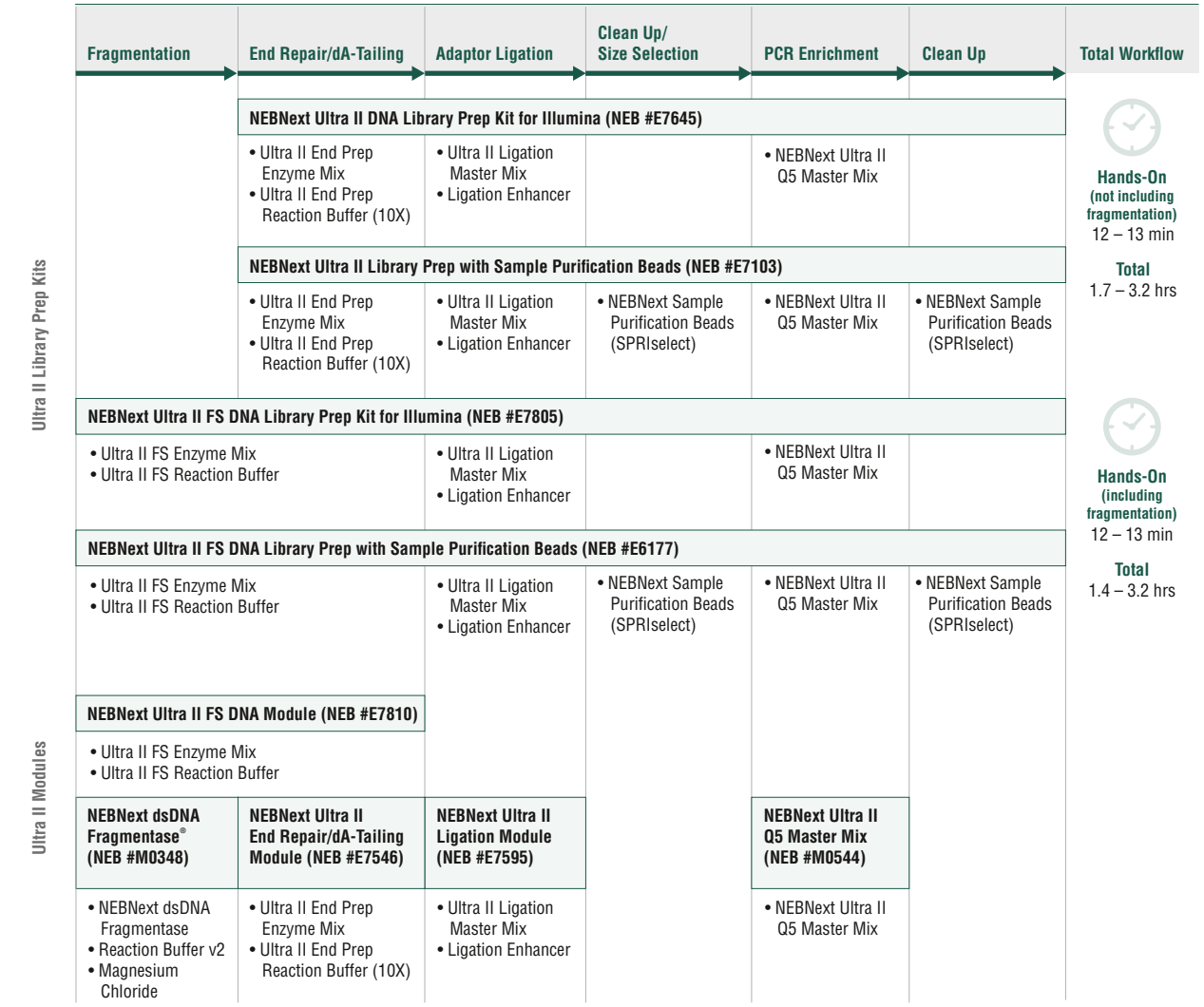
NEBNext Ultra II provides the highest and most uniform coverage of difficult sequence regions.
A: *Indexed libraries were prepared from 100 ng of Human NA19240 genomic DNA using a PCR-free workflow or the library prep kits shown, following manufacturers' recommendations. The PCR-free library was prepared using NEBNext Ultra II. Libraries were sequenced on the Illumina NextSeq® 500. 420 million reads were randomly extracted from each dataset, to produce an average coverage of 10X. Reads were mapped to the GRCh37 reference genome using Bowtie 2.2.4. Reads on each region were counted using bedtools v2.19.1. The number of reads overlapping distinct difficult, low-coverage regions of the human genome (1) are shown for each library. Ultra II provides the highest and most uniform coverage of these difficult regions, and provides the coverage closest to that obtained with a PCR-free protocol.*

B: *From the 420 million 75 bp reads randomly extracted from each dataset, 10X coverage was expected. The percentage of difficult regions covered at ≥ 10X is shown for each library prep kit and for the PCR-free workflow. Ultra II provides the highest percentage of reads at ≥ 10X coverage and also provides the coverage closest to that obtained with a PCR-free protocol. (1) Aird, D. et al. (2011). Analyzing and minimizing PCR amplification bias in Illumina sequencing libraries. Genome Biology 12(2), R18.*

NEBNext Ultra II DNA Reagents for Illumina Sequencing

NEBNext Ultra II Kits for DNA are available with or without integrated enzymatic DNA fragmentation. Note that adaptors and primers are supplied separately. In addition to stringent QCs on individual components, the NEBNext DNA kits are also functionally validated by preparation of a library, followed by Illumina sequencing.

Input Ultra II DNA Workflow: 500 pg – 1 µg Ultra II FS DNA Workflow: 100 pg – 0.5 µg



NEBNext Enzymatic Methyl-seq

NEW
NEBNext Enzymatic Methyl-seq Kit
#E7120S 24 reactions
#E7120L 96 reactions

NEW
NEBNext Enzymatic Methyl-seq Conversion Module
#E7125S 24 reactions
#E7125L 96 reactions

NEW
NEBNext Q5U™ Master Mix
#M0597S 50 reactions
#M0597L 250 reactions

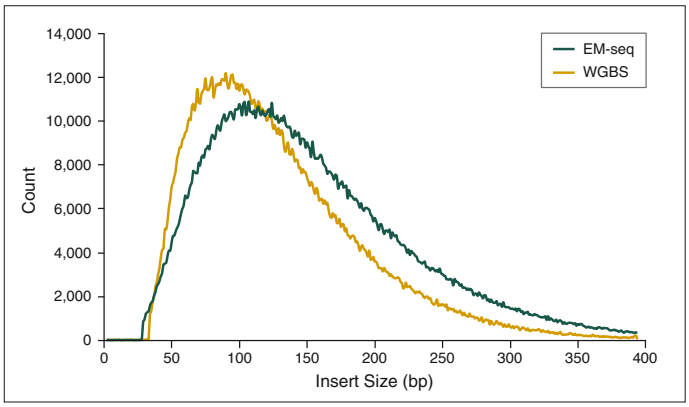
NEW
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)
#E7140S 24 reactions
#E7140L 96 reactions

- *Superior sensitivity of detection of 5-mC and 5-hmC*
- *Larger library insert sizes*
- *More uniform GC coverage*
- *Greater mapping efficiency*
- *High-efficiency library preparation*

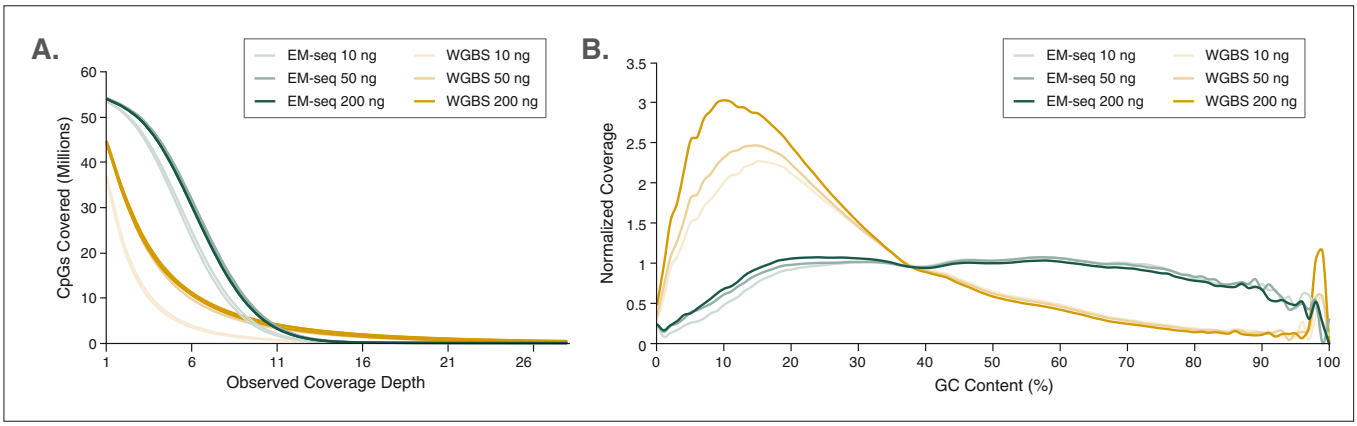
NEBNext Enzymatic Methyl-seq is an enzymatic alternative to bisulfite conversion with superior performance. For more information, including extensive performance data, visit **NEBNext.com**.

Description: While bisulfite sequencing has been the gold standard for the study of DNA methylation, this conversion treatment is damaging to DNA, resulting in DNA fragmentation, loss and GC bias. The NEBNext Enzymatic Methyl-seq Kit (EM-seq™) provides an enzymatic alternative to whole genome bisulfite sequencing (WGBS), combined with high-efficiency streamlined library preparation suitable for Illumina sequencing.

The highly effective EM-seq enzymatic conversion minimizes damage to DNA and, in combination with the supplied NEBNext Ultra II library preparation workflow reagents, results in high quality libraries that enable superior detection of 5-mC and 5-hmC from fewer sequencing reads.



NEBNext Enzymatic Methyl-seq libraries have larger insert sizes *50 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris® S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold™ kit for bisulfite conversion. Libraries were sequenced on an Illumina MiSeq (2 x 76 bases) and insert sizes were determined using Picard 2.18.14. The normalized frequency of each insert size was plotted, illustrating that library insert sizes are larger for EM-seq than for WGBS, and indicating that EM-seq does not damage DNA as bisulfite treatment does in WGBS.*



EM-seq identifies more CpGs than WGBS, at lower sequencing coverage depth with superior uniformity of GC coverage. *10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold Kit for bisulfite conversion. Libraries were sequenced on an Illumina NovaSeq® 6000 (2 x 100 bases). Reads were aligned to hg38 using bwa-meth 0.2.2.*

A: *Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads, and each top and bottom strand CpGs were counted independently, yielding a maximum of 56 million possible CpG sites. EM-seq identifies more CpGs at lower depth of sequencing.*

B: *GC coverage was analyzed using Picard 2.17.2 and the distribution of normalized coverage across different GC contents of the genome (0-100%) was plotted. EM-seq libraries have significantly more uniform GC coverage, and lack the AT over-representation and GC under-representation typical of WGBS libraries.*

NEBNext Ultra II Library Prep Kits for RNA

NEBNext Ultra II Directional
RNA Library Prep Kit for Illumina
#E7760S 24 reactions
#E7760L 96 reactions

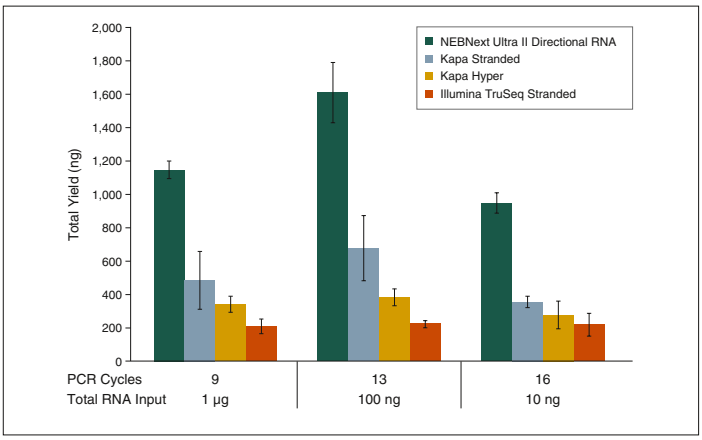
NEBNext Ultra II Directional RNA
Library Prep with Sample Purification Beads
#E7765S 24 reactions
#E7765L 96 reactions

NEBNext Ultra II RNA Library Prep Kit
for Illumina
#E7770S 24 reactions
#E7770L 96 reactions

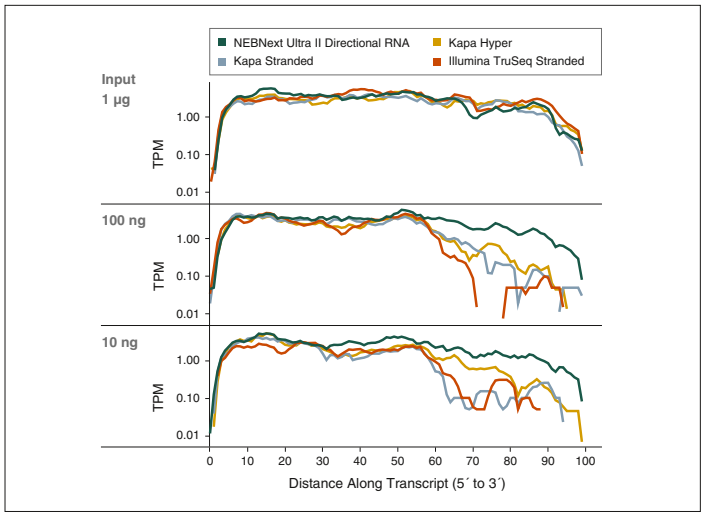
NEBNext Ultra II RNA Library Prep
with Sample Purification Beads
#E7775S 24 reactions
#E7775L 96 reactions

See ordering information for
NEBNext Ultra II modules.

Do you need increased sensitivity and specificity from your RNA-seq experiments? Do you have ever-decreasing amounts of input RNA? To address these challenges, our next generation of RNA library prep kits have been reformulated at each step, resulting in several fold higher yields of high quality libraries and enabling use of lower input amounts and fewer PCR cycles. The kits have streamlined, automatable workflows and are available for directional (strand-specific, using the "dUTP method") and non-directional library prep, with the option of SPRISelect beads for size-selection and clean-up steps.



NEBNext Ultra II Directional RNA produces the highest yields, from a range of input amounts. *Poly(A)-containing mRNA was isolated from 10 ng, 100 ng and 1 µg of Universal Human Reference RNA (Agilent #740000) and libraries were made using the NEBNext Ultra II Directional RNA kit, Kapa Stranded mRNA-Seq kit, Kapa mRNA HyperPrep kit and Illumina TruSeq Stranded mRNA Kit. The input RNA amount and number of PCR cycles are indicated. Library yields from an average of three replicates are shown. Error bars indicate standard deviation. Library yields were assessed using the Agilent® Bioanalyzer®.*



Uniformity of Coverage across the DAM1 transcript. *Poly(A)-containing mRNA was isolated from 10 ng, 100 ng and 1 µg of Universal Human Reference RNA (Agilent #740000) and libraries were made using the NEBNext Ultra II Directional RNA kit, Kapa Stranded mRNA-Seq kit, Kapa mRNA HyperPrep kit and Illumina TruSeq Stranded mRNA Kit. Coverage across transcript ENST00000369541.3 (DAM1) was assessed by mapping reads directly to the transcriptome (Hisat 2.0.3) and assessing coverage using bedtools cov in 100 bins along the transcript length. Libraries prepared using the NEBNext Ultra II Directional RNA Kit provided superior coverage across the transcript at 100 ng and 10 ng input amounts.*

- *Get more of what you need, with the highest library yields*
- *Generate high quality libraries with limited amounts of RNA:*
  - *10 ng–1µg Total RNA (polyA mRNA workflow)*
  - *5 ng–1µg (rRNA depletion workflow)*
- *Minimize bias, with fewer PCR cycles required*
- *Maximize the flexibility to order reagents for your specific workflow needs*
  - *Directional (strand-specific, using the "dUTP method") and non-directional workflow options available*
  - *rRNA Depletion and poly(A) mRNA Isolation reagents are available separately*
  - *Adaptors and primers for multiplexing, in 12- and 96-index formats, are available separately*
- *Save time with streamlined workflows, reduced hands-on time, and automation compatibility*
- *Enjoy the reliability of the gold standard SPRIselect size selection and clean-up beads, supplied in just the amounts you need*
- *Rely on robust performance, even with low quality RNA*

Visit **UltraIIRNA.com** to learn more
and to view performance data

View the NEBNext
Ultra II Directional
RNA Workflow.

NEBNext Ultra II RNA Reagents for Illumina Sequencing

NEBNext Ultra II RNA Kits are available for directional (strand-specific) and non-directional library preparation, and for bulk RNA and single cell samples. These kits utilize streamlined workflows and have been designed for performance with input amounts as low as 5 ng. Note that reagents for rRNA depletion and poly(A) mRNA enrichment are supplied separately, as are adaptors and primers. In addition to stringent QCs on individual components, the NEBNext RNA kits are functionally validated by preparation of a library, followed by Illumina sequencing.

Input Poly(A) mRNA Workflow: 10 ng – 1 µg rRNA Depletion Workflow: 5 ng – 1 µg

	mRNA Isolation/ rRNA Depletion	mRNA Fragmentation	First Strand cDNA Synthesis	Second Strand cDNA Synthesis	End Repair/ dA Tailing	Adaptor Ligation	Size Selection	PCR Enrichment	Clean Up	Total Workflow
Ultra II Directional Kits		**NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760)**								Hands-On 27 min Total 5.5 – 5.7 hrs * 6.6 – 6.8 hrs **
		NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads (NEB #7765)								
		• First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent		• Second Strand Synthesis Reaction Buffer with dUTP Mix • Second Strand Synthesis Enzyme Mix • Nuclease-free Water	• End Prep Enzyme Mix • End Repair Reaction Buffer	• Ultra II Ligation Master Mix • Ligation Enhancer • Adaptor Dilution Buffer	• NEBNext Sample Purification Beads (SPRIselect) – NEB #E7765 only	• NEBNext Ultra II Q5 Master Mix	• NEBNext Sample Purification Beads (SPRIselect) – NEB #E7765 only	
Ultra II Non-directional Kits		**NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770)**								Hands-On 27 min Total 5.5 – 5.7 hrs * 6.6 – 6.8 hrs **
		NEBNext Ultra II RNA Library Prep with Sample Purification Beads (NEB #E7775)								
		• First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent		• Second Strand Synthesis Reaction Buffer • Second Strand Synthesis Enzyme Mix • Nuclease-free Water	• End Prep Enzyme Mix • End Repair Reaction Buffer	• Ultra II Ligation Master Mix • Ligation Enhancer • Adaptor Dilution Buffer	• NEBNext Sample Purification Beads (SPRIselect) – NEB #E7775 only	• NEBNext Ultra II Q5 Master Mix	• NEBNext Sample Purification Beads (SPRIselect) – NEB #E7775 only	
Ultra II Modules	**rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E6310, #E6350)**	**Magnesium RNA Fragmentation Module (NEB #E6150)**	**Ultra II RNA First Strand Synthesis Module (NEB #E7771)**	**Ultra II Directional RNA Second Strand Synthesis Module (NEB #E7550)**	**NEBNext Ultra II End Repair/ dA-Tailing Module (NEB #E7546)**	**NEBNext Ultra II Ligation Module (NEB #E7595)**		**NEBNext Ultra II Q5 Master Mix (NEB #M0544)**		
	• RNase H/RNase H Reaction Buffer • rRNA Depletion Solution • Probe Hybridization Buffer • DNase I/DNase I Reaction Buffer • Nuclease-free Water • NEBNext RNA Sample Purification Beads (NEB #E6350 only)	• RNA Fragmentation Buffer • RNA Fragmentation Stop Solution	• First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent	• Second Strand Synthesis Enzyme Mix • Second Strand Synthesis Reaction Buffer with dUTP	• Ultra II End Prep Enzyme Mix • Ultra II End Prep Reaction Buffer	• Ultra II Ligation Master Mix • Ligation Enhancer		• NEBNext Ultra II Q5 Master Mix		
	Globin & rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E7750, #E7755)			**Ultra II Non-directional RNA Second Strand Synthesis Module (NEB #E6111)**						
	• RNase H/RNase H Reaction Buffer • Globin & rRNA Depletion Solution • Probe Hybridization Buffer • DNase I/DNase I Reaction Buffer • Nuclease-free Water • NEBNext RNA Sample Purification Beads (NEB #E7775 only)			• Second Strand Synthesis Enzyme Mix • Second Strand Synthesis Reaction Buffer						
	Poly(A) mRNA Magnetic Isolation Module (NEB #E7490)									
	• Oligo d(T)25 beads • RNA Binding Buffer • Wash Buffer • Nuclease-free Water									

* Including poly(A) mRNA isolation
** Including rRNA depletion

NEBNext Single Cell/Low Input RNA Library Prep

NEW
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina
#E6420S 24 reactions
#E6420L 96 reactions

NEW
NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module
#E6421S 24 reactions
#E6421L 96 reactions

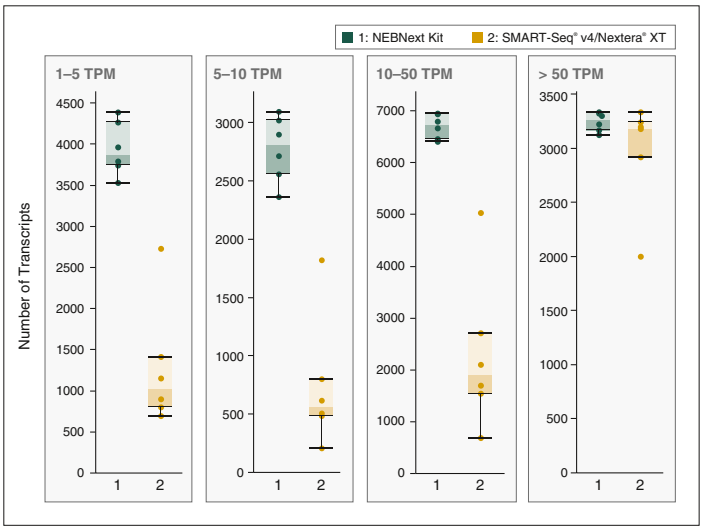
NEW
NEBNext Single Cell Lysis Module
#E5530S 96 reactions

- *Generate the highest yields of high-quality full-length transcript sequencing libraries from single cells, or as little as 2 pg–200 ng total RNA*
- *Experience unmatched detection of low abundance transcripts*
- *Rely on consistent transcript detection for a wide range of input amounts and sample types*
- *Obtain full-length, uniform transcript coverage, regardless of input amount or sample type*
- *Use with cultured or primary cells, or total RNA*
- *Save time with a fast, streamlined workflow, minimal handling steps and hands-on time*
  - *Single-tube protocol from cell lysis to cDNA*
  - *Enzymatic DNA fragmentation, end repair and dA-tailing reagents in a single enzyme mix, with a single protocol, regardless of GC content*
- *Available with or without library construction reagents*

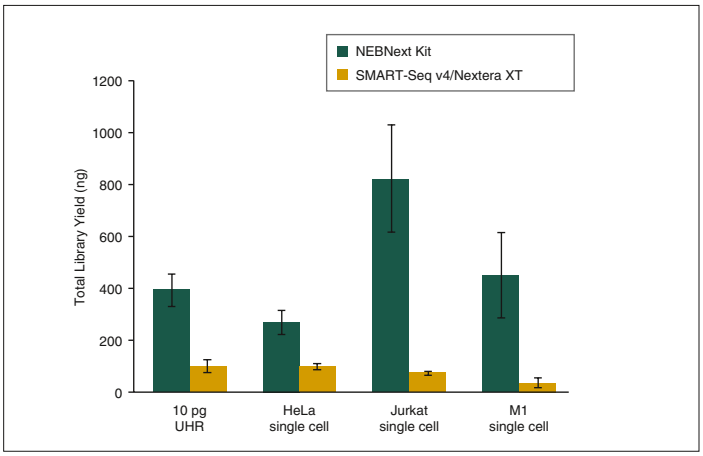
Description: The unique workflow of the NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina meets the demand for a highly sensitive, yet robust method that consistently generates high-quality, full-length transcript sequencing data from a single cell or ultra-low input RNA.

Optimized cDNA synthesis and amplification steps incorporate template switching, and utilize a unique protocol and suite of reagents.

cDNAs are generated directly from single cells or 2 pg–200 ng RNA, and even low-abundance transcripts are represented in the high yields of cDNA obtained. This is followed by library construction that incorporates the Ultra II FS enzymatic DNA fragmentation/end repair/dA-tailing mix in a simple and efficient workflow.



Increased transcript detection with the NEBNext Single Cell/Low Input RNA Library Prep Kit *Sequencing libraries were generated from Jurkat single cells (6 replicates) using the NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech # 634891) plus the Nextera XT DNA Library Prep Kit (Illumina #FC-131-1096). Libraries were sequenced on an Illumina NextSeq 500 using paired-end mode (2 x 76 bp). TPM = Transcripts per Kilobase Million. Each dot represents the number of transcripts identified at the given TPM range, and each box represents the median, first and third quartiles per replicate and method. Salmon 0.6 was used for read mapping and quantification of all GENCODE v25 transcripts. Panels show the number of transcripts detected within the following TPM ranges: 1-5, 5-10, 10-50 and > 50 TPM. Increased identification of low abundance transcripts is observed with the NEBNext libraries.*



Higher library yields with the NEBNext Single Cell/Low Input RNA Library Prep Kit *Sequencing libraries were generated from HeLa, Jurkat and M1 single cells or 10 pg of Universal Human Reference (UHR) RNA (Agilent #740000) with recommended amounts of ERCC RNA Spike-In Mix I (Thermo Fisher Scientific® #4456740). The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech #634891) plus the Nextera® XT DNA Library Prep Kit (Illumina #FC-131-1096) were used. Error bars indicate standard deviation for 6-11 replicates. For the NEBNext workflow ~80% of the cDNA was used as input into sequencing library preparation, and libraries were amplified with 8 PCR cycles. For the SMART-Seq v4/Nextera XT workflow, as recommended, 125 pg of cDNA was used as input in sequencing library preparation and 12 PCR cycles were used for amplification. Error bars indicate standard deviation for 6-11 replicates.*

NEBNext Small RNA Library Prep Kits

NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)
#E7300S 24 reactions
#E7300L 96 reactions

NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)
#E7580S 24 reactions
#E7580L 96 reactions

NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)
#E7560S 96 reactions

NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)
#E7330S 24 reactions
#E7330L 96 reactions

For adenylation of custom ssDNA adaptors, the 5´ DNA Adenylation Kit is available (NEB #E2610).

The novel NEBNext Small RNA workflow has been optimized to minimize adaptor-dimers while producing high-yield, high-diversity libraries. Adaptors and primers are included in the Small RNA kits, and multiplexing options are available. The Multiplex kit contains index primers, and the Multiplex-Compatible kit enables use with your own barcode primers.

In addition to stringent QCs on individual components, the NEBNext Small RNA kits are functionally validated by library preparation of a Small RNA library, followed by Illumina sequencing. Reagent lots are also reserved specifically for inclusion in NEBNext kits. Most of these components are available in master mix format, reducing the number of vials provided in the kits, and reducing pipetting steps.

Input 100 ng – 1 µg

3´ Adaptor Ligation	Primer Hybridization	5´ Adaptor Ligation	First Strand Synthesis	PCR Enrichment	Size Selection	Total Workflow
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1 NEB #E7300, Set 2 NEB #E7580)						
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp® *Taq* 2X Master Mix • SR Primer • Index Primers 1–12 (Set 1) • Index Primers 13–24 (Set 2)	• Gel Loading Dye, Blue (6X) • Quick-Load® pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	**Hands-On Time** 30 min **Total Time** 6 hrs
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48) (NEB #E7560)						
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp *Taq* 2X Master Mix • SR Primer • NEBNext Index 1-48 Primers for Illumina	• Gel Loading Dye, Blue (6X) • Quick-Load pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	**Hands-On Time** 30 min **Total Time** 6 hrs
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) (NEB #E7330)						
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp *Taq* 2X Master Mix • SR Primer • Index Primer 1	• Gel Loading Dye, Blue (6X) • Quick-Load pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	**Hands-On Time** 30 min **Total Time** 6 hrs

Isabel has been a Technical Support Scientist at NEB for over 3 years. She is a member of the NEB Running Club and the Hostess of the NEB Internal Tech Support Blog.

NEBNext Adaptors & Primers for Illumina

NEW
NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs)
#E6440S 96 rxns (96 indices)
#E6440L 384 rxns (96 indices)

NEBNext Multiplex Oligos for Illumina
(Dual Index Primers Set 1)
#E7600S 96 rxns (8 x 12 indices)

NEW
NEBNext Multiplex Oligos for Illumina
(Dual Index Primers Set 2)
#E7780S 96 rxns (8 x 12 indices)

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 1)
#E7335S 24 rxns (12 indices)
#E7335L 96 rxns (12 indices)

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 2)
#E7500S 24 rxns (12 indices)
#E7500L 96 rxns (12 indices)

NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)
#E7710S 24 rxns (12 indices)
#E7710L 96 rxns (12 indices)

NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)
#E7730S 24 rxns (12 indices)
#E7730L 96 rxns (12 indices)

NEBNext Multiplex Oligos for Illumina
(96 Index Primers)
#E6609S 96 rxns (96 indices)
#E6609L 384 rxns (96 indices)

NEBNext Multiplex Oligos for Illumina
(Methylated Adaptor, Index Primers Set 1)
#E7535S 24 rxns (12 indices)
#E7535L 96 rxns (12 indices)

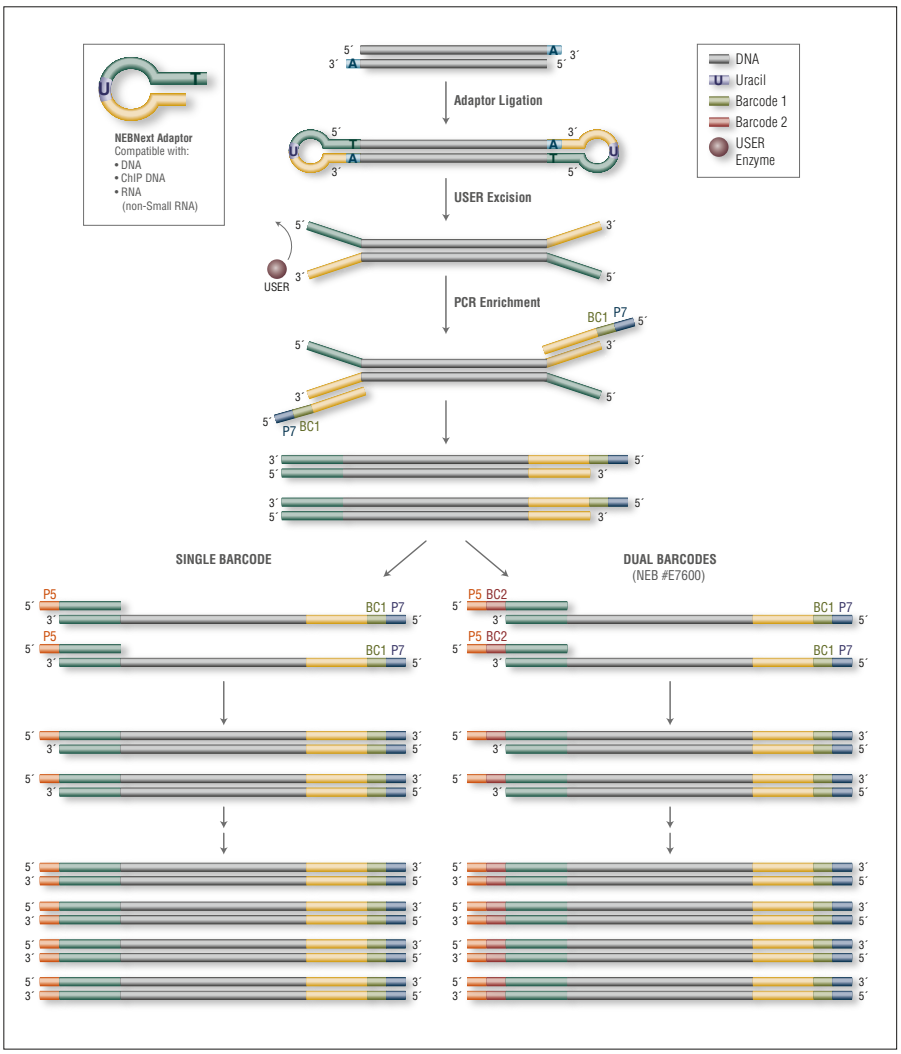
NEW
NEBNext Adaptor Dilution Buffer
#B1430S 1 x 9.6 ml

Description: Designed for use in library prep for DNA, ChIP DNA and RNA (but not Small RNA), the NEBNext Adaptors enable high-efficiency adaptor ligation and high library yields, with minimized adaptor-dimer formation. Incorporating a novel hairpin loop structure, the NEBNext Adaptor ligates with increased efficiency to end-repaired, dA-tailed DNA. The loop contains a U, which is removed by treatment with USER® Enzyme (a combination of UDG and Endo VIII), to open up the loop and make it available as a substrate for PCR. During PCR, barcodes can be incorporated by use of the NEBNext index primers, thereby enabling multiplexing. NEBNext Oligos can be used with NEBNext products, and with other standard Illumina-compatible library preparation protocols.

Single or dual barcode primer options are available. Unique dual index primer pairs are available to address the "index hopping" seen with certain Illumina sequencig instruments.

Functional Validation: Each set is functionally validated by construction of libraries, followed by Illumina sequencing.

- *Increased ligation efficiency*
- *Minimized adaptor-dimer formation*
- *Increased library yields*
- *Increased sample identification specificity (dual barcodes)*
- *Unique dual index pairs enable detection of barcode hopping*
- *Large number of barcodes/indices available*
- *Index pooling guidelines and sample sheets are provided*



NEBNext rRNA Depletion Kits (Human/Mouse/Rat)

NEBNext rRNA Depletion Kit (Human/Mouse/Rat)

#E6310S	6 reactions
#E6310L	24 reactions
#E6310X	96 reactions

NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads

#E6350S	6 reactions
#E6350L	24 reactions
#E6350X	96 reactions

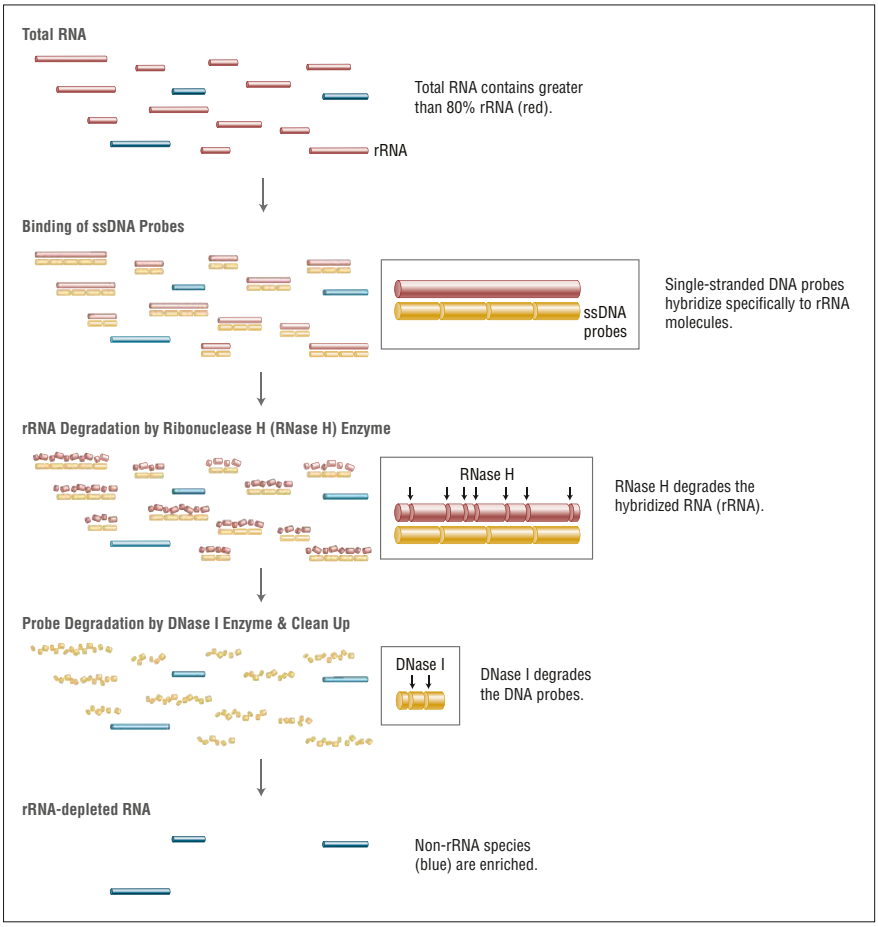
- *A single kit that performs reliably well for all of your RNA samples:*
  - *FFPE (degraded) or high-quality (intact) RNA*
  - *10 ng to 1 µg input amounts*
- *Remove > 95% of rRNA, and obtain more relevant sequence reads from your sample*
- *Obtain a more complete transcriptome picture through retention of noncoding & incomplete RNAs that are lost with oligo d(T) poly(A) mRNA enrichment methods*
- *Suitable for use with human, mouse or rat samples*
- *Easily integrated upstream of any downstream random-primed cDNA synthesis protocol*
- *Enjoy the reliability of the gold standard RNAClean beads, supplied in just the amounts you need*

Description: The NEBNext rRNA Depletion Kit (Human/Mouse/Rat) employs an RNase H-based method (1,2) to deplete both cytoplasmic (5S rRNA, 5.8S rRNA, 18S rRNA and 28S rRNA) and mitochondrial ribosomal RNA (12S rRNA and 16S rRNA) from human total RNA preparations. This product is suitable for both intact and degraded RNA (e.g., FFPE RNA). The resulting rRNA-depleted RNA is suitable for RNA-Seq, random-primed cDNA synthesis, or other downstream RNA analysis applications. This kit is now available with or without RNAClean® beads.

(1) Adiconis, X. et al (2013) *Nature Methods*, 10, 623–629.
(2) Morlon, J.D. et al (2012) *PLoS One*, 77 e42882.

The rRNA Depletion Kit Includes:

- RNase H
- RNase H Reaction Buffer (10X)
- NEBNext rRNA Depletion Solution
- NEBNext Probe Hybridization Buffer
- DNase I (RNase-free)
- DNase I Reaction Buffer
- Nuclease-free Water
- NEBNext RNA Sample Purification Beads (#E6350)



NEBNext rRNA Depletion Kit (Human/Mouse/Rat) Workflow

NEBNext Poly(A) mRNA Magnetic Isolation Module

#E7490S	24 reactions
#E7490L	96 reactions

Description: The NEBNext Poly(A) mRNA Magnetic Isolation Module is designed to isolate intact poly(A)+ RNA from previously-isolated total RNA. The technology is based on the coupling of Oligo $d(T)_{25}$ to 1 µm paramagnetic beads which are then used as the solid support for the direct binding of poly(A)+ RNA. Thus, the procedure permits the manual processing of multiple samples and can be adapted for automated high-throughput applications. Additionally, magnetic separation technology permits elution of intact mRNA in small volumes eliminating the need for precipitating the poly(A)+ transcripts in the eluent. Intact poly(A)+ RNA which is fully representative of the mRNA population of the original sample can be obtained in less than one hour.

The mRNA Magnetic Isolation Module Includes:

- NEBNext Oligo $d(T)_{25}$ Beads
- NEBNext RNA Binding Buffer (2X)
- NEBNext Wash Buffer
- Nuclease-free Water
- NEBNext Tris Buffer

NEBNext Globin & rRNA Depletion Kits

NEW
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)

#E7750S	6 reactions
#E7750L	24 reactions
#E7750X	96 reactions

NEW
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads

#E7755S	6 reactions
#E7755L	24 reactions
#E7755X	96 reactions

- *Efficient, specific depletion of globin mRNA and rRNA*
- *Suitable for low-quality or high-quality RNA*
- *Compatible with a broad range of input amounts: 10 ng–1 µg*
- *Optional integration with poly(A) mRNA isolation workflows for removal of globin RNAs, rRNAs, and noncoding RNAs*
- *Fast workflow: 2 hours, with less than 10 minutes hands-on time*
- *Available with optional RNAClean beads*

Description: The great majority of RNA in blood samples is comprised of globin mRNA as well as cytoplasmic and mitochondrial ribosomal RNAs (rRNA). These highly abundant RNA species can conceal the biological significance of less abundant transcripts, and so their efficient and specific removal is desirable.

The NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) employs the NEBNext RNase H-based RNA depletion workflow to deplete the following:

- Globin mRNA (HBA1/2, HBB, HBD, HBM, HBG1/2, HBE1, HBQ1 and HBZ)
- Cytoplasmic rRNA (5S, 5.8S, 18S and 28S)
- Mitochondrial rRNA (12S and 16S)

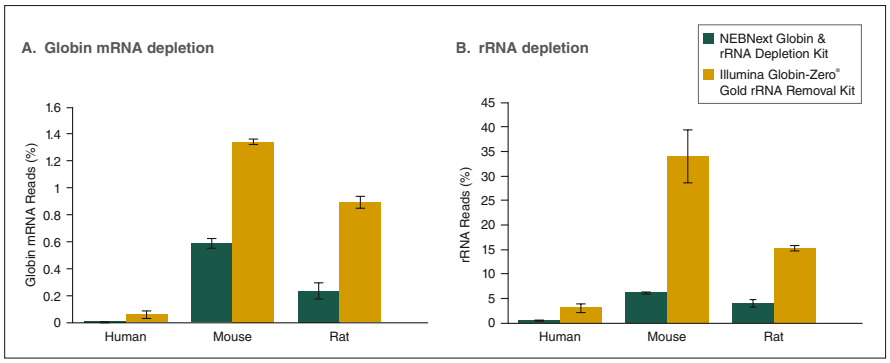
The kit is effective with human, mouse and rat total RNA preparations, both intact and degraded. The resulting depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis.

This kit can also be used following poly(A) mRNA enrichment (e.g., using the NEBNext poly(A) mRNA Magnetic Isolation Module, NEB #E7490), so that the final depleted RNA contains only mRNA of interest and no non-coding RNA.

This kit is available with or without RNAClean beads.

The Globin & rRNA Depletion Kits Include:

- RNase H
- RNase H Reaction Buffer (10X)
- NEBNext Globin & rRNA Depletion Solution
- NEBNext Probe Hybridization Buffer
- DNase I (RNase-free)
- DNase I Reaction Buffer
- Nuclease-free Water
- NEBNext RNA Sample Purification Beads (#E7785)



NEBNext Globin & rRNA Depletion Kit efficiently removes Globin mRNA and rRNA. *Ribosomal RNA (rRNA) and globin mRNA were depleted from Human, Mouse, and Rat Whole Blood Total RNA (100 ng) using the NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E7750) or the Globin-Zero® Gold rRNA Removal Kit (Illumina #GZG1224). Libraries were prepared from the depleted RNA using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina and sequenced on an Illumina NextSeq® instrument (2 x 75 bp). Reads were down sampled to 20 million reads per sample for analysis, and were identified as globin mRNA (A) or rRNA (B) using Mirabait (6 or more, 25-mers). The data represents an average of 3-4 replicates. Error bars indicate standard error.*

NEW
NEBNext Magnetic Separation Rack

#S1515S	24 tubes (0.2 ml)

- *Fast separations in purification and size-selection steps in next generation sequencing workflows*
- *Small-scale separation of magnetic particles*
- *Anodized aluminum rack with Neodymium Iron Boron (NdFeB) rare earth magnets, the most powerful commercially available*
- *24 tube capacity: 8- and 12-strip 0.2 ml PCR tubes or individual 0.2 ml PCR tubes*

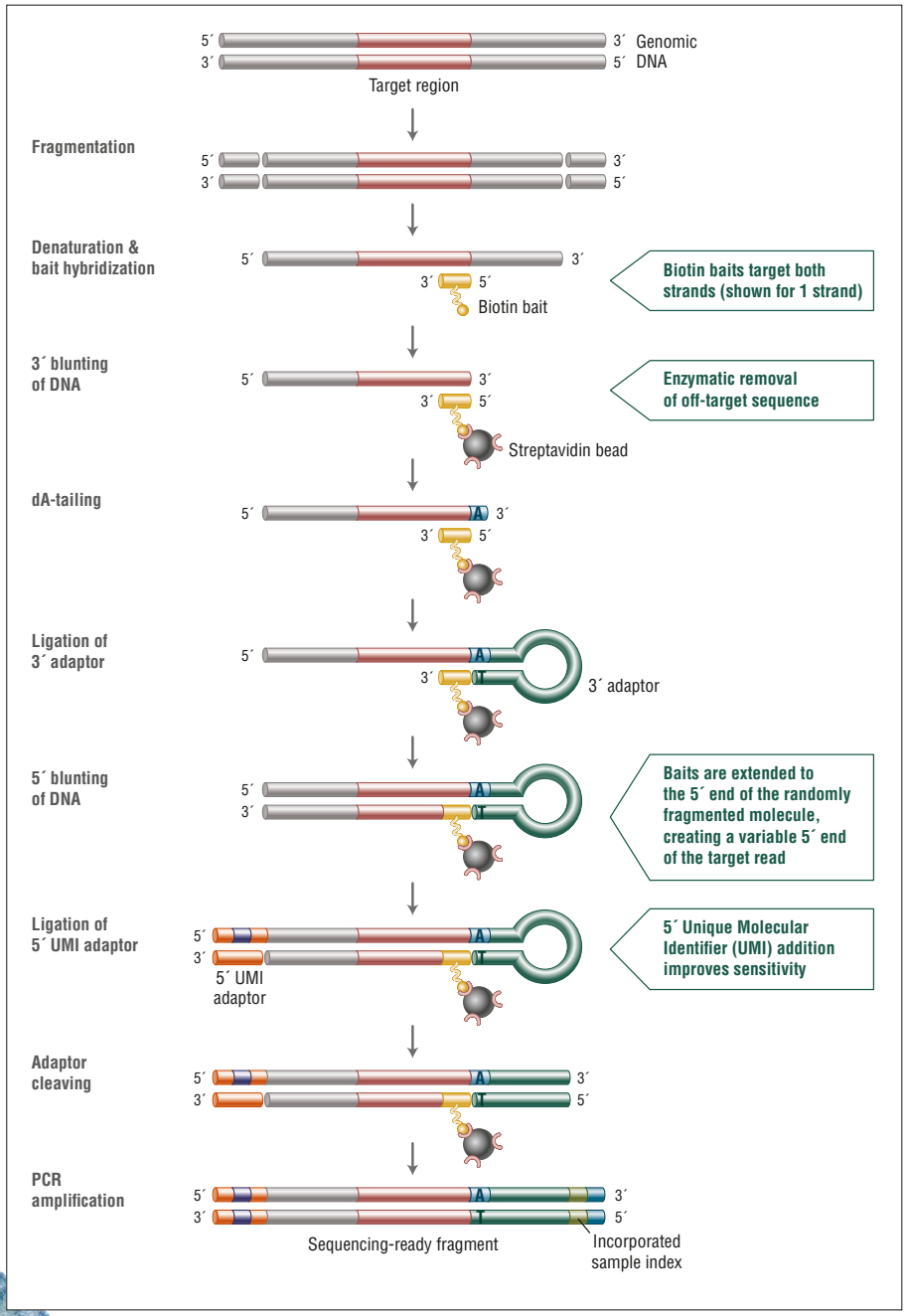
Description: Next generation sequencing library preparation workflows include magnetic bead-based purification and size-selection steps and it is important for library yield and quality that bead separation be highly efficient and fast.

The NEBNext Magnetic Separation Rack was designed for this application and contains rare earth Neodymium Iron Boron (NdFeB) magnets, the most powerful commercially available magnets, in an anodized aluminium rack. The rack holds 24 0.2 ml tubes, and is compatible with single tubes or strip tubes.

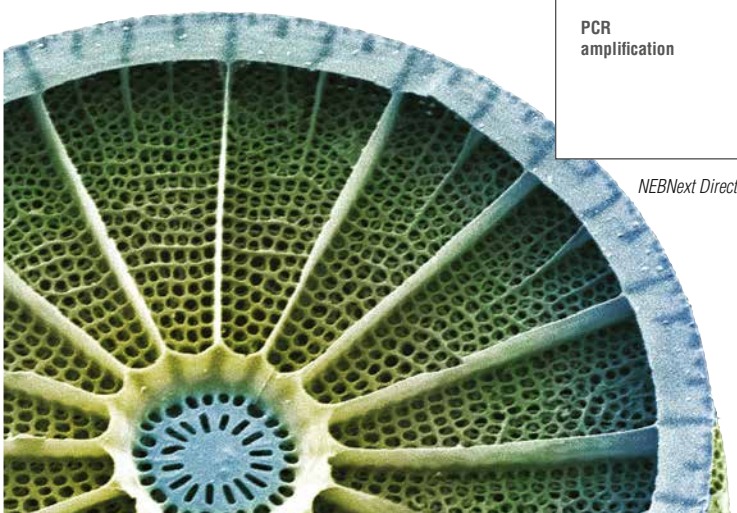
NEBNext Direct® – Target Enrichment for NGS

NEBNext Direct enables highly specific target enrichment of genomic regions of interest. This innovative approach to target enrichment balances the speed and precision of multiplexed PCR-based approaches with the content scalability typical of hybridization-based methods.

This flexibility allows a singular workflow for assays ranging from single gene tests to comprehensive panels including hundreds of genes. Regardless of sample type or assay content, NEBNext Direct allows you to enrich your targets with precision.



NEBNext Direct employs a fast hybridization-based workflow that couples capture with library preparation



View the NEBNext Direct Workflow.

NEBNext Direct Cancer HotSpot Panel

#E7000S	8 reactions
#E7000L	24 reactions
#E7000X	96 reactions

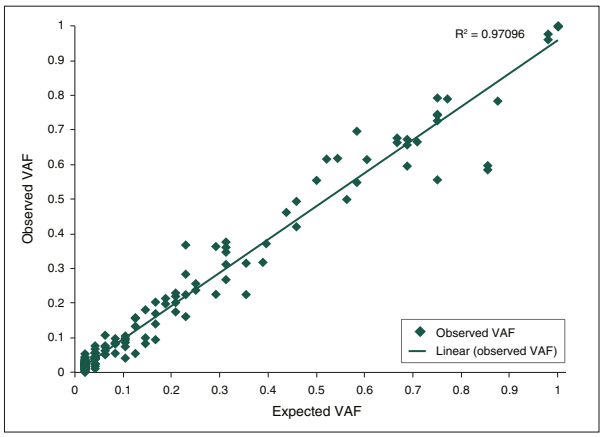
- *Generate a higher percentage of your sequencing reads aligning to your targets*
- *Eliminate the need to over-sequence, reducing cost per sample*
- *Obtain uniform sequencing of all targets, regardless of GC content*
- *Save time with a 1-day workflow that combines enrichment with library preparation*
- *Generate high quality libraries with limited input amounts and degraded DNA samples, including FFPE and ctDNA*
- *Distinguish molecular duplicates, reducing false positive variants and improving sensitivity*

Visit **NEBNextDirect.com** for more information, including additional performance data

Description: Using a novel approach to target enrichment, the NEBNext Direct Cancer Hotspot Panel enables highly specific hybridization-based capture of 190 common cancer targets from 50 genes. The NEBNext Direct technology offers significant advantages over both traditional in-solution hybridization and multiplex PCR protocols. Target enrichment is combined with library preparation, reducing processing time and minimizing sample loss. Ideal for automation, NEBNext Direct enables deep sequencing of genomic regions of interest for the discovery and identification of low frequency variants from challenging sample types.

TARGETS INCLUDE REGIONS FROM THE FOLLOWING CANCER-RELATED GENES				
ABL1	EGFR	GNAQ	KRAS	PTPN11
AKT1	ERBB2	GNAS	MET	RB1
ALK	ERBB4	HNF1A	MLH1	RET
APC	EZH2	HRAS	MPL	SMAD4
ATM	FBXW7	IDH1	NOTCH1	SMARCB1
BRAF	FGFR1	IDH2	NPM1	SMO
CDH1	FGFR2	JAK2	NRAS	SRC
CDKN2A	FGFR3	JAK3	PDGFRA	STK11
CSF1R	FLT3	KDR	PIK3CA	TP53
CTNNB1	GNA11	KIT	PTEN	VHL

For research use only, not intended for diagnostic use.



The NEBNext Direct Cancer HotSpot Panel demonstrates the ability to accurately detect a range of nucleic acid variants. *This figure shows the expected versus observed variant allele frequencies (VAF) across the range of well-characterized variants present in a pool of 24 HapMap samples screened against the NEBNext Direct Cancer HotSpot Panel. 100 ng of input DNA was used, samples were sequenced on the Illumina MiSeq using 2 x 75 bp sequencing, and standard data analysis and variant calling algorithms were used. We were able to successfully detect 100% of the 168 truth variants present across a range of 2–100% VAF. The high degree of linearity across this broad dynamic range demonstrates the ability of the NEBNext Direct Cancer HotSpot Panel to accurately predict variant allele frequencies across a broad dynamic range.*

NEW

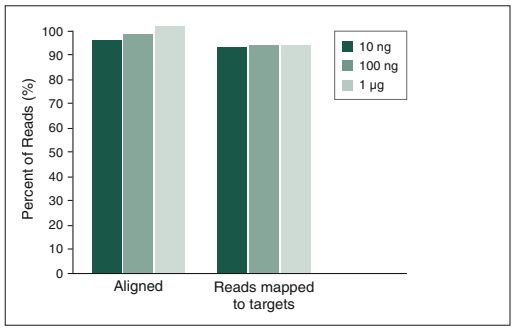
NEBNext Direct BRCA1/BRCA2 Panel

#E6627S	8 reactions
#E6627L	24 reactions
#E6627X	96 reactions

- *Generate a higher percentage of your sequencing reads aligning to your targets*
- *Eliminate the need to over-sequence, reducing cost per sample*
- *Obtain uniform sequencing of all targets, regardless of GC content*
- *Save time with a 1-day workflow that combines enrichment with library preparation*
- *Generate high quality libraries with limited input amounts and degraded DNA samples, including FFPE and ctDNA*
- *Distinguish molecular duplicates, reducing false positive variants and improving sensitivity*

Visit **NEBNextDirect.com** for more information, including additional performance data

Description: NEBNext Direct employs a unique hybridization-based enrichment workflow that hybridizes baits directly to genomic DNA, without the need for upfront library preparation. The BRCA1/BRCA2 panel demonstrates extremely high specificity and unmatched coverage uniformity across a wide range of DNA inputs, allowing highly sensitive calling of germline and somatic variants while maximizing sequencer efficiency.



The NEBNext Direct BRCA1/BRCA2 Panel delivers highly efficient enrichment of BRCA1 and BRCA2 coding regions with a high percentage of reads mapping to targets. *This histogram shows the percent of reads aligned to the human genome, and the percent of reads mapped to the targets included in the BRCA1/BRCA2 Panel across different input DNA amounts. 10 ng, 100 ng and 1 µg of purified genomic DNA was enriched using the NEBNext Direct BRCA1/BRCA2 Panel. Sequencing reads were generated on an Illumina® MiSeq with 2 x 75 bp reads, 8 bp Sample ID and 12 bp unique molecular identifier. Sequencing read alignments were performed with BWA-MEM, and PCR duplicates were filtered using the UMIs.*

NEW

NEBNext Direct Custom Ready Panels

#E6631S	8 reactions
#E6631L	24 reactions
#E6631X	96 reactions

Visit **www.neb.com/E6631** to learn more and request a quote.

- *Choose from a single gene to hundreds of genes.*
- *Experience unmatched specificity and coverage uniformity*
- *Eliminate synthesis and optimization steps, for a faster turnaround*
- *Improve sensitivity with our Unique Molecular Identifier (UMI)*
- *Generate results in one day with our automation-friendly workflow*

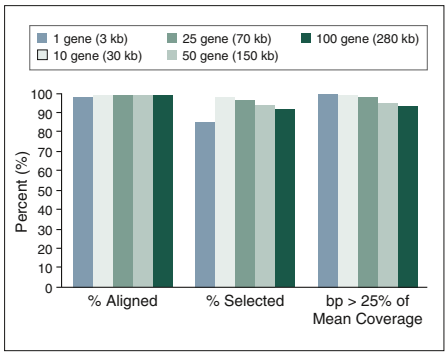
Panels can be designed and ordered by visiting **www.neb.com/CustomReadyPanelForm**

Description: Employing the unique NEBNext Direct hybridization-based enrichment method, NEBNext Direct Custom Ready Panels allow rapid customization of targeted gene panels for Illumina sequencing. Select from a list of human genes where baits have been carefully designed and optimized to provide complete coverage of the full coding (exon) regions. High quality panels can be designed by you and rapidly delivered, from any combination of genes.

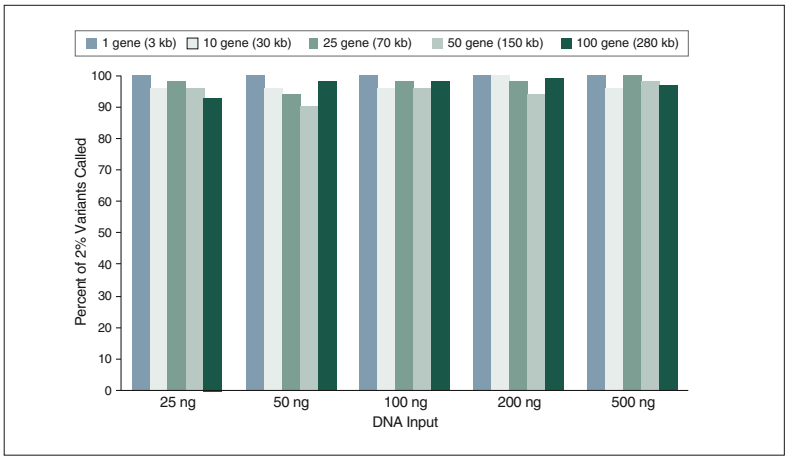
The genes available through the NEBNext Direct Custom Ready offering are continually updated, and currently include those associated with a variety of translational research areas, including cancer, neurological disorders, cardiological disease, autism, severe combined immunodeficiency, cystic fibrosis and the recommended genes for incidental findings by the American College of Medical Genetics. The full list of genes currently available can be found at www.neb.com/CustomReadyPanelForm.

NEBNext Direct Custom Ready Panels can include anywhere from a single specific gene up to 1.5 megabases of total target territory. There are no limitations on genes that can be combined together in a panel. Each panel is tested prior to shipment, and sequencing results are returned through a custom Performance Report.

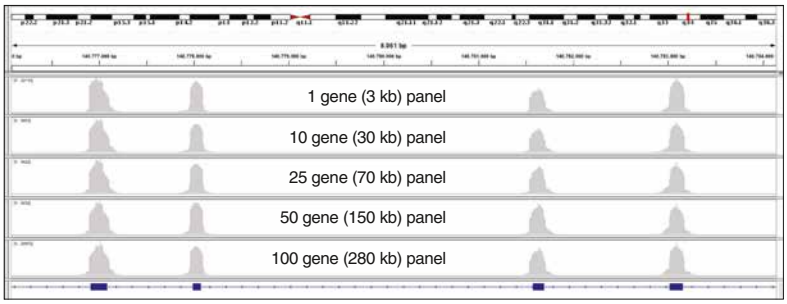
Bait sets for each gene included in the panel have undergone a rigorous development and optimization process to maximize specificity and target coverage uniformity.



NEBNext Direct Custom Ready Panels demonstrate optimum performance across a wide range of panel sizes. *Key target enrichment metrics demonstrate consistent performance across a range of panel sizes. 100 ng of DNA was tested against panels of 1, 10, 25, 50 and 100 genes, and sequenced using Illumina paired-end 150 bp sequencing. Larger panels included all genes present in smaller panels.*



Sensitivity in detection of variants across panel size and DNA input amount. *24 HapMap samples were blended to create a range of variant allele frequencies (VAF) down to 2%. 25, 50, 100, 200 and 500 ng of this blended DNA was enriched using NEBNext Direct Custom Ready Panels of 1, 10, 25, 50 and 100 genes. Larger panels were inclusive of the genes in smaller panels. Resulting libraries were sequenced using 2 x 150 bp Illumina sequencing and variants were called using Mutect and Vardict variant calling algorithms.*



NEBNext Direct Custom Ready Panels demonstrate retention of target behavior across panel sizes. *IGV image of coverage profile for 4 BRAF exons included in panels of 1, 10, 25, 50 and 100 genes, demonstrates consistent target behavior with the addition of gene targets. 100 ng of DNA was used as input for NEBNext enrichment using the 5 panels, including the BRAF gene. Libraries were sequenced on an Illumina 2 x 150 basepair sequencing.*

NEBNext Microbiome DNA Enrichment Kit

#E2612S 6 reactions
#E2612L 24 reactions

- *Effective separation of microbial DNA from contaminating host DNA*
- *Fast, simple protocol*
- *Compatible with downstream applications including next generation sequencing on all platforms, qPCR and end point PCR*
- *Suitable for a wide range of sample types*
- *No requirement for live cells*
- *Captured host DNA can also be eluted and retained*

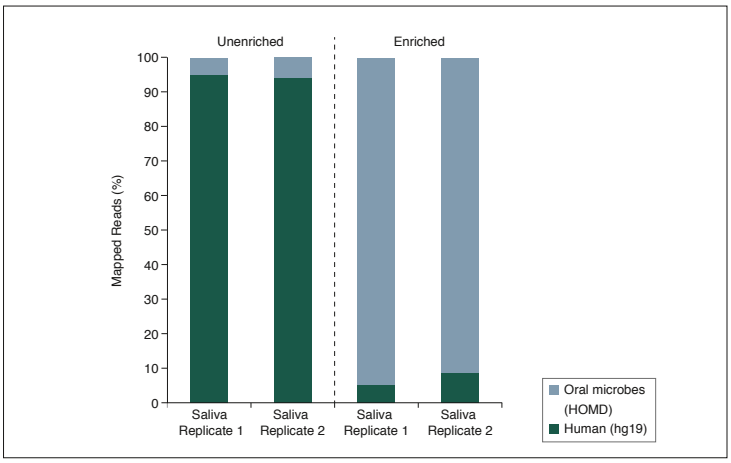
Description: The NEBNext Microbiome DNA Enrichment Kit facilitates separation of microbial DNA from methylated host DNA (including human) by selective binding and removal of the CpG methylated host DNA (1).

Functional Validation: Each set of reagents are functionally validated by enriching *E. coli* DNA from a mixture of *E. coli* and human DNA. Enrichment is evaluated through library construction and sequencing of the enriched sample on an Illumina sequencer.

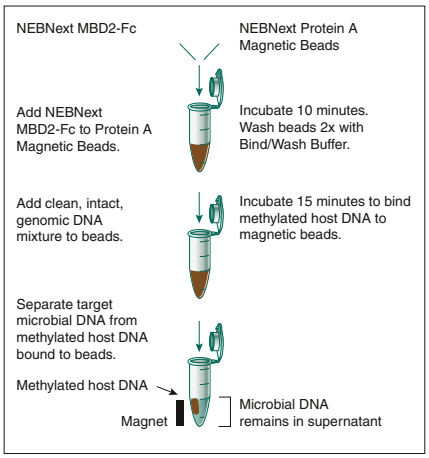
The Microbiome Enrichment Kit Includes:

– NEBNext MBD2-Fc Protein
– NEBNext Protein A Magnetic Beads
– NEBNext Bind/Wash Buffer (5X)
– 16S RNA Universal Bacteria Control Primers
– RPL30 Human DNA Control Primers

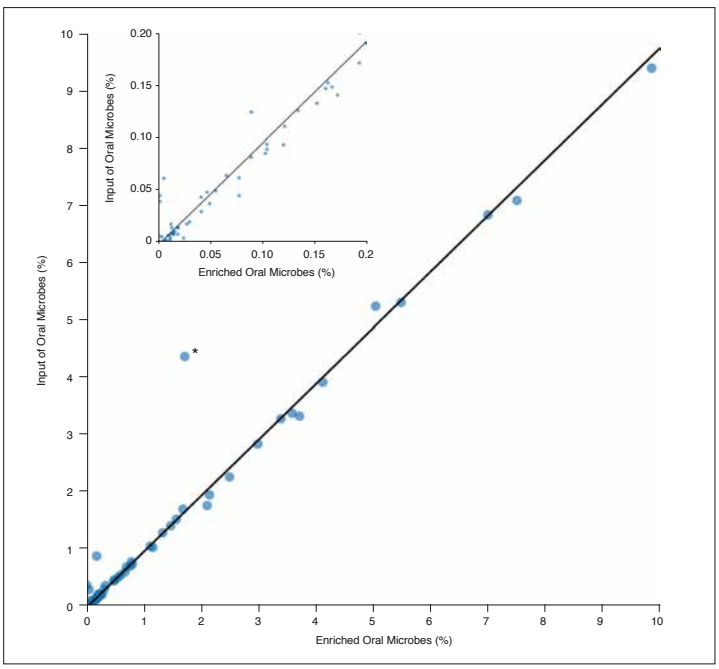
(1) Feehery, G.R. et al. (2013) *PLoS One*, 8: e76096.
(2) Chen, T., et al. (2010) *Database*, Vol. 2010, Article ID baq013, doi: 10.1093/database/baq013
(3) Langmead, B., et al. (2009) *Genome Biol.* 10:R25 doi:10.1186/gb-2009-10-3-r25



Salivary Microbiome DNA Enrichment. *DNA was purified from pooled human saliva DNA (Innovative Research) and enriched using the NEBNext Microbiome DNA Enrichment Kit. Libraries were prepared from unenriched and enriched samples and sequenced on the SOLiD 4 platform. The graph shows percentages of 500 M–537 M SOLiD™4 50 bp reads that mapped to either the Human reference sequence (hg19) or to a microbe listed in Human Oral Microbiome Database (HOMD)[2]. (Because the HOMD collection is not comprehensive, ~80% of reads in the enriched samples do not map to either database.) Reads were mapped using Bowtie 0.12.7[3] with typical settings (2 mismatches in a 28 bp seed region, etc.).* SOLiD™4 is a registered trademark of Life Technologies, Inc.



NEBNext Microbiome DNA Enrichment Kit workflow.



Microbiome Diversity is Retained after Enrichment with the NEBNext Microbiome DNA Enrichment Kit. *DNA was purified from pooled human saliva DNA (Innovative Research) and enriched using the NEBNext Microbiome DNA Enrichment Kit. Libraries were prepared from unenriched and enriched samples, followed by sequencing on the SOLiD 4 platform. The graph shows a comparison between relative abundance of each bacterial species listed in HOMD[2] before and after enrichment with the NEBNext Microbiome DNA Enrichment Kit. Abundance is inferred from the number of reads mapping to each species as a percentage of all reads mapping to HOMD. High concordance continues even to very low abundance species (inset). We compared 501M 50 bp SOliD 4 reads in the enriched dataset to 537M 50 bp SOLiD 4 reads in the unenriched dataset. Reads were mapped using Bowtie 0.12.7[3] with typical settings (2 mismatches in a 28 bp seed region, etc).*

** Niesseria flavescens – This organism may have unusual methylation density, allowing it to bind the enriching beads at a low level. Other Niesseria species (N. mucosa, N. sicca and N. elognata) are represented, but do not exhibit this anomalous enrichment.*

NEBNext FFPE DNA Repair Mix

#M6630S 24 reactions
#M6630L 96 reactions

- *Construct high-quality NGS libraries from FFPE DNA samples*
- *Use upstream of library prep for any NGS platform*
- *No alteration of DNA sequence*
- *Rely on NEB's NGS validation process for FFPE DNA library prep*

Description: Archiving of clinical materials as Formalin-Fixed, Paraffin-Embedded (FFPE) samples is a common practice. However, the methods used for fixation and storage significantly damage and compromise the quality of nucleic acids from these samples. As a result, it can be challenging to obtain useful information, including high quality sequence data, especially when sample amounts are limited. The NEBNext FFPE DNA Repair Mix is a cocktail of enzymes formulated to repair DNA, and specifically optimized and validated for repair of FFPE DNA samples. The FFPE DNA Repair Mix increases library yield and overall library success rates, without introduction of bias.

Functional Validation: Each lot is functionally validated by repair of FFPE DNA followed by library construction and Illumina sequencing.

For the most up-to-date product and pricing information, visit **NEBNext.com**

FFPE DAMAGE TYPE	REPAIRED BY THE FFPE DNA ENZYME REPAIR MIX?
Deamination of cytosine to uracil	Yes
Nicks and gaps	Yes
Oxidized bases	Yes
Blocked 3´ ends	Yes
DNA fragmentation	No
DNA-protein crosslinks	No

Table 1: *Types of FFPE DNA damage and ability to be repaired by the NEBNext FFPE DNA Repair Mix.*

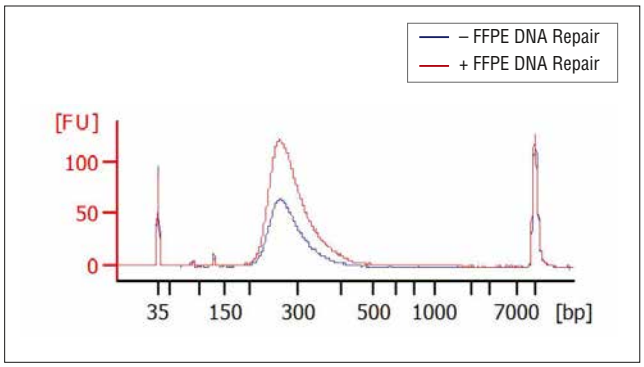


Figure 1: Effect of FFPE DNA Repair Mix on library yields. *An example of Agilent Bioanalyzer traces of libraries prepared from stomach tumor FFPE DNA that was treated with the FFPE DNA Repair Mix, or was untreated, before library construction. Yield improvements of 101% to 458% have been observed.*

NEBNext dsDNA Fragmentase®

37° 65°

#M0348S 50 reactions
#M0348L 250 reactions

Companion Product:

NEBNext dsDNA Fragmentase Reaction Buffer v2
#B0349S 6 x 1 ml

- *Generation of dsDNA fragments for sequencing on next generation sequencing platforms*
- *Generation of dsDNA fragments for libraries*

Description: NEBNext dsDNA Fragmentase generates dsDNA breaks in a time-dependent manner to yield 50–1,000 bp DNA fragments depending on reaction time. NEBNext dsDNA Fragmentase contains two enzymes, one randomly generates nicks on dsDNA and the other recognizes the nicked site and cuts the opposite DNA strand across from the nick, producing dsDNA breaks. The resulting DNA fragments contain short overhangs, 5´-phosphates, and 3´-hydroxyl groups. The random nicking activity of NEBNext dsDNA Fragmentase has been confirmed by preparing libraries for next-generation sequencing. A comparison of the sequencing results between gDNA prepared with NEBNext dsDNA fragmentase and with mechanical shearing demonstrates that the NEBNext dsDNA Fragmentase does not introduce any detectable bias during the sequencing library preparation and no difference in sequence coverage is observed using the two methods.

Source: NEBNext dsDNA Fragmentase is composed of endonucleases isolated from two different *E. coli* sources: one construct expresses a fusion protein consisting of *E. coli* maltose binding protein and *Vibrio vulnificus* nuclease mutant protein; the other expresses a fusion protein consisting of maltose binding protein and T7 endonuclease mutant protein.

Reaction Conditions: 1X NEBNext dsDNA Fragmentase Reaction Buffer v2, supplemented with 100 µM $MgCl_2$, when required. Incubate at 37°C.

1X NEBNext dsDNA Fragmentase Reaction Buffer v2:
20 mM Tris-HCl
10 mM $MgCl_2$
50 mM NaCl
0.15% Triton X-100
pH 7.5 @ 25°C

Reagents Supplied with Enzyme:
10X NEBNext dsDNA Fragmentase Reaction Buffer v2
200 mM $MgCl_2$

Heat Inactivation: 65°C for 15 minutes in the presence of 50 mM DTT

MINELUTE® is a registered trademark of the Qiagen Group.

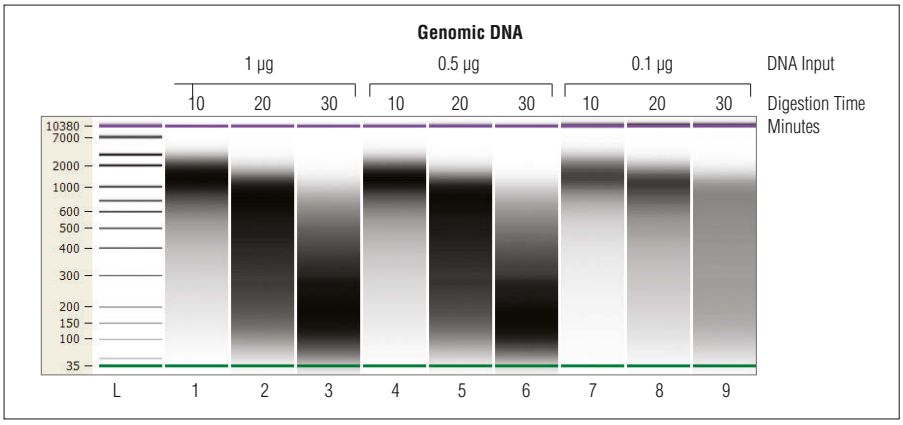


Figure 1: *Fragmentation of E. coli gDNA. E. coli gDNA was fragmented with NEBNext dsDNA Fragmentase for the indicated times and purified on MinElute® columns.*

Chaithanya has been with NEB for 5 years, and is currently a Development Scientist. His work focuses on the development of new tools for NGS sample preparation. When he isn't busy at the bench, Chaithanya enjoys photography and a good cricket match.

NEBNext Ultra II Q5® Master Mix

#M0544S	50 reactions
#M0544L	250 reactions

Additional Products:

NEBNext Q5 HotStart HiFi PCR Master Mix

#M0543S	50 reactions
#M0543L	250 reactions

NEBNext High-Fidelity 2X PCR Master Mix

#M0541S	50 reactions (50 µl)
#M0541L	250 reactions (50 µl)

- *Next generation sequencing library preparation*
- *High-fidelity amplification*
- *Uniform GC coverage*
- *Improves sequencing library coverage of known difficult regions of the human genome*

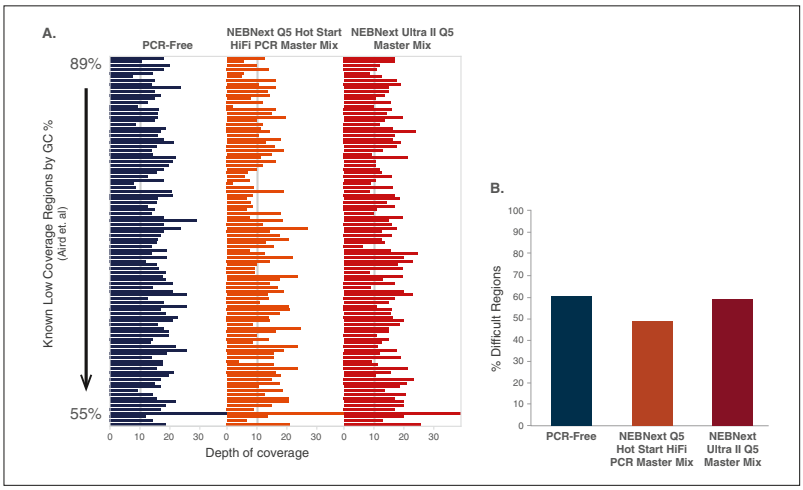
Description: The NEBNext Ultra II Q5 Master Mix is a new formulation of Q5 DNA Polymerase that has been optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries. This formulation further improves the uniformity of amplification of libraries, including superior performance with GC-rich regions.

The polymerase component of the master mix, Q5 High-Fidelity DNA Polymerase, is a novel thermostable DNA polymerase that possesses 3´→5´ exonuclease activity, and is fused to a processivity-enhancing Sso7d domain. Q5 also has the highest fidelity available (> 100-fold higher than that of *Taq* DNA Polymerase and ~12-fold higher than that of *Pyrococcus furiosus* (Pfu) DNA Polymerase), resulting in ultra-low error rates.

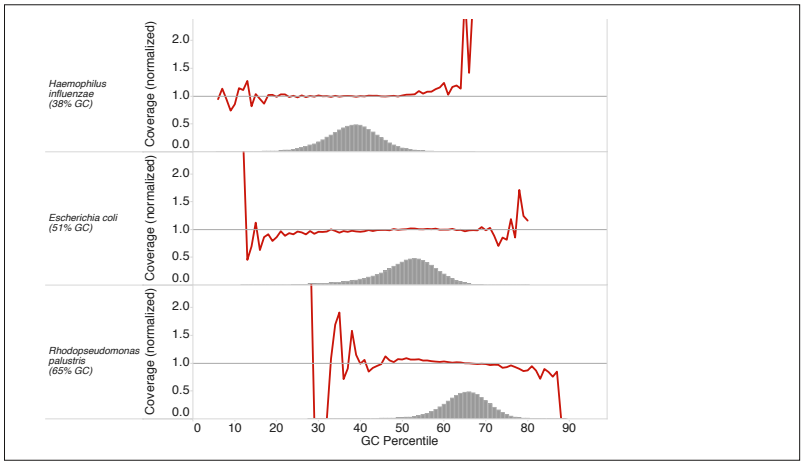
The NEBNext Ultra II Q5 Master Mix is an aptamer-based hot start formulation that allows convenient room temperature reaction set up. The convenient 2X master mix format contains dNTPs, Mg^{++} and a proprietary buffer, and requires only the addition of primers and DNA template for robust amplification. NEBNext Ultra II Q5 Master Mix is also included in the NEBNext Ultra II DNA Library Prep Kit for Illumina.

Source: An *E. coli* strain that carries the Q5 High-Fidelity DNA Polymerase gene.

Reaction Conditions: NEBNext Ultra II Q5 Master Mix, DNA template and 1 µM primers in a total reaction volume of 50 µl.



NEBNext Ultra II Q5 Master Mix provides improved coverage of known low-coverage regions of the human genome. *Libraries were prepared from Human NA19240 genomic DNA. One library was not amplified. The other two libraries were amplified using 5 cycles of PCR with NEBNext Q5 Hot Start HiFi PCR Master Mix (NEB #M0543) or with NEBNext Ultra II Q5 Master Mix (NEB #M0544). Libraries were sequenced on an Illumina NextSeq 500. 420 million 75 bp reads were randomly extracted from each dataset, representing an average coverage of 10X. Reads were mapped to the GRCh37 reference genome using Bowtie 2.2.4. Reads on each region were counted using bedtools v2.19.1.*
A: *The number of reads overlapping distinct low coverage regions of the human genome (1) are shown for each library.*
B: *From the 420 million 75 bp reads randomly extracted from each dataset, 10X coverage was expected. The % of difficult regions covered at > 10X are shown for each library. The NEBNext Ultra II Q5 Master Mix provides improved coverage of these known low coverage regions, without drop-outs, and shows similar coverage to the unamplified sample.*
(1) Popatov, V. and Ong, J.L. (2017). Examining Sources of Error in PCR by Single-Molecule Sequencing. PLoS ONE. 12(1):e0169774.



NEBNext Ultra II Q5 Master Mix provides uniform GC coverage for microbial genomic DNA with a broad range of GC composition. *Libraries were made using 100 ng of the genomic DNAs shown and the NEBNext Ultra II DNA Library Prep Kit. Libraries were amplified using the NEBNext Ultra II Q5 Master Mix, and sequenced on an Illumina MiSeq. GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library. NEBNext Ultra II Q5 Master Mix provides uniform GC coverage regardless of the GC content of the DNA.*

NEBNext Library Quant Kit for Illumina

NEBNext Library Quant Kit for Illumina
#E7630S 100 reactions
#E7630L 500 reactions

Companion Product:

NEBNext Library Dilution Buffer
#B6118S 7.5 ml

- *Provides more accurate and reproducible quant values than alternative methods and kits*
- *Compatible with libraries with a broad range of insert sizes and GC content, made by a variety of methods*
- *Requires only 4 standards, allowing more libraries to be quantitated per kit*
- *Supplied with a convenient, Library Dilution Buffer*
- *The NEBNext Library Quant Master Mix requires only the addition of primers*
- *Utilizes a single extension time for all libraries, regardless of insert size*
- *Library quant values can be easily calculated using NEB's online tool, at NEBioCalculator.neb.com*
- *ROX is included in the kit, for use with qPCR instruments that require a reference dye for normalization*

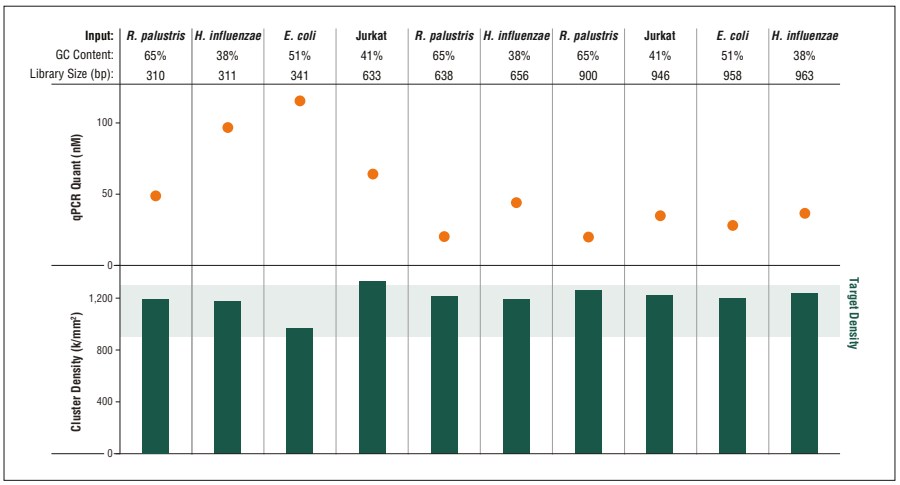
Description: Accurate quantitation of next-generation sequencing libraries is essential for maximizing data output and quality from each sequencing run. For Illumina sequencing specifically, accurate quantitation of libraries is critical to achieve optimal cluster densities, a requirement for optimal sequence performance. qPCR is considered to be the most accurate and effective method of library quantitation, providing considerably higher consistency and reproducibility than electrophoresis or spectrophotometry, which measure total nucleic acid concentration. Amplification-based methods quantitate only those molecules that contain both adaptor sequences, thereby providing a more accurate estimate of the concentration of the library molecules that can be sequenced.

The NEBNext Library Quant Kit delivers significant improvements to qPCR-based library quantitation for next gen sequencing. The NEBNext Library Quant Kit for Illumina contains components that are optimized for qPCR-based quantitation of libraries prepared for Illumina next-generation sequencing platforms. The NEBNext Library Quant Kit contains primers which target the P5 and P7 Illumina adaptor sequences and a set of high-quality, pre-diluted DNA standards to enable reliable quantitation of diluted DNA libraries between 150–1000 bp.

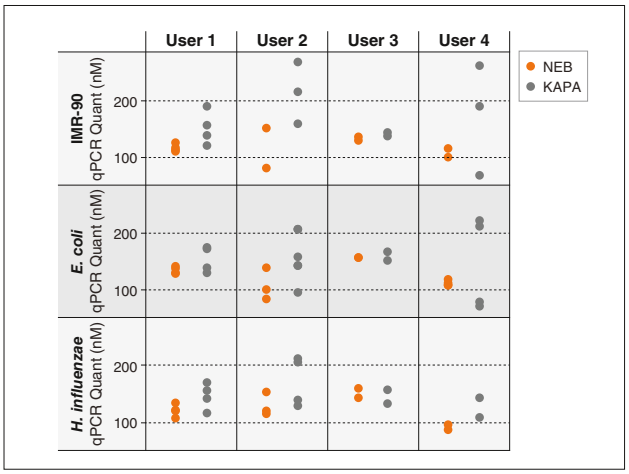
The Library Quant Kit Includes:

– NEBNext Library Quant Master Mix
– NEBNext Library Quant Primer Mix
– NEBNext Library Quant DNA Standards 1–4
– ROX (Low) and ROX (High)
– NEBNext Library Dilution Buffer

	Reagent Preparation	Library Dilution	Set Up	qPCR	Data Analysis	Total Workflow
Hands-On	5 min.	10 min.	25 min.	1 min.	10 min.	**51 min.**
Total	5 min.	10 min.	25 min.	60 min.	10 min.	**1 hr. 45 min.**



With NEBNext, optimal cluster density is achieved from quantitated libraries with a broad range of library size and GC content. Libraries of 310–963 bp from the indicated sources were quantitated using the NEBNext Library Quant Kit, then diluted to 8 pM and loaded onto a MiSeq (v2 chemistry; MCS v2.4.1.3). Library concentrations ranged from 7–120 nM, and resulting raw cluster density for all libraries was 965–1300 k/mm² (ave. =1199). Optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit for all library sizes.



Three 340–400 bp libraries were quantitated by 4 different users 2–4 times using either the NEBNext or Kapa Library Quantification Kit (Universal). A notable improvement in quantitation consistency was observed for concentrations determined by the NEBNext Kit (orange) versus those from the Kapa kit (gray).

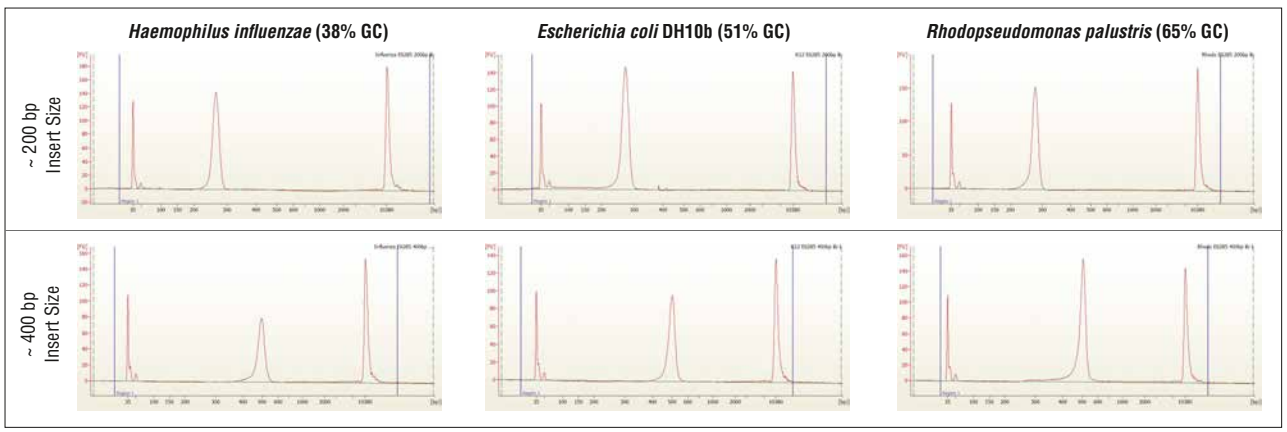
NEBNext Reagents for Ion Torrent™: DNA Library Preparation

NEBNext kits are available for DNA library preparation for Ion Torrent, with or without enzymatic DNA fragmentation. In addition to stringent QCs on individual components, the NEBNext DNA kits are functionally validated by library preparation of a genomic DNA library, followed by Ion Torrent sequencing. Reagent lots are reserved specifically for inclusion in NEBNext kits. Most of these reagents are provided in master mix format, reducing the number of vials provided in the kits, and reducing pipetting steps. Adaptors and primers for singleplex libraries are supplied in the kits. For multiplexed libraries, the Ion XPress™ Barcode Adaptors from Thermo Fisher Scientific can be used.

Input 10 ng – 1 µg*

Fragmentation	End Repair	Adaptor Ligation/Fill-In	PCR Enrichment	Total Workflow
NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent (NEB #E6285)				
• DNA Fragmentation Master Mix • DNA Fragmentation Reaction Buffer	• End Repair Enzyme Mix • End Repair Reaction Buffer (10X)	• Adaptors for Ion Torrent • T4 DNA Ligase • T4 DNA Ligase Buffer for Ion Torrent (10X) • *Bst* 2.0 WarmStart® DNA Polymerase	• Primers for Ion Torrent • NEBNext Q5 Hot Start HiFi PCR Master Mix	**Hands-On Time** 12 min. **Total Time** 110 min. – 133 min.
NEBNext Fast DNA Library Prep Set for Ion Torrent (NEB #E6270)				
	• End Repair Enzyme Mix • End Repair Reaction Buffer (10X)	• Adaptors for Ion Torrent • T4 DNA Ligase • T4 DNA Ligase Buffer for Ion Torrent (10X) • *Bst* 2.0 WarmStart DNA Polymerase	• Primers for Ion Torrent • NEBNext Q5 Hot Start HiFi PCR Master Mix	**Hands-On Time** 12 min. **Total Time** 110 min. – 133 min.

**Note that a minimum of 100 ng is recommended when used in conjunction with Ion Express Barcode Adaptors.*



Varying GC Content Libraries. *0.5 µg of DNA from 3 different genomes with varying GC content were used to construct 200 bp and 400 bp libraries using the NEBNext Fast DNA Fragmentation and Library Prep Set for Ion Torrent, analyzed by the Agilent Bioanalyzer.*

NEBNext Reagents for DNA Library Preparation – Ordering Information

Illumina Platform:

KITS FOR ILLUMINA DNA LIBRARY PREPARATION		NEB #	SIZE
DNA & ChIP	NEBNext Ultra II DNA Library Prep Kit for Illumina	E7645S/L	24/96 rxns
	NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S/L	24/96 rxns
	NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 rxns
	NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	E6177S/L	24/96 rxns
	NEBNext Ultra DNA Library Prep Kit for Illumina	E7370S/L	24/96 rxns
	NEBNext DNA Library Prep Master Mix Set for Illumina	E6040S/L	12/60 rxns
	NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina	E6240S/L	12/60 rxns
	NEBNext Enzymatic Methyl-seq Kit	E7120S/L	24/96 rxns
	NEBNext Enzymatic Methyl-seq Conversion Module	E7125S/L	24/96 rxns
MODULES & ENZYMES		**NEB #**	**SIZE**
DNA & ChIP	NEBNext FFPE DNA Repair Mix	M6630S/L	24/96 rxns
	NEBNext Microbiome DNA Enrichment Kit	E2612S/L	6/24 rxns
	NEBNext Ultra II FS DNA Module	E7810S/L	24/96 rxns
	NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 rxns
	NEBNext Ultra II Ligation Module	E7595S/L	24/96 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 rxns
	NEBNext Ultra End Repair/dA-Tailing Module	E7442S/L	24/96 rxns
	NEBNext Ultra Ligation Module	E7445S/L	24/96 rxns
	NEBNext dsDNA Fragmentase	M0348S/L	50/250 rxns
	NEBNext End Repair Module	E6050S/L	20/100 rxns
	NEBNext dA-Tailing Module	E6053S/L	20/100 rxns
	NEBNext Quick Ligation Module	E6056S/L	20/100 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
	NEBNext Q5U Master Mix	M0597S/L	50/250 rxns
	NEBNext dsDNA Fragmentase Reaction Buffer v2	B0349S	6 ml
ADAPTORS & PRIMERS		**NEB #**	**SIZE**
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1)	E7535S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 rxns
	NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	E7140S/L	24/96 rxns
	NEBNext Adaptor Dilution Buffer	B1430S	1 x 9.6 ml
TARGET ENRICHMENT		**NEB #**	**SIZE**
	NEBNext Direct Cancer HotSpot Panel	E7000S/L/X	8/24/96 rxns
	NEBNext Direct BRCA1/BRCA2 Panel	E6627S/L/X	8/24/96 rxns
	NEBNext Direct Custom Ready Panels	E6631S/L/X	8/24/96 rxns
LIBRARY QUANTITATION		**NEB #**	**SIZE**
	NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 rxns
	NEBNext Library Dilution Buffer	B6118S	7.5 ml

Ion Torrent Platform:

PRODUCTS FOR DNA LIBRARY PREPARATION		NEB #	SIZE
DNA	NEBNext Fast DNA Library Prep Set for Ion Torrent	E6270SL	50 rxns
	NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent	E6285SL	50 rxns

Suitable for Any Sequencing Platform:

DNA ENRICHMENT		NEB #	SIZE
DNA	NEBNext Microbiome DNA Enrichment Kit	E2612S/L	6/24 rxns
DNA REPAIR		**NEB #**	**SIZE**
DNA	NEBNext FFPE DNA Repair Mix	M6630S/L	24/96 rxns
MODULES & ENZYMES		**NEB #**	**SIZE**
DNA	NEBNext dsDNA Fragmentase	M0348S/L	50/250 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
	NEBNext dsDNA Fragmentase Reaction Buffer v2	B0349S	6 ml
MAGNETIC SEPARATION		**NEB #**	**SIZE**
	NEBNext Magnetic Separation Rack	S1515S	24 tubes

Bo is a Research Scientist and has been with NEB for more than 3 years. When she is not busy at the bench, Bo is an avid swimmer.

NEBNext Reagents for RNA Library Preparation – Ordering Information

Illumina Platform:

KITS FOR ILLUMINA RNA LIBRARY PREPARATION		NEB #	SIZE
Directional RNA	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 rxns
	NEBNext Ultra Directional RNA Library Prep Kit for Illumina	E7420S/L	24/96 rxns
Non-directional RNA	NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 rxns
	NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 rxns
	NEBNext Ultra RNA Library Prep Kit for Illumina	E7530S/L	24/96 rxns
Small RNA	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)	E7300S/L	24/96 rxns
	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)	E7580S/L	24/96 rxns
	NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)	E7560S	96 rxns
	NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)	E7330S/L	24/96 rxns
Single Cell	NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24/96 rxns
MODULES & ENZYMES		**NEB #**	**SIZE**
RNA	NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 rxns
	NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
	NEBNext Magnesium RNA Fragmentation Module	E6150S	200 rxns
	NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Second Strand Synthesis Module	E7550S/L	24/96 rxns
	NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module	E6111S/L	20/100 rxns
	NEBNext RNA First Strand Synthesis Module	E7525S/L	24/96 rxns
	NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module	E6421S/L	24/96 rxns
	NEBNext Single Cell Lysis Module	E5530S	96 rxns
	NEBNext Globin & rRNA Depletion Kit	E7750S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 rxns
DNA	NEBNext Ultra End Repair/dA-Tailing Module	E7442S/L	24/96 rxns
	NEBNext Ultra Ligation Module	E7445S/L	24/96 rxns
	NEBNext End Repair Module	E6050S/L	20/100 rxns
	NEBNext dA-Tailing Module	E6053S/L	20/100 rxns
	NEBNext Quick Ligation Module	E6056S/L	20/100 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
ADAPTORS & PRIMERS		**NEB #**	**SIZE**
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 rxns
	NEBNext Adaptor Dilution Buffer	B1430S	1 x 9.6 ml
LIBRARY QUANTITATION		**NEB #**	**SIZE**
	NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 rxns
	NEBNext Library Dilution Buffer	B6118S	7.5 ml

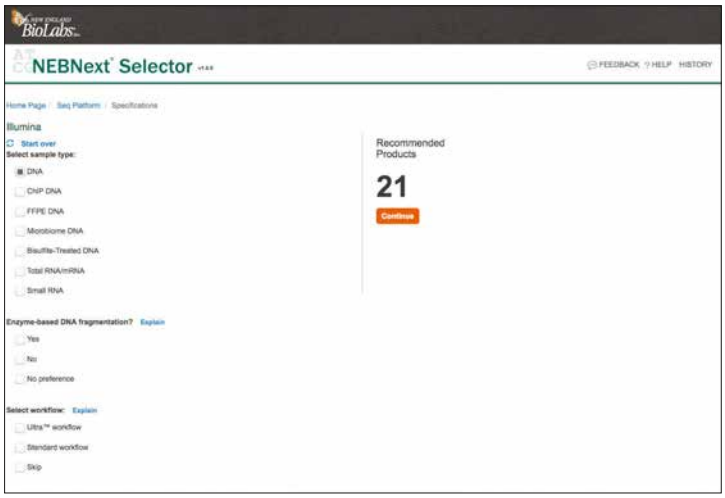
Suitable for Any Sequencing Platform:

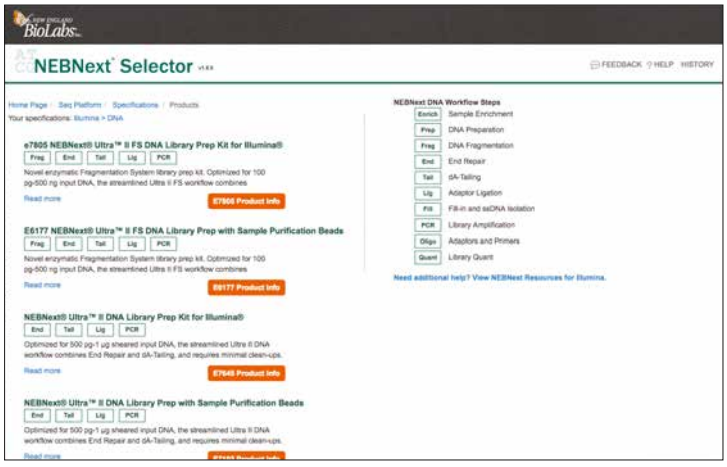
MODULES & ENZYMES		NEB #	SIZE
RNA	NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 rxns
	NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
	NEBNext Magnesium RNA Fragmentation Module	E6150S	200 rxns
	NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns
	NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module	E6111S/L	20/100 rxns
	NEBNext Globin & rRNA Depletion Kit	E7750S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 rxns
MAGNETIC SEPARATION		**NEB #**	**SIZE**
	NEBNext Magnetic Separation Rack	S1515S	24 tubes

Featured Online Tools

NEBNext Selector

Use this tool to guide you through selection of NEBNext reagents for next generation sequencing sample preparation. Try it out at **NEBNextSelector.neb.com**





Bee foraging on a blue thistle (*Eryngium planum*) in a garden.
Credit: Frédéric Collin, Getty Images

The Threat to Pollinators

In 2006, beekeepers were reporting huge losses in their honeybee colonies — 30-90% colony loss, up from 15-20% in previous years. This phenomenon was termed Colony Collapse Disorder (CCD), because worker bees would leave their colony, never to return, ultimately causing the entire colony to collapse.

It has since been generally agreed upon that many different environmental factors can cause CCD. Farming practices no longer include cover crops of clover and alfalfa — which are highly nutritious for bees — and the land is now used to grow large monocrops. Infection with Varroa mite leaves the bees immunocompromised and more susceptible to infection with other diseases. Overuse of neurotoxic pesticides (specifically, neonicotinoids) causes death, or in lower doses, disorientation that affects the bees' ability to fly back to their hive.

Furthermore, honeybees are often treated like livestock and are overworked. They are shipped from farm to farm to pollinate vast monocrops, without time to rejuvenate before they are moved on to the next crop that requires pollination. Additionally, like any managed livestock, there are high levels of disease among honeybee colonies, because of the density in which they are kept, and pathogen spillover to other species of wild pollinators is common.

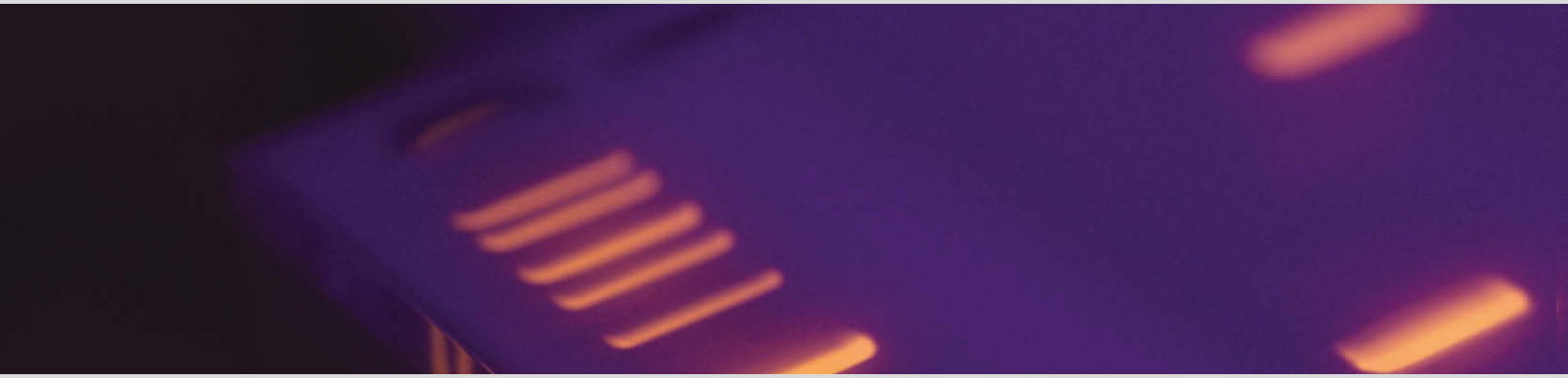
The observation in the past couple of years is that these losses are beginning to plateau. However, this could be due to more controlled breeding of honeybees to meet agricultural demands rather than addressing the environmental triggers. Did we avoid a catastrophic extinction, or is there a broader threat that remains for the health of our pollinators?

There are 20,000 species of bees in the world responsible for pollinating one third of the world's crops, which equates to two to six billion dollars in global agriculture every year. Not only do our fruits and vegetables rely on pollination to eventually make it to our table, but bees also pollinate alfalfa hay, which feeds farm animals.

Pollination of 50% of crops worldwide requires the services of not just honeybees, but a whole host of wild pollinators, including butterflies, wasps, beetles and many other bee species. Truly efficient pollination requires all of these pollinators working together — at different times of the day and on crops best suited to their abilities. For example, tomato plants are either wind pollinated or require particular strong-winged bees, such as bumblebees, to perform "buzz pollination", or sonication, whereby the bee vibrates at a high frequency to release the pollen. Loss of these pollinators means that in some farming areas, tomato plants need to be hand pollinated.

While there has been much discussion about protecting honeybees, we should in fact be thinking about the well-being of ALL the crop pollinators. Being mindful about farming practices, pesticide use and habitat disruption can help to ensure the protection and proliferation of these important workers.

Markers & Ladders (DNA, RNA & Protein)



A wide range of ladders and markers to meet your macromolecule quantification needs.

New England Biolabs provides a wide range of ladders and markers, featuring exceptional quality, uniform band intensities, convenient band spacing and easy-to-identify reference bands.

Double-stranded DNA markers are available for conventional electrophoresis. Conventional electrophoresis markers (size range: ~10 to 2.3 x 10^4 bp) include: HindIII and BstEII digests of Lambda DNA, BstNI and MspI digests of pBR322 DNA, and a HaeIII digest of ϕX174 DNA.

We also supply a series of DNA ladders ranging from 25 bp to 48.5 kb. Our 100 bp DNA ladder, 1 kb DNA Ladder and 1 kb Plus DNA Ladder are available in several formats: Conventional, Quick-Load® using either non-fluorescing purple dye or bromophenol blue as a tracking dye, and TriDye™ containing three dyes to track gel migration. Our Quick-Load Purple DNA ladders utilize our purple loading dye that improves band visibility by casting no UV shadow and are supplied with a vial of purple dye.

Our RNA ladders and markers have a size range of 17 to 9,000 bases. Both ssRNA ladders are supplied with 2X sample buffer and feature a higher intensity fragment to serve as a reference band. The dsRNA ladders are suitable for use as a size size standard in dsRNA and RNAi analysis on both denaturing polyacrylamide and agarose gels.

For your protein analysis needs, NEB offers a selection of highly pure protein standards. Sizes range from 10–250 kDa, which is ideal for accurate molecular weight determination for a wide range of expressed proteins.

Featured Products

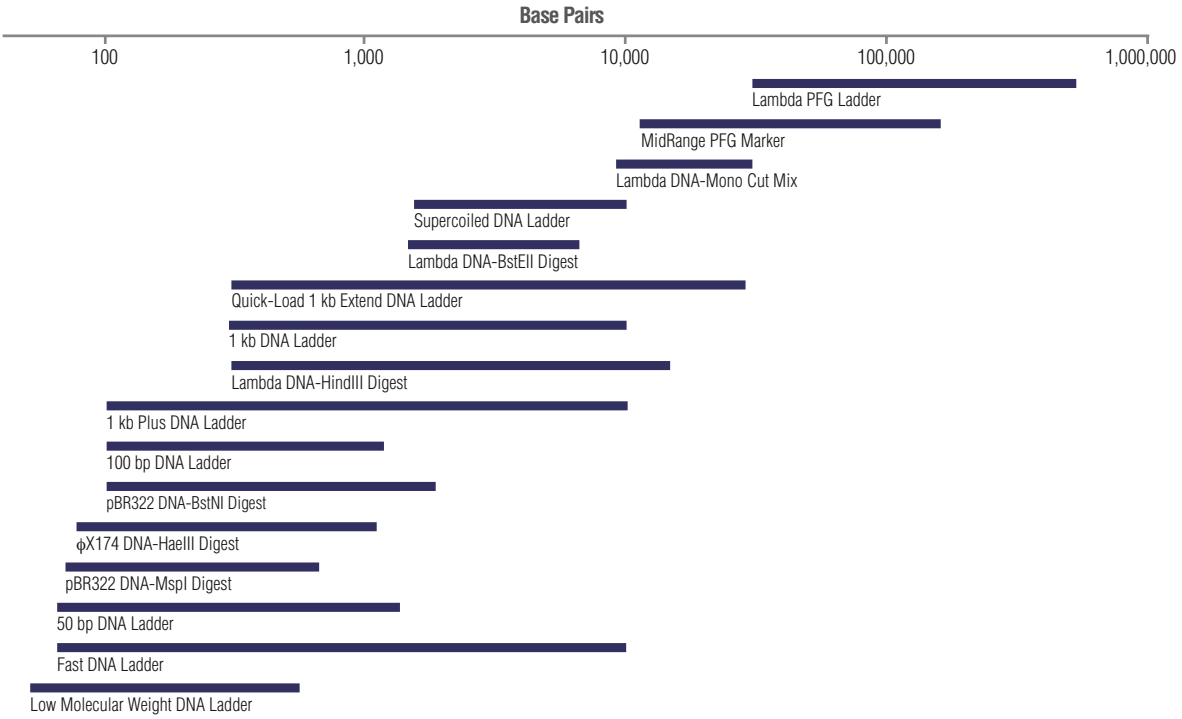
168 **Quick-Load Purple 1 kb Plus DNA Ladder**

171 **Color Prestained Protein Standard, Broad Range (10–250 kDa)**

Featured Tools & Resources

Visit www.neb.com/DNAladders to find selection charts for NEB's DNA markers and ladders.

Size Ranges of DNA Ladders from NEB

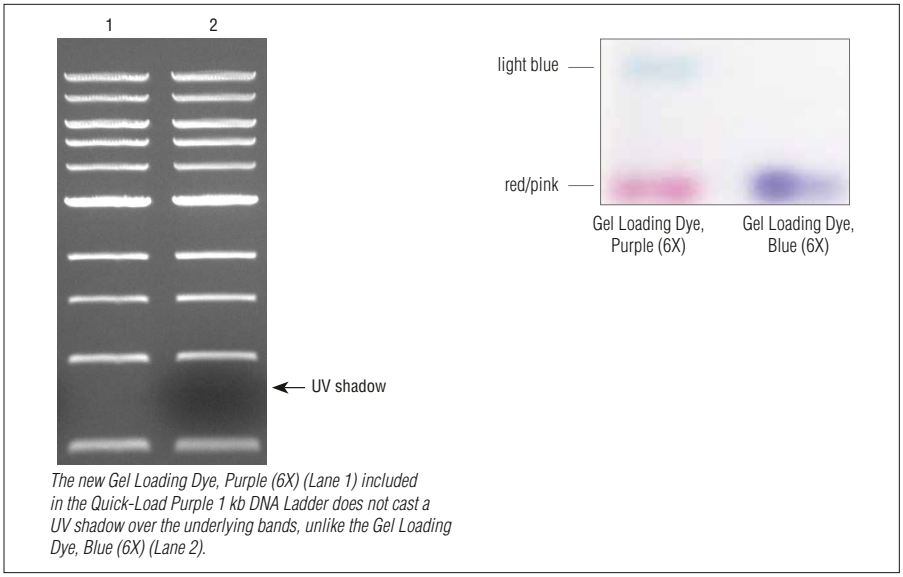


Purple Loading Dye

Gel Loading Dye, Purple (6X)
#B7024S 4.0 ml

Gel Loading Dye, Purple (6X), no SDS
#B7025S 4.0 ml

Our Gel Loading Dye, Purple (6X) (with and without SDS) is now supplied with all unstained DNA Ladders, sharpens bands and eliminates the UV shadow seen with other dyes. These pre-mixed loading buffers contain a combination of two dyes, Dye 1 (pink/red) and Dye 2 (blue). The red dye serves as the tracking dye for both agarose and non-denaturing polyacrylamide gel electrophoresis. The two dyes separate upon electrophoresis; the red band is the major indicator and migrates similarly to Bromophenol Blue on agarose gels. Specifically chosen, this dye does not leave a shadow under UV light. EDTA is also included to chelate magnesium (up to 10 mM) in enzymatic reactions, thereby stopping the reaction. The dyes also contain Ficoll, which creates brighter and tighter bands when compared to glycerol loading dyes. Gel Loading Dye, Purple (6X) contains SDS, which often results in sharper bands, as some restriction enzymes are known to remain bound to DNA following cleavage.



The new Gel Loading Dye, Purple (6X) (Lane 1) included in the Quick-Load Purple 1 kb DNA Ladder does not cast a UV shadow over the underlying bands, unlike the Gel Loading Dye, Blue (6X) (Lane 2).

DNA Ladders

1 kb DNA Ladder
#N3232S 200 gel lanes
#N3232L 1,000 gel lanes

100 bp DNA Ladder
#N3231S 100 gel lanes
#N3231L 500 gel lanes

1 kb Plus DNA Ladder
#N3200S 100-200 gel lanes
#N3200L 500-1,000 gel lanes

50 bp DNA Ladder
#N3236S 100-200 gel lanes
#N3236L 500-1,000 gel lanes

Low Molecular Weight DNA Ladder
#N3233S 100 gel lanes
#N3233L 500 gel lanes

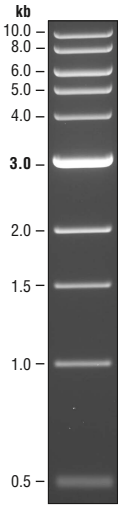
Fast DNA Ladder
#N3238S 50-200 gel lanes

PCR Marker
#N3234S 100 gel lanes
#N3234L 500 gel lanes

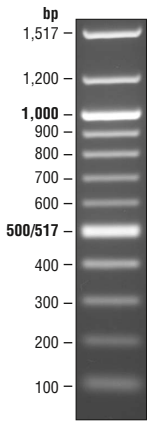
NEB offers a variety of DNA Ladders with sizes ranging from 25 bp to 48.5 kb for use in agarose gel electrophoresis.

- Stable at room temperature
- Sharp, uniform bands
- Easy-to-identify reference bands
- Supplied with 1 vial of Gel Loading Dye, Purple (6X), no SDS
- Can be used for sample quantification (see www.neb.com for mass values)

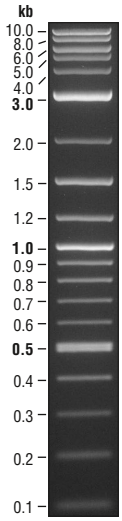
Concentration: 1 kb Plus and 50 bp DNA Ladders are supplied at 1,000 µg/ml. 1 kb, 100 bp and Low Molecular Weight DNA Ladders are supplied at 500 µg/ml. PCR Marker is supplied at 300 µg/ml. Fast DNA Ladder is supplied at 25 µg/ml in ready-to-load format.



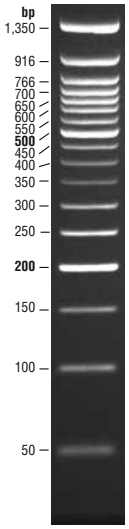
1 kb DNA Ladder
1 kb DNA Ladder visualized by ethidium bromide staining on a 0.8% TAE agarose gel.



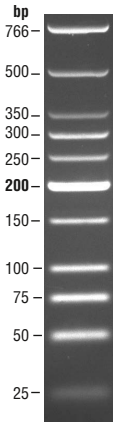
100 bp DNA Ladder
100 bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel.



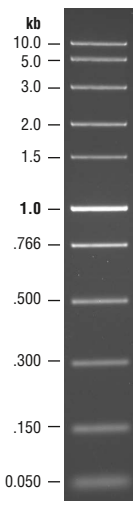
1 kb Plus DNA Ladder
1 kb Plus DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel.



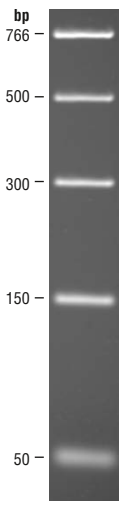
50 bp DNA Ladder
1.0 µg of 50 bp DNA Ladder visualized by ethidium bromide staining on a 3% TBE agarose gel.



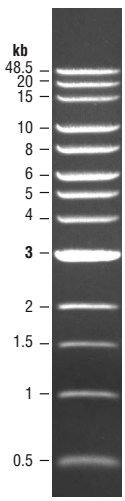
Low Molecular Weight DNA Ladder
0.5 µg of LMW DNA Ladder visualized by ethidium bromide staining on a 3% TBE agarose gel.



Fast DNA Ladder*
1.2% TBE agarose gel.



PCR Marker
0.3 µg of PCR Marker visualized by ethidium bromide staining on a 1.8% TBE agarose gel.



Quick-Load 1 kb Extend DNA Ladder
0.6% TBE agarose gel.

Usage Notes: Dilution of these markers is recommended in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O.

Individual DNA Fragments, as seen in the 1 kb DNA Ladder, are available on request.

Contact **info@neb.com** for more information.

* Fast DNA Ladder can be used for fast electrophoresis systems as well as standard electrophoresis. It is in a ready-to-load format with a xylene cyanol dye.

DNA Ladders in Convenient Pre-mixed Formats

Quick-Load® Purple Formats:

Quick-Load Purple 1 kb Plus DNA Ladder*
#N0550S 125-250 gel lanes
#N0550L 375-750 gel lanes

Quick-Load Purple 1 kb DNA Ladder*
#N0552S 125 gel lanes
#N0552L 375 gel lanes

Quick-Load Purple 100 bp DNA Ladder*
#N0551S 125 gel lanes
#N0551L 375 gel lanes

Quick-Load Purple 50 bp DNA Ladder*
#N0556S 125-250 gel lanes

Quick-Load Purple Low Molecular Weight DNA Ladder*
#N0557S 125 gel lanes

* Supplied with 1 vial of Gel Loading Dye, Purple (6X), no SDS.

TriDye™ Formats:

TriDye 1 kb Plus DNA Ladder
#N3270S 125-250 gel lanes

TriDye 1 kb DNA Ladder
#N3272S 125 gel lanes

TriDye 100 bp DNA Ladder
#N3271S 125 gel lanes

Quick-Load Formats:

Quick-Load 1 kb Plus DNA Ladder
#N0469S 125-250 gel lanes

Quick-Load 1 kb DNA Ladder
#N0468S 125 gel lanes
#N0468L 375 gel lanes

Quick-Load 1 kb Extend DNA Ladder
#N3239S 125 gel lanes

Quick-Load 100 bp DNA Ladder
#N0467S 125 gel lanes
#N0467L 375 gel lanes

- Ready-to-load
- Stable at 25°C
- Uniform band intensities
- Easy-to-identify reference bands
- Defined mass profile for sample quantification

Our 1 kb Plus, 1 kb and 100 bp DNA Ladders are offered in four formats. Choose from the conventional ladder, the Quick-Load version using either non-fluorescing, purple dye or bromophenol blue as a tracking dye, or TriDye containing three dyes to facilitate monitoring of gel migration.

PFG Ladders

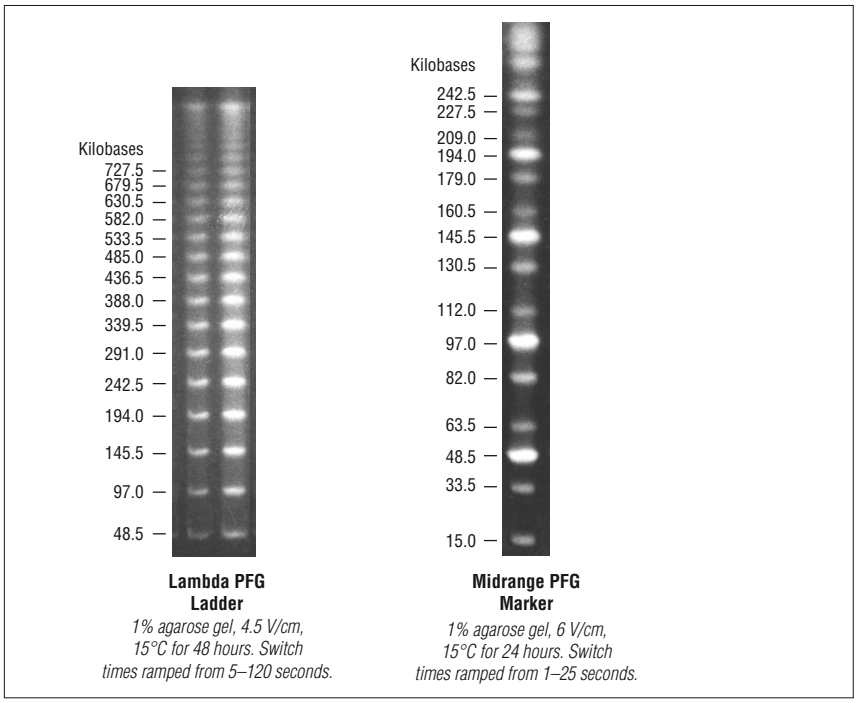
Lambda PFG Ladder
#N0341S 50 gel lanes

NEW
Midrange PFG Marker
#N0342S 50 gel lanes

The Lambda PFG Ladder consists of one gelsyringe dispenser, sufficient for 50 gel lanes. Successively larger concatemers of lambda DNA (cI857 ind 1 Sam7) are embedded in 1% LMP agarose. This product is designed to be used as size markers for pulsed-field gel electrophoresis (PFG). Size range: 48.5–1,018 kb.

MidRange PFG Marker consists of concatemers of λ DNA isolated from the bacteriophage λ (cI857 ind1 Sam7) mixed with XhoI digested λ DNA embedded in 1% LMP agarose and supplied in a gelsyringe dispenser. XhoI produces fragments of 15.0 and 33.5 kb. These fragments anneal to and form concatemers with intact λ DNA. It is designed for use as a size marker for pulsed field gel electrophoresis (PFG). Size range: 15-291 kb.

Concentration: 50 µg/ml.



Lambda PFG Ladder
1% agarose gel, 4.5 V/cm, 15°C for 48 hours. Switch times ramped from 5–120 seconds.

Midrange PFG Marker
1% agarose gel, 6 V/cm, 15°C for 24 hours. Switch times ramped from 1–25 seconds.

Usage Recommendations

Lambda PFG Ladder: The photograph represents the pulsed field gel separation of Lambda PFG Ladders using a CHEF apparatus, for 48 hours at 15°C in 0.5X TBE made with Milli-Q® water allowing resolution of 15 to 21 Lambda PFG Ladder bands.

Midrange PFG Marker: The photograph represents the pulsed field gel separation of Mid Range PFG Marker using a CHEF apparatus, for 24 hours at 15°C in 0.5X TBE made with Milli-Q water.

Usage Notes for PFG Ladders: Recommended plug sizes are from 5-10 µl. A 10 µl plug (one small graduation on the GelSyringe volume scale) contains approximately 0.5 µg of DNA. Each gelsyringe yields 50+ plugs.

Extrude agarose from gelsyringe carefully and slice plugs from the end with a sharp blade. One plug is sufficient for one lane of a gel. Place the plug at the front of the well and seal with molten agarose just above gelling temperature (~42–45°C). Allow no bubbles to form.

Melting plugs will cause denaturation of concatemers.

Never attach the agarose plugs to the gel comb before the gel is poured. Heat from the solidifying gel will cause the Lambda concatemers to denature.

Conventional DNA Markers

Lambda DNA-Mono Cut Mix
#N3019S 100 gel lanes

Lambda DNA-HindIII Digest
#N3012S 150 gel lanes
#N3012L 750 gel lanes

Lambda DNA-BstEII Digest
#N3014S 150 gel lanes

ϕX174 DNA-HaeIII Digest
#N3026S 50 gel lanes
#N3026L 250 gel lanes

pBR322 DNA-BstNI Digest
#N3031S 50 gel lanes
#N3031L 250 gel lanes

pBR322 DNA-MspI Digest
#N3032S 50 gel lanes
#N3032L 250 gel lanes

NEB offers a wide range of double-stranded DNA molecular weight markers for conventional agarose gel electrophoresis. These standards have a size range of approximately 10–23,000 base pairs.

The typical pattern generated by each of the conventional markers is shown below. The number of fragments generated by each marker, as well as the specific fragment sizes for each of the conventional markers can be found on the datacard, as well as our website, www.neb.com.

