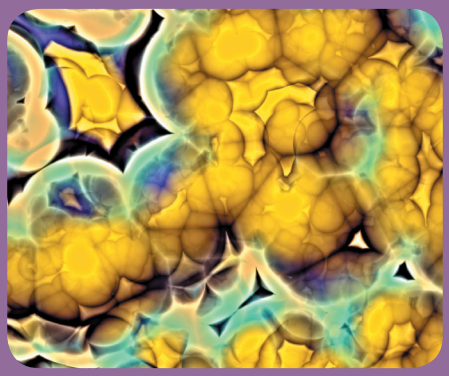
motif|vations

THE NEWSLETTER OF ACTIVE MOTIF
APRIL 2016 | VOLUME 17 NUMBER 1

CANCER EPIGENETICS EDITION

ACTIVE MOTIF®

Enabling Epigenetics Research



IN THIS ISSUE

Epigenetics & Cancer

Epigenetics, or the heritable changes in gene function that occur independent of DNA sequence, has become a major focus of development and disease research. Countless studies link epigenetic alterations to developmental processes, including X-chromosome inactivation, genomic imprinting, axis patterning and differentiation, as well as to diseases such as cancer, chromosomal instabilities and mental retardation. Because genetics alone does not provide an adequate explanation for the complexity of these processes, a deeper understanding of the role of epigenetics is crucial to unraveling the underlying mechanisms responsible for regulating development and disease.

DNA methylation & gene regulation
DNA methylation is an important regulator of gene expression and chromosome conformation. It occurs at the cytosine bases of eukaryotic DNA, which are converted to 5-methyl-cytosine (5-mC) by DNA methyltrans-ferase enzymes. DNA methylation occurs almost exclusively in the context of CpG dinucleotides that are relatively rare in the mammalian genome and tend to be clustered in CpG islands. Approximately 60% of gene promoters are associated with CpG islands and are normally unmethylated. CpG methylation of gene promoters is usually associated with transcriptional silencing, which can occur through a number of mechanisms, including the recruit-ment of methyl binding domain (MBD) proteins. DNA methylation is involved in a number of cellular functions, such as embryonic development, genetic imprinting, X chromosome inactivation and control of gene expression. Alter-ations in normal DNA methylation patterns, either local upregulation at promoters resulting in silencing of specific cell cycle regulatory or tumor suppressor genes, or a global decrease in DNA methylation, are hallmarks of various types of cancer.

Methylation & the cancer epigenome
Changes in DNA methylation have been well characterized in cancer. In general, the cancer epigenome is marked by global DNA hypomethylation and promoter-specific DNA hyper-methylation, which often leads to silencing of tumor suppressor genes. This link to cancer is supported by the finding that mutations in DNMT3A are found in acute myeloid leukemia, and there are currently two FDA approved cancer drugs that target DNMTs. The recently characterized DNA modifica-tion 5-hydroxymethylcytosine (5-hmC) has also been linked to cancer. Both a reduction in 5-hmC and in expression of the TET enzymes that convert 5-mC to 5-hmC have been reported in breast, liver, lung, prostate and pancreatic cancer.

Tools for DNA methylation analysis
Active Motif offers a number of products specific for DNA methylation research, including kits and antibodies to enrich for DNA fragments that contain 5-mC and 5-hmC. Our DNA methylation-related antibodies have been validated for various applications, including methylated DNA immunoprecipitation (MeDIP) and immunofluorescence.

For complete details on all of our DNA methylation products, or to download a free copy of our new DNA Methylation & Cancer eBook, please visit us at www.activemotif.com/dnamt.

The role of non-coding RNAs in cancer

The discovery of non-coding RNAs (ncRNAs) and their role in gene regulation represents a significant advance in cancer research. These ncRNAs interact with DNA, RNA or protein molecules and/or some combination thereof, acting as essential regulators in chromatin organization, and transcriptional and post-transcriptional regulation. The functional relevance of ncRNAs is particularly evident for microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), which have been associated with all stages of cancer including diagnosis, staging, progression, prognosis and response to treatment. Their misexpression confers upon a cancer cell the capacity for tumor initiation, growth and metastasis, making ncRNAs attractive therapeutic targets and potentially useful diagnostic tools.

