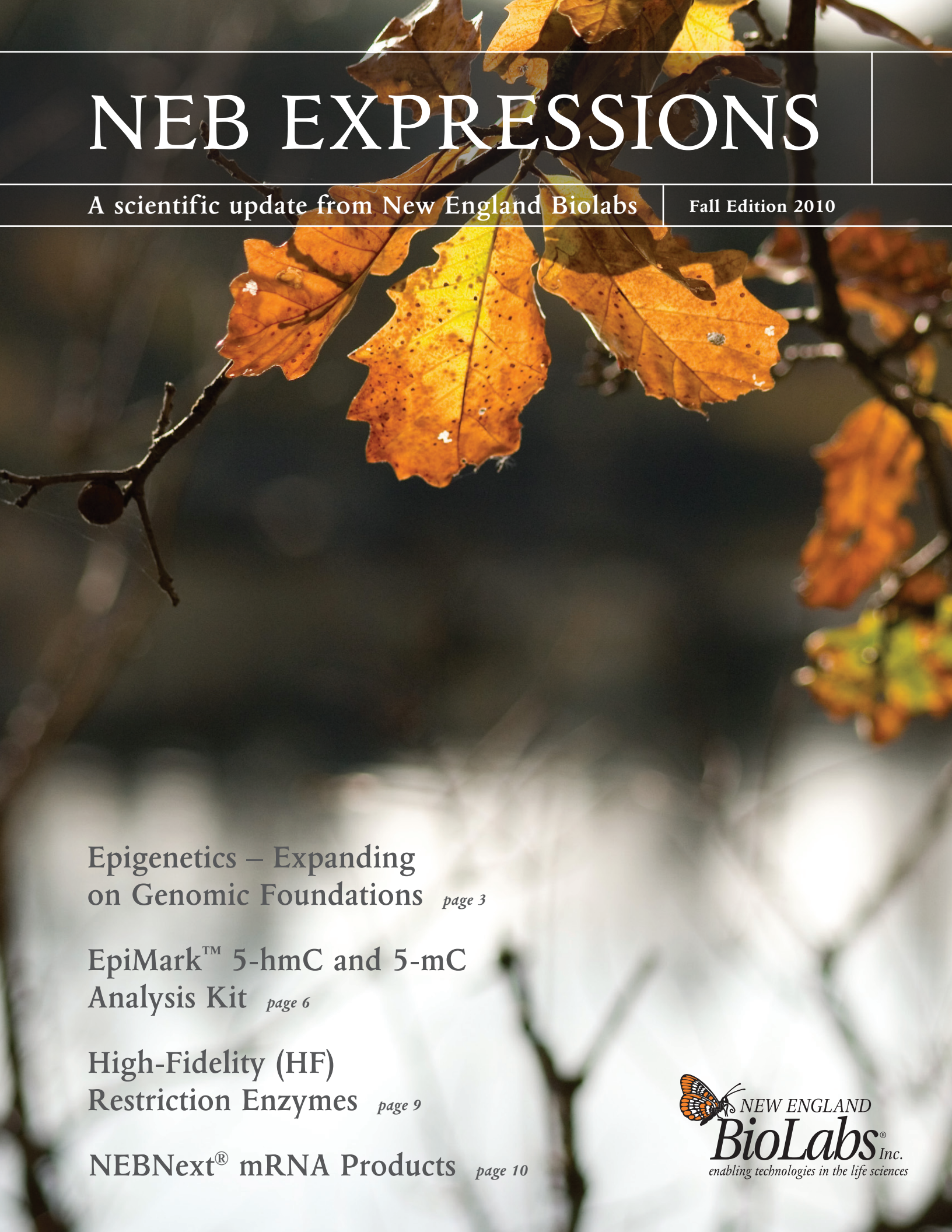
NEB EXPRESSIONS

A scientific update from New England Biolabs | Fall Edition 2010



NEW ENGLAND BioLabs® Inc.
enabling technologies in the life sciences

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Upcoming Tradeshows

Visit the NEB booth at the following meetings:

- **Society for Glycobiology**
  November 7–10, 2010
  St. Petersburg, FL
  www.glycobiology.org
- **Society for Neuroscience**
  November 13–17, 2010
  San Diego, CA
  www.sfn.org/am2010/
- **ASCB** – December 11–15, 2010
  Philadelphia, PA
  www.ascb.org/meetings/ascb-annual-meetings.html

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A Letter from NEB

Fall is a time of change in New England, as evidenced by cooler weather, brightly colored leaves and shorter days. At New England Biolabs, we have been thinking a lot about change as it relates to epigenetic studies, and are pleased to be launching a suite of products validated for epigenetics research. As researchers explore the intricacies of the molecular basis of epigenetic modifications, the causes of epigenetic change and mechanisms of its inheritance, NEB is committed to developing high quality reagents to enable and accelerate this work.

In this issue, we introduce the EpiMark™ 5-hmC and 5-mC Analysis Kit, which uses two methylation-sensitive restriction enzyme isoschizomers followed by PCR analysis to identify 5-hydroxymethyl-C (5-hmC) and 5-methyl-C (5-mC) within a specific locus. As interest in the role of 5-hmC in the epigenome grows, the availability of techniques to distinguish 5-mC from 5-hmC becomes more important. Additionally, our expertise in enzymology has led to the discovery of a family of restriction enzymes that discriminate between methylation states within a specific cut site. Methylation-dependent cleavage enables the fragmentation of DNA that can be sequenced and used for mapping epigenetic modifications.

