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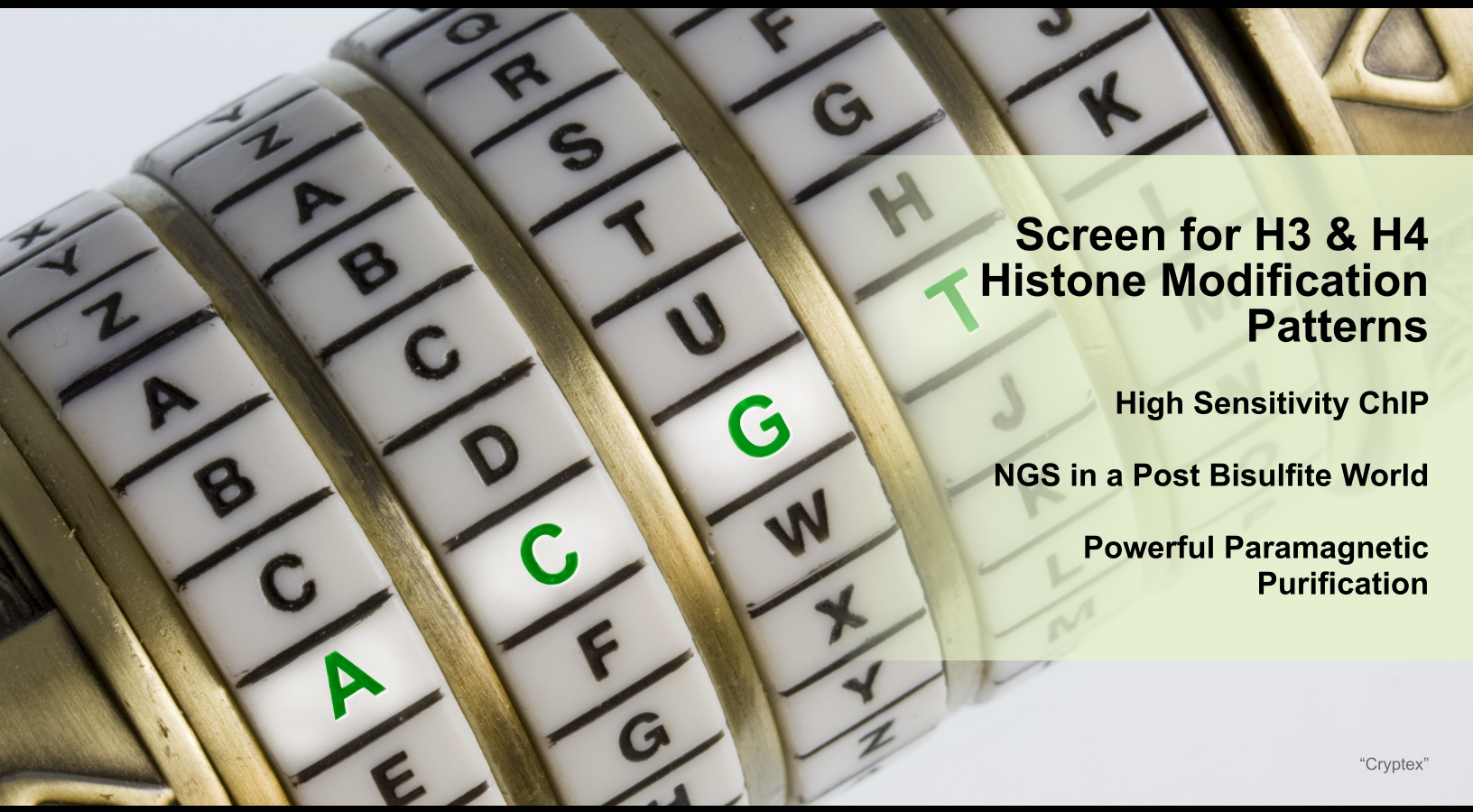
THE DECODER

A NEWSLETTER FOR EPIGENETIC RESEARCHERS

2014 • Issue 3



Screen for H3 & H4 Histone Modification Patterns

High Sensitivity ChIP

NGS in a Post Bisulfite World

Powerful Paramagnetic Purification

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PROGRESS in DNA Methylation Analysis Technology:

Selecting the Right Tool for the Job

Recent developments in DNA methylation analysis technologies have given researchers even more to consider when choosing the right method of analysis for their particular study. See what the latest tools and technologies offer and review some more popular epigenetic techniques.

