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THE DECODER

A NEWSLETTER FOR EPIGENETIC RESEARCHERS

2016 • Issue 4

An Easier Means to Studying Global DNA Methylation

Multiplex Identification of Histone Modifications

m6A RNA Methylation ELISA

Microplate-based ChIP

"Rubik's Cube"

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APPLYING NEXT GENERATION SEQUENCING TO EPIGENETICS

An overview of the integration of next generation sequencing into epigenetic research as its ecosystem of complementary technologies continues to mature.

Applying Next Generation Sequencing to Epigenetics

Next-generation sequencing (NGS) is a method of non-Sanger-based high throughput DNA sequencing in which millions or even billions of DNA strands can be sequenced in parallel, thereby minimizing the need for the fragment-cloning methods that are often used in Sanger sequencing of genomes as well as yielding significantly more throughput. There are several modern technologies that exist for the typical NGS – these players include Illumina, Ion Torrent, Roche 454, SOLiD, and Pacific Biosciences. The emergence of NGS has provided epigenetic researchers an important tool to gain detailed and comprehensive insight into epigenetic modifications in the genomes of many species and cell types by generating and exploring substantially more data.

Genome-Wide DNA Methylation Identification

DNA methylation has always played a major role in epigenetics and it is unsurprising that it was one of the first mechanisms to be evaluated on NGS platforms for epigenetic research. Many NGS-based methods for the genome-wide detection of DNA methylation have been developed over the past decade. These methods primarily include reduced representation bisulfite sequencing (RRBS), whole genome bisulfite sequencing (WGBS), and methylated DNA immunoprecipitation sequencing. MeDIP-Seq needs only small amount of DNA, but the analysis resolution of these methods is not high (around 100 bases). In contrast, RRBS or WGBS allow genome-wide or whole genome DNA methylation to be analyzed at single base-resolution but requires a relatively large amount of DNA (> 1 µg). Most recently, a post-bisulfite DNA tagging method was developed as an important improvement of bisulfite-based RRBS or WGBS [1]. In this method, DNA is first bisulfite converted followed by adaptor ligation and then sequenced. This improvement allows for use of small amounts of DNA from a few cells, even a single cell (scWGBS), with high resolution at single-base resolution level [2].

"NGS-based analysis of epigenetic changes has dramatically increased our ability to survey epigenetic markers genome wide"

Genome-Wide Chromatin (or Histone Modification) Analysis

Another popular application in NGS-based epigenetic research is chromatin immunoprecipitation sequencing (ChIP-seq), which can be utilized to investigate whether a specific protein, transcription factor, or histone binds to specific sequences of a gene in living cells at genome-wide level. In recent years, the multi-day ChIP method itself was significantly improved in assay speed (fast ChIP), throughput (96-well plate format or "matrix" ChIP), enrichment efficiency (carrier ChIP), and precise binding location (ChIP-exo), involving less amounts of cells/tissues at higher resolution and throughput and more accurate positioning of protein-DNA binding while reducing protocol time. A new ChIP-Seq method, single cell ChIP-seq, was also developed for the analysis of transcription factors and site-specific histone modifications at single cell but genome-wide scale [3].

Fig. 1: With the release of their latest MiniSeq™ System, Illumina® continues to be the dominant player in the NGS instrumentation market.

Moreover, NGS can be applied to genome-wide chromatin accessibility detection. Chromatin accessibility approaches directly measure the effect of chromatin structure modifications on gene transcription by separating the genome with enzymes or chemicals and isolating either the accessible or protected locations. Genome-wide chromatin accessibility identification is dependent on NGS analysis. Current genome-wide high throughput chromatin accessibility assays mainly include digestion enzyme-based MNase-seq, DNase-seq, and ATAC-seq, as well as chemical-based FAIRE-seq. Most recently, two new methods, single cell ATAC-seq and THS-seq were established to enable an increase in the sensitivity of chromatin accessibility assay with use of much fewer input cells [4,5].

Epigenetic Editing

There has been significant interest as of late in the scientific community towards genome editing. Currently, the major groups of DNA binding proteins that have been predominantly used for genome editing are Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR) sequences with CRISPR-Associated Protein 9 (Cas9). The CRISPR/Cas9 system consists of two components. First, the Cas9 protein is a nuclease. Second, the CRISPR/Cas9 system consists of a synthetic guide RNA (sgRNA). The Cas9 nuclease, as a dedicated effector enzyme, cleaves DNA when guided by sgRNA sequences. This system can be adapted into a type of genetic engineering, epigenetic editing, in which the epigenome is modified at specific sites using engineered molecules targeted to those sites.