In the realm of sequencing, the demand for high quality sample preparation becomes increasingly important, as higher quality and quantity of data becomes more desirable. In this issue, we also introduce NEBNext® reagents for mRNA sample preparation for next-gen sequencing. These reagents lower the cost of mRNA sample prep for the Illumina® platform.

Wishing you continued success in your research,

New England Biolabs

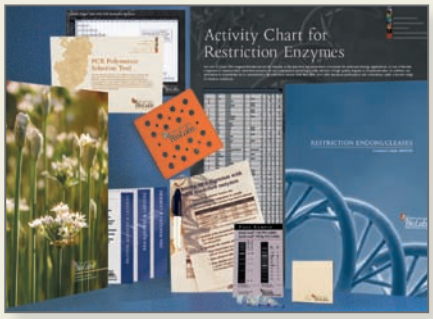
New Student Starter Pack

Start the academic year off on the right path with the New Student Starter Pack from NEB. The Starter Pack contains a selection of technical literature, laboratory aids and product samples that support your research. Starter Packs are available through December 31, 2010 to all new research students.

Visit **www.neb.com/starterpack** to request your Starter Pack today.

Starter Pack Includes*:

- Catalog and Technical Reference
- Quickload™ DNA Ladder Samples
- Activity Chart for Restriction Enzymes
- Ladder/Marker Reference Cards
- Restriction Enzyme Technical Guide
- Double Digest Card
- PCR Polymerase Selection Tool Card
- Microfuge Tube Floatie
- NEB Quick Protocol Cards with pen
- Post-it® notepad



Throughout the course of the year, special offers will be sent only to Starter Pack recipients, so sign-up and watch for these exciting offers from NEB.

**Offer valid while supplies last*

Epigenetics – Expanding on Genomic Foundations

Epigenetics is the study of heritable changes in the phenotype of a cell or organism that are not caused by its genotype. The molecular basis of an epigenetic profile arises from covalent modifications of protein and DNA components of chromatin. The epigenetic profile of a cell often dictates cell fate, as well as mammalian development, aging and disease. Epigenetic changes may persist for the remainder of a cell's life, but may also last for multiple generations in a lineage. Here, we provide an overview of the molecular basis for epigenetics and methods for studying DNA methylation.

Maureen Young, Ph.D., Science Writer, Austin, TX

Histone Modifications

In eukaryotes, chromatin is organized into nucleosome core particles that consist of approximately 147 bp of DNA and an octamer of histones (typically, two each of the core histones: H2A, H2B, H3 and H4) (1). The linker histone, H1, can further condense chromatin by binding to linker DNA between the nucleosome core particles. In mammals, chromatin can be generally classified as condensed, transcriptionally silent heterochromatin, or less-condensed, transcriptionally active euchromatin. Most genomic DNA is heterochromatin, which constitutes telomeres, pericentric regions and areas rich in repetitive sequences.

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 (Table 1), they occur primarily on the N-terminal tail. Some of these changes are enzymatically reversible. In general, the biological significance of all these modifications is not well understood, but the modifications are known to influence transcription, DNA repair, DNA replication and chromatin condensation. A "histone code" hypothesis is being tested by researchers to determine if combinations of histone modifications can be used to predict changes in gene expression (2-3). A comprehensive list of histone-modifying enzymes can be found in a review of mammalian epigenetic mechanisms by Kim et al (4). New England Biolabs offers a selection of unmodified, recombinant human histones that function as substrates for histone-modifying enzymes and can assemble into octamers (see page 8).

Figure 1: The Epigenetic Code

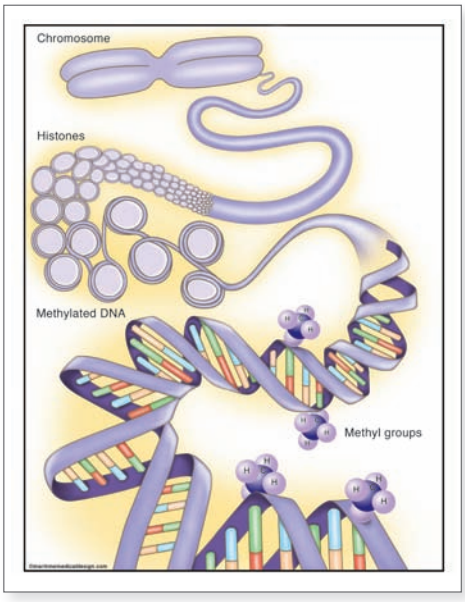


Table 1: Epigenetic Modifications

Types of Amino Acid Modifications of Core Histones	
Lysine	Methylation, Acetylation, Ubiquitination, Sumoylation, ADP-Ribosylation
Arginine	Methylation
Serine	Phosphorylation
Threonine	Phosphorylation
Types of Methylated DNA Bases	
C5-methylcytosine	
C5-hydroxymethylcytosine	
N4-methylcytosine (bacteria)	
N6-methyladenine (not found in mammals)	

DNA Methylation

DNA can be modified by methylation of adenine and cytosine bases in a wide variety of prokaryotes and eukaryotes (Table 1). In prokaryotes, DNA methylation is involved in the 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, flowering, imprinting, mammalian X chromosome inactivation, carcinogenesis, complex diseases and aging.

