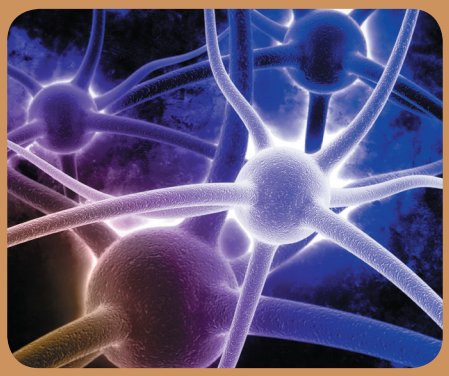
motif|vations

THE NEWSLETTER OF ACTIVE MOTIF
OCTOBER 2015 | VOLUME 16 NUMBER 2

NEUROEPIGENETICS EDITION

ACTIVE MOTIF®
Enabling Epigenetics Research



IN THIS ISSUE

New to Neuroepigenetics? We Can Help.

There is increasing evidence that several neurodevelopmental, neurodegenerative and psychiatric disorders are, in part, caused by aberrant epigenetic modifications. As a result, researchers in neurogenetics are now beginning to investigate the mechanisms underlying epigenetic regulation in neuronal gene expression and how this impacts normal brain function. The transition into epigenetics research does not have to be intimidating because there are an increasing number of tools available to help researchers obtain their desired results.

Various epigenetic modifications are found in neurons

These modifications include classical epigenetic marks such as DNA methylation and histone modifications. Other epigenetic mechanisms in play include histone subunit exchange, cytosine methylation variants and non-coding RNAs. In recent years, there has been a rapid increase in the number of publications suggesting a role for epigenetics in regulating the Central Nervous System (CNS) and a growing body of evidence linking epigenetic mechanisms to neural development, plasticity and CNS disorders, such as schizophrenia and drug addiction. More than a dozen neurological syndromes so far have been linked to mutations in single genes that encode DNA methyltransferase and histone modifying enzymes. This list includes neurological disorders that appear in early childhood (Rett syndrome) or later in life, such as Hereditary Sensory and Autonomic Neuropathy Type 1 (HSAN1).

The epigenetic tool kit

The development of methods such as chromatin immunoprecipitation (ChIP) and DNA methylation enrichment techniques, along with Next-Generation Sequencing, is allowing researchers to get a better picture of the genome-wide changes associated with neurological and other diseases. There are a number of techniques that are key to epigenetic analysis. These include:

- *Antibodies* to discriminate between the multiple post-translational modifications on the histone tails, and to detect methylated DNA
- *ChIP and ChIP-Seq* techniques to enable scientists to link specific states of chromatin to individual gene loci in a cell to understand how genes are regulated
- *Global and locus-specific methods* to enable researchers to analyze shifts in DNA methylation status of repetitive elements or at specific promoters
- *Bisulfite conversion* of methylated DNA prior to sequencing to allow detection of methylated cytosines

"What Roles do Epigenetic Mechanisms Play in Neural Development and Diseases of the Nervous System?"

Using the right tools for ChIP
ChIP is a technically challenging method. Researchers who are not experts in ChIP techniques are best served using well-validated and reliable kits to perform these assays. Active Motif has developed a number of kits and accessory reagents tailored to aid researchers with their ChIP experiments. These kits and associated antibodies have been used in hundreds of labs and cited in over 1,000 papers in peer-reviewed journals. For the full list of kits and reagents for ChIP, visit us at www.activemotif.com/chip.

"How do MicroRNAs Regulate Gene Expression in Neurons?"

MicroRNAs in neural function
MicroRNAs (miRNAs) are regulators of gene expression that are implicated in nearly all biological processes, including neural development and dysfunction. miRNAs regulate neural specification and also contribute to neurodegenerative and psychiatric conditions such as Fragile X syndrome and drug addiction. While miRNAs are abundant in neurons, their biological roles are still emerging.

Active Motif's LightSwitch™ portfolio of products includes over 12,000 3'UTR constructs, miRNA mimics and inhibitors, and custom services to ensure you have all the functional genomics tools at your disposal for the study of miRNA function. To learn more about LightSwitch™ miRNA products, visit us at www.activemotif.com/lightswitch.

What is the role of DNA methylation in brain function?
DNA methylation of genes is found in all human tissues, including the brain. There are several methylation variants, and one of these, 5-hmC, is highly expressed in the brain relative to other tissues. This has led to speculation that 5-hmC may have a unique biological role in the CNS. Also, recent studies have shown a correlation between DNA methylation levels and behavioral disorders such as anxiety and depression. In addition, mutations in genes involved in DNA methylation (*e.g.* MeCP2) are linked to neurological diseases. However, the mechanism by which DNA methylation exerts these effects is not fully understood.

