For over 35 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. EpiMark® validated reagents simplify epigenetics research and expand the potential for biomarker discovery.

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, lifestyle, toxin exposure) (1). Cross-talk between histone modifications, DNA methylation or RNAi pathways are being studied in such areas as cancer, X chromosome inactivation, and imprinting.

Table 1: A selection of biological events and diseases influenced by epigenetics

<table>
<thead>
<tr>
<th>CELLULAR PROCESSES</th>
<th>Gene Regulation</th>
<th>Chromatin Structure</th>
<th>Flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic Development/Cell Fate</td>
<td>Mammalian X</td>
<td>Protection from Transposable Elements</td>
<td></td>
</tr>
<tr>
<td>Aging</td>
<td>Imprinting</td>
<td>Host Defense Systems</td>
<td></td>
</tr>
<tr>
<td>CANCER</td>
<td>Breast Cancer</td>
<td>Lung Cancer</td>
<td>Colon Cancer</td>
</tr>
<tr>
<td></td>
<td>T Cell Cutaneous Lymphoma</td>
<td>Myelodysplastic Syndrome</td>
<td>Leukemia</td>
</tr>
<tr>
<td></td>
<td>Type II Diabetes</td>
<td>Autoimmune Disease</td>
<td>Systemic Lupus Erythematosis</td>
</tr>
<tr>
<td></td>
<td>Schizophrenia</td>
<td>Autism</td>
<td>Fragile X Syndrome</td>
</tr>
<tr>
<td></td>
<td>Angelman’s Syndrome</td>
<td>Prader-Willi Syndrome</td>
<td>Rett Syndrome</td>
</tr>
<tr>
<td></td>
<td>α-Thalassemia</td>
<td>Hypertrophic Cardiomyopathy</td>
<td>Long QT Syndrome</td>
</tr>
<tr>
<td>OTHER DISEASES</td>
<td>Beckwith-Wiedemann Syndrome</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References
NEB offers a suite of EpiMark validated reagents to study DNA and histone modifications. Visit [www.epimark.com](http://www.epimark.com) for the latest list of reagents available from NEB.

### EpiMark Validated Reagents for Epigenetic Studies

#### HISTONES (Pages 4–6)

<table>
<thead>
<tr>
<th>Histone H1</th>
<th>Human, Recombinant</th>
<th>Histone H3.3</th>
<th>Human, Recombinant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histone H2A</td>
<td>Human, Recombinant</td>
<td>H4 Human</td>
<td>Recombinant</td>
</tr>
<tr>
<td>Histone H2B</td>
<td>Human, Recombinant</td>
<td>EpiMark Nucleosome Assembly Kit</td>
<td></td>
</tr>
<tr>
<td>Histone H3.1</td>
<td>Human, Recombinant</td>
<td>Histone H2A/H2B Dimer</td>
<td></td>
</tr>
<tr>
<td>Histone H3.2</td>
<td>Human, Recombinant</td>
<td>Histone H3.1/H4 Tetramer</td>
<td></td>
</tr>
</tbody>
</table>

#### HISTONE/PROTEIN METHYLTRANSFERASES (Page 8)

<table>
<thead>
<tr>
<th>G9a Methyltransferase</th>
<th>HEN1 mRNA Methyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRMT1 Methyltransferase</td>
<td>Human DNA (cytosine-5) Methyltransferase (Dnmt1)</td>
</tr>
<tr>
<td>SET7 Methyltransferase</td>
<td></td>
</tr>
</tbody>
</table>

#### RESTRICTION ENZYMES (Pages 12–13)

| DpnI | LpnPl |
| DpnII | MspI |
| FspEl | MspII |
| HpaII |

#### 5-HYDROXYMETHYLCYTOSINE ANALYSIS (Pages 14–15)

| EpiMark 5-hmC and 5-mC Analysis Kit | T4 Phage β-Glucosyltransferase |

#### DNA METHYLATION ANALYSIS (Page 16–17)

| EpiMark Bisulfite Conversion Kit | EpiMark Hot Start Taq DNA Polymerase |
| EpiMark Methylated DNA Enrichment Kit |

#### DNA METHYLTRANSFERASES (Pages 18–19)

| Human DNA (cytosine-5) Methyltransferase (Dnmt1) | dam Methyltransferase |
| CpG Methyltransferase (M.SsII) | BamH1 Methyltransferase |
| CpG Methyltransferase (M.CviPI) | MspI/Hal Methyltransferase |
| HpaII Methyltransferase | TaqI Methyltransferase |
| MspI Methyltransferase | AluI Methyltransferase |
| EcoRI Methyltransferase | HaeIII Methyltransferase |

#### SAMPLE PREP FOR NEXT GEN SEQUENCING (Page 20)

| NEBNext ChIP-Seq Sample Prep Reagent Set 1 | NEBNext ChIP-Seq Sample Prep Master Mix Set 3 |
| NEBNext ChIP-Seq Sample Prep Master Mix Set 1 |

#### ANTIBODIES (Page 21)

| Dnmt1 Amino-terminal | Dnmt3B Carboxy-terminal |
| Dnmt3A Amino-terminal |

#### CONTROL DNAs (Page 21)

| Jurkat Genomic DNA | CpG Methylated NIH 3T3 Genomic DNA |
| CpG Methylated Jurkat Genomic DNA | HeLa Genomic DNA |
| 5-Aza-dc–Treated Jurkat Genomic DNA | CpG Methylated HeLa Genomic DNA |
| NIH 3T3 Mouse Genomic DNA |
Chromatin and Histones

In eukaryotes, chromatin is organized into nucleosome core particles (NCPs) that consist of approximately 147 bp of DNA and an octamer complex made up of two molecules of each histone (H2A, H2B, H3 and H4). The linker histone H1 further condenses chromatin by binding to DNA between the nucleosome core particles (1). Chromatin can be generally classified as condensed, transcriptionally silent heterochromatin or less-condensed, transcriptionally active euchromatin. The dynamic nature of the chromatin predicts different conformational forms exist in the nucleus at a given time. Furthermore, chromatin structure is influenced by the modification of DNA or histones that comprise it and by its transcriptional state (2). Although, most genomic DNA is believed to be packed into heterochromatin (telomeres, pericentric regions and areas rich in repetitive sequences), looping of large stretches of chromatin from a chromosome to generate local secondary structure poised for transcription is observed (3).

New England Biolabs offers a selection of unmodified, recombinant human histones that function as substrates for histone-modifying enzymes. Seven human histones, including three histone H3 variants, have been individually cloned in *E. coli* expression vectors and then purified from *E. coli* cell extracts. Mass spectrometry analysis demonstrates that these histones are free of post-translational modifications. To aid in studying intact nucleosomes, NEB also offers the EpiMark Nucleosome Assembly Kit. The precise mixing of preformed recombinant Human Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer generates a human histone octamer, and in the presence of DNA, forms nucleosomes (4,5). Enzymes that are unable to modify individual histone or DNA may be active on these nucleosome core particles, the histone dimer or the histone tetramer (6).

EpiMark Nucleosome Assembly Kit

This kit contains the components necessary to form an unmodified recombinant human nucleosome using experimental DNA of interest 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 at high salt, followed by dialysis down to low salt to form 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 better substrates 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.

