Epigenetics

UNDERSTANDING HISTONE & DNA MODIFICATIONS

Update 2015/16

NEW ENGLAND BioLabs® GmbH

Epigenetics

For over 40 years, New England Biolabs has been committed to understanding the mechanisms of restriction and methylation of DNA. This expertise in enzymology has led to the development of a suite of validated products for epigenetics research. These unique solutions to study DNA and histone modifications are designed to address some of the challenges of the current methods. 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, life style, 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.

TOOLS & RESOURCES

Visit www.epimark.com to find:

- An interactive tutorial explaining the phenomenon of epigentics at the molecular level
- Videos from NEB scientists discussing the concept of epigenetics
- Videos and tutorials from NEB scientists explaining methods for 5-hmC and 5-mC detection and quantitation

INTERACTIVE TUTORIAL EXPLAINING THE PHENOMENON OF EPIGENETICS AT THE MOLECULAR LEVEL

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

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

References
1. Tost, J. (2010) *Mol. Biotechnol.* 44, 71-81.

NEB offers a suite of EpiMark validated reagents to study DNA and histone modifications. Visit www.epimark.com for the latest list of reagents available from NEB.

EpiMark Validated Reagents for Epigenetic Studies

HISTONES (Pages 4–6)	
Histone $H1^0$ Human, Recombinant	Histone H3.3 Human, Recombinant
Histone H2A Human, Recombinant	H4 Human, Recombinant
Histone H2B Human, Recombinant	EpiMark Nucleosome Assembly Kit
Histone H3.1 Human, Recombinant	Histone H2A/H2B Dimer
Histone H3.2 Human, Recombinant	Histone H3.1/H4 Tetramer
HISTONE/PROTEIN METHYLTRANSFERASES (Page 8)	
G9a Methyltransferase	5-methyl-dCTP
PRMT1 Methyltransferase	SET8 Methyltransferase
SET7 Methyltransferase	
RESTRICTION ENZYMES (Pages 12–13)	
AbaSI	HpaII
DpnI	LpnPI
DpnII	MspI
FspEI	MspJI
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.SssI)	BamHI Methyltransferas
GpC Methyltransferase (M.CviPI)	HhaI Methyltransferase
HpaII Methyltransferase	*Taq*I Methyltransferase
MspI Methyltransferase	AluI Methyltransferase
EcoRI Methyltransferase	HaeIII Methyltransferase
SAMPLE PREP FOR NEXT GEN SEQUENCING (Page 20)	
NEBNext® ChIP-Seq Library Prep Master Mix Set for Illumina®	NEBNext® Ultra™ DNA Library Prep Kit for Illumina
NEBNext ChIP-Seq Library Prep Reagent Set for Illumina	NEBNext Modules (see Page 23)
CONTROL DNAs (Page 21)	
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).

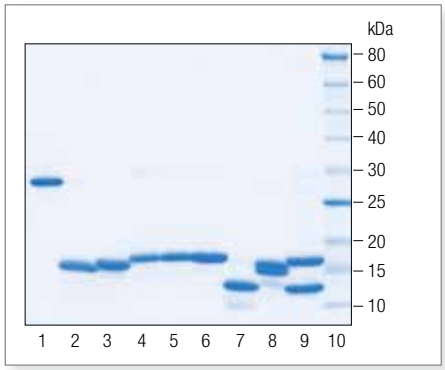
APPLICATIONS

- 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

TOOLS & RESOURCES

- Visit **www.epimark.com** for more information on histone modifications

Highly Purified Histones from NEB



Experience the purity of Histones from NEB.
SDS-PAGE analysis of the histones available from NEB.
1. Histone H1° (NEB #M2501) 1 µg
2. Histone H2A (NEB #M2502) 1 µg
3. Histone H2B (NEB #M2505) 1 µg
4. Histone H3.1 (NEB #M2503) 1 µg
5. Histone H3.2 (NEB #M2506) 1 µg
6. Histone H3.3 (NEB #M2507) 1 µg
7. Histone H4 (NEB #M2504) 1 µg
8. Histone H2A/H2B Dimer (NEB #M2508) 2 µg
9. Histone H3.1/H4 Tetramer (NEB #M2509) 2 µg
10. NEB Protein Ladder (NEB #P7703)

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

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2. Kim, J.K., Samaranayake, M. and Pradhan, S. (2009) *Cell. Mol. Life Sci.*, 66, 596–612.
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Recombinant Human Histones

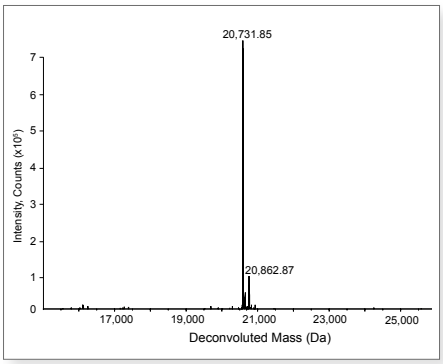
Histone H1°

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

Mass Spectroscopy Analysis of Histone H1° Human, Recombinant



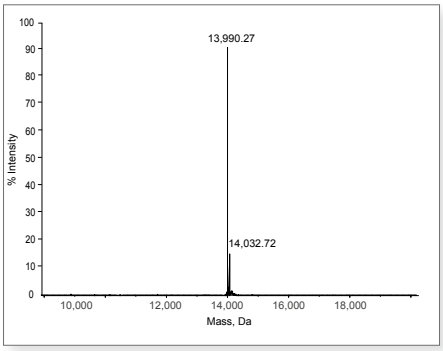
Histones H2A & H2B

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).

