Now includes NEBNext® Enzymatic Methyl-seq (EM-seq™) v2 and NEBNext Enzymatic 5hmC-seq (E5hmC-seq™)

Epigenetics

UNDERSTANDING DNA & HISTONE MODIFICATIONS

NEW ENGLAND Biolabs®

Epigenetics

For over 50 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. NEBNext® Enzymatic Methyl-seq (EM-seq™) and 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 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.

TOOLS & RESOURCES

Visit NEBNext.com to learn:

How EM-seq compares to bisulfite sequencing

How EM-seq minimizes DNA damage and produces high-quality, high-diversity libraries

What more sensitive detection of 5mC and 5hmC means for your methylome analysis

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 5hmC and 5mC detection and quantitation

hm m
CCGTCGGACCGCG
SODIUM BISULFITE METHOD
☑ Greater DNA damage
EM-SEQ METHOD
☑ Minimizes DNA damage
☑ Superior performance
• More sensitive detection of 5mC and 5hmC

NEBNEXT ENZYMATIC METHYL-SEQ WORKFLOW VIDEO

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References

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Reagents for Epigenetic Studies

DNA METHYLATION ANALYSIS (Page 4–8)	
NEBNext Enzymatic Methyl-seq v2 Kit	EpiMark Methylated DNA Enrichment Kit
NEBNext Enzymatic Methyl-seq v2 Conversion Module	EpiMark Hot Start *Taq* DNA Polymerase
NEBNext Enzymatic 5hmC-seq Kit	T4 Phage β-Glucosyltransferase
NEBNext Enzymatic 5hmC-seq Conversion Module	
NEBNext LV Unique Dual Index Primers Sets 1–5	
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HpaII	
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NEBNext® Ultra™ II DNA Library Prep Kit for Illumina	
NEBNext UltraExpress® DNA Library Prep Kit	
DNA METHYLTRANSFERASES (Pages 14–15)	
CpG Methyltransferase (M.SssI)	EcoRI Methyltransferase
GpC Methyltransferase (M.CviPI)	HaeIII Methyltransferase
AluI Methyltransferase	HhaI Methyltransferase
BamHI Methyltransferas	HpaII Methyltransferase
dam Methyltransferase	MspI Methyltransferase
EcoGII Methyltransferase	*Taq*I Methyltransferase

Visit **www.NEB.com** for the full list of reagents available for epigentic studies.

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.

TOOLS & RESOURCES

Visit **NEBNext.com** for more information on NEBNext Enzymatic Methyl-seq, an enzyme-based alternative to bisulfite sequencing

DNA Methylation in Mammals

DNA methylation in mammals primarily occurs on the fifth carbon of the cytosine base (5-methylcytosine, 5mC, see Table 1) of CpG dinucleotides, and approximately 70% to 80% of CpG dinucleotides are methylated in somatic cells. However, 5mC at CpA, CpT and CpC sequences have been found in genomic DNA from mouse embryonic stem cells, and 5mC 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.

Methods for Studying DNA Methylation

Study of the DNA methylation patterns on genomic DNA had, until recently, taken one of three approaches – pretreatment with sodium bisulfite, restriction enzymes, or a methylated DNA-binding affinity matrix – with sodium bisulfite treatment and bisulfite sequencing being the gold standard for analysis at the single base level. In 2019, NEB introduced a groundbreaking new method, NEBNext Enzymatic Methyl-seq (EM-seq), which offered myriad advantages over methylome analysis with sodium bisulfite pretreatment. Recently, NEB introduced an updated version of EM-seq, EM-seq v2, expanding the dynamic range down to 100 pg. This was followed by the release of E5hmC-seq, expanding the utility of the technology to discretely identify 5hmC.

These techniques are compared and contrasted in Table 2 (next page). Both bisulfite treatment and EM-seq can reveal the methylation status of every cytosine residue in the genome, and they are therefore amenable to massively parallel sequencing methods. Methyl-specific differential cleavage of DNA requires restriction enzymes, that are either methylation sensitive or methylation dependent, to fragment genomic DNA for subsequent analysis. This method offers lower resolution data due to the requirement of a range of enzyme recognition sequences and the risk for incomplete digestion. Finally, affinity-based methods use methylated DNA binding proteins or antibodies to enrich the experimental DNA sample for methylated DNA to be analyzed in subsequent steps.