DNA methylation in mammals primarily occurs on the fifth carbon of the cytosine base (5 methylcytosine, 5-mC) of CpG dinucleotides. Approximately 70% to 80% of CpG dinucleotides are methylated in somatic cells. However, 5-mC at CpA, CpT and CpC sequences have also 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) was discovered in mouse embryonic stem cells, Purkinje neurons and granule neurons (5-6). 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. The study of 5-hmC has been hampered, because it cannot be distinguished from 5-mC in many assays (e.g., enzymatic digestion, bisulfite treatment). Recently, an enzymatic method based on radiolabeled glucosylation of 5-hmC by a bacteriophage β-glucosyltransferase, was used to detect and quantitate 5-hmC in a sequence independent manner (7). Scientists at New England Biolabs have developed an enzymatic method that does not require the use of radioactivity, which is based on treatment of the DNA with T4 Phage β-glucosyltransferase, followed by cleavage with methylation-sensitive restriction enzymes (MspI and HpaII). This forms the basis of the EpiMark™ 5-hmC and 5-mC Analysis Kit (NEB #E3317, see page 6 for more information). Additionally, while MeCP2 (a methylcytosine-binding protein, MBP) and antibodies against 5-mC specifically bind to 5-mC, similar reagents with specificities for 5-hmC are only just being developed.

Role of DNA Methylation in Mammals

DNA methylation in mammals influences a wide range of developmental and pathological processes. DNA methylation is required for normal embryonic development and survival of differentiated cells (8–10). Early in development, the paternal genome is actively demethylated,

and the maternal genome is subsequently demethylated, potentially through a passive mechanism. Methylation then increases in the blastocyst to generate the methylation patterns observed in adults. In addition, genomic imprinting, which is the specific expression of a paternal or maternal gene in placental mammals and is necessary for normal embryonic, neonatal and neurological growth, is mediated by DNA methylation and noncoding RNAs. These imprints are established during sperm and egg development by DNA methyltransferase Dnmt3A and are maintained by Dnmt1 isoforms. X inactivation, which is the process of mammalian dosage compensation of X-linked genes, is also mediated by DNA methylation and noncoding RNA.

Many cancers involve generalized, genomewide hypomethylation and local hypermethylation of CpG islands associated with promoters (reviewed in 4, 11). Demethylation of long interspersed nuclear elements (LINEs), which is a family of repetitive DNA sequences, occur early in some cancers, and the degree of LINE methylation is often correlated with the degree of malignancy. Cancer patients can vary in the frequency of methylation changes, and those with hypermethylation of multiple genes are proposed to have a CpG island methylator phenotype (CIMP), which could impact diagnosis, treatment and outcomes. In cancer, epigenetic changes are more frequent than genetic mutations and have resulted in cancer-specific biomarker discovery (e.g., Septin 9 for colorectal cancer). Although the significance of each epigenetic change is not clear, hundreds to thousands of genes can be epigenetically silenced by DNA methylation.

Increasing evidence suggests that epigenetic and genetic abnormalities also contribute to the development of other complex diseases such as type II diabetes, schizophrenia, autoimmune disease, hypertrophic cardiomyopathy, long QT syndrome and autism. Epigenetic mechanisms may help explain some features of complex diseases, including late onset, gender effects, parent-of-origin effects, phenotypic differences between monozygotic twins and fluctuation of symptoms.

Epigenetics in Clinical Applications

Epigenetic modifications and enzymes have the potential to be the basis of new therapeutics and diagnostic tests for diseases or syndromes with epigenetic components. So far, a histone deacetylase inhibitor and two DNA methyltransferase inhibitors (azanucleoside drugs) have been approved by the United States Food and Drug Administration to treat T cell cutaneous lymphoma and myelodysplastic syndrome, respectively. Additional drug candidates that inhibit histone deacetylases and DNA methyltransferases are in development (12–14), as are histone methyltransferase inhibitors and DNA methylation inhibitors that do not require incorporation into DNA like the azanucleoside drugs. The utility of combination therapies and development of more specific, targeted therapies remain areas of interest. In addition, because cancers are frequently associated with hypermethylated tumor suppressor genes and because tumor-derived DNA is present in various, easily accessible body fluids, methylated DNA could be a biomarker for detecting some cancers (15–18). Epigenetic therapies and biomarkers have also been studied and developed for systemic lupus erythematosis.

Methods to Study DNA Methylation

There are three main approaches for studying DNA methylation today. These are based on pretreating genomic DNA with either sodium bisulfite, restriction enzymes or a methylated DNA-binding affinity matrix (Table 2, page 5). Briefly, using sodium bisulfite to convert unmethylated cytosines to uracil, as opposed to 5-mC, 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 easy-to-use and not as harsh as those required for bisulfite methods; however, the resolution of the data is limited by the availability of enzyme recognition sequence. Finally, affinity-based methods use methylated DNA binding proteins or antibodies to enrich the experimental DNA sample 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 to quantify modified nucleobases in complex DNA. While HPLC is quantitative and reproducible, it requires large amounts of DNA and is often unsuitable for high throughput applications. MALDI-TOF MS is also quantitative, but is more 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 (19). For more information about several of these methods, visit www.neb.com/epigenetics.