EpiMark Nucleosome Assembly Kit ................................................................. E5350S

Applications of NEB Histones:

- Purification and characterization of enzymes that modify histone proteins
- Formation of unmodified nucleosome core particles, which may be modified by enzymes that are inactive on individual histones or DNA

Highly Purified Histones from NEB

Visit www.epimark.com for more information on histone modifications
Recombinant Human Histones

**Histone H1°**

Histone H1 acts on the linker region of polynucleosome DNA to condense the chromatin into structures of ~30 nm (1) and is not necessary for octamer or nucleosome core particle formation.

Eight different histone H1 proteins have been identified in the human genome (2). Histone H1° is a non replication-dependent histone that is highly expressed in cells that have terminally differentiated (3). Recombinant human histone H1 from NEB is expressed in *E. coli* using the H1F0 or H1Fv gene (Genbank accession number: X03473).

Histone H1° Human, Recombinant ................................................................................................... M2501S

**Histones H2A & H2B**

Histone H2A interacts with histone H2B to form the H2A/H2B heterodimer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer (1,4). Histones H2A and H2B are modified by various enzymes and have been shown to be important in gene transcription (5).

Recombinant human histones H2A and H2B are expressed in *E. coli* using the HIST3H2A gene (Genbank accession number: AY131974) and the HIST2H2BE or H2BFQ gene (Genbank accession number: AY131979), respectively. NEB also offers the preformed histone H2A/H2B dimer. This is generated by refolding the denatured, purified subunits H2A and H2B, followed by gel filtration.

Histone H2A Human, Recombinant ................................................................. M2502S
Histone H2B Human, Recombinant ................................................................. M2505S
Histone H2A/H2B Dimer Human, Recombinant ........................................... M2508S

References
Histones H3 & H4

Histone H3 interacts with histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer (1,2).

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 (3). Histone H3.2, an H3 variant that is found in all eukaryotes, except budding yeast, is replication-dependent and is associated with gene silencing (4). 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 (5). It has been shown to be enriched in covalent modifications associated with gene activation (4,6).

Recombinant human histones H3.1, H3.2 and H3.3 are synthesized in E. coli using the HIST1H3A or H3FA gene (Genbank accession number: AF531274), HIST2H3A or HIST2H3C gene (Genbank accession number: BC130637) and H3F3A or H3F3B gene (Genbank accession number: AK311905), respectively. Recombinant human histone H4 is synthesized in E. coli using the HIST2H4 gene (Genbank accession number: AF525682). NEB also offers preformed recombinant histone H3.1/H4 tetramer. This is generated by refolding the denatured, purified subunits H3.1 and H4, followed by gel filtration.

Histone H3.1 Human, Recombinant ................................................................. M2503S
Histone H3.2 Human, Recombinant ................................................................. M2506S
Histone H3.3 Human, Recombinant ................................................................. M2507S
Histone H4 Human, Recombinant ................................................................. M2504S
Histone H3.1/H4 Tetramer Human, Recombinant ........................................... M2509S

References
Histone Modifications

The core histones consist of a globular C-terminal domain and an unstructured N-terminal tail. Although a variety of modifications occur throughout the histone protein (see Table 1), they occur primarily on the N-terminal tail (1-5). Through their potential combinatorial modification on a given histone and its reversibility, these modifications dynamically restrict or recruit numerous other proteins or protein complexes onto chromatin (5). The study of their roles in gene regulation (6), cellular stress events (6), aging and DNA repair (7) is revealing the multiple functions of histone modifications in determining the fate of a cell. Additional variability is incorporated into the system by histone variants. Acting individually or in conjunction with DNA modification, histone modifications and histone variants are thought to establish an epigenetic code or epigenetic signature for gene regulation (5).

Table 1: Types of Histone Modifications

<table>
<thead>
<tr>
<th>AMINO ACID</th>
<th>MODIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>Methylation, Acetylation, Ubiquitination, Sumoylation, ADP-Ribosylation</td>
</tr>
<tr>
<td>Arginine</td>
<td>Methylation</td>
</tr>
<tr>
<td>Serine</td>
<td>Phosphorylation</td>
</tr>
<tr>
<td>Threonine</td>
<td>Phosphorylation</td>
</tr>
</tbody>
</table>

Methods for Studying Histone Modifications

One of the most widely used methods for studying histone modifications in vivo is chromatin immunoprecipitation (ChIP). In brief, protein and DNA are generally cross-linked by formaldehyde treatment. After the chromatin is fragmented by sonication, antibodies specific for a histone modification or chromatin binding protein are used to immunoprecipitate the DNA. The histones from NEB can be used as carrier chromatin in CChIP (Carrier Chromatin Immunoprecipitation) assays (8). For large-scale analyses, the isolated DNA can be analyzed on a microarray (ChIP-chip) or by sequencing (ChIP-seq, see page 20). The limitations of traditional ChIP (e.g., quality of the antibody, bias from fixation and fragmentation, and interference from other histone-binding proteins) are partially addressed by alternative methods, such as N-ChIP (Native-ChIP), biotin-tag affinity purification, and DamID (reviewed in 9).

References
Histone Methyltransferases

Lysine or arginine residues in histones undergo enzymatic methylation via the attachment of one, two or three methyl groups. The timing of the appearance of these modifications is often dynamic and will depend on the signaling condition of the cell. Histone modifications participate in transcription, repair, replication and chromatin condensation. NEB offers a selection of protein methyltransferases specific for histone H3.1, H3.2, H3.3 and histone H4.

G9a Methyltransferase

G9a methyltransferase methylates lysine 9 (Lys 9) of histone H3 (1-3). Methylation occurs at the ε amino group of lysine residues. Methylation of histone H3 Lys 9 is a hallmark of silent chromatin and is globally distributed throughout the heterochromatic regions, such as centromeres and telomeres (4,5). The G9a enzyme from NEB is expressed from mouse G9a cDNA (1,2).

Human PRMT1 Methyltransferase

PRMT1 is a major protein arginine methyltransferase (6). It specifically methylates arginine 3 (Arg 3) of histone H4. Furthermore, methylation of histone H4 at Arg 3 facilitates transcriptional activation by nuclear hormone receptors (7). In addition, the ordered cooperative functions of PRMT1, p300 and CARM1 in transcriptional activation by p53 is observed on the GADD45 gene following ectopic p53 expression and/or UV irradiation (8). The PRMT1 enzyme from NEB is expressed from rat PMRT1 cDNA.

SET7 Methyltransferase

SET7 Methyltransferase methylates lysine 4 (Lys 4) of histone H3 (9). Methylation occurs at the ε amino group of lysine residues (10,11). Di- and tri- methylation of histone H3 Lys 4 is a hallmark of transcriptionally active chromatin and is globally distributed (12,13). The SET7 enzyme from NEB is expressed from human SET7 cDNA.

Human DNA (cytosine-5) Methyltransferase (DNMT1)

DNMT1 methylates cytosine residues in hemimethylated DNA at 5′…CG…3′ sites (14,15). Mammalian DNA methylation afforded by DNMT1 is involved in carcinogenesis, embryonic development and several other biological functions (16-18).