A wide variety of analytical and enzymatic methods may be employed downstream of methyl-enrichment steps to characterize genomic DNA. Analytical methods, including high-performance liquid chromatography (HPLC) and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), are routinely used to quantify modified nucleobases in complex DNA. Though HPLC is quantitative and reproducible, it is poorly suited to high-throughput applications due to a requirement for high input amounts, although recent work has lowered the minimum input to nanogram levels (1). MALDI-TOF MS is both quantitative and amenable to higher throughput applications. Other downstream methylome analysis methods include end-point PCR, real-time PCR, primer extension, single-stranded conformational polymorphism assays, blotting, microarrays, and sequencing. Selecting a method(s) will depend on your sample size and experimental goals (2, see also www.epimark.com).

Table 1: 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-hydroxymethyl-cytosine	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*

References

1. Song, L., et al (2005) *Anal. Chem.*, 77, 504–510.
2. Laird, P.W. (2010) *Nat. Rev. Genet.* 11, 191–203.

Table 2: Approaches for Studying DNA Methylation

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	APPLICATION
NEBNext Enzymatic Methyl-seq (EM-seq) v2	EM-seq v2 is a method for detection of 5mC and 5hmC at single-base resolution. In a two-step conversion process, TET2 and an oxidation enhancer protect modified cytosines from downstream deamination. TET2 enzymatically oxidizes 5mC and 5hmC through a cascade reaction into 5-carboxycytosine [5-methylcytosine (5mC) → 5-hydroxymethylcytosine (5hmC) → 5-formylcytosine (5fC) → 5- carboxycytosine (5caC)]. This protects 5mC and 5hmC from deamination. 5hmC can also be protected from deamination by glucosylation to form 5ghmc using the oxidation enhancer. Then, APOBEC deaminates cytosines but does not affect 5caC and 5ghmC. Comparison of sequence information between the reference genome and EM-seq DNA can provide single-nucleotide resolution information about cytosine methylation patterns.	• Superior sensitivity of detection of 5mC and 5hmC, from as low as 100 pg • High mapping efficiency with uniform GC coverage • Gentle enzymatic process/minimal DNA damage • More CpG data with fewer sequencing runs than WGBS • Works with damaged DNA (e.g., FFPE) • Faster workflow than WGBS • Resolution at the nucleotide level • Automated analysis • Gives % mC at a specific site	• Cannot distinguish between 5mC and 5hmC • Intensive downstream analysis (same as WGBS)	• Whole genome (or single locus) methylation analysis
NEBNext Enzymatic 5hmC-seq (E5hmC-seq)	Enzymatic 5hmC-seq (E5hmC-seq™) is a method for detection of 5hmC at the single base level. 5hmC is detected using a two-step enzymatic conversion workflow that minimizes damage to DNA and allows discrimination of 5hmC from both cytosine and 5mC, after Illumina® sequencing. The kit has an input range of 0.1–200 ng and includes NEBNext Ultra™ II library prep reagents and the E5hmC-seq Adaptor.	• High sensitivity of detection of 5hmC • 0.1–200 ng input range • Even GC coverage • High-efficiency library preparation • E5hmC-seq and EM-seq data can be combined • Conversion module also available separately (NEB #E3365)	• May require deeper sequencing (100x)	• Whole genome 5hmC analysis
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 5mC intact. The uracils are amplified as thymines, and 5mC 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 5mC-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 5mC and 5hmC	• 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 5mC. 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 5mC 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

DNA Methylome Analysis

The methylome comprises the total of methyl marks attached to the cytosine bases within a genome. Analyzing the complete methylome requires tools that enable the reliable quantitation of methylated cytosines, in most cases requiring the conversion of methylated cytosines into other structures before deamination and sequence comparison.

NEBNext Enzymatic Methyl-seq (EM-seq) v2

NEBNext Enzymatic Methyl-seq is a high-performance enzyme-based alternative to bisulfite conversion for methylome analysis using Illumina sequencing. With the expanded input range of the NEBNext Enzymatic Methyl-seq v2 Kit, as little as 100 pg of input DNA can be used. The protocol has also been streamlined to minimize cleanup steps and reduce workflow time.