New England Biolabs offers a suite of validated products for epigenetics research. These solutions to study DNA and histone modifications are designed to address some of the challenges of current methods.

EpiMark™ validated reagents include:

- New methylation-dependent restriction enzymes
- A novel 5-hmC and 5-mC analysis kit
- Methyltransferases (DNA and Protein)
- Histones
- Genomic DNAs

For more information, see page 8 or visit **www.neb.com/epigenetics**

Future Prospects and Challenges

The epigenetic code is hypothesized to be the combined effects of histone modifications and DNA methylation on gene expression (Table 1). While the genetic code in an individual is the same in every cell, the epigenetic code could be tissue- and cell-specific and may change over time because of aging, disease or environmental stimuli (e.g., nutrition, life style, toxin exposure) (reviewed in 20). Cross-talk between histone modifications, DNA methylation or RNAi pathways are being studied in such areas as cancer, X inactivation and imprinting.

Studying the timing and changes in epigenetic modifications during development and disease has many challenges. In addition, epigenome maps are still being assembled for most organisms. Advances in research methodologies must address issues such as the reduction in sample size requirements for histone modification studies and biomarker detection, development of better antibodies, development of more reagents and methods that can distinguish 5-mC and 5-hmC, improving highly parallel DNA analyses, and developing computational tools to organize and integrate diverse epigenomic data.

Table 2. Brief comparison of current methods to pretreat genomic DNA for methylation analysis.

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES
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 PCRs. 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	• Requires micrograms of DNA input • Harsh chemical treatment of DNA can lead to its damage • Potentially incomplete conversion of DNA • Cannot distinguish 5-mC and 5-hmC • Multi-step protocol
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
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
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

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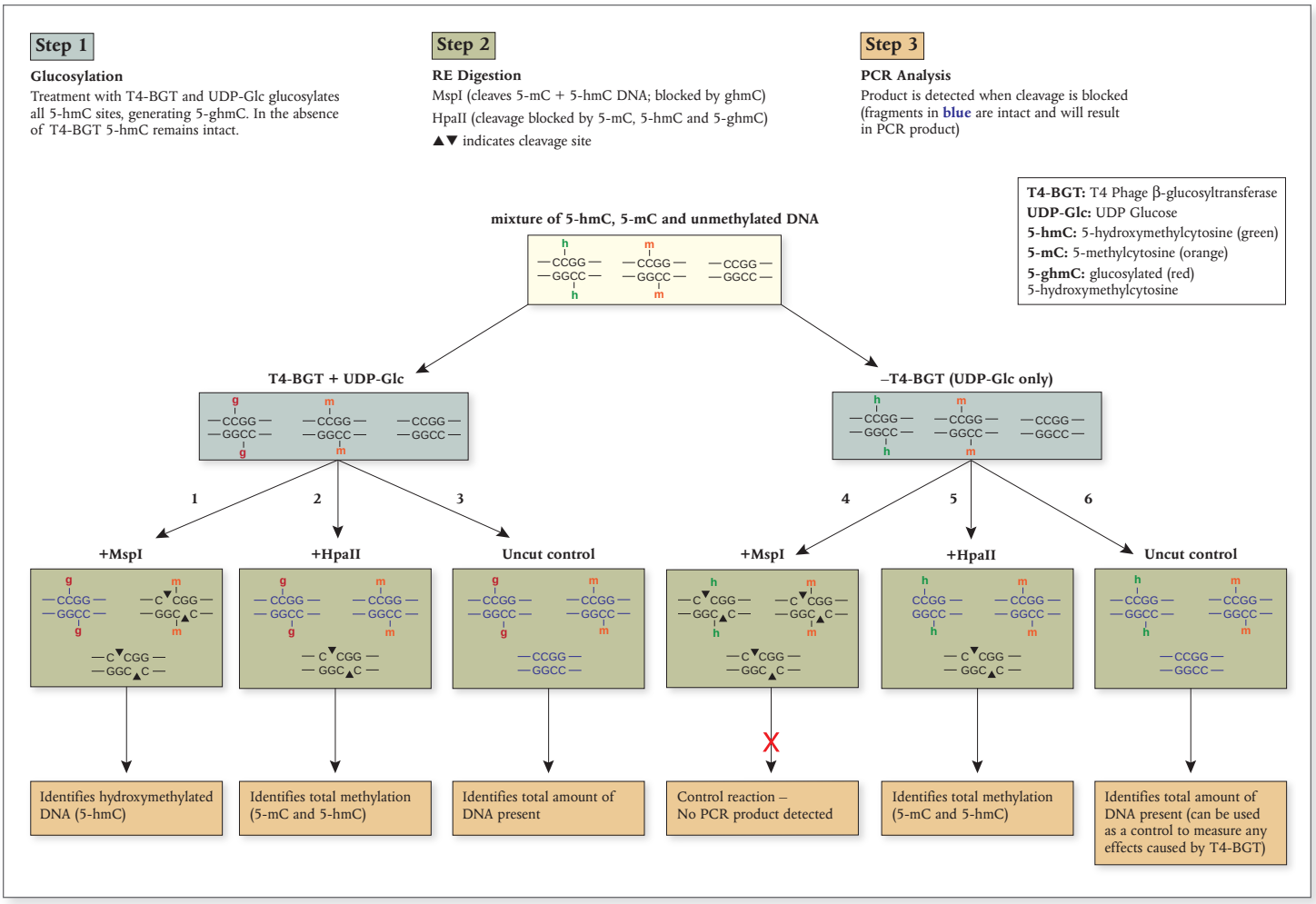
Breakthrough in 5-hmC Quantitation for Epigenetics