References
DNA Modifications

DNA can be modified by methylation of cytosine and adenine bases in a wide variety of prokaryotes and eukaryotes (see Table 2). In prokaryotes, DNA methylation is involved in determination of DNA-host specificity, virulence, DNA repair, chromosome replication and segregation, cell cycle regulation and gene expression. In higher eukaryotes, DNA methylation is involved in gene regulation, chromatin structure, differentiation, imprinting, mammalian X chromosome inactivation, carcinogenesis, complex diseases and aging.

DNA Methylation in Mammals

DNA methylation in mammals primarily occurs on the fifth carbon of the cytosine base (5-methylcytosine, 5-mC, see sidebar) of CpG dinucleotides, and approximately 70% to 80% of CpG dinucleotides are methylated in somatic cells. However, 5-mC at CpA, CpT and CpC sequences have been found in genomic DNA from mouse embryonic stem cells, and 5-mC at CpA sequences are thought to regulate enhancers in mouse brain. Of note, while DNA methylation in mammals primarily occurs at CpG dinucleotides, DNA methylation in plants may occur at CpG, CpHpG and CpHpH sequences, where H is adenine, cytosine, or thymine.

Recently, 5-hydroxymethylcytosine (5-hmC, see sidebar) was discovered in mouse embryonic stem cells, Purkinje neurons and granule neurons (1,2). The role of this modified base is not known, but it may be involved in demethylation or it may influence chromatin structure and local transcriptional activity by either recruiting selective 5-hmC-binding proteins or excluding proteins that specifically bind 5-mC.

Table 2: Types of DNA Modifications

<table>
<thead>
<tr>
<th>METHYLATED BASE</th>
<th>ORGANISM</th>
<th>DNA METHYLATION SEQUENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5-methylcytosine</td>
<td>Bacteria</td>
<td>Varies (e.g., CCAGG, CCTGG)</td>
</tr>
<tr>
<td></td>
<td>Some Fungi, Some Insects, Mammals, Plants</td>
<td>CpG, CpH<em>pG, CpH</em>pH</td>
</tr>
<tr>
<td></td>
<td>Plants</td>
<td>C5-methylcytosine</td>
</tr>
<tr>
<td>C5-hydroxymethylcytosine</td>
<td>Bacteriophages</td>
<td>Varies (e.g., CCGG, GATC), Some contain only modified cytosines</td>
</tr>
<tr>
<td></td>
<td>Mammals</td>
<td>CpG, CpH<em>pG, CpH</em>pH</td>
</tr>
<tr>
<td>N4-methylcytosine</td>
<td>Bacteria</td>
<td>Varies (e.g., CTCTTC, CCCGGG)</td>
</tr>
<tr>
<td>N6-methyladenine</td>
<td>Bacteria, Bacteriophages, Archaea, Protists, Some Fungi, Plants</td>
<td>Varies (e.g., GATC, GANTC, GAAGAG)</td>
</tr>
</tbody>
</table>

* = Adenine, Cytosine, or Thymine

References

DNA METHYLATION

Methods for Studying DNA Methylation

There are three main approaches for studying DNA methylation. These are based on pre-treating genomic DNA with either sodium bisulfite, restriction enzymes or a methylated DNA-binding affinity matrix (Table 3, next page). Briefly, using sodium bisulfite to convert unmethylated cytosines to uracil, as opposed to 5-methylcytosine, which is refractory to bisulfite-mediated deamination, is the gold standard for assessing DNA methylation. This is partly because this technique can reveal the methylation status of every cytosine residue and is amenable to massively parallel sequencing methods. Differential enzymatic cleavage of DNA relies on methylation-sensitive or methylation-dependent restriction enzymes fragmenting genomic DNA for subsequent analysis. Reaction conditions used for restriction enzyme-based methods are not as harsh as those required for bisulfite methods; however, the resolution of the data is limited by the enzyme recognition sequence and the completeness of digestion. Finally, affinity-based methods use methylated DNA binding proteins or antibodies to enrich the experimental DNA sample for methylated DNA for subsequent analysis.

A wide variety of analytical and enzymatic downstream methods can be used to characterize genomic DNA. Analytical methods, such as high-performance liquid chromatography (HPLC) and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), have been used routinely to quantify modified nucleobases in complex DNA. While HPLC is quantitative and reproducible, it typically requires large amounts of DNA and is often unsuitable for high throughput applications. However, recent work to improve the method has shown that nanogram amounts can be used (2). MALDI-TOF MS is also quantitative but amenable to high throughput applications. Other downstream methylation detection methods include end-point PCR, real-time PCR, primer extension, single-stranded conformational polymorphism assays, blotting, microarrays and sequencing. Choosing which method(s) to use largely depends on the experimental sample size and the goals of the experiments (1; see also www.epimark.com).

NEB offers a selection of EpiMark validated products to aid in DNA methylation studies. These include methylation sensitive and methylation dependent restriction enzymes, as well as a kit that utilizes these methylation sensitive restriction enzymes to identify 5-mC and 5-hmC at a specific loci. NEB also offers a kit that selectively binds and enriches double-stranded CpG methylated DNA from fragmented genomic DNA. For bisulfite conversion, NEB supplies a EpiMark validated kit and a polymerase that is ideal for bisulfite converted DNA. Additionally, DNA methyltransferases are available and can be used for modification studies. At the sequencing level, NEB offers reagents for DNA sample preparation for ChIP-Seq analysis.

Visit www.epimark.com for more information on DNA methylation and methods of analysis

References
## Table 3: Approaches for Studying DNA Methylation

<table>
<thead>
<tr>
<th>METHOD</th>
<th>DESCRIPTION</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
<th>APPLICATION</th>
</tr>
</thead>
</table>
| Sodium Bisulfite Conversion   | Treatment of denatured DNA (i.e., single-stranded DNA) with sodium bisulfite leads to deamination of unmethylated cytosine residues to uracil, leaving 5-mC intact. The uracils are amplified as thymines, and 5-mC residues are amplified as cytosines in PCR. Comparison of sequence information between the reference genome and bisulfite-treated DNA can provide single-nucleotide resolution information about cytosine methylation patterns. | • Resolution at the nucleotide level  
• Works on 5-mC-containing DNA  
• Automated analysis  
• Gives % mC at a specific site | • Requires micrograms of DNA input, depending on downstream processes  
• DNA is often damaged  
• Multi-step protocol  
• Potentially incomplete conversion of DNA  
• Intensive downstream analysis  
• Cannot distinguish 5-mC and 5-hmC | • Whole genome or a single DNA locus methylation analysis                                                                 |
| Sequence-Specific Enzyme Digestion | Restriction enzymes are used to generate DNA fragments for methylation analysis. Some restriction enzymes are methylation-sensitive (i.e., digestion is impaired or blocked by methylated DNA). When used in conjunction with an isoschizomer that has the same recognition site, but is methylation insensitive, information about methylation status can be obtained. Additionally, the use of methylation-dependent restriction enzymes (i.e., requires methylated DNA for cleavage to occur) can be used to fragment DNA for sequencing analysis. | • High enzyme turnover  
• Well-studied  
• Easy-to-use  
• Availability of recombinant enzymes | • Determination of methylation status is limited by the enzyme recognition site  
• Overnight protocols  
• Lower throughput | • Southern blots using MspI/ HpaII                                                                                           |
| Methylated DNA Immunoprecipitation | Fragmented genomic DNA (restriction enzyme digestion or sonication) is denatured and immunoprecipitated with antibodies specific for 5-mC. The enriched DNA fragments can be analyzed by PCR for locus-specific studies or by microarrays (MeDIP-chip) and massively parallel sequencing (MeDIP-seq) for whole genome studies. | • Relatively fast  
• Compatible with array-based analysis  
• Applicable for high throughput sequencing | • Dependent on antibody specificity  
• May require more than one 5-mC for antibody binding  
• Requires DNA denaturation  
• Resolution depends on the size of the immunoprecipitated DNA and for microarray experiments; depends on probe design  
• Data from repeat sequences may be overrepresented | • Immuno affinity capture                                                                                   |
| Methylated DNA-Binding Proteins | Instead of relying on antibodies for DNA enrichment, affinity-based assays use proteins that specifically bind methylated or unmethylated CpG sites in fragmented genomic DNA (restriction enzyme digestion or sonication). The enriched DNA fragments can be analyzed by PCR for locus-specific studies or by microarrays and massively parallel sequencing for whole genome studies. | • Well-studied  
• Does not require denaturation  
• Compatible with array-based analysis  
• Applicable for high throughput sequencing | • May require high DNA input  
• May require a long protocol  
• Requires salt elutions  
• Does not give single base methylation resolution data | • Capture of methylated DNA                                                                                     |
Methylation-Sensitive Restriction Enzymes