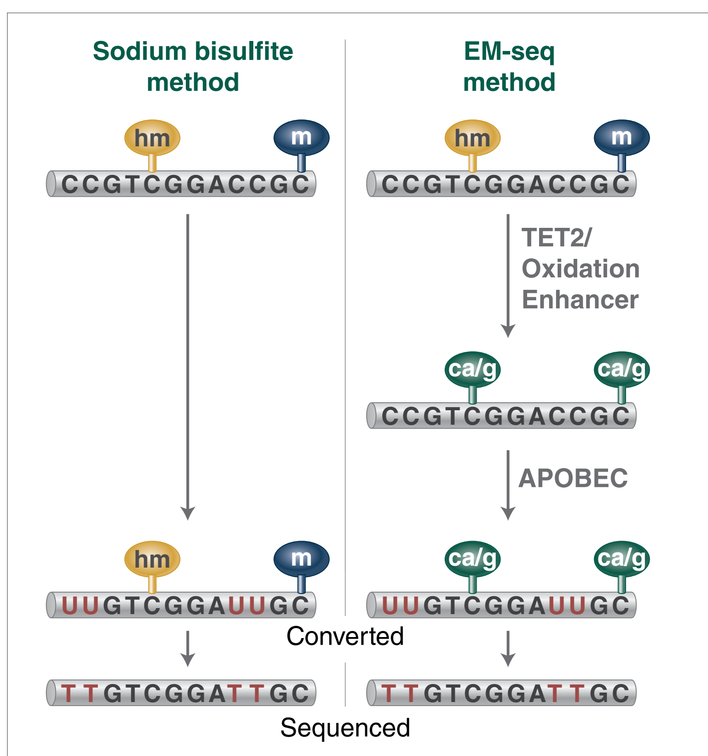
EM-seq is a two-step enzymatic conversion process to detect modified cytosines. In the first step, TET2 and T4-BGT protect modified cytosines from downstream deamination. TET2 enzymatically oxidizes 5mC and 5hmC, and T4-BGT glucosylates 5hmC.

The second enzymatic step uses APOBEC to deaminate unmodified cytosines to uracils, but 5mC and 5hmC protected in the first step are not deaminated.

This is followed by amplification using a NEBNext master mix formulation of Q5U® (a modified version of Q5® High-Fidelity DNA Polymerase), and sequencing on the Illumina platform. The consistently high conversion performance and minimized DNA damage with the EM-seq protocol, in combination with highly efficient Ultra II library prep, result in superior detection of CpGs with fewer sequencing reads.

NEBNext Enzymatic Methyl-seq v2 Kit E8015S/L
NEBNext Enzymatic Methyl-seq v2 Conversion Module E8020S/L

EM-seq and sodium bisulfite conversion methods



ADVANTAGES

- Superior sensitivity of detection of 5mC and 5hmC
- 0.1 ng - 200 ng input range
- Detection of more CpGs with fewer sequencing reads
- Even GC coverages
- High performance library preparation and larger library insert sizes
- Index primers supplied separately
- High-efficiency library preparation
- Enzymatic fragmentation of DNA compatible with EM-seq workflows can be achieved using NEBNext UltraShear® (NEB #M7634).

What users are saying:

"*As an ISO17025-accredited laboratory, we need reliable and reproducible kits and protocols. After thorough benchmarking of NEBNext EM-seq v2, interrogating 5mC, the standard bisulfite conversion method lost its reference status for our methylation analysis. The NEBNext E5hmC-seq kit for detecting 5hmC specifically, are now our method of choice to study both modifications*"

– Marta Gut, Ph.D.,
Head of Sequencing Unit
CNAG

"*We've been testing EM-seq on a variety of inputs, platforms, and samples, and it shows more even coverage across CpG islands, the whole genome, and also greater detection of CpG sites across the genome vs. WGBS.*"

– Christopher Mason,
Weill Cornell Medical School
New York

"*Whole genome bisulfite sequencing is the workhorse technique in our laboratory and we have tested range of different kits. NEB's EM-seq Kit provides an excellent alternative that causes far less damage to the DNA and results in larger fragments which make the process of sequencing more cost effective. We found that the kit also produces libraries with very low biases in nucleotide coverage and methylation estimates.*"

– Duncan Sproul,
MRC Human Genetics Unit
Edinburgh

NEBNext Enzymatic 5hmC-seq (E5hmC-seq™) Kit

Typically, the modified cytosines 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) are detected by sequencing of Illumina libraries generated using the NEBNext EM-seq enzyme-based workflow or bisulfite conversion. However, these methods cannot differentiate between 5mC and 5hmC.