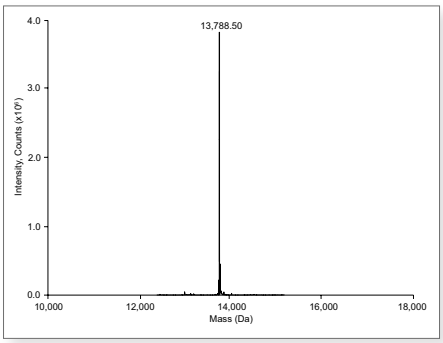
Recombinant human histones H2A and H2B are expressed in *E. coli* using the HIST3H2A gene (Genbank accession number: AY131974) and the HIST2H2BE or H2BFQ gene (Genbank accession number: AY131979), respectively. NEB also offers the preformed histone H2A/H2B dimer. This is generated by refolding the denatured,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

Mass Spectroscopy Analysis of Histone H2A Human, Recombinant



Mass Spectroscopy Analysis of Histone H2B Human, Recombinant



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2. Gongidi, P., et al. (2002) *Genomics*, 80, 487–497.
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Histones H3 & H4

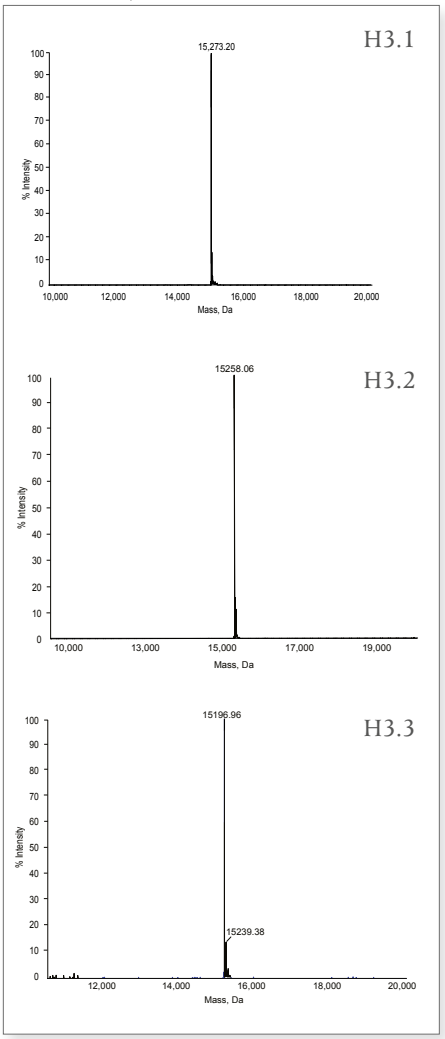
Histone H3 interacts with histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer (1,2).

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).

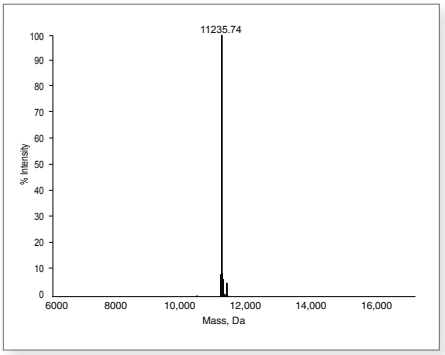
Recombinant human histones H3.1, H3.2 and H3.3 are synthesized in *E. coli* using the HIST1H3A or H3FA gene (Genbank accession number: AF531274), HIST2H3A or HIST2H3C gene (Genbank accession number: BC130637) and H3F3A or H3F3B gene (Genbank accession number: AK311905), respectively. Recombinant human 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

Mass Spectroscopy Analysis of Histone H3 Human, Recombinant



Mass Spectroscopy Analysis of Histone H4 Human, Recombinant



References
1. Kornberg, R.D. (1977) *Annu. Rev. Biochem.*, 46, 931–954.
2. van Holde, K.E. (1989) *Chromatin*, 1–497.
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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

AMINO ACID	MODIFICATION
Lysine	Methylation, Acetylation, Ubiquitination, Sumoylation, ADP-Ribosylation
Arginine	Methylation
Serine	Phosphorylation
Threonine	Phosphorylation

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).

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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.

APPLICATIONS

- Purification and characterization of enzymes that modify histone proteins
- Octamer modification studies
- Carrier Chromatin Immunoprecipitation (CChIP)

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.

SET8 Methyltransferase

SET8 (PR-Set7) Methyltransferase mono-methylates lysine 20 of histone H4 (H4-K20) at the ε amino group of lysine residues. SET8-mediated histone H4 methylation is implicated in genome replication and stability; and plays an important role in the nodal pathways of embryo development.

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).

G9a Methyltransferase	M0235S
Human PRMT1 Methyltransferase	M0221S
SET7 Methyltransferase	M0233S
SET8 Methyltransferase	M0428S
Human DNA (cytosine-5) Methyltransferase (DNMT1)	M0230S/L

References
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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