PROGRESS IN DNA Methylation Analysis Technology:

Selecting The Right Tool For The Job

DNA methylation occurs by the covalent addition of a methyl group (CH3) at the 5-carbon of the cytosine ring by DNA methyltransferases (DNMTs), resulting in 5-methylcytosine (5-mC). When a CpG island in the promoter region of a gene is methylated, expression of the gene is repressed [1]. A number of human diseases such as cancer are known to occur when DNA methylation is not properly established and/or maintained. DNA methylation is a dynamic and reversible process which can result in several intermediate modification forms. 5-mC converted from cytosine by DNMTs can be hydroxylated to 5-hydroxymethylcytosine (5-hmC) by TET enzymes. 5-hmC is further modified to 5-formylcytosine (5-fC) and 5-carboxycytosine (5-caC) by the same TET enzymes [2, 3]. Analysis of DNA methylation is critical to understanding and targeting DNA methylation–associated changes, which would help to identify DNA methylation markers and their biological regulation.

Recently, a number of new methods were developed for profiling genome-wide and region-specific DNA methylation. These methods have enabled epigenetic variation to be connected to phenotypic consequences on an unprecedented scale and at single-base resolution. For example, CRISPR/Cas9-based epigenetic editing has been engineered for targeted rewriting of epigenetic marks to modulate expression of selected target genes, allowing functional methylation analysis of those genes. Although cost, minimum sample input requirements, accuracy, rapidity and throughput are important considerations, choosing the right method is most important for success of the analysis. Here we discuss the principles of these recently developed methods and provide suggestions for choosing appropriate methods for DNA methylation analysis specifically required for different studies.

Oxidative Bisulfite-Sequencing: Identify and Distinguish 5-mC from 5-hmC

5-hmC content is found to be less than 0.7% of total nucleotides in human and mouse tissues [4] and barely detected in most cultured cell lines. 5-hmC is regarded as an intermediate of DNA demethylation and associated with expressed genes and has a potential role in gene activation. Like 5-mC, 5-hmC remains the same after bisulfite-conversion of cytosine to uracil (C to U), which makes 5-hmC indistinguishable from 5-mC. As a consequence, all 5-hmC will be misread as 5-mC by bisulfite-sequencing, resulting in incorrect identification of DNA methylation status since both 5-mC and 5-hmC are included. Booth et al., established a method called oxidative bisulfite-sequencing (oxBS-Seq) to enable 5-hmC to be distinguished from 5-mC [5]. This method is based on chemical oxidation (KRuO4) of 5-hmC into 5-fC which can be converted to uracil by bisulfite treatment, leaving only 5-mC to be detected as cytosine by sequencing. Compared to bisulfite-sequencing without an oxidation step, which yields 5-mC + 5-hmC, oxidative bisulfite-sequencing allows specific 5-hmC bases to be identified after quantitative subtraction (Fig. 1) [6].

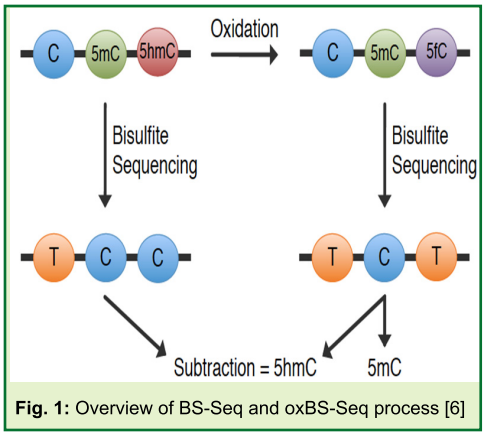

Fig. 1: Overview of BS-Seq and oxBS-Seq process [6]

Post-Bisulfite Sequencing: Genome-Wide Methylation Assessment at Single Cell Level

Traditional bisulfite-sequencing methods such as WGBS require large amounts of DNA (>1 µg) as input material, which is difficult to prepare from limited biological samples such as tumor biopsy samples, early embryos, embryonic tissues and circulating DNA. These methods also require that DNA is first sheared and then ligated to adaptors followed by bisulfite conversion (post-ligation bisulfite conversion). This procedure causes most of the DNA fragments contained in the adaptor-DNA fragment constructs to be broken, thereby forming mono-tagged templates that will be removed during library enrichment. Thus, incomplete coverage and bias can occur when performing whole genome bisulfite-sequencing. To improve traditional WGBS, post-bisulfite sequencing methods have been established [7, 8]. The assay principle is that DNA is first treated with bisulfite salt and then the bisulfite-treated DNA (which is in single-stranded form) is converted to double-stranded DNA to be directly used for ligation followed by PCR amplification (Fig. 2). The prepared library DNA is then used for sequencing. The major advantage of post-bisulfite sequencing is that it ensures maximum yields from minimum amounts of starting material which can be as few as a single cell [9].