Some restriction enzymes are methylation-sensitive (i.e., digestion is impaired or blocked by methylated DNA). When used in conjunction with an isoschizomer that has the same recognition site but is methylation insensitive, information about methylation status can be obtained. Table 4A lists methylation sensitive restriction enzymes that can be used in epigenetic studies.

Table 4A: Methylation Sensitive Restriction Enzymes

<table>
<thead>
<tr>
<th>ENZYMES</th>
<th>METHYLATION SENSITIVITY</th>
<th>SEQUENCE</th>
<th>NEB #</th>
<th>ISOSCHIZOMER</th>
</tr>
</thead>
<tbody>
<tr>
<td>DpnII</td>
<td>Cleaves dam sites** which lack adenomethylation and is blocked by complete dam methylation and probably by hemi-methylation</td>
<td>5’…GATC…3’ 3’…CTAG…5’</td>
<td>R0543</td>
<td>MboI DpnII</td>
</tr>
<tr>
<td>HpaII</td>
<td>Will not cleave methylated CpG sites</td>
<td>5’…CGCG…3’ 3’…GGCC…5’</td>
<td>R0171</td>
<td>MspI</td>
</tr>
<tr>
<td>MspI</td>
<td>Not methylation sensitive</td>
<td>5’…CGCG…3’ 3’…GGCC…5’</td>
<td>R0106</td>
<td>HpaII</td>
</tr>
</tbody>
</table>

** dam sites: methylation at the N6 position of the adenine in the sequence GATC (GmATC).

Applications of Methylation-Sensitive Restriction Enzymes

- Differentiation of methylation patterns

Protocol for Restriction Enzyme Digestion:

1. Add the following components to a sterile microcentrifuge tube (restriction enzyme should be added last):

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>25 µl REACTION</th>
<th>50 µl REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>0.5 µg</td>
<td>1 µg</td>
</tr>
<tr>
<td>10X NEBuffer</td>
<td>2.5 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>100X BSA* (if needed)</td>
<td>0.25 µl</td>
<td>0.5 µl</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>to 25 µl</td>
<td>to 50 µl</td>
</tr>
<tr>
<td>Restriction Enzyme**</td>
<td>5 units</td>
<td>10 units</td>
</tr>
</tbody>
</table>

* BSA can be diluted in 1X buffer. If BSA is required for either enzyme, it should be added to the reaction.

** Restriction enzymes can be diluted using the recommended diluent buffer.

2. Gently mix the reaction by pipetting up and down and microfuge briefly.

3. Incubate at the recommended temperature for 1 hour or 5 minutes for Time-Saver qualified restriction enzymes (see www.neb.com/TimeSaver for more information).

4. Terminate the reaction by heat inactivation or DNA purification according to product recommendations.

Methylation-Dependent Restriction Enzymes

Some restriction enzymes are dependent on methylation or hydroxymethylation for cleavage to occur, making them particularly useful for DNA methylation studies.

McrBC

McrBC is an endonuclease which only cleaves DNA containing methylcytosine (5-methylcytosine, 5-hydroxymethylcytosine or N4 methylcytosine) on one or both strands (2). McrBC will not act upon unmethylated DNA (3) and will not recognize HpaII/MspI sites (CCGG) in which the internal cytosine is methylated. 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 (4).

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 (5). Therefore, the enzyme does not produce defined DNA ends upon cleavage. Also, 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 and a smeared, rather than a sharp, banding pattern is produced.

Applications of McrBC

- CpG methylation studies (6–10)
- Methylated cytosine detection
- Methylated DNA enrichment (11)
**MspJI Family of Restriction Enzymes**

Scientists at NEB recently identified the MspJI family of restriction enzymes, which are dependent on methylation and hydroxymethylation for cleavage to occur (12). 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.

Table 4B: Methylation Dependent Restriction Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Methylation Sensitivity</th>
<th>Sequence</th>
<th>NEB #</th>
<th>Isoschizomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>DpnI</td>
<td>Cleaves fully-adenomethylated dam sites (hemimethylated dam sites 60X more slowly)</td>
<td>$5'...G\ AT\ C...3'$ $3'...C\ TA\ G...5'$</td>
<td>R0176</td>
<td>DpnII</td>
</tr>
<tr>
<td>FspEI</td>
<td>Cleaves DNA containing 5-methylcytosine and 5-hydroxymethylcytosine</td>
<td>$5'...C\ C\ (N)<em>{13}...3'$ $3'...G\ G\ (N)</em>{13}...5'$</td>
<td>R0662</td>
<td>N/A</td>
</tr>
<tr>
<td>LpnPI</td>
<td>Cleaves DNA containing 5-methylcytosine and 5-hydroxymethylcytosine</td>
<td>$5'...C\ C\ D\ G\ (N)<em>{13}...3'$ $3'...G\ H\ C\ (N)</em>{14}...5'$</td>
<td>R0663</td>
<td>N/A</td>
</tr>
<tr>
<td>McrBC</td>
<td>Cleaves DNA containing 5-methylcytosine, 5-hydroxymethylcytosine or N4-methylcytosine on one or both strands</td>
<td>$5'...PumC(N40-3000)PumC...3'$ Optimum spacing is N55-103</td>
<td>R0272</td>
<td>N/A</td>
</tr>
<tr>
<td>MspJI (I)</td>
<td>Cleaves DNA containing 5-methylcytosine and 5-hydroxymethylcytosine</td>
<td>$5'...C\ N\ N\ R\ (N)<em>{13}...3'$ $3'...G\ N\ N\ Y\ (N)</em>{13}...5'$</td>
<td>R0661</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**References**


Advantages of the MspJI Family

- Specificity to epigenetically relevant DNA modifications (5-mC and 5-hmC)
- Easy-to-use protocols (enzymatic digestion followed by gel extraction)
- Less harsh than bisulfite conversion
- Simplified data analysis

Simplify DNA methylation analysis with MspJI

-MspJI recognizes methylated and hydroxymethylated DNA and cleaves out 32 bp fragments for downstream sequencing analysis. Overnight digestion of 1 µg of genomic DNA from various sources with or without MspJI is shown. Note: Yeast DNA does not contain methylated DNA, therefore no 32-mer is detected.