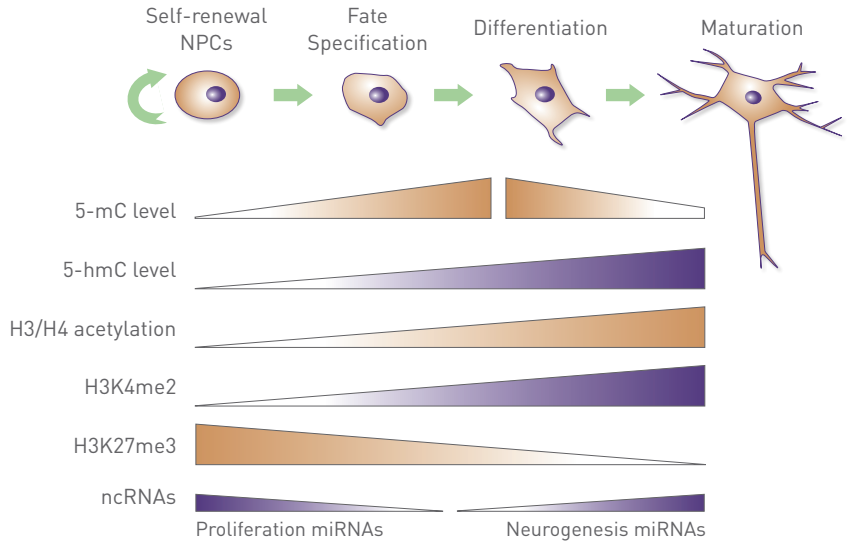
Tools for DNA methylation analysis
Active Motif offers a number of products specific for this area of research, including kits and antibodies that enrich for DNA fragments that contain 5-mC and 5-hmC. Our DNA methylation related antibodies have been validated for various applications including ChIP, methylated DNA immunoprecipitation (MeDIP) and immunofluorescence. For complete details on all our DNA methylation products, visit www.activemotif.com/dnamt.

Epigenetic techniques require validated antibodies
One of the greatest challenges for epigenetics research has been the lack of available antibodies that show proper specificity and that have been validated for use in techniques such as ChIP and ChIP-Seq. Histones have multiple post-translational modifications along the N-terminal tail portion that are involved in gene regulation and disease. Antibodies need to be able to clearly differentiate between these multiple modifications. It is a major challenge to develop antibodies with sufficient specificity that will also perform in demanding techniques such as ChIP.

Active Motif has established a validation program for its antibodies that includes ChIP-Seq testing, ChIP validation, as well as a test on the specificity of our histone antibodies with a unique Modified™ Histone Peptide Array (Catalog No. 13001). For the full list of our epigenetics antibodies please visit www.activemotif.com/abs.

Let us do the work for you - Active Motif Epigenetic Services
Generating high quality, interpretable data from ChIP experiments can be challenging as it requires optimized protocols for various cell types and knowledge of cell type-specific binding sites. Add in the technical and bioinformatics challenges associated with generating whole-genome data sets, and ChIP-Seq may literally be beyond your reach. Our Epigenetic Services team provides a wide variety of ChIP services. This makes it possible for you to utilize our expertise and research tools without having to be an expert in the techniques yourself. To find out more, or to get a quote, please go to www.activemotif.com/services.

GLOBAL EPIGENETIC SHIFTS DURING NEUROGENESIS



NEW

Improve the Success of RNA Applications with Subcellular Isolation of High-Quality RNA

RNA molecules exist at different levels of maturation and processing within a cell at any given time. Such heterogeneity can bias downstream analysis and data interpretation. Unlike other commercial RNA isolation kits, Active Motif's RNA Subcellular Isolation Kit enables isolation of RNA from specific subcellular fractions, greatly improving the accuracy of gene expression profiling by reverse transcription quantitative PCR (RT-qPCR) or RNA-Seq. Additionally, subcellular isolation can be used to localize long non-coding RNAs (lncRNAs) to their site of action which can provide important insights into their functional role.

RNA Subcellular Isolation Kit

- Separates nuclear & cytoplasmic RNA without cross-contamination
- Works with cell or tissue samples
- Spin columns included for RNA purification
- Isolates RNA sizes 75-7000 nt
- Purified RNA validated for use in RT-qPCR and RNA-Seq

Minimize sample heterogeneity with cellular fractionation

Traditional methods for RNA analysis often utilize total RNA which consists of a mixture of intronic RNA originating from immature transcripts in the nucleus, mature RNA from the cytoplasm and a variety of non-coding RNAs. Such heterogeneity can bias downstream analysis and data interpretation by reducing the sensitivity of detection for low abundance transcripts and the ability to study RNA processing dynamics. Total RNA also lacks information regarding RNA localization.

To overcome these challenges, Active Motif has developed the RNA Subcellular Isolation Kit to efficiently isolate separate nuclear and cytoplasmic fractions of RNA for downstream analysis. This method can be used to isolate both long and short RNA sequences from cells or tissue without cross-contamination or the use of phenolic compounds.

How does it work?

Lysis Buffer is added to cell or tissue samples. The lysate is then separated by centrifugation, with the supernatant containing cytoplasmic RNA and the pellet containing nuclear RNA. A guanidine-based buffer and ethanol are added to each RNA fraction before loading onto individual spin columns. Following a wash step to remove proteins, the RNA fractions are eluted and quantified.

The RNA Subcellular Isolation Kit can be used in combination with Active Motif's new DNase I Treatment Kit to remove any genomic DNA contamination from the purified RNA sample.

What's in the box?

Each kit contains enough reagents to perform either 15 cytoplasmic and 15 nuclear RNA isolations, or 30 whole-cell RNA isolations.

For more information, please visit us at www.activemotif.com/rna-iso.