New England Biolabs is pleased to introduce the EpiMark™ 5-hmC and 5-mC Analysis Kit, a simple and robust method for the identification and quantitation of 5-hydroxymethylcytosine (5-hmC) and 5-methylcytosine (5-mC) within a specific DNA locus (Figure 1, page 7). This enzymatic approach utilizes the differential methylation sensitivity of the isoschizomers MspI and HpaII in a simple 3-step protocol (Table 1). The EpiMark Kit is the first commercially available PCR-based assay to reproducibly identify and quantitate the presence of 5-hmC, and expands the potential for new biomarker discovery.

Advantages

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

Table 1: Overview of 5-hmC and 5-mC identification using the EpiMark 5-hmC and 5-mC Analysis Kit



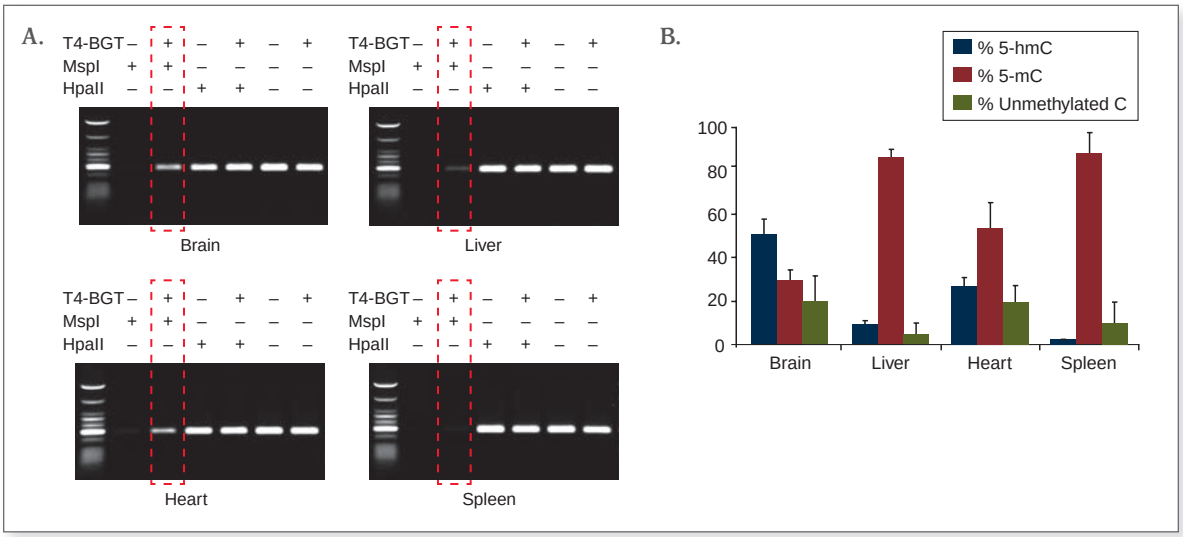
Ordering Information

PRODUCT	NEB #	SIZE
EpiMark™ 5-hmC and 5-mC Analysis Kit	E3317S	20 reactions
T4 Phage β-glucosyltransferase	M0357S/L	500/2,500 units

Also available:

NEB also offers **T4 Phage β-glucosyltransferase (T4-BGT)**, as a stand alone enzyme for the glucosylation of 5-hmC in DNA.

Figure 1: Analysis of the different methylation states in Balb/C mouse tissue samples (locus 12) using the EpiMark 5-hmC and 5-mC Analysis Kit.

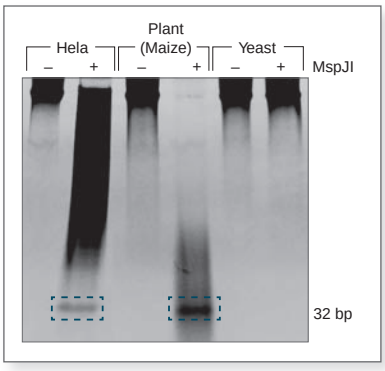


A) Endpoint PCR of the 6 different reactions needed for methylation analysis. The boxed lanes indicate the presence of 5-hmC. B) Real time PCR data was used to determine amounts of 5-hmC and 5-mC present. The results demonstrate a variation in 5-hmC levels in the tissue sources indicated.