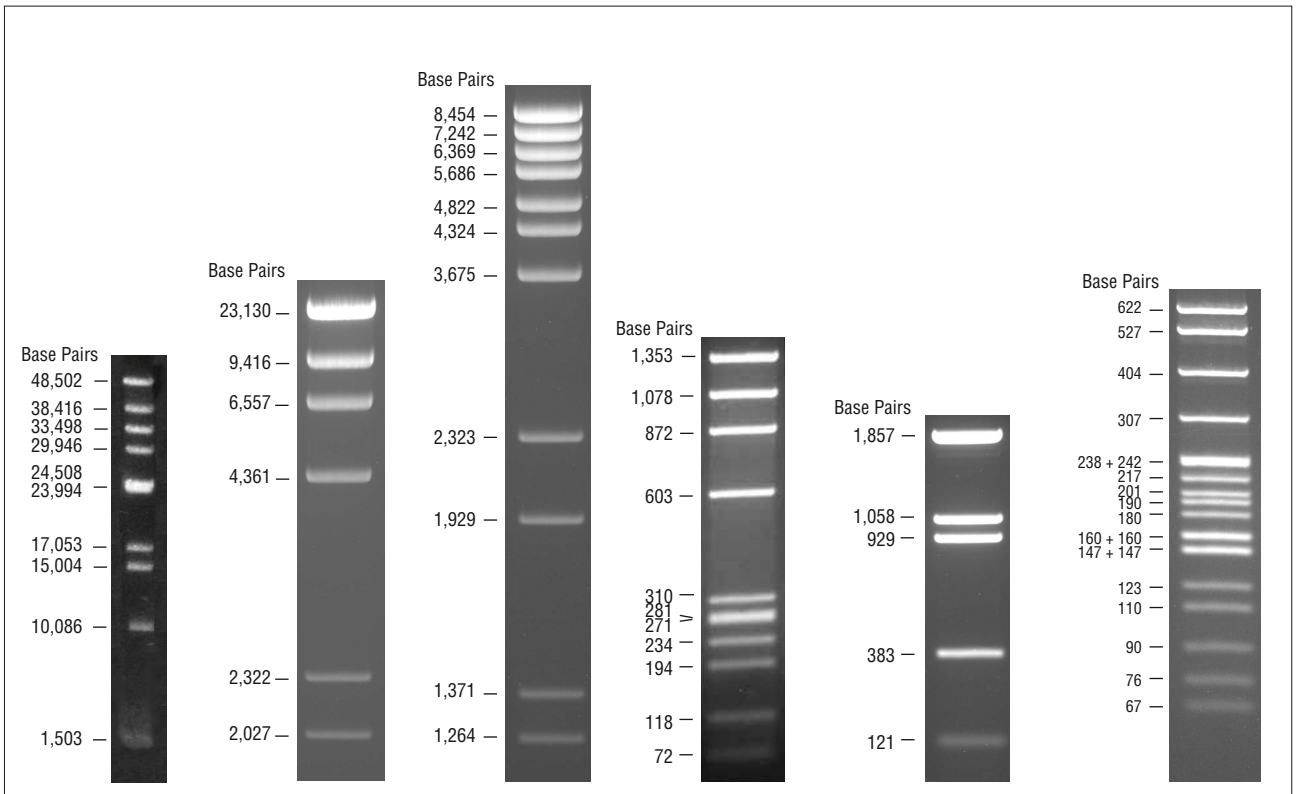
In addition, the conventional markers can be used for mass approximation. See the datacard or website for DNA mass information.

The Lambda DNA-Mono Cut Mix is best separated by Pulsed Field Gel Electrophoresis. It is designed to be used as an RFLP marker on Southern blots because it provides an uncomplicated gel pattern.

Concentration: pBR322 DNA-BstNI Digest, pBR322 DNA-MspI Digest and ϕX174 DNA-HaeIII Digest are supplied at 1,000 µg/ml. Lambda DNA-BstEII Digest, Lambda DNA-HindIII Digest and Lambda DNA-Mono Cut Mix are supplied at 500 µg/ml.

Usage Recommendation: Dilution of these markers is recommended in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O.

The cohesive ends of fragments 1 and 4 of the Lambda DNA-HindIII and Lambda DNA-BstEII Digests can be separated by heating to 60°C for 3 minutes.



Lambda DNA-Mono Cut Mix
PFGE separation of 0.5 µg of Lambda DNA-Mono Cut Mix. 1% agarose gel, 0.5X TBE, 6 V/cm, 15°C for 20 hours. Switch times ramped from 0.5–1.5 seconds.

Lambda DNA-HindIII Digest
0.5 µg of Lambda DNA-HindIII Digest visualized by ethidium bromide staining. 1.0% agarose gel.

Lambda DNA-BstEII Digest
0.5 µg of Lambda DNA-BstEII Digest visualized by ethidium bromide staining. 1.0% agarose gel.

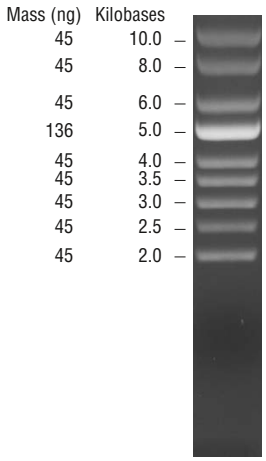
ϕX174 DNA-HaeIII Digest
0.5 µg of ϕX174 DNA-HaeIII Digest visualized by ethidium bromide staining. 1.7% agarose gel.

pBR322 DNA-BstNI Digest
0.5 µg of pBR322 DNA-BstNI Digest visualized by ethidium bromide staining. 1.4% agarose gel.

pBR322 DNA-MspI Digest
0.5 µg of pBR322 DNA-MspI Digest visualized by ethidium bromide staining. 3% agarose gel.

Supercoiled DNA Ladder

#N0472S 100 gel lanes



Supercoiled DNA Ladder
0.5 µg/lane.
0.8% TAE agarose gel.

The Supercoiled DNA ladder contains 9 proprietary supercoiled plasmids, ranging in size from 2 to 10 kb, that are suitable for use as supercoiled molecular weight standards for agarose electrophoresis. The 5 kb plasmid has an increased intensity to serve as a reference band.

Source: The 9 proprietary plasmids are purified, phenol extracted and equilibrated to 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

Concentration: 500 µg/ml.

Notes: This ladder may contain some traces of nicked DNA and dimers above the 10 kb plasmid. To minimize nicking of the supercoiled DNA, always use sterile pipette tips and avoid multiple freeze-thaw cycles. The migration of supercoiled plasmids in agarose gels can change depending on agarose concentration, buffer and electrophoresis conditions. Dilute in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O.

Usage Recommendation: Centrifuge briefly and mix gently before use. We recommend loading 0.5 µg (1 µl) of the Supercoiled DNA Ladder diluted in sample buffer. This ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size. The approximate mass of DNA in each of the bands in our Supercoiled DNA ladder is as follows (assuming a 0.5 µg loading):

Band	Base Pairs	DNA Mass
1	10,000	45 ng
2	8,000	45 ng
3	6,000	45 ng
4	**5,000**	**136 ng**
5	4,000	45 ng
6	3,500	45 ng
7	3,000	45 ng
8	2,500	45 ng
9	2,017	45 ng

RNA Markers & Ladders

dsRNA Ladder
#N0363S 25 gel lanes

microRNA Marker
#N2102S 100 gel lanes

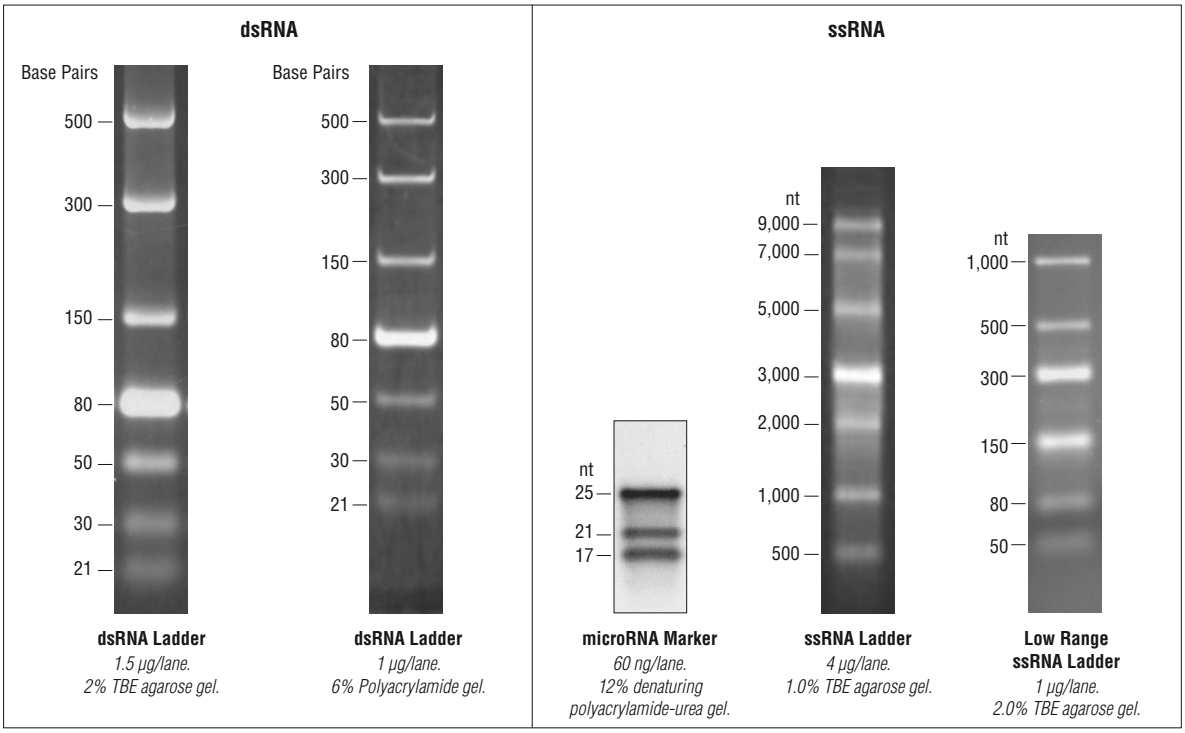
ssRNA Ladder
#N0362S 25 gel lanes

Low Range ssRNA Ladder
#N0364S 100 gel lanes

NEB offers several RNA Markers and Ladders with a size range from 17 to 9,000 bases. The Low Range ssRNA Ladder and the ssRNA Ladder are suitable for use as RNA size standards on denaturing or native gels. Both are supplied with RNA Loading Dye (2X) (NEB #B0363) and feature a higher intensity fragment to serve as a reference band. The microRNA Marker, provided in a ready-to-load denaturing solution, is ideally used as a size marker on denaturing polyacrylamide gels or northern blots and is best visualized stained with SYBR®-Gold. It is supplied with a 3´-biotinylated 21-mer oligonucleotide probe that can be labeled with γ32-P-ATP and T4 PNK (NEB #M0201). The ds RNA Ladder is suitable for use as a size standard in dsRNA and RNAi analysis on both polyacrylamide and agarose gels.

Concentration: Low Range ssRNA Ladder and dsRNA Ladder are supplied at 500 µg/ml. ssRNA Ladder is supplied at 2,000 µg/ml. MicroRNA Marker is supplied at 12 ng/µl.

SYBR® is a registered trademark of Molecular Probes, Inc.



dsRNA Ladder
1.5 µg/lane.
2% TBE agarose gel.

dsRNA Ladder
1 µg/lane.
6% Polyacrylamide gel.

microRNA Marker
60 ng/lane.
12% denaturing polyacrylamide-urea gel.

ssRNA Ladder
4 µg/lane.
1.0% TBE agarose gel.

Low Range ssRNA Ladder
1 µg/lane.
2.0% TBE agarose gel.

Protein Standards

NEW
Unstained Protein Standard, Broad Range (10–200 kDa)
#P7717S 150 gel lanes
#P7717L 750 gel lanes

NEW
Color Prestained Protein Standard, Broad Range (10–250 kDa)
#P7719S 150 gel lanes
#P7719L 750 gel lanes

NEW
Blue Prestained Protein Standard, Broad Range (11–250 kDa)
#P7718S 150 gel lanes
#P7718L 750 gel lanes

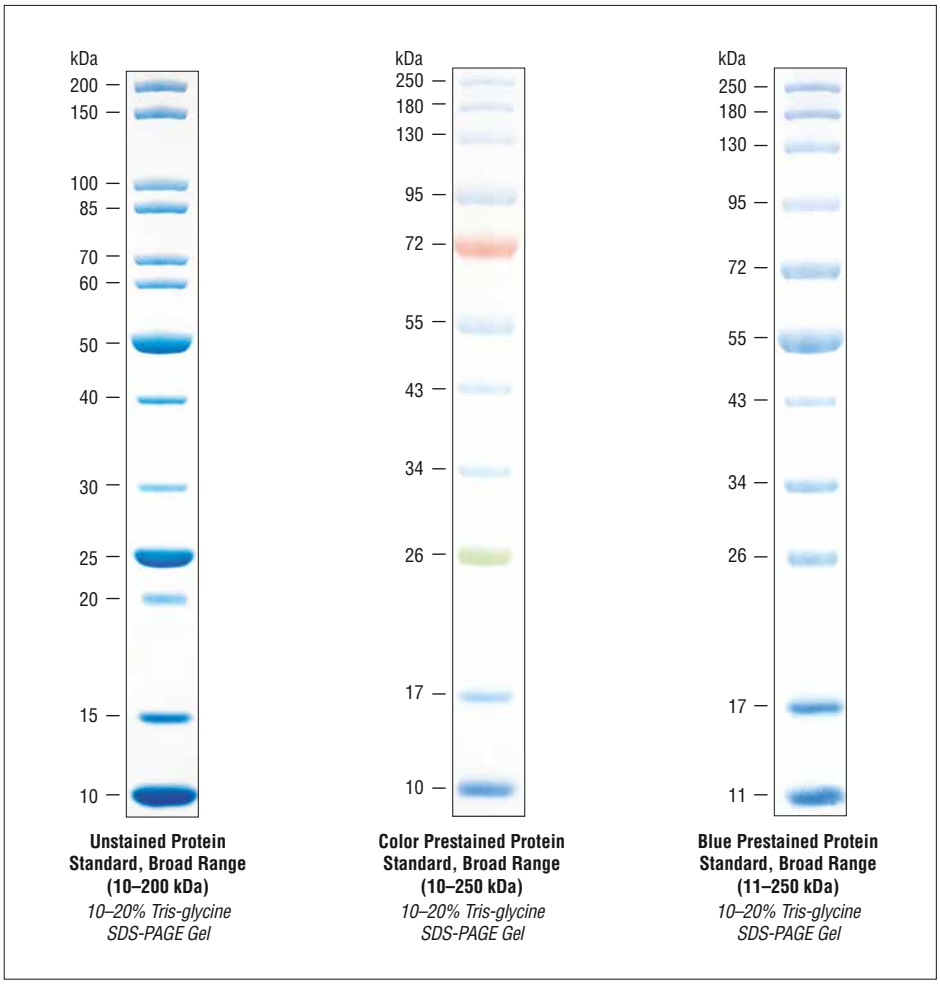
Companion Products:

Blue Loading Buffer Pack
3X Blue Loading Buffer (8 ml)
30X Reducing Agent (1 ml)
#B7703S

NEB offers a selection of highly pure protein standards available as unstained, blue prestained or color prestained (containing two colored reference bands for easy identification). Sizes range from 10 to 250 kDa which is ideal for calculating molecular weight determination for a wide range of expressed proteins. NEB protein standards provide uniform band intensities, convenient band spacing and easy-to-identify reference bands.

Recommended Load Volume: 3 µl

Note: For calculating molecular weight determinations, use NEB's Unstained Protein Standard, Broad Range.



Unstained Protein Standard, Broad Range (10–200 kDa)
10–20% Tris-glycine SDS-PAGE Gel

Color Prestained Protein Standard, Broad Range (10–250 kDa)
10–20% Tris-glycine SDS-PAGE Gel

Blue Prestained Protein Standard, Broad Range (11–250 kDa)
10–20% Tris-glycine SDS-PAGE Gel

Large blue butterfly (*Maculinea arion*) on a Common Centaury (*Centaurium erythraea*) flower.
Credit: Ross Hoddinott/ Nature Picture Library, Getty Images

The Ripple Effect

The removal or extinction of one species in an ecosystem can create gaps in the food chain. This gap can cause a “ripple” throughout the ecosystem, sequentially threatening different species due to disturbances in their food source, habitat or specialized relationships with other species.

In the late 1970s, the Large Blue Butterfly (*Maculinear arion*) was declared extinct in Britain. This beautiful, rare species was highly sought after in the 19th century by butterfly enthusiasts, and consequently, its numbers declined. Conservation efforts to fend off collectors and preserve Large Blue colonies began in the 1920s, and part of this effort included the removal of grazing sheep.

The Large Blue has a complex, predatory relationship with the red ant, *Myrmica sabuleti*. The butterfly lays its eggs on the leaves of wild thyme plants, which is where the caterpillar feeds before eventually dropping to the ground. It deceptively hunches its body so that it appears to be the size of the ant larvae and produces a pheromone which mimics that of the red ant grub. The pheromone attracts the red ant, fooling it into adopting the caterpillar and transporting it to its underground nest where it feeds on ant larvae. The caterpillar will only survive in the nest of the *Myrmica sabuleti*; all other red ant species in this environment will recognize it as an impostor and kill it. The caterpillars go as far as to mimic the sound of the Queen ants so that the worker ants will feed and clean them. At least 230 ant larvae and 354 ant workers are required to guarantee the survival of just one Large Blue Butterfly.

Researcher Jeremy Thomas carefully observed the last surviving colony of Large Blue Butterflies from 1972 until it disappeared in 1977. He pieced together the butterfly’s intricate, exploitative relationship with the red ant and also concluded that a ripple throughout the ecosystem began with the seemingly minor modification of removing grazing sheep. This habitat felt a ripple when the grass grew longer and the soil temperature decreased by just 1 or 2°C, resulting in an unsuitable habitat for the temperature- and humidity-sensitive red ant. The red ant population plummeted, and this left its predator, the very visible and appreciated Large Blue, without its food source. The Large Blue’s lifecycle was no longer viable now that the incredibly specialized environment in which it had survived, was gone.

A habitat recovery project involved re-establishing grazing animals on over 100 sites. Large Blue larvae were imported from Sweden and placed in red ant nests in the 1980s and 1990s. A period of optimization of grazing conditions was required before the red ant, and then the Large Blue, thrived.

In recent years, the number of insects in Britain and Europe has declined more than plants and birds. Thomas’ findings highlighted the need to focus on the ecological conditions that cause insect numbers to decrease and to integrate their needs with modern land use. The intensive research project to restore the habitat of the Large Blue was the first effort to reverse the decrease in numbers of an endangered insect species, which has helped other declining insect populations, and hopefully will help to prevent other such “ripple effects”.

Genome Editing

Programmable nucleases for your applications.

Easily changing the sequence specificity of a DNA binding protein enables many new possibilities for the detection and manipulation of DNA, including for genome editing – creating targeted changes in the DNA of living cells. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) -associated (Cas) nucleases have been adapted from bacterial immune systems into useful tools for biotechnology. Cas nucleases including Cas9 and Cas12a, are attractive for genome editing because they are easily programmable with a single guide RNA or crRNA/tracrRNA to introduce a double-stranded break at a specific target. The resultant breaks are repaired by cellular machinery, in some applications using an exogenous repair template.

The ease of programming Cas nucleases, and their conversion into nicking endonucleases, or DNA binding proteins without nuclease activity has expanded their use to include delivering a specific cargo to a locus for applications including visualization, activation, repression and base editing. Furthermore, Cas nuclease and their variants are useful tools for the detection and manipulation of DNA *in vitro*. .

Featured Products

178 EnGen Mutation Detection Kit

179 EnGen sgRNA Synthesis Kit, *S. pyogenes*

177 EnGen Lba Cas12a (Cpf1)

177 EnGen Sau Cas9

Featured Tools & Resources

Visit www.neb.com/GenomeEditing for more information, including our feature article and latest brochure.

Learn more about genome editing.

FEATURED PRODUCTS SUPPORTING CRISPR WORKFLOWS

RH Recombinant Enzyme

Featured NEB Products Supporting CRISPR Workflows

New England Biolabs provides reagents to support a broad variety of CRISPR/Cas genome editing approaches. From introduction of Cas and single guide RNA (sgRNA) on plasmids, to direct introduction of Cas ribonucleoprotein (RNP) and detection of edits using next generation sequencing or enzymatic mutation detection, NEB® provides reagents that simplify and shorten genome editing workflows.

PRODUCT	CRISPR/Cas9 APPLICATION	NEB #	SIZE
EnGen® Spy Cas9 NLS	*in vitro* cleavage of dsDNA. Genome engineering by direct introduction of active ribonucleoproteins	M0646T/M	400/2,000 pmol
EnGen Mutation Detection Kit	Determination of the targeting efficiency of genome editing protocols	E3321S	25 rxns
EnGen sgRNA Synthesis Kit, *S. pyogenes*	Generation of microgram quantities of custom sgRNA	E3322S	20 rxns
NEW **EnGen Spy Cas9 Nickase**	*in vitro* nicking of dsDNA. Genome engineering by direct introduction of active nickase complexes.	M0650S/T	70/400 pmol
NEW **EnGen Spy dCas9 (SNAP-tag®)**	Programmable binding of DNA. Compatible with SNAP-tag substrates for visualization and enrichment.	M0652S/T	70/400 pmol
NEW **EnGen Lba Cas12a (Cpf1)**	*in vitro* cleavage of dsDNA. Genome engineering by direct introduction of active nuclease complexes. Recognizes 5´-TTTN PAM.	M0653S/T	70/2,000 pmol
NEW **EnGen Sau Cas9**	*in vitro* cleavage of dsDNA. Genome engineering by direct introduction of active nuclease complexes. Recognizes 5´-NNGRRT-3´ PAM.	M0654S/T	70/400 pmol
Cas9 Nuclease, *S. pyogenes*	*in vitro* cleavage of dsDNA. Genome engineering by direct introduction of active ribonucleoproteins.	M0386S/T/M	70/400/ 2,000 pmol
Monarch® Total RNA Miniprep Kit	Purification of total RNA, with a binding capacity of up to 100 µg	T2010S	50 preps
Q5® Site-Directed Mutagenesis Kit (with or without competent cells)	Insertion of target sequence into a Cas9-sgRNA construct and modification of HDR templates	E0554S/E0552S	10 rxns
Q5 High-Fidelity DNA Polymerases	High-fidelity construct generation for use with CRISPR workflows and for sequencing	Multiple	Multiple
NEBuilder® HiFi DNA Assembly Master Mix	Single-tube, isothermal generation of the Cas9-sgRNA construct and HDR templates	E2621S/L/X	10/50/250 rxns
NEBuilder HiFi DNA Assembly Cloning Kit	Single-tube, isothermal generation of the Cas9-sgRNA construct and HDR templates	E5520S	10 rxns
HiScribe T7 ARCA mRNA Kit (with or without tailing)	Generation of Cas9 mRNA with ARCA cap	E2060S/E2065S	20 rxns
HiScribe T7 High Yield RNA Synthesis Kit	Generation of sgRNA and Cas9 mRNA	E2040S	50 rxns
HiScribe T7 Quick High Yield RNA Synthesis Kit	Generation of sgRNA and Cas9 mRNA	E2050S	50 rxns
T7 Endonuclease I	Determination of the editing efficiency of genome editing experiments	M0302S/L	250/1,250 units

Programmable Cas Nucleases

The highest efficiency strategy for genome editing with CRISPR/Cas nucleases is direct introduction of Cas/guide RNA complexes. This method further simplifies CRISPR/Cas workflows and has been reported to increase on-target editing activity and reduce off-target events. NEB provides purified Cas9 nucleases from *S. pyogenes* and *S. aureus*, and Cas12a (Cpf1) nuclease from *Lachnospiraceae* bacterium ND2006. In addition, NEB provides variants of Cas9 from *S. pyogenes*, including nicking endonuclease and endonuclease deficient versions.

PRODUCT	NEB #	FEATURES	SIZE
Cas9 Nuclease, *S. pyogenes*	M0386S/T/M	• Ideal for *in vitro* digestion of dsDNA • Compatible with EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322) and the EnGen Mutation Detection Kit (NEB #E3321) • For help with oligo design, try EnGen sgbNA Template Oligo Designer – now included in the NEBioCalculator® Tool	70/300/600 pmol
EnGen Spy Cas9 NLS	M0646T/M	• Ideal for direct introduction of Cas9/sgRNA complexes • Dual NLS for improved transport to the nucleus • Compatible with EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322) and the EnGen Mutation Detection Kit (NEB #E3321)	400/2,000 pmol
NEW **EnGen Spy Cas9 Nickase**	M0650S/T	• Variant of Cas9 nuclease differing by a point mutation (D10A) in the RuvC nuclease domain • Capable of generating nicks, but not cleaving DNA • DNA double strand breaks can be generated, with reduced off-target cleavage, by targeting two sites with EnGen Cas9 Nickase in close proximity • Compatible with the EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322)	70/400 pmol
NEW **EnGen Spy dCas9 (SNAP-tag)**	M0652S/T	• An inactive mutant of Cas9 nuclease that retains programmable DNA binding activity • The N-terminal SNAP-tag allows for covalent attachment of fluorophores, biotin, and a number of other conjugates useful for visualization and target enrichment • Compatible with the EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322)	70/400 pmol
NEW **EnGen Lba Cas12a (Cpf1)**	M0653S/T	• T-rich TTTN PAM sequence opens up additional genomic regions for targeting • Shorter, 40-44 base guide RNA • Two nuclear localization signals for improved transport to the nucleus • 5´ overhanging termini on cleavage products • Active from 16 to 48°C • Maintains activity at lower temperatures than the *Acidaminococcus* orthologs, permitting editing in ectothermic organisms such as zebra fish and *xenopus* • High concentration enzyme can be used for microinjection, electroporation and lipofection	70/2,000 pmol
NEW **EnGen Sau Cas9**	M0654S/T	• 5´-NNGRRT-3´ PAM • Dual NLS for improved transport to nucleus • High concentration enzyme can be used for microinjection electroporation and lipofection • Cleaves 3 bases upstream of PAM, blunt-ended cleavage	70/400 pmol

EnGen Mutation Detection Kit

#E3321S 25 reactions

Companion Products:

Gel Loading Dye, Purple (6X), no SDS
#B7025S 4 ml

Quick-Load Purple 1 kb Plus DNA Ladder
#N0550S 250 gel lanes
#N0550L 750 gel lanes

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 reactions
#M0494L 500 reactions

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 reactions
#T1030L 250 reactions

EnGen Spy Cas9 NLS
#M0646T 400 pmol
#M0646M 2,000 pmol

EnGen Spy Cas9 Nickase
#M0650S 70 pmol
for high (20X) concentrationn
#M0650T 400 pmol

EnGen Spy dCas9 (SNAP-tag)
#M0652S 70 pmol
for high (20X) concentration
#M0652T 400 pmol

Cas9 Nuclease, *S.pyogenes*
#M0386S 70 pmol
for high (20X) concentration
#M0386T 400 pmol
for high (20X) concentration
#M0386M 2,000 pmol

EnGen Lba Cas12a (Cpf1)
#M0653S 70 pmol
for high (100X) concentration
#M0653T 2,000 pmol

EnGen Sau Cas9
#M0654S 70 pmol
for high (20X) concentration
#M0654T 400 pmol

T7 Endonuclease I
#M0302S 250 reactions
#M0302L 1,250 reactions

- *T7 Endonuclease-based detection of genome editing events*

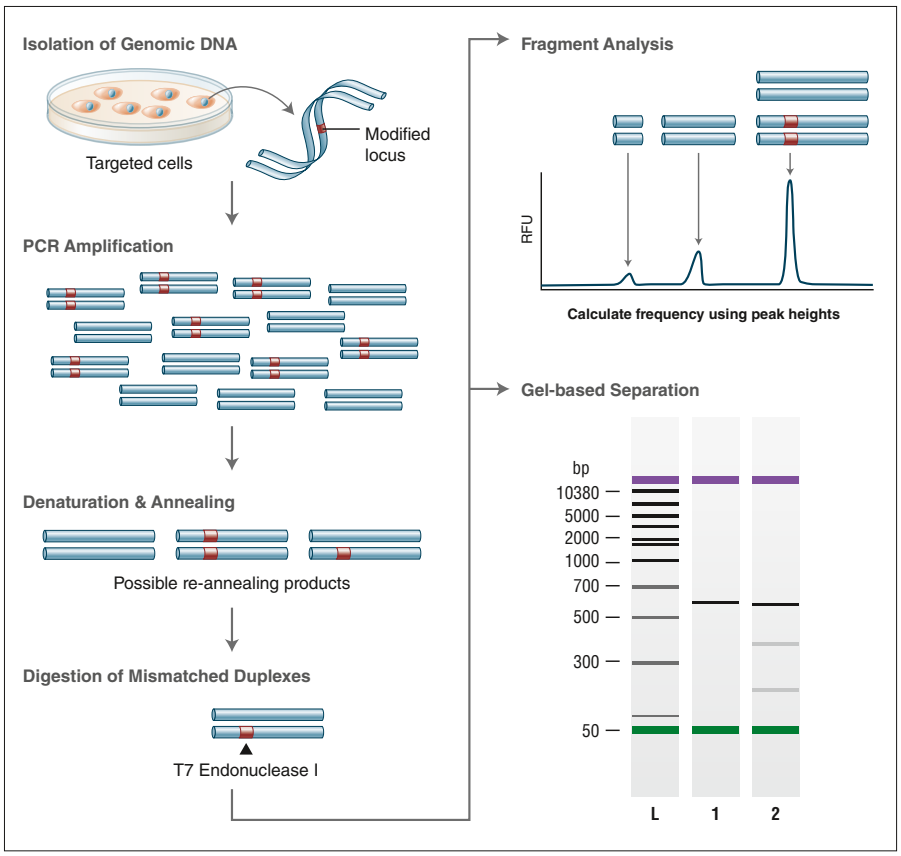
Description: The EnGen Mutation Detection Kit provides reagents for detection of on-target genome editing events. In the first step, regions targeted for genome editing (i.e. CRISPR/Cas9, TALENs, Zinc-finger Nucleases) are amplified using Q5 Hot Start High-Fidelity 2X Master Mix. Upon denaturation and re-annealing, heteroduplexes are formed when mutations from insertions and deletions (indels) are present in the amplicon pool. In the second step, annealed PCR products are digested with EnGen T7 Endonuclease I, a structure-specific enzyme that will recognize mismatches larger than 1 base. Both strands of the DNA are cut when a mismatch is present, which results in the formation of smaller fragments. Analysis of the resulting fragments provides an estimate of the efficiency of the genome editing experiments.

The EnGen Mutation Detection Kit includes a Control Template and Primer Mix that can be used as a control for the PCR reaction and T7 Endonuclease I digestion. The Control Template and Primer Mix provided contains two plasmids and primers that when amplified, denatured and re-annealed will form heteroduplexes that contain a 10-base insertion. This structure is a substrate for T7 Endonuclease I. The digestion of the 600 bp heteroduplex containing amplicon yields products of 200 bp and 400 bp. 600 bp parental homoduplexes are uncleaved, and are easily distinguished from cleaved heteroduplexes when separated and visualized by agarose gel electrophoresis or fragment analysis instrument.

The protocol has been optimized so that PCR products generated by the Q5 Hot Start High-Fidelity 2X Master Mix can be introduced directly into the T7 Endonuclease I digestion without the need for purification. Digestion of the heteroduplex is complete in only 15 minutes, and Proteinase K is included to stop the reaction efficiently. Additional Q5 Hot Start High-Fidelity 2X Master Mix is also included to allow for optimization of target site amplification before digestion.

The EnGen Mutation Kit Includes:

- Q5 Hot Start High-Fidelity 2X Master Mix
- NEBuffer 2
- EnGen T7 Endonuclease I
- Control Template and Primer Mix
- Proteinase K., Molecular Biology Grade
- Quick-Load® Purple 1 kb Plus DNA Ladder
- Gel Loading Dye, Purple (6X), no SDS



Genomic DNA is amplified with primers bracketing the modified locus. PCR products are then denatured and re-annealed yielding three classes of possible structures. Duplexes containing a mismatch greater than one base are digested by T7 Endonuclease I. The DNA is then electrophoretically separated and fragment analysis is used to estimate targeting efficiency.

EnGen sgRNA Synthesis Kit, *S. pyogenes*

#E3322S 20 reactions

Companion Products:

EnGen Spy Cas9 NLS
#M0646T 400 pmol
#M0646M 2,000 pmol

EnGen Spy Cas9 Nickase
#M0650S 70 pmol
for high (20X) concentration
#M0650T 400 pmol

EnGen Spy dCas9 (SNAP-tag)
#M0652S 70 pmol
for high (20X) concentration
#M0652T 400 pmol

Cas9 Nuclease, *S.pyogenes*
#M0386S 70 pmol
for high (20X) concentration
#M0386T 400 pmol
for high (20X) concentration
#M0386M 2,000 pmol

RNA Loading Dye, (2X)
#B0363S 4 ml

Alkaline Phosphatase, Calf Intestinal (CIP)
#M0290S 1,000 units
#M0290L 5,000 units

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *Rapid generation of microgram quantities of sgRNAs in less than one hour*

NEBioCalculator®

Configure target-specific DNA oligos design for use with the EnGen sgRNA Synthesis Kit, *S. pyogenes* with our oligo design tool accessible through NEBioCalculator® at **NEBioCalculator.neb.com**

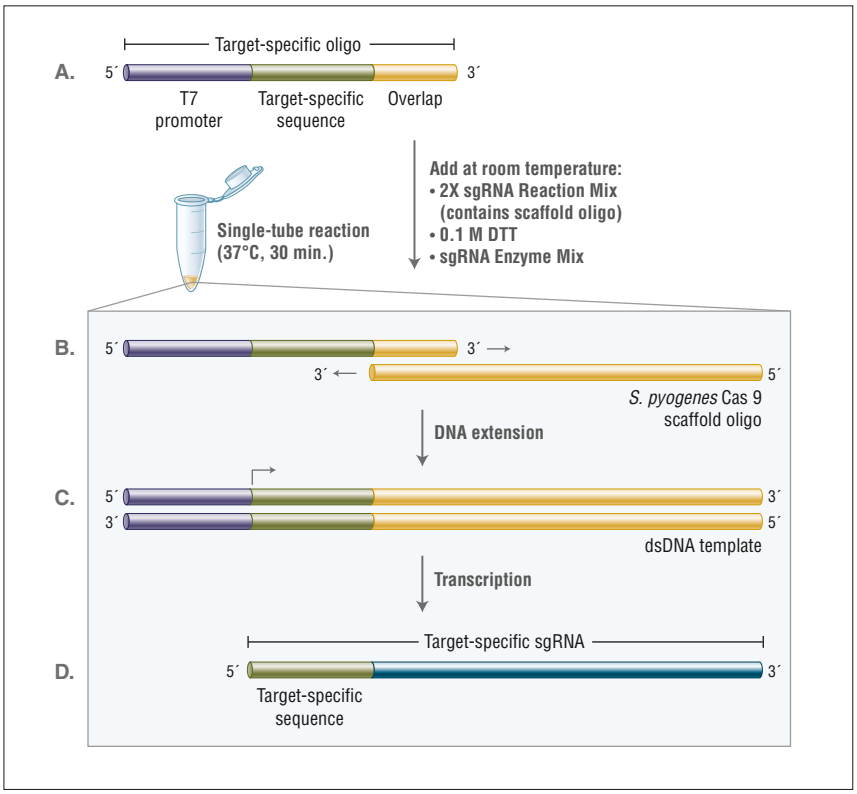
Description: The EnGen sgRNA Synthesis Kit, *S. pyogenes* provides a simple and quick method for transcribing high yields of sgRNA in a single 30 minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user.

In nature, *S. pyogenes* Cas9 is programmed with two separate RNAs, the crRNA and tracrRNA. The crRNA, or CRISPR RNA sequence contains approximately 20 nucleotides of homology complementary to the strand of DNA opposite and upstream of a PAM (Protospacer Adjacent Motif) (NGG) sequence. The tracrRNA, or transactivating crRNA, contains partial complementary sequence to the crRNA as well as the sequence and secondary structure that is recognized by Cas9. These sequences have been adapted for use in the lab by combining the tracrRNA and crRNA into one long single guide RNA (sgRNA) species capable of complexing with Cas9 to recognize and cleave the target DNA.

The EnGen sgRNA Synthesis Kit, *S. pyogenes* combines an *S. pyogenes* Cas9-specific Scaffold Oligo (included in the EnGen 2X sgRNA Reaction Mix) that is partially complementary to the target-specific oligos designed by the user. The two oligos anneal at the overlapping region and are filled in by the DNA polymerase, creating a double-stranded DNA (dsDNA) template for transcription. Synthesis of the dsDNA template and transcription of RNA occur in a single reaction, resulting in the generation of a functional sgRNA.

The EnGen sgRNA Synthesis Kit, *S. pyogenes* Includes:

- EnGen sgRNA Enzyme Mix
- EnGen 2X sgRNA Reaction Mix, *S. pyogenes*
- DNase I (RNase-free)
- EnGen sgRNA Control Oligo, *S. pyogenes*
- DTT (0.1 M)



A. *The target-specific oligo contains the T7 promoter sequence, ~20 nucleotides of target-specific sequence and a 14 nucleotide overlap sequence complementary to the S. pyogenes Cas9 Scaffold Oligo supplied in the reaction mix. Target-specific oligos (or EnGen sgRNA Control Oligo, S. pyogenes) are mixed with the EnGen 2X sgRNA Reaction Mix (NTPs, dNTPs, S. pyogenes Cas9 Scaffold Oligo), 0.1 M DTT and the EnGen sgRNA Enzyme Mix (DNA and RNA polymerases) at room temperature.* ***B.*** *At 37°C the two oligos anneal at the 14 nucleotide overlap region of complementarity.* ***C.*** *The DNA polymerase extends both oligos from their 3´ ends creating a double-stranded DNA template.* ***D.*** *The RNA polymerase recognizes the double-stranded DNA of the T7 promoter and initiates transcription. The resulting sgRNA contains the target-specific/crRNA sequence as well as the tracrRNA. All steps occur in a single reaction during a 30 minute incubation at 37°C.*

Dutch Harbor, Amaknak Island, Aleutian Islands, Alaska, USA is the largest fisheries port in the United States.
Credit: Jacob Maentz, Getty Images

The Downstream Effects of Overfishing

Fishing has been a mainstay throughout the history of civilization. However, the methods, equipment and extent of fishing have drastically changed in order to feed an overpopulated world. As a result, we are rapidly depleting our oceans of what was once thought to be an endless supply of fish. What is not as readily understood is that gaps in the food web created by overfishing certain species, and the currently-used industrial fishing methods, such as bottom trawling, are destroying entire ecosystems.

The global fishing industry is worth nearly 250 billion dollars per year, which equates to 90 million tons of fish. Eighty percent of commercial fish stocks have been declared overexploited, yet this is not reflected in the variety of fish available in local markets. This is due to the fact that industrial-scale fishing efforts have moved further offshore and to deeper depths of the ocean to find new sources. “Super trawlers” the size of ocean liners use probes, radar, sonar, helicopters and spotter planes to hunt down marine life. Nets that can measure 40 km long catch targeted species of fish, as well as non-targeted species (“bycatch”), such as seabirds, turtles and dolphins, which are thrown back into the sea, often dead or dying.

Modern fishing techniques completely devastate ocean habitats. Bottom trawlers drag nets along the ocean floor, destroying rich, complex ecosystems and communities of invertebrates on the sea bed as they comb for scallops and shrimp. They leave behind a barren landscape of sand and gravel. The disregard for entire ecosystems also extends to coastal areas where mangroves are destroyed for shrimp fishing, leaving local coastal communities without storm protection, a natural form of water filtration, or nursery habitats for marine life.

Large deep-water fish, such as Bluefin tuna, are commercially very valuable and are aggressively targeted. They take longer to reach sexual maturity and do not reproduce as often. They are unable to replenish themselves fast enough to meet demand and consequently, younger fish that have not yet reached spawning age are targeted. Depleting the large and predatory fish significantly affects food web dynamics. The smaller, more resilient fish remain, and eventually, the range of fish observed are smaller and less diverse.

The Aleutian Islands, off the coast of northern Alaska, saw the effects of overfishing on an entire ecosystem when baleen and sperm whales were overfished, removing a food source for Orcas. This forced the Orcas to feed on less calorie-rich animals, including otters. The primary prey of otters is sea urchin, which in turn, feed on kelp. The decline in otters led to a drastic increase in kelp grazing by urchin, and the destruction of kelp forests. When nesting fish do not have the protection of kelp, their larvae are vulnerable to predation. In the case of the Aleutian Islands, this ultimately led to the collapse of the fishing industry.

Overfishing affects the balance of marine ecosystems and the livelihood of millions of people. As awareness grows, steps are being taken to protect our oceans. Marine reserves, or “no fishing” zones are being established so that fish stocks can recover. Organizations such as the Marine Stewardship Council certify sustainably caught fish, indicating to conscientious consumers the products that support restoration of marine life. Supermarket chains are taking on a vital role offering sustainably caught seafood to customers. These measures give hope that with even greater awareness we may just be able to restore this essential resource.

RNA Reagents

A broad portfolio of reagents to support RNA research.

RNA molecules play a multitude of cellular roles in all kingdoms of life, perhaps reflecting the hypothetical, prehistoric RNA world. RNA is an essential carrier of genetic information, can scaffold molecular interactions, catalyze chemical reactions, and influences gene expression.

In the last several years, our understanding of RNAs as regulatory molecules in the cell has dramatically changed. Many new classes of small and large non-coding RNAs, with largely unexplored functions, have been reported, ushering in a renaissance of RNA-focused research in biology.

Small RNAs play a major role in the post-transcriptional regulation of gene expression in eukaryotic and prokaryotic organisms. Small RNAs play central roles in CRISPR pathways of adaptive immunity. Researchers have capitalized on these pathways to enable analyses of gene function not previously possible.

Introducing RNA, and in particular mRNA, into cells is an exciting new platform for inducing phenotypic changes in cells with implications for therapy, vaccines, and research applications.

New England Biolabs continues its strong tradition of providing high quality reagents to support RNA research. Our expanding range of products includes tools for the synthesis, processing, cleanup, isolation, analysis, amplification, copying and cloning of RNA molecules. Further, all NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

Featured Products

186 HiScribe™ T7 ARCA mRNA Kit (with tailing)

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188 Hi-T7™ RNA Polymerase

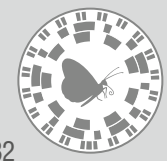
Featured Tools & Resources

184 Avoiding Ribonuclease Contamination

195 Reported Activities for RNA Ligases

View our video tutorial describing high yield *in vitro* synthesis of both capped and uncapped mRNA.

View our video for avoiding ribonuclease contamination.



Learn more about RNA modifications.

RI Recombinant Enzyme

Avoiding Ribonuclease Contamination

RNA is more susceptible to degradation than DNA, due to the ability of the 2´ hydroxyl groups to act as nucleophiles. Many ribonucleases (RNases) bypass the need for metal ions by taking advantage of the 2´ hydroxyl group as a reactive species. These RNases are resistant to metal chelating agents and, some of them, like RNase A family enzymes, can survive prolonged boiling or autoclaving. RNase A-type enzymes rely on active site histidine residues for catalytic activity (1) and can be inactivated by the histidine-specific alkylating agent diethyl pyrocarbonate (DEPC).

Sources of RNase Contamination:

RNases are found in all cell types and organisms from prokaryotes to eukaryotes. These enzymes generally have very high specific activity, meaning tiny amounts of contamination in an RNA sample is sufficient to destroy the RNA. The major sources of RNase contamination in a typical laboratory include:

- Aqueous solutions, reagents used in experiments
- Environmental exposure RNases are in the air, on most surfaces and on dust
- Contact with skin

Laboratory Precautions (2,3):

New England Biolabs' enzymes certified for RNA work have been purified to be free of ribonucleases. However, it is possible to reintroduce RNases during the course of experimentation from various sources. RNase contamination can be prevented by following a few common sense laboratory procedures:

- Always wear gloves during an experiment and change them often, especially after contact with skin, hair or other potentially RNase-contaminated surfaces, such as doorknobs, keyboards and animals.
- Use RNase-free solutions. Use RNase-free certified, disposable plasticware and filter tips whenever possible.
- Maintain a separate area for RNA work. Carefully clean the surfaces.
- Decontaminate glassware by baking at 180°C or higher for several hours or by soaking in freshly prepared 0.1% (v/v) DEPC in water or ethanol for 1 hour, followed by draining and autoclaving. Autoclaving will destroy any unreacted DEPC which can otherwise react with other proteins and RNA.
- Decontaminate polycarbonate or polystyrene materials (e.g., electrophoresis tanks) by soaking in 3% hydrogen peroxide for 10 minutes. Remove peroxide by extensively rinsing with RNase-free water prior to use.

Preparation of Solutions (2,3):

Preparation of solutions using the following suggestions can help prevent RNase contamination:

- As an alternative to the historic use of DEPC, which can inhibit enzymatic reactions if not completely removed, we have found that Milli-Q® (Millipore) purified water is sufficiently free of RNases for most RNA work.
- DEPC treatment of solutions is accomplished by adding 1 ml DEPC (Sigma) per liter of solution, stirring for 1 hour, and autoclaving for one hour to remove any remaining DEPC. [Note: Compounds with primary amine groups (e.g., Tris) which will react with DEPC, cannot be DEPC-treated. Other compounds, which are not stable during autoclaving, cannot be DEPC-treated].
- Solutions and buffers (e.g. DTT, nucleotides, manganese salts) should be prepared by dissolving the solid (highest available purity) in autoclaved DEPC-treated or Milli-Q water and passing the solution through a 0.22 µm filter to sterilize.

Inhibitors of Ribonucleases:

RNA can also be protected from RNase activity by using one of the following RNase inhibitors:

- RNase Inhibitor, Murine, (NEB #M0314) is a recombinant protein RNase inhibitor of murine origin. Like human and porcine RNAse, it is a specific inhibitor of RNases A, B and C, but is more stable due to improved resistance to oxidation (4). The inhibitor requires low concentration of DTT (< 1 mM) to maintain activity, making it ideal for reactions where low DTT concentration is required (e.g., real-time RT-PCR).
- RNase Inhibitor, Human Placenta, (NEB #M0307), a recombinant protein of human placental origin, is a specific inhibitor for RNases A, B and C. Similar to the Murine RNase Inhibitor, it is compatible with many enzymatic reactions involving RNA (e.g., *in vitro* transcription, RT-PCR, ligation, etc.).
- Ribonucleoside Vanadyl Complex (NEB #S1402) is a transition-state analog inhibitor of RNase A-type enzymes with K_i = 1 X 10^{-5} M. This complex is compatible with many RNA isolation procedures, but it should not be used in the presence of EDTA. The complex also inhibits many other enzymes used in RNA work (5).

References:

(1) Fersht, A.R. (1977) *Enzyme Structure and Mechanism* Freeman, Reading, PA, 325–329.
(2) Blumberg, D.D. (1987) *Methods Enzymol.,* 152, 20–24.
(3) Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual,* (2nd ed.), (pp. 7.3–7.5). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
(4) Kim, B.M. et al. (1999) *Protein Science,* 8, 430–434.
(5) Berger, S.L. (1987) *Methods Enzymol.,* 152, 227–234.

Find tips for avoiding RNA contamination.

HiScribe™ T7 High Yield RNA Synthesis Kits

HiScribe T7 High Yield RNA Synthesis Kit
#E2040S 50 reactions

HiScribe T7 Quick High Yield RNA Synthesis Kit
#E2050S 50 reactions

- *Synthesis of long and short RNA transcripts*
- *Incorporation of modified nucleotides*
- *Incorporation of labeled nucleotides*
- *Generation of capped RNA using cap analogs*
- *Synthesis of radioactively labeled probes with high or low specific activity*

Description: NEB's HiScribe T7 High Yield RNA Synthesis Kits offer robust *in vitro* RNA transcription of many kinds of RNA, including internally labeled and co-transcriptionally capped transcripts. Utilizing T7 RNA Polymerase, these kits achieve efficient transcription with small amounts of template, and can generate up to 180 µg per reaction, or up to 30–40 µg of capped RNA using cap analog. RNA generated can be used in a variety of applications, including RNA structure/function studies, ribozyme biochemistry, probes for RNase protection assays and hybridization-based blots, anti-sense RNA and RNAi experiments, microarray analysis, microinjection, and *in vitro* translation and RNA vaccines.

The HiScribe T7 High Yield RNA Synthesis Kit is an extremely flexible system, with separate NTPs included for flexible reaction setup. The HiScribe T7 Quick High Yield RNA Synthesis Kit is provided in master mix format for fast reaction setup.

The HiScribe T7 High Yield RNA Synthesis Kit Includes:
- T7 RNA Polymerase Mix
- T7 Reaction Buffer (10X)
- ATP, GTP, UTP, CTP (100 mM)
- FLuc Control Template

The HiScribe T7 Quick High Yield RNA Synthesis Kit Includes:
- T7 RNA Polymerase Mix
- NTP Buffer Mix
- FLuc Control Template
- DNase I (RNase-free)
- LiCl Solution

HiScribe SP6 RNA Synthesis Kit

#E2070S 50 reactions

- *Synthesis of long and short RNA transcripts*
- *Incorporation of modified nucleotides*
- *Incorporation of labeled nucleotides*
- *Generation of capped RNA using cap analogs*
- *Synthesis of radioactively labeled probes with high or low specific activity*

Use Monarch RNA Cleanup Kits to purify your synthesized RNA, see page 130–131.

Description: The HiScribe SP6 RNA Synthesis Kit is designed for the *in vitro* transcription of RNA using SP6 RNA Polymerase. This kit is suitable for synthesis of high yield RNA transcripts and allows for incorporation of cap analogs (not included) or modified nucleotides (not included) to obtain capped, biotin-labeled or dye-labeled RNA. The kit is also capable of synthesizing high specific activity radiolabeled RNA for use as probes or targets.

RNA synthesized from this kit is suitable for many applications including RNA structure and function studies, ribozyme biochemistry, probes for RNase protection or gel shift assays, hybridization-based blots, anti-sense RNA or RNAi experiments, microarray analysis, microinjection and *in vitro* translation studies.

This kit contains sufficient reagents for 50 reactions of 25 µl each. Each standard reaction yields ≥ 80 µg of RNA from 1 µg SP6 Control Template DNA. Each kit can yield ≥ 4 mg of RNA.

The HiScribe SP6 RNA Synthesis Kit Includes:
- SP6 Reaction Buffer (10X)
- ATP (Tris), GTP (Tris), UTP (Tris), CTP (Tris), (50 mM)
- SP6 Control Template
- SP6 RNA Polymerase Mix
- DNase I (RNase-free)
- LiCl Solution

Companion Products:

DNase I (RNase-Free)
#M0303S 1,000 units
#M0303L 5,000 units

RNA Loading Dye (2X)
#B0363S 4 ml

Vaccinia Capping System
#M2080S 400 units

Q5® Hot Start High-Fidelity DNA Polymerase
#M0493S 100 units
#M0493L 500 units

Monarch® PCR & DNA Cleanup Kit
#T1030S 50 preps
#T1030L 250 preps

Monarch RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Low Range ssRNA Ladder
#N0364S 25 gel lanes

E. coli Poly(A) Polymerase
#M0276S 100 units
#M0276L 500 units

mRNA Cap 2´-O-Methyltransferase
#M0366S 2,000 units

3´-O-Me-m^{7}G(5´)ppp(5´)G RNA Cap Structure Analog
#S1411S 1 µmol
#S1411L 5 µmol

G(5´)ppp(5´)A RNA Cap Structure Analog
#S1406S 1 µmol
#S1406L 5 µmol

G(5´)ppp(5´)G RNA Cap Structure Analog
#S1407S 1 µmol
#S1407L 5 µmol

m^{7}G(5´)ppp(5´)G RNA Cap Structure Analog
#S1404S 1 µmol
#S1404L 5 µmol

m^{7}G(5´)ppp(5´)A RNA Cap Structure Analog
#S1405S 1 µmol
#S1405L 5 µmol

HiScribe T7 ARCA mRNA Kits

HiScribe T7 ARCA mRNA Kit
#E2065S 20 reactions

HiScribe T7 ARCA mRNA Kit (with tailing)
#E2060S 20 reactions

Companion Products:

DNase I (RNase-Free)
#M0303S 1,000 units
#M0303L 5,000 units

RNA Loading Dye (2X)
#B0363S 4 ml

- *Synthesis of capped and tailed mRNA*
- *Incorporation of modified nucleotides*
- *Template removal and mRNA purification reagents included*

Description: Most eukaryotic mRNAs require a 7-methyl guanosine (m7G) cap structure at the 5´ end and a Poly(A) tail at the 3´ end to be efficiently translated. By using a DNA template encoding a poly(A) tail, the HiScribe T7 ARCA mRNA Kit can be used to synthesize capped and tailed mRNAs. The cap structure is added to the mRNA by co-transcriptional incorporation of Anti-Reverse Cap Analog (ARCA, NEB #S1411) using T7 RNA Polymerase. The transcription reaction can be set up easily by combining the ARCA/NTP Mix, T7 RNA Polymerase Mix and a suitable DNA template. The kit also allows for partial incorporation of 5mCTP, Pseudo-UTP and other modified nucleotides into mRNA. mRNAs synthesized with the kit can be used for cell transfection, microinjection, *in vitro* translation and RNA vaccines. Poly(A) tail is incorporated during the transcription reaction. The kit also includes DNase I and LiCl for DNA template removal and quick mRNA purification.

The HiScribe T7 ARCA mRNA Kit (with tailing) is designed for quick production of ARCA capped and poly(A) tailed mRNA *in vitro* from templates without encoded poly(A) tails.

The HiScribe T7 ARCA mRNA Kit Includes:
- T7 RNA Polymerase Mix
- ARCA/NTP Mix
- DNase I (RNase-free)
- LiCl Solution
- CLuc Control Template

The HiScribe T7 ARCA mRNA Kit (with tailing) Includes:
- T7 RNA Polymerase Mix
- ARCA/NTP Mix
- DNase I (RNase-free)
- *E. coli* Poly(A) Polymerase
- Poly(A) Polymerase Reaction Buffer
- LiCl Solution
- CLuc Control Template

Advantages:
- Quick reaction setup and streamlined protocol
- Enables partial incorporation of 5mCTP, Pseudo-UTP and other modified CTP and UTP
- Ultra-high quality components ensure mRNA integrity

EnGen® sgRNA Synthesis Kit, *S. pyogenes*

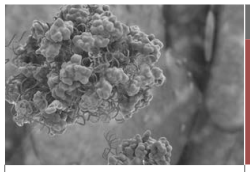
See page 179 for more information.

#E3322S 20 reactions

The EnGen sgRNA Synthesis Kit, *S. pyogenes* provides a simple and quick method for transcribing high yields of sgRNA in a single 30 minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user.

NEB's RNA Research Division works to discover, understand and develop enzymes to streamline RNA workflows. Several of its team members are pictured here.

Recommended HiScribe RNA Synthesis Kits by Application



		T7 KITS				SP6 KITS
	APPLICATION	**HiScribe T7 High Yield RNA Synthesis Kit** (#E2040)	**HiScribe T7 Quick High Yield RNA Synthesis Kit** (#E2050)	**HiScribe T7 ARCA mRNA Kit** (#E2065)	**HiScribe T7 ARCA mRNA (with Tailing)** (#E2060)	**HiScribe SP6 RNA Synthesis Kit** (#E2070)
Probe labeling	Fluorescent labeling: FAM, Cyanine (Cy) dyes, etc. • Fluorescent *in situ* hybridization (FISH)		✔			✔
	Non-fluorescent labeling: Biotin, Digoxigenin • *In situ* hybridization • Blot hybridization with secondary detection • Microarray		✔			✔
	High specific activity radiolabeling • Blot hybridization • RNase protection	✔				✔
mRNA & RNA for transfection	Streamlined mRNA synthesis with ARCA co-transcriptional capping and enzymatic poly(A) tailing • Transfection • Microinjection • *In vitro* translation				✔	
	Streamlined ARCA capped RNA synthesis • Template encoded poly(A) tails • Non polyadenylated transcripts • Transfection • Microinjection • *In vitro* translation			✔		
	Co-transcriptional capping with alternate cap analogs • Transfection • Microinjection • *In vitro* translation		✔			✔
	Post-transcriptional capping with Vaccinia Capping System • Transfection • Microinjection • *In vitro* translation	✔	✔			✔
	Complete substitution of NTPs: 5-mC, pseudouridine, etc. • Post-transcriptional capping with Vaccinia mRNA Capping System	✔				✔
	Partial substitution of NTPs: 5-mC, pseudouridine, etc.		✔	✔	✔	✔
	Unmodified RNA		✔			✔
	Hairpins, short RNA, dsRNA • Gene knockdown		✔			✔
Structure, function, & binding studies	Complete substitution of NTPs • Aptamer selection • Isotopic labeling	✔				✔
	Partial substitution of one or more NTPs • Aptamer selection • Structure determination		✔			✔
	Unmodified RNA • SELEX • Structure determination		✔			✔

RNA Polymerases

RR 37°

T3 RNA Polymerase
#M0378S 5,000 units

T7 RNA Polymerase
#M0251S 5,000 units
#M0251L 25,000 units

SP6 RNA Polymerase
#M0207S 2,000 units
#M0207L 10,000 units

NEW
Hi-T7 RNA Polymerase
#M0658S 5,000 units

- *Radiolabeled RNA probe preparation*
- *RNA generation for in vitro translation*
- *RNA generation for studies of RNA structure, processing and catalysis*

Description: Initiation of transcription with T3, T7 and SP6 RNA Polymerases is highly specific for the T3, T7 and SP6 phage promoters, respectively. Cloning vectors have been developed which direct transcription from the T3, T7 or SP6 promoter through polylinker cloning sites. These vectors allow *in vitro* synthesis of defined RNA transcripts from a cloned DNA sequence.

Hi-T7 RNA Polymerase is an engineered, thermoactive T7 RNA Polymerase. Hi-T7 uses T7 RNA Polymerase Promoters. It increases capping efficiency and eliminates dsRNA by-product formation during synthesis.

Reaction Conditions: 1X RNAPol Reaction Buffer. Supplement with 0.5 mM each ATP, UTP, GTP, CTP (not included) and DNA template containing the appropriate promoter. Incubate at 37°C (T3, T7 and SP6) or 50°C (Hi-T7).

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol ATP into an acid-insoluble material in 1 hour at 37°C or 50°C for Hi-T7. Unit assay conditions can be found at www.neb.com.

Concentration: T3 RNA Polymerase: 50,000 units/ml. T7 RNA Polymerase: 50,000 units/ml. SP6 RNA Polymerase: 20,000 units/ml. Hi-T7 RNA Polymerase: 50,000 units/ml.

E. coli Poly(A) Polymerase

RR 37° 65°

#M0276S 100 units
#M0276L 500 units

Companion Products:

Adenosine-5´ Triphosphate (ATP)
#P0756S 1 ml
#P0756L 5 ml

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

- *Labeling of RNA with ATP or cordycepin 5´-triphosphate*
- *Poly(A) tailing of RNA for cloning or affinity purification*
- *Enhances translation of RNA transferred into eukaryotic cells*

Description: *E. coli* Poly(A) Polymerase catalyzes the template independent addition of AMP from ATP to the 3´ end of RNA.

Reaction Conditions: 1X Poly(A) Polymerase Reaction Buffer. Supplement with 1 mM ATP. Incubate at 37°C. May heat inactivate at 65°C for 20 minutes.

Reagents Supplied with Enzyme:
10X Poly(A) Polymerase Reaction Buffer
10 mM ATP

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of AMP into RNA in a 20 µl volume in 10 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Poly(U) Polymerase

RR 37° 65°

#M0337S 60 units

Companion Products:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

Ribonucleotide Solution Set
#N0450S 10 µmol of each
#N0450L 50 µmol of each

- *Labeling of RNA with UTP*
- *Poly(U) tailing of RNA for cloning*

Description: Poly(U) Polymerase catalyzes the template-independent addition of UMP from UTP or AMP from ATP to the 3´ end of RNA.

Reaction Conditions: 1X NEBuffer 2. Supplement with 0.5 mM UTP (not supplied). Incubate at 37°C. May heat inactivate at 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that incorporates 1 nmol of UMP into RNA in a 50 µl volume in 10 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 2,000 units/ml

Notes: Poly(U) Polymerase in NEBuffer 2 will incorporate UMP or AMP from UTP or ATP into RNA. Tailing length of poly(U) varies with UTP and primer concentration. Poly(U) Polymerase is highly processive under low primer concentrations (< 100 pmol).

E. coli RNA Polymerase, Core Enzyme & Holoenzyme

37°

E. coli RNA Polymerase, Core Enzyme
#M0550S 100 units

E. coli RNA Polymerase, Holoenzyme
#M0551S 50 units

- *RNA synthesis from E. coli promoter*
- *Transcription initiation studies*
- *In vitro translation with PURExpress*

Description: *E. coli* RNA Polymerase Core Enzyme consists of 5 subunits designated α, α, β´, β, and ω. The enzyme is free of sigma factor and does not initiate specific transcription from bacterial and phage DNA promoters. The enzyme retains the ability to transcribe RNA from nonspecific initiation sequences. Addition of sigma factors will allow the enzyme to initiate RNA synthesis from specific bacterial and phage promoters. The core enzyme has a molecular weight of approximately 400 kDa.

E. coli RNA Polymerase Holoenzyme is the core enzyme saturated with sigma factor 70. The Holoenzyme initiates RNA synthesis from sigma 70 specific bacterial and phage promoters.

Reaction Conditions: 1X *E. coli* RNA Polymerase Reaction Buffer, 0.5 mM of each rNTP and DNA template. Incubate at 37°C.

Unit Definition: One unit is the amount of enzyme required to incorporate 1 nmol of NTP into RNA in 10 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 1,000 units/ml

Ribonucleotides

Ribonucleotide Solution Set
#N0450S 10 µmol of each
#N0450L 50 µmol of each

Ribonucleotide Solution Mix
#N0466S 10 µmol of each
#N0466L 50 µmol of each

Description:
Ribonucleotide Solution Set:
The set consists of four separate solutions of ATP, GTP, CTP and UTP, pH 7.5, as sodium salts. Each nucleotide is supplied at 100 mM.

Ribonucleotide Solution Mix:
A buffered equimolar solution of ribonucleotide triphosphates: rATP, rCTP, rGTP and rUTP, pH 7.5, as sodium salts. Each nucleotide is supplied at a concentration of 25 mM (total rNTP concentration equals 100 mM).

Note: To ensure maximum activity upon long-term storage, aliquot and store at –80°C.

Pyrophosphatases

RR

Pyrophosphatase, Inorganic (*E. coli*)
#M0361S 10 units
#M0361L 50 units

Pyrophosphatase, Inorganic (yeast)
#M2403S 10 units
#M2403L 50 units

Thermostable Inorganic Pyrophosphatase
#M0296S 250 units
#M0296L 1,250 units

- *Increasing RNA yield in transcription reactions*
- *Enhancing DNA replication*

Description: Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate.

$P_2O_7^{-4} + H_2O \rightarrow 2HPO_4^{-2}$

Source: Pyrophosphatase, Inorganic (*E. coli*) is prepared from a clone of the *E. coli* inorganic pyrophosphatase gene.

Pyrophosphatase, Inorganic (yeast) is an *E. coli* strain containing a genetic fusion of the *Saccharomyces cerevisiae ppa* gene and the gene coding for *Mycobacterium xenopi* GyrA intein. Developed by BioHelix Corporation, now a wholly owned subsidiary of Quidel Corporation, and produced at New England Biolabs.

Thermostable Inorganic Pyrophosphatase is an *E. coli* strain carrying a plasmid encoding a pyrophosphatase from the extreme thermophile *Thermococcus litoralis*.

Unit Definition: One unit is defined as the amount of enzyme that will generate 1 µmol of phosphate per minute from inorganic pyrophosphate under standard reaction conditions. Unit assay conditions can be found at www.neb.com.

Concentration: Pyrophosphatase, Inorganic (*E. coli*) and Pyrophosphatase, Inorganic (yeast) 100 units/ml. Thermostable Inorganic Pyrophosphatase 2,000 units/ml.

Thermostable Inorganic Pyrophosphatase retains 100% activity after incubation at 100°C for 4 hours.

Vaccinia Capping System

RR 37°

#M2080S 400 units

Companion Product:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

- *Capping mRNA prior to in vivo or in vitro translation*
- *Labeling 5´ end of mRNA*

Description: Based on the Vaccinia Virus Capping Enzyme, the Vaccinia Capping System provides the necessary components to add 7-methylguanylate cap structures (Cap 0) to the 5´ end of RNA. In eukaryotes, these terminal cap structures are involved in stabilization, transport and translation of mRNAs. Enzymatic production of capped RNA is an easy way to improve the stability and translational competence of RNA used for *in vitro* translation, transfection and microinjection. Alternatively, use of labeled GTP in a reaction provides a convenient way to label any RNA containing a 5´ terminal triphosphate.

This single enzyme is composed of two subunits (D1 and D12) and has three enzymatic activities (RNA triphosphatase and guanylyltransferase by the D1 subunit and guanine methyltransferase by the D12 subunit), all necessary for addition of a complete Cap 0 structure, m^7Gppp(5´)N. All capped structures are added in the proper orientation, unlike co-transcriptional addition of some cap analogs.

Reaction Conditions: 1X Capping Buffer. Supplement with 0.5 mM GTP and 0.1 mM SAM. Incubate at 37°C.

Reagents Supplied:
Vaccinia Capping Enzyme
Capping Buffer (10X)
GTP Solution (10 mM)
SAM Solution (32 mM)

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 10 pmol of (α-^{32}P) GTP into an 80 nucleotide transcript in 1 hour at 37°C.

Concentration: 10,000 units/ml

mRNA Cap 2´-*O*-Methyltransferase

NEB RR 37°

#M0366S 2,000 units

- *Enhances translation of RNA*
- *Improving mRNA expression during microinjection and transfection*

Description: mRNA Cap 2´-*O*-Methyltransferase adds a methyl group at the 2´-O position of the first nucleotide adjacent to the cap structure at the 5´ end of the RNA. The enzyme utilizes S-adenosylmethionine (SAM) as a methyl donor to methylate capped RNA (Cap 0) resulting in a Cap 1 structure.

Reaction Conditions: 1X Capping Buffer. Supplement with 0.2 mM SAM (supplied). Incubate at 37°C.

Reagents Supplied:
Capping Buffer (10X)
SAM (32 mM)

Unit Definition: One unit is defined as the amount of enzyme required to methylate 10 pmoles of 80 nt long capped RNA transcript in 1 hour at 37°C.

Concentration: 50,000 units/ml

RNA Cap Analog Selection Chart

The 5´ terminal m^7G cap present on most eukaryotic mRNAs promotes translation, *in vitro*, at the initiation level. For most RNAs, the cap structure increases stability, decreases susceptibility to exonuclease degradation, and promotes the formation of mRNA initiation complexes. Certain prokaryotic mRNAs with 5´ terminal cap structures are translated as efficiently as eukaryotic mRNA in a eukaryotic cell-free protein synthesizing system. Splicing of certain eukaryotic substrate RNAs has also been observed to require a cap structure.

PRODUCT	APPLICATION	SIZE
Anti-Reverse Cap Analog 3´-O-Me-m^7G(5´) ppp(5´)G (#S1411S/L)	• Produces 100% translatable capped transcripts • Co-transcriptional capping with T7 (NEB #M0251), SP6 (NEB #M0207) and T3 RNA polymerases • Synthesis of m^7G capped RNA for *in vitro* splicing assays • Synthesis of m^7G capped RNA for transfection or microinjection	1/5 µmol
Standard Cap Analog m^7G(5´)ppp(5´)G (#S1404S/L)	• Co-transcriptional capping with T7, SP6 and T3 RNA polymerases • Synthesis of m^7G capped RNA for *in vitro* splicing assays • Synthesis of m^7G capped RNA for transfection or microinjection	1/5 µmol
Unmethylated Cap Analog G (5´)ppp(5´)G (#S1407S/L)	• Co-transcriptional capping with T7, SP6 and T3 RNA polymerases • Synthesis of unmethylated G capped RNA	1/5 µmol
Methylated Cap Analog for A +1 sites m^7G(5´)ppp(5´)A (#S1405S/L)	• Co-transcriptional capping with T7 RNA polymerase from the phi2.5 promoter that contains an A at the transcription initiation site • Synthesis of m^7G capped RNA for *in vitro* splicing assays • Synthesis of m^7G capped RNA for transfection or microinjection	1/5 µmol
Unmethylated Cap Analog for A +1 sites G(5´)ppp(5´)A (#S1406S/L)	• Co-transcriptional capping with T7 RNA polymerase from the phi2.5 promoter that contains an A at the transcription initiation site • Synthesis of unmethylated G capped RNA • Synthesis of A capped RNA	1/5 µmol

3´-Desthiobiotin-GTP & 3´-Biotin-GTP

3´-Desthiobiotin-GTP
#N0761S 0.5 µmol

NEW
3´-Biotin-GTP
#N0760S 0.5 µmol

Description: 3´-Desthiobiotin-GTP or 3´-Biotin GTP are guanosine triphosphate (GTP) analogs which are modified at their 3´ position with desthiobiotin or biotin, respectively. When used with the Vaccinia Capping System, (NEB #M2080) these reagents enable affinity tagging of RNA triphosphate ends. Tagged RNAs are enriched by binding to Hydrophilic Streptavidin Magnetic Beads (NEB #S1421). Desthiobiotin-tagged RNAs can be eluted with free biotin. This approach is used in Cappable-seq, a method developed at NEB for directly enriching the 5´-ends of primary transcripts (1).

Reference:
(1) Ettwiller, L. et al. (2016) *BMC Genomics*, 17,199.

NEW yDcpS

RR NEB U 37° 70°

#M0463S 4,000 units

- *mRNA decapping, enabling recapping with tagged-GTP analogs*
- *Biotinylation of 5´ ends of primary transcripts*
- *Recappable-seq*

Description: yDcpS decapping enzyme from *S. cerevisiae* hydrolyzes the phosphodiester bond between the gamma and beta phosphates of m^7G capped mRNA, leaving behind a diphosphorylated 5´ end and m^7GMP. yDcpS is capable of decapping full length mRNAs and the diphosphorylated 5´ end it leaves behind is suitable for recapping using Vaccinia Capping Enzyme.

Reaction Conditions: 1X yDcpS Reaction Buffer. Incubate at 37°C.

Concentration: 200,000 units/ml

cDNA Synthesis Selection Chart

cDNA SYNTHESIS	FEATURES	SIZE
KITS AND MIXES		
NEW **LunaScript® RT SuperMix Kit** (NEB #E3010)	• Ideal for cDNA synthesis in a two-step RT-qPCR workflow • Single tube supermix contains random hexamer and oligo-dT primers, dNTPs, Murine RNase Inhibitor, and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol	25/100 rxns
ProtoScript® II First Strand cDNA Synthesis Kit (NEB #E6560)	• Generates cDNA at least 10 kb in length • Contains ProtoScript II Reverse Transcriptase, an enzyme with increased thermostability and reduced RNase H activity • Convenient 2-tube kit includes dNTPs, Oligo-dT primer and Random Primer Mix	30/150 rxns
ProtoScript First Strand cDNA Synthesis Kit (NEB #E6300)	• Generates cDNA at least 5 kb in length • Contains M-MuLV Reverse Transcriptase • Convenient 2-tube kit includes dNTPs, Oligo-dT primer and Random Primer Mix	30/150 rxns
NEW **Template Switching RT Enzyme Mix** (NEB #M0466)	• Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction • Enzyme mix and buffer are optimzed for efficient template switching • RT enzyme mix includes RNase Inhibitor • High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA • Robust and simple workflow for 5´ Rapid Amplification of cDNA Ends (RACE) • Retains the complete 5´ end of transcripts for 2nd Strand cDNA Synthesis	20/100 rxns
STANDALONE REAGENTS		
ProtoScript II Reverse Transcriptase (NEB #M0368) An alternative to SuperScript® II	• RNase H⁻ mutant of M-MuLV Reverse Transcriptase with increased thermostability and reduced RNase H activity • Increased reaction temperatures (37–50°C)	4,000/10,000/40,000 units
M-MuLV Reverse Transcriptase (NEB #M0253)	• Robust reverse transcriptase for a variety of templates • Standard reaction temperatures (37–45°C)	10,000/50,000 units
AMV Reverse Transcriptase (NEB #M0277)	• Robust reverse transcriptase for a broad temperature range (37–52°C) • Can be used for templates requiring higher reaction temperatures	200/1,000 units
WarmStart® RTx Reverse Transcriptase (NEB #M0380)	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection	50/250 rxns

For PCR amplification of first strand cDNA see page 194.
For RT-PCR kits please see page 72.
For RT-qPCR kits please see page 71.

ProtoScript® II Reverse Transcriptase

RR 42° 65°

#M0368S 4,000 units
#M0368L 10,000 units
#M0368X 40,000 units

Companion Products:

RNase H
#M0297S 250 units
#M0297L 1,250 units

NEW
Monarch Total RNA Miniprep Kit
#T2010S 50 preps

For a complete listing of Deoxynucleotide Solutions, see page 79.

- *Efficient reverse transcription from different starting RNA amounts*
- *Increased thermostability*
- *Generates cDNA up to 10 kb or more*

Description: ProtoScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 50°C, providing higher specificity, higher yield of cDNA and more full length cDNA product, up to 12 kb in length. This product was formerly known as M-MuLV Reverse Transcriptase RNase H$^-$.

Reaction Conditions: 1X ProtoScript II Reverse Transcriptase Reaction Buffer, 10 mM DTT, 200 units M-MuLV (RNase H$^-$), supplemented with 0.5 mM dNTPs (not included) and 5 µM $dT_{23}VN$ (not included). Incubate at 42°C for 50 minutes. If random primers are used, a 10 minute incubation at room temperature is recommended before transferring to 42°C. May heat inactivate at 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 10 minutes at 37°C using poly(rA)•oligo(dT)18 as template. Unit assay conditions can be found at www.neb.com.

Concentration: 200,000 units/ml

NEW LunaScript® RT SuperMix Kit

 RR PCR HS NO

See page 70 for more information.

#E3010S 25 reactions
#E3010L 100 reactions

LunaScript RT SuperMix Kit is an optimized master mix containing all the necessary components for first strand cDNA synthesis in the context of a two-step RT-qPCR workflow. It features the thermostable Luna Reverse Transcriptase, which supports cDNA synthesis at elevated temperatures. Murine RNase Inhibitor is also included to protect template RNA from degradation. The LunaScript RT SuperMix Kit contains random hexamer and poly-dT primers, allowing for even coverage across the length of the RNA targets.

M-MuLV Reverse Transcriptase

RR 65°

#M0253S 10,000 units
#M0253L 50,000 units

Companion Product:

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *cDNA synthesis*
- *RNA Sequencing*
- *RT-PCR*

Description: Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase is an RNA-directed DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template. M-MuLV Reverse Transcriptase lacks 3´→ 5´ exonuclease activity.

Reaction Conditions: 1X M-MuLV Reverse Transcriptase Reaction Buffer. Supplement with dNTPs (not included). Incubate at 37–42°C. May heat inactivate at 65°C for 20 minutes.

Unit Definition: One unit is the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble material in 10 minutes at 37°C using poly(rA)•oligo(dT) as template primer. Unit assay conditions can be found at www.neb.com.

Concentration: 200,000 units/ml

AMV Reverse Transcriptase

NO

#M0277S 200 units
#M0277L 1,000 units

Companion Product:

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *cDNA synthesis*
- *RNA Sequencing*
- *RT-PCR*

Description: Avian Myeloblastosis Virus (AMV) Reverse Transcriptase is an RNA-directed DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template.

Source: Avian Myeloblastosis Virus (AMV)

Reaction Conditions: 1X AMV Reverse Transcriptase Reaction Buffer. Supplement with dNTPs (not included). Incubate at 37–42°C.

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37°C using poly(rA)•oligo(dT) as template primer. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 and 25,000 units/ml

Storage Note: Once thawed, store at –20°C. Repeated freeze thaw cycles will inactivate the enzyme. Aliquots can be stored for longer periods at –70°C.

WarmStart® RTx Reverse Transcriptase

#M0380S 50 reactions
#M0380L 250 reactions

Companion Product:

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *RT-LAMP*
- *cDNA Synthesis*
- *RT reactions requiring room temperature setup*

For more information on products for LAMP, see pages 74.

Description: WarmStart RTx Reverse Transcriptase is a unique *in silico*-designed, RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template. RTx is a robust enzyme for RNA detection in amplification reactions and is particularly well suited for use in Loop-mediated Isothermal Amplification (LAMP). The WarmStart property enables high-throughput applications, room-temperature setup, and increases the consistency and specificity of amplification reactions. RTx contains intact RNase H activity.

Reaction Conditions: 1X Isothermal Amplification Buffer, template, primer, dNTPs and 0.25–0.5 µl of WarmStart RTx Reverse Transcriptase in a reaction volume of 25 µl. Incubate at 50–55°C for cDNA synthesis or directly at 65°C for One-step RT-LAMP. May heat inactivate at 80°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 20 minutes at 50°C using poly(rA)•oligo(dT)18 as template.

Concentration: 15,000 units/ml

NEW

Template Switching RT Enzyme Mix

#M0466S 20 reactions
#M0466L 100 reactions

Companion Products:

NEBNext High-Fidelity 2X PCR Master Mix
#M0541S 50 reactions
#M0541L 250 reactions

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 rxns (2 x 1.25 ml)
#M0494L 500 rxns (10 x 1.25 ml)
#M0494X 500 rxns (1 x 12.5 ml)

NEB PCR Cloning Kit
#E1202S 20 reactions

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

LongAmp Hot Start *Taq* 2X Master Mix
#M0533S 100 reactions
#M0533L 500 reactions

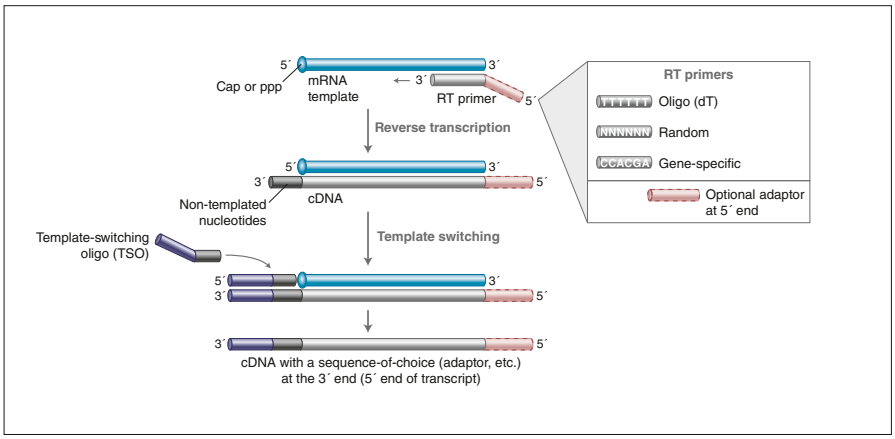
- Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction
- High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA
- Robust and simple workflow for 5´-Rapid Amplification of cDNA Ends (RACE)
- Retains the complete 5´ end of transcripts for 2nd Strand cDNA Synthesis

Description: Template switching reverse transcription (RT) incorporates a universal adaptor sequence to the 3´-end of cDNA. This convenient feature can be utilized in several downstream applications:

- cDNA synthesis and amplification in a one-tube reaction
- 5´ Rapid Amplification of cDNA Ends (RACE)
- 2nd strand cDNA synthesis that keeps the 5´ end of the transcripts intact

The Template Switching RT Enzyme Mix is optimized for efficient template switching during the RT reaction. The enzyme mix contains RNase Inhibitor in a specially formulated buffer, making reactions easy to setup with no additives needed. It is highly sensitive and specific and can generate RNA-seq libraries from as little as 2 pg of human total RNA or 5´-RACE from 10 ng of total RNA, both with minimal background.

Reagents Supplied:
Template Switching RT Enzyme Mix (10X)
Template Switching RT Buffer (4X)



Primers for cDNA Synthesis

Oligo d($N)_n$ primers are used for the priming and sequencing of mRNA adjacent to the 3´-poly A tail or tailed cDNA. Note: #S1316 does not contain a 5´-phosphate.

Reference: References for properties and applications of these products can be found at www.neb.com.

PRODUCT	NEB #	SIZE
Random Primer 6 (5´d(N_6)3´) ~14.6 nmol	S1230S	1.0 A_{260} unit
Random Primer 9 (5´d(N_9)3´) ~11.6 nmol	S1254S	1.0 A_{260} unit
Oligo d($T)_{23}$ VN	S1327S	1.0 A_{260} unit
Random Primer Mix	S1330S	100 µl (60 µM)
Oligo d($T)_{18}$ mRNA Primer	S1316S	5.0 A_{260} units

ProtoScript II First Strand cDNA Synthesis Kit

RR

#E6560S 30 reactions
#E6560L 150 reactions

Companion Products:

polyA Spin mRNA Isolation Kit
#S1560S 8 isolations

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

Random Primer Mix
#S1330S 100 µl (60 µM)

Oligo $d(T)_{23}VN$*
#S1327S 1.0 A_{260} unit

*Note: V = A, G or C, and N = A, G, C or T

- *Enzyme and reaction mixes add flexibility to reaction setup*
- *Suitable for any PCR format*
- *Efficient reverse transcription from different starting RNA amounts*
- *Generates cDNA at least 10 kb*

Description: ProtoScript II First Strand cDNA Synthesis Kit features two optimized mixes, ProtoScript II Enzyme Mix and ProtoScript II Reaction Mix. The enzyme mix combines ProtoScript II Reverse Transcriptase and Murine RNase Inhibitor, while the reaction mix contains dNTPs and an optimized buffer. ProtoScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 50°C, providing higher specificity and higher yield of cDNA.

The kit also provides two optimized primers for reverse transcription and nuclease-free water. An anchored Oligo-dT primer [$d(T)_{23}VN$] forces the primer to anneal to the beginning of the poly(A) tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs. The first strand cDNA product generated is up to 10 kb.

The ProtoScript II First Strand cDNA Synthesis Kit Includes:

– 10X ProtoScript II Enzyme Mix
– 2X ProtoScript II Reaction Mix
– Random Primer Mix (60 µM), Oligo $d(T)_{23}VN$ Primer (50 µM)** and Nuclease-free Water

**Oligo $d(T)_{23}$ VN and Random Primer Mix contain 1 mM dNTP

For robust amplification of a wide range of DNA templates, we recommend One*Taq*® or Q5® High-Fidelity DNA Polymerases.

ProtoScript First Strand cDNA Synthesis Kit

RR

#E6300S 30 reactions
#E6300L 150 reactions

Companion Products:

polyA Spin mRNA Isolation Kit
#S1560S 8 isolations

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

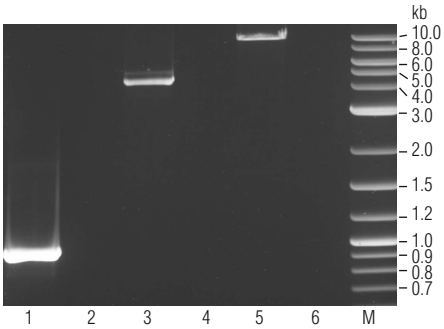
- *Enzyme and reaction mixes add flexibility to reaction setup*
- *Suitable for any PCR format*
- *Efficient reverse transcription from different starting RNA amounts*
- *Generates cDNA at least 5 kb*

Description: ProtoScript First Strand cDNA Synthesis Kit features two optimized mixes, ProtoScript Enzyme Mix and Protoscript Reaction Mix. Protoscript Enzyme Mix combines M-MuLV Reverse Transcriptase and RNase Inhibitor, Murine, while Protoscript Reaction Mix contains dNTPs and an optimized buffer. The kit also contains two optimized primers for reverse transcription and nuclease-free water. An anchored oligo-dT primer [$d(T)_{23}VN$] forces the primer to anneal to the beginning of the polyA tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA template including both mRNAs and non-polyadenylated RNAs. The first strand cDNA product generated is over 13.0 kb. This product was formerly known as M-MuLV First Strand cDNA Synthesis Kit.

The ProtoScript First Strand cDNA Synthesis Kit Includes:

– 10X ProtoScript Enzyme Mix
– 2X ProtoScript Reaction Mix
– Random Primer Mix (60 µM), Oligo $d(T)_{23}VN$ Primer (50 µM)** and Nuclease-free Water

**Oligo $d(T)_{23}$ VN and Random Primer Mix contain 1 mM dNTP



First Strand cDNA Synthesis with the ProtoScript Kit. *Reactions were performed at 42°C using 2 µg of human spleen total RNA. Negative control reactions (-RT) were carried out with 1X ProtoScript Reaction Mix. A fraction of the first strand cDNA product was used to amplify sequences specific for three different messenger RNAs using 1X LongAmp® Taq 2X Master Mix (NEB #M0287). Lane 1: 1.1 kb of beta-actin gene. Lane 2: -RT control of 1.1 kb of beta-actin gene. Lane 3: 4.7 kb of Xrn-1 gene. Lane 4: -RT control of 4.7 kb of Xrn-1 gene. Lane 5: 9.8 kb of guanine nucleotide exchange factor p532. Lane 6: -RT control of 9.8 kb of guanine nucleotide exchange factor p532. Marker M is 1 kb Plus DNA Ladder (NEB #N3200).*

RT-PCR & RT-qPCR Kits

RR See pages 71–72 for more information.

Luna® Universal One-Step RT-qPCR Kit

#E3005S	200 reactions
#E3005L	500 reactions
#E3005X	1,000 reactions
#E3005E	2,500 reactions

Luna Universal Probe One-Step RT-qPCR Kit

#E3006S	200 reactions
#E3006L	500 reactions
#E3006X	1,000 reactions
#E3006E	2,500 reactions

One*Taq*® One-Step RT-PCR Kit

#E5315S	30 reactions

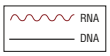
One*Taq* RT-PCR Kit

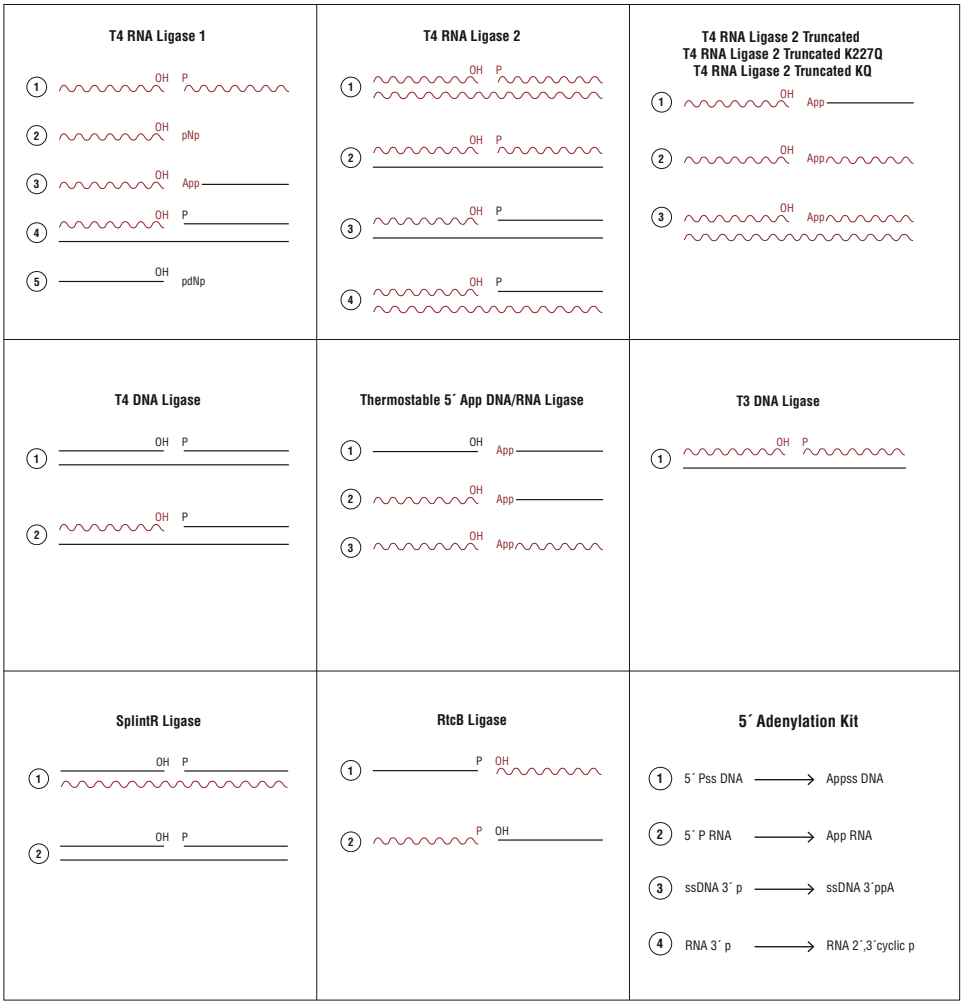
#E5310S	30 reactions

RNA Ligase Activity Chart

NEB offers a variety of ligases for DNA and RNA research. Many of these enzymes are recombinant and all offer the quality and value you have come to expect from our products. The chart below highlights reported activities of our T4 ligases ranked by application. A substrate-based selection chart for DNA ligases can be found on page 94.

Reported Activities and Applications for T4 Ligases

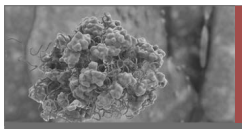




The ligation activities depicted have been reported, but may require optimized reaction conditions.

RNA Ligase Selection Chart

RNA Ligase Selection Chart

	T4 RNA Ligase 1	T4 RNA Ligase 2	T4 RNA Ligase 2 Truncated	T4 RNA Ligase 2, Truncated K227Q	T4 RNA Ligase 2, Truncated KQ	Thermostable 5´ App DNA/RNA Ligase	5´ Adeny-lation Kit	SplintR® Ligase	RtcB Ligase
RNA APPLICATIONS									
Ligation of nicks in dsRNA		★★★							
Labeling of 3´ termini of RNA	★★★		★	★	★	★			
Ligation of ssRNA to ssRNA	★★★								
Ligation of preadenylated adaptors to RNA	★★		★★	★★	★★★	★★			
5´ Adenylation							★★★		
Ligation of 3´ P and 5´ OH of ssRNA									★★★
DNA APPLICATIONS									
Ligation of preadenylated adaptors to ssDNA						★★★			
DNA/RNA APPLICATIONS									
Joining of RNA & DNA in a ds-structure		★★							
ssDNA Ligation with RNA Splint		★★						★★★	
Ligation of RNA and DNA with 3´ P and 5´ OH									★★
NGS APPLICATIONS									
NGS Library Prep ssRNA-ssDNA (ligation)	▲		▲	▲	▲				
NGS Library Prep ssRNA-ds-Adaptor splinted ligation		▲							
FEATURES									
Thermostable						✔	✔		
Recombinant	✔	✔	✔	✔	✔	✔	✔	✔	✔

★★★ Optimal, recommended ligase for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
▲ Please consult the specific NGS protocol to determine the optimal enzyme for your needs

Harriet is the Chief IP Counsel for NEB and has been with the company for 17 years. When she is not working, Harriet enjoys traveling with a good history book.

T4 RNA Ligase 1 (ssRNA Ligase)

T4 RNA Ligase 1 (ssRNA Ligase)
#M0204S 1,000 units
#M0204L 5,000 units

T4 RNA Ligase 1 (ssRNA Ligase) High Concentration
#M0437M 5,000 units

Companion Products:

Adenosine 5´ -Triphosphate (ATP)
#P0756S 1 ml
#P0756L 5 ml

Universal miRNA Cloning Linker
#S1315S 5 µg

- *Ligation of ss-RNA and DNA*
- *Labeling of 3´-termini of RNA with 5´-[^{32}P] pCp*
- *Inter- and intramolecular joining of RNA and DNA molecules*
- *Synthesis of ss-oligodeoxyribonucleotides*
- *Incorporation of unnatural amino acids into proteins*

Description: Catalyzes ligation of a 5´ phosphoryl-terminated nucleic acid donor to a 3´ hydroxyl-terminated nucleic acid acceptor through the formation of a 3´→ 5´ phosphodiester bond with hydrolysis of ATP to AMP and PPi. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer. Supplement with 1 mM ATP. Incubate at 25°C. May heat inactivate at 65°C for 15 minutes or boiling for 2 minutes.

Notes on Use: Addition of DMSO to 10% (v/v) is required for pCp ligation.

Reagents Supplied with Enzyme:
10X T4 RNA Ligase Reaction Buffer
10 mM ATP (with NEB #M0204) or 100 mM ATP (with NEB #M0437) 50% PEG 8000

Unit Definition: One unit is defined as the amount of enzyme required to convert 1 nmol of 5´-[^{32}P] rA16 into a phosphate resistant form in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 or 30,000 units/ml

T4 RNA Ligase 2 (dsRNA Ligase)

#M0239S 150 units
#M0239L 750 units

- *Cohesive-end adaptor ligation*
- *Best choice for ligating nicks in dsRNA*
- *Suitable for ligating 3´ OH of RNA to 5´ phosphate of DNA in a DNA/RNA hybrid*

Description: T4 RNA Ligase 2, also known as T4 Rnl2 (gp24.1), has both intermolecular and intramolecular RNA strand-joining activity. Unlike T4 RNA Ligase 1 (NEB #M0204), T4 RNA Ligase 2 is much more active joining nicks on double stranded RNA than on joining the ends of single stranded RNA. The enzyme requires an adjacent 5´ phosphate and 3´ OH for ligation. The enzyme can also ligate the 3´ OH of RNA to the 5´ phosphate of DNA in a double stranded structure.

Reaction Conditions: 1X T4 RNA Ligase 2 Reaction Buffer. Incubate at 37°C. May heat inactivate at 80°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to ligate 0.4 µg of an equimolar mix of a 23-mer and 17-mer RNAs in a total reaction volume of 20 µl in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

T4 RNA Ligase 2, truncated

#M0242S 2,000 units
#M0242L 10,000 units

Companion Product:

Universal miRNA Cloning Linker
#S1315S 5 µg

- *Ligate a pre-adenylated DNA or RNA sequence tag to any RNA 3´end*
- *Join a single-stranded adenylated primer to small RNAs for cDNA library creation*
- *Join a single-stranded adenylated primer to RNA for strand-specific cDNA library construction*

Description: T4 RNA Ligase 2, truncated (T4 Rnl2tr) specifically ligates the pre-adenylated 5´ end of DNA or RNA to the 3´ end of RNA. The enzyme does not require ATP, but does need the pre-adenylated substrate. T4 Rnl2tr is expressed from a plasmid in *E. coli* which encodes the first 249 amino acids of the full-length T4 RNA Ligase 2. Unlike the full-length ligase, T4 Rnl2tr cannot ligate the phosphorylated 5´ end of RNA or DNA to the 3´ end of RNA. This enzyme, also known as Rnl2 (1–249), has been used for optimized linker ligation for the cloning of microRNAs. This enzyme reduces background ligation, because it can only use pre-adenylated linkers.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer. Incubate at 25°C. May heat inactivate at 65°C for 20 minutes.

Reagents Supplied with Enzyme:
10X T4 RNA Ligase Reaction Buffer
50% PEG 8000

Unit Definition: 200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer [DNA Universal miRNA Cloning Linker (NEB #S1315)] in a total reaction volume of 20 µl in 1 hour at 25°C. Unit assay conditions can be found at www.neb.com.

Concentration: 200,000 units/ml

T4 RNA Ligase 2, truncated K227Q and truncated KQ

RR 25° 65°

T4 RNA Ligase 2, truncated K227Q

#M0351S	2,000 units
#M0351L	10,000 units

T4 RNA Ligase 2, truncated KQ

#M0373S	2,000 units
#M0373L	10,000 units

Companion Product:

Universal miRNA Cloning Linker

#S1315S	5 µg

- *Ligate a pre-adenylated DNA or RNA sequence tag to any RNA 3´ end*
- *Join a single stranded adenylated primer to small RNAs for cDNA library creation*
- *Join a single stranded adenylated primer to RNA for strand-specific cDNA library construction*

Description: T4 RNA Ligase 2, truncated KQ (T4 Rnl2tr KQ) specifically ligates the pre-adenylated 5´ end of DNA or RNA to the 3´ OH end of RNA. The enzyme does not use ATP for ligation, but requires pre-adenylated linkers.

T4 Rnl2tr KQ is a double-point mutant of T4 RNA Ligase 2, truncated (NEB #M0242). Mutation of K227 in T4 RNA Ligase 2 reduces enzyme lysyl adenylation. K227Q reduces the formation of undesired ligation products (concatemers and circles) by T4 Rnl2tr, by reducing the trace activity of T4 Rnl2tr in transfer of adenylyl groups from linkers to the 5´-phosphates of input RNAs. Mutation of R55K in T4 Rnl2tr K227Q increases the ligation activity of the enzyme to levels similar to T4 Rnl2tr.

The exclusion of ATP, use of pre-adenylated linkers, and the reduced enzyme lysyl adenylation activity provide the lowest possible background in ligation reactions. This enzyme has been used for optimized linker ligation for high-throughput sequencing library construction of small RNAs.

Source: Expressed as an MBP fusion from a plasmid in *E. coli* which encodes the first 249 amino acids of the full length T4 RNA Ligase 2. T4 Rnl2tr K227Q has a lysine to glutamine mutation at position 227. T4 Rnl2tr KQ has an arginine to lysine and lysine to glutamine mutation at positions 55 and 227, respectively.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer. Incubate at 25°C. May heat inactivate at 65°C for 20 minutes.

Reagents Supplied with Enzyme:
10X T4 RNA Ligase Reaction Buffer
50% PEG 8000

Unit Definition: 200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA [Universal miRNA Cloning Linker (NEB #S1315)] in a total reaction volume of 20 µl in 1 hour at 25°C. Unit assay conditions can be found at www.neb.com.

Concentration: 200,000 units/ml

RtcB Ligase

🦋 RR 37°

#M0458S 25 reactions

- *Ligate ssRNA or ssDNA with a 3´-phosphate or a 2´,3´-cyclic phosphate to the 5´-OH of ssRNA*
- *Circularization of ssRNA with compatible ends*

This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Description: RtcB Ligase from *E. coli* joins single stranded RNA with a 3´-phospate or 2´,3´-cyclic phosphate to another RNA with a 5´ -hydroxyl. Ligation requires both GTP and $MnCl_2$ and proceeds through a 3´-guanylate intermediate. With substrates having a 2´,3´-cyclic phosphate end, hydrolysis to a 3´-phosphate precedes 3´ end activation with GMP and ligation.

Source: RtcB Ligase is expressed as His-tagged fusion in *E. coli*.

Reaction Conditions: 1X RtcB Reaction Buffer. Supplement with 0.1 mM GTP and 1 mM $MnCl_2$ (supplied). Incubate at 37°C.

Reagents Supplied with Enzyme:
10X RtcB Reaction Buffer
$MnCl_2$ (10 mM)
GTP (10 mM)

Concentration: 15 µM

Thermostable 5´ AppDNA/RNA Ligase

🦋 RR 65° NEB

#M0319S	10 reactions
#M0319L	50 reactions

Companion Product:

Universal miRNA Cloning Linker

#S1315S	5 µg

- *Ligation of ssDNA to an adenylated DNA linker for NGS library construction*
- *Ligation of an adenylated linker to RNA at elevated temperatures for small RNA NGS library construction*

Description: Thermostable 5´ App DNA/RNA Ligase is a point mutant of catalytic lysine of RNA ligase from *Methanobacterium thermoautotrophicum*. This enzyme is ATP independent. It requires a 5´ pre-adenylated linker for ligation to the 3´-OH end of either RNA or single stranded DNA (ssDNA). The enzyme is also active in ligation of RNA with 2´-*O*-methylated 3´ end to 5´-adenylated linkers. The optimal temperature for ligation reaction is 60–65°C. The mutant ligase is unable to adenylate the 5´-phosphate of RNA or ssDNA, which reduces the formation of undesired ligation products (concatemers and circles).

The ability of the ligase to function at 65°C might reduce the constraints of RNA secondary structure in RNA ligation experiments.

Reaction Conditions: 1X NEBuffer 1. Incubate at 65°C.

Reagents Supplied with Enzyme:
10X NEBuffer 1
10X $MnCl_2$

Concentration: 20 µM

Usage Note: For optimal ligation of ssDNA to preadenylated linkers, we recommend using NEBuffer 1 supplemented with manganese (supplied).

5´ DNA Adenylation Kit

RR 65°

#E2610S 10 reactions
#E2610L 50 reactions

- *Enzymatic 5´ adenylation of ss-DNA linkers for next gen sequencing*
- *One-step reaction gives quantitative adenylation*
- *Simpler than existing chemical and enzymatic methods*
- *Reduces need for purification of reaction product*

Description: The 5´ DNA Adenylation Kit is a simple and efficient enzymatic method for generating 5´-adenylated DNA oligonucleotides using *Mth* RNA ligase, ATP and single stranded 5´-phosphorylated DNA. The kit is optimized to produce the adenylated DNA intermediate with or without 3´ terminator. The 5´ DNA adenylation kit routinely generates greater than 95% conversion of pDNA to AppDNA. This highly efficient process eliminates the need for gel isolation of the product and increases overall yield.

The 5´ DNA Adenylation Kit Includes:
– *Mth* RNA Ligase (Recombinant)
– 5´ DNA Adenylation Reaction Buffer (10X)
– 1 mM ATP

Note: The low turnover of the enzyme requires an approximately equimolar concentration of the enzyme and the oligonucleotide substrate. Adenylated DNA linkers can be used for 3´-end ligation of RNA in cDNA library preparation for next generation sequencing protocols.

SplintR® Ligase

RR 25° 65°

#M0375S 1,250 units
#M0375L 6,250 units

- *Ligation of adjacent, single-stranded DNA splinted by a complimentary RNA*
- *Characterization of miRNAs and mRNAs, including SNPs*

Description: SplintR Ligase, also known as PBCV-1 DNA Ligase or *Chlorella* virus DNA Ligase, efficiently catalyzes the ligation of adjacent, single-stranded DNA oligonucleotides splinted by a complementary RNA strand. This previously unreported activity may enable novel approaches for characterization of miRNAs and mRNAs, including SNPs. SplintR is ideally suited for many target enrichment workflows with applications in next-generation sequencing and molecular diagnostics. The robust activity of the enzyme and its affinity for RNA-splinted DNA substrates (apparent Km = 1 nM) enable sub-nanomolar detection of unique RNA species within a complex mixture, making SplintR ligase a superior choice for demanding RNA detection technologies.

Reaction Conditions: 1X SplintR Ligase Reaction Buffer. Incubate at 25°C. May heat inactivate at 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme needed to ligate (to 50% completion) 2 picomoles of a tripartite FAM-labeled DNA:RNA hybrid substrate in a 20 µl reaction at 25°C in 15 minutes in 1X SplintR Ligase Reaction Buffer. Unit assay conditions can be found at www.neb.com.

Concentration: 25,000 units/ml

RNA 5´ Pyrophosphohydrolase (RppH)

RR 37°

#M0356S 200 units

- *Conversion of 5´-triphosphate RNA to monophosphate RNA*
- *Preparation of 5´-phosphate RNA for ligation*
- *Characterization of RNA 5´ ends*

Description: The bacterial RNA 5´ Pyrophosphohydrolase (RppH) removes pyrophosphate from the 5´ end of triphosphorylated RNA to leave a 5´ monophosphate RNA. The RppH protein was also known as NudH/YgdP which can split diadenosine penta-phosphate to ADP and ATP.

Reaction Conditions: 1X NEBuffer 2. Incubate at 37°C.

Reagents Supplied with Enzyme:
10X NEBuffer 2

Unit Definition: One unit is defined as the amount of enzyme that converts 1 µg 300-mer RNA transcript into an XRN-1 digestible RNA in 30 minutes at 37°C.

Concentration: 5,000 units/ml

5´ Deadenylase

RR 30° 70°

#M0331S 1,000 units

- *Deadenylation of 5´ end of DNA and RNA*
- *Aprataxin-dependent DNA repair assay*
- *Analysis of dinucleoside tetraphosphate*

Description: Yeast 5´ Deadenylase is a member of the HIT (histidine triad) family of proteins and specifically a member of the Hint branch. It is the yeast orthologue of aprataxin. Mutations in human aprataxin have been known to be involved in the neurological disorder known as ataxia oculomotor apraxia-1. The human protein has been shown to resolve abortive ligation intermediates by removing the AMP at the 5´ end of DNA (AMP-DNA hydrolase activity). It also repairs DNA damage at 3´ ends by removing 3´-phosphate and 3´-phosphoglycolate. Human aprataxin acts on small molecules, such as nucleotide polyphosphates diadenosine tetraphosphate (AppppA) and lysyl-AMP.

The 5´ Deadenylase is encoded by the *HNT3* gene of *S. cerevisiae*. NEB has shown this protein is capable of deadenylation from 5´ end of DNA and RNA, leaving the phosphate at 5´ end. It also cleaves AppppA into ATP and AMP. Its activity on lysyl-AMP is not detectable.

Reaction Conditions: 1X NEBuffer 1 and 5–50 pmol adenylated DNA (AMP-DNA) in 20 µl. Incubate at 30°C. May heat inactivate at 70°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to remove 10 pmoles of AMP from a 5´-adenylated DNA oligo in 10 minutes at 30°C.

Concentration: 50,000 units/ml

RNase I_f

RR 37° 70°

#M0243S 5,000 units
#M0243L 25,000 units

- *Eliminates RNA from DNA and protein preparations*
- *Degradation of single-stranded RNA to mono-, di- and trinucleotides*
- *Used in ribonuclease protection assays*

Description: Ribonuclease I_f (RNase I_f) is an RNA endonuclease which will cleave at all RNA dinucleotide bonds leaving a 5´ hydroxyl and 2´, 3´ cyclic monophosphate. It has a preference for single-stranded RNA over double-stranded RNA. RNase I_f is a recombinant protein fusion of RNase I (from *E. coli*) and maltose-binding protein. It has identical activity to RNase I.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C. May heat inactivate at 70°C for 20 minutes.

Notes on Use: RNase I_f will not degrade DNA. It has a strong preference for single-stranded RNA over double-stranded RNA.

Unit Definition: One unit is defined as the amount of enzyme required to fully digest 1 picomole of synthetic ssRNA 33-mer in a total reaction volume of 10 µl in 15 minutes in 1X NEBuffer 3 as visualized on a 20% acrylamide gel (40:1 Bis) stained with SYBR® Gold. Unit assay conditions can be found at www.neb.com.

Concentration: 50,000 units/ml

SYBR® is a registered trademark of Molecular Probes, Inc.

RNase H

RR 37° 65°

#M0297S 250 units
#M0297L 1,250 units

- *Removal of poly(A) tails of mRNA hybridized to poly(dT)*
- *Removal of mRNA during second strand cDNA synthesis*

Description: Ribonuclease H (RNase H) is an endoribonuclease which specifically hydrolyzes the phosphodiester bonds of RNA which is hybridized to DNA. This enzyme does not digest single- or double-stranded DNA.

Reaction Conditions: 1X RNase H Reaction Buffer. Incubate at 37°C. May heat inactivate at 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 20 picomoles of a fluorescently labelled 50 base pair RNA-DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

NEW

Thermostable RNase H

RR 50° No

#M0523S 250 units

- *Higher stringency RNA structure mapping and site-specific RNA cleavage*
- *Removal of poly(A) tails from mRNA hybridized to oligo(dT)*
- *Removal of mRNA during second strand cDNA synthesis*
- *Component of isothermal amplification methods*

Description: Thermostable RNase H specifically recognizes and cleaves the phosphodiester bonds of an RNA strand in an RNA-DNA hybrid while leaving the DNA strand intact. This thermostable nuclease exhibits the same enzymatic properties as *E. coli* RNase H, but is active at much higher temperatures.

Reaction Conditions: 1X RNase H Reaction Buffer. Incubate at 50°C.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 40 picomoles of a fluorescently labelled 25 base pair RNA-DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 50°C. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

RNase HII

RR 37° No

#M0288S 250 units
#M0288L 1,250 units

- *Nicking of products generated with a polymerase that will incorporate ribonucleotides*
- *Generation of a double-stranded break at the site of an incorporated ribonucleotide when used with T7 Endo I*
- *Degradation of the RNA portion of Okazaki fragments or other RNA-DNA hybrids*

Description: Ribonuclease HII (RNase HII) is an endoribonuclease that preferentially nicks 5´ to a ribonucleotide within the context of a DNA duplex. The enzyme leaves 5´ phosphate and 3´ hydroxyl ends. RNase HII will also nick at multiple sites along the RNA portion of an Okazaki fragment.

Source: An *E. coli* strain containing a genetic fusion of the RNase HII gene (rnhB) from *E. coli* and the gene coding for maltose binding protein (MBP). Following affinity chromatography, RNase HII is cleaved from the fusion construct by Factor Xa and then purified away from both MBP and Factor Xa. RNase HII cleaved from MBP has four additional amino acids at its N-terminus (Ile-Ser-Glu-Phe).

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to yield a fluorescence signal consistent with the nicking of 100 pmol of synthetic double-stranded DNA substrate containing a single ribonucleotide near the quencher of a fluorophore/quencher pair in 30 minutes at 37°C in 1X ThermoPol Reaction Buffer. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Note: Incubation with 0.1% SDS is sufficient to inactivate RNase HII.

Phosphorylation and Dephosphorylation

See pages 98–100 for more information.

Quick Dephosphorylation Kit
#M0508S 100 units
#M0508L 500 units

Antarctic Phosphatase
#M0289S 1,000 units
#M0289L 5,000 units

Alkaline Phosphatase Calf Intestinal (CIP)
#M0290S 1,000 units
#M0290L 5,000 units

Shrimp Alkaline Phosphatase (rSAP)
#M0371S 500 units
#M0371L 2,500 units

T4 Polynucleotide Kinase
#M0201S 500 units
#M0201L 2,500 units

ShortCut® RNase III

RR 37° 65°

#M0245S 200 units
#M0245L 1,000 units

- *Generates siRNAs for RNA interference studies*
- *Gene silencing*
- *Target validation*
- *Removal of long dsRNAs*

Description: ShortCut RNase III converts long double-stranded RNA into a heterogeneous mix of short (18–25 bp) interfering RNAs (siRNA) suitable for RNA interference in mammalian cells. 1.5 units (1 µl) of ShortCut RNase III is sufficient to convert 1 µg of dsRNA into siRNA.

Source: An *E. coli* strain containing a genetic fusion of the *E. coli* RNase III gene (rnc) and the gene coding for maltose binding protein (MBP).

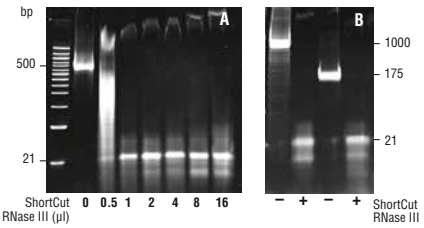
Reaction Conditions: 1X ShortCut RNase III Reaction Buffer. Supplement with 20 mM $MnCl_2$ (supplied). Incubate at 37°C.

Unit Definition: One unit is the amount of enzyme required to digest 1 µg of dsRNA to siRNA in a total reaction volume of 50 µl in 20 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 2,000 units/ml

Advantages of the ShortCut RNase III:

- Make effective siRNAs against any gene target
- Heterogeneous population of siRNA ensures silencing of target gene
- From DNA template to transfection in just 1 day
- Eliminates trial and error approach of synthetic siRNA



siRNA production by ShortCut RNase III: *A) Varying amounts of ShortCut RNase III were incubated with 2 µg of a 500 bp dsRNA for 20 minutes. B) dsRNA fragments (1 kb and 175 bp) were digested with ShortCut RNase III. Digests were analyzed by 20% TBE polyacrylamide gel electrophoresis.*

XRN-1

RR 37° 70°

#M0338S 20 units
#M0338L 100 units

- *Removal of RNA containing 5´ monophosphate from an RNA mixture*

Description: XRN-1 is a highly processive 5´ to 3´ exoribonuclease, requiring a 5´ monophosphate. It also acts on 5´ monophosphate ssDNA with reduced efficiency.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C. May heat inactivate at 70°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme that digests 1 µg of phosphorylated yeast RNA in 60 minutes at 37°C.

Concentration: 1,000 units/ml

Exonuclease T

#M0265S 250 units
#M0265L 1,250 units

- *Generate blunt ends in DNA or RNA with 3´ overhangs*

Description: Exonuclease T (Exo T), also known as RNase T, is a single-stranded RNA or DNA specific nuclease that requires a free 3´ terminus and removes nucleotides in the 3´→ 5´ direction. Exo T can be used to generate blunt ends from RNA or DNA having 3´ extensions.

Source: Exonuclease T is overexpressed and purified as a C-terminal fusion to maltose-binding protein (MBP). MBP is removed from Exo T by Factor Xa cleavage and then purified. When cleaved from MBP has an additional amino acid on the N-terminus and a Phe instead of a Met (i.e. Glu-Phe-Exo T instead of Met-Exo T).

Reaction Conditions: 1X NEBuffer 4. Incubate at 25°C. May heat inactivate at 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 0.1 nmol of TCA soluble nucleotides from 1 nmol of [^{3}H]-labeled polythymidine in a total reaction volume of 100 µl in 30 minutes at 25°C in 1X NEBuffer 4 with 1 nmol [^{3}H]-labeled polythymidine DNA.

Concentration: 5,000 units/ml

Usage Note: Exo T is has different activity on RNA vs. DNA. For RNA, 1 unit of Exo T is required to completely digest 1.0 pmol of rA20 under standard reaction condition as measured by gel electrophoresis.

NEW

Nucleoside Digestion Mix

#M0649S 50 reactions

- *Convenient one-step protocol*
- *Digests both DNA and RNA to single nucleosides*
- *Low-glycerol formulation significantly reduces glycerol-induced ion suppression during MS analysis*

Description: The Nucleoside Digestion Mix is a mixture of enzymes that provides a convenient one-step method to generate single nucleosides from DNA or RNA. Optimized for quantitative analysis by liquid chromatography-mass spectrometry (LC-MS), this reagent eliminates the need for sequential multi-step, time-consuming digestion protocols. The Nucleoside Digestion Mix digests ssDNA, dsDNA, DNA/RNA hybrids and RNA (except mRNA cap structures) containing epigenetically modified (m5C, hm5C, f5C, ca5C, m4C, m6A, etc.), unnatural, or damaged bases. Moreover, the low-glycerol formulation (< 1%) significantly reduces glycerol-induced ion suppression during mass spectrometry analysis.

Reaction Conditions: 1X Nucleoside Digestion Mix Reaction Buffer. Incubate at 37°C. May heat inactivate at 65°C for 20 minutes.

DNase I (RNase-free)

#M0303S 1,000 units
#M0303L 5,000 units

- *Degradation of DNA template in transcription reactions*
- *Removal of contaminating genomic DNA from RNA samples*
- *DNase I footprinting*
- *Nick Translation*

Description: DNase I (RNase-free) is an endonuclease that nonspecifically cleaves DNA to release di-, tri- and oligonucleotide products with 5´-phosphorylated and 3´-hydroxylated ends. DNase I acts on single- and double-stranded DNA, chromatin and RNA:DNA hybrids.

Reaction Conditions: 1X DNase I Reaction Buffer. Incubate at 37°C. May heat inactivate at 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme which will completely degrade 1 µg of pBR322 DNA in a total reaction volume of 50 µl in 10 minutes at 37°C.

Complete degradation is defined as the reduction of the majority of DNA fragments to tetranucleotides or smaller.

Concentration: 2,000 units/ml

Note: EDTA should be added to a final concentration of 5 mM to protect RNA from being degraded during enzyme inactivation.

RNase Inhibitor, Murine

#M0314S 3,000 units
#M0314L 15,000 units

- *Inhibits common eukaryotic RNases*
- *Compatible with Taq Polymerase, AMV or M-MuLV Reverse Transcriptases*
- *cDNA synthesis & RT-PCR*
- *In vitro transcription/translation*
- *Enzymatic RNA labeling reaction*

RNase Inhibitor, Murine has significantly improved resistance to oxidation compared to human & porcine RNase inhibitors.

Description: RNase Inhibitor, Murine is a 50 kDa recombinant protein of murine origin. It specifically inhibits RNases A, B and C by binding noncovalently in a 1:1 ratio with high affinity. It is not effective against RNase 1, RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. No inhibition of polymerase activity is observed when used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

Recombinant RNase Inhibitor, Murine does not contain the pair of cysteines identified in the human version that are very sensitive to oxidation and lead to inactivation of the inhibitor. As a result, RNase Inhibitor, Murine has significantly improved resistance to oxidation compared to the human/porcine RNase inhibitors, and is stable at low DTT concentrations (< 1 mM). This makes it ideal for reactions where high concentration DTT is adverse to the reaction (e.g., RT-qPCR).

Unit Definition: One unit is defined as the amount of RNase Inhibitor, Murine required to inhibit the activity of 5 ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2´, 3´-cyclic monophosphate by RNase A.

Concentration: 40,000 units/ml

RNase Inhibitor, Human Placenta

RR

#M0307S 2,000 units
#M0307L 10,000 units

- *Inhibits common eukaryotic RNases*
- *Compatible with Taq Polymerase, AMV or M-MuLV Reverse Transcriptases*
- *Active over a broad pH range (pH 5–8)*
- *cDNA synthesis reactions*
- *In vitro transcription/translation*

Description: RNase Inhibitor, Human Placenta is a recombinant human placental protein which specifically inhibits ribonucleases (RNases) A, B and C. It is not effective against RNase 1, RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. In addition, no inhibition of polymerase activity is observed when RNase Inhibitor, Human Placenta is used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

The 50 kDa protein inhibits RNases by binding noncovalently in a 1:1 ratio with an association constant greater than 10^{14}.

Unit Definition: One unit is defined as the amount of RNase Inhibitor, Human Placenta required to inhibit the activity of 5 ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2´, 3´-cyclic monophosphate by RNase A.

Concentration: 40,000 units/ml

Ribonucleoside Vanadyl Complex

#S1402S 10 ml (200 mM)

Ribonucleoside Vanadyl Complex is an equimolar mixture of all four ribonucleosides, complexed with oxovanadium IV by a modification of the procedures by Berger (1). Each lot of the complex is assayed for oxovanadium V content and inhibition of ribonuclease activity.

Vanadium complexes are used in mRNA purifications as exogenous ribonuclease inhibitors. They are compatible with cell lysis techniques and with sucrose gradient fractionation of cytoplasmic components.

Reference:

(1) Berger, S.L. and Birkenmeier, C.S. (1979) *Biochemistry* 18, 5143–5149.

NEBNext® Reagents for RNA Library Preparation

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina
#E7760S 24 rxns
#E7760L 96 rxns

NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads
#E7765S 24 rxns
#E7765L 96 rxns

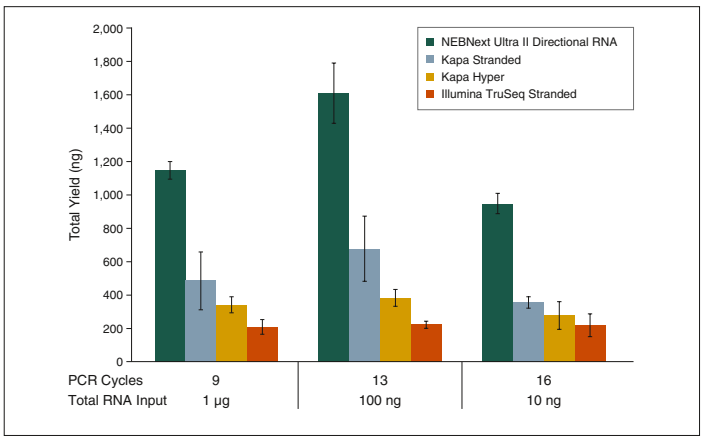
NEBNext Ultra II RNA Library Prep Kit for Illumina
#E7770S 24 rxns
#E7770L 96 rxns

NEBNext Ultra II RNA Library Prep with Sample Purification Beads
#E7775S 24 rxns
#E7775L 96 rxns

NEW
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina
#E6420S 24 rxns
#E6420L 96 rxns

NEW
NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module
#E6421S 24 rxns
#E6421L 96 rxns

Do you need increased sensitivity and specificity from your RNA-seq experiments? Do you have ever-decreasing amounts of input RNA? To address these challenges, our next generation of RNA library prep kits have been reformulated at each step, resulting in several fold higher yields of high quality libraries and enabling use of lower input amounts, including single cells, and fewer PCR cycles. The kits have streamlined, automatable workflows and are available for directional (strand-specific, using the "dUTP method") and non-directional library prep, with the option of SPRISelect beads for size-selection and clean-up steps.



NEBNext Ultra II Directional RNA produces the highest yields, from a range of input amounts.
Poly(A)-containing mRNA was isolated from 10 ng, 100 ng and 1 µg of Universal Human Reference RNA (Agilent #740000) and libraries were made using the NEBNext Ultra II Directional RNA kit, Kapa™ Stranded mRNA-Seq kit, Kapa mRNA HyperPrep kit and Illumina® TruSeq® Stranded mRNA Kit. The input RNA amount and number of PCR cycles are indicated. Library yields from an average of three replicates are shown. Error bars indicate standard deviation. Library yields were assessed using the Agilent® Bioanalyzer®.

rRNA depletion and poly(A) mRNA isolation reagents are available separately. More information on NEBNext reagents for RNA library preparation can be found on pages 142–148.

EpiMark® N6-Methyladenosine Enrichment Kit

Epi

#E1610S 20 reactions

- *Enrichment for m6A modified RNA in immunoprecipitation protocols*
- *Enriched RNA can be used directly for next gen sequencing or RT-qPCR*

Description: The EpiMark N6-Methyladenosine Enrichment Kit contains a rabbit monoclonal antibody specific for N6-Methyladenosine (m6A). The kit also contains two control RNAs, one with m6A modification (*Gaussia* luciferase) and one without (*Cypridina* luciferase) to monitor enrichment and depletion. The GLuc RNA control was transcribed in the presence of 20% m6ATP and 80% ATP.

This kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR. Modified RNA is isolated from a fragmented RNA sample by binding to the N6-Methyladenosine antibody attached to Protein G Magnetic Beads. After multiple wash and clean-up steps, the enriched RNA is eluted in nuclease-free water and is ready for further analysis.

The EpiMark N6-Methyladenosine Enrichment Kit Includes:

– N6-Methyladenosine Antibody

– m6A Control RNA (100 nM)

– Unmodified Control RNA (100 nM)

N6-Methyladenosine Antibody is produced by Cell Signaling Technology, Inc. and sold by New England Biolabs, Inc.

p19 siRNA Binding Protein

RR

#M0310S 1,000 units

Companion Products:

Chitin Resin
#S6651S 20 ml
#S6651L 100 ml

Chitin Magnetic Beads
#E8036S 5 ml
#E8036L 25 ml

- *High affinity binding of siRNAs*
- *Affinity purification of siRNA with chitin magnetic beads*

Description: The p19 siRNA Binding Protein (19 kDa) from the plant Carnation Italian Ringspot Virus (CIRV) binds siRNAs with nanomolar affinity. The dimeric protein preferentially binds 21 -nucleotide siRNAs with a 2 -nucleotide 3´ extension and a 5´phosphate. The protein binds RNA in a size-dependent and sequence-independent manner. If the siRNAs are 4 bases longer, the affinity for the protein is reduced about 100 fold. When p19 siRNA Binding Protein is expressed in plants it suppresses RNA interference.

Source: p19 siRNA Binding Protein is cloned and expressed in *E. coli* as a fusion protein with an amino terminal MBP (Maltose-binding protein) and a carboxy terminal CBD (Chitin binding domain).

Unit Definition: One unit is defined as amount of protein that binds to 10 ng of siRNA at 25°C in 1 hour. Unit assay conditions can be found at www.neb.com.

