

# NEB expressions

a scientific update

## in this issue

**2-4 Feature Article:** The effect of nucleic acid modifications on digestion by DNA exonucleases.

**5-7 Focus:** Useful endo- and exonucleases and more.

**8-10 10 years of NEBNext Innovation:**  
Celebrate with us / New Products:  
– Globin & RNA Depletion Kits  
– NEBNext EM-seq: The new alternative to bisulfite sequencing!

**11 Conservation of Biodiversity:**  
It's not easy being a right whale

**12 Special prices:**  
– Luna Universal qPCR reagents

**13-15 Free sample:**  
– New proteases & protein markers  
– Introducing Monarch gDNA purification kits

**16 Get free NEB Starter Pack!**  
– Start your career off on the right path

## The effect of nucleic acid modifications on digestion by DNA exonucleases

by Greg Lobman, Ph.D., New England Biolabs, Inc.

New England Biolabs offers a wide variety of exonucleases with a range of nucleotide structure specificity. Exonucleases can be active on ssDNA and/or dsDNA, initiate from the 5' end and/or the 3' end of polynucleotides, and can also act on RNA. Exonucleases have many applications in molecular biology, including removal of PCR primers, cleanup of plasmid DNA and production of ssDNA from dsDNA. In this article, we explore the activity of commercially available exonucleases on oligonucleotides that have chemical modifications added during phosphoramidite synthesis, including phosphorothioate diester bonds, 2'-modified riboses, modified bases, and 5' and 3' end modifications. We discuss how modifications can be used to selectively protect some polynucleotides from digestion *in vitro*, and which modifications will be cleaved like natural DNA. This information can be helpful for designing primers that are stable to exonucleases, protecting specific strands of DNA, and preparing oligonucleotides with modifications that will be resistant to rapid cleavage by common exonuclease activities.

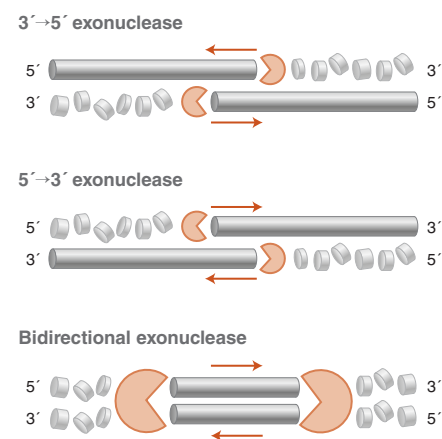
The ability of nucleases to hydrolyze phosphodiester bonds in nucleic acids is among the earliest nucleic acid enzyme activities to be characterized (1-6). Endonucleases cleave internal phosphodiester bonds, while exonucleases, the focus of this article, must begin at the 5' or 3' end of a nucleic acid strand and cleave the bonds sequentially (Figure 1). Exonucleases may be DNA or RNA specific, and can act on single-stranded or double-stranded nucleic acids, or both. Double-strand specific exonucleases may initiate at blunt ends, nicks, or short single-stranded 5' or 3' overhangs, though most exonucleases are active on a subset of these structures. For a summary of the substrate specificity of exonucleases available from NEB, view our newly-updated selection chart, Properties of Exonucleases and Non-specific Endonucleases, at [go.neb.com/ExosEndos](http://go.neb.com/ExosEndos).

A variety of DNA exonucleases have been characterized from many different organisms; *in vivo*, these enzymes play critical roles in polynucleotide repair, recycling, error correction, and protection from exogenous DNA (6-8). *In vitro*, exonucleases are used in many applications where it is desirable to remove certain nucleic acids. For example, Exonuclease V (RecBCD) (Exo V, NEB #M0345) is often used to remove contaminating linear ssDNA and dsDNA from plasmid preparations (4,9); T7 Exonuclease (T7 Exo, NEB #M0263) can be used to generate 3' overhangs in DNA (4, 10, 11); Exonuclease I (Exo I, NEB #M0293), Thermolabile Exonuclease I (NEB #M0568) or Exonuclease VII (Exo VII, NEB #M0379) can be used to eliminate ssDNA PCR primers, leaving double-stranded products undigested (12, 13), and Lambda Exonuclease (Lambda Exo, NEB #M0262) can be used to convert dsDNA to ssDNA for a variety of applications (14-16). More information on common applications of exonucleases available from NEB can be found in our selection chart, Common Applications of Exonucleases and Non-specific Endonucleases, at [go.neb.com/ExosEndos](http://go.neb.com/ExosEndos), (also see page 8).

What about cases where you only want to degrade some of the ssDNA in a reaction? Or, when you want to make ssDNA from a dsDNA substrate, but which strand is degraded matters greatly? What about cases where the ends of your nucleic acids are modified—will exonucleases still digest the substrate, or cleave the modification? Several methods depend on selective protection of polynucleotides, such as protection of primers from degradation by polymerase exonuclease domains (17), selective protection of one strand of a DNA duplex for the production of ssDNA (14-16), and the protection of polynucleotides from degradation by serum nucleases, as in the case of RNA interference drugs (18, 19). In each of these cases, it is critical to understand the influence of modifications on exonuclease activity—which modifications inhibit nucleotide cleavage and which do not.

Recently, researchers at NEB have worked to characterize the interaction between exonucleases and modified polynucleotides, as part of a broader effort to gain deeper insight into the sequence and structural determinants of nuclease activity and specificity. In an effort to catalog the modifications that inhibit exonuclease digestion, we treated polynucleotides containing a range of modifications (including non-standard bases, sugars and backbone chemistries) with exonucleases under the recommended *in vitro* reaction conditions. This article will summarize data from the literature, as well as the key results from NEB's work related to understanding the activity of exonucleases on chemically modified polynucleotides. We will focus on the most widely used—and most successful—method for blocking nuclease activity, the phosphorothioate bond (20-23), but will also discuss the use of other modifications to inhibit nuclease activity, as well as which modifications have little to no effect on exonuclease digestion.

Figure 1:  
Examples of exonuclease directionality

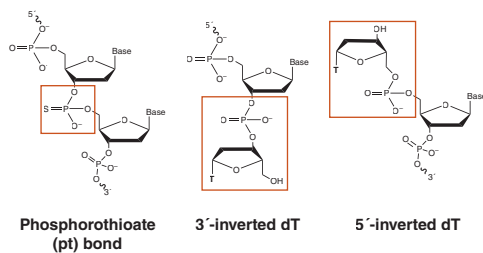


Pictured are double stranded exonucleases with a 3' to 5' polarity (top), a 5' to 3' polarity (middle), and a bidirectional nuclease (bottom).

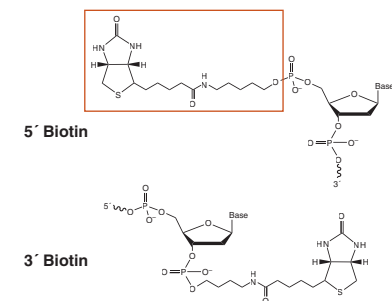
### Phosphorothioate linkages

A phosphorothioate (pt) bond is a phosphodiester linkage where one of the two non-bridging oxygens has been replaced by a sulfur (Figure 2). This modification has been used for decades to inhibit nuclease phosphodiesterase and phosphoryl transferase activities, as well as for gaining mechanistic insights into these enzymes (20, 23). Chemically, the substitution of oxygen with sulfur does not dramatically change the reactivity of the bond, and pt-containing polynucleotides can still function in many enzymatic reactions. In a typical phosphodiester bond, the two non-bridging oxygens are chemically equivalent. When one of these oxygens is replaced by sulfur, however, the phosphorus is now connected to four distinct groups, rendering it a chiral center with two possible configurations referred to as “S<sub>p</sub>” and “R<sub>p</sub>” (Figure 3). It is this key feature that confers resistance for the majority of

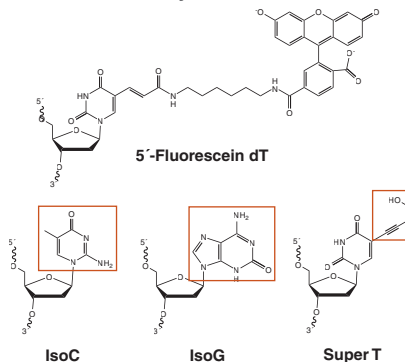
### Modified phosphodiester (pt bonds) block exonuclease activity



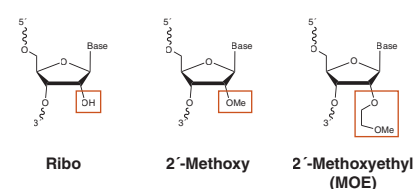
### 5' and 3' end modification does not block exonuclease activity



### Modified bases do not inhibit exonuclease activity



### Sugar modifications inhibit most exonuclease activity



nuclease enzymes studied; one configuration will react at rates similar to a phosphodiester, while the other is significantly inhibitory or completely unreactive. Isomer reactivity varies from enzyme to enzyme, and different pt isomers can inhibit enzymes that catalyze the same reaction (e.g., phosphoryl transfer). For example, DNA Polymerase I (DNA Pol I, NEB #M0209) can incorporate deoxynucleotide triphosphates with a pt ester at the  $\alpha$  phosphate (dNTP $\alpha$ S), allowing formation of pt-bonded polynucleotides. However, it can only react with  $S_p$  configured dNTP $\alpha$ S molecules, and does so with inversion of the stereocenter to form exclusively  $R_p$ -configured pt bonds in the product. Conversely, the 3'  $\rightarrow$  5' exo activity of this polymerase cleaves  $R_p$ , but not  $S_p$  configured bonds (20). Alternatively, the 3'  $\rightarrow$  5' exo activity of *E. coli* Exonuclease III (Exo III, NEB #M0206) cleaves  $S_p$ , but not  $R_p$  configured pt bonds (24). Therefore, DNA created from the incorporation of dNTP $\alpha$ S by DNA Pol I is highly resistant to exonuclease cleavage by Exo III (25).