Protocol for Genomic DNA Digestion (MspJI):

1. Set up the following reaction in a sterile microcentrifuge tube (it is important to add the recommended amount of MspJI last):

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>STANDARD REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA (0.5 to 1 µg)</td>
<td>1-5 µl</td>
</tr>
<tr>
<td>10X NEBuffer 4</td>
<td>3 µl</td>
</tr>
<tr>
<td>BSA</td>
<td>1 µl</td>
</tr>
<tr>
<td>MspJI</td>
<td>0.5–1 µl (2 to 4 units)</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>to 30 µl</td>
</tr>
</tbody>
</table>

2. Incubate at 37°C for 16 hours.

www.neb.com
5-Hydroxymethylcytosine and 5-methylcytosine Identification and Quantification

EpiMark 5-hmC and 5-mC Analysis Kit

The EpiMark 5-hmC and 5-mC Analysis Kit can be used to analyze and quantitate 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) within a specific locus. The kit distinguishes 5-mC from 5-hmC by the addition of glucose to the hydroxyl group of 5-hmC via an enzymatic reaction utilizing T4 phage β-glucosyltransferase (T4-BGT). When 5-hmC occurs in the context of CCGG, this modification converts a cleavableMspI site to a noncleavable one.

Overview of 5-hmC and 5-mC identification using the EpiMark 5-hmC and 5-mC Analysis Kit

**Glucosylation**
Treatment with T4-BGT and UDP-Glc glucosylates all 5-hmC sites, generating 5-ghmC. In the absence of T4-BGT 5-hmC remains intact.

**RE Digestion**
MspI (cleaves 5-mC + 5-hmC DNA; blocked by ghmC)
HpaII (cleavage blocked by 5-mC, 5-hmC and 5-ghmC)

**PCR Analysis**
Product is detected when cleavage is blocked (fragments in blue are intact and will result in PCR product)

Advantages:
- Reproducible quantitation of 5-hmC and 5-mC
- Easy-to-use protocols
- Compatible with existing techniques
- Amenable to high throughput

EpiMark 5-hmC and 5-mC Analysis Kit.........................................................................................E3317S
**T4 Phage β-glucosyltransferase**

T4 Phage β-glucosyltransferase (T4-BGT) is also available as a stand-alone enzyme for the glucosylation of 5-hmC in DNA. This is the same enzyme included in the EpiMark 5-hmC and 5-mC Analysis Kit.

T4 Phage β-glucosyltransferase ................................................................. M0357S

Glucosylation with T4-BGT

![Glucosylation Diagram](image)

C5-hydroxymethylcytosine  Glucosylated 5-hydroxymethylcytosine

Treatment of DNA with T4-BGT and UDP-Glc glucosylates all 5-hydroxymethylcytosine (5-hmC) sites, generating glucosylated 5-hydroxymethylcytosine (5-ghmC).

**Applications of T4 BGT**

- Glucosylation of 5-hmC in DNA (1)
- Immunodetection of 5-hmC in DNA (3)
- Labeling of 5-hmC by incorporation of [3H]- or [14C]- glucose into 5-hmC-containing DNA acceptor after incubation with [3H]- or [14C]- UDP-Glc (4)
- Detection of 5-hmC in DNA by protection from endonuclease cleavage

**References**

Enrichment of Methylated DNA

EpiMark Methylated DNA Enrichment Kit

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 beads coupled with 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:

Step I. Fragment genomic DNA by sonication, nebulization or enzymatic treatment to an average size of less than 1,000 bp

Step II. Generation of bead mixture by combining MBD2a-Fc, Protein A Magnetic Beads and 1X Bind/Wash Reaction Buffer

Step III. Capture of methylated CpG DNA by incubation with MBD2a-Fc/Protein A Magnetic Bead mixture

Step IV. Elute enriched methylated CpG DNA from beads

In the final step, enriched fractions are eluted in small volumes, simplifying downstream applications, including adaptor ligation for next generation sequencing.

Advantages:

- Increased sensitivity
- Easy-to-use protocol yields enriched methylated DNA in less than 2 hours
- Amenable to downstream applications, including next generation sequencing
- Suitable for low levels of input DNA

Comparison of the EpiMark Methylated DNA Enrichment Kit to other commercially available DNA enrichment kits

The Epimark Methylated DNA Enrichment Kit shows excellent signal-to-noise, even at very low levels of input DNA.
Bisulfite Conversion

EpiMark Bisulfite Conversion Kit

Bisulfite conversion, the most common technique for determining the methylation status of DNA, 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.

EpiMark Bisulfite Conversion Kit ................................................................. E3318S

Overview of bisulfite conversion

Advantages:
• Complete conversion of unmodified cytosines to uracil
• Easy-to-use protocol ensures reliable and consistent results
• All reagents, including purification columns, are provided

EpiMark Hot Start Taq DNA Polymerase

EpiMark Hot Start Taq DNA Polymerase is a mixture of Taq DNA Polymerase and a temperature sensitive, aptamer-based inhibitor. This inhibitor binds reversibly to the enzyme, inhibiting polymerase activity below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits PCR reactions to be assembled at room temperature and eliminates an activation step. This aptamer-based hot start activity combined with the supplied reaction buffer, that has been optimized for amplification of converted DNA, makes EpiMark Hot Start Taq an excellent choice for use on bisulfite-treated DNA.

EpiMark Hot Start Taq DNA Polymerase ................................................................. M0490S/L

EpiMark Kit enables complete DNA conversion

1 µg of genomic DNA was bisulfite-treated using the EpiMark Bisulfite Conversion Kit, and 2 µl of eluted DNA was analyzed by end-point PCR using EpiMark Hot Start Taq. Amplification with primer pairs for bisulfite converted DNA (lanes 1, 3, and 5), or with primer pairs for unconverted DNA (lanes 2, 4, and 6) were performed. Lanes 2, 4, and 6 show no amplification product, indicating complete conversion.
DNA Methyltransferases

NEB offers a selection of DNA methyltransferases that can be used to generate methylated DNA at specific sites for gene expression studies. Our selection includes CpG methyltransferases, which is especially useful for studying CpG methylation effects.