Subcellular RNA Expression Analysis

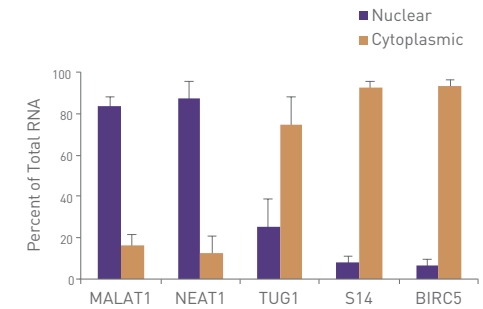


Figure 1: **Analysis of subcellular RNA fractions by RT-qPCR provides insight into RNA localization.** Nuclear, cytoplasmic and total RNA were isolated from HeLa cells using the RNA Subcellular Isolation Kit. Purified RNA was then subjected to RT-qPCR. Each subcellular fraction was plotted as a percentage of total RNA. The data shows localization of lncRNAs, MALAT1, NEAT1 and TUG1, that is consistent with published RNA-FISH data. Ribosomal protein (S14) and mRNA (BIRC5) are both shown to localize to the cytoplasm, as expected and consistent with previously reported results.

Product	Format	Catalog No.
RNA Subcellular Isolation Kit	30 rxns	25501
DNase I Treatment Kit	30 rxns	25502

NEW

Service for Quantitation of Histone Post-translational Modifications in Multiplex

Active Motif's Histone PTM Quantitation Service gives you access to ground-breaking technology that enables high-throughput multiplexed screening of histone modifications. The service uses Luminex® xMAP® magnetic bead-based multiplexing technology to simultaneously measure multiple histone modification targets in a single reaction. This first-of-its-kind multiplex epigenetic assay generates more data using less sample material than traditional Western blot, mass spectrometry or ELISA methods and enables you to analyze both specific and off-target effects in your sample simultaneously.

HISTONE PTM QUANTITATION SERVICES

Simply submit frozen cell pellets and we will:

1. Prepare cell lysates
2. Perform Luminex® histone quantitation
3. Perform normalization to Histone H3
4. Perform analysis and delivery of data

Simultaneously screen specific and off-target effects in the same sample

Profiling variances in histone post-translational modification (PTM) levels in clinical or compound-treated samples using traditional Western blot or ELISA techniques requires large numbers of cells from samples that may already be in scarce supply. Traditional assays are also limited by low throughput and the inability to simultaneously analyze specific and off-target effects. Our Histone PTM Quantitation Service provides a quantitative and highly sensitive method to enable screening of variances in histone modification levels in multiplex across clinical or compound-treated samples.

How does it work?

The service utilizes the same Luminex technology as our Histone H3 PTM Multiplex Assay to enable multiplexing and quantitation of histone PTM targets (Figure 1). The end-to-end, customizable service includes sample preparation, assay design and experimental execution, data analysis and support. Simply submit your samples and our expert scientists will consult with you to formulate an appropriate experimental design.

Service Features:

- Minimal cell requirements (250K cells are sufficient to quantify 12 histone modifications)
- Detects ≥ 25% difference between samples
- Controls include total H3 and samples containing known PTM changes

For more complete information, visit www.activemotif.com/services-luminex.

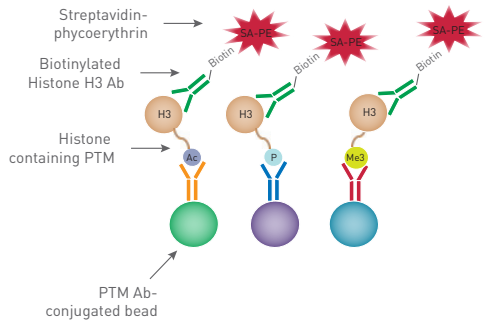


Figure 1: **Schematic of histone PTM multiplexing technology.** Histone-specific modifications in the N-terminus are captured using antibodies conjugated to fluorescent-labeled magnetic beads. Each bead emits a unique fluorescent signal to enable detection of multiple targets within the same well. A biotinylated H3 C-terminal antibody is used to capture histones. Streptavidin-phycoerythrin is then added to bind biotin and produce a signal. A Luminex® instrument is used to read signals and decipher both the bead identity and the number of binding events pertaining to each histone modification.

AVAILABLE TARGETS	
H3 pan-ac	H3K56ac
H3K4me3	H3S10ph
H3K9ac	H3T11ph
H3K9me1	H3K27ac
H3K9me2	H3K27me2
H3K9me3	H3K27me3

To inquire about services or to receive a quote, contact **sales@activemotif.com**

NEW

Quantitative ELISA for the Detection of Global 5-hmC

Hydroxymethylation is known to play a role in transcriptional regulation and embryonic development. Research has indicated it may also serve as a prognostic indicator in certain cancers and neurodegenerative disorders. To better understand the influence of changes in global 5-hydroxymethylcytosine (5-hmC) levels on normal and diseased states, Active Motif has developed the Global 5-hmC Quantification Kit. Obtain the same quantitative capabilities as mass spectrometry, but with higher throughput and lower sample requirements.

Simplified global 5-hmC quantification
Gene-specific analysis techniques do not provide information about the global levels of DNA hydroxymethylation within a genome. Chromatographic methods followed by mass spectrometry are commonly used for global DNA methylation analysis. Although they are highly quantitative and reproducible, these methods are labor-intensive, require special equipment and expertise and large amounts of high quality DNA. Active Motif's new Global 5-hmC Quantification Kit enables you to obtain the same quantitative results using less sample material and in a higher throughput and easier-to-use format than mass spectrometry (Figure 1).