Molecular Weight: 67 kDa

Concentration: 10,000 units/ml

Magnetic mRNA Isolation Kit

#S1550S 25 isolations

Component Sold Separately:

Oligo $d(T)_{25}$ Magnetic Beads
#S1419S 25 mg

Companion Products:

6-tube Magnetic Separation Rack
#S1506S 6 tubes (1.5 ml)

12-tube Magnetic Separation Rack
#S1509S 12 tubes (1.5 ml)

- *Suitable for automated high-throughput applications*
- *Eliminates need for organic solvents*
- *No need to precipitate poly(A)+ transcripts in eluent*
- *Obtain intact poly(A)+ RNA in < 1 hour*
- *Negligible gDNA contamination*

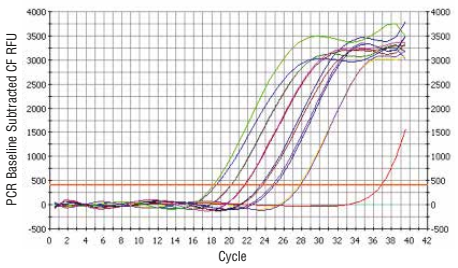
Description: The New England Biolabs Magnetic mRNA Isolation Kit is designed to isolate intact poly(A)$^+$ RNA from cells and tissue without requiring phenol or other organic solvents. The technology is based on the coupling of Oligo $d(T)_{25}$ to 1 µm paramagnetic beads which are then used as the solid support for the direct binding of poly(A)$^+$ RNA. Thus, the procedure permits the manual processing of multiple samples and can be adapted for automated high-throughput applications. Additionally, magnetic separation technology permits elution of intact mRNA in small volumes eliminating the need for precipitating the poly(A)$^+$ transcripts in the eluent. Intact poly(A)$^+$ RNA which is fully representative of the mRNA population of the original sample can be obtained in less than one hour. Oligo $d(T)_{25}$ Magnetic Beads can be reused up to three times and the researcher has the option of eluting the isolated mRNA or using the bound dT DNA as a primer in a first-strand cDNA reaction.

The Magnetic mRNA Isolation Kit Includes:

– Oligo $d(T)_{25}$ Magnetic Beads

– Lysis/Binding Buffer

– Wash Buffer I, II, III, Elution Buffer



Consistency and wide isolation range are demonstrated by poly(A)$^+$ RNA isolation from duplicate samples of decreasing numbers of HEPG2 cells (5 x 10^5 to 1 x 10^3) by direct lysis/binding in microtiter plates followed by mRNA isolation with the magnetic method. 1/10th of isolated mRNA is converted to oligo (dT) primed cDNA using ProtoScript M-MuLV First Strand cDNA Synthesis Kit (NEB #E6300) and qPCR done with validated primers for the peptidylpropyl isomerase, a low-abundance housekeeping gene.

Oligo $d(T)_{25}$ Magnetic Beads

#S1419S 25 mg

Companion Product:

96-well Microtiter Plate Magnetic Separation Rack
#S1511S 96 well

Description: An affinity matrix for the small-scale isolation of mRNA from crude cell lysates and tissue. The isolation occurs through the hybridization of covalently coupled oligo $d(T)_{25}$ to the poly(A) region present in most eukaryotic mRNAs. Applications include direct mRNA isolation following lysis and second-round purification of previously isolated total RNA. The magnetic separation technology is scalable and permits elution of intact mRNA in small volumes eliminating the need for precipation of the isolated mRNA. Beads can be reused up to three times, and the researcher has the option of eluting the isolated mRNA or using the bound $d(T)_{25}$ as a primer in a first-strand cDNA reaction.

Beads are supplied as a 5 mg/ml suspension in phosphate buffer (PBS) (pH 7.4), containing 0.05% Tween-20 and 0.05% NaN_3.

Support Matrix: 1 µm nonporous superparamagnetic microparticles.

Binding Capacity: 1 mg of Oligo $d(T)_{25}$ Beads will bind 10 µg of poly(A)$^+$ RNA.

Oligo $(dT)_{25}$ Cellulose Beads

#S1408S 250 mg

Description: An affinity matrix used for the isolation of mRNA containing polyadenylic (poly A) regions. This matrix consists of oligo $(dT)_{25}$ covalently coupled to a cross-linked cellulose bead.

Support Matrix: A cross-linked cellulose bead.

Binding Capacity: > 400 O.D. per gram of cellulose

polyA Spin™ mRNA Isolation Kit

#S1560S 8 isolations

Companion Product:

Oligo $d(T)_{25}$ Cellulose Beads
#S1408S 250 mg

Description: The polyA Spin Kit is a rapid and convenient alternative for the purification of full-length poly(A)$^+$ eukaryotic messenger (mRNA) from samples of total RNA. Poly(A)$^+$ RNA selection is made by affinity chromatography using spin columns prepackaged with NEB's Oligo $(dT)_{25}$-Cellulose Beads (NEB #S1408). Its high binding capacity and rapid hybridization kinetics make it an ideal support for chromatographic poly(A)$^+$ RNA selection. Intact mRNA can be isolated in as little as forty minutes from multiple eukaryotic cell lysates or samples of total RNA from various sources, including fungi, plants and animal tissue.

Reagents sufficient for the isolation and subsequent precipitation of poly(A)$^+$ RNA from eight samples of as much as 1 mg of total RNA are provided. The isolated RNA can be used for *in vitro* translation, preparation of cDNA libraries, northern analysis, subtractive hybridization or differential display.

The polyA Spin Kit Includes:

- Prepacked Oligo dT_{25}-Cellulose Beads
- Sterile microfuge recovery tubes
- Wash Buffer, Elution Buffer, Glycogen, NaCl and NaOAc

Streptavidin Magnetic Beads

#S1420S 5 ml (20 mg)

Companion Products:

6-Tube Magnetic Separation Rack
#S1506S 6 tubes (1.5 ml)

96-well Microtiter Plate Magnetic Separation Rack
#S1511S 96 well

Description: Streptavidin Magnetic Beads are 1 µm superparamagnetic particles covalently coupled to a highly pure form of streptavidin. The beads can be used to capture of biotin labeled substrates including antigens, antibodies and nucleic acids. The strength of the biotin-streptavidin interaction coupled with low non-specific binding permits captured substrates to be useful as ligands in subsequent experiments including mRNA isolation and the capture of primary or secondary antibodies.

Beads are supplied as a 4 mg/ml suspension in phosphate buffer (PBS) (pH 7.4) containing 0.1% BSA, 0.05% Tween-20 and 0.05% NaN_3.

Support Matrix: 1 µm non-porous superparamagnetic microparticle.

Binding Capacity: The beads will bind greater than 1000 pmol of free biotin per mg and greater than 500 pmol of single-stranded 25 bp biotinylated oligonucleotide per mg.

NEW

Monarch Kits for Cleanup & Isolation

See pages 130–131 for more information.

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

Monarch RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

The Monarch Total RNA Miniprep Kit is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, tough-to-lyse samples, such as bacteria, yeast, and plant, can be processed with additional steps that enhance lysis. Cleanup of enzymatic reactions or purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has high quality metrics, including $A_{260/280}$ and $A_{260/230}$ ratios ≥ 1.8, high RIN scores, and minimal residual gDNA. Captured RNA ranges in size from full-length rRNAs down to intact miRNAs. Additionally, differential binding conditions allow selective capture or exclusion of the sub-200 nucleotide RNA pool that includes miRNA, 5S rRNA, and tRNA. Purified RNA is suitable for downstream applications, such as RT-qPCR, cDNA synthesis, RNA-seq, Northern blot analysis, etc.

The Monarch RNA Cleanup Kits provide a fast and simple silica spin column-based solution for RNA cleanup and concentration after any enzymatic reaction (including *in vitro* transcription, DNase I treatment, capping and labeling) and after other purification methods such as phenol/chloroform extraction. The Monarch RNA Cleanup Kits are available in 3 different binding capacities: 10 µg, 50 µg and 500 µg. Each kit contains unique columns, all designed to prevent buffer retention and ensure no carryover of contaminants, enabling low-volume elution of highly-pure RNA. Following the standard protocol, RNA ≥ 25 nt is purified with this kit; however, a modified protocol is available to enable the binding of RNA as small as 15 nt (including miRNAs).

The Monarch Total RNA Miniprep Kit Includes:

– Monarch gDNA Removal Columns
– Monarch RNA Purification Columns
– Monarch Collection Tubes II
– Monarch DNA/RNA Protection Reagent (2X)
– Monarch RNA Lysis Buffer
– Monarch Proteinase K
– Monarch Proteinase K Resuspension Buffer
– Monarch Proteinase K Reaction Buffer
– Monarch DNase I
– Monarch DNase I Reaction Buffer
– Monarch RNA Priming Buffer
– Monarch RNA Wash Buffer (5X)
– Monarch Nuclease-free Water

The Monarch RNA Cleanup Kits Include:

– Monarch RNA Cleanup Columns (10, 50 or 500 µg)
– Monarch RNA Cleanup Binding Buffer
– Monarch RNA Cleanup Wash Buffer
– Monarch Collection Tubes II
– Nuclease-free Water

Claire has been with NEB for just over a year as a Quality Systems & Documentation Specialist. She is already an active member of the NEB community, having participated on the Raffle Committee, Education Committee, and in the Gloucester BioTech Academy/ NEB Mentorship Program.

RNA Markers & Ladders

dsRNA Ladder
#N0363S 25 gel lanes

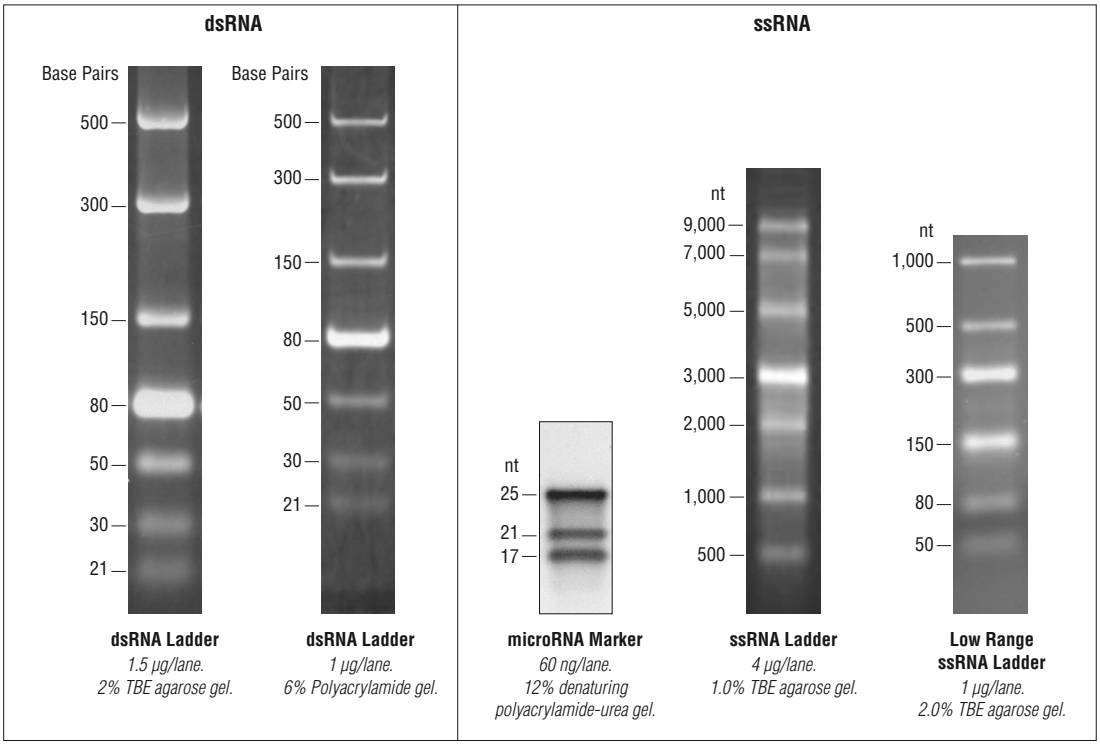
microRNA Marker
#N2102S 100 gel lanes

ssRNA Ladder
#N0362S 25 gel lanes

Low Range ssRNA Ladder
#N0364S 100 gel lanes

NEB offers several RNA Markers and Ladders with a size range from 17 to 9,000 bases. The ssRNA ladders are suitable for use as RNA size standards on denaturing or native gels. Both are supplied with 2X Loading Buffer and feature a higher intensity fragment to serve as a reference band. The microRNA Marker, supplied in a ready-to-load denaturing solution, is ideally used as a size marker on polyacrylamide gels or Northern blots and is best visualized stained with ssRNA fluorescent dyes. It is supplied with a 3´-biotinylated 21-mer oligonucleotide probe that can also be labeled with [γ-^{32}P] ATP and T4 PNK (NEB #M0201). The ds RNA Ladder is suitable for use as a size standard in dsRNA analysis on both polyacrylamide and agarose gels.

Concentration: Low Range ssRNA Ladder and dsRNA Ladder are supplied at 500 µg/ml. ssRNA Ladder is supplied at 2,000 µg/ml. MicroRNA Marker is supplied at 12 ng/µl.



dsRNA Ladder
1.5 µg/lane.
2% TBE agarose gel.

dsRNA Ladder
1 µg/lane.
6% Polyacrylamide gel.

microRNA Marker
60 ng/lane.
12% denaturing polyacrylamide-urea gel.

ssRNA Ladder
4 µg/lane.
1.0% TBE agarose gel.

Low Range ssRNA Ladder
1 µg/lane.
2.0% TBE agarose gel.

RNA Loading Dye (2X)

#B0363S 4 ml

Description: The RNA Loading Dye, (2X) is a premixed loading dye for use with denaturing and non-denaturing PAGE/agarose gels.

RNA Loading Dye Composition:

1X RNA Loading Dye:

47.5% Formamide, 0.01% SDS, 0.01% Bromophenol Blue, 0.005% xylene cyanol and 0.5 mM EDTA.

Universal miRNA Cloning Linker

#S1315S 0.83 nmol

Companion Product:

T4 RNA Ligase 2, truncated KQ
#M0373S 2,000 units
#M0373L 10,000 units

This 5-adenylated, 3´ -blocked oligoribonucleotide can be used for cloning short RNAs according to the procedure of Bartel (1). RNA ligase recognizes the "activated" adenylated oligo and covalently links (ligates) its 5´ end to the 3´ OH of a second single stranded sequence in the absence of ATP. In a mixture of nucleic acids use of the 5´ adenylated, 3´ blocked oligo with T4 RNA Ligase 2, truncated, T4 RNA Ligase 2, truncated K227Q or T4 RNA Ligase 1 (w/o ATP) results in ligation of the target oligo only.

The sequence of the adenylated DNA oligo is 5´-rAppCTGTAGGCACCATCAAT-NH_2 3´.

Reference:

(1) Lau et al. (2001) *Science*, 294, 858–856.

The first oil to appear in the marshes from the 2010 Gulf Oil Spill, Venice, Louisiana, United States.
Credit: Carrie Vonderhaar/ Ocean Futures Society, Getty Images

Microbes Cleaning Up Our Mess

In a world where we consume 90 million barrels of oil every day, spills at oil rigs are inevitable. In fact, according to the U.S. Environmental Protection Agency, there are 70 spills per day — most are insignificant. However, in 2010, 4.9 million barrels of crude oil spewed from the BP Macondo well, located in the Gulf of Mexico, over a period of 87 days. This unfortunate event was termed the Deepwater Horizon spill, and is the largest marine oil spill in history.

Extensive damage to marine ecosystems resulted from the spill, including heavy oiling of four of the five endangered, protected turtle species that live and breed in the Gulf of Mexico. Additionally, many marine mammals that live in the Gulf of Mexico experienced reproductive failure and organ damage following the spill.

Large fire booms were used to surround the slick and burn off approximately 5% of the oil. Chemical dispersants broke 40% of the oil into tiny droplets suspended under the ocean surface in a plume that measured 161 km (100 miles) long. Scientists were surprised to find that the clean-up occurred at a much faster rate than predicted, because it was aided by hydrocarbon-degrading microorganisms. Further, the use of chemical dispersants, while controversial due to their toxicity, actually aided this process by generating smaller size oil droplets (and a larger surface area) for what was estimated to be a bloom of 100 sextillion microbes.

Background levels of oil exist in many marine ecosystems due to slow seepage from underwater reservoirs. Luckily, a well-established community of bacteria capable of degrading the compounds contained in oil is also present. The short doubling time of these bacteria and their ability for horizontal gene transfer allow for the rapid proliferation of this diverse community following a spill. These hydrocarbon-degraders use the oil as an energy source. Each microbe population has a distinct set of hydrocarbon degradation genes with specificity for breaking down different constituents of the oil, and they proliferate at different time points following a spill, depending on their specificity. Following degradation of the oil, the microbes stop rapidly dividing and are consumed by other organisms further up the food chain.

Oil from the Deepwater Horizon spill washed into wetlands and marshes all along the northern Gulf of Mexico, and heavily oiled a coastal plant named *Spartina alterniflora*, commonly known as smooth cordgrass. Cordgrass is a foundational plant that stabilizes land and minimizes erosion. Scientists found that in addition to the presence of oil in the plant tissues, the composition of endophytes living symbiotically in cordgrass roots showed an increase in oil-degrading bacteria. It is well known that these bacteria can degrade oil in oceans and on beaches, but what researchers are trying to establish is whether the bacteria continue to process the oil inside the plant tissue, and if so, which bacteria are most efficient at accomplishing this. Scientists are also exploring whether the plant roots can deliver hydrocarbon-degrading bacteria to the buried oil in the marsh. This raises the possibility for bacterial inoculation of cordgrass, which can then be planted and used to help restore coastal areas exposed to an oil spill.

The Deepwater Horizon oil spill was catastrophic to marine habitats. What resulted from the close study of these habitats was the identification and understanding of a diverse community of microorganisms, which are highly evolved to deal with their specialized environment and when necessary, can clean up the mess we make. Yet another reason to appreciate microbes.

Protein Expression & Purification Technologies

NEB offers an array of solutions for robust expression of your target protein.

At first glance, recombinant protein expression looks quite simple. Essentially, DNA encoding a target protein is cloned downstream of a promoter in an expression vector. This vector is then introduced into a host cell, and the cell's protein synthesis machinery produces the desired protein. In practice, however, protein expression can be very challenging because so many factors may influence the process. For example, each protein folds in its own unique manner, a process that may be influenced by the choice of expression host. Similarly, some proteins require post-translational modifications or proper insertion into a biological membrane. Finally, some proteins may have an activity that is detrimental to the host. Thus, no single solution exists for successful production of all recombinant proteins. Instead, it is beneficial to have access to a wide range of expression tools, and a willingness to explore multiple approaches to better one's chances for success.

NEB offers an array of expression systems offering different advantages, enabling you to choose the strategy that best suits your protein expression and purification needs. Many share a compatible polylinker, enabling the gene of interest to be easily shuffled between systems. Additionally, a selection of competent cells is available for *in vitro* expression of difficult-to-express proteins.

Featured Products

216 PURExpress® *In Vitro* Protein Synthesis Kit

217 Competent Cells for Protein Expression

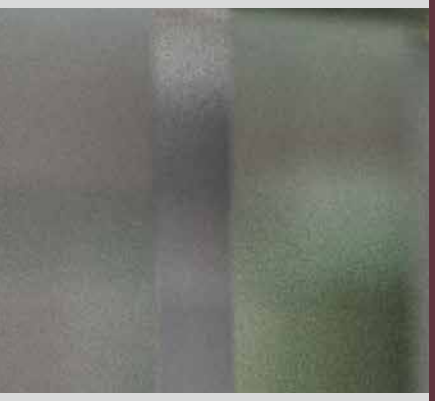
Featured Tools & Resources

212 Protein Expression Selection Chart

361 Enhancing Efficiency

362 Protein Expression with T7 Express Strains

Did you know you can find application notes using our expression systems on our website? Choose the applications tab at www.neb.com to learn more.



Protein Expression & Purification Selection Chart

NEB offers an array of expression systems with different advantages, enabling you to choose the strategy that best suits your protein expression and purification needs. Many share a compatible polylinker, enabling the gene of interest to be easily shuffled between systems. Additionally, a selection of competent cells is available for *in vitro* expression of difficult-to-expess proteins.

APPLICATION	KIT	ADVANTAGES
High Yield Expression	pMAL™ Protein Fusion and Purification System	Substantial yields (up to 100 mg/L) in more than 75% cases tested; uses the strong P_{tac} promoter
	K. lactis Protein Expression Kit	Uses the strong *LAC4* promoter; multiple integrations of plasmid results in higher yield
	IMPACT™ Kit	Uses the T7 promoter for high level regulated expression
Cell-free Expression	PURExpress® *In Vitro* Protein Synthesis Kit	Quickly generates analytical amounts of protein
Co-expression of Multiple Proteins	*K. lactis* Protein Expression Kit	Easily co-express 2–4 proteins
	PURExpress *In Vitro* Protein Synthesis Kits	Bicistronic constructs or multiple plasmids with appropriate transcription and translation regulatory elements can be used
Enhanced Solubility	pMAL Protein Fusion and Purification System	Fusion to MBP enhances solubility of proteins in *E.coli* *
	K. lactis Protein Expression Kit	Utilizes *K. lactis* eukaryotic folding pathway
Affinity Tag Chromatography	IMPACT Kit	Utilizes an intein-chitin binding domain (CBD) tag on either the N- or C- terminus, offering single-step purification
	pMAL Protein Fusion and Purification System	Fusion to MBP allows for purification on amylose resin
	K. lactis Protein Expression Kit	Vectors are sold separately that generate fusions to MBP allowing for purification on amylose resin
Post-translational Modification	*K. lactis* Protein Expression Kit	Secretion of both *N*- and *O*- glycosylated proteins
Periplasmic Expression	pMAL Protein Fusion and Purification System	Periplasmic expression enhances folding of proteins with disulfide bonds
Secreted Expression	*K. lactis* Protein Expression Kit	Eliminates cell lysis step, simplifying purification
Toxic Proteins	*K. lactis* Protein Expression Kit	Secretion of protein from the cell
	IMPACT Kit	Can express the toxic gene in two pieces and ligate proteins together using intein mediated protein ligation (IPL)
	pMAL Protein Fusion and Purification System	Can export toxic proteins into periplasmic space
	PURExpress *In Vitro* Protein Synthesis Kits	Cell-free environment not affected by "toxicity" of expressed protein
Protein Modification	IMPACT Kit	Generates proteins with reactive ends (N-terminal cysteine and/or C-terminal thioester) allowing for labeling or ligation of proteins or peptides.
	PURExpress *In Vitro* Protein Synthesis Kits	Allows introduction of modified, unnatural or labeled amino acids
No Additional Amino Acid Residues	IMPACT Kit	Start of native protein is fused adjacent to site of intein cleavage
	pMAL Protein Fusion and Purification System	Start of protein is fused adjacent to protease site

*Kapust and Waugh (1999) *Protein Science*, 8, 1668–1674.

pMAL™ Protein Fusion and Purification System

#E8200S

Components Sold Separately:

Amylose Resin
#E8021S 15 ml
#E8021L 100 ml

Factor Xa Protease
#P8010S 50 µg
#P8010L 250 µg

pMAL-p5X Vector
#N8109S 10 µg

pMAL-c5X Vector
#N8108S 10 µg

- *Reliable expression: substantial yields (up to 100 mg/L) in more than 75% of the cases tested*
- *Expression in either the cytoplasm or periplasm: folding of proteins with disulfide bonds can be enhanced by expression in the periplasm or expression in the cytoplasm of SHuffle® cells*
- *Enhanced solubility: MBP fusion proteins demonstrate improved solubility when expressed in E. coli*
- *Gentle elution with maltose: no detergents or harsh denaturants*

For a more detailed description and a restriction map of the pMAL-p5X vector, including the MCS, see page 374. See our website, www.neb.com, for pMAL sequence files.

Description: In the pMAL Protein Fusion and Purification System, the cloned gene is inserted into a pMAL vector downstream from the *malE* gene, which encodes maltose-binding protein (MBP). This results in the expression of a MBP-fusion protein. The technique uses the strong P_{tac} promoter and the translation initiation signals of MBP to express large amounts of the fusion protein. The fusion protein is then purified by a one-step affinity purification specific for MBP (Figure 1).

The system uses the pMAL vectors which include a sequence coding for the recognition site of a specific protease adjacent to the multiple cloning site. This allows the protein of interest to be cleaved from MBP after purification, without adding any vector-derived residues to the protein. For this purpose, the polylinker includes a restriction site superimposed on the sequence coding for the site of the specific protease. This is where the gene of interest is inserted.

Expression from the pMAL vectors yields up to 100 mg fusion protein from a liter of culture. In most cases, the expressed protein is soluble, as fusion to MBP has been proven to enhance the solubility of proteins expressed in *E. coli*. While no expression system works with every cloned gene, the pMAL Protein Fusion and Purification System gives substantial yields of protein in more than 75% of the cases tested.

In pMAL-c5X, the *malE* signal sequence is deleted, resulting in cytoplasmic expression of the fusion protein. The pMAL-p5X Vector contains the *malE* signal sequence directing the fusion protein through the cytoplasmic membrane into the periplasm.

References: References for properties and applications of this product can be found at www.neb.com.

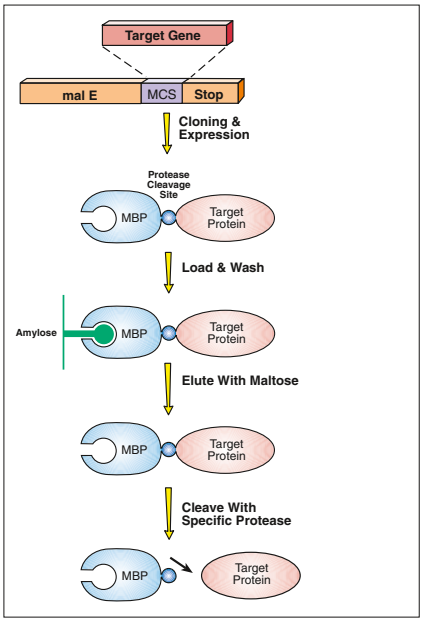


Figure 1: *Schematic of the pMAL System.*

The pMAL Protein Fusion and Purification System includes:

- pMAL-c5X, pMAL-p5X
- Amylose Resin (binding capacity > 4 mg/ml volume)
- Factor Xa Protease
- MBP5 (marker for SDS-polyacrylamide gels)
- MBP5-paramyosin-ΔSal (control protein for Factor Xa cleavage)
- *E. coli* ER2523 (NEB Express)

pMAL Companion Products

Amylose Resin
#E8021S 15 ml
#E8021L 100 ml

Amylose Resin High Flow
#E8022S 15 ml
#E8022L 100 ml

Description: Affinity matrix used for isolation of proteins fused to maltose-binding protein. It is a composite amylose/agarose bead. Amylose Resin High Flow is a more rigid bead, suitable for use in automated chromatography systems.

Binding Capacity: Amylose Resin and Amylose Resin High Flow: > 4 mg MBP5-paramyosin ΔSal fusion protein/ml of bed volume.

Amylose Magnetic Beads
#E8035S 25 mg

Anti-MBP Magnetic Beads
#E8037S 10 mg

Description: Affinity matrices for the small-scale isolation and purification of MBP-fusion proteins. Amylose or monoclonal Anti-MBP are covalently coupled to a paramagnetic particle through a linkage that is stable and leak resistant over a wide pH range.

Binding Capacity: 1 mg of Amylose Magnetic Beads will bind ≥ 10 µg of MBP-fusion protein.

1 mg of Anti-MBP Magnetic Beads will bind 5 µg of MBP-paramyosin fusion protein.

Anti-MBP Monoclonal Antibody
#E8032S 0.05 ml
#E8032L 0.25 ml

Description: Anti-MBP Monoclonal Antibody is a murine anti-maltose-binding protein antibody, isotype IgG2a. It is purified from tissue culture supernatant by protein A affinity chromatography.

IMPACT™ Kit

#E6901S

Components Sold Separately:

pTXB1 Vector
#N6707S 10 µg

pTYB21 Vector
#N6709S 10 µg

Chitin Resin
#S6651S 20 ml
#S6651L 100 ml

Anti-CBD Monoclonal Antibody
#E8034S 0.05 ml

pTWIN1 Vector
#N6951S 10 µg
(not included with the kit)

- *Single-column purification without the use of proteases*
- *Produce target protein without vector–derived amino acids*
- *Fusion to either N- or C-terminus of target protein*
- *Ligation and labeling of recombinant proteins*
- *Isolation of proteins with or without N-terminal methionine*

For a more detailed description and a restriction map of the pTXB1 and pTYB21 vectors, including the MCS, see page 378–379; visit www.neb.com for sequence files.

Description: The IMPACT (Intein Mediated Purification with an Affinity Chitin-binding Tag) Kit utilizes engineered protein splicing elements (inteins) to purify recombinant proteins by a single column (Figure 1). This kit distinguishes itself from other protein fusion systems by its ability to separate a recombinant protein from the affinity tag without the use of a protease.

The IMPACT Kit allows fusion of a tag consisting of an intein and the chitin binding domain (CBD) from *Bacillus circulans*, to either the C-terminus (pTXB1) or the N-terminus (pTYB21) of a target protein (Figure 2). In the presence of thiols, such as DTT, the intein undergoes specific self-cleavage which releases the target protein. The pTXB1 vector can also be used to express a protein with a C-terminal thioester for use in Intein-mediated Protein Ligation (IPL). The IPL reaction, also referred to as expressed protein ligation, allows for ligation of a peptide or a protein with a N-terminal cysteine to a bacterially expressed protein with a C-terminal thioester through a native peptide bond for use in protein labeling and semisynthesis. For more information on the IMPACT System and IPL, visit www.neb.com.

pTXB1 is a *E. coli* expression vector that utilizes a mini-intein from the *Mycobacterium xenopi gyrA* gene [*Mxe* GyrA intein; 22 kDa]. This intein has been modified and combined with the CBD to create an affinity tag which can be bound to chitin beads (NEB #S6651). Release of the target protein is induced by thiol-reagents such as DTT or 2-mercaptoethanesulfonic acid (for ligation).

The pTYB21 vector allows for the fusion of the intein tag containing the *Saccharomyces cerevisiae* (*Sce*) *VMA*1 intein and CBD to the N-terminus of the target protein.

pTWIN1 Vector is available separately and enables isolation of proteins with an N-terminal cysteine and/or a C-terminal thioester. The polylinker is designed for the in-frame fusion of a target gene between the modified *Ssp* DnaB and *Mxe* GyrA inteins. The presence of the CBD facilitates purification.

References: References for properties and applications of this product can be found at www.neb.com.

The IMPACT Kit Includes:

– *E. coli* expression vectors: pTXB1, pTYB21 and pMXB10 Control Vector

– Chitin Beads: Affinity matrix (binding capacity=2 mg/ml)

– Anti-CBD Monoclonal Antibody

– 1, 4-Dithiothreitol (DTT) & SDS Loading Buffer

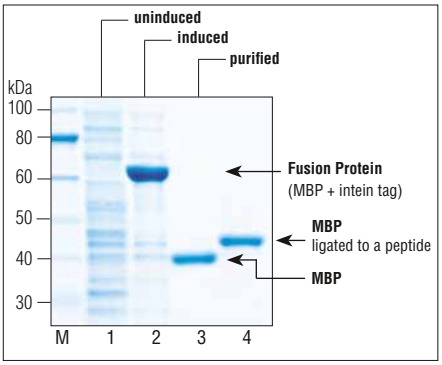


Figure 1: *Purification of Maltose Binding Protein (MBP) in a single affinity purification step: Lane 1: uninduced cell extract. Lane 2: induced cell extract showing expressed fusion protein. Lane 3: MBP fraction eluted after inducing cleavage overnight at 4°C. Lane 4: MBP ligated to a peptide containing an N-terminal cysteine. Marker M is the protein ladder.*

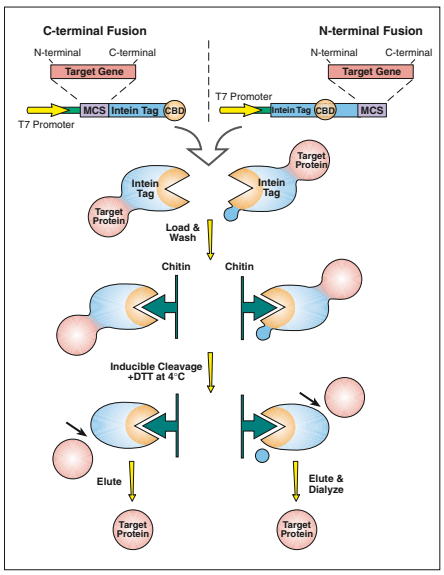


Figure 2: *Schematic of the IMPACT System.*

Table 1: *Guide to IMPACT vectors and applications.*

VECTORS	SITE OF TARGET PROTEIN FUSION	INTEIN TAG (KDA)	RECOMMENDED CLONING SITES[a]	PREFERRED RESIDUES AT CLEAVAGE SITE[b]	METHOD OF CLEAVAGE[c,d]	APPLICATIONS
pTXB1	C-terminus	Mxe GyrA intein (28)	NdeI-SapI/SpeI	Y, F, O, N, T, K, A, H, M (Unfavorable residues-S, P, D, G)	DTT (or MESNA) pH 8.0-8.5, 4°C	Purification; C-terminal thioester for ligation and modification
pTYB21	N-terminus	Sce VMA1 intein (56)	SapI/BsmI/NdeI-PstI	A, Q, M, G, L, N, W, F, Y (Unfavorable residues-P, S, C, T, R)	DTT[d] pH 8.0-8.5, 25°C	Purification
pTWIN1	C-terminus (Intein 2)	Mxe GyrA intein (28)	NdeI-SapI/SpeI	M, Y, F, LEM (Unfavorable residues-S, P, E, D)	DTT (or MESNA) pH 8.0-8.5, 4°C	Purification; C-terminal thioester for ligation and modification

[a] NEBuilder HiFi DNA Assembly Cloning Kit (NEB #E5520) can be used to generate construct without the use of restriction enzymes. [b] Actual cleavage efficiency is dependent on the adjacent residues as well as the folding of the fusion protein. [c] Dithiothreitol (DTT) is used only for protein purification. 2-mercaptoethanesulfonic acid (MESNA) is used for isolation of proteins possessing a C-terminal thioester for ligation, labeling and cyclization. [d] Cysteine can be used in the place of DTT.

K. lactis Protein Expression Kit

#E1000S

Components Sold Separately:

SacII

#R0157S	2,000 units
#R0157L	10,000 units

Yeast Medium Pack

#B9017S	12 g

K. lactis GG799 Competent Cells

#C1001S	5 transformation reactions

- *Clone and express genes toxic to E. coli*
- *Simultaneous expression of multiple genes*
- *No expensive antibiotics or methanol required*
- *Easy-to-use protocols for those inexperienced with yeast systems*
- *Attractive commercial sublicensing*

Restriction map for pKLAC2 can be found on page 373; sequence files are available at www.neb.com.

Description: The *K. lactis* Protein Expression Kit provides an easy method for expressing a gene of interest in the yeast *Kluyveromyces lactis*. The gene is cloned into the integrative pKLAC-series of vectors, and may be expressed intracellularly or secreted. The *K. lactis* system offers several advantages over other yeast and bacterial expression systems. Abundant overexpression of protein is achieved through high culture densities as well as the ability to integrate multiple copies of the vector. The pKLAC-series of vectors use a strong *LAC4* promoter, which has been modified to lack expression in *E. coli*, making this system useful for expressing toxic genes. For the selection of transformants, no expensive antibiotics are required. In addition, no methanol is required in growth media. Finally, the *K. lactis* system can express post-translationally modified proteins, making it a useful alternative to bacterial expression systems.

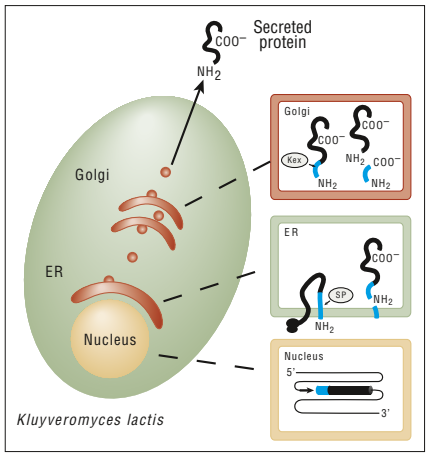
pKLAC2 is a general purpose expression vector. Using this vector, proteins may be produced intracellularly or may be fused to the *K. lactis* α-mating factor sequence for secreted expression. Vector pKLAC2 contains an MCS that is compatible with other expression systems available from NEB.

GG799 competent cells are provided in the *K. lactis* Protein Expression Kit. GG799 cells are characterized by very high cell density growth and efficient expression of foreign proteins. GG799 cells have no genetic modifications.

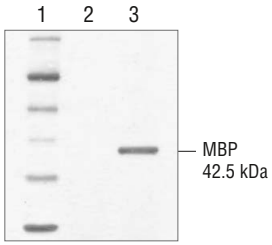
The *K. lactis* Protein Expression Kit Includes:

- pKLAC2 Vector and pKLAC1-*malE* Control Plasmid
- SacII
- Integration Primer Set
- CutSmart® Buffer
- *K. lactis* GG799 Competent Cells and Transformation Reagent
- Yeast Medium Powder & Acetamide Solution

References: References for properties and applications of these products can be found at www.neb.com.



Secreted protein processing. *In the nucleus, an integrated expression vector encoding a fusion between the α-MF domain (blue) and a desired protein (black) is expressed. A signal peptide in the α-MF domain directs entry of the fusion protein into the endoplasmic reticulum (ER) and is removed by signal peptidase (SP). The fusion protein is transported to the Golgi apparatus where the Kex protease removes the α-MF domain. The protein of interest is then secreted from the cell.*



Protein Expression in K. lactis. *SDS-polyacrylamide gel electrophoresis separation of secreted recombinant maltose-binding protein (MBP) detected directly in peptone rich growth medium by Coomassie staining. Lane 1: Protein Molecular Weight Markers. Lane 2: spent culture medium (15 µl) from wild-type K. lactis cells. Lane 3: spent culture medium (15 µl) from K. lactis cells harboring an integrated expression cassette containing the E. coli malE gene.*

Companion Products:

Enterokinase, light chain

#P8070S	480 units
#P8070L	2,560 units

BstXI

#R0113S	1,000 units
#R0113L	5,000 units

One*Taq* DNA Polymerase

#M0480S	200 units
#M0480L	1,000 units
#M0480X	5,000 units

One*Taq* Hot Start DNA Polymerase

#M0481S	200 units
#M0481L	1,000 units
#M0481X	5,000 units

Deoxynucleotide (dNTP) Solution Mix

#N0447S	8 µmol of each*
#N0447L	40 µmol of each

*Available in 4 x 0.2 ml aliquots

PURExpress® *In Vitro* Protein Synthesis Kits

PURExpress *In Vitro* Protein Synthesis Kit
#E6800S 10 reactions
#E6800L 100 reactions

PURExpress Δ Ribosome Kit
#E3313S 10 reactions

PURExpress Δ (aa, tRNA) Kit
#E6840S 10 reactions

PURExpress Δ RF123 Kit
#E6850S 10 reactions

Companion Product:

PURExpress Disulfide Bond Enhancer
#E6820S 50 reactions

E. coli Ribosome
#P0763S 1 mg

- *Generation of analytical amounts of proteins for further characterization*
- *Confirmation of open reading frames*
- *Generation of truncated proteins to identify active domains and functional residues*
- *Introduction of modified, unnatural or labeled amino acids (NEB #E6840, #E6850)*
- *tRNA structure and function studies (NEB #E6840)*
- *Ribosome structure and function studies (NEB #E3313, #P0763)*
- *Release factor function studies/ ribosome display (NEB #E6850)*
- *Epitope mapping*

PURExpress is based on the PURE system technology originally developed by Dr. Takuya Ueda at the University of Tokyo and commercialized as the **PURESYSTEM™** by Biocomber (Tokyo, Japan).

PURESYSTEM™ is a trademark of Post Genome Institute.

Description: A rapid method for gene expression analysis, PURExpress is a novel cell-free transcription/translation system reconstituted from the purified components necessary for *E. coli* translation. The nuclease-free and protease-free nature of the PURExpress platform preserves the integrity of DNA and RNA templates/complexes and results in proteins that are free of modification and degradation. Transcription and translation are carried out in a one-step reaction, and require the mixing of only two tubes. With results available in a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput technologies.

Advantages:

- Suitable for circular or linear DNA template
- Visualize synthesized protein directly on a Coomassie stained gel
- Protein expression in approximately 2 hours
- Transcription/translation components can be removed by affinity chromatography

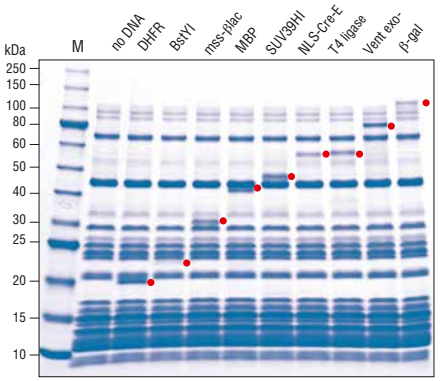
PURExpress Disulfide Bond Enhancer: This proprietary blend of proteins and buffer components is designed to correctly fold target proteins with multiple disulfide bonds produced in PURExpress reactions or *E. coli* S30 extracts. Added at the beginning of a reaction, the components assist with the oxidation of cysteine thiols and correcting mis-oxidized substrates, increasing yield of soluble and functionally active protein.

PURExpress Δ Ribosome Kit: The ribosomes are removed from this kit, allowing users to add their own modified ribosomes for studies on protein translation. Control ribosomes (sufficient for 2 reactions) are provided in a separate tube.

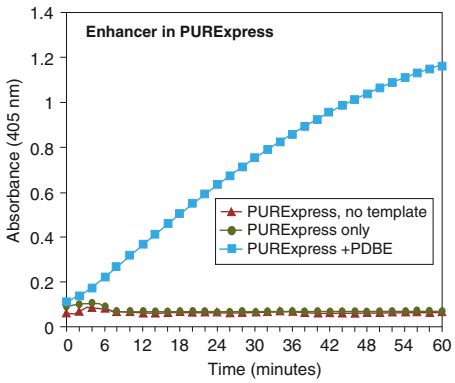
PURExpress Δ RF123 Kit: Release factors are involved in termination of protein translation by recognizing the stop codons in an mRNA sequence. In a ribosome display experiment using PURExpress, lack of release factors could stabilize the ternary complex of mRNA-ribosome-nascent protein. As a result, the cDNA recovery could be higher. In this kit, the three release factors are supplied separately, allowing users to perform a protein synthesis reaction/ribosome display experiment with/without release factors of their choice.

PURExpress Δ (aa, tRNA) Kit: The tRNA and amino acids are supplied separately in this kit, allowing users to run a protein synthesis reaction by adding modified amino acids and tRNA mixtures to the reaction.

***E. coli* Ribosome:** The 70S *E. coli* Ribosome consists of a small subunit (30S) and a large subunit (50S). This preparation of ribosomes is highly active in NEB's PURExpress Protein Synthesis Kit (NEB #E6800), and can be used in ribosome structure and function studies, as a target for drug screening and as starting material for isolation of native ribosomal RNAs (5S, 16S, 23S). *E. coli* Ribosome is supplied as a 33.3 mg/ml solution.



Protein expression using the PURExpress In Vitro Protein Synthesis Kit. *25 µl reactions containing 250 ng template DNA and 20 units RNase Inhibitor were incubated at 37°C for 2 hours. 2.5 µl of each reaction was analyzed by SDS-PAGE using a 10–20% Tris-glycine gel. The red dot indicates the protein of interest. Marker M is the Protein Ladder.*



PURExpress Disulfide Bond Enhancer. *(PDBE) promotes proper folding of active vtPA. Reactions were set up according to PURExpress specifications with the vtPA template DNA. After a 2 hour incubation at 37°C, 5 µl of each reaction was used in an activity assay and cleavage of the chromogenic substrate was monitored for one hour.*

PURExpress Kit Components

IN VITRO PROTEIN SYNTHESIS (E6800S)	Δ RIBOSOME (E3313S)	Δ RF 123 (E6850S)	Δ (aa, tRNA) (E6840S)
Solution A	Solution A	Solution A	Solution A (minus aa and tRNA)
Solution B	Factor Mix	Solution B (minus RF1, RF2 and RF3)	Solution B
Control (DHFR) template	Control (DHFR) template	Control (DHFR) template	Control (DHFR) template
	Control Ribosomes	RF1, RF2 and RF3	Amino Acid Mixture
			E. coli tRNA

Competent Cell Expression Strain Selection Chart

- *Free of animal products*
- *T1 phage resistance (fhuA2)*
- *B strains are deficient in proteases Lon and OmpT*
- *A variety of convenient formats*
- *Bulk sales capabilities with custom packaging*

NEB offers a wide selection of competent cell strains designed for the expression of a variety of proteins. Proteins with multiple disulfide bonds are correctly oxidized to significantly higher yields with SHuffle® strains. Tunable T7 expression is achieved with Lemo21(DE3), an ideal strain for difficult targets including membrane proteins. NiCo21(DE3) can be used for expression and purification of His-tagged proteins. NEB Express and T7 Express are offered with varying levels of expression control. Several strains are available with added control by *lacI*^{q}. Only NEB offers exceptional control of T7 expression from the *lysY* gene, which is ideal for proteins that are difficult to express or toxic to the cell. Each strain is provided with a protocol for optimal expression.

For more information see pages 229–233.

Expression Strains

CHARACTERISTICS	STRAIN	NEB #	SIZE
• Versatile non-T7 expression strain • Protease deficient	NEB Express Competent *E. coli**	C2523H/I	20 x 0.05 ml/6 x 0.2 ml
• Control of IPTG induced expression from P_{tac}, P_{lac} and P_{trc} • Protease deficient	NEB Express I^q Competent *E. coli*	C3037I	6 x 0.2 ml
• Most popular T7 expression strain • Protease deficient	T7 Express Competent *E. coli*	C2566H/I	20 x 0.05 ml/6 x 0.2 ml
• T7 expression • Protease deficient • Better reduction of basal expression	T7 Express *lysY* Competent *E. coli*	C3010I	6 x 0.2 ml
• T7 expression • Protease deficient • Highest level of expression control	T7 Express *lysY/I*^{q} Competent *E. coli*	C3013I	6 x 0.2 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • Protease deficient/B strain	SHuffle® Express Competent *E. coli*	C3028J	12 x 0.05 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression • Protease deficient/B strain	SHuffle T7 Express Competent *E. coli*	C3029J	12 x 0.05 ml
• T7 expression • Protease deficient/B strain • Tightly controlled expression of toxic proteins • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	SHuffle T7 Express *lysY* Competent *E. coli*	C3030J	12 x 0.05 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression/K12 strain	SHuffle T7 Competent *E. coli*	C3026J	12 x 0.05 ml
• Routine expression for non-T7 Vectors • Protease deficient	BL21 Competent *E. coli*	C2530H	20 x 0.05 ml
• Routine T7 Expression • Protease deficient	BL21(DE3) Competent *E. coli*	C2527H/I	20 x 0.05 ml/6 x 0.2 ml
• Tunable T7 Expression for difficult targets • Protease deficient	Lemo21(DE3) Competent *E. coli*	C2528J	12 x 0.05 ml
• Expression and purification of His-tagged proteins • Protease deficient	NiCo21(DE3) Competent *E. coli*	C2529H	20 x 0.05 ml

Note: Store Competent Cells at –80°C. Once thawed, do not refreeze. Storage at –20°C will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above –80°C, even if they do not thaw.

* NEB Express is the recommended strain for the pMAL Protein Fusion and Purification System.

Magnetic Affinity Matrices

Magnetic particles are ideally suited for applications involving high-throughput proteomic screening, small-scale protein isolation, immunomagnetic isolations or cell separation experiments. Magnetic affinity purification of tagged proteins, antigens, antibodies and nucleic acids can be done conveniently and quickly, with minimal time necessary for separation of the solid-phase from solution. In addition, immobilized substrates remain biologically active and can be eluted in small volumes or serve as ligands in subsequent pull-down or target interaction experiments involving DNA or proteins.

- No centrifugation required; matrix can be regenerated without loss of binding capacity.
- Minimal sample loss during pipetting because magnetic beads concentrate at the side of the tube instead of the bottom.

Immunomagnetic Isolation

Protein A Magnetic Beads
#S1425S 1 ml

Protein G Magnetic Beads
#S1430S 1 ml

- *Small-scale purification or immunoprecipitation of IgG species*
- *No centrifugation required*
- *Regenerate matrix without binding capacity loss*

Description: Protein A and Protein G Magnetic Beads are affinity matrices for the small-scale isolation and purification of immunoglobulins. They exhibit high affinity for subclasses of IgG from many mammalian species including human, rabbit and mouse. Truncated recombinant forms of Protein A and Protein G are covalently coupled to a nonporous superparamagnetic particle. These truncated proteins exhibit higher unit binding for the IgG Fc region and lower non-specific binding. The affinity for IgG varies with species and subclasses of IgG within a species (see table).

The proteins are coupled through a linkage that is stable and leak resistant over a wide pH range. This permits the immunomagnetic purification of IgG from ascites, serum or cell culture supernatants; after which, the matrix can be re-generated without loss of binding capacity. Protein A and Protein G magnetic beads can also be used to immunoprecipitate target proteins from crude cell lysates using selected primary antibodies. In addition, specific antibodies can be chemically cross-linked to the Protein A or Protein G coated surface to create a reusable immunprecipitation bead, avoiding co-elution of antibody with target antigen.

Supplied as a 1 ml suspension in PBS Buffer.

Support Matrix: 2 µm nonporous superparamagnetic microparticle

Binding Capacity: 1 ml of Protein A or Protein G Magnetic Beads binds > 280 µg of Human IgG.

References: References for enzyme properties and applications for these products can be found at www.neb.com.

Affinity of Protein A/G for IgG Species:

Species	Immunoglobulin	Binding to Protein A	Binding to Protein G
Human	IgG (normal)	++++	++++
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	–	++++
	IgG4	++++	++++
	IgM	–	–
	IgA	–	–
	IgE	–	–
Mouse	IgG1	+	++++
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	++	+++
Rat	IgG1	–	+
	IgG2a	–	++++
	IgG2b	–	++
	IgG2c	+	++
Goat	IgG	+/-	++
Rabbit	IgG	++++	+++
Sheep	IgG	+/-	++

Magnetic Beads

Streptavidin Magnetic Beads
#S1420S 5 ml

Hydrophilic Streptavidin Magnetic Beads
#S1421S 5 ml

Description: Streptavidin Magnetic Beads are 1 µm superparamagnetic particles covalently coupled to a highly pure form of streptavidin. The beads can be used to capture biotin labeled substrates including antigens, antibodies and nucleic acids. The strength of the biotin-streptavidin interaction, an association constant (Ka) of 10^{15} M^{-1}, coupled with the low non-specific binding of streptavidin, permits captured substrates to be useful as ligands in subsequent experiments including mRNA isolation and the capture of primary or secondary antibodies.

Hydrophilic Streptavidin Magnetic Beads have a modified bead surface generated by using a unique combination of blocking reagent to give better consistency, much lower non-specific binding and improved handling properties resulting in superior signal to noise in applications involving DNA immunoprecipitations.

Beads are supplied as a 4 mg/ml suspension in phosphate buffer (PBS).

Support Matrix: 1 µm nonporous superparamagnetic microparticle (#S1420) or 2 µM nonporous superparamagnetic microparticle (#S1421).

Binding Capacity: S1420 will bind > 1000 pmol of free biotin per mg or > 500 pmol of ss-25 bp biotinylated oligonucleotide per mg. S1421 will bind > 800 pmol of free biotin per mg or > 400 pmol of ss-25 bp biotinylated oligonucleotide per mg.

References: References for enzyme properties and applications for these products can be found at www.neb.com.

Immobilized Secondary Antibodies

Goat Anti-Rabbit IgG Magnetic Beads
#S1432S 20 mg

Goat Anti-Mouse IgG Magnetic Beads
#S1431S 20 mg

Goat Anti-Rat IgG Magnetic Beads
#S1433S 20 mg

Goat Anti-Rabbit IgG Magnetic Beads: An affinity matrix for the small-scale immunomagnetic separation and purification of rabbit IgGs. Goat Anti-Rabbit IgG is covalently coupled to a nonporous superparamagnetic particle. This secondary antibody binds the heavy chain of all rabbit IgG subclasses and is suitable for immunoassays that employ a rabbit IgG primary polyclonal antibody. Cell separations and sorting can be accomplished using rabbit IgG antibody to defined cell surface antigens

Goat Anti-Mouse IgG Magnetic Beads: An affinity matrix for the small-scale immunomagnetic separation and purification of mouse IgGs. Anti-Mouse IgG is covalently coupled to a nonporous superparamagnetic particle. This secondary antibody binds the heavy chain of mouse IgG and is suitable for immunoassays that employ a mouse IgG primary monoclonal antibody. Cell separations and sorting can be accomplished using a mouse IgG antibody to defined cell surface antigens.

Goat Anti-Rat IgG Magnetic Beads: An affinity matrix for the small-scale immunomagnetic separation and purification of rat IgG's. Anti-Rat IgG is covalently coupled to a 1µm nonporous superparamagnetic particle. This secondary antibody binds the Fc portion of all monoclonal rat IgG subclasses and is suitable for immunoassays that employ a rat IgG primary monoclonal antibody. Cell separations and sorting can be accomplished using a rat IgG antibody to defined cell surface antigens.

Supplied as a 1 ml suspension in PBS Buffer.

Support Matrix: 1 µm nonporous superparamagnetic microparticle.

Binding Capacity: 1 mg will bind 5 µg of IgG.

Magnetic Protein Purification

Chitin Magnetic Beads
#E8036S 5 ml
#E8036L 25 ml

Amylose Magnetic Beads
#E8035S 25 mg

NEW
Ni-NTA Magnetic Beads
#S1423S 1 ml
#S1423L 5 ml

Companion Product:

Anti-MBP Magnetic Beads
#E8037S 10 mg

Chitin Magnetic Beads: An affinity matrix for the small-scale isolation of target proteins fused to a chitin binding domain (CBD). Chitin beads have been prepared having a magnetite core. This permits the magnetic isolation of CBD-fusion proteins from cell culture supernatants; after which, the matrix can be re-generated without loss of binding capacity. Immobilized fusion proteins can be used in subsequent experiments to capture target proteins from crude cell lysates that interact with the immobilized fusion protein.

Chitin Magnetic Beads are supplied as a 50:50 (v/v) suspension in water containing 20% ethanol.

Amylose Magnetic Beads: An affinity matrix for the small-scale isolation and purification of MBP-fusion proteins. Amylose is covalently coupled to a superparamagnetic particle through a linkage that is stable and leak resistant over a wide pH range. This permits the isolation of MBP-fusion proteins from cell culture supernatants. Immobilized fusion proteins can be used in subsequent experiments to capture target proteins from crude cell lysates that interact with the immobilized MBP-fusion protein.

Amylose Magnetic Beads are supplied as a 25 mg/ml suspension in water containing 20% ethanol.

Ni-NTA Magnetic Beads: An affinity matrix for the small-scale isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins in manual or automated formats. Immobilized Metal Affinity Chromatography (IMAC) purifications employing Ni-NTA (nickel-nitrilotriacetic acid) magnetic beads can be performed under native or denaturing conditions, which permit efficient binding and purification of insoluble proteins, proteins that aggregate in inclusion bodies, or proteins that possess a tertiary structure that sequester the polyhistidine affinity tag. Immobilized His-tagged proteins can be used in subsequent experiments to pull-down proteins that may interact with the immobilized protein.

Support Matrix: Chitin Magnetic Beads are approximately 10–100 µm superparamagnetic microparticle.

Amylose Magnetic Beads are 1–10 µm superparamagnetic microparticle.

Ni-NTA Magnetic Beads are spherical, agarose-based super-paramagnetic microparticles ranging in size from 20–100 µm.

Binding Capacity: 1 ml of Chitin Magnetic Beads will bind 2 mg of CBD fusion protein.

1 mg of Amylose Magnetic Beads will bind 10 µg of MBP-fusion protein.

Ni-NTA: Varies with target, typically ≥ 7.5 mg His-tagged fusion protein/ml bed volume.

Magnetic Racks

6-Tube Magnetic Separation Rack
#S1506S 6 tubes (1.5 ml)

12-Tube Magnetic Separation Rack
#S1509S 12 tubes (1.5 ml)

50 ml Magnetic Separation Rack
#S1507S 4 tubes (50 ml)

96-Well Microtiter Plate Magnetic Separation Rack
#S1511S 96-well

Description: The Magnetic Separation Racks are designed to be used for small-scale separations using magnetic particles. The magnets are located on the sides of the racks, resulting in minimal sample loss during pipetting.

Magnets: Neodymium rare earth permanent magnets.

Dimensions: 2-Tube Rack (1" x 2" x 1¾"), 6-Tube Rack (3" x 2" x 1¾"), 12-Tube Rack (5½" x 2" x 1¾"), 50 ml Rack (3½" x 4¼" x 3½") and 96-Well Microtiter Plate Rack (5½" x 1¼" x 3¾").

SNAP-Capture Magnetic Beads

See page 283 for more information.

#S9145S 2 ml

Elwha River Restoration,
Glines Canyon Dam, Washington State.
Credit: Joel Rogers, Getty Images

The Effect of Dams on River Systems

Dams have substantially contributed to urban development and industrialization. Historically, they were considered a sign of a thriving civilization, providing hydroelectricity, irrigation, flood control and a supply of water to nearby towns. However, they come with an environmental cost, and aging dams have limited capability to meet the energy needs of a modern world.

Essentially, dams obstruct the flow of rivers — there are 84,000 dams in the USA that are three feet high or greater, blocking 600,000 miles of river. They also have a profound effect on the river ecosystem. Upstream of a dam, the water is stagnant. Sediment, rocks and wood that would normally flow downstream and shape the landscape, build up and affect the coastline ecosystem. Weeds and algae proliferate and reservoir depth results in colder water, which in turn reduces the amount of oxygen and changes the nutrient composition for marine life. The dam effectively turns the river into a lake.

The Elwha and Glines Canyon River Dams were built on the Elwha River, which mostly lies within Olympic National Park in Washington, USA, in 1913 and 1927, respectively. They were built in order to harness the power of the river to generate electricity. At the time, they energized economic growth in the region, but 100 years later they provided only a minimal amount of the electricity needs of the district. The cost of keeping the dams outweighed the benefits, and they were removed between 2011 and 2013. The removal of the dams was two decades in planning. This gave scientists the opportunity to document the surrounding ecosystem before and after the demolition, so that the feasibility of other dam removal projects could be assessed.

The two dams blocked wild salmon runs, and this had a profound effect, not only on salmon numbers but also on the surrounding ecosystem upstream of the dam. Salmon are a keystone species. They thrive and grow on marine nutrients in the sea, and when they return to the river and travel upstream to spawn, they transport valuable nutrients to the wildlife. If they die or are killed by other animals in the ecosystem, such as bears, otter and eagles, these nutrients are distributed into the surrounding vegetation. When the salmon disappeared, so did the animals that relied on them for survival.

The Elwha River was choked with 33 tons of sediment — dismantling the dams reshaped 13 miles of the Elwha River and expanded the river delta at the Pacific Ocean. Wood and sediment reshaped the shore — beaches, kelp beds and eelgrass beds flourished. Willows were soon thriving by the river. Moss grew and created a microclimate for other plants. The dams had prevented Pacific Salmon from reaching 90% of their habitat, and now they were returning to parts of the river that had not seen salmon in 100 years.

This massive project has provided a wealth of information for ecologists. Not only do we better understand the effects of putting dams in place, but we can now predict the outcome of future dam removal projects. Not every dam is obsolete, but as the outdated and unsafe dams are coming down, the flow of rivers and valuable ecosystems are being restored.

Competent Cells

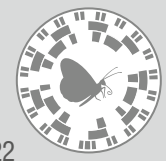
NEB has a competent cell strain for your needs.

Choose the right cells for your cloning and protein expression applications from NEB's portfolio of high efficiency competent cell strains.

For cloning experiments, choose from a variety of formats, including chemical and electrocompetent. These *E. coli* strains are T1 phage resistant and are Endonuclease I-deficient for high-quality plasmid preparations. Additionally, all competent cells from NEB are free of animal products.

NEB also offers a wide variety of competent cell strains ideal for many protein expression applications. These strains address the needs of difficult protein expression control, toxic protein expression and cytoplasmic disulfide bond formation. NEB Express, T7 Express and SHuffle® strains are available with varying levels of control. I^q strains feature added control from increased supply of Lac repressor (*lacI*^{q}). Only NEB offers the exceptional control of expression from the *lysY* gene that reduces basal expression from T7 strains without inhibiting IPTG-induced expression. Our Lemo21(DE3) strain features tunable T7 expression for difficult targets. Each strain is provided with a detailed protocol for optimal expression.

NO DRY ICE CHARGES
with Competent Cells from NEB



Find tips for successful transformation.

Featured Products

226 NEB Cloning Competent *E. coli* Sampler

228 NEB Stable Competent *E. coli*

229 BL21 Competent *E. coli*

232 SHuffle Express Competent *E. coli*

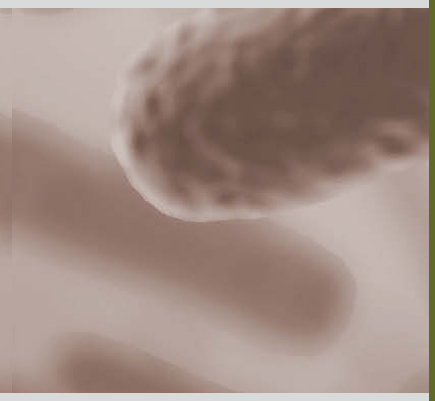
Featured Tools & Resources

361 Enhancing Transformation Efficiency

362 Protein Expression with T7 Express Strains

349 Troubleshooting Guide for Cloning

Visit www.neb.com to find additional online tools, including our Competitor Cross-reference Tool for comparing NEB strains to other commercially available strains.



Strain Properties

There are many properties to consider when choosing a strain for your experiments.

Requirements such as plasmid preparation, blue/white screening, proper disulfide bond formation and fast colony growth necessitate specific strain choices. The following selection chart highlights the characteristics of NEB's strains to help select the optimal strain for a particular experiment.

CAUTION: Chemically Competent *E. coli* contain DMSO, a hazardous material. Review the MSDS before handling.

Cloning Strain Properties

STRAIN PROPERTIES	TRANSFORMATION EFFICIENCY (cfu/µg)(1)	STRAIN	T1 PHAGE RESISTANT	BLUE/WHITE SCREENING	*lacI*^{q}	*lysY*	COLONIES VISIBLE AFTER 6.5 HRS.	*endA*$^-$(2)	PROTEASE DEFICIENT(3)	F´	*recA*$^-$	T7 RNA POLYMERASE	CYTOPLASMIC DISULFIDE BOND FORMATION(4)	DRUG RESISTANCE(5)
NEB Turbo	1–3 x 10^9	K12	●	●	●	–	●	●	–	●	–	–	–	nit
NEB 5-alpha	1–3 x 10^9	K12	●	●	–	–	–	●	–	–	●	–	–	none
NEB 5-alpha F´ I^q	1–3 x 10^9	K12	●	●	●	–	–	●	–	●	●	–	–	tet
NEB 10-beta	1–3 x 10^9	K12	●	●	–	–	–	●	–	–	●	–	–	str
***dam*$^-$/*dcm*$^-$**	1–3 x 10^6	K12	●	–	–	–	–	●	–	–	–	–	–	cam, str, nit
NEB Stable	1–3 x 10^9	K12	●	●	●	–	–	●	–	●	●	–	–	tet, str

Protein Expression Strain Properties

NEB Express	0.6–1 x 10^9	B	●	–	–	–	–	●	●	–	–	–	–	nit
NEB Express I^q	0.6–1 x 10^9	B	●	–	●	–	–	●	●	–	–	–	–	cam, nit
T7 Express	0.6–1 x 10^9	B	●	–	–	–	–	●	●	–	–	●	–	nit
T7 Express *lysY*	0.6–1 x 10^9	B	●	–	–	●	–	●	●	–	–	●	–	cam, nit
T7 Express *lysY*/I^q	0.6–1 x 10^9	B	●	–	●	●	–	●	●	–	–	●	–	cam, nit
SHuffle® Express	1 x 10^7	B	●	–	●	–	–	●	●	–	–	–	●	spec(6), nit
SHuffle T7 Express	1 x 10^7	B	●	–	●	–	–	●	●	–	–	●	●	spec(6), nit
SHuffle T7 Express *lysY*	1 x 10^7	B	●	–	●	●	–	●	●	–	–	●	●	cam, spec(6), nit
SHuffle T7	1 x 10^6	K12	●	–	●	–	–	–	–	●	–	●	●	str, spec, nit
BL21	1–5 x 10^7	B	●	–	–	–	–	–	●	–	–	–	–	none
BL21(DE3)	1–5 x 10^7	B	●	–	–	–	–	–	●	–	–	●	–	none
Lemo21(DE3)	1–3 x 10^7	B	●	–	–	●	–	–	●	–	–	●	–	cam
NiCo21(DE3)	1–5 x 10^7	B	●	–	–	–	–	–	●	–	–	●	–	none

(1) TE are given high-quality for high efficiency chemically competent strains. TE for electrocompetent strains are 1-4 x 10^{10} cfu/µg. TE for subcloning strains are >1 x 10^6 cfu/µg.
(2) Important for high-quality plasmid preparation.
(3) Lacks Lon and OmpT protease activity.
(4) Constitutively expresses a chromosomal copy of the disulfide bond isomerase DsbC.
(5) nit = nitrofurantoin, tet = tetracycline, cam = chloramphenicol, str = streptomycin, spec = spectinomycin
(6) Resistance to low levels of streptomycin may be observed.

NEBcloner®

For help with choosing the right competent cell strain, try NEBcloner at **NEBcloner.neb.com**

Convenient Formats

NEB provides these superior competent cell strains in several formats for your convenience. Most are available as 50 µl single-use transformation tubes and many are available in larger, 200 µl tubes for multiple simultaneous reactions. Our most popular cloning strains are available as electrocompetent cells. NEB 5-alpha is also available in a lower efficiency, subcloning format for substantial value, as well as in a 96-well plate, 384-well plate and striptube formats.

Cloning Formats

FORMATS	50 µl SINGLE-USE (H,J-FORMATS)	200 µl TUBES (I-FORMAT)	ELECTROCOMPETENT (K-FORMAT)	SUBCLONING (F-FORMAT)	96-WELL PLATE (P-FORMAT)	384-WELL PLATE (R-FORMAT)	8-TUBE STRIPS (U-FORMAT)	OUTGROWTH MEDIUM & CONTROL PLASMID INCLUDED
NEB Turbo	●	●	●	–	–			●
NEB 5-alpha	●	●	●	●	●	●	●	●
NEB 5-alpha F´ I^q	●	●	–	–	–			●
NEB 10-beta	●	●	●	–	–			●
dam⁻/*dcm*⁻	●	●	–	–				●
NEB Stable	●	●	–	–	–			●
NEB Express	●	●	–	–	–			●
NEB Express I^q	–	●	–	–	–			–
T7 Express	●	●	–	–	–			●
T7 Express *lysY*	–	●	–	–	–			–
T7 Express *lysY*/I^q	–	●	–	–	–			–
SHuffle® Express	●	–	–	–	–			–
SHuffle T7 Express	●	–	–	–	–			–
SHuffle T7 Express *lysY*	●	–	–	–	–			–
SHuffle T7	●	–	–	–	–			–
BL21	●	–	–	–	–			●
BL21(DE3)	●	●	–	–	–			●
Lemo21(DE3)	●	–	–	–	–			●(1)
NiCo21(DE3)	●	–	–	–	–			●

(1) Rhamnose solution is provided instead of SOC, control plasmid is included.

Competitor Cross Reference

Using another competent cell strain? Try our competitor cross reference tool to find out which NEB strain is compatible.

Learn how to perform a transformation.

NEW

NEB Cloning Competent *E. coli* Sampler

#C1010S 8 tubes

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Outgrowth medium and control plasmid included*
- *Value pricing*
- *Free of animal products*

Description: A sample pack of four cloning strains of *E. coli* suitable for high efficiency transformation.

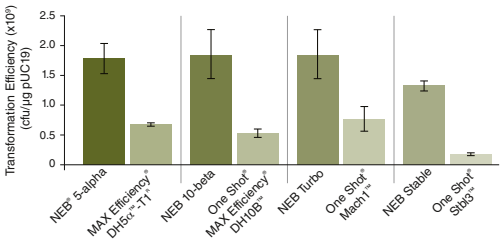
Please refer to the individual datacards for each reagent's recommended use and storage conditions.

Transformation Efficiency:
1–3 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*)

The Sampler Includes:

- NEB Turbo Competent *E. coli* (High Efficiency)
- NEB 5-alpha Competent *E. coli* (High Efficiency)
- NEB 10-beta Competent *E. coli* (High Efficiency)
- NEB Stable Competent *E. coli* (High Efficiency)
- SOC Outgrowth Medium
- NEB 10-beta/Stable Outgrowth Medium
- pUC19 Vector



Benefit from high transformation efficiencies: *Transformation efficiences were compared using manufacturers' recommended protocols. Values shown are the average of triplicate experiments.*

NEB Turbo Competent *E. coli*

NEB Turbo Competent *E. coli*
(High Efficiency)
#C2984H 20 x 0.05 ml
#C2984I 6 x 0.2 ml

NEB Turbo Electrocompetent *E. coli*
#C2986K 6 x 0.1 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Tight expression control (lacI^q)*
- *Colonies visible after 6.5 hours*
- *Isolate DNA after 4 hrs growth*
- *5 minute transformation protocol with Amp^R plasmids*
- *Clone toxic genes*
- *Free of animal products*

Description: *E. coli* cells featuring fast colony growth (6.5 hours) and tight expression control.

Genotype: F´ *proA$^+$B$^+$ lacI^q ΔlacZM15 / fhuA2 Δ(lac-proAB) glnV galK16 galE15 R(zgb-210::Tn10)*Tet^S *endA1 thi-1 Δ(hsdS-mcrB)5*

Features:

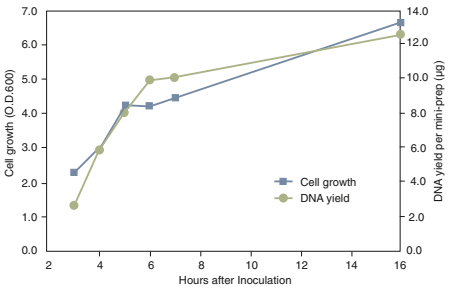
- Suitable for blue/white screening
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations

Transformation Efficiency:
High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA

Electrocompetent: > 1 x 10^{10} cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet



Miniprep DNA can be prepared from a single overnight colony after inoculation and only 3 hours growth. DNA yield doubles after an additional hour of growth.

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

NEB Turbo Transformation: *With NEB Turbo, colonies are visible after only 8 hours. Ligation products were transformed into 50 µl of NEB Turbo Competent E. coli and plated on LB/Amp. Plates were incubated for 8 hours, 10 hours and 12 hours at 37°C. NEB Turbo features fast colony growth and blue/white selection to simplify cloning experiments.*

What is the difference between chemical transformation and electroporation?

NEB 10-beta Competent *E. coli*

NEB 10-beta Competent *E. coli*
(High Efficiency)

#C3019H	20 x 0.05 ml
#C3019I	6 x 0.2 ml

NEB 10-beta Electrocompetent *E. coli*

#C3020K	6 x 0.1 ml

Companion Product:

NEB 10-beta/Stable Outgrowth Medium

#B9035S	4 x 25 ml

- *Clone large plasmids and BACs*
- *DH10B derivative*
- *Free of animal products*

Description: A DH10B derivative suitable for a wide range of applications, including large plasmid and BAC cloning.

Genotype: *Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14- ϕ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL* (Str^R) *rph spoT1 Δ(mrr-hsdRMS-mcrBC)*

Features:

- Efficient transformation of methylated DNA derived from eukaryotic sources or unmethylated DNA derived from PCR, cDNA and many other sources
- Suitable for blue/white screening without IPTG
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)

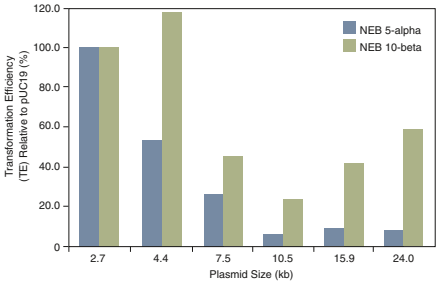
Transformation Efficiency:
High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA

Electrocompetent: > 2 x 10^{10} cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Str

Sensitivity: Amp, Cam, Kan, Nit, Spec, Tet

Reagents Supplied:
NEB 10-beta/Stable Outgrowth Medium
pUC19 Control DNA



Effect of Plasmid Size on Transformation Efficiency: *NEB 10-beta chemically competent cells are more efficiently transformed with large plasmids than NEB 5-alpha cells. The difference in TE between the two cell lines increases with the size of the plasmid being transformed.*

NEB 5-alpha Competent *E. coli*

NEB 5-alpha Competent *E. coli*
(High Efficiency)

#C2987H	20 x 0.05 ml
#C2987I	6 x 0.2 ml
#C2987P	1 x 96 well plate
#C2987R	1 x 384 well plate
#C2987U	96 x 50 µl/tube

NEB 5-alpha Competent *E. coli*
(Subcloning Efficiency)*

#C2988J	6 x 0.4 ml

NEB 5-alpha Electrocompetent *E. coli*

#C2989K	6 x 0.1 ml

Companion Product:

SOC Outgrowth Medium

#B9020S	4 x 25 ml

- *DH5α derivative*
- *Free of animal products*

Description: A DH5α derivative and versatile *E. coli* cloning strain.

Genotype: *fhuA2Δ(argF-lacZ)U169 phoA glnV44 ϕ80Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*

Features:

- Efficient transformation of unmethylated DNA derived from PCR, cDNA and many other sources (*hsdR*)
- Suitable for blue/white screening
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)

Transformation Efficiency:
High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA

Subcloning Efficiency: > 1 x 10^6 cfu/µg pUC19 DNA

Electrocompetent: > 1 x 10^{10} cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

* NEB 5-alpha Competent *E. coli* (Subcloning Efficiency) is not supplied with SOC Outgrowth Medium or pUC19 Control DNA.

NEB 5-alpha F´I^q Competent *E. coli*

NEB 5-alpha F´I^q Competent *E. coli*
(High Efficiency)

#C2992H	20 x 0.05 ml
#C2992I	6 x 0.2 ml

Companion Product::

SOC Outgrowth Medium

#B9020S	4 x 25 ml

- *Tight expression control (lacI^q)*
- *F´ Strain with extremely high TE*
- *DH5α derivative*
- *Free of animal products*

Description: An F´ *E. coli* strain with extremely high transformation efficiency suitable for toxic gene cloning.

Genotype: F´ *proA$^+$B$^+$ lacI^q Δ(lacZ)M15 zzf::Tn10* (Tet^R) */ fhuA2Δ(argF-lacZ)U169 phoA glnV44 ϕ80 Δ(lacZ)M15 gyrA96 recA1 endA1 thi-1 hsdR17*

Features:

- Efficient transformation of unmethylated DNA derived from PCR, cDNA and many other sources (*hsdR*)
- Suitable for blue/white screening
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)
- Suitable for propagation of M13 clones

Transformation Efficiency:
High Efficiency: 1–3 x 10^9 cfu/µg

Resistance: T1 phage (*fhuA2*), Tet

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

NEB Stable Competent *E. coli*

NEB Stable Competent *E. coli*
(High Efficiency)

#C3040H	20 x 0.05 ml
#C3040I	6 x 0.2 ml

Companion Product:

NEB 10-beta/Stable Outgrowth Medium

#B9035S	4 x 25 ml

- *T1 phage resistance (fhuA)*
- *Free of animal products*
- *Carries endA mutation (isolated plasmids are free of EndoI)*

Description: Chemically competent *E. coli* cells suitable for high efficiency transformation and isolation of plasmid clones containing repeat elements.

Genotype: F´ *proA*$^{+}$*B*$^{+}$ *lacI*^{q} *Δ(lacZ)M15 zzf::Tn10* (Tet^{R})*/Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14- ϕ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL* (Str^{R}) *rph spoT1 Δ(mrr-hsdRMS-mcrBC)*

Features:

- Activity of nonspecific endonuclease I (*endA1*) abolished for highest quality plasmid preparations
- Rapid growth *recA* strain

Applications:

- Cloning unstable inserts
- Isolating and propagating retroviral/lentiviral clones
- Compatible with Gibson Assembly® Reactions, as well as ligation reactions

Transformation Efficiency:
1–5 x 10^{8} cfu/µg pUC19 DNA (NEB #C3040H)

> 1 x 10^{8} cfu/µg pUC19 DNA (NEB #C3040I)

Resistance: T1 phage (*fhuA*), Str, Tet

Sensitivity: Amp, Cam, Kan, Nit, Spec

Reagents Supplied:
NEB 10-beta/Stable Outgrowth Medium
pUC19 Control DNA

dam^{-} / *dcm*$^{-}$ Competent *E. coli*

#C2925H	20 x 0.05 ml
#C2925I	6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium

#B9020S	4 x 25 ml

- *Isolate plasmids free of Dam and Dcm methylation*
- *Free of animal products*

Description: Methyltransferase deficient *E. coli* cells suitable for growth of plasmids free of Dam and Dcm methylation.

Genotype: *ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10)* Tet^{S} *endA1 rspL136* (Str^{R}) *dam13::Tn9* (Cam^{R}) *xylA-5 mtl-1 thi-1 mcrB1 hsdR2*

Features:

- Allows for propagation of plasmids free of Dam and Dcm methylation
- Activity of nonspecific endonuclease I (*endA1*) abolished for highest quality plasmid preparations

Transformation Efficiency:
1–3 x 10^{6} cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA31*), Cam, Nit, Str

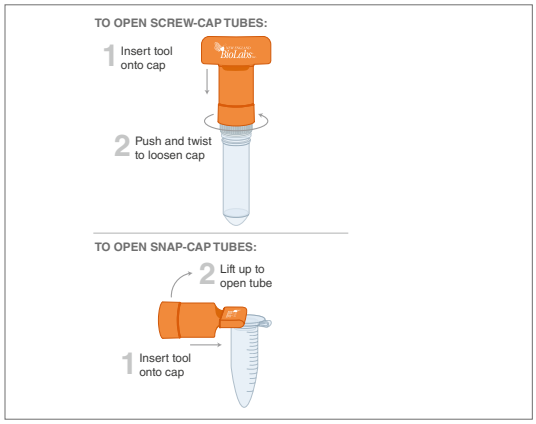
Sensitivity: Amp, Kan, Spec, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

NEB Tube Opener

#C1008S 2 units

Description: Use to open a variety of microcentrifuge tubes. Can be used for snap-on caps or screw-on caps.



BL21 Competent *E. coli*

#C2530H 20 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Ideal for P_{lac}, P_{tac}, P_{trc}, ParaBAD expression vectors*
- *Resistance to phage T1 (fhuA2)*
- *Protease deficient*
- *Free of animal products*

Description: Widely used non-T7 expression *E. coli* strain. Suitable for transformation and protein expression. This strain does not express the T7 RNA Polymerase.

Genotype: *fhuA2 [lon] ompT gal [dcm] ΔhsdS*

Features:

- Deficient in proteases Lon and OmpT

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

BL21(DE3) Competent *E. coli*

#C2527H 20 x 0.05 ml
#C2527I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Routine T7 expression*
- *Free of animal products*
- *Protease deficient B strain*

Description: Widely used T7 expression *E. coli* strain.

Genotype: *fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*

Features:

- Deficient in proteases Lon and OmpT
- Resistant to phage T1 (*fhuA2*)

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

Lemo21(DE3) Competent *E. coli*

#C2528J 12 x 0.05 ml

- *Expression of difficult targets*
- *Membrane protein expression*
- *Ideal for periplasmic expression*
- *Expression of toxic proteins*
- *Proteins with solubility issues*

Description: Lemo21(DE3) Competent *E. coli* is a tunable T7 expression strain designed for the expression of challenging proteins. A derivative of BL21(DE3), Lemo21(DE3) offers the host features of this popular expression strain, with the added benefit of being able to control expression levels by varying the level of T7 lysozyme (*lysY*), the natural inhibitor of T7 RNA Polymerase. The fine control of expression makes Lemo21(DE3) ideal for membrane proteins, toxic proteins, secreted proteins and proteins prone to insoluble expression.

Genotype: *fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS/* pLemo(Cam^R) *λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*
pLemo = pACYC184-*PrhaBAD-lysY*

Features:

- Enhanced BL21(DE3) derivative
- Fine control of expression
- Greatest range of expression of any T7 strain (0-2,000 µM rhamnose)
- Potential elimination of inclusion body formation

Transformation Efficiency:
High Efficiency: 1–3 x 10^7 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Cam

Sensitivity: Amp, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
L-rhamnose solution
pUC19 control DNA

NiCo21(DE3) Competent *E. coli*

#C2529H 20 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Superior alternative to BL21(DE3) for routine protein expression*
- *Improved purity of target proteins isolated by IMAC*
- *Free of animal products*

Description: Poly-histidine tagged recombinant proteins that are isolated by immobilized metal affinity chromatography (IMAC) are often contaminated with significant amounts of endogenous *E. coli* metal binding proteins. The protein expression strain NiCo21(DE3) has been engineered to minimize *E. coli* protein contamination of IMAC fractions: GlmS is mutated to eliminate binding to IMAC resins and three other proteins (SlyD, ArnA and Can) are tagged to enable rapid removal by chitin affinity chromatography.

Genotype: *can::CBD fhuA2 [lon] ompT gal (λ DE3) [dcm] arnA::CBD slyD::CBD glmS6Ala ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*

Features:

- Identical growth characteristics as BL21(DE3)
- Deficient in proteases Lon and OmpT

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

NEB Express Competent *E. coli*

NEB Express Competent *E. coli*
(High Efficiency)
#C2523H 20 x 0.05 ml
#C2523I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Enhanced BL21 derivative ideal for P_{lac}, P_{tac}, P_{trc} expression vectors*
- *Fast growth from colonies*
- *Free of animal products*
- *Protease deficient*

Description: A versatile non-T7 expression *E. coli* strain. NEB Express is the recommended host strain for pMAL protein fusion and purification system.

Genotype: *fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA/Δ(mcrC-mrr)114::IS10*

Features:

- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA

Transformation Efficiency:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Nit

Sensitivity: Amp, Cam, Kan, Tet, Spec, Str

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

NEB Express I^q Competent *E. coli*

NEB Express I^q Competent *E. coli*
(High Efficiency)
#C3037I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Enhanced BL21 derivative ideal for P_{lac}, P_{tac}, P_{trc}, P_{T5} expression vectors*
- *Better control of IPTG induced expression with non-T7 plasmids*
- *Fast growth from colonies*
- *$lacI^q$* reduces basal expression
- *Protease deficient*
- *Free of animal products*

Description: *E. coli* cells featuring control of IPTG induced expression with non-T7 plasmids.

Genotype: MiniF *$lacI^q$* (Cam^R) / *fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA/Δ(mcrC-mrr)114::IS10*

Features:

- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- Ideal for controlled protein expression from pUC19 and pUC19 derivatives

Transformation Efficiency:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

T7 Express Competent *E. coli*

T7 Express Competent *E. coli*
(High Efficiency)
#C2566H 20 x 0.05 ml
#C2566I 6 x 0.2 ml

Companion Product:
SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Enhanced BL21 derivative*
- *Popular T7 expression strain*
- *Fast growth from colonies*
- *Free of animal products*

Description: Enhanced BL21 *E. coli* derivative for T7 expression.

Genotype: *fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr)114::IS10*

Features:
- T7 RNA Polymerase in the *lac* operon - no λ prophage
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA

Transformation Efficiency:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

T7 Express *lysY* Competent *E. coli*

T7 Express lysY Competent *E. coli*
(High Efficiency)
#C3010I 6 x 0.2 ml

Companion Product:
SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Enhanced BL21 derivative*
- *T7 Lysozyme for expression control*
- *Clone toxic genes*
- *Fast growth from colonies*
- *Free of animal products*

Description: Enhanced BL21 *E. coli* derivative for T7 expression with enhanced reduction of basal expression.

Genotype: MiniF *lysY (*Cam^R*) / fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr)114::IS10*

Features:
- T7 RNA Polymerase in the *lac* operon - no λ prophage
- Control of T7 RNA Polymerase by T7 lysozyme allows potentially toxic genes to be expressed
- LysY is a variant of T7 lysozyme lacking amidase activity, thus cells are not susceptible to lysis during induction
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- No Cam requirement

Transformation Efficiency:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

T7 Express *lysY/I^q* Competent *E. coli*

T7 Express lysY/I^q Competent *E. coli*
(High Efficiency)
#C3013I 6 x 0.2 ml

Companion Product:
SOC Outgrowth Medium
#B9020S 4 x 25 ml

- *Enhanced BL21 derivative*
- *Tight control of expression (lacI^q)*
- *Highest level of expression control*
- *Clone toxic genes*
- *Fast growth from colonies*
- *Free of animal products*

Description: Enhanced BL21 *E. coli* derivative with highest level of T7 expression control.

Genotype: MiniF *lysY lacI^q*(Cam^R*) / fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr)114::IS10*

Features:
- T7 RNA Polymerase in the *lac* operon - no λ prophage
- Tight control of expression by *lacI^q* allows potentially toxic genes to be cloned
- Control of T7 RNA Polymerase by T7 lysozyme allows toxic genes to be expressed
- LysY is a variant of T7 lysozyme lacking amidase activity, thus cells are less susceptible to lysis during induction
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- No Cam requirement

Transformation Efficiency:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

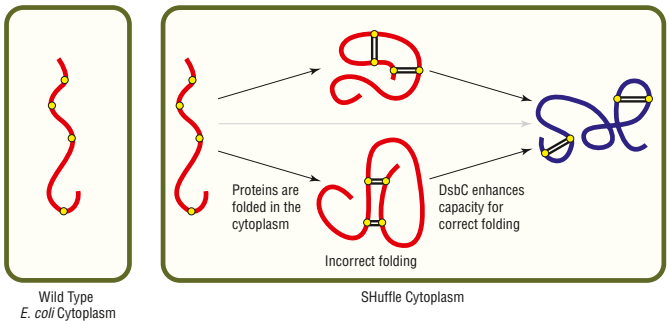
Disulfide Bonds

Features of Shuffle® Strains:

- Engineered *E. coli* K12 or B strains promote disulfide bond formation in the cytoplasm
- Constitutively expresses a chromosomal copy of the disufide bond isomerase DsbC
- DsbC promotes the correction of mis-oxidized proteins into their correct form
- The cytoplasmic DsbC is a chaperone that can also assist in the folding of proteins that do not require disulfide bonds
- Alternative expression strain for proteins that do not fold in wild-type *E. coli*, independent of redox state

SHuffle strains from NEB are engineered *E. coli* strains capable of expressing proteins with increasing disulfide bond complexity in the cytoplasm. SHuffle strains express the disulfide bond isomerase DsbC within the cytoplasm. DsbC isomerizes mis-oxidized substrates into their correctly folded state greatly enhancing the fidelity of disulfide bond formation. Cytoplasmic expression also results in significantly higher protein yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle strains are sensitive to kan, amp, tet and in most cases, cam, which makes them able to express proteins from a wide variety of expression vectors offering greater versatility in experimental design.

References:
References for properties and applications for these products can be found at www.neb.com.



Disulfide bond formation in the cytoplasm of wild type E. coli is not favorable, while SHuffle is capable of correctly folding proteins with multiple disulfide bonds in the cytoplasm.

SHuffle Express Competent *E. coli*

#C3028J 12 x 0.05 ml

- *Folds disulfide bonded proteins in the cytoplasm*
- *Protease deficient*
- *Enhanced BL21 derivative*
- *Free of animal products*

Description: *E. coli* cells with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: *fhuA2 [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC* (Spec^R, *lacI^q*) *ΔtrxB sulA11 R(mcr-73-::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA1 Δgor Δ(mcrC-mrr)114::IS10*

Transformation Efficiency:
1 x 10^7 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Nit, Str*, Spec

Sensitivity: Amp, Cam, Kan, Tet

*Resistance to low levels of streptomycin may be observed.

SHuffle T7 Express Competent *E. coli*

#C3029J 12 x 0.05 ml

- *Folds disulfide bonded proteins in the cytoplasm*
- *T7 expression*
- *Protease deficient B strain*
- *Enhanced BL21 derivative*
- *Free of animal products*

Description: T7 Expression *E. coli* strain with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

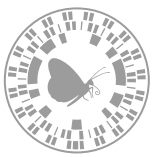
Genotype: *fhuA2 lacZ::T7 gene1 [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC* (Spec^R, *lacI^q*) *ΔtrxB sulA11 R(mcr-73-::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA1 Δgor Δ(mcrC-mrr)114::IS10*

Transformation Efficiency: 1 x 10^7 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Nit, Str*, Spec

Sensitivity: Amp, Cam, Kan, Tet

*Resistance to low levels of streptomycin may be observed.



What is a disulfide bond?

SHuffle T7 Competent *E. coli*

#C3026J 12 x 0.05 ml

- *Folds disulfide bonded proteins in the cytoplasm*
- *T7 expression*
- *K12 strain*
- *Free of animal products*

Description: T7 Expression *E. coli* strain with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: F´ *lac, pro, lacI^q / Δ(ara-leu)7697 araD13 fhuA2 lacZ::T7 gene1 Δ(phoA)PvuII phoR ahpC* galE (or U) galK λatt::pNEB3-r1-cDsbC* (Spec^R, *lacI^q*) *ΔtrxB rpsL150*(Str^R) *Δgor Δ(malF)3*

Transformation Efficiency: 1 x 10^6 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Nit, Spec, Str*

Sensitivity: Amp, Cam, Kan, Tet

*Resistance to low levels of streptomycin may be observed.

SHuffle T7 Express *lysY* Competent *E. coli*

#C3030J 12 x 0.05 ml

- *Express toxic proteins (lysY)*
- *Folds disulfide bonded proteins in the cytoplasm*
- *T7 expression*
- *Protease deficient B strain*
- *Free of animal products*
- *Enhanced BL21 derivative*

Description: *E. coli* strain with tight T7 Expression control and enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: MiniF *lysY* (Cam^R) */ fhuA2 lacZ::T7 gene1 [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC* (Spec^R, *lacI^q*) *ΔtrxB sulA11 R(mcr-73-::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δgor Δ(mcrC-mrr)114::IS10*

Transformation Efficiency:
1 x 10^7 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Cam, Nit, Str*, Spec

Sensitivity: Amp, Kan, Tet

*Resistance to low levels of streptomycin may be observed.

How do I express my protein in SHuffle cells?

Currently there are two SHuffle cell lines available from NEB; SHuffle (NEB #C3026) based on *E. coli* K12, and SHuffle Express (NEB #C3028, #C3029, #C3030) based on *E. coli* B.

We recommend testing both B and K12 expression strains, as we do see variability in expression depending on the protein of interest (Table 1). If T7 expression is not necessary, then we recommend comparing NEB #C3026 and #C3028. If T7 expression is necessary, test NEB #C3026 and #C3029. If T7-driven expression of a protein is toxic, switch to a non-leaky lysY version (NEB #C3030). Once the strain is chosen expression conditions should be optimized. This can include temperature as well as auto expression (1).

View our online tutorial for tips on setting up reactions with SHuffle.

Table 1. Percentage of relative solubility of various proteins using SHuffle (K12 and B strains):

PROTEIN	RELATIVE % SOLUBLE		# CYSTEINES
	K12	B	
Gluc	65	100	10
Urokinase	60	100	24
vtPA	5	100	12
BSA	100	0	35
Polymerase	100	0	0
Nuclease	100	10	4

Results are determined based on protein levels detected by SDS-PAGE (not shown)

Reference:

(1) Ke, N. and Berkmen, M. (2014) *Current Protocols Molecular Biology* 16.1B.21.

Taking Molecular Tools to the Jungle

Climate change, habitat destruction and pollution are causing accelerated species extinction, particularly in tropical ecosystems. Extinction rates that were one species/million/year have increased to 100–1,000 species/million/year. Unfortunately, most of these species have yet to be identified. With an incomplete understanding of species diversity, it is difficult to know where to direct conservation efforts and resources. This knowledge gap has prompted an urgency to catalog as much biodiversity as possible, as quickly as possible.

DNA barcoding is a standardized method of identification that utilizes a short region of the mitochondrial cytochrome c oxidase I (COI) gene in animals (and various other sequences in plants, fungi and protists) to document species quickly and inexpensively. The mutation rate of mitochondrial DNA over relatively short evolutionary periods reflects the diversity between species. It should be noted that barcoding is distinct from, and does not supersede, specialized taxonomic identification of subtle anatomical differences between species, but the combination of molecular and morphological data improves the characterization and delimitation of species.

Biologists have used barcoding in large projects, such as the Census of Marine Life, a 10-year study that assessed the biodiversity and distribution of the Earth's aquatic ecosystems. This study identified 190,000 species, including 6,000 potentially new species. Scientists discovered new habitats, symbiotic relationships and microbial biospheres. They found species that are in decline and new examples of ecosystem resilience.

The colossal effort to document all of the organisms on our planet before they become extinct is a race against time, and it presents researchers with logistical hurdles related to cost and sampling. First, the countries with the greatest biodiversity, such as tropical regions, are often the countries that do not have abundant research resources dedicated to conducting this work. Second, the laws that govern the international transportation of biological materials from biodiverse regions to resource-rich areas can cause delays that compromise sample integrity or prevent sample transportation altogether.

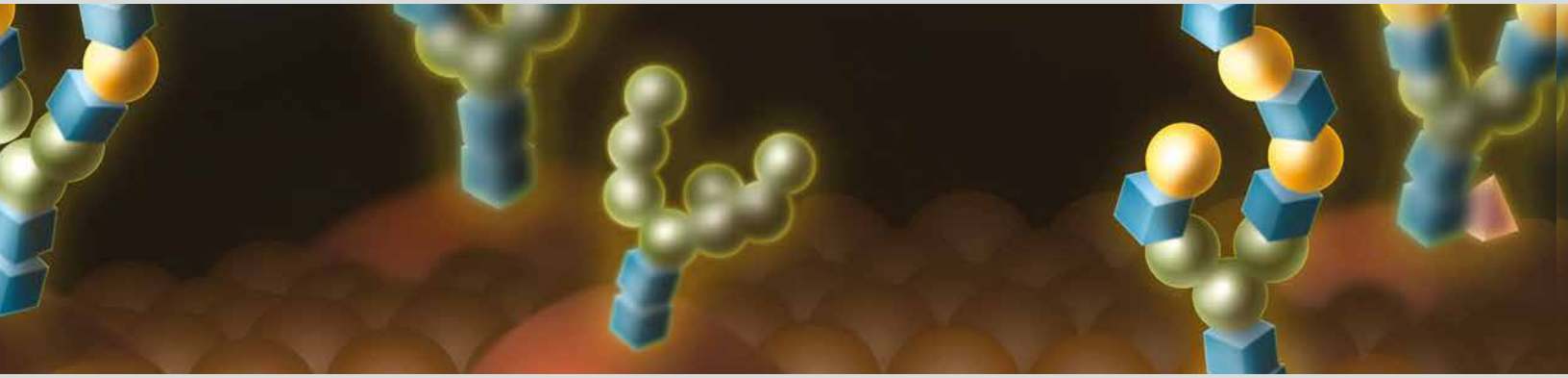
These limitations have necessitated the development of small, portable sequencing tools and technologies that can be used on-site. Conservation scientists can now transport a small DNA sequencing platform, PCR thermocycler, microcentrifuge, reagents and a laptop in a backpack to remote regions, where they can rapidly and cost-effectively extract DNA, amplify and sequence barcodes. This process can be carried out within 24 hours of sample collection, accelerating data acquisition significantly.

Collecting genetic information at the source allows easy documentation of information regarding species health, geographic distribution, hybrid zones, as well as the identification of new species. Rapid access to this information can help focus conservation efforts, and guide the allocation of appropriate resources when planning for species conservation. Geographical areas of highest biological value can be identified and protected, and laws can be implemented to preserve the most endangered species.

Documenting organisms in their habitats is a less invasive, expedient method that can assist conservationists in protecting our planet's immense, yet diminishing, biodiversity.

Scientists walk through the Amazon rainforest.
Credit: R. Tyler Gross, Getty Images

Glycobiology & Protein Tools



Trust NEB's expertise in enzymology when you need glycobiology reagents.

Glycobiology

Proteomics, the systematic study of proteins in biological systems, has expanded the knowledge of protein expression, modification, interaction and function. However, in eukaryotic cells, the majority of proteins are post-translationally modified (1). A common post-translational modification, essential for cell viability, is the attachment of glycans. Glycosylation defines the adhesive properties of glycoconjugates, and it is largely through glycan–protein interactions that cell–cell and cell–pathogen contacts occur, a fact that accentuates the importance of glycobiology.

Glycomics, the study of glycan expression in biological systems, relies on effective enzymatic and analytical techniques for correlation of glycan structure with function. Glycobiology is a small but rapidly growing field in biology, with relevance to biomedicine, biotechnology, biofuels and basic research. Glycan molecules modulate many other processes important for cell and tissue differentiation, metabolic and gene regulation, protein activity, protein clearance, transport and more (2-9).

Protein Tools

Not only are proteins a major structural component of living systems, they can also be effector molecules whose states determine downstream activities. Therefore, studying the protein complement within a cell can reveal the mechanisms behind many of the cell's responses to its environment. Given the vast number of applications for protein analysis, several tools and methods for its study exist; determining the correct method for your application is paramount to success.

Phage display technology is an *in vitro* screening technique for identifying ligands for proteins and other macromolecules. At the crux of phage display technology is the ability to express peptide or protein sequences as fusions to the coat proteins of a bacteriophage. Libraries of phage-displayed peptides or proteins are thereby physically linked to their encoding nucleic acid, allowing selection of binding partners for myriad target types by iterative rounds of *in vitro* panning and amplification, followed by DNA sequencing.

All NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

Featured Products

241 Rapid™ PNGase F

243 *N*-Glycan Sequencing Kit

254 Thermolabile Proteinase K

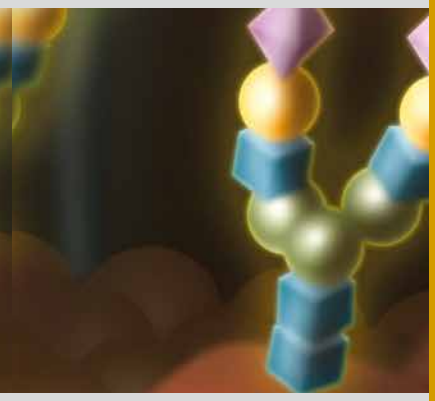
Featured Tools & Resources

Protein Tools & Glycomics Overview

Glycobiology Unit Conversion Chart

Visit www.NEBglycosidase.com to view our online tutorial on *N*- and *O*-linked glycosylation.

(1) Khoury G.A. et al. (2011) *Scientific Reports* 1: 90. PMID: 22034591.
(2) Varki A. (1993) *Glycobiology*, 3, 97–130. PMID: 8490246.
(3) Zhao Y.Y. et al. (2008) *Cancer Sci.* 99, 1304–1310. PMID: 18492092.
(4) Zhao Y. et al. (2008) *FEBS J.* 275, 1939–1948. PMID: 18384383.
(5) Skropeta D. (2009) *Bioorg. Med. Chem.* 17, 2645–2653. PMID: 19285412.
(6) Neu U. et al. (2011) *Curr. Opin. Struct. Biol.* 21, 610–618.PMID: 21917445.
(7) Cerliani J.P. et al. (2011) *J. Clin. Immunol.* 31, 10–21. PMID: 21184154.
(8) Aarnoudse C.A. et al. (2006) *Curr. Opin. Immunol.* 18, 105–111. PMID: 16303292.
(9) Arnold J.N. (2006) *Immunol. Lett.* 106, 103–110. PMID: 16814399.



RH Recombinant Enzyme

Glycosidases

- *Enabling Novel Technologies*
- *Unique Specifications*
- *Exceptional Value*
- *High Purity*

NEB offers a selection of endoglycosidases and exoglycosidases for glycobiology research. Many of these reagents are recombinant, and all undergo several quality control assays, enabling us to provide products with lower unit cost, high purity, and reduced lot-to-lot variation.

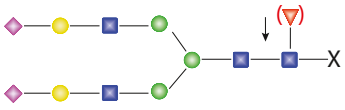
All of our glycosidases are tested for contaminants. Since *p*-nitrophenyl-glycosides are not hydrolyzed by some exoglycosidases, we use only fluorescently-labeled oligosaccharides to screen for contaminating glycosidases.

NEB's glycosidases are provided with 10X buffer to ensure optimal activity. Using more than one glycosidase simultaneously is a common timesaving procedure. Selecting the best buffer to provide reaction conditions that optimize enzyme activity is an important consideration.

Reaction Buffer Compositions:
Visit www.neb.com for details.

Endo F2

#P0772S 480 units



- *Removal of complex biantennary N-linked glycans from glycoproteins and glycopeptides*
- *Useful for determining N-glycosylation sites*

Description: Endo F2 is a highly specific recombinant endoglycosidase which cleaves within the chitobiose core of asparagine-linked complex biantennary and high mannose oligosaccharides from glycoproteins and glycopeptides. Endo F2 cleaves biantennary glycans at a rate approximately 20 times greater than high mannose glycans. The activity of Endo F2 is identical on biantennary structures with and without core fucosylation. However, Endo F2 is not active on hybrid or tri- and tetra-antennary oligosaccharides. Endo F2 is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Source: Cloned from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*) and expressed in *E. coli*.

Reagents Supplied:
10X GlycoBuffer 4

Molecular Weight: 39,800 daltons

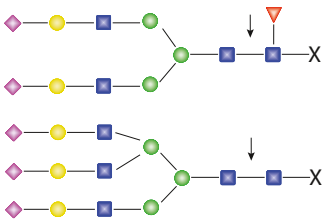
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the carbohydrate from 10 µg Porcine Fibrinogen in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 units/ml

Heat Inactivation: 65°C for 10 minutes

Endo F3

#P0771S 240 units



- *Removal of complex biantennary and triantennary N-linked glycans from glycoproteins and glycopeptides*
- *Useful for determining N-glycosylation sites*

Description: Endo F3 is a highly specific recombinant endoglycosidase which cleaves within the chitobiose core of asparagine-linked fucosylated-biantennary and triantennary complex oligosaccharides from glycoproteins. Endo F3 is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Source: Cloned from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*) and expressed in *E. coli*.

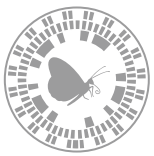
Reagents Supplied:
10X GlycoBuffer 4

Molecular Weight: 38,800 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the carbohydrate from 10 µg Porcine Fibrinogen in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 units/ml

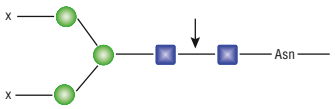
Heat Inactivation: 65°C for 10 minutes



Find an overview of glycobiology.

Endo D

#P0742S 1,500 units
#P0742L 7,500 units



X= (H or oligosaccharide)

- *Removal of paucimannose N-linked glycans from glycoproteins and glycopeptides*
- *Useful for determining N-glycosylation sites*

Description: Endo D, also known as Endoglycosidase D, is a recombinant glycosidase, which cleaves within the chitobiose core of paucimannose *N*-linked glycans, with or without extensions in the antennae.

Endo D is tagged with a chitin binding domain (CBD) for easy removal from a reaction, and is supplied glycerol-free for optimal performance in HPLC and MS intensive methods.

Source: A truncated Endo D gene cloned from *Streptococcus pneumoniae* and expressed in *E. coli* as a fusion to chitin binding domain.

Reagents Supplied:
10X DTT
10X GlycoBuffer 2

Molecular Weight: 140,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of glycosidase-trimmed (trimannosyl core) Fetuin in 1 hour at 37°C in a total reaction volume of 10 µl.

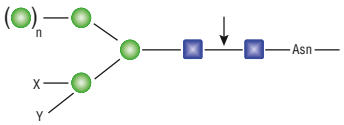
Concentration: 50,000 units/ml

Heat Inactivation: 65°C for 10 minutes

Endoglycosidase H

Endo H
#P0702S 10,000 units
#P0702L 50,000 units

Endo H_f
#P0703S 100,000 units
#P0703L 500,000 units



Endo H and Endo H_f cleave only high mannose structures (n = 2–150, x = $(Man)_{1-2}$, y = H) and hybrid structures (n = 2, x and/or y = AcNeu-Gal-GlcNAc).

- *Removal of high mannose N-glycans from glycoproteins*

Description: Endoglycosidase H is a recombinant glycosidase which cleaves within the chitobiose core of high mannose and some hybrid oligosaccharides from *N*-linked glycoproteins.

Endo H_f is a recombinant protein fusion of Endoglycosidase H and maltose binding protein. It has identical activity to Endo H.

Source: Endo H and Endo H_f have been cloned from *Streptomyces plicatus* and overexpressed in *E. coli.*

Reaction Conditions:
Denature glycoprotein in 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. Incubate in 1X GlycoBuffer 3 at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X Glycoprotein Denaturing Buffer
10X GlycoBuffer 3

Molecular Weight:
Endo H: 29,000 daltons
Endo H_f: 70,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.

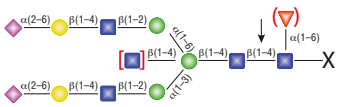
Concentration:
Endo H concentration: 500,000 units/ml
Endo H_f concentration: 1,000,000 units/ml

Usage Notes: Enzymatic activity is not affected by SDS.

To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

Endo S

#P0741S 6,000 units
#P0741L 30,000 units



- *Removal of N-glycans from native IgG*
- *Useful for determining N-glycosylation sites*

Description: Endo S is an endoglycosidase with a uniquely high specificity for removing *N*-linked glycans from the chitobiose core of the heavy chain of native IgG Endo S is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol-free for optimal performance in HPLC- and MS-intensive methods.

Source: Endo S is cloned from *Streptococcus pyogenes* and overexpressed as a fusion to the chitin binding domain in *E. coli.*

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 136,000 daltons

Unit Definition: 5 µg of IgG in 1X GlycoBuffer 1 are incubated with two-fold dilutions of Endo S for 1 hour at 37°C. Separation of reaction products is visualized by SDS-PAGE.

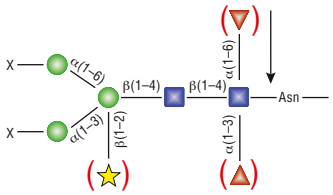
Concentration: 200,000 units/ml

Heat Inactivation: 55°C for 10 minutes

PNGase A

37° 65°

#P0707S 150 units
#P0707L 750 units



PNGase A hydrolyzes N-glycan chains from glycoproteins/peptides regardless of the presence of xylose or fucose. [x = H or Man or GlcNAc].

- *Removal of N-linked glycans from glycoproteins*

Description: PNGase A is a recombinant amidase, which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and short complex oligosaccharides such as those found in plant and insect cells from *N*-linked glycoproteins and glycopeptides. PNGase A differs from PNGase F in that it cleaves *N*-linked glycans with or without α(1,3)-linked core fucose residues.

Source: Cloned from *Oryza sativa* (rice) and expressed in *Pichia pastoris*.

Reaction Conditions:
Denature 1 µg of recombinant Avidin in 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. After the addition of NP-40 and GlycoBuffer 3, two-fold dilutions of PNGase A are added and the reaction mix is incubated for 1 hour at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X Glycoprotein Denaturing Buffer
10X GlycoBuffer 3
10% NP-40

Molecular Weight: 63,800 daltons

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 1 µg of denatured recombinant Avidin produced in Maize in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 5,000 units/ml

Usage Notes: PNGase A is active on both glycoproteins and glycopeptides.

PNGase A cannot cleave larger *N*-glycans such as those from Fetuin, Fibrinogen, IgG, Lactoferrin and Transferrin.

PNGase A is able to cleave high mannose *N*-glycan structures from Man 3 up to Man 9.

PNGase F & PNGase F, Recombinant

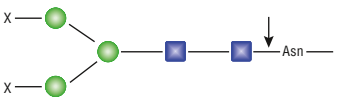
RR 37° 75°

PNGase F
#P0704S 15,000 units
#P0704L 75,000 units

PNGase F (Glycerol-free)
#P0705S 15,000 units
#P0705L 75,000 units

PNGase F, Recombinant
#P0708S 15,000 units
#P0708L 75,000 units

PNGase F (Glycerol-free), Recombinant
#P0709S 15,000 units
#P0709L 75,000 units



PNGase F hydrolyzes nearly all types of N-glycan chains from glycopeptides/proteins [x = H or oligosaccharide].

- *Removal of N-linked glycans from glycoproteins*

Description: Peptide-N-Glycosidase F, also known as PNGase F, is an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid and complex oligosaccharides from *N*-linked glycoproteins. A glycerol-free version of PNGase F is also offered for HPLC methods.

Source: NEB #P0704 and NEB #P0705 are purified from *Flavobacterium meningosepticum*.

NEB #P0708 and NEB #P0709 are cloned from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum)* and expressed in *E. coli*.

Reaction Conditions:
Denature glycoprotein in 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
10X Glycoprotein Denaturing Buffer
10X GlycoBuffer 2
10% NP-40

Molecular Weight: 36,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 500,000 units/ml

Usage Notes: Since PNGase F activity is inhibited by SDS, it is essential to have NP-40 present in the reaction mixture.

To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

After addition of NP-40 and Glycobuffer 2, two-fold dilutions of PNGase F are added and the reaction mix is incubated for 1 hour at 39°C.

Companion Products:

RNase B (control substrate)
#P7817S 250 µg

Endoglycosidase Reaction Buffer Pack
10X GlycoBuffer 2, 10X GlycoBuffer 3,
10X Glycoprotein Denaturing Buffer,
10% NP-40 (1 ml of each)
#B0701S

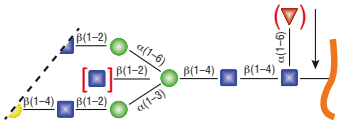
Rapid™ PNGase F & Rapid PNGase F (non-reducing format)

Rapid PNGase F
#P0710S 50 reactions

Rapid PNGase F (non-reducing format)
#P0711S 50 reactions

Companion Product:

Rapid PNGase F Antibody Standard
#P6043S 250 ng



- *Complete deglycosylation of antibodies and fusion proteins in minutes*
- *Release of all N-glycans rapidly and without bias*
- *Optimal activity is ensured for 12 months, if stored properly*
- *Purified to > 95% homogeneity, as determined by SDS-PAGE*

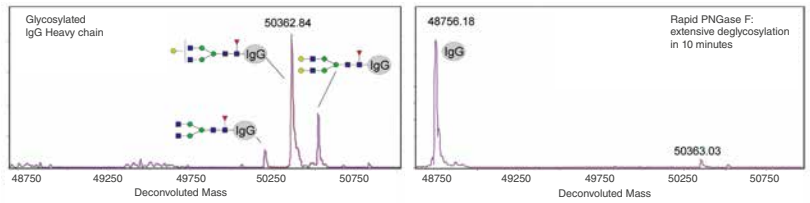
Description: Rapid PNGase F is an improved reagent that allows the complete and rapid deglycosylation of antibodies and fusion proteins in minutes. All *N*-glycans are released rapidly and without bias, and are ready to be prepared for downstream chromatography or mass spectrometry analysis. Rapid PNGase F creates an optimized workflow which reduces processing time without compromising sensitivity or reproducibility.

Developed for proteomic applications, Rapid PNGase F (non-reducing format) enables complete and rapid deglycosylation while preserving disulfide bonds. This facilitates high throughput proteomics applications and methods for antibody characterization by mass spectrometry such as intact mass analysis. Rapid PNGase F (non-reducing format) combines the advantages of Rapid PNGase F (fast processing time), with non-reducing conditions, preserving quaternary structure.

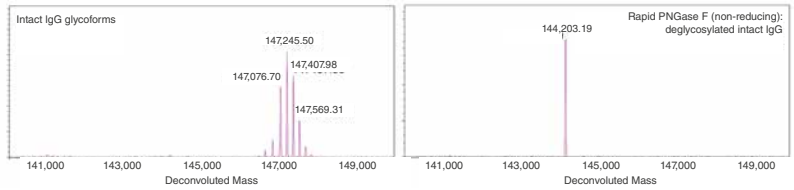
Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
Rapid PNGase F
Rapid PNGase F Reaction Buffer (5X)
Rapid PNGase F (non-reducing format)
Rapid PNGase F (non-reducing format) Buffer (5X)

Specificity: Rapid PNGase F cleaves all complex, hybrid and high-mannose type glycans from antibodies and related proteins. Core α1-3 fucosylation (found in immunoglobulins expressed in plant or insect cells) is resistant to both PNGase F and Rapid PNGase F.



ESI-TOF analysis of an antibody before and after treatment with Rapid PNGase F.



ESI-TOF analysis of an antibody before and after treatment with Rapid PNGase F (non-reducing format).

Remove-iT® PNGase F

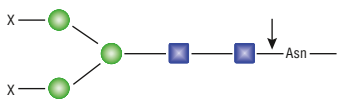
#P0706S 6,750 units
#P0706L 33,750 units

Companion Products:

Chitin Magnetic Beads
#E8036S 5 ml
#E8036L 25 ml

6-Tube Magnetic Separation Rack
#S1506S 6 tubes

12-Tube Magnetic Separation Rack
#S1509S 12 tubes



Remove-iT PNGase F hydrolyzes nearly all types of N-glycan chains from glycopeptides/proteins [x = H or oligosaccharide].

- *Removal of N-linked glycans from glycoproteins*

Description: Remove-iT PNGase F is an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from *N*-linked glycoproteins. Remove-iT PNGase F is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Source: Purified from *Flavobacterium meningosepticum.*

Reaction Conditions:
Denature glycoprotein in 1X DTT (40 mM) at 55°C for 10 minutes. Incubate in 1X GlycoBuffer 2 for 1 hour at 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
10X DTT
10X GlycoBuffer 2

Molecular Weight: 41,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 5 µg of DTT denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl

Concentration: 225,000 units/ml

Usage Notes: Using typical RNase B denaturing conditions with NEB Glycoprotein Denaturing Buffer containing SDS and DTT, Remove-iT PNGase F yields a higher concentration of 500,000 U/ml.

If using Remove-iT PNGase F under typical PNGase F denaturing conditions, it is essential to have NP-40 in the reaction mixture, as Remove-iT PNGase F is inhibited by SDS. It is not known why this non-ionic detergent counteracts the SDS inhibition.

Removal of Remove-iT PNGase F from the deglycosylation reaction can be scaled up linearly with larger volumes of chitin magnetic beads.

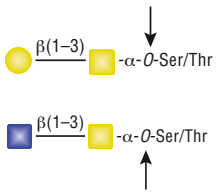
O-Glycosidase

#P0733S 2,000,000 units
#P0733L 10,000,000 units

Companion Products:

O-Glycosidase & Neuraminidase Bundle
#E0540S 1 bundle

α2-3,6,8 Neuraminidase
#P0720S 2,000 units
#P0720L 10,000 units



- *Removal of Core 1 and Core 3 O-linked disaccharide glycans from glycoproteins*

Description: *O*-Glycosidase, also known as Endo-α-*N*-Acetylgalactosaminidase, catalyzes the removal of Core 1 and Core 3 *O*-linked disaccharides from glycoproteins.

Source: Cloned from *Enterococcus faecalis* and expressed in *E. coli*.

Reagents Supplied:
10X Glycoprotein Denaturing Buffer
10X GlycoBuffer 2
10% NP-40

Molecular Weight: 147,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to remove 0.68 nmol of *O*-linked disaccharide from 5 mg of neuraminidase-digested, non-denatured fetuin in 1 hour at 37°C in a total reaction volume of 100 µl (1 unit of both *O*-Glycosidase and PNGase F will remove equivalent molar amounts of *O*-linked disaccharides and *N*-linked oligosaccharides, respectively).

Concentration: 40,000,000 units/ml

Heat Inactivation: 65°C for 10 minutes

Usage Note: Remove sialic acids if present

Protein Deglycosylation Mix II

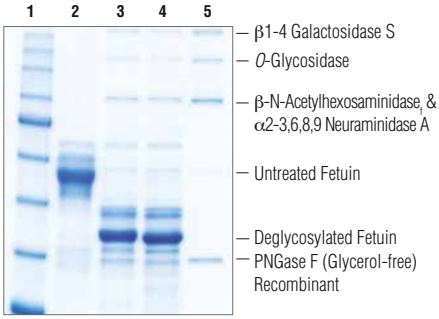
#P6044S 20 reactions

- *Fast reaction setup*
- *Enzyme mixture ensures effective deglycosylation of N-and O-linked glycans*
- *Can be used under native and reducing conditions*
- *Enzymatic deglycosylation leaves intact core structures suitable for mass spectrometry analysis*

Description: The Protein Deglycosylation Mix II contains all of the enzymes, reagents and controls necessary to remove almost all *N*-linked and simple *O*-linked glycans, as well as some complex *O*-linked glycans. This mix contains enzymes sufficient for 20 reactions or the cleavage of as much as 2 mg of glycoprotein.

Reagents Supplied:
Deglycosylation Mix Buffer 1 (10X)
Deglycosylation Mix Buffer 2 (10X)

Deglycosylation Enzyme Mix II:
PNGase F (Glycerol-free), Recombinant: 10,000 units/vial
O-Glycosidase: 80,000 units/vial
α2-3,6,8,9 Neuraminidase A: 400 units/vial
β1-4 Galactosidase S: 960 units/vial
β-*N*-Acetylhexosaminidase$_f$: 300 units/vial



Enzymatic Deglycosylation of Bovine Fetuin under both native (10X Deglycosylation Mix Buffer 1) and reducing (10X Deglycosylation Mix Buffer 2) conditions. 20 µg reactions were loaded onto a 10-20% Tris-glycine SDS-PAGE gel. Lane 1: Color Prestained Protein Standard, Broad Range (11-245 kDa), Lane 2: 20 µg untreated Fetuin control, Lane 3: 20 µg Fetuin deglycosylated under native conditions with Deglycosylation Mix Buffer 1, Lane 4: 20 µg Fetuin deglycosylated under reducing conditions with Deglycosylation Mix Buffer 2, Lane 5: 5 µl Protein Deglycosylation Mix II

Fetuin

#P6042S 500 µg

Description: Fetuin is a glycoprotein containing sialylated *N*-linked and *O*-linked glycans that can be used as a positive control for endoglycosidase enzymes.

Source: Fetal Calf Serum

Molecular Weight: 64 kDa

Concentration: 10 mg/ml

Note: 500 µg is enough for approximately 20 reactions. Due to heterogeneous glycosylation, Fetuin runs as a doublet on an SDS-PAGE gel.

NEW

N-Glycan Sequencing Kit

RR 37°

#E0577S 20 reactions

- *Recombinant enzymes with no detectable endoglycosidase or other exoglycosidase contaminating activities*
- *Compatible with N-linked glycans released from a variety of CHO and murine derived antibodies, as well as N-linked glycans released from other glycoproteins*
- *Simultaneous digestion with other exoglycosidases*
- *Optimal activity and stability for up to 12 months*

Description: The *N*-Glycan Sequencing Kit consists of seven well characterized, highly pure, recombinant exoglycosidase enzymes selected to simplify the process of characterizing typical *N*-linked glycan structures.

The *N*-Glycan Sequencing Kit Includes:

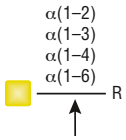
- α2-3,6,8,9 Neuraminidase A
- α2-3 Neuraminidase S
- β-*N*-Acetylglucosaminidase S
- β1-4 Galactosidase S
- α1-3,4,6 Galactosidase
- α1-2,4,6 Fucosidase O
- α1-2,3,6 Mannosidase
- Zinc
- GlycoBuffer 1

Deglycosylation	PCA or 2AB Labeling	HILIC Clean Up	Dry Glycans	Exoglycosidase Digestion
10 minutes	45 minutes	20 minutes	30 mins-1.5 hrs	3 hours
using PNGase F	by reductive amination with a fluorophore label		to concentrate and remove volatile solvents	

α-*N*-Acetylgalactosaminidase

BSA RR BSA 37° 65°

#P0734S 3,000 units
#P0734L 15,000 units



Description: α-*N*-Acetylgalactosaminidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α-linked D-*N*-Acetylgalactosamine residues from oligosaccharides and *N*-glycans attached to proteins.

Source: Cloned from *Chryseobacterium meningosepticum* and expressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

Molecular Weight: 47,000 daltons

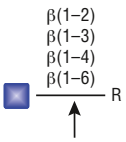
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-*N*-Acetylgalactosamine from 1 nmol of (GalNAcα1-3)(Fucα1-2)Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 20,000 units/ml

β-*N*-Acetylglucosaminidase S

RR 37° NG

#P0744S 100 units
#P0744L 500 units



- *Removal of bisecting β-GlcNAc residues*

Description: β-*N*-Acetylglucosaminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing β-*N*-Acetylglucosamine residues from oligosaccharides.

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Incubate at 37°C.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 125,000 daltons

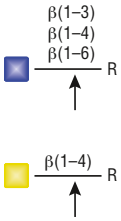
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, non-reducing β-*N*-Acetylglucosamine from 1 nmol GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 4,000 units/ml

β-*N*-Acetylhexosaminidase$_f$

RR 37° 75°

#P0721S 500 units
#P0721L 2,500 units



- *Active only on linear substrates*

Description: β-*N*-Acetylhexosaminidase$_f$ is a recombinant protein fusion of β-*N*-Acetylhexosaminidase and maltose binding protein with identical activity to β-*N*-Acetylhexosaminidase. It catalyzes the hydrolysis of terminal β-D-*N*-Acetylgalacto-samine and glucosamine residues from oligosaccharides.

Source: Cloned from *Streptomyces plicatus* and overexpressed in *E. coli.*

Reaction Conditions: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 100,000 daltons

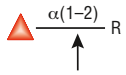
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal β-D-*N*-Acetylgalactosamine from 1 nmol of GalNAcβ1-4Galβ1-4Glc-AMC, in 1 hour at 37°C in 10 µl volume.

Concentration: 5,000 units/ml

α1-2 Fucosidase

BSA RR BSA 37° 65°

#P0724S 1,000 units
#P0724L 5,000 units



- *Active only on linear substrates*

Description: α1-2 Fucosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of linear α1-2 linked L-fucopyranosyl residues from oligosaccharides. In this case, a linear substrate is defined as having no branching on the adjacent residue.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli.*

Reaction Conditions: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

Molecular Weight: 70,000 daltons

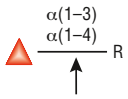
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the α-L-fucose from 1 nmol of Fucα1-2Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 20,000 units/ml

α1-3,4 Fucosidase

BSA RR BSA 37° 65°

#P0769S 200 units
#P0769L 1,000 units



Description: α1-3,4 Fucosidase, (also known as AMF) is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-3 and α1-4 linked fucose residues from oligosaccharides and glycoproteins.