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>NEB #</th>
<th>SEQUENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYTOSINE-C5 METHYLTRANSFERASES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human DNA (cytosine-5) Methyltransferase (DNMT1)</td>
<td>M0230S/L</td>
<td>CH₃ 5’... C G...3’ Human DNMT1 3’... G C...5’</td>
</tr>
<tr>
<td>C CpG Methyltransferase (M.SssI)</td>
<td>M0226S/L</td>
<td>CH₃ 5’... C G...3’ 3’... G C...5’</td>
</tr>
<tr>
<td>GpC Methyltransferase (M.CviPI)</td>
<td>M0227S/L</td>
<td>CH₃ 5’... G C...3’ 3’... C G...5’</td>
</tr>
<tr>
<td>AluI Methyltransferase</td>
<td>M0220S/L</td>
<td>CH₃ 5’... A G C T...3’ 3’... T C G A...5’</td>
</tr>
<tr>
<td>HaeIII Methyltransferase</td>
<td>M0224S/L</td>
<td>CH₃ 5’... G G C C...3’ 3’... C C G G...5’</td>
</tr>
<tr>
<td>Hhal Methyltransferase</td>
<td>M0217S/L</td>
<td>CH₃ 5’... G G C G...3’ 3’... C C G G...5’</td>
</tr>
<tr>
<td>HpaII Methyltransferase</td>
<td>M0214S/L</td>
<td>CH₃ 5’... C C G G...3’ 3’... G G C C...5’</td>
</tr>
<tr>
<td>MspI Methyltransferase</td>
<td>M0215S/L</td>
<td>CH₃ 5’... C C G G...3’ 3’... G G C C...5’</td>
</tr>
<tr>
<td><strong>CYTOSINE-N4 METHYLTRANSFERASE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BamHI Methyltransferase</td>
<td>M0221S/L</td>
<td>CH₃ 5’... G G A T T C...3’ 3’... C C T A G G...5’</td>
</tr>
<tr>
<td><strong>ADENINE-N6 METHYLTRANSFERASES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dam Methyltransferase</td>
<td>M0222S/L</td>
<td>CH₃ 5’... G A T C...3’ 3’... C T A G...5’</td>
</tr>
<tr>
<td>EcoRI Methyltransferase</td>
<td>M0211S/L</td>
<td>CH₃ 5’... G A A T T C...3’ 3’... C T T A G...5’</td>
</tr>
<tr>
<td>TaqI Methyltransferase</td>
<td>M0219S/L</td>
<td>CH₃ 5’... T C G A...3’ 3’... A G C T...5’</td>
</tr>
</tbody>
</table>

Applications

- Blocking restriction enzyme cleavage
- Generating positive control DNA samples for methylation-specific PCR or bisulfite sequencing experiments
- Studying CpG methylation-dependent gene expression [CpG Methyltransferase (M.SssI), NEB# M0226]
- Probing sequence-specific contacts within the major groove of DNA
- Nucleosome footprinting
- Uniform [³H]-labeling of DNA
- Altering the physical properties of DNA [e.g., methylcytosines lower the free energy of Z-DNA formation (1), increase the helical pitch of DNA (2), alter the kinetics of cruciform extrusion (3) and decrease reactivity to hydrazine (4)]
Genomic DNA Methylation Using CpG Methyltransferase (M. SssI)

CpG Methyltransferase (M. SssI) may be useful for studying the function of cytosine methylation in higher eukaryotes as its specificity mimics the pattern of modification found in their genomes (1). In contrast to the mammalian enzymes (2,3), both unmethylated and hemi-methylated DNA substrates are methylated with equal efficiency by this CpG methyltransferase (4), making it a more useful tool for modifying DNA.

CpG Methyltransferase can be used to block cleavage by a variety of restriction endonucleases whose recognition sites either contain the sequence CG, or overlap the dinucleotide. It should be noted that DNAs methylated by the CpG Methyltransferase are subject to Mcr and Mrr restriction in E. coli, and thus should be transformed into Mcr- Mrr- E. coli strains.

The high density of CpG dinucleotides in DNA substrates should be taken into account when methylating DNAs in vitro. For example, lambda DNA (48,502 bp) contains 3, 112 CpG sites, and thus a 0.1 mg DNA/ml solution is 19 µM with respect to methyl acceptor sites for the methyltransferase. This is significant because the recommended concentration of methyl donor, S-adenosylmethionine (SAM, AdoMet), is 160 µM, an 8-fold excess over acceptor sites. First, the SAM concentration remains high enough to drive the reaction. Second, potential end-product inhibition, arising from S-adenosyl-L-homocysteine (SAH, AdoHcy) generated during the reaction, is limited.

Protocol:

1. For the standard reaction in step 2, dilute SAM to 1600 µM using the supplied 32 mM stock. (1 µl SAM, 19 µl Nuclease-free water).

2. Add the following to a sterile microcentrifuge tube, in the order listed:

<table>
<thead>
<tr>
<th>Nuclease-free water</th>
<th>14 µl</th>
<th>220 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X NEBuffer 2</td>
<td>2 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>SAM</td>
<td>2 µl from step 1</td>
<td>10 µl (32 µM SAM)</td>
</tr>
<tr>
<td>Genomic DNA</td>
<td>1 µl (1 µg)</td>
<td>200 µl (500µg/ml λ DNA)</td>
</tr>
<tr>
<td>CpG methylase (M. SssI)</td>
<td>1 µl (4 U/µl)</td>
<td>20 µl (20 U/µl)</td>
</tr>
</tbody>
</table>

3. Mix by pipetting up and down at least six times.

4. Incubate for one hour at 37°C.

5. Stop the reaction by heating at 65°C for 20 minutes.

6. DNA can be purified by phenol extraction followed by ethanol precipitation or by using a commercial DNA purification kit. For long-term storage at -20°C, suspend in TE.

Tips:

- MgCl₂ is not required as a cofactor. In the presence of Mg²⁺, methylation by M. SssI becomes distributive rather than processive and also exhibits topoisomerase activity (5).
- Adding more AdoMet after 4 hours can improve results, and using more enzyme for less time may improve methylation. Methylation reactions, however, are greatly affected by AdoHcy (6), which is a by-product of the methylation reaction and binds more tightly to methylases than does AdoMet. Inhibition by AdoHcy greatly reduces the reaction rate.
- The incubation time can be increased to 4 hours. Overnight incubations do not give significant increases in methylation.
- The volume of DNA can be increased to 5 µl. When using more dilute DNA, increase the reaction volume to 50 µl. Using too much DNA volume in the reaction can cause inhibition by changing the pH or salt concentration of the reaction.
- Up to 4 µg of DNA can be methylated in a 20 µl reaction. The SAM concentration should be adjusted to 640 µM. Concentrated Ssal (NEB #M0226M) (1 µl of 20,000 U/ml) should be used.
- The protocol can also be used for other types of DNA, including plasmids and purified PCR products.

References
Sample Preparation for ChIP-Seq

NEBNext Reagents

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 the NEBNext ChIP-Seq Library Prep Reagent Set and Master Mix Set for Illumina, and the NEBNext ChIP-Seq Library Prep Set for SOLiD. With similar workflows to the NEBNext DNA Library Prep Kits, the ChIP-Seq kits require lower amounts of input DNA and are compatible with commercially available ChIP kits.

NEBNext ChIP-Seq Library Prep Reagent Set for Illumina ................................................... E6200S/L
NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina ............................................. E6240S/L
NEBNext ChIP-Seq Library Prep Set for SOLiD .................................................................... E6260S/L

Advantages:

- Enzymes and buffers are provided at concentrations and volumes suitable for the included protocols. Reagents are available in master mix formats for additional convenience.
- Each set is functionally validated by preparation of a library that is sequenced using the appropriate platform (Illumina or SOLiD)
- Additional quality controls ensure maximum quality and purity
- Value pricing

For a complete list of NEBNext reagents for sample prep of DNA or RNA for next generation sequencing, visit www.NEBNext.com.
Human DNA (cytosine-5) Methyltransferase Antibodies

There are three functional DNMTs in mammals. DNMT1 is a maintenance enzyme that methylates hemi-methylated CpG dinucleotides in mammalian genomes. The amino terminus (N-terminus) of human DNMT1 has been shown to bind proliferating cell nuclear antigen (PCNA) and histone deacetylase (1,2). Deletion of this region stimulates DNA methylation (3). In neoplastic transformed cells methylation pattern is abnormal, leading to the speculation that DNMT1 may be involved in such processes (4). DNMT3A and DNMT3B are de novo mammalian methyltransferases that are architecturally similar to DNMT1 (5). The amino terminus of human DNMT3A contains various motifs that recruit transcriptional repressors (6). The carboxy terminus (C-terminus) of human DNMT3B contains the conserved catalytic motifs and is responsible for catalytic function. DNMT3A and DNMT3B cooperate with other DNA methyltransferases to maintain DNA methylation (7,8). For more information about DNMTs, see page 8.