CRISPR/Cas9 has already been applied to investigation of epigenetic mechanisms, by targeting MeCP2 and DNMTs. The system was recently used for experimentation in human embryonic stem cells to create precise knockout deletions in the DNMTs. Another important progress in this field is to identify effective transfection of CRISPR/Cas9 with anti-Cas9 and to identify the genome-wide on/off target effect with Cas9 ChIP-seq or degenome-seq method [6,7]. The Cas9 ChIP-seq utilizes a Cas9 antibody to capture Cas9 targeted sequences and examine genome-wide CRISPR binding specificity at gRNA-specific and gRNA-independent sites for two guide RNAs with NGS. The degenome-seq profiles genome-wide Cas9 off-target effects by in vitro Cas9-digested whole-genome sequencing.

RNA Modification Detection

RNA modifications are changes to the chemical composition of ribonucleic acid (RNA) molecules post-synthesis that have the potential to alter function or stability. Major epigenetic RNA modifications include N6-methyladenosine (m6A or 6mA) RNA methylation and 5-methylcytosine (5mC) RNA methylation, although other functional

RNA modification such as RNA 5-hydroxymethylcytosine (5hmC) has also been observed. For transcriptome-wide profiling of m6A, MeRIP-seq was developed by combining immunocapture with an m6A antibody and massively parallel sequencing. However, this method only localizes m6A residues to transcript regions of 100–200 nt length but cannot identify precise m6A positions on a transcriptome-wide level [8]. Another method, MiCLIP-seq, was established, which can be used for transcriptome-wide identification of m6A at

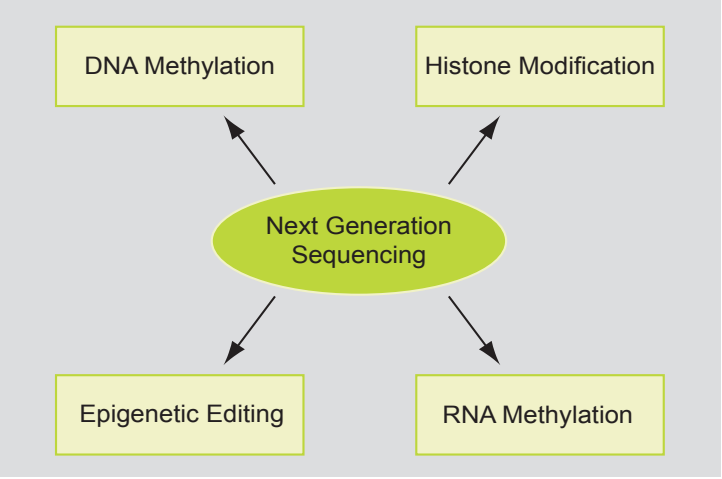

Fig. 2: An overview of notable NGS applications in epigenetics.

single nucleotide resolution level [9]. In addition, transcriptome-wide 5-mC RNA-methylation can be detected by combining bisulfite treatment of RNA, cDNA synthesis followed by NGS [10].

Summary

It is without a question that the rapid development of computer technology and processing power has allowed NGS development and adoption to flourish by enabling researchers to crunch "big data", a feat that would not have been able to be accomplished a decade ago. NGS-based analysis of epigenetic changes has dramatically increased our ability to survey epigenetic markers genome wide, especially for DNA methylation and chromatin/histone modifications. Advances in NGS applications for epigenetics would allow for a better understanding of the epigenetic processes in gene regulation. As NGS standardization and scientific consensus matures — specifically regarding library preparation methods, bioinformatics analysis, and an ecosystem of reagents and tools built around the techniques – epigenetic scientists can focus more on applications that will eventually lead to the identification of reliable markers and the development of targeted therapies.