Phosphorothioates can block many, but not all, exonucleases. To block exonuclease cleavage, the pt bonds must be placed at the end(s) where the enzyme initiates, e.g., the 5' end for Lambda Exo and the 3' end for Exo III. It is important to note that a single pt bond is insufficient to fully protect an oligonucleotide from exonuclease digestion. When the pt bond is installed via an oxidation step during phosphoramidite synthesis, a nearly equal amount of each isomer ( $S_p$  and  $R_p$ ) is formed at each pt linkage (20). Since most enzymes can cleave one of these isomers, a single chemically

installed pt will protect only half the molecules from digestion by a given exonuclease. Thus, it is typically recommended that 3–6 pt bonds be used to block exonuclease digestion, to prevent this read-through. One might expect that because each bond is a 50:50 mixture of isomers, when presented with 5 consecutive isomers, a given enzyme could cleave the first bond on half the molecules, then half of the molecules that had the first bond hydrolyzed would have the second hydrolyzed, and so on, such that there would be a range of partially degraded products. In practice, it has been reported (and confirmed by recent results at NEB) that five consecutive pt bonds completely block all exonuclease activity at all pt bond positions (16). The exact reasons for this are not currently known, but it is likely that exonucleases engage multiple bases at once, and the net effect of the isomeric mixture somehow prevents the active site from properly organizing around bonds that are the normally cleavable pt isomer.

There are several commonly used exonucleases that are not blocked even by 5 consecutive pt bonds; for example, Exo V, Exo VII and T5 Exonuclease (T5 Exo, NEB #M0363) all can cleave, leaving short oligos instead of cutting at every bond in a series, and thus can digest DNA by skipping over termini blocked by multiple pt bonds and cleaving at the first phosphodiester (5, 26). Importantly, any enzyme with endonuclease activity, like DNase I, will simply ignore the ends and degrade the polynucleotides from the inside out (unless every phosphodiester bond is replaced by a phosphorothioate). Keeping these important exceptions in

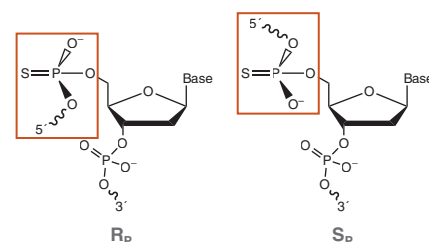
mind, phosphorothioate bonds remain the most generally applicable (and relatively inexpensive) way to protect oligonucleotides from digestion by exonucleases. For a complete list of DNA exonucleases and their interaction with pt bonds, view our selection chart, Activity of Exonucleases and Non-Specific Endonucleases, at [go.neb.com/ExosEndos](http://go.neb.com/ExosEndos).

## 2'-modified nucleosides

Generally, DNA exonucleases do not digest RNA portions of oligonucleotides, though RNA is itself susceptible to RNases and nonspecific hydrolysis. We have further found that hybridizing RNA to DNA strands does not block the activity of dsDNA exonucleases on the DNA strand. Hybridization of ssDNA to RNA will block the activity of ssDNA exonucleases as effectively as hybridization to dsDNA. Additionally, certain 2'-O-modified riboses, are both stable to spontaneous hydrolysis and offer strong resistance to exonuclease activity (27). 2'-O-methyl and 2'-O-methoxyethyl (MOE) nucleosides, which contain bulky substituents off the sugar ring, have been shown to grant strong resistance to nucleases and additionally increase the strength of annealing to complementary DNA and RNA. These features have found utility in antisense nuclease strategies, to make oligonucleotides that are both resistant to degradation and able to bind tightly to target RNAs.

These sugar modifications also work *in vitro* to block exonuclease activity quite strongly. Our studies have found that, while a single terminal MOE nucleoside only weakly inhibits exonuclease activity, three successive MOE modifications provide enhanced resistance to many exonucleases, including Exo I, Exo III, Lambda Exo, RecJ (NEB #M0264) and polymerase exonucleases, such as that of DNA Polymerase I, Large (Klenow) Fragment (NEB #M0210). Similar to pt bonds, several exonucleases can digest through these regions, notably T5 Exo, T7 Exo, Exo V, Exo VII and Exo VIII. Overall, exonuclease inhibition by MOE is quite strong, but pt bonds are more effective and are typically cheaper to prepare and incorporate. However, if for some reason the pt chemistry is not desired, 2'-O-modified ribose moieties are a viable alternative.

Figure 3: Chirality of phosphorothioate bonds



## Other 5'/3' end modifications

Several other modifications, such as the inverted deoxythymidine bases and dideoxynucleotides (Figure 2) have been reported to suppress serum nuclease activity when appended to the end of synthetic oligonucleotides (27). Many other modifications may be attached through “linkers” at either the 5' or 3' end, including fluorescent tags, biotin or other affinity labels, or reactive groups for attachment to beads or surfaces. These linkers are typically connected to the 5' or 3' end via a phosphodiester, but what is the interaction of these modified ends with exonucleases?

We have surveyed a range of these modifications in typical *in vitro* exonuclease assays. In general, while many provide modest inhibition as compared to a 5'-phosphate, all exonucleases tested could cleave all modifications connected through phosphodiester bonds. Interestingly, this poor inhibition held true for the inverted dT modifications, which have been reported to grant extra stability versus degradation by serum exonucleases for aptamers and other modified oligonucleotides. In our hands, 3'-inverted dT blocked only the relatively weak 3'→5' exonuclease activity of DNA Polymerase I, Large (Klenow) Fragment (NEB #M0210) and Exonuclease T (Exo T, NEB #M0265), but did not block more active exonucleases such as in T7 DNA Polymerase (NEB #M0274), Exo I or Exo III. Similarly, 5'-inverted dT partially inhibited only Lambda Exo activity, which is known to require a 5'-phosphate for efficient initiation. Other 5'→3' exonucleases were not significantly inhibited by this modification, showing complete digestion after a one-hour incubation under the recommended usage conditions.

Figure 4: Designing oligonucleotides with nuclease-resistant modifications

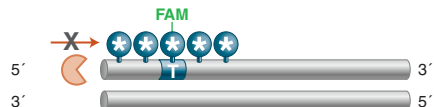
### A. End fluorescein (FAM)-labeled DNA



### B. pt bonds



### C. Internal FAMdT surrounded by pt bonds



(A) End fluorescein (FAM) labeled-DNA is rapidly degraded by exonucleases. (B) pt bonds between nucleotides prevent the DNA strand from being degraded, but the end label can still be cleaved. (C) An internal FAMdT surrounded by pt bonds will prevent the exonuclease from removing the label.

We do not recommend 5'/3' end modification as a good strategy for producing nucleotides resistant to exonuclease degradation *in vitro*. Researchers should be aware that these modifications will be cleaved by the majority of exonucleases, potentially leading to the loss of fluorescent labels and affinity tags. If a modification stable to exonuclease activity is needed, a better strategy is to use internal labels connected to the 5-methyl position of dT (e.g., Fluorescein dT, Figure 2). If these modified dT bases are used near the end of an oligo, they can be protected with surrounding pt bonds (Figure 4). The linkage to the base is not susceptible to enzymatic cleavage, and the pt bonds will protect the backbone from digestion, as described above.

## Base modifications

None of the exonucleases available from NEB were significantly inhibited by modified bases under the conditions we tested. Modifications tested included 5-methyl-substituted dT (e.g., Fluorescein dT), deoxyuridine, the Tm-enhancing “super T,” and the non-natural base pair isoG:isoC (Figure 2) (28). All modified substrates were digested completely by all the exonucleases tested. Some modifications showed weak blockage, pausing at the modification site before completely degrading the substrate. For several exonucleases tested, modified dT bases with large modifications off the 5-methyl position (Figure 2) showed a buildup of partially-digested intermediates, apparently stalling just before the modification; in no case did this resistance approach the inhibition seen for 2' MOE sugars or pt linkages.