Methylation-Dependent Restriction Enzymes

Many restriction enzymes are sensitive to DNA methylation states. Cleavage can be blocked or impaired when a particular base in the recognition site is modified (see www.neb.com/MeSensitivity for a complete list of methylation sensitive restriction enzymes). Scientists at NEB recently identified the MspJI family of restriction enzymes, which are dependent on methylation and hydroxymethylation for cleavage to occur (1). These enzymes excise 32 base pair fragments containing a centrally located 5-hmC or 5-mC modified residue that can be extracted and sequenced. Due to the known position of this epigenetic modification, bisulfite conversion is not required prior to downstream analysis. These EpiMark™ validated*, methylation-dependent restriction enzymes expand the potential for mapping epigenetic modifications and simplify the study of DNA methylation. Additionally, they provide an opportunity to better understand the role of 5-hydroxymethylcytosine in the genome.

Simplify DNA methylation analysis with MspJI



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

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

1. Zheng, Y. et al. (2010) A unique family of Mrr-like modification dependent restriction endonucleases. Nucl. Acids. Res. 38, 5527-5534.

NEB Tools – Enzyme Finder

To select restriction enzymes by name, sequence, overhang or type, visit www.neb.com/enzymefinder.

Ordering Information

PRODUCT	RECOGNITION SITE**	NEB #	SIZE
FspEI	C^{m}C (12/16)	R0662S/L	200/1,000 units
LpnPI	C^{m}CDG (10/14)	R0663S/L	200/1,000 units
MspJI	^{m}CNNR (9/13)	R0661S/L	200/1,000 units

* *The EpiMark suite of products has been validated for use in an epigenetics application.*

** *D=A or G or T (not C)*
R=A or G

Additional EpiMark™ Validated Products

In addition to the new methylation-dependent restriction enzymes and kit for 5-hmC and 5-mC identification, NEB offers a selection of DNA methyltranferases that can be used to generate methylated DNA at specific sites for gene expression studies. Genomic DNAs are also available with or without modification for use as controls.

For histone studies, choose from a selection of unmodified recombinant histones for use as substrates for the purification and characterization of histone modifying proteins. These highly pure proteins can also assemble into octamers. Several protein methyltransferases are also available for the specific methylation of histones.

Ordering Information

PRODUCT	NEB #	SIZE
METHYLTRANSFERASES		
G9a Methyltransferase	M0235S	100 units
PRMT1 Methyltransferase	M0234S	50 units
SET7 Methyltransferase	M0233S	100 units
Human DNA (cytosine-5) Methyltransferase (Dnmt1)	M0230S/L	50/250 units
CpG Methyltransferase (M.SssI)	M0226S/L/X	100/500/2,500 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
CONTROL DNA		
Jurkat Genomic DNA	N4001S	15 µg
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
CpG Methylated NIH3T3 Mouse Genomic DNA	N4005S	15 µg
HeLa Genomic DNA	N4006S	15 µg
CpG Methylated HeLa Genomic DNA	N4007S	15 µg
HISTONES		
$H1^0$ 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
ADDITIONAL RESTRICTION ENZYMES FOR EPIGENETIC ANALYSIS		
MspI	R0106S/L/M/T	5,000/25,000 units
HpaII	R0171S/L/M	2,000/10,000 units
DpnI	R0176S/L	1,000/5,000 units
DpnII	R0543S/L	1,000/5,000 units
McrBC	M0272S/L	500/2,500 units

Applications of Methyltransferases

- Positive controls for methylation specific PCR or bisulfite sequences
- CpG-methylated gene expression studies
- Nucleosome footprinting

Applications of Control DNAs

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

Applications of Histones

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

Return to **www.neb.com/epigenetics** frequently to view the latest EpiMark validated products from NEB.

Recent Advances in Restriction Enzyme Research

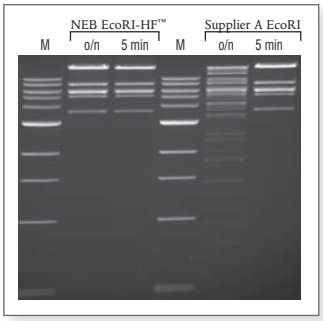
High Fidelity (HF) Restriction Enzymes

As part of our ongoing commitment to the study and improvement of restriction enzymes, NEB continues to develop its line of High-Fidelity (HF) restriction enzymes. These engineered enzymes have the same specificity as their established counterparts with the benefit of reduced star activity, and are available at the same price. They bring a new level of flexibility when choosing reaction conditions, including volume, incubation time and buffer compatibility.

Why Choose an HF Enzyme?

In addition to reduced star activity, HF restriction enzymes work optimally in NEBuffer 4, which has the highest level of enzyme compatibility and will simplify double digest reactions. They are also Time-Saver qualified and digest substrate DNA in five minutes (for more information on Time-Saver qualification, see www.neb.com). In order to distinguish these engineered enzymes, the letters -HF™ have been added to the restriction enzyme name. These enzymes are packaged with purple labels to distinguish them from our existing enzymes.

EcoRI-HF shows significantly reduced star activity, as compared to other enzymes that digest DNA in 5 minutes.



EcoRI-HF shows no star activity when used in 5 minutes or overnight. 50 µl reactions were set up using 1 µg of Lambda DNA, 1 µl of enzyme and recommended reaction buffer. Digests were incubated at 37°C. Marker M is the 1kb DNA Ladder (NEB# N3232).

High-Fidelity (HF) Restriction Enzymes:

- Engineered for performance
- Dramatically reduced star activity (up to 500-fold in some cases)
- 100% Activity in a single buffer
- Time-Saver qualified for 5 minute digests
- Value – Same price as established enzymes

Visit **www.neb.com/HF** for the most up-to-date list of HF Enzymes available from NEB.