Polyclonal antibodies from NEB are produced by immunizing rabbits with a KLH-coupled synthetic peptide corresponding to residues in the DNMT peptides.

Human DNA Methyltransferase (DNMT1) Amino-terminal Antibody ........................................ M0231S/L
Human DNA Methyltransferase (DNMT3A) Amino-terminal Antibody .................................... M0229S
Human DNA Methyltransferase (DNMT3B) Carboxy-terminal Antibody ............................... M0232S

Methylated and Hypomethylated DNA

Positive and negative control DNAs are especially important for studies using sensitive PCR-based assays. NEB offers three sets of genomic DNA that are untreated or treated with CpG Methylase (M. SsoI), which methylates cytosine residues (C5) within the double-stranded dinucleotides recognition sequence 5′…CG…3′. The methylation-positive DNAs are extensively tested for complete methylation by an additional methyl group transfer assay and methylation-specific PCR.

A partially demethylated DNA control has also been created by treating Jurkat cells with a potent methyltransferase inhibitor (5-Aza-2-deoxycytidine, 5-Aza-dc). Hypomethylation is verified using bisulfite conversion and sequencing to analyze a section of intergenic (IGS) repetitive DNA, which is normally highly methylated.

Jurkat Genomic DNA ............................................................................................................. N4001S
CpG Methylated Jurkat Genomic DNA ...................................................................................... N4002S
5-Aza-dc–Treated Jurkat Genomic DNA ............................................................................... N4003S
NIH 3T3 Mouse Genomic DNA ............................................................................................. N4004S
CpG Methylated NIH 3T3 Genomic DNA ............................................................................. N4005S
HeLa Genomic DNA ........................................................................................................... N4006S
CpG Methylated HeLa Genomic DNA ................................................................................... N4007S

Applications of DNMT Antibodies:
- Western blotting (M0231S/L, M0229S, M0232S)
- Immunocytochemistry (M0229S)
- Immunoprecipitation (M0231S/L, M0232S)

Applications for Genomic DNAs:
- PCR
- SNP analysis
- Southern blotting
- Genomic DNA library construction
- Methylation-specific PCR (MSP)
- Bisulfite sequencing
- Methylation-sensitive single-nucleotide primer extension (ms-SNUPE)
- Combined bisulfite restriction analysis (COBRA)
- Bisulfite treatment and PCR single-stranded confirmation polymorphism analysis (Bisulfite-PCR-SSCP/BiPS)

References
5-hydroxymethylcytosine (5-hmC) – A nucleotide originally discovered in bacteriophage genomes that was recently identified in mice (embryonic stem cells and neural cells). The role of this modified base is not known, but it may be involved in demethylation or it may influence chromatin structure and local transcriptional activity.

5-methylcytosine (5-mC) – The primary form of methylated DNA in mammals, which most commonly occurs at CpG dinucleotides.

ChIP – Chromatin immunoprecipitation is the most commonly used method for analyzing histone modifications. Antibodies specific for histone modifications are used to precipitate DNA fragments for subsequent analysis.

COBRA – Combined Bisulfite Restriction Analysis, which involves digesting PCR amplicons from untreated and bisulfite-treated DNA with methylation-sensitive or -insensitive restriction enzymes. The resulting DNA fragments are electroblotted, hybridized to radiolabeled oligonucleotides and quantitated by densitometry.

CpG Island – Cluster of CpG dinucleotides in a DNA region that is defined using computational methods. Although most CpG dinucleotides are methylated, those in CpG islands are often unmethylated and upstream of gene coding sequences.

DNA Methylation – DNA can be modified by methylation of adenine and cytosine bases in a wide variety of prokaryotes and eukaryotes. In higher eukaryotes, DNA methylation is involved in gene regulation, chromatin structure, differentiation, imprinting, carcinogenesis, complex diseases and aging.

DNA Methyltransferases – Enzymes that use S-adenosylmethionine as the methyl donor to generate methylated adenines and cytosines. Some are specific for hemi-methylated DNA (maintenance enzymes), and some are specific for unmethylated DNA (de novo enzymes).

Euchromatin – In mammals, transcriptionally active chromatin, which is less condensed than heterochromatin.

Glucosyltransferase – Enzymes that transfer glucosyl groups from one compound to another. The T4 phage enzyme specifically transfers glucose to 5-hydroxymethylcytosine (5-hmC) and can be used in epigenetic studies to distinguish 5-hmC from 5-methylcytosine (5-mC).

HELP Assay – HpaII tiny fragment enrichment by ligation-mediated PCR involves digesting genomic DNA with either a methylation-sensitive enzyme (e.g., HpaII) or its methylation-insensitive isoschizomer (e.g., MspI). The digestion products are ligated with oligonucleotide pairs forming cohesive ends with restriction enzyme recognition sequences, becoming the template for ligation-mediated PCR.

Heterochromatin – Transcriptionally silent and highly condensed chromatin in mammals.

HRM – High Resolution Melting Analysis is a real-time PCR-based method that relies on a temperature-dependent release of fluorescent dyes that were intercalated into untreated and bisulfite-treated DNA samples. The rate of DNA melting reflects differences in the C:T content determined by the level of methylation.

Imprinting – An epigenetic process such that only the maternal allele or only the paternal allele are expressed. Less than one percent of mammalian genes are imprinted.

Linker Histone – Histone H1 binds to linker DNA between nucleosome core particles and helps to further condense chromatin.

MeDIP – Methylated DNA Immunoprecipitation is used to enrich for methylated DNA fragments for subsequent analysis.

Methylation-dependent Restriction Enzyme – Restriction enzyme that requires DNA methylation for cleavage.

Methylation-sensitive Restriction Enzyme – Restriction enzyme whose activity is blocked or impaired by DNA methylation.

MIRA – Methylated CpG Island Recovery Assay involves using the MBD2/MBD3L1 complex (a high-affinity, methylated DNA-binding protein complex) to enrich methylated DNA fragments for subsequent analysis.

MS-SnuPE – Methylation-Sensitive single nucleotide Primer Extension involves analyzing untreated and bisulfite-treated DNA based on primer extension assays using bisulfite-specific primers that anneal to the sequence immediately before the CpG of interest. The primer extends one base pair into the C or T, using DNA polymerase terminating deoxynucleotides (dNTPs), and the ratio of C to T can then be determined quantitatively using radioactive or fluorescent dNTPs, sequencing, MALDI-TOF mass spectrometry and/or HPLC.

MS-SSCA – Methylation-Sensitive Single-Strand Conformation Analysis, which involves analyzing untreated and bisulfite-treated DNA by assessing differential migration of single-stranded DNA containing the CpG sites of interest through nondenaturing gels. The C to T content will vary with methylation status.