Source: Cloned from the sweet almond tree (*Prunus dulcis*) and expressed in *Pichia pastoris.*

Reaction Conditions: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

Molecular Weight: 56,000 daltons

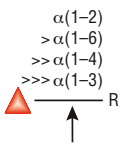
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the α-fucose from 1 nmol of Galβ1-4GlcNAcβ1-3(Fucα1-3)Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 4,000 units/ml

α1-2,3,4,6 Fucosidase

BSA RR BSA 37° 100°

#P0748S 400 units
#P0748L 2,000 units



Description: α1-2,3,4,6 Fucosidase is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-2, α1-3,α1-4 and α1-6 linked L-fucopyranosyl residues from oligosaccharides.

Source: Cloned from bovine kidney and expressed in *E. coli.*

Reaction Conditions: 1X Glycobuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 100°C for 10 minutes.

Reagents Supplied:
10X Glycobuffer 1
100X BSA

Molecular Weight: 51,800 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the α-L-fucose from 1 nmol of Fucα1-2Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

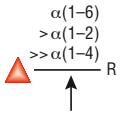
Concentration: 8,000 units/ml

Cloned at NEB | RR Recombinant Enzyme | BSA Requires BSA | Enzymes for Innovation | 65° Heat Inactivation

NEW

α1-2,4,6 Fucosidase O

#P0749S 400 units
#P0749L 2,000 units



Description: α1-2,4,6 Fucosidase O is a broad specificity exoglycosidase that catalyzes the hydrolysis of terminal α1-2, α1-4 and α1-6 linked fucose residues from oligosaccharides. α1-2,4,6 Fucosidase O cleaves α1-6 fucose residues more efficiently than other linkages.

Source: Cloned from *Omnitrophica* bacterium and expressed in *E. coli*.

Reaction Conditions: 1X Glycobuffer 1. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X Glycobuffer 1

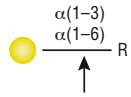
Molecular Weight: 49,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the fucose from 1 nmol of G0F from human IgG [GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc(Fucα1-6)-AMAC], in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 2,000 units/ml

α1-3,6 Galactosidase

#P0731S 100 units
#P0731L 500 units



Description: α1-3, 6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-3, 6 linked D-galactopyranosyl residues from oligosaccharides.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

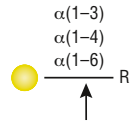
Molecular Weight: 70,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, α-D-galactose from 1 nmol Galα1-3Galβ1-4Gal-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 4,000 units/ml

α1-3,4,6 Galactosidase

#P0747S 200 units
#P0747L 1,000 units



Description: α1-3,4,6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-3, α1-4 and α1-6 linked D-galactopyranosyl residues from oligosaccharides.

Source: Cloned from green coffee bean and expressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

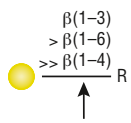
Molecular Weight: 39,700 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, α-D-galactose from 1 nmol Galα1-3Galβ1-4Gal-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 units/ml

β1-3 Galactosidase

#P0726S 500 units
#P0726L 2,500 units



Description: β1-3 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-3 and, at a much lower rate, β1-6 linked D-galactopyranosyl residues from oligosaccharides. The approximate kinetic data show > 100-fold preference for β1-3 over β1-6 linkages and > 500-fold preference from β1-3 over β1-4 linkages.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

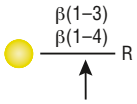
Molecular Weight: 66,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal β-D-galactose from 1 nmol of Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 10,000 units/ml

β1-3,4 Galactosidase

#P0746S 400 units
#P0746L 2,000 units



Description: β1-3,4 Galactosidase, cloned from bovine testis and also known as BTG, is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal β1-3 and β1-4 linked galactose residues from oligosaccharides.

Source: Cloned from bovine testis and expressed in *Pichia pastoris.*

Reaction Conditions: 1X GlycoBuffer 4. Incubate at 37°C.Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 4

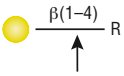
Molecular Weight: 71,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, β-D-galactose from 1 nmol Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 units/ml

β1-4 Galactosidase S

#P0745S 400 units
#P0745L 2,000 units



Description: β1-4 Galactosidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-4 linked galactose residues from oligosaccharides.

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli.*

Reaction Conditions: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

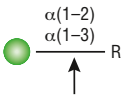
Molecular Weight: 231,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, β-D-galactose from 1 nmol Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 units/ml

α1-2,3 Mannosidase

#P0729S 640 units
#P0729L 3,200 units



Description: α1-2,3 Mannosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-2 and α1-3 linked D-mannopyranosyl residues from oligosaccharides.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli.*

Reaction Conditions: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 55°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

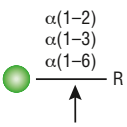
Molecular Weight: 90,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-Mannose from 1 nmol of Manα1-3Manβ1-4GlcNAc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 32,000 units/ml

α1-2,3,6 Mannosidase

#P0768S 80 units
#P0768L 400 units



Description: α1-2,3,6 Mannosidase, cloned from Jack Bean, and also known as JBM, is a broad specificity exoglycosidase that catalyzes the hydrolysis of terminal α1-2, α1-3 and α1-6 linked mannose residues from oligosaccharides. α1-2,3,6 Mannosidase has a slight preference for α1-2 mannose residues over α1-3 and α1-6 mannose residues.

Source: Cloned from *Canavalia ensiformis* (Jack Bean) and expressed in *Pichia pastoris.*

Reaction Conditions: 1X GlycoBuffer 4 and 1X Zinc. Incubate at 37°C. Heat inactivation: 95°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 4
10X Zinc

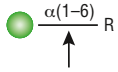
Molecular Weight: 110,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal mannose from 1 nmol of Man(α1,3)-Man(β1,4)-GlcNAc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 2,000 units/ml

α1-6 Mannosidase

#P0727S 800 units
#P0727L 4,000 units



Description: α1-6 Mannosidase is a highly specific exoglycosidase that removes unbranched α1-6 linked D-mannopyranosyl residues from oligosaccharides. When used in conjunction with α1-2,3 Mannosidase, the α1-6 Mannosidase will cleave α1-6 Mannose residues from branched carbohydrate substrates.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

Molecular Weight: 58,000 daltons

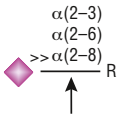
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-mannose from 1 nmol of Manα1-6Manα1-6Man-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 40,000 units/ml

Note: *p*-nitrophenyl-α-D-mannopyranoside is NOT a substrate for this enzyme.

α2-3,6,8 Neuraminidase

#P0720S 2,000 units
#P0720L 10,000 units



- *Active from pH 4.5 to 8.5*

Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). This Neuraminidase catalyzes the hydrolysis of α2-3, α2-6 and α2-8 linked N-acetylneuraminic acid residues from glycoproteins and oligosaccharides.

Source: Cloned from *Clostridium perfringens* and overexpressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 43,000 daltons

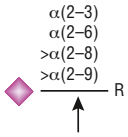
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol of Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 5 minutes at 37°C in a total reaction volume of 10 µl.

Concentration: 50,000 units/ml

Note: This enzyme shows a preference for α2,3 and α2,6 linkages over α2,8 linkages.

α2-3,6,8,9 Neuraminidase A

#P0722S 800 units
#P0722L 4,000 units



- *Removes branched sialic acid residues that are linked to an internal residue*

Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). α2-3,6,8,9 Neuraminidase A catalyzes the hydrolysis of all linear and branched non-reducing terminal sialic acid residues from glycoproteins and oligosaccharides. The enzyme releases α2-3 and α2-6 linkages at a slightly higher rate than α2-8 and α2-9 linkages.

Source: Cloned from *Arthrobacter ureafaciens* and expressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

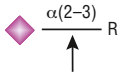
Molecular Weight: 100,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 20,000 units/ml

α2-3 Neuraminidase S

#P0743S 400 units
#P0743L 2,000 units



Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). α2-3 Neuraminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of α2-3 linked N-acetyl-neuraminic acid residues from glycoproteins and oligosaccharides.

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 74,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the α-Neu5Ac from 1 nmol of Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 units/ml

IdeZ Protease (IgG-specific)

#P0770S 4,000 units

human IgG1, IgG3, IgG4: CPAPELLG▼GPSVF
human IgG2: CPAPPVA▼GPSVF
murine IgG2a: CPAPNLLG▼GPSVF
murine IgG3: CPPGNILG▼GPSVF

- *Complete fragmentation of antibodies and immunoglobulin fusion proteins in 30 minutes under native conditions*

Description: IdeZ Protease (IgG-specific) is a recombinant antibody specific protease cloned from *Streptococcus equi* subspecies *zooepidemicus* that recognizes all human, sheep, monkey, and rabbit IgG subclasses, specifically cleaving at a single recognition site below the hinge region, yielding a homogenous pool of F(ab´)2 and Fc fragments. IdeZ Protease more effectively cleaves murine IgG2a than IdeS.

Source: Cloned from *Streptococcus equi* subspecies *zooepidemicus* and expressed in *E. coli*.

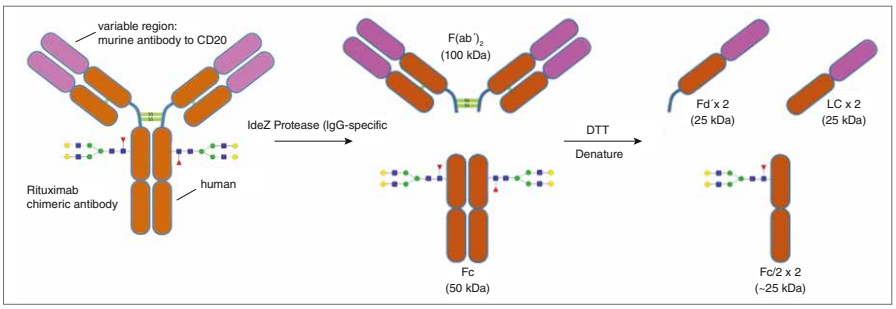
Reaction Conditions:
1X GlycoBuffer 2. Incubate at 37°C.
Heat inactivation: 65°C for 10 minutes.

Reagents Supplied with Enzyme:
10X GlycoBuffer 2

Molecular Weight: 35,578 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of 1 µg of human IgG, in 15 minutes at 37°C in a total reaction volume of 10 µl.

Concentration: 80,000 units/ml

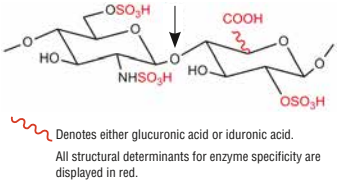


Digestion of IgG with IdeZ Protease (IgG-specific), followed by denaturation.

Angela, Alycia and Anne have worked at NEB for 9, 9 and 30 years, respectively. This mother/daughter trio has worked in various departments within the company and are well-loved by the NEB community.

Bacteroides Heparinase I

#P0735S 240 units
#P0735L 600 units



Denotes either glucuronic acid or iduronic acid.
All structural determinants for enzyme specificity are displayed in red.

- *Degradation of heparin and heparan sulfate glycosaminoglycans*

Description: *Bacteroides* Heparinase I cloned from *Bacteroides eggerthii*, also called Heparin Lyase I, is active on heparin and the highly sulfated domains of heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Source: Cloned from *Bacteroides eggerthii* and expressed in *E. coli*.

Reaction Conditions:
10 µg heparin substrate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H_2O in a total reaction volume of 100 µl. Incubate reaction at 30°C.
Heat inactivation: 100°C for 1 minute.

Reagents Supplied:
10X *Bacteroides* Heparinase Reaction Buffer

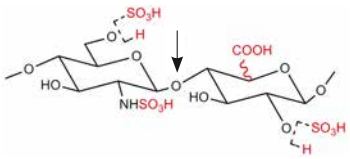
Molecular Weight: 42,000 daltons

Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from porcine mucosal heparin per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl. For unit assay conditions, visit www.neb.com.

Concentration: 12,000 units/ml

Bacteroides Heparinase II

#P0736S 80 units
#P0736L 200 units



Denotes either glucuronic acid or iduronic acid.
All structural determinants for enzyme specificity are displayed in red.

- *Degradation of heparin and heparan sulfate glycosaminoglycans*

Description: *Bacteroides* Heparinase II cloned from *Bacteroides eggerthii*, also called Heparin Lyase II, is active on heparin and heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Source: Cloned from *Bacteroides eggerthii* and expressed in *E. coli*.

Reaction Conditions:
10 µg heparin substrate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H_2O in a total reaction volume of 100 µl. Incubate reaction at 30°C.
Heat inactivation: 100°C for 1 minute.

Reagents Supplied:
10X *Bacteroides* Heparinase Reaction Buffer

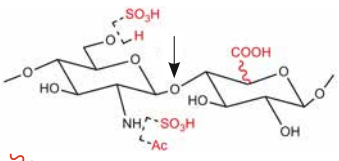
Molecular Weight: 86,000 daltons

Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from porcine mucosal heparin per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl. For unit assay conditions, visit www.neb.com.

Concentration: 4,000 units/ml

Bacteroides Heparinase III

#P0737S 14 units
#P0737L 35 units



Denotes either glucuronic acid or iduronic acid.
All structural determinants for enzyme specificity are displayed in red.

- *Degradation of heparan sulfate glycosaminoglycans*

Description: *Bacteroides* Heparinase III cloned from *Bacteroides eggerthii*, also called Heparin Lyase III, is active on heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Source: Cloned from *Bacteroides eggerthii* and expressed in *E. coli*.

Reaction Conditions:
10 µg heparan sulfate substrate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H_2O in a total reaction volume of 100 µl. Incubate reaction at 30°C.
Heat inactivation: 100°C for 1 minute.

Reagents Supplied:
10X *Bacteroides* Heparinase Reaction Buffer

Molecular Weight: 75,000 daltons

Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from heparan sulfate per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl. For unit assay conditions, visit www.neb.com.

Concentration: 700 units/ml

Ph.D.™ Phage Display Peptide Library Kits

Ph.D.-7 Kit
10 Panning Experiments
#E8100S

Ph.D.-12 Kit
10 Panning Experiments
#E8110S

Ph.D.-C7C Kit
10 Panning Experiments
#E8120S

Components Sold Separately:

Ph.D.-12 Library
50 Panning Experiments
#E8111L

The Ph.D. Kits Include:

- *Sufficient Phage Display Library for 10 separate panning experiments, complexity of 10^9 clones*
- *28 gIII Sequencing Primer (100 pmol)*
- *96 gIII Sequencing Primer (100 pmol)*
- *Host E. coli strain ER2738*
- *Control Target (Streptavidin) and Elutant (Biotin)*
- *Detailed Protocols*

Description: Phage display describes a selection technique in which a library of peptide or protein variants is expressed on the outside of a phage virion, while the genetic material encoding each variant resides on the inside. This creates a physical linkage between each variant protein sequence and the DNA encoding it, which allows rapid partitioning based on binding affinity to a given target molecule (antibodies, enzymes, cell-surface receptors, etc.) by an *in vitro* selection process called panning. In its simplest form (Figure 1), panning is carried out by incubating a library of phage-displayed peptides with a plate (or bead) coated with the target, washing away the unbound phage, and eluting the specifically-bound phage. The eluted phage is then amplified and taken through additional binding/amplification cycles to enrich the pool in favor of binding sequences. After 3–4 rounds, individual clones are characterized by DNA sequencing and ELISA.

New England Biolabs offers 3 pre-made random peptide libraries, as well as the cloning vector M13KE for construction of custom libraries. The pre-made libraries consist of linear heptapeptide (Ph.D.-7) and dodecapeptide (Ph.D.-12) libraries, as well as a disulfide-constrained heptapeptide (Ph.D.-C7C) library. All of the libraries have complexities in excess of 2 billion independent clones. The randomized peptide sequences in all three libraries are expressed at the N-terminus of the minor coat protein pIII, resulting in a valency of 5 copies of the displayed peptide per virion.

The Ph.D. libraries have been used for myriad applications, including epitope mapping (Figure 2), identification of protein-protein contacts and enzyme inhibitors and discovery of peptide ligands for GroEL, HIV, semiconductor surfaces and small-molecule fluorophores and drugs.

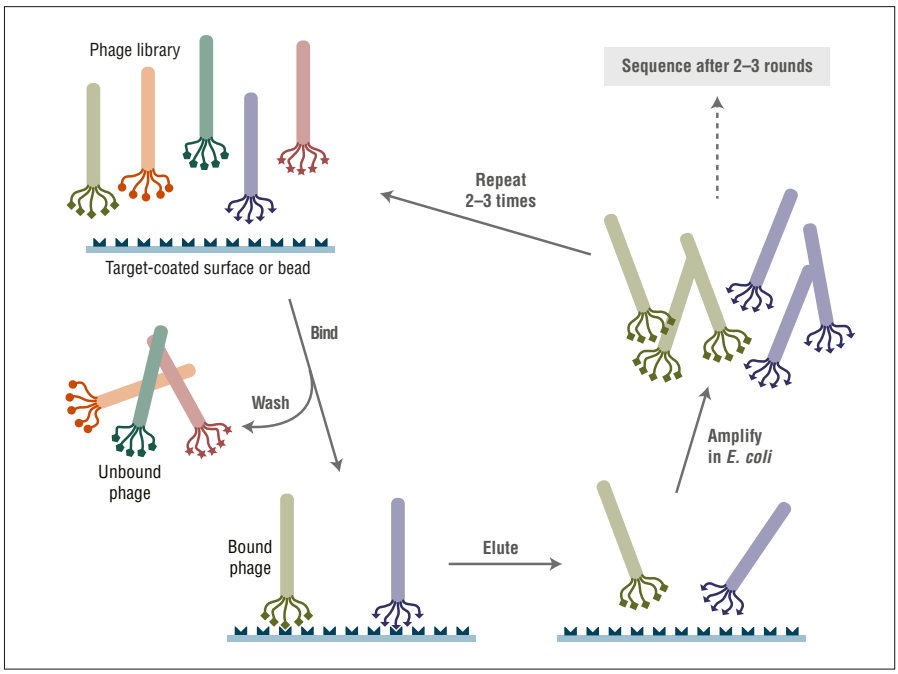


Figure 1: Routine Phage Display Workflow. *Round 1 : Incubate 10^{11} pfu Ph.D. library + target incubation, wash away non-binders, elute bound phage, enrich selected phage with amplification in E. coli. Carry out 3-4 rounds of selection and then proceed with sequencing and/or phage-ELISA.*

β-endorphin:

Y	G	G	F	M	T	S	E	K	Q	T	P…

1st round sequences:

Y	G	W	I	S	P	P	L	H	L	P	T
Y	Q	P	D	N	P	S	R	Q	I	A	N
Y	W	P	A	H	I	R	A	V	P	M	I
R	L	D	D	I	K	N	T	L	A	F	S
S	S	D	V	Y	S	L	Y	P	F	I	M
E	F	F	P	H	P	M	L	H	N	S	R
D	N	W	P	Y	R	P	S	F	S	L	S
S	H	N	T	Y	S	A	P	R	P	S	A
S	L	L	H	Y	A	S	S	L	S	L	M
F	N	Q	N	A	E	P	F	S	S	R	P
H	P	R	Q	L	L	H	H	P	L	S	P

2nd round sequences:

Y	G	G	F	L	I	G	L	Q	D	A	S
Y	G	G	F	H	Y	K	E	T	G	A	L
Y	Q	P	D	N	P	S	R	Q	I	A	N
V	Y	C	Y	I	N	Q	S	M	I	G	N
H	H	D	T	E	Y	R	T	T	Q	L	S
N	L	K	F	P	T	N	P	K	A	M	W
L	P	N	L	T	W	A	L	M	P	R	A
D	N	W	P	Y	R	P	S	F	S	L	S
S	H	N	T	Y	S	A	P	R	P	S	A
S	L	L	H	Y	A	S	S	L	S	L	M
V	T	M	N	T	K	T	P	G	P	M	P

3rd round sequences:

Y	G	G	F	M	T	T	P	S	H	V	P
Y	G	G	F	M	T	T	P	S	H	V	P
Y	G	G	F	I	S	Q	T	Q	H	Y	S
Y	G	G	F	I	S	Q	T	Q	H	Y	S
Y	G	G	F	G	N	S	L	V	M	P	V
Y	G	G	F	S	M	P	F	L	P	A	L
Y	G	A	F	D	V	T	T	G	V	T	S
Y	G	V	F	N	P	H	Y	L	P	S	L
A	P	S	T	D	K	Q	A	T	M	P	L
A	S	V	A	V	S	S	R	Q	D	A	A

Figure 2: Epitope mapping of an anti-β-endorphin monoclonal antibody with the Ph.D.-12 library.
The Ph.D.-12 library was panned against anti-β-endorphin antibody 3-E7 in solution (10 nM antibody), followed by affinity capture of the antibody-phage complexes onto Protein A-agarose (rounds 1 and 3) or Protein G-agarose (round 2). Bound phage were eluted with 0.2 M glycine-HCl, pH 2.2. Selected sequences from each round are shown aligned with the first 12 residues of β-endorphin; consensus elements are boxed. The results clearly show that the epitope for this antibody spans the first 7 residues of β-endorphin, and that the bulk of the antibody-antigen binding energy is contributed by the first 4 residues (YGGF), with some flexibility allowed in the third position. Additionally, the conserved position of the selected sequences within the 12 residue window indicates that the free α-amino group of the N-terminal tyrosine is part of the epitope.

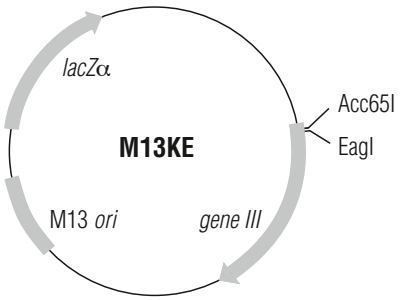
Ph.D. Peptide Display Cloning System

Ph.D. Peptide Display Cloning System

20 µg M13KE gIII Cloning Vector
16 µg Extension Primer

#E8101S

Description: The Ph.D. Peptide Display Cloning System facilitates the display of custom peptide libraries on the surface of bacteriophage M13 as coat protein fusions, creating a physical linkage between each displayed peptide and its encoding DNA sequence. Peptide ligands for a variety of targets can then be selected by the straightforward method of panning. The supplied display vector M13KE is an M13 derivative with cloning sites engineered for N-terminal pIII fusion, resulting in a valency of 5 displayed peptides per virion. The use of a phage vector, rather than a phagemid, simplifies the intermediate amplification steps, since neither antibiotic selection nor helper phage superinfection are required. Since displayed proteins longer than 20–30 amino acids have a deleterious effect on the infectivity function of pIII in phage vectors, **this vector is suitable only for the display of short peptides.** Included with the cloning vector is an insert extension primer as well as a detailed protocol for cloning a peptide library into M13KE.



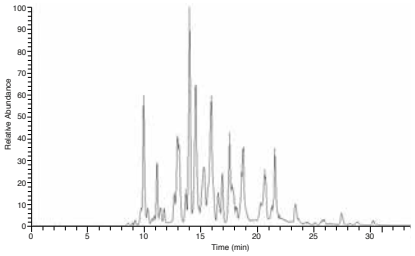
Trypsin-digested BSA MS Standard (CAM Modified)

#P8108S 500 pmol

- *Standardization range: 500 to 2400 Da*

Description: A complex mixture of peptides produced by Trypsin digestion of Bovine Serum Albumin (BSA) that was reduced and alkylated with Iodoacetimide (CAM modified). This peptide mixture can be used to test a Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) or Electrospray Ionization (ESI) mass spectrometer (TOF, Q-TOF or Ion Trap).

Source: BSA (GENBANK P02769) was digested using Trypsin-ultra, Mass Spectrometry Grade, (NEB #P8101).



One hundred fmol of resuspended peptide mix may be analyzed by reverse phase liquid chromatography with on-line MS/MS analysis, for example with a Proxeon EASY-nLC and by Orbitrap Mass Spectrometer. Both analytical methods reveal a range of peptides in the standard. At least sixty percent sequence coverage is seen after database search, with greater than 15 unique peptides being identified.

Trypsin-ultra™, Mass Spectrometry Grade

#P8101S 100 µg

Lys/Arg▼XXX

- *Digestion of proteins for proteomic analysis by mass spectrometry*
- *Protein and peptide identification*
- *TPCK treatment eliminates chymotryptic activity*
- *Free of contaminating proteases*

Description: Trypsin-ultra, Mass Spectrometry Grade is a serine endopeptidase. It selectively cleaves peptide bonds C-terminal to lysine and arginine residues. Trypsin-ultra, Mass Spectrometry Grade is treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inactivate any remaining chymotryptic activity. It is modified by acetylation of the ε-amino groups of lysine residues to prevent autolysis. Trypsin-ultra, Mass Spectrometry Grade cleaves at Lys-Pro and Arg-Pro bonds at a much slower rate than other amino acid residues.

Source: Isolated from bovine (*Bos taurus*) pancreas.

Reaction Conditions:
1X Trypsin Ultra Reaction Buffer. Incubate at 37°C.

Reagents Supplied with Enzyme:
2X Trypsin-ultra Reaction Buffer

Molecular Weight: 23,675 daltons

Reconstitution: Trypsin-ultra, Mass Spectrometry Grade should be reconstituted by the addition of 20–200 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

Notes: Can be stored frozen in solution at –20°C for up to 1 week. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

Sue is a member of our NEBNext Production team, and has worked at NEB for over 25 years! Sue has many interests outside of NEB, including sewing, biking, fishing and spending time with her grandchildren.

Endoproteinase GluC

#P8100S 50 µg

XX-Glu▼XX

- *Ideal for proteomic analysis by mass spectrometry*
- *Protein & peptide identification*
- *Free of contaminating proteases. Produced from a protease-deficient Bacillus subtilis strain*

Description: Endoproteinase GluC (*Staphylococcus aureus* Protease V8) is a serine proteinase which selectively cleaves peptide bonds C-terminal to glutamic acid residues. Endoproteinase GluC also cleaves at aspartic acid residues at a rate 100–300 times slower than at glutamic acid residues.

Source: *Staphylococcus aureus* Protease V8 gene cloned and expressed in *Bacillus subtilis.*

Reaction Conditions:
1X GluC Reaction Buffer. Incubate at 37°C.

Reagents Supplied with Enzyme:
2X GluC Reaction Buffer

Molecular Weight: 29,849 daltons

Reconstitution: Endoproteinase GluC should be reconstituted by the addition of 50–500 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

Note: Can be stored frozen in solution at –20°C for up to 2 weeks. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

Endoproteinase AspN

#P8104S 50 µg

XX▼Asp-XXX

- *Ideal for proteomic analysis by mass spectrometry*
- *Free of contaminating proteases*
- *Best suited for peptide identification*

Description: Endoproteinase AspN (flavastacin) is a zinc metalloendopeptidase which selectively cleaves peptide bonds N-terminal to aspartic acid residues.

Source: Purified from *Flavobacterium menigosepticum.*

Reaction Conditions:
1X AspN Reaction Buffer. Incubate at 37°C.

Reagents Supplied with Enzyme:
2X AspN Reaction Buffer

Molecular Weight: 40,089.9 daltons

Reconstitution: Endoproteinase AspN should be reconstituted by the addition of 50–500 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

Notes: Can be stored frozen in solution at –20°C for up to 2 weeks. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

Endoproteinase LysC

#P8109S 20 µg

XX-Lys▼XXX

- *Ideal for proteomic analysis by mass spectrometry*
- *Free of contaminating proteases*
- *Best suited for peptide identification*

Description: LysC is a serine endoproteinase, isolated from *Lysobacter enzymogenes*, that cleaves peptide bonds C-terminal to lysine residues. LysC is a sequencing grade enzyme and is suitable for proteomics and glycobiology applications.

Note: Resuspend in 200 µl double-distilled water to make 100 ng/µl LysC solution in 10 mM Tris-HCl, pH 8.0. The solution can be stored at 4°C for several days or in single-use aliquots at –20°C for several months.

Source: Isolated from *Lysobacter enzymogenes.*

Molecular Weight: 30,000 daltons

Reconstitution: Endoproteinase LysC should be reconstituted in 200 µl double-distilled water to make a 100 ng/µl solution in 10 mM Tris-HCl, pH 8.0. Rapid autolysis is a function of enzyme concentration.

Proteinase K, Molecular Biology Grade

#P8107S 2 ml

- *Isolation of plasmid and genomic DNA*
- *Isolation of RNA*
- *Inactivation of RNases, DNases and enzymes in reactions*

Description: Proteinase K is a subtilisin-related serine protease that will hydrolyze a variety of peptide bonds.

Source: *Engyodontium album (Tritirachium album)*

Reaction Conditions: Proteinase K is active in a wide range of buffers including all NEB specific restriction endonuclease buffers. It is highly active between pH 7.5 and 12.0 and temperatures between 20 and 60°C. Proteinase K is also active in chelating agents such as EDTA and activity is stimulated in up to 2% SDS or 4 M urea. Calcium is important for thermostability of Proteinase K but it is not required for catalysis, therefore Proteinase K is also active in buffers containing chelating agents such as EDTA.

Molecular Weight: 28,900 daltons

Unit Definition: One unit will digest urea-denatured hemoglobin at 37°C (pH 7.5) per minute to produce equal absorbance as 1.0 µmol of L-tyrosine using Folin & Ciocalteu's phenol reagent.

Concentration: 800 units/ml

NEW

Thermolabile Proteinase K

#P8111S 30 units

- *Isolation of plasmid and genomic DNA*
- *Isolation of RNA*
- *Inactivation of RNases, DNases and enzymes in reactions*
- *Removal of enzymes from DNA to improve cloning efficiency*
- *PCR purification*

Description: Thermolabile Proteinase K is an engineered, subtilisin-related serine protease that will hydrolyze a variety of peptide bonds.

Source: Cloned from *Engyodontium album (Tritirachium album)*, mutagenized to increase thermolability of the enzyme and expressed in *K. lactis*.

Molecular Weight: 29,000 daltons

Reaction Conditions: Thermolabile Proteinase K is active in a wide range of buffers. It is highly active between pH 7.0-9.5 and temperatures 20-40°C. It is active in chelating agents such as EDTA and activity is stimulated in up to 1% SDS.

Unit Definition: One unit is defined as the amount of enzyme required to release 1.0 µmol of 4-nitroaniline per minute from N-Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide at 25°C, in a total reaction volume of 105 µl.

Concentration: 120 units/ml

Factor Xa Protease

#P8010S 50 µg
#P8010L 250 µg

Ile-Glu/Asp-Gly-Arg▼

Description: Factor Xa cleaves after the arginine residue in its preferred cleavage site Ile-(Glu or Asp)-Gly-Arg. It will sometimes cleave at other basic residues, depending on the substrate conformation. The most common secondary site, among those that have been sequenced, is Gly-Arg. There seems to be a correlation between proteins that are unstable in *E. coli* and those that are cleaved by Factor Xa at secondary sites; this may indicate that these proteins are in a partially unfolded state. Factor Xa will not cleave a site followed by proline or arginine.

Source: Purified from bovine plasma and activated by treatment with the activating enzyme from Russell's viper venom.

Molecular Weight: The predominant form of Factor Xa has a molecular weight of approximately 43,000 daltons, consisting of two disulfide-linked chains of approximately 27,000 daltons and 16,000 daltons. On SDS-PAGE, the reduced chains have apparent molecular weights of 30,000 daltons and 20,000 daltons.

Unit Definition: 1 µg of Factor Xa will cleave 50 µg of test substrate to 95% completion in 6 hours or less. Unit assay conditions can be found at www.neb.com.

Concentration: 1 mg/ml

Removal: Factor Xa will bind specifically to benzamidine-agarose (e.g., GE Life Sciences #17-5143-02).

Enterokinase, light chain

RR

#P8070S 480 units
#P8070L 2,560 units

Asp-Asp-Asp-Asp-Lys▼

Description: Enterokinase is a specific protease that cleaves after the lysine at its cleavage site, Asp-Asp-Asp-Asp-Lys. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate. Enterokinase will not cleave a site followed by proline.

Source: Purified from *K. lactis* containing a clone of the light chain of the bovine enterokinase gene.

Molecular Weight: The molecular weight of the light chain of enterokinase is 26,300 daltons. Its apparent molecular weight on SDS-PAGE is 31 kDa.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 25 µg of a MBP-EK-paramyosin-ΔSal substrate to 95% completion in 16 hours at 25°C in a total reaction volume of 25 µl. Unit assay conditions can be found at www.neb.com.

Concentration: 16,000 U/ml

Removal: Enterokinase will bind specifically to trypsin inhibitor agarose (e.g., Sigma T-0637).

Furin

RR

#P8077S 50 units
#P8077L 250 units

Arg-X-X-Arg▼

Description: Furin is an ubiquitous subtilisin-like proprotein convertase. It is the major processing enzyme of the secretory pathway and is localized in the trans-golgi network. Substrates of Furin include blood clotting factors, serum proteins and growth factor receptors such as the insulin-like growth factor receptor. The minimal cleavage site is Arg-X-X-Arg▼. However, the enzyme prefers the site Arg-X-(Lys/Arg)-Arg▼. An additional arginine at the P6 position appears to enhance cleavage. Furin is inhibited by EGTA, α1-Antitrypsin Portland and polyarginine compounds.

Note: The ability to cleave a particular substrate appears to depend on its tertiary structure as well as on the amino acids immediately surrounding the cleavage site.

Source: Isolated from *Spodoptera frugiperda* (Sf9) cells infected with recombinant baculovirus carrying truncated human furin (kindly provided by R. Fuller).

Molecular Weight: The calculated molecular weight of truncated human furin is 52,700 daltons. Its apparent molecular weight in SDS-PAGE gels is 57,000 daltons.

Unit Definition: One unit is defined as the amount of furin required to cleave 25 µg of a MBP-FN-paramyosin-ΔSal substrate to 95% completion in 6 hours at 25°C in a total reaction volume of 25 µl. Unit assay conditions can be found at www.neb.com.

Concentration: 2,000 units/ml

NEW

TEV Protease

RR 30° 65°

#P8112S 1,000 units

ENLYFQ▼(G/S)

Description: TEV Protease, also known as Tobacco Etch Virus (TEV) Protease, is a highly specific cysteine protease that recognizes the amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser) and cleaves between the Gln and Gly/Ser residues. It is often used for the removal of affinity purification tags such as maltose-binding protein (MBP) or poly-histidine from fusion proteins. TEV Protease has a 7xHis-tag for easy removal from a reaction using nickel affinity resins and has been engineered for greater performance.

Source: Cloned from Tobacco Etch Virus and expressed in *E. coli*.

Molecular Weight: 28,000 daltons

Unit Definition: 1 unit of TEV Protease will cleave 2 µg of MBP-fusion protein, MBP5-TEV-paramyosin ΔSal, to 95% completion in a total reaction volume of 10 µl in 1 hour at 30°C in 50 mM Tris-HCl (pH 7.5 @ 25°C) with 0.5 mM EDTA and 1 mM DTT.

Concentration: 10,000 units/ml

Properties of Protein Phosphatases from NEB

The significance of protein phosphorylation in regulating the function and activity of protein factors and enzymes is now well established. Analysis of the presence of such phosphorylation, and its attendant effects, is often aided by removal of the protein phosphate groups by phosphatases.

Lambda Protein Phosphatase (Lambda PP)

RR 30° 65°

#P0753S 20,000 units
#P0753L 100,000 units

Companion Products:

p-Nitrophenylphosphate (PNPP)
Non-specific substrate for protein, alkaline and acid phosphatases

#P0757S 1 ml
#P0757L 5 ml

Sodium Orthovanadate (Vanadate)
General inhibitor for protein phosphotyrosyl specific phosphatases

#P0758S 1 ml
#P0758L 5 ml

Description: Lambda Protein Phosphatase (Lambda PP) is a Mn^{2+}-dependent protein phosphatase with activity towards phosphorylated serine, threonine and tyrosine residues. Lambda-PPase is active on phosphorylated histidine residues.

Source: Isolated from a strain of *E. coli* that carries the bacteriophage lambda ORF221 open reading frame (kindly provided by Dr. D. Barford).

Reaction Conditions:
1X NEBuffer for Protein MetalloPhosphatases (PMP). Supplement with 1 mM $MnCl_2$. Incubate at 30°C. Heat inactivation: 65°C for 1 hour in the presence of 50 mM EDTA.

Reagents Supplied with Enzyme:
10X NEBuffer for Protein MetalloPhosphatases (PMP)
10X $MnCl_2$ (10 mM)

Molecular Weight: 25,000 daltons

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 nmol of *p*-Nitrophenyl Phosphate (50 mM, NEB #P0757) in 1 minute at 30°C in a total reaction volume of 50 µl.

Concentration: 400,000 units/ml

Lydia has been a member of the Marketing Communications Team for over 3 years. She is also the host of the NEB podcast, Lessons from Lab & Life.

Protein Kinases

The reversible addition of phosphate groups to proteins is important for the transmission of signals within eukaryotic cells and, as a result, protein phosphorylation and dephosphorylation regulate many diverse cellular processes. As the number of known protein kinases has increased at an ever-accelerating pace, it has become more challenging to determine which protein kinases interact with which substrates in the cell. The determination of consensus phosphorylation site motifs by amino acid sequence alignment of known substrates has proven useful in this pursuit. These motifs can be helpful for predicting phosphorylation sites for specific protein kinases within a potential protein substrate.

Since the determinants of protein kinase specificity involve complex 3-dimensional interactions, these motifs, short amino-acid sequences describing the primary structure around the phosphoacceptor residue, are a significant over-simplification of the issue. They do not take into account possible secondary and tertiary structural elements, or determinants from other polypeptide chains or from distant locations within the same chain. Furthermore, not all of the residues described in a particular specificity motif may carry the same weight in determining recognition and phosphorylation by the kinase. As a consequence, they should be used with some caution.

On the other hand, many of the residues within these consensus sequences have in fact proven to be crucial recognition elements, and the very simplicity of these motifs has made them useful in the study of protein kinases and their substrates. In addition to the prediction of phosphorylation sites, short synthetic oligopeptides based on consensus motifs are often excellent substrates for protein kinase activity assays.

The table below summarizes the specificity motifs for protein kinases that are available from NEB. Phosphoacceptor residue is indicated in red, amino acids which can function interchangeably at a particular residue are separated by a slash (/), and residues which do not appear to contribute strongly to recognition are indicated by an "X".

Visit **www.neb.com** for more information on protein kinases from NEB, including detailed information on recognition determinants.

PROTEIN KINASE	NEB #	RECOGNITION DETERMINANT	SIZE
cAMP-dependent Protein Kinase (PKA), Catalytic Subunit	P6000S/L	R - R - X - S / T Y Y= hydrophobic residue	100,000/500,000 units
Casein Kinase II (CK2)	P6010S/L	S - X - X - E / D	10,000/50,000 units

D = aspartic acid, E = glutamic acid, R = arginine, S = serine, T = threonine, Y = tyrosine, X = any amino acid

Note: More specific information on recognition determinants for each kinase can be found on the corresponding product page at www.neb.com.

Companion Product:

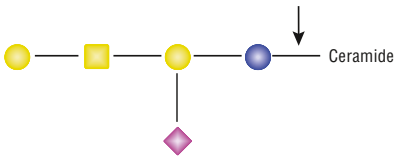
Adenosine-5´ Triphosphate (ATP)

#P0756S	1.0 ml
#P0756L	5.0 ml

NEW

Endoglycoceramidase I (EGCase I)

#P0773S 150 mU



This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Description: Endoglycoceramidase I (ECGase I) catalyzes the hydrolysis of the β-glycosidic linkage between oligosaccharides and ceramides in various glycosphingolipids. One unit of *R. triatomea* EGCase I is defined as the amount of enzyme required to hydrolyze 1 µmol of ganglioside GM1a per minute at 37°C.

Source: EGCase I is isolated from a strain of *E. coli*, which contains the cloned EGCase I gene from *Rhodococcus triatomea*.

Reaction Conditions: 1X EGCase I Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:
10X EGCase I Reaction Buffer

Molecular Weight: 50,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to hydrolyze 1 µmol of ganglioside GM1a per minute at 37°C.

Concentration: 6 units/ml

Timber Wolf (*Canis lupus*) in snow,
Yellowstone National Park, Wyoming, USA.
Credit: Ralf Kistowski, Minden Pictures

The Importance of the Top Predator

The collapse of an ecosystem, if we are not observant, can take decades to fully realize and understand. In the mid-1990s, the banks of the Lamar River in Yellowstone National Park, USA, were eroded, streams had widened, willow shoots that had previously flourished by the river in the 1920s were nonexistent, aspen trees were not regenerating and beavers were scarce. How is it that the collapse of this ecosystem could be the result of an event that occurred in the 1920s?

The case in Yellowstone National Park began as an investigation into the disappearance of aspen trees. Scientists examined the age of the remaining trees by drilling into the cores and establishing age versus diameter relationships. They discovered that the aspen tree had not regenerated in the past 70 years, and by back-dating, it was realized that this 70-year hiatus correlated with the disappearance of the wild wolf from Yellowstone. The downstream effects that occurred as a result of the sudden absence of the wild wolves highlights the complexity of a balanced ecosystem.

A top predator, also known as an apex predator, is at the head of the food chain. In the early 1900s, Yellowstone's top predator, the wild wolf, was viewed as a threat and was hunted until it was completely eliminated from the park by the 1920s.

Pieces of the puzzle started to fit together when scientists examined the effect of unchecked population growth of elk, the primary prey of the wild wolf. Vegetation, including aspen and willow tree saplings, were overgrazed by the elk. The riverside willow had provided material for beavers to build their dams, and in turn, the dams provided more water and nutrients for growth of the riverside vegetation. Additionally, the beaver's protective dams had tempered the seasonal changes in the river flow. With no material to build dams, the beavers also disappeared.

In 1995, in a highly controversial move, 31 wild wolves were relocated from Canada to Yellowstone. Their movement and behavior were observed as they hunted elk and deer. The elk carcasses not only fed the wolves, but also coyotes, ravens, magpies, eagles and finally, insects.

The elk were also tracked, and it was observed that they avoided the gorges and valleys where they were easy prey. With less elk and deer grazing on willow and aspen, the long-gone vegetation started to regenerate; more berries and insects followed, and then various bird species. Beaver families moved back into the area and used the willow to build their dams, which created a habitat for otter, muskrat and reptiles. The wolves also killed coyotes, and so the rabbit and mice populations grew, which fed hawks, foxes, badgers and weasels. The regeneration of the riverside vegetation stabilized the river banks against erosion, and subsequently, the rivers narrowed and became more fixed in their course. Pools began to form, creating habitats for other organisms.

The Yellowstone example gives insight into the trophic cascade that can result from removing a key organism from a balanced landscape and ecosystem, and while a top predator kills certain species, the downstream effects give life to many other species. The regeneration of this amazing ecosystem shows the incredible ability of a vast biome to restore itself, and the lessons learned here could lead to better predator management decisions in other locations.

Epigenetics

Simplify your epigenetics research with EpiMark® validated products.

Epigenetics is the study of heritable changes in the phenotype of a cell or organism that are not encoded in the DNA of the genome. The molecular basis of an epigenetic profile arises from covalent modifications of the protein and DNA components of chromatin. The epigenetic profile of a cell often dictates cell memory and cell fate and, thus influences mammalian development.

The epigenetic code is hypothesized to be the combined effects of histone modifications and DNA methylation on gene expression. While the genetic code for an individual is the same in every cell, the epigenetic code is tissue- and cell-specific, and may change over time as a result of aging, disease or environmental stimuli (e.g., nutrition, life style, toxin exposure). Cross-talk between histone modifications, DNA methylation or RNAi pathways are being studied in such areas as cancer, X chromosome inactivation, and imprinting.

For over 40 years, New England Biolabs has been committed to understanding the mechanisms of restriction and methylation of DNA. This expertise in enzymology has led to the development of a suite of validated products for epigenetics research. These unique solutions to study DNA and histone modifications are designed to address some of the challenges of the current methods. All NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

Featured Products

262 EpiMark 5-hmC and 5-mC Analysis Kit

263 EpiMark Methylated DNA Enrichment Kit

267 EpiMark Nucleosome Assembly Kit

273 NEBNext Methyl-seq

Featured Tools & Resources

Videos of NEB Scientists Discussing Epigenetics

Epigenetics-related FAQs

Feature Articles

Visit www.EpiMark.com to view an interactive tutorial explaining the phenomenon of epigenetics at the molecular level.

Find an interactive tutorial on epigenetics.

RR Recombinant Enzyme

EpiMark® 5-hmC and 5-mC Analysis Kit

#E3317S 20 reactions

- *Reproducible quantitation of 5-hmC and 5-mC within a specific loci*
- *Easy-to-follow protocols*
- *Compatible with existing techniques (PCR)*
- *Amenable to high throughput*

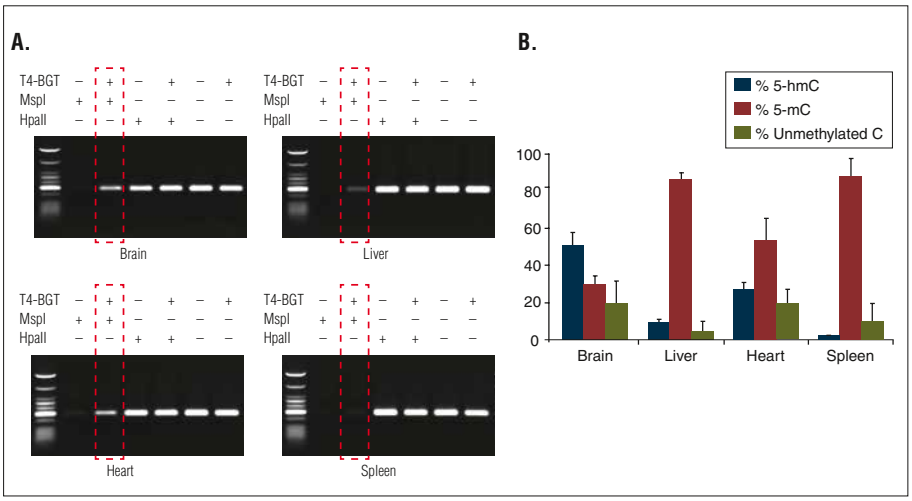
Visit **EpiMark.com** to view a video tutorial for this kit.

Description: The EpiMark 5-hmC and 5-mC Analysis Kit is a simple and robust method for the identification and quantitation of 5-hydroxymethylcytosine (5-hmC) and 5-methylcytosine (5-mC) within a specific DNA locus. This enzymatic approach utilizes the differential methylation sensitivity of the isoschizomers MspI and HpaII in a simple 3-step protocol.

Briefly, genomic DNA is treated with T4 phage β-glucosyltransferase and UDP-glucose, which glucosylates all 5-hmC present. DNA is digested with MspI and HpaII, two isoschizomers with different methylation sensitivity. Endpoint or real time PCR can then be used to identify and quantitate the different methylation states. Designed to simplify methylation analysis, the EpiMark Kit expands the potential for new biomarker discovery.

The EpiMark 5-hmC and 5-mC Analysis Kit Includes:

- T4 Phage β-glucosyltransferase
- UDP-Glucose
- MspI
- HpaII
- Proteinase K
- Control DNA (unmodified, 5-mC and 5-hmC)
- Forward and reverse control primer mix
- NEBuffer 4



Analysis of the different methylation states in Balb/C mouse tissue samples (locus 12) using the EpiMark 5-hmC and 5-mC Analysis Kit. *A) Endpoint PCR of the 6 different reactions needed for methylation analysis. The boxed lanes indicate the presence of 5-hmC. B) Real time PCR data was used to determine amounts of 5-hmC and 5-mC present. The results demonstrate a variation in 5-hmC levels in the tissue sources indicated.*

T4 Phage β-glucosyltransferase

#M0357S 500 units
#M0357L 2,500 units

- *Glucosylation of 5-hydroxymethylcytosine in DNA*
- *Immunodetection of 5-hydroxymethylcytosine in DNA*
- *Labeling of 5-hydroxymethylcytosine residues by incorporation of [^{3}H]- or [^{14}C]-glucose into 5-hmC-containing DNA acceptor after incubation with [^{3}H]- or [^{14}C]-UDP-Glc*
- *Detection of 5-hydroxymethylcytosine in DNA by protection from endonuclease cleavage*

Learn about 5-hmC detection in Balb/C brain tissue.

Description: T4 Phage β-glucosyltransferase specifically transfers the glucose moiety of uridine diphosphoglucose (UDP-Glc) to the 5-hydroxymethylcytosine (5-hmC) residues in double-stranded DNA, making beta-glucosyl-5-hydroxymethylcytosine.

Reaction Conditions: 1X NEBuffer 4 and 40 µM UDP-Glucose. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied with Enzyme:
10X NEBuffer 4
50X UDP-Glucose (2 mM)

Unit Definition: One unit is defined as the amount of enzyme required to protect 0.5 µg T4gt-DNA against cleavage by MfeI restriction endonuclease.

Concentration: 10,000 units/ml

EPIGENETICS

Cloned at NEB | Recombinant Enzyme | EpiMark Validated | Optimal Buffer | Requires BSA | Incubation Temperature

EpiMark Methylated DNA Enrichment Kit

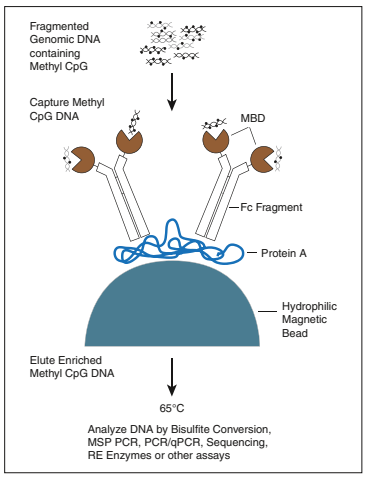
Epi

#E2600S 25 reactions

- *High-affinity binding provides greater sensitivity*
- *Elution in a small volume simplifies downstream applications*
- *Easy-to-follow protocol yields enriched fractions in less than 2 hours*
- *Enriched methylated DNA fragments can be easily ligated to double-stranded adaptors for next generation sequencing*
- *Highly pure product from a wide range of input DNA concentrations*

Description: The EpiMark Methylated DNA Enrichment Kit enables the enrichment of double-stranded CpG methylated DNA based on CpG methylation density. It utilizes the methyl-CpG binding domain of human MBD2a protein as a capture agent. The protein is fused to the Fc tail of human IgG1 (MBD2a-Fc), which is coupled to Protein A Magnetic Beads (MBD2a-Fc/Protein A Bead). This stable complex will selectively bind double-stranded methylated CpG containing DNA. The high binding affinity of the MBD2a-Fc coupled beads and optimized reagents increases sensitivity and accuracy. This kit contains all the individual components necessary to achieve enrichment in less than two hours using a four step process.

Enrichment Workflow



The EpiMark Methylated DNA Enrichment Kit Includes:

- MBD2-Fc protein
- Protein A Magnetic Beads
- Bind/wash buffer
- High-salt elution buffer
- Fragmented HeLa DNA
- Line element primers for methylated-locus controls
- RPL30 primers for unmethylated locus contols
- MirA locus control primers

EpiMark N6-Methyladenosine Enrichment Kit

Epi See page 204 for more information.

#E1610S 20 reactions

Description: The EpiMark N6-Methyladenosine Enrichment Kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR. Modified RNA is isolated from a fragmented RNA sample by binding to the N6-Methyladenosine antibody attached to Protein G Magnetic Beads. After multiple wash and clean-up steps, the enriched RNA is eluted in nuclease-free water and is ready for further analysis.

EpiMark Bisulfite Conversion Kit

Epi

#E3318S 48 reactions

- *Complete conversion of unmodified cytosine to uracil*
- *Easy-to-follow protocol*
- *Reliable and consistent results*
- *Purification columns included*

Description: This technique can reveal the methylation status of every cytosine residue, and it is amenable to massively parallel sequencing methods. Bisulfite conversion involves the conversion of unmodified cytosines to uracil, leaving the modified bases 5-mC and 5-hmC. The EpiMark Bisulfite Conversion Kit is designed for the detection of methylated cytosine, using a series of alternating cycles of thermal denaturation, followed by incubation with sodium bisulfite. This kit includes all the reagents necessary for complete bisulfite conversion, including spin columns. Amplification of bisulfite-treated samples can then be performed using EpiMark Hot Start *Taq* DNA Polymerase.

The EpiMark Bisulfite Conversion Kit Includes:

- Sodium metabisulfite
- Solubilization buffer
- Desulphonation reaction buffer
- EpiMark spin columns with 2 ml collection tubes
- Binding buffer
- Wash buffer
- Elution buffer

EpiMark Hot Start *Taq* DNA Polymerase

RR Epi See page 68 for more information.

#M0490S 100 reactions
#M0490L 500 reactions

Description: EpiMark Hot Start *Taq* DNA Polymerase is a mixture of *Taq* DNA Polymerase and a temperature sensitive, aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits assembly of reactions at room temperature. An advantage of the aptamer-based hot start mechanism is that it does not require a separate high temperature incubation step to activate the enzyme. The advanced aptamer-based hot-start activity coupled with the supplied optimized reaction buffer makes the EpiMark Hot Start *Taq* DNA Polymerase an excellent choice for use on bisulfite-converted DNA.

dilA Diluent Buffer 65° Heat Inactivation

Methylation-Dependent Restriction Enzymes

- *Specificity to epigenetically-relevant DNA modifications (5-mC and 5-hmC)*
- *Easy-to-follow protocols*
- *Less harsh than bisulfite conversion*
- *Simplified data analysis*

The EpiMark Suite of products has been validated for use in epigenetics applications. Visit **EpiMark.com** for more information.

Many restriction enzymes are sensitive to DNA methylation states. Cleavage can be blocked or impaired when a particular base in the recognition site is modified. The MspJI family of restriction enzymes are dependent on methylation and hydroxymethylation for cleavage to occur (1). These enzymes excise 32-base pair DNA fragments containing a centrally located 5-hmC or 5-mC modified residue that can be extracted and sequenced. Due to the known position of this epigenetic modification, bisulfite conversion is not required prior to downstream analysis.

These EpiMark validated, methylation-dependent restriction enzymes expand the potential for mapping epigenetic modifications and simplify the study of DNA methylation. Additionally, they provide an opportunity to better understand the role of 5-hydroxymethylcytosine in the genome.

Reference:

(1) Cohen-Karni, D. et al. (2011) *PNAS*, 108, 11040–11045.

AbaSI

CutSmart · Recombinant Enzyme · EpiMark Validated · Diluent C · 25°C · Heat Inactivation 65°C

#R0665S 1,000 units

5´...^{ghm}C $N_{11\text{-}13}$▼$N_{9\text{-}10}$ G...3´
3´..... G $N_{9\text{-}10}$▲ $N_{11\text{-}13}$ ^{x}C...5´

^{x}C = ^{ghm}C, ^{hm}C, ^{m}C or C

Description: AbaSI is a DNA modification-dependent endonuclease that recognizes 5-glucosylhydroxymethylcytosine (^{ghm}C) in double-stranded DNA and cleaves 11–13 bases 3´ from the modified C leaving a 2–3 base 3´ overhang. The enzyme only cleaves if there is a G residue 20–23 nucleotides 3´ from the modified C. AbaSI also recognizes 5-hydroxymethylcytosine (^{hm}C) at a much lower efficiency. It does not recognize DNA with 5-methylcytosine (^{m}C) or unmodified cytosine.

Reaction Conditions: 1X CutSmart Buffer + 1 mM DTT (supplied), 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

FspEI

CutSmart · Recombinant Enzyme · EpiMark Validated · Diluent B · 37°C · Heat Inactivation 80°C

#R0662S 200 units

5´... C^{m}C $(N)_{12}$▼...3´
3´... G G $(N)_{16}$▲...5´

Description: FspEI is a modification-dependent endonuclease which recognizes C^{m}C sites and generates a double-stranded DNA break on the 3´ side of the modified cytosine at N_{12}/N_{16}. Recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC).

Reaction Conditions: 1X CutSmart Buffer + 1X Enzyme Activator Solution (supplied), 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Note: Star activity may result from extended digestion.

LpnPI

CutSmart · Recombinant Enzyme · EpiMark Validated · Diluent B · 37°C · Heat Inactivation 65°C

#R0663S 200 units

5´... C^{m}C D G $(N)_{10}$▼...3´
3´... G G H C $(N)_{14}$▲...5´

Description: LpnPI is a modification-dependent endonuclease which recognizes C^{m}CDG sites and generates a double-stranded DNA break on the 3´ side of the modified cytosine at N_{10}/N_{14}. Recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC).

Reaction Conditions: 1X CutSmart Buffer + 1X Enzyme Activator Solution (supplied), 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Note: Star activity may result from extended digestion.

MspJI

CutSmart · Recombinant Enzyme · EpiMark Validated · Diluent B · 37°C · Heat Inactivation 65°C

#R0661S 200 units
#R0661L 1,000 units

5´...^{m}C N N R $(N)_{9}$▼...3´
3´... G N N Y $(N)_{13}$▲...5´

Description: MspJI is a modification-dependent endonuclease that recognizes ^{m}CNNR sites and generates a double-stranded DNA break on the 3´ side of the modified cytosine at N_9/N_{13}. The recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC).

Reaction Conditions: 1X CutSmart Buffer + 1X Enzyme Activator Solution (supplied), 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Note: Star activity may result from extended digestion.

Cloned at NEB · Recombinant Enzyme · EpiMark Validated · Optimal Buffer · Requires BSA · Incubation Temperature · Diluent Buffer · Heat Inactivation

McrBC

RR Epi NEB2 BSA dilB 37° 65°

#M0272S 500 units
#M0272L 2,500 units

5´...Pu^{m}C ($N_{40–3000}$) Pu^{m}C... 3´

- *Determination of the methylation state of CpG dinucleotides*
- *Detection of cytosine methylated DNA*

Description: McrBC is an endonuclease that cleaves DNA containing methylcytosine* on one or both strands. McrBC will not act upon unmethylated DNA. Sites on the DNA recognized by McrBC consist of two half-sites of the form (G/A)^{m}C. These half-sites can be separated by up to 3 kb, but the optimal separation is 55–103 base pairs. McrBC requires GTP for cleavage, but in the presence of a non-hydrolyzable analog of GTP, the enzyme will bind to methylated DNA specifically, without cleavage. McrBC will act upon a pair of Pu^{m}CG sequence elements, thereby detecting a high proportion of methylated CpGs, but will not recognize HpaII/MspI sites (CCGG) in which the internal cytosine is methylated.

* 5-methylcytosine, 5-hydroxymethylcytosine or N4-methylcytosine

Reaction Conditions: 1X NEBuffer 2 + 200 µg/ml BSA + 1 mM GTP, 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied with Enzyme:
10X NEBuffer 2
100X BSA
100X GTP (100 mM)
Control Plasmid DNA

Unit Definition: One unit is defined as the amount of enzyme required to cleave 0.5 µg of a plasmid containing multiple McrBC sites in 1 hour at 37°C in a total reaction volume of 50 µl.

Concentration: 10,000 units/ml

Note: McrBC makes one cut between each pair of half-sites, cutting close to one half-site or the other, but cleavage positions are distributed over several base pairs approximately 30 base pairs from the methylated base. Therefore, when multiple McrBC half-sites are present in DNA (as is the case with cytosine-methylated genomic DNA), the flexible nature of the recognition sequence results in an overlap of sites, producing a smeared, rather than a sharp, banding pattern.

Additional Restriction Enzymes for Epigenetic Analysis

See pages 34, 38, 41 for more information.

Visit pages 334–336 for a complete list of methylation-sensitive restriction enzymes.

Methylation sensitive restriction enzymes can be used to generate fragments for further analysis. When used in conjunction with an isoschizomer that has the same recognition site, but is methylation insensitive, information about methylation status can be obtained.

DpnI
#R0176S 1,000 units
#R0176L 5,000 units

DpnII
#R0543S 1,000 units
#R0543L 5,000 units
for high (5X) concentration
#R0543T 1,000 units
#R0543M 5,000 units

HpaII
#R0171S 2,000 units
#R0171L 10,000 units
for high (5X) concentration
#R0171M 10,000 units

MspI
#R0106S 5,000 units
#R0106L 25,000 units
for high (5X) concentration
#R0106T 5,000 units
#R0106M 25,000 units

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

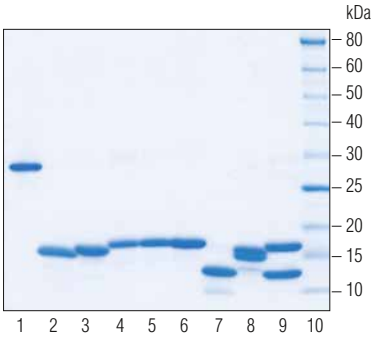
Histones

- *Purification and characterization of enzymes that modify histone proteins*
- *Octamer and nucleosome modification studies*
- *Carrier chromatin immunoprecipitation (CChIP)*
- *High throughput studies*

In eukaryotes, nuclear DNA is assembled into chromatin by nucleoprotein complexes. The primary unit of chromatin, a nucleosome core particle (NCP), is an octamer complex made up of two molecules each of Histone H2A, H2B, H3 and H4 and approximately 147 base pairs of nuclear DNA. Histone H1 further condenses the DNA by binding the linker segments between NCP complexes (1,2). Histones undergo diverse post-translational modification including acetylation, phosphorylation, mono-, di- or tri-methylation, ubiquitination, isomerization and ADP-ribosylation. Through their potential combinatorial sequences on a given histone and their reversibility, these modifications dynamically restrict or recruit numerous other proteins or protein complexes onto chromatin (3). The study of their roles in gene regulation (4), cellular stress events (4), aging and DNA repair (5) is revealing the multiple functions of histone modifications in the fate of a cell. Additional variability is incorporated into the system by histone variants. Acting individually or combinatorially in conjunction with DNA modification, histone modifications and histone variants are thought to establish an epigenetic code or epigenetic mechanism of gene regulation (3).

In total, seven human histones, including three histone H3 variants (see alignment below), have been individually cloned and expressed in *E. coli* and then highly purified from cell extracts at NEB. Mass spectrometry analysis demonstrates that these recombinant histones are free of post-translational modifications. These histones are ideal substrates for the purification and characterization of histone modifying enzymes.

To aid in studying intact nucleosomes, we now offer the EpiMark Nucleosome Assembly Kit. The precise mixing of a 2:1 ratio of Histone H2A/H2B Dimer to Histone H3.1/H4 Tetramer generates a recombinant human histone octamer, and in the presence of DNA forms nucleosomes (7,8). Enzymes that are unable to modify individual histones or DNA may be active on these nucleosome core particles, the histone dimer, or the histone tetramer (9,10). The NCPs also may be used as carrier chromatin in CChIP (carrier chromatin immunoprecipitation) assays (11). The recombinant human histone dimer and recombinant human histone tetramer are also available as separate products.



Experience the purity of Histones from NEB, *SDS-PAGE analysis of the histones available from NEB.*

1: Histone H1° (NEB #M2501) 1 µg
2: Histone H2A (NEB #M2502) 1 µg
3: Histone H2B (NEB #M2505) 1 µg
4: Histone H3.1 (NEB #M2503) 1 µg
5: Histone H3.2 (NEB #M2506) 1 µg
6: Histone H3.3 (NEB #M2507) 1 µg
7: Histone H4 (NEB #M2504) 1 µg
8: Histone H2A/H2B Dimer (NEB #M2508) 2 µg
9: Histone H3.1/H4 Tetramer (NEB #M2509) 2 µg
10: NEB Protein Ladder

References:

(1) Kornberg, R.D. (1977) *Annu. Rev. Biochem.*, 46, 931–954.
(2) van Holde, K.E. (1989) *Chromatin*, 1–497.
(3) Kim, J.K., Samaranayake, M. and Pradhan, S. (2009) *Cell. Mol. Life Sci.*, 66, 596–612.
(4) Huang, J. et al. (2006) *Nature*, 444, 629–632.
(5) Pahlich, S., Zakaryan, R.P. and Gehring, H. (2006) *Biochim. Biophys. Acta.*, 1764, 1890–1903.
(6) Hake, S.B. et al. (2006) *J. Biol. Chem.*, 281, 559–568.
(7) Luger et al. (1999) *Methods in Mol. Biol.*, 119, 1–16.
(8) Comb, D. and Mersha, F. unpublished observations.
(9) Li, Yan et al. (2009) *J. Biol. Chem.*, 284, 34283–34295.
(10) Fung, J. et al. (2002) *Current Biology*, 12, 1086–1099.
(11) O'Neill et al. (2006) *Nature Genetics*, 38, 835–841.


```
        10         20         30         40         50         60         70
ARTKQTARKS TGGKAPRKQL ATKAARKSAP ATGGVKKPHR YRPGTVALRE IRRYQKSTEL LIRKLPFQRL   histone H3.1
ARTKQTARKS TGGKAPRKQL ATKAARKSAP ATGGVKKPHR YRPGTVALRE IRRYQKSTEL LIRKLPFQRL   histone H3.2
ARTKQTARKS TGGKAPRKQL ATKAARKSAP STGGVKKPHR YRPGTVALRE IRRYQKSTEL LIRKLPFQRL   histone H3.3
        80         90        100        110        120        130
VREIAQDFKT DLRFQSSAVM ALQEACEAYL VGLFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA         histone H3.1
VREIAQDFKT DLRFQSSAVM ALQEASEAYL VGLFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA         histone H3.2
VREIAQDFKT DLRFQSAAIG ALQEASEAYL VGLFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA         histone H3.3
```

Sequence alignment of Human Histone variants H3.1, H3.2 and H3.3. *Human Histone H3.1, 3.2 and 3.3 vary by only a few amino acids (changes are highlighted in **red**), but are associated with different biological functions (6).*

EPIGENETICS

Cloned at NEB | Recombinant Enzyme | EpiMark Validated | Optimal Buffer | Requires BSA | Incubation Temperature | Diluent Buffer | Heat Inactivation

EpiMark Nucleosome Assembly Kit

#E5350S 20 reactions

Components Sold Separately:

Histone H3.1/H4 Tetramer Human, Recombinant
#M2509S 1 nmol

Histone H2A/H2B Dimer Human, Recombinant
#M2508S 2 nmol

Nucleosome Control DNA
#N1202S 0.2 nmol

- *Highly pure, recombinant system*
- *Pre-formed histone dimer and tetramer complexes simplify octamer formation*
- *Components stable for one year*
- *Ideal for ChIP Assay, HAT Assay and enzyme modification assays (e.g., methylation studies)*

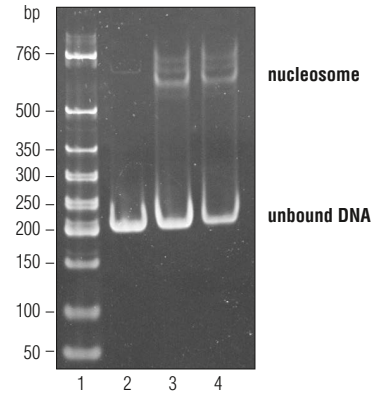
Description: This kit contains the components necessary to form an unmodified recombinant human nucleosome using your own target DNA or the supplied control DNA. The protocol requires the mixing of already formed and purified recombinant human Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer in the presence of DNA in high salt, followed by dialysis down to low salt, to make nucleosomes. One tetramer associates with two dimers to form the histone octamer on the DNA, generating a nucleosome. A method for assaying nucleosome formation by gel shift assay is also provided. These nucleosomes may serve as a better substrate for enzymes that are inactive on the DNA or one of the core histones alone. Each described reaction creates nucleosomes from ~50 pmol of a 208 bp DNA and may be scaled depending on the experiment.

Histone H2A/H2B Dimer Human, Recombinant is generated by refolding the denatured, purified subunits H2A and H2B, followed by gel filtration. Histone H3.1/H4 Tetramer Human, Recombinant is generated by refolding the denatured, purified subunits H3.1 and H4, followed by gel filtration. Both the dimer and tetramer are highly pure and are available separately for histone modification studies.

The Nucleosome Control DNA is a 208 base pair fragment from *Lytechinus variegates* 5SrDNA, and can be used for mononucleosome formation.

The EpiMark Nucleosome Assembly Kit Includes:

– Histone H2A/H2B Dimer
– Histone H3.1/H4 Tetramer
– Control DNA



Gel shift assay to visualize nucleosome formation.
Samples from nucleosome assembly reactions were run on 6% polyacrylamide gel in 0.5X TBE. Lane 1: Low Molecular Weight DNA Ladder (NEB #N3233). Lane 2: Nucleosome Control DNA. Lane 3: 0.5:1 ratio of Octamer to DNA. Lane 4: 1:1 ratio of Octamer* to DNA.*

**Octamer = 2:1 mix of Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer.*

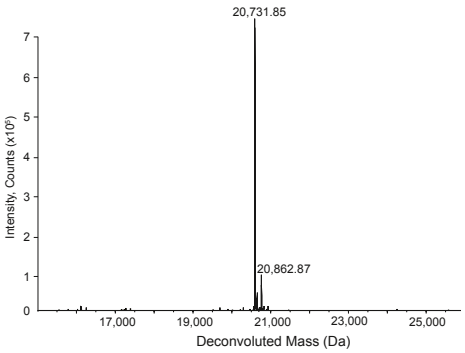
Histone H1⁰ Human, Recombinant

#M2501S 100 µg

Description: Histone H1 acts on the linker region of polynucleosome DNA to condense the chromatin into structures of ~30 nm. It is not necessary for octamer or nucleosome core particle formation. Eight different Histone H1 proteins have been identified in the human genome. Histone H1⁰ is a non replication-dependent histone that is highly expressed in terminally differentiated cells.

Synonyms: Histone H1.0, Histone H1(0), Histone H1'

Concentration: 1 mg/ml



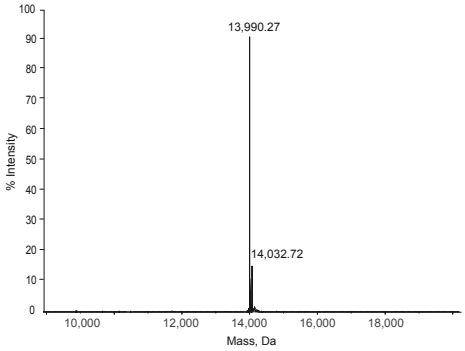
Mass Spectrometry Analysis of Histone H1⁰ Human, Recombinant. The average mass calculated from primary sequence is 20731.53 Da.

Histone H2A Human, Recombinant

#M2502S 100 µg

Description: Histone H2A combines with Histone H2B to form the H2A-H2B heterodimer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. Histone H2A is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H2A Human, Recombinant. The average mass calculated from primary sequence is 13990.28 Da.

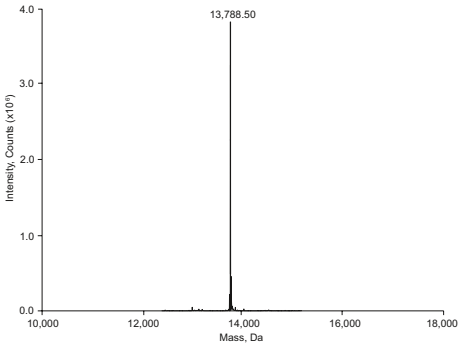
Histone H2B Human, Recombinant

#M2505S 100 µg

Description: Histone H2B combines with Histone H2A to form the H2A-H2B heterodimer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. Histone H2B is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Synonyms: Histone H2B/8, Histone H2B.1, Histone H2B-GL105

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H2B Human, Recombinant. The average mass calculated from primary sequence is 13788.97 Da.

Histone H3.1 Human, Recombinant

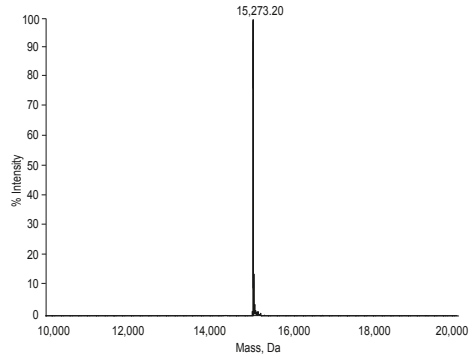
#M2503S 100 µg

Description: Histone H3 combines with Histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. It is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Histone H3.1, an H3 variant that has thus far only been found in mammals, is replication dependent and is associated with gene activation and gene silencing.

Synonyms: Histone H3/a

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H3.1 Human, Recombinant. The average mass calculated from primary sequence is 15272.89 Da.

Histone H3.2 Human, Recombinant

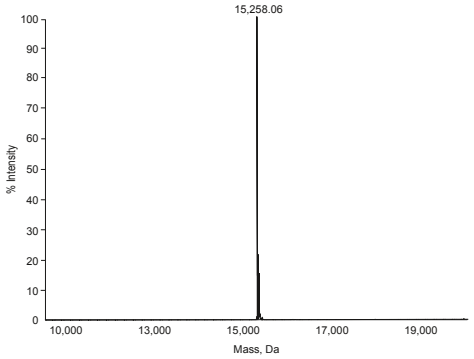
#M2506S 100 µg

Description: Histone H3 combines with Histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. It is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Histone H3.2, an H3 variant that is found in all eukaryotes except budding yeast, is replication dependent and is associated with gene silencing.

Synonyms: Histone H3/m, H3/o

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H3.2 Human, Recombinant. The average mass calculated from primary sequence is 15258.06 Da.

Histone H3.3 Human, Recombinant

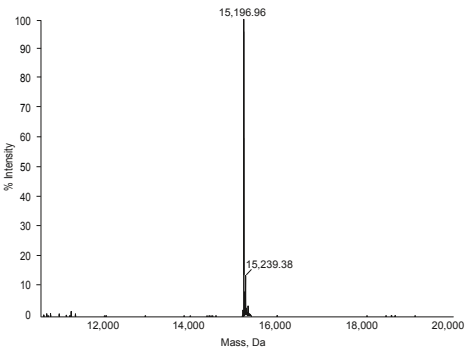
#M2507S 100 µg

Description: Histone H3 combines with Histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. It is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Histone H3.3, an H3 variant that is found in all eukaryotes from yeast to human, is replication and cell cycle phase independent and is the most common H3 in non-dividing cells. It has been shown to be enriched in covalent modifications associated with gene activation.

Synonyms: Histone H3.3A, H3F3, H3.3B

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H3.3 Human, Recombinant. The average mass calculated from primary sequence is 15176.96 Da.

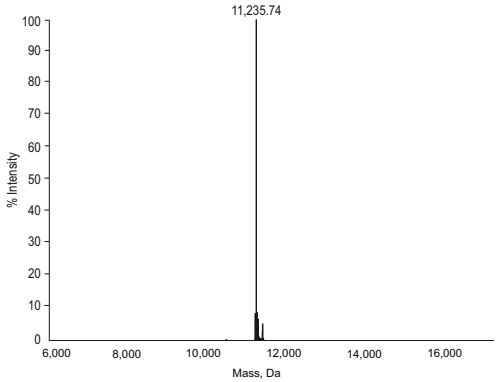
Histone H4 Human, Recombinant

#M2504S 100 µg

Description: Histone H3 combines with Histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. Histone H4 is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Synonyms: For HIST2H4 gene: H4/N, H4F2, H4FN

Concentration: 1 mg/ml

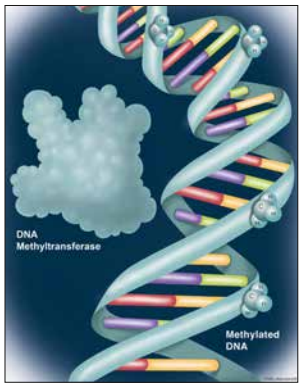


Mass Spectrometry Analysis of Histone H4 Human, Recombinant. The average mass calculated from primary sequence is 11236.15 Da.

DNA Methyltransferases

RR

See pages 114-116 for more information.



CpG Methyltransferase (M.SssI)
#M0226S 100 units
#M0226L 500 units
for high (5X) concentration
#M0226M 500 units

GpC Methyltransferase (M.CviPI)
#M0227S 200 units
#M0227L 1,000 units

AluI Methyltransferase
#M0220S 100 units

BamHI Methyltransferase
#M0223S 100 units
#M0223L 500 units

dam Methyltransferase
#M0222S 500 units
#M0222L 2,500 units

EcoGII Methyltransferase
#M0603S 200 units

EcoRI Methyltransferase
#M0211S 10,000 units

HaeIII Methyltransferase
#M0224S 500 units

HhaI Methyltransferase
#M0217S 1,000 units

HpaII Methyltransferase
#M0214S 100 units

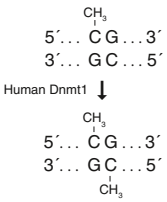
MspI Methyltransferase
#M0215S 100 units

*Taq*I Methyltransferase
#M0219S 1,000 units

Human DNA (cytosine-5) Methyltransferase (Dnmt1)

RR Epi SAM 37° 65°

#M0230S 50 units
#M0230L 250 units



Description: Dnmt1 methylates cytosine residues in hemimethylated DNA at 5´...CG...3´. Mammalian Dnmt1 is believed to be involved in carcinogenesis, embryonic development and several other biological functions. The bulk of the methylation takes place during DNA replication in the S-phase of the cell cycle.

Reaction Conditions: 1X Dnmt1 Reaction Buffer. Supplement with 100 µg/ml BSA and 160 µM S-adenosylmethionine. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied with Enzyme:
10X Dnmt1 Reaction Buffer
100X BSA
32 mM S-adenosylmethionine (SAM)

Unit Definition: One unit is the amount of enzyme required to catalyze the transfer of 1 pmol of methyl group to poly dI.dC substrate in a total reaction volume of 25 µl in 30 minutes at 37°C.

Concentration: 2,000 units/ml

Note: For DNA modification and protection applications, M.SssI (NEB #M0226) is preferred because it efficiently methylates both unmethylated and hemimethylated DNA substrates.

HeLa Genomic DNA

#N4006S 15 µg

- *PCR, SNP analysis and southern blotting*
- *Genomic DNA library construction*
- *Control DNA for Methylation-specific PCR (MSP), Bisulfite sequencing, Methylation-sensitive Single-Nucleotide Primer Extension (MS-SNuPE,) Combined Bisulfite Restriction Analysis (COBRA), Bisulfite treatment and PCR-single strand Confirmation Polymorphism Analysis (Bisulfite-PCR-SSCP/BiPS)*

Description: HeLa (cervix adenocarcinoma) cells were grown to confluency in DMEM plus 10% fetal bovine serum. Genomic DNA was isolated by a standard genomic purification protocol, phenol extracted and equilibrated to 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA.

Note: NEB is one of the only suppliers of this product.

$A_{260}/_{280}$ Ratio: 1.87

5-methyl-dCTP

#N0356S 1 µmol in 0.1 ml

- *Generation of fully methylated cytosine-substituted DNA*

Description: Cytosine modification at carbon 5 (C5) represents an important epigenetic modification. It is also believed to be the starting substrate for the Ten-Eleven Translocation (TET) family of enzymes and their associated oxidation pathways. 5-methyl-dCTP offers the ability to enzymatically make defined fully methylated cytosine-substituted DNA, which can be used for a variety of biochemical and cellular applications.

5-methyl-dCTP (2´-deoxy-5-methylcytidine 5´-triphosphate) is supplied as a 10 mM solution at pH 7. Nucleotide concentration is determined by measurements of absorbance at 260 nm.

Formula: $C_{10}H_{15}N_3O_{13}P_3$ (free acid)

Concentration: 10 mM solution

Molecular weight: 481.1 (acid form)

Diluent Compatibility: Can be diluted using sterile distilled water, preferably Milli-Q® water, or can be diluted using sterile TE [10 mM Tris-HCl, 1 mM EDTA (pH 7.5)]

NEB's Golf Committee has been organizing our charity golf tournament for over 10 years, with the proceeds benefiting Ipswich High School students. Pictured are committee members Kari, Deana, Ted, Tanya and Karen.

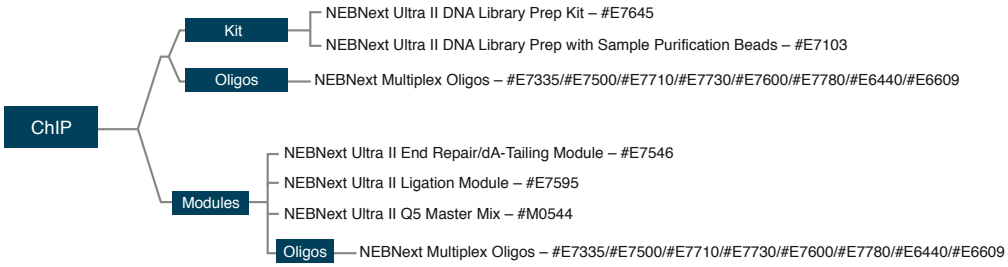
Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

NEBNext® Reagents for ChIP-Seq Library Preparation

Epi

NEBNext reagents are a series of highly pure reagents that facilitate library preparation of DNA or RNA for downstream applications, such as next generation sequencing and expression library construction. These reagents undergo stringent quality controls and functional validation, ensuring maximum yield, convenience and value.

For sample preparation of a ChIP-Seq DNA library, NEB offers kits, oligos and modules that support standard or fast workflows. To decide which products to choose, use the selection chart below. For more information on NEBNext reagents for library preparation, see pages 134–161.



Katrina is a Production Planner and has been at NEB for over 4 years. In this role, she works with many departments within NEB to ensure timely product release. She is quite active in the NEB community, and is a member of the NEB Soccer Club, Running Club and Holiday Raffle Committee.

Cloned at NEB | RR Recombinant Enzyme | Epi EpiMark Validated | NEB4 Optimal Buffer | BSA Requires BSA | 37° Incubation Temperature | diA Diluent Buffer | 65° Heat Inactivation

NEBNext Enzymatic Methyl-seq

NEW
NEBNext Enzymatic Methyl-seq Kit
#E7120S 24 reactions
#E7120L 96 reactions

NEW
NEBNext Enzymatic Methyl-seq Conversion Module
#E7125S 24 reactions
#E7125L 96 reactions

NEW
NEBNext Q5U Master Mix
#M0597S 50 reactions
#M0597L 250 reactions

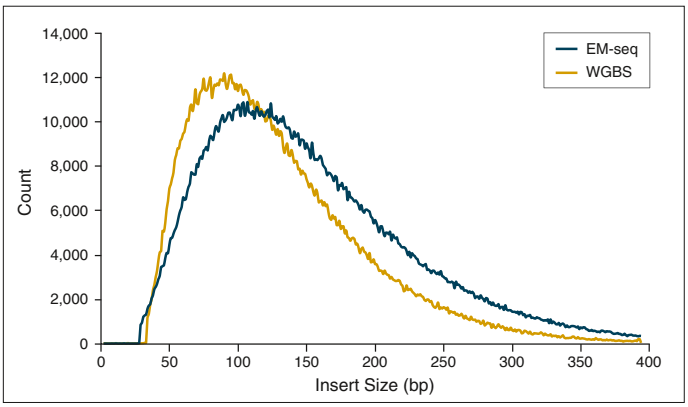
NEW
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)
#E7140S 24 reactions
#E7140L 96 reactions

- *Superior sensitivity of detection of 5-mC and 5-hmC*
- *Larger library insert sizes*
- *More uniform GC coverage*
- *Greater mapping efficiency*
- *High-efficiency library preparation*

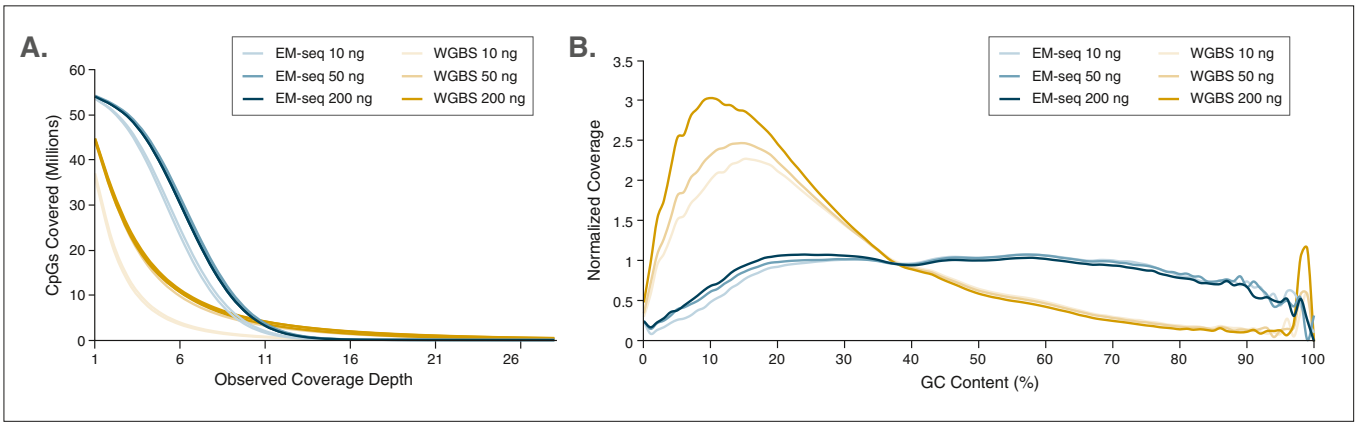
NEBNext Enzymatic Methyl-seq is an enzymatic alternative to bisulfite conversion with superior performance. For more information, including extensive performance data, visit **NEBNext.com**.

Description: While bisulfite sequencing has been the gold standard for the study of DNA methylation, this conversion treatment is damaging to DNA, resulting in DNA fragmentation, loss and GC bias. The NEBNext Enzymatic Methyl-seq Kit (EM-seq™) provides an enzymatic alternative to whole genome bisulfite sequencing (WGBS), combined with high-efficiency streamlined library preparation suitable for Illumina sequencing.

The highly effective EM-seq enzymatic conversion minimizes damage to DNA and, in combination with the supplied NEBNext Ultra II library preparation workflow reagents, results in high quality libraries that enable superior detection of 5-mC and 5-hmC from fewer sequencing reads.



NEBNext Enzymatic Methyl-seq libraries have larger insert sizes *50 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris® S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold™ kit for bisulfite conversion. Libraries were sequenced on an Illumina MiSeq (2 x 76 bases) and insert sizes were determined using Picard 2.18.14. The normalized frequency of each insert size was plotted, illustrating that library insert sizes are larger for EM-seq than for WGBS, and indicating that EM-seq does not damage DNA as bisulfite treatment does in WGBS.*



EM-seq identifies more CpGs than WGBS, at lower sequencing coverage depth with superior uniformity of GC coverage. *10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold Kit for bisulfite conversion. Libraries were sequenced on an Illumina NovaSeq® 6000 (2 x 100 bases). Reads were aligned to hg38 using bwa-meth 0.2.2.*

A: *Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads, and each top and bottom strand CpGs were counted independently, yielding a maximum of 56 million possible CpG sites. EM-seq identifies more CpGs at lower depth of sequencing.*

B: *GC coverage was analyzed using Picard 2.17.2 and the distribution of normalized coverage across different GC contents of the genome (0-100%) was plotted. EM-seq libraries have significantly more uniform GC coverage, and lack the AT over-representation and GC under-representation typical of WGBS libraries.*

Brazil Nut (Bertholletia excelsa) flowers and buds on tree, Brazil
Credit: Silvestre Silva, Minden Pictures

The Importance and Fragility of the Amazon Biome

The expanse and biodiversity of the world's largest rainforest is hard to fathom. The Amazon covers almost 7 million km^2 (2.7 million miles^2) in the northern region of South America. The Amazon River flows for 6,000 km (3,700 miles) from Peru, across Brazil, and into the Atlantic. The uniqueness of the biodiversity in the Amazon is matched nowhere else on Earth. It is home to one in 10 species on our planet, and 25% of all terrestrial species. There are 400 billion trees that belong to 16,000 different species. In Brazil alone, there are 100 different species of monkeys; nine new species have been discovered in the last decade. Dozens of plant and animal species are still being discovered every year.

Plant and animal species form intricate, cooperative relationships with each other that have evolved over millions of years. In the Amazon, adaption is the key to survival. For example, the Brazil nut tree cannot be cultivated outside of the Amazon because of its incredibly specialized, mutualistic relationship with large-bodied bees strong enough to open its petal and pollinate other flowers, the agouti who disperses the Brazil nut and sustains a healthy population of Brazil nut trees, and the scent of a specific forest-dwelling orchard that male bees use to attract females.

The Amazon is often referred to as the lungs of our planet — the trees help to regulate Earth's atmosphere by "breathing" in carbon dioxide and releasing oxygen, thereby keeping the Earth cooler. It is estimated that 40% of human-made carbon dioxide is cleared by rainforests.

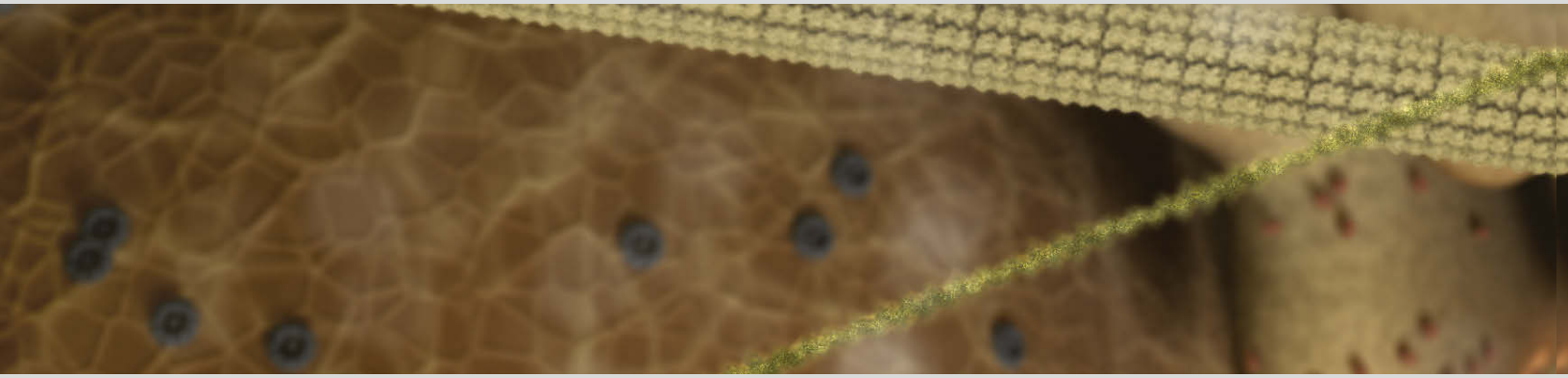
The Amazon regulates local and global weather patterns by absorbing heat and releasing water vapor via photosynthesis into the atmosphere, seeding the clouds with rain. The Amazon River carries 20% of the world's water to the sea.

Unfortunately, the Amazon is under constant threat by humans who are logging and farming the land, mining its resources, damming its rivers, and destroying indigenous lands and cultures. Deforestation has the Amazon at the "tipping point", with just over 80% of trees remaining. This drastic reduction in the number of trees reduces the amount of moisture released into the atmosphere, thereby jeopardizing the rainfall patterns and the replenishment of the rivers. The act of cutting down the trees releases stored carbon into the atmosphere, contributing to global warming. The immense plant diversity of the rainforest is being replaced with monocrops, such as soy or palm, and the animal diversity replaced by a single species, such as grazing cattle.

Substantial efforts are being made to protect large swaths of forest. In Brazil, armed guards protect against illegal logging, and while this activity has decreased from 25,000 km^2/year (15,500 miles^2) to just over 6,000 km^2/year (3,700 miles^2), it is almost impossible to guard an area that is larger than India. Governments are expanding protected areas — for example, the Colombian government expanded protection around Chiribiquete National Park to incorporate an area that is home to three uncontacted and isolated tribes and more than 200,000 paintings of pre-Columbian art. Further, technology is being carefully introduced to contacted tribes to give them the control to map and manage over 70 million acres of ancestral rainforest.

Protecting the entire forest from human destruction is a seemingly impossible feat. However, global awareness of the significance of this rainforest, its biological, cultural and economic riches, can turn the tide and ensure its survival for generations to come.

Cellular Analysis



Novel tools to study expression & function of proteins.

Cell imaging analysis can use fluorescent dyes, fluorophore-labeled molecules or recombinant protein plasmid systems. Recombinant protein labeling systems and bioluminescent reporter systems are among the most sensitive fluorescence methods for imaging expression, transport, co-localization and degradation in either fixed or living cells. Protein labeling systems offer many advantages. For example, color changes can be easily implemented by using different substrates. Protein labeling systems can involve the use of tag-specific antibodies or antibodies to separate epitopes engineered into a plasmid tag system for detection. Protein labeling systems can be used with non-cell permeable substrates to enable the specific imaging of cell surface targets. This strategy is not possible with bioluminescent recombinant systems. In living cells, protein labeling substrates can be introduced and followed in cells over time. Two separate cellular targets can also be imaged simultaneously, using protein labeling systems with mutually exclusive, tag-specific fluorescent substrates.

Studies of protein expression, interactions and structure, often use reporter systems to introduce and select for gene targets in cells. Reporter genes confer drug resistance, bioluminescence or fluorescence properties in the cells into which they are introduced. Typical reporter studies link reporter genes directly to a promoter region of interest, the function of which can be monitored by the reporter activity. Protein fusion tagging is used to detect subcellular localization, degradation, protein-protein interactions, etc. Typical fusion tags are fluorescent proteins (e.g., eGFP) or small protein epitopes (e.g., FLAG, Myc HA) which can be detected by fluorescence FACS or western blots. New generations of reporter gene systems expand the range of applications and enhance experimental possibilities.

Featured Products

279 SNAP-Cell® Starter Kit

280 SNAP-Cell 647-SiR

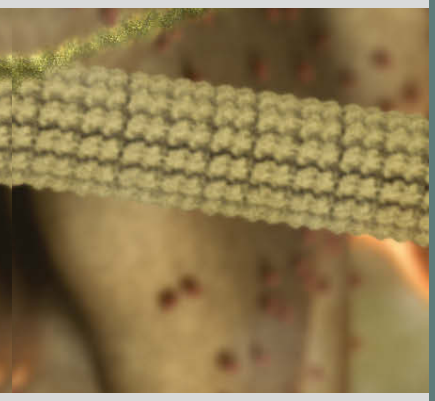
280 SNAP-Surface® 649

Featured Tools & Resources

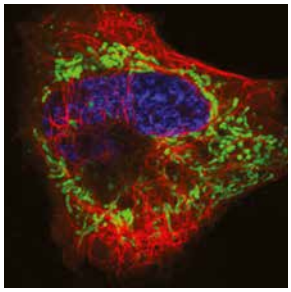
Troubleshooting Guide for SNAP-tag Technology

Application Notes

Videos and Tutorials: SNAP-tag Technology



Cellular Imaging & Analysis



Live HeLa cell transfected with pSNAP$_f$-tubulin and pCLIP$_f$-Cox8A (mitochondrial cytochrome oxidase 8A). Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

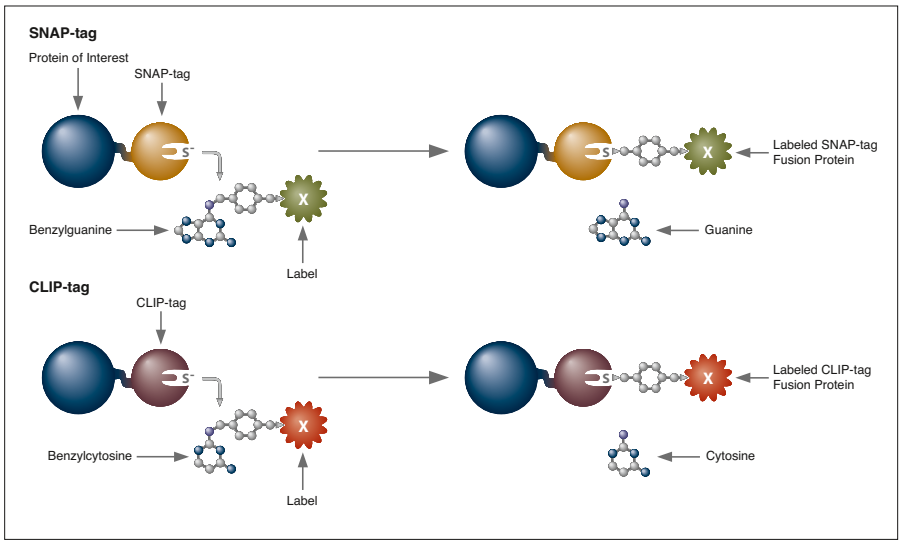
Features of SNAP-tag and CLIP-tag:

- *Clone and express once, then use with a variety of substrates*
- *Non-toxic to living cells*
- *Wide selection of fluorescent substrates*
- *Highly specific covalent labeling*
- *Simultaneous dual labeling*

Applications of SNAP-tag and CLIP-tag:

- *Simultaneous dual protein labeling inside or on the surface of live cells*
- *Protein localization and translocation*
- *Pulse-chase experiments*
- *Receptor internalization studies*
- *Selective cell surface labeling*
- *Protein pull down assays*
- *Protein detection in SDS-PAGE*
- *Flow cytometry*
- *High throughput binding assays in microtiter plates*
- *Biosensor interaction experiments*
- *FRET-based binding assays*
- *Single molecule labeling*
- *Super-resolution microscopy*

New England Biolabs offers an innovative technology for studying the function and localization of proteins in live and fixed cells. Covalent protein labeling brings simplicity and versatility to the imaging of mammalian proteins in live cells, as well as the ability to capture proteins *in vitro*. The creation of a single genetic construct generates a fusion protein which, when covalently attached to a variety of fluorophores, biotin or beads, provides a powerful tool for studying proteins. For added flexibility, NEB offers two systems in which the protein is labeled by a self-labeling fusion protein (SNAP-tag® and CLIP-tag™).



Protein labeling with SNAP-tag (gold) and CLIP-tag (purple). *The SNAP- or CLIP-tag is fused to the protein of interest (blue). Labeling occurs through covalent attachment to the tag, releasing either a guanine or a cytosine moeity.*

SNAP-tag and CLIP-tag – Self-Labeling Tag Technology

The SNAP- and CLIP-tag protein labeling systems enable the specific, covalent attachment of virtually any molecule to a protein of interest. There are two steps to using this system: cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice. The SNAP-tag is based on the human O^6-alkylguanine-DNA-alkyltransferase (hAGT), a DNA repair protein. SNAP-tag substrates are fluorophores, biotin or beads conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag. CLIP-tag is a modified version of SNAP-tag, engineered to react with benzylcytosine rather than benzylguanine derivatives. When used in conjunction with SNAP-tag, CLIP-tag enables the orthogonal and complementary labeling of two proteins simultaneously in the same cells.

SNAP-Cell®: SNAP-Cell labels are cell-permeant and uniquely suited for the labeling of SNAP-tag fusion proteins inside living or fixed cells, on cell surfaces or *in vitro*. These labels are spread across the visible spectrum, ranging from blue to red. Non-fluorescent cell-permeable blocking agent is also available.

SNAP-Surface®: SNAP-Surface labels are non-cell-permeant and routinely used to label SNAP-tag fusion proteins on the surface of living cells, in fixed cells or *in vitro*. These labels are spread across the visible spectrum and include the photostable AlexaFluor® dyes and a variety of other commonly used fluorophores. Non-fluorescent, non-cell-permeable blocking agent is also available.

CLIP-Cell™: CLIP-Cell labels are cell-permeant and uniquely suited for the labeling of CLIP-tag fusion proteins inside living or fixed cells, on cell surfaces or *in vitro*. The CLIP-tag is a derivative of the SNAP-tag that reacts with orthogonal substrates, allowing simultaneous labeling of two expressed proteins with different fluorophores. Non-fluorescent cell-permeable blocking agent is also available.

CLIP-Surface™: CLIP-Surface labels are non-cell permeant and routinely used to label CLIP-tag fusion proteins on the surface of living cells, in fixed cells or *in vitro*. The CLIP-tag is a derivative of the SNAP-tag that reacts with orthogonal substrates, allowing simultaneous labeling of two expressed proteins with different fluorophores. The labels include fluorophores at commonly used areas of the visible spectrum, such as 488, 547 and 647 nm.

Find an overview of SNAP-tag labeling.

Comparison of SNAP-tag/CLIP-tag Technologies to GFP

While SNAP/CLIP-tag technologies are complementary to GFP, there are several applications for which SNAP- and CLIP-tag self-labeling technologies are advantageous.

APPLICATION	SNAP-tag/CLIP-tag	GFP AND OTHER FLUORESCENT PROTEINS
Time-resolved fluorescence	Fluorescence can be initiated upon addition of label	Color is genetically encoded and always expressed. Photoactivatable fluorescent proteins require high intensity laser light, which may activate undesired cellular pathways (e.g., apoptosis)
Pulse-chase analysis	Labeling of newly synthesized proteins can be turned off using available blocking reagents (e.g., SNAP-Cell® Block)	Fluorescence of newly synthesized proteins cannot be specifically quenched to investigate dynamic processes
Ability to change colors	A single construct can be used with different fluorophore substrates to label with multiple colors	Requires separate cloning and expression for each color
Surface specific labeling	Can specifically label subpopulation of target protein expressed on cell surface using non-cell permeant substrates	Surface subpopulation cannot be specifically visualized
Single molecule detection	Conjugation with high quantum yield and photostable fluorophores	Fluorescent proteins are generally less bright and photobleach quicker than most organic fluorophores
Visualizing fixed cells	Resistant to fixation; strong labeling	Labile to fixation; weak labeling
Pull-down studies	"Bait" proteins can be covalently captured on BG beads	Requires anti-GFP antibody to non-covalently capture "bait" protein, complicating downstream analysis
Live animal imaging	Cell permeable far-red dye available, permitting deep tissue visualization	Signal is easily quenched by fixation (whole-mount specimens or thick sections); limited spectral flexibility and weaker fluorescence

SNAP-tag Starter Kit

SNAP-Cell® Starter Kit
#E9100S 1 set

- *Detailed yet easy to follow labeling protocol*
- *Robust, well characterized fluorophores included*
- *Control plasmid included; expressed proteins have well defined subcellular localization*
- *Examine protein localization either inside cells or on the surface of cells*
- *Intracellular labeling*
- *Cell surface labeling*
- *In vitro analysis*

To enable researchers to quickly and easily begin using our protein labeling system, our Starter Kit includes all the components necessary to covalently attach either a red or a green fluorophore to SNAP-tag fusion proteins in living cells, fixed cells or *in vitro*. The SNAP-Cell Starter Kit contains a mammalian expression plasmid (pSNAP$_f$) encoding the SNAP-tag flanked by restriction sites for cloning a gene of interest, and two cell-permeable fluorescent SNAP-tag substrates. A positive control plasmid (pSNAP$_f$-Cox8A), encoding a SNAP-tagged protein (cytochrome c oxidase 8A) with a well-characterized mitochondrial localization, is also included. Lastly, a negative control "blocking agent" (SNAP-Cell Block) is included that interacts with the SNAP-tag, but is not fluorescent. There are two steps to using this system: subcloning and expression of the protein of interest as a SNAP$_f$ fusion, and labeling of the fusion with the SNAP-tag substrate of choice.

Fluorophores

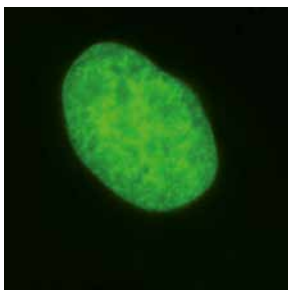
Each of the fluorophores have been extensively validated and selected for their brightness and stability. Furthermore, they have been assessed for cell permeability.

SNAP-Cell TMR-Star is a photostable red fluorescent substrate that can be used to label SNAP-tag fusion proteins inside living cells or fixed cells, on cell surfaces, or *in vitro*. This cell-permeant substrate is based on tetramethylrhodamine and suitable for imaging with standard rhodamine filter sets. When covalently bound to SNAP-tag proteins, it has an excitation maximum at 554 nm and an emission maximum at 580 nm.

SNAP-Cell 505-Star is a photostable green fluorescent substrate that can be used to label SNAP-tag fusion proteins inside living cells or fixed cells, on cell surfaces, or *in vitro*. This cell-permeant substrate is suitable for imaging with standard fluorescein filter sets. When covalently bound to SNAP-tag proteins, it has an excitation maximum at 504 nm and an emission maximum at 532 nm.

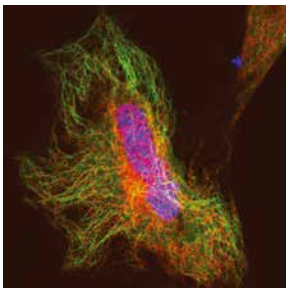
The SNAP-Cell Starter Kit Includes:

– pSNAP$_f$ Vector
– pSNAP$_f$-Cox8A Control Plasmid
– SNAP-Cell 505-Star
– SNAP-Cell TMR-Star
– SNAP-Cell Block



SNAP-Cell: *Live CHO-K1 cells transiently transfected with pSNAP$_f$-H2B. Cells were labeled with SNAP-Cell 505-Star (green) for 15 minutes at 37°C, 5% CO_2.*

Fluorescent Substrates for Protein Labeling



Live HeLa cell transfected with pSNAP$_f$-ER (endoplasmic reticulum) and pCLIP$_f$-tubulin. Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

- *Fluorescently label SNAP-tag or CLIP-tag fusions for cellular imaging*
- *Labels span fluorescent imaging spectrum from aqua (430 nm) to far-red (647+ nm) wavelengths*
- *Cell-permeable and non-cell-permeable labels available*

NEB offers a large selection of fluorescent labels (substrates) for SNAP-tag and CLIP-tag fusion proteins. SNAP-tag substrates consist of a fluorophore conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker, while CLIP-tag substrates consist of a fluorophore conjugated to a cytosine leaving group via a benzyl linker. These substrates will label their respective tags without the need for additional enzymes. Cell-permeant substrates (SNAP- and CLIP-Cell) are suitable for both intracellular and cell-surface labeling, whereas non-cell-permeant substrates (SNAP- and CLIP-Surface) are specific for fusion proteins expressed on the cell surface only.

SELF-LABELING TAG					
	APPLICATIONS	NEB #	EXCITATION*	EMISSION*(1)	SIZE
SNAP-tag	**Cell-Permeable**				
	SNAP-Cell 430	S9109S	421	444,484	50 nmol
	SNAP-Cell 505-Star	S9103S	504	532	50 nmol
	SNAP-Cell Oregon Green®	S9104S	490	514	50 nmol
	SNAP-Cell TMR-Star	S9105S	554	580	30 nmol
	SNAP-Cell 647-SiR	S9102S	645	661	30 nmol
	Non-cell-permeable				
	SNAP-Surface Alexa Fluor® 488	S9129S	496	520	50 nmol
	SNAP-Surface 488	S9124S	506	526	50 nmol
	SNAP-Surface Alexa Fluor 546	S9132S	558	574	50 nmol
	SNAP-Surface 549	S9112S	560	575	50 nmol
	SNAP-Surface 594	S9134S	606	626	50 nmol
	SNAP-Surface Alexa Fluor 647	S9136S	652	670	50 nmol
	SNAP-Surface 649	S9159S	655	676	50 nmol
CLIP-tag	APPLICATIONS	NEB #	EXCITATION*	EMISSION*(1)	SIZE
	Cell-Permeable				
	CLIP-Cell 505	S9217S	504	532	50 nmol
	CLIP-Cell TMR-Star	S9219S	554	580	30 nmol
	Non-cell-permeable				
	CLIP-Surface 488	S9232S	506	526	50 nmol
	CLIP-Surface 547	S9233S	554	568	50 nmol
	CLIP-Surface 647	S9234S	660	673	50 nmol

* Excitation and emission values determined experimentally for labeled protein tag.
(1) Colors are based on the electromagnetic spectrum. Actual color visualization may vary.

This table lists all currently available fluorescent substrates for SNAP-tag and CLIP-tag, along with excitation and emission wavelengths (determined from a labeled fusion tag, rather than the unreacted substrate).

Blocking Agents

- *Irreversible blocking*
- *Ideal for pulse-chase applications*

Blocking agents are non-fluorescent substrates that block the reactivity of the SNAP- or CLIP-tag intracellularly (SNAP-Cell Block and CLIP-Cell Block) or on the surface of live cells (SNAP-Surface Block and CLIP-Cell Block). They can be used to generate inactive controls in live cell and *in vitro* labeling experiments performed with SNAP- or CLIP-tag fusion proteins.

SNAP- and CLIP-Cell Block are highly membrane permeant and once inside the cell react with the SNAP- or CLIP-tag, irreversibly inactivating them for subsequent labeling steps.

SNAP-Surface Block also reacts with the SNAP-tag irreversibly, inactivating it for subsequent labeling steps. This blocker is largely membrane impermeant essentially limiting blocking to cell surface-exposed SNAP-tags.

PRODUCT	NEB #	APPLICATION	SIZE
SNAP-Cell Block	S9106S	Block SNAP-tag inside live cells and *in vitro*	100 nmol
CLIP-Cell Block	S9220S	Block CLIP-tag inside or on the surface of live cells and *in vitro*	100 nmol
SNAP-Surface Block	S9143S	Block SNAP-tag on the surface of live cells and *in vitro*	200 nmol

Anti-SNAP-tag Antibody, Polyclonal

#P9310S 100 µl

Description: The Anti-SNAP-tag Antibody (Polyclonal) can be used in Western blots with SNAP-tag and CLIP-tag proteins. Polyclonal antibodies are produced from the immunization of rabbit with purified recombinant SNAP-tag protein and affinity purified using SNAP-BG resin.

Sensitivity: 5 ng of SNAP-tag per load in Western blotting.

Recommended Dilution: 1:1000

SNAP-tag Purified Protein

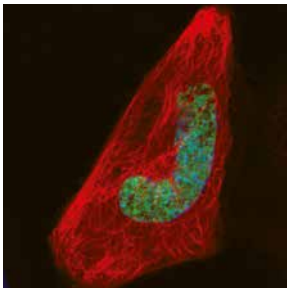
#P9312S 50 µg

Description: SNAP-tag Purified Protein can be used as a positive control for *in vitro* labeling with various SNAP-tag fluorescent substrates. The coding sequence of SNAP-tag was cloned into a pTXB1 derived *E. coli* T7 expression vector. SNAP-tag protein was expressed and purified according to the instructions in the IMPACT™ kit manual (NEB #E6901). The purified SNAP-tag protein was dialyzed into 1X phosphate buffered saline (PBS) solution containing 1 mM DTT at 1 mg/ml (50 µM) and stored at -80°C.

Molecular Weight: 19,694

Chris has been at NEB for over 12 years and is currently a member of the Production Team. Chris is a fitness enthusiast and enjoys running, obstacle course racing, endurance events—NEB is lucky to have him as a member of the Gym Committee.

Cloning Vectors



Live HeLa cell transfected with pSNAP$_f$-tubulin and pCLIPf-H2B constructs generated using pSNAP$_f$ and pCLIP$_f$ vectors. Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

- *Vectors for mammalian and bacterial expression available*

Vectors are available for SNAP-tag and CLIP-tag fusion protein expression and labeling in mammalian and bacterial systems as part of the starter kits. The mammalian SNAP$_f$ and CLIP$_f$ vectors express faster-reacting variants of the SNAP- and CLIP-tags than previously available vectors. Improved polylinker sequences both upstream and downstream from the tag allow expression of the tag on either end of the protein of interest, under control of the CMV promoter. SNAP$_f$-tag and CLIP$_f$-tag expression vectors contain a neomycin resistance (NeoR) gene for selection of stable transfectants, together with an IRES element for efficient expression of both the fusion protein and NeoR. Codon usage has been optimized for mammalian expression. Control plasmids encoding fusion proteins that are localized to the nucleus (H2B), mitochondria (Cox8A) and cell surface (ADRβ2, NK1R) are also available through Addgene.

The bacterial expression vector pSNAP-tag(T7)-2 includes cloning sites both upstream and downstream from the SNAP-tag, which is under control of the T7 promoter. Codon usage in the SNAP-tag gene has been optimized for *E. coli* expression.

Source: Isolated from an *E. coli* strain by a standard plasmid purification procedure. Plasmids have been purified free of endotoxins for efficient transfection

Concentration: 500 µg/ml

Restriction Map: For a more detailed description and restriction map of pSNAP$_f$ Vector, see page 389. See www.neb.com for sequence and map files for all expression and control plasmids.

PRODUCT	NEB #	FEATURES	SIZE
pSNAP$_f$ Vector	N9183S	stable and transient mammalian expression	20 µg
pSNAP-tag(T7)-2 Vector	N9181S	bacterial expression under T7 control	20 µg
pCLIP$_f$ Vector	N9215S	stable and transient mammalian expression	20 µg

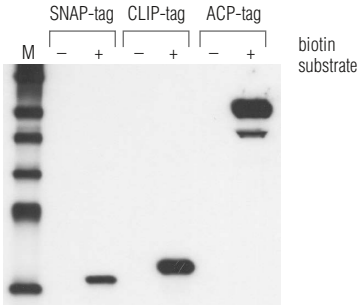
Biotin Labels

SNAP-Biotin®
#S9110S 50 nmol

CLIP-Biotin
#S9221S 50 nmol

- *Label SNAP-tag and CLIP-tag fusions with biotin*
- *Compatible with a variety of streptavidin conjugates*
- *Attach to streptavidin surfaces on microtiter plates*

For optimal flexibility with existing technologies, biotinylated labels are available for studies using streptavidin platforms. Cell-permeant (SNAP-Biotin and CLIP-Biotin) substrates are based on biotin with an amidocaproyl linker. Biotin labels are suitable for applications such as biotinylation of fusion proteins for detection with streptavidin fluorophore conjugates or labeling in solution for analysis by SDS-PAGE/ Western blot. Biotin labels are also used for capture with streptavidin surfaces for binding and interaction studies.



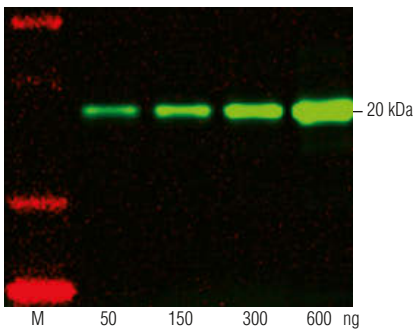
Western blot analysis of biotin labeling reactions using anti-Biotin Antibody (CST #7075). SNAP-tag, CLIP-tag, and ACP-tag-MBP (5 µM) labeled with a biotin-containing substrate (10 µM). Marker M is Biotinylated protein ladder (CST #7727).

Vista Label

SNAP-Vista® Green
#S9147S 50 nmol

- *Label protein fusions in cell lysates, or as purified proteins*
- *Detect proteins on SDS-PAGE*
- *Use with standard gel documentation equipment*

SNAP-Vista Green fluorescent substrate can be used to label SNAP-tag fusions in cell lysates or as purified proteins for detection by SDS-PAGE. The substrate is optimal for visualization using a laser based gel scanner.

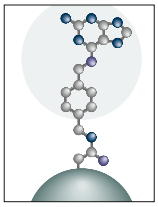


Typical SDS-PAGE of SNAP-Vista Green labeled proteins visualized using a gel scanner (Tyhoon 9400).

SNAP-Capture

- *Selectively capture SNAP-tag fusion proteins from solution*
- *Ideal for protein pull-down experiments or proteomic analysis*

SNAP-Capture products are agarose or magnetic agarose beads coupled to a benzylguanine substrate, used to selectively capture and immobilize SNAP-tag fusion proteins from solution. These beads have a high loading capacity for SNAP-tag fusion proteins and show very low non-specific adsorption of proteins from a complex lysate, making them especially suitable for pull-down applications.



Substrate structure on SNAP-Capture Pull-Down Resin

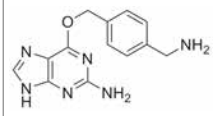
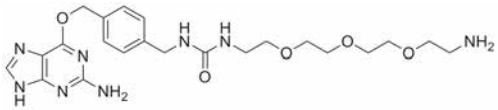
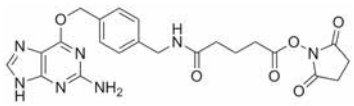
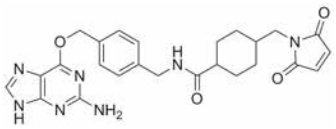
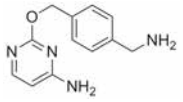
PRODUCT	NEB #	SIZE
SNAP-Capture Pull-Down Resin	S9144S	2 ml
SNAP-Capture Magnetic Beads	S9145S	2 ml

Building Blocks

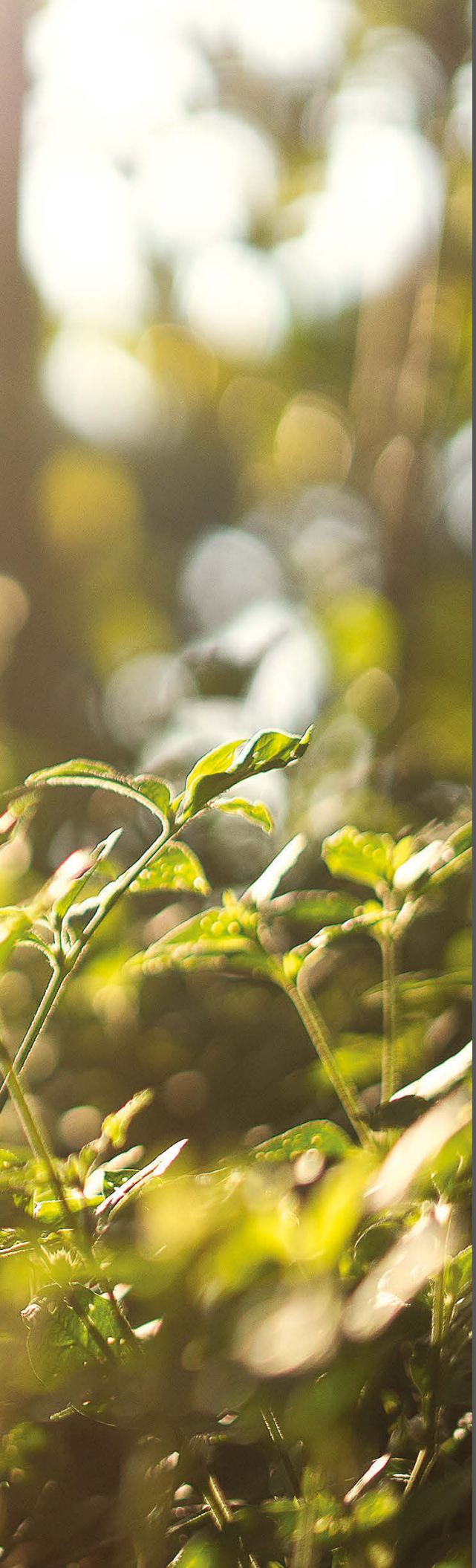
- *Synthesize new SNAP-tag and CLIP-tag substrates*
- *Make surfaces for protein immobilization*
- *Attach novel molecules or ligands to proteins*
- *Create custom substrates for protein labeling*

For advanced users with novel probes interested in working with SNAP-tag and CLIP-tag labeling technologies, building blocks are available for linkage of the core benzylguanine (BG) and benzylcytosine (BC) moieties to activated esters, primary amines and thiol groups. A variety of functional groups allows the choice of chemical coupling approaches to suit the molecule or surface to be coupled. Couple onto surfaces such as Biacore® chips or microarrays for specific protein immobilization. Couple onto peptides, proteins and DNA oligomers. Couple onto new fluorophores or affinity reagents for specific protein labeling. Labeling is gentle, precise, and versatile: one label is covalently bound under biological conditions in a defined position.

References:
References for enzyme properties and applications for this product can be found at www.neb.com.

PRODUCT	NEB #	STRUCTURE	APPLICATION	SIZE
BG-NH2	S9148S		SNAP-tag substrate. Suitable for linkage to NHS esters and other activated carboxylic esters.	2 mg
BG-PEG-NH2	S9150S		SNAP-tag substrate. PEG-linker gives superior flexibility. Particularly suited for immobilization on solid surfaces.	2 mg
BG-GLA-NHS	S9151S		SNAP-tag substrate. Activated as NHS ester. Reacts with primary amines.	2 mg
BG-Maleimide	S9153S		SNAP-tag substrate. Activated as maleimide. Reacts with thiols.	2 mg
BC-NH2	S9236S		CLIP-tag substrate. Suitable for linkage to NHS esters and other activated carboxylic esters.	2 mg

A hand in nature.
Credit: skaman306,
Getty Images



Backyard Biodiversity

Habitat loss due to human development is the primary cause of diminishing biodiversity. Living in urban areas furnished with exotic plant species and manicured lawns fragments ecosystems, and it leads to a disconnect between our everyday activities and the unintended consequences that they have on nature. As a result, biodiversity is often treated as a commodity. But, there are simple things that can be done to help preserve biodiversity — starting with learning how to share our living space with the organisms that are so essential to our existance.

Whether you live in a house with a yard or an apartment with a balcony, you can contribute to creating continuity in nature by bringing back the plants that were once naturally found there — this supports the safe travel of animals between core habitats as they search for cover and forage for food and water. A “wildlife corridor” that incorporates many vegetative layers and provides food, water and shelter will be more resilient to perturbations.

Native plants are adapted to local conditions and are easier to maintain, particularly in arid regions, so leave the native plant species undisturbed. Landscape using native trees and vegetation, and remove invasive plant species. Also, plants protect themselves by producing distasteful or toxic chemicals, and native insects that have evolved with specific native plant lineages develop a tolerance for and only eat, these plants. Non-native plants produce different chemicals, which can be detrimental to the native insects. Systemic pesticides such as neonicotinoids should always be avoided, as these pesticides persist in all parts of the plant and can poison the pollinators.

Fragmented habitats and pesticides leave pollinators malnourished. However, nectar from a variety of native flowering plants that bloom throughout the season attracts many pollinators — birds and beneficial insects — which keep the pests at bay without the use of pesticides. In addition to nectar-producing flowers, plants that feed butterfly larvae are also important.

Leaving wooded areas to age and decompose on their own offers significant benefit to many species. A dead tree may provide shelter or a perch for woodpeckers and other birds, frogs and lizards. A pile of rocks or logs can serve as a home for chipmunks or toads. Decomposing logs provide a habitat for insects and worms to thrive, and nutrients for the soil, encouraging plant growth. Insects and worms then pass the energy from plants to non-plant eating animals further up the food chain, such as spiders, birds and amphibians.

A tree hollow takes up to 150 years to naturally develop and is essential for nesting and breeding. Nesting boxes for birds, bees and bats can help alleviate the shortage of hollows. A birdbath, pond, or a carefully planned rain garden will attract birds and aquatic wildlife, such as frogs and dragonflies.