## Conclusion

We have evaluated a variety of chemical modifications for their inhibition of exonuclease activity at the 5' and 3' ends of oligonucleotides. Broadly, the phosphorothioate modification, one of the more well-known used modifications to block nuclease cleavage, remains the most effective choice to protect oligonucleotides from degradation. However, one must be careful to use multiple pt bonds, place them at the correct end of the oligonucleotide to match the polarity of the exonucleases used, and be aware that several exonucleases can read-through or bypass terminal pt bonds; your choice of nucleases is as important as the modifications used. Aside from pt bonds, MOE nucleotides are the next best choice for providing nuclease resistance *in vitro*, with similar caveats to pt bonds. The vast majority of end modifications, including affinity tags and fluorophores, as well as internal non-standard bases, provide little, if any, nuclease resistance, and will be cleaved completely *in vitro*.

## References

- Lehman, I. R., and Nussbaum, A. L. (1964) *J. Biol. Chem.* 239, 2628-2636.
- Nichols, N. M. (2011) *Curr. Protoc. Mol. Biol.* Chapter 3, Unit3.12.
- Richardson, C. C., Lehman, I. R., and Kornberg, A. (1964) *J. Biol. Chem.* 239, 251-258.
- McReynolds, L. A., and Nichols, N. M. (2011) *Curr. Protoc. Mol. Biol.* Chapter 3, Unit 3.11.
- Lovett, S. T. (2011) *EcoSal Plus* 4.
- Yang, W. (2011) *Q. Rev. Biophys.* 44, 1-93.
- Bebenek, A., and Ziuzia-Graczyk, I. (2018) *Curr. Genet.* 64, 985-996.
- Tsutakawa, S. E., Lafrance-Vanasse, J., and Tainer, J. A. (2014) *DNA Repair (Amst)*. 19, 95-107.
- Karu, A. E., MacKay, V., Goldmark, P. J., and Linn, S. (1973) *J. Biol. Chem.* 248, 4874-4884.
- Kerr, C., and Sadowski, P. D. (1972) *J. Biol. Chem.* 247, 305-310.
- Straus, N. A., and Zagursky, R. J. (1991) *Biotechniques*. 10, 376-384.
- Li, H. H., Cui, X. F., and Arnheim, N. (1991) *Nucleic Acids Res.* 19, 3139-3141.
- Enzymatic PCR Cleanup using Exonuclease I and Shrimp Alkaline Phosphatase. New England Biolabs, Ipswich, MA.
- Civit, L., Fragoso, A., and O'Sullivan, C. K. (2012) *Anal. Biochem.* 431, 132-138.
- Murgha, Y. E., Rouillard, J. M., and Gulari, E. (2014) *PLoS One* 9, e94752.
- Nikiforov, T. T., Rendle, R. B., Kotewicz, M. L., and Rogers, Y. H. (1994) *PCR Methods Appl.* 3, 285-291.
- Skerra, A. (1992) *Nucleic Acids Res.* 20, 3551-3554.
- Evers, M. M., Toonen, L. J., and van Roon-Mom, W. M. (2015) *Adv. Drug Deliv. Rev.* 87, 90-103.
- Lundin, K. E., Gissberg, O., and Smith, C. I. (2015) *Hum. Gene Ther.* 26, 475-485.
- Eckstein, F. (1985) *Annu. Rev. Biochem.* 54, 367-402.
- Spitzer, S., and Eckstein, F. (1988) *Nucleic Acids Res.* 16, 11691-11704.
- Eckstein, F., and Gish, G. (1989) *Trends Biochem Sci* 14, 97-100.
- Eckstein, F. (2014) *Nucleic Acid Ther.* 24, 374-387.
- Putney, S. D., Benkovic, S. J., and Schimmel, P. R. (1981) *Proc. Natl. Acad. Sci. U S A*, 78, 7350-7354.
- Yang, Z., Sismour, A. M., and Benner, S. A. (2007) *Nucleic Acids Res.* 35, 3118-3127.
- Sayers, J. R., and Eckstein, F. (1990) *J. Biol. Chem.* 265, 18311-18317.
- Kratschmer, C., and Levy, M. (2017) *Nucleic Acid Ther.* 27, 335-344.
- Piccirilli, J. A., Krauch, T., Moroney, S. E., and Benner, S. A. (1990) *Nature*, 343, 33-37.

# SOMETHING TO CHEW ON

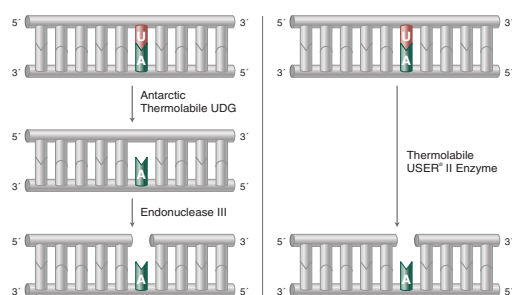


Exonucleases and endonucleases are used in many of today's molecular biology workflows and applications. Did you know that NEB offers the largest supply of these important tools, and has a team of experts studying the function and optimization of these enzymes? We also offer several helpful tools to help you find the best enzyme to facilitate your work, including selection charts, recommended applications, usage guidelines and more.

## Featured DNA Repair Enzymes and Structure-specific Endonucleases

### Thermolabile USER® II Enzyme

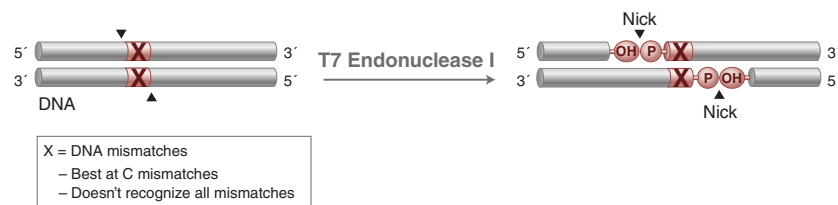
Thermolabile USER (Uracil-Specific Excision Reagent) II Enzyme generates a single nucleotide gap at the location of a uracil residue. It can be 100% inactivated at temperatures >65°C, streamlining workflows and enabling DNA to be used directly in downstream applications.



*Thermolabile USER (Uracil-Specific Excision Reagent) II is a unique blend of two enzymes: Antarctic Thermolabile Uracil DNA glycosylase (UDG) and Endonuclease III. Antarctic UDG catalyzes the excision of a uracil base, forming an abasic (apyrimidinic) site while leaving the phosphodiester backbone intact. Endonuclease III hydrolyses the phosphodiester backbone at the 3' and 5' sides of the abasic site. Thermolabile USER II Enzyme can also be completely heat inactivated after 10 minutes at 65°C.*

### T7 Endonuclease I

T7 Endonuclease I recognizes and cleaves non-perfectly matched DNA, cruciform DNA structures, Holliday structures or junctions, heteroduplex DNA and more slowly, nicked double-stranded DNA. The cleavage site is at the first, second or third phosphodiester bond that is 5' to the mismatch. The protein is the product of T7 gene 3.



X = DNA mismatches  
 - Best at C mismatches  
 - Doesn't recognize all mismatches

### Thermostable FEN1

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.



## Visit [go.neb.com/EndosExos](http://go.neb.com/EndosExos) to:

- Access the full list of exonucleases and endonucleases available from NEB
- View extensive selection charts and activity information
- Request copies of our new exonuclease poster and endonuclease magnet
- View our webinar: Exonucleases and endonucleases as molecular tools

### Applications include:

- Directional RNA-seq
- NEBNext® adaptor cleavage
- USER cloning

### Ordering information:

PRODUCT	NEB #	SIZE
Thermolabile USER II Enzyme	M5508S/L	50/250 units

### Application:

- Determining genome targeting efficiency (access protocol via the protocol tab at [www.neb.com/M0302](http://www.neb.com/M0302))

### Ordering information:

PRODUCT	NEB #	SIZE
T7 Endonuclease I	M0302S/L	250/1,250 units

### Applications:

- Base excision repair

### Ordering information:

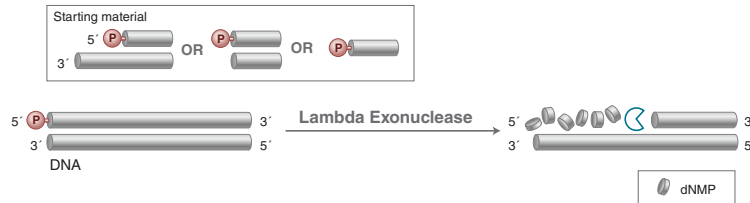
PRODUCT	NEB #	SIZE
Thermostable FEN1	M0645S	1,600 units



## Featured Exonucleases

### Lambda Exonuclease

Lambda Exonuclease catalyzes the removal of nucleotides from linear or nicked double-stranded DNA in the 5' to 3' direction. This enzyme is highly processive. The preferred substrate is 5'-phosphorylated double-stranded DNA, although non-phosphorylated substrates are degraded at a greatly reduced rate.