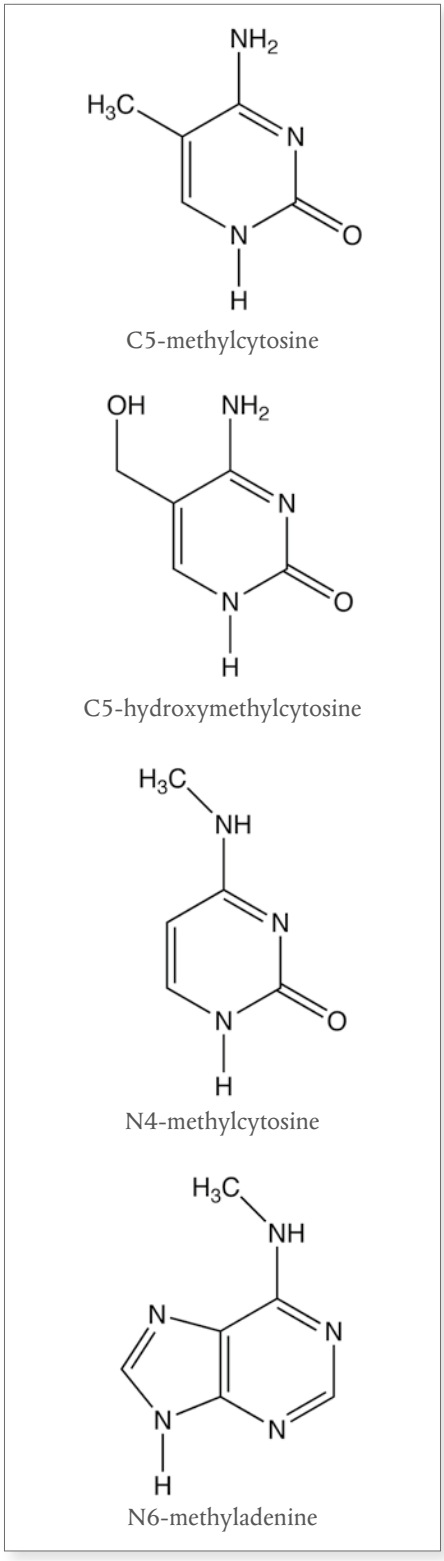
DNA methylation in mammals primarily occurs on the fifth carbon of the cytosine base (5-methylcytosine, 5-mC, see sidebar) of CpG dinucleotides, and approximately 70% to 80% of CpG dinucleotides are methylated in somatic cells. However, 5-mC at CpA, CpT and CpC sequences have been found in genomic DNA 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

METHYLATED BASE	ORGANISM	DNA METHYLATION SEQUENCE
C5-methylcytosine	Bacteria	Varies (e.g., CCAGG, CCTGG)
	Some Fungi, Some Insects, Mammals	CpG, CpH*pG, CpH*pH
	Plants	CpG, CpH*pG, CpH*pH
C5-hydroxymethylcytosine	Bacteriophages	Varies (e.g., CCGG, GATC); Some contain only modified cytosines
	Mammals	CpG, CpH*pG, CpH*pH
N4-methylcytosine	Bacteria	Varies (e.g., CTCTTC, CCCGGG)
N6-methyladenine	Bacteria, Bacteriophages, Archaea, Protists, Some Fungi, Plants	Varies (e.g., GATC, GANTC, GAAGAG)

* = *Adenine, Cytosine, or Thymine*



Types of DNA modifications.

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Methods for Studying DNA Methylation

There are three main approaches for studying DNA methylation. These are based on pretreating 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.

TOOLS & RESOURCES

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

References
1. Laird, P.W. (2010) Nat. Rev. Genet. 11, 191–203.
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Table 3: Approaches for Studying DNA Methylation

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	APPLICATION
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

	METHYLATION SENSITIVITY	SEQUENCE	NEB#	ISOSCHIZOMER
DpnII	Cleaves dam sites** which lack adenomethylation and is blocked by complete dam methylation and probably by hemi-methylation	5′...▼GATC...3′ 3′...CTAG▲...5′	R0543	MboI DpnII
HpaII	Will not cleave methylated CpG sites	5′...C▼CGG...3′ 3′...GGC▲C...5′	R0171	MspI
MspI	Not methylation sensitive	5′...C▼CGG...3′ 3′...GGC▲C...5′	R0106	HpaII

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

APPLICATIONS

- Differentiation of methylation patterns

RESTRICTION ENZYME DIGESTION PROTOCOL

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

COMPONENT	25 µl REACTION	50 µl REACTION
DNA	0.5 µg	1 µg
10X NEBuffer	2.5 µl	5 µl
Nuclease-free water	to 25 µl	to 50 µl
Restriction Enzyme*	5 units	10 units

** 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 resriction 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

- 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 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

	METHYLATION SENSITIVITY	SEQUENCE	NEB #	ISOSCHIZOMER
AbaSI (1, 2, 3)	Recognizes 5-glucosylhydroxy-methylcytosine (^{ghm}C) in double-stranded DNA and cleaves 11–13 bases 3′ from the modified C	5′...^{ghm}C N_{11-13} N_{9-16} G...3′ 3′.....G N_{9-10} N_{11-13} ^{x}C...5′ ^{x}C = ^{ghm}C, ^{hm}C, ^{m}C or C	R0665	N/A
DpnI	Cleaves fully-adenomethylated dam** sites (hemi-adenomethylated dam sites 60X more slowly). Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.	CH_3 5′... G A T C ...3′ 3′... C T A G ...5′ CH_3	R0176	DpnII
FspEI (6)	Cleaves DNA containing 5-methylcytosine and 5-hydroxymethylcytosine	5′... C ^{m}C $(N)_{12}$ ...3′ 3′... G G $(N)_{16}$ ...5′	R0662	N/A
LpnPI (6)	Cleaves DNA containing 5-methylcytosine and 5-hydroxymethylcytosine	5′... C ^{m}C D G $(N)_{10}$ ▼...3′ 3′... G G H C $(N)_{14}$ ▲...5′	R0663	N/A
McrBC	Cleaves DNA containing 5-methylcytosine, 5-hydroxymethylcytosine or N4-methylcytosine on one or both strands.	5′...PumC(N40-3000)PumC...3′ Optimum spacing is N55-103 Pu = A or G Cleavage site is between the half-sites and ~30 bp from one of the half-sites	M0272	N/A
MspJI (4,5,6,7,8)	Cleaves DNA containing 5-methylcytosine and 5-hydroxymethylcytosine	5′... ^{m}C N N R $(N)_{9}$ ▼...3′ 3′... G N N Y $(N)_{13}$ ▲...5′	R0661	N/A