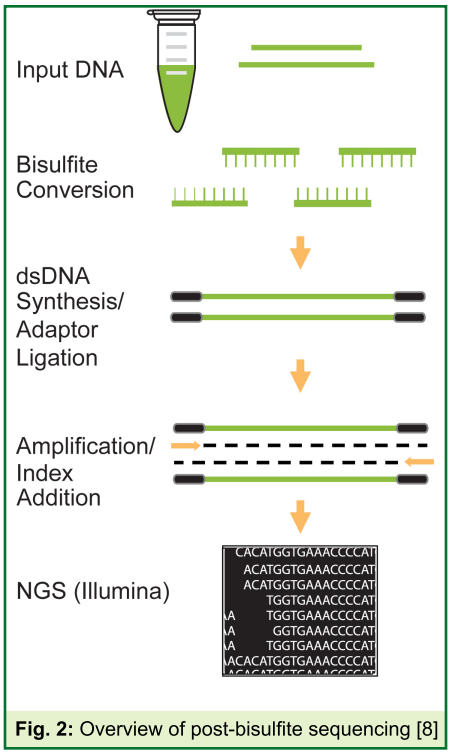

Fig. 2: Overview of post-bisulfite sequencing [8]

Recently, a number of new methods were developed for profiling genome-wide and region-specific DNA methylation.

Targeted Methylation-Sequencing: Flexible Targeted Gene Panels or Massive Genome Regions

Current DNA methylation assays are limited in the flexibility and efficiency of characterizing a large number of genomic targets. Targeted methylation-sequencing was developed to solve this problem. This technique can be carried out using bisulfite-PCR amplification, ligation capture, bisulfite padlock probe (BSPP) capture or liquid hybridization followed by bisulfite-sequencing.

Bisulfite-PCR amplification of target loci requires that the primers are designed based on the bisulfite converted reference genome. PCR amplification can be multiplexed for simultaneous assay of hundreds to thousands of targets by performing multiple singleplex PCR amplifications in emulsion droplets, allowing a maximum of 20,000 loci to be targeted with 99% sensitivity and 90% specificity. Ligation capture such as mTACL [10] is based on selected enrichment of target genome regions to reduce the complexity of the genomic DNA to be analyzed.

The capture is achieved using segments of DNA complementary to the targets, except that all of the thymidines (T) have been substituted with uridines (U). These "dU capture probes" (dU probes) contain sequences complementary to the targets to be analyzed, flanked by two common regions shared by all dU probes. The common regions can match PCR primers used later for bisulfite-sequencing or barcoded primers for PCR amplification and pyrosequencing. 100% sensitivity and 90% specificity can be achieved. BSPP capture relies on a padlock probe-based capture of bisulfite-converted DNA [11]. The padlock probe contains two short capture sequences which are selected to be complementary to bisulfite converted DNA with minimal overlap with CG dinucleotides. A unique feature of BSPP is that probes can be pooled into a single capturing reaction. Over 97% specificity with BSPP has been demonstrated. Liquid hybridization based capture for bisulfite-sequencing (LHC-BS) [12] involves hybridization of preligated target DNA fragments into biotinylated oligonucleotides followed by affinity enrichment selection, bisulfite conversion, PCR amplification and sequencing (Fig. 3). The specificity of LHC-BS is between 70%–80%, heavily dependent on oligonucleotide design, and is generally lower than the specificity achieved with other ligation-based target enrichment methods. However, LHXC-BS has more uniform target coverage compared to ligation capture and BSPP capture, which translates to better sensitivity and cost efficiency. Recent commercially available LHC-BS methods have been developed such as SureSelect^{XT} MethylSeq™ and SeqCap EZ™.

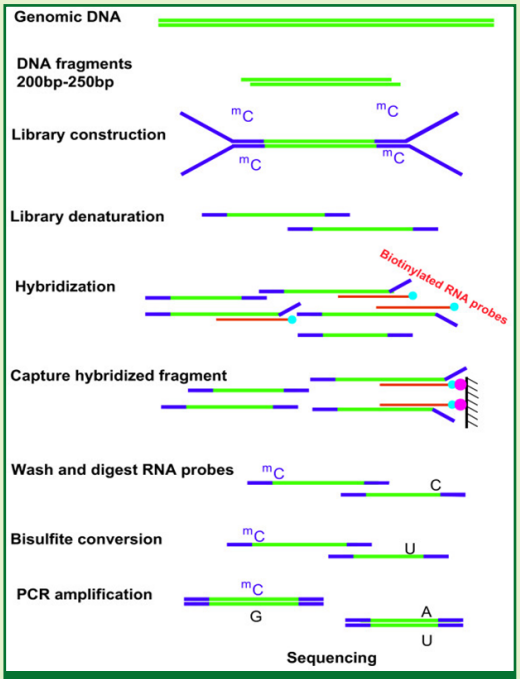

Fig. 3. LHC-BS assay overview [12]

Epigenetic Editing: Targeted Rewriting of Epigenetic Marks for Functional Methylation/Demethylation Analysis of Targeted Genes

Epigenetic editing is a type of genetic engineering recently developed in which the epigenome is modified at specific sites using engineered molecules targeted to those sites (as opposed to whole-genome modifications). The mechanism results in a change of the status of DNA methylation at target loci: removal or addition of a methyl group on cytosine. Epigenetic editing could address a bigger technology bottleneck in epigenetics than high throughput sequencing; that is, it allows researchers to determine the exact biological role of an epigenetic modification at the site in question.