#### Application:

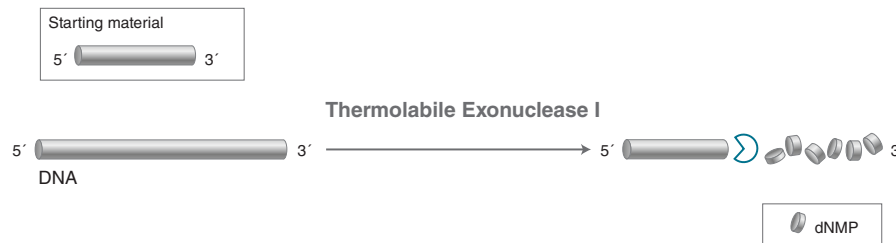
- Conversion of linear double-stranded DNA to single-stranded DNA via preferred activity on 5'-phosphorylated ends

#### Ordering information:

PRODUCT	NEB #	SIZE
Lambda Exonuclease	M0262S/L	1,000/5,000 units

### Thermolabile Exonuclease I

Thermolabile Exonuclease I catalyzes the removal of nucleotides from linear single-stranded DNA in the 3' to 5' direction. Unlike Exonuclease I (NEB #M0293), Thermolabile Exonuclease I can be heat inactivated at 80°C in one minute.



#### Applications:

- 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 linear single-stranded DNA, leaving behind double-stranded DNA

#### Ordering information:

PRODUCT	NEB #	SIZE
Thermolabile Exonuclease I	M0568S/L	3,000/15,000 units

### Exonuclease V (RecBCD)

Exonuclease V (RecBCD) is a DNA specific exonuclease that also acts as an endonuclease on single-stranded DNA. It initiates at both 5' and 3' termini of linear double-stranded DNA and cleaves linear double-stranded DNA in both the 3' to 5' and 5' to 3' directions. It requires ATP in the reaction.



#### Applications:

- Degradation of contaminating linear DNA in plasmid samples
- Removal of residual gDNA after purification of low copy plasmids

#### Ordering information:

PRODUCT	NEB #	SIZE
Exonuclease V (RecBCD)	M0345S/L	1,000/5,000 units

## Featured Resources

Request your copy of our endonuclease card or exonuclease poster at

[www.neb.com/ExosEndosRequest](http://www.neb.com/ExosEndosRequest)

or contact your local distributor!

Find the NEB Exonuclease Best Suited for your Application

Starting material: DOUBLE-STRANDED SINGLE-STRANDED

What's your intended ending material(s)?\*\*

Recommended enzyme(s):

NEB ENGLAND BIO LABS

WIPFEEB INC. WIPFEEB INC. WIPFEEB INC.



## Common Applications for Exonucleases and Endonucleases

Not sure which exonuclease or endonuclease to choose?  
Find the right enzyme for your application using the table below.

Application	Recommended Enzyme(s)	NEB #
Removal of 3' overhangs	Quick Blunting Kit	E1201S/L
5' overhang fill-in treatment	Quick Blunting Kit	E1201S/L
Removal of ss primers for nested PCR reactions	Thermolabile Exonuclease I	M0568S/L
Removal of primers post PCR prior to DNA sequencing or SNP detection	Exonuclease I Thermolabile Exonuclease I (1) Exonuclease VII (2)	M0293S/L M0568S/L M0379S/L
Mapping positions of introns in genomic DNA	Exonuclease VII	M0379S/L
Removal of primers with or without 3' or 5' terminal phosphorothioate bonds	Exonuclease VII	M0379S/L
Generating ssDNA from linear dsDNA:		
If 5' → 3' polarity required	Lambda Exonuclease (3)	M0262S/L
If 3' → 5' polarity required	Exonuclease III (4)	M0206S/L
Preparation of nested deletions in double-stranded DNA	Exonuclease III ( <i>E. coli</i> ) plus Exonuclease VII	M0206S/L M0379S/L
Site-directed mutagenesis	Exonuclease III ( <i>E. coli</i> ) (5) T7 Exonuclease (6)	M0206S/L M0263S/L
Nick-site extension	T7 Exonuclease	M0263S/L
Degradation of denatured DNA from alkaline-based plasmid purification methods for improving DNA cloning	T5 Exonuclease	M0363S/L
Removal of chromosomal/linear DNA in plasmid preparations	T5 Exonuclease (7) Exonuclease V (RecBCD) (8)	M0363S/L M0345S/L
Removal of unligated products (linear dsDNA) from ligated circular double-stranded DNA	T5 Exonuclease (9) Exonuclease V (RecBCD) (10)	M0363S/L M0345S/L
Removal of residual gDNA after purification of low copy plasmid	Exonuclease V (RecBCD)	M0345S/L
Removal of contaminating DNA from RNA samples	DNase I	M0303S/L
Conversion of single-stranded DNA or RNA to 5'-mononucleotides	Nuclease P1	M0660S
Analysis of base composition, potential damage and modification of nucleic acids	Nuclease P1	M0660S
Progressive shortening of both ends of double-stranded DNA	Nuclease BAL-31	M0213S
Preparation of double-stranded DNA fragments with 5'-OH and 3'-phosphate	Micrococcal Nuclease	M0247S
Degradation of nucleic acids (both DNA and RNA) in crude cell-free extracts	Micrococcal Nuclease	M0247S
Preparation of rabbit reticulocyte	Micrococcal Nuclease	M0247S
Chromatin Immunoprecipitation (ChIP) analysis	Micrococcal Nuclease	M0247S

### Notes:

1. Rapid heat inactivation versus Exonuclease I
2. For 3' chemically modified primers
3. Strand targeted for removal requires one 5' end with phosphate
4. Strand targeted for removal requires a 5' overhang, a blunt end, or a 3' overhang (with less than 4 bases)
5. Removes nicked-strand DNA from 3' to 5'
6. Removes nicked-strand DNA from 5' to 3'
7. Degrades linear ss + dsDNA, nicked DNA
8. Degrades linear ss + dsDNA: preferred as Exo V will save nicked plasmids resulting in higher yields especially for low-copy number plasmid prep
9. Only the unnicked form of ligated circular double-stranded DNA remains
10. Both nicked and unnicked form of ligated circular double-stranded DNA remains

FREE  
SAMPLE\*



## 10 YEARS OF NEBNEXT® – INNOVATION

# It's time to celebrate!

For 10 years, NEB has helped advance next generation sequencing (NGS) by streamlining sample prep workflows, minimizing inputs, and improving library yield and quality.

As sequencing technologies improve and applications expand, the need for compatibility with ever-decreasing input amounts and sub-optimal sample quality grows. Scientists must balance reliability and performance with faster turnaround, higher throughput and automation compatibility. Our NEBNext portfolio addresses these challenges and includes solutions for DNA and RNA sample prep from a wide range of sample types.

- Reaching far beyond standard library prep, NEBNext reagents continue to innovate with:
- a novel enzymatic alternative to bisulfite sequencing
- solutions for FFPE DNA and enzymatic DNA fragmentation
- customized target enrichment with fast turnaround

NEBNext products continue to set the bar for quality and flexibility. All reagents are extensively QC'd at the individual component and kit levels, while product formats are designed for workflow customization. From individual kits to bulk and custom configurations, we've got you covered.

If you still aren't convinced, why not see for yourself? NEBNext reagents have been cited in over 5,000 peer-reviewed publications to date.

**As we celebrate 10 years of NEBNext, we would like to thank you for making NEBNext part of your workflows, and we are excited to continue to exceed your expectations for NGS sample prep innovation.**

You are:

- **New to NEBNext?**
- **Or an experienced user,** but want to test one of the new NEBNext workflow solutions?

Find the NEBNext product that is best suited for your application:

[NEBNextSelector.neb.com](http://NEBNextSelector.neb.com)



Get started with a **free sample\*** at [NEBNext.com](http://NEBNext.com) or contact your local distributor!

\* limited offer. Ask your local distributor for details.





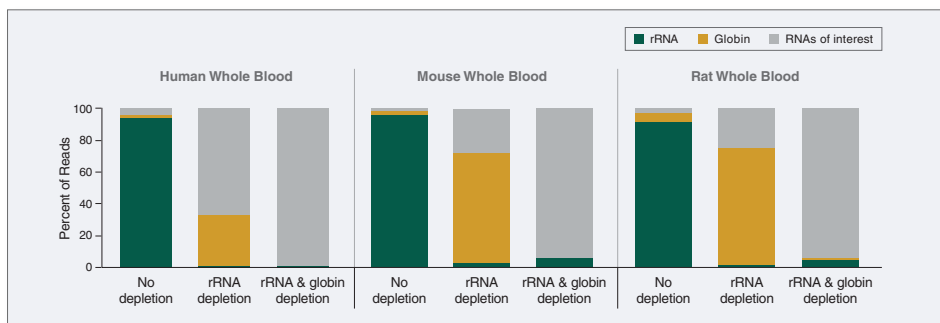
## NEBNext® Globin & rRNA Depletion Kits (Human/Mouse/Rat)

Abundant RNAs can conceal the biological significance of less abundant transcripts, and so their efficient and specific removal is desirable. In blood samples, the great majority of RNA is comprised of rRNA and globin mRNA. Utilizing the RNase H-based method, the NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) depletes specifically globin mRNA (HBA1/2, HBB, HBD, HBM, HBG1/2, HBE1, HBQ1 and HBZ), cytoplasmic rRNA (5S, 5.8S, 18S, 28S, ITS and ETS) and mitochondrial rRNA (12S, 16S).