In addition to our own backyards, one can consider getting involved with regional ecological restoration efforts. Good places to look for opportunities are land trusts, wildlife foundations, native plant societies, government agencies (e.g., Forest Service, Fish & Wildlife), and environmental organizations. Protecting habitats before they have been damaged is the best form of biodiversity conservation and is most successfully implemented by government regulations.

As human development and urban expansion continues to increase, we are outcompeting other species for space on Earth. Educating ourselves on how we can co-exist and provide wildlife with water, food, cover and a safe place to raise their young in our own surroundings can go a long way in conserving biodiversity.

Reference Appendix

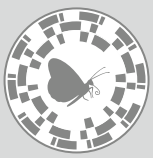
Technical Support – for scientists, by scientists

As a partner to the scientific community, New England Biolabs is committed to providing top quality tools and scientific expertise. This philosophy still stands, and has led to long-standing relationships with many of our fellow scientists. NEB's commitment to scientists is the same regardless of whether or not they purchase product from NEB: their ongoing research is supported by our catalog, website and technical staff.

NEB's technical support model is unique as it utilizes most of the scientists at NEB. Several of our product lines have designated technical support scientists assigned to servicing customers in those application areas. Any questions regarding a product could be dealt with by one of the technical support scientists, the product manager who manufactures it, the product development scientist who optimizes it, or a researcher who uses the product in their daily research. As such, customers are supported by scientists and often experts in the product or its application.

To access technical support:

- Call 1-800-632-7799 (Monday – Friday: 9:00 am - 6:00 pm EST)
- Submit an online form at www.neb.com/techsupport
- Email info@neb.com
- International customers can contact a local NEB subsidiary or distributor. For more information see inside back cover.

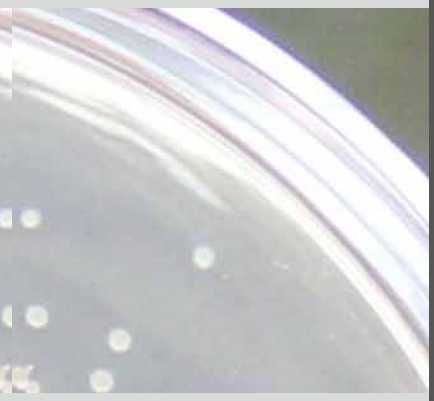


Learn more about NEB's tech support program.

Featured Tools & Resources

View NEB TV Episode #22 to learn more about our Technical Support program.

Visit the Tools & Resources tab at www.neb.com to find additional online tools, video tech tips and tutorials to help you in your research.



Online Interactive Tools

Use the Tools & Resources tab at www.neb.com to access our growing selection of interactive technical tools. These tools can also be accessed directly in the footer of every web page.

NEB scientists are often involved in the development of online tools that will aid in their research. We are now making these tools and in some circumstances, the source code, available for you to evaluate. To learn more, visit **www.neb.com/NEBetaTools.**

Online Tools

Competitor Cross-Reference Tool

Use this tool to select another company's product and find out which NEB product is compatible. Choose either the product name or catalog number from the available selections, and this tool will identify the recommended NEB product.

DNA Sequences and Maps Tool

With the DNA Sequences and Maps Tool, find the nucleotide sequence files for commonly used molecular biology tools, including plasmid, viral and bacteriophage vectors.

Double Digest Finder

Use this tool to guide your reaction buffer selection when setting up double digests, a common timesaving procedure. Choosing the right buffers will help you to avoid star activity and loss of product.

Enzyme Finder

Use this tool to select restriction enzymes by name, sequence, overhang or type. Enter your sequence using single letter code nomenclature, and Enzyme Finder will identify the right enzyme for the job.

Glycan Analyzer

Use this tool to interpret ultra or high pressure liquid chromatography (UPLC/HPLC) N-glycan profiles following exoglycosidase digestions.

NEB Golden Gate Assembly Tool

Use this tool to assist with in silico DNA construct design for Golden Gate DNA assembly. It enables the accurate design of primers with appropriate Type IIS restriction sites and overlaps, quick import of sequences in many formats and export of the final assembly, primers and settings.

NEBaseChanger®

NEBaseChanger can be used to design primers specific to the mutagenesis experiment you are performing using the Q5® Site-Directed Mutagenesis Kit. This tool will also calculate a recommended custom annealing temperature based on the sequence of the primers by taking into account any mismatches.

NEBNext® Selector

Use this tool to guide you through the selection of NEBNext reagents for next generation sequencing sample preparation.

NEBcutter® V2.1

Identify the restriction sites within your DNA sequence using NEBcutter. Choose between Type II and commercially available Type III restriction enzymes to digest your DNA. NEBcutter will indicates cut frequency and methylation-state sensitivity.

NEBioCalculator®

Use this tool for your scientific calculations and conversions for DNA and RNA. Options include conversion of mass to moles, ligation amounts, conversion of OD to concentration, dilution and molarity. Additional features include sgRNA template oligo design and qPCR library quantification.

NEBcloner®

Use this tool to find the right products and protocols for each step (digestion, end modification, ligation, transformation and mutagenesis) of your next traditional cloning experiment. Also, find other relevant tools and resources to enable protocol optimization.

NEBuilder® Assembly Tool

Use this tool to design primers for your DNA assembly reaction, based on the entered fragment sequences and the polymerase being used for amplification.

PCR Fidelity Estimator

Estimate the percentage of correct DNA copies (those without base substitution errors) per cycle of PCR for selected DNA polymerases.

PCR Selector

Use this tool to help select the right DNA polymerase for your PCR setup. Whether your amplicon is long, complex, GC-rich or present in a single copy, the PCR selection tool will identify the perfect DNA polymerase for your reaction.

Tm Calculator

Determine the optimal annealing temperature for your amplicon with our Tm Calculator. Simply input your DNA polymerase, primer concentration and your primer sequence, and the Tm Calculator will guide you to successful reaction conditions.

Thermostable Ligase Reaction Temperature Calculator

This tool will help you estimate an optimal reaction temperature to minimize mismatch for thermostable ligation of two adjacent ssDNA probes annealed to a template.

Online Tools (continued)

Read Coverage Calculator

This tool allows for easy calculation of values associated with read coverage in NGS protocols.

Additional Databases

Polbase®

Polbase is a repository of biochemical, genetic and structural information about DNA Polymerases.

REBASE®

Use this tool as a guide to the ever-changing landscape of restriction enzymes. REBASE, the Restriction Enzyme DataBASE, is a dynamic, curated database of restriction enzymes and related proteins.

Mobile Apps

NEB Tools for iPhone®, iPad® or Android®

NEB Tools brings New England Biolabs' most popular web tools to your iPhone, iPad or Android devices.

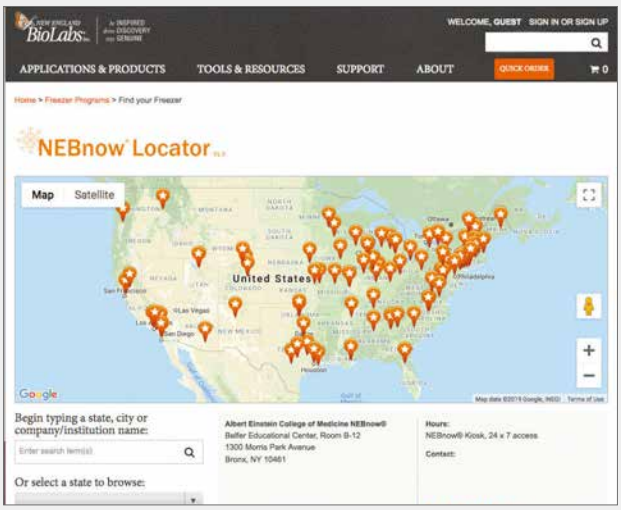
- Use Enzyme Finder to select a restriction enzyme by category or recognition sequence, or search by name to find information on any NEB enzyme. Sort your results so they make sense to you, then email them to your inbox or connect directly to www.neb.com.
- Use Double Digest Finder to determine buffer and reaction conditions for experiments requiring two restriction enzymes.
- Use Tm Calculator to calculate annealing temperatures for your PCR reaction.
- Also included are several popular calculators from the NEBioCalculator web app.

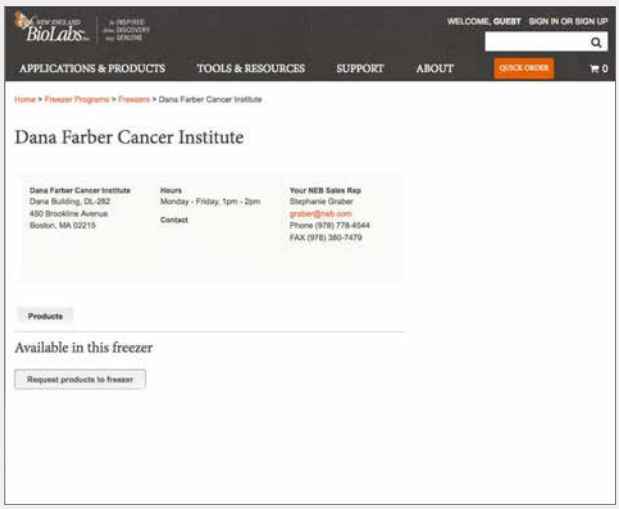
When using either of these tools, look for CutSmart®, HF® and Time-Saver™ enzymes for the ultimate in convenience. NEB Tools enables quick and easy access to the most requested restriction enzyme information, and allows you to plan your experiments from anywhere.

Looking for a Freezer Program?

NEBnow® Locator

NEBnow Freezer Programs are ideally suited for researchers in academics and industry looking for on-site access to the world's finest restriction enzymes and related products. NEB freezers offer you convenience, flexibility and value.





Optimizing Restriction Enzyme Reactions

While standard recommended reaction conditions are a good place to start, in some cases, optimization may be necessary to achieve the best results. Depending on the enzyme(s) being used, variables such as incubation time, number of enzyme units used, and reaction temperature should be tested to find the optimal reaction conditions for your substrate DNA and enzyme(s) of choice.

Protocol: Restriction Enzyme Reactions

	STANDARD PROTOCOL	TIME-SAVER PROTOCOL
DNA	up to 1 µg	up to 1 µg
10X NEBuffer	5 µl (1X)	5 µl (1X)
Restriction Enzymes	10 units*	1 µl
Total Volume	50 µl	50 µl
Incubation Temperature	Enzyme-dependent	Enzyme-dependent
Incubation Time	60 minutes	5–15 minutes**

*Sufficient to digest all types of DNAs.
**Time-Saver qualified enzymes can also be incubated overnight with no star activity.

TOOLS & RESOURCES

Visit NEBRestrictionEnzymes.com to find:

- Online tutorials for setting up restriction enzyme digests
- Tips to avoid star activity
- Restriction Enzyme Performance Chart
- Troubleshooting guide
- Access to NEB's online tools, including: **Enzyme Finder, Double Digest Finder** and **NEBcloner**

TIPS FOR OPTIMIZATION

Enzyme

- Keep on ice when not in the freezer
- Should be the last component added to reaction
- Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- In general, we recommend 5–10 units of enzyme per µg DNA, and 10–20 units per µg of genomic DNA in a 1 hour digest

Star Activity

- Unwanted cleavage that can occur when enzyme is used under sub-optimal conditions, such as:
  – Too much enzyme present
  – Too long of an incubation time
  – Using a non-recommended buffer
  – Glycerol concentrations above 5%
- Star activity can be reduced by using a High-Fidelity (HF®) enzyme, reducing incubation time, using a Time-Saver™ enzyme or increasing reaction volume

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents and salts. Spin column purification readily accomplishes this; extra washes during purification can also help.
- Methylation of DNA can affect digestion with certain enzymes. For more information about methylation visit **www.neb.com/methylation**

Buffer

- Use at a 1X concentration
- BSA is included in NEBuffer 1.1, 2.1, 3.1 and CutSmart® Buffer. No additional BSA is needed.
- Restriction enzymes that do not require BSA for optimal activity are not adversely affected if BSA is present in the reaction

Reaction Volume

- A 50 µl reaction volume is recommended for digestion of up to 1 µg of substrate. This helps maintain salt levels introduced by miniprepped DNA low enough that they don't affect enzyme activity.
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol
- Additives in the restriction enzyme storage buffer (e.g., glycerol, salt), as well as contaminants found in the substrate solution (e.g., salt, EDTA or alcohol), can be problematic in smaller reaction volumes

	RESTRICTION ENZYME*	DNA	10X NEBUFFER
10 µl rxn**	1 unit	0.1 µg	1 µl
25 µl rxn	5 units	0.5 µg	2.5 µl
50 µl rxn	10 units	1 µg	5 µl

* Restriction enzymes can be diluted using the recommended diluent buffer when smaller amounts are needed

** 10 µl rxns should not be incubated for longer than 1 hour to avoid evaporation.

Incubation Time

- Incubation time for the Standard Protocol is 1 hour. Incubation for the Time-Saver Protocol is 5–15 minutes.
- Visit **www.neb.com/timesaver** for list of Time-Saver qualified enzymes
- It is possible, with many enzymes, to use fewer units and digest for up to 16 hours. For more information, visit **www.neb.com**

Storage

- Storage at –20°C is recommended for most restriction enzymes. For a few enzymes, storage at –80°C is recommended. Visit **www.neb.com** for storage information.
- 10X NEBuffers should be stored at –20°C

Stability

- The expiration date is found on the label
- Long term exposure to temperatures above –20°C should be minimized whenever possible

Double Digestion

Digesting a DNA substrate with two restriction enzymes simultaneously (double digestion) is a common timesaving procedure. Over 210 restriction enzymes are 100% active in CutSmart Buffer, making double digestion simple. If you are using an enzyme that is not supplied with CutSmart Buffer, the Performance Chart for Restriction Enzymes (pages 293–298) rates the percentage activity of each restriction endonuclease in the four standard NEBuffers.

Setting up a Double Digestion

- Double digests with CutSmart restriction enzymes can be set up in CutSmart Buffer. Otherwise, choose an NEBuffer that results in the most activity for both enzymes. If star activity is a concern, consider using one of our High-Fidelity (HF) enzymes.
- Set up reaction according to recommended protocol (see page 290). The final concentration of glycerol in any reaction should be less than 5% to minimize the possibility of star activity (see page 300). For example, in a 50 µl reaction, the total amount of enzyme added should not exceed 5 µl.
- If two different incubation temperatures are necessary, choose the optimal reaction buffer and set up reaction accordingly. Add the first enzyme and incubate at the desired temperature. Then, heat inactivate the first enzyme, if it can be heat inactivated, add the second enzyme and incubate at the recommended temperature.
- Depending on an enzyme's activity rating in a non-optimal NEBuffer, the number of units or incubation time may be adjusted to compensate for the slower rate of cleavage.

Setting up a Double Digestion with a Unique Buffer (designated "U")

- NEB currently supplies three enzymes with unique buffers: EcoRI, SspI and DpnII. In most cases, DpnII requires a sequential digest. Note that EcoRI and SspI have HF versions (NEB #R3101 and NEB #R3132, respectively) which is supplied with CutSmart Buffer.

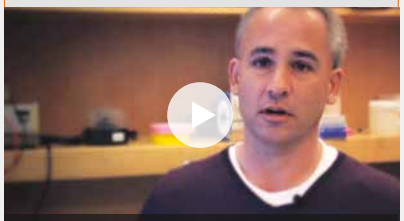
Setting up a Sequential Digestion

- If there is no buffer in which the two enzymes exhibit > 50% activity, a sequential digest can be performed.
- Set up a reaction using the restriction endonuclease that has the lowest salt concentration in its recommended buffer and incubate to completion.
- Adjust the salt concentration of the reaction (using a small volume of a concentrated salt solution) to approximate the reaction conditions of the second restriction endonuclease.
- Add the second enzyme and incubate to complete the second reaction.
- Alternatively, a spin column can be used to isolate the DNA prior to the second reaction.

TOOLS & RESOURCES

Visit www.neb.com/nebtools for:

- Help choosing double digest conditions using NEB's, **Double Digest Finder** and **NEBcloner®**



TIPS FOR SETTING UP DOUBLE DIGESTS

Types of Restriction Enzymes

Restriction enzymes are traditionally classified into four types on the basis of subunit composition, cleavage position, sequence specificity and cofactor requirements. However, amino acid sequencing has uncovered extraordinary variety among restriction enzymes and revealed that at the molecular level there are many more than four different types.

Type I Enzymes are complex, multisubunit, combination restriction-and-modification enzymes that cut DNA at random far from their recognition sequences. Type I enzymes are of considerable biochemical interest, but they have little practical value since they do not produce discrete restriction fragments or distinct gel-banding patterns.

Type II Enzymes cut DNA at defined positions close to or within their recognition sequences. They produce discrete restriction fragments and distinct gel banding patterns, and they are the only class used in the laboratory for DNA analysis and gene cloning. Rather than forming a single family of related proteins, Type II enzymes are a collection of unrelated proteins of many different sorts. Type II enzymes frequently differ so utterly in amino acid sequence from one another, and indeed from every other known protein, that they exemplify the class of rapidly evolving proteins that are often indicative of involvement in host-parasite interactions.

Type III Enzymes are also large combination restriction-and-modification enzymes. They cleave outside of their recognition sequences and require two such sequences in opposite orientations within the same DNA molecule to accomplish cleavage; they rarely yield complete digests.

Type IV Enzymes recognize modified, typically methylated DNA and are exemplified by the McrBC and Mrr systems of *E. coli*.

TOOLS & RESOURCES

Visit the video library at www.neb.com to find:

- Tutorials on Type I, II and III restriction enzymes

TYPE I, II AND III RESTRICTION ENZYMES

View double digest protocol.

Restriction Enzyme Troubleshooting Guide

PROBLEM	CAUSE	SOLUTION
Few or no transformants	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove any contaminants that may inhibit the enzyme • When digesting a PCR fragment, make sure to have at least 6 nucleotides between the recognition site and the end of the DNA molecule
The digested DNA ran as a smear on an agarose gel	The restriction enzyme(s) is bound to the substrate DNA	• Lower the number of units • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA
	Nuclease contamination	• Use fresh, clean running buffer and a fresh agarose gel • Clean up the DNA
Incomplete restriction enzyme digestion	Cleavage is blocked by methylation	• DNA isolated from a bacterial source may be blocked by Dam and Dcm methylation • DNA isolated from eukaryotic source may be blocked by CpG methylation • Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • If the enzyme is inhibited by Dam or Dcm methylation, grow the plasmid in a *dam-/dcm-* strain (NEB #C2925)
	Salt inhibition	• Enzymes that have low activity in salt-containing buffers (NEBuffer 3.1) may be salt sensitive, so clean up the DNA prior to digestion • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. To prevent this, DNA solution should be no more than 25% of total reaction volume.
	Inhibition by PCR components	• Clean up the PCR fragment prior to restriction digest
	Using the wrong buffer	• Use the recommended buffer supplied with the restriction enzyme
	Too few units of enzyme used	• Use at least 3–5 units of enzyme per µg of DNA
	Incubation time was too short	• Increase the incubation time
	Digesting supercoiled DNA	• Some enzymes have a lower activity on supercolied DNA. Increase the number of enzyme units in the reaction.
	Presence of slow sites	• Some enzymes can exhibit slower cleavage towards specific sites. Increase the incubation time, 1–2 hours is typically sufficient.
	Two sites required	• Some enzymes require the presence of two recognition sites to cut efficiently
	DNA is contaminated with an inhibitor	• Assay substrate DNA in the presence of a control DNA. Control DNA will not cleave if there is an inhibitor present. Miniprep DNA is particularly susceptible to contaminants. • Clean DNA with a spin column, resin or drop dialysis, or increase volume to dilute contaminant
Extra bands in the gel	If larger bands than expected are seen in the gel, this may indicate binding of the enzyme(s) to the substrate	• Lower the number of units in the reaction • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the substrate
	Star activity	• Use the recommended buffer supplied with the restriction enzyme • Decrease the number of enzyme units in the reaction • Make sure the amount of enzyme added does not exceed 10% of the total reaction volume. This ensures that the total glycerol concentration does not exceed 5% v/v. • Decrease the incubation time. Using the minimum reaction time required for complete digestion will help prevent star activity. • Try using a High-Fidelity (HF) restriction enzyme. HF enzymes have been engineered for reduced star activity.
	Partial restriction enzyme digest	• Enzymes that have low activity in salt-containing buffers (e.g., NEBuffer 3.1) may be salt sensitive. Make sure to clean up the DNA prior to digestion. • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. To prevent this, DNA solution should be no more than 25% of total reaction volume. • Clean-up the PCR fragment prior to restriction digest • Use the recommended buffer supplied with the restriction enzyme • Use at least 3–5 units of enzyme per µg of DNA and digest the DNA for 1–2 hours

Performance Chart for Restriction Enzymes

New England Biolabs supplies > 210 restriction enzymes that are 100% active in a single buffer, CutSmart. This results in increased efficiency, flexibility and ease-of-use, especially when performing double digests.

This performance chart summarizes the activity information of NEB restriction enzymes. To help select the best conditions for double digests, this chart shows the optimal (supplied) NEBuffer and approximate activity in the four standard NEBuffers for each enzyme. Note that BSA is included in all NEBuffers, and is not provided as a separate tube. In addition, this performance chart shows recommended reaction temperature, heat-inactivation temperature, recommended diluent buffer, methylation sensitivity and whether the enzyme is Time-Saver qualified (e.g., cleaves substrate in 5–15 minutes under recommended conditions, and can be used overnight without degradation of DNA).

Chart Legend

U Supplied with a unique reaction buffer that is different from the four standard NEBuffers. The compatibility with the four standard NEBuffers is indicated in the chart.

RR Recombinant

e Engineered enzyme for maximum performance

Time-Saver qualified

2 Site Indicates that the restriction enzyme requires two or more sites for cleavage

SAM Supplied with a separate vial of S-adenosylmethionine (SAM). To obtain 100% activity, SAM should be added to the 1X reaction mix as specified on the product data card.

dcm dcm methylation sensitivity

dam dam methylation sensitivity

CpG CpG methylation sensitivity

Activity Notes (see last column)

FOR STAR ACTIVITY

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.

FOR LIGATION AND RECUTTING

a. Ligation is less than 10%

b. Ligation is 25% – 75%

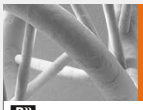
c. Recutting after ligation is < 5%

d. Recutting after ligation is 50% – 75%

e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

			ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
RR			AatII	CutSmart	< 10	50*	50	100	37°	80°	B	Lambda		CpG	
RR			AbaSI	CutSmart	25	50	50	100	25°	65°	C	T4 wt Phage			e
RR			AccI	CutSmart	50	50	10	100	37°	80°	A	Lambda		CpG	
RR			Acc65I	3.1	10	75*	100	25	37°	65°	A	pBC4	dcm	CpG	
RR			AciI	CutSmart	< 10	25	100	100	37°	65°	A	Lambda		CpG	d
RR			AclI	CutSmart	< 10	< 10	< 10	100	37°	No	B	Lambda		CpG	
RR			AcuI	CutSmart + SAM	50	100	50	100	37°	65°	B	Lambda			1, b, d
RR			AfeI	CutSmart	25	100	25	100	37°	65°	B	pXba		CpG	
RR			AflII	CutSmart	50	100	10	100	37°	65°	A	phiX174			
RR			AflIII	3.1	10	50	100	50	37°	80°	B	Lambda			
RR			AgeI	1.1	100	75	25	75	37°	65°	C	Lambda		CpG	
RR		e	AgeI-HF	CutSmart	100	50	10	100	37°	65°	A	Lambda		CpG	
RR			AhdI	CutSmart	25	25	10	100	37°	65°	A	Lambda		CpG	a
RR			AleI-v2	CutSmart	< 10	< 10	< 10	100	37°	80°	B	Lambda		CpG	
RR			AluI	CutSmart	25	100	50	100	37°	80°	B	Lambda			b
RR			AlwI	CutSmart	50	50	10	100	37°	No	A	Lambda dam-	dam		1, b, d
RR			AlwNI	CutSmart	10	100	50	100	37°	80°	A	Lambda	dcm		
RR			ApaI	CutSmart	25	25	< 10	100	25°	65°	A	pXba	dcm	CpG	
RR			ApaLI	CutSmart	100	100	10	100	37°	No	A	Lambda HindIII		CpG	
RR			ApeKI	3.1	25	50	100	10	75°	No	B	Lambda		CpG	
RR			ApoI	3.1	10	75	100	75	50°	80°	A	Lambda			
RR		e	ApoI-HF	CutSmart	10	100	10	100	37°	80°	B	Lambda			
RR			AscI	CutSmart	< 10	10	10	100	37°	80°	A	Lambda		CpG	
RR			AseI	3.1	< 10	50*	100	10	37°	65°	B	Lambda			3
RR			AsiSI	CutSmart	100	100	25	100	37°	80°	B	pXba (Xho digested)		CpG	2, b
RR			AvaI	CutSmart	< 10	100	25	100	37°	80°	A	Lambda		CpG	
RR			AvaII	CutSmart	50	75	10	100	37°	80°	A	Lambda	dcm	CpG	
RR			AvrII	CutSmart	100	50	50	100	37°	No	B	Lambda HindIII			
RR			BaeI	CutSmart + SAM	50	100	50	100	25°	65°	A	Lambda		CpG	e
RR			BaeGI	3.1	75	75	100	25	37°	80°	A	Lambda			
RR			BamHI	3.1	75*	100*	100	100*	37°	No	A	Lambda			3
RR		e	BamHI-HF	CutSmart	100	50	10	100	37°	No	A	Lambda			
RR			BanI	CutSmart	10	25	< 10	100	37°	65°	A	Lambda	dcm	CpG	1

Performance Chart for Restriction Enzymes (continued)



ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
BanII	CutSmart	100	100	50	100	37°	80°	A	Lambda			2
BbsI	2.1	100	100	25	75	37°	65°	B	Lambda			
BbsI-HF	CutSmart	10	10	10	100	37°	65°	B	Lambda			
BbvI	CutSmart	100	100	25	100	37°	65°	B	pBR322			3
BbvCI	CutSmart	10	100	50	100	37°	No	B	Lambda		CpG	1, a
BccI	CutSmart	100	50	10	100	37°	65°	A	pXba			3, b
BceAI	3.1	100*	100*	100	100*	37°	65°	A	pBR322		CpG	1
BcgI	3.1 + SAM	10	75*	100	50*	37°	65°	A	Lambda	dam	CpG	e
BciVI	CutSmart	100	25	< 10	100	37°	80°	C	Lambda			b
BclI	3.1	50	100	100	75	50°	No	A	Lambda dam-	dam		
BclI-HF	CutSmart	100	100	10	100	37°	65°	B	Lambda dam-	dam		
BcoDI	CutSmart	50	75	75	100	37°	No	B	Lambda		CpG	
BfaI	CutSmart	< 10	10	< 10	100	37°	80°	B	Lambda			2, b
BfuAI	3.1	< 10	25	100	10	50°	65°	B	Lambda		CpG	3
BglI	3.1	10	25	100	10	37°	65°	B	Lambda		CpG	
BglII	3.1	10	10	100	< 10	37°	No	A	Lambda			
BlpI	CutSmart	50	100	10	100	37°	No	A	Lambda			d
BmgBI	3.1	< 10	10	100	10	37°	65°	B	Lambda		CpG	3, b, d
BmrI	2.1	75	100	75	100*	37°	65°	B	Lambda HindIII			b
BmtI	3.1	100	100	100	100	37°	65°	B	pXba			2
BmtI-HF	CutSmart	50	100	10	100	37°	65°	B	pXba			
BpmI	3.1	75	100	100	100	37°	65°	B	Lambda			2
Bpu10I	3.1	10	25	100	25	37°	80°	B	Lambda			3, b, d
BpuEI	CutSmart + SAM	50*	100	50*	100	37°	65°	B	Lambda			d
BsaI	CutSmart	75*	75	100	100	37°	65°	B	pXba	dcm	CpG	3
BsaI-HFv2	CutSmart	100	100	100	100	37°	80°	B	pXba	dcm	CpG	
BsaAI	CutSmart	100	100	100	100	37°	No	C	Lambda		CpG	
BsaBI	CutSmart	50	100	75	100	60°	80°	B	Lambda dam-	dam	CpG	2
BsaHI	CutSmart	50	100	100	100	37°	80°	C	Lambda	dcm	CpG	
BsaJI	CutSmart	50	100	100	100	60°	80°	A	Lambda			
BsaWI	CutSmart	10	100	50	100	60°	80°	A	Lambda			
BsaXI	CutSmart	50*	100*	10	100	37°	No	C	Lambda			e
BseRI	CutSmart	100	100	75	100	37°	80°	A	Lambda			d
BseYI	3.1	10	50	100	50	37°	80°	B	Lambda		CpG	d
BsgI	CutSmart + SAM	25	50	25	100	37°	65°	B	Lambda			d
BsiEI	CutSmart	25	50	< 10	100	60°	No	A	Lambda		CpG	
BsiHKAI	CutSmart	25	100	100	100	65°	No	A	Lambda			
BsiWI	3.1	25	50*	100	25	55°	65°	B	phiX174		CpG	
BsiWI-HF	CutSmart	50	100	10	100	37°	No	B	phiX174		CpG	
BsII	CutSmart	50	75	100	100	55°	No	A	Lambda	dcm	CpG	b
BsmI	CutSmart	25	100	< 10	100	65°	80°	A	Lambda			
BsmAI	CutSmart	50	100	100	100	55°	No	B	Lambda		CpG	
BsmBI	3.1	10	50*	100	25	55°	80°	B	Lambda		CpG	
BsmFI	CutSmart	25	50	50	100	65°	80°	A	pBR322	dcm	CpG	1
BsoBI	CutSmart	25	100	100	100	37°	80°	A	Lambda			
Bsp1286I	CutSmart	25	25	25	100	37°	65°	A	Lambda			3
BspCNI	CutSmart + SAM	100	75	10	100	25°	80°	A	Lambda			b
BspDI	CutSmart	25	75	50	100	37°	80°	A	Lambda	dam	CpG	
BspEI	3.1	< 10	10	100	< 10	37°	80°	B	Lambda dam-	dam	CpG	
BspHI	CutSmart	< 10	50	25	100	37°	80°	A	Lambda	dam		
BspMI	3.1	10	50*	100	10	37°	65°	B	Lambda			

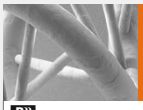
a. Ligation is less than 10%
b. Ligation is 25% – 75%
c. Recutting after ligation is < 5%
d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY	NOTE(S)
BspQI	3.1	100*	100*	100	100*	50°	80°	B	Lambda		3
BsrI	3.1	< 10	50	100	10	65°	80°	B	phiX174		b
BsrBI	CutSmart	50	100	100	100	37°	80°	A	Lambda	CpG	d
BsrDI	2.1	10	100	75	25	65°	80°	A	Lambda		3, d
BsrFI-v2	CutSmart	25	25	0	100	37°	No	C	pBR322	CpG	
BsrGI	2.1	25	100	100	25	37°	80°	A	Lambda		
BsrGI-HF	CutSmart	10	100	100	100	37°	80°	A	Lambda		
BssHII	CutSmart	100	100	100	100	50°	65°	B	Lambda	CpG	
BssSI-v2	CutSmart	10	25	< 10	100	37°	No	B	Lambda		
BstAPI	CutSmart	50	100	25	100	60°	80°	A	Lambda	CpG	b
BstBI	CutSmart	75	100	10	100	65°	No	A	Lambda	CpG	
BstEII	3.1	10	75*	100	75*	60°	No	A	Lambda		3
BstEII-HF	CutSmart	< 10	10	< 10	100	37°	No	A	Lambda		
BstNI	3.1	10	100	100	75	60°	No	A	Lambda		a
BstUI	CutSmart	50	100	25	100	60°	No	A	Lambda	CpG	b
BstXI	3.1	< 10	50	100	25	37°	80°	B	Lambda	dcm	3
BstYI	2.1	25	100	75	100	60°	No	A	Lambda		
BstZ17I-HF	CutSmart	100	100	10	100	37°	No	A	Lambda	CpG	
Bsu36I	CutSmart	25	100	100	100	37°	80°	C	Lambda HindIII		b
BtgI	CutSmart	50	100	100	100	37°	80°	B	pBR322		
BtgZI	CutSmart	10	25	< 10	100	60°	80°	A	Lambda	CpG	3, b, d
BtsI-v2	CutSmart	100	100	25	100	55°	No	A	Lambda		
BtsIMutI	CutSmart	100	50	10	100	55°	80°	A	pUC19		b
BtsCI	CutSmart	10	100	25	100	50°	80°	B	Lambda		
Cac8I	CutSmart	50	75	100	100	37°	65°	B	Lambda	CpG	b
ClaI	CutSmart	10	50	50	100	37°	65°	A	Lambda dam-	dam CpG	
CspCI	CutSmart + SAM	10	100	10	100	37°	65°	A	Lambda		e
CviAII	CutSmart	50	50	10	100	25°	65°	C	Lambda		
CviKI-1	CutSmart	25	100	100	100	37°	No	A	pBR322		1, b
CviQI	3.1	75	100*	100	75*	25°	No	C	Lambda		b
DdeI	CutSmart	75	100	100	100	37°	65°	B	Lambda		
DpnI	CutSmart	100	100	75	100	37°	80°	B	pBR322	CpG	b
DpnII	U	25	25	100*	25	37°	65°	B	Lambda dam-	dam	
DraI	CutSmart	75	75	50	100	37°	65°	A	Lambda		
DraIII-HF	CutSmart	< 10	50	10	100	37°	No	B	Lambda	CpG	b
DrdI	CutSmart	25	50	10	100	37°	65°	A	pUC19	CpG	3
EaeI	CutSmart	10	50	< 10	100	37°	65°	A	Lambda	dcm CpG	b
EagI	3.1	10	25	100	10	37°	65°	B	pXba	CpG	
EagI-HF	CutSmart	25	100	100	100	37°	65°	B	pXba	CpG	
EarI	CutSmart	50	10	< 10	100	37°	65°	B	Lambda	CpG	b, d
EciI	CutSmart	100	50	50	100	37°	65°	A	Lambda	CpG	2
Eco53kI	CutSmart	100	100	< 10	100	37°	65°	A	pXba	CpG	3, b
EcoNI	CutSmart	50	100	75	100	37°	65°	A	Lambda		b
EcoO109I	CutSmart	50	100	50	100	37°	65°	A	Lambda HindIII	dcm	3
EcoP15I	3.1 + ATP	75	100	100	100	37°	65°	A	pUC19		e
EcoRI	U	25	100*	50	50*	37°	65°	C	Lambda	CpG	
EcoRI-HF	CutSmart	10	100	< 10	100	37°	65°	C	Lambda	CpG	
EcoRV	3.1	10	50	100	10	37°	80°	A	Lambda	CpG	
EcoRV-HF	CutSmart	25	100	100	100	37°	65°	B	Lambda	CpG	
Esp3I	CutSmart	100	100	< 10	100	37°	65°	B	Lambda	CpG	
FatI	2.1	10	100	50	50	55°	80°	A	pUC19		

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.

Performance Chart for Restriction Enzymes (continued)



ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
FauI	CutSmart	100	50	10	100	55°	65°	A	Lambda		CpG	3, b, d
Fnu4HI	CutSmart	< 10	< 10	< 10	100	37°	No	A	Lambda		CpG	a
FokI	CutSmart	100	100	75	100	37°	65°	A	Lambda	dcm	CpG	3, b, d
FseI	CutSmart	100	75	< 10	100	37°	65°	B	pBC4	dcm	CpG	
FspI	CutSmart	10	100	10	100	37°	No	C	Lambda		CpG	b
FspEI	CutSmart	< 10	< 10	< 10	100	37°	80°	B	pBR322	dcm		1, e
HaeII	CutSmart	25	100	10	100	37°	80°	A	Lambda		CpG	
HaeIII	CutSmart	50	100	25	100	37°	80°	A	Lambda			
HgaI	1.1	100	100	25	100	37°	65°	A	phiX174		CpG	1
HhaI	CutSmart	25	100	100	100	37°	65°	A	Lambda		CpG	
HincII	3.1	25	100	100	100	37°	65°	B	Lambda		CpG	
HindIII	2.1	25	100	50	50	37°	80°	B	Lambda			2
HindIII-HF	CutSmart	10	100	10	100	37°	80°	B	Lambda			
HinfI	CutSmart	50	100	100	100	37°	80°	A	Lambda		CpG	
HinP1I	CutSmart	100	100	100	100	37°	65°	A	Lambda		CpG	
HpaI	CutSmart	< 10	75*	25	100	37°	No	A	Lambda		CpG	1
HpaII	CutSmart	100	50	< 10	100	37°	80°	A	Lambda		CpG	
HphI	CutSmart	50	50	< 10	100	37°	65°	B	Lambda	dam	CpG	b, d
Hpy99I	CutSmart	50	10	< 10	100	37°	65°	A	Lambda		CpG	
Hpy166II	CutSmart	100	100	50	100	37°	65°	C	pBR322		CpG	
Hpy188I	CutSmart	25	100	50	100	37°	65°	A	pBR322	dam		1, b
Hpy188III	CutSmart	100	100	10	100	37°	65°	B	pUC19	dam	CpG	3, b
HpyAV	CutSmart	100	100	25	100	37°	65°		Lambda		CpG	3, b, d
HpyCH4III	CutSmart	100	25	< 10	100	37°	65°	A	Lambda			b
HpyCH4IV	CutSmart	100	50	25	100	37°	65°	A	pUC19		CpG	
HpyCH4V	CutSmart	50	50	25	100	37°	65°	A	Lambda			
I-CeuI	CutSmart	10	10	10	100	37°	65°	B	pBHS ScaI-linearized			
I-SceI	CutSmart	10	50	25	100	37°	65°	B	pGPS2 NotI-linearized			
KasI	CutSmart	50	100	50	100	37°	65°	B	pBR322		CpG	3
KpnI	1.1	100	75	< 10	50	37°	No	A	pXba			1
KpnI-HF	CutSmart	100	25	< 10	100	37°	No	A	pXba			
LpnPI	CutSmart	< 10	< 10	< 10	100	37°	65°	B	pBR322			1, e
MboI	CutSmart	75	100	100	100	37°	65°	A	Lambda dam-	dam	CpG	
MboII	CutSmart	100*	100	50	100	37°	65°	C	Lambda dam-	dam		b
MfeI	CutSmart	75	50	10	100	37°	No	A	Lambda			2
MfeI-HF	CutSmart	75	25	< 10	100	37°	No	A	Lambda			
MluI	3.1	10	50	100	25	37°	80°	A	Lambda		CpG	
MluI-HF	CutSmart	25	100	100	100	37°	No	A	Lambda		CpG	
MluCI	CutSmart	100	10	10	100	37°	No	A	Lambda			
MlyI	CutSmart	50	50	10	100	37°	65°	A	Lambda			b, d
MmeI	CutSmart + SAM	50	100	50	100	37°	65°	B	phiX174		CpG	b, c
MnlI	CutSmart	75	100	50	100	37°	65°	B	Lambda			b
MscI	CutSmart	25	100	100	100	37°	80°	C	Lambda	dcm		
MseI	CutSmart	75	100	75	100	37°	65°	A	Lambda			
MslI	CutSmart	50	50	< 10	100	37°	80°	A	Lambda			
MspI	CutSmart	75	100	50	100	37°	No	A	Lambda			
MspA1I	CutSmart	10	50	10	100	37°	65°	B	Lambda		CpG	
MspJI	CutSmart	< 10	< 10	< 10	100	37°	65°	B	pBR322			1, e
MwoI	CutSmart	< 10	100	100	100	60°	No	B	Lambda		CpG	
NaeI	CutSmart	25	25	< 10	100	37°	No	A	pXba		CpG	b
NarI	CutSmart	100	100	10	100	37°	65°	A	pXba		CpG	

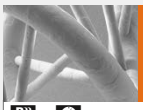
a. Ligation is less than 10%
b. Ligation is 25% – 75%
c. Recutting after ligation is < 5%
d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
Nb.BbvCI	CutSmart	25	100	100	100	37°	80°	A	pUB			e
Nb.BsmI	3.1	< 10	50	100	10	65°	80°	A	pBR322			e
Nb.BsrDI	CutSmart	25	100	100	100	65°	80°	A	pUC19			e
Nb.BssSI	3.1	10	100	100	25	37°	No	B	pUC19			e
Nb.BtsI	CutSmart	75	100	75	100	37°	80°	A	phiX174			e
NciI	CutSmart	100	25	10	100	37°	No	A	Lambda		CpG	b
NcoI	3.1	100	100	100	100	37°	80°	A	Lambda			
NcoI-HF	CutSmart	50	100	10	100	37°	80°	B	Lambda			
NdeI	CutSmart	75	100	100	100	37°	65°	A	Lambda			
NgoMIV	CutSmart	100	50	10	100	37°	No	A	pXba		CpG	1
NheI	2.1	100	100	10	100	37°	65°	C	Lambda HindIII		CpG	
NheI-HF	CutSmart	100	25	< 10	100	37°	80°	C	Lambda HindIII		CpG	
NlaIII	CutSmart	< 10	< 10	< 10	100	37°	65°	B	phiX174			
NlaIV	CutSmart	10	10	10	100	37°	65°	B	pBR322	dcm	CpG	
NmeAIII	CutSmart + SAM	10	10	< 10	100	37°	65°	B	phiX174			c
NotI	3.1	< 10	50	100	25	37°	65°	C	pBC4		CpG	
NotI-HF	CutSmart	25	100	25	100	37°	65°	A	pBC4		CpG	
NruI	3.1	< 10	10	100	10	37°	No	A	Lambda	dam	CpG	b
NruI-HF	CutSmart	0	25	50	100	37°	No	A	Lambda	dam	CpG	
NsiI	3.1	10	75	100	25	37°	65°	B	Lambda			
NsiI-HF	CutSmart	< 10	20	< 10	100	37°	80°	B	Lambda			
NspI	CutSmart	100	100	< 10	100	37°	65°	A	Lambda			
Nt.AlwI	CutSmart	10	100	100	100	37°	80°	A	pUC101 dam-dcm-	dam		e
Nt.BbvCI	CutSmart	50	100	10	100	37°	80°	A	pUB		CpG	e
Nt.BsmAI	CutSmart	100	50	10	100	37	65°	A	pBR322		CpG	e
Nt.BspQI	3.1	< 10	25	100	10	50°	80°	B	pUC19			e
Nt.BstNBI	3.1	0	10	100	10	55°	80°	A	T7			e
Nt.CviPII	CutSmart	10	100	25	100	37°	65°	A	pUC19		CpG	e
PacI	CutSmart	100	75	10	100	37°	65°	A	pNEB193			
PaeR7I	CutSmart	25	100	10	100	37°	No	A	Lambda HindIII		CpG	
PciI	3.1	50	75	100	50*	37°	80°	B	pXba			
PflFI	CutSmart	25	100	25	100	37°	65°	A	pBC4			b
PflMI	3.1	0	100	100	50	37°	65°	A	Lambda	dcm		3, b, d
PI-PspI	U	10	10	10	10	65°	No	B	pAKR XmnI			
PI-SceI	U	10	10	10	10	37°	65°	B	pBSvdeX XmnI			
PleI	CutSmart	25	50	25	100	37°	65°	A	Lambda		CpG	b, d
PluTI	CutSmart	100	25	< 10	100	37°	65°	A	pXba		CpG	b
PmeI	CutSmart	< 10	50	10	100	37°	65°	A	Lambda		CpG	
PmlI	CutSmart	100	50	< 10	100	37°	65°	A	Lambda HindIII		CpG	
PpuMI	CutSmart	< 10	< 10	< 10	100	37°	No	B	Lambda HindIII	dcm		
PshAI	CutSmart	25	50	10	100	37°	65°	A	Lambda		CpG	
PsiI	CutSmart	10	100	10	100	37°	65°	B	Lambda			3
PspGI	CutSmart	25	100	50	100	75°	No	A	T7	dcm		3
PspOMI	CutSmart	10	10	< 10	100	37°	65°	B	pXba	dcm	CpG	
PspXI	CutSmart	< 10	100	25	100	37°	No	B	Lambda HindIII		CpG	
PstI	3.1	75	75	100	50*	37°	80°	C	Lambda			
PstI-HF	CutSmart	10	75	50	100	37°	No	C	Lambda			
PvuI	3.1	< 10	25	100	< 10	37°	No	B	pXba		CpG	
PvuI-HF	CutSmart	25	100	100	100	37°	No	B	pXba		CpG	
PvuII	3.1	50	100	100	100*	37°	No	B	Lambda			
PvuII-HF	CutSmart	< 10	< 10	< 10	100	37°	No	B	Lambda			

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.

Performance Chart for Restriction Enzymes (continued)



ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS 1.1	2.1	3.1	CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
RsaI	CutSmart	25	50	< 10	100	37°	No	A	Lambda		CpG	
RsrII	CutSmart	25	75	10	100	37°	65°	C	Lambda		CpG	
SacI	1.1	100	50	10	100	37°	65°	A	Lambda HindIII			
SacI-HF	CutSmart	10	50	< 10	100	37°	65°	A	Lambda HindIII		CpG	
SacII	CutSmart	10	100	10	100	37°	65°	A	pXba		CpG	
SalI	3.1	< 10	< 10	100	< 10	37°	65°	A	Lambda HindIII		CpG	
SalI-HF	CutSmart	10	100	100	100	37°	65°	A	Lambda HindIII		CpG	
SapI	CutSmart	75	50	< 10	100	37°	65°	B	Lambda			
Sau3AI	1.1	100	50	10	100	37°	65°	A	Lambda		CpG	b
Sau96I	CutSmart	50	100	100	100	37°	65°	A	Lambda	dcm	CpG	
SbfI	CutSmart	50	25	< 10	100	37°	80°	A	Lambda			3
SbfI-HF	CutSmart	50	25	< 10	100	37°	80°	B	Lambda			
ScaI-HF	CutSmart	100	100	10	100	37°	80°	B	Lambda			
ScrFI	CutSmart	100	100	100	100	37°	65°	C	Lambda	dcm	CpG	2, a
SexAI	CutSmart	100	75	50	100	37°	65°	A	pBC4 dcm-	dcm		3, b, d
SfaNI	3.1	< 10	75	100	25	37°	65°	B	phiX174		CpG	3, b
SfcI	CutSmart	75	50	25	100	37°	65°	B	Lambda			3
SfiI	CutSmart	25	100	50	100	50°	No	C	pXba	dcm	CpG	
SfoI	CutSmart	50	100	100	100	37°	No	B	Lambda HindIII	dcm	CpG	
SgrAI	CutSmart	100	100	10	100	37°	65°	A	Lambda		CpG	1
SmaI	CutSmart	< 10	< 10	< 10	100	25°	65°	B	Lambda HindIII		CpG	b
SmlI	CutSmart	25	75	25	100	55°	No	A	Lambda			b
SnaBI	CutSmart	50	50	10	100	37°	80°	A	T7		CpG	1
SpeI	CutSmart	75	100	25	100	37°	80°	C	Adenovirus-2			
SpeI-HF	CutSmart	25	50	10	100	37°	80°	C	pXba			
SphI	2.1	100	100	50	100	37°	65°	B	Lambda			2
SphI-HF	CutSmart	50	25	10	100	37°	65°	B	Lambda			
SrfI	CutSmart	10	50	0	100	37°	65°	B	pNEB193-SrfI		CpG	
SspI	U	50	100	50	50	37°	65°	C	Lambda			
SspI-HF	CutSmart	25	100	< 10	100	37°	65°	B	Lambda			
StuI	CutSmart	50	100	50	100	37°	No	A	Lambda	dcm		
StyD4I	CutSmart	10	100	100	100	37°	65°	B	Lambda	dcm	CpG	
StyI	3.1	10	25	100	10	37°	65°	A	Lambda			b
StyI-HF	CutSmart	25	100	25	100	37°	65°	A	Lambda			
SwaI	3.1	10	10	100	10	25°	65°	B	pXba			b, d
Taq$^{\alpha}$I	CutSmart	50	75	100	100	65°	80°	B	Lambda	dam		
TfiI	CutSmart	50	100	100	100	65°	No	C	Lambda		CpG	
TseI	CutSmart	75	100	100	100	65°	No	B	Lambda		CpG	3
Tsp45I	CutSmart	100	50	< 10	100	65°	No	A	Lambda			
TspMI	CutSmart	50*	75*	50*	100	75°	No	B	pUCAdeno		CpG	d
TspRI	CutSmart	25	50	25	100	65°	No	B	Lambda			
Tth111I	CutSmart	25	100	25	100	65°	No	B	pBC4			b
XbaI	CutSmart	< 10	100	75	100	37°	65°	A	Lambda HindIII dam-	dam		
XcmI	2.1	10	100	25	100	37°	65°	C	Lambda			2
XhoI	CutSmart	75	100	100	100	37°	65°	A	Lambda HindIII			b
XmaI	CutSmart	25	50	< 10	100	37°	65°	A	pXba		CpG	3
XmnI	CutSmart	50	75	< 10	100	37°	65°	A	Lambda			b
ZraI	CutSmart	100	25	10	100	37°	80°	B	Lambda		CpG	

a. Ligation is less than 10%
b. Ligation is 25% – 75%
c. Recutting after ligation is < 5%
d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

Activity of Enzymes at 37°C

Listed below is the percentage of activity exhibited at 37°C for enzymes that have an optimal incubation temperature higher (thermophiles) or lower (25°C) than 37°C.

ENZYME	OPTIMAL TEMP. (°C)	% ACTIVITY AT 37°C
AbaSI	25°	0
ApaI	25°	100*
ApeKI	75°	10
ApoI	50°	50
BaeI	25°	20
BclI	50°	50
BfuAI	50°	50
BsaBI	60°	20
BsaJI	60°	20
BsaWI	60°	20
BsiEI	60°	30
BsiHKAI	65°	5
BsiWI	55°	50
BslI	55°	30
BsmAI	55°	50
BsmBI	55°	20
BsmFI	65°	50
BsmI	65°	20

ENZYME	OPTIMAL TEMP. (°C)	% ACTIVITY AT 37°C
BspCNI	25°	75
BspQI	50°	10
BsrI	65°	20
BsrDI	65°	30
BssHII	50°	75
BstAPI	60°	10
BstBI	65°	10
BstEII	60°	50
BstNI	60°	30
BstUI	60°	20
BstYI	60°	30
BtgZI	60°	75
BtsI-v2	55°	75
BtsCI	50°	50
BtsIMutI	55°	N/A
CviAII	25°	20
CviQI	25°	10
FatI	55°	20

ENZYME	OPTIMAL TEMP. (°C)	% ACTIVITY AT 37°C
FauI	55°	20
MwoI	60°	10
Nb.BsmI	65°	25
Nb.BsrDI	65°	75
Nt.BspQI	50°	80
Nt.BstNBI	55°	10
PI-PspI	65°	5
PspGI	75°	10
SfiI	50°	10
SmaI	25°	50
SmlI	55°	10
SwaI	25°	50
TaqαI	65°	10
TfiI	65°	10
TseI	65°	20
Tsp45I	65°	10
TspMI	75°	20
TspRI	65°	10
Tth111I	65°	10

*ApaI has 100% activity at 37°C, however the half-life of this enzyme at 37°C is only 30 minutes.

Activity of DNA Modifying Enzymes in CutSmart Buffer

A selection of DNA modifying enzymes were assayed in CutSmart Buffer, in lieu of their supplied buffers. Functional activity was compared to the activity in its supplied buffer, plus required supplements. Reactions were set up according to the recommended reaction conditions, with CutSmart Buffer replacing the supplied buffer.

ENZYME	ACTIVITY IN CUTSMART	REQUIRED SUPPLEMENTS
Alkaline Phosphatase (CIP)	+++	
Antarctic Phosphatase	+++	Requires Zn^{2+}
Bst DNA Polymerase	+++	
CpG Methyltransferase (M. SssI)	+++	
DNA Polymerase I	+++	
DNA Polymerase I, Large (Klenow) Fragment	+++	
DNA Polymerase Klenow Exo$^-$	+++	
DNase I (RNase-free)	+++	Requires Ca^{2+}
E. coli DNA Ligase	+++	Requires NAD
Endonuclease III (Nth), recombinant	+++	
Endonuclease VIII	+++	
Exonuclease I	+++	
Exonuclease III	+++	
Exonuclease VII	+++	
Exonuclease V (Rec BCD)	+++	Requires ATP
GpC Methyltransferase (M. CviPI)	+	Requires DTT
Lambda Exonuclease	++	
McrBC	+++	

ENZYME	ACTIVITY IN CUTSMART	REQUIRED SUPPLEMENTS
Micrococcal Nuclease	+++	Requires Ca^{2+}
Nuclease Bal-31	+++	
phi29 DNA Polymerase	+++	
Quick Dephosphorylation Kit	+++	
$RecJ_f$	+++	
Shrimp Alkaline Phosphatase (rSAP)	+++	
T3 DNA Ligase	+++	Requires ATP + PEG
T4 DNA Ligase	+++	Requires ATP
T4 DNA Polymerase	+++	
T4 Phage β-glucosyltransferase (T4-BGT)	+++	
T4 Polynucleotide Kinase	+++	Requires ATP + DTT
T4 PNK (3´ phosphatase minus)	+++	Requires ATP + DTT
T5 Exonuclease	+++	
T7 DNA Ligase	+++	Requires ATP + PEG
T7 DNA Polymerase (unmodified)	+++	
T7 Exonuclease	+++	
Thermolabile ExoI	+++	
USER Enzyme, recombinant	+++	

+++ full functional activity
++ 50–100% functional activity
+ 0–50% functional activity

Tips for Avoiding Star Activity

Under non-standard reaction conditions, some restriction enzymes are capable of cleaving sequences which are similar but not identical to their defined recognition sequence. This altered specificity has been termed "star activity". Although the propensity for star activity varies, the vast majority of enzymes from New England Biolabs will not exhibit star activity when used under recommended conditions in their supplied NEBuffers. If an enzyme has been reported to exhibit star activity, it will be indicated in the product entry found in the catalog, on the supplied card and on our website.

CONDITIONS THAT CONTRIBUTE TO STAR ACTIVITY	STEPS THAT CAN BE TAKEN TO INHIBIT STAR ACTIVITY
High glycerol concentration (> 5% v/v)	Restriction enzymes are stored in 50% glycerol, therefore the amount of enzyme added should not exceed 10% of the total reaction volume. Use the standard 50 µl reaction volume to reduce evaporation during incubation.
High concentration of enzyme/µg of DNA ratio (varies with each enzyme, usually 100 units/µg)	Use the fewest units possible to achieve digestion. This avoids overdigestion and reduces the final glycerol concentration in the reaction.
Non-optimal buffer	Whenever possible, set up reactions in the recommended buffer. Buffers with differing ionic strengths and pHs may contribute to star activity.
Prolonged reaction time	Use the minimum reaction time required for complete digestion. Prolonged incubation may result in increased star activity, as well as evaporation.
Presence of organic solvents [DMSO, ethanol (4), ethylene glycol, dimethylacetamide, dimethylformamide, sulphalane (5)]	Make sure the reaction is free of any organic solvents, such as alcohols, that might be present in the DNA preparation.
Substitution of Mg^{2+} with other divalent cations (Mn^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+})	Use Mg^{2+} as the divalent cation. Other divalent cations may not fit correctly into the active site of the restriction enzyme, possibly interfering with proper recognition.

Note: The relative significance of each of these altered conditions will vary from enzyme to enzyme.

New England Biolabs recommends setting up restriction enzyme digests in a 50 µl reaction volume. However, different methods may require smaller reaction volumes. When performing restriction enzyme digests in smaller reaction volumes, extra care must be taken to follow the steps listed above to avoid star activity. Alternatively, using our line of **High Fidelity (HF) restriction enzymes** will allow greater flexibility in reaction setup. Please visit **www.neb.com/HF** frequently to learn about new additions to the HF restriction enzyme product line.

Reference:

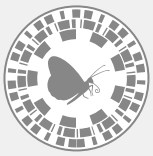
(1) Nasri, M. and Thomas, D. (1986) *Nucleic Acids Res.* 14, 811.

TOOLS & RESOURCES

Visit NEBRestrictionEnzymes.com to find:

- Online tutorials on how to avoid star activity, and for setting up restriction enzyme digests
- The full list of HF enzymes available
- Troubleshooting guides

Giron has been with NEB for over 2 years as a Development Scientist. Giron's family lives in Germany and he frequently travels to spend time with them. Giron is also an experienced spiritual teacher and feng shui consultant, as well as a skilled violinist.



Learn about the benefits of HF enzymes.

High-Fidelity (HF) Restriction Enzymes

As part of our ongoing commitment to the study and improvement of restriction enzymes, NEB offers a line of High-Fidelity (HF) restriction enzymes. These engineered enzymes have the same specificity as the native enzymes, are all active in CutSmart Buffer and have reduced star activity. Star activity, or off-target cleavage, is an intrinsic property of restriction enzymes. Most restriction enzymes will not exhibit star activity when used under recommended reaction conditions. However, for enzymes that have reported star activity, extra caution must be taken to set up reactions according to the recommended conditions to avoid unwanted cleavage.

Many techniques such as cloning, genotyping, mutational analysis, mapping, probe preparation, sequencing and methylation detection employ a wide range of reaction conditions and require the use of enzymes under suboptimal conditions. HF enzymes with reduced star activity offer increased flexibility to reaction setup and help maximize results under a wide range of conditions.

In addition to reduced star activity, all of these engineered enzymes work optimally in CutSmart Buffer, which has the highest level of enzyme compatibility and will simplify double digest reactions. They are all Time-Saver qualified and digest substrate DNA in 5–15 minutes, and can also be incubated overnight without degredation of DNA. HF enzymes are available at the same price as the native enzymes and are supplied with purple loading dye.

Visit **www.neb.com/HF** to learn more about HF enzymes.

TOOLS & RESOURCES

Visit NEBRestrictionEnzymes.com to find:

- The full list of HF enzymes available
- Online tutorials on how to avoid star activity and setting up digests using the Time-Saver protocol

Reduced Star Activities of HF Enzymes

The following table indicates the number of units of HF enzyme that can be used compared to the native enzyme before any significant star activity is detected. The HF Factor refers to the X-fold increase in fidelity that is achieved by choosing an HF enzyme. This data clearly illustrates the flexibility that is offered by using an HF restriction enzyme.

PRODUCT NAME	PRODUCT NUMBER	BUFFER†	MAXIMUM UNITS WITH NO STAR ACTIVITY*	HF FACTOR
AgeI-HF	#R3552	CutSmart	≥ 250	≥ 8
AgeI	#R0552	1.1	32	
ApoI-HF	#R3566	CutSmart	500	25
ApoI	#R0566	3.1	20	
BamHI-HF	#R3136	CutSmart	≥ 4,000	≥ 125
BamHI	#R0136	3.1	32	
BbsI-HF	#R3539	CutSmart	≥ 500	≥ 4
BbsI	#R0539	2.1	120	
BclI-HF	#R3160	CutSmart	500	16
BclI	#R0160	3.1	32	
BmtI-HF	#R3658	CutSmart	1,000,000	62,500
BmtI	#R0658	3.1	32	
BsaI-HFv2	#R3733	CutSmart	500	16
BsaI	#R0535	CutSmart	32	
BsiWI-HF	#R3553	CutSmart	100	1
BsiWI	#R0553	3.1	100	
BsrGI-HF	#R3575	CutSmart	≥ 2,000	≥ 62
BsrGI	#R0575	2.1	16	
BstEII-HF	#R3162	CutSmart	> 2,000	> 125
BstEII	#R0162	3.1	16	
BstZ17I-HF	#R3594	CutSmart	500	25
BstZ17I**	N/A	CutSmart	20	
DraIII-HF	#R3510	CutSmart	≥ 2,000	≥ 1,000
DraIII**	N/A	3.1	2	
EagI-HF	#R3505	CutSmart	500	2
EagI	#R0505	3.1	250	
EcoRI-HF	#R3101	CutSmart	16,000	64
EcoRI	#R0101	U	250	
EcoRV-HF	#R3195	CutSmart	≥ 64,000	≥ 64
EcoRV	#R0195	3.1	1,000	
HindIII-HF	#R3104	CutSmart	≥ 500,000	≥ 2,000
HindIII	#R0104	2.1	250	
KpnI-HF	#R3142	CutSmart	≥ 1,000,000	≥ 62,500
KpnI	#R0142	1.1	16	
MfeI-HF	#R3589	CutSmart	≥ 500	≥ 16
MfeI	#R0589	CutSmart	32	

PRODUCT NAME	PRODUCT NUMBER	BUFFER†	MAXIMUM UNITS WITH NO STAR ACTIVITY*	HF FACTOR
MluI-HF	#R3198	CutSmart	≥ 4,000	2
MluI	#R0198	3.1	≥ 2,000	
NcoI-HF	#R3193	CutSmart	≥ 64,000	≥ 530
NcoI	#R0193	3.1	120	
NheI-HF	#R3131	CutSmart	≥ 32,000	≥ 266
NheI	#R0131	2.1	120	
NotI-HF	#R3189	CutSmart	≥ 64,000	≥ 16
NotI	#R0189	3.1	4,000	
NruI-HF	#R3192	CutSmart	≥ 32,000	64
NruI	#R0192	3.1	≥ 500	
NsiI-HF	#R3127	CutSmart	≥ 8,000	2
NsiI	#R0127	3.1	≥ 4,000	
PstI-HF	#R3140	CutSmart	4,000	33
PstI	#R0140	3.1	120	
PvuI-HF	#R3150	CutSmart	≥ 16,000	≥ 32
PvuI	#R0150	3.1	500	
PvuII-HF	#R3151	CutSmart	500	32
PvuII	#R0151	3.1	16	
SacI-HF	#R3156	CutSmart	≥ 32,000	≥ 266
SacI	#R0156	1.1	120	
SalI-HF	#R3138	CutSmart	≥ 32,000	≥ 8,000
SalI	#R0138	3.1	4	
SbfI-HF	#R3642	CutSmart	250	32
SbfI	#R0642	CutSmart	8	
ScaI-HF	#R3122	CutSmart	250	62
ScaI**	N/A	3.1	4	
SpeI-HF	#R3133	CutSmart	≥ 8,000	≥ 16
SpeI	#R0133	CutSmart	500	
SphI-HF	#R3182	CutSmart	8,000	250
SphI	#R0182	2.1	32	
SspI-HF	#R3132	CutSmart	500	16
SspI	#R0132	U	32	
StyI-HF	#R3500	CutSmart	4,000	125
StyI	#R0500	3.1	32	

† Wild type enzymes were tested in supplied buffer for comparisons.
* Wei, H. et al (2008) *Nucleic Acids Reseach* 36, e50.
** No longer available.

Time-Saver Qualified Restriction Enzymes

Whether you are quickly screening large numbers of clones or setting up overnight digests, you will benefit from the high quality of our enzymes. Typically, a restriction digest involves the incubation of 1 µl of enzyme with 1 µg of purified DNA in a final volume of 50 µl for 1 hour. However, to speed up the screening process, choose one of NEB's enzymes that are Time-Saver qualified. Over 185 of our enzymes will digest 1 µg of substrate DNA in 5-15 minutes using 1 µl of enzyme under recommended reaction conditions, and can also be used safely in overnight digestions. Unlike other suppliers, there is no special formulation, change in concentration or need to buy more expensive, new lines of enzymes to achieve digestion in 5-15 minutes. Nor do you have to worry if you incubate too long.

In an effort to provide you with as much information as possible, NEB has tested all of its enzymes on unit assay substrate as well as plasmid substrate and PCR Fragments. We recommend that this data be used as a guide, as it is not definitive for all plasmids. Restriction enzymes can often show site preference, presumably determined by the sequence flanking the recognition site. In addition, supercoiled DNA may have varying rates of cleavage. For more information, visit **www.neb.com/TimeSaver**. Note that there are some enzymes indicated below that can cut in 5-15 minutes, but cannot be incubated overnight. These are not Time-Saver qualified.

Since all of our enzymes are rigorously tested for nuclease contamination, you can also safely set up digests for long periods of time without sample degradation. Only NEB Time-Saver qualified enzymes offer power and flexibility – the power to digest in 5-15 minutes and the flexibility to withstand overnight digestions with no loss of substrate.

TOOLS & RESOURCES

Visit www.neb.com/TimeSaver to find:

- The full list of Time-Saver qualified restriction enzymes available
- Online tutorials on using Time-Saver qualified enzymes to speed up restriction enzyme digests

Chart Legend

- ● digests in 5 minutes
- ■ digests in 15 minutes
- ▲ not completely digested in 15 minutes

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
AatII	■	▲	●
AccI	■	▲	▲
Acc65I	●	▲	●
AciI	●	●	●
AclI	●	■	▲
AcuI	■	▲	▲
AflIII	●	●	●
AgeI-HF	●	●	●
AhdI	●	●	■
AluI	●	▲	●
AlwNI	●	●	▲
ApaI	●	●	●
ApaLI	●	●	▲
ApeKI	●	■	▲
ApoI	●	●	●
ApoI-HF	■	■	▲
AscI	●	●	NT
AseI	●	●	NT
AvaI	●	▲	▲
AvaII	●	●	●
AvrII	●	NT	NT
BaeI	■	●	▲
BaeGI	●	▲	▲
BamHI	●	●	▲
BamHI-HF	●	●	●
BbsI	■	▲	▲
BbsI-HF	■	▲	▲
BbvI	●	▲	▲
BccI	■	▲	▲
BceAI	■	■	▲
BciVI	●	■	▲
BclI	●	▲	▲
BclI-HF	■	▲	▲
BcoDI	●	●	▲

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
BfuAI	●	●	▲
BfuCI	■	▲	●
BglI	●	●	▲
BglII	●	■	▲
BlpI	●	●	●
BmgBI	●	●	▲
BmrI	■	▲	■
BmtI-HF	●	●	▲
BpuEI	●	●	▲
BsaI	●	●	■
BsaI-HFv2	■	■	▲
BsaAI	●	●	■
BsaHI	■	■	●
BsaWI	■	▲	▲
BsaXI	●	▲	▲
BseRI	●	●	■
BsgI	●	●	▲
BsiEI	●	▲	▲
BsiHKAI	●	●	▲
BsiWI	●	●	▲
BsiWI-HF	■	■	▲
BsmI	●	■	■
BsmI	●	●	▲
BsmAI	●	▲	●
BsmBI	■	▲	▲
BsmFI	●	●	▲
BsoBI	●	■	●
Bsp1286I	●	●	▲
BspCNI	■	▲	▲
BspEI	●	▲	▲
BspHI	■	●	●
BspQI	●	●	▲
BsrI	●	■	▲
BsrBI	●	■	▲
BsrDI	●	■	▲

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
BsrFI-v2	■	▲	▲
BsrGI	■	▲	▲
BsrGI-HF	■	■	▲
BssHII	●	▲	▲
BssSI-v2	■	▲	▲
BstBI	●	●	▲
BstEII	●	●	▲
BstEII-HF	●	●	●
BstNI	●	●	▲
BstUI	●	●	▲
BstXI	●	●	▲
BstYI	■	●	▲
BstZ17I-HF	■	■	▲
Bsu36I	■	▲	■
BtgI	●	●	■
BtsI-v2	■	■	■
BtsCI	●	■	▲
Cac8I	■	▲	▲
ClaI	●	●	▲
CspCI	●	●	▲
CviAII	■	●	●
CviQI	●	●	●
DdeI	●	■	■
DpnI	●	●	▲
DpnII	■	▲	●
DraI	●	●	■
DraIII-HF	●	●	▲
DrdI	■	●	●
EagI	●	▲	▲
EagI-HF	■	■	▲
EarI	■	■	▲
Eco53KI	●	●	■
EcoNI	●	■	●
Eco0109I	●	▲	▲
EcoP15I	■	▲	▲

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
EcoRI	●	●	▲
EcoRI-HF	●	●	●
EcoRV	●	●	▲
EcoRV-HF	●	●	▲
Esp3I	■	■	▲
Fnu4HI	●	■	■
FokI	●	●	●
FseI	●	●	▲
FspI	■	▲	■
HaeII	■	▲	▲
HaeIII	●	●	●
HgaI	■	▲	▲
HhaI	●	■	▲
HincII	■	▲	●
HindIII-HF	●	●	●
HinfI	●	●	●
HinP1I	●	▲	●
HpaII	●	●	▲
HphI	●	▲	▲
Hpy166II	●	●	●
HpyAV	●	●	NT
HpyCH4IV	●	●	●
HpyCH4V	●	●	●
KpnI	●	●	●
KpnI-HF	●	●	●
MboI	●	▲	●
MboII	●	●	●
MfeI	●	●	●
MfeI-HF	●	●	●
MluI	●	●	●
MluI-HF	●	●	▲
MluCI	●	●	▲
MlyI	●	▲	●
MmeI	●	●	▲
MnlI	●	●	■
MseI	■	■	●

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
MslI	●	●	●
MspI	●	●	●
MspA1I	●	●	●
MwoI	■	▲	▲
NciI	●	●	●
NcoI	●	■	▲
NcoI-HF	●	●	●
NdeI	●	●	▲
NgoMIV	■	●	▲
NheI	●	■	▲
NheI-HF	●	●	■
NlaIII	■	▲	■
NmeAIII	●	▲	▲
NotI	●	●	▲
NotI-HF	●	●	●
NruI	●	■	▲
NruI-HF	■	■	▲
NsiI	●	●	●
NsiI-HF	●	●	■
NspI	●	■	▲
PacI	●	●	●
PaeR7I	●	▲	▲
PflFI	●	■	▲
PflMI	●	▲	▲
PmeI	●	■	NT
PmlI	●	▲	▲
PpuMI	●	▲	▲
PshAI	■	■	■
PstI	●	●	●
PstI-HF	●	●	●
PvuI	●	▲	●
PvuI-HF	●	●	●
PvuII	●	●	▲
PvuII-HF	●	●	▲
RsaI	●	●	●
SacI	●	●	▲

ENZYME	UNIT ASSAY	SUBSTRATE PLASMID	SUBSTRATE PCR
SacI-HF	●	●	●
SacII	●	▲	▲
SalI	●	■	▲
SalI-HF	●	●	▲
SapI	■	▲	▲
SbfI	●	●	▲
SbfI-HF	●	●	▲
ScaI-HF	●	●	▲
SfaNI	▲	▲	■
SfiI	●	▲	▲
SfoI	●	●	●
SmaI	●	■	■
SpeI	●	●	●
SpeI-HF	■	■	▲
SphI	●	●	▲
SphI-HF	●	●	▲
SrfI	■	■	▲
SspI	●	●	▲
SspI-HF	●	●	▲
StuI	■	▲	▲
StyI	■	▲	▲
StyI-HF	●	●	▲
StyD4I	■	▲	▲
SwaI	■	▲	▲
Taq$^{\alpha}$I	●	●	▲
TfiI	■	●	▲
TseI	■	▲	▲
TspMI	●	■	▲
TspRI	●	■	▲
Tth111I	■	■	▲
XbaI	●	●	▲
XhoI	●	●	▲
XmaI	■	▲	■
XmnI	●	●	▲

Learn more about Time-Saver qualified enzymes.

Cross Index of Recognition Sequences

Sequences at the top of each column are written 5´ to 3´ according to convention. Open squares at the left of each row are place holders for nucleotides within a restriction enzyme recognition sequence; arrowheads indicate the point of cleavage.

Sequences of complementary strands and their cleavage sites are implied.

blue type = enzymes that recognize only one sequence

black type = enzymes that recognize multiple sequences (degenerate)

Palindromic Tetra- and Hexa-Nucleotide Recognition Sequences

	AATT	ACGT	AGCT	ATAT	CATG	CCGG	CGCG	CTAG	GATC	GCGC	GGCC	GTAC	TATA	TCGA	TGCA	TTAA
▼□□□□	MluCI				FatI				BfuCI DpnII MboI Sau3AI							
□▼□□□		HpyCH2IV			CviAII	MspI HpaII		BfaI		HinP1I		Csp6I CviQI		TaqI		MseI
□□▼□□			AluI CviKI-1				BstUI		DpnI		HaeIII PhoI CviKI-1	RsaI			HpyCH4V	
□□□▼□									BstKTI	HhaI						
□□□□▼		TaiI			NlaIII											
A▼□□□□T	ApoI ●		HindIII ●		PciI AflIII	AgeI ● BsrFI BsaWI	MluI ● AflIII	SpeI ●	BglII BstYII			TatI				
A□▼□□□T		AclI												ClaI BspDI		AseI
A□□▼□□T				SspI ●						AfeI	StuI	ScaI ●				
A□□□▼□T																
A□□□□▼T					NspI					HaeII					NsiI ●	
C▼□□□□G	MfeI ●				NcoI ● StyI ● BtgI BsaJI	TspMI XmaI AccI AvaI	BsaJI BtgI	AvrII BsaJI StyI ●			EagI ● EaeI	BsiWI ●	SfcI	PaeR7I XhoI AvaI	SfcI	AflII SmlI
C□▼□□□G				NdeI		BsaJI BsoBI BmeT110I								BsoBI SmlI BmeT110I		
C□□▼□□G		PmlI BsaAI	PvuII ● MspA1I			SmaI	MspA1I									
C□□□▼□G							SacII		PvuI ● BsiEI		BsiEI					
C□□□□▼G															PstI ●	
G▼□□□□C	EcoRI ● ApoI ●					NgoMIV BsrFI	BssHII	NheI ●	BamHI ● BstYI	KasI BanI	PspOMI	Acc65I BanI		SalI ●	ApaLI	
G□▼□□□C		BsaHI								NarI BsaHI			AccI	AccI		
G□□▼□□C		ZraI	EcI136II Eco53KI	EcoRV ●	Cac8I	NaeI Cac8I	Cac8I	Cac8I	NlaIV	SfoI NlaIV	NlaIV	NlaIV	BstZ17I ● Hpy8I Hpy166II	HincII Hpy8I Hpy166II	Hpy8I Hpy166II	HpaI HincII Hpy8I Hpy166II
G□□□▼□C																
G□□□□▼C		AatII	SacI ● BanII BsiHKAI Bsp1286I		SphI ● NspI			BmtI ●		PluTI BbeI HaeII	ApaI BanII BaeGI Bsp1286I	KpnI ●			BaeGI Bsp1286I BsiHKAI	
T▼□□□□A					BspHI	BspEI BsaWI AcoI		XbaI	BclI ●		EaeI	BsrGI ● TatI				
T□▼□□□A					Hpy188III	Hpy188III	Hpy188III	Hpy188III						BstBI		
T□□▼□□A		SnaBI BsaAI					NruI ●			FspI	MscI		PsiI			DraI
T□□□▼□A																
T□□□□▼A																

● HF (High-Fidelity) versions of these enzymes are available for simplified reactions and reduced star activity, at no additional cost. See page 301.

APPENDIX

Palindromic Penta-Nucleotide Recognition Sequences

	AA □ TT	AC □ GT	AG □ CT	AT □ AT	CA □ TG	CC □ GG	CG □ CG	CT □ AG	GA □ TC	GC □ GC	GG □ CC	GT □ AC	TA □ TA	TC □ GA	TG □ CA	TT □ AA
▼□□ N □□						BssKI StyD4I						MaeIII				
□□▼ N □□								DdeI	HinfI		Sau96I					
□□ N▼□□						ScrFI				Fnu4HI	BmgT120I					
□□ N □□▼		HpyCH4III												Hpy188I		
□□ N ▼□□											FmuI					
□□ N □□▼																
▼□□ A T □□						PspGI										
□□▼ A T □□									TfiI	ApeKI TseI	AvaII					
□□ A T▼□□						BstNI										
□□ A T ▼□□																
□□ A T □□▼							Hpy99I									
▼□□ G C □□												Tsp45I				
□□▼ G C □□																
□□ G C▼□□						NciI										
□□ G C ▼□□																
□□ G C □□▼										TauI BspUI						
□□ G C □□▼																

Single Letter Code:

R = A or G
K = G or T
H = A or C or T
V = A or C or G
N = A or C or G or T
Y = C or T
S = C or G
M = A or C
W = A or T
B = C or G or T
D = A or G or T

In addition, see homing endonucleases on pages 55–56.

Note:

Enzymes marked with a "▲" are available from NEB.

● HF (High-Fidelity) versions of these enzymes are available for simplified reactions and reduced star activity, at no additional cost. See page 301.

Enzymes marked with a "⊗" are not currently commercially available.

SPECIFICITIES GREATER THAN 6 BASES

AarI	CACCTGC(4/8)
Aba6411II⊗	CRRTAAG
AbaCIII⊗	CTATCAV
AbsI	CC/TCGAGG
AcoY31II⊗	TAGCRAB
AhyRBAHI⊗	GCYYGAC
AhyYL17I⊗	YAAMGAG
AjuI	(7/12)GAANNNNNNNTTGG(11/6)
AloI	(7/12)GAACNNNNNNTCC(12/7)
AlwFI⊗	GAAAYNNNNNRTG
AquIV⊗	GRGGAAG(19/17)
ArsI	(8/13)GACNNNNNNTTYG(11/6)
▲ AscI	GG/CGCGCC
▲ AsiSI	GCGAT/CGC
Asp103I⊗	CGRAGGC
AspJHL3II⊗	CGCCCAG
AspNIH4III⊗	AAGAACB
Asp114pII⊗	AGCABCC
▲ BaeI	(10/15)ACNNNNGTAYC(12/7)
BarI	(7/12)GAAGNNNNNNTAC(12/7)
Bbr57III⊗	GTRAAYG
▲ BbvCI	CCTCAGC(-5/-2)
BkrAM31DI⊗	RTTAAATM
Ble402II⊗	GRAGCAG
Bsp460III⊗	CGCGCAG
▲ BspQI	GCTCTTC(1/4)
CalB3II⊗	GRTTRAG
Cbo67071IV⊗	GCRGAAG
CcrNAIII⊗	CGACCAG
Cdi81III⊗	GCMGAAG
Cgl13032II⊗	ACGABGG
Cly7489II⊗	AAAAGRG

SPECIFICITIES GREATER THAN 6 BASES (CONT.)

▲ CspCI	(11/13)CAANNNNNGTGG(12/10)
Ecl35734I⊗	GAAAYTC
Eco4465II⊗	GAAABCC
Eco43896II⊗	CRARCAG
▲ FseI	GGCCGG/CC
FspAI	RTGC/GCAY
FspPK15I⊗	GARGAAG
GauT27I⊗	CGCGCAGG
Jma19592II⊗	GRGCRAC
KfiI	GG/GWCCC
Kpn156V⊗	CRTGATT
Lmo370I⊗	AGCGCCG
Lsp6406VI⊗	CRAGCAC
MaqI⊗	CRTTGAC(21/19)
MauBI	CG/CGCGCG
Mcr10I⊗	GAAGNNNNNCTC
MkaDII⊗	GAGAYGT
MreI	CG/CCGGCG
MspSC27II⊗	CCGCGAC
MteI	GCGC/NGCGC
MtuHN878II⊗	CACGCAG
NhaXI⊗	CAAGRAG
▲ NotI ●	GC/GGCCGC
NpeUS61II⊗	GATCGAC
▲ PacI	TTAAT/TAA
Pal408I⊗	CCRTGAG
PasI	CC/CWGGG
PflPt14I⊗	RGCCCAC
PfrJS12V⊗	GGCGGAG
PinP23II⊗	CTRKCAG
PliMI⊗	CGCCGAC
▲ PmeI	GTTT/AAAC
PpiI⊗	(7/12)GAACNNNNNCTC(13/8)
PpiP13II⊗	CGCRGAC
▲ PpuMI	RG/GWCCY
Pse18267I⊗	RCCGAAG
PspOMII⊗	CGCCCAR(20/18)
▲ PspXI	VC/TCGAGB
PsrI	(7/12)GAACNNNNNNTAC(12/7)
Pst145I⊗	CTAMRAG
Pst273I⊗	GATCGAG
Rba2021I⊗	CACGAGH
RceI⊗	CATCGAC(20/18)
RpaI⊗	GTYGGAG(11/9)
RpaBI⊗	CCCGCAG(20/18)
RpaB5I⊗	CGRGGAC(20/18)
RpaTI⊗	GRTGGAG
RspPBTS2III⊗	CTTCGAG

SPECIFICITIES GREATER THAN 6 BASES (CONT.)

▲ RsrII	CG/GWCCG
▲ SapI	GCTCTTC(1/4)
▲ SbfI ●	CCTGCA/GG
SdeOSI⊗	(11/13)GACNNNNRTGA(12/10)
▲ SexAI	A/CCWGGT
▲ SfiI	GGCCNNNN/NGGCC
▲ SgrAI	CR/CCGGYG
SgrDI	CG/TCGACG
SmaUMH8I⊗	GCGAACB
Sno506I⊗	GGCCGAG
SpoDI⊗	GCGGRAG
▲ SrfI	GCCC/GGGC
Sse8647I⊗	AG/GWCCT
Ssp714II⊗	CGCAGCG
SstE37I⊗	CGAAGAC(20/18)
Sth20745III⊗	GGACGAC
▲ SwaI	ATTT/AAAT
TspARh3I⊗	GRACGAC
UbaF9I⊗	TACNNNNNRTGT
UbaF12I⊗	CTACNNNGTC
UbaF13I⊗	GAGNNNNNNCTGG
Vtu19109I⊗	CACRAYC

INTERRUPTED PALINDROMES

AgsI	TTS/AA
▲ AhdI	GACNNN/NNGTC
▲ AleI	CACNN/NNGTG
AlfI⊗	(10/12)GCANNNNNNTGC(12/10)
▲ AlwNI	CAGNNN/CTG
ApaBI⊗	GCANNNNN/TGC
▲ ApeKI	G/CWGC
▲ AvaII	G/GWCC
BdaI⊗	(10/12)TGANNNNNNTCA(12/10)
▲ BglI	GCCNNNN/NGGC
BisI	GC/NGC
▲ BlpI	GC/TNAGC
BlsI	GCN/GC
BpII	(8/13)GAGNNNNNCTC(13/8)
▲ BsaBI	GATNN/NNATC
▲ BsaJI	C/CNNGG
▲ BsaWI	W/CCGGW
▲ BsiHKAI	GWGCW/C
▲ BsmI	CCNNNNN/NNGG
▲ BstAPI	GCANNNN/NTGC
▲ BstEII ●	G/GTNACC
▲ BstNI	CC/WGG
▲ BstXI	CCANNNNN/NTGG

INTERRUPTED PALINDROMES (CONT.)	
▲ Bsu36I	CC/TNAGG
BthCI⊗	GCNG/C
▲ Cac8I	GCN/NGC
CjuI⊗	CAYNNNNNRTG
▲ DdeI	C/TNAG
Dde51507I⊗	CCWGG
▲ DraIII ●	CACNNN/GTG
▲ DrdI	GACNNNN/NNGTC
EcoHI⊗	/CCSGG
▲ EcoNI	CCTNN/NNNAGG
▲ Eco0109I	RG/GNCCY
FaiI	(8/13)AAGNNNNNCTT(13/8)
FmuI⊗	GGNC/C
▲ Fnu4HI	GC/NGC
HaeI⊗	WGG/CCW
HgiEII⊗	ACCNNNNNNGGT
▲ HinfI	G/ANTC
▲ Hpy99I	CGWCG/
▲ Hpy166II	GTN/NAC
▲ Hpy188I	TCN/GA
▲ Hpy188III	TC/NNGA
▲ HpyCH4III	ACN/GT

INTERRUPTED PALINDROMES (CONT.)	
HpyUM032XIII⊗	CYANNNNNNNTRG
HsoII⊗	(8/14)CAYNNNNNRTG(14/8)
KfiI	GG/GWCCC
MaeIII	/GTNAC
MjaIV⊗	GTNNAC
▲ MsiI	CAYNN/NNRTG
MteI	GCGC/NGCGC
▲ MwoI	GCNNNNN/NNGC
▲ NciI	CC/SGG
NhoI⊗	GCWGC
▲ NlaIV	GGN/NCC
PasI	CC/CWGGG
PcsI	WCGNNNN/NNNCGW
Pfl8569I⊗	GCN/NGC
▲ PflFI	GACN/NNGTC
▲ PflMI	CCANNNN/NTGG
PfoI	T/CCNGGA
▲ PpuMI	RG/GWCCY
▲ PshAI	GACNN/NNGTC
Psp03I⊗	GGWC/C
▲ PspGI	/CCWGG
PssI⊗	RGGNC/CY

INTERRUPTED PALINDROMES (CONT.)	
▲ RsrII	CG/GWCCG
▲ Sau96I	G/GNCC
▲ ScrFI	CC/NGG
SetI	ASST/
▲ SexAI	A/CCWGGT
▲ SfiI	GGCCNNNN/NGGCC
Sse8647I⊗	AG/GWCCT
▲ StyI	C/CWWGG
▲ StyD4I	/CCNGG
TatI	W/GTACW
TauI	GCSG/C
▲ TfiI	G/AWTC
▲ TseI	G/CWGC
▲ Tsp45I	/GTSAC
▲ TspRI	CASTGNN/
TssI⊗	GAGNNNCTC
▲ Tth111I	GACN/NNGTC
UnbI⊗	/GGNCC
VpaK11AI⊗	/GGWCC
▲ XcmI	CCANNNNN/NNNNTGG
▲ XmnI	GAANN/NNTTC

Multiple Recognition Sequences

TOOLS & RESOURCES

Visit the Tools & Resources tab at NEB.com to find:

- Access to our online tool **NEBcutter**, for help with restriction enzyme mapping

Single Letter Code:

R = A or G	Y = C or T	M = A or C
K = G or T	S = C or G	W = A or T
H = A or C or T		B = C or G or T
V = A or C or G		D = A or G or T
N = A or C or G or T		

Note:

Enzymes marked with a "▲" are available from NEB. Enzymes marked with a "⊗" are not currently commercially available.

MULTIPLE RECOGNITION SEQUENCES	
Aba6411II⊗	CRRTAAG
AbaCIII⊗	CTATCAV
AbaUMB2I⊗	YCCGSS
▲ AccI	GT/MKAC
Aco12261II⊗	CCRGAG
AcoY31II⊗	TAGCRAB
▲ AflIII	A/CRYGT
AgsI	TTS/AA
AhyRBAHI⊗	GCYYGAC
AhyYL17I⊗	YAAMGAG
AlwFI⊗	GAAAYNNNNNRTG
▲ ApeKI	G/CWGC
▲ ApoI	R/AATTY
AquIV⊗	GRGGAAG(19/17)
ArsI	(8/13)GACNNNNNNTTYG(11/6)
Asp103I⊗	CGRAGGC
AspBHI⊗	YSCNS(8/12)
AspNIH4III⊗	AAGAACB
Asp114pII⊗	AGCABCC
Asu14238IV⊗	CGTRAC
AteTI⊗	GGGRAG
▲ AvaI	C/YCGRG

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
▲ AvaII	G/GWCC
Awo1030IV⊗	GCCRAG
▲ BaeI	(10/15)ACNNNNGTAYC(12/7)
▲ BaeGI	GKGCM/C
▲ BanI	G/GYRCC
▲ BanII	GRGCY/C
BanLI⊗	RTCAGG
Bbr11I⊗	GGRCAG
Bbr57III⊗	GTRAAYG
BkrAM31DI⊗	RTTAAATM
Ble402II⊗	GRAGCAG
BmgI⊗	GKGCCC
▲ BsaAI	YAC/GTR
▲ BsaHI	GR/CGYC
▲ BsaWI	W/CCGGW
▲ BsiEI	CGRY/CG
▲ BsiHKAI	GWGCW/C
▲ BsoBI	C/YCGRG
▲ Bsp1286I	GDGCH/C
▲ BsrFI	R/CCGGY
▲ BstNI	CC/WGG
▲ BstYI	R/GATCY
▲ BtgI	C/CRYGG
CalB3II⊗	GRTTRAG
Cba16038I⊗	CCTNAYNC
Cbo67071IV⊗	GCRGAAG
CchII⊗	GGARGA(11/9)
Cch467III⊗	GNGAAAY
Cco14983V⊗	GGGTDA
Cco14983VI⊗	GCYGA
Cdi81III⊗	GCMGAAG
Cfupf3II⊗	GARCAG
Cgl13032II⊗	ACGABGG
Cje265V⊗	GKAAGC
Cje54107III⊗	GKAAYC
CjeFV⊗	GGRCA
CjeNIII⊗	GKAAYG(19/17)
CjeNV⊗	CCYGA
CjuI⊗	CAYNNNNNRTG
CjuII⊗	CAYNNNNNCTC
Cly7489II⊗	AAAAGRG

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
▲ CviKI-1	RG/CY
Dde51507I⊗	CCWGG
▲ EaeI	Y/GGCCR
Ecl35734I⊗	GAAAYTC
Eco4465II⊗	GAAABCC
Eco43896II⊗	CRARCAG
EcoBLMcrX⊗	RCSRC(-3/-2)
EcoE1140I⊗	ACCYAC
EcoHI⊗	/CCSGG
Eco57MI⊗	CTGRAG(16/14)
▲ Eco0109I	RG/GNCCY
FaiI	YA/TR
Fco1691IV⊗	GCVGAG
FspAI	RTGC/GCAY
FspPK15I⊗	GARGAAG
GdiII⊗	CGGCCR(-5/-1)
HaeI⊗	WGG/CCW
▲ HaeII	RGCGC/Y
HaeIV⊗	(7/13)GAYNNNNNRTC(14/9)
Hin4I⊗	(8/13)GAYNNNNNVTC(13/8)
▲ HincII	GTY/RAC
▲ Hpy99I	CGWCG/
Hpy99XIV⊗	GGWTAA
Hpy99XIV-mut1⊗	GGWCNA
Hpy99XXII⊗	TCANNNNNNTRG
Hpy300XI⊗	CCTYNA
HpyAXVI-mut1⊗	CRTTAA
HpyAXVI-mut2⊗	CRTCNA
HpyUM032XIII⊗	CYANNNNNNNTRG
HpyUM032XIII-mut1⊗	CYANNNNNNNTTC
HsoII⊗	(8/14)CAYNNNNNRTG(14/8)
Jma19592II⊗	GRGCRAC
Jsp2502II⊗	GRNGAAT
KfiI	GG/GWCCC
Kor51II⊗	RTCGAG
Kpn156V⊗	CRTGATT
KpnNH25III⊗	CTRGAG
KpnNIH50I⊗	GCYAAG
Lba2029III⊗	CYAAANG
LlaG50I⊗	CCGTKA
Lmo911II⊗	TAGRAG

Multiple Recognition Sequences (continued)

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
Lpl1004II⊗	AGGRAG
LpnI⊗	RGC/GCY
▲ LpnPI	CCDG(10/14)
Lsp6406VI⊗	CRAGCAC
MaqI⊗	CRTTGAC(21/19)
MkaDII⊗	GAGAYGT
▲ MmeI	TCCRAC(20/18)
▲ MsII	CAYNN/NNRTG
▲ MspA1I	CMG/CKG
MspI7II⊗	ACGRAG
▲ MspJI	CNNR(9/13)
▲ NciI	CC/SGG
NhaXI⊗	CAAGRAG
NhoI+	GCWGC
Nli3877I⊗	CYCGR/G
NmeDI⊗	(12/7)RCCGGY(7/12)
▲ NspI	RCATG/Y
OspHL35III⊗	YAGGAG
Pal408I⊗	CCRTGAG
PasI	CC/CWGGG
PcsI	WCGNNNN/NNNCGW
PflPt14I⊗	RGCCCAC
Pin17FIII⊗	GGYGAB
PinP23II⊗	CTRKCAG
PpiP13II⊗	CGCRGAC

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
▲ PpuMI	RG/GWCCY
Pse18267I⊗	RCCGAAG
Psp03I⊗	GGWC/C
▲ PspGI	/CCWGG
PspOMII⊗	CGCCCAR(20/18)
PspPRI⊗	CCYCAG(15/13)
▲ PspXI	VC/TCGAGB
PssI⊗	RGGNC/CY
Pst145I⊗	CTAMRAG
Pst14472I⊗	CNYACAC
PsuGI⊗	BBCGD
Rba2021I⊗	CACGAGH
RdeGBIII⊗	(9/11)TGRYCA(11/9)
RlaI⊗	VCW
Rmu369III⊗	GGCYAC
RpaI⊗	GTYGGAG(11/9)
RpaB5I⊗	CGRGGAC(20/18)
RpaTI⊗	GRTGGAG
▲ RsrII	CG/GWCCG
Sba460II⊗	GGNGAYG
SdeAI⊗	CAGRAG(21/19)
SdeOSI⊗	(11/13)GACNNNNRTGA(12/10)
SenSARA26III⊗	ACRCAG
SetI	ASST/
▲ SexAI	A/CCWGGT

MULTIPLE RECOGNITION SEQUENCES (CONT.)	
▲ SfcI	C/TRYAG
▲ SgrAI	CR/CCGGYG
SgrTI⊗	CCDS(10/14)
SmaUMH8I⊗	GCGAACB
▲ SmlI	C/TYRAG
SpoDI⊗	GCGGRAG
Sse8647I⊗	AG/GWCCT
▲ StyI	C/CWWGG
SurP32aII⊗	ACRGAG
TatI	W/GTACW
TauI	GCSG/C
▲ TfiI	G/AWTC
▲ TseI	G/CWGC
TsoI⊗	TARCCA(11/9)
▲ Tsp45I	/GTSAC
TspARh3I⊗	GRACGAC
▲ TspRI	CASTGNN/
Tth111II⊗	CAARCA(11/9)
UbaF9I⊗	TACNNNNNRTGT
Van9116I⊗	CCKAAG
Vdi96II⊗	GNCYTAG
VpaK11AI⊗	/GGWCC
Vtu19109I⊗	CACRAYC
WviI⊗	CACRAG(21/19)

Nonpalindromic Recognition Sequences

Single Letter Code:

R = A or G
K = G or T
H = A or C or T
V = A or C or G
N = A or C or G or T
Y = C or T
S = C or G
M = A or C
W = A or T
B = C or G or T
D = A or G or T

Note:

Enzymes marked with a "▲" are available from NEB.

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Enzymes marked with a "⊗" are not currently commercially available.

NONPALINDROMIC SEQUENCES	
AarI	CACCTGC(4/8)
Aba6411II⊗	CRRTAAG
AbaB8342IV⊗	CATTAG
AbaCIII⊗	CTATCAV
▲ AbaSI	C(11/9)
AbaUMB2I⊗	YCCGSS
Acc65V⊗	GACGCA
AceIII⊗	CAGCTC(7/11)
AchA6III⊗	AGCCAG
▲ AciI	CCGC(-3/-1)
Aco12261II⊗	CCRGAG
AcoY31II⊗	TAGCRAB
▲ AcuI	CTGAAG(16/14)
Adh6U21I⊗	GAANCAG
AhyRBAHI⊗	GCYYGAC
AhyYL17I⊗	YAAMGAG
AjuI	(7/12)GAANNNNNNNTTGG(11/6)
AloI	(7/12)GAACNNNNNNTCC(12/7)
▲ AlwI	GGATC(4/5)
AlwFI⊗	GAAAYNNNNNRTG
AmaCSI⊗	GCTCCA(11/9)
ApyPI⊗	ATCGAC(20/18)
AquII⊗	GCCGNAC(20/18)
AquIII⊗	GAGGAG(20/18)
AquIV⊗	GRGGAAG(19/17)
ArsI	(8/13)GACNNNNNNTTYG(11/6)
Asp103I⊗	CGRAGGC
AspBHI⊗	YSCNS(8/12)
AspDUT2V⊗	GNGCAAC
AspJHL3II⊗	CGCCCAG
AspNIH4III⊗	AAGAACB

NONPALINDROMIC SEQUENCES (CON'T)	
AspSLV7III⊗	GTCTCA
Asp114pII⊗	AGCABCC
Asu14238IV⊗	CGTRAC
AteTI⊗	GGGRAG
Awo1030IV⊗	GCCRAG
▲ BaeI	(10/15)ACNNNNGTAYC(12/7)
Bag18758I⊗	CCCGAG
BanLI⊗	RTCAGG
BarI	(7/12)GAAGNNNNNNTAC(12/7)
Bbr11I⊗	GGRCAG
Bbr52II⊗	GGCGAG
Bbr57III⊗	GTRAAYG
▲ BbsI	GAAGAC(2/6)
▲ BbvI	GCAGC(8/12)
▲ BbvCI	CCTCAGC(-5/-2)
▲ BccI	CCATC(4/5)
Bce3081I⊗	TAGGAG
▲ BceAI	ACGGC(12/14)
BceSIV⊗	(7/5)GCAGC(9/11)
BcefI⊗	ACGGC(12/13)
▲ BcgI	(10/12)CGANNNNNNTGC(12/10)
▲ BciVI	GTATCC(6/5)
▲ BcoDI	GTCTC(1/5)
BfaSII⊗	GANGGAG
▲ BfuAI	ACCTGC(4/8)
BkrAM31DI⊗	RTTAAATM
Ble402II⊗	GRAGCAG
BloAII⊗	GAGGAC
BmeDI⊗	C(2/0)
BmgI⊗	GKGCCC
▲ BmgBI	CACGTC(-3/-3)

NONPALINDROMIC SEQUENCES (CON'T)	
▲ BmrI	ACTGGG(5/4)
▲ BpmI	CTGGAG(16/14)
▲ Bpu10I	CCTNAGC(-5/-2)
▲ BpuEI	CTTGAG(16/14)
▲ BsaI ●	GGTCTC(1/5)
▲ BsaXI	(9/12)ACNNNNNCTCC(10/7)
BsbI⊗	CAACAC(21/19)
BscAI⊗	GCATC(4/6)
BscGI⊗	CCCGT
BseMII	CTCAG(10/8)
▲ BseRI	GAGGAG(10/8)
▲ BseYI	CCCAGC(-5/-1)
▲ BsgI	GTGCAG(16/14)
▲ BsmI	GAATGC(1/-1)
▲ BsmAI	GTCTC(1/5)
▲ BsmBI	CGTCTC(1/5)
▲ BsmFI	GGGAC(10/14)
Bsp24I⊗	(8/13)GACNNNNNNTGG(12/7)
Bsp460III⊗	CGCGCAG
Bsp3004IV⊗	CCGCAT
▲ BspCNI	CTCAG(9/7)
BspD6I⊗	GAGTC(4/6)
BspGI⊗	CTGGAC
▲ BspMI	ACCTGC(4/8)
BspNCI⊗	CCAGA
▲ BspQI	GCTCTTC(1/4)
▲ BsrI	ACTGG(1/-1)
▲ BsrBI	CCGCTC(-3/-3)
▲ BsrDI	GCAATG(2/0)
▲ BssSI	CACGAG(-5/-1)
▲ BtgZI	GCGATG(10/14)

NONPALINDROMIC SEQUENCES (CON'T)	
▲ BtsI	GCAGTG(2/0)
▲ BtsIMutI	CAGTG(2/0)
▲ BtsCI	GGATG(2/0)
Cal14237I⊗	GGTTAG
CalB3II⊗	GRTTRAG
Cau10061II⊗	GTTAAT
Cba13II⊗	AGGAAT
Cba16038I⊗	CCTNAYNC
Cbo67071IV⊗	GCRGAAG
CchII⊗	GGARGA(11/9)
CchIII⊗	CCCAAG(20/18)
Cch467III⊗	GNGAAAY
Cco14983V⊗	GGGTDA
Cco14983VI⊗	GCYGA
CcrNAIII⊗	CGACCAG
CdiI⊗	CATCG(-1/-1)
Cdi81III⊗	GCMGAAG
Cdi11397I⊗	GCGCAG
CdpI⊗	GCGGAG(20/18)
Cdu23823II⊗	GTGAAG
Cfupf3II⊗	GARCAG
Cgl13032I⊗	GGCGCA
Cgl13032II⊗	ACGABGG
CjeI⊗	(8/14)CCANNNNNNGT(15/9)
Cje265V⊗	GKAAGC
Cje54107III⊗	GKAAYC
CjeFIII⊗	GCAAGG
CjeFV⊗	GGRCA
CjeNII⊗	GAGNNNNNGT
CjeNIII⊗	GKAAYG(19/17)
CjeNV⊗	CCYGA
CjePI⊗	(7/13)CCANNNNNNNTC(14/8)
CjeP659IV⊗	CACNNNNNNNGAA
CjuII⊗	CAYNNNNNCTC
Cla11845III⊗	GCGAA
Cly7489II⊗	AAAAGRG
Cma23826I⊗	CGGAAG
Csp2014I⊗	GGAGGC
▲ CspCI	(11/13)CAANNNNNGTGG(12/10)
CstMI⊗	AAGGAG(20/18)
DraRI⊗	CAAGNAC(20/18)
DrdII⊗	GAACCA
▲ EarI	CTCTTC(1/4)
▲ EciI	GGCGGA(11/9)
Ecl234I⊗	CGGNAAG
Ecl35734I⊗	GAAAYTC
Eco4465II⊗	GAAABCC
Eco43896II⊗	CRARCAG
EcoBLMcrX⊗	RCSRC(-3/-2)
EcoE1140I⊗	ACCYAC
Eco57MI⊗	CTGRAG(16/14)
EcoMVII⊗	CANCATC
EcoNIH6II⊗	ATGAAG
Eli8509II⊗	CCGGAG
EsaSSI⊗	GACCAC
▲ Esp3I	CGTCTC(1/5)
Esp3007I⊗	CAGAAG
Exi27195I⊗	GCCGAC
▲ FauI	CCCGC(4/6)
Fco1691IV⊗	GCVGAG
FinI⊗	GGGAC
▲ FokI	GGATG(9/13)
▲ FspEI	CC(12/16)
FspPK15I⊗	GARGAAG
FtnUV⊗	GAAACA
GauT27I⊗	CGCGCAGG
Gba708II⊗	ATGCAC
GdiII⊗	CGGCCR(-5/-1)
GsaI	CCCAGC(-1/-5)
HaeIV⊗	(7/13)GAYNNNNNRTC(14/9)

NONPALINDROMIC SEQUENCES (CON'T)	
HauII⊗	TGGCCANNNNNNNNNN/
HbaII⊗	GCCCAG
HdeNY26I⊗	CGANNNNNNTCC
HdeZA17I⊗	GCANNNNNNTCC
▲ HgaI	GACGC(5/10)
Hin4I⊗	(8/13)GAYNNNNNVTC(13/8)
▲ HphI	GGTGA(8/7)
Hpy99XIII⊗	GCCTA
Hpy99XIV⊗	GGWTAA
Hpy99XIV-mut1⊗	GGWCNA
Hpy99XXII⊗	TCANNNNNNTRG
Hpy300XI⊗	CCTYNA
▲ HpyAV	CCTTC(6/5)
HpyAXIV⊗	GCGTA
HpyAXVI-mut1⊗	CRTTAA
HpyAXVI-mut2⊗	CRTCNA
HpyUM032XIII-mut1⊗	CYANNNNNNNTTC
HpyUM032XIV⊗	GAAAG
HpyUM037X⊗	GTGGNAG, TNGGNAG
Jma19592I⊗	GTATNAC
Jma19592II⊗	GRGCRAC
Jsp2502II⊗	GRNGAAT
Kor51II⊗	RTCGAG
Kpn156V⊗	CRTGATT
KpnNH25III⊗	CTRGAG
KpnNIH30III⊗	GTTCNAC
KpnNIH50I⊗	GCYAAG
Lba2029III⊗	CYAAANG
Lde4408II⊗	ACAAAG
LlaG50I⊗	CCGTKA
LmnI	GCTCC(1/-1)
Lmo370I⊗	AGCGCCG
Lmo911II⊗	TAGRAG
Lpl1004II⊗	AGGRAG
▲ LpnPI	CCDG(10/14)
Lra68I⊗	GTTCNAG
LsaDS4I⊗	TGGAAT
Lsp48III⊗	AGCACC
Lsp6406VI⊗	CRAGCAC
MaqI⊗	CRTTGAC(21/19)
Mba11I⊗	AGGCGA
▲ MboII	GAAGA(8/7)
Mcr10I⊗	GAAGNNNNNCTC
MkaDII⊗	GAGAYGT
▲ MlyI	GAGTC(5/5)
▲ MmeI	TCCRAC(20/18)
▲ MnlI	CCTC(7/6)
MspI7II⊗	ACGRAG
▲ MspJI	CNNR(9/13)
MspSC27II⊗	CCGCGAC
MtuHN878II⊗	CACGCAG
NalI45188II⊗	ACCAGC
Nbr128II⊗	ACCGAC
NgoAVII⊗	GCCGC(7/7)
NgoAVIII⊗	(12/14)GACNNNNNTGA(13/11)
NhaXI⊗	CAAGRAG
NlaCI⊗	CATCAC(19/17)
▲ NmeAIII	GCCGAG(21/19)
NpeUS61II⊗	GATCGAC
OspHL35III⊗	YAGGAG
PacIII⊗	GTAATC
Pac19842II⊗	CCTTGA
PaI408I⊗	CCRTGAG
Pba2294I⊗	GTAAG
PcaII⊗	GACGAG
Pcr308II⊗	CCAAAG
Pdi8503III⊗	CCGGNAG
Pdu1735I⊗	CACCAC
PenI⊗	GCAGT
Pfl1108I⊗	TCGTAG

NONPALINDROMIC SEQUENCES (CON'T)	
PflPt14I⊗	RGCCCAC
PfrJS12IV⊗	TANAAG
PfrJS12V⊗	GGCGGAG
PfrJS15III⊗	CTTCNAC
Pin17FIII⊗	GGYGAB
PinP23II⊗	CTRKCAG
PinP59III⊗	GAAGNAG
PlaDI⊗	CATCAG(21/19)
▲ PleI	GAGTC(4/5)
PliMI⊗	CGCCGAC
PpiI⊗	(7/12)GAACNNNNNCTC(13/8)
PpiP13II⊗	CGCRGAC
Pse18267I⊗	RCCGAAG
Psp0357II⊗	GCGAAG
PspOMII⊗	CGCCCAR(20/18)
PspPRI⊗	CCYCAG(15/13)
PsrI	(7/12)GAACNNNNNNTAC(12/7)
Pst145I⊗	CTAMRAG
Pst273I⊗	GATCGAG
Pst14472I⊗	CNYACAC
PsuGI⊗	BBCGD
Rba2021I⊗	CACGAGH
RceI⊗	CATCGAC(20/18)
RdeGBI⊗	CCGCAG
RdeGBII⊗	ACCCAG(20/18)
RflFIII⊗	CGCCAG
RlaI⊗	VCW
RlaII⊗	ACACAG(20/18)
RleAI⊗	CCCACA(12/9)
Rmu369III⊗	GGCYAC
RpaI⊗	GTYGGAG(11/9)
RpaBI⊗	CCCGCAG(20/18)
RpaB5I⊗	CGRGGAC(20/18)
RpaTI⊗	GRTGGAG
Rsp008IV⊗	ACGCAG
Rsp008V⊗	GCCCAT
RspPBTS2III⊗	CTTCGAG
Rtr1953I⊗	TGANNNNNNTGA
Saf8902III⊗	CAATNAG
▲ SapI	GCTCTTC(1/4)
Sba460II⊗	GGNGAYG
Sbo46I⊗	TGAAC
ScoDS2II⊗	GCTAAT
SdeAI⊗	CAGRAG(21/19)
SdeOSI⊗	(11/13)GACNNNNRTGA(12/10)
Sen17963III⊗	CCAAAC
SenA1673III⊗	GNGGCAG
SenSARA26III⊗	ACRCAG
SenTFIV⊗	GATCAG
▲ SfaNI	GCATC(5/9)
SgeI	CNNGNNNNNNNNN/
SgrTI⊗	CCDS(10/14)
SimI⊗	GGGTC(-3/0)
SmaUMH5I⊗	CTTGAC
SmaUMH8I⊗	GCGAACB
Sno506I⊗	GGCCGAG
SpnRII⊗	TCGAG
SpoDI⊗	GCGGRAG
Ssp714II⊗	CGCAGCG
Ssp6803IV⊗	GAAGGC
SspD5I⊗	GGTGA(8/8)
SstE37I⊗	CGAAGAC(20/18)
Sth132I⊗	CCCG(4/8)
Sth20745III⊗	GGACGAC
SthSt3II⊗	GAAGT
StsI⊗	GGATG(10/14)
SurP32aII⊗	ACRGAG
TaqII	GACCGA(11/9)
TaqIII⊗	CACCCA(11/9)
TsoI⊗	TARCCA(11/9)

Nonpalindromic Recognition Sequences (continued)

NONPALINDROMIC SEQUENCES (CON'T)	
TspARh3I⊗	GRACGAC
TspDTI	ATGAA(11/9)
TspGWI	ACGGA(11/9)
TstI⊗	(8/13)CACNNNNNNTCC(12/7)
TsuI⊗	GCGAC
Tth111II⊗	CAARCA(11/9)
UbaF9I⊗	TACNNNNNRTGT
UbaF11I⊗	TCGTA
UbaF12I⊗	CTACNNNGTC
UbaF13I⊗	GAGNNNNNNCTGG

NONPALINDROMIC SEQUENCES (CON'T)	
UbaF14I⊗	CCANNNNNTCG
UbaPI⊗	CGAACG
Van9116I⊗	CCKAAG
Vdi96II⊗	GNCYTAG
Vtu19109I⊗	CACRAYC
WviI⊗	CACRAG(21/19)
Xca85IV⊗	TACGAG
YkrI⊗	C(10/9)
Yps3606I⊗	CGGAAG

Alphabetized List of NEB Recognition Sequences

All restriction enzyme recognition specificities and recommended enzymes available from New England Biolabs are listed below. For enzymes that recognize non-palindromic sequences, the complementary sequence of each strand is listed. For example, CCTC(7/6) and (6/7)GAGG both represent an MnlI site. New entries are listed in **bold** type.

All recognition sequences are written 5´ to 3´ using the single letter code nomenclature with the point of cleavage indicated by a "/".

Numbers in parentheses indicate point of cleaveage for non-palindromic enzymes. For example, GGTCTC(1/5) indicates cleavage at: 5´ ...GGTCTCN/...3´
3´ ...CCAGAGNNNNN/...5´

AA/CGTT	AclI
A/AGCTT	HindIII-HF
AAT/ATT	SspI-HF
/AATT	MluCI
A/CATGT	PciI
A/CCGGT	AgeI-HF
ACCTGC(4/8)	BfuAI
ACCTGC(4/8)	BspMI
A/CCWGGT	SexAI
A/CGCGT	MluI-HF
ACGGC(12/14)	BceAI
A/CGT	HpyCH4IV
ACN/GT	HpyCH4III
(10/15)ACNNNNGTAYC(12/7)	BaeI
(9/12)ACNNNNNCTCC(10/7)	BsaXI
A/CRYGT	AflIII
A/CTAGT	SpeI-HF
ACTGG(1/-1)	BsrI
ACTGGG(5/4)	BmrI
A/GATCT	BglII
AGC/GCT	AfeI
AG/CT	AluI
AGG/CCT	StuI
AGT/ACT	ScaI-HF
AT/CGAT	BspDI
AT/CGAT	ClaI
ATGCA/T	NsiI-HF
AT/TAAT	AseI
ATTT/AAAT	SwaI
C(11/9)	AbaSI
(11/13)CAANNNNNGTGG(12/10)	CspCI
C/AATTG	MfeI-HF
CACGAG(-5/-1)	BssSI-v2
CACGTC(-3/-3)	BmgBI
CAC/GTG	PmlI
CACNNN/GTG	DraIII-HF
CACNN/NNGTG	AleI-v2
(0/2)CACTG	BtsIMutI
(0/2)CACTGC	BtsI-v2
CAG/CTG	PvuII-HF
CAGNNN/CTG	AlwNI
CAGTG(2/0)	BtsIMutI
CASTGNN/	TspRI
CA/TATG	NdeI
(0/2)CATCC	BtsCI
(13/9)CATCC	FokI
(14/10)CATCGC	BtgZI
C/ATG	CviAII

/CATG	FatI
CATG/	NlaIII
(0/2)CATTGC	BsrDI
CAYNN/NNRTG	MslI
CC(12/16)	FspEI
(10/12)CCACNNNNNTTG	CspCI
(-1/1)CCAGT	BsrI
CCANNNNN/NNNNTGG	XcmI
CCANNNNN/NTGG	BstXI
CCANNNN/NTGG	PflMI
CCATC(4/5)	BccI
C/CATGG	NcoI-HF
CCCAGC(-5/-1)	BseYI
(4/5)CCCAGT	BmrI
CCCGC(4/6)	FauI
CCC/GGG	SmaI
C/CCGGG	TspMI
C/CCGGG	XmaI
CCDG(10/14)	LpnPI
CCGC(-3/-1)	AciI
CCGC/GG	SacII
CCGCTC(-3/-3)	BsrBI
C/CGG	HpaII
C/CGG	MspI
CC/NGG	ScrFI
/CCNGG	StyD4I
C/CNNGG	BsaJI
CCNNNNN/NNGG	BslI
C/CRYGG	BtgI
CC/SGG	NciI
C/CTAGG	AvrII
CCTC(7/6)	MnlI
CCTCAGC(-5/-2)	BbvCI
CCTGCA/GG	SbfI-HF
CCTNAGC(-5/-2)	Bpu10I
CC/TNAGG	Bsu36I
CCTNN/NNNAGG	EcoNI
CCTTC(6/5)	HpyAV
CC/WGG	BstNI
/CCWGG	PspGI
C/CWWGG	StyI-HF
(10/12)CGANNNNNNTGC(12/10)	BcgI
CGAT/CG	PvuI-HF
CG/CG	BstUI
C/GGCCG	EagI-HF
CG/GWCCG	RsrII
CGRY/CG	BsiEI
C/GTACG	BsiWI

CGTCTC(1/5)	BsmBI
CGTCTC(1/5)	**Esp3I**
CGWCG/	Hpy99I
(14/10)CHGG	LpnPI
CMG/CKG	MspA1I
CNNR(9/13)	MspJI
CR/CCGGYG	SgrAI
C/TAG	BfaI
(14/16)CTCAAG	BpuEI
CTCAG(9/7)	BspCNI
(14/16)CTCCAG	BpmI
(8/10)CTCCTC	BseRI
C/TCGAG	PaeR7I
C/TCGAG	XhoI
(19/21)CTCGGC	NmeAIII
(-1/-5)CTCGTG	BssSI-v2
CTCTTC(1/4)	EarI
CTGAAG(16/14)	AcuI
(7/9)CTGAG	BspCNI
(14/16)CTGCAC	BsgI
CTGCA/G	PstI-HF
CTGGAG(16/14)	BpmI
C/TNAG	DdeI
C/TRYAG	SfcI
C/TTAAG	AflII
(14/16)CTTCAG	AcuI
CTTGAG(16/14)	BpuEI
C/TYRAG	SmlI
C/YCGRG	AvaI
C/YCGRG	BsoBI
(9/11)G	AbaSI
GAAGA(8/7)	MboII
GAAGAC(2/6)	BbsI-HF
(4/1)GAAGAG	EarI
(4/1)GAAGAGC	BspQI
(4/1)GAAGAGC	SapI
(5/6)GAAGG	HpyAV
GAANN/NNTTC	XmnI
GAATGC(1/-1)	BsmI
G/AATTC	EcoRI-HF
GACGC(5/10)	HgaI
GACGT/C	AatII
GAC/GTC	ZraI
(-3/-3)GACGTG	BmgBI
GACN/NNGTC	PflFI
GACN/NNGTC	Tth111I
GACNN/NNGTC	PshAI
GACNNN/NNGTC	AhdI

GACNNNN/NNGTC	DrdI
(5/5)GACTC	MlyI
(5/4)GACTC	PleI
(5/1)GAGAC	BcoDI
(5/1)GAGAC	BsmAI
(5/1)GAGACC	BsaI
(5/1)GAGACG	BsmBI
(5/1)GAGACG	**Esp3I**
(-3/-3)GAGCGG	BsrBI
GAG/CTC	Eco53kI
GAGCT/C	SacI-HF
(6/7)GAGG	MnlI
GAGGAG(10/8)	BseRI
GAGTC(5/5)	MlyI
GAGTC(4/5)	PleI
G/ANTC	HinfI
GAT/ATC	EcoRV-HF
GA/TC	DpnI
/GATC	DpnII
/GATC	MboI
/GATC	Sau3AI
(5/4)GATCC	AlwI
(9/5)GATGC	SfaNI
(5/4)GATGG	BccI
GATNN/NNATC	BsaBI
G/AWTC	TfiI
GCAATG(2/0)	BsrDI
GCAGC(8/12)	BbvI
(8/4)GCAGGT	BfuAI
(8/4)GCAGGT	BspMI
GCAGTG(2/0)	BtsI-v2
(10/12)GCANNNNNNTCG	BcgI
GCANNNN/NTGC	BstAPI
GCATC(5/9)	SfaNI
GCATG/C	SphI-HF
(-1/1)GCATTC	BsmI
GCCC/GGGC	SrfI
GCCGAG(21/19)	NmeAIII
GCC/GGC	NaeI
G/CCGGC	NgoMIV
(14/12)GCCGT	BceAI
GCCNNNN/NGGC	BglI
GCGAT/CGC	AsiSI
GCGATG(10/14)	BtgZI
GCG/C	HhaI
G/CGC	HinP1I
G/CGCGC	BssHII
(-1/-3)GCGG	AciI

GC/GGCCGC	NotI-HF
(6/4)GCGGG	FauI
(10/5)GCGTC	HgaI
GC/NGC	Fnu4HI
GCN/NGC	Cac8I
GCNNNNN/NNGC	MwoI
GCTAG/C	BmtI-HF
G/CTAGC	NheI-HF
GCTCTTC(1/4)	BspQI
GCTCTTC(1/4)	SapI
(-2/-5)GCTGAGG	BbvCI
(12/8)GCTGC	BbvI
(-1/-5)GCTGGG	BseYI
GC/TNAGC	BlpI
(-2/-5)GCTNAGG	Bpu10I
G/CWGC	ApeKI
G/CWGC	TseI
GDGCH/C	Bsp1286I
(16/12)GG	FspEI
(7/10)GGAGNNNNNGT	BsaXI
(5/6)GGATAC	BciVI
GGATC(4/5)	AlwI
G/GATCC	BamHI-HF
GGATG(2/0)	BtsCI
GGATG(9/13)	FokI
GG/CC	HaeIII

GGCCGG/CC	FseI
GGCCNNNN/NGGCC	SfiI
G/GCGCC	KasI
GG/CGCC	NarI
GGCGC/C	PluTI
GGC/GCC	SfoI
GG/CGCGCC	AscI
GGCGGA(11/9)	EciI
GGGAC(10/14)	BsmFI
GGGCC/C	ApaI
G/GGCCC	PspOMI
G/GNCC	Sau96I
GGN/NCC	NlaIV
G/GTACC	Acc65I
GGTAC/C	KpnI-HF
GGTCTC(1/5)	BsaI-HFv2
GGTGA(8/7)	HphI
G/GTNACC	BstEII-HF
G/GWCC	AvaII
G/GYRCC	BanI
GKGCM/C	BaeGI
GR/CGYC	BsaHI
GRGCY/C	BanII
(7/12)GRTACNNNNGT	BaeI
G/TAC	CviQI
GT/AC	RsaI

GTA/TAC	BstZ17I
GTATCC(6/5)	BciVI
(14/10)GTCCC	BsmFI
G/TCGAC	SalI-HF
GTCTC(1/5)	BcoDI
GTCTC(1/5)	BsmAI
(6/2)GTCTTC	BbsI-HF
G/TGCAC	ApaLI
GTGCAG(16/14)	BsgI
GT/MKAC	AccI
GTN/NAC	Hpy166II
/GTSAC	Tsp45I
GTT/AAC	HpaI
GTTT/AAAC	PmeI
(18/20)GTYGGA	MmeI
GTY/RAC	HincII
GWGCW/C	BsiHKAI
R/AATTY	ApoI
RCATG/Y	NspI
R/CCGGY	BsrFI-v2
R/GATCY	BstYI
RGCGC/Y	HaeII
RG/CY	CviKI-1
RG/GNCCY	EcoO109I
RG/GWCCY	PpuMI
TAC/GTA	SnaBI

(7/8)TCACC	HphI
T/CATGA	BspHI
(9/11)TCCGCC	EciI
T/CCGGA	BspEI
TCCRAC(20/18)	MmeI
T/CGA	Taq$^{\alpha}$I
TCG/CGA	NruI-HF
TCN/GA	Hpy188I
TC/NNGA	Hpy188III
T/CTAGA	XbaI
(7/8)TCTTC	MboII
T/GATCA	BclI
TG/CA	HpyCH4V
TGC/GCA	FspI
TGG/CCA	MscI
T/GTACA	BsrGI-HF
T/TAA	MseI
TTAAT/TAA	PacI
TTA/TAA	PsiI
TT/CGAA	BstBI
TTT/AAA	DraI
VC/TCGAGB	PspXI
W/CCGGW	BsaWI
YAC/GTR	BsaAI
Y/GGCCR	EaeI
(13/9)YNNG	MspJI

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Isoschizomers

Restriction enzymes that recognize the same sequence are isoschizomers. The first example discovered is called a prototype, and all subsequent enzymes that recognize the same sequence are isoschizomers of the prototype. The list below contains isoschizomers for commercially-available restriction endonucleases. It also specifies which isoschizomer is available from New England Biolabs.

All recognition sequences are written 5´ to 3´ using the single letter code nomenclature with the point of cleavage indicated by a "/".

Numbers in parentheses indicate point of cleavage for non-palindromic enzymes. For example, GGTCTC(1/5) indicates cleavage at: 5´ ...GGTCTCN/...3´
3´ ...CCAGAGNNNNN/...5´

Isoschizomers with alternative cleavage sites (neoschizomers) are indicated with a "^". Enzymes that are not currently commercially available are indicated with a "⊗". For more information on isoschizomers, visit **REBASE.neb.com**

Neoschizomers are a subset of isoschizomers that recognize the same sequence, but cleave at different positions from the prototype. Thus, AatII (recognition sequence: GACGT↓C) and ZraI (recognition sequence: GAC↓GTC) are neoschizomers of one another, while HpaII (recognition sequence: C↓CGG) and MspI (recognition sequence: C↓CGG) are isoschizomers. Analogous designations are not appropriate for methyltransferases, where the differences between enzymes are not so easily defined and usually have not been well characterized.