Early studies of epigenetic editing utilized a Zinc finger system [13] or TALE system [14] to introduce DNMT/TET enzymes to the specific loci for targeted DNA methylation/demethylation. The most recently developed, CRISPR/Cas9 system is now used for overcoming some of the limitations of previous tools. In the CRISPR/Cas9 system, histone or DNA modifying enzymes are fused to Cas9 mutants without nuclease activity (dCas9), which means these enzymes will not cleave DNA but can still be recruited by gRNAs to target specific DNA sites to modulate epigenetic status (Fig. 4) [15]. Such CRISPR/Cas9-based epigenetic editing will be able to not only alter DNA methylation status, but also influence chromatin states and histone modifications, which would allow access to an entirely new repertoire of regulation of gene function.

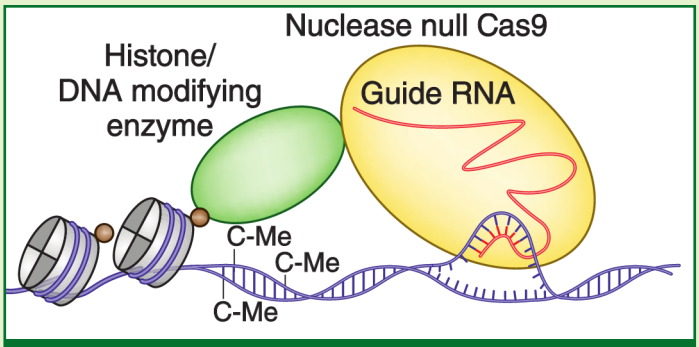

Fig. 4. CRISPR/Cas9-mediated targeted DNA methylation/demethylation [15]

Selection of the Right Tool for Your Study

The growing number of new sequencing-based methods for DNA methylation analysis makes it necessary for one to know the appropriate steps to take and how to choose the most suitable method for a study since the majority of methods require a big investment in time, capital, equipment and resources.

Here are some suggestions:

(1) Start with a simple pre-screen of global DNA methylation and hydroxymethylation status in your samples. With the use of a quick global DNA methylation or hydroxymethylation assay, you can know if your sample contains 5-mC or 5-hmC and how much. Then you can decide exactly what the next step is and which method should be used. Such global DNA methylation or hydroxymethylation assay kits are commercially available from Epigentek. (2) If you find your sample contains both 5-mC and 5-hmC, oxidative bisulfite-sequencing could be used, which would help to identify and distinguish 5-mC from 5-hmC on a genome-wide scale at single base resolution. If only 5-mC is detected with little or no 5-hmC, conventional WGBS or RRBS can be performed. (3) If the sample amount is limited or single cell level analysis is desired, post-bisulfite sequencing may meet this requirement. Post-bisulfite sequencing kits are now commercially available. (4) For analyzing methylation/hydroxymethylation of specific gene panels such as a cancer gene panel or multiple genomic regions, targeted bisulfite-sequencing or targeted oxidative bisulfite-sequencing is a good choice. (5) For determining the function of specific methylation sites on a loci or the role of an epigenetic modification on a specific gene, CRISPR/Cas9-based epigenetic editing would be applicable.

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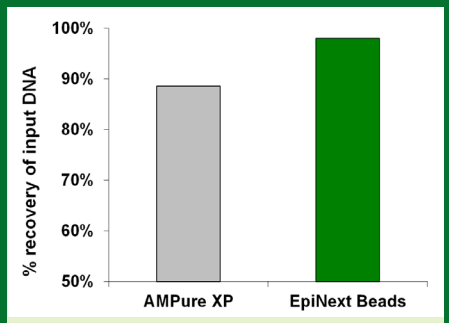
NEXT-GEN Sequencing

Next-generation sequencing technologies have increased data output volumes and lowered the cost of DNA sequencing beyond what is possible with standard dye-terminator methods. This has lead to a significant advancement of understanding of several epigenetic mechanisms, namely genome-wide DNA methylation and protein-DNA interaction.

Powerful Paramagnetic Purification

Achieving a high recovery of purified DNA or selected DNA fragments is an essential first step for many genomic-based downstream applications such as PCR, sequencing, cloning, microarray, and DNA fragment analysis, regardless of the platform used. One effective way to prepare DNA samples is by using a magnetic-bead-based isolation solution. Free of hazardous chemicals, this method of DNA separation renders high quality, efficient yields with excellent sample concentration.