As with EM-seq, NEBNext Enzymatic 5hmC-seq (E5hmC-seq) uses an enzyme-based method to specifically detect 5hmC, without the DNA damage typical of bisulfite-based methods, and this sensitive method can be used with as little as 0.1 ng of input DNA.

In a two-step process, T4-BGT glucosylates 5hmC, providing protection from deamination by APOBEC in the next step. T4-BGT does not protect 5mC or unmethylated cytosines, which are deaminated by APOBEC to uracils. This is followed by amplification using a NEBNext master mix formulation of Q5U (a modified version of Q5 High-Fidelity DNA Polymerase), and sequencing on the Illumina platform.

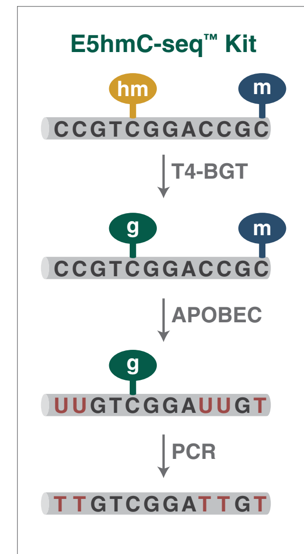
Bioinformatic analysis tools used for EM-seq and for bisulfite sequencing can also be used for E5hmC-seq. E5hmC-seq data can be subtracted from EM-seq data, thereby allowing determination of the precise location of individual 5mC and 5hmC sites.

NEBNext Enzymatic 5hmC-seq Kit E3350S/L
NEBNext Enzymatic 5hmC-seq Conversion Module E3365S/L

ADVANTAGES

- High sensitivity of detection of 5hmC
- 0.1 ng - 200 ng input range
- Even GC coverages
- High-efficiency library preparation
- E5hmC-seq and EM-seq data can be combined

E5hmC-seq conversion method



NEBNext UltraShear®

Enzymatic fragmentation of DNA as part of the library prep workflow provides many advantages compared to mechanical shearing. However, specialized fragmentation reagents are required for enzymatic shearing in order to maintain methylation marks on samples for methylome analysis or for use with challenging samples such as FFPE DNA.

NEBNext UltraShear is a mix of enzymes that has been designed and optimized to fragment these sample types upstream of library preparation. This improves library quality and allows retention of methylation marks.

NEBNext UltraShear M7634S/L

ADVANTAGES

- Compatible with methylation analysis workflows, including NEBNext Enzymatic Methyl-seq v2 Kit (NEB #E8015)
- Compatible with FFPE DNA
- Fast workflow with minimal hands-on time
- For methylation analysis, improves library yields, CpG coverage and sequencing metrics
- For FFPE DNA, increases usable reads and coverage uniformity and decreases artificial mutation frequency

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.

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

T4 Phage β-glucosyltransferase

T4 Phage β-glucosyltransferase (T4-BGT) is also available as a stand-alone enzyme for the glucosylation of 5hmC in DNA.