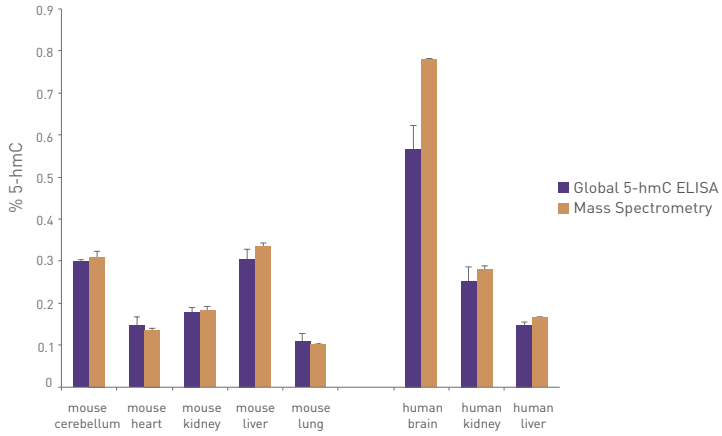


Figure 1: **The Global 5-hmC ELISA generates comparable 5-hmC quantification to mass spectrometry using 10-fold less sample material.** The Global 5-hmC ELISA Kit was used to determine the % 5-hmC in genomic DNA isolated from mouse and human tissues. Genomic DNA was tested in the range of 20 ng/well for human brain to up to 50 ng/well for all other DNA samples. The %5-hmC was calculated for each sample using the included DNA standards. The data was compared to mass spectrometry data obtained from 500 ng of the same DNA samples. Results show the Global 5-hmC ELISA provides equivalent quantification using only a fraction of the starting material.

How does it work?
Genomic DNA of interest is enzymatically digested and heat denatured before addition to coated 96-well plates. Unbound DNA fragments are then washed away. A 5-hydroxymethylcytosine antibody and a secondary antibody conjugated to horseradish peroxidase (HRP) are used for detection of the hydroxymethylated fragments. The colorimetric readout is then easily quantified by spectrophotometry. Results are compared to the included DNA standards to extrapolate the percent 5-hmC in the DNA sample (Figure 1).

Global 5-hmC ELISA features

- Direct detection assay is specific for 5-hmC DNA
- Requires only 20-50 ng per well of starting DNA
- Includes DNA standards for quantification of 5-hmC levels
- Detects as little as 0.02% 5-hmC
- Higher throughput quantification than mass spectrometry

What's in the box?
Each kit contains an optimized protocol and all the reagents necessary to perform DNA fragmentation, plate coating, capture and colorimetric detection. DNA standards with known levels of 5-hmC are included for standard curve generation and sample quantification.

For more information, please visit www.activemotif.com/global-hmc.

Product	Format	Catalog No.
Global 5-hmC Quantification Kit	1 x 96 rxns	55018

NEW

Determine the Genome-wide Distribution of 5-Hydroxymethylcytosine

Because 5-hydroxymethylcytosine (5-hmC) is known to play a major role in the Central Nervous System (CNS) in the regulation of development, neuroplasticity and disease, it has gained prominence in neuroepigenetic research. Therefore, tools to accurately detect and quantify 5-hmC are highly warranted. A recent study from the laboratory of Francois Fuks utilized Active Motif's Hydroxymethyl Collector™-Seq assay to investigate the impact of oxidative stress on TET-mediated hydroxymethylation. Their results show, for the first time, that oxidative assaults deeply modify the hydroxymethylome.

Profile changes in 5-hmC

5-hmC is highly abundant in the CNS where it regulates various processes. High 5-hmC levels in the developing brain are associated with maintenance of active transcription. In adult neurons, alternating DNA methylation and demethylation are observed in response to neuronal activity that suggests 5-hmC is involved in cognitive function and development of psychiatric disorders such as stress-induced PTSD. Also, aberrant 5-hmC distribution is linked to the etiopathology of various neurodegenerative disorders, including Alzheimer's and Huntington's disease.

Active Motif's new Hydroxymethyl Collector™-Seq Kit is designed specifically to determine the genome-wide localization of 5-hmC in your samples (Figure 1).

Genome-wide 5-hmC pattern changes in response to oxidative stress

In a recent study, Delatte *et al.* investigated the impact of oxidative stress on TET-mediated hydroxymethylation. To determine the genome-wide 5-hmC distribution in neuroblastoma cells that had been subjected to oxidative stress, they utilized Active Motif's Hydroxymethyl Collector™-Seq assay (hmC-Seq). Interestingly, the cells subjected to oxidative stress displayed a significant global decrease in 5-hmC as well as local differential hmC patterns at genes important for a protective response to oxidative stress, in agreement with previous mass spectra and dot blot (5-hmC antibody, Catalog No. 39769) results. Further, hmC-Seq in mice lacking the glutathione peroxidases 1 and 2 confirmed and extended the above findings to show restructuring of the hydroxymethylome in response to oxidative stress *in vivo*.

Further, Delatte *et al.* observed an unexpected high proportion of differentially hydroxymethylated microRNA (miRNA) coding sequences. The miRNAs displaying the most robust change in 5-hmC were those with target mRNAs of toxicogenomic pathways involved in oxidative stress. In summary, these results suggests that TET1 may play a role in protecting cells against oxidative stress, and the resulting changes in 5-hmC pattern occur, not only in DNA regions that code for proteins, but also miRNAs.