MSCC Assay – Methylation-Sensitive Cut Counting Assay involves using methylation-sensitive enzymes to fragment genomic DNA for massively parallel sequencing.

MSP – Methylation-Specific PCR involves analyzing untreated and bisulfite-treated DNA using two sets of PCR primer pairs that target the unaltered, methylated sequence and the converted, unmethylated sequence.

Nucleosome Core Particles – Organizational unit of chromatin that consists of ~147 bp of DNA and an octamer of histones (typically, two each of the core histones).

qAMP – Quantitative Analysis by Methylation-sensitive PCR involves using methylation-sensitive enzymes to fragment genomic DNA for quantitative analysis by real-time PCR.

RRBS – Reduced Representation Bisulfite Sequencing is a method involving sequencing of untreated and bisulfite-treated DNA fragments that were adapter-ligated and size-selected after restriction enzyme digestion.

Sodium Bisulfite Conversion – Process of treating genomic DNA with sodium bisulfite to convert (i.e., deaminate) unmethylated cytosines to uracil (methylcytosines are not converted). A comparison of sodium bisulfite-treated and untreated DNA after PCR amplification provides information about the methylation status of the DNA, because uracils amplify as thymines and methylated cytosines amplify as cytosines.

X-inactivation – Mammalian mechanism for dosage compensation for X chromosome genes in females. In placental mammals, the randomly-inactivated X chromosome is packaged as heterochromatin.
## ORDERING INFORMATION

To learn how these products can help you to better understand epigenetic changes, visit [www.epimark.com](http://www.epimark.com).

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>NEB #</th>
<th>SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>METHYLATION ANALYSIS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MspI</td>
<td>R0661S/L</td>
<td>200/1,000 units</td>
</tr>
<tr>
<td>LpnPl</td>
<td>R0663S/L</td>
<td>250/1,250 units</td>
</tr>
<tr>
<td>FspEI</td>
<td>R0662S/L</td>
<td>250/1,250 units</td>
</tr>
<tr>
<td>EpiMark™ 5-hmC and 5-mC Analysis Kit</td>
<td>E3317S</td>
<td>20 reactions</td>
</tr>
<tr>
<td>EpiMark™ Bisulfite Conversion Kit</td>
<td>E3318S</td>
<td>48 reactions</td>
</tr>
<tr>
<td>EpiMark™ Hot Start Taq DNA Polymerase</td>
<td>M0490S/L</td>
<td>100/500 reactions</td>
</tr>
<tr>
<td>EpiMark™ Methylated DNA Enrichment Kit</td>
<td>E2600S</td>
<td>25 reactions</td>
</tr>
<tr>
<td>5-Methyl-dCTP</td>
<td>N0356S</td>
<td>1 μmol</td>
</tr>
<tr>
<td><strong>METHYLTRANSFERASES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G9a Methyltransferase</td>
<td>M0235S</td>
<td>100 units</td>
</tr>
<tr>
<td>SET7 Methyltransferase</td>
<td>M0233S</td>
<td>100 units</td>
</tr>
<tr>
<td>Human DNA (cytosine-5) Methyltransferase (Dnmt1)</td>
<td>M0230S/L</td>
<td>50/250 units</td>
</tr>
<tr>
<td>CpG Methyltransferase (M.SssI)</td>
<td>M0226S/M/L</td>
<td>100/500/2,500 units</td>
</tr>
<tr>
<td>GpC Methyltransferase (M.CviPI)</td>
<td>M0227S/L</td>
<td>200/1,000 units</td>
</tr>
<tr>
<td>HpaII Methyltransferase</td>
<td>M0214S/L</td>
<td>100/500 units</td>
</tr>
<tr>
<td>MspI Methyltransferase</td>
<td>M0215S/L</td>
<td>100/500 units</td>
</tr>
<tr>
<td>EcoRI Methyltransferase</td>
<td>M0211S/L</td>
<td>10,000/50,000 units</td>
</tr>
<tr>
<td>dam Methyltransferase</td>
<td>M0222S/L</td>
<td>500/2,500 units</td>
</tr>
<tr>
<td>BamHI Methyltransferase</td>
<td>M0223S/L</td>
<td>100/500 units</td>
</tr>
<tr>
<td>HhaI Methyltransferase</td>
<td>M0217S/L</td>
<td>1,000/5,000 units</td>
</tr>
<tr>
<td>TaqI Methyltransferase</td>
<td>M0219S/L</td>
<td>1,000/5,000 units</td>
</tr>
<tr>
<td>AluI Methyltransferase</td>
<td>M0220S/L</td>
<td>100/500 units</td>
</tr>
<tr>
<td>HaeIII Methyltransferase</td>
<td>M0224S/L</td>
<td>500/2,500 units</td>
</tr>
<tr>
<td><strong>CONTROL DNA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jurkat Genomic DNA</td>
<td>N4001S</td>
<td>15 μg</td>
</tr>
<tr>
<td>CpG Methylated Jurkat Genomic DNA</td>
<td>N4002S</td>
<td>15 μg</td>
</tr>
<tr>
<td>5-Aza-dc Treated Jurkat Genomic DNA</td>
<td>N4003S</td>
<td>15 μg</td>
</tr>
<tr>
<td>NIH 3T3 Mouse Genomic DNA</td>
<td>N4004S</td>
<td>15 μg</td>
</tr>
<tr>
<td>CpG Methylated NIH3T3 Mouse Genomic DNA</td>
<td>N4005S</td>
<td>15 μg</td>
</tr>
<tr>
<td>HeLa Genomic DNA</td>
<td>N4006S</td>
<td>15 μg</td>
</tr>
<tr>
<td>CpG Methylated HeLa Genomic DNA</td>
<td>N4007S</td>
<td>15 μg</td>
</tr>
<tr>
<td><strong>HISTONES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpiMark Nucleosome Assembly Kit</td>
<td>E5150S</td>
<td>20 reactions</td>
</tr>
<tr>
<td>H1a Human, Recombinant</td>
<td>M2501S</td>
<td>100 μg</td>
</tr>
<tr>
<td>H2A Human, Recombinant</td>
<td>M2502S</td>
<td>100 μg</td>
</tr>
<tr>
<td>H2B Human, Recombinant</td>
<td>M2505S</td>
<td>100 μg</td>
</tr>
<tr>
<td>H3.1 Human, Recombinant</td>
<td>M2503S</td>
<td>100 μg</td>
</tr>
<tr>
<td>H3.2 Human, Recombinant</td>
<td>M2506S</td>
<td>100 μg</td>
</tr>
<tr>
<td>H3.3 Human, Recombinant</td>
<td>M2507S</td>
<td>100 μg</td>
</tr>
<tr>
<td>H4 Human, Recombinant</td>
<td>M2504S</td>
<td>100 μg</td>
</tr>
<tr>
<td>Histone H3.1/H4 Tetramer Human, Recombinant</td>
<td>M2509S</td>
<td>1 nmol</td>
</tr>
<tr>
<td>Histone H2A/H2B Dimer Human, Recombinant</td>
<td>M2508S</td>
<td>1 nmol</td>
</tr>
<tr>
<td>Nucleosome Control DNA</td>
<td>N1202S</td>
<td>0.2 nmol</td>
</tr>
</tbody>
</table>