The kit is effective with human, mouse and rat total RNA preparations, both intact and degraded.

When only mRNA (and not non-coding RNA) is of interest, the Globin & rRNA Depletion Kits can be used following poly(A) mRNA enrichment (e.g. using the NEBNext poly(A) mRNA Magnetic Isolation Module NEB #E7490).

### Depletion of globin mRNA and ribosomal RNA enriches for RNAs of interest across species



### Advantages

- Superior depletion of abundant RNAs, with retention of RNAs of interest
- Suitable for low-quality (e.g., FFPE) and high-quality RNA
- Compatible with a broad range of input amounts: 10 ng–1 µg
- Fast workflow: 2 hours, with less than 10 minutes hands-on time
- Depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis applications
- Available with optional Agencourt® RNAClean® XP Beads for RNA Purification

Fig. left: RNA-seq libraries were prepared from untreated and depleted RNA using the NEBNext Ultra II RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). See all details at [www.neb.com/E7750](http://www.neb.com/E7750)

### Consistent depletion of globin mRNA and rRNA across species and across inputs

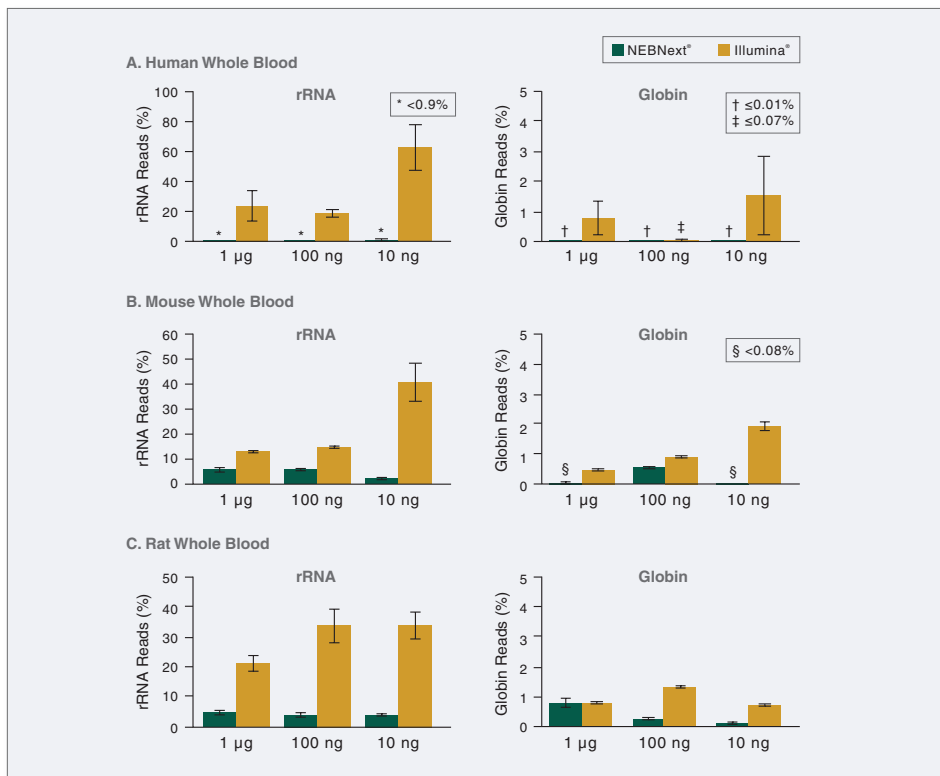
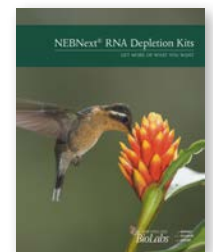


Fig. left: Human, mouse and rat whole blood total RNA (1 µg, 100 ng and 10 ng) was depleted of rRNA and globin mRNA using the NEBNext Globin & rRNA Depletion Kit or Globin-Zero® Gold rRNA Depletion Kit (Illumina). See all details at [www.neb.com/E7750](http://www.neb.com/E7750)

Request your free NEBNext RNA Depletion brochure from your local distributor!



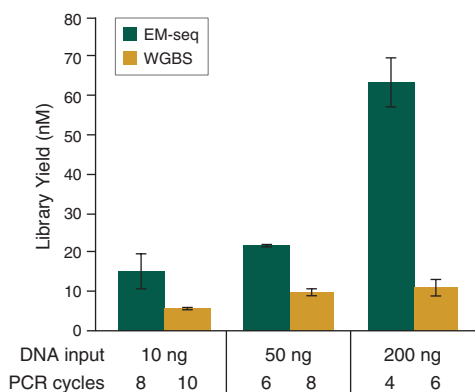
PRODUCTS	NEB #	SIZE
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S/L/X	6/24/96 rxns
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 rxns
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns
NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 rxns



## There's a new alternative to bisulfite sequencing – Introducing NEBNext® Enzymatic Methyl-seq (EM-seq™)

While whole genome bisulfite sequencing (WGBS) has been the gold standard for methylome analysis, it also damages DNA, resulting in fragmentation, loss and bias. In contrast, EM-seq enzymatic conversion minimizes damage to DNA and, in combination with the supplied NEBNext Ultra™ II Illumina® library preparation reagents, produces high quality libraries that enable superior detection of 5mC and 5hmC from fewer sequencing reads.

### EM-seq produces higher yields than WGBS using fewer PCR cycles



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. For all input amounts, EM-seq library yields were higher, and fewer PCR cycles were required, suggesting greater DNA loss in the WGBS protocol. Error bars indicate standard deviation.

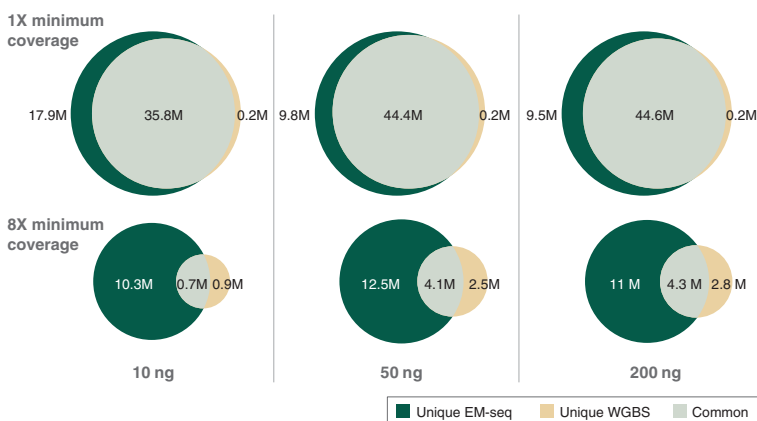


### Advantages

- Superior sensitivity of detection of 5mC and 5hmC
- Achieve greater mapping efficiency
- Generate more uniform GC coverage
- Detect more CpGs with fewer sequence reads
- Experience uniform dinucleotide distribution
- Obtain larger library insert sizes
- Conversion module also available separately

\*Visit [www.NEBNext.com](http://www.NEBNext.com) to learn more and request a sample, or contact your local distributor!

### EM-seq identifies more CpGs than WGBS, at lower sequencing coverage depth



Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads. The number of unique and common CpGs identified by EM-seq and WGBS at 1x and 8x minimum coverage for each input amount are shown. EM-seq covers at least 20% more CpGs than WGBS at 1x minimum coverage threshold. The difference in CpG coverage increases to two-fold at 8x minimum coverage threshold.



Request your free NEBNext EM-Seq brochure from your local distributor!

PRODUCTS	NEB #	SIZE
NEBNext Enzymatic Methyl-seq Kit	E7120S/L	24/96 rxns
NEBNext Enzymatic Methyl-seq Conversion Module	E7125S/L	24/96 rxns
NEBNext Q5U™ Master Mix	M0597S/L	50/250 rxns
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	E7140S/L	24/96 rxns

# It's Not Easy Being a Right Whale

Guest Article written by Emily Greenbalgh, New England Aquarium

## 411: The predicted number of right whales remaining in the North Atlantic population.

These behemoths of the sea were once called the “right whales to hunt”, because they swam close to shore, produced high yields of whale oil and baleen, and—thanks to their thick blubber—floated when killed. But now, rather than whalers, the population faces threats from humans in an ever-increasing urbanized ocean. Due primarily to human impacts, the population of these endangered whales has been in decline since 2010. Entanglement in fishing gear, changes in food distribution due to climate change, busy shipping lanes, and ocean noise are just some of the challenges facing the species.

It was humans who nearly destroyed the right whale population, and it is humans who are striving now to save it from extinction. In 1980, New England Aquarium scientists discovered a group of 25 North Atlantic right whales in Canada's Bay of Fundy. At the time, the species was thought to be almost extinct. In the nearly four decades since that startling discovery, dedicated researchers have been fighting to save this iconic species.