** dam sites: methylation at the N6 position of the adenine in the sequence GATC (GmATC).
H = A or C or T, not G
D = A or G or T, not C

ADVANTAGES

- 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

GENOMIC DNA DIGESTION (MspJI) PROTOCOL

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

COMPONENT	STANDARD REACTION
DNA (0.5 to 1 µg)	1–5 µg
10X NEBuffer 4	3 µl
BSA	1 µl
MspJI	0.5–1 µl (2 to 4 units)
Nuclease-free water	to 30 µl

2. Incubate at 37°C for 16 hours.

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5-Hydroxymethylcytosine and 5-methylcytosine Identification and Quantification

EpiMark 5-hmC and 5-mC Analysis Kit

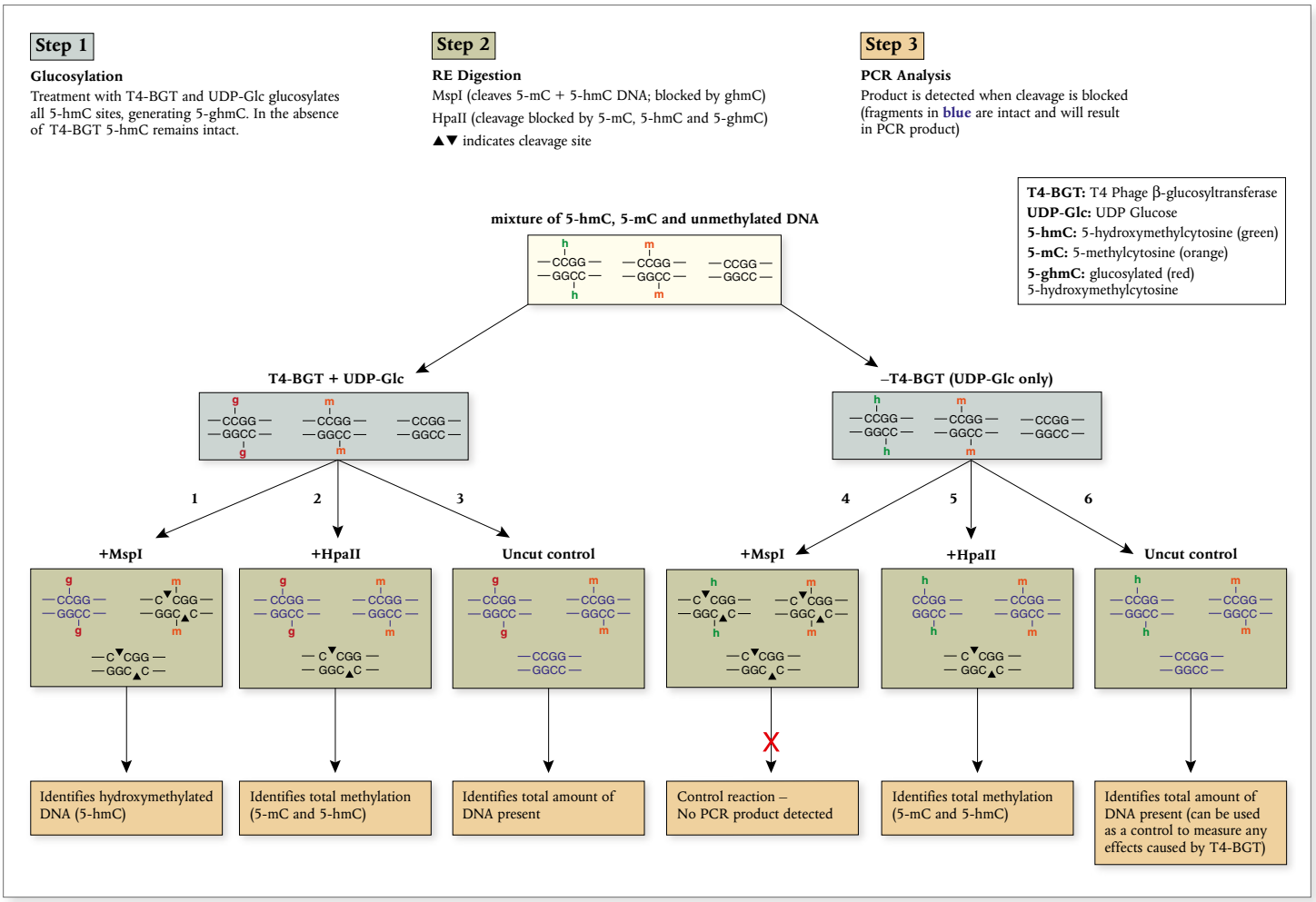
The EpiMark 5-hmC and 5-mC Analysis Kit can be used to analyze and quantitate 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) within a specific locus. The kit distinguishes 5-mC from 5-hmC by the addition of glucose to the hydroxyl group of 5-hmC via an enzymatic reaction utilizing T4 phage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