Epigentek offers the **EpiNext™ DNA Purification HT System**, also called "EpiNext beads" *(Cat. # P-1063),* which utilizes magnetic bead technology for high throughput DNA or PCR amplicon purification and DNA size selection. DNA or PCR amplicons bind tightly to the beads and excess primers, adaptors, nucleotides, salts, enzymes, and PCR inhibiting substances can be removed by simply washing the beads. The system can also be used for concentrating DNA and is suitable for selectively and consistently capturing DNA fragments or PCR amplicons that are 100 bps or larger in size.



Percentage of recovery of input DNA fragments by AMPure® XP* beads vs EpiNext™ beads.

*Agencourt® AMPure® is a registered trademark of Beckman Coulter, Inc.

Tools in the Lab:

EpiNext™ High-Sensitivity DNA Library Preparation Kit (Illumina) *(Cat. # P-1053)*

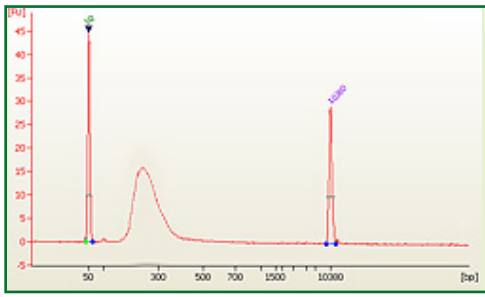
Prepare a DNA library from input as low as 0.2 ng of DNA for use in next-generation sequencing applications. Both non-barcoded (singleplexed) and barcoded (multiplexed) DNA libraries can be constructed quickly with reduced bias.

EpiNext™ NGS Barcode (Index) Set-12 *(Cat. # P-1060)*

Construct multiplex DNA/RNA libraries used for next-generation sequencing with the Illumina platform including GAIIx, HiSeq and MiSeq. This product includes 12 DNA barcodes, each of which contain an index and flow cell binding sequence and can be attached to the sample insert during library preparation.

EpiNext™ Post-Bisulfite DNA Library Preparation Kit (Illumina) *(Cat. # P-1055)*

This kit allows you to prepare a DNA library -- after successful bisulfite conversion -- for various Illumina platform-based bisulfite sequencing (bisulfite-seq) assays, such as whole genome bisulfite sequencing (WGBS), oxidative bisulfite sequencing (oxBs-seq), reduced representation bisulfite sequencing (RRBS), and other bisulfite-based next-generation sequencing applications.



Size distribution of library fragments as demonstrated by a post-bisulfite DNA library constructed using the **EpiNext™ Post-Bisulfite DNA Library Preparation Kit** from 10 ng of input DNA.

EpiNext™ DNA Size Selection Kit *(Cat. # P-1059)*

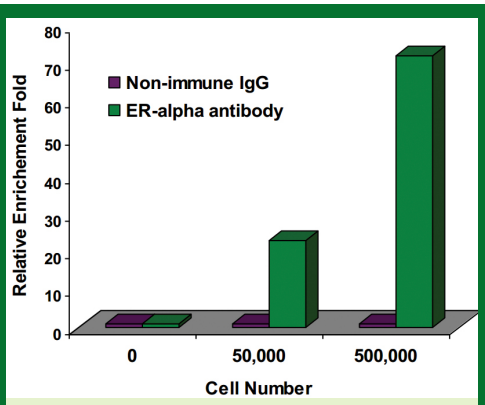
This kit is a complete set of optimized reagents for quick removal of DNA fragments of <150 bps for library preparation in next-generation sequencing applications. The kit utilizes magnetic bead technology and is suitable for Illumina, Life Technologies (SOLiD), Ion Torrent, and Roche/454 platforms. It can also be used for removing undesired larger DNA fragments by optimizing the bead to DNA volume ratio accordingly. The indicated number of reactions can be performed for a standard 50 µl solution input DNA sample.

CHROMATIN Studies

Chromatin structures are regulated by various mechanisms including histone modification and chromatin remodeling, which involve the binding of transcription factors. By using tools such as chromatin immunoprecipitation, it is possible to gain further insight into the dynamic interactions between transcription proteins and components of chromatin, and to ultimately understand their roles in cellular functions such as gene transcription and epigenetic silencing.

Maximum Sensitivity ChIP

Chromatin immunoprecipitation (ChIP) is an advantageous tool for studying protein-DNA interactions. It allows for the detection of a specific protein bound to a specific gene sequence in living cells using PCR (ChIP-PCR), microarrays (ChIP-chip), or sequencing (ChIP-seq). ChIP analysis requires that ChIPed DNA contains minimal background in order to reliably identify true TF-enriched regions. High background in ChIP is mainly caused by ineffective wash buffers, insufficient cross-link reversal, inappropriate DNA fragment length, and residual RNA interference. To effectively capture TF/DNA complexes, which are often in low abundance, an ideal ChIP method requires having maximum sensitivity with minimized background levels. This method should also be able to enrich highly abundant protein/DNA complexes using a small amount of cells or tissues in a high throughput format. Epigentek's microplate-based **ChromaFlash™ High-Sensitivity ChIP Kit** *(Cat. # P-2027)* is designed to achieve these goals by maximizing sensitivity and minimizing non-specific background signals.