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
AanI	TTA/TAA	PsiI	R0657	TTA/TAA	PsiI
AarI	CACCTGC(4/8)				
AasI	GACNNNN/NNGTC	DrdI	R0530	GACNNNN/NNGTC	DrdI, DseDI
AatII	GACGT/C	AatII	R0117	GACGT/C	ZraI^
		ZraI^	R0659	GAC/GTC	
Aba6411II ⊗	CRRTAAG				
AbaB8342IV ⊗	CATTAG				
AbaCIII ⊗	CTATCAV				
AbaSI	C(11/9)	AbaSI	R0665	C(11/9)	
AbaUMB2I ⊗	YCCGSS				
AbsI	CC/TCGAGG				
AccI	GT/MKAC	AccI	R0161	GT/MKAC	FbII, XmiI
AccII	CG/CG	BstUI	R0518	CG/CG	Bsh1236I, BspFNI, BstFNI, BstUI, MvnI
AccIII	T/CCGGA	BspEI	R0540	T/CCGGA	Aor13HI, BseAI, Bsp13I, BspEI, Kpn2I, MroI
Acc16I	TGC/GCA	FspI	R0135	TGC/GCA	FspI, NsbI
Acc36I	ACCTGC(4/8)	BfuAI	R0701	ACCTGC(4/8)	BfuAI, BspMI, BveI
		BspMI	R0502	ACCTGC(4/8)	
Acc65I	G/GTACC	Acc65I	R0599	G/GTACC	Asp718I, KpnI^, KpnI-HF^
		KpnI-HF^	R3142	GGTAC/C	
Acc65V ⊗	GACGCA				
AccB1I	G/GYRCC	BanI	R0118	G/GYRCC	BanI, BshNI, BspT107I
AccB7I	CCANNNN/NTGG	PflMI	R0509	CCANNNN/NTGG	PflMI, Van91I
AccBSI	CCGCTC(-3/-3)	BsrBI	R0102	CCGCTC(-3/-3)	BsrBI, MbiI
AceIII ⊗	CAGCTC(7/11)				
AchA6III ⊗	AGCCAG				
AciI	CCGC(-3/-1)	AciI	R0551	CCGC(-3/-1)	BspACI, SsiI
AclI	AA/CGTT	AclI	R0598	AA/CGTT	Psp1406I
AclWI	GGATC(4/5)	AlwI	R0513	GGATC(4/5)	AlwI, BspPI
AcoI	Y/GGCCR	EaeI	R0508	Y/GGCCR	EaeI
Aco12261II ⊗	CCRGAG				
AcoY31II ⊗	TAGCRAB				
AcsI	R/AATTY	ApoI-HF	R3566	R/AATTY	ApoI, ApoI-HF, XapI,
AcuI	CTGAAG(16/14)	AcuI	R0641	CTGAAG(16/14)	Eco57I
AcvI	CAC/GTG	PmlI	R0532	CAC/GTG	BbrPI, Eco72I, PmaCI, PmlI, PspCI
AcyI	GR/CGYC	BsaHI	R0556	GR/CGYC	BsaHI, BssNI, BstACI, Hin1I, Hsp92I
AdeI	CACNNN/GTG	DraIII-HF	R3510	CACNNN/GTG	DraIII, DraIII-HF
Adh6U21I ⊗	GAANCAG				
AfaI	GT/AC	CviQI^	R0639	G/TAC	Csp6I^, CviQI^, RsaI, RsaNI^
		RsaI	R0167	GT/AC	
AfeI	AGC/GCT	AfeI	R0652	AGC/GCT	Aor51HI, Eco47III
AfiI	CCNNNNN/NNGG	BslI	R0555	CCNNNNN/NNGG	Bsc4I, BseLI, BslI
AflII	C/TTAAG	AflII	R0520	C/TTAAG	BfrI, BspTI, BstAFI, MspCI, Vha464I
AflIII	A/CRYGT	AflIII	R0541	A/CRYGT	
AgeI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, AsiGI, BshTI, CspAI, PinAI
AgsI	TTS/AA				
AhaIII ⊗	TTT/AAA	DraI	R0129	TTT/AAA	DraI
AhdI	GACNNN/NNGTC	AhdI	R0584	GACNNN/NNGTC	BmeRI, DriI, Eam1105I
AhlI	A/CTAGT	SpeI-HF	R3133	A/CTAGT	BcuI, SpeI, SpeI-HF
AhyRBAHI ⊗	GCYYGAC				
AhyYL17I ⊗	YAAMGAG				
AjiI	CACGTC(-3/-3)	BmgBI	R0628	CACGTC(-3/-3)	BmgBI, BtrI
AjnI	/CCWGG	BstNI^	R0168	CC/WGG	BciT130I^, BseBI^, BstNI^, Bst2UI^, EcoRII, MvaI^, Psp6I, PspGI
		PspGI	R0611	/CCWGG	
AjuI	(7/12)GAANNNNNNNTTGG(11/6)				
AleI	CACNN/NNGTG	AleI	R0634	CACNN/NNGTG	OliI

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
AlfI ⊗	(10/12)GCANNNNNNTGC(12/10)				
AloI	(7/12)GAACNNNNNNTCC(12/7)				
AluI	AG/CT	AluI	R0137	AG/CT	AluBI
AluBI	AG/CT	AluI	R0137	AG/CT	AluI
AlwI	GGATC(4/5)	AlwI	R0513	GGATC(4/5)	AclWI, BspPI
Alw21I	GWGCW/C	BsiHKAI	R0570	GWGCW/C	Bbv12I, BsiHKAI
Alw26I	GTCTC(1/5)	BcoDI	R0542	GTCTC(1/5)	BcoDI, BsmAI, BstMAI
		BsmAI	R0529	GTCTC(1/5)	
Alw44I	G/TGCAC	ApaLI	R0507	G/TGCAC	ApaLI, VneI
AlwFI ⊗	GAAAYNNNNNRTG				
AlwNI	CAGNNN/CTG	AlwNI	R0514	CAGNNN/CTG	CaiI, PstNI
Ama87I	C/YCGRG	AvaI	R0152	C/YCGRG	AvaI, BmeT110I, BsiHKCI, BsoBI, Eco88I
		BsoBI	R0586	C/YCGRG	
AmaCSI ⊗	GCTCCA(11/9)				
Aor13HI	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, BseAI, Bsp13I, BspEI, Kpn2I, MroI
Aor51HI	AGC/GCT	AfeI	R0652	AGC/GCT	AfeI, Eco47III
AoxI	/GGCC				
ApaI	GGGCC/C	ApaI	R0114	GGGCC/C	Bsp120I^, PspOMI^
		PspOMI^	R0653	G/GGCCC	
ApaBI ⊗	GCANNNNN/TGC	BstAPI^	R0654	GCANNNN/NTGC	BstAPI^
ApaLI	G/TGCAC	ApaLI	R0507	G/TGCAC	Alw44I, VneI
ApeKI	G/CWGC	ApeKI	R0643	G/CWGC	TseI
		TseI	R0591	G/CWGC	
ApoI	R/AATTY	ApoI-HF^	R3566	R/AATTY	AcsI, XapI, ApoI, ApoI-HF^
ApyPI ⊗	ATCGAC(20/18)				
AquII ⊗	GCCGNAC(20/18)				
AquIV ⊗	GRGGAAG(19/17)				
ArsI	(8/13)GACNNNNNNTTYG(11/6)				
AscI	GG/CGCGCC	AscI	R0558	GG/CGCGCC	PalAI, SgsI
AseI	AT/TAAT	AseI	R0526	AT/TAAT	PshBI, VspI
AsiGI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, BshTI, CspAI, PinAI
AsiSI	GCGAT/CGC	AsiSI	R0630	GCGAT/CGC	RgaI, SfaAI, SgfI
Asp103I ⊗	CGRAGGC				
Asp700I	GAANN/NNTTC	XmnI	R0194	GAANN/NNTTC	MroXI, PdmI, XmnI
Asp718I	G/GTACC	Acc65I	R0599	G/GTACC	Acc65I, KpnI^, KpnI-HF^
		KpnI-HF^	R3142	GGTAC/C	
AspA2I	C/CTAGG	AvrII	R0174	C/CTAGG	AvrII, BlnI, XmaJI
AspBHI ⊗	YSCNS(8/12)				
AspDUT2V ⊗	GNGCAAC				
AspJHL3II ⊗	CGCCCAG				
AspLEI	GCG/C	HhaI	R0139	GCG/C	BstHHI, CfoI, HhaI, Hin6I^, HinP1I^, HspAI^
		HinP1I^	R0124	G/CGC	
AspNIH4III ⊗	AAGAACB				
AspS9I	G/GNCC	Sau96I	R0165	G/GNCC	BmgT120I, Cfr13I, PspPI, Sau96I
AspSLV7III ⊗	GTCTCA				
Asp114pII ⊗	AGCABCC				
AsuI ⊗	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, BmgT120I, Cfr13I, PspPI, Sau96I
AsuII	TT/CGAA	BstBI	R0519	TT/CGAA	Bpu14I, Bsp119I, BspT104I, BstBI, NspV, SfuI
Asu14238IV ⊗	CGTRAC				
AsuC2I	CC/SGG	NciI	R0196	CC/SGG	BcnI, BpuMI, NciI
AsuHPI	GGTGA(8/7)	HphI	R0158	GGTGA(8/7)	HphI
AsuNHI	G/CTAGC	BmtI-HF^	R3658	GCTAG/C	BmtI^, BmtI-HF^, BspOI^, NheI, NheI-HF
		NheI-HF	R3131	G/CTAGC	
AteTI ⊗	GGGRAG				
AvaI	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, BmeT110I, BsiHKCI, BsoBI, Eco88I
		BsoBI	R0586	C/YCGRG	
AvaII	G/GWCC	AvaII	R0153	G/GWCC	Bme18I, Eco47I, SinI, VpaK11BI
AvaIII ⊗	ATGCAT	NsiI-HF	R3127	ATGCA/T	EcoT22I, Mph1103I, NsiI, NsiI-HF, Zsp2I
AvrII	C/CTAGG	AvrII	R0174	C/CTAGG	AspA2I, BlnI, XmaJI
Awo1030IV ⊗	GCCRAG				
AxyI	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	Bse21I, Bsu36I, Eco81I
B					
BaeI	(10/15)ACNNNNGTAYC(12/7)	BaeI	R0613	(10/15)ACNNNNGTAYC(12/7)	
BaeGI	GKGCM/C	BaeGI	R0708	GKGCM/C	BseSI, BstSLI
Bag18758I ⊗	CCCGAG				

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
BalI	TGG/CCA	MscI	R0534	TGG/CCA	MlsI, MluNI, Mox20I, MscI, Msp20I
BamHI	G/GATCC	BamHI-HF	R3136	G/GATCC	BamHI, BamHI-HF
BanI	G/GYRCC	BanI	R0118	G/GYRCC	AccB1I, BshNI, BspT107I
BanII	GRGCY/C	BanII	R0119	GRGCY/C	Eco24I, EcoT38I, FriOI
BanLI ⊗	RTCAGG				
BarI	(7/12)GAAGNNNNNNTAC(12/7)				
BauI	CACGAG(-5/-1)	BssSI-v2	R0680	CACGAG(-5/-1)	BssSI, BssSI-v2, Bst2BI
Bbr11I ⊗	GGRCAG				
Bbr52II ⊗	GGCGAG				
Bbr57III ⊗	GTRAAYG				
BbrPI	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, Eco72I, PmaCI, PmlI, PspCI
BbsI	GAAGAC(2/6)	BbsI-HF	R3539	GAAGAC(2/6)	BbsI, BbsI-HF, BpiI, BstV2I
BbvI	GCAGC(8/12)	BbvI	R0173	GCAGC(8/12)	BseXI, BstV1I, Lsp1109I
BbvII ⊗	GAAGAC(2/6)	BbsI-HF	R3539	GAAGAC(2/6)	BbsI, BbsI-HF, BpiI, BstV2I
Bbv12I	GWGCW/C	BsiHKAI	R0570	GWGCW/C	Alw21I, BsiHKAI
BbvCI	CCTCAGC(-5/-2)	BbvCI	R0601	CCTCAGC(-5/-2)	
BccI	CCATC(4/5)	BccI	R0704	CCATC(4/5)	
Bce83I ⊗	CTTGAG(16/14)	BpuEI	R0633	CTTGAG(16/14)	BpuEI
Bce3081I ⊗	TAGGAG				
BceAI	ACGGC(12/14)	BceAI	R0623	ACGGC(12/14)	
BcefI ⊗	ACGGC(12/13)	BceAI^	R0623	ACGGC(12/14)	BceAI^
BcgI	(10/12)CGANNNNNNTGC(12/10)	BcgI	R0545	(10/12)CGANNNNNNTGC(12/10)	
BciT130I	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BseBI, BstNI, Bst2UI, EcoRII^, MvaI, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
BciVI	GTATCC(6/5)	BciVI	R0596	GTATCC(6/5)	BfuI, BsuI
BclI	T/GATCA	BclI-HF	R3160	T/GATCA	FbaI, Ksp22I, BclI, BclI-HF
BcnI	CC/SGG	NciI	R0196	CC/SGG	AsuC2I, BpuMI, NciI
BcoDI	GTCTC(1/5)	BcoDI	R0542	GTCTC(1/5)	Alw26I, BsmAI, BstMAI
		BsmAI	R0529	GTCTC(1/5)	
BcuI	A/CTAGT	SpeI-HF	R3133	A/CTAGT	AhlI, SpeI, SpeI-HF
BdaI ⊗	(10/12)TGANNNNNNTCA(12/10)				
BetI ⊗	W/CCGGW	BsaWI	R0567	W/CCGGW	BsaWI
BfaI	C/TAG	BfaI	R0568	C/TAG	FspBI, MaeI, SspMI, XspI
BfaSII ⊗	GANGGAG				
BfiI ⊗	ACTGGG(5/4)	BmrI	R0600	ACTGGG(5/4)	BmrI, BmuI
BfmI	C/TRYAG	SfcI	R0561	C/TRYAG	BstSFI, SfcI
BfoI	RGCGC/Y	HaeII	R0107	RGCGC/Y	BstH2I, HaeII
BfrI	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BspTI, BstAFI, MspCI, Vha464I
BfuI	GTATCC(6/5)	BciVI	R0596	GTATCC(6/5)	BciVI, BsuI
BfuAI	ACCTGC(4/8)	BfuAI	R0701	ACCTGC(4/8)	Acc36I, BspMI, BveI
		BspMI	R0502	ACCTGC(4/8)	
BglI	GCCNNNN/NGGC	BglI	R0143	GCCNNNN/NGGC	
BglII	A/GATCT	BglII	R0144	A/GATCT	
BinI ⊗	GGATC(4/5)	AlwI	R0513	GGATC(4/5)	AclWI, AlwI, BspPI
BisI	GC/NGC				BlsI^, GluI, PkrI^
BkrAM31DI ⊗	RTTAAATM				
Ble402II ⊗	GRAGCAG				
BlnI	C/CTAGG	AvrII	R0174	C/CTAGG	AspA2I, AvrII, XmaJI
BloAII ⊗	GAGGAC				
BlpI	GC/TNAGC	BlpI	R0585	GC/TNAGC	Bpu1102I, Bsp1720I
BlsI	GCN/GC				BisI^, GluI^, PkrI
BmcAI	AGT/ACT	ScaI-HF	R3122	AGT/ACT	ScaI, ScaI-HF, ZrmI
Bme18I	G/GWCC	AvaII	R0153	G/GWCC	AvaII, Eco47I, SinI, VpaK11BI
Bme1390I	CC/NGG	ScrFI	R0110	CC/NGG	BmrFI, BstSCI^, MspR9I, ScrFI, StyD4I^
		StyD4I^	R0638	/CCNGG	
BmeRI	GACNNN/NNGTC	AhdI	R0584	GACNNN/NNGTC	AhdI, DriI, Eam1105I
BmeT110I	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, AvaI, BsiHKCI, BsoBI, Eco88I
		BsoBI	R0586	C/YCGRG	
BmgI ⊗	GKGCCC				
BmgBI	CACGTC(-3/-3)	BmgBI	R0628	CACGTC(-3/-3)	AjiI, BtrI
BmgT120I	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, Cfr13I, PspPI, Sau96I
BmiI	GGN/NCC	NlaIV	R0126	GGN/NCC	BspLI, NlaIV, PspN4I
BmrI	ACTGGG(5/4)	BmrI	R0600	ACTGGG(5/4)	BmuI
BmrFI	CC/NGG	ScrFI	R0110	CC/NGG	Bme1390I, BstSCI^, MspR9I, ScrFI, StyD4I^
		StyD4I^	R0638	/CCNGG	
BmsI	GCATC(5/9)	SfaNI	R0172	GCATC(5/9)	LweI, SfaNI

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
BmtI	GCTAG/C	BmtI-HF^	R3658	GCTAG/C	BmtI, BmtI-HF^, AsuNHI^, BspOI, NheI^, NheI-HF^
		NheI-HF^	R3131	G/CTAGC	
BmuI	ACTGGG(5/4)	BmrI	R0600	ACTGGG(5/4)	BmrI
BoxI	GACNN/NNGTC	PshAI	R0593	GACNN/NNGTC	BstPAI, PshAI
BpiI	GAAGAC(2/6)	BbsI-HF	R3539	GAAGAC(2/6)	BbsI, BbsI-HF, BstV2I
BpiI	(8/13)GAGNNNNNCTC(13/8)				
BpmI	CTGGAG(16/14)	BpmI	R0565	CTGGAG(16/14)	GsuI
Bpu10I	CCTNAGC(-5/-2)	Bpu10I	R0649	CCTNAGC(-5/-2)	
Bpu14I	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bsp119I, BspT104I, BstBI, NspV, SfuI
Bpu1102I	GC/TNAGC	BlpI	R0585	GC/TNAGC	BlpI, Bsp1720I
BpuEI	CTTGAG(16/14)	BpuEI	R0633	CTTGAG(16/14)	
BpuMI	CC/SGG	NciI	R0196	CC/SGG	AsuC2I, BcnI, NciI
BsaI	GGTCTC(1/5)	BsaI-HFv2	R3733	GGTCTC(1/5)	BsaI, BsaI-HFv2, Bso31I, BspTNI, Eco31I
Bsa29I	AT/CGAT	BspDI	R0557	AT/CGAT	BseCI, BshVI, BspDI, Bsu15I, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
BsaAI	YAC/GTR	BsaAI	R0531	YAC/GTR	BstBAI, Ppu21I
BsaBI	GATNN/NNATC	BsaBI	R0537	GATNN/NNATC	Bse8I, BseJI
BsaHI	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BssNI, BstACI, Hin1I, Hsp92I
BsaJI	C/CNNGG	BsaJI	R0536	C/CNNGG	BseDI, BssECI
BsaWI	W/CCGGW	BsaWI	R0567	W/CCGGW	
BsaXI	(9/12)ACNNNNNCTCC(10/7)	BsaXI	R0609	(9/12)ACNNNNNCTCC(10/7)	
BsbI ⊗	CAACAC(21/19)				
Bsc4I	CCNNNNN/NNGG	BsiI	R0555	CCNNNNN/NNGG	AfiI, BseLI, BsiI
BscGI ⊗	CCCGT				
Bse1I	ACTGG(1/-1)	BsrI	R0527	ACTGG(1/-1)	BseNI, BsrI
Bse8I	GATNN/NNATC	BsaBI	R0537	GATNN/NNATC	BsaBI, BseJI
Bse21I	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	AxyI, Bsu36I, Eco81I
Bse118I	R/CCGGY	BsrFI-v2	R0682	R/CCGGY	BsrFI-v2, BssAI, Cfr10I
BseAI	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, Bsp13I, BspEI, Kpn2I, MroI
BseBI	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BciT130I, BstNI, Bst2UI, EcoRII^, MvaI, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
BseCI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BshVI, BspDI, Bsu15I, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
BseDI	C/CNNGG	BsaJI	R0536	C/CNNGG	BsaJI, BssECI
Bse3DI	GCAATG(2/0)	BsrDI	R0574	GCAATG(2/0)	BseMI, BsrDI
BseGI	GGATG(2/0)	BtsCI	R0647	GGATG(2/0)	BstF5I, BtsCI, FokI^
		FokI^	R0109	GGATG(9/13)	
BseJI	GATNN/NNATC	BsaBI	R0537	GATNN/NNATC	BsaBI, Bse8I
BseLI	CCNNNNN/NNGG	BsiI	R0555	CCNNNNN/NNGG	AfiI, Bsc4I, BsiI
BseMI	GCAATG(2/0)	BsrDI	R0574	GCAATG(2/0)	Bse3DI, BsrDI
BseMII	CTCAG(10/8)	BspCNI^	R0624	CTCAG(9/7)	BspCNI^
BseNI	ACTGG(1/-1)	BsrI	R0527	ACTGG(1/-1)	Bse1I, BsrI
BsePI	G/CGCGC	BssHII	R0199	G/CGCGC	BssHII, PauI, PteI
BseRI	GAGGAG(10/8)	BseRI	R0581	GAGGAG(10/8)	
BseSI	GKGCM/C	BaeGI	R0708	GKGCM/C	BaeGI, BstSLI
BseXI	GCAGC(8/12)	BbvI	R0173	GCAGC(8/12)	BbvI, BstV1I, Lsp1109I
BseX3I	C/GGCCG	EagI-HF	R3505	C/GGCCG	BstZI, EagI, EagI-HF, EclXI, Eco52I
BseYI	CCCAGC(-5/-1)	BseYI	R0635	CCCAGC(-5/-1)	GsaI^, PspFI
BsgI	GTGCAG(16/14)	BsgI	R0559	GTGCAG(16/14)	
Bsh1236I	CG/CG	BstUI	R0518	CG/CG	AccII, BspFNI, BstFNI, BstUI, MvnI
Bsh1285I	CGRY/CG	BsiEI	R0554	CGRY/CG	BsiEI, BstMCI
BshFI	GG/CC	HaeIII	R0108	GG/CC	BsnI, BspANI, BsuRI, HaeIII
BshNI	G/GYRCC	BanI	R0118	G/GYRCC	AccB1I, BanI, BspT107I
BshTI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, AsiGI, CspAI, PinAI
BshVI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BspDI, Bsu15I, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
BsiI ⊗	CACGAG(-5/-1)	BssSI-v2	R0680	CACGAG(-5/-1)	BauI, BssSI-v2, Bst2BI
BsiEI	CGRY/CG	BsiEI	R0554	CGRY/CG	Bsh1285I, BstMCI
BsiHKAI	GWGCW/C	BsiHKAI	R0570	GWGCW/C	Alw21I, Bbv12I
BsiHKCI	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, AvaI, BmeT110I, BsoBI, Eco88I
		BsoBI	R0586	C/YCGRG	
BsiSI	C/CGG	HpaII	R0171	C/CGG	HapII, HpaII, MspI
		MspI	R0106	C/CGG	
BsiWI	C/GTACG	BsiWI-HF	R3553	C/GTACG	BsiWI, BsiWI-HF, Pfl23II, PspLI
BsiYI ⊗	CCNNNNN/NNGG	BsiI	R0555	CCNNNNN/NNGG	AfiI, Bsc4I, BseLI, BsiI

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
BslI	CCNNNNN/NNGG	BslI	R0555	CCNNNNN/NNGG	AfiI, Bsc4I, BseLI
BslFI	GGGAC(10/14)	BsmFI	R0572	GGGAC(10/14)	BsmFI, FaqI
BsmI	GAATGC(1/-1)	BsmI	R0134	GAATGC(1/-1)	Mva1269I, PctI
BsmAI	GTCTC(1/5)	BcoDI	R0542	GTCTC(1/5)	Alw26I, BcoDI, BstMAI
		BsmAI	R0529	GTCTC(1/5)	
BsmBI	CGTCTC(1/5)	BsmBI	R0580	CGTCTC(1/5)	Esp3I
		Esp3I	R0734	CGTCTC(1/5)	
BsmFI	GGGAC(10/14)	BsmFI	R0572	GGGAC(10/14)	BslFI, FaqI
BsnI	GG/CC	HaeIII	R0108	GG/CC	BshFI, BspANI, BsuRI, HaeIII
Bso31I	GGTCTC(1/5)	BsaI-HFv2	R3733	GGTCTC(1/5)	BsaI, BsaI-HFv2, BspTNI, Eco31I
BsoBI	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, AvaI, BmeT110I, BsiHKCI, Eco88I
		BsoBI	R0586	C/YCGRG	
Bsp13I	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, BspEI, Kpn2I, MroI
Bsp19I	C/CATGG	NcoI-HF	R3193	C/CATGG	NcoI, NcoI-HF
Bsp24I ⊗	(8/13)GACNNNNNNTGG(12/7)				
Bsp68I	TCG/CGA	NruI-HF	R3192	TCG/CGA	BtuMI, NruI, NruI-HF, RruI
Bsp119I	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, BspT104I, BstBI, NspV, SfuI
Bsp120I	G/GGCCC	ApaI^	R0114	GGGCC/C	ApaI^, PspOMI
		PspOMI	R0653	G/GGCCC	
Bsp143I	/GATC	DpnII	R0543	/GATC	BssMI, BstKTI^, BstMBI, DpnII, Kzo9I, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
Bsp460III ⊗	CGCGCAG				
Bsp1286I	GDGCH/C	Bsp1286I	R0120	GDGCH/C	MhlI, SduI
Bsp1407I	T/GTACA	BsrGI-HF	R3575	T/GTACA	BsrGI, BsrGI-HF, BstAUI
Bsp1720I	GC/TNAGC	BlpI	R0585	GC/TNAGC	BlpI, Bpu1102I
Bsp3004IV ⊗	CCGCAT				
BspACI	CCGC(-3/-1)	AciI	R0551	CCGC(-3/-1)	AciI, SsiI
BspANI	GG/CC	HaeIII	R0108	GG/CC	BshFI, BsnI, BsuRI, HaeIII
BspCNI	CTCAG(9/7)	BspCNI	R0624	CTCAG(9/7)	BseMII^
BspDI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BshVI, Bsu15I, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
BspEI	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, Bsp13I, Kpn2I, MroI
BspFNI	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BstFNI, BstUI, MvnI
BspGI ⊗	CTGGAC				
BspHI	T/CATGA	BspHI	R0517	T/CATGA	CciI, PagI
BspLI	GGN/NCC	NlaIV	R0126	GGN/NCC	BmiI, NlaIV, PspN4I
BspLU11I ⊗	A/CATGT	PciI	R0655	A/CATGT	PciI, PscI
BspMI	ACCTGC(4/8)	BfuAI	R0701	ACCTGC(4/8)	Acc36I, BfuAI, BveI
		BspMI	R0502	ACCTGC(4/8)	
BspMII ⊗	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, Bsp13I, BspEI, Kpn2I, MroI
BspMAI	CTGCA/G	PstI-HF	R3140	CTGCA/G	PstI, PstI-HF
BspNCI ⊗	CCAGA				
BspOI	GCTAG/C	BmtI-HF	R3658	GCTAG/C	AsuNHI^, BmtI, BmtI-HF, NheI^, NheI-HF^
		NheI-HF^	R3131	G/CTAGC	
BspPI	GGATC(4/5)	AlwI	R0513	GGATC(4/5)	AclWI, AlwI
BspQI	GCTCTTC(1/4)	BspQI	R0712	GCTCTTC(1/4)	LguI, PciSI, SapI
		SapI	R0569	GCTCTTC(1/4)	
BspTI	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BfrI, BstAFI, MspCI, Vha464I
BspT104I	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, Bsp119I, BstBI, NspV, SfuI
BspT107I	G/GYRCC	BanI	R0118	G/GYRCC	AccB1I, BanI, BshNI
BspTNI	GGTCTC(1/5)	BsaI-HFv2	R3733	GGTCTC(1/5)	BsaI, BsaI-HFv2, Bso31I, Eco31I
BsrI	ACTGG(1/-1)	BsrI	R0527	ACTGG(1/-1)	Bse1I, BseNI
BsrBI	CCGCTC(-3/-3)	BsrBI	R0102	CCGCTC(-3/-3)	AccBSI, MbiI
BsrDI	GCAATG(2/0)	BsrDI	R0574	GCAATG(2/0)	Bse3DI, BseMI
BsrFI	R/CCGGY	BsrFI-v2	R0682	R/CCGGY	Bse118I, BsrFI-v2, BssAI, Cfr10I
BsrGI	T/GTACA	BsrGI-HF	R3575	T/GTACA	BsrGI, BsrGI-HF, Bsp1407I, BstAUI
BssAI	R/CCGGY	BsrFI-v2	R0682	R/CCGGY	Bse118I, BsrFI-v2, Cfr10I
BssECI	C/CNNGG	BsaJI	R0536	C/CNNGG	BsaJI, BseDI
BssHII	G/CGCGC	BssHII	R0199	G/CGCGC	BsePI, PauI, PteI
BssMI	/GATC	DpnII	R0543	/GATC	Bsp143I, BstKTI^, BstMBI, DpnII, Kzo9I, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
BssNI	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BsaHI, BstACI, Hin1I, Hsp92I
BssNAI	GTA/TAC	BstZ17I-HF	R3594	GTA/TAC	Bst1107I, BstZ17I, BstZ17I-HF
BssSI	CACGAG(-5/-1)	BssSI-v2	R0680	CACGAG(-5/-1)	BauI, BssSI-v2, Bst2BI
BssT1I	C/CWWGG	StyI-HF	R3500	C/CWWGG	Eco130I, EcoT14I, ErhI, StyI, StyI-HF

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
Bst6I	CTCTTC(1/4)	EarI	R0528	CTCTTC(1/4)	Eam1104I, EarI
Bst1107I	GTA/TAC	BstZ17I-HF	R3594	GTA/TAC	BssNAI, BstZ17I, BstZ17I-HF
BstACI	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BsaHI, BssNI, Hin1I, Hsp92I
BstAFI	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BfrI, BspTI, MspCI, Vha464I
BstAPI	GCANNNN/NTGC	BstAPI	R0654	GCANNNN/NTGC	
BstAUI	T/GTACA	BsrGI-HF	R3575	T/GTACA	Bsp1407I, BsrGI, BsrGI-HF
BstBI	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, Bsp119I, BspT104I, NspV, SfuI
Bst2BI	CACGAG(-5/-1)	BssSI-v2	R0680	CACGAG(-5/-1)	BauI, BssSI, BssSI-v2
BstBAI	YAC/GTR	BsaAI	R0531	YAC/GTR	BsaAI, Ppu21I
Bst4CI	ACN/GT	HpyCH4III	R0618	ACN/GT	HpyCH4III, TaaI
BstC8I	GCN/NGC	Cac8I	R0579	GCN/NGC	Cac8I
BstDEI	C/TNAG	DdeI	R0175	C/TNAG	DdeI, HpyF3I
BstDSI	C/CRYGG	BtgI	R0608	C/CRYGG	BtgI
BstEII	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, BstPI, Eco91I, EcoO65I, PspEI
BstENI	CCTNN/NNNAGG	EcoNI	R0521	CCTNN/NNNAGG	EcoNI, XagI
BstF5I	GGATG(2/0)	BtsCI	R0647	GGATG(2/0)	BseGI, BtsCI, FokI^
		FokI^	R0109	GGATG(9/13)	
BstFNI	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BspFNI, BstUI, MvnI
BstH2I	RGCGC/Y	HaeII	R0107	RGCGC/Y	BfoI, HaeII
BstHHI	GCG/C	HhaI	R0139	GCG/C	AspLEI, CfoI, HhaI, Hin6I^, HinP1I^, HspAI^
		HinP1I^	R0124	G/CGC	
BstKTI	GAT/C	DpnII^	R0543	/GATC	Bsp143I^, BssMI^, BstMBI^, DpnII^, Kzo9I^, MboI^, NdeII^, Sau3AI^
		MboI^	R0147	/GATC	
		Sau3AI^	R0169	/GATC	
BstMAI	GTCTC(1/5)	BcoDI	R0542	GTCTC(1/5)	Alw26I, BcoDI, BsmAI
		BsmAI	R0529	GTCTC(1/5)	
BstMBI	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, DpnII, Kzo9I, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
BstMCI	CGRY/CG	BsiEI	R0554	CGRY/CG	Bsh1285I, BsiEI
BstMWI	GCNNNNN/NNGC	MwoI	R0573	GCNNNNN/NNGC	HpyF10VI, MwoI
BstNI	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BciT130I, BseBI, Bst2UI, EcoRII^, MvaI, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
BstNSI	RCATG/Y	NspI	R0602	RCATG/Y	NspI, XceI
BstPI	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, Eco91I, EcoO65I, PspEI
BstPAI	GACNN/NNGTC	PshAI	R0593	GACNN/NNGTC	BoxI, PshAI
BstSCI	/CCNGG	ScrFI^	R0110	CC/NGG	Bme1390I^, BmrFI^, MspR9I^, ScrFI^, StyD4I
		StyD4I	R0638	/CCNGG	
BstSFI	C/TRYAG	SfcI	R0561	C/TRYAG	BfmI, SfcI
BstSLI	GKGCM/C	BaeGI	R0708	GKGCM/C	BaeGI, BseSI
BstSNI	TAC/GTA	SnaBI	R0130	TAC/GTA	Eco105I, SnaBI
BstUI	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BspFNI, BstFNI, MvnI
Bst2UI	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BciT130I, BseBI, BstNI, EcoRII^, MvaI, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
BstV1I	GCAGC(8/12)	BbvI	R0173	GCAGC(8/12)	BbvI, BseXI, Lsp1109I
BstV2I	GAAGAC(2/6)	BbsI-HF	R3539	GAAGAC(2/6)	BbsI, BbsI-HF, BpiI
BstXI	CCANNNNN/NTGG	BstXI	R0113	CCANNNNN/NTGG	
BstX2I	R/GATCY	BstYI	R0523	R/GATCY	BstYI, MflI, PsuI
BstYI	R/GATCY	BstYI	R0523	R/GATCY	BstX2I, MflI, PsuI
BstZI	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, EagI, EagI-HF, EclXI, Eco52I
BstZ17I	GTA/TAC	BstZ17I-HF	R3594	GTA/TAC	BssNAI, Bst1107I, BstZ17I, BstZ17I-HF
BsuI	GTATCC(6/5)	BciVI	R0596	GTATCC(6/5)	BciVI, BfuI
Bsu15I	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BshVI, BspDI, BsuTUI, ClaI
		ClaI	R0197	AT/CGAT	
Bsu36I	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	AxyI, Bse21I, Eco81I
BsuRI	GG/CC	HaeIII	R0108	GG/CC	BshFI, BsnI, BspANI, HaeIII
BsuTUI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BshVI, BspDI, Bsu15I, ClaI
		ClaI	R0197	AT/CGAT	
BtgI	C/CRYGG	BtgI	R0608	C/CRYGG	BstDSI
BtgZI	GCGATG(10/14)	BtgZI	R0703	GCGATG(10/14)	
BtrI	CACGTC(-3/-3)	BmgBI	R0628	CACGTC(-3/-3)	AjiI, BmgBI
BtsI	GCAGTG(2/0)	BtsI-v2	R0667	GCAGTG(2/0)	
BtsIMutI	CAGTG(2/0)	BtsIMutI	R0664	CAGTG(2/0)	
BtsCI	GGATG(2/0)	BtsCI	R0647	GGATG(2/0)	BseGI, BstF5I, FokI^
		FokI^	R0109	GGATG(9/13)	

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
BtuMI	TCG/CGA	NruI-HF	R3192	TCG/CGA	Bsp68I, NruI, NruI-HF, RruI
BveI	ACCTGC(4/8)	BfuAI	R0701	ACCTGC(4/8)	Acc36I, BfuAI, BspMI
		BspMI	R0502	ACCTGC(4/8)	
C					
Cac8I	GCN/NGC	Cac8I	R0579	GCN/NGC	BstC8I
CaiI	CAGNNN/CTG	AlwNI	R0514	CAGNNN/CTG	AlwNI, PstNI
Cal14237I ⊗	GGTTAG				
CalB3II ⊗	GRTTRAG				
CauII ⊗	CC/SGG	NciI	R0196	CC/SGG	AsuC2I, BcnI, BpuMI, NciI
Cau10061II ⊗	GTTAAT				
Cba13II ⊗	AGGAAT				
Cba16038I ⊗	CCTNAYNC				
Cbo67071IV ⊗	GCRGAAG				
CchII ⊗	GGARGA(11/9)				
CchIII ⊗	CCCAAG(20/18)				
Cch467III ⊗	GNGAAAY				
CciI	T/CATGA	BspHI	R0517	T/CATGA	BspHI, PagI
CciNI	GC/GGCCGC	NotI-HF	R3189	GC/GGCCGC	NotI, NotI-HF
Cco14983V ⊗	GGGTDA				
Cco14983VI ⊗	GCYGA				
CcrNAIII ⊗	CGACCAG				
CdiI ⊗	CATCG(-1/-1)				
Cdi81III ⊗	GCMGAAG				
Cdi11397I ⊗	GCGCAG				
CdpI ⊗	GCGGAG(20/18)				
Cdu23823II ⊗	GTGAAG				
CfoI	GCG/C	HhaI	R0139	GCG/C	AspLEI, BstHHI, HhaI, Hin6I^, HinP1I^, HspAI^
		HinP1I^	R0124	G/CGC	
CfrI ⊗	Y/GGCCR	EaeI	R0508	Y/GGCCR	AcoI, EaeI
Cfr9I	C/CCGGG	SmaI^	R0141	CCC/GGG	SmaI^, TspMI, XmaI
		TspMI	R0709	C/CCGGG	
		XmaI	R0180	C/CCGGG	
Cfr10I	R/CCGGY	BsrFI-v2	R0682	R/CCGGY	Bse118I, BsrFI-v2, BssAI
Cfr13I	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, BmgT120I, PspPI, Sau96I
Cfr42I	CCGC/GG	SacII	R0157	CCGC/GG	KspI, SacII, Sfr303I, SgrBI
Cfupf3II ⊗	GARCAG				
CgI13032I ⊗	GGCGCA				
CgI13032II ⊗	ACGABGG				
CjeI ⊗	(8/14)CCANNNNNNGT(15/9)				
Cje265V ⊗	GKAAGC				
Cje54107III ⊗	GKAAYC				
CjeFIII ⊗	GCAAGG				
CjeFV ⊗	GGRCA				
CjeNII ⊗	GAGNNNNNGT				
CjeNIII ⊗	GKAAYG(19/17)				
CjeNV ⊗	CCYGA				
CjePI ⊗	(7/13)CCANNNNNNNTC(14/8)				
CjeP659IV ⊗	CACNNNNNNNGAA				
CjuI ⊗	CAYNNNNNRTG				
CjuII ⊗	CAYNNNNNCTC				
ClaI	AT/CGAT	BspDI	R0557	AT/CGAT	Bsa29I, BseCI, BshVI, BspDI, Bsu15I, BsuTUI
		ClaI	R0197	AT/CGAT	
Cla11845III ⊗	GCGAA				
Cly7489II ⊗	AAAAGRG				
Cma23826I ⊗	CGGAAG				
CpoI	CG/GWCCG	RsrII	R0501	CG/GWCCG	CspI, RsrII, Rsr2I
CseI	GACGC(5/10)	HgaI	R0154	GACGC(5/10)	HgaI
CsiI	A/CCWGGT	SexAI	R0605	A/CCWGGT	MabI, SexAI
CspI	CG/GWCCG	RsrII	R0501	CG/GWCCG	CpoI, RsrII, Rsr2I
Csp6I	G/TAC	CviQI	R0639	G/TAC	AfaI^, CviQI, RsaI^, RsaNI
		RsaI^	R0167	GT/AC	
Csp2014I ⊗	GGAGGC				
CspAI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, AsiGI, BshTI, PinAI
CspCI	(11/13)CAANNNNNGTGG(12/10)	CspCI	R0645	(11/13)CAANNNNNGTGG(12/10)	
CstMI ⊗	AAGGAG(20/18)				

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
CviAII	C/ATG	CviAII	R0640	C/ATG	FaeI^, FatI^^, Hin1II^, Hsp92II^, NlaIII^
		FatI^^	R0650	/CATG	
		NlaIII^	R0125	CATG/	
CviJI	RG/CY	CviKI-1	R0710	RG/CY	CviKI-1
CviKI-1	RG/CY	CviKI-1	R0710	RG/CY	CviJI
CviQI	G/TAC	CviQI	R0639	G/TAC	AfaI^, Csp6I, RsaI^, RsaNI
		RsaI^	R0167	GT/AC	
CviRI ⊗	TG/CA	HpyCH4V	R0620	TG/CA	HpyCH4V
D					
DdeI	C/TNAG	DdeI	R0175	C/TNAG	BstDEI, HpyF3I
Dde51507I ⊗	CCWGG				
DinI	GGC/GCC	KasI^	R0544	G/GCGCC	EgeI, EheI, KasI^, Mly113I^^, NarI^^, PluTI^^^, SfoI, SspDI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI	R0606	GGC/GCC	
DpnI	GA/TC	DpnI	R0176	GA/TC	MalI
DpnII	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, Kzo9I, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
DraI	TTT/AAA	DraI	R0129	TTT/AAA	
DraII ⊗	RG/GNCCY	EcoO109I	R0503	RG/GNCCY	EcoO109I
DraIII	CACNNN/GTG	DraIII-HF	R3510	CACNNN/GTG	AdeI, DraIII, DraIII-HF
DraRI ⊗	CAAGNAC(20/18)				
DrdI	GACNNNN/NNGTC	DrdI	R0530	GACNNNN/NNGTC	AasI, DseDI
DrdII ⊗	GAACCA				
DriI	GACNNN/NNGTC	AhdI	R0584	GACNNN/NNGTC	AhdI, BmeRI, Eam1105I
DsaI ⊗	C/CRYGG	BtgI	R0608	C/CRYGG	BstDSI, BtgI
DseDI	GACNNNN/NNGTC	DrdI	R0530	GACNNNN/NNGTC	AasI, DrdI
E					
EaeI	Y/GGCCR	EaeI	R0508	Y/GGCCR	AcoI
EagI	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, BstZI, EagI, EagI-HF, EclXI, Eco52I
Eam1104I	CTCTTC(1/4)	EarI	R0528	CTCTTC(1/4)	Bst6I, EarI
Eam1105I	GACNNN/NNGTC	AhdI	R0584	GACNNN/NNGTC	AhdI, BmeRI, DriI
EarI	CTCTTC(1/4)	EarI	R0528	CTCTTC(1/4)	Bst6I, Eam1104I
EciI	GGCGGA(11/9)	EciI	R0590	GGCGGA(11/9)	
Ecl136II	GAG/CTC	Eco53kI	R0116	GAG/CTC	EcoICRI, Eco53kI, Psp124BI^, SacI^, SacI-HF^, SstI^
		SacI-HF^	R3156	GAGCT/C	
Ecl234I ⊗	CGGNAAG				
Ecl35734I ⊗	GAAAYTC				
EclXI	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, BstZI, EagI, EagI-HF, Eco52I
Eco24I	GRGCY/C	BanII	R0119	GRGCY/C	BanII, EcoT38I, FriOI
Eco31I	GGTCTC(1/5)	BsaI-HFv2	R3733	GGTCTC(1/5)	BsaI, BsaI-HFv2, Bso31I, BspTNI
Eco32I	GAT/ATC	EcoRV-HF	R3195	GAT/ATC	EcoRV, EcoRV-HF
Eco47I	G/GWCC	AvaII	R0153	G/GWCC	AvaII, Bme18I, SinI, VpaK11BI
Eco47III	AGC/GCT	AfeI	R0652	AGC/GCT	AfeI, Aor51HI
Eco52I	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, BstZI, EagI, EagI-HF, EclXI
Eco53kI	GAG/CTC	Eco53kI	R0116	GAG/CTC	Ecl136II, EcoICRI, Psp124BI^, SacI^, SacI-HF^, SstI^
		SacI-HF^	R3156	GAGCT/C	
Eco57I	CTGAAG(16/14)	AcuI	R0641	CTGAAG(16/14)	AcuI
Eco72I	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, BbrPI, PmaCI, PmlI, PspCI
Eco81I	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	AxyI, Bse21I, Bsu36I
Eco88I	C/YCGRG	AvaI	R0152	C/YCGRG	Ama87I, AvaI, BmeT110I, BsiHKCI, BsoBI
		BsoBI	R0586	C/YCGRG	
Eco91I	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, BstPI, EcoO65I, PspEI
Eco105I	TAC/GTA	SnaBI	R0130	TAC/GTA	BstSNI, SnaBI
Eco130I	C/CWWGG	StyI-HF	R3500	C/CWWGG	BssT1I, EcoT14I, ErhI, StyI, StyI-HF
Eco147I	AGG/CCT	StuI	R0187	AGG/CCT	PceI, SseBI, StuI
Eco4465II ⊗	GAAABCC				
Eco43896II ⊗	CRARCAG				
EcoBLMcrX ⊗	RCSRC(-3/-2)				
EcoE1140I ⊗	ACCYAC				
EcoICRI	GAG/CTC	Eco53kI	R0116	GAG/CTC	Ecl136II, Eco53kI, Psp124BI^, SacI^, SacI-HF^, SstI^
		SacI-HF^	R3156	GAGCT/C	
Eco57MI ⊗	CTGRAG(16/14)				
EcoMVII ⊗	CANCATC				

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
EcoNI	CCTNN/NNNAGG	EcoNI	R0521	CCTNN/NNNAGG	BstENI, XagI
EcoNIH6II ⊗	ATGAAG				
Eco065I	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, BstPI, Eco91I, PspEI
Eco0109I	RG/GNCCY	Eco0109I	R0503	RG/GNCCY	
EcoRI	G/AATTC	EcoRI-HF	R3101	G/AATTC	EcoRI, EcoRI-HF
EcoRII	/CCWGG	BstNI^	R0168	CC/WGG	AjnI, BciT130I^, BseBI^, BstNI^, Bst2UI^, MvaI^, Psp6I, PspGI
		PspGI	R0611	/CCWGG	
EcoRV	GAT/ATC	EcoRV-HF	R3195	GAT/ATC	Eco32I, EcoRV, EcoRV-HF
EcoT14I	C/CWWGG	StyI-HF	R3500	C/CWWGG	BssT1I, Eco130I, ErhI, StyI, StyI-HF
EcoT22I	ATGCA/T	NsiI-HF	R3127	ATGCA/T	Mph1103I, NsiI, NsiI-HF, Zsp2I
EcoT38I	GRGCY/C	BanII	R0119	GRGCY/C	BanII, Eco24I, FriOI
Eco53kI	GAG/CTC	Eco53kI	R0116	GAG/CTC	Ecl136II, EcoICRI, Psp124BI^, SacI^, SacI-HF^, SstI^
		SacI-HF^	R3156	GAGCT/C	
EgeI	GGC/GCC	KasI^	R0544	G/GCGCC	DinI, EheI, KasI^, Mly113I^^, NarI^^, PluTI^^^, SfoI, SspDI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI	R0606	GGC/GCC	
EheI	GGC/GCC	KasI^	R0544	G/GCGCC	DinI, EgeI, KasI^, Mly113I^^, NarI^^, PluTI^^^, SfoI, SspDI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI	R0606	GGC/GCC	
Eli8509II ⊗	CCGGAG				
ErhI	C/CWWGG	StyI-HF	R3500	C/CWWGG	BssT1I, Eco130I, EcoT14I, StyI, StyI-HF
EsaSSI ⊗	GACCAC				
EspI ⊗	GC/TNAGC	BlpI	R0585	GC/TNAGC	BlpI, Bpu1102I, Bsp1720I
Esp3I	CGTCTC(1/5)	BsmBI	R0580	CGTCTC(1/5)	BsmBI
		Esp3I	R0734	CGTCTC(1/5)	
Esp3007I ⊗	CAGAAG				
Exi27195I ⊗	GCCGAC				
F					
FaeI	CATG/	CviAII^	R0640	C/ATG	CviAII^, FatI^^, Hin1II, Hsp92II, NlaIII
		FatI^^	R0650	/CATG	
		NlaIII	R0125	CATG/	
FaiI	YA/TR				
FalI	(8/13)AAGNNNNNCTT(13/8)				
FaqI	GGGAC(10/14)	BsmFI	R0572	GGGAC(10/14)	BslFI, BsmFI
FatI	/CATG	CviAII^	R0640	C/ATG	CviAII^, FaeI^^, Hin1II^^, Hsp92II^^, NlaIII^^
		FatI	R0650	/CATG	
		NlaIII^^	R0125	CATG/	
FauI	CCCGC(4/6)	FauI	R0651	CCCGC(4/6)	
FauNDI	CA/TATG	NdeI	R0111	CA/TATG	NdeI
FbaI	T/GATCA	BclI-HF	R3160	T/GATCA	BclI, BclI-HF, Ksp22I
FbII	GT/MKAC	AccI	R0161	GT/MKAC	AccI, XmiI
Fco1691IV ⊗	GCVGAG				
FinI ⊗	GGGAC	BsmFI	R0572	GGGAC(10/14)	BslFI, BsmFI, FaqI
FnuDII ⊗	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BspFNI, BstFNI, BstUI, MvnI
Fnu4HI	GC/NGC	Fnu4HI	R0178	GC/NGC	Fsp4HI, SatI
FokI	GGATG(9/13)	BtsCI^	R0647	GGATG(2/0)	BseGI^, BstF5I^, BtsCI^
		FokI	R0109	GGATG(9/13)	
FriOI	GRGCY/C	BanII	R0119	GRGCY/C	BanII, Eco24I, EcoT38I
FseI	GGCCGG/CC	FseI	R0588	GGCCGG/CC	RigI
FspI	TGC/GCA	FspI	R0135	TGC/GCA	Acc16I, NsbI
FspAI	RTGC/GCAY				
FspBI	C/TAG	BfaI	R0568	C/TAG	BfaI, MaeI, SspMI, XspI
FspEI	CC(12/16)	FspEI	R0662	CC(12/16)	
Fsp4HI	GC/NGC	Fnu4HI	R0178	GC/NGC	Fnu4HI, SatI
FspPK15I ⊗	GARGAAG				
FtnUV ⊗	GAAACA				
G					
GauT27I ⊗	CGCGCAGG				
Gba708II ⊗	ATGCAC				
GdiII ⊗	CGGCCR(-5/-1)				
GlaI	GC/GC				
GluI	GC/NGC				BisI, BlsI^, PkrI^
GsaI	CCCAGC(-1/-5)	BseYI^	R0635	CCCAGC(-5/-1)	BseYI^, PspFI^
GsuI	CTGGAG(16/14)	BpmI	R0565	CTGGAG(16/14)	BpmI

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
H					
HaeI ⊗	WGG/CCW				
HaeII	RGCGC/Y	HaeII	R0107	RGCGC/Y	BfoI, BstH2I
HaeIII	GG/CC	HaeIII	R0108	GG/CC	BshFI, BsnI, BspANI, BsuRI
HaeIV ⊗	(7/13)GAYNNNNNRTC(14/9)				
HapII	C/CGG	HpaII	R0171	C/CGG	BsiSI, HpaII, MspI
		MspI	R0106	C/CGG	
HbaII ⊗	GCCCAG				
HdeNY26I ⊗	CGANNNNNNTCC				
HdeZA17I ⊗	GCANNNNNNTCC				
HgaI	GACGC(5/10)	HgaI	R0154	GACGC(5/10)	CseI
HgiAI ⊗	GWGCW/C	BsiHKAI	R0570	GWGCW/C	Alw21I, Bbv12I, BsiHKAI
HgiCI ⊗	G/GYRCC	BanI	R0118	G/GYRCC	AccB1I, BanI, BshNI, BspT107I
HgiEII ⊗	ACCNNNNNNGGT				
HgiJII ⊗	GRGCY/C	BanII	R0119	GRGCY/C	BanII, Eco24I, EcoT38I, FriOI
HhaI	GCG/C	HhaI	R0139	GCG/C	AspLEI, BstHHI, CfoI, Hin6I^, HinP1I^, HspAI^
		HinP1I^	R0124	G/CGC	
Hin1I	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BsaHI, BssNI, BstACI, Hsp92I
Hin1II	CATG/	CviAII^	R0640	C/ATG	CviAII^, FaeI, FatI^^, Hsp92II, NlaIII
		FatI^^	R0650	/CATG	
		NlaIII	R0125	CATG/	
Hin4I ⊗	(8/13)GAYNNNNNVTC(13/8)				
Hin4II ⊗	CCTTC(6/5)	HpyAV	R0621	CCTTC(6/5)	HpyAV
Hin6I	G/CGC	HhaI^	R0139	GCG/C	AspLEI^, BstHHI^, CfoI^, HhaI^, HinP1I, HspAI
		HinP1I	R0124	G/CGC	
HinP1I	G/CGC	HhaI^	R0139	GCG/C	AspLEI^, BstHHI^, CfoI^, HhaI^, Hin6I, HspAI
		HinP1I	R0124	G/CGC	
HincII	GTY/RAC	HincII	R0103	GTY/RAC	HindII
HindII	GTY/RAC	HincII	R0103	GTY/RAC	HincII
HindIII	A/AGCTT	HindIII-HF	R3104	A/AGCTT	HindIII, HindIII-HF
HinfI	G/ANTC	HinfI	R0155	G/ANTC	
HpaI	GTT/AAC	HpaI	R0105	GTT/AAC	KspAI
HpaII	C/CGG	HpaII	R0171	C/CGG	BsiSI, HapII, MspI
		MspI	R0106	C/CGG	
HphI	GGTGA(8/7)	HphI	R0158	GGTGA(8/7)	AsuHPI
Hpy8I	GTN/NAC	Hpy166II	R0616	GTN/NAC	Hpy166II
Hpy99I	CGWCG/	Hpy99I	R0615	CGWCG/	
Hpy99XIII ⊗	GCCTA				
Hpy99XIV ⊗	GGWTAA				
Hpy99XIV-mut1 ⊗	GGWCNA				
Hpy99XXII ⊗	TCANNNNNNTRG				
Hpy166II	GTN/NAC	Hpy166II	R0616	GTN/NAC	Hpy8I
Hpy178III ⊗	TC/NNGA	Hpy188III	R0622	TC/NNGA	Hpy188III
Hpy188I	TCN/GA	Hpy188I	R0617	TCN/GA	
Hpy188III	TC/NNGA	Hpy188III	R0622	TC/NNGA	
Hpy300XI ⊗	CCTYNA				
HpyAV	CCTTC(6/5)	HpyAV	R0621	CCTTC(6/5)	
HpyAXIV ⊗	GCGTA				
HpyAXVI-mut1 ⊗	CRTTAA				
HpyAXVI-mut2 ⊗	CRTCNA				
HpyCH4III	ACN/GT	HpyCH4III	R0618	ACN/GT	Bst4CI, TaaI
HpyCH4IV	A/CGT	HpyCH4IV	R0619	A/CGT	HpySE526I, MaeII, TaiI^
HpyCH4V	TG/CA	HpyCH4V	R0620	TG/CA	
HpyF3I	C/TNAG	DdeI	R0175	C/TNAG	BstDEI, DdeI
HpyF10VI	GCNNNNN/NNGC	MwoI	R0573	GCNNNNN/NNGC	BstMWI, MwoI
HpySE526I	A/CGT	HpyCH4IV	R0619	A/CGT	HpyCH4IV, MaeII, TaiI^
HpyUM032XIII ⊗	CYANNNNNNNTRG				
HpyUM032XIII-mut1 ⊗	CYANNNNNNNTTC				
HpyUM032XIV ⊗	GAAAG				
HpyUM037X ⊗	GTGGNAG, TNGGNAG				
Hsp92I	GR/CGYC	BsaHI	R0556	GR/CGYC	AcyI, BsaHI, BssNI, BstACI, Hin1I
Hsp92II	CATG/	CviAII^	R0640	C/ATG	CviAII^, FaeI, FatI^^, Hin1II, NlaIII
		FatI^^	R0650	/CATG	
		NlaIII	R0125	CATG/	

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V = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
HspAI	G/CGC	HhaI^	R0139	GCG/C	AspLEI^, BstHHI^, CfoI^, HhaI^, Hin6I, HinP1I
		HinP1I	R0124	G/CGC	
J					
Jma19592I ⊗	GTATNAC				
Jma19592II ⊗	GRGCRAC				
Jsp2502II ⊗	GRNGAAT				
K					
KasI	G/GCGCC	KasI	R0544	G/GCGCC	DinI^, EgeI^, EheI^, Mly113I^^, NarI^^, PluTI^^^, SfoI^, SspDI
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI^	R0606	GGC/GCC	
KflI	GG/GWCCC				
Kor51II ⊗	RTCGAG				
KpnI	GGTAC/C	Acc65I^	R0599	G/GTACC	Acc65I^, Asp718I^, KpnI, KpnI-HF
		KpnI-HF	R3142	GGTAC/C	
Kpn2I	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, Bsp13I, BspEI, MroI
Kpn156V ⊗	CRTGATT				
KpnNH25III ⊗	CTRGAG				
KpnNIH30III ⊗	GTTCNAC				
KpnNIH50I ⊗	GCYAAG				
KroI	G/CCGGC				
KspI	CCGC/GG	SacII	R0157	CCGC/GG	Cfr42I, SacII, Sfr303I, SgrBI
Ksp22I	T/GATCA	BclI-HF	R3160	T/GATCA	BclI, BclI-HF, FbaI
Ksp632I ⊗	CTCTTC(1/4)	EarI	R0528	CTCTTC(1/4)	Bst6I, Eam1104I, EarI
KspAI	GTT/AAC	HpaI	R0105	GTT/AAC	HpaI
Kzo9I	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, DpnII, MboI, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
L					
Lba2029III ⊗	CYAAANG				
Lde4408II ⊗	ACAAAG				
LguI	GCTCTTC(1/4)	BspQI	R0712	GCTCTTC(1/4)	BspQI, PciSI, SapI
		SapI	R0569	GCTCTTC(1/4)	
LlaG50I ⊗	CCGTKA				
LmnI	GCTCC(1/-1)				
Lmo370I ⊗	AGCGCCG				
Lmo911II ⊗	TAGRAG				
Lpl1004II ⊗	AGGRAG				
LpnPI	CCDG(10/14)	LpnPI	R0663	CCDG(10/14)	
Lra68I ⊗	GTTCNAG				
LsaDS4I ⊗	TGGAAT				
Lsp48III ⊗	AGCACC				
Lsp1109I	GCAGC(8/12)	BbvI	R0173	GCAGC(8/12)	BbvI, BseXI, BstV1I
Lsp6406VI ⊗	CRAGCAC				
LweI	GCATC(5/9)	SfaNI	R0172	GCATC(5/9)	BmsI, SfaNI
M					
MabI	A/CCWGGT	SexAI	R0605	A/CCWGGT	CsiI, SexAI
MaeI	C/TAG	BfaI	R0568	C/TAG	BfaI, FspBI, SspMI, XspI
MaeII	A/CGT	HpyCH4IV	R0619	A/CGT	HpyCH4IV, HpySE526I, TaiI^
MaeIII	/GTNAC				
MalI	GA/TC	DpnI	R0176	GA/TC	DpnI
MaqI ⊗	CRTTGAC(21/19)				
MauBI	CG/CGCGCG				
Mba11I ⊗	AGGCGA				
MbiI	CCGCTC(-3/-3)	BsrBI	R0102	CCGCTC(-3/-3)	AccBSI, BsrBI
MboI	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, DpnII, Kzo9I, NdeII, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
MboII	GAAGA(8/7)	MboII	R0148	GAAGA(8/7)	
McrI ⊗	CGRY/CG	BsiEI	R0554	CGRY/CG	Bsh1285I, BsiEI, BstMCI
Mcr10I ⊗	GAAGNNNNNCTC				
MfeI	C/AATTG	MfeI-HF	R3589	C/AATTG	MunI, MfeI, MfeI-HF
MflI	R/GATCY	BstYI	R0523	R/GATCY	BstX2I, BstYI, PsuI
MhlI	GDGCH/C	Bsp1286I	R0120	GDGCH/C	Bsp1286I, SduI
MjaIV ⊗	GTNNAC	Hpy166II	R0616	GTN/NAC	Hpy8I, Hpy166II
MkaDII ⊗	GAGAYGT				
MlsI	TGG/CCA	MscI	R0534	TGG/CCA	BalI, MluNI, Mox20I, MscI, Msp20I

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
MluI	A/CGCGT	MluI-HF	R3198	A/CGCGT	MluI, MluI-HF
MluCI	/AATT	MluCI	R0538	/AATT	Sse9I, TasI
MluNI	TGG/CCA	MscI	R0534	TGG/CCA	BalI, MlsI, Mox20I, MscI, Msp20I
MlyI	GAGTC(5/5)	MlyI	R0610	GAGTC(5/5)	PleI^, PpsI^, SchI
		PleI^	R0515	GAGTC(4/5)	
Mly113I	GG/CGCC	KasI^	R0544	G/GCGCC	DinI^^, EgeI^^, EheI^^, KasI^, NarI, PluTI^^^, SfoI^^, SspDI^
		NarI	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI^^	R0606	GGC/GCC	
MmeI	TCCRAC(20/18)	MmeI	R0637	TCCRAC(20/18)	
MnlI	CCTC(7/6)	MnlI	R0163	CCTC(7/6)	
Mox20I	TGG/CCA	MscI	R0534	TGG/CCA	BalI, MlsI, MluNI, MscI, Msp20I
Mph1103I	ATGCA/T	NsiI-HF	R3127	ATGCA/T	EcoT22I, NsiI, NsiI-HF, Zsp2I
MreI	CG/CCGGCG				
MroI	T/CCGGA	BspEI	R0540	T/CCGGA	AccIII, Aor13HI, BseAI, Bsp13I, BspEI, Kpn2I
MroNI	G/CCGGC	NaeI^	R0190	GCC/GGC	NaeI^, NgoMIV, PdiI^
		NgoMIV	R0564	G/CCGGC	
MroXI	GAANN/NNTTC	XmnI	R0194	GAANN/NNTTC	Asp700I, PdmI, XmnI
MscI	TGG/CCA	MscI	R0534	TGG/CCA	BalI, MlsI, MluNI, Mox20I, Msp20I
MseI	T/TAA	MseI	R0525	T/TAA	SaqAI, Tru1I, Tru9I
MslI	CAYNN/NNRTG	MslI	R0571	CAYNN/NNRTG	RseI, SmiMI
MspI	C/CGG	HpaII	R0171	C/CGG	BsiSI, HapII, HpaII
		MspI	R0106	C/CGG	
Msp20I	TGG/CCA	MscI	R0534	TGG/CCA	BalI, MlsI, MluNI, Mox20I, MscI
MspA1I	CMG/CKG	MspA1I	R0577	CMG/CKG	
MspCI	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BfrI, BspTI, BstAFI, Vha464I
MspI7II ⊗	ACGRAG				
MspJI	CNNR(9/13)	MspJI	R0661	CNNR(9/13)	
MspR9I	CC/NGG	ScrFI	R0110	CC/NGG	Bme1390I, BmrFI, BstSCI^, ScrFI, StyD4I^
		StyD4I^	R0638	/CCNGG	
MspSC27II ⊗	CCGCGAC				
MssI	GTTT/AAAC	PmeI	R0560	GTTT/AAAC	PmeI
MstI ⊗	TGC/GCA	FspI	R0135	TGC/GCA	Acc16I, FspI, NsbI
MteI	GCGC/NGCGC				
MtuHN878II ⊗	CACGCAG				
MunI	C/AATTG	MfeI-HF	R3589	C/AATTG	MfeI, MfeI-HF
MvaI	CC/WGG	BstNI	R0168	CC/WGG	AjnI^, BciT130I, BseBI, BstNI, Bst2UI, EcoRII^, Psp6I^, PspGI^
		PspGI^	R0611	/CCWGG	
Mva1269I	GAATGC(1/-1)	BsmI	R0134	GAATGC(1/-1)	BsmI, PctI
MvnI	CG/CG	BstUI	R0518	CG/CG	AccII, Bsh1236I, BspFNI, BstFNI, BstUI
MwoI	GCNNNNN/NNGC	MwoI	R0573	GCNNNNN/NNGC	BstMWI, HpyF10VI
N					
NaeI	GCC/GGC	NaeI	R0190	GCC/GGC	MroNI^, NgoMIV^, PdiI
		NgoMIV^	R0564	G/CCGGC	
NaI45188II ⊗	ACCAGC				
NarI	GG/CGCC	KasI^	R0544	G/GCGCC	DinI^^, EgeI^^, EheI^^, KasI^, Mly113I, PluTI^^^, SfoI^^, SspDI^
		NarI	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI^^	R0606	GGC/GCC	
Nbr128II ⊗	ACCGAC				
NciI	CC/SGG	NciI	R0196	CC/SGG	AsuC2I, BcnI, BpuMI
NcoI	C/CATGG	NcoI-HF	R3193	C/CATGG	Bsp19I, NcoI, NcoI-HF
NdeI	CA/TATG	NdeI	R0111	CA/TATG	FauNDI
NdeII	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, DpnII, Kzo9I, MboI, Sau3AI
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
NgoAVII ⊗	GCCGC(7/7)				
NgoAVIII ⊗	(12/14)GACNNNNNTGA(13/11)				
NgoMIV	G/CCGGC	NaeI^	R0190	GCC/GGC	MroNI, NaeI^, PdiI^
		NgoMIV	R0564	G/CCGGC	
NhaXI ⊗	CAAGRAG				
NheI	G/CTAGC	BmtI-HF^	R3658	GCTAG/C	AsuNHI, BmtI^, BmtI-HF^, BspOI^, NheI, NheI-HF
		NheI-HF	R3131	G/CTAGC	
NhoI ⊗	GCWGC				

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
NlaIII	CATG/	CviAII^	R0640	C/ATG	CviAII^, FaeI, FatI^^, Hin1II, Hsp92II
		FatI^^	R0650	/CATG	
		NlaIII	R0125	CATG/	
NlaIV	GGN/NCC	NlaIV	R0126	GGN/NCC	BmiI, BspLI, PspN4I
NlaCI ⊗	CATCAC(19/17)				
NmeAIII	GCCGAG(21/19)	NmeAIII	R0711	GCCGAG(21/19)	
NmuCI	/GTSAC	Tsp45I	R0583	/GTSAC	TseFI, Tsp45I
NotI	GC/GGCCGC	NotI-HF	R3189	GC/GGCCGC	CciNI, NotI, NotI-HF
NpeUS61II ⊗	GATCGAC				
NruI	TCG/CGA	NruI-HF	R3192	TCG/CGA	Bsp68I, BtuMI, NruI, NruI-HF, RruI
NsbI	TGC/GCA	FspI	R0135	TGC/GCA	Acc16I, FspI
NsiI	ATGCA/T	NsiI-HF	R3127	ATGCA/T	EcoT22I, NsiI, NsiI-HF, Mph1103I, Zsp2I
NspI	RCATG/Y	NspI	R0602	RCATG/Y	BstNSI, XceI
NspV	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, Bsp119I, BspT104I, BstBI, SfuI
NspBII ⊗	CMG/CKG	MspA1I	R0577	CMG/CKG	MspA1I
O					
OliI	CACNN/NNGTG	AleI-v2	R0685	CACNN/NNGTG	AleI, AleI-v2
OspHL35III ⊗	YAGGAG				
P					
PacI	TTAAT/TAA	PacI	R0547	TTAAT/TAA	
PacIII ⊗	GTAATC				
Pac19842II ⊗	CCTTGA				
PaeI	GCATG/C	SphI-HF	R3182	GCATG/C	SphI, SphI-HF
PaeR7I	C/TCGAG	PaeR7I	R0177	C/TCGAG	Sfr274I, SlaI, XhoI
		XhoI	R0146	C/TCGAG	
PagI	T/CATGA	BspHI	R0517	T/CATGA	BspHI, CciI
Pal408I ⊗	CCRTGAG				
PalAI	GG/CGCGCC	AscI	R0558	GG/CGCGCC	AscI, SgsI
PasI	CC/CWGGG				
PauI	G/CGCGC	BssHII	R0199	G/CGCGC	BsePI, BssHII, PteI
Pba2294I ⊗	GTAAG				
PcaII ⊗	GACGAG				
PceI	AGG/CCT	StuI	R0187	AGG/CCT	Eco147I, SseBI, StuI
PciI	A/CATGT	PciI	R0655	A/CATGT	PscI
PciSI	GCTCTTC(1/4)	BspQI	R0712	GCTCTTC(1/4)	BspQI, LguI, SapI
		SapI	R0569	GCTCTTC(1/4)	
Pcr308II ⊗	CCAAAG				
PcsI	WCGNNNN/NNNCGW				
PctI	GAATGC(1/-1)	BsmI	R0134	GAATGC(1/-1)	BsmI, Mva1269I
PdiI	GCC/GGC	NaeI	R0190	GCC/GGC	MroNI^, NaeI, NgoMIV^
		NgoMIV^	R0564	G/CCGGC	
Pdi8503III ⊗	CCGGNAG				
PdmI	GAANN/NNTTC	XmnI	R0194	GAANN/NNTTC	Asp700I, MroXI, XmnI
Pdu1735I ⊗	CACCAC				
PenI ⊗	GCAGT				
PfeI	G/AWTC	TfiI	R0546	G/AWTC	TfiI
Pfl23II	C/GTACG	BsiWI-HF	R3553	C/GTACG	BsiWI, BsiWI-HF, PspLI
Pfl1108I ⊗	TCGTAG				
Pfl8569I ⊗	GCN/NGC				
PflFI	GACN/NNGTC	PflFI	R0595	GACN/NNGTC	PsyI, Tth111I
		Tth111I	R0185	GACN/NNGTC	
PflMI	CCANNNN/NTGG	PflMI	R0509	CCANNNN/NTGG	AccB7I, Van91I
PflPt14I ⊗	RGCCCAC				
PfoI	T/CCNGGA				
PfrJS12IV ⊗	TANAAG				
PfrJS12V ⊗	GGCGGAG				
PfrJS15III ⊗	CTTCNAC				
PinAI	A/CCGGT	AgeI-HF	R3552	A/CCGGT	AgeI, AgeI-HF, AsiGI, BshTI, CspAI
Pin17FIII ⊗	GGYGAB				
PinP23II ⊗	CTRKCAG				
PinP59III ⊗	GAAGNAG				
PkrI	GCN/GC				BisI^, BlsI, GluI^
PleI	GAGTC(4/5)	MlyI^	R0610	GAGTC(5/5)	MlyI^, PpsI, SchI^
		PleI	R0515	GAGTC(4/5)	
Ple19I	CGAT/CG	PvuI-HF	R3150	CGAT/CG	PvuI, PvuI-HF
PliMI ⊗	CGCCGAC				

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
PluTI	GGCGC/C	KasI^	R0544	G/GCGCC	DinI^^, EgeI^^, EheI^^, KasI^, Mly113I^^^, NarI^^^, SfoI^^, SspDI^
		NarI^^^	R0191	GG/CGCC	
		PluTI	R0713	GGCGC/C	
		SfoI^^	R0606	GGC/GCC	
PmaCI	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, BbrPI, Eco72I, PmlI, PspCI
PmeI	GTTT/AAAC	PmeI	R0560	GTTT/AAAC	MssI
PmlI	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, BbrPI, Eco72I, PmaCI, PspCI
PpiI ⊗	(7/12)GAACNNNNNCTC(13/8)				
PpiP13II ⊗	CGCRGAC				
PpsI	GAGTC(4/5)	MlyI^	R0610	GAGTC(5/5)	MlyI^, PleI, SchI^
		PleI	R0515	GAGTC(4/5)	
Ppu21I	YAC/GTR	BsaAI	R0531	YAC/GTR	BsaAI, BstBAI
PpuMI	RG/GWCCY	PpuMI	R0506	RG/GWCCY	Psp5II, PspPPI
PscI	A/CATGT	PciI	R0655	A/CATGT	PciI
Pse18267I ⊗	RCCGAAG				
PshAI	GACNN/NNGTC	PshAI	R0593	GACNN/NNGTC	BoxI, BstPAI
PshBI	AT/TAAT	AseI	R0526	AT/TAAT	AseI, VspI
PsiI	TTA/TAA	PsiI	R0657	TTA/TAA	AanI
Psp5II	RG/GWCCY	PpuMI	R0506	RG/GWCCY	PpuMI, PspPPI
Psp6I	/CCWGG	BstNI^	R0168	CC/WGG	AjnI, BciT130I^, BseBI^, BstNI^, Bst2UI^, EcoRII, MvaI^, PspGI
		PspGI	R0611	/CCWGG	
Psp0357II ⊗	GCGAAG				
Psp1406I	AA/CGTT	AclI	R0598	AA/CGTT	AclI
Psp124BI	GAGCT/C	Eco53kI^	R0116	GAG/CTC	Ecl136II^, EcoICRI^, Eco53kI^, SacI, SacI-HF, SstI
		SacI-HF	R3156	GAGCT/C	
PspCI	CAC/GTG	PmlI	R0532	CAC/GTG	AcvI, BbrPI, Eco72I, PmaCI, PmlI
PspEI	G/GTNACC	BstEII-HF	R3162	G/GTNACC	BstEII, BstEII-HF, BstPI, Eco91I, EcoO65I
PspFI	CCCAGC(-5/-1)	BseYI	R0635	CCCAGC(-5/-1)	BseYI, GsaI^
PspGI	/CCWGG	BstNI^	R0168	CC/WGG	AjnI, BciT130I^, BseBI^, BstNI^, Bst2UI^, EcoRII, MvaI^, Psp6I
		PspGI	R0611	/CCWGG	
PspLI	C/GTACG	BsiWI-HF	R3553	C/GTACG	BsiWI, BsiWI-HF, Pfl23II
PspN4I	GGN/NCC	NlaIV	R0126	GGN/NCC	BmiI, BspLI, NlaIV
PspOMI	G/GGCCC	ApaI^	R0114	GGGCC/C	ApaI^, Bsp120I
		PspOMI	R0653	G/GGCCC	
PspOMII ⊗	CGCCCAR(20/18)				
PspPI	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, BmgT120I, Cfr13I, Sau96I
PspPPI	RG/GWCCY	PpuMI	R0506	RG/GWCCY	PpuMI, Psp5II
PspPRI ⊗	CCYCAG(15/13)				
PspXI	VC/TCGAGB	PspXI	R0656	VC/TCGAGB	
PsrI	(7/12)GAACNNNNNNTAC(12/7)				
PstI	CTGCA/G	PstI-HF	R3140	CTGCA/G	BspMAI, PstI, PstI-HF
Pst145I ⊗	CTAMRAG				
Pst273I ⊗	GATCGAG				
Pst14472I ⊗	CNYACAC				
PstNI	CAGNNN/CTG	AlwNI	R0514	CAGNNN/CTG	AlwNI, CaiI
PsuI	R/GATCY	BstYI	R0523	R/GATCY	BstX2I, BstYI, MflI
PsuGI ⊗	BBCGD				
PsyI	GACN/NNGTC	PflFI	R0595	GACN/NNGTC	PflFI, Tth111I
		Tth111I	R0185	GACN/NNGTC	
PteI	G/CGCGC	BssHII	R0199	G/CGCGC	BsePI, BssHII, PauI
PvuI	CGAT/CG	PvuI-HF	R3150	CGAT/CG	Ple19I, PvuI, PvuI-HF
PvuII	CAG/CTG	PvuII-HF	R3151	CAG/CTG	PvuII, PvuII-HF
R					
Rba2021I ⊗	CACGAGH				
RceI ⊗	CATCGAC(20/18)				
RdeGBI ⊗	CCGCAG				
RdeGBII ⊗	ACCCAG(20/18)				
RdeGBIII ⊗	(9/11)TGRYCA(11/9)				
RflFIII ⊗	CGCCAG				
RgaI	GCGAT/CGC	AsiSI	R0630	GCGAT/CGC	AsiSI, SfaAI, SgfI
RigI	GGCCGG/CC	FseI	R0588	GGCCGG/CC	FseI
RlaI ⊗	VCW				
RlaII ⊗	ACACAG(20/18)				
RleAI ⊗	CCCACA(12/9)				
Rmu369III ⊗	GGCYAC				

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ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
RpaI ⊗	GTYGGAG(11/9)				
RpaBI ⊗	CCCGCAG(20/18)				
RpaB5I ⊗	CGRGGAC(20/18)				
RpaTI ⊗	GRTGGAG				
RruI	TCG/CGA	NruI-HF	R3192	TCG/CGA	Bsp68I, BtuMI, NruI, NruI-HF
RsaI	GT/AC	CviQI^	R0639	G/TAC	AfaI, Csp6I^, CviQI^, RsaNI^
		RsaI	R0167	GT/AC	
RsaNI	G/TAC	CviQI	R0639	G/TAC	AfaI^, Csp6I, CviQI, RsaI^
		RsaI^	R0167	GT/AC	
RseI	CAYNN/NNRTG	MslI	R0571	CAYNN/NNRTG	MslI, SmiMI
Rsp008IV ⊗	ACGCAG				
Rsp008V ⊗	GCCCAT				
RspPBTS2III ⊗	CTTCGAG				
RsrII	CG/GWCCG	RsrII	R0501	CG/GWCCG	CpoI, CspI, Rsr2I
Rsr2I	CG/GWCCG	RsrII	R0501	CG/GWCCG	CpoI, CspI, RsrII
Rtr1953I ⊗	TGANNNNNNTGA				
S					
SacI	GAGCT/C	Eco53kI^	R0116	GAG/CTC	Ecl136II^, EcoICRI^, Eco53kI^, Psp124BI, SacI, SacI-HF, SstI
		SacI-HF	R3156	GAGCT/C	
SacII	CCGC/GG	SacII	R0157	CCGC/GG	Cfr42I, KspI, Sfr303I, SgrBI
Saf8902III ⊗	CAATNAG				
SalI	G/TCGAC	SalI-HF	R3138	G/TCGAC	SalI, SalI-HF
SanDI ⊗	GG/GWCCC				KflI
SapI	GCTCTTC(1/4)	BspQI	R0712	GCTCTTC(1/4)	BspQI, LguI, PciSI
		SapI	R0569	GCTCTTC(1/4)	
SaqAI	T/TAA	MseI	R0525	T/TAA	MseI, Tru1I, Tru9I
SatI	GC/NGC	Fnu4HI	R0178	GC/NGC	Fnu4HI, Fsp4HI
SauI ⊗	CC/TNAGG	Bsu36I	R0524	CC/TNAGG	AxyI, Bse21I, Bsu36I, Eco81I
Sau96I	G/GNCC	Sau96I	R0165	G/GNCC	AspS9I, BmgT120I, Cfr13I, PspPI
Sau3AI	/GATC	DpnII	R0543	/GATC	Bsp143I, BssMI, BstKTI^, BstMBI, DpnII, Kzo9I, MboI, NdeII
		MboI	R0147	/GATC	
		Sau3AI	R0169	/GATC	
Sba460II ⊗	GGNGAYG				
SbfI	CCTGCA/GG	SbfI-HF	R3642	CCTGCA/GG	SbfI, SbfI-HF, SdaI, Sse8387I
Sbo46I ⊗	TGAAC				
ScaI	AGT/ACT	ScaI-HF	R3122	AGT/ACT	BmcAI, ScaI, ScaI-HF, ZrmI
SchI	GAGTC(5/5)	MlyI	R0610	GAGTC(5/5)	MlyI, PleI^, PpsI^
		PleI^	R0515	GAGTC(4/5)	
ScoDS2II ⊗	GCTAAT				
ScrFI	CC/NGG	ScrFI	R0110	CC/NGG	Bme1390I, BmrFI, BstSCI^, MspR9I, StyD4I^
		StyD4I^	R0638	/CCNGG	
SdaI	CCTGCA/GG	SbfI-HF	R3642	CCTGCA/GG	SbfI, SbfI-HF, Sse8387I
SdeAI ⊗	CAGRAG(21/19)				
SdeOSI ⊗	(11/13)GACNNNNRTGA(12/10)				
SduI	GDGCH/C	Bsp1286I	R0120	GDGCH/C	Bsp1286I, MhlI
SecI ⊗	C/CNNGG	BsaJI	R0536	C/CNNGG	BsaJI, BseDI, BssECI
Sen17963III ⊗	CCAAAC				
SenA1673III ⊗	GNGGCAG				
SenSARA26III ⊗	ACRCAG				
SenTFIV ⊗	GATCAG				
SetI	ASST/				
SexAI	A/CCWGGT	SexAI	R0605	A/CCWGGT	CsiI, MabI
SfaAI	GCGAT/CGC	AsiSI	R0630	GCGAT/CGC	AsiSI, RgaI, SgfI
SfaNI	GCATC(5/9)	SfaNI	R0172	GCATC(5/9)	BmsI, LweI
SfcI	C/TRYAG	SfcI	R0561	C/TRYAG	BfmI, BstSFI
SfeI ⊗	C/TRYAG	SfcI	R0561	C/TRYAG	BfmI, BstSFI, SfcI
SfiI	GGCCNNNN/NGGCC	SfiI	R0123	GGCCNNNN/NGGCC	
SfoI	GGC/GCC	KasI^	R0544	G/GCGCC	DinI, EgeI, EheI, KasI^, Mly113I^^, NarI^^, PluTI^^^, SspDI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI	R0606	GGC/GCC	
Sfr274I	C/TCGAG	PaeR7I	R0177	C/TCGAG	PaeR7I, SlaI, XhoI
		XhoI	R0146	C/TCGAG	
Sfr303I	CCGC/GG	SacII	R0157	CCGC/GG	Cfr42I, KspI, SacII, SgrBI
SfuI	TT/CGAA	BstBI	R0519	TT/CGAA	AsuII, Bpu14I, Bsp119I, BspT104I, BstBI, NspV
SgeI	CNNGNNNNNNNNN/				
SgfI	GCGAT/CGC	AsiSI	R0630	GCGAT/CGC	AsiSI, RgaI, SfaAI

Isoschizomers (continued)

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
SgrAI	CR/CCGGYG	SgrAI	R0603	CR/CCGGYG	
SgrBI	CCGC/GG	SacII	R0157	CCGC/GG	Cfr42I, KspI, SacII, Sfr303I
SgrDI	CG/TCGACG				
SgrTI ⊗	CCDS(10/14)				
SgsI	GG/CGCGCC	AscI	R0558	GG/CGCGCC	AscI, PalAI
SimI ⊗	GGGTC(-3/0)				
SinI	G/GWCC	AvaII	R0153	G/GWCC	AvaII, Bme18I, Eco47I, VpaK11BI
SlaI	C/TCGAG	PaeR7I	R0177	C/TCGAG	PaeR7I, Sfr274I, XhoI
		XhoI	R0146	C/TCGAG	
SmaI	CCC/GGG	SmaI	R0141	CCC/GGG	Cfr9I^, TspMI^, XmaI^
		TspMI^	R0709	C/CCGGG	
		XmaI^	R0180	C/CCGGG	
SmaUMH5I ⊗	CTTGAC				
SmaUMH8I ⊗	GCGAACB				
SmiI	ATTT/AAAT	SwaI	R0604	ATTT/AAAT	SwaI
SmiMI	CAYNN/NNRTG	MslI	R0571	CAYNN/NNRTG	MslI, RseI
SmlI	C/TYRAG	SmlI	R0597	C/TYRAG	SmoI
SmoI	C/TYRAG	SmlI	R0597	C/TYRAG	SmlI
SnaI ⊗	GTATAC	BstZ17I-HF	R3594	GTA/TAC	BssNAI, Bst1107I, BstZ17I, BstZ17I-HF
SnaBI	TAC/GTA	SnaBI	R0130	TAC/GTA	BstSNI, Eco105I
Sno506I ⊗	GGCCGAG				
SpeI	A/CTAGT	SpeI-HF	R3133	A/CTAGT	AhlI, BcuI, SpeI, SpeI-HF
SphI	GCATG/C	SphI-HF	R3182	GCATG/C	PaeI, SphI, SphI-HF
SplI ⊗	C/GTACG	BsiWI-HF	R3553	C/GTACG	BsiWI, BsiWI-HF, Pfl23II, PspLI
SpnRII ⊗	TCGAG				
SpoDI ⊗	GCGGRAG				
SrfI	GCCC/GGGC	SrfI	R0629	GCCC/GGGC	
Sse9I	/AATT	MluCI	R0538	/AATT	MluCI, TasI
Sse232I ⊗	CG/CCGGCG				MreI
Sse8387I	CCTGCA/GG	SbfI-HF	R3642	CCTGCA/GG	SbfI, SbfI-HF, SdaI
Sse8647I ⊗	AG/GWCCT				
SseBI	AGG/CCT	StuI	R0187	AGG/CCT	Eco147I, PceI, StuI
SsiI	CCGC(-3/-1)	AciI	R0551	CCGC(-3/-1)	AciI, BspACI
SspI	AAT/ATT	SspI-HF	R3132	AAT/ATT	SspI, SspI-HF
Ssp714II ⊗	CGCAGCG				
Ssp6803IV ⊗	GAAGGC				
SspDI	G/GCGCC	KasI	R0544	G/GCGCC	DinI^, EgeI^, EheI^, KasI, Mly113I^^, NarI^^, PluTI^^^, SfoI^
		NarI^^	R0191	GG/CGCC	
		PluTI^^^	R0713	GGCGC/C	
		SfoI^	R0606	GGC/GCC	
SspMI	C/TAG	BfaI	R0568	C/TAG	BfaI, FspBI, MaeI, XspI
SstI	GAGCT/C	Eco53kI^	R0116	GAG/CTC	Ecl136II^, EcoICRI^, Eco53kI^, Psp124BI, SacI, SacI-HF
		SacI-HF	R3156	GAGCT/C	
SstE37I ⊗	CGAAGAC(20/18)				
Sth132I ⊗	CCCG(4/8)				
Sth20745III ⊗	GGACGAC				
SthSt3II ⊗	GAAGT				
StuI	AGG/CCT	StuI	R0187	AGG/CCT	Eco147I, PceI, SseBI
StyI	C/CWWGG	StyI-HF	R3500	C/CWWGG	BssT1I, Eco130I, EcoT14I, ErhI, StyI, StyI-HF
StyD4I	/CCNGG	ScrFI^	R0110	CC/NGG	Bme1390I^, BmrFI^, BstSCI, MspR9I^, ScrFI^
		StyD4I	R0638	/CCNGG	
SurP32aII ⊗	ACRGAG				
SwaI	ATTT/AAAT	SwaI	R0604	ATTT/AAAT	SmiI
T					
TaaI	ACN/GT	HpyCH4III	R0618	ACN/GT	Bst4CI, HpyCH4III
TaiI	ACGT/	HpyCH4IV^	R0619	A/CGT	HpyCH4IV^, HpySE526I^, MaeII^
TaqI	T/CGA	TaqI	R0149	T/CGA	
TaqII	GACCGA(11/9)				
TaqIII ⊗	CACCCA(11/9)				
TasI	/AATT	MluCI	R0538	/AATT	MluCI, Sse9I
TatI	W/GTACW				
TauI	GCSG/C				
TfiI	G/AWTC	TfiI	R0546	G/AWTC	PfeI
Tru1I	T/TAA	MseI	R0525	T/TAA	MseI, SaqAI, Tru9I
Tru9I	T/TAA	MseI	R0525	T/TAA	MseI, SaqAI, Tru1I

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ENZYME	SEQUENCE	NEB ENZYME	NEB #	SEQUENCE	OTHER ISOSCHIZOMERS
TscAI	CASTGNN/	TspRI	R0582	CASTGNN/	TspRI
TseI	G/CWGC	ApeKI	R0643	G/CWGC	ApeKI
		TseI	R0591	G/CWGC	
TseFI	/GTSAC	Tsp45I	R0583	/GTSAC	NmuCI, Tsp45I
TsoI ⊗	TARCCA(11/9)				
Tsp45I	/GTSAC	Tsp45I	R0583	/GTSAC	NmuCI, TseFI
TspARh3I ⊗	GRACGAC				
Tsp4CI ⊗	ACN/GT	HpyCH4III	R0618	ACN/GT	Bst4CI, HpyCH4III, TaaI
TspDTI	ATGAA(11/9)				
TspEI ⊗	/AATT	MluCI	R0538	/AATT	MluCI, Sse9I, TasI
TspGWI	ACGGA(11/9)				
TspMI	C/CCGGG	SmaI^	R0141	CCC/GGG	Cfr9I, SmaI^, XmaI
		TspMI	R0709	C/CCGGG	
		XmaI	R0180	C/CCGGG	
TspRI	CASTGNN/	TspRI	R0582	CASTGNN/	TscAI
TssI ⊗	GAGNNNCTC				
TstI ⊗	(8/13)CACNNNNNNTCC(12/7)				
TsuI ⊗	GCGAC				
Tth111I	GACN/NNGTC	PflFI	R0595	GACN/NNGTC	PflFI, PsyI
		Tth111I	R0185	GACN/NNGTC	
Tth111II ⊗	CAARCA(11/9)				
U					
UbaF9I ⊗	TACNNNNNRTGT				
UbaF11I ⊗	TCGTA				
UbaF12I ⊗	CTACNNNGTC				
UbaF13I ⊗	GAGNNNNNNCTGG				
UbaF14I ⊗	CCANNNNNTCG				
UbaPI ⊗	CGAACG				
V					
Van91I	CCANNNN/NTGG	PflMI	R0509	CCANNNN/NTGG	AccB7I, PflMI
Van9116I ⊗	CCKAAG				
Vdi96II ⊗	GNCYTAG				
Vha464I	C/TTAAG	AflII	R0520	C/TTAAG	AflII, BfrI, BspTI, BstAFI, MspCI
VneI	G/TGCAC	ApaLI	R0507	G/TGCAC	Alw44I, ApaLI
VpaK11BI	G/GWCC	AvaII	R0153	G/GWCC	AvaII, Bme18I, Eco47I, SinI
VspI	AT/TAAT	AseI	R0526	AT/TAAT	AseI, PshBI
Vtu19109I ⊗	CACRAYC				
W					
WviI ⊗	CACRAG(21/19)				
X					
XagI	CCTNN/NNNAGG	EcoNI	R0521	CCTNN/NNNAGG	BstENI, EcoNI
XapI	R/AATTY	ApoI-HF	R3566	R/AATTY	AcsI, ApoI, ApoI-HF
XbaI	T/CTAGA	XbaI	R0145	T/CTAGA	
Xca85IV ⊗	TACGAG				
XceI	RCATG/Y	NspI	R0602	RCATG/Y	BstNSI, NspI
XcmI	CCANNNNN/NNNNTGG	XcmI	R0533	CCANNNNN/NNNNTGG	
XhoI	C/TCGAG	PaeR7I	R0177	C/TCGAG	PaeR7I, Sfr274I, SlaI
		XhoI	R0146	C/TCGAG	
XhoII ⊗	R/GATCY	BstYI	R0523	R/GATCY	BstX2I, BstYI, MflI, PsuI
XmaI	C/CCGGG	SmaI^	R0141	CCC/GGG	Cfr9I, SmaI^, TspMI
		TspMI	R0709	C/CCGGG	
		XmaI	R0180	C/CCGGG	
XmaIII ⊗	C/GGCCG	EagI-HF	R3505	C/GGCCG	BseX3I, BstZI, EagI, EagI-HF, EclXI, Eco52I
XmaJI	C/CTAGG	AvrII	R0174	C/CTAGG	AspA2I, AvrII, BlnI
XmiI	GT/MKAC	AccI	R0161	GT/MKAC	AccI, FblI
XmnI	GAANN/NNTTC	XmnI	R0194	GAANN/NNTTC	Asp700I, MroXI, PdmI
XspI	C/TAG	BfaI	R0568	C/TAG	BfaI, FspBI, MaeI, SspMI
Y					
Yps3606I ⊗	CGGAAG				
Z					
ZraI	GAC/GTC	AatII^	R0117	GACGT/C	AatII^
		ZraI	R0659	GAC/GTC	
ZrmI	AGT/ACT	ScaI-HF	R3122	AGT/ACT	BmcAI, ScaI, ScaI-HF
Zsp2I	ATGCA/T	NsiI-HF	R3127	ATGCA/T	EcoT22I, Mph1103I, NsiI, NsiI-HF

Survival in a Reaction

Restriction enzymes vary with respect to their ability to maintain activity in a reaction over an extended period of time.

+++	Enzyme is active > 8 hours	N/A	Not Available
++	Enzyme is active 4–8 hours		
+	Enzyme is active 2–4 hours		
–	No benefit from digesting over 1 hour		

While most routine restriction digests are incubated for one hour or less at 37°C, there are certain applications that require the addition of less than 1 unit/µg of DNA and increasing the reaction time beyond one hour. The table below can be used as a guide when low levels of enzyme and extended reaction times are needed.

For example, 1 unit of AatII can be used to digest 8 µg of DNA in a 16 hour digest (+++).

Extended activity was determined by performing the restriction endonuclease unit assay, using a 16 hour incubation in place of the standard 1 hour digestion. After the 16 hour digestion, extended activity enzymes (+++) required only 0.13 units to completely digest 1 µg of DNA. Intermediate activity enzymes required either 0.25 (++) or 0.50 (+) units for complete digestion over this extended incubation time. Finally, enzymes marked (–) required 1.0 unit for complete digestion, the same amount of enzyme required for a 1 hour digestion.

Note: Reaction temperature is 37°C, unless otherwise noted.

ENZYME	SURVIVAL
AatII	+++
AbaSI @25°C	N/A
AccI	+++
Acc65I	+
AciI	–
AclI	+
AcuI	–
AfeI	++
AflII	+++
AflIII	+++
AgeI	+
AgeI-HF	++
AhdI	+++
AleI-v2	N/A
AluI	+++
AlwI	+++
AlwNI	+++
ApaI @25°C	+++
ApaLI	+++
ApeKI @75°C	–
ApoI @50°C	+++
ApoI-HF	+++
AscI	–
AseI	+++
AsiSI	+++
AvaI	+
AvaII	+++
AvrII	+++
BaeI @25°C	+
BaeGI	+++
BamHI	+
BamHI-HF	+
BanI	+++
BanII	+++
BbsI	++
BbsI-HF	–
BbvI	++
BbvCI	+++
BccI	+
BceAI	+++
BcgI	++
BciVI	++
BclI @50°C	+
BclI-HF	N/A
BcoDI	+++
BfaI	+
BfuAI @50°C	++
BglI	+++
BglII	++
BlpI	+
BmgBI	–
BmrI	++
BmtI	+++
BmtI-HF	+++
BpmI	–

ENZYME	SURVIVAL
Bpu10I	+
BpuEI @25°C	+
BsaI	–
BsaI-HFv2	++
BsaAI	++
BsaBI @60°C	+++
BsaHI	+++
BsaJI @60°C	+++
BsaWI @60°C	+++
BsaXI	++
BseRI	+
BseYI	+
BsgI	+
BsiEI @60°C	++
BsiHKAI @65°C	–
BsiWI @55°C	–
BsiWI-HF	+++
BslI @55°C	+
BsmI @65°C	+++
BsmAI @55°C	+++
BsmBI @55°C	+
BsmFI @65°C	+++
BsoBI	+++
Bsp1286I	+
BspCNI @25°C	–
BspDI	–
BspEI	+++
BspHI	++
BspMI	+++
BspQI @50°C	+++
BsrI @65°C	++
BsrBI	+
BsrDI @65°C	+
BsrFI-v2	+++
BsrGI	+++
BsrGI-HF	+++
BssHII @50°C	++
BssSI-v2	+++
BstAPI @60°C	++
BstBI @65°C	+++
BstEII @60°C	–
BstEII-HF	–
BstNI @60°C	–
BstUI @60°C	+++
BstXI @55°C	+
BstYI @60°C	++
BstZ17I-HF	++
Bsu36I	+++
BtgI	+
BtgZI @60°C	–
BtsI-v2 @55°C	+++
BtsMutI @55°C	N/A
BtsCI @50°C	+
Cac8I	+++
ClaI	+

ENZYME	SURVIVAL
CspCI	+++
CviAII @25°C	–
CviKI-1	++
CviQI @25°C	++
DdeI	+++
DpnI	+++
DpnII	+++
DraI	+
DraIII-HF	+++
DrdI	+++
EaeI	+++
EagI	+++
EagI-HF	+++
EarI	++
EciI	–
Eco53kI	++
EcoNI	+++
EcoO109I	+++
EcoP15I	–
EcoRI	+++
EcoRI-HF	++
EcoRV	+
EcoRV-HF	+++
Esp3I	N/A
FatI @55°C	–
FauI @55°C	–
Fnu4HI	+++
FokI	++
FseI	–
FspI	+++
FspEI	+++
HaeII	–
HaeIII	+++
HgaI	–
HhaI	++
HincII	+++
HindIII	+++
HindIII-HF	+++
HinfI	+++
HinP1I	+++
HpaI	++
HpaII	+++
HphI	+++
Hpy99I	–
Hpy166II	+
Hpy188I	+++
Hpy188III	++
HpyAV	–
HpyCH4III	+++
HpyCH4IV	+++
HpyCH4V	+++
I-CeuI	++
I-SceI	++
KasI	–
KpnI	++

ENZYME	SURVIVAL
KpnI-HF	+
LpnPI	–
MboI	+++
MboII	+
MfeI	++
MfeI-HF	++
MluI	+++
MluI-HF	+++
MluCI	–
MlyI	–
MmeI	–
MnlI	++
MscI	+++
MseI	+++
MslI	++
MspI	+
MspA1I	++
MspJI	+++
MwoI @60°C	+++
NaeI	–
NarI	+++
Nb.BbvCI	+++
Nb.BsmI @65°C	++
Nb.BsrDI @65°C	++
Nb.BssSI	+++
Nb.BtsI	++
NciI	+
NcoI	+++
NcoI-HF	+++
NdeI	++
NgoMIV	++
NheI	++
NheI-HF	+++
NlaIII	+
NlaIV	+
NmeAIII	–
NotI	++
NotI-HF	+++
NruI	+++
NruI-HF	+++
NsiI	+++
NsiI-HF	+++
NspI	++
Nt.AlwI	+++
Nt.BbvCI	+++
Nt.BsmAI	+++
Nt.BspQI @50°C	++
Nt.BstNBI @55°C	+
Nt.CviPII	–
PacI	+++
PaeR7I	+++
PciI	++
PflFI	+++
PflMI	+
PI-PspI @65°C	+++

Survival in a Reaction (continued)

ENZYME	SURVIVAL
PI-SceI	+++
PhoI @75°C	–
PleI	+++
PluTI	N/A
PmeI	–
PmlI	+
PpuMI	+++
PshAI	–
PsiI	++
PspGI @75°C	++
PspOMI	+++
PspXI	+++
PstI	+
PstI-HF	+++
PvuI	+++
PvuI-HF	+++
PvuII	+++

ENZYME	SURVIVAL
PvuII-HF	+++
RsaI	++
RsrII	+++
SacI	+++
SacI-HF	+++
SacII	+++
SalI	+++
SalI-HF	+++
SapI	+++
Sau3AI	+
Sau96I	+++
SbfI	+
SbfI-HF	+++
ScaI-HF	++
ScrFI	+++
SexAI	++
SfaNI	++

ENZYME	SURVIVAL
SfcI	–
SfiI @50°C	++
SfoI	–
SgrAI	+++
SmaI @25°C	–
SmlI @55°C	++
SnaBI	+
SpeI	++
SpeI-HF	N/A
SphI	+
SphI-HF	+
SrfI	+++
SspI	++
SspI-HF	+++
StuI	+++
StyI	++
StyI-HF	+++

ENZYME	SURVIVAL
StyD4I	+
SwaI @25°C	++
Taq$^{\alpha}$I @65°C	+
TfiI @65°C	++
TliI @16°C	++
TseI @65°C	+
Tsp45I @65°C	+
TspMI @75°C	+
TspRI @65°C	+++
Tth111I @65°C	++
XbaI	+++
XcmI	+++
XhoI	+++
XmaI	+++
XmnI	++
ZraI	–

Cleavage of Supercoiled DNA

Restriction enzymes cleave different DNA substrates with varying efficiency. Restriction enzymes were tested for their ability to cleave various plasmids (pBR322, pUC19 and pLITMUS) under standard reaction conditions. Single sites were tested on each of these plasmids, depending on availability, and average values were taken when there was more than one data point available. Lambda DNA was used as the standard (1 unit to cleave in all cases).

ENZYME	UNITS TO CLEAVE PLASMID
AatII	3
AflIII	1
AhdI	1
AccI	4
Acc65I	1
AflII	2
AgeI	1
AlwNI	2
ApaI	1
ApoI	1
AseI	0.3
AvaI	10
AvrII	1
BaeI	3
BamHI	3
BanII	1
BglII	8
BpmI	1
BsaI	2
BsaAI	20
BsaXI	2
BsiWI	3
BsgI	1
BsmI	1
BspDI	1
BspEI	1
BspMI	**
BspQI	3
BsrFI	2

ENZYME	UNITS TO CLEAVE PLASMID
BsrGI	1
BssHI	4
BtgI	5
ClaI	5
EagI	10
Eco0109I	8
EcoNI	3
EcoRI	3
EcoRV	1
HincII	4
HindIII	5
KasI	4
KpnI	2
MluI	2
NarI	10
NcoI	1
NdeI	3
NgoMIV	2
NheI	5
NruI	1
NsiI	1
PciI	3
PsiI	3
PstI	1
PvuI	2
PvuII	2
SacI	5
SalI	10
SapI	1

ENZYME	UNITS TO CLEAVE PLASMID
ScaI	20
SmaI	1
SnaBI	1
SpeI	1
SphI	3
SspI	4
StuI	3
StyI	4
TliI	2
TspMI	1
Tth111I	2
XbaI	2
XhoI	10
XmnI	5

** Requires two copies of its recognition sequence for cleavage to occur.

Generating New Cleavage Sites

New restriction sites can be generated by ligation of DNA fragments with compatible ends. These ends may be generated by:

1. Cleavage followed by fill-in of 5´ overhangs to generate blunt ends.
2. Cleavage with two restriction enzymes that produce blunt ends.
3. Cleavage with two restriction enzymes that produce compatible overhangs.

Compatible ends, generated by each of the above methods, can be ligated to produce DNA sequences that often contain useful restriction endonuclease sites. Generation of these sites is detailed in the following tables.

Recleavable Filled-in 5´ Overhangs

The table below lists a selection of restriction enzymes that generate 5´ overhangs which, if filled-in and ligated, result in new restriction sites. The combinations listed were identified by computer analysis, and have not necessarily been confirmed by experimentation. For a more complete listing visit our website, www.neb.com.

Restriction enzymes that have degenerate recognition specificities (e.g., recognize more than one sequence) have been excluded from this list. Where isoschizomers exist, only one member of each set is listed. Only commercially available enzymes have been listed.

Example:

EcoRI Fragments
5´...G AATTC...3´
3´...CTTAA G...5´

Fill-in and Ligate →

XmnI and AseI Sites
5´...GAATTAATTC...3´
3´...CTTAATTAAG...5´

ENZYME	CLEAVAGE SITE	AFTER FILL-IN/ LIGATION	RECLEAVED BY
Acc65I	G/GTACC	GGTACGTACC	BsaAI, HpyCH4IV, RsaI, **SnaBI**[6]
AciI	C/CGC	CCGCGC	(AciI), BstUI, HhaI
AclI	AA/CGTT	AACGCGTT	AflIII, BstUI, **MluI**[6]
AflII	C/TTAAG	CTTAATTAAG	MseI[2], **PacI**[8], MluCI
AgeI	A/CCGGT	ACCGGCCGGT	BsiEI, (BsrFI)[2], EaeI, **EagI**[6], HaeIII, HpaII
ApaLI	G/TGCAC	GTGCATGCAC	Cac8I, NlaIII, NspI, **SphI**[6]
AscI	GG/CGCGCC	GGCGCGCGCGCC	(BssHII)[2], BstUI, Cac8I, HhaI
AvrII	C/CTAGG	CCTAGCTAGG	AluI, (BfaI)[2]
BamHI	G/GATCC	GGATCGATCC	AlwI, **ClaI**[6], (DpnII[2]), TaqI
BclI	T/GATCA	TGATCGATCA	**ClaI**[6], (DpnII[2]), TaqI
BfaI	C/TAG	CTATAG	SfcI
BglII	A/GATCT	AGATCGATCT	**ClaI**[6], DpnII[2], TaqI
BsiWI	C/GTACG	CGTACGTACG	BsaAI, (BsiWI), HpyCH4IV, RsaI, **SnaBI**[6]
BspDI/ClaI	AT/CGAT	ATCGCGAT	BstUI, **NruI**[6]
BspEI	T/CCGGA	TCCGGCCGGA	BsiEI, EaeI, **EagI**[6], HaeIII, (HpaII)[2]
BspHI	T/CATGA	TCATGCATGA	(NlaIII)[2], **NsiI**[6]
BsrGI	T/GTACA	TGTACGTACA	BsaAI, (RsaI)[2], **SnaBI**[6]
BssHII	G/CGCGC	GCGCGCGCGC	(BssHII), BstUI, Cac8I, HhaI
BstBI	TT/CGAA	TTCGCGAA	BstUI, **NruI**[6]
DpnII/MboI/Sau3AI	/GATC	GATCGATC	**ClaI**[6], (DpnII), TaqI
EagI	C/GGCCG	CGGCCGGCCG	BsiEI, BsrFI, Cac8I, EaeI[2], (EagI[2]), **FseI**[8], HaeIII, HpaII, NaeI
EcoRI	G/AATTC	GAATTAATTC	**AseI**[6], MseI, MluCI, **XmnI**[6]
FatI	/CATG	CATGCATG	**BrfBI**[6], HpyCH4V , (FatI[2])
HinP1I	G/CGC	GCGCGC	**BssHII**[6], BstUI, Cac8I, (HhaI)
HindIII	A/AGCTT	AAGCTAGCTT	AluI, BfaI, Cac8I, **NheI**[6]
HpaII/MspI	C/CGG	CCGCGG	AciI, BsaJI, BstUI, BtgI, MspA1I, **SacII**[6]
HpyCH4IV	A/CGT	ACGCGT	AflIII, BstUI, **MluI**[6]
KasI	G/GCGCC	GGCGCGCGCC	**(BssHII**[6]**)**[2], (BstUI)[2], Cac8I, (HhaI)[2]
MfeI	C/AATTG	CAATTAATTG	**AseI**[6], MluCI[2]
MluI	A/CGCGT	ACGCGCGCGT	**BssHII**[6], BstUI, Cac8I, (HhaI)[2]
MluCI	/AATT	AATTAATT	**AseI**[6], MseI, (MluCI[2])
NarI	GG/CGCC	GGCGCGCC	**AscI**[8], BssHII, BstUI, Cac8I, HhaI
NcoI	C/CATGG	CCATGCATGG	NlaIII, **NsiI**[6]
NgoMIV	G/CCGGC	GCCGGCCGGC	BsiEI, BsrFI, Cac8I, EaeI, **EagI**[6], HaeIII, HpaII, (NgoMIV[2])
NheI	G/CTAGC	GCTAGCTAGC	AluI, BfaI, Cac8I, (NheI)
NotI	GC/GGCCGC	GCGGCCGGCCGC	AciI, BsiEI, BsrFI, Cac8I, EaeI, **(EagI**[6]**)**[2], Fnu4HI, **FseI**[8], HaeIII, HpaII, **NaeI**[6]
PaeR7I/XhoI	C/TCGAG	CTCGATCGAG	BsiEI, DpnII, **PvuI**[6], (TaqI[2])
PciI	A/CATGT	ACATGCATGT	HpyCH4V , (NlaIII[2]), **NsiI**[6]
PspOMI	G/GGCCC	GGGCCGGCCC	BsrFI, Cac8I, **FseI**[8], HaeIII, HpaII, NaeI, Sau96I
PspXI	VC/TCGAGB	VCTCGATCGAGB	**PvuI**[6], (TaqI[2])
SalI	G/TCGAC	GTCGATCGAC	BsiEI, DpnII, **PvuI**[6], TaqI
SpeI	A/CTAGT	ACTAGCTAGT	AluI, (BfaI)[2]
TaqI	T/CGA	TCGCGA	BstUI, **NruI**[6]
XbaI	T/CTAGA	TCTAGCTAGA	AluI, BfaI
XmaI	C/CCGGG	CCCGGCCGGG	BsiEI, EaeI, **EagI**[6], HaeIII, HpaII, NciI, ScrFI

Table Notes

Enzymes in **bold** have recognition sequences of 6 or 8 bases. Sequence length is indicated by superscript (e.g., **AscI**[8] = 8-base cutter).

Enzymes in parentheses indicate that the new sequence is still a substrate for the original enzyme.

A superscript 2 indicates that two identical sites have been generated within the filled-in/ligated sequence. For example, fill-in/ligation of AflII generates the sequence CTTAATTAAG which contains two MseI sites (TTAA).

Recleavable Blunt Ends

The table below lists a selection of blunt-end cutters that produce recleavable ligation products. The combinations listed were identified by computer analysis, and although we have tried to ensure their accuracy, they have not necessarily been confirmed by experimentation. For a more complete listing visit our website, **www.neb.com**

Enzymes that have degenerate recognition sequences (e.g., recognize more than one sequence) are followed by a specific sequence in parentheses and are only listed if a non-degenerate equivalent does not exist. Be aware that these degenerate enzymes will cleave sequences in addition to the one listed. Where isoschizomers exist, only one member of each set is listed. Only commercially available enzymes are shown.

ENZYME	LIGATED TO	RECLEAVED BY
AfeI (AGC/GCT)	BsrBI, MspA1I (CMG/CGG) BstZ17I EcoRV SfoI FspI NaeI SmaI	HpaII AluI SfaNI HaeII, HhaI HhaI AciI, Fnu4HI AciI
AluI (AG/CT)	BsrBI, MspA1I (CMG/CGG) BstZ17I MspA1I (CMG/CTG), PvuII EcoRV SfoI	AciI RsaI AluI MboI HaeIII
BsaAI (YAC/GTR) (CAC/GTR) (TAC/GTR) (TAC/GTR)	BsrBI, MspA1I (CMG/CGG) PmlI PmlI, SnaBI SnaBI	HpaII BsaAI, PmlI BsaAI BsaAI, SnaBI
BstUI (CG/CG)	BsrBI, MspA1I (CMG/CGG) BstZ17I EcoRV SfoI NruI	AciI, BstUI RsaI MboI HaeIII BstUI
BstZ17I (GTA/TAC)	AluI, BsrBI, BstUI, HaeIII, MscI, MspA1I (CMG/CKG), NruI, PvuII, StuI AfeI HincII (GTY/GAC) SspI	RsaI AluI AccI MluCI
DraI (TTT/AAA)	HincII (GTY/AAC), HpaI NruI PmeI, SwaI	MseI TaqI DraI, MseI
EcoRV (GAT/ATC)	AluI, BsrBI, BstUI, MspA1I (CMG/CKG), PvuII HaeIII, MscI, StuI AfeI, SfoI, FspI NruI	MboI AlwI, MboI SfaNI MboI, TaqI
FspI (TGC/GCA)	BsrBI, MspA1I (CMG/CGG) AfeI, SfoI EcoRV NaeI SmaI	HpaII HhaI SfaNI AciI, Fnu4HI AciI
HaeIII/PhoI (GG/CC)	BsrBI, MspA1I (CMG/CGG) BstZ17I MscI, StuI EcoRV SfoI HincII (GTY/GAC)	AciI RsaI HaeIII AlwI, MboI HaeIII, Sau96I BsmFI
HpaI (GTT/AAC)	DraI, PmeI HincII (GTY/AAC) HincII (GTY/GAC) NruI	MseI HincII, HpaI, MseI HincII TaqI
HincII (GTC/RAC) (GTC/RAC) (GTC/RAC) (GTT/RAC) (GTC/RAC) (GTT/RAC) (GTT/RAC) (GTC/RAC)	BsrBI, MspA1I (CMG/CGG) BstZ17I HaeIII, MscI, StuI DraI, PmeI HpaI HpaI NruI RsaI, ScaI	HpaII AccI BsmFI MseI HincII HincII, HpaI, MseI TaqI Tsp45I
MscI (TGG/CCA)	BsrBI, MspA1I (CMG/CGG) BstZ17I HaeIII, StuI EcoRV SfoI HincII (GTY/GAC)	AciI RsaI HaeIII AlwI, MboI HaeIII, Sau96I BsmFI
MspA1I (CAG/CKG) (CCG/CKG) (CCG/CKG) (CAG/CKG) (CCG/CKG) (CMG/CKG) (CCG/CKG) (CCG/CKG) (CAG/CKG) (CCG/CKG)	AluI AluI, HaeIII, MscI, StuI BsaAI, FspI, HincII (GTY/GAC), PmlI, SnaBI BsrBI, PvuII BsrBI BstZ17I BstUI, NruI AfeI EcoRV SfoI SfoI NaeI PvuII SmaI	AluI AciI HpaII AciI, MspA1I AciI, BsaJI, BstUI, MspA1I, Sac II RsaI AciI, BstUI HpaII MboI HaeIII HaeIII, HpaII HpaII, NciI, ScrFI AluI, MspA1I, PvuII BsaJI, HpaII, NciI, ScrFI
NaeI (GCC/GGC)	BsrBI, MspA1I (CMG/CGG), SmaI AfeI, SfoI, FspI	HpaII, NciI, ScrFI AciI, Fnu4HI
NruI (TCG/CGA)	BsrBI, MspA1I (CMG/CGG) BstZ17I BstUI DraI, HincII (GTY/AAC), HpaI, PmeI, RsaI, ScaI, SspI EcoRV SfoI	AciI, BstUI RsaI BstUI TaqI MboI, TaqI HaeIII
PmeI (GTTT/AAAC)	DraI, SwaI HincII (GTY/AAC), HpaI NruI	DraI, MseI MseI TaqI
PmlI (CAC/GTG)	BsaAI (YAC/GTA), SnaBI BsaAI (YAC/GTG) BsrBI, MspA1I (CMG/CGG) BmgBI	BsaAI BsaAI, PmlI HpaII PmlI
PvuII (CAG/CTG)	AluI BsrBI, MspA1I (CMG/CGG) BstZ17I EcoRV SfoI MspA1I (CMG/CTG)	AluI AciI, MspA1I RsaI MboI HaeIII AluI, MspA1I, PvuII
RsaI (GT/AC)	HincII (GTY/GAC) NruI ScaI	Tsp45I TaqI RsaI
ScaI (AGT/ACT)	HincII (GTY/GAC) NruI RsaI	Tsp45I TaqI RsaI
SfoI (GGC/GCC)	AluI, BstUI, MspA1I (CMG/CKG), NruI, PvuII BsrBI, MspA1I (CMG/CGG) HaeIII, MscI, StuI AfeI EcoRV FspI NaeI SmaI	HaeIII HaeIII, HpaII HaeIII, Sau96I HaeII, HhaI SfaNI HhaI AciI, Fnu4HI AciI
SmaI (CCC/GGG)	BsrBI, MspA1I (CMG/CGG) AfeI, SfoI, FspI NaeI	BsaJI, HpaII, NciI, ScrFI AciI HpaII, NciI, ScrFI
SnaBI (TAC/GTA)	BsaAI (YAC/GTA) BsaAI (YAC/GTG), PmlI BsrBI, MspA1I (CMG/CGG)	BsaAI, SnaBI BsaAI HpaII
SspI (AAT/ATT)	BstZ17I NruI	MluCI TaqI
StuI (AGG/CCT)	BsrBI, MspA1I (CMG/CGG) BstZ17I HaeIII, MscI EcoRV SfoI HincII (GTY/GAC)	AciI RsaI HaeIII AlwI, MboI HaeIII, Sau96I BsmFI
SwaI (ATTT/AAAT)	DraI, PmeI	DraI, MseI

Compatible Cohesive Ends

Restriction enzymes that produce compatible cohesive ends often produce recleavable ligation products. The combinations listed were identified by computer analysis, and have not necessarily been confirmed by experimentation.

Where isoschizomers exist, only one member of each set is listed. A selection of enzymes available from New England Biolabs has been listed. For a more complete listing visit our website, **www.neb.com**

Enzymes that have degenerate recognition sequences (e.g., recognize more than one sequence) are followed by a specific sequence in parentheses and are only listed if a non-degenerate equivalent does not exist. Be aware that these degenerate enzymes will cleave sequences in addition to the one listed. A "—" denotes a ligation product that cannot be recleaved.

ENZYME	LIGATED TO	RECLEAVED BY
Acc65I (G/GTACC)	BanI (G/GTACC) BsiWI, BsrGI	Acc65I, BanI, KpnI, NlaIV, RsaI RsaI
AccI (GT/CGAC) (GT/CGAC)	AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI ClaI, BstBI, TaqI	— TaqI
AciI (C/CGC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI BsaHI (GR/CGCC), HinP1I, NarI HpaII	— AciI HpaII
AclI (AA/CGTT)	AccI (GT/CGAC), AciI, ClaI, BstBI, HinP1I, HpaII, NarI, TaqI	—
AgeI (A/CCGGT)	AvaI (C/CCGGG), XmaI BsaWI, BspEI BsrFI (A/CCGGT), SgrAI (CA/CCGGTG) NgoMIV	HpaII, NciI, ScrFI BsaWI, HpaII AgeI, BsaWI, BsrFI, HpaII BsrFI, HpaII
ApaI (GGGCC/C)	BanII (GGGCC/C), Bsp1286I (GGGCC/C)	ApaI, BanII, Bsp120I, Bsp1286I, HaeIII, NlaIV, Sau96I
ApaLI (G/TGCAC)	SfcI (C/TGCAG)	BsgI
ApoI (A/AATTY) (G/AATTY) (R/AATTY)	EcoRI EcoRI MfeI, MluCI	ApoI, MluCI ApoI, EcoRI, MluCI MluCI
AscI (GG/CGCGCC)	MluI BssHII	BstUI, HhaI BssHII, BstUI, Cac8I, HhaI
AseI (AT/TAAT)	BfaI, Csp6I, NdeI MseI	— MseI
AsiSI (GCGAT/CGC)	BsiEI (CGAT/CG) PacI PvuI	DpnII, PvuI MseI DpnII, PvuI
AvaI (C/CCGGG) (C/TCGAG) (C/TCGAG) (C/CCGGG)	AgeI, BsaWI, BspEI, BsrFI (R/CCGGY), NgoMIV, SgrAI (CR/CCGGYG) XhoI SalI XmaI	HpaII, NciI, ScrFI AvaI, TaqI, XhoI TaqI AvaI, BsaJI, HpaII, NciI, ScrFI, SmaI
AvaII (G/GWCC)	PpuMI (RG/GACCY) RsrII PpuMI (RG/GTCCY)	AvaII, NlaIV, Sau96I AvaII, Sau96I AvaII, BsmFI, NlaIV, Sau96I
AvrII (C/CTAGG)	NheI, SpeI, XbaI StyI (C/CTAGG)	BfaI AvrII, BfaI, BsaJI, StyI
BamHI (G/GATCC)	BclI, DpnII BglII, BstYI (R/GATCY) BstYI (G/GATCC)	AlwI, DpnII AlwI, BstYI, DpnII AlwI, BamHI, BstYI, DpnII, NlaIV
BanI (G/GTACC) (G/GCGCC) (G/GTACC)	Acc65I KasI BsiWI, BsrGI	Acc65I, BanI, KpnI, NlaIV, RsaI BanI, BsaHI, HaeII, HhaI, KasI, NarI, NlaIV RsaI
BanII (GGGCC/C) (GAGCT/C)	ApaI, Bsp1286I (GGGCC/C) Bsp1286I (GAGCT/C), SacI	ApaI, BanII, Bsp1286I, HaeIII, NlaIV, Sau96I AluI, BanII, BsiHKAI, Bsp1286I, SacI
BclI (T/GATCA)	BamHI, BstYI (R/GATCY) BglII, MboI	AlwI, DpnII DpnII
BfaI (C/TAG)	AseI, Csp6I, MseI, NdeI	—
BglII (A/GATCT)	BamHI, BstYI (R/GATCY) BclI, DpnII	AlwI, BstYI, DpnII DpnII
BsaHI (GR/CGYC) (GA/CGYC) (GG/CGYC) (GG/CGYC) (GA/CGYC) (GG/CGYC)	AccI (GT/CGAC), ClaI, BstBI, TaqI AciI, HinP1I AciI, HinP1I HpaII NarI NarI	— HgaI HhaI AciI BsaHI, HgaI BanI, BsaHI, HaeII, HhaI, NarI, NlaIV
BsaWI (W/CCGGW)	AgeI, BsrFI (R/CCGGY), SgrAI (CR/CCGGYG) AvaI (C/CCGGG), XmaI BspEI BsrFI (R/CCGGY), NgoMIV NgoMIV	AgeI, BsaWI, BsrFI, HpaII HpaII, NciI, ScrFI BsaWI, BspEI, HpaII BsrFI, HpaII HpaII
BsiEI (CGAT/CG) (CGAT/CG) (CGGC/CG)	PacI PvuI SacII	MseI BsiEI, DpnII, PvuI AciI
BsiHKAI (GTGCA/C)	Bsp1286I (GTGCA/C) Bsp1286I (GAGCA/C) Bsp1286I (GAGCT/C), SacI NsiI PstI, SbfI	BsiHKAI, Bsp1286I BsiHKAI, Bsp1286I AluI, BanII, BsiHKAI, Bsp1286I, SacI — BsgI
BsiWI (C/GTACG)	Acc65I, BanI (G/GTACC), BsrGI	RsaI
Bsp1286I (GGGCC/C) (GTGCA/C) (GGGCC/C) (GAGCT/C) (GWGCW/C) (GTGCA/C) (GTGCA/C)	ApaI, BanII (GGGCC/C) BsiHKAI BanII (GGGCC/C) BanII (GAGCT/C), BsiHKAI, SacI BsiHKAI NsiI PstI, SbfI	ApaI, BanII, Bsp1286I, HaeIII, NlaIV, Sau96I ApaLI, BsiHKAI, Bsp1286I BanII, Bsp1286I AluI, BanII, BsiHKAI, Bsp1286I, SacI BsiHKAI, Bsp1286I — BsgI
BspEI (T/CCGGA)	AgeI, BsaWI, BsrFI (R/CCGGY), SgrAI (CR/CCGGYG) AvaI (C/CCGGG), XmaI BsaWI BsrFI (R/CCGGY), NgoMIV	BsaWI, HpaII HpaII, NciI, ScrFI BsaWI, BspEI, HpaII HpaII
BspHI (T/CATGA)	FatI, NcoI, PciI	FatI, NlaIII
BsrFI (A/CCGGY) (G/CCGGY) (R/CCGGY) (A/CCGGY) (R/CCGGY) (G/CCGGY) (CR/CCGGYG)	AgeI, BsaWI AgeI, BsaWI, NgoMIV AvaI (C/CCGGG), XmaI BsaWI, BspEI BsaWI, BspEI NgoMIV SgrAI	AgeI, BsaWI, BsrFI, HpaII BsrFI, HpaII HpaII, NciI, ScrFI BsaWI, HpaII HpaII BsrFI, Cac8I, HpaII, NaeI BsrFI, HpaII
BsrGI (T/GTACA)	Acc65I, BanI (G/GTACC), BsiWI	RsaI
BssHII (G/CGCGC)	MluI AscI	BstUI, HhaI BssHII, BstUI, Cac8I, HhaI
BstBI (TT/CGAA)	AccI (GT/CGAC), ClaI, TaqI AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI —

ENZYME	LIGATED TO	RECLEAVED BY
BstYI (A/GATCY) (G/GATCY) (R/GATCY) (G/GATCY) (A/GATCY)	BamHI, BglII BamHI BclI, DpnII BclI, DpnII BglII	AlwI, BstYI, DpnII AlwI, BamHI, BstYI, DpnII, NlaIV DpnII AlwI, DpnII BglII, BstYI, DpnII
ClaI (AT/CGAT)	AccI (GT/CGAC), BstBI, TaqI AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI —
DpnII/MboI/ Sau3AI (/GATC)	BamHI, BstYI (R/GATCC) BclI, BglII, BstYI (R/GATCY)	AlwI, DpnII DpnII
EaeI (Y/GGCCR) (C/GGCCR) (T/GGCCR) (C/GGCCR) (T/GGCCR)	PspOMI EagI EagI NotI NotI	HaeIII, Sau96I BsiEI, EaeI, EagI, HaeIII EaeI, HaeIII AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII AciI, EaeI, Fnu4HI, HaeIII
EagI (C/GGCCG)	PspOMI EaeI (Y/GGCCR) EaeI (C/GGCCG) NotI	HaeIII, Sau96I EaeI, HaeIII BsiEI, EaeI, EagI, HaeIII AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII
EcoRI (G/AATTC)	ApoI (G/AATTC) ApoI (R/AATTY) MfeI, MluCI	ApoI, EcoRI, MluCI ApoI, MluCI MluCI
FatI (/CATG)	BspHI, NcoI, PciI	FatI, NlaIII
HinP1I (G/CGC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI AciI, BsaHI (GR/CGCC), NarI BsaHI (GR/CGTC) HpaII	— HhaI HgaI AciI
HpaII/MspI (C/CGG)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI AciI, BsaHI (GR/CGCC), HinP1I, NarI	— AciI
KasI (G/GCGCC)	BanI (G/GCGCC)	BanI, BsaHI, HaeII, HhaI, KasI, NarI, NlaIV
MfeI (C/AATTG)	ApoI (R/ATTTY), EcoRI, MluCI	MluCI
MluI (A/CGCGT)	AscI, BssHII	BstUI, HhaI
MluCI (/AATT)	ApoI (R/AATTY), EcoRI, MfeI	MluCI
MseI (T/TAA)	AseI BfaI, Csp6I, NdeI	MseI —
NarI (GG/CGCC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI AciI, HinP1I BsaHI (GR/CGCC) BsaHI (GR/CGTC) HpaII	— HhaI BanI, BsaHI, HaeII, HhaI, NarI, NlaIV BsaHI, HgaI AciI
NcoI (C/CATGG)	BspHI, FatI, PciI	FatI, NlaIII
NdeI (CA/TATG)	AseI, BfaI, Csp6I, MseI	—
NgoMIV (G/CCGGC)	AgeI, BsaWI, BsrFI (R/CCGGY), SgrAI AvaI (C/CCGGG), XmaI BsaWI, BspEI BsrFI (R/CCGGC), SgrAI	BsrFI, HpaII HpaII, NciI, ScrFI HpaII BsrFI, Cac8I, HpaII, NaeI
NheI (G/CTAGC)	AvrII, SpeI, StyI (C/CTAGG), XbaI	BfaI
NlaIII (CATG/)	SphI, NspI	NlaIII
NotI (GC/GGCCGC)	PspOMI EagI EaeI (Y/GGCCR)	AciI, EaeI, Fnu4HI, HaeIII AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII AciI, BsiEI, EaeI, Fnu4HI, HaeIII

ENZYME	LIGATED TO	RECLEAVED BY
NsiI (ATGCA/T)	BsiHKAI (GTGCA/C), Bsp1286I (GTGCA/C), PstI, SbfI	—
NspI (RCATG/Y)	NlaIII, SphI	NlaIII
PacI (TTAAT/TAA)	AsiSI BsiEI (CGAT/CG), PvuI	MseI
PciI (A/CATGT)	BspHI, FatI, NcoI	FatI, NlaIII
PluTI (GGCGC/C)	HaeII	HaeII
PpuMI (RG/GWCCY) (GG/GTCCY) (GG/GACCY)	AvaII, RsrII AvaII, RsrII AvaII, RsrII	AvaII, Sau96I AvaII, BsmFI, NlaIV, Sau96I AvaII, NlaIV, Sau96I
PspOMI (G/GGCCC)	EaeI (Y/GGCCR), EagI NotI	HaeIII, Sau96I AciI, Fnu4HI, HaeIII, Sau96I
PspXI (VC/TCGAGB)	XhoI, TliI SalI	XhoI, TliI TaqI
PstI (CTGCA/G)	BsiHKAI, Bsp1286I (GTGCA/C) NsiI SbfI	BsgI — PstI
PvuI (CGAT/CG)	AsiSI PacI BsiEI (CGAT/CG)	DpnII, PvuI MseI BsiEI, DpnII, PvuI
RsrII (CG/GWCCG)	AvaII, PpuMI (RG/GACCY) PpuMI (RG/GACCY) PpuMI (RG/GTCCY)	AvaII, Sau96I AvaII, NlaIV, Sau96I AvaII, BsmFI, NlaIV, Sau96I
SacI (GAGCT/C)	BanII (GAGCT/C), BsiHKAI, Bsp1286I (GAGCT/C)	AluI, BanII, BsiHKAI, Bsp1286I, SacI
SacII (CCGC/GG)	BsiEI (CGGC/CG)	AciI
SalI (G/TCGAC)	PspXI, XhoI	TaqI
SbfI (CCTGCA/GG)	BsiHKAI, Bsp1286I (GTGCA/C) NsiI PstI	BsgI — PstI
SfcI (C/TGCAG)	ApaLI	BsgI
SgrAI (CR/CCGGYG)	See BsrFI	
SpeI (A/CTAGT)	AvrII, NheI, StyI (C/CTAGG), XbaI	BfaI
SphI (GCATG/C)	NlaIII, NspI	NlaIII
StyI (C/CTAGG) (C/CATGG)	AvrII NheI, SpeI, XbaI BspHI NcoI	AvrII, BfaI, BsaJI, StyI BfaI NlaIII BsaJI, NcoI, NlaIII, StyI
TaqI (T/CGA)	AccI (GT/CGAC), ClaI, BstBI AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI —
XbaI (T/CTAGA)	AvrII, NheI, SpeI, StyI (C/CTAGG)	BfaI
XhoI (TliI) (C/TCGAG)	PspXI SalI	XhoI, TliI TaqI
XmaI (C/CCGGG)	AgeI, BsaWI, BspEI, BsrFI, NgoMIV, SgrAI AvaI (C/CCGGG)	HpaII, NciI, ScrFI AvaI, BsaJI, HpaII, NciI, ScrFI, SmaI, XmaI

Dam (G^mATC), Dcm (C^mCWGG) and CpG (^mCG) Methylation

DNA methyltransferases (MTases) that transfer a methyl group from S-adenosylmethionine to either adenine or cytosine residues are found in a wide variety of prokaryotes and eukaryotes. Methylation should be considered when digesting DNA with restriction endonucleases because cleavage can be blocked or impaired when a particular base in the recognition site is methylated.