EpiMark 5-hmC and 5-mC Analysis Kit E3317S

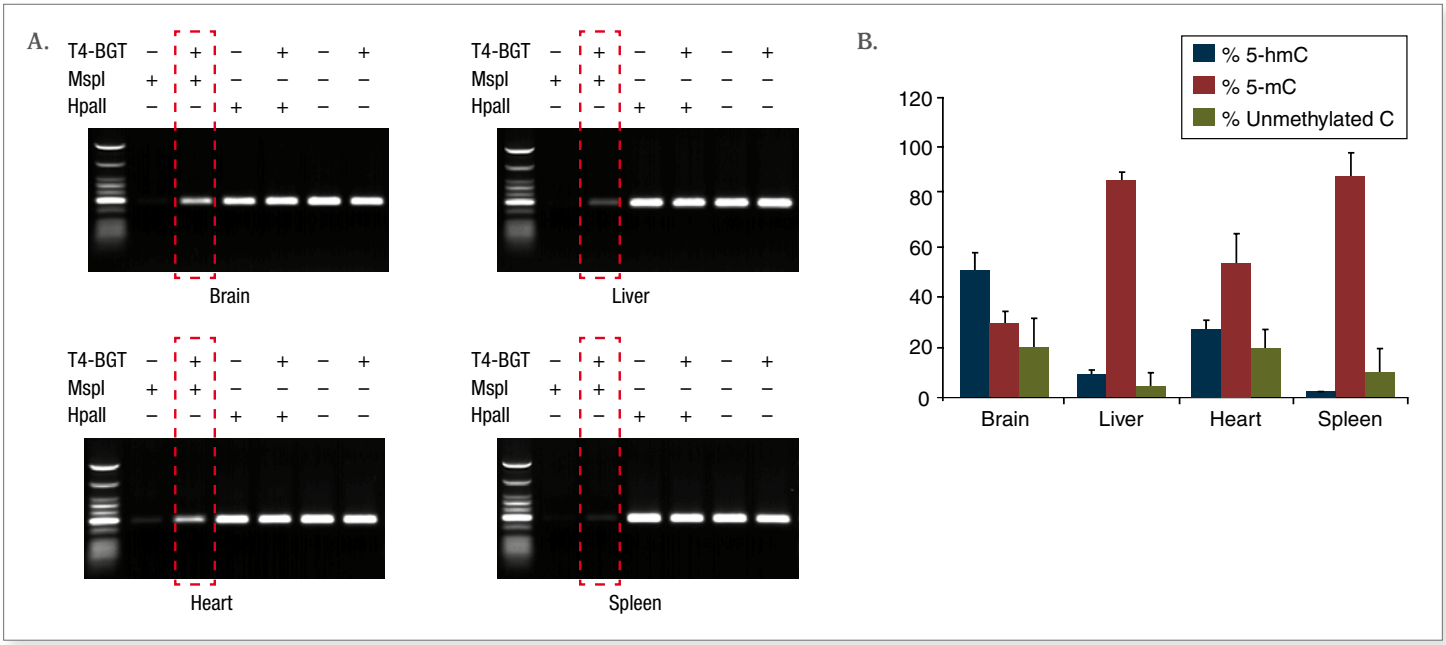
ADVANTAGES

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

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



Analysis of the different methylation states in Balb/C mouse tissue samples using the EpiMark 5-hmC and 5-mC Analysis Kit



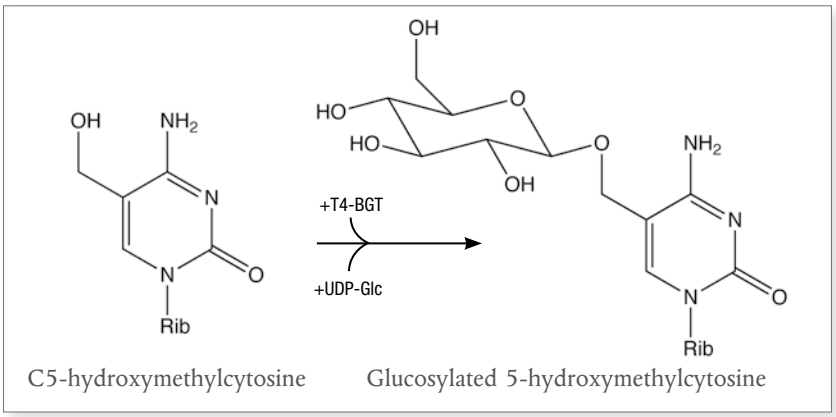
A) Endpoint PCR of the 6 different reactions needed for methylation analysis. The boxed lanes indicate the presence of 5-hmC. B) Real-time PCR data was used to determine amounts of 5-hmC and 5-mC present. The results demonstrate a variation in 5-hmC levels in the tissue sources indicated.

T4 Phage β-glucosyltransferase

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



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

APPLICATIONS

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

References
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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

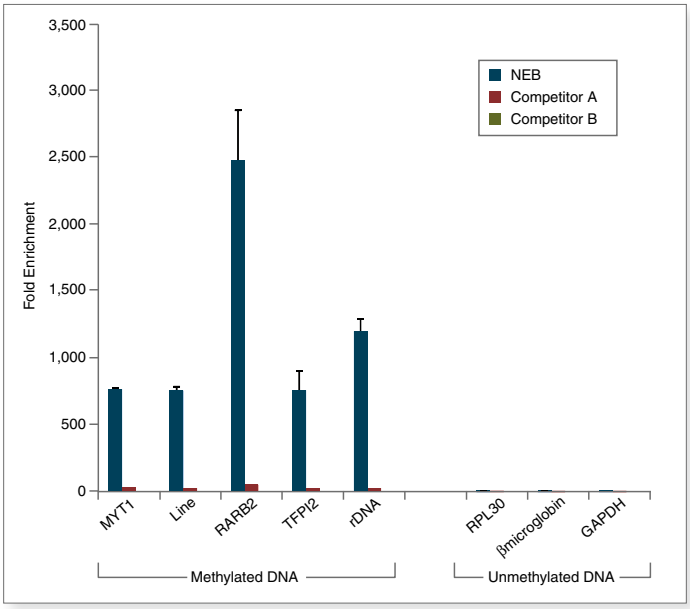
In the final step, enriched fractions are eluted in small volumes, simplifying downstream applications, including adaptor ligation for next generation sequencing.