1. Miura F et al: Nucleic Acids Res. 2012;40(17):e136
2. Farlik M et al: Cell Rep. 2015;10(8):1386-97
3. Rotem A et al: Nat Biotechnol. 2015;33(11):1165-72
4. Cusanovich D et al: Science. 2015;348(6237):910-914
5. Sos BC et al: Genome Biol. 2016;17(1):20
6. O'Geen H et al: Nucleic Acids Res. 2015;43(6):3389-404
7. Kim D et al: Nat Methods. 2015;12(3):237-43, 1p following 243
8. Dominissini D et al: Nat Protoc. 2013;8:176-89
9. Linder B et al: Nat Methods. 2015;12(8):767-72
10. Schaefer M: Methods Enzymol. 2015;560:297-329

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www.epigentek.com/newsletter

MEASURING GLOBAL CHANGES IN DNA METHYLATION

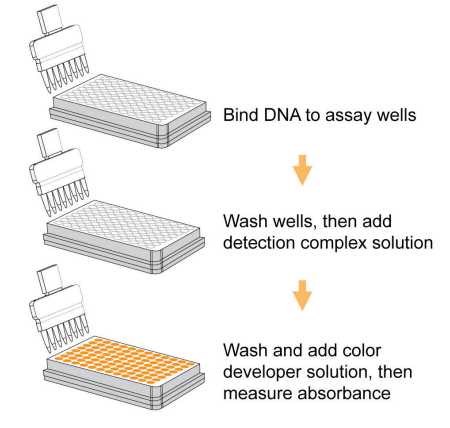
Changes in genomic DNA methylation levels have been observed during development, aging and in various human diseases. In addition, dietary and environmental influences have been shown to influence DNA methylation patterns. Measuring global DNA methylation is important because the extent of methylation can be associated with functional consequences such as genomic instability, mutations, or altered gene expression. While 5-methylcytosine (5-mC) has been found to be a major modification of cytosine, it has also been found that 5-hydroxymethylcytosine (5-hmC) occurs in mammalian DNA and may be an intermediate in the demethylation of 5-mC.

Accurately Measure Global 5-mC & 5-hmC in DNA in Just 2 Hours

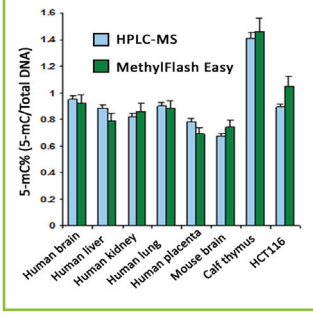
Our **MethylFlash™ Global DNA Methylation and Hydroxymethylation ELISA Easy Kits** are each a complete set of optimized buffers and reagents to colorimetrically quantify global DNA methylation or hydroxymethylation in a simplified, "one-step" ELISA reaction. As a fourth generation technology of EpiGentek's popular global DNA methylation technique, these are a further refinement of the predecessor MethylFlash kits by improving upon speed, simplicity, sensitivity, and reproducibility.

- **Fast** - Reduced steps allow you to finish in only 2 hours
- **Robust** - Improved kits have a greater "signal window" with reduced variation between replicates
- **Convenient** - Inherently low background noise without the need for DNA denaturation and plate blocking steps
- **Sensitive** - Detection limit can be as low as 0.05% methylated or 0.01% hydroxymethylated DNA from 100 ng of input DNA
- **Specific** - No cross-reactivity to unmethylated or other modified cytosines
- **Universal** - Included positive and negative controls allow for detection of DNA methylation or hydroxymethylation in any species from either single-stranded or double-stranded input DNA
- **Accurate** - Optimized positive controls that can be fractionalized in percentage scale, allowing the assays to be more accurate and highly comparable with HPLC-MS analysis
- **Flexible** - Strip-well microplate format for manual or high throughput analysis

Cat.#	Product Name
P-1030	MethylFlash™ Global DNA Methylation (5-mC) ELISA Easy Kit (Colorimetric)
P-1032	MethylFlash™ Global DNA Hydroxymethylation (5-hmC) ELISA Easy Kit (Colorimetric)



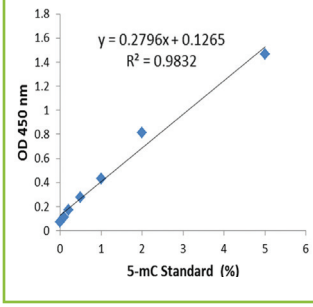
▲ Schematic procedure for the MethylFlash™ Global DNA Methylation (5-mC) ELISA Easy Kit (Cat. #P-1030).



◀ Accurate quantification of 5-mC content of various DNA samples from different species using the MethylFlash™ Global DNA Methylation (5-mC) ELISA Easy Kit (Cat. #P-1030). The results are closely correlated with those obtained by HPLC-MS.

How are these new kits different from our previous MethylFlash kits?

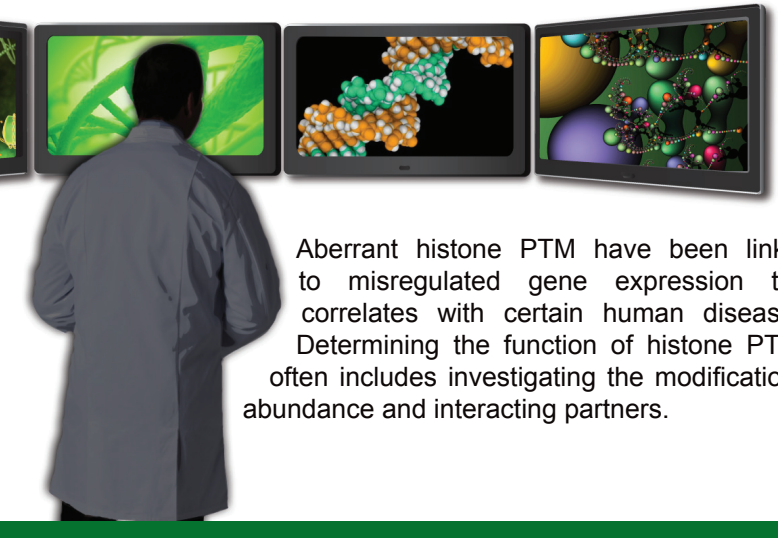
The **MethylFlash Easy kits**, while still retaining the many advantageous features of the previous kits, utilizes a "one-step" ELISA reaction to eliminate the need for multiple sample and antibody binding steps. The protocols of these new kits are simplified, reducing the procedure times by 50% versus the previous MethylFlash kits. Some adjustments were made to also reduce the CV% between wells. The greater "signal window" of each kit, along with adjusted calculation formulas, makes these new kits very accurate. In a nutshell, MethylFlash Easy kits are faster, easier, more robust, and more accurate.



◀ An example of an optimal standard curve generated with 5-mC standard control using the MethylFlash™ Global DNA Methylation (5-mC) ELISA Easy Kit (Cat. #P-1030).

IDENTIFY HISTONE MODIFICATIONS

Histone proteins are subject to a variety of modifications, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. Recognized as post-translational modification (PTM), histone modifications are critical for regulating chromatin structure and function, which can then affect DNA-related processes such as transcriptional activation/inactivation, chromosome packaging, and DNA damage/repair.



Aberrant histone PTM have been linked to misregulated gene expression that correlates with certain human diseases. Determining the function of histone PTMs often includes investigating the modification's abundance and interacting partners.

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

Histone Modification Multiplex Assays

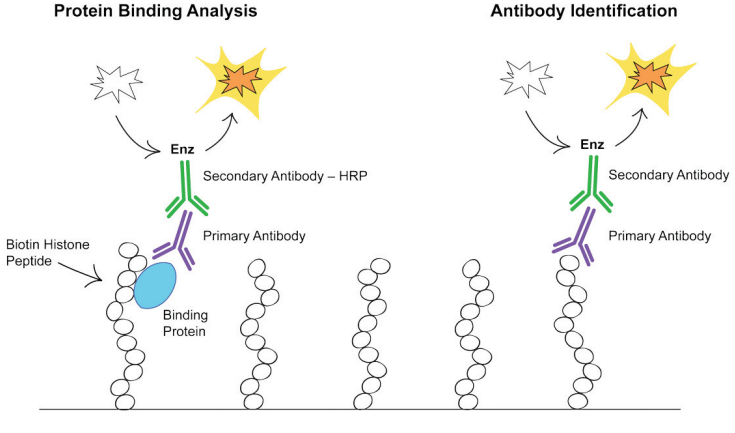
Histone H3 Modification Multiplex Assay Kit
Cat. #P-3100
Simultaneously screen and measure 21 different, well-characterized histone H3 modifications in 2.5 hours, including histone H3K4, H3K9, H3K27, H3K36, H3K79 methylation, histone H3K9, H3K14, H3K18, H3K56 acetylation, and more.

Histone H4 Modification Multiplex Assay Kit
Cat. #P-3102
In a simple 2.5 hour procedure, simultaneously detect and quantify 10 different histone H4 modifications, including histone H4K5, H4K8, H4K12, H4K16, H4K20, H4R3, and more.

See All Our Histone Modification Products Online:
www.epigentek.com/histone-modifications

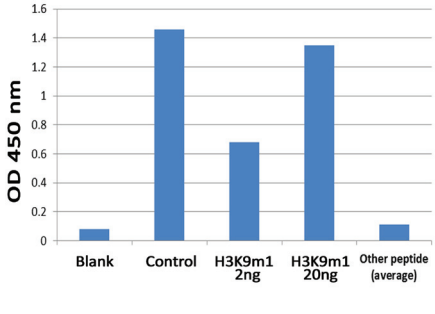
SCREEN FOR HISTONE H3 & H4/H2A/H2B SPECIFICITY

The **Pre-Sure™ Histone H3 & H4/H2A/H2B Peptide Array ELISA Kits** are each a complete set of optimized reagents designed to rapidly examine the selectivity and specificity of antibodies against 46 different histone modifications in a simple, ELISA-like format with use of a standard microplate reader. These kits are also suitable for identifying substrates of histone modifying enzymes as well as for analyzing specificity of histone binding proteins.



The Pre-Sure™ Kits have the following advantages:

- Quick and efficient 1 hour and 45 minute procedure.
- High quality peptides (over 95% pure), coated in two concentrations, allow for the screening of both strong and weak reactive antibodies, enzymes, and histone binding proteins.
- The most comprehensive variety of histone H3 and H4/H2A/H2B modifications.
- Easy and convenient ELISA-like format.
- Simple, reliable, and consistent assay conditions.



Representative specificity identification of histone H3 antibodies using the Pre-Sure™ Histone H3 Peptide Array ELISA Kit (Cat. #P-3104). The Histone H3 Array was probed with H3K9me1 Polyclonal Antibody (Cat. #A-4034; 1 µg/mL). Peptides and control were visualized using a goat anti-rabbit IgG-HRP and a color development system.

See Our Histone Peptide Array ELISA Kits Online:
www.epigentek.com/histone-peptide-array

Cat.#	Product Name
P-3104	Pre-Sure™ Histone H3 Peptide Array ELISA Kit
P-3106	Pre-Sure™ Histone H4/H2A/H2B Peptide Array ELISA Kit

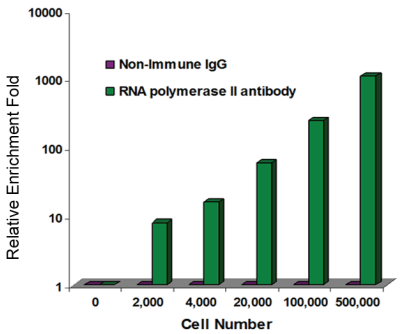
RELIABLY IMMUNOPRECIPITATE CHROMATIN

Chromatin immunoprecipitation (ChIP) is a common antibody-based method for determining the location of DNA binding sites on the genome for a particular protein of interest. This technique is a convenient means for studying protein-DNA interactions that occur inside the nucleus of cells and for understanding cellular processes. Downstream applications of ChIP include ChIP-sequencing, ChIP-PCR, and ChIP-on-chip (microarrays).

EpiGentek's microplate-based **ChromaFlash™ High-Sensitivity ChIP Kit** *(Cat. #P-2027)* is a complete set of optimized reagents to successfully carry out a chromatin immunoprecipitation procedure in a high throughput format starting from mammalian cells or tissues. The highly specific and sensitive kit is suitable for selective enrichment of a chromatin fraction containing specific DNA sequences using various mammalian cells/tissues. The optimized protocol and kit components reduce non-specific background ChIP levels to allow capture of low abundance protein/transcription factors and increased specific enrichment of target protein/DNA complexes. The target protein bound DNA prepared with the ChromaFlash™ High-Sensitivity ChIP Kit can be used for various downstream applications including PCR (ChIP-PCR), microarrays (ChIP-on-chip), and sequencing (ChIP-Seq).

- Fast and easy 5 hour procedure
- Low cell input of just 2,000 cells
- Flexible strip microplate format for manual or high throughput with 24-48 reactions each time
- High specificity and sensitivity with minimal background
- High enrichment ratio with positive to negative ratio >500
- High selectivity and capture efficiency with strong binding of any IgG subtype antibodies within a wide pH range regardless if they are in monoclonal or polyclonal form

Cat.#	Product Name
P-2027	ChromaFlash™ High-Sensitivity ChIP Kit



High abundance protein enrichment: Sheared chromatin isolated from different numbers of MBD-231 cells was used for ChIP-qPCR analysis of RNA polymerase II enrichment in GAPDH promoters using the ChromaFlash™ High-Sensitivity ChIP Kit (Cat. #P-2027) and the EpiQuik™ Quantitative PCR Fast Kit (Cat. #P-1029).

More Microplate-based ChIP Kits

EpiNext ChIP-Seq High-Sensitivity Kit
All-in-one ChIP with ChIP-seq library preparation

EpiQuik Plant ChIP Kit
ChIP optimized specifically for plant samples

EpiQuik Chromatin Immunoprecipitation (ChIP) Kit
Our heavily-cited, classic microplate-based ChIP kit

Cat.#	Product Name
P-2030	EpiNext™ ChIP-Seq High-Sensitivity Kit
P-2014	EpiQuik™ Plant ChIP Kit
P-2002	EpiQuik™ Chromatin Immunoprecipitation (ChIP) Kit

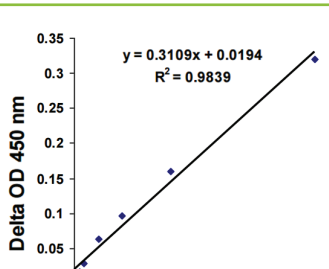
See All Our Microplate-based ChIP Kits Online:
www.epigentek.com/chip

DIRECTLY MEASURE M6A RNA METHYLATION

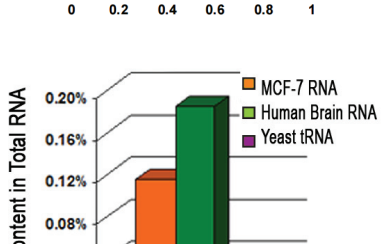
N6-methyladenosine (m6A or 6mA) is the most common and abundant modification in RNA molecules present in eukaryote, accounting for more than 80% of all RNA base methylations, and exists in various species. The relative abundance of m6A in mRNA transcripts has been shown to affect RNA metabolism processes such as splicing, nuclear export, translation ability and stability, and RNA transcription. The dynamic and reversible chemical m6A modification in RNA adds a new dimension to the developing picture of post-transcriptional regulation of gene expression and may also serve as a novel epigenetic marker of profound biological significance.

Expand upon RNA methylation research by quantifying the "fifth RNA base" N6-methyladenosine (m6A) directly in RNA samples with our new **EpiQuik™ m6A RNA Methylation Quantification Kit** *(Cat. #P-9005)*:

- Simple, <4 hour ELISA-like procedure
- Sensitive detection limit of as low as 10 pg of m6A
- High specificity to m6A without cross-reactivity to unmethylated adenosine
- Universally suitable for any species
- Quantify m6A from both mRNA and ncRNA such as tRNA, rRNA and snRNA



m6A standard control was added into the assay wells at different concentrations and then measured with the EpiQuik™ m6A RNA Methylation Quantification Kit (Colorimetric) (Cat. #P-9005).

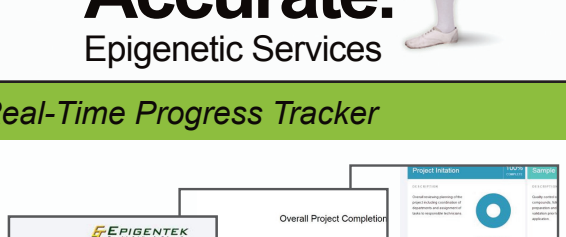


Quantification of m6A RNA methylation in different samples. 200 ng of RNA isolated from different tissues or cells were added into the assay wells and the m6A contained in RNA was measured using the EpiQuik™ m6A RNA Methylation Quantification Kit (Cat. #P-9005).

Cat.#	Product Name
P-9005	EpiQuik™ m6A RNA Methylation Quantification Kit

EPIGENETIC NGS SERVICES

Real-Time Progress Tracker



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Expand your research capabilities with our comprehensive and accurate lab services for Methyl-Seq Bisulfite Sequencing and ChIP Sequencing.

NGS Bisulfite Sequencing (Methyl-Seq)
Genome-wide, base resolution DNA methylation analysis.

- Publication ready results
- Complete conversion (>99.9%) and maximum recovery of bisulfite-converted DNA
- Low input of 0.5 ng of DNA
- High yield of the constructed DNA library, low error rates
- Real-time progress tracker

ChIP Sequencing (ChIP-Seq)
Map genome-wide histone modifications or identify transcription factor and co-factor binding sites.

- Publication ready results
- High enrichment ratio of positive to negative control >500
- High sensitivity and flexibility allows both non-barcoded and barcoded DNA library preparation
- Access to hundreds of ChIP-grade antibodies
- Low sample input of 50,000 cells
- Real-time progress tracker

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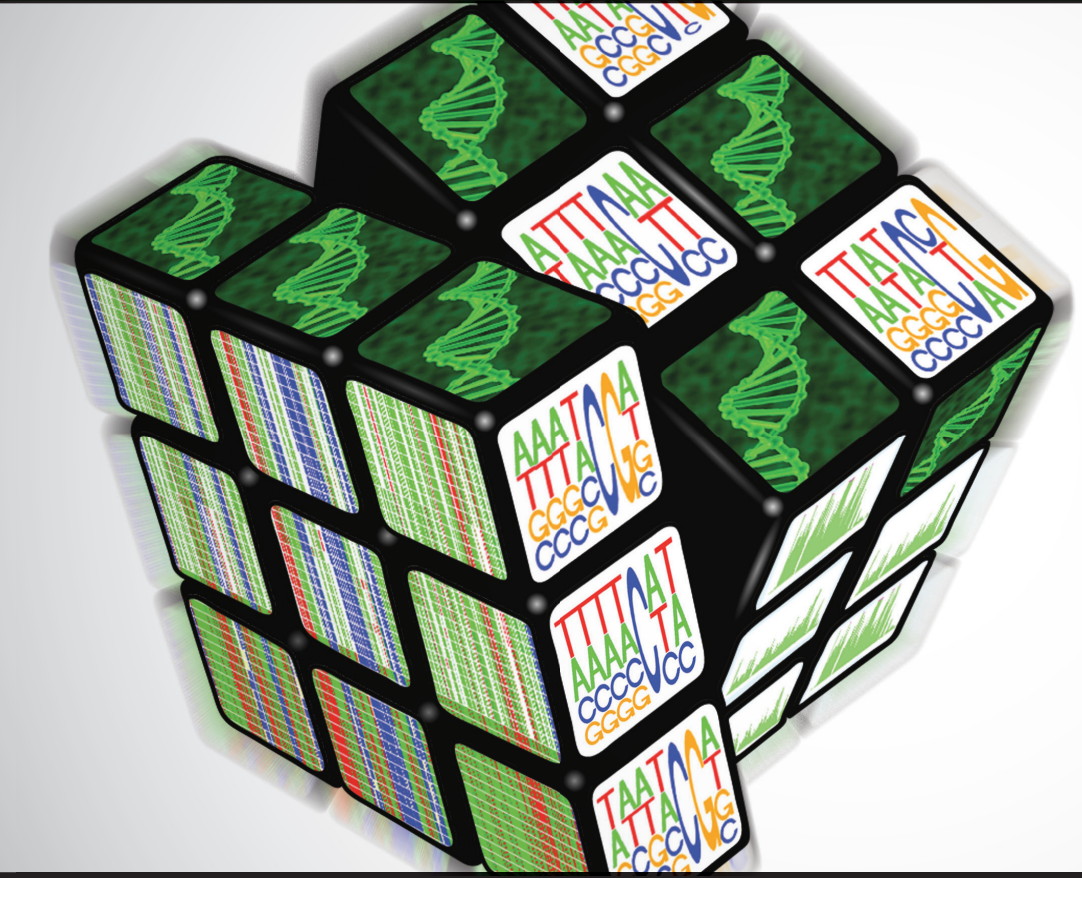

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