In addition to Hydroxymethyl Collector™-Seq, Active Motif offers a comprehensive portfolio of 5-hmC products, including antibodies, kits for 5-hmC enrichment, assay-ready enzymes and custom services to aid in this area of research.

This article is a summary of the research published in *Sci Rep.*: Delatte *et al.*, 2015.

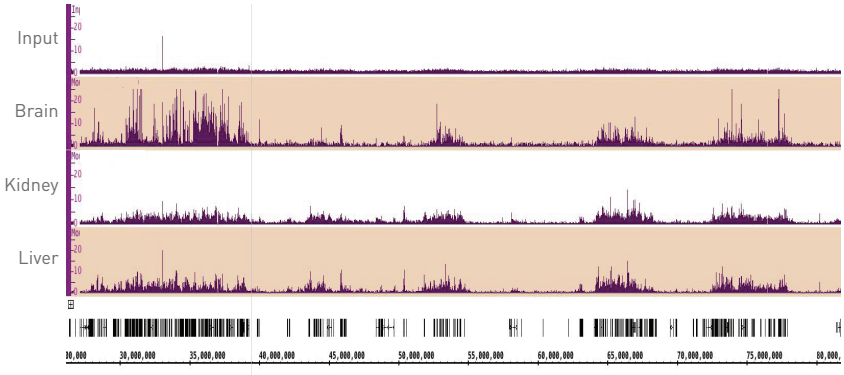


Figure 1: Hydroxymethyl Collector-Seq was performed on 5 µg each of mouse brain, mouse kidney and mouse liver genomic DNA and submitted for Next-Generation Sequencing to identify the localization of 5-hmC within each sample.

Product	Format	Catalog No.
Hydroxymethyl Collector™-Seq	25 rxns	55019
5-Hydroxymethylcytosine (5-hmC) antibody (pAb)	100 µl	39769

Tools to Enable Insight into the Transcriptional Programs of Neurogenesis

The transcriptional programs of neural progenitor cells change dynamically during neurogenesis. While most research has focused on identifying changes in these gene regulatory networks and the underlying mechanism causing them, it is equally important to determine the actual functional impact of these changes or events. In a recent study performed by the laboratory of Dr. Linda Richards at the Queensland Brain Institute in Australia, numerous tools from the Active Motif product line were utilized to investigate and functionally validate the epigenetic and transcriptional mechanisms regulating neural progenitor cell self-renewal during cortical development.

NFIB-mediated repression of EZH2 regulates cortical development

The chromatin-modifying protein Enhancer of zeste homolog 2 (EZH2) has recently emerged as a central player in promoting progenitor cell self-renewal during cortical development. However, the mechanism by which EZH2 itself is regulated remains unclear. Furthermore, the nuclear factor I (NFI) family of transcription factors has been implicated in driving neural progenitor cell differentiation. Thus, researchers from the laboratory of Dr. Linda Richards at the Queensland Brain Institute set out to determine the role of transcription factor nuclear factor IB (NFIB) in this process. Utilizing a suite of molecular and bioinformatics tools, they identified the transcriptional repression of *EZH2* by NFIB to be an important component of the process of neural progenitor cell differentiation during cortical development.

For more detailed information,visit www.jneurosci.org/content/34/8/2921.

The role of NFIB in neural progenitor cell differentiation

To determine the underlying mechanism of NFIB-mediated neural progenitor cell differentiation, the laboratory of Dr. Richards performed a microarray screen to analyze differential expression in the hippocampus of *NFIB*-/- mice. Interestingly, targets involved in the modulation of chromatin structure were highly enriched in the *NFIB*-/- compared to wild-type mice, suggesting that NFIB may influence chromatin structure during development. Furthermore, an *in silico* screen searching for the high-affinity NFI-binding site (TTGGC(N5)GCCAA) in the 2 kb region upstream of the transcription start site (TSS) of all known Polycomb Repressive Complex (PRC) genes identified *EZH2* as having two putative NFI-binding motifs in its basal promoter (Figure 1).

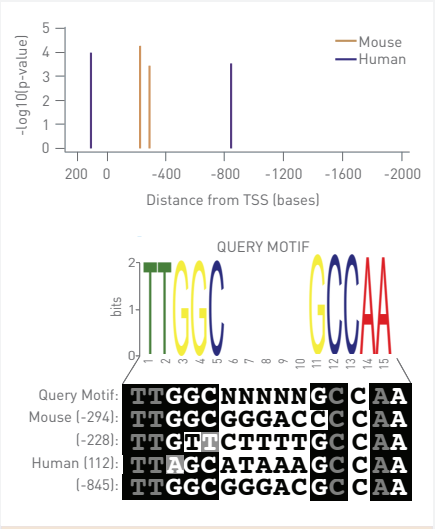


Figure 1: An *in silico* screen for predicted NFI-binding sites identified conserved potential NFI-binding sites within the proximal promoter of the mouse *EZH2* and the human *EZH2* gene.