EpiMark Methylated DNA Enrichment Kit E2600S

ADVANTAGES

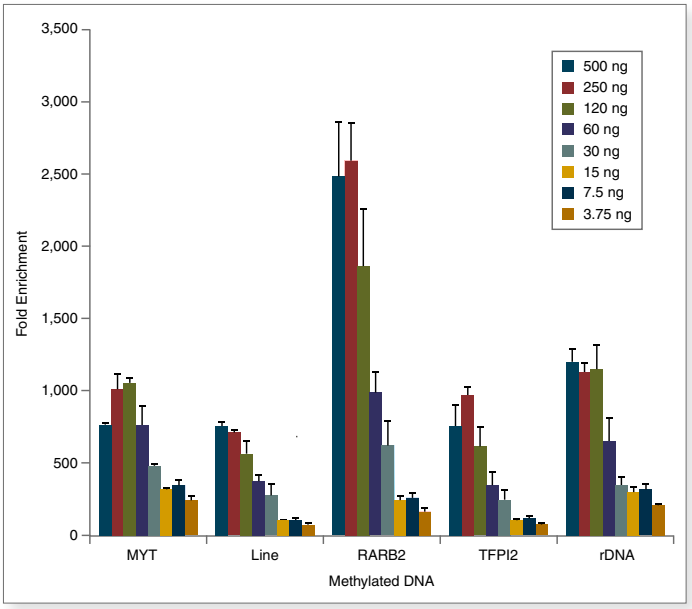
- Increased sensitivity
- Easy-to-use protocol yields enriched methylated DNA in less than 2 hours
- Amenable to downstream applications, including next generation sequencing
- Suitable for low levels of input DNA

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



Analysis of five known methylated loci and three known unmethylated loci in HeLa cell DNA using the EpiMark Methylated DNA Enrichment Kit and two other commercially available kits that also utilize MBD as a capture agent; results illustrate the sensitivity of the EpiMark Kit. Enrichment was performed according to manufacturers' recommendations.

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



Analysis of five known methylated loci in HeLa cell DNA using the EpiMark Methylated DNA Enrichment Kit was performed using the supplied protocols with varying amounts of input DNA.

Bisulfite Conversion

EpiMark Bisulfite Conversion Kit

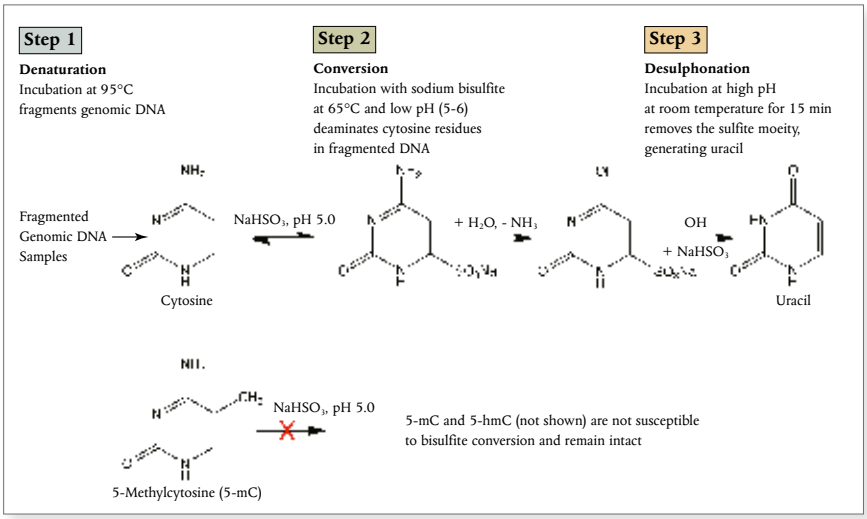
Bisulfite conversion, the most common technique for determining the methylation status of DNA, involves the conversion of unmodified cytosines to uracil, leaving the modified bases (5-mC and 5-hmC). The EpiMark Bisulfite Conversion Kit is designed for the detection of methylated cytosine, using a series 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

ADVANTAGES

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

Overview of bisulfite conversion

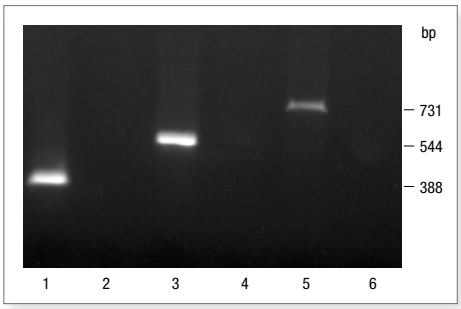


EpiMark Hot Start *Taq* DNA Polymerase

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

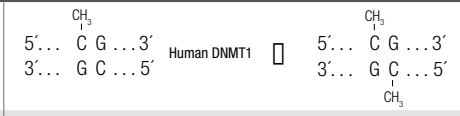
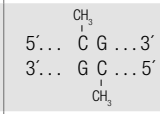
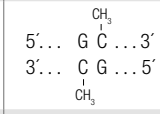
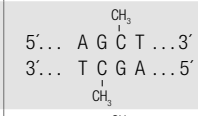
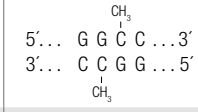
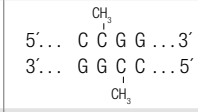
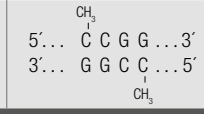
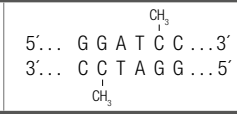
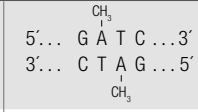
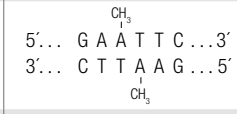
EpiMark Kit enables complete DNA conversion



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.