*“The species is resilient. We know they can rebound if we just stop killing them.”*

– Dr. Scott Kraus, Vice President of the New England Aquarium's Anderson Cabot Center for Ocean Life

For nearly 40 years, members of the Aquarium's right whale research team have been dedicated to ensuring these whales have the opportunity to survive. Fieldwork, including 39 years of uninterrupted surveys in the Bay of Fundy, has provided invaluable data about right whale behavior, habitat use, and human impacts on the population. The Anderson Cabot Center Right Whale Program also oversees

the North Atlantic Right Whale Catalog (<http://rwcatalog.neaq.org/Terms.aspx>), a tremendous collaborative effort of more than 300 individuals and organizations.

Right whales are identifiable by callosities, the natural patches on the top of their heads, as well as scars or markings on their bodies. Scientists can recognize individual whales from these marks in thousands of photographs, connecting important information about the population, such as location, mortality, health, and reproductive success. The Right Whale Catalog, which is linked with human impact studies, visual health assessments, and genetic and hormone analysis, is the cornerstone of right whale research.

Genetic samples from biopsies have also helped scientists estimate the original size of the right whale population before commercial hunting, and even shed light on how few calving-age females there were during the population's lowest point. About 80% of the right whale population has been sampled, and between the genetic database and the Right Whale Catalog, scientists know entire family histories for many of these whales.

This cornerstone has allowed dedicated scientists and researchers to build an array of methods to try to save this species. Since 1999, researchers from the Aquarium's Anderson Cabot Center have been collecting right whale fecal samples. By examining the levels of glucocorticoid hormones in these samples, Senior Scientist Rosalind Rolland, D.V.M. and her team were able to determine the stress levels of living whales for the first time. From 1999 to 2014, scientists examined samples from 125 different right whales – 113 healthy whales, six chronically entangled in fishing gear, one that was live-stranded, and five killed quickly by vessel strikes.

The robust samples allowed Rolland and her team to create a baseline of hormone levels in normal, healthy whales and compare those levels to animals under stress. The researchers found “sky-high hormone levels” in whales entangled in fishing gear.

“These levels showed stress from extreme physical trauma,” said Rolland. “It's an animal welfare issue.”

How many of the right whales are undergoing this trauma? A total of 83% of endangered North Atlantic right whales show signs of entanglement, and 59% have been entangled more than once. Entanglements now surpass ship strikes as the main threat to the right whale population.

According to Anderson Cabot Center scientists, only a third of severely entangled female whales survive, and those that do survive are less likely to have calves. When in good condition, a right whale can give birth every three years. But with all the threats they are facing now, the birth rate for the population has dropped to an average 10 years between births - births are simply not outpacing deaths.

*“Because they're a long-lived species, the right whales can weather short-term events. We have to give them the opportunity.”*

– Philip Hamilton, Research Scientist

Rolland and other scientists on the Aquarium's Marine Stress and Ocean Health team built on their pioneering fecal hormone research by studying stress hormone levels in whale blow, baleen, blubber and blood. The data collected from these samples include hormone levels, DNA, marine biotoxins and pathogens, to name just a few—and this helps paint a picture of the overall health of each animal.

Knowing how humans are affecting right whale populations is a key factor to protecting them in the future. “We just have to keep from killing them, both directly and indirectly,” said Rolland.

Scientists are working with partners across industry, scientific institutions, and the U.S. and Canadian governments to try to save the species from the brink of extinction. From supporting the implementation of ropeless fishing technology to speaking against offshore oil and gas development on our coasts, the right whale community is fighting hard to ensure these iconic whales not just survive, but thrive.



LEFT: “FDR” was photographed in the Bay of Fundy in 2016, entangled in a large amount of fishing gear. He was successfully disentangled by the Campobello Whale Rescue Team. Photographed by Jerry Conway, Campobello Whale Rescue Team. Permit: Collected under Permits issued by Canadian DFO under the Species at Risk Act.

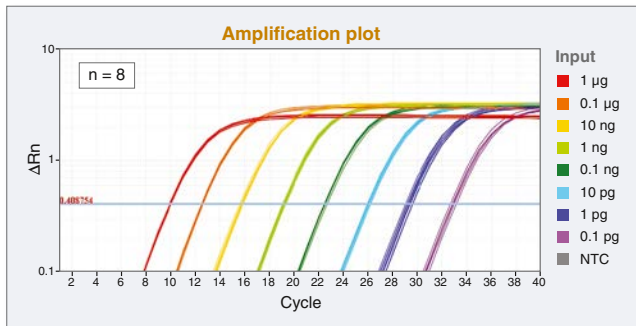
ABOVE: “Caterpillar” was struck by a vessel, which left large propeller cuts on her body. Caterpillar was photographed in the Bay of Fundy on August 5, 2007 by Amy Knowlton, ACCOL, New England Aquarium. Permit: Collected under Permits issued by Canadian DFO under the Species at Risk Act.



## LUNA Universal qPCR & RT-qPCR Reagents

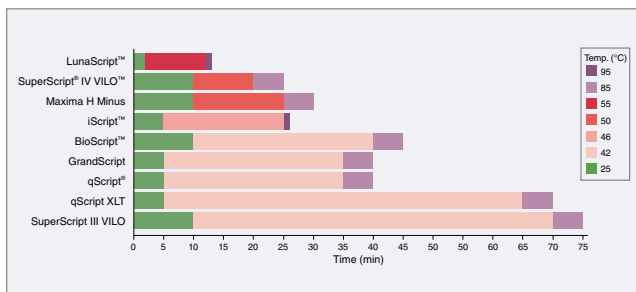
New England Biolabs offers a bright, new choice for your qPCR and one-step, or two-step RT-qPCR. Luna products have been optimized for robust performance on diverse sample sources and target types. Available for dye-based or probe-based detection, Luna qPCR master mix has been formulated with a unique 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 (NEB #M0372).

**SPECIAL PRICE\***  
until 31.01.2020



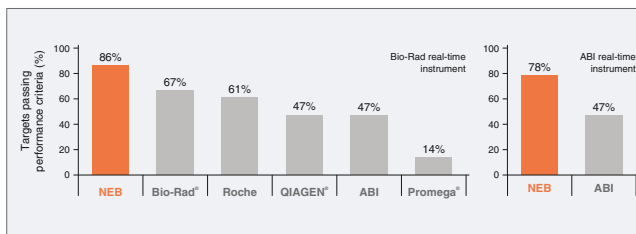
### Luna Universal One-Step RT-qPCR Kit offers exceptional sensitivity, reproducibility & RT-qPCR performance

RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit (Input: 1 μg – 0.1 pg Jurkat total RNA); NTC = non-template control



### The LunaScript RT SuperMix Kit offers the shortest available first-strand cDNA synthesis protocol

Comparison of recommended protocols for cDNA synthesis. The LunaScript RT SuperMix Kit requires the shortest reaction time and tolerates elevated temperatures.



### Evaluation of different dye-based qPCR reagents demonstrates the robustness and specificity of Luna

qPCR reagents from NEB and other manufacturers were tested across 16–18 qPCR targets varying in abundance, length and %GC according to manufacturer's specifications. Bar graph indicates % of targets that met MIQE criteria. Please see all details and explanations at [www.neb.com/M3003](http://www.neb.com/M3003).

## Advantages

- Best-in-Class performance utilizing unique (designer-) enzyme technology
- Excellent specificity, sensitivity, accuracy and reproducibility
- Validated reagents following the MIQE-guidelines for "publication-grade qPCR"
- Superior reliability: tested on a broad range of sample sources (%GC, abundance, length)
- Convenient master mix formats and user-friendly protocols simplify reaction setup
- Non-interfering, visible tracking dye helps to eliminate pipetting errors
- Compatible with all leading instrument platforms

\* [miqe.gene-quantification.info](http://miqe.gene-quantification.info)

© Clin Chem. 2009 Apr;55(4):611-22. doi: 10.1373/clinchem.2008.112797. Epub 2009 Feb 26.

It's that easy to find the best Luna product for your application:

		2 Select your detection method	
		Dye-based	Probe-based
1 Select your target	Genomic DNA or cDNA	Luna Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)
	RNA	One-Step: Luna Universal One-Step RT-qPCR Kit (NEB #E3005) Two-Step: LunaScript Supermix Kit (NEB #E3010) & Luna Universal qPCR Master Mix (NEB #M3003)	One-Step: Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006) Two-Step: LunaScript Supermix Kit (NEB #E3010) & Luna Universal Probe qPCR Master Mix (NEB #M3004)

## Special Price! Order now!

\*Please ask your local distributor for details and a free sample!