Low abundance protein enrichment: Sheared chromatin isolated from different numbers of MCF-7 cells was used for ChIP-qPCR analysis of ER-a enrichment in TFF1 promoters using the **ChromaFlash™ High-Sensitivity ChIP Kit** *(Cat. # P-2027)*and the **EpiQuik™ Quantitative PCR Fast Kit** *(Cat. # P-1029).*

Tools in the Lab:

ChromaFlash™ Chromatin Extraction Kit *(Cat. # P-2001)*

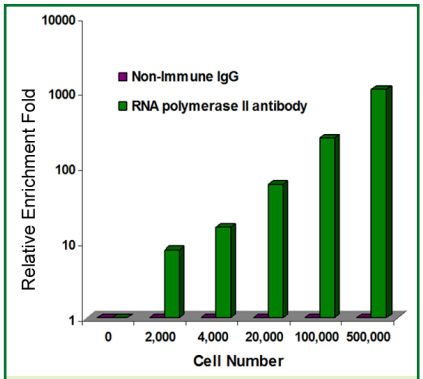
Isolate chromatin or DNA-protein complex from mammalian cells or tissues in a simple and rapid format. Chromatin prepared by this kit can be used in a variety of chromatin immunoprecipitation methods. The isolated chromatin can also be used in other chromatin-related applications such as in vitro protein-DNA binding assays and nuclear enzyme assays.

EpiSonic™ NGS Sonication System *(Cat. # EQC-2000)*

Multi-sample sonication instrument for use in a wide range of biological applications, in particular for DNA and chromatin shearing. This completely digital instrument allows for simultaneous optimized processing of 1 to 12 samples at small volume, ideal for NGS applications, and can be easily integrated into existing lab workflows.

EpiNext™ ChIP-Seq High-Sensitivity Kit (Illumina) *(Cat. # P-2030)*

Carry out a successful ChIP-Seq starting from mammalian cells or tissues with a single kit. This kit is designed to selectively enrich a chromatin fraction containing specific DNA sequences from various species, particularly mammals, and to prepare a ChIP-Seq library for next-generation sequencing using Illumina platforms such as Illumina Genome Analyzer II, HiSeq and MiSeq systems.



Highly sensitive ChIP: The sheared chromatin isolated from different amounts of MBD-231 cells was used for ChIP-qPCR analysis of RNA polymerase II enrichment in GAPDH promoters.

HISTONE H3 & H4 Modification Patterns

Histones are primary protein components of eukaryotic chromatin and play a role in gene regulation. H3 and H4 histones have tails protruding from the nucleosome that can be modified post-translationally to alter the histone's interactions with DNA and nuclear proteins, leading to epigenetic changes for regulating many normal and disease-related processes.

Screen and measure up to 21 different histone H3 or 10 different histone H4 modification patterns in a single ELISA-like kit.

EpiQuik™ Histone H3 Modification Multiplex Assay Kit (Colorimetric) - Simultaneously screen and measure 21 different, well-characterized histone H3 modifications in 2 hours and 30 min.

H3K4me1	H3K4me2	H3K4me3	H3K9me1	H3K9me2
H3K9me3	H3K27me1	H3K27me2	H3K27me3	H3K36me1
H3K36me2	H3K36me3	H3K79me1	H3K79me2	H3K79me3
H3K9ac	H3K14ac	H3K18ac	H3K56ac	H3ser10P
H3ser28P				

EpiQuik™ Histone H4 Modification Multiplex Assay Kit (Colorimetric) - In a simple 2 hour and 30 min procedure, simultaneously detect and quantify nearly all histone H4 modifications (10 different types).

H4K5ac	H4K8ac	H4K12ac	H4K16ac	H4R3m2a
H4R3m2s	H4K20m1	H4K20m2	H4K20m3	H4ser1

Cat.#	Product Name
P-3100	EpiQuik™ Histone H3 Modification Multiplex Assay Kit
P-3102	EpiQuik™ Histone H4 Modification Multiplex Assay Kit

HATs & HDACs

The balance between protein acetylation and deacetylation controls several physiological and pathological cellular processes, and the enzymes involved in the maintenance of this equilibrium—acetyltransferases (HATs) and deacetylases (HDACs)—have been widely studied. Presently, evidence obtained in this field suggests that the dynamic acetylation equilibrium is mostly maintained through the physical and functional interplay between HAT and HDAC activities.