To determine whether NFIB is able to regulate the transcription of *EZH2*, researchers first investigated whether NFIB could actually bind to these predicted sites within the *EZH2* promoter. Using EMSA, they determined that a protein from nuclear extracts derived from embryonic cortical tissue bound to one of the two predicted NFI binding sites (-228). Supershifting of the -228 probe with an anti-NFIB antibody indicated that this protein complex contained NFIB (Figure 2, left). EMSA experiments using a probe in which the -228 site had been mutated did not exhibit the same band as the wild-type -228 probe, indicating that NFIB could no longer bind this sequence (Figure 2, right).

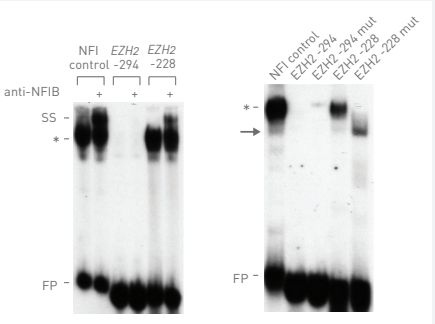


Figure 2: EMSAs performed with mouse brain nuclear extracts and probes for (left) NFI control (lanes 1-2) and the -294 (lanes 3-4) or the -228 (lanes 5-6) consensus sites. (Right) NFI control (lane 1) and the -294 (lane 2), mutated -294 sequence (lane 3), -228 (lane 4), or mutated -228 (lane 5) consensus sites.

To learn about Active Motif's Gelshift™ Chemiluminescent EMSA Assay, visit www.activemotif.com/gelshift.

NFIB directly regulates *EZH2* transcriptional activity

To confirm the binding of NFIB to the -228 site of the *EZH2* basal promoter, chromatin immunoprecipitation (ChIP) was performed using Active Motif's ChIP-IT® Express Kit on wild-type embryonic cortical samples. ChIP confirmed the enrichment of NFIB at the *EZH2* promoter region containing the predicted NFI-binding site (Figure 3).

To learn more about Active Motif ChIP-IT Express Kits, please visit www.activemotif.com/chipit.

To determine whether NFIB could functionally regulate *EZH2* promoter-driven gene expression, the researchers performed luciferase assays using the LightSwitch™ Dual Assay Kit. A 461 base pair region of the mouse *EZH2* promoter containing the putative NFI motif at -228 was cloned upstream of the luciferase gene. As depicted in Figure 4, NFIB is able to repress *EZH2* promoter-driven transcription *in vitro*. Further, this repression was abolished when the NFI-binding site at -228 in the *EZH2* promoter was mutated.

For more information on LightSwitch™ products & services, please visit us at www.activemotif.com/lightswitch.

NFIB represses *EZH2* expression in primary cortical cells

To address whether the observed upregulation of *EZH2* led to increased repression of its downstream targets, such as the *INK4A* locus, ChIP was performed against H3K27me3 using Active Motif's Histone H3K27me3 antibody (Catalog No. 39155). H3K27me3 levels were significantly enriched at exons 1 and 2 of the *INK4A* locus in *NFIB*$^{-/-}$ cortical tissue, consistent with the elevated levels of EZH2 in these mice. Further, the proximal promoter regions of the *INK4B* (*p15, CDKN2B*), *INK4C* (*p18, CDKN2C*), and *INK4D* (*p19, CDKN2D*) loci carried significantly reduced levels of H3K27me3 (Figure 5). Collectively, these data show that NFIB represses *EZH2* expression in cortical cells *in vitro* and that the elevation of *EZH2* expression in *NFIB*$^{-/-}$ mice promotes epigenetic changes to key downstream target genes *in vivo*.

For more complete information on all available ChIP validated antibodies, visit www.activemotif.com/chipabs.

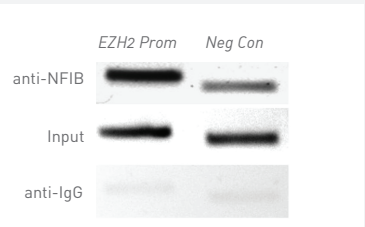


Figure 3: ChIP performed on tissue from mouse wild-type cortex with antibodies against NFIB (Catalog No. 39091) or IgG control (Catalog No. 53011). PCR was performed with primers encompassing the predicted NFI-binding site within the *EZH2* promoter.

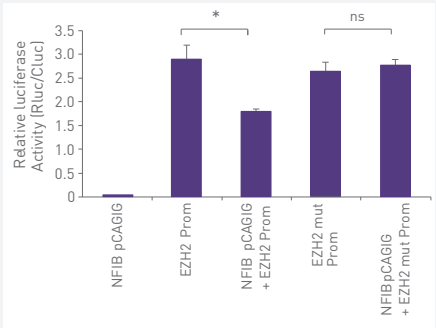


Figure 4: LightSwitch Dual Assay reporter assay performed in Neuro2A cells. NFIB expression construct alone did not elicit luciferase activity (NFIB pCAGIG), whereas the *EZH2* promoter luciferase construct displayed high levels of luciferase. Cotransfection of the NFIB expression and EZH2 promoter luciferase constructs yielded significantly reduced levels of luciferase. Mutating the NFI-binding site at -228 in the *EZH2* promoter abolished NFIB-mediated repression of luciferase activity. ns: Not Significant. *p<0.05, ANOVA.