PRODUCT	RECOGNITION SITE	NEB #
AgeI-HF™	5′... A▼CCGGT...3′ 3′... TGGCC▲A...5′	#R3552S/L
BamHI-HF™	5′... G▼GATCC...3′ 3′... CCTAG▲G...5′	#R3136S/L/T/M
BsaI-HF™	5′... GGTCTC $(N)_1$▼...3′ 3′... CCAGAG $(N)_5$▲...5′	#R3535S/L
EagI-HF™	5′... C▼GGCCG...3′ 3′... GCCGG▲C...5′	#R3505S/L/M
EcoRI-HF™	5′... G▼AATTC...3′ 3′... GCCGG▲C...5′	#R3101S/L/T/M
EcoRV-HF™	5′... GAT▼ATC...3′ 3′... CTA▲TAG...5′	#R3195S/L/T/M
KpnI-HF™	5′... GGTAC▼C...3′ 3′... C▲CATGG...5′	#R3142S/L/M
MfeI-HF™	5′... C▼AATTG...3′ 3′... GTTAA▲C...5′	#R3589S/L
NcoI-HF™	5′... C▼CATGG...3′ 3′... GGTAC▲C...5′	#R3193S/L/M
NheI-HF™	5′... G▼CTAGC...3′ 3′... CGATC▲G...5′	#R3131S/L/M
NotI-HF™	5′... GC▼GGCCGC...3′ 3′... CGCCGG▲CG...5′	#R3189S/L/M
PstI-HF™	5′... CTGCA▼G...3′ 3′... G▲ACGTC...5′	#R3140S/L/T/M
PvuII-HF™	5′... CAG▼CTG...3′ 3′... GTC▲GAC...5′	#R3151S/L/M
SacI-HF™	5′... GAGCT▼C...3′ 3′... C▲TCGAG...5′	#R3156S/L
SalI-HF™	5′... G▼TCGAC...3′ 3′... CAGCT▲G...5′	#R3138S/L/T/M
SbfI-HF™	5′... CCTGCA▼GG...3′ 3′... GG▲ACGTCC...5′	#R3642S/L
ScaI-HF™	5′... AGT▼ACT...3′ 3′... TCA▲TGA...5′	#R3122S/L/M
SphI-HF™	5′... GCATG▼C...3′ 3′... C▲GTACG...5′	#R3182S/L/M
SspI-HF™	5′... AAT▼ATT...3′ 3′... TTA▲TAA...5′	#R3132S/L/M
StyI-HF™	5′... C▼CWWGG...3′ 3′... GGWWC▲C...5′	#R3500S/L

New Reagents for mRNA Sample Preparation for Next Gen Sequencing

New England Biolabs has expanded its line of reagents for sample preparation upstream of next generation sequencing with the introduction of the NEBNext® mRNA Sample Prep Reagent Set 1 and Master Mix Set 1. These new products include enzymes, buffers and protocols for the conversion of purified mRNA to DNA and subsequent library construction. The reagents are suitable for non-directional mRNA library preparation for sequencing using the Illumina® GAIIx, GAIIe and HiSeq™2000 instruments, as well as for the preparation of expression libraries. Both sets provide substantial cost savings, and the master mix set further streamlines the workflow by reducing the number of vials and pipetting steps.

Reagents supplied in the NEBNext mRNA products

Workflow for mRNA Sample Prep	NEBNext® mRNA Sample Prep Reagent Set 1	NEBNext® mRNA Sample Prep Master Mix Set 1	NEBNext® mRNA Second Strand Synthesis Module
Ordering Information	E6100S/L 10/50 reactions	E6110S/L 10/50 reactions	E6111S/L 20/100 reactions
mRNA Fragmentation	• NEBNext RNA Fragmentation Buffer • RNA Fragmentation Stop Solution • Nuclease-Free Water	• NEBNext RNA Fragmentation Buffer • RNA Fragmentation Stop Solution • Nuclease-Free Water	
Precipitation of Fragmented mRNA	• Linear Acrylamide • Nuclease-Free Water	• Linear Acrylamide • Nuclease-Free Water	
First Strand cDNA Synthesis*	• First Strand Synthesis Reaction Buffer • Random Primers • Murine RNase Inhibitor	• First Strand Synthesis Reaction Buffer • Random Primers • Murine RNase Inhibitor	
Second Strand cDNA Synthesis	• Nuclease-Free Water • Second Strand Synthesis Reaction Buffer** • Second Strand Synthesis Enzyme Mix	• Nuclease-Free Water • Second Strand Synthesis Reaction Buffer** • Second Strand Synthesis Enzyme Mix	• Second Strand Synthesis Reaction Buffer** • Second Strand Synthesis Enzyme Mix
End Repair	• T4 DNA Polymerase • T4 Polynucleotide Kinase • T4 DNA Pol I, Large (Klenow) Fragment • Phosphorylation Reaction Buffer • Deoxynucleotide Solution Mix • Nuclease-Free Water	• NEBNext End Repair Enzyme Mix • NEBNext End Repair Reaction Buffer • Nuclease-Free Water	
dA Tailing	• NEBuffer 2 for Klenow Fragment (3´→5´ exo⁻) • Deoxyadenosine 5´- Triphosphate (dATP) • Klenow Fragment (3´→5´ exo-)	• NEBNext dA-Tailing Reaction Buffer • Klenow Fragment (3´→5´ exo-)	
Adaptor Ligation	• Quick T4 DNA Ligase • Quick Ligation Reaction Buffer (2X)	• Quick T4 DNA Ligase • NEBNext Quick Ligation Reaction Buffer (5X)	
PCR Enrichment of Adaptor-Ligated cDNA Library	• Phusion® High-Fidelity DNA Polymerase† • Phusion HF Buffer (5X) • Deoxynucleotide Solution Mix • Nuclease-Free Water	• Phusion® High-Fidelity DNA Polymerase† • Phusion HF Buffer (5X) • Deoxynucleotide Solution Mix • Nuclease-Free Water	