Direct Measurement of Histone Acetyltransferases (HATs)

HATs are enzymes that modify N-terminal lysine residues of histones by the addition of an acetyl group. HAT activation or inhibition is vital in several diseases including neurodegenerative disorders and cancer. Common procedures indirectly measure HAT activity by detecting free CoA or CoA-SH. Using a novel assay principal, HAT activity can now be directly measured to produce highly accurate results.

Get the HAT Kit Solution

Epigentek offers the **EpiQuik™ HAT Activity/Inhibition Assay Kit** *(Cat. # P-4003).* This kit is a ready-to-use set of essential components needed to measure histone acetyltransferase activity/inhibition safely and quickly. It is suitable for measuring histone acetyltransferase activity/inhibition from a broad range of species including mammalian cells/tissues, plants, and bacteria.

A Simple Yet Innovative Approach for Quantifying All HDACs

Histone deacetylases (HDACs) are tightly involved in development of human cancer, transcriptional repression of gene expression, cell cycle regulation, and cell proliferation. HDAC inhibition displays significant effects on apoptosis, cell cycle arrest, and differentiation in cancer cells. The entire family of HDACs, 1-11, can be easily quantified using an ELISA-like process. Epigentek offers several HDAC assay kits to suit your research needs.

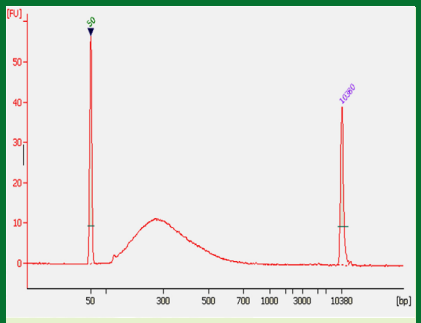
Cat.#	Product Name
P-4005	EpiQuik™ HDAC1 Assay Kit (Colorimetric)
P-4006	EpiQuik™ HDAC2 Assay Kit (Colorimetric)
P-4040	EpiQuik™ HDAC3 Assay Kit (Colorimetric)
P-4042	EpiQuik™ HDAC4 Assay Kit (Colorimetric)
P-4044	EpiQuik™ HDAC5 Assay Kit (Colorimetric)
P-4046	EpiQuik™ HDAC6 Assay Kit (Colorimetric)
P-4048	EpiQuik™ HDAC7 Assay Kit (Colorimetric)
P-4007	EpiQuik™ HDAC8 Assay Kit (Colorimetric)
P-4050	EpiQuik™ HDAC9 Assay Kit (Colorimetric)
P-4052	EpiQuik™ HDAC10 Assay Kit (Colorimetric)
P-4054	EpiQuik™ HDAC11 Assay Kit (Colorimetric)
P-4034	Epigenase™ HDAC Activity/Inhibition Direct Assay Kit
P-4036	Epigenase™ Universal SIRT Activity/Inhibition Assay Kit

DNA Methylation & Demethylation

DNA methylation was the first epigenetic mark to be discovered, involving the addition of a methyl group to the 5 position of cytosine by DNA methyltransferases, and can be inherited through cell division. DNA hydroxymethylation, caused by oxidation of 5-methylcytosine through the TET family of enzymes, was further discovered to be involved in switching genes on and off. DNA methylation plays an important role in normal human development and is associated with the regulation of gene expression, tumorigenesis, and other genetic and epigenetic diseases.

Upgrade Your Library Methyl-Seq

Traditional methods of performing whole genome bisulfite sequencing are time consuming and require large amounts of input material. With the **EpiNext™ High-Sensitivity Bisulfite-Seq Kit (Illumina)** *(Cat. # P-1056A)* the entire process is streamlined and optimized for your convenience. The kit combines bisulfite conversion and library preparation together, allowing you less steps in the process, plus you'll get your constructed library in only 6 ½ hours.



Size distribution of library fragments. Post-bisulfite DNA library was prepared from 10 ng of human placenta DNA using the **EpiNext™ High-Sensitivity Bisulfite-Seq Kit.**

▲ **EpiNext™ High-Sensitivity Bisulfite-Seq Kit** *(Cat. # P-1056A)*

Tools in the Lab:

Methylamp™ DNA Modification Kit *(Cat. # P-1001)*

Convert DNA so that 5-methylcytosines can be detected in downstream applications, for the purpose of gene-specific DNA methylation analysis.

BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit *(Cat. # P-1050)*

Fast, high throughput DNA bisulfite conversion. The kit uses a unique liquid conversion solution and magnetic binding bead clean-up process to quickly generate bisulfite-converted DNA for higher yields via a 96-well microplate in just 1 hour and 20 min. A magnetic bead based approach via microplates also allows for automation workflows .