Conclusion

Using analysis of *NFIB*$^{-/-}$ mice and a suite of molecular and bioinformatics techniques, the laboratory of Dr. Richards were able to show that *EZH2* is a target for transcriptional repression by NFIB, suggesting that the deficits in neural progenitor cell differentiation evident within *NFIB*$^{-/-}$ mice may be due, at least in part, to the loss of NFIB-mediated repression of this epigenetic regulator. This study is just one example of how the Active Motif suite of gene regulation products and services can accelerate your functional genomics research.

This article is a summary of the research performed by Dr. Richards at the Queensland Brain Institute in Australia, published in *J Neurosci.*: Piper *et al.*, 2014.

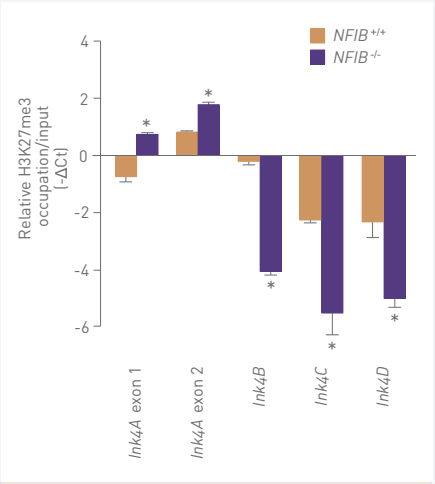


Figure 5: ChIP-IT Express performed using Histone H3K27me3 antibody (Catalog No. 39155) on chromatin isolated from *NFIB*+/+ and *NFIB* -/- cortices. *NFIB*-/- mice exhibited significantly elevated levels of H3K27me3 at exons 1 and 2 of the *INK4A* locus, but significantly reduced levels at the *INK4B*, *INK4C*, and *INK4D* loci. *p = 0.05, Wilcoxon rank-sum test.

Product	Format	Catalog No.
LightSwitch™ Custom Cloning	1 GoClone	32051
LightSwitch™ Custom Mutagenesis	1 GoClone	32052
LightSwitch™ Dual Assay Kit	100 assays	32035
LightSwitch™ Luciferase Assay Kit	100 assays	32031
LightSwitch™ ACTB Promoter Control	5 µg	32003
Gelshift™ Chemiluminescent EMSA	100 rxns	37341
ChIP-IT® Express Kit	25 rxns	53008

Perform ChIP Without Protein-specific Antibodies using Tag-ChIP-IT®

Can't find a suitable antibody for chromatin immunoprecipitation (ChIP)? Don't limit your ChIP experiments based on antibody availability. Active Motif's new Tag-ChIP-IT system enables you to create a fusion protein expressing Active Motif's unique AM-tag sequence that offers several advantages over FLAG, GFP and HA tags for ChIP. Simply clone your protein of interest into the provided vector and our system provides everything else you need to analyze the AM-tag fusion protein by ChIP.

No ChIP-validated antibody for your protein of interest? No problem!
ChIP analysis is often hindered by the lack of available antibodies capable of recognizing fixed protein targets, or the inability of antibodies to distinguish between protein isoforms. Active Motif has developed the Tag-ChIP-IT Kit to enable ChIP without the use of a target-specific antibody. Tag-ChIP-IT utilizes a unique AM-tag that, unlike traditional FLAG, GFP or HA tags, is specifically optimized for ChIP. Furthermore, we have designed a high specificity antibody to the tag to optimize pull-down efficiency during immunoprecipitation (IP).

The AM-tag is engineered to have minimal cross-reactivity with mammalian samples. Additionally, in contrast to larger GFP and FLAG tags, the smaller footprint of the AM-tag reduces obstruction of the DNA binding domain. The AM-tag design also maximizes exposure during the IP reaction to increase the enrichment efficiency of low abundance transcription factors for more reliable and consistent ChIP results (Figure 1).

Tag-ChIP-IT advantages

- Ideal for targets lacking ChIP-qualified antibodies
- Specifically designed for ChIP
  Distinguishes between isoforms
- Improved sensitivity & specificity over FLAG, GFP and HA tags

Tag-ChIP-IT Identifies Estrogen Receptor (ER) Binding Motifs

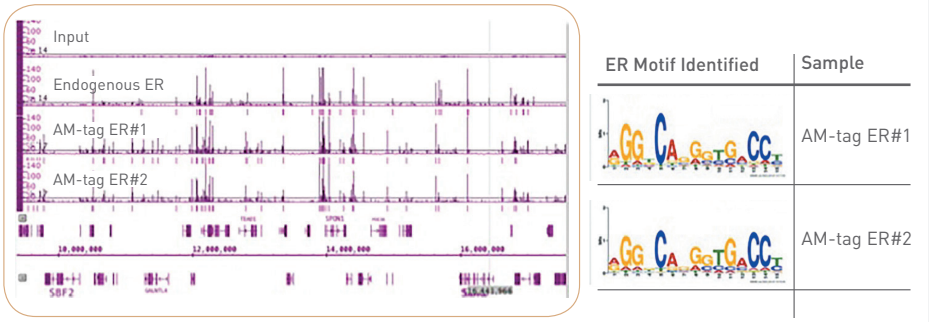


Figure 1: ER cDNA was cloned into pAM_1C Empty Vector and transiently transfected into cells. Cells were induced with estradiol and chromatin was harvested and Tag-ChIP performed using the Tag-ChIP-IT Kit. Following cross-link reversal, enriched DNA was submitted for Next-Generation Sequencing. Data was compared to published ChIP-Seq results using an anti-ER antibody in the same cell line and induction conditions. ChIP-Seq data shows the same ER peak profile with the AM-tag ChIP as endogenous ER. Detected binding sites were further evaluated for binding motifs. Results show the ER motif was identified in both Tag-ChIP-IT samples.