T4 Phage β-glucosyltransferase... M0357S

APPLICATIONS

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

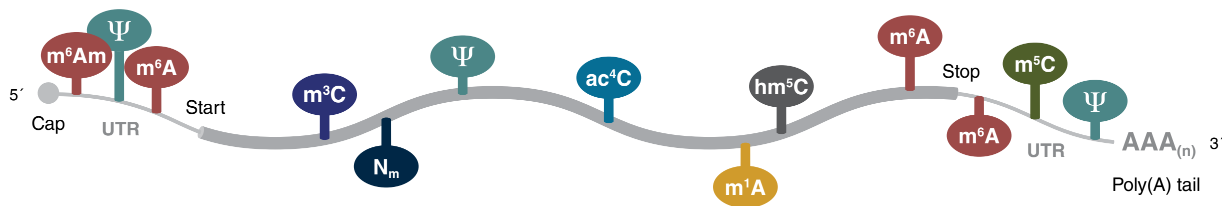
EpiMark Hot Start *Taq* DNA Polymerase

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

RNA Modifications

The epitranscriptome encompasses RNA modifications that influence RNA structure and function and regulate gene expression. More than a hundred and fifty distinct RNA modifications have been discovered in nature. Post-transcriptional regulatory markers occur in messenger RNAs (mRNAs), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), circular RNAs (circRNAs), micro RNAs (miRNA), and long non-coding RNAs (lncRNAs). Liquid Chromatography Mass Spectrometry (LC-MS) is utilized to quantify the abundance of m5C, hm5C, f5C, ca5C, m4C, m6A, pseudouridine (Ψ), and many other modified RNA nucleotides. Next generation sequencing (NGS) can identify some of the modified RNA nucleotides in context of epitranscriptome-wide analyses. Epitranscriptomics is expanding with new profiling methods and discoveries of promising biomarkers and therapeutic targets.



RNA modifications detectable with NGS or mass spectrometry for epitranscriptomic studies.

RNA Methylation

Just as epigenetic information can be conveyed via DNA modifications, so too can it be conveyed via RNA modifications. Common modifications of messenger RNAs (mRNAs) include methylation of cytosines and adenosines. The most common mRNA modification in mammals is N6-methyladenosine (m6A), and it is thought to be involved in RNA stability, splicing, transport, and tolerance (1,2).

EpiMark® N6-Methyladenosine Enrichment Kit

The EpiMark N6-Methyladenosine Enrichment Kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR. The kit contains a rabbit monoclonal antibody specific for N6-Methyladenosine (m6A). The kit also contains two control RNAs, one with m6A modification (*Gaussia* luciferase) and one without (*Cypridina* luciferase) to monitor enrichment and depletion. The GLuc RNA control was transcribed in the presence of 20% m6ATP and 80% ATP. This kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR. Modified RNA is isolated from a fragmented RNA sample by binding to the N6-Methyladenosine antibody attached to Protein G Magnetic Beads. After multiple wash and clean-up steps, the enriched RNA is eluted in nuclease-free water and is ready for further analysis.

EpiMark N6-Methyladenosine Enrichment Kit E1610S

ADVANTAGES

- Complete protocol for enrichment of m6A-modified – RNA and analysis by RT-qPCR included
- RNA controls (m6A modified and unmodified RNA) enable monitoring of enrichment and depletion
- Antibody supplied in a ready-to-use solution form

References

1. Bokar, J.A. (2005) *Fine-tuning of RNA Functions by Modification and Editing*, Springer-Verlag, Berlin pp. 141–178.
2. Kariko, K., Buckstein, M., Ni, H. and Weissman, D. (2005), *Immunity*, 23, pp. 165–175.

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.

APPLICATIONS

- Differentiation of methylation patterns

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´. . .▼G A T C . . . 3´ 3´. . . C T A G▲. . . 5´	R0543	MboI DpnII
HpaII	Will not cleave methylated CpG sites	5´. . . C▼C G G . . . 3´ 3´. . . G G C▲C . . . 5´	R0171	MspI
MspI	Not methylation sensitive	5´. . . C▼C G G . . . 3´ 3´. . . G G C▲C . . . 5´	R0106	HpaII

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

MspJI Family of Restriction Enzymes

Scientists at NEB identified the MspJI family of restriction enzymes, which are dependent on methylation and hydroxymethylation for cleavage to occur (6). These enzymes excise DNA fragments containing a centrally located 5hmC or 5mC 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.