MicroRNAs in cancer

MicroRNAs are important epigenetic regulators of gene expression and have been shown to play a role in numerous biological processes, such as cellular signaling, cell differentiation, growth, development and apoptosis. Mutations and improper regulation of miRNAs have been linked to a variety of physiological disorders, such as cancer and heart disease (Figure 1). The recent emergence of miRNAs has been one of the defining developments in cancer biology. Many studies have demonstrated how miRNAs impact cancer progression through controlling expression of their target mRNAs to facilitate tumor growth, invasion, angiogenesis and immune response. The explosion of knowledge since their discovery in 1993 has brought forward new diagnostic and therapeutic opportunities. Clinical trials utilizing miRNA profiling for patient prognosis and clinical response are now underway, and the first miRNA mimic entered the clinic for cancer therapy in 2013.

Tools for miRNA analysis

Inherent to the rapid advancements and general excitement surrounding miRNA discoveries is the need for applicable and validated experimental tools to enable researchers to accurately study the expression and biological function of miRNAs. There are a number of diverse methodologies available for miRNA detection and target identification, as well as to determine the functional effects of a specific miRNA of interest. These include computational tools, expression and proteomics assays, 3'UTR reporter assays and chromatin immunoprecipitation (ChIP) based techniques. Active Motif offers a wide selection of tools to enable researchers to identify mRNA targets and elucidate the functional relevance of a specific miRNA, including the LightSwitch™ Luciferase Reporter Assay System. 3'UTR reporter assays have become an important component of thorough miRNA target studies because they provide functional evidence for, and quantitate the effects of, specific miRNA-3'UTR interactions in a cell-based system. For complete details on all our miRNA products, or to download a free copy of our new microRNAs in Cancer eBook, please visit us at www.activemotif.com/ncRNAs-and-cancer.

Let us do the work for you - Active Motif Epigenetic Services

Our Epigenetic Services team provides a wide variety of DNA Methylation and Gene Regulation services. For more complete information, or to get a quote, visit www.activemotif.com/services.

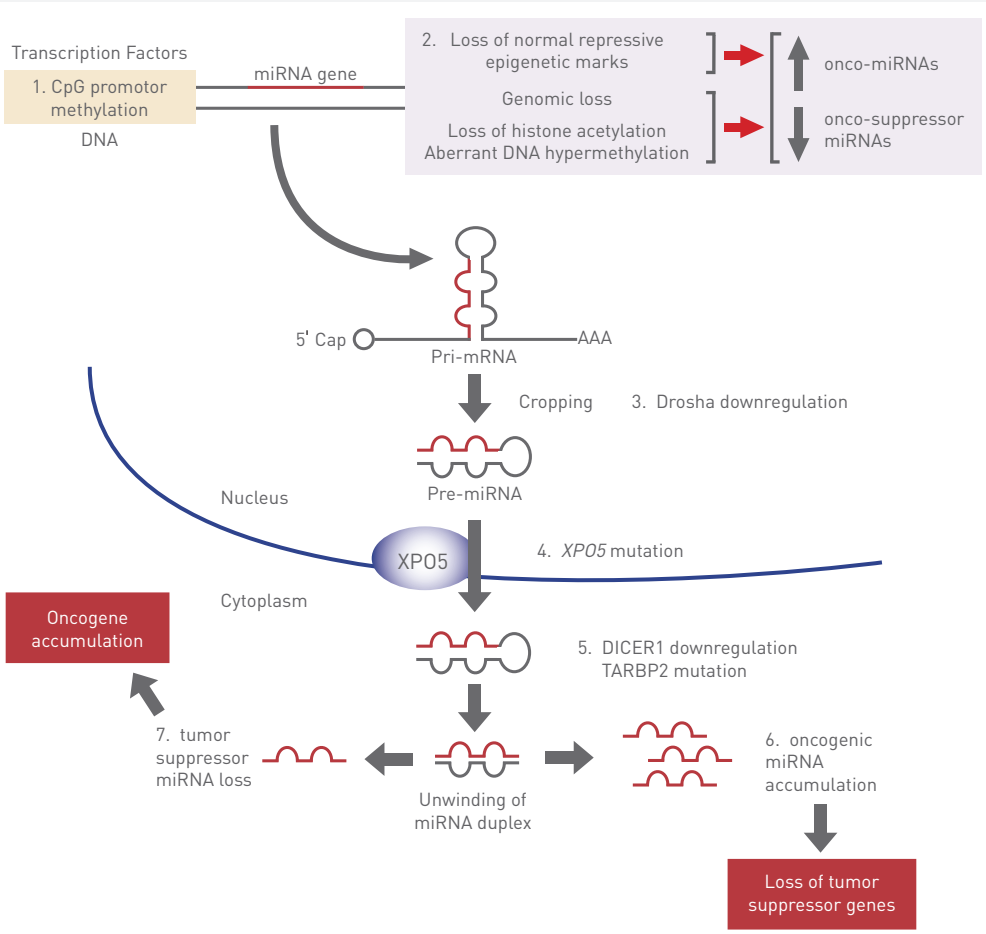


Figure 1: **DNA methylation and miRNA mysregulation in cancer.**
A schematic representation of alterations in DNA methylation and in the miRNA biogenesis pathway that commonly lead to cancer development (adapted from Bertoli G. *et al.* (2015). *Theranostics* 5, 1122–43).