PRODUCT	NEB #	SEQUENCE
CYTOSINE-C5 METHYLTRANSFERASES		
Human DNA (cytosine-5) Methyltransferase (DNMT1)	M0230S/L	
CpG Methyltransferase (M.SssI)	M0226S/L	
GpC Methyltransferase (M.CviPI)	M0227S/L	
AluI Methyltransferase	M0220S/L	
HaeIII Methyltransferase	M0224S/L	
HhaI Methyltransferase	M0217S/L	
HpaII Methyltransferase	M0214S/L	
MspI Methyltransferase	M0215S/L	
CYTOSINE-N4 METHYLTRANSFERASE		
BamHI Methyltransferase	M0223S/L	
ADENINE-N6 METHYLTRANSFERASES		
***dam* Methyltransferase**	M0222S/L	
EcoRI Methyltransferase	M0211S/L	
***Taq*I Methyltransferase**	M0219S/L	

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 [^{3}H]-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. Reducing the DNA concentration (<0.02 mg/ml) gives two advantages. 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:

	STANDARD REACTION	REPRESENTATIVE LARGE-SCALE REACTION
Nuclease-free water	14 µl	220 µl
10X NEBuffer 2	2 µl	50 µl
SAM	2 µl from step 1	10 µl (32 µM SAM)
Genomic DNA	1 µl (1 µg)	200 µl (500 µg/ml λ DNA)
CpG methylase (M. SssI)	1 µl (4 U/µl)	20 µl (20 U/µl)

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

- $MgCl_2$ is not required as a cofactor. In the presence of Mg^{2+}, 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 SssI (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.

TOOLS & RESOURCES

Visit www.neb.com to find:

- A protocol for labeling genomic DNA with [^{3}H] using methyltransferases

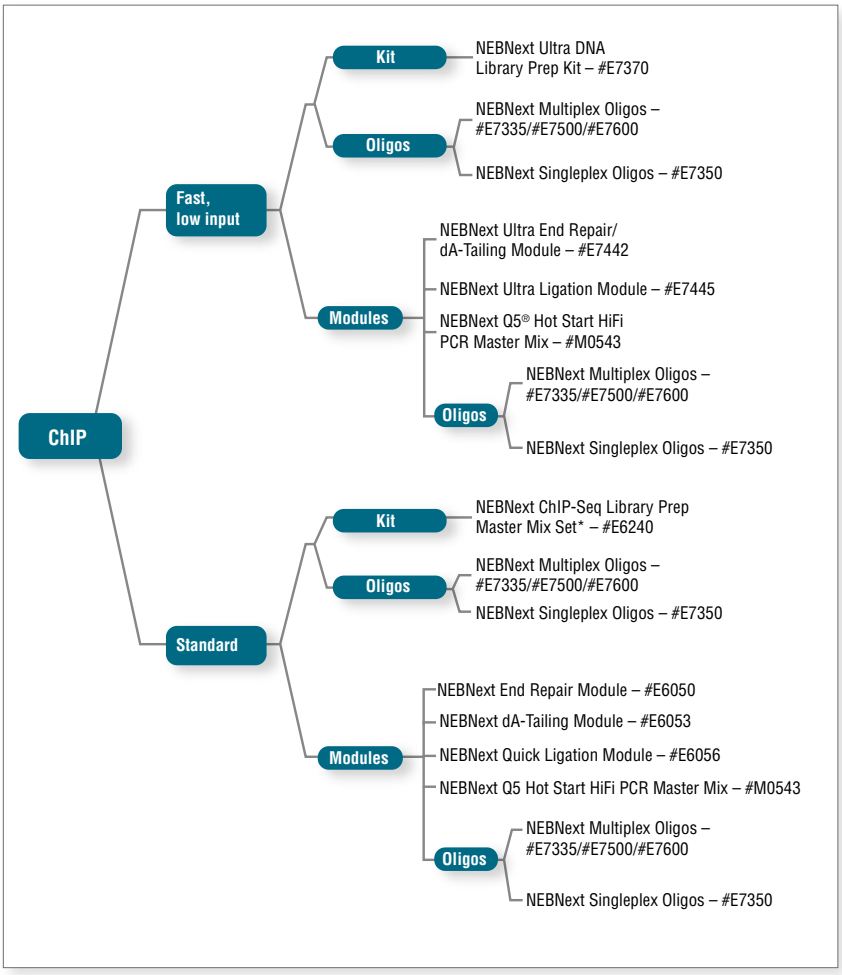
References
1. Forney, J.A. and Jack, W.E. (1991) *NEB Transcript*, 3(1), 5.
2. Matsuo, K. et al. (1994) *Nucl. Acids Res.*, 22, 5354–5359.
3. Doerfler, W. (1983) *Ann. Rev. Biochem.*, 52, 93–124.
4. Ohmori, H. et al. (1978) *Nucl. Acids Res.*, 5, 1479–1485.
5. Renbaum, P. et al. (1990) *Nucl. Acids Res.*, 18, 1145–1152.
6. Murchie, A.I. and Lilley, D.M. (1989) *J. Mol. Biol.*, 205, 593–602.