ADVANTAGES

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

Table 4B: Methylation Dependent Restriction Enzymes

	METHYLATION SENSITIVITY	SEQUENCE	NEB #	ISOSCHIZOMER
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 (on A) 5´. . . G A▼T C . . . 3´ 3´. . . C T▲A G . . . 5´ CH_3 (on A)	R0176	DpnII
MspJI (1–5)	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

References

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2. Zheng, Y., et al. (2010) *Nucl. Acids. Res.*, doi;10, 1093/nar/gkq327
3. Cohen-Karni, D., et al. (2011) PNAS, 108 (27) 11040–11045 http://www.pnas.org/content/108/27/11040.long
4. Horton, J., et al. (2012) *Nucl. Acids. Res.*, 40 (19) 9763–9773 http://nar.oxfordjournals.org/content/40/19/9763
5. Horton, J., et al. (2014) *Nucl. Acids. Res.*, 42 (19) 12092–12101
6. Gowher, H. et al. (2000) *EMBO J.*, 19, 6918–6923

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		
CpG Methyltransferase (M.SssI)	M0226S/L	CH_3 5´... CG...3´ 3´... GC...5´ CH_3
GpC Methyltransferase (M.CviPI)	M0227S/L	CH_3 5´... GC...3´ 3´... CG...5´ CH_3
AluI Methyltransferase	M0220S/L	CH_3 5´... AGCT...3´ 3´... TCGA...5´ CH_3
HaeIII Methyltransferase	M0224S/L	CH_3 5´... GGCC...3´ 3´... CCGG...5´ CH_3
HhaI Methyltransferase	M0217S/L	CH_3 5´... GCGC...3´ 3´... CGCG...5´ CH_3
HpaII Methyltransferase	M0214S/L	CH_3 5´... CCGG...3´ 3´... GGCC...5´ CH_3
MspI Methyltransferase	M0215S/L	CH_3 5´... CCGG...3´ 3´... GGCC...5´ CH_3
CYTOSINE-N4 METHYLTRANSFERASE		
BamHI Methyltransferase	M0223S/L	CH_3 5´... GGATCC...3´ 3´... CCTAGG...5´ CH_3
ADENINE-N6 METHYLTRANSFERASES		
dam **Methyltransferase**	M0222S/L	CH_3 5´... GATC...3´ 3´... CTAG...5´ CH_3
EcoGII Methyltransferase	M0603S	CH_3 5´... A...3´ 3´... T...5´
EcoRI Methyltransferase	M0211S/L	CH_3 5´... GAATTC...3´ 3´... CTTAAG...5´ CH_3
***Taq*I Methyltransferase**	M0219S/L	CH_3 5´... TCGA...3´ 3´... AGCT...5´ CH_3

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

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).

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

References

1. Kornberg, R.D. (1977) *Annu. Rev. Biochem.* 46, 931–954.
2. Kim, J.K., Samaranayake, M. and Pradhan, S. (2009) *Cell. Mol. Life Sci.*, 66, 596–612.
3. Gilbert et al., (2004) *Cell* 118, 555-566.

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. 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. The study of their roles in gene regulation, cellular stress events, aging and DNA repair (1,2) 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 (3).

Table 1: Types of Histone Modifications

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

References

1. Huang, J. et al. (2006) *Nature*, 444, 629–632.
2. Pahlich, S., Zakaryan, R.P. and Gehring, H. (2006) *Biochim. Biophys. Acta.*, 1764, 1890–1903.
3. Kim, J.K., Samaranayake, M. and Pradhan, S. (2009) *Cell. Mol. Life Sci.*, 66, 596–612.

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 (1). For large-scale analyses, the isolated DNA can be analyzed on a microarray (ChIP-chip) or by sequencing (ChIP-seq). 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 2).

References

1. O'Neill, L.P., et al. (2006) *Nat. Genet.*, 38, 835–841.
2. Bernstein, B.E., et al. (2007) *Cell* 128, 669-681.

Library 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.