RNA Analysis Techniques to Advance our Understanding of the Transcriptome

Technological advances in the field of RNA analysis have expanded our understanding of the transcriptome beyond messenger RNA (mRNA) to introduce new classes of RNAs that include short and long non-coding RNA (ncRNA). These non-coding RNAs vary in cellular localization and appear to have unique regulatory functions. Combining RNA analysis with high quality RNA isolation from subcellular fractions, such as with Active Motif's RNA Subcellular Isolation Kit, or with next-generation sequencing (NGS) by utilizing Active Motif's RNA Sequencing (RNA-Seq) Service, enables you to achieve a greater understanding of RNA function in your biological system.

High quality RNA fractions improve downstream RNA analysis

Active Motif's RNA Subcellular Isolation Kit is designed 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.

Traditional methods fall short for RNA analysis because they often isolate 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. The RNA Subcellular Isolation Kit reduces this complexity to improve downstream analysis and also provides information regarding RNA localization.

What's in the box?

Each kit contains enough reagents to perform 15 cytoplasmic and 15 nuclear RNA isolations. For more information, visit www.activemotif.com/rna-iso.

RNA Subcellular Isolation Kit

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

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

RNA-Seq Service – get the most out of your RNA-Seq experiment

Active Motif provides a comprehensive RNA-Seq Service that includes everything from isolation of RNA to data analysis. Combine our worldwide leading ChIP-Seq Service with RNA-Seq to gain an even greater understanding of your biological system.

RNA-Seq is the most widely used technique for studying gene expression changes in response to treatment and disease, but it also provides much more. With sequence data from an RNA-Seq experiment it is possible to obtain, among other things, information about splice variants, gene fusions and SNPs. It is, therefore, important to design RNA-Seq experiments according to the goals in mind. Should you use paired-end or single-end sequencing? What length of sequencing read is needed, and how deep should you go?

Is polyA selection or rRNA depletion appropriate for your experiment? Active Motif understands and offers solutions for all the experimental variables. We will consult with you and provide specific recommendations on experimental design that precisely align with the goals of your research.

RNA-Seq Service Features

- PolyA selection or rRNA reduction
- Directional library preparation
- 50bp or 100bp paired-end reads
- QC performed using Bioanalyzer
- Data analysis options (TOPHAT, CUFFLINKS, CUFFCOMPARE, CUFFDIFF)

NEW

Tools to Study Nrf2 Transcription Factor Activation

Nrf2 is an important transcription factor (TF) in the defense against oxidative stress. Much investment has been made in developing Nrf2 agonists for the treatment of diseases such as multiple sclerosis, diabetes and cancer. Like most TFs, Nrf2 activation is dynamically regulated, involving controlled protein degradation and nuclear translocation. Active Motif offers a range of products for Nrf2 research, including TransAM®, a simple, plate-based assay to screen for Nrf2 activation, a chromatin immunoprecipitation (ChIP) validated Nrf2 antibody and compatible ChIP kit, and a luciferase reporter assay system along with pre-cloned Nrf2-related reporter vectors.

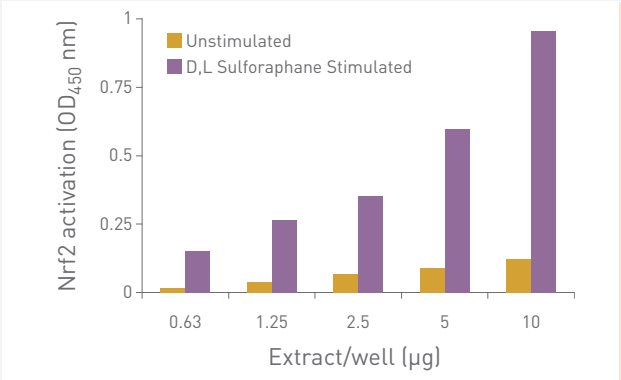


Figure 1: Nrf2 activation is detected using the TransAM Nrf2 Kit. Active Motif's Nuclear Extract Kit was used to prepare nuclear lysates from unstimulated HepG2 cells and cells that were stimulated for 24 hours with 10 µM D,L-Sulforaphane. The extracts were assayed using a range of 0.625 to 10 µg per well to show the linearity of the assay. Robust Nrf2 activation is clearly detectable across all conditions.