Prokaryotic Methylation

In prokaryotes, MTases have most often been identified as elements of restriction/modification systems that act to protect host DNA from cleavage by the corresponding restriction endonuclease. Most laboratory strains of *E. coli* contain three site-specific DNA methyltransferases.

- Dam methyltransferases– methylation at the N^6 position of the adenine in the sequence GATC (1,2).
- Dcm methyltransferases– methylation at the C^5 position of cytosine in the sequences CCAGG and CCTGG (1,3).
- EcoKI methylase– methylation of adenine in the sequences AAC(N^6A)GTGC and GCAC(N^6A)GTT.

Some or all of the sites for a restriction endonuclease may be resistant to cleavage when isolated from strains expressing the Dam or Dcm MTase if the methylase recognition site overlaps the endonuclease recognition site. For example, plasmid DNA isolated from *dam*$^+$ *E. coli* is completely resistant to cleavage by MboI, which cleaves at GATC sites.

Not all DNA isolated from *E. coli* is methylated to the same extent. While pBR322 DNA is fully modified (and is therefore completely resistant to MboI digestion), only about 50% of λ DNA Dam sites are methylated, presumably because the methylase does not have the opportunity to methylate the DNA fully before it is packaged into the phage head. As a result, enzymes blocked by Dam or Dcm modification will yield partial digestion patterns with λ DNA.

Restriction sites that are blocked by Dam or Dcm methylation can be un-methylated by cloning your DNA into a *dam*$^-$, *dcm*$^-$ strain of *E. coli*, such as *dam*$^-$/*dcm*$^-$ Competent *E. coli* (NEB #C2925).

Restriction sites can also be blocked if an overlapping site is present. In this case, part of the Dam or Dcm sequence is generated by the restriction enzyme sequence, followed by the flanking sequence. This situation should also be considered when designing restriction enzyme digests.

Eukaryotic Methylation

CpG MTases, found in higher eukaryotes (e.g., Dnmt1), transfer a methyl group to the C^5 position of cytosine residues. Patterns of CpG methylation are heritable, tissue specific and correlate with gene expression. Consequently, CpG methylation has been postulated to play a role in differentiation and gene expression (4).

Note: The effects of CpG methylation are mainly a concern when digesting eukaryotic genomic DNA. CpG methylation patterns are not retained once the DNA is cloned into a bacterial host.

Methylation Sensitivity

The table below summarizes methylation sensitivity for NEB restriction enzymes, indicating whether or not cleavage is blocked or impaired by Dam, Dcm or CpG methylation if or when it overlaps each recognition site. This table should be viewed as a guide to the behavior of the enzymes listed rather than an absolute indicator. **Consult REBASE (http://rebase.neb.com/rebase/), the restriction enzyme database, for more detailed information and specific examples upon which these guidelines are based.**

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Legend

●	Not Sensitive
■	Blocked
□ ol	Blocked by Overlapping
□ scol	Blocked by Some Combinations of Overlapping
◆	Impaired
◇ ol	Impaired by Overlapping
◇ scol	Impaired by Some Combinations of Overlapping

Single Letter Code

R = A or G	Y = C or T	M = A or C
K = G or T	S = C or G	W = A or T
H = A or C or T	B = C or G or T	V = A or C or G
D = A or G or T	N = A or C or G or T	

ENZYME	SEQUENCE	Dam	Dcm	CpG
AatII	GACGT/C	●	●	■
AbaSI	^{m}C(11/9)	●	●	●
AccI	GT/MKAC	●	●	□ ol
Acc65I	G/GTACC	●	□ scol	□ scol
AciI	CCGC(-3/-1)	●	●	■
AclI	AA/CGTT	●	●	■
AcuI	CTGAAG(16/14)	●	●	●
AfeI	AGC/GCT	●	●	■
AflII	C/TTAAG	●	●	●
AflIII	A/CRYGT	●	●	●
AgeI	A/CCGGT	●	●	■
AgeI-HF	A/CCGGT	●	●	■
AhdI	GACNNN/NNGTC	●	●	◇ scol
AleI-v2	CACNN/NNGTG	●	●	◇ scol
AluI	AG/CT	●	●	●
AlwI	GGATC(4/5)	■	●	●
AlwNI	CAGNNN/CTG	●	□ ol	●
ApaI	GGGCC/C	●	□ ol	□ ol
ApaLI	G/TGCAC	●	●	□ ol
ApeKI	G/CWGC	●	●	□ ol
ApoI	R/AATTY	●	●	●
ApoI-HF	R/AATTY	●	●	●
AscI	GG/CGCGCC	●	●	■
AseI	AT/TAAT	●	●	●
AsiSI	GCGAT/CGC	●	●	■
AvaI	C/YCGRG	●	●	■
AvaII	G/GWCC	●	□ ol	□ ol
AvrII	C/CTAGG	●	●	●
BaeI	(10/15)ACNNNNGTAYC(12/7)	●	●	□ scol

ENZYME	SEQUENCE	Dam	Dcm	CpG
BaeGI	GKGCM/C	●	●	●
BamHI	G/GATCC	●	●	●
BamHI-HF	G/GATCC	●	●	●
BanI	G/GYRCC	●	□ scol	□ scol
BanII	GRGCY/C	●	●	●
BbsI	GAAGAC(2/6)	●	●	●
BbsI-HF	GAAGAC(2/6)	●	●	●
BbvI	GCAGC(8/12)	●	●	●
BbvCI	CCTCAGC(-5/-2)	●	●	◇ ol
BccI	CCATC(4/5)	●	●	●
BceAI	ACGGC(12/14)	●	●	■
BcgI	(10/12)CGANNNNNNTGC(12/10)	◇ ol	●	□ scol
BciVI	GTATCC(6/5)	●	●	●
BclI	T/GATCA	■	●	●
BclI-HF	T/GATCA	■	●	●
BcoDI	GTCTC(1/5)	●	●	◇ scol
BfaI	C/TAG	●	●	●
BfuAI	ACCTGC(4/8)	●	●	◇ ol
BglI	GCCNNNN/NGGC	●	●	□ scol
BglII	A/GATCT	●	●	●
BlpI	GC/TNAGC	●	●	●
BmgBI	CACGTC(-3/-3)	●	●	■
BmrI	ACTGGG(5/4)	●	●	●
BmtI	GCTAG/C	●	●	●
BmtI-HF	GCTAG/C	●	●	●
BpmI	CTGGAG(16/14)	●	●	●
Bpu10I	CCTNAGC(-5/-2)	●	●	●
BpuEI	CTTGAG(16/14)	●	●	●
BsaI	GGTCTC(1/5)	●	◇ scol	□ scol

ENZYME	SEQUENCE	Dam	Dcm	CpG
BsaI-HFv2	GGTCTC(1/5)	●	◇ scol	□ scol
BsaAI	YAC/GTR	●	●	■
BsaBI	GATNN/NNATC	□ ol	●	□ scol
BsaHI	GR/CGYC	●	□ scol	■
BsaJI	C/CNNGG	●	●	●
BsaWI	W/CCGGW	●	●	●
BsaXI	(9/12)ACNNNNNCTCC(10/7)	●	●	●
BseRI	GAGGAG(10/8)	●	●	●
BseYI	CCCAGC(-5/-1)	●	●	□ ol
BsgI	GTGCAG(16/14)	●	●	●
BsiEI	CGRY/CG	●	●	■
BsiHKAI	GWGCW/C	●	●	●
BsiWI	C/GTACG	●	●	■
BsiWI-HF	C/GTACG	●	●	■
BslI	CCNNNNN/NNGG	●	□ scol	□ scol
BsmI	GAATGC(1/-1)	●	●	●
BsmAI	GTCTC (1/5)	●	●	□ scol
BsmBI	CGTCTC(1/5)	●	●	■
BsmFI	GGGAC(10/14)	●	□ ol	□ ol
BsoBI	C/YCGRG	●	●	●
Bsp1286I	GDGCH/C	●	●	●
BspCNI	CTCAG(9/7)	●	●	●
BspDI	AT/CGAT	□ ol	●	■
BspEI	T/CCGGA	□ ol	●	◆
BspHI	T/CATGA	◇ ol	●	●
BspMI	ACCTGC(4/8)	●	●	●
BspQI	GCTCTTC(1/4)	●	●	●
BsrI	ACTGG(1/-1)	●	●	●
BsrBI	CCGCTC(-3/-3)	●	●	□ scol
BsrDI	GCAATG(2/0)	●	●	●
BsrFI-v2	R/CCGGY	●	●	■
BsrGI	T/GTACA	●	●	●
BsrGI-HF	T/GTACA	●	●	●
BssHII	G/CGCGC	●	●	■
BssSI-v2	CACGAG(-5/-1)	●	●	●
BstAPI	GCANNNN/NTGC	●	●	□ scol
BstBI	TT/CGAA	●	●	■
BstEII	G/GTNACC	●	●	●
BstEII-HF	G/GTNACC	●	●	●
BstNI	CC/WGG	●	●	●
BstUI	CG/CG	●	●	■
BstXI	CCANNNNN/NTGG	●	□ scol	●
BstYI	R/GATCY	●	●	●
BstZ17I-HF	GTA/TAC	●	●	□ scol
Bsu36I	CC/TNAGG	●	●	●
BtgI	C/CRYGG	●	●	●
BtgZI	GCGATG(10/14)	●	●	◆
BtsI-v2	GCAGTG(2/0)	●	●	●
BtsCI	GGATG(2/0)	●	●	●
BtsIMutI	CAGTG(2/0)	●	●	●
Cac8I	GCN/NGC	●	●	□ scol
ClaI	AT/CGAT	□ ol	●	■
CspCI	(11/13)CAANNNNNGTGG(12/10)	●	●	●
CviAII	C/ATG	●	●	●
CviKI-1	RG/CY	●	●	●
CviQI	G/TAC	●	●	●
DdeI	C/TNAG	●	●	●
DpnI	GA/TC	●	●	□ ol
DpnII	/GATC	■	●	●
DraI	TTT/AAA	●	●	●
DraIII-HF	CACNNN/GTG	●	●	◇ ol
DrdI	GACNNNN/NNGTC	●	●	□ scol
EaeI	Y/GGCCR	●	□ ol	□ ol

ENZYME	SEQUENCE	Dam	Dcm	CpG
EagI	C/GGCCG	●	●	■
EagI-HF	C/GGCCG	●	●	■
EarI	CTCTTC(1/4)	●	●	◇ ol
EciI	GGCGGA(11/9)	●	●	□ scol
Eco53kI	GAG/CTC	●	●	□ scol
EcoNI	CCTNN/NNNAGG	●	●	●
Eco0109I	RG/GNCCY	●	□ ol	●
EcoP15I	CAGCAG(25/27)	●	●	●
EcoRI	G/AATTC	●	●	□ scol
EcoRI-HF	G/AATTC	●	●	□ scol
EcoRV	GAT/ATC	●	●	◇ scol
EcoRV-HF	GAT/ATC	●	●	◇ scol
Esp3I	CGCCTC(1/5)	●	●	■
FatI	/CATG	●	●	●
FauI	CCCGC(4/6)	●	●	■
Fnu4HI	GC/NGC	●	●	□ ol
FokI	GGATG(9/13)	●	◇ ol	◇ ol
FseI	GGCCGG/CC	●	◇ scol	■
FspI	TGC/GCA	●	●	■
FspEI	C5mCNNNNNNNNNNN	●	●	●
HaeII	RGCGC/Y	●	●	■
HaeIII	GG/CC	●	●	●
HgaI	GACGC(5/10)	●	●	■
HhaI	GCG/C	●	●	■
HinclI	GTY/RAC	●	●	□ scol
HindIII	A/AGCTT	●	●	●
HindIII-HF	A/AGCTT	●	●	●
HinfI	G/ANTC	●	●	□ scol
HinP1I	G/CGC	●	●	■
HpaI	GTT/AAC	●	●	□ scol
HpaII	C/CGG	●	●	■
HphI	GGTGA(8/7)	■	■	●
Hpy99I	CGWCG/	●	●	■
Hpy166II	GTN/NAC	●	●	□ ol
Hpy188I	TCN/GA	□ ol	●	●
Hpy188III	TC/NNGA	□ ol	●	□ ol
HpyAV	CCTTC(6/5)	●	●	◇ ol
HpyCH4III	ACN/GT	●	●	●
HpyCH4IV	A/CGT	●	●	■
HpyCH4V	TG/CA	●	●	●
KasI	G/GCGCC	●	●	■
KpnI	GGTAC/C	●	●	●
KpnI-HF	GGTAC/C	●	●	●
LpnPI	C5mCDGNNNNNNNNN	●	●	●
MboI	/GATC	■	●	◇ ol
MboII	GAAGA(8/7)	□ ol	●	●
MfeI	C/AATTG	●	●	●
MfeI-HF	C/AATTG	●	●	●
MluI	A/CGCGT	●	●	■
MluI-HF	A/CGCGT	●	●	■
MluCI	/AATT	●	●	●
MlyI	GAGTC(5/5)	●	●	●
MmeI	TCCRAC(20/18)	●	●	□ ol
MnlI	CCTC(7/6)	●	●	●
MscI	TGG/CCA	●	□ ol	●
MseI	T/TAA	●	●	●
MslI	CAYNN/NNRTG	●	●	●
MspI	C/CGG	●	●	●
MspA1I	CMG/CKG	●	●	□ ol
MspJI	5mCNNRNNNNNNNNN	●	●	●
MwoI	GCNNNNN/NNGC	●	●	□ scol
NaeI	GCC/GGC	●	●	■
NarI	GG/CGCC	●	●	■

Dam (G^{m}ATC), Dcm (C^{m}CWGG) and CpG (^{m}CG) Methylation (continued)

ENZYME	SEQUENCE	Dam	Dcm	CpG
Nb.BbvCI	CCTCAGC(none/-2)	●	●	●
Nb.BsmI	GAATGC(none/-1)	●	●	●
Nb.BsrDI	GCAATG(none/0)	●	●	●
Nb.BssSI	CACGAG(none/-1)	●	●	●
Nb.BtsI	GCAGTG(none/0)	●	●	●
NciI	CC/SGG	●	●	◇ ol
NcoI	C/CATGG	●	●	●
NcoI-HF	C/CATGG	●	●	●
NdeI	CA/TATG	●	●	●
NgoMIV	G/CCGGC	●	●	■
NheI	G/CTAGC	●	●	□ scol
NheI-HF	G/CTAGC	●	●	□ scol
NlaIII	CATG/	●	●	●
NlaIV	GGN/NCC	●	□ ol	□ ol
NmeAIII	GCCGAG(21/19)	●	●	●
NotI	GC/GGCCGC	●	●	■
NotI-HF	GC/GGCCGC	●	●	■
NruI	TCG/CGA	□ ol	●	■
NruI-HF	TCG/CGA	□ ol	●	■
NsiI	ATGCA/T	●	●	●
NsiI-HF	ATGCA/T	●	●	●
NspI	RCATG/Y	●	●	●
Nt.AlwI	GGATC(4/none)	■	●	●
Nt.BbvCI	CCTCAGC(-5/none)	●	●	□ scol
Nt.BsmAI	GTCTCN(1/none)	●	●	■
Nt.BspQI	GCTCTTC(1/none)	●	●	●
Nt.BstNBI	GAGTC(4/none)	●	●	●
Nt.CviPII	CCD(-3/none)	●	●	■
PacI	TTAAT/TAA	●	●	●
PaeR7I	C/TCGAG	●	●	■
PciI	A/CATGT	●	●	●
PflFI	GACN/NNGTC	●	●	●
PflMI	CCANNNN/NTGG	●	□ ol	●
PleI	GAGTC(4/5)	●	●	□ scol
PluTI	GGCGC/C	●	●	■
PmeI	GTTT/AAAC	●	●	□ scol
PmlI	CAC/GTG	●	●	■
PpuMI	RG/GWCCY	●	□ ol	●
PshAI	GACNN/NNGTC	●	●	□ scol
PsiI	TTA/TAA	●	●	●
PspGI	/CCWGG	●	■	●
PspOMI	G/GGCCC	●	◇ scol	□ ol
PspXI	VC/TCGAGB	●	●	◆
PstI	CTGCA/G	●	●	●
PstI-HF	CTGCA/G	●	●	●
PvuI	CGAT/CG	●	●	■
PvuI-HF	CGAT/CG	●	●	■
PvuII	CAG/CTG	●	●	●
PvuII-HF	CAG/CTG	●	●	●

ENZYME	SEQUENCE	Dam	Dcm	CpG
RsaI	GT/AC	●	●	□ scol
RsrII	CG/GWCCG	●	●	■
SacI	GAGCT/C	●	●	●
SacI-HF	GAGCT/C	●	●	□ scol
SacII	CCGC/GG	●	●	■
SalI	G/TCGAC	●	●	■
SalI-HF	G/TCGAC	●	●	■
SapI	GCTCTTC(1/4)	●	●	●
Sau3AI	/GATC	●	●	□ ol
Sau96I	G/GNCC	●	□ ol	□ ol
SbfI	CCTGCA/GG	●	●	●
SbfI-HF	CCTGCA/GG	●	●	●
ScaI-HF	AGT/ACT	●	●	●
ScrFI	CC/NGG	●	□ ol	□ ol
SexAI	A/CCWGGT	●	■	●
SfaNI	GCATC(5/9)	●	●	◇ scol
SfcI	C/TRYAG	●	●	●
SfiI	GGCCNNNN/NGGCC	●	◇ ol	□ scol
SfoI	GGC/GCC	●	□ scol	■
SgrAI	CR/CCGGYG	●	●	■
SmaI	CCC/GGG	●	●	■
SmlI	C/TYRAG	●	●	●
SnaBI	TAC/GTA	●	●	■
SpeI	A/CTAGT	●	●	●
SpeI-HF	A/CTAGT	●	●	●
SphI	GCATG/C	●	●	●
SphI-HF	GCATG/C	●	●	●
SrfI	GCCC/GGGC	●	●	■
SspI	AAT/ATT	●	●	●
SspI-HF	AAT/ATT	●	●	●
StuI	AGG/CCT	●	□ ol	●
StyI	C/CWWGG	●	●	●
StyI-HF	C/CWWGG	●	●	●
StyD4I	/CCNGG	●	□ ol	◇ ol
SwaI	ATTT/AAAT	●	●	●
Taq$^{\alpha}$I	T/CGA	□ ol	●	●
TfiI	G/AWTC	●	●	□ scol
TseI	G/CWGC	●	●	□ scol
Tsp45I	/GTSAC	●	●	●
TspMI	C/CCGGG	●	●	■
TspRI	NNCASTGNN/	●	●	●
Tth111I	GACN/NNGTC	●	●	●
XbaI	T/CTAGA	□ ol	●	●
XcmI	CCANNNNN/NNNNTGG	●	●	●
XhoI	C/TCGAG	●	●	◆
XmaI	C/CCGGG	●	●	◆
XmnI	GAANN/NNTTC	●	●	●
ZraI	GAC/GTC	●	●	■

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

General Guidelines for PCR Optimization

New England Biolabs offers a diverse group of DNA Polymerases for PCR-based applications. Specific recommendations for PCR optimization can be found in the product literature or on the individual product webpages. However, these general guidelines will help to ensure success using New England Biolabs' PCR enzymes.

SETUP GUIDELINES

DNA Template

- Use high quality, purified DNA templates whenever possible. Please refer to specific product information for amplification from unpurified DNA (e.g., colony PCR or direct PCR).
- For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction
- For higher complexity templates (e.g., genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction
- Higher DNA concentrations tend to decrease amplicon specificity, particularly for high numbers of cycles

Primers

- Primers should typically be 20–30 nucleotides in length, with 40–60% GC Content
- Primer Tm values should be determined with NEB's Tm Calculator (**TmCalculator.neb.com**)
- Primer pairs should have Tm values that are within 5°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between the primers
- Higher than recommended primer concentrations may decrease specificity
- When engineering restriction sites onto the end of primers, 6 nucleotides should be added 5´ to the site
- Annealing temperatures should be determined according to specific enzyme recommendations. *Please note that Q5® and Phusion®* annealing temperature recommendations are unique.*
- Final concentration of each primer should be 0.05–1 µM in the reaction. Please refer to the more detailed recommendations for each specific enzyme.
- When amplifying products > 20 kb in size, primers should be ≥ 24 nucleotides in length with a GC content above 50% and matched Tm values above 60°C
- To help eliminate primer degradation and subsequent non-specific product formation, use a hot-start enzyme (e.g., One*Taq*® Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

Magnesium Concentration

- Optimal Mg^{2+} concentration is usually 1.5–2.0 mM for most PCR polymerases
- Most PCR buffers provided by NEB already contain sufficient levels of Mg^{2+} at 1X concentrations.
- NEB offers a variety of Mg-free reaction buffers to which supplemental Mg^{2+} can be added for applications that require complete control over Mg^{2+} concentration
- Further optimization of Mg^{2+} concentration can be done in 0.2-1 mM increments, if necessary. For some specific applications, the enzyme may require as much as 6 mM Mg^{2+} in the reaction.
- Excess Mg^{2+} may lead to spurious amplification; Insufficient Mg^{2+} concentrations may cause reaction failure

Deoxynucleotides

- Ideal dNTP concentration is typically 200 µM of each, however, some enzymes may require as much as 400 µM each. Please refer to specific product literature for more detailed recommendations.
- Excess dNTPs can chelate Mg^{2+} and inhibit the polymerase
- Lower dNTP concentration can increase fidelity, however, yield is often reduced
- The presence of uracil in the primer, template, or deoxynucleotide mix will cause reaction failure when using archaeal PCR polymerases. Use One*Taq* or *Taq* DNA Polymerases for these applications.

Enzyme Concentration

- Optimal enzyme concentration in the reaction is specific to each polymerase. Please see the product literature for specific recommendations.
- In general, excess enzyme can lead to amplification failure, particularly when amplifying longer fragments

Starting Reactions

- Unless using a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase), assemble all reaction components on ice
- Add the polymerase last, whenever possible
- Transfer reactions to a thermocycler that has been pre-heated to the denaturation temperature. Please note that pre-heating the thermocycler is not necessary when using a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase).

CYCLING GUIDELINES

Denaturation

- Optimal denaturation temperature ranges from 94°–98°C and is specific to the polymerase in the reaction. Please refer to product information for recommended conditions.
- Avoid longer or higher temperature incubations unless required due to high GC content of the template
- For most PCR polymerases, denaturation of 5–30 seconds is recommended during cycling
- NEB's aptamer-based hot start enzymes do not require additional denaturation steps to activate the enzymes

Annealing

- Primer Tm values should be determined using the NEB Tm Calculator (**TmCalculator.neb.com**)
- For PCR polymerases other than Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase*, annealing temperatures are usually set at 2°–5°C below the lowest Tm of the primer pair
- When using Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase*, annealing temperatures should be set at 0°–3°C above the lowest Tm of the primer pair. Please refer to the product literature for detailed recommendations.
- Non-specific product formation can often be avoided by optimizing the annealing temperature or by switching to a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)
- Annealing temperatures can be optimized by doing a temperature gradient PCR, starting at 5°C below the lowest Tm of the primer pair
- Ideally, primer Tm values should be less than the extension temperature. However, if Tm values are calculated to be greater than the extension temperature, a two-step PCR program (combining annealing and extension into one step) can be employed.

Extension

- Extension temperature recommendations range from 65°–72°C and are specific to each PCR polymerase. Please refer to the product literature for specific recommendations.
- Extension rates are specific to each PCR polymerase. In general, extension rates range from 15–60 seconds per kb. Please refer to the recommendations for each specific product.
- Longer than recommended extension times can result in higher error rates, spurious banding patterns and/or reduction of amplicon yields

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

PCR Troubleshooting Guide

The following guide can be used to troubleshoot PCR reactions. Additional tips for optimizing reactions can be found in the technical reference section of our website, **www.neb.com.**

PROBLEM	POSSIBLE CAUSE	SOLUTION
Sequence errors	Low fidelity polymerase	• Choose a higher fidelity polymerase such as Q5 High-Fidelity (NEB #M0491) or Phusion (NEB #M0530)* DNA Polymerases
	Suboptimal reaction conditions	• Reduce number of cycles • Decrease extension time
	Unbalanced nucleotide concentrations	• Prepare fresh deoxynucleotide mixes
	Template DNA has been damaged	• Start with a fresh template • Try repairing DNA template with the PreCR® Repair Mix (NEB #M0309) • Limit UV exposure time when analyzing or excising PCR product from the gel
	Desired sequence may be toxic to host	• Clone into a non-expression vector • Use a low-copy number cloning vector
Incorrect product size	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (**TmCalculator.neb.com**)
	Mispriming	• Verify that primers have no additional complementary regions within the template DNA
	Improper Mg^{2+} concentration	• Adjust Mg^{2+} concentration in 0.2–1 mM increments
	Nuclease contamination	• Repeat reactions using fresh solutions
No product	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (**TmCalculator.neb.com**) • Test an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer
	Poor primer specificity	• Verify that oligos are complementary to proper target sequence
	Insufficient primer concentration	• Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions
	Missing reaction component	• Repeat reaction setup
	Suboptimal reaction conditions	• Optimize Mg^{2+} concentration by testing 0.2–1 mM increments • Thoroughly mix Mg^{2+} solution and buffer prior to adding to the reaction • Optimize annealing temperature by testing an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor template quality	• Analyze DNA via gel electrophoresis before and after incubation with Mg^{2+} • Check 260/280 ratio of DNA template
	Presence of inhibitor in reaction	• Further purify starting template by alcohol precipitation, drop dialysis or commercial clean up kit • Decrease sample volume
	Insufficient number of cycles	• Rerun the reaction with more cycles
	Incorrect thermocycler programming	• Check program, verify times and temperatures
	Inconsistent thermocycler block temperature	• Test calibration of heating block
	Contamination of reaction tubes or solutions	• Autoclave empty reaction tubes prior to use to eliminate biological inhibitors • Prepare fresh solutions or use new reagents
	Complex template	• Use Q5 High-Fidelity (NEB #M0491) or One*Taq* DNA Polymerase (NEB #M0482) • For GC-rich templates, use One*Taq* DNA Polymerase (NEB #M0480) with One*Taq* GC Reaction Buffer (plus One*Taq* High GC Enhancer, if necessary) or Q5 High-Fidelity DNA Polymerase (NEB #M0491) with the High GC Enhancer • For longer templates, we recommend LongAmp® *Taq* DNA Polymerase (NEB #M0323), Q5 or Q5 Hot Start High Fidelity DNA Polymerase (NEB #M0493)
Multiple or non-specific products	Premature replication	• Use a hot start polymerase, such as Q5 Hot Start High-Fidelity (NEB #M0493) or One*Taq* Hot Start (NEB #M0481) DNA Polymerases • Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature
	Primer annealing temperature too low	• Recalculate primer Tm values using the NEB Tm Calculator (**TmCalculator.neb.com**) • Increase annealing temperature
	Incorrect Mg^{2+} concentration	• Adjust Mg^{2+} in 0.2–1 mM increments
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer • Avoid GC-rich 3´ ends
	Excess primer	• Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions.
	Contamination with exogenous DNA	• Use positive displacement pipettes or non-aerosol tips • Set-up dedicated work area and pipettor for reaction setup • Wear gloves during reaction setup
	Incorrect template concentration	• For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction • For higher complexity templates (e.g., genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

Optimization Tips for Luna® qPCR

TIPS FOR OPTIMIZATION

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**. The following tips can be used to help optimize qPCR. For RT-qPCR guidelines, please see page 340.

Target Selection

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible

DNA Template

- Use high quality, purified DNA templates whenever possible. Luna qPCR is compatible with DNA samples prepared through typical nucleic acid purification methods.
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Generally, useful concentrations of standard and unknown material will be in the range of 10^6 copies to 1 copy. For gDNA samples from large genomes, (e.g., human, mouse) a range of 50–1 pg of gDNA is typical. For small genomes, adjust as necessary using 10^6–1 copy input as an approximate range. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution.
- To generate cDNA, use of the LunaScript® RT SuperMix Kit (NEB #E3010) is recommended. Up to 1 µg total RNA, 1 µg mRNA or 100 ng specific RNA can be used in a 20 µl reaction.
- cDNA does not need to be purified before addition to the Luna reaction but should be diluted at least 1:20 before addition to qPCR

Primers

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's TmCalculator (**TmCalculator.neb.com**) using the Hot Start *Taq* setting
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- Optimal primer concentration for dye-based experiments (250 nM) is lower than for probe-based experiments (400 nM). If necessary, the primer concentration can be optimized between 100–500 nM for dye-based qPCR or 200–900 nM for probe-based experiments.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification.
- For cDNA targets, it is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA
- Primers designed to target intronic regions can ensure amplification exclusively from genomic DNA

Hydrolysis Probes

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

Multiplexing

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR.
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

Cycling Conditions

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the hot start nature of the polymerase, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio®)
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

Reaction Setup

- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization

Assay Performance

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

Optimization Tips for Luna One-Step RT-qPCR

TIPS FOR OPTIMIZATION

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**. The following tips can be used to help optimize your one-step RT-qPCR. For qPCR guidelines (DNA/cDNA starting material), please see page 339.

Target Selection

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible
- Target sequences containing significant secondary structure should be avoided

RNA Template

- Use high quality, purified RNA templates whenever possible. Luna qPCR is compatible with RNA samples prepared through typical nucleic acid purification methods.
- Prepared RNA should be stored in an EDTA-containing buffer (e.g., 1X TE) for long-term stability
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Treatment of RNA samples with DNase I (NEB #M0303) may minimize amplification from genomic DNA contamination
- Generally, useful concentrations of standard and unknown material will be in the range of 10^8 copies to 10 copies. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution. For total RNA, Luna One-Step Kits can provide linear quantitation over an 8-order input range of 1 µg–0.1 pg. For most targets, a standard input range of 100 ng–10 pg total RNA is recommended. For purified mRNA, input of ≤ 100 ng is recommended. For *in vitro*-transcribed RNA, input of ≤ 10^9 copies is recommended.

Primers

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's TmCalculator. (**TmCalculator.neb.com**) using the Hot Start *Taq* setting.
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- The optimal primer concentration for dye-based experiments and probe-based experiments is 400 nM. If necessary, the primer concentration can be optimized between 100–900 nM.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification
- It is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA

Hydrolysis Probes

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

Multiplexing

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR.
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

Reverse Transcription

- The default reverse transcription temperature is 55°C
- For difficult targets, the temperature of reverse transcription may be increased to 60°C for 10 minutes
- Due to the WarmStart feature of the Luna RT, reverse transcription temperatures lower than 50°C are not recommended

Cycling Conditions

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the dual WarmStart/Hot Start feature of the Luna kits, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio).
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

Reaction Setup

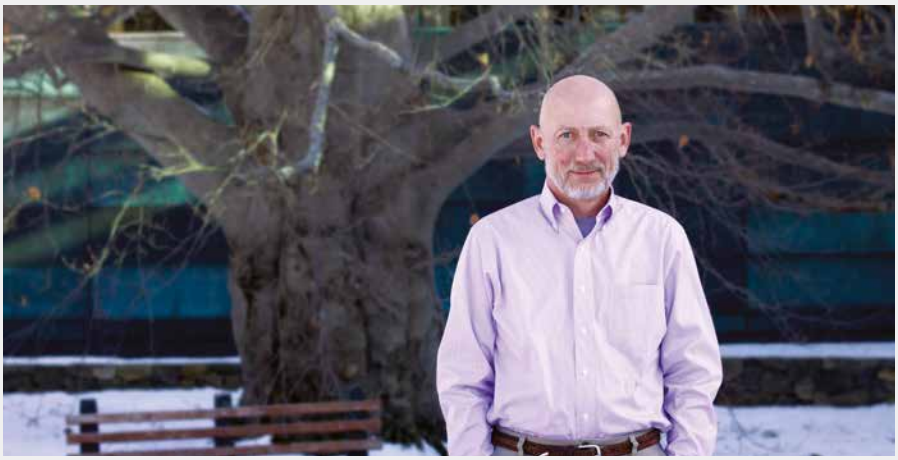
- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- A no Luna RT control should be conducted to guarantee amplification is specific for RNA input and not due to genomic DNA contamination
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization

Assay Performance

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template.
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

Luna qPCR Troubleshooting Guide

PROBLEM	PROBABLE CAUSE(S)	SOLUTION(S)
qPCR traces show low or no amplification	Reagent omitted from qPCR assay Reagent added improperly to qPCR assay	• Verify all steps of the protocol were followed correctly
	Incorrect cycling protocol	• Refer to the proper qPCR cycling protocol in product manual
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings on the qPCR instrument
	DNA template or reagents are contaminated or degraded	• Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in this user manual • Rerun the qPCR assay with fresh reagents • Confirm template input amount
Inconsistent qPCR traces for triplicate data	Improper pipetting during qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler. • Exclude problematic trace(s) from data analysis.
	Poor mixing of reagents during qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
DNA standard curve has a poor correlation coefficient/efficiency of the DNA standard curve falls outside the 90–110% range	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems
	Improper pipetting during qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed correctly
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
	Threshold is improperly set for the qPCR traces	• Ensure the threshold is set in the exponential region of qPCR traces • Refer to the real-time instrument user manual to manually set an appropriate threshold
Melt curve shows different peaks for low input samples	Non-template amplification is occurring Infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks	• Compare melt curve of NTC to samples • Redesign primers with a Tm of 60°C or use our Tm calculator to determine the optimal annealing temperature of the primers • Perform a primer matrix analysis to determine optimal primer concentrations
No template control qPCR trace shows amplification, NTC C_q is close to or overlapping lower copy standards	Reagents are contaminated with carried-over products of previous qPCR (melt curve of NTC matches melt curve of higher input standards)	• Replace all stocks and reagents • Clean equipment and setup area with a 10% chlorine bleach • Consider use of 0.2 U/µl Antarctic Thermolabile UDG to eliminate carryover products
	Primers produce non-specific amplification (melt curve of NTC does not match melt curve of higher input standards)	• Redesign primers with a Tm of 60°C or use qPCR primer design software



Mark has been with NEB for over 35 years and currently serves as our Senior Network Engineer, keeping our communications running smoothly.

Luna One-Step RT-qPCR Troubleshooting Guide

PROBLEM	PROBABLE CAUSE(S)	SOLUTION(S)
qPCR traces show low or no amplification	Incorrect RT step temperature or RT step omitted	• For typical use, a 55°C RT step temperature is optimal for the Luna WarmStart Reverse Transcriptase.
	Incorrect cycling protocol	• Refer to the proper RT-qPCR cycling protocol in product manual
	Reagent omitted from RT-qPCR assay Reagent added improperly to RT-qPCR assay	• Verify all steps of the protocol were followed correctly
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings on the qPCR instrument
	RNA template or reagents are contaminated or degraded	• Prepare high quality RNA without RNase/DNase contamination • Confirm template input amount • Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in product manual • Rerun the RT-qPCR assay with fresh reagents
Inconsistent qPCR traces for triplicate data	Improper pipetting during RT-qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates.	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler • Exclude problematic trace(s) from data analysis
	Poor mixing of reagents during RT-qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
Standard curve has a poor correlation coefficient/efficiency of the standard curve falls outside the 90–110% range	Cycling protocol is incorrect	• Refer to the proper RT-qPCR cycling protocol in product manual • Use a 55°C RT step temperature • For ABI instruments, use a 1 minute 60°C annealing/extension step
	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems
	Improper pipetting during RT-qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed correctly
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
	Threshold is improperly set for the qPCR traces	• Ensure the threshold is set in the exponential region of qPCR traces • Refer to the real-time instrument user manual to manually set an appropriate threshold
Melt curve shows different peaks for low input samples	Non-template amplification is occurring Infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks	• Compare melt curve of NTC to samples • Redesign primers with a Tm of 60°C or use our Tm calculator to determine the optimal annealing temperature of the primers • Perform a primer matrix analysis to determine optimal primer concentrations
No template control qPCR trace shows amplification/NTC C_q is close to or overlapping lower copy standards	Reagents are contaminated with carried-over products of previous qPCR (Melt curve of NTC matches melt curve of higher input standards)	• Replace all stocks and reagents • Clean equipment and setup area with a 10% chlorine bleach • Consider use of 0.2 U/µl Antarctic Thermolabile UDG to eliminate carryover products
	Primers produce non-specific amplification (Melt curve of NTC does not match melt curve of higher input standards)	• Redesign primers with a Tm of 60°C or use qPCR primer design software
Amplification in No-RT control	RNA is contaminated with genomic DNA	• Treat sample with DNase I • Redesign amplicon to span exon-exon junction

Cleavage Close to the End of DNA Fragments

Annealed 5´ FAM-labeled oligos were incubated with the indicated enzyme (10 units/ 1pmol oligo) for 60 minutes at the recommended incubation temperature and NEBuffer. The digest was run on a TBE acrylamide gel and analyzed by fluorescent imaging. The double stranded oligos were designed to have the indicated number of base pairs from the end followed by the recognition sequence and an additional 12 bases. In some cases asymmetric cleavage was observed and interpreted as a negative result. Asymmetric cleavage decreased with increasing base pairs from the end.

Note: As a general rule and for enzymes not listed below, 6 base pairs should be added on on either side of the recognition site to cleave efficiently.

The extra bases should be chosen so that palindromes and primer dimers are not formed. In most cases there is no requirement for specific bases.

Chart Legend

–	0%	+	0–20%
++	20–50%	+++	50–100%
NT	not tested		

	BASE PAIRS FROM END				
ENZYME	1 bp	2 bp	3 bp	4 bp	5 bp
AciI	–	+	+	++	+++
AgeI	+++	+++	+++	+++	+++
AgeI-HF	++	+++	+++	+++	+++
AluI	–	+++	+++	+++	+++
ApaI	+++	+++	+++	+++	+++
AscI	+++	+++	+++	+++	+++
AvrII	++	++	+++	+++	+++
BamHI	+	++	+++	+++	+++
BamHI-HF	+	+	+++	+++	+++
BbsI-HF	+++	+++	+++	+++	+++
BclI-HF	–	–	+++	+++	+++
BglII	++	+++	+++	+++	+++
BmtI	+++	+++	+++	+++	+++
BmtI-HF	+++	+++	+++	+++	+++
BsaI	+++	+++	+++	+++	+++
BsaI-HFv2	+++	+++	+++	+++	+++
BsiWI	++	+++	+++	+++	+++
BsiWI-HF	+++	+++	+++	+++	+++
BsmBI	+++	+++	+++	+++	+++
BsrGI	+++	+++	+++	+++	+++
BssHII	+	+++	+++	+++	+++
BstZ17I-HF	+	+++	+++	+++	+++
ClaI	–	–	+	+++	+++
DdeI	+++	+++	+++	+++	+++
DpnI	–	++	++	NT	NT
DraIII-HF	+++	+++	+++	+++	+++
EagI	++	+++	+++	+++	+++
EagI-HF	+	+++	+++	+++	+++
EcoRI	+	+	++	++	+++
EcoRI-HF	+	+	++	+++	+++
EcoRV	++	++	++	++	+++
EcoRV-HF	+	++	++	++	+++
Esp3I	+++	+++	+++	+++	+++
FseI	+	++	+++	+++	+++
HindIII	–	+	+++	+++	+++
HindIII-HF	–	+	+++	+++	+++
HpaI	+++	+++	+++	+++	+++
KpnI	+	+++	+++	+++	+++
KpnI-HF	+	+++	+++	+++	+++
MfeI	+	++	+++	+++	+++
MfeI-HF	+	++	+++	+++	+++
MluI	+	++	+++	+++	+++
MseI	+++	+++	+++	+++	+++

	BASE PAIRS FROM END				
ENZYME	1 bp	2 bp	3 bp	4 bp	5 bp
NcoI	–	++	+++	+++	+++
NcoI-HF	+	++	+++	+++	+++
NdeI	+	+	+++	+++	+++
NheI	+	++	+++	+++	+++
NheI-HF	++	++	+++	+++	+++
NlaIII	++	+++	+++	+++	+++
NotI	++	++	++	++	++
NotI-HF	++	++	++	++	++
NsiI	+	+	+++	+++	+++
NspI	–	–	+	+	+++
PacI	+++	+++	+++	+++	+++
PciI	+++	+++	+++	+++	+++
PmeI	+++	+++	+++	+++	+++
PstI	+	+++	+++	+++	+++
PstI-HF	++	+++	+++	+++	+++
PvuI	+++	+++	+++	+++	+++
PvuI-HF	+++	+++	+++	+++	+++
PvuII	++	++	++	+++	+++
PvuII-HF	–	++	++	+++	+++
RsaI	+	+++	+++	+++	+++
SacI	–	++	+++	+++	+++
SacI-HF	–	+	+++	+++	+++
SacII	+++	+++	+++	+++	+++
SalI	–	++	+++	+++	+++
SalI-HF	–	++	+++	+++	+++
SapI	+++	+++	+++	+++	+++
Sau3AI	+++	+++	+++	+++	+++
SbfI	++	+++	+++	+++	+++
SbfI-HF	++	+++	+++	+++	+++
ScaI-HF	+	+++	+++	+++	+++
SfiI	+++	+++	+++	+++	+++
SmaI	+++	+++	+++	+++	+++
SpeI	+	++	++	++	++
SpeI-HF	+	++	++	++	++
SphI	+++	+++	+++	+++	+++
SphI-HF	++	++	+++	+++	+++
SspI	+	+++	+++	+++	+++
SspI-HF	+	+++	+++	+++	+++
StuI	+++	+++	+++	+++	+++
StyI	+	++	+++	+++	+++
StyI-HF	+	+++	+++	+++	+++
XbaI	++	++	++	++	++
XhoI	++	++	++	+++	+++
XmaI	+++	+++	+++	+++	+++

Activity of Restriction Enzymes in PCR Buffers

Frequently, a PCR product must be digested with restriction enzymes. For convenience, digestion can be performed directly in the PCR mix without any purification of the DNA. This table summarizes the activity of restriction enzymes on the DNA in *Taq,* Phusion*, One*Taq* and LongAmp *Taq* PCR mixes. 50 µl reactions containing 5 units of restriction enzyme were incubated at the appropriate temperature for 1 hour in a PCR mix containing the following: 1 µg DNA, 1 unit of DNA Polymerase and 1X ThermoPol Reaction Buffer, Standard *Taq* Reaction Buffer, Phusion HF Buffer, One*Taq* Standard Reaction Buffer or LongAmp *Taq* Reaction Buffer. Reactions were supplemented with 200 µM dNTPs. Enzyme activity was analyzed by gel electrophoresis.

Notes: The polymerase is still active and can alter the ends of DNA fragments after cleavage, affecting subsequent ligation. Primers containing the restriction enzyme recognition site can act as competitive inhibitors in the cleavage reaction. The use of restriction enzymes under non-optimal conditions may increase the likelihood of star activity. If any problems are encountered, the DNA should be purified by spin column or phenol/chloroform extraction followed by alcohol precipitation.

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

Chart Legend

Cleavage in extension mix with 5 units of enzyme:

+++	complete cleavage	++	~ 50% cleavage
+	~ 25% cleavage	–	no cleavage

** It has been shown that the addition of 1X Restriction Enzyme Buffer may help to improve the ability of some enzymes to cleave.

ENZYME	*Taq* IN THERMOPOL RXN BUFFER	Q5 IN Q5 BUFFER**	PHUSION IN PHUSION HF BUFFER	ONE*Taq* IN ONE*Taq* RXN BUFFER	LONGAMP *Taq* IN LONGAMP *Taq* RXN BUFFER
AatII	<++	<+	+	++	+
AccI	<++	<+	<+	+++	+++
Acc65I	+++	<+	<+	<+	+
AciI	++	++	+++	+++	+++
AclI	+++	<+	<+	+++	+++
AcuI	+++	<+	++	+++	+++
AfeI	+++	<+	++	+++	+++
AflII	+	<+	<+	+	<+
AflIII	<+	+++	+	<+	<+
AgeI	+++	+	+++	+++	<+
AgeI-HF	+++	<+	++	+++	+++
AhdI	<+	–	–	<+	<+
AleI-v2	–	–	–	+	+
AluI	+++	+	+++	+++	+++
AlwI	–	<+	<+	<+	<+
AlwNI	<++	+	+++	<+	+
ApaI	+++	<+	<+	<+	–
ApaLI	+++	<+	<+	+++	+++
ApeKI	<++	++	+++	<+	+
ApoI	+++	++	+++	++	+++
ApoI-HF	+++	+	++	+++	+++
AscI	+++	<+	<+	<+	–
AseI	+++	<+	+	++	++
AsiSI	+++	<+	++	+++	+++
AvaI	+++	<+	+++	+++	+
AvaII	+++	<+	++	+++	+++
AvrII	+++	<+	<+	+++	+++
BaeGI	+++	<+	+++	+++	+++
BaeI	–	<+	++	<+	<+
BamHI	+++	<+	+++	+++	+++
BamHI-HF	+++	<+	–	<+	++
BanI	+++	<+	+++	+++	+++
BanII	+++	<+	+++	+++	+++
BbsI	+++	<+	<+	+++	+++
BbsI-HF	+	–	–	–	+
BbvCI	+++	–	–	<+	<+
BbvI	+++	<+	++	+++	+++
BccI	<+	<+	<+	<+	<+
BceAI	<+	<+	++	+	<+
BcgI	<+	<+	+	++	++
BciVI	–	–	–	<+	–
BclI	+++	++	+++	+++	+++
BclI-HF	+++	–	–	+	+
BcoDI	<+	<+	+	+	<+
BfaI	–	<+	–	–	–
BfuAI	<++	–	+	<+	–
BglI	<+	++	+	<+	<+
BglII	<+	+	++	<+	<+
BlpI	<++	<+	<+	<+	–
BmgBI	–	++	+	<+	<+
BmrI	<++	<+	+++	+++	+++
BmtI	+++	<+	++	+++	+++
BmtI-HF	++	<+	+	++	+++
BpmI	<+	<+	+++	<++	<++
BpuEI	+++	–	++	<++	<++
Bpu10I	<+	<+	+++	++	+++
BsaAI	+++	++	+++	+++	+++
BsaBI	+	<+	++	++	+++

ENZYME	*Taq* IN THERMOPOL RXN BUFFER	Q5 IN Q5 BUFFER**	PHUSION IN PHUSION HF BUFFER	ONE*Taq* IN ONE*Taq* RXN BUFFER	LONGAMP *Taq* IN LONGAMP *Taq* RXN BUFFER
BsaHI	+++	+	+++	+++	+++
BsaI-HFv2	+	<+	+	+	++
BsaJI	+++	<+	++	+++	+++
BsaWI	<++	<+	++	+	+
BsaXI	<++	<+	<+	<+	<+
BseRI	+++	<+	++	++	+
BseYI	+++	++	++	+++	+++
BsgI	<+	<+	+	<+	<+
BsiEI	+++	<+	++	++	++
BsiHKAI	–	++	+	–	–
BsiWI	+++	<+	+++	+++	+++
BsiWI-HF	–	–	–	–	–
BslI	+++	++	+++	+++	+++
BsmAI	+++	++	+++	<+	<+
BsmBI	<++	+	++	<+	<+
BsmFI	<+	+++	++	+	+
BsmI	+++	+	<+	+++	+
BsoBI	+++	+++	+++	++	+++
BspCNI	<+	<+	+	–	–
BspDI	<++	<+	++	+++	+++
BspEI	–	<+	<+	–	–
BspHI	+++	<+	+++	+++	+++
Bsp1286I	<+	<+	<+	<+	<+
BspMI	+++	<+	++	<+	<+
BspQI	+	++	+++	+++	+++
BsrBI	+++	<+	+	+++	+++
BsrDI	<+	<+	+	<+	<+
BsrFI-v2	<+	–	–	–	–
BsrGI	<+	+	+++	<+	+++
BsrI	+++	<+	+++	++	+++
BssHII	+++	<+	+	+++	+++
BssSI-v2	+++	–	+	+++	+++
BstAPI	+++	<+	++	+++	+++
BstBI	+++	++	+++	+++	+++
BstEII	+++	<+	<+	+++	+++
BstEII-HF	+++	<+	<+	++	++
BstNI	+++	<+	<+	<+	<+
BstUI	+++	<+	<+	+++	+
BstXI	<++	+	+	+	<+
BstYI	+++	<+	<+	++	+
BstZ17I-HF	+++	–	+	+++	+++
Bsu36I	<+	<+	<+	<+	+
BtgI	+++	<+	+	<+	<+
BtgZI	+++	+	++	++	++
BtsI-v2	+++	–	+	+++	+++
BtsCI	+++	<+	<+	+++	+++
Cac8I	+++	<+	<+	+++	++
ClaI	++	<+	<+	<+	++
CspCI	<+	–	+	<+	<+
CviAII	+++	<+	+	+++	+++
CviKI-1	+++	<+	++	+++	+++
CviQI	+++	+	+++	++	+++
DdeI	+++	++	+	+++	+++
DpnI	+++	++	+++	++	++
DpnII	+++	++	+++	+++	++
DraI	+++	<+	+++	+++	+++
DraIII-HF	++	++	+++	++	++
DrdI	+++	<+	+++	+++	+++

ENZYME	*Taq* IN THERMOPOL RXN BUFFER	Q5 IN Q5 BUFFER**	PHUSION IN PHUSION HF BUFFER	ONE*Taq* IN ONE*Taq* RXN BUFFER	LONGAMP *Taq* IN LONGAMP *Taq* RXN BUFFER
EaeI	+++	<+	–	<+	<+
EagI	<+	+++	+++	+++	+++
EagI-HF	+	<+	+	++	++
EarI	+++	<+	+++	+	<+
EciI	<+	++	+++	<++	<++
Eco53kI	+++	<+	<+	+++	+++
EcoNI	+++	<+	+	+++	+++
EcoO109I	+++	<+	–	<+	+
EcoP15I	<+	<+	+	<+	+
EcoRI	+	<+	+++	–	–
EcoRI-HF	+++	<+	+	+++	+++
EcoRV	<+	<+	+	–	<+
EcoRV-HF	+	<+	<+	+	++
Esp3I	+++	-	+++	+	+++
FatI	++	<+	+++	<+	+++
FauI	+	<+	++	+++	++
Fnu4HI	+++	<+	<+	++	+
FokI	+++	+	+	+++	+++
FseI	+	<+	++	+++	–
FspI	<++	<+	+	+	+
HaeII	+++	<+	+++	+++	+++
HaeIII	+++	<+	+++	+++	+++
HgaI	<+	<+	+	<++	<++
HhaI	+++	<+	+++	+++	+++
HincII	+++	<+	<+	+++	+++
HindIII	+++	<+	+	++	+++
HindIII-HF	+++	<+	<+	+++	+++
HinfI	+++	+++	+++	+	+++
HinP1I	+++	+	+++	+++	+++
HpaI	+++	<+	+++	+++	+++
HpaII	+++	<+	<+	<+	<+
HphI	<++	<+	<+	<+	<+
HpyAV	+++	–	++	+	++
HpyCH4III	<++	<+	+	<++	<++
HpyCH4IV	+++	<+	<+	+++	+++
HpyCH4V	+++	<+	<+	+++	+++
Hpy99I	+++	–	+	<+	<+
Hpy188I	+++	<+	+	++	++
Hpy166II	+++	+	++	+++	+++
Hpy188III	+	<+	<+	+	<+
KasI	+++	<+	+++	+++	–
KpnI	+++	++	+	++	<+
KpnI-HF	++	–	++	<+	<+
MboI	+++	<+	+++	+++	+++
MboII	+++	+	++	+	+
MfeI	+++	<+	<+	+++	+
MfeI-HF	+	–	–	+++	<+
MluCI	+	<+	<+	++	+
MluI	+++	++	++	++	++
MluI-HF	++	–	++	++	++
MlyI	+++	+	++	<+	+
MmeI	<+	–	++	<+	<+
MnlI	+++	+	+	+	+
MscI	<+	<+	+	<+	<+
MseI	<+	<+	<+	<+	<+
MslI	+++	<+	+	+++	++
MspA1I	+++	<+	+++	++	+++
MspI	+++	<+	+++	++	+++
MwoI	+++	+++	+++	++	+++
NaeI	<+	<+	+	<+	<+
NarI	–	<+	++	+++	+++
NciI	+++	<+	<+	+	<+
NcoI	+++	<+	+	++	++
NcoI-HF	+++	<+	–	++	+
NdeI	<++	++	+++	++	<+
NgoMIV	–	<+	+	<+	<+
NheI	+++	<+	<+	+++	+++
NheI-HF	+++	<+	–	++	++
NlaIII	<+	<+	+	++	<+
NlaIV	+++	<+	+++	+++	+++
NmeAIII	<+	–	+++	<+	<+
NotI	++	<+	+	<+	<+
NotI-HF	+++	<+	<+	<+	+

ENZYME	*Taq* IN THERMOPOL RXN BUFFER	Q5 IN Q5 BUFFER**	PHUSION IN PHUSION HF BUFFER	ONE*Taq* IN ONE*Taq* RXN BUFFER	LONGAMP *Taq* IN LONGAMP *Taq* RXN BUFFER
NruI	++	+	+	++	++
NruI-HF	++	–	–	+	–
NsiI	+++	+	+++	++	+
NsiI-HF	+++	++	+++	+++	+++
NspI	+++	<+	<+	+++	++
PacI	+++	<+	<+	++	+++
PaeR7I	+++	<+	<+	+++	+++
PciI	<+	<+	–	–	–
PflFI	+++	<+	<+	<+	+
PflMI	+	<+	+++	++	+++
PleI	+++	<+	<+	<+	<+
PluTI	+++	<+	+	+++	+++
PmeI	+++	<+	<+	+++	+++
PmlI	–	–	–	+	<+
PpuMI	+++	<+	+++	+++	+++
PshAI	+++	<+	<+	<+	<+
PsiI	+++	<+	<+	<+	+++
PspGI	+++	+++	+++	+++	+++
PspOMI	+++	<+	+	+++	+++
PspXI	+++	<+	++	+++	+++
PstI	++	+	+	<+	<+
PstI-HF	+++	<+	++	++	+
PvuI	<+	<+	+++	–	<+
PvuI-HF	+++	<+	+++	++	+++
PvuII	+++	<+	+	+++	+++
PvuII-HF	+	–	–	<+	<+
RsaI	+++	<+	++	+++	+++
RsrII	<++	–	–	<+	<+
SacI	+++	<+	+	++	++
SacI-HF	+++	<+	<+	<+	++
SacII	+++	<+	+++	++	+
SalI	<+	+	++	–	–
SalI-HF	+	<+	+++	+	+++
SapI	<++	<+	++	++	++
Sau3AI	+++	<+	<+	<+	<+
Sau96I	<++	+	+	+++	+++
SbfI	<++	<+	+	<+	+++
SbfI-HF	+	–	–	<+	<+
ScaI-HF	+	<+	<+	–	–
ScrFI	+++	+++	+++	+++	+++
SexAI	+++	<+	+++	+++	+++
SfaNI	–	<+	++	<++	<++
SfcI	+++	<+	<+	+	+
SfiI	+++	–	–	+++	+++
SfoI	+++	<+	+++	+	+++
SgrAI	<++	<+	++	+	+++
SmaI	+++	<+	++	+++	+++
SmlI	<+	<+	+	+	+
SnaBI	<+	<+	<+	+++	+++
SpeI	+++	+	<+	+++	+++
SpeI-HF	+++	–	<+	+++	+++
SphI	+++	+	++	<+	<+
SphI-HF	+++	<+	+	+++	+++
SrfI	<+	<+	+++	+	++
SspI-HF	++	<+	+	+++	+++
StuI	+++	<+	<+	+++	+++
StyD4I	<++	<+	+	<+	<+
StyI	<+	+	<+	<+	<+
StyI-HF	+	<+	<+	++	+++
SwaI	<+	<+	<+	<+	+++
Taq^α^I	+++	<+	+	+++	+++
TfiI	<++	<+	<+	++	++
TseI	+++	+++	+++	+++	+++
Tsp45I	+++	–	–	+	<+
TspMI	+++	<+	+	+++	+++
TspRI	+	<+	<+	+++	+++
Tth111I	+++	<+	++	<+	+
XbaI	+++	–	<+	++	++
XcmI	+++	<+	+	+++	+++
XhoI	<+	<+	+++	++	+++
XmaI	+++	<+	+	–	–
XmnI	+++	<+	<+	++	+++
ZraI	+++	<+	<+	++	+

Molecular cloning has traditionally used restriction enzymes to excise a fragment from source DNA, and to linearize a plasmid vector, while creating compatible ends. After purification, insert and vector are ligated to form a recombinant vector, which is transformed into an *E. coli* host. Alternatively, PCR can been used to generate both the vector and insert, which can be joined using a variety of techniques, such as standard DNA ligation, enzymatic joining using a recombinase or topoisomerase, or homologous recombination.

Regardless of the method chosen, the process can be made more efficient and successful by following good practices in the lab. The following tips will help improve the success of your cloning experiments.

1. Take the time to plan your experiments

Pay attention to the junction sequences and the effect on reading frames of any translated sequences. Check both the vector and insert for internal restriction sites (we recommend NEBcutter at **NEBcutter.neb.com**) prior to designing PCR primers that contain similar sites to those used for cloning. Verify that the antibiotic selective marker in the vector is compatible with the chosen host strain.

2. Start with clean DNA at the right concentration

Ensure that your source DNA is free of contaminants, including nucleases and unwanted enzymatic activities. Use commercially-available spin columns to purify starting DNA, (e.g., Monarch Plasmid Miniprep Kit, NEB #T1010 for DNA plasmids, Monarch PCR & DNA Cleanup Kit, NEB #T1030 for DNA Fragments). Completely remove solvents, such as phenol, chloroform and ethanol, prior to manipulation of the DNA. Elute DNA from the spin columns with salt-free buffer to prevent inhibition of the downstream steps, either restriction digestion or PCR amplification. Use a sufficient amount of DNA for the technique being used. Preparative restriction digests often require between 0.2 –2.0 µg, while single nanogram amounts are usually sufficient for DNA being used as a PCR template.

3. Perform your restriction digests carefully

The reaction volume should be compatible with the downstream step (e.g., smaller than the volume of the well of an agarose gel used to resolve the fragments). For a typical cloning reaction, this is often between 20–50 µl. The volume of restriction enzyme(s) added should be no more than 10% of the total reaction volume, to ensure that the glycerol concentration stays below 5%; this is an important consideration to minimize star activity (unwanted cleavage).

4. Mind your ends

DNA ends prepared for cloning by restriction digest are ready for ligation without further modification, assuming the ends to be joined are compatible. If the ends are non-compatible, they can be modified using blunting reagents, phosphatases, etc.

DNA ends prepared by PCR for cloning may have a 3´ addition of a single adenine (A) residue following amplification using *Taq* DNA Polymerase (NEB #M0273). High-fidelity DNA polymerases, such as Q5 (NEB #M0491), leave blunt ends. PCR using standard commercial primers produces non-phosphorylated fragments, unless the primers were 5´ phosphorylated. The PCR product may need to be kinase treated to add a 5´ phosphate prior to ligation with a dephosphorylated vector.

5. Clean up your DNA prior to vector:insert joining

This can be done with gel electrophoresis or column purification (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030). Isolating the desired DNA from unwanted parent vectors and/or other DNA fragments can dramatically improve your cloning results.

Confirm digested DNA on an agarose gel prior to ligation. For a single product, run a small amount of the digest, and then column purify to capture the remainder (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030). When multiple fragments are produced and only one is to be used, resolve the fragments on a gel and excise the desired fragment under UV light. Using longwave (365 nm) UV light will minimize any radiation-induced DNA damage to the fragment. Recover the DNA fragment from the agarose slice using a gel extraction kit (e.g., Monarch DNA Gel Extraction Kit, NEB #T1020) or β-Agarase I (NEB #M0392).

6. Quantitate your isolated material

Simple quantitation methods, such as gel electrophoresis with mass standards or spectroscopic quantitation on low-input spectrophotometers (such as a NanoSpec®), ensure that the proper amount of material is used for the downstream joining reaction.

7. Follow the manufacturer's guidelines for the joining reaction

For traditional cloning, follow the guidelines specified by the ligase supplier. If a 3:1 molar ratio of insert to vector is recommended, try this first for best results. Using a 3:1 mass ratio is not the same thing (unless the insert and vector have the same mass). Ligation usually proceeds quickly and, unless your cloning project requires the generation of a high-complexity library that benefits from the absolute capture of every possible ligation product, long incubation times are not necessary.

Follow the manufacturer's guidelines for the joining reactions in PCR cloning and seamless cloning. If you are performing a cloning protocol for the first time, adhere to the recommended protocol for optimal results.

8. Use competent cells that are suited to your needs

While some labs prepare their own competent cells "from scratch" for transformations, the levels of competence achieved rarely matches the high levels attained with commercially-available competent cells. Commercially-available competent cells save time and resources, and make cloning more reproducible.

Find an overview of traditional cloning.

Traditional Cloning Quick Guide

PREPARATION OF INSERT AND VECTORS

Insert From a Plasmid Source

- Digest plasmid with the appropriate restriction enzymes to produce a DNA fragment that can be cloned directly into a vector. Unidirectional cloning is achieved with restriction enzymes that produce non-compatible ends.

Insert From a PCR Product

- Design primers with appropriate restriction sites to clone unidirectionally into a vector
- Addition of 6 bases upstream of the restriction site is sufficient for digestion with most enzymes
- If fidelity is a concern, choose a proofreading polymerase such as Q5 High-Fidelity DNA Polymerase (NEB #M0491)
- Visit **www.NEBPCRPolymerases.com** for additional guidelines for PCR optimization
- Purify PCR product by running the DNA on an agarose gel and excising the band or by using a spin column (e.g., Monarch® DNA Gel Extraction Kit, NEB #T1020, Monarch PCR & DNA Cleanup Kit, NEB #T1030)
- Digest with the appropriate restriction enzyme

Standard Restriction Enzyme Protocol

DNA	1 µg
10X NEBuffer	5 µl (1X)
Restriction Enzyme	10 units is sufficient, generally 1 µl is used
Nuclease-free Water	To 50 µl
Incubation Time	1 hour*
Incubation Temperature	Enzyme dependent

** Can be decreased by using a Time-Saver qualified enzyme*

Time-Saver Restriction Enzyme Protocol

DNA	1 µg
10X NEBuffer	5 µl (1X)
Restriction Enzyme	1 µl
Nuclease-free Water	To 50 µl
Incubation Time	5–15 minutes*
Incubation Temperature	Enzyme dependent

** Time-Saver qualified enzymes can also be incubated overnight with no star activity*

Insert from Annealed Oligos

- Annealed oligos can be used to introduce a fragment (e.g., promoter, polylinker, etc.)
- Anneal two complementary oligos that leave protruding 5´ or 3´ overhangs for ligation into a vector cut with appropriate enzymes
- Non-phosphorylated oligos can be phosphorylated using T4 Polynucleotide Kinase (NEB #M0201)

Typical Annealing Reaction

Primer	1 µg
10X T4 Ligase Buffer	5 µl
Nuclease-free Water	To 50 µl
Incubation	85°C for 10 minutes, cool slowly (30-60 min.)

Vector

- Digest vector with appropriate restriction enzymes. Enzymes that leave non-compatible ends are ideal as they prevent vector self-ligation

DEPHOSPHORYLATION

- Dephosphorylation is sometimes necessary to prevent self-ligation. NEB offers four products for dephosphorylation of DNA:
- The Quick Dephosphorylation Kit (NEB #M0508), Shrimp Alkaline Phosphatase (rSAP) (NEB #M0371) and Antarctic Phosphatase (AP) (NEB #M0289) are heat-inactivatable phosphatases. They work in all NEBuffers, but AP requires supplementation with Zn^{2+}
- Calf Intestinal Phosphatase (CIP) (NEB #M0290) will function under many different conditions and in most NEBuffers. However, CIP cannot be heat inactivated and requires a purification step (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) before ligation.

Dephosphorylation of 5´ ends of DNA Using the Quick Dephosphorylation Kit

DNA	1 pmol of DNA ends
10X CutSmart Buffer	2 µl
Quick CIP	1 µl
Nuclease-free Water	To 20 µl
Incubation	37°C for 10 minutes
Heat Inactivation	80°C for 2 minutes

Note: Scale larger reaction volumes proportionally.

BLUNTING

- In some instances, the ends of the insert or vector require blunting
- PCR with a proofreading polymerase will leave a predominantly blunt end
- T4 DNA Polymerase (NEB #M0203) or Klenow (NEB #M0210) will fill in a 5´ overhang and chew back a 3´ overhang
- The Quick Blunting Kit (NEB #E1201) is optimized to blunt and phosphorylate DNA ends for cloning in less than 30 minutes
- Analyze agarose gels with longwave UV (360 nM) to minimize UV exposure that may cause DNA damage

Blunting with the Quick Blunting Kit

DNA	Up to 5 µg
10X Blunting Buffer	2.5 µl
dNTP Mix (1 mM)	2.5 µl
Blunt Enzyme Mix	1 µl
Nuclease-free Water	To 25 µl
Incubation	room temperature; 15 min for RE-digested DNA; 30 min for sheared/nebulized DNA or PCR products*
Heat Inactivation	70°C for 10 minutes

** PCR-generated DNA must be purified before blunting using a purification kit (NEB #T1030), phenol extraction/ethanol precipitation, or gel extraction (NEB #T1020).*

PHOSPHORYLATION

- For ligation to occur, at least one of the DNA ends (insert or vector) should contain a 5´ phosphate
- Primers are usually supplied non-phosphorylated; therefore, the PCR product will not contain a 5´ phosphate
- Digestion of DNA with a restriction enzyme will always produce a 5´ phosphate
- A DNA fragment can be phosphorylated by incubation with T4 Polynucleotide Kinase (NEB #M0201)

Phosphorylation With T4 PNK

DNA (20 mer)	1–2 µg
10X T4 PNK Buffer	5 µl
10 mM ATP	5 µl (1 mM final conc.)
T4 PNK	1 µl (10 units)
Nuclease-free Water	To 50 µl
Incubation	37°C for 30 minutes

PURIFICATION OF VECTOR AND INSERT

- Purify the vector and insert by either running the DNA on an agarose gel and excising the appropriate bands or by using a spin column, such as Monarch DNA Gel Extraction Kit or PCR & DNA Cleanup Kit (NEB #T1020 or T1030)
- DNA can also be purified using β-Agarase I (NEB #M0392) with low melt agarose, or an appropriate spin column or resin
- Analyze agarose gels with longwave UV (360 nM) to minimize UV exposure that may cause DNA damage

LIGATION OF VECTOR AND INSERT

- Use a molar ratio of 1:3 vector to insert. Use NEBioCalculator to calculate molar ratios.
- If using T4 DNA Ligase (NEB #M0202) or the Quick Ligation Kit (NEB #M2200), thaw and resuspend the Ligase Buffer at room temp. If using Ligase Master Mixes, no thawing is necessary.
- The Quick Ligation Kit (NEB #M2200) is optimized for ligation of both sticky and blunt ends
- Instant Sticky-end Ligase Master Mix (NEB #M0370) is optimized for instant ligation of sticky/cohesive ends
- Blunt/TA Ligase Master Mix (NEB #M0367) is optimized for ligation of blunt or single base overhangs, which are the more challenging type of ends for T4 DNA Ligase
- Following ligation, chill on ice and transform
- DO NOT heat inactivate when using the Quick Ligation Buffer or Ligase Master Mixes, as this will inhibit transformation
- Electroligase (NEB #M0369) is optimized for ligation of both sticky and blunt ends and is compatible with electroporation (i.e., no cleanup step required)
- Improved Golden Gate Assembly can be achieved by selecting high fidelity overhangs [Potapov, V. et al. (2018) *ACS Synth. Biol.* 7(11), 2665–2674.

Ligation with the Quick Ligation Kit

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	To 50 ng
2X Quick Ligation Buffer	10 µl
Quick T4 DNA Ligase	1 µl
Nuclease-free Water	20 µl (mix well)
Incubation	Room temperature for 5 minutes

Ligation with Instant Sticky-end Ligase Master Mix

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	None

Ligation with Blunt/TA Ligase Master Mix

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	Room temperature for 15 minutes

TRANSFORMATION

- To obtain tranformants in 8 hrs., use NEB Turbo Competent *E. coli* (NEB #C2984)
- If recombination is a concern, then use the *recA*⁻ strains NEB 5-alpha Competent *E. coli* (NEB #C2987), NEB-10 beta Competent *E. coli* (NEB #C3019) or NEB Stable Competent *E. coli* (NEB #C3040)
- NEB-10 beta Competent *E. coli* works well for constructs larger than 5 kb
- NEB Stable Competent *E. coli* (NEB #C3040) can be used for constructs with repetitive sequences such as lentiviral constructs
- If electroporation is required, use NEB 5-alpha (NEB #C2989) or NEB 10-beta (NEB #C3020) Electrocompetent *E. coli*
- Use pre-warmed selection plates
- Perform several 10-fold serial dilutions in SOC for plating

Transformation with NEB 5-alpha Competent *E. coli*

DNA	1–5 µl containing 1 pg – 100 ng of plasmid DNA
Competent *E. coli*	50 µl
Incubation	On ice for 30 minutes
Heat Shock	Exactly 42°C for exactly 30 seconds
Incubation	On ice for 5 minutes Add 950 µl room temperature SOC 37°C for 60 minutes, with shaking

Troubleshooting Guide for Cloning

We strongly recommend running the following controls during transformations. These controls may assist in identifying which step(s) in the cloning workflow has failed.

- Transform 100 pg – 1 ng of uncut vector to check cell viability, calculate transformation efficiency and verify the antibiotic resistance of the plasmid.
- Transform the cut vector to determine the amount of background due to undigested plasmid. The number of colonies in this control should be < 1% of the number of colonies in the uncut plasmid control transformation (from control #1).
- Transform a vector only ligation reaction. The ends of the vector should not be able to re-ligate because either they are incompatible (e.g., digested with two restriction enzymes that do not generate compatible ends) or the 5´ phosphate group has been removed in a dephosphorylation reaction (e.g., blunt ends treated with rSAP). This control transformation should yield the same number of colonies as control #2.
- Digest vector DNA with a single restriction enzyme, re-ligate and transform. The ends of the vector DNA should be compatible and easily joined during the ligation reaction, resulting in approximately the same number of colonies as control #1.

The cloning workflow often benefits from an accurate quantitation of the amount of DNAs that are being worked with. We recommend quantification of DNAs whenever possible.

PROBLEM	CAUSE	SOLUTION
Few or no transformants	Cells are not viable	• Transform an uncut plasmid (e.g., pUC19) and calculate the transformation efficiency of the competent cells. If the transformation efficiency is low (< 10^4) re-make the competent cells or consider using commercially available high efficiency competent cells.
	Incorrect antibiotic or antibiotic concentration	• Confirm antibiotic and antibiotic concentration
	DNA fragment of interest is toxic to the cells	• Incubate plates at lower temperature (25–30°C). • Transformation may need to be carried out using a strain that exerts tighter transcriptional control over the DNA fragment of interest (e.g., NEB 5-alpha F´ I^q Competent *E. coli* (NEB #C2992))
	If using chemically competent cells, the wrong heat-shock protocol was used	• Follow the manufacturer's specific transformation protocol (Note: going above the recommended temperature during the heat shock can result in competent cell death)
	If using electrocompetent cells, PEG is present in the ligation mix	• Clean up DNA by drop dialysis prior to transformation with Monarch PCR & DNA Cleanup Kit (NEB #T1030) • Try NEB's ElectroLigase (NEB #M0369)
	If using electrocompetent cells, arcing was observed or no voltage was registered	• Clean up the DNA prior to the ligation step • Tap the cuvette to get rid of any trapped air bubbles • Be sure to follow the manufacturer's specified electroporation parameters
	Construct is too large	• Select a competent cell strain that can be transformed efficiently with large DNA constructs (≥ 10 kb, we recommend trying NEB 10-beta Competent *E. coli* (NEB #C3019)) • For very large constructs (> 10 kb), consider using electroporation
	Construct may be susceptible to recombination	• Select a *recA*- strain such as NEB 5-alpha (NEB #C2987), NEB 10-beta (NEB #C3019) or NEB Stable (NEB #C3040) Competent *E. coli*
	The insert comes directly from mammalian or plant DNA and contains methylated cytosines, which are degraded by many *E. coli* strains	• Use a strain that is deficient in McrA, McrBC and Mrr, such as NEB 10-beta Competent *E. coli*
	Too much ligation mixture was used	• Use < 5 µl of the ligation reaction for the transformation
	Inefficient ligation	• Make sure that at least one fragment being ligated contains a 5´ phosphate moiety • Vary the molar ratio of vector to insert from 1:1 to 1:10. Use NEBiocalculator to calculate molar ratios • Purify the DNA to remove contaminants such as salt and EDTA with Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) • ATP will degrade after multiple freeze-thaws; repeat the ligation with fresh buffer • Heat inactivate or remove the phosphatase prior to ligation • Ligation of single base-pair overhangs (most difficult) may benefit from being carried out with Blunt/TA Master Mix (NEB #M0367), Quick Ligation Kit (NEB #M2200) or concentrated T4 DNA Ligase (NEB #M0202) • Test the activity of the ligase by carrying out a ligation control with Lambda-HindIII digested DNA (NEB #N0312)
	Inefficient phosphorylation	• Purify the DNA prior to phosphorylation with Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030). Excess salt, phosphate or ammonium ions may inhibit the kinase. • If the ends are blunt or 5´ recessed, heat the substrate/buffer mixture for 10 minutes at 70°C. Rapidly chill on ice before adding the ATP and enzyme, then incubate at 37°C. • ATP was not added. Supplement the reaction with 1 mM ATP, as it is required by T4 Polynucleotide Kinase (NEB #M0201) • Alternatively, use 1X T4 DNA Ligase Buffer (contains 1 mM ATP) instead of the 1X T4 PNK Buffer

Troubleshooting Guide for Cloning (continued)

PROBLEM	CAUSE	SOLUTION
Few or no transformants	Inefficient blunting	• Heat inactivate or remove the restriction enzymes prior to blunting • Clean up the PCR fragment prior to blunting with Monarch PCR & DNA Cleanup Kit (NEB #T1030) • Sonicated gDNA should be blunted for at least 30 minutes • Do not use > 1 unit of enzyme/µg of DNA • Do not incubate for > 15 minutes • Do not incubate at temperatures > 12°C (for T4 DNA Polymerase, NEB #M0203) or > 24°C (for Klenow, NEB #M0210) • Make sure to add a sufficient amount of dNTPs to the reaction (33 µM each dNTP for DNA Polymerase I, Large (Klenow) Fragment, NEB #M0210 and 100 µM each dNTP for T4 DNA Polymerase, NEB #M0203). • When using Mung Bean Nuclease (NEB #M0250), incubate the reaction at room temperature. Do not use > 1 unit of enzyme/µg DNA or incubate the reaction > 30 minutes.
	Inefficient A-Tailing	• Clean up the PCR prior to A-tailing. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). High-fidelity enzymes will remove any non-templated nucleotides.
	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove any contaminants that may inhibit the enzyme. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • When digesting a PCR fragment, make sure to have at least 6 nucleotides between the recognition site and the end of the DNA molecule
Colonies don't contain a plasmid	Antibiotic level used was too low	• Increase the antibiotic level on plates to the recommended amount • Use fresh plates with fresh antibiotics
	Satellite colonies were selected	• Choose large, well-established colonies for analysis
Colonies contain the wrong construct	Recombination of the plasmid has occurred	• Use a *recA*$^-$ strain such NEB 5-alpha, NEB 10-beta or NEB Stable Competent *E. coli*
	Incorrect PCR amplicon was used during cloning	• Optimize the PCR conditions • Gel purify the correct PCR fragment. NEB recommends the Monarch DNA Gel Extraction Kit (NEB #T1020).
	Internal recognition site was present	• Use NEBcutter to analyze insert sequence for presence of an internal recognition site
	DNA fragment of interest is toxic to the cells	• Incubate plates at lower temperature (25–30°C) • Transformation may need to be carried out using a strain that exerts tighter transcriptional control of the DNA fragment of interest (e.g., NEB 5-alpha F´ I^q Competent *E. coli*)
	Mutations are present in the sequence	• Use a high-fidelity polymerase (e.g., Q5 High-Fidelity DNA Polymerase, NEB #M0491) • Re-run sequencing reactions
Too much background	Inefficient dephosphorylation	• Heat inactivate or remove the restriction enzymes prior to dephosphorylation
	Kinase is present/active	• Heat inactivate the kinase after the phosphorylation step. Active kinase will re-phosphorylate the dephosphorylated vector.
	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the restriction enzyme(s) to be sure it is not inhibited by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove contaminants. (e.g., too much salt). NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
	Antibiotic level is too low	• Confirm the correct antibiotic concentration
Ran the ligation on a gel and saw no ligated product	Inefficient ligation	• Make sure at least one DNA fragment being ligated contains a 5´ phosphate • Vary the molar ratios of vector to insert from 1:1 to 1:10. Use NEBioCalculator to calculate molar ratios. • Purify the DNA to remove contaminants such as salt and EDTA. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • ATP will degrade after multiple freeze-thaws; repeat the ligation with fresh buffer • Heat inactivate or remove the phosphatase prior to ligation • Ligation of single base-pair overhangs (most difficult) may benefit from being carried out with Blunt/TA Master Mix, Quick Ligation Kit or concentrated T4 DNA Ligase • Test the activity of the ligase by carrying out a ligation control with Lambda-HindIII digested DNA
The ligated DNA ran as a smear on an agarose gel	The ligase is bound to the substrate DNA	• Treat the ligation reaction with Proteinase K (NEB #P8107) prior to running on a gel
The digested DNA ran as a smear on an agarose gel	The restriction enzyme(s) is bound to the substrate DNA	• Lower the number of units • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA
	Nuclease contamination	• Use fresh, clean running buffer • Use a fresh agarose gel • Clean up the DNA. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030).

PROBLEM	CAUSE	SOLUTION
Incomplete restriction enzyme digestion	Cleavage is blocked by methylation	• DNA isolated from a bacterial source may be blocked by Dam and Dcm methylation • DNA isolated from eukaryotic source may be blocked by CpG methylation • Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • If the enzyme is inhibited by Dam or Dcm methylation, grow the plasmid in a dam-/dcm- strain (NEB #C2925)
	Salt inhibition	• Enzymes that have low activity in salt-containing buffers (NEBuffer 3.1) may be salt sensitive, so clean up the DNA prior to digestion. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch kits (NEB #T1010, #T1020, #T1030) use columns that have been designed to minimize salt carry over into the eluted DNA, so using them can minimize this issue. To prevent this, DNA solution should be no more than 25% of total reaction volume.
	Inhibition by PCR components	• Clean up the PCR fragment prior to restriction digest. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
	Using the wrong buffer	• Use the recommended buffer supplied with the restriction enzyme
	Too few units of enzyme used	• Use at least 3–5 units of enzyme per µg of DNA
	Incubation time was too short	• Increase the incubation time
	Digesting supercoiled DNA	• Some enzymes have a lower activity on supercolied DNA. Increase the number of enzyme units in the reaction.
	Presence of slow sites	• Some enzymes can exhibit slower cleavage towards specific sites. Increase the incubation time, 1–2 hours is typically sufficient.
	Two sites required	• Some enzymes require the presence of two recognition sites to cut efficiently. For more information, visit the table "Restriction Enzymes Requiring Multi-sites" on neb.com.
	DNA is contaminated with an inhibitor	• Assay substrate DNA in the presence of a control DNA. Control DNA will not cleave if there is an inhibitor present. Miniprep DNA is particularly susceptible to contaminants. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • Clean DNA with a spin column, with Monarch PCR & DNA Cleanup Kit (NEB #T1030), resin or drop dialysis, or increase volume to dilute contaminant
Extra bands in the gel	If larger bands than expected are seen in the gel, this may indicate binding of the enzyme(s) to the substrate	• Lower the number of units in the reaction • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the substrate
	Star activity	• Use the recommended buffer supplied with the restriction enzyme • Decrease the number of enzyme units in the reaction • Make sure the amount of enzyme added does not exceed 10% of the total reaction volume. This ensures that the total glycerol concentration does not exceed 5% v/v • Decrease the incubation time. Using the minimum reaction time required for complete digestion will help prevent star activity. • Try using a High-Fidelity (HF) restriction enzyme. HF enzymes have been engineered for reduced star activity.
	Partial restriction enzyme digest	• Enzymes that have low activity in salt-containing buffers (e.g., NEBuffer 3.1) may be salt sensitive. Make sure to clean up the DNA prior to digestion. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch kits (NEB #T1010, #T1020, #T1030) use columns that have been designed to minimize salt carry over into the eluted DNA, so using them can minimize this issue. To prevent this, DNA solution should be no more than 25% of total reaction volume • Clean-up the PCR fragment prior to restriction digest. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • Use the recommended buffer supplied with the restriction enzyme • Use at least 3–5 units of enzyme per µg of DNA • Digest the DNA for 1–2 hours
No PCR fragment amplified	Used the wrong primer sequence	• Double check the primer sequence
	Incorrect annealing temperature	• Use the NEB Tm calculator to determine the correct annealing temperature (**www.neb.com/TmCalculator**)
	Incorrect extension temperature	• Each polymerase type has a different extension temperature requirement. Follow the manufacturer's recommendations.
	Too few units of polymerase	• Use the recommended number of polymerase units based on the reaction volume
	Incorrect primer concentration	• Each polymerase has a different primer concentration requirement. Make sure to follow the manufacturer's recommendations.
	Mg^{2+} levels in the reaction are not optimal	• Titrate the Mg^{2+} levels to optimize the amplification reaction. Follow the manufacturer's recommendations.
	Difficult template	• With difficult templates, try different polymerases and/or buffer combinations
The PCR reaction is a smear on a gel	If bands are larger than expected it may indicate binding of the enzyme(s) to the DNA	• Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA
Extra bands in PCR reaction	Annealing temperature is too low	• Use the NEB Tm calculator to determine the annealing temperature of the primers
	Mg^{2+} levels in the reaction are not optimal	• Titrate the Mg^{2+} levels to optimize the amplification reaction. Make sure to follow the manufacturer's recommendations.
	Additional priming sites are present	• Double check the primer sequence and confirm it does not bind elsewhere in the DNA template
	Formation of primer dimers	• Primer sequence may not be optimal. Additional primers may need to be tested in the reaction.
	Incorrect polymerase choice	• Try different polymerases and/or buffer combinations

Optimization Tips for Your Cloning Reactions

New England Biolabs offers a wide selection of reagents for your cloning experiments. For more information, visit **ClonewithNEB.com**. The following tips can be used to help optimize each step in your cloning workflow. Tips for restriction enzyme digestion and amplification can be found on pages 290 and 337, respectively.

cDNA SYNTHESIS

Starting Material

- Intact RNA of high purity is essential for generating cDNA for cloning applications
- Total RNA or mRNA can be used in the reverse transcription reaction. Total RNA is generally sufficient for cDNA synthesis reactions. However, if desired, mRNA can be easily obtained using a PolyA Spin mRNA Isolation Kit (NEB #S1560) or Magnetic mRNA Isolation Kit (NEB #S1550).
- The amount of RNA required for cDNA cloning depends on the abundance of the transcript-of-interest. In general, 1 ng to 1 µg total RNA or 0.1–100 ng mRNA are recommended.

Product Selection

- Streamline your reaction setup by using the ProtoScript II First Strand cDNA Synthesis Kit (NEB #E6560). This kit combines ProtoScript II Reverse Transcriptase (NEB #M0360), a thermostable M-MuLV (RNase H^-) Reverse Transcriptase, and recombinant RNase Inhibitor in an enzyme Master Mix, along with a separate Reaction Mix containing dNTPs. Additionally, the kit contains two optimized reverse transcription primer mixes.

Yield

- ProtoScript II Reverse Transcriptase is capable of generating cDNA of more than 10 kb up to 48°C. We recommend 42°C for routine reverse transcription.
- You can increase the yield of a long cDNA product by doubling the amount of enzyme and dNTPs

Additives

- For most RT-PCR reactions, RNase H treatment is not required. But for some difficult amplicons or sensitive assays, add 2 units of *E. coli* RNase H to the reaction and incubate at 37°C for 20 minutes

PHOSPHORYLATION

Enzyme

- T4 Polynucleotide Kinase (NEB #M0201) and T4 DNA Ligase (NEB #M0202) can be used together in the T4 DNA Ligase Buffer
- T4 Polynucleotide Kinase is inhibited by high levels of salt (50% inhibition by 150 mM NaCl), phosphate (50% inhibition by 7 mM phosphate) and ammonium ions (75% inhibited by 7 mM $(NH_4)_2SO_4$)
- If using T4 Polynucleotide Kinase and working with 5´-recessed ends, heat the reaction mixture for 10 min at 70°C, chill rapidly on ice before adding the ATP (or Ligase Buffer containing ATP) and enzyme, then incubate at 37°C

Additives

- The addition of PEG 8000 (up to 5%) can improve results

DEPHOSPHORYLATION

Enzyme

- When dephosphorylating a fragment following a restriction enzyme digest, a DNA clean up step is required if the restriction enzyme(s) used is NOT heat inactivatable. We recommend the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
- When working with the Quick Dephosphorylation Kit (NEB #M0508), rSAP (NEB #M0371) or AP (NEB #M0289), which are heat-inactivatable enzymes, a DNA clean-up step after dephosphorylation is not necessary prior to the ligation step. However, when using CIP (NEB #M0290), a clean-up step (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) prior to ligation is necessary.

Additives

- AP requires the presence of Zn^{2+} in the reaction, so don't forget to supplement the reaction with 1X Antarctic Phosphatase Reaction Buffer when using other NEBuffers

BLUNTING/END REPAIR

Enzyme

- Make sure that you choose the correct enzyme to blunt your fragment. The Quick Blunting Kit (NEB #E1201), T4 DNA Polymerase (NEB #M0203) and DNA Polymerase I, Large (Klenow) Fragment (NEB #M0210) will fill 5´ overhangs and degrade 3´ overhangs. Mung Bean Nuclease (NEB #M0250) degrades 5´ overhangs.
- T4 DNA Polymerase and DNA Polymerase I, Large (Klenow) Fragment are active in all NEBuffers. Please remember to add dNTPs.

Clean-up

- When trying to blunt a fragment after a restriction enzyme digestion, if the restriction enzyme(s) used are heat inactivable, then a clean-up step prior to blunting is not needed. Alternatively, if the restriction enzyme(s) used are not heat inactivable, a DNA clean-up step is recommended prior to blunting.
- When trying to blunt a fragment amplified by PCR, a DNA clean-up step is necessary prior to the blunting step to remove the nucleotides and polymerase
- When trying to dephosphorylate a fragment after the blunting step, you will need to add a DNA clean-up step (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) after the blunting and before the addition of the phosphatase

Temperature

- When trying to blunt a fragment with Mung Bean Nuclease, the recommended temperature of incubation is room temperature, since higher temperatures may cause sufficient breathing of the dsDNA ends that the enzyme may degrade some of the dsDNA sequence. The number of units to be used and time of incubation may be determined empirically to obtain best results.

Heat Inactivation

- Mung Bean Nuclease reactions should not be heat inactivated. Although Mung Bean Nuclease can be inactivated by heat, this is not recommended because the DNA begins to "breathe" before the Mung Bean Nuclease is inactivated and undesirable degradation occurs at breathing sections. Purify DNA by phenol/chloroform extraction and ethanol precipitation or spin column purification [e.g., Monarch PCR & DNA Cleanup Kit (NEB #T1030)].

A-TAILING

- If the fragment to be tailed has been amplified with a high-fidelity polymerase, the DNA needs to be purified prior to the tailing reaction. For this we recommend the Monarch PCR & DNA Cleanup Kit (NEB T1030). Otherwise, any high-fidelity polymerase present in the reaction will be able to remove any non-templated nucleotides added to the end of the fragments.

DNA LIGATION

Reaction Buffers

- T4 DNA Ligase Buffer (NEB #B0202) should be thawed on the bench or in the palm of your hand, and not at 37°C (to prevent breakdown of ATP)
- Once thawed, T4 DNA Ligase Buffer should be placed on ice
- Ligations can be performed in any of the four standard restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer (NEB #B0201) supplemented with 1 mM ATP
- When supplementing with ATP, use ribo-ATP (NEB #P0756). Deoxyribo-ATP will inhibit ligation.
- Before ligation, completely inactivate restriction enzyme by heat inactivation, spin column (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) or Phenol/EtOH purification

DNA

- Either heat inactivate (AP, SAP, Quick Dephosphorylation Kit) or remove phosphatase (CIP) before ligation
- Keep total DNA concentration between 1–10 µg/ml
- Vector:Insert molar ratios between 1:1 and 1:10 are optimal for single insertions. Use NEBioCalculator at **NEBioCalculator.neb.com** to calculate molar ratios.
- For cloning more than one insert, we recommend the NEBuilder® HiFi DNA Assembly Master Mix (NEB #E2621) or Cloning Kit (NEB #E5520)
- If you are unsure of your DNA concentration, perform multiple ligations with varying ratios

Ligase

- For cohesive-end ligations, standard T4 DNA Ligase. Instant Sticky-end Ligase Master Mix or the Quick Ligation Kit are recommended.
- For blunt and single-base overhangs the Blunt/TA Ligase Master Mix is recommended.
- For ligations that are compatible with electroporation, Electroligase is recommended
- Standard T4 DNA Ligase can be heat inactivated at 65°C for 20 minutes
- Do not heat inactivate the Quick Ligation Kit or the ligase master mixes

Transformation

- Add between 1–5 µl of ligation mixture to competent cells for transformation
- Extended ligation with PEG causes a drop off in transformation efficiency
- Electroporation is recommended for larger constructs (> 10,000 bp). Dialyze samples or use a spin column first if you have used the Quick Ligation Kit or ligase master mixes
- For ligations that are compatible with electroporation, Electroligase is recommended.

TRANSFORMATION

Thawing

- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming above 0°C decreases efficiency

DNA

- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency

Incubation & Heat Shock

- Incubate on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes this step is shortened.
- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended, except when using BL21 (NEB #C2530) which requires exactly 10 seconds.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes this step is shortened.
- SOC and NEB 10-beta/Stable Outgrowth Medium give 2-fold higher transformation efficiency than LB medium
- Incubation with shaking or rotation results in 2-fold higher transformation efficiency

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on transformation efficiency
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA Contaminants to Avoid

CONTAMINANT	REMOVAL METHOD
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify (e.g., Monarch PCR & DNA Cleanup Kit) or phenol/chloroform extract and ethanol precipitate

Troubleshooting Guide for DNA Cleanup & Plasmid Purification using Monarch® Kits

PROBLEM	PRODUCT	POSSIBLE CAUSE	SOLUTION
No DNA purified	Monarch Plasmid Miniprep Kit (NEB #T1010)	Buffers added incorrectly	• Add buffers in the correct order so that the sample is bound, washed and eluted in the correct sequence • Ensure ethanol was added to Plasmid Wash Buffer 2
		Plasmid loss during culture growth	• Ensure proper antibiotic and concentration was used to maintain selection during culture growth
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Ethanol not added to wash buffer	• Ensure the proper amount of ethanol was added to Monarch DNA Wash Buffer
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)		
Low DNA yield	Monarch Plasmid Miniprep Kit (NEB #T1010)	Incomplete lysis	• Pellet must be completely resuspended before addition of Plasmid Lysis Buffer (B2) – color should change from light to dark pink • Avoid using too many cells; this can overload the column. If culture volume is larger than recommended, scale up buffers B1-B3.
		Plasmid loss during culture growth	• Ensure proper antibiotic and concentration was used to maintain selection during culture growth
		Low-copy plasmid selected	• Increase amount of cells processed and scale buffers accordingly
		Lysis of cells during growth	• Harvest culture during transition from logarithmic growth to stationary phase (~12-16 hours)
		Incomplete neutralization	• Invert tube several times until color changes to yellow
		Incomplete elution	• Deliver Elution Buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of plasmids > 10 kb, heat the DNA Elution Buffer to 50°C and extend incubation time to 5 minutes
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Buffers added incorrectly	• Be sure that buffers have been reconstituted correctly and that reagents have been added in the correct order
		Gel slice not fully dissolved	• Undissolved agarose may clog the column and interfere with binding. Incubate in Monarch Gel Dissolving Buffer for proper time and temperature.
		Gel dissolved above 60°C	• Dissolve gel slice in specified range (37-55°C). Higher temperatures can denature DNA
		Incomplete elution during preparation	• Deliver Elution Buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of DNA > 10 kb, heat the Elution Buffer to 50°C and extend incubation time to 5 minutes • Multiple rounds of elution can also be performed
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)	Buffers added incorrectly	• Be sure that buffers have been reconstituted correctly and that reagents have been added in the correct order
		Incomplete elution during preparation	• Deliver Elution Buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of DNA > 10 kb, heat the Elution Buffer to 50°C and extend incubation time to 5 minutes • Multiple rounds of elution can also be performed
Low DNA quality	Monarch Plasmid Miniprep Kit (NEB #T1010)	Plasmid degradation	• Be cautious of strains with high levels of endogenous endonuclease (e.g., HB101 and JM 100 series)
		Plasmid is denatured	• Limit incubation with Plasmid Lysis Buffer (B2) to two minutes, as NaOH in the buffer can denature the plasmid
		gDNA contamination	• Use careful inversion mixing after cell lysis to avoid shearing of host cell chromosomal DNA. Do not vortex.
		RNA contamination	• Incubate sample in neutralization buffer for the full 2 minutes. For cell culture volumes > 3 ml, increase the spin after neutralization to 5 minutes.
		Improper storage	• Elute DNA in DNA Elution Buffer or nuclease-free water, and store at -20°C. Do not store in solutions containing magnesium.
Low DNA performance	Monarch Plasmid Miniprep Kit (NEB #T1010)	Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Excessive salt in sample	• Use both plasmid wash buffers and do not skip wash steps
		Excessive carbohydrate has been carried over	• Avoid strains with high amounts of endogenous carbohydrate (e.g., HB101 and JM 100 series). Be sure to follow protocol and include Plasmid Wash Buffer 1 step.
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Gel slice not fully dissolved	• Undissolved agarose may leach salts into the eluted DNA
		Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Trace amounts of salts have been carried over	• Ensure column tip does not come in contact with new tube for elution
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)	Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Trace amounts of salts have been carried over	• Ensure column tip does not come in contact with new tube

Guidelines for Choosing Sample Input Amounts When Using the Monarch Genomic Purification Kit

Genomic DNA yield, purity and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield, purity, and DIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Genomic DNA Purification Kit. It is very important not to overload the column and the buffer system when extracting and purifying gDNA, as DNA yields, purity, integrity, and length may suffer.

SAMPLE TYPE	RECOMMENDED INPUT AMOUNT	TYPICAL YIELD (µg)	DIN	MAXIMUM INPUT AMOUNT
TISSUE*				
Tail (mouse)	10 mg	12–20	8.5–9.5	25 mg
Ear (mouse)	10 mg	18–21	8.5–9.5	10 mg
Liver (mouse and rat)	10 mg	15–30	8.5–9.5	15 mg
Kidney (mouse)	10 mg	10–25	8.5–9.5	10 mg
Spleen (mouse)	10 mg	30–70	8.5–9.5	10 mg
Heart (mouse)	10 mg	9–10	8.5–9.5	25 mg
Lung (mouse)	10 mg	14–20	8.5–9.5	15 mg
Brain (mouse and rat)	10 mg	4–10	8.5–9.5	12 mg
Muscle (mouse and rat)	10 mg	4–7	8.5–9.5	25 mg
Muscle (deer)	10 mg	5	8.5–9.5	25 mg
BLOOD**				
Human (whole)	100 µl	2.5–4	8.5–9.5	100 µl
Mouse	100 µl	1–3	8.5–9.5	100 µl
Rabbit	100 µl	3–4	8.5–9.5	100 µl
Pig	100 µl	3.5–5	8.5–9.5	100 µl
Guinea pig	100 µl	3–8	8.5–9.5	100 µl
Cow	100 µl	2–3	8.5–9.5	100 µl
Horse	100 µl	4–7	8.5–9.5	100 µl
Dog	100 µl	2–4	8.5–9.5	100 µl
Chicken (nucleated)	10 µl	30–45	8.5–9.5	10 µl
CELLS				
HeLa	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
HEK293	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
NIH3T3	1 x 10^6 cells	6–7.5	9.0–9.5	5 x 10^6 cells
BACTERIA				
E. coli (Gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
Rhodobacter sp. (Gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
B. cereus (Gram-positive)	2 x 10^9 cells	6–9	8.5–9.0	2 x 10^9 cells
ARCHAEA				
T. kodakarensis	2 x 10^9 cells	3–5	8.5–9.0	2 x 10^9 cells
YEAST				
S. cerevisiae	5 x 10^7 cells	0.5–0.6	8.5–9.0	5 x 10^7 cells
SALIVA/BUCCAL CELLS***				
Saliva (human)	200 µl	2–3	7.0–8.0	500 µl
Buccal swab (human)	1 swab	5–7	6.0–7.0	1 swab

* Tissue gDNA yields are shown for frozen tissue powder, frozen tissue pieces and RNAlater-stabilized tissue pieces. Though frozen tissue powder results in highly-intact gDNA, lower yields can be expected than when using frozen or RNAlater-stabilized tissue pieces. Residual nuclease activity in tissue pieces will cut the gDNA, resulting in a slightly smaller overall size; however, this gDNA is optimal for silica-based purification.

** Human whole blood samples stabilized with various anticoagulants (e.g., EDTA, citrate and heparin) and various counter-ions were evaluated and results were comparable in all cases. Additionally, all indicated blood samples were tested both as fresh and frozen samples, yielding comparable results. Human samples were donated by healthy individuals; yields from unhealthy donors may differ.

*** Buccal swabs and saliva samples partially consist of dead cell material with degraded gDNA. Therefore, the purified gDNA from those samples will naturally have lower DIN values.

Troubleshooting Guide for Genomic DNA Purification using the Monarch Kit

PROBLEM	CAUSE	SOLUTION
LOW YIELD		
Cells	Frozen cell pellet was thawed and/or resuspended too abruptly	• Thaw cell pellets slowly on ice and flick tube several times to release the pellet from bottom of tube. Use cold PBS, and resuspend gently by pipetting up and down 5–10 times until pellet is dissolved
	Cell Lysis Buffer was added concurrently with enzymes	• Add Proteinase K and RNase A to sample and mix well before adding the Cell Lysis Buffer
Blood	Blood was thawed, allowing for DNase activity	• Keep blood samples frozen and add Proteinase K, RNase A and Blood Lysis Buffer directly to the frozen samples
	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Formation of hemoglobin precipitates	• Species with high hemoglobin content (e.g., guinea pig) may accumulate insoluble hemoglobin complexes that clog the membrane. Reduce Proteinase K lysis time from 5 to 3 minutes.
Tissue	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will destroy the DNA before the Proteinase K can lyse the tissue.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
	Sample was not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Genomic DNA was degraded (common in DNase-rich tissues)	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
	Column is overloaded with DNA	• Some organ tissues (e.g., spleen, kidney, liver) are extremely rich in genomic DNA. Using inputs larger than recommended will result in the formation of tangled, long-fragment gDNA that cannot be eluted from the silica membrane. Reduce the amount of input material.
	Incorrect amount of Proteinase K added	• Most samples are digested with 10 µl Proteinase K, but for brain, kidney and ear clips, use 3 µl.
DNA DEGRADATION		
Tissue	Tissue samples were not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will degrade the DNA before Proteinase K can lyse the tissue.
	High DNase content of soft organ tissue	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
Blood	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Blood was thawed, allowing for DNase activity	• Keep frozen blood samples frozen and add enzymes and lysis buffer directly to the frozen samples
SALT CONTAMINATION		
	Guanidine Thiocyanate salt from the binding buffer was carried over into the eluate	• When transferring the lysate/binding buffer mix, avoid touching the upper column area with the pipet tip and always pipet carefully onto the silica membrane. • Avoid transferring any foam that may have been present in the lysate; foam can enter into the cap area of the spin column. • Close the caps gently to avoid splashing the mixture into the upper column area and move the samples with care in and out of the centrifuge. • If salt contamination is a concern, invert the columns a few times (or vortex briefly) with gDNA Wash Buffer as indicated in the protocol.
PROTEIN CONTAMINATION		
Tissue	Incomplete digestion	• Cut samples to the smallest possible pieces. Incubate sample in the lysis buffer for an extra 30 minutes to 3 hours to degrade any remaining protein complexes.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small, indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge the lysate at maximum speed for 3 minutes as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
Blood	High hemoglobin content	• Some blood samples (e.g., horse) are rich in hemoglobin, evidenced by their dark red color. Extend lysis time by 3–5 minutes for best purity results.
	Formation of hemoglobin precipitates	• Species with high hemoglobin content (e.g., guinea pig) may accumulate insoluble hemoglobin complexes that clog the membrane. Reduce Proteinase K lysis from 5 to 3 minutes.
RNA CONTAMINATION		
Tissue	Too much input material	• DNA-rich tissues (e.g., spleen, liver and kidney) will become very viscous during lysis and may inhibit RNase A activity. Do not use more than the recommended input amount.
	Lysis time was insufficient	• Extend lysis time by 30 minutes to 3 hours after the tissue piece has completely dissolved
TISSUE DIGESTION TAKES TOO LONG		
	Tissue pieces too large	• Cut tissue pieces to the smallest possible size or grind with liquid nitrogen before starting lysis
	Tissue pieces are stuck to bottom of tube	• Vortex to release pieces from the tube bottom, and immediately after adding Proteinase K and Tissue Lysis Buffer
	Too much starting material	• Use recommended input amount
TISSUE LYSATE APPEARS TURBID		
	Formation of indigestible fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
RATIO $A_{260}/A_{230} > 2.5$		
	Slight variations in EDTA concentration in eluates	• EDTA in elution buffer may complex with cations like Mg^{2+} and Ca^{2+} samples present in genomic DNA, which may lead to higher than usual A_{260}/A_{230} ratio. In some cases, this ratio exceeds a value of 3.0 and is consistent with highly pure samples. In these cases, the elevated value does not have any negative effect on downstream applications.

Guidelines for Choosing Sample Input Amounts When Using the Monarch Total RNA Miniprep Kit

RNA yield, purity, and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield, purity, and RIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Total RNA Miniprep Kit. It is very important not to overload the column when extracting and purifying RNA, as yields, purity and integrity may suffer.

SAMPLE TYPE[1]		INPUT	AVERAGE YIELD (µg)	OBSERVED RIN	MAXIMUM STARTING MATERIAL
CULTURED CELLS					
HeLa		1 x 10^6 cells	12–15	9-10	1 x 10^7 cells
HEK 293		1 x 10^6 cells	12–14	9-10	1 x 10^7 cells
NIH3T3		1 x 10^6 cells	8–12	9-10	1 x 10^7 cells
MAMMALIAN BLOOD[2]					
Human	Fresh	200 µl	0.5–1.0	7-8	3 ml
	Frozen	200 µl	0.5–1.0	7-8	3 ml
	Stabilized	200 µl	0.5–1.0	7-8	3 ml
Rat	Frozen	100 µl	5.6	9	1 ml*
BLOOD CELLS					
PBMC (isolated from 5 ml whole blood)		5 ml	3	7	1 x 10^7 cells
TISSUE					
Rat liver	Frozen pulverized	10 mg	25	8–9	20 mg
	Stabilized solid	10 mg	50–60	8–9	20 mg
Rat spleen (stabilized solid with bead homogenizer)		10 mg	40–50	9	20 mg
Rat kidney (frozen pulverized)		10 mg	7–10	9	50 mg
Rat brain	Frozen pulverized	10 mg	2–3	8–9	50 mg
	Stabilized solid	10 mg	0.5–1.5	8–9	50 mg
	Stabilized solid with bead homogenizer	10 mg	5–8	8–9	50 mg
Rat muscle (frozen pulverized)		10 mg	2–3	8–9	50 mg
Mouse muscle	Frozen pulverized	10 mg	3	8–9	50 mg
	Powder with bead homogenizer	10 mg	5	7–8	50 mg
	Stabilized solid with bead homogenizer	10 mg	8–10	9	50 mg
Mouse heart (stabilized solid w/bead homogenizer)		10 mg	5–6	8–9	50 mg
YEAST					
S. cerevisiae	Frozen with bead homogenizer	1 x 10^7 cells	50	9–10**	5 x 10^7 cells
	Fresh with Zymolyase®	1 x 10^7 cells	60	9**	5 x 10^7 cells
BACTERIA					
E. coli	Frozen	1 x 10^9 cells	5	10	1 x 10^9 cells
	Frozen with bead homogenizer	1 x 10^9 cells	10	10	1 x 10^9 cells
	Frozen with lysozyme	1 x 10^9 cells	70	10	1 x 10^9 cells
B. cereus	Frozen with lysozyme	1 x 10^8 cells	20–30	9	1 x 10^9 cells
	Frozen with bead homogenizer	1 x 10^8 cells	8	9–10	1 x 10^9 cells
PLANT					
Corn leaf (frozen pulverized with bead homogenizer)		100 mg	45	8	100 mg
Tomato leaf (frozen pulverized with bead homogenizer)		100 mg	30	8	100 mg

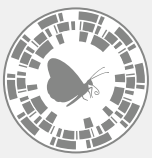
[1] RNA for other blood samples, including drosophila, zebrafish embryos/larvae, plasma, serum, saliva, buccal swabs and nucleated blood have been successfully purified with this kit; protocols are available in the product manual.

[2] A protocol for nucleated blood (e.g., birds, reptiles) is also available.

* Mouse blood also has a maximum input of 1 ml.

** *S.cerevisiae* total RNA was run on an Agilent® Nano 600 Chip using plant assay.

Find tips for RNA purification using Monarch.



Troubleshooting Guide for Total RNA Extraction & Purification Using Monarch Kits

PROBLEM	CAUSE	SOLUTION
Clogged column	Insufficient sample disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Protection Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample-specific protocols in the product manual.
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts on page 357.
Low RNA yield	Incomplete elution	• After addition of Nuclease-free Water (NEB #B1500) to column matrix, incubate 5-10 min at room temperature and then centrifuge to elute • Perform a second elution (note: this will dilute sample)
	Sample is degraded	• Store input sample at -80°C prior to use • Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage
	Insufficient disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample specific protocol in the product manual. • For Proteinase K treated samples, doubling Proteinase K (from 5% to 10%) may lead to an increase in RNA yield
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts on page 357.
RNA degradation	Starting material not handled/stored properly	• Store input sample at -80°C prior to use. Degradation of RNA may occur if sample is not flash frozen or protected by a preservation reagent. Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage.
	Deviation from the stated protocol may expose RNA to unwanted RNase activities	• Refer to the General Guidelines for working with RNA in the product manual
	RNase contamination of eluted materials or kit buffers may have occurred	• See General Guidelines for working with RNA in the product manual for advice on reducing risks of contamination
Low OD ratios	Low $A_{260/280}$ values indicate residual protein in the purified sample	• Ensure the Proteinase K step was utilized for the recommended time. Ensure samples have no debris prior to addition of ethanol and loading onto RNA Purification Column.
	Low $A_{260/230}$ values indicate residual guanidine salts have been carried over during elution	• Ensure wash steps are carried out prior to eluting sample. Use care to ensure the tip of the column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer.
DNA contamination	Genomic DNA not removed by column	• Perform optional on-column DNase I treatment to remove unwanted gDNA from lysed sample • Perform in-tube/off-column DNase I treatment to remove gDNA
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts on page 357.
Low performance of RNA in downstream steps	Salt and/or ethanol carryover has occurred	• Use care to ensure the tip of the RNA Purification Column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. • Be sure to spin the RNA Purification Column for 2 minutes following the final wash with RNA Wash Buffer • When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer • Add additional wash step and/or extend spin time for final wash
Unusual spectrophotometric readings	RNA concentration is too low for spectrophotometric analysis	• For more concentrated RNA, elute with 30 µl of nuclease-free water • Increase amount of starting material (within kit specifications). See Guidelines for Choosing Sample Input Amounts on page 357.
	Silica fines in eluate	• Re-spin eluted samples and pipet aliquot from the top of the liquid to ensure the $A_{260/230}$ is unaffected by possible elution of silica particles

Genetic Markers

A *genotype* indicates the genetic state of the DNA in an organism. It is a theoretical construct describing a genetic situation that explains the observed properties (phenotype, see below) of a strain. *E. coli* genotypes list only genes that are defective (1). If a gene is not mentioned, then it is not known to be mutated*, **. Prophages and plasmids that were present in the original K-12 strain (F, λ, e14, rac) are normally listed only if absent. However, for simplicity, we have not listed λ except when it is present, and we have listed F and its variants in all cases. Parentheses or brackets surround a prophage or plasmid when listed. Genes are given three-letter, lower-case, italicized names (e.g., *dam*) that are intended to be mnemonics suggesting the function of the gene (here, **D**NA **a**denine **m**ethylase). If the same function is affected by several genes, the different genes are distinguished with uppercase italic letters (e.g., *recA, recB, recC, recD* all affect **rec**ombination). Proper notation omits superscript + or – in a genotype, but these are sometimes used redundantly for clarity, as with F´ *lac*$^-$ *proA*$^+$*B*$^+$. Deletion mutations are noted as Δ, followed by the names of deleted genes in parentheses, [e.g., Δ(*lac-pro*)]. All genes between the named genes are also deleted. Specific mutations are given allele numbers that are usually italic arabic numerals (e.g., *hsdR17*) and may be characterized as *am*=amber (UAG) mutation or *ts*= inactive at high temperature, as appropriate. Some common alleles [e.g., Δ*(lac-pro)X111*] break the rules. If two strains' genotypes list a gene with the same allele number, they should carry exactly the same mutation.

The *phenotype* of a strain is an observable behavior, e.g., Lac$^-$ fails to grow on lactose as a sole carbon source. Phenotypes are capitalized and in Roman type, and the letters are always followed by superscript + or – (or sometimes r, resistant, or s, sensitive). Although phenotypes do not, strictly speaking, belong in a genotype, they are sometimes included following the genotype designation when the former is not obvious from the latter [e.g., *rpsL104* (Str^r)–gene name from **r**ibosomal **p**rotein, **s**mall subunit, S12, confers resistance to streptomycin].

Some common genes of interest are described below and on the next page; a catalogue of genetically defined genes can be found in reference 2 and on the very useful internet site maintained by the *E. coli* Genetic Stock Center (CGSC) at Yale University <http://cgsc.biology.yale.edu/>. Additional information from CGSC can be obtained from curator Mary Berlyn by e-mail <cgsc@yale.edu>.

* Most *E. coli* laboratory strains have been heavily mutagenized over forty years of study, and different lines may carry different, so far undiscovered, mutations that may or may not affect your situation. For this reason, it is sometimes useful to try more than one line or strain background in your experiments.

** *E. coli* B and its derivatives are naturally Lon$^-$ and Dcm$^-$. We have listed this in brackets even though it is the wild type state for these strains.

References

(1) Demerec et al. (1966) *Genetics,* 54, 61–76.
(2) Berlyn, M.K.B. (1996). In F. C. Niedhardt et al. (Ed.), *Escherichia coli and Salmonella: cellular and molecular biology,* (2nd ed.), Vol. 2, (pp. 1715–1902). ASM Press.
(3) Raleigh, E.A. et al. (1991) *J. Bacteriol.,* 173, 2707–2709.

dam Endogenous adenine methylation at GATC sequences is abolished. *dam* strains have a high recombination frequency, express DNA repair functions constitutively, and are poorly transformed by Dam-modified plasmids. Used for making DNA susceptible to cleavage by some restriction enzymes (e.g., BclI).

dcm Endogenous cytosine methylation at CCWGG sequences is abolished. Used for making DNA susceptible to cleavage by some restriction enzymes (e.g., AvaII).

dnaJ One of several "chaperonins" is inactive. This defect has been shown to stabilize certain mutant proteins expressed in *E. coli.*

dut dUTPase activity is abolished. This mutation, in combination with *ung*, allows incorporation of uracil into DNA. Used for oligonucleotide mutagenesis.

endA Activity of nonspecific Endonuclease I is abolished. DNA preparations are thought to be of higher quality when prepared from *endA* strains.

e14 An excisable prophage-like element, present in K-12 but missing from many derivatives. e14 carries the *mcrA* gene among others, therefore e14$^-$ strains are McrA$^-$.

F A low-copy number self-transmissible plasmid. F´ factors carry portions of the *E. coli* chromosome, most notably the *lac* operon and *proAB* on F´ *lac-proA*$^+$*B*$^+$.

fhuA An iron uptake receptor is mutated. This mutation confers resistance to phage T1 (ferric hydroxamate uptake). Former name is *tonA.*

gal The ability to metabolize galactose is abolished.

glnV See ***supE***.

gyrA A point mutation in DNA gyrase, subunit A. This mutation confers resistance to the antibiotic nalidixic acid.

hflA This mutation results in high frequency lysogenization by λ.

hsdR, DNA that does not contain methylation

hsdS of certain sequences is recognized as foreign by EcoKI or EcoBI and restricted (degraded). These enzymes recognize different sequences and are encoded by different alleles of *hsdRMS. hsdR* mutations abolish restriction but not protective methylation (r$^-$m$^+$), while *hsdS* mutations abolish both (r$^-$m$^-$). DNA made in the latter will be restricted when introduced into a wild-type strain.

lacIq The *lac* repressor is overproduced, turning off expression from P*lac* more completely.

lacZ β-galactosidase activity is abolished.

lacZ::T7gene 1 The phage T7 RNA polymerase (= gene 1) is inserted into the lacZ gene.

lacY Lactose permease activity is abolished.

Δ(*lac*) = deletion; there are four common deletions involving *lac*:

Δ(*lacZ*)M15 expresses a fragment that complements the *lac* α-fragment encoded by many vectors. These vectors will yield blue color on X-Gal only if the host carries ΔM15.

ΔU169, ΔX111, and ΔX74 all delete the entire *lac* operon from the chromosome, in addition to varying amounts of flanking DNA. ΔX111 deletes *proAB* as well, so that the cell requires proline for growth on minimal medium, unless it also carries F´ *lac proA*$^+$*B*$^+$.

lon Activity of a protease responsible for degrading aberrant proteins is abolished. Some eukaryotic proteins are stabilized in *lon* strains. *E. coli* B naturally lacks Lon.