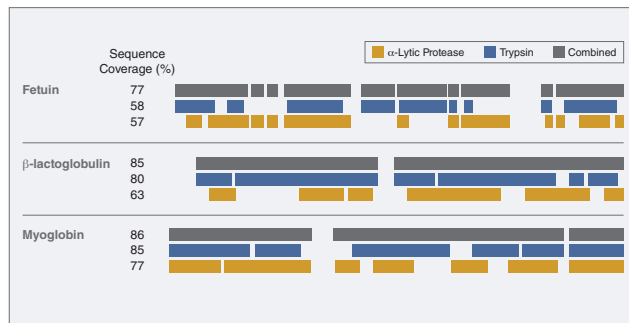
PRODUCTS	NEB #	SIZE
Luna Universal qPCR Master Mix	M3003S/L/X/E	200/500/1.000/2.500 rxns
Luna Universal Probe qPCR Master Mix	M3004S/L/X/E	200/500/1.000/2.500 rxns
Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/1.000/2.500 rxns
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/1.000/2.500 rxns
LunaScript RT SuperMix Kit	E3010S/L	25/100 rxns

Learn more on [LUNAqPCR.com](http://LUNAqPCR.com)

## New Proteases

### a-Lytic Protease

a-Lytic Protease (aLP) cleaves after Threonine (T), Alanine (A), Serine (S) and Valine (V) residues. Its specificity makes it an orthogonal and alternative protease to others commonly used in proteomics applications, including trypsin and chymotrypsin. Peptides generated by aLP are of similar average length to those of Trypsin. a-Lytic Protease can be used alone or in combination with other proteases to yield increased sequence coverage.



**a-Lytic Protease can be used alone or in combination with other proteases to yield increased sequence coverage**

*Comparison of sequence coverage for three protein standards after parallel digestion using Trypsin (blue) and a-Lytic Protease (gold). The combined data set (grey) results in overlapping peptides and increased sequence coverage.*

### Thermolabile Proteinase K

Thermolabile Proteinase K is an engineered, subtilisin-related serine protease that will hydrolyze a variety of peptide bonds. In contrast to native Proteinase K, this variation of the enzyme is inactivated when heated for 10 minutes at 55°C.

#### Applications include:

- Isolation of plasmid and genomic DNA and RNA
- Inactivation of RNases, DNases and enzymes in reactions
- Removal of enzymes from DNA to improve cloning efficiency
- PCR purification

PRODUCT	NEB #	SIZE
Thermolabile Proteinase K	P8111S	30 u
Trypsin-ultra, Mass Spectrometry Grade	P8101S	100 µg
α-Lytic Protease	P8113S/L	20/100 µg

# PROTEIN MARKERS FROM NEB

**Built for perfect migration & “ready-to-load”**

**FREE SAMPLE\***

### Advantages

- Suitable for a wide range of proteins (from 10 to 250 kDa)
- “ready-to-load” – no heating prior to gel loading required
- Uniform band intensities
- Easy-to-identify reference bands
- Shelf life: 24 months at -20°C; 12 months at 4°C; 2 weeks at RT (25 °C)

#### Ordering information:

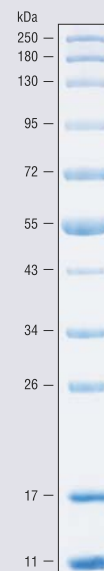
PRODUCT	NEB #	SIZE
Unstained Protein Standard, Broad Range (10-200 kDa)	P7717S/L	150 / 750 lanes
Blue Prestained Protein Standard, Broad Range (11-250 kDa)	P7718S/L	100 / 500 lanes
Color Prestained Protein Standard, Broad Range (10-250 kDa)	P7719S/L	100 / 500 lanes



Unstained Protein Standard, Broad Range (10-200 kDa)  
10-20% Tris-glycine SDS-PAGE Gel  
NEB #P7717



Color Prestained Protein Standard, Broad Range (10-250 kDa)  
10-20% Tris-glycine SDS-PAGE Gel  
NEB #P7719



Blue Prestained Protein Standard, Broad Range (11-250 kDa)  
10-20% Tris-glycine SDS-PAGE Gel  
NEB #P7718

\* To request your free sample of the Color Prestained Protein Standard (#P7719), please visit [www.neb.com/p7719](http://www.neb.com/p7719), or contact your local distributor!

# MIGRATE TO MONARCH

## Monarch<sup>®</sup> Genomic DNA Purification Kit

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, minimal residual RNA, and is suitable for downstream applications, such as endpoint PCR, qPCR and library prep for next generation sequencing (NGS).

### Monarch gDNA Purification Workflow: It's that easy!



## Advantages

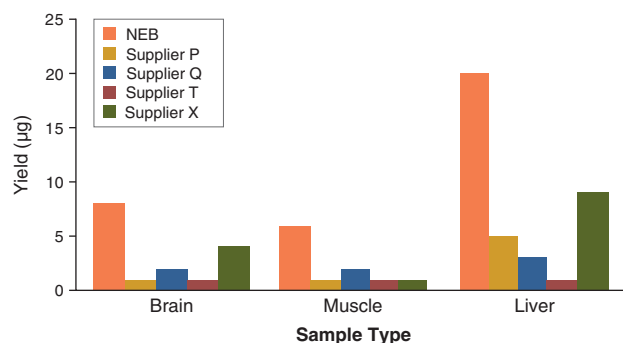
- Use with a wide variety of sample types
- Generate higher yields, especially with tough samples (e.g., brain and muscle)
- Isolate longer DNA (peak size > 50 kb), which is ideal for long-read sequencing platforms
- Effectively remove RNA (< 1% residual RNA)
- Includes RNase A
- Save time with fast protocols, efficient lysis steps and minimal hands on time

## Product Feedback:

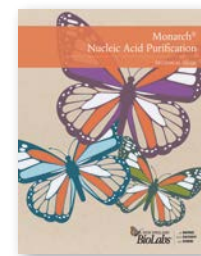
“The very rapid and easy protocol provided high quality DNA from difficult to lyse biological samples for Illumina and PacBio DNA sequencing. The use of environmentally friendly packaging was also a plus for me.

Barton Slatko, Senior Research Scientist, Genome Biology

### The Monarch Genomic DNA Purification Kit provides excellent yields for difficult tissue types

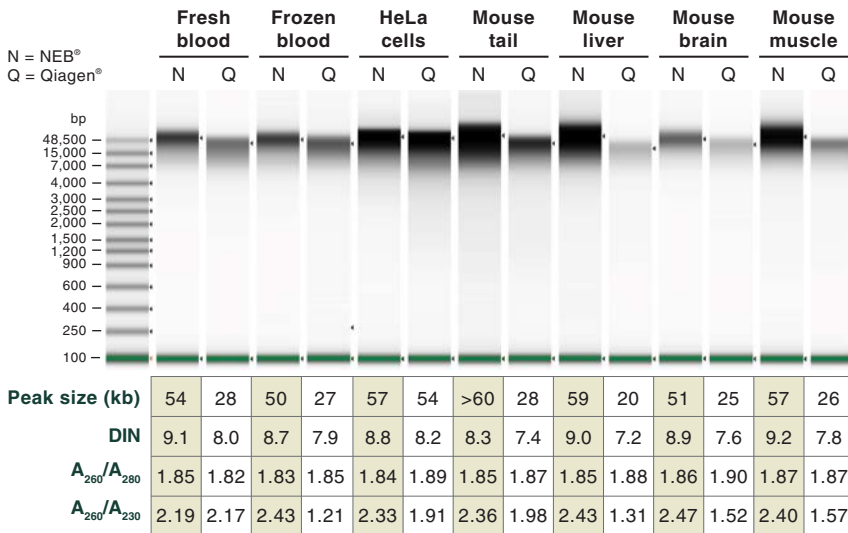


Duplicate 10 mg samples of RNAlater<sup>®</sup>-stabilized rat tissue were cut to small pieces and subsequently lysed and purified according to the protocols provided for each kit. Optional RNase A steps were included. Elution was carried out with 100 µl elution buffer provided in the respective kits. Yields displayed are averages of the duplicate samples, and represent the genomic DNA yield after correcting for the RNA content as determined by LC-MS. Results indicate that the Monarch Genomic DNA Purification Kit provides excellent yields for a wide range of tissues, which can be problematic for other commercial kits.



Request your free Monarch DNA & RNA Purification brochure from your local distributor!

The Monarch Genomic DNA Purification Kit provides excellent yields of higher quality, higher molecular weight DNA than the Qiagen® DNeasy® Blood & Tissue Kit



Request your free sample from your local distributor!