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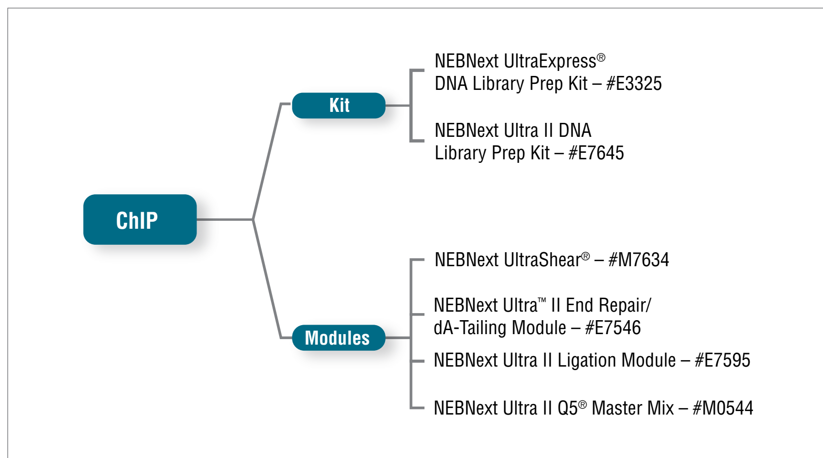

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- Convenient formats include kits and modules
- All reagents undergo stringent quality controls, plus sequencing validation
- Value pricing

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NEBNext Enzymatic Methyl-seq v2 Conversion Module	E8020S/L	24/96 reactions
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	E7140S/L	24/96 reactions
NEBNext Enzymatic 5hmC-seq Kit	E3350S/L	24/96 reactions
NEBNext Enzymatic 5hmC-seq Conversion Module	E3365S/L	24/96 reactions
NEBNext UltraShear	M7634S/L	24/96 reactions
EpiMark Hot Start *Taq* DNA Polymerase	M0490S/L	100/500 reactions
EpiMark N6-Methyladenosine Enrichment Kit	E1610S	20 reactions
EpiMark Methylated DNA Enrichment Kit	E2600S	25 reactions
DpnI	R0176S/L	1,000/5,000 units
DpnII	R0543S/T/L/M	1,000/1,000/5,000/5,000 units
HpaII	R0171S/M/L	2,000/10,000/10,000 units
MspI	R0106S/T/M/L	5,000/5,000/25,000/25,000 units
MspJI	R0661S/L	200/1,000 units
5-Methyl-dCTP	N0356S	1 µmol
METHYLTRANSFERASES & ANTIBODIES		
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
EcoGII Methyltransferase	M0603S	200 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
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NEBNext Ultra II Ligation Module	E7595S/L	24/96 reactions
NEBNext Ultra™ II Q5 Master Mix	M0594S/L/X	50/250/500 reactions
NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S/L	24/96 reactions
NEBNext Magnetic Separation Rack	S1515S	24 tubes

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NEBNext LV Unique Dual Index Primer Sets (2A, 2B)	E3390S E3392S	24 rxns
NEBNext LV Unique Dual Index Primer Sets (1, 2, 3, 4, 5)	E3400S E3402S E3404S E3406S E3408S	96 rxns
NEBNext Multiplex Oligos for Illumina 96 Unique Dual Index Primer Pairs Sets (1, 2, 3, 4, 5)	E6440S/L E6442S/L E6444S/L E6446S/L E6448S/L	96/384 rxns 96/384 rxns 96/384 rxns 96/384 rxns 96/384 rxns
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 rxns
NEBNext Multiplex Oligos for Illumina Index Primers Sets (1, 2, 3, 4)	E7335S/L E7500S/L E7710S/L E7730S/L	24/96 rxns
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
NEBNext Adaptor Dilution Buffer	B1430S	1 x 9.6 ml

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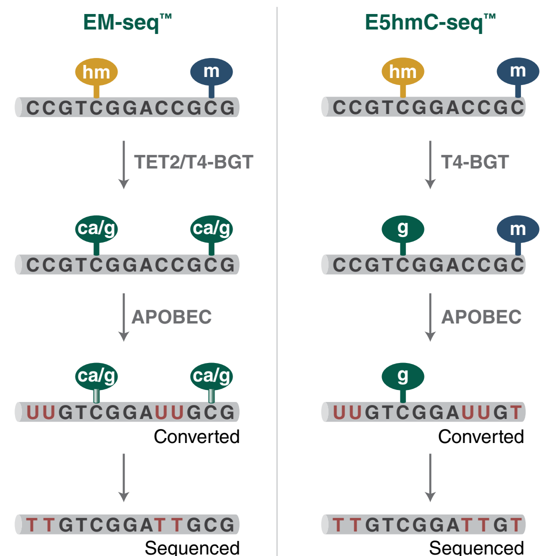

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