EpiQuik™ DNMT Activity/Inhibition Assay Ultra Kit (Colorimetric) *(Cat. # P-3009)*

Colorimetrically measure DNA methyltransferase activity or inhibition in less than 4 hours on a 96-stripwell microplate. The kit is ready-to-use and provides all the essential components needed to carry out a successful DNMT activity/inhibition experiment without the need for radioactivity or any special equipment.

Epigenase™ 5mC-Hydroxylase TET Activity/ Inhibition Assay Kit *(Cat. # P-3086)*

Measure activity/inhibition of total cytosine oxygenase TET enzymes in nuclear extracts or purified TET isoforms (TETs 1-3) from a broad range of species.

THE MOST **Popular** PROTOCOL FOR AN **Accurate** ASSAY

Global DNA Methylation ELISA

View Our MethylFlash™ Global DNA Methylation Kits Online at:
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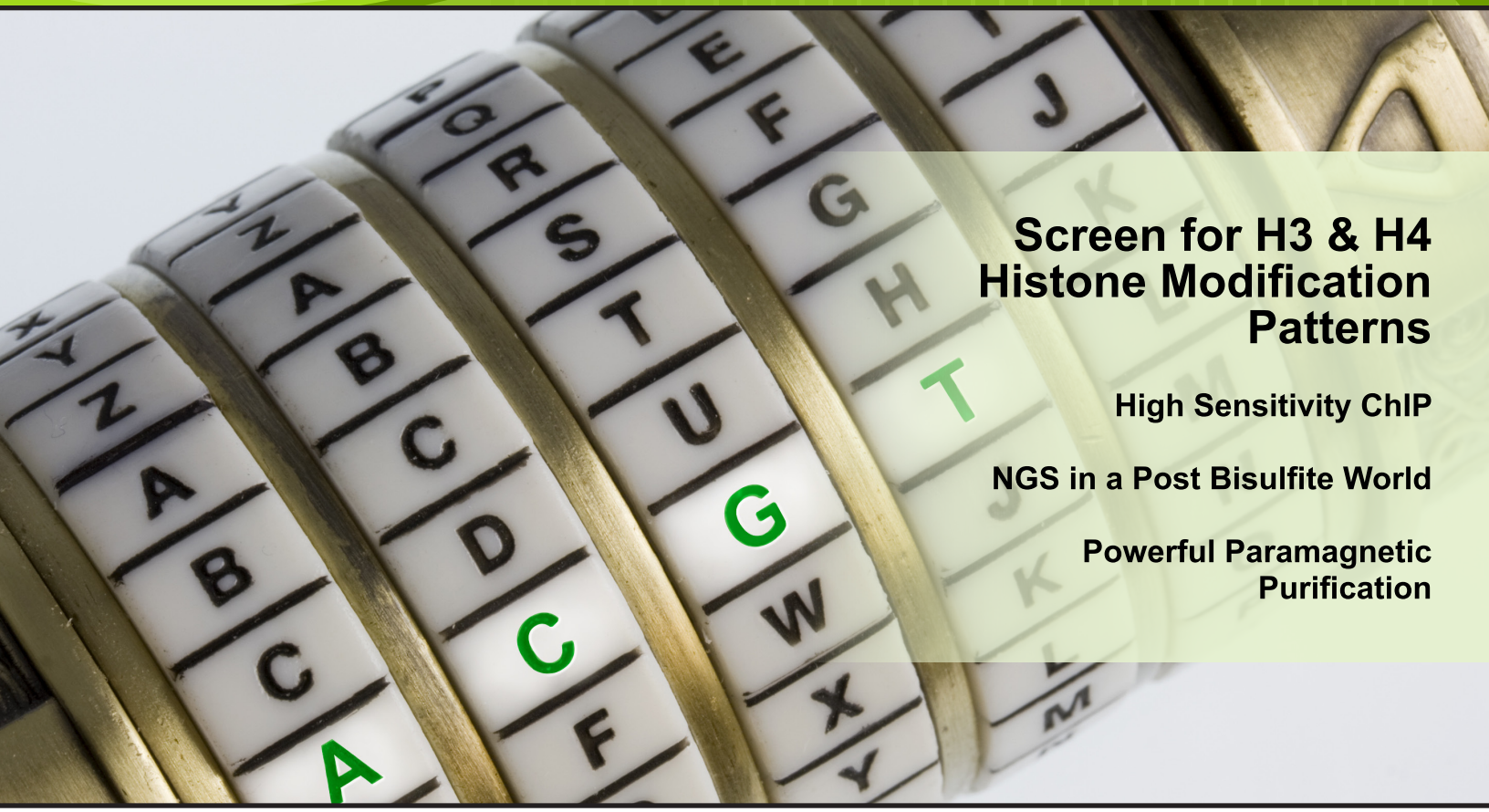

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