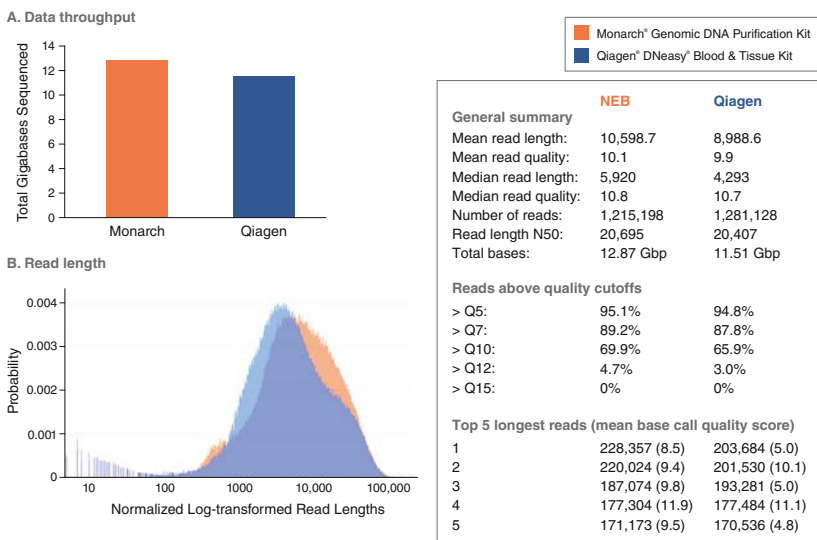
Visit [NEBmonarch.com](http://NEBmonarch.com) to:

- View the full portfolio of products for DNA & RNA purification
- Find out what our customers are saying about Monarch kits
- Learn how Monarch kits are sustainably designed

Agilent Technologies® 4200 TapeStation® Genomic DNA ScreenTape was used for analysis of blood, cultured cell, and tissue samples purified using the relevant protocols of the Monarch Genomic DNA Purification Kit and the Qiagen DNeasy Blood & Tissue Kit. gDNA was eluted in 100 µl and 1/100 of the eluates (~1 µl) was loaded on a Genomic DNA ScreenTape. Starting materials used: 100 µl fresh human whole blood, 100 µl frozen pig blood, 1x10<sup>6</sup> HeLa cells and 10 mg frozen tissue powder. Monarch-purified gDNA samples typically show peak sizes 50 – 70 kb and DINs of ~9. DNeasy-purified gDNA peak sizes are typically <30 kb with DINs ~7-8. DNeasy kits produce lower yields and low A260/230 ratios for liver, brain, muscle and frozen blood.



The Monarch Genomic DNA Purification Kit generates high quality DNA for nanopore sequencing



HeLa cell genomic DNA was extracted using either the Monarch Genomic DNA Purification Kit or the Qiagen DNeasy Blood & Tissue Kit. One microgram of purified DNA was used to prepare Oxford Nanopore Technology (ONT) sequencing libraries following the ONT 1D Ligation Sequencing Kit (SQK-LSK109) protocol without DNA fragmentation. Libraries were loaded on a GridION (Flow cell R9.4.1) and the data was collected for 48 hrs. Libraries produced using the Monarch gDNA exceeded the Qiagen libraries on common sequencing metrics including: A. total sequencing data collected, B. read length. Data was generated using NanoComp (Bioinformatics, Volume 34, Issue 15, 1 August 2018, Pages 2666–2669).

PRODUCTS	NEB #	SIZE
Monarch Genomic DNA Purification Kit	T3010S/L	50/150 preps

COLUMNS AVAILABLE SEPARATELY

Monarch gDNA Purification Columns	T3017L	100 columns and 200 tubes
Monarch Collection Tubes II	T2018L	100 tubes

BUFFERS AND REAGENTS AVAILABLE SEPARATELY

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

NEW TO MOLECULAR BIOLOGY?

# Start your career off on the right path. Get your free NEB Starter Pack!

The free NEB Starter Pack is available to all research students, PhD students, PostDocs, PIs etc. who are Rookies to Molecular Biology and who are willing to join the NEB mailing list.

## The NEB Starter Pack contains\*:

- NEB Catalog & Technical Reference 2019/20 incl. Buffer Poster
- Voucher for 20% discount on your first purchase of any NEB product incl. free NEBCool lab bench cooler
- NEB notepad
- NEB Pull Reel Key Ring
- Free Sample of Quick-Load DNA Ladder
- Year planning calendar poster 2020
- Tech-Guide "Molecular Cloning"
- Guide PCR Reagents
- RNA Poster
- NEB Pencil
- Water bath floatie



Register at [www.neb-online.eu/starterpack](http://www.neb-online.eu/starterpack)

\*Offer available through to Jan. 31<sup>st</sup>, 2020, or while stocks last. Limited to one pack/person. Content may vary from those shown. The 20% discount voucher/offer is void where prohibited by regional or institutional laws or regulations. Please ask your local distributor for details and availability.



Now catalog incl. Augmented Reality content – Download free NEB-AR App for Android or iOS to explore and discover!

## Already an experienced Molbiol Pro?

You don't need the NEB Starter Pack? You still need the updated NEB Catalog & Technical Reference 2019/20, do you? Request your free copy at your local distributor!

## Your local NEB distributor:

BeNeLux:  
BIOKÉ  
Tel: (+31) 71 720 0220  
(BE: 0800 - 71640)  
Fax: (+31) 71 891 0019  
info@bioke.com  
www.bioke.com

BULGARIA  
ELTA 90M Ltd.  
Tel: (+359) 2 983 96 49  
Fax: (+359) 2 9832211  
office@elta90.eu  
www.elta90.com

CROATIA:  
DIAGNOSTICA SKALPELI D.O.O.  
Free call: 800-315911  
Tel: (+385) 1 377 8484  
Fax: (+385) 1 377 8585  
skalpeli@skalpeli.hr  
www.skalpeli.hr

CZECH REPUBLIC:  
BIOTECH A.S.  
Tel: (Toll Free) 0800 124683  
Fax: 02 72701742  
info@ibitech.cz  
www.ibitech.cz

DENMARK:  
BioNordika Denmark A/S  
DENMARK  
Tel: (39) 56 20 00  
Fax: (39) 56 19 42  
info@bionordika.dk  
www.bionordika.dk

FINLAND:  
BioNordika Oy  
Tel: (+358) 207 410 270  
info@bionordika.fi  
www.bionordika.fi

GREECE:  
BIOLINE SCIENTIFIC  
Tel: (0030) 210 522 6547  
Fax: (0030) 210 524 4744  
info@bioline.gr  
www.bioline.gr

HUNGARY:  
KVALITEX SCIENTIFIC  
Phone: (1) 340 4700  
Fax: (1) 339 8274  
info@kvalitex.hu  
www.kvalitex.hu

ITALY:  
EUROCLONE S.P.A.  
Free call: 800-315911  
Tel: +39 02 38195.1  
Fax: +39 02 3810145  
info@euroclone.it  
www.euroclongroup.it

ISRAEL:  
ORNAT LTD.  
Tel: (+972) 8 947 7077  
Fax: (+972) 8 936 3034  
ornatbio@ornat.co.il  
www.ornat.co.il

NORWAY:  
BioNordika AS  
Tel +47 23 03 58 00  
Fax +47 23 03 58 01  
info@bionordika.no  
www.bionordika.no

POLAND:  
Lab-JOT Ltd, Sp.z o.o, Sp.k.  
Stacjonarny: +48 22 335 988 4  
Komórkowy: +48 606 338 879  
Fax: +48 22 335 981 9  
biuro@labjot.com  
www.labjot.com

PORTUGAL:  
Werfen  
Tel: +351 214 247 312  
Fax: +351 21 417 26 74  
dac-pt@werfen.com  
www.pt.werfen.com

ROMANIA:  
ELTA 90 MEDICAL RESEARCH SRL  
Tel: +40 21 2322694  
Fax: +40 21 2322696  
office@elta90mr.ro  
www.elta90mr.ro

RUSSIA:  
SkyGen LLC  
Tel: +7 495 215 02 22  
info@skygen.com  
www.skygen.com

SERBIA:  
ALFA GENETICS D.O.O.  
Tel: 00381 11 3291219  
Fax: 00381 11 2761281  
office@alfagenetics.rs  
www.alfagenetics.rs

SPAIN:  
Werfen  
Tel: +34 902 20 30 90  
Fax: +34 902 22 33 66  
customerservice-es@werfen.com  
www.es.werfen.com

SLOVAK REPUBLIC  
BioTech s.r.o.  
Tel: +421 2 54 77 44 88  
Fax: +421 2 32 20 10 48  
info@ibitech.sk  
www.ibitech.sk

SLOVENIA:  
Mikro+Polo d.o.o.  
Tel: +386 2 614 33 00  
Fax: +386 2 614 33 20  
info@mikro-polo.si  
www.mikro-polo.com

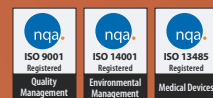
SOUTH AFRICA:  
INQABA BIOTEC  
Tel: +27 123 43 58 29  
Fax: +27 86 677 8409  
info@inqababiotec.co.za  
www.inqababiotec.co.za

SWEDEN, ESTONIA, LATVIA,  
LITHUANIA:  
BIONORDIKA SWEDEN AB  
Tel: +46 8 30 60 10  
Fax: +46 8 30 60 15  
info@bionordika.se  
www.bionordika.se

SWITZERLAND:  
BIOCONCEPT  
Tel: +41 (0)61 486 80 80  
Fax: +41 (0)61 486 80 00  
info@bioconcept.ch  
www.bioconcept.ch

TURKEY:  
EKA BIOLAB TEKNOLOJİ  
Tel: +90/312 248 13 24  
Email: info@ekabiolab.com  
www.ekabiolab.com

For contacts in other countries, please visit [www.neb.com/international-support](http://www.neb.com/international-support)



# neb.com