Genetic Markers (continued)

lysY The lysozyme gene from the T7 bacteriophage is mutated. The mutation K128Y eliminates lysozyme activity, but the mutant protein still binds to and inhibits T7 RNA polymerase.

malB The *malB* region encompasses the genes *malEFG* and *malK lamB malM*. Δ(*malB*) deletes most or all of this region and eliminates expression of Maltose Binding Protein (MalE).

mcrA, mcrBC A restriction system that requires methyl cytosine is abolished. DNA containing methylcytosine in some sequences is restricted by Mcr$^+$. *dcm*-modified DNA is not restricted by Mcr$^+$. Δ(*mcrC-mrr*) deletes six genes: *mcrC-mcrB-hsdS-hsdM-hsdR-mrr*; *mcrA* is lost with e14.

mrr A restriction system that requires cytosine or adenine methylation is abolished; however, *dam*$^-$, *dcm*$^-$ or EcoKI-modified DNA is not restricted by Mrr$^+$. The methylcytosine-dependent activity is also known as McrF (3).

mtl The ability to metabolize the sugar alcohol mannitol is abolished.

ompT Activity of outer membrane protease (protease VII) is abolished.

phoA Activity of alkaline phosphatase is abolished.

prc See ***tsp***.

recA Homologous recombination is abolished; particularly desirable when working with sequences containing direct repeats > 50 bp.

recB, recC Exonuclease and recombination activity of Exonuclease V is abolished. Homologous recombination is much reduced in *recB recC* strains that are not also *sbcB* or *sbcA*. Stability of inverted repeat sequences is enhanced in *recB recC* strains, especially if they are also *sbcB sbcC*. Plasmid replication may be aberrant.

recD Exonuclease activity of ExoV is abolished, but recombination activity is elevated. Inverted repeat sequences in λ can be propagated in *recD* strains. Plasmid replication is aberrant.

recF Plasmid-by-plasmid homologous recombination is abolished.

recJ Plasmid-by-plasmid homologous recombination is abolished.

relA1 Lacks ppGpp synthesis during the stringent response to amino acid starvation; activity of ATP:GTP 3´-pyrophosphotransferase (EC2.7.6.5) is abolished.

rfbD Lacks functional TDP-rhamnose synthetase, and thus does not synthesize the cell surface O-antigen.

rpoH (also known as *htpR)* Lack of this heat-shock transcription factor abolishes expression of some stress-induced protease activities in addition to *lon*. Some cloned proteins are more stable in *rpoH*am *sup*Cts strains at high temperature.

sbcB Exo I activity is abolished. Strains carrying *recB recC* and *sbcB* are usually also *sbcC*. These quadruple mutant strains are recombination-proficient and propagate inverted repeats in λ, but plasmid replication is aberrant.

sbcC Usually found with *recB recC sbcB*. However, strains carrying *sbcC* alone are recombination-proficient and stably propagate inverted repeats both in λ and in plasmids.

sulA Mutations in this gene allows cells to divide and recover from DNA damage in a lon mutant background (suppressor of Lon).

supC(ts) A thermosensitive tyrosine-inserting ochre (UAA) and amber (UAG) suppressor tRNA. Nonsense mutations in the same strain are suppressed only at low temperatures. Now called ***tyrT***.

supE A glutamine-inserting amber (UAG) suppressor tRNA; required for growth of some phage vectors. Now called ***glnV***.

supF A tyrosine-inserting amber (UAG) suppressor tRNA; required for lytic growth of S7 or S100 λ phage, such as λgt11. Now called ***tyrT***.

thi-1 The ability to synthesize thiamine is abolished (vitamin B1).

traD The self-transmissibility of the F factor is severely reduced.

tsp A periplasmic protease that may degrade secreted or cytoplasmically overexpressed proteins after lysis is abolished. Now called ***prc***.

tsx Confers resistance to bacteriophage T6.

tyrT See ***supC, supF***.

ung Uracil N-glycosylase activity is abolished. Uracil incorporated into DNA is removed by Ung$^+$, leaving baseless site. See *dut*.

xyl The ability to metabolize the sugar xylose is abolished.

(P1) The cell carries a P1 prophage. Such strains express the P1 restriction system.

(P2) The cell carries a P2 prophage. This allows selection against Red$^+$ Gam$^+$ λ (Spi$^-$ selection).

(φ80) The cell carries the lambdoid prophage φ80. A defective φ80 prophage carrying the *lac* M15 deletion is present in some strains.

(Mu) Mu prophage; Mud means the phage is defective.

Enhancing Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1 µg of plasmid into a given volume of competent cells. However, 1 µg of plasmid is rarely transformed. Instead, efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. Transformation Efficiency (TE) is calculated as: TE = Colonies/µg/Dilution. Efficiency calculations can be used to compare cells or ligations. Our recommended protocols and tips are presented here to help you achieve maximum results.

Recommended Protocols

High Efficiency Transformation Protocol

1. Thaw cells on ice for 10 minutes
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 30 minutes
4. Heat shock at 42°C for 10–30 seconds or according to recommendations. For BL21, use exactly 10 seconds.
5. Place on ice for 5 minutes
6. Add 950 µl of room temperature SOC or NEB 10-beta/Stable Outgrowth Medium.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Mix cells without vortexing and perform several 10-fold serial dilutions in SOC or NEB 10-beta/Stable Outgrowth Medium.
9. Spread 50–100 µl of each dilution onto pre-warmed selection plates and incubate overnight at 37°C (30°C for SHuffle® strains) or according to recommendations

5 Minute Transformation Protocol

(10% efficiency compared to above protocol)

1. Thaw cells in your hand
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 2 minutes
4. Heat shock at 42°C for 30 seconds or according to recommendations.
5. Place on ice for 2 minutes
6. Add 950 µl of room temperature SOC or NEB 10-beta/Stable Outgrowth Medium. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37-42°C. (30°C for SHuffle strains) NOTE: Selection using antibiotics other than ampicillin may require some outgrowth prior to plating.

Transformation Tips

Thawing

- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming above 0°C decreases efficiency

Incubation of DNA with Cells on Ice

- Incubate on ice for 30 minutes. Expect a 2-fold loss in TE for every 10 minutes this step is shortened.

Heat Shock

- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in TE for every 15 minutes this step is shortened.
- SOC gives 2-fold higher TE than LB medium
- Incubation with shaking or rotation results in 2-fold higher TE

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on TE
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA

- DNA should be purified and resuspended in water or TE Buffer
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency
- Purification by either a spin column or phenol/chloroform extraction and ethanol precipitation is ideal
- The optimal amount of DNA is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

DNA Contaminants to Avoid

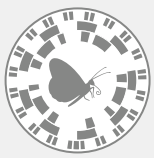
CONTAMINANT	REMOVAL METHOD
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g., ligase)	Column purify or phenol/ chloroform extract and ethanol precipitate

Electroporation Tips

NEB Turbo (NEB #C2986), NEB 5-alpha (NEB #C2989) and NEB 10-beta (NEB #C3020) Competent *E. coli* Strains are available as electrocompetent cells. The following tips will help maximize transformation efficiencies.

- Pre-chill electroporation cuvettes and microcentrifuge tubes on ice
- Thaw cells on ice and suspended well by carefully flicking the tubes
- Once DNA is added, electroporation can be carried out immediately. It is not necessary to incubate DNA with cells. The maximum recommended volume of a DNA solution to be added is 2.5 µl. Addition of a large volume of DNA decreases transformation efficiency.
- DNA should be purified and suspended in water or TE. Transformation efficiency is > 10-fold lower for ligation mixtures than the control pUC19 plasmid due to the presence of ligase and salts. If used directly, ligation reactions should be heat-inactivated at 65°C for 20 min and then diluted 10-fold. For optimal results, spin columns are recommended for clean up of ligation reactions.
- Electroporation conditions vary with different cuvettes and electroporators. If you are using electroporators not specified in the protocol, you may need to optimize the electroporation conditions. Cuvettes with 1mm gap are recommended (e.g., BTX Model 610/613 and Bio-Rad #165-2089). Higher voltage is required for cuvettes with 2 mm gap.
- Arcing may occur due to high concentration of salts or air bubbles
- It is essential to add recovery medium to the cells immediately after electroporation. One minute delay can cause a 3-fold reduction in efficiency.
- Cold and dry selection plates lead to lower transformation efficiency. Pre-warm plates at 37°C for 1 hour. Using 37°C pre-warmed recovery medium increases the efficiency by about 20%.
- Refreeze unused cells in a dry ice/ethanol bath for 5 min and then store at -80°C. Do not use liquid nitrogen. Additional freeze-thaw cycles result in lower transformation efficiency.

Find tips for successful transformation.



T7 Protein Expression

1. Transform expression plasmid into a T7 expression strain. Plate out on antibiotic selection plates and incubate overnight at 37°C (24 hours at 30°C for SHuffle strains).
2. Resuspend a single colony in 10 ml liquid culture with antibiotic
3. Incubate at 37°C until OD_{600} reaches 0.4–0.6
4. Induce with 40 µl of a 100 mM stock of IPTG (final conc. = 0.4 mM) and induce for 2 hours at 37°C (4 hours at 30°C or 16°C overnight for SHuffle strains)
5. Check expression by Coomassie stained protein gel, Western Blot or activity assay. Check expression in the total cell extract (soluble + insoluble) and the soluble fraction alone.
6. For large scale, inoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 37°C (30°C for SHuffle strains) until OD_{600} reaches 0.4–0.6. Add IPTG to 0.4 mM. Induce 2 hours at 37°C or 15°C overnight (4 hours at 30°C or 16°C overnight for SHuffle strains).

Troubleshooting Tips

No Colonies or No Growth in Liquid Culture

- Even though T7 expression is tightly regulated, there may be a low level of basal expression in the T7 Express host. If toxicity of the expressed protein is likely, transformation of the expression plasmid should be carried out in a more tightly controlled expression strain:
  - In *I^q* strains over-expression of the *LacI^q* repressor reduces basal expression of the T7 RNA polymerase
  - In *lysY* strains, mutant T7 lysozyme is produced which binds to T7 RNA polymerase, reducing basal expression of the target protein. Upon induction, newly made T7 RNA polymerase titrates out the lysozyme and results in expression of the target protein.
- Incubation at 30°C or room temperature may also alleviate toxicity issues
- Check antibiotic concentration (test with control plasmid)

No Protein Visible on Gel or No Activity

- Check for toxicity - the cells may have eliminated or deleted elements in the expression plasmid. If this is the case, test *I^q* and/or *lysY* strains to reduce basal expression.
- Culture cells for protein induction. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, significantly fewer colonies will be seen on plates containing antibiotic (indicating that the plasmid has been lost) compared to plates without antibiotic.

Induced Protein is Insoluble

T7 expression often leads to very high production of protein that can result in the target protein becoming insoluble. In this case:

- Induce at lower temperatures (12–15°C overnight)
- Reduce IPTG concentration to 0.01 mM – 0.1 mM
- Induce for less time (as little as 15 minutes)
- Induce earlier in growth (OD_{600} = 0.3 or 0.4)

Lori is the Senior Executive Assistant to Jim Ellard, NEB's CEO, and Donald Comb, NEB's Founder. Lori is very involved in NEB's Educational Course Support Program, providing reagents to college and high school teaching labs at no charge.

DNA/RNA Input Guidelines for NGS Library Prep

DNA SAMPLE INPUT GUIDELINES

Integrity of DNA

- Start with as high quality DNA as possible. The quality of the input material directly affects the quality of the library. Absorbance measurements can be used as an indication of DNA purity. Ideally, the ratio of the absorbance at 260 nm to 280 nm should be between 1.8–2.0. However, measurements can be affected by the presence of RNA or small nucleic acid fragments. A DNA Integrity Number can be determined using the Agilent TapeStation® and qPCR-based methods can also provide a measurement of DNA integrity.

Quantitation of DNA

- It is important to quantify accurately the DNA sample prior to library construction. Fluorescence-based detection which utilizes dsDNA-specific dyes, such as the Qubit® from Life Technologies, is more accurate than UV spectrometer-based measurements, as the presence of RNA or other contaminants can result in overestimation of the amount of the DNA sample.

RNA SAMPLE INPUT GUIDELINES

Integrity of RNA

- It is important to start with high quality RNA. The use of degraded RNA can result in low yield or failure to generate libraries. We recommend determining RNA quality using the RNA Integrity Number (RIN) estimated by the Agilent® Bioanalyzer® or similar instrumentation. Ideally, the RNA sample should have a RIN value higher than 7, enabling use of poly(A) mRNA or rRNA depletion protocols. Degraded RNA with RIN values as low as 1-2 can be used if specific protocols are followed.
- RNA should be completely free of DNA. DNase digestion of the purified RNA with RNase-free DNase is recommended.

Quantitation of RNA

- It is important to quantify accurately the RNA sample prior to library construction. The concentration can be estimated with the Agilent Bioanalyzer on a pico or nano chip. Alternatively, RNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer such as a NanoDrop®. However, free nucleotides or other organic compounds routinely used to extract RNA will also absorb UV light near 260 nm and will result in an over-estimation of the RNA concentration.

BEAD-BASED CLEAN-UPS AND SIZE SELECTION

Integrity of DNA

- Be careful not to disturb the bead pellet when transferring material
- Be sure to vortex the beads just before use – they should be a uniform suspension
- Do not over-dry the beads. This can make resuspension difficult and reduce yield.
- Bead-based clean-ups and size-selection are explained in animations and videos available on our website
- Use a magnet that is strong enough to separate the beads completely and quickly

INDICES

- Open only one index primer vial at a time, to minimize the risk of contamination
- When you are using a subset of the indices supplied in a kit, or using indices from more than one kit, it is important to optimize the combination of indices used, to ensure balanced sequencing reads.

We provide recommendations for NEBNext index combinations at **NEBNext.com**.

Labeling with SNAP-tag® Technology-Troubleshooting Guide

APPLICATION	PROBLEM	CAUSE	SOLUTION
Cellular Labeling	No labeling	Fusion protein not expressed	• Verify transfection • Check expression of fusion protein via Western blot or SDS-PAGE with Vista Green label
	Weak labeling	Poor expression and/or insufficient exposure of fusion protein to substrate	• Increase substrate concentration • Increase incubation time
		Rapid turnover of fusion protein	• Analyze samples immediately or fix cells directly after labeling • Label at lower temperature (4°C or 16°C)
	High background	Non-specific binding of substrates	• Reduce substrate concentration and/or incubation time • Allow final wash step to proceed for up to 2 hours • Include fetal calf serum or BSA during labeling
	Signal strongly reduced after short time	Instability of fusion protein	• Fix cells • Switch tag from N-terminus to C-terminus or vice versa
		Photobleaching	• Add commercially available anti-fade reagent • Reduce illumination time and/or intensity
Labeling in Solution	Precipitation	Insoluble fusion	• Test from pH 5.0 to 10.0 • Optimize salt concentration [50 to 250 mM] • Add 0.05 to 0.1% Tween 20
	Weak or no labeling	Exhaustive labeling has not been achieved	• Increase incubation time to 2 hrs at 25°C or 24 hrs at 4°C • Reduce the volume of protein solution labeled • Check expression of fusion protein via SDS-PAGE with Vista Green label
	Loss of activity	Instability of fusion protein	• Reduce labeling time • Decrease labeling temperature (4°C or 16°C)

Cellular Imaging & Analysis FAQs

Q. *How does SNAP-tag® labeling differ from using GFP fusion proteins?*

A. GFP and SNAP-tag are both valuable technologies used to visualize proteins in live cells. GFP is an intrinsically fluorescent protein derived from *Aequorea victoria* while SNAP-tag is derived from hAGT, a human DNA repair protein. In contrast to GFP, the fluorescence of SNAP-tag fusions can be readily turned on with the addition of a variety of fluorescent probes added directly to the culture media. Substituting different fluorophores or other functionalities (biotin, magnetic beads, blocking agents) requires no new cloning or expression, merely incubation of the appropriate substrate with cells, cell lysates or recombinant proteins.

Q. *What is the difference between SNAP- and CLIP-tag™?*

A. SNAP-tag and CLIP-tag are both derived from O^6 -alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag recognizes O^6-labeled benzylguanine substrates while CLIP-tag recognizes O^2-labeled benzylcytosine substrates. Each tag transfers the label from the substrate to itself, resulting in specific covalent labeling. In creating the tags, hAGT has been engineered to no longer interact with DNA, but rather with derivatives of the free benzylguanine or benzylcytosine substrates. The tags exhibit no cross-reactivity with one another, enabling researchers to simultaneously label fusion proteins containing SNAP- and CLIP-tags with different fluorophores in live cells.

Q. *Can I clone my protein as a fusion to the N- or C-terminus of the tags?*

A. Yes. SNAP- and CLIP-tags can be fused to either the N- or C-terminus of a protein of interest. However, to label surface proteins on the outside of cells, the SNAP-tag or CLIP-tag must be cloned so that it is oriented to the extracellular surface of the plasma membrane. In this orientation, the tag is accessible to its fluorophore conjugated substrate.

Q. *Are the substrates toxic to cells?*

A. No toxicity has been noted by proliferation or viability assays when using up to 20 µM substrate for 2 hours. Most of the substrates can be incubated with cells for 24 hours up to a concentration of 20 µM without significant toxicity.

Q. *How stable is the labeled protein in mammalian cells?*

A. The stability of the tagged protein in the cell is dependent upon the stability of protein of interest. Labeled SNAP-tag fusion protein has been detected for up to 2 days in mammalian cells.

Q. *Are SNAP-tag substrates stable to fixation?*

A. Yes. SNAP-tag substrates are derived from organic fluorophores which are stable to fixation. Fluorescently-labeled SNAP-tag fusion proteins do not lose signal intensity in contrast to some GFP spectral variants. After labeling the SNAP-tag fusion proteins, the cells can be fixed with standard fixation methods such as para-formaldehyde, ethanol, methanol, methanol/acetone etc. without loss of signal.

Q. *What conditions are recommended for SNAP-tag labeling in vitro?*

A. The SNAP-tag labeling reaction is tolerant of a wide range of buffers. The requirements of the fusion partner should dictate the buffer selected. The following buffer guidelines are recommended: pH between 5.0 and 10.0, monovalent salts (e.g. sodium chloride) between 50 mM and 250 mM and at least 1 mM DTT. Non-ionic detergents can be added to 0.5% v/v if required, but SDS and other ionic detergents should be avoided entirely because they inhibit the activity of the SNAP-tag. Metal chelating reagents (e.g., EDTA and EGTA) also inhibit SNAP-tag activity and should be avoided.

Frequencies of Restriction Sites in Sequenced DNAs

The table below summarizes the frequencies with which restriction enzyme sites occur in eleven commonly used DNA molecules. Detailed restriction maps can be found on subsequent pages. The sites listed in these tables were identified by computer analyses of published sequences. Although we have tried to ensure their accuracy, the sites have not necessarily been confirmed by experimentation. When the same specificity is displayed by several enzymes, the site is listed by the name of the enzyme that is available from New England Biolabs. Other enzymes with the same specificity are listed in the table of isoschizomers on page 311–327. Enzymes not available from NEB are listed with an (x). If NEB offers an HF version of that enzyme, it is indicated by a red dot (•). Recognition sequences are written 5´ to 3´.

ENZYME	SITE	ADENO-2	LAMBDA	M13MP18	pBR322	pKLAC2	pMAL-P5X	pSNAP$_F$	pTXB1	pTYB21	pUC19	T7
AarI (x)	CACCTGC	9	12	0	0	0	0	1	0	0	0	5
AatII	GACGTC	3	10	0	1	0	0	5	1	0	1	1
AccI	GTMKAC	17	9	1	2	5	1	2	5	3	1	33
Acc65I	GGTACC	8	2	1	0	0	0	2	0	1	1	5
AciI	CCGC	582	516	42	67	81	81	75	102	102	34	199
AclI	AACGTT	3	7	2	4	2	5	3	12	13	2	19
AcuI	CTGAAG	23	40	0	2	8	4	5	2	2	2	1
AfeI	AGCGCT	13	2	1	4	0	2	0	1	1	0	0
AflII	CTTAAG	4	3	0	0	0	0	0	0	0	0	19
AflIII	ACRYGT	25	20	3	1	4	2	5	3	4	1	23
AgeI (•)	ACCGGT	5	13	0	0	1	0	1	1	0	0	2
AhdI	GACNNNNNGTC	9	9	0	1	1	2	1	2	2	1	14
AleI	CACNNNNGTG	10	20	1	0	1	0	1	0	2	0	8
AluI	AGCT	158	143	27	17	38	28	27	30	31	16	140
AlwI	GGATC	35	58	3	12	18	12	20	15	17	10	1
AlwNI	CAGNNNCTG	25	41	1	1	5	2	4	1	2	1	15
ApaI	GGGCCC	12	1	0	0	0	1	1	1	1	0	0
ApaLI	GTGCAC	7	4	0	3	3	6	4	4	4	3	1
ApeKI	GCWGC	179	199	10	21	27	25	20	27	26	12	116
ApoI (•)	RAATTY	29	58	11	0	19	5	3	5	6	1	13
AscI	GGCGCGCC	2	2	0	0	0	0	1	0	0	0	0
AseI	ATTAAT	3	17	7	1	5	4	7	10	10	3	12
AsiSI	GCGATCGC	1	0	0	0	0	0	0	0	0	0	0
AvaI	CYCGRG	40	8	2	1	2	1	2	3	1	1	4
AvaII	GGWCC	73	35	1	8	7	9	6	6	7	2	54
AvrII	CCTAGG	2	2	0	0	0	0	1	0	0	0	3
BaeGI	GKGCMC	45	10	1	3	8	8	8	6	5	3	16
BaeI	ACNNNNGTAYC	5	10	3	0	1	0	0	0	1	0	3
BamHI (•)	GGATCC	3	5	1	1	1	1	1	1	1	1	0
BanI	GGYRCC	57	25	7	9	7	4	8	8	7	4	33
BanII	GRGCYC	57	7	2	2	5	2	5	3	4	1	1
BbsI (•)	GAAGAC	27	24	0	3	3	3	2	4	5	0	38
BbvCI	CCTCAGC	9	7	2	0	0	0	0	0	0	0	10
BbvI	GCAGC	179	199	10	21	27	25	20	27	26	12	116
BccI	CCATC	62	145	14	9	22	16	8	14	20	3	121
BceAI	ACGGC	80	115	7	3	13	11	8	13	12	2	47
BcgI	CGANNNNNNTGC	10	28	0	3	6	4	1	4	6	1	19
BciVI	GTATCC	9	26	0	2	3	4	3	4	4	2	23
BclI (•)	TGATCA	5	8	0	0	2	2	1	1	2	0	1
BcoDI	GTCTC	60	37	5	3	11	8	4	8	9	4	95
BfaI	CTAG	54	13	5	5	19	3	14	8	8	4	60
BfuAI	ACCTGC	39	41	3	1	5	4	4	2	3	1	18
BfuCI (x)	GATC	87	116	6	22	35	23	31	24	27	15	6
BglI	GCCNNNNNGGC	20	29	1	3	3	1	7	2	2	2	2
BglII	AGATCT	11	6	1	0	1	1	2	0	1	0	1
BlpI	GCTNAGC	8	6	0	0	0	1	0	1	1	0	20
BmgBI	CACGTC	15	17	0	0	1	1	2	0	0	0	8
BmrI	ACTGGG	22	4	1	5	2	5	6	11	11	2	6
BmtI (•)	GCTAGC	4	1	0	1	4	0	1	1	1	0	1
BpmI	CTGGAG	32	25	2	4	3	4	1	5	6	1	23
Bpu10I	CCTNAGC	23	19	4	1	0	1	2	0	2	0	39
BpuEI	CTTGAG	19	13	4	6	7	5	9	7	9	4	56
BsaI (•)	GGTCTC	18	2	0	1	3	2	2	2	1	1	29
BsaAI	YACGTR	22	14	5	1	4	0	3	2	4	0	35
BsaBI	GATNNNNATC	2	21	2	1	2	2	1	1	2	0	7
BsaHI	GRCGYC	44	40	1	6	6	5	8	12	8	3	8
BsaJI	CCNNGG	234	105	9	8	18	10	17	15	16	5	85
BsaWI	WCCGGW	28	81	6	5	8	7	5	8	6	3	32

Frequencies of Restriction Sites (continued)

ENZYME	SITE	ADENO-2	LAMBDA	M13MP18	pBR322	pKLAC2	pMAL-P5X	$pSNAP_F$	pTXB1	pTYB21	pUC19	T7
BsaXI	ACNNNNNCTCC	29	19	4	0	3	1	1	2	3	1	12
BseRI	GAGGAG	63	19	1	0	2	0	3	0	0	0	13
BseYI	GCTGGG	31	32	3	2	3	4	5	4	4	1	29
BsgI	GTGCAG	34	41	0	1	4	6	3	5	4	0	21
BsiEI	CGRYCG	50	22	3	7	11	8	6	9	7	5	17
BsiHKAI	GWGCWC	38	28	3	8	8	9	9	7	7	5	24
BsiWI (•)	CGTACG	4	1	0	0	0	1	0	1	0	0	0
BslI	CCNNNNNNNGG	216	176	17	20	18	16	26	31	27	6	90
BsmI	GAATGC	10	46	1	1	3	1	5	1	0	0	15
BsmBI	CGTCTC	21	14	1	1	2	2	0	2	2	2	16
BsmFI	GGGAC	59	38	2	4	4	1	5	4	4	0	46
BsoBI	CYCGRG	40	8	2	1	2	1	2	3	1	1	4
Bsp1286I	GDGCHC	105	38	5	10	16	11	15	11	10	5	40
BspCNI	CTCAG	75	80	24	7	10	10	9	20	23	5	142
BspDI	ATCGAT	2	15	2	1	2	0	0	0	0	0	3
BspEI	TCCGGA	8	24	0	1	1	2	0	1	1	0	0
BspHI	TCATGA	3	8	1	4	2	1	2	2	2	3	13
BspMI	ACCTGC	39	41	3	1	5	4	4	2	3	1	18
BspQI	GCTCTTC	7	10	0	1	2	1	3	1	1	1	4
BspUI(x)	GCSGC	232	181	7	21	25	18	27	22	23	7	40
BsrI	ACTGG	86	110	19	18	23	26	19	32	30	11	118
BsrBI	CCGCTC	28	17	4	2	6	4	6	9	9	3	17
BsrDI	GCAATG	14	44	3	2	7	4	4	4	4	2	18
BsrFI	RCCGGY	40	61	1	7	9	2	6	11	7	1	3
BsrGI (•)	TGTACA	5	5	1	0	1	0	1	1	2	0	13
BssHII	GCGCGC	52	6	0	0	1	2	2	1	1	0	1
BssKI (x)	CCNGG	233	185	11	16	25	27	28	46	42	12	11
BssSI	CACGAG	11	8	0	3	5	3	4	2	4	3	31
BstAPI	GCANNNNNTGC	20	34	0	2	3	2	0	3	2	1	12
BstBI	TTCGAA	1	7	0	0	2	0	0	0	1	0	7
BstEII (•)	GGTNACC	10	13	0	0	1	1	0	1	1	0	1
BstNI	CCWGG	136	71	7	6	15	14	19	19	19	5	2
BstUI	CGCG	303	157	17	23	26	31	19	41	35	10	65
BstXI	CCANNNNNNTGG	10	13	0	0	2	4	1	4	3	0	11
BstYI	RGATCY	22	21	2	8	12	9	11	10	12	7	2
BstZ17I	GTATAC	3	3	0	1	3	0	1	1	1	0	8
Bsu36I	CCTNAGG	7	2	1	0	1	1	1	0	0	0	30
BtgI	CCRYGG	82	46	2	2	4	3	6	1	3	0	26
BtgZI	GCGATG	23	45	4	3	4	6	6	4	3	0	24
BtsI	GCAGTG	22	34	1	2	7	5	5	4	4	3	20
BtsCI	GGATG	78	150	4	12	20	17	7	12	12	5	97
Cac8I	GCNNGC	285	238	28	31	33	32	41	49	45	14	104
ClaI	ATCGAT	2	15	2	1	2	0	0	0	0	0	3
CspCI	CAANNNNNGTGG	6	7	1	0	1	0	1	0	0	0	9
CviAII	CATG	183	181	14	26	38	23	21	23	29	11	148
CviKI-1	RGCY	680	692	103	73	131	86	119	112	116	45	562
CviQI	GTAC	83	113	19	3	15	7	14	6	10	3	168
DdeI	CTNAG	97	104	30	8	17	11	11	20	26	6	282
DpnI	GATC	87	116	6	22	35	23	31	24	27	15	6
DpnII	GATC	87	116	6	22	35	23	31	24	27	15	6
DraI	TTTAAA	12	13	5	3	5	1	6	3	3	3	9
DraIII (•)	CACNNNGTG	10	10	1	0	2	0	3	1	1	0	16
DrdI	GACNNNNNNGTC	6	3	1	2	5	2	2	4	4	2	11
EaeI	YGGCCR	70	39	3	6	10	5	15	4	5	3	2
EagI	CGGCCG	19	2	0	1	4	1	2	2	2	0	0
EarI	CTCTTC	29	34	2	2	11	6	4	3	4	3	46
EciI	GGCGGA	29	32	2	4	6	6	8	9	11	3	2
Eco53KI	GAGCTC	16	2	1	0	2	1	2	0	1	1	0
EcoNI	CCTNNNNNAGG	10	9	0	1	3	0	0	2	1	0	1
EcoO109I	RGGNCCY	44	3	0	4	1	2	5	1	2	1	22
EcoP15I	CAGCAG	50	72	4	7	7	10	6	6	5	3	36
EcoRI (•)	GAATTC	5	5	1	1	1	1	1	1	1	1	0
EcoRV (•)	GATATC	9	21	0	1	1	1	1	1	1	0	0
Esp3I	CGTCTC	21	14	1	1	2	2	0	2	2	2	16
FatI	CATG	183	181	14	26	38	23	21	23	29	11	148

ENZYME	SITE	ADENO-2	LAMBDA	M13MP18	pBR322	pKLAC2	pMAL-P5X	$pSNAP_F$	pTXB1	pTYB21	pUC19	T7
FauI	CCCGC	147	90	10	10	14	17	11	28	28	5	24
Fnu4HI	GCNGC	411	380	17	42	52	43	47	49	49	19	156
FokI	GGATG	78	150	4	12	20	17	7	12	12	5	97
FseI	GGCCGGCC	3	0	0	0	0	0	0	0	0	0	0
FspI	TGCGCA	17	15	1	4	3	2	2	1	1	2	7
HaeII	RGCGCY	76	48	6	11	6	9	3	7	7	3	26
HaeIII	GGCC	216	149	15	22	31	23	36	34	36	11	68
HgaI	GACGC	87	102	7	11	10	12	7	20	18	4	70
HhaI	GCGC	375	215	26	31	36	39	27	41	39	17	103
HinP1I	GCGC	375	215	26	31	36	39	27	41	39	17	103
HincII	GTYRAC	25	35	1	2	9	7	4	7	6	1	61
HindIII (•)	AAGCTT	12	6	1	1	1	1	4	0	1	1	0
HinfI	GANTC	72	148	26	10	31	9	11	16	20	6	218
HpaI	GTTAAC	6	14	0	0	3	1	1	1	2	0	18
HpaII	CCGG	171	328	18	26	32	25	24	50	40	13	58
HphI	GGTGA	99	168	18	12	15	19	14	21	21	7	102
Hpy99I	CGWCG	61	102	8	9	14	9	13	18	14	5	29
Hpy166II	GTNNAC	116	125	10	8	29	20	13	27	28	5	199
Hpy188I	TCNGA	80	170	31	15	24	19	17	19	26	10	153
Hpy188III	TCNNGA	103	185	28	19	32	22	25	27	29	13	173
HpyAV	CCTTC	84	106	14	10	24	14	11	16	18	6	110
HpyCH4III	ACNGT	122	187	31	14	25	20	15	18	17	8	174
HpyCH4IV	ACGT	83	143	22	10	21	10	19	23	26	5	170
HpyCH4V	TGCA	207	273	18	21	39	28	30	26	25	13	116
KasI	GGCGCC	20	1	1	4	1	1	1	1	1	1	2
KpnI (•)	GGTACC	8	2	1	0	0	0	2	0	1	1	5
MboI	GATC	87	116	6	22	35	23	31	24	27	15	6
MboII	GAAGA	113	130	10	11	38	15	14	14	17	8	140
MfeI (•)	CAATTG	4	8	0	0	2	1	2	1	1	0	8
MluI (•)	ACGCGT	5	7	0	0	0	1	2	2	1	0	1
MluCI	AATT	87	189	62	8	43	22	19	31	44	7	79
MlyI	GAGTC	40	61	8	4	17	5	6	11	10	4	115
MmeI	TCCRAC	25	18	3	4	8	3	5	4	4	2	33
MnlI	CCTC	397	262	62	26	56	24	41	35	39	13	342
MscI	TGGCCA	17	18	1	1	0	1	2	0	1	0	2
MseI	TTAA	115	195	63	15	41	24	23	32	41	13	207
MslI	CAYNNNNRTG	35	62	3	7	10	10	6	7	9	3	38
MspA1I	CMGCKG	95	75	4	6	14	11	8	10	11	6	35
MspI	CCGG	171	328	18	26	32	25	24	50	40	13	58
MwoI	GCNNNNNNNGC	391	347	19	34	33	30	42	41	35	13	170
NaeI	GCCGGC	13	1	1	4	3	0	2	5	5	0	0
NarI	GGCGCC	20	1	1	4	1	1	1	1	1	1	2
NciI	CCSGG	97	114	4	10	10	13	9	27	23	7	9
NcoI (•)	CCATGG	20	4	0	0	1	1	3	0	1	0	1
NdeI	CATATG	2	7	3	1	1	1	1	1	1	1	7
NgoMIV	GCCGGC	13	1	1	4	3	0	2	5	5	0	0
NheI (•)	GCTAGC	4	1	0	1	4	0	1	1	1	0	1
NlaIII	CATG	183	181	14	26	38	23	21	23	29	11	148
NlaIV	GGNNCC	178	82	18	24	22	14	20	22	24	11	99
NmeAIII	GCCGAG	17	8	0	3	2	3	2	2	3	1	14
NotI (•)	GCGGCCGC	7	0	0	0	1	1	1	1	1	0	0
NruI (•)	TCGCGA	5	5	0	1	1	0	1	1	0	0	3
NsiI (•)	ATGCAT	9	14	0	0	6	0	1	0	0	0	8
NspI	RCATGY	41	32	6	4	9	3	5	5	6	3	24
Nt.BstNBI	GAGTC	40	61	8	4	17	5	6	11	10	4	115
Nt.CviPII	CCD	4148	4641	570	457	806	570	609	716	743	251	3575
PacI	TTAATTAA	1	0	1	0	0	0	1	0	0	0	1
PaeR7I	CTCGAG	6	1	0	0	1	0	1	1	0	0	0
PciI	ACATGT	9	2	3	1	3	1	2	1	1	1	6
PflFI	GACNNNGTC	12	2	0	1	1	1	1	1	2	0	1
PfiMI	CCANNNNNTGG	18	14	0	2	3	1	5	2	3	0	8
PhoI (x)	GGCC	216	149	15	22	31	23	36	34	36	11	68
PleI	GAGTC	40	61	8	4	17	5	6	11	10	4	115
PluTI	GGCGCC	20	1	1	4	1	1	1	1	1	1	2
PmeI	GTTTAAAC	1	2	0	0	0	0	1	1	1	0	2
PmlI	CACGTG	10	3	0	0	0	0	1	0	1	0	1

Frequencies of Restriction Sites (continued)

ENZYME	SITE	ADENO-2	LAMBDA	M13MP18	pBR322	pKLAC2	pMAL-P5X	$pSNAP_F$	pTXB1	pTYB21	pUC19	T7
PpuMI	RGGWCCY	23	3	0	2	1	2	1	0	0	0	12
PshAI	GACNNNNGTC	2	7	0	1	1	0	0	1	2	0	6
PsiI	TTATAA	4	12	2	0	2	1	1	1	1	0	5
PspGI	CCWGG	136	71	7	6	15	14	19	19	19	5	2
PspOMI	GGGCCC	12	1	0	0	0	1	1	1	1	0	0
PspXI	VCTCGAGB	3	1	0	0	0	0	1	1	0	0	0
PstI	CTGCAG	30	28	1	1	3	1	4	1	1	1	0
PvuI (•)	CGATCG	7	3	1	1	3	2	1	1	1	2	0
PvuII (•)	CAGCTG	24	15	3	1	3	3	3	3	3	2	3
RsaI	GTAC	83	113	19	3	15	7	14	6	10	3	168
RsrII	CGGWCCG	2	5	0	0	0	1	1	0	0	0	1
SacI (•)	GAGCTC	16	2	1	0	2	1	2	0	1	1	0
SacII	CCGCGG	33	4	0	0	2	0	1	1	1	0	0
SalI (•)	GTCGAC	3	2	1	1	1	1	1	4	1	1	0
SapI	GCTCTTC	7	10	0	1	2	1	3	1	1	1	4
Sau3AI	GATC	87	116	6	22	35	23	31	24	27	15	6
Sau96I	GGNCC	164	74	4	15	14	20	21	26	28	6	79
SbfI (•)	CCTGCAGG	3	5	1	0	1	1	1	0	1	1	0
ScaI (•)	AGTACT	5	5	0	1	2	1	2	1	2	1	4
ScrFI	CCNGG	233	185	11	16	25	27	28	46	42	12	11
SexAI	ACCWGGT	9	5	0	0	3	0	0	0	0	0	0
SfaNI	GCATC	85	169	7	22	18	20	17	23	19	8	96
SfcI	CTRYAG	47	38	7	4	9	4	10	6	7	4	48
SfiI	GGCCNNNNNGGCC	3	0	0	0	0	0	1	0	0	0	1
SfoI	GGCGCC	20	1	1	4	1	1	1	1	1	1	2
SgrAI	CRCCGGYG	6	6	0	1	0	0	0	1	0	0	0
SmaI	CCCGGG	12	3	1	0	1	0	1	0	0	1	0
SmlI	CTYRAG	29	17	4	6	8	5	10	8	9	4	75
SnaBI	TACGTA	0	1	1	0	1	0	1	0	0	0	13
SpeI (•)	ACTAGT	3	0	0	0	0	0	1	1	1	0	2
SphI (•)	GCATGC	8	6	1	1	2	0	2	2	2	1	0
SspI (•)	AATATT	5	20	6	1	6	2	1	3	5	1	6
StuI	AGGCCT	11	6	0	0	1	0	0	1	1	0	1
StyI (•)	CCWWGG	44	10	0	1	4	1	4	2	4	0	36
StyD4I	CCNGG	233	185	11	16	25	27	28	46	42	12	11
SwaI	ATTTAAAT	1	0	1	0	0	0	1	1	1	0	1
TaqI	TCGA	50	121	12	7	32	16	15	22	20	4	111
TatI(x)	WGTACW	19	24	5	2	5	1	8	3	6	2	37
TfiI	GAWTC	32	87	18	6	14	4	5	5	10	2	103
TseI	GCWGC	179	199	10	21	27	25	20	27	26	12	116
Tsp45I	GTSAC	73	81	9	9	9	7	5	12	11	4	108
TspMI	CCCGGG	12	3	1	0	1	0	1	0	0	1	0
TspRI	CASTG	83	119	9	11	22	14	16	16	14	10	94
Tth111I	GACNNNGTC	12	2	0	1	1	1	1	1	2	0	1
XbaI	TCTAGA	5	1	1	0	1	0	1	1	1	1	3
XcmI	CCANNNNNNNNNTGG	14	12	0	0	1	3	0	3	4	0	8
XhoI	CTCGAG	6	1	0	0	1	0	1	1	0	0	0
XmaI	CCCGGG	12	3	1	0	1	0	1	0	0	1	0
XmnI	GAANNNNTTC	5	24	2	2	3	1	3	7	8	1	12
ZraI	GACGTC	3	10	0	1	0	0	5	1	0	1	1

Lambda

48,502 base pairs
GenBank Accession #: NC_001416
See page 118 for ordering information.

There are no restriction sites for the following enzymes: AsiSI, FseI, I-CeuI, I-SceI, NotI, PI-PspI, PI-SceI, PacI, SfiI, SpeI, SrfI(x), SwaI

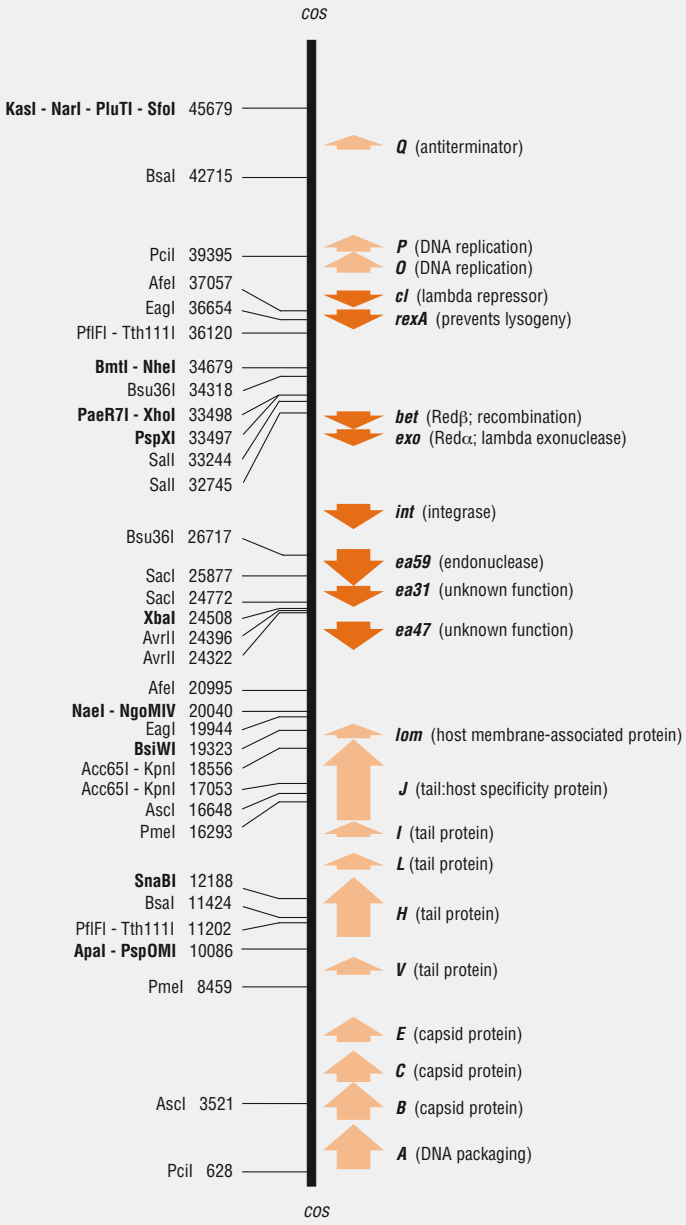
(x) = enzyme not available from NEB

Lambda (λ) is a large, temperate *E. coli* bacteriophage with a linear, largely double-stranded DNA genome (1-5). At each end, the 5´ strand overhangs the 3´ strand by 12 bases. These single-stranded overhangs are complementary and anneal to form a *cos* site following entry into a host cell. Once annealed, the genome is a circular, completely double-stranded molecule which serves as a template for rolling-circle replication.

Many laboratory strains of lambda are derivatives of the strain λ *c*I857*ind*1 *Sam*7, which contains four point mutations relative to the wild type strain. The *ind*1 mutation in the *c*I gene creates a new HindIII site at 37584 not present in the wild type. All lambda products sold by NEB are λ *c*I857*ind*1 *Sam*7.

Numbering of the genome sequence begins at the first (5´-most) base of the left end (bottom of diagram below) and continues rightward from late genes *nu1* and *A* towards the early genes. The map below shows the positions of all known ORFs larger than 200 codons.

Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.



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(1) Echols, H. and Murialdo, H. (1978) *Microbiol. Rev.,* 42, 577–591.
(2) Szybalski, E.H. and Szybalski, W. (1979) *Gene,* 7, 217–270
(3) Daniels, D.L., de Wet, J.R. and Blattner, F.R. (1980) *J. Virol.,* 33, 390–400.
(4) Sanger, F., Coulson, A.R., Hong, G.F., Hill, D.F. and Petersen, G.B. (1982) *J. Mol. Biol.,* 162, 729–773.
(5) Daniels, D.L. et al. (1983). In R.W. Hendrix, J.W. Roberts, F.W. Stahl and R.A. Weisberg (Eds.), *Lambda II: Appendix,* New York: Cold Spring Harbor Press.

mp1

6163

```
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCC
                                                MetThrMetIleThrAspSerLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnArg ⟶ LacZ
```

mp2

6163

```
                                                EcoRI
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCC
                                                MetThrMetIleThrAsnSerLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnArg ⟶ LacZ
```

mp7/pUC7

6163

```
                                                                                AccI                AccI
                                                                               HincII              HincII
                                                EcoRI      BamHI              SalI      PstI      SalI      BamHI      EcoRI
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTCCCCGGATCCGTCGACCTGCAGGTCGACGGATCCGGGGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCG
                                                MetThrMetIleThrAsnSerProAspProSerThrCysArgSerThrAspProGlyAsnSerLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGly ⟶ LacZ
```

mp8/pUC8

6163

```
                                                                                AccI
                                                                               HincII
                                                EcoRI  SmaI  BamHI              SalI      PstI            HindIII
                                                       XmaI
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTCCCGGGGATCCGTCGACCTGCAGCCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCG
                                                MetThrMetIleThrAsnSerArgGlySerValAspLeuGlnProSerLeuAlaLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGly ⟶ LacZ
```

mp9/pUC9

6534

```
                                                                                AccI
                                                                               HincII
                                                HindIII      PstI              SalI      BamHI  SmaI
                                                                                         XmaI   EcoRI
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTGGCTGCAGGTCGACGGATCCCCGGGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCG
                                                MetThrMetIleThrProSerLeuAlaAlaGlyArgArgIleProGlyAsnSerLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGly ⟶ LacZ
```

mp10/pUC12

6164

```
                                                                                                AccI
                                                                                               HincII
                                                EcoRI  SacI        SmaI                        SalI      PstI          HindIII
                                                              XmaI  BamHI  XbaI
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGCCCGGGGATCCTCTAGAGTCGACCTGCAGCCCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCG
                                                MetThrMetIleThrAsnSerSerSerProGlyAspProLeuGluSerThrCysSerProSerLeuAlaLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGly ⟶ LacZ
```

mp11/pUC13

6164

```
                                                                                AccI
                                                                               HincII                                  SmaI
                                                HindIII      PstI              SalI      XbaI      BamHI              XmaI      SacI      EcoRI
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTGGGCTGCAGGTCGACTCTAGAGGATCCCCGGGCGAGCTCGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCG
                                                MetThrMetIleThrProSerLeuGlyCysArgSerThrLeuGluAspProArgAlaSerSerAsnSerLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGly ⟶ LacZ
```

mp18/pUC18

6164

```
                                                                                                AccI
                                                                                               HincII
                                                EcoRI  SacI  KpnI        SmaI                  SalI      PstI      SphI      HindIII
                                                             XmaI  BamHI      XbaI
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCG
                                                MetThrMetIleThrAsnSerSerSerValProGlyAspProLeuGluSerThrCysArgHisAlaSerLeuAlaLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGly ⟶ LacZ
```

mp19/pUC19

6164

```
                                                                                AccI
                                                                               HincII                                  SmaI
                                                HindIII  SphI      PstI        SalI      XbaI      BamHI              XmaI  KpnI      SacI      EcoRI
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCG
                                                MetThrMetIleThrProSerLeuHisAlaCysArgSerThrLeuGluAspProArgValProSerSerAsnSerLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGly ⟶ LacZ
```

M13mp18

GenBank Accession #: X02513
Revised sequence file available at www.neb.com.
See page 118 for ordering information.

There are no restriction sites for the following enzymes: AarI(x), AatII, AcuI, AflII, AgeI, AhdI, ApaI, ApaLI, AscI, AsiSI, AvrII, BbsI, BcgI, BciVI, BclI, BlpI, BmgBI, BmtI, BsaI, BsgI, BsiWI, BspEI, BspQI, BssHII, BssSI, BstAPI, BstBI, BstEII, BstXI, BstZ17I, EagI, EcoNI, EcoO109I, EcoRV, FseI, FspAI(x), HpaI, I-CeuI, I-SceI, MfeI, MluI, NcoI, NheI, NmeAIII, NotI, NruI, NsiI, PI-PspI, PI-SceI, PaeR7I, PflFI, PflMI, PmeI, PmlI, PpuMI, PshAI, PspOMI, PspXI, RsrII, SacII, SanDI(x), SapI, ScaI, SexAI, SfiI, SgrAI, SpeI, SrfI(x), StuI, StyI, Tth111I, XcmI, XhoI, ZraI

(x) = enzyme not available from NEB

M13 is a filamentous *E. coli* bacteriophage specific for male (F factor-containing) cells. Its genome is a circular, single-stranded DNA molecule 6407 bases in length, and contains 10 genes. A double-stranded form (RF) arises as an intermediate during DNA replication.

The M13mp phage vectors, derived from M13, contain the *lacZ*α gene and differ from each other by the cloning sites embedded within it. The location of cloning sites inside this gene allows screening for insertions using α-complementation. The map of M13mp18, whose multiple cloning site (MCS) was later employed to construct the plasmid pUC19, is shown below; sequences of the MCS region from other M13mp vectors are shown on the previous page. M13mp19 is identical to M13mp18 except that the MCS region (6231-6288) is inverted.

The complete nucleotide sequences of M13mp18 and M13mp19 have recently been determined at New England Biolabs (1), resulting in several nucleotide changes relative to the previous sequence data (2,3).

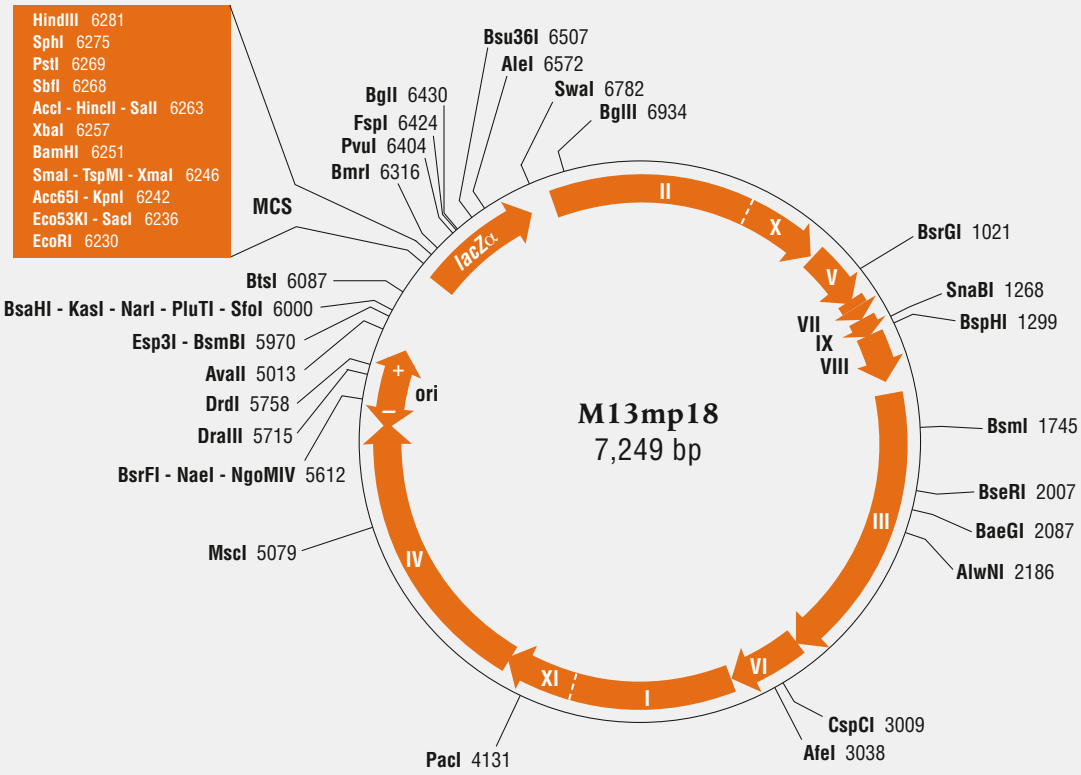
Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

M13 origin of replication arrows indicate the direction of synthesis of both the (+) and (–) strands.

Feature	Description	Coordinates
gene II	replication	6848-831 (cw)
gene X	replication	496-831
gene V	replication	843-1106
gene VII	minor coat protein	1108-1209
gene IX	minor coat protein	1206-1304
gene VIII	major coat protein	1301-1522
gene III	minor coat protein	1578-2852
gene VI	minor coat protein	2855-3193
gene I	phage assembly	3195-4241
gene XI (I*)	phage assembly	3915-4241
gene IV	phage assembly	4219-5499
ori	M13 origin (+) of replication	5487-5867
*lacZ*α	for α-complementation	6216-6722
MCS	multiple cloning site	6230-6286

(cw) = clockwise



References

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pBR322

GenBank Accession #: J01749
See page 118 for ordering information.

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AflII, AgeI, AleI, ApaI, ApuI, AscI, AsiSI, AvrII, BaeI, BbvCI, BclI, BglII, BlpI, BmgBI, BsaXI, BseRI, BsiWI, BsrGI, BssHII, BstBI, BstEII, BstXI, Bsu36I, CspCI, DraIII, Eco53KI, FseI, HpaI, I-CeuI, I-SceI, KpnI, MfeI, MluI, NcoI, NotI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PmeI, PmlI, PsiI, PspOMI, PspXI, RsrII, SacI, SacII, SanDI(x), SbfI, SexAI, SfiI, SmaI, SnaBI, SpeI, SrfI(x), StuI, SwaI, TspMI, XbaI, XcmI, XhoI, XmaI

(x) = enzyme not available from NEB

pBR322 is an *E. coli* plasmid cloning vector containing the origin of replication from pMB1 (a plasmid in the ColE1 compatibility group; 1–3). The *rop* gene product, which regulates plasmid replication by stabilizing the interaction between RNAI and RNAII transcripts, maintains the copy number at about 20 per cell. However, pBR322 can be amplified with chloramphenicol or spectinomycin (4).

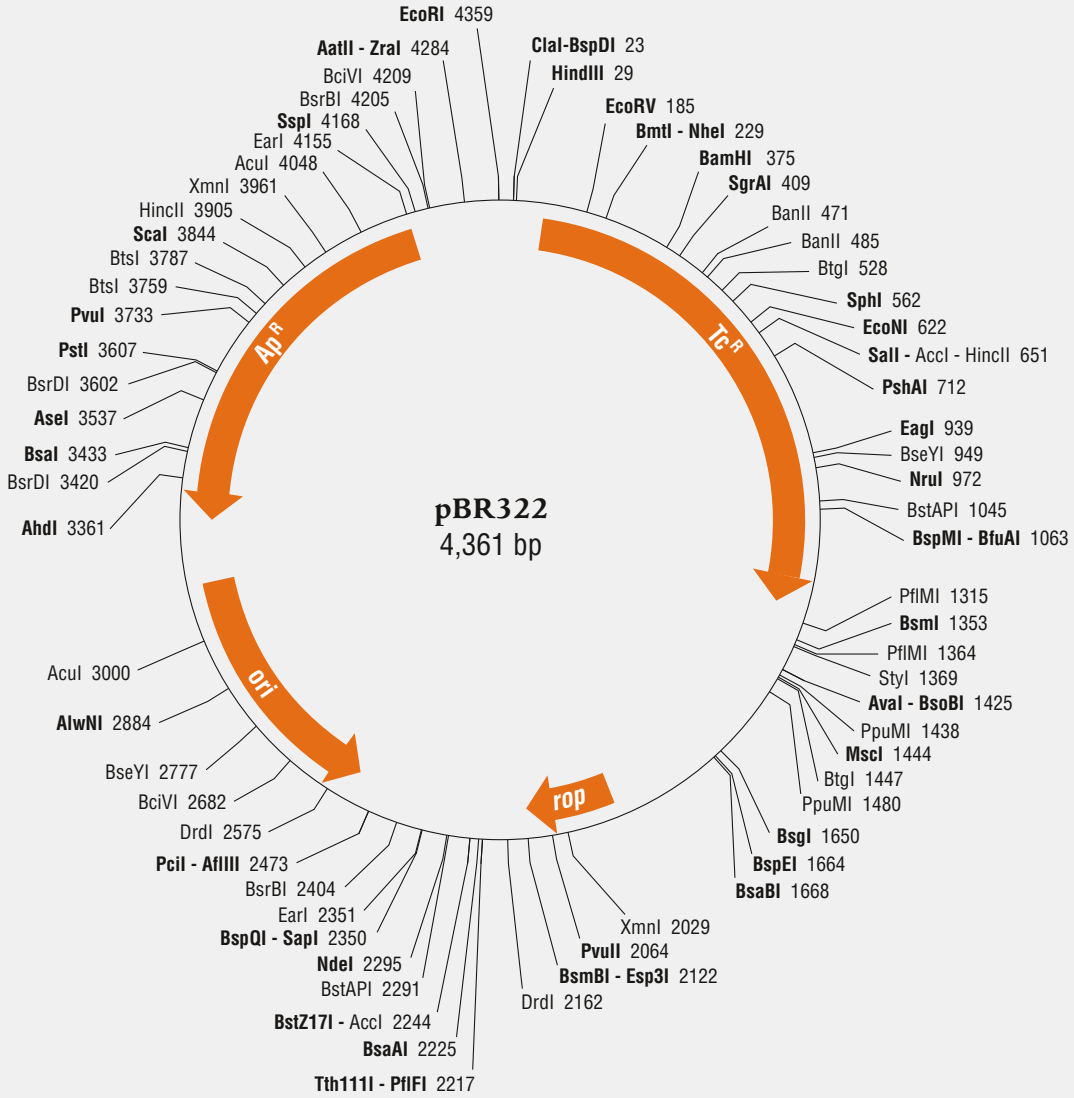
Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.

Feature	Coordinates	Source
tet (Tc^R)	86-1276	pSC101
bla (Ap^R)	4153-3293	*Tn3*
rop	1915-2106	pMB1
origin	3122-2534	pMB1

ori = origin of replication
Ap = ampicillin, Tc = tetracycline



References

(1) Bolivar, F. et al. (1977) *Gene,* 2, 95–113.
(2) Sutcliffe, J.G. (1979) *Cold Spring Harb. Symp. Quant. Biol.,* 43, 77–90.
(3) Watson, N. (1988) *Gene,* 70, 399–403.
(4) Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, (2nd ed.), Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

pKLAC2

GenBank Accession #: EU196354
See page 215 for ordering information.

There are no restriction sites for the following enzymes: AarI(x), AatII, Acc65I, AfeI, AflII, ApaI, AscI, AsiSI, AvrII, BbvCI, BlpI, Bpu10I, BsiWI, FseI, FspAI(x), I-CeuI, I-SceI, KpnI, MluI, MscI, PacI, PI-PspI, PI-SceI, PmeI, PmlI, PspOMI, PspXI, RsrII, SanDI(x), SfiI, SgrAI, SpeI, SrfI(x), SwaI, ZraI

(x) = enzyme not available from NEB

pKLAC2 is an expression vector capable both of replication in *E. coli* and stable integration into the genome of the yeast *Kluyveromyces lactis* (1). It is designed for high-level expression of recombinant protein in *K. lactis* using the *K. lactis* Protein Expression Kit (NEB #E1000). pKLAC2 contains a universal multiple cloning site (MCS) that is compatible with all NEB expression systems.

In *E. coli*, it replicates using the pMB1 origin of replication from pBR322 (although the *rop* gene is missing) and carries the *bla* (Ap^R) marker for selection with ampicillin. Upon transformation of *K. lactis* GG799 competent cells (NEB #C1001), SacII- or BstXI-linearized pKLAC2 integrates into the *K. lactis* chromosome at the *LAC4* locus. Yeast transformants can be selected using the acetamidase selectable marker (*amdS*), which is expressed from the yeast *ADH1* promoter. Acetamidase expressed from pKLAC2 permits transformed cells to utilize acetamide as a sole nitrogen source on defined medium (2).

The multiple cloning site (MCS) is positioned to allow translational fusion of the *K. lactis* α-mating factor secretion domain (α-MF) to the N-terminus of the recombinant target protein. This directs the fusion protein to the general secretory pathway, but the α-MF domain is cleaved off in the Golgi apparatus by the Kex protease, resulting in secretion of the recombinant protein alone.

Expression of the recombinant fusion protein is driven by the *K. lactis LAC4* promoter, which has been modified to be transcriptionally silent in *E. coli* (1). This facilitates the cloning of proteins that are toxic to *E. coli*. This promoter is split such that when pKLAC2 is cleaved with SacII or BstXI, the recombinant protein and selectable marker are flanked by the two halves of the promoter. When these ends recombine with the *LAC4* promoter in the *K. lactis* chromosome, the result is integration of the recombinant fusion protein (driven by the *LAC4* promoter) and *amdS* upstream of the *LAC4* gene (driven by a duplicate copy of the *LAC4* promoter) (2).

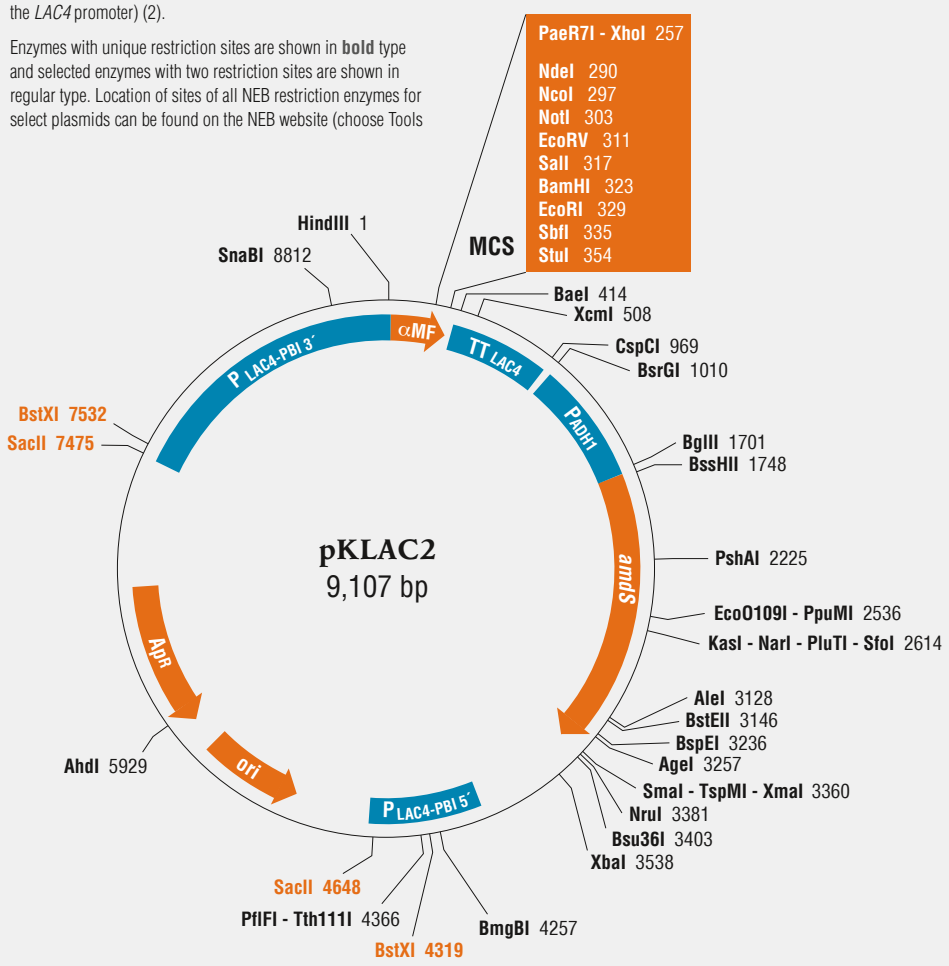
Enzymes with unique restriction sites are shown in **bold** type and selected enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Components of coordinated regions are indented below the region itself.

pMB1 origin of replication coordinates include the region from the –35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. Promoter and transcription terminator coordinates represent cloned regions and not necessarily the precise functional elements.

Feature	Coordinates	Source
expression region:		
α-mating factor		
leader sequence	14-349	*K. lactis*
MCS	257-354	–
LAC4 TT region	371-953	*K. lactis*
AdH1 promoter region	1010-1712	*S. cerevisiae*
amdS	1713-3359	*A. nidulans*
LAC4 promoter		
region (5´ end)	4068-4648	*K. lactis*
origin	5102-5690	pMB1
bla (Ap^R)	6721-5861	Tn3
LAC4 promoter		
region (3´ end)	7475-9107	*K. lactis* (modified)

ori = origin of replication
Ap = ampicillin
TT = transcription terminator



References

(1) Colussi, P.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 7092–7098.
(2) van Ooyen, A.J. et al. (2006) *FEMS Yeast Res.*, 6, 381–392.

pMAL-p5X

Sequence file available at www.neb.com.
See page 213 for ordering information.

Feature	Coordinates	Source
lacI^q	81-1163	*E. coli*
P_{tac}	1406-1433	–
expression ORF	1528-2832	–
malE	1528-2703	*E. coli*
MCS	2709-2832	–
bla (Ap^R)	3162-4022	*Tn3*
origin	4110-4698	pMB1
rop	5068-5259	pMB1

There are no restriction sites for the following enzymes: AarI(x), AatII, Acc65I, AflII, AgeI, AleI, AscI, AsiSI, AvrII, BaeI, BbvCI, BmtI, BsaAI, BseRI, BspDI, BsrGI, BstBI, BstZ17I, ClaI, CspCI, DraIII, EcoNI, FseI, I-CeuI, I-SceI, KpnI, NaeI, NgoMIV, NheI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PmeI, PmlI, PshAI, PspXI, SacII, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SphI, SrfI(x), StuI, SwaI, TspMI, XbaI, XhoI, XmaI, ZraI

(x) = enzyme not available from NEB

pMAL-p5X is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the pMAL Protein Fusion and Purification System (NEB #E8200) (1–3). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *E. coli* maltose binding protein (MBP, encoded by the *malE* gene) to the N-terminus of the cloned target protein. The pMAL-p5 and -c5 series of vectors differs from the -p4 and -c4 series in that they contain a universal multiple cloning site (MCS) that is compatible with other NEB expression systems and is followed by stop codons in all three reading frames. In addition, *lacZ*α and the M13 origin have been removed. In these vectors, MBP has been engineered for tighter binding to amylose. This allows easy purification of the fusion protein, and the MBP domain can be subsequently removed using Factor Xa protease (3).

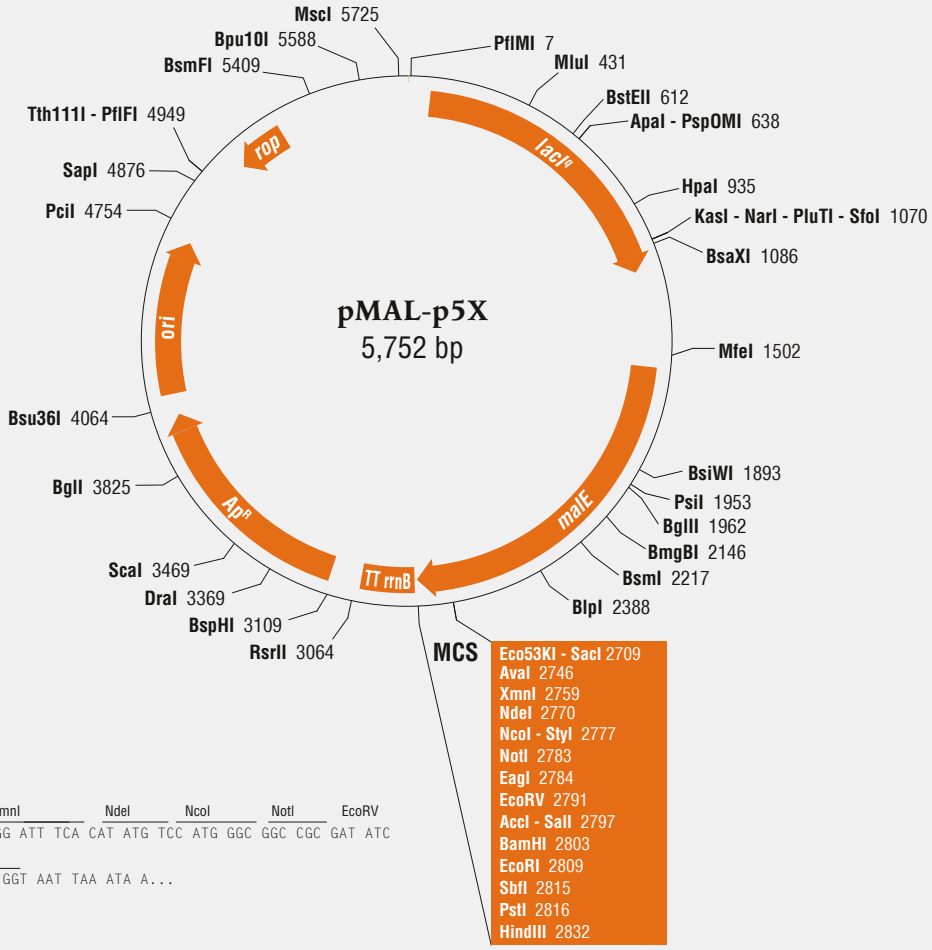
Transcription of the gene fusion is controlled by the inducible "tac" promoter (P_{tac}). Basal expression from P_{tac} is minimized by the binding of the Lac repressor, encoded by the *lacI*^{q} gene, to the *lac* operator immediately downstream of P_{tac}. A portion of the *rrnB* operon containing two terminators, derived from the vector pKK233-2, prevents transcription originating from P_{tac} from interfering with plasmid functions.

pMAL-c5-series vectors are identical to the pMAL-p5-series vectors above except for a deletion of the *malE* signal sequence (nt 1531-1605) (1).

Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

The pMB1 origin of replication includes the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point (labeled "ori") and the rop gene, which controls expression of the RNAII transcript. *bla* (Ap^R) gene coordinates include the signal sequence.



pMAL-p5X Polylinker:

```
          AvaI                  XmnI        NdeI      NcoI      NotI      EcoRV
5´ malE...CTC GGG ATC GAG GGA AGG ATT TCA CAT ATG TCC ATG GGC GGC CGC GAT ATC
   SalI     BamHI    EcoRI     SbfI
GTC GAC GGA TCC GAA TTC CCT GCA GGT AAT TAA ATA A...
```

pMAL-p5E Polylinker:

```
          AvaI                           KpnI      NdeI      NcoI      NotI
5´ malE...CTC GGG GAT GAC GAT GAC AAG GTA CCG CAT ATG TCC ATG GGC GGC CGC
   EcoRV    SalI     BamHI    EcoRI     SbfI
GAT ATC GTC GAC GGA TCC GAA TTC CCT GCA GGT AAT TAA ATA A...
```

References

(1) Guan, C. et al. (1987) *Gene*, 67, 21–30.
(2) Maina, C.V. et al. (1988) *Gene*, 74, 365–373.
(3) Riggs, P.D. (1992). In F.M. Ausubel, et al. (Eds.), *Current Prot. in Molecular Biol.* New York: John Wiley & Sons, Inc.

pMiniT 2.0

Sequence available at www.neb.com
See page 91 for more information.

Feature	Coordinates	Source
Constitutive promoter	1-214	pNK2138
SP6 promoter	479-496	SP6
Toxic minigene	541-549	–
Synthetic T7 promoter	619-602	T7
bla (Ap^R)	733-1593	*Tn3*
origin	1764-2352	pUC19

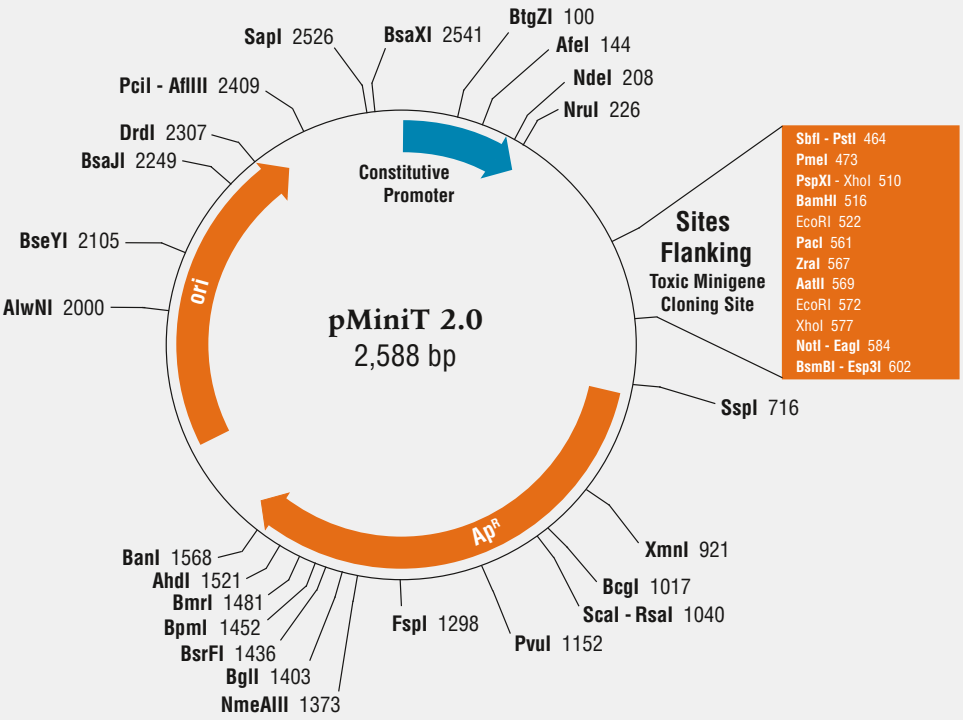
pMiniT 2.0 is an *E. coli* plasmid cloning vector designed for cloning blunt-ended or single-base overhang PCR products, or amplicons, using the NEB PCR Cloning Kit (NEB #E1202, #E1203). The pMiniT2.0 also enables *in vitro* transcription using SP6 and T7 promoters. It is compatible with Golden Gate Assembly as the BsaI site has been removed from the Ampicillin resistance gene.

In *E. coli*, it replicates using the pMB1 origin of replication from pUC19 and carries the *bla* (ApR) marker for selection with ampicillin. pMiniT2.0 contains a toxic minigene that is under the control of a constitutive promoter. If the pMiniT 2.0 vector recircularizes without an insert, the toxic minigene it will cause lethal inhibition of protein synthesis and no colony will result. If the pMiniT 2.0 Vector carries an insert, a colony will grow.

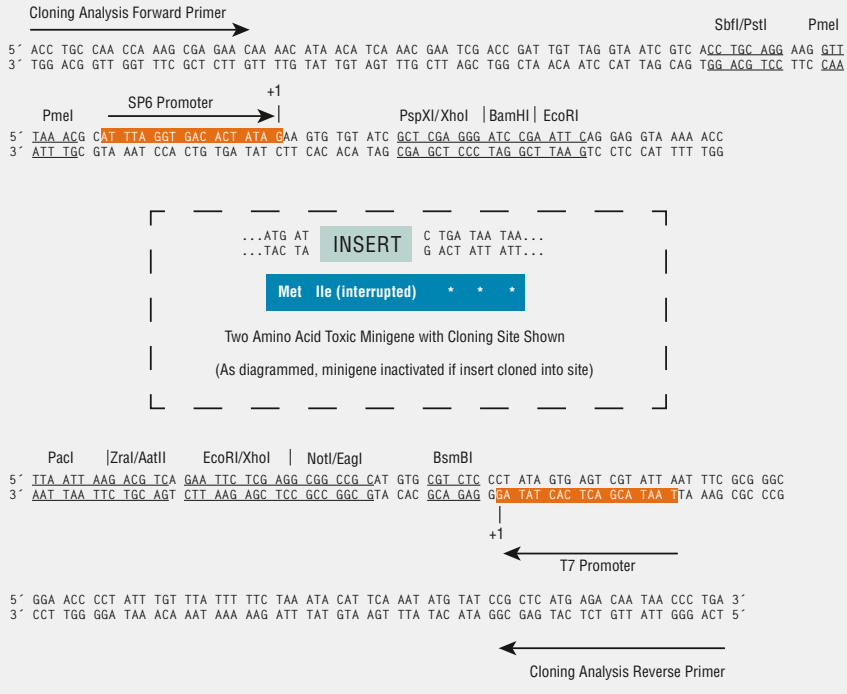
The map shown below displays the construct formed if no insert is present. Unique restriction sites are shown in **bold**. Additional restriction sites that can be used for subcloning are also shown. Expanded box below shows location of sequencing primers, restriction sites for subcloning or linearization for *in vitro* transcription, RNA Polymerase promoter sequences and placement of insertion site within the toxic minigene.

There are no restriction sites for the following enzymes: AbsI(x), Acc65I, AccI, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BanII, BarI(x), BbsI, BbvCI, BclI, BglII, BlpI(x), BmgBI, BmtI, BpiI(x), Bpu10I, BsaI, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstAPI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, ClaI, CspCI, DraIII, Eco53kI, EcoNI, EcoO109I, EcoRV, FalI(x), FseI, FspAI(x), HincII, HindIII, HpaI, KasI, KfiI(x), KpnI, MauBI(x), MfeI, MluI, MreI(x), MscI, MteI(x), NaeI, NarI, NcoI, NgoMIV, NheI, NsiI, PasI(x), PflFI, PflMI, PfoI(x), PluTI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PsrI(x), PvuII, RsrII, SacI, SacII, SalI, SexAI, SfiI, SfoI, SgrAI, SgrDI(x), SmaI, SnaBI, SpeI, SphI, SrfI(x), StuI, StyI, SwaI, TspMI, Tth111I, XbaI, XcmI, XmaI

(x) = enzyme not available from NEB



Features within Sequence Flanking the Toxic Minigene/Cloning Site:



pNEB206A

Sequence file available at www.neb.com.

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AccI, AfeI, AflII, AgeI, AleI, ApaI, AsiSI, AvaI, AvrII, BaeI, BbsI, BclI, BfuAI, BglII, BlpI, BmgBI, BmtI, BsaAI, BsaBI, BsgI, BsiWI, BsmFI, BsmI, BsoBI, BspDI, BspEI, BspMI, BsrGI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FseI, FspAI(x), HincII, HpaI, I-CeuI, I-SceI, KpnI, MfeI, MluI, MscI, NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PI-PspI, PI-SceI, PaeR7I, PflFI, PflMI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, RsrII, SacII, SalI, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SphI, SrfI(x), StuI, StyI, SwaI, TspMI, Tth111I, XcmI, XhoI, XmaI

(x) = enzyme not available from NEB

pNEB206A is an *E. coli* plasmid vector designed for fast and efficient cloning of PCR products to be used in conjunction with USER Enzyme (NEB #M5505; 1). It is derived from pNEB193 containing the high-copy pUC19 origin of replication and *lacZ*α gene for screening of insertions at the cloning site using α-complementation (2).

The plasmid is supplied in a linearized form 2,706 bp in length (with bp 438-453 excised from the circular form), flanked by two noncomplementary 8-base 3´ overhangs at the intended cloning site. Amplification with deoxyuridine-containing primers and subsequent treatment (as defined in the protocol "Cloning with USER Enzyme" found on our website), results in PCR products with 5´ overhangs complementary to those in pNEB206A. These products can be directionally cloned into pNEB206A at high efficiency without the use of restriction enzymes or DNA ligase, forming recombinant circular molecules.

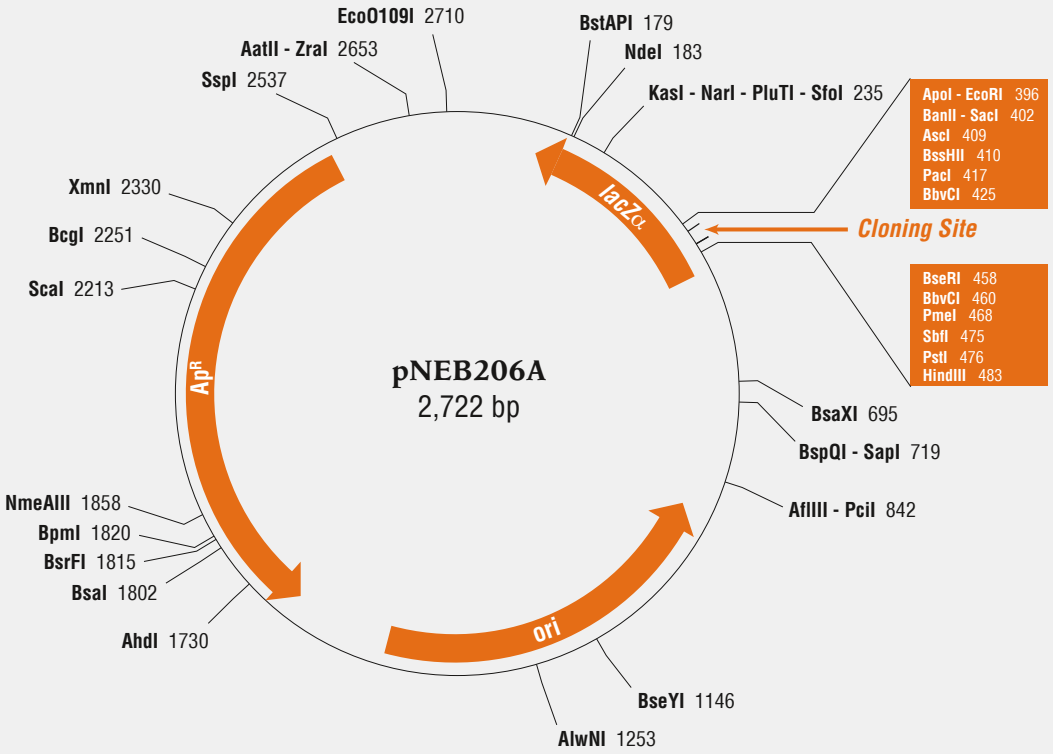
Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence. Coordinates on the map and in the tables refer to the 2,722 bp circular plasmid prior to linearization and can be used to calculate relative distances.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence. Cloning site coordinates include those bases in the circular form that are single-stranded in or missing from the supplied linear form.

Feature	Coordinates	Source
*lacZ*α	505-146	–
cloning site	430-461	–
origin	1491-903	pUC19
bla (Ap^R)	2522-1662	*Tn3*

ori = origin of replication
Ap = ampicillin



pNEB206A (linearized form) cloning site:

```
          Eco53KI
            SacI        BssHII
    EcoRI           AscI        PacI        BbvCI
agtgAATTCGAGCTCAGGCGCGCCTTAATTAAGCTGAGGGAAAGT
tcacTTAAGCTCGAGTCCGCGCGGAATTAATTCGACT
      .           .           .           .
      400         410         420         430

...S  N  S  S  L  R  A  K  I  L  S  L  S  L
```

```
                                  SbfI
      BbvCI     PmeI        PstI      HindIII
        TCAGCGTTTAAACCCTGCAGGAAGCTTGGcgtaatcatggtcat
TACAGAGGAGTCGCAAATTTGGGACGTCCTTCGAACCgcattagtaccagta
      .           .           .           .           .
      460         470         480         490         500

T  E  E  A  N  L  G  Q  L  F  S  P  T  I  M  T  M
```

*lacZ*α translational start

References

(1) Bitinaite, J. and Vaiskunaite, R. (2003) unpublished observations.
(2) Yanisch-Perron, C. et al. (1985) *Gene,* 33, 103–119.

pSNAP$_f$

Sequence file available at www.neb.com.
See page 282 for ordering information.

There are no restriction sites for the following enzymes: AbsI(x), AfeI, AflII, AjuI(x), AlfI(x), AloI(x), AsiSI, BaeI, BarI(x), BbvCI, BlpI, BpiI(x), BsiWI, BsmBI, BspDI, BspEI, BstAPI, BstBI, BstEII, ClaI, EcoNI, Esp3I, FseI, FspAI(x), KfiI(x), MauBI(x), MreI(x), PasI(x), PfoI(x), PshAI, PsrI(x), SexAI, SgrAI, SrfI(x), StuI, XcmI

(x) = enzyme not available from NEB

pSNAP$_f$ Vector is a mammalian expression plasmid intended for the cloning and stable or transient expression of SNAP-tag® protein fusions in mammalian cells. This plasmid encodes SNAP$_f$, a SNAP-tag protein, which is expressed under control of the CMV promoter. SNAP$_f$ is an improved version of the SNAP-tag which exhibits faster labeling kinetics. The SNAP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag is a small protein based on human O6-alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag substrates are derivatives of benzyl purines and benzyl pyrimidines. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag. Use of this system involves two steps: sub-cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice. Further details are provided with the SNAP-Cell Starter Kit (NEB #E9100) and SNAP-Surface Starter Kit (NEB #E9120).

Codon usage of the gene is optimized for expression in mammalian cells. pSNAP$_f$ contains two multiple cloning sites to allow cloning of the fusion partner as a fusion to the N- or C-terminus of the SNAP-tag. The expression vector has an Internal Ribosome Entry Site (IRES) and a neomycin resistance gene downstream of the SNAP-tag for the efficient selection of stable transfectants.

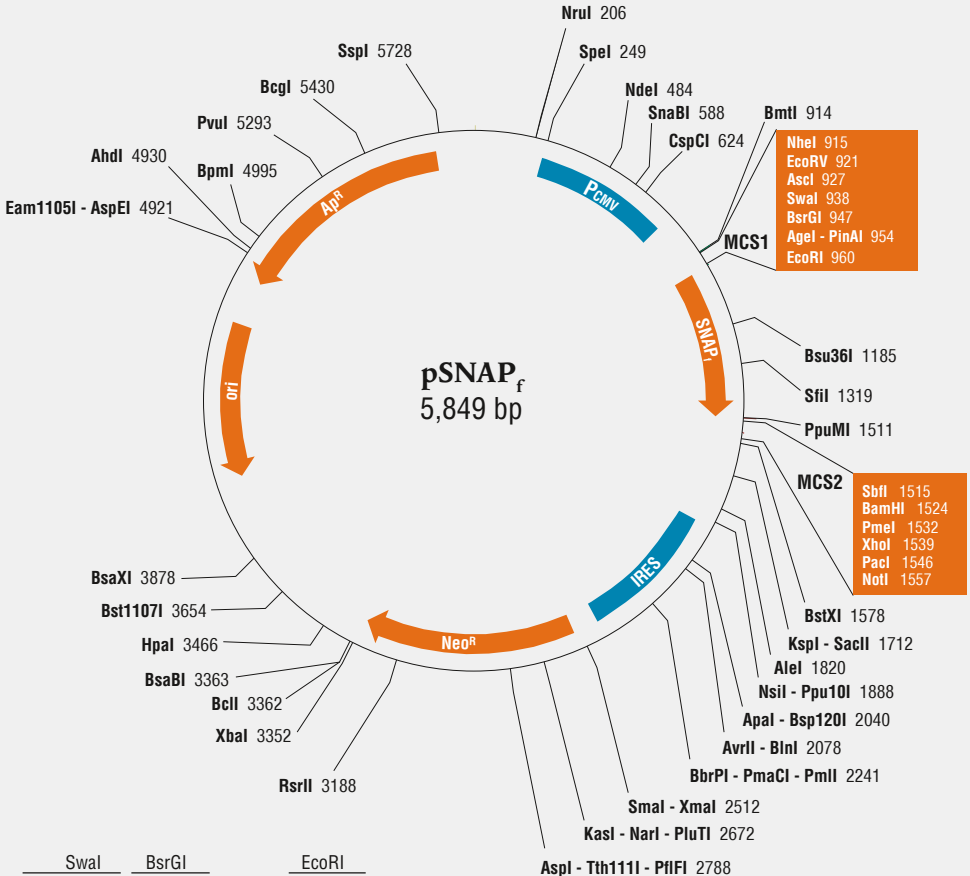
Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes for select plasmid can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pUC19 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.

Feature	Coordinates	Source
CMV promoter	251-818	–
expression region	915-1564	–
MCS1	915-965	–
SNAP$_f$	969-1514	–
MCS2	1515-1564	–
IRES	1910-2500	*ECMV*
Neo^R	2536-3339	*Tn5*
origin	4094-4682	pUC19
bla (Ap^R)	4853-5713	*Tn3*

ori = origin of replication
Ap = ampicillin
Neo = neomycin
IRES = internal ribosomal entry site



MCS1

```
    NheI        AscI        SwaI      BsrGI          EcoRI
...GCTAGC GATATCGGCG CGCCAGCATT TAAATCTGTA CAGACCGGTG AATTC
   CGATCG CTATAGCCGC GCGGTCGTAA ATTTAGACAT GTCTGGCCAC TTAAG...
```

MCS2

```
      SbfI      BamHI      PmeI      XhoI      PacI      NotI
...CCTGCA GGCGGATCCG CGTTTAAACT CGAGGTTAAT TAATGAGCGG CCGC
   GGACGT CCGCCTAGGC GCAAATTTGA GCTCCAATTA ATTACTCGCC GGCG...
```

pTXB1

Sequence file available at www.neb.com.
See page 214 for ordering information.

Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-6558	–
MCS	5722-5775	–
Mxe GyrA intein	5776-6369	*M. xenopi*
CBD	6400-6558	*B. circulans*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AflII, AleI, AscI, AsiSI, AvrII, BaeI, BbvCI, BglII, BmgBI, Bpu10I, BseRI, BspDI, BstBI, Bsu36I, ClaI, CspCI, Eco53KI, FseI, FspAI(x), HindIII, I-CeuI, I-SceI, KpnI, MscI, NcoI, NsiI, PI-PspI, PI-SceI, PacI, PmlI, PpuMI, RsrII, SacI, SanDI(x), SbfI, SexAI, SfiI, SmaI, SnaBI, SrfI(x), TspMI, XmaI

(x) = enzyme not available from NEB

pTXB1 is an *E. coli* plasmid cloning vector designed for recombinant protein expression, purification, and ligation using the IMPACT™ Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTXB1 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *Mxe* GyrA intein tag to the C-terminus of the cloned target protein (2,3). The chitin binding domain (CBD) from *B. circulans*, fused to the C-terminus of the intein, facilitates purification of the intein-target protein precursor.

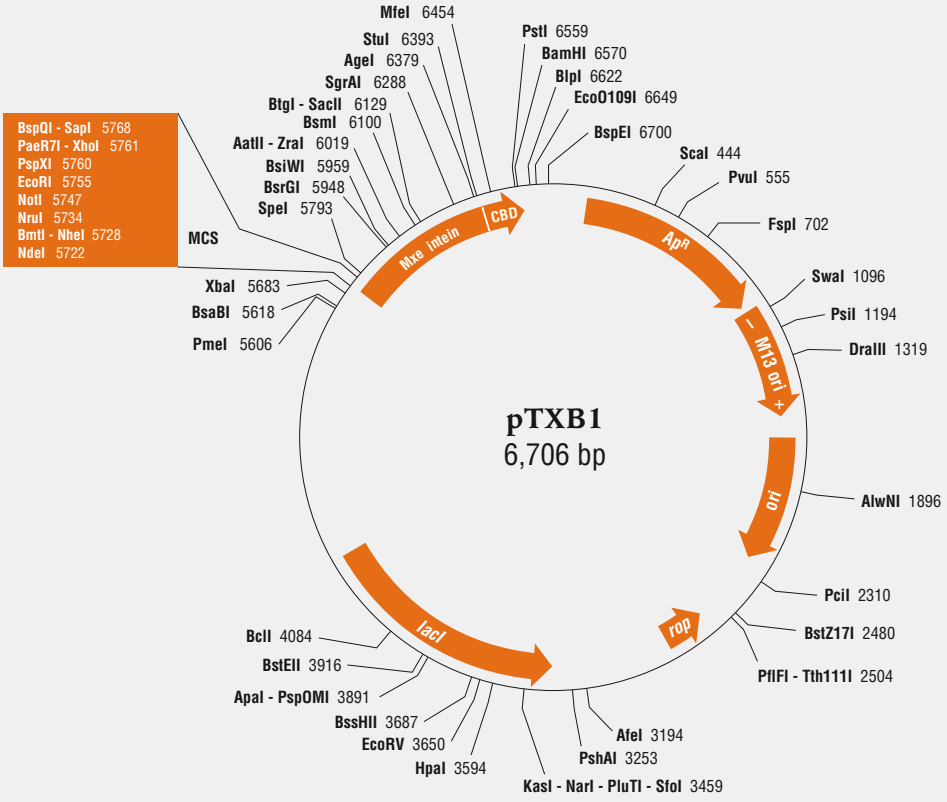
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the *lacI* gene, to the *lac* operator immediately downstream of the T7 promoter (4). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (ϕ10).

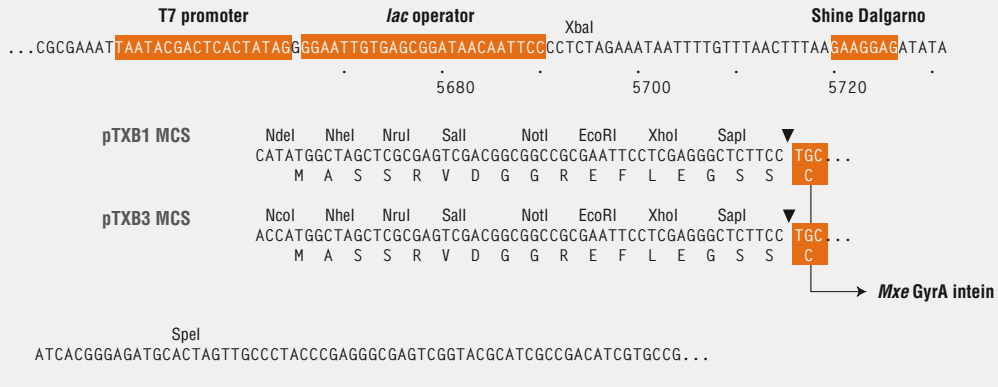
pTXB1 and pTXB3 are identical except for the MCS regions: pTXB1 contains an NdeI site, and pTXB3 an NcoI site, overlapping the initiating methionine codon of the intein fusion gene. The N-terminal cysteine residue ("Cys$_1$") of the intein is shaded.

Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.





References

(1) Chong, S. et al. (1997) *Gene*, 192, 271–281.
(2) Evans, T.C., Benner and Xu, M.-Q. (1998) *Protein Sci.*, 7, 2256–2264.
(3) Southworth, M.W. et al. (1999) *Biotechniques*, 27, 110–120.
(4) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.*, 219, 45–59.

pTYB21

Sequence file available at www.neb.com.
See page 214 for ordering information.

Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-7368	–
MCS	7301-7361	–
Sce VMA intein	5770-7299	*S. cerevisiae*
CBD	6595-6747	*B. circulans*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), AatII, AflII, AgeI, AscI, AsiSI, AvrII, BbvCI, BmgBI, BseRI, BsiWI, BsmI, BspDI, Bsu36I, ClaI, CspCI, FseI, FspAI(x), I-CeuI, I-SceI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PpuMI, PspXI, RsrII, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SrfI(x), TspMI, XhoI, XmaI, ZraI

(x) = enzyme not available from NEB

pTYB21 is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the IMPACT™ Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTYB21 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *Sce* VMA intein tag to the N-terminus of the cloned target protein (2). The chitin binding domain (CBD) from *B. circulans*, facilitates purification of the intein-target protein precursor.

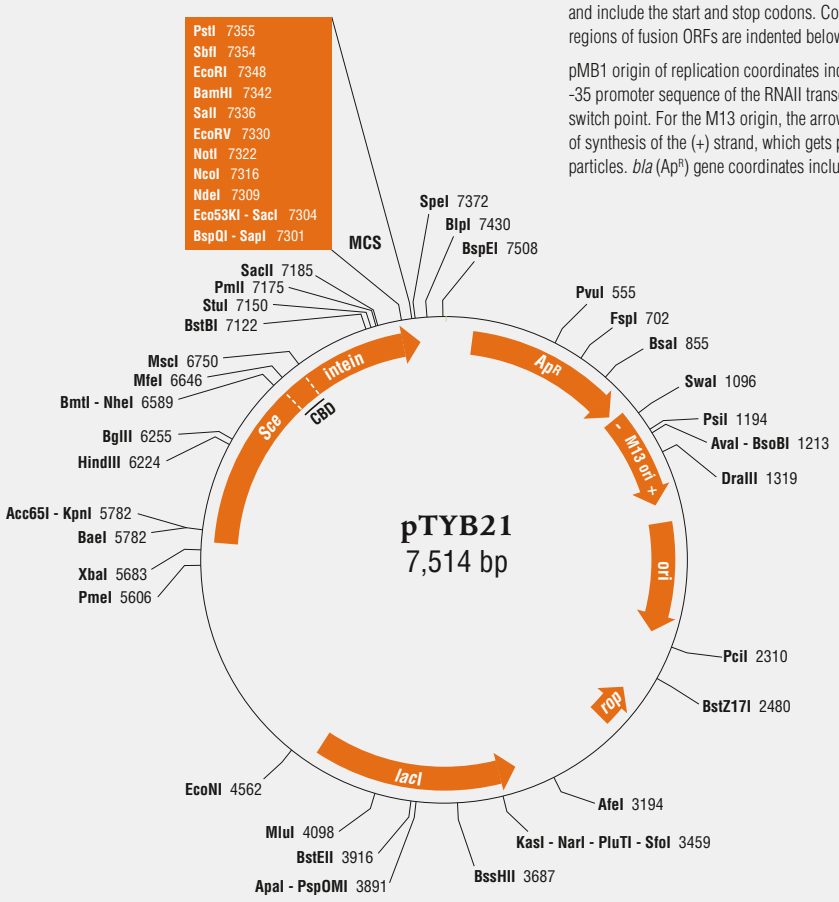
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the *lacI* gene, to the *lac* operator immediately downstream of the T7 promoter (3). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (φ10).

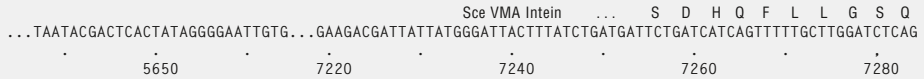
pTYB21 contains a SapI site which allows for cloning of a target gene without any extra amino acids. pTYB22 is identical to pTYB21 except for the MCS regions (see below). pTYB22 contains an NdeI site overlapping the initiating methionine codon of the intein fusion gene. pTYB21 differs from pTYB11 in that it contains a universal MCS that is compatible with all NEB expression systems.

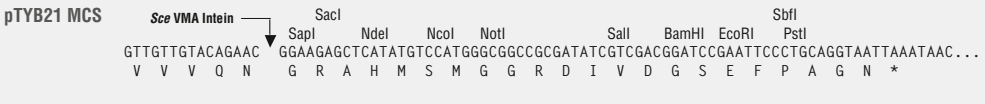
Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (Technical Reference > DNA Sequences and Maps). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.







References

(1) Chong et al. (1996) *J. Biol. Chem.*, 271, 22159–22168
(2) Chong et al. (1998) *NAR*, 26, 5109–5115.
(3) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.*, 219, 45–59.

pUC19

GenBank Accession #: L09137
See page 118 for ordering information.

Feature	Coordinates	Source
*lacZ*α	469-146	–
origin	1455-867	pMB1 (mutant)
bla (Ap^R)	2486-1626	*Tn3*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), AfeI, AflII, AgeI, AleI, ApaI, AscI, AsiSI, AvrII, BaeI, BbsI, BbvCI, BclI, BglII, BlpI, BmgBI, BmtI, Bpu10I, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FseI, FspAI(x), HpaI, I-CeuI, I-SceI, MfeI, MluI, MscI, NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PflFI, PflMI, PmeI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, RsrII, SacII, SanDI(x), SexAI, SfiI, SgrAI, SnaBI, SpeI, SrfI(x), StuI, StyI, SwaI, Tth111I, XcmI, XhoI

(x) = enzyme not available from NEB

pUC19 is a small, high-copy number *E. coli* plasmid cloning vector containing portions of pBR322 and M13mp19 (1). It contains the pMB1 origin of replication from pBR322, but it lacks the *rop* gene and carries a point mutation in the RNAII transcript (G 2975 in pBR322 to A 1308 in pUC19; 2). These changes together result in a temperature-dependent copy number of about 75 per cell at 37°C and > 200 per cell at 42°C (2,3). The multiple cloning site (MCS) is in frame with the *lacZ*α gene, allowing screening for insertions using α-complementation.

pUC18 is identical to pUC19 except that the MCS region (nt 397-454) is inverted.

pNEB193 is also identical to pUC19 except for the addition of several restriction endonuclease sites to the MCS. Its total length is 2713 bp.

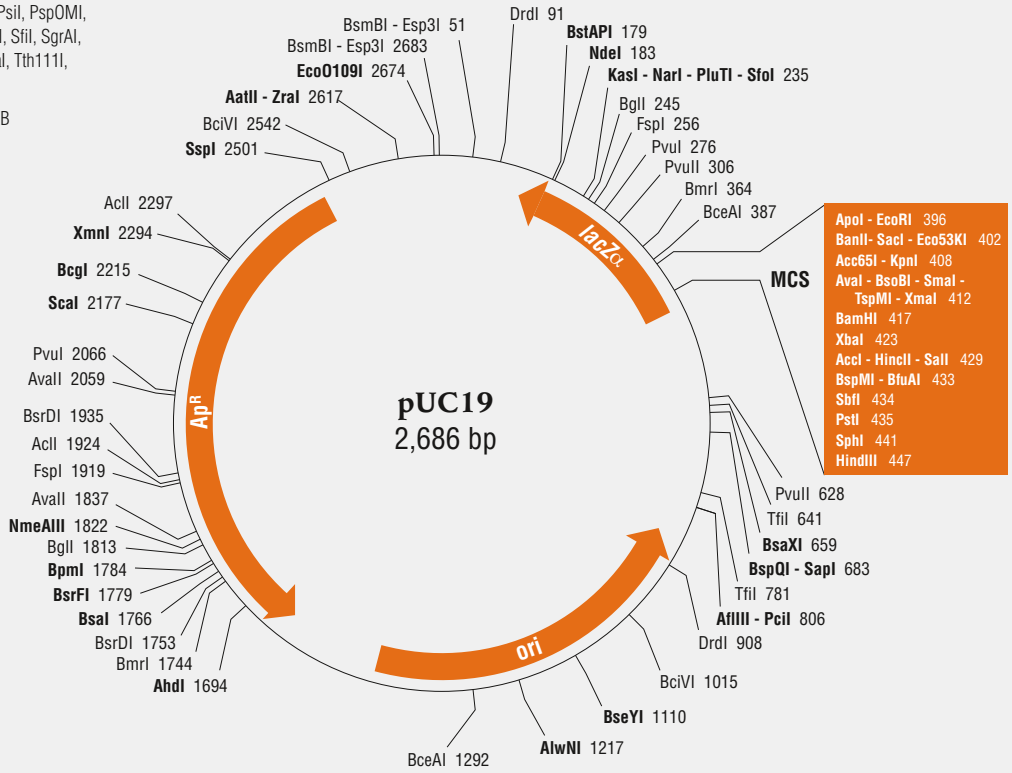
Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.

References

(1) Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene,* 33, 103–119.
(2) Lin-Chao, S., Chen, W.-T. and Wong, T.-T. (1992) *Mol. Microbiol.,* 6, 3385–3393
(3) Miki, T. et al. (1987) *Protein Eng.,* 1, 327–332.



pUC19 MCS

```
          SacI        SmaI        XbaI        SbfI
   EcoRI        KpnI       BamHI       SalI    PstI    SphI   HindIII
agtgAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
      .           .           .           .       .       .       .
     400         410         420         430     440     450     460

...S  N  S  S  P  V  R  P  D  E  L  T  S  R  C  A  H  L  S  P  T  I  M  T  M
```

*lacZ*α translational start

pNEB193 MCS

```
          SacI        SmaI    BssHII          PacI                          SbfI
   EcoRI        KpnI        AscI       BamHI            XbaI   SalI    PmeI    PstI   SphI   HindIII
agtgAATTCGAGCTCGGTACCCGGGGGCGCGCCGGATCCTTAATTAAGTCTAGAGTCGACTGTTTAAACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
      .           .           .           .           .       .       .       .       .       .       .
     400         410         420         430         440     450     460     470     480     490

...S  N  S  S  P  V  R  P  R  A  P  D  K  I  L  D  L  T  S  Q  K  F  R  C  A  H  L  S  P  T  I  M  T  M
```

*lacZ*α translational start

T7

39,937 base pairs
GenBank Accession #: NC_001604
Not currently available from NEB.

There are no restriction sites for the following enzymes: AfeI, ApaI, AscI, AsiSI, BamHI, BsiWI, BspEI, EagI, Eco53KI, EcoRI, EcoRV, FseI, HindIII, I-CeuI, I-SceI, NaeI, NgoMIV, NotI, PI-PspI, PI-SceI, PaeR7I, PspOMI, PspXI, PstI, PvuI, SacI, SacII, SalI, SbfI, SexAI, SgrAI, SmaI, SphI, SrfI(x), TspMI, XhoI, XmaI

(x) = enzyme not available from NEB

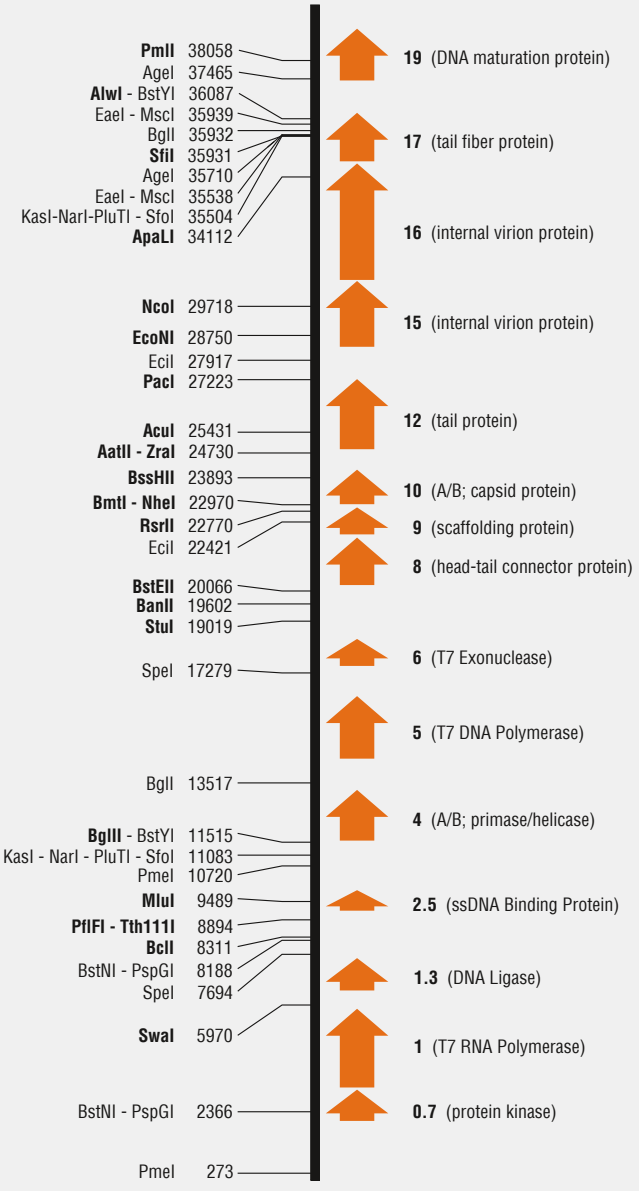
T7 is a lytic *E. coli* bacteriophage with a linear, double-stranded DNA genome containing 56 genes (1-4). Genes are classified as early or late based on the order of transcription in the infected host and their dependence on host or phage RNA polymerase.

Numbering of the sequence begins at the first (5´-most) base of the left end (bottom of the diagram below) and continues rightward (upward) in the direction of early to late genes. The map below shows the positions of all known ORFs larger than 200 codons.

Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

References

(1) Oakley, J.L. and Coleman, J.E. (1977) *Proc. Natl. Acad. Sci.* USA, 74, 4266–4270.
(2) Stahl, S.J. and Zinn, K. (1981) *J. Mol. Biol.,* 148, 481–485.
(3) Dunn, J.J. and Studier, F.W. (1981) *J. Mol. Biol.,* 148, 303–330.
(4) Dunn, J.J. and Studier, F.W. (1983) *J. Mol. Biol.,* 166, 477–535.



The Genetic Code

	A Ala	R Arg	N Asn	D Asp	C Cys	Q Gln	E Glu	G Gly	H His	I Ile	L Leu	K Lys	M Met	F Phe	P Pro	S Ser	T Thr	W Trp	Y Tyr	V Val	
5′	GCA C G U	CGA C G U or AGA G	AAC U	GAC U	UGC U	CAA G	GAA G	GGA C G U	CAC U	AUA C U	CUA C G U or UUA G	AAA G	AUG	UUC U	CCA C G U	UCA C G U or AGC U	ACA C G U	UGG	UAC U	GUA	C G U 3′

Second Position

First Position (5′end)	U	C	A	G	Third Position (3′ end)
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

Termination Signals

UAA (Ochre)
UAG (Amber)
UGA (Opal)

Single Letter Code

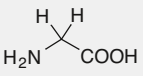
A = adenosine
C = cytidine
G = guanosine
T = thymidine
U = uridine

B = C or G or T
D = A or G or T
H = A or C or T
K = G or T
M = A or C
N = A or C or G or T
R = A or G
S = C or G
V = A or C or G
W = A or T
Y = C or T

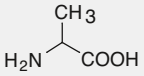
Amino Acid Structures

Each amino acid is accompanied by its three- and one-letter code, residue molecular weight (actual molecular weight minus water) and side-chain pK_a where appropriate.

SMALL

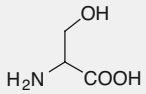


Glycine (Gly, G)
MW: 75.07

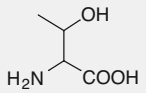


Alanine (Ala, A)
MW: 89.09

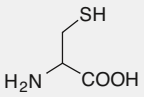
NUCLEOPHILIC



Serine (Ser, S)
MW: 105.09, pK_a ~ 16

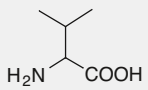


Threonine (Thr, T)
MW: 119.1, pK_a ~ 16

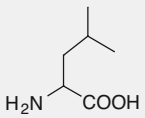


Cysteine (Cys, C)
MW: 121.2, pK_a = 8.18

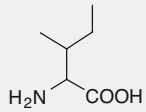
HYDROPHOBIC



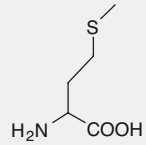
Valine (Val, V)
MW: 117.1



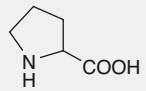
Leucine (Leu, L)
MW: 131.2



Isoleucine (Ile, I)
MW: 131.2

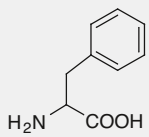


Methionine (Met, M)
MW: 149.2

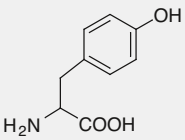


Proline (Pro, P)
MW: 115.1

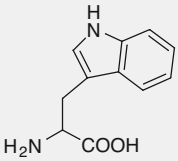
AROMATIC



Phenylalanine (Phe, F)
MW: 165.2

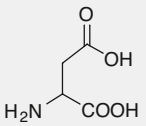


Tyrosine (Tyr, Y)
MW: 181.2, pK_a = 10.46

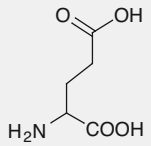


Tryptophan (Trp, W)
MW: 204.2

ACIDIC

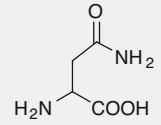


Aspartic Acid (Asp, D)
MW: 133.1, pK_a = 3.9

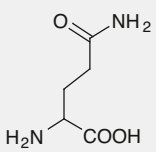


Glutamic Acid (Glu, E)
MW: 147.1, pK_a = 4.07

AMIDE

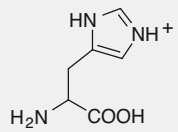


Asparagine (Asn, N)
MW: 132.1

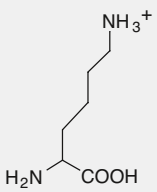


Glutamine (Gln, Q)
MW: 146.1

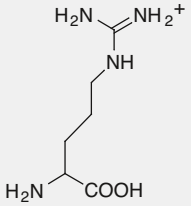
BASIC



Histidine (His, H)
MW: 155.2, pK_a = 6.04



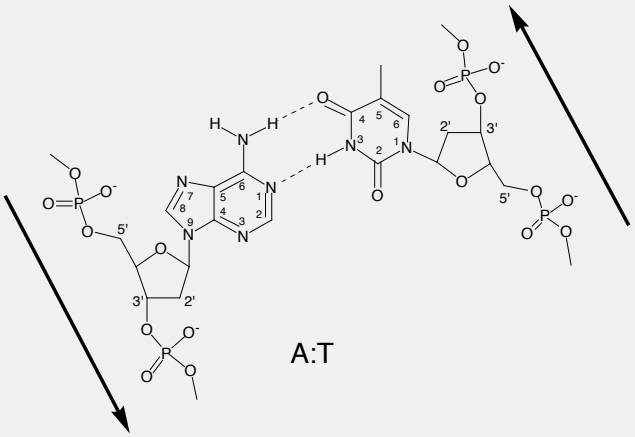
Lysine (Lys, K)
MW: 146.2, pK_a = 10.79

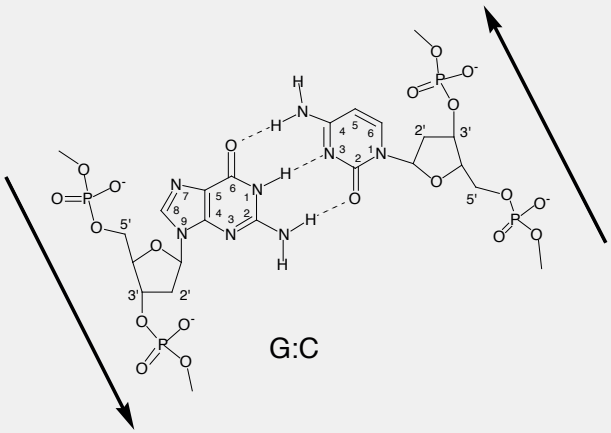


Arginine (Arg, R)
MW: 174.2, pK_a = 12.48

DNA Base Pairs

The structures of the adenosine:thymidine and guanosine:cytidine base pairs are shown in the context of the ribose phosphodiester backbones. The numbering schemes of the ribose and nucleotide moieties are indicated. Arrows indicate the polarity of each strand from 5´ to 3´.





Nucleic Acid Data

Average weight of a DNA basepair (sodium salt) = 650 daltons

1.0 A_{260} unit ds DNA = 50 µg/ml = 0.15 mM (in nucleotides)
1.0 A_{260} unit ss DNA = 33 µg/ml = 0.10 mM (in nucleotides)
1.0 A_{260} unit ss RNA = 40 µg/ml = 0.11 mM (in nucleotides)

MW of a double-stranded DNA molecule = (# of base pairs) x (650 daltons/base pair)
Moles of ends of a double-stranded DNA molecule = 2 x (grams of DNA) / (MW in daltons)
Moles of ends generated by restriction endonuclease cleavage:
a) circular DNA molecule: 2 x (moles of DNA) x (number of sites)
b) linear DNA molecule: 2 x (moles of DNA) x (number of sites) + 2 x (moles of DNA)

1 µg of 1000 bp DNA = 1.52 pmol = 9.1×10^{11} molecules
1 µg of pUC18/19 DNA (2686 bp) = 0.57 pmol = 3.4×10^{11} molecules
1 µg of pBR322 DNA (4361 bp) = 0.35 pmol = 2.1×10^{11} molecules
1 µg of M13mp18/19 DNA (7249 bp) = 0.21 pmol = 1.3×10^{11} molecules
1 µg of λ DNA (48502 bp) = 0.03 pmol = 1.8×10^{10} molecules

1 pmol of 1000 bp DNA = 0.66 µg
1 pmol of pUC18/19 DNA (2686 bp) = 1.77 µg
1 pmol of pBR322 DNA (4361 bp) = 2.88 µg
1 pmol of M13mp18/19 DNA (7249 bp) = 4.78 µg
1 pmol of λ DNA (48502 bp) = 32.01 µg

1.0 kb DNA = coding capacity for 333 amino acids ≈ 37,000 dalton protein
10,000 dalton protein ≈ 270 bp DNA
50,000 dalton protein ≈ 1.35 kb DNA

Isotope Data

Isotope	Particle Emitted	Half Life				
^{14}C	β	5,730 years	1 Ci	=	1,000 mCi
^{3}H	β	12.3 years	1 mCi	=	1,000 µCi
^{125}I	γ	60 days	1 µCi	=	2.2×10^{6} disintegrations/minute
^{32}P	β	14.3 days	1 Becquerel	=	1 disintegration/second
^{33}P	β	25 days	1 µCi	=	3.7×10^{4} Becquerels
^{35}S	β	87.4 days	1 Becquerel	=	2.7×10^{-5} µCi

Acids and Bases

Compound	Formula	Molecular Weight	Specific Gravity	% by Weight	Conc Reagent Molarity
Acetic acid, glacial	CH_3COOH	60.0	1.05	99.5	17.4
Formic acid	$HCOOH$	46.0	1.20	90	23.4
Hydrochloric acid	HCl	36.5	1.18	36	11.6
Nitric acid	HNO_3	63.0	1.42	71	16.0
Perchloric acid	$HClO_4$	100.5	1.67	70	11.6
Phosphoric acid	H_3PO_4	98.0	1.70	85	18.1
Sulfuric acid	H_2SO_4	98.1	1.84	96	18.0
Ammonium hydroxide	NH_4OH	35.0	0.90	28	14.8
Potassium hydroxide	KOH	56.1	1.52	50	13.5
Sodium hydroxide	$NaOH$	40.0	1.53	50	19.1
β-mercaptoethanol	$HSCH_2CH_2OH$	78.1	1.11	100	14.3

Protein Data

Bacterial Cells: *E. coli* or *Salmonella typhimurium*

	Cell Data	per cell	per liter at 10^9 cells per ml
Theoretical maximum yield for a 1 liter culture	Wet Weight	9.5×10^{-13} g	0.95 g
(10^9 cells /ml) if protein of interest is:	Dry Weight	2.8×10^{-13} g	0.28 g
0.1% of total protein: 150 µg/liter	Total Protein	1.55×10^{-13} g	0.15 g
2.0% of total protein: 3 mg/liter	Volume	1.15 μm^3 = 1 femtoliter	
50.0% of total protein: 75 mg/liter	Protein Conc. in the cell: 135 mg/ml		

Common Plasmid Gene Products

Gene	Gene Product # of Residues	Molecular Weight (daltons)
tet (pBR322)	401	43,267
amp (pBR322, bla)	286	31,515
kan (pACYC177, nptI)	264	29,047
cam (pACYC184, cat)	219	25,663
*lacZ*α (pUC19)	107	12,232
lacZ	1,023	116,351

Nucleotide Physical Properties

Compound	Molecular Weight	λ max (pH 7.0)	Absorbance at λ max 1 M solution (pH 7.0)
ATP	507.2	259	15,400
CTP	483.2	271	9,000
GTP	523.2	253	13,700
UTP	484.2	262	10,000
dATP	491.2	259	15,200
dCTP	467.2	271	9,300
dGTP	507.2	253	13,700
dTTP	482.2	267	9,600

pH vs Temperature for Tris Buffer

pH of Tris Buffer (0.05 M)		
5°C	25°C	37°C
7.76	7.20	6.91
7.89	7.30	7.02
7.97	7.40	7.12
8.07	7.50	7.22
8.18	7.60	7.30
8.26	7.70	7.40
8.37	7.80	7.52
8.48	7.90	7.62
8.58	8.00	7.71
8.68	8.10	7.80
8.78	8.20	7.91
8.88	8.30	8.01
8.98	8.40	8.10
9.09	8.50	8.22
9.18	8.60	8.31
9.28	8.70	8.42

Agarose Gel Resolution

% Gel	Optimum Resolution for Linear DNA (kb)
0.5	30 to 1.0
0.7	12 to 0.8
1.0	10 to 0.5
1.2	7 to 0.4
1.5	3 to 0.2

Common Buffer Chart

The following chart lists some of the common buffers used in biology. The useful buffer range is the pK_a ± 0.5–1 pH unit.
The buffering capacity decreases beyond this range.

COMMON NAME	pK_a AT 25°C	MOLECULAR WEIGHT	CHEMICAL FORMULA	CHEMICAL NAME
Phosphate	2.12	98.00	$H_3PO_4^-$	–
Acetate	4.76	60.00	CH_3CO_2H or $C_2H_4O_2$	–
MES	6.15	195.20	$C_6H_{13}NO_4S$	2-(*N*-morpholino)ethanesulfonic acid
PIPES	6.76	302.40	$C_8H_{18}N_2O_6S_2$	piperazine-N,N´-bis(2-ethanesulfonic acid)
Imidazole	6.95	68.08	$C_3H_4N_2$	1,3-Diaza-2,4-cyclopentadiene
MOPS	7.20	209.30	$C_7H_{15}NO_4S$	3-(*N*-morpholino)propanesulfonic acid
Phosphate	7.21	97.00	$H_2PO_4^-$	–
TES	7.40	229.20	$C_6H_{15}NO_6S$	N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid
HEPES	7.48	238.30	$C_8H_{18}N_2O_4S$	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
Tricine	8.05	179.20	$C_6H_{13}NO_5$	N-(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine
Tris	8.06	121.14	$C_4H_{11}NO_3$	Tris(hydroxymethyl)methylamine
Bicine	8.35	163.20	$C_6H_{13}NO_4$	N,N-bis(2-hydroxyethyl)glycine
TAPS	8.43	243.30	$C_7H_{17}NO_6S$	N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid
Phosphate	12.67	96.00	HPO_4^{2-}	–

NEB expanded its manufacturing footprint by opening a facility in Rowley, MA for production of GMP-grade materials. Pictured here are several of the team members.

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Environmental stewardship is one of the founding principles of NEB. We feel it is important to protect and preserve our natural resources, including biodiversity. In addition to the content found in this catalog, we invite you to take a look at other NEB resources that address this topic.

Visit **www.neb.com/EnvironmentalPhilosophy** and download our Social and Environmental Responsibility Brochure.

Visit **www.neb.com/NEBTV** view our NEB TV episodes that discuss the conservation of biodiversity (Episode 25) and green laboratory practices (Episode 22).

Visit **www.neb.com/NEBpodcast** and listen to our podcast featuring a group of sustainable science experts that participated in a panel discussion during our Go Green Symposium (Episode 12).

Visit **LabConscious.com**, a community dedicated to promoting green lab initiatives to reduce waste, use green chemistry, conserve water and save energy. Join the discussion!

For 45 years, the New England Biolabs catalog has been a resource for scientists around the world. This catalog features a collection of mini-reviews that discuss conservation of biodiversity. As part of this effort, NEB will offer support to the organization below that protects biodiversity.

ClimatePartner

Reforestation and preservation activities:

Safe Habitat for Chimpanzees – Project 1169

Kibale National Park is one of the last remaining tropical forest blocks in Uganda. It harbours the greatest variety of primates found anywhere in East Africa. It is home to almost 1,500 chimpanzees, Uganda's largest population of this endangered species. The variety of further species, like forest elephants, wild cats, birds and plants represent the intact biodiversity.

Through reforestation and preservation activities, the project helps protect biodiversity and mitigate climate change on a total area of 10,000 hectares.

To learn more please visit: **https://fpm.climatepartner.com/project/1169/en**

Environmental Philosophy & the NEB Catalog

The NEB Catalog & Technical Reference is printed with sustainability in mind.

- The catalog is printed on Forest Stewardship Council (FSC) Certified, recycled paper.
- The printing facility is also FSC Certified.
- The facility uses environmental-friendly inks and coatings

As with previous catalogs, we have achieved "ClimatePartner" certification by working with the ClimatePartner Company, a world-leading provider of carbon reduction solutions. This means that the unavoidable CO_2 generated by the printing and distribution of this catalog have been reduced to net zero through verified carbon offset projects. Offsets will be used to support Safe Habitat for Chimpanzees.

More information on carbon neutral production as well as details about the Biomass project Nr 1067 supported by this NEB catalog can be found at **www.climatepartner.com**.

Using 30% post-consumer fiber instead of virgin fiber for this catalog resulted in the following:

- 760 trees saved
- 3,000 lbs of solid waste not generated
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- 430,000 pounds of greenhouse gas emissions avoided
- 340,000,000 British Thermal Units (BTUs) of energy not consumed

Note: Statistics and calculations are based on the Environmental Paper Network Paper Calculator v3.2 and US-print production.

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videos, tutorials and immersive experiences by scanning the page using the AR app.