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 kits, oligos and modules that support standard or fast workflows. To decide which products to choose, use the selection chart below.



**Set of individual reagents is also available. See ordering information.*

ADVANTAGES

- Validated for ChIP-Seq
- Fast high-performance workflows with minimal hands-on-time
- Convenient formats include kits and modules
- All reagents undergo stringent quality controls, plus sequencing validation
- Value pricing

TOOLS & RESOURCES

Visit www.NEBNext.com to find:

- Complete list of NEBNext reagents for sample prep of DNA or RNA for next generation sequencing
- Protocols & FAQs
- Online tutorials to help with product selection, general handling tips and more
- NEBNext citations
- The latest NEBNext brochures

WHY CHOOSE NEBNEXT REAGENTS FOR NGS LIBRARY PREP?

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. SssI*), 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.

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

APPLICATIONS

- 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)

Glossary of Epigenetic Terms

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, mammalian X chromosome inactivation, 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 dideoxynucleotides (ddNTPs), and the ratio of C to T can then be determined quantitatively using radioactive or fluorescent ddNTPs, 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 thymidines 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

PRODUCT	NEB #	SIZE
METHYLATION ANALYSIS		
AbaSI	R0665S	1,000 units
DpnI	R0176S/L	1,000/5,000 units
DpnII	R0543S/T/L/M	1,000/1,000/5,000/5,000 units
FspEI	R0662S/L	200/1,000 units
HpaII	R0171S/M/L	2,000/10,000/10,000 units
LpnPI	R0663S/L	200/1,000 units
MspI	R0106S/T/M/L	5,000/5,000/25,000/25,000 units
MspJI	R0661S/L	200/1,000 units
EpiMark 5-hmC and 5-mC Analysis Kit	E3317S	20 reactions
EpiMark Bisulfite Conversion Kit	E3318S	48 reactions
EpiMark Hot Start *Taq* DNA Polymerase	M0490S/L	100/500 reactions
EpiMark Methylated DNA Enrichment Kit	E2600S	25 reactions
5-Methyl-dCTP	N0356S	1 µmol
McrBC	M0272S/L	500/2,500 units
METHYLTRANSFERASES & ANTIBODIES		
G9a Methyltransferase	M0235S	100 units
PRMT1 Methyltransferase	M0221S	50 units
SET7 Methyltransferase	M0233S	100 units
SET8 Methyltransferase	M0428S	100 units
Human DNA (cytosine-5) Methyltransferase (Dnmt1)	M0230S/L	50/250 units
CpG Methyltransferase (M.SssI)	M0226S/M/L	100/500/500 units
GpC Methyltransferase (M.CviPI)	M0227S/L	200/1,000 units
HpaII Methyltransferase	M0214S/L	100/500 units
MspI Methyltransferase	M0215S/L	100/500 units
EcoRI Methyltransferase	M0211S/L	10,000/50,000 units
dam Methyltransferase	M0222S/L	500/2,500 units
BamHI Methyltransferase	M0223S/L	100/500 units
HhaI Methyltransferase	M0217S/L	1,000/5,000 units
TaqI Methyltransferase	M0219S/L	1,000/5,000 units
AluI Methyltransferase	M0220S/L	100/500 units
HaeIII Methyltransferase	M0224S/L	500/2,500 units
SAMPLE PREP FOR ChIP-SEQ		
NEBNext ChiP-Seq Library Prep Master Mix Set for Illumina	E6240S/L	12/60 reactions
NEBNext ChiP-Seq Library Prep Reagent Set for Illumina	E6200S/L	12/60 reactions
NEBNext Ultra DNA Library Prep Kit for Illumina	E7370S/L	24/96 reactions
NEBNext DNA Library Prep Master Mix Set for Illumina	E6040S/L	12/60 reactions
NEBNext dsDNA Fragmentase®	M0348S/L	50/250 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 reactions
NEBNext Singleplex Oligos for Illumina	E7350S/L	12/60 reactions

PRODUCT	NEB #	SIZE
NEBNext Ultra™ End Repair/dA-Tailing Module	E7442S/L	24/96 reactions
NEBNext Ultra Ligation Module	E7445S/L	24/96 reactions
NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 reactions
NEBNext End Repair Module	E6050S/L	20/100 reactions
NEBNext dA-Tailing Module	E6053S/L	20/100 reactions
NEBNext Quick Ligation Module	E6056S/L	20/100 reactions
CONTROL DNA		
CpG Methylated Jurkat Genomic DNA	N4002S	15 µg
5-Aza-dc Treated Jurkat Genomic DNA	N4003S	15 µg
NIH 3T3 Mouse Genomic DNA	N4004S	15 µg
HeLa Genomic DNA	N4006S	15 µg
CpG Methylated HeLa Genomic DNA	N4007S	15 µg
HISTONES		
EpiMark Nucleosome Assembly Kit	E5350S	20 reactions
$H1^0$ Human, Recombinant	M2501S	100 µg
H2A Human, Recombinant	M2502S	100 µg
H2B Human, Recombinant	M2505S	100 µg
H3.1 Human, Recombinant	M2503S	100 µg
H3.2 Human, Recombinant	M2506S	100 µg
H3.3 Human, Recombinant	M2507S	100 µg
H4 Human, Recombinant	M2504S	100 µg
Histone H3.1/H4 Tetramer Human, Recombinant	M2509S	1 nmol
Histone H2A/H2B Dimer Human, Recombinant	M2508S	2 nmol
Nucleosome Control DNA	N1202S	0.2 nmol

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