Screening multiple samples?
Active Motif's TransAM® Nrf2 Kit includes a 96-stripwell plate to which an oligomer corresponding to the antioxidant response element (ARE) has been immobilized. Nuclear extracts, prepared using Active Motif's Nuclear Extract Kit, are added to each well. Activated Nrf2 binds the ARE consensus sequence, while unbound proteins are washed away. Detection is achieved using an Nrf2 primary antibody and HRP-conjugated secondary antibody along with colorimetric detection reagents. The assay signal is easily measured by OD 450nm using a microplate reader (Figure 1). In addition to Nrf2, TransAM is available for over 40 transcription factor targets. For more information and a complete list of TransAM products, go to www.activemotif.com/transam.

Active Motif also offers cell-based screening assays using our LightSwitch™ Luciferase Reporter Assay System that combine optimized transfection and luciferase assay reagents along with a large selection of Nrf2 responsive luciferase reporter constructs. These include a variety of synthetic ARE elements and pre-cloned reporter vectors containing the promoters of Nrf2 responsive genes such as *NQO1* and *HMOX1*.

Interested in monitoring Nrf2 activation across the genome?
ChIP-Seq is now the most widely used technique to study transcription factor binding. To facilitate your ChIP-Seq studies of Nrf2, Active Motif provides a Nrf2 ChIP-Seq validated antibody and our best-selling ChIP-IT® High Sensitivity Kit (Figure 2). Or let Active Motif Epigenetic Services do the experiment for you. As the ChIP Experts™, with over 10,000 samples processed and the highest level of expertise of any service provider, you can expect the best results. For more information about our custom ChIP-Seq services, visit www.activemotif.com/services.

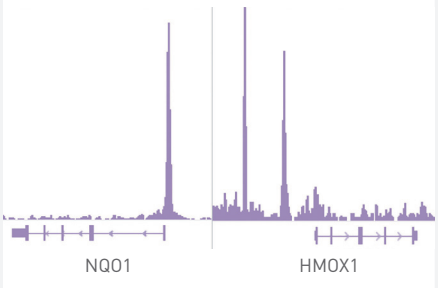


Figure 2: ChIP-Seq was performed using Active Motif's Nrf2 antibody and ChIP-IT High Sensitivity Kit. Select peaks are presented and show Nrf2 binding in the promoters of known Nrf2 responsive genes.

Product	Format	Catalog No.
TransAM® Nrf2 Kit	1 x 96 rxns	50296
Nuclear Extract Kit	100 rxns	40010
ARE synthRE	5 µg	32108
NRF2 (NFE2L2) antibody (pAb)	100 µl	61599
ChIP-IT® High Sensitivity Kit	16 rxns	53040

Practical Guide to Chromatin Immunoprecipitation Techniques

Chromatin immunoprecipitation (ChIP) is the most widely utilized technique for analysis of protein-DNA interactions and histone modifications. Given the importance of ChIP and ChIP-Seq data sets for development and disease research, obtaining high quality data is crucial. However, ChIP can be technically challenging, and researchers who are not experts in ChIP techniques are best served using well-validated and reliable kits to perform these assays. As the ChIP experts®, Active Motif has developed a number of highly innovative kits and reagents tailored to aid researchers with their ChIP experiments and to overcome many of the common challenges researchers encounter when applying this technique.

TOPICS

Performing ChIP on epigenetic modifications

As a leader in providing innovative solutions for ChIP, Active Motif has developed a highly comprehensive portfolio of ChIP products and services to make ChIP technologies accessible to researchers at any level of expertise, and to also provide cutting-edge technologies to address many of the common limitations experienced by ChIP users when analyzing chromatin function.

Traditional ChIP and ChIP-Seq are extremely informative methods for identification and profiling of chromatin interactions with ubiquitous features, for example chromatin modifying proteins (*e.g.* p300, HDACs), histone modifications (*e.g.* H3K4me3, H3K27me3, H3K27ac) and transcriptional machinery (*e.g.* RNA pol II). When conducting ChIP analysis, the most important aspects of your ChIP protocol are that it be reproducible, sensitive and efficient.

For performing ChIP on relatively abundant proteins such as histones, Active Motif's ChIP-IT® Express kits provide the ideal solution. ChIP-IT Express kits use magnetic beads to streamline the ChIP protocol, allowing the ChIP procedure to be completed in a single day. The reduced hands-on time and use of ChIP-grade magnetic