Advantages

- **Convenient formats** – Enzymes, buffers and nucleotides are included, at appropriate concentrations and in appropriate volumes, and are available in master mix format. Large and small sizes are available.
- **Functional validation** – Each set is validated together by preparation of a library from an accepted RNA reference sample that is sequenced on the Illumina platform.
- **Stringent quality controls**
- **Value pricing**

Applications

- **mRNA sample preparation for Illumina sequencing using the GAIIx, GAIIe and HiSeq™ 2000 instruments**
- **Expression library construction**

* *Reverse Transcriptase required but not included. See product manuals for further information.*

** *For applications requiring alternative concentrations of deoxynucleotides, NEBNext Second Strand Synthesis (dNTP-free) Reaction Buffer is available (NEB #B6117S).*

NEBNext® Reagents for DNA Sample Preparation

Reagents are available as reagent sets, master mixes or modules for the three leading next generation sequencing platforms, and offer convenience and customization of workflow. All reagents undergo stringent quality controls and functional validation by sequencing, ensuring maximum performance, convenience and value. Additionally, NEB offers NEBNext® dsDNA Fragmentase™, an enzyme-based solution for the fragmentation of DNA.

Ordering Information

PRODUCT	NEB #	SIZE	COMPATIBLE SEQUENCING PLATFORM
NEBNext® DNA Sample Prep Reagent Set 1	E6000S/L	10/50 rxns	Illumina®
NEBNext® DNA Sample Prep Master Mix Set 1	E6040S/L	10/50 rxns	Illumina
NEBNext® Quick DNA Sample Prep Reagent Set 2	E6080S/L	10/50 rxns	454
NEBNext® Quick DNA Sample Prep Master Mix Set 2	E6090S/L	10/50 rxns	454
NEBNext® DNA Sample Prep Reagent Set 2	E6020S/L	10/50 rxns	454
NEBNext® DNA Sample Prep Master Mix Set 2	E6070S/L	10/50 rxns	454
NEBNext® DNA Sample Prep Master Mix Set 3	E6060S/L	10/50 rxns	SOLiD
NEBNext® End Repair Module	E6050S/L	20/100 rxns	Illumina, 454, SOLiD™
NEBNext® dA-Tailing Module	E6053S/L	20/100 rxns	Illumina
NEBNext® Quick Ligation Module	E6056S/L	20/100 rxns	Illumina, 454, SOLiD
NEBNext® Fill-in and ssDNA Isolation Module	E6071S/L	20/100 rxns	454
NEBNext® dsDNA Fragmentase™	M0348S/L	50/250 rxns	Illumina, 454, SOLiD

Advantages

- **Convenient formats** – All of the required enzymes, buffers and nucleotides are included, many available in master mix format. Modules offer the ability to customize sample preparation.
- **Functional validation** – Each reagent set or module is functionally validated by preparation of a genomic DNA library that is sequenced using the appropriate sequencing platform (Illumina, Roche/454 or SOLiD) and by preparation of an expression library or single-stranded DNA.
- **Stringent quality controls** – Additional QCs ensure maximum quality and purity.
- **Value pricing**

FAQ Spotlight

Q: How do I choose which NEBNext product to use with my sequencing platform?

A: *For DNA sample preparation, NEBNext reagents are available for the three major sequencing platforms. Customers can choose from reagent sets, master mixes or modules depending on their preference and interest in customization. Each reagent set or master mix name includes a number that corresponds to a specific platform. NEBNext products ending in a "1" are used with the Illumina platform, "2" are used with the Roche/454 platform and "3" are used with the SOLiD platform.*
For RNA sample preparation, NEBNext reagent sets and master mixes are available for the Illumina platform only at this time. RNA reagents compatible with the GS FLX, GS FLX Titanium and SOLiD platforms will be available soon.

Q: The NEBNext product manuals refer to the individual reagents as being "Lot Controlled". What does this mean?

A: *Each set of reagents is functionally validated together through construction of a library from genomic DNA or an accepted RNA reference sample that is then sequenced on the appropriate platform. Once a new lot of any of the reagents is introduced into the set, the set is re-validated by library construction and sequencing.*

NEW ENGLAND BioLabs® Inc.
enabling technologies in the life sciences

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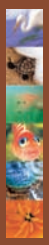

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RNA ANALYSIS
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GENE EXPRESSION & CELLULAR ANALYSIS

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