How does it work?
Use the pAM_1C Empty Vector to clone your protein of interest in-frame with the C-terminal AM-tag. Alternatively, the AM-tag sequence can be cloned into your expression vector of choice. Following transfection and expression of your tagged protein, the Tag-ChIP-IT Kit can be used to isolate chromatin and perform immunoprecipitations using AM-Tag antibody specific for the AM-tag.

What's in the box?
The Tag-ChIP-IT Kit contains all the buffers and reagents necessary for ChIP analysis using the AM-tag. For cloning, the pAM_1C Empty Vector is available separately. For more information, visit www.activemotif.com/tagchip.

Product	Format	Catalog No.
Tag-ChIP-IT® Kit	16 rxns	53022
pAM_1C Empty Vector	20 µg	53023
pAM_1C_JunD Vector	50 µg	53044
AM-Tag antibody	100 µg	61677
FuGENE® HD Transfection Reagent	0.2 ml	32042

Enhance the Reproducibility of Any Probe Sonicator with the EpiShear™ Cooled Sonication Platform

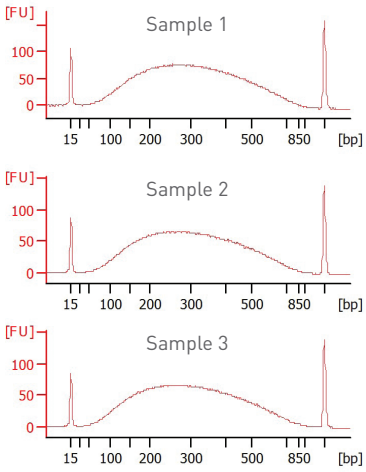
The most critical factor of sonication is reproducibility. The EpiShear Cooled Sonication Platform enhances sample-to-sample reproducibility by enabling you to precisely position the probe at the same depth in the sample every sonication. Also included is a Tube Cooler that ensures that the sample stays cold throughout the entire sonication, eliminating the need to move samples to an ice bucket between pulses. Use with any programmable sonicator like our EpiShear™ Probe Sonicator. Simply set your parameters, press START, then walk away.

Achieve better consistency when shearing chromatin or DNA
Ensure sample-to-sample reproducibility from shearing when preparing samples for ChIP. Once you determine the optimal settings for sonication, recreate them every time with the EpiShear Cooled Sonication Platform.

How does it work?
The EpiShear Cooled Sonication platform is designed to be used with Active Motif's Tube Coolers, which are available in sizes that fit microfuge, 15 ml and 50 ml tubes. The Tube Coolers keep the sample cold during sonication to save time spent moving samples to an ice bath to prevent overheating.

The durable stainless steel and aluminum platform includes a hand crank, a height counter and a vertical alignment tool. The supplied vertical alignment tool is used to determine the depth of the probe inside the tube when it is in the Cooler. Once the appropriate adjustments have been made to ensure proper probe height within the tube, you can record and use the same setting for all future experiments to ensure consistency between samples. Each unit is supplied in a sound enclosure to reduce the noise of sonication. To learn more and for ordering information, visit www.activemotif.com/platform.

The EpiShear™ Cooled Sonication Platform



Sample	Average	Range (bp)
1	325 bp	55-1088
2	322 bp	46-988
3	320 bp	54-1012

Figure 1: **Bioanalyzer data confirms reproducibility.**
Three different samples of sheared chromatin were prepared from HeLa cells using the EpiShear Probe Sonicator (1/8" probe) and the ChIP-IT® Express Kit. Each sample was sonicated using the identical settings on the Cooled Sonication Platform. Analysis performed using an Agilent 2100 Bioanalyzer shows that all 3 samples were sheared to near identical average sizes and comparable size ranges. (The 15 bp and 1500 bp peaks in the graphs above are DNA markers that correspond to the green and purple bars present in the gels.)

Product	Format	Catalog No.
EpiShear™ Cooled Sonication Platform, 1.5 ml	1 platform	53080
EpiShear™ Cooled Sonication Platform, 15 ml	1 platform	53081
EpiShear™ Cooled Sonication Platform, 30 ml	1 platform	53082
EpiShear™ Probe Sonicator	110V	53051
	230V	53052

PRSRT STD
US POSTAGE
PAID
PERMIT #94
SAN DIEGO, CA

NORTH AMERICA

Toll Free: 877 222 9543
Direct: 760 431 1263
Fax: 760 431 1351
sales@activemotif.com
tech_service@activemotif.com

Customer Service:
order@activemotif.com

JAPAN

Direct: +81 (0)3 5225 3638
Fax: +81 (0)3 5261 8733
japantech@activemotif.com

EUROPE

GERMANY 0800/181 99 10
UNITED KINGDOM 0800/169 31 47
FRANCE 0800/90 99 79
OTHER COUNTRIES, DIRECT +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
eurotech@activemotif.com

CHINA

Hotline: 400 018 8123
Direct: +86 21 2092 6090
techchina@activemotif.com

ACTIVE MOTIF®

Enabling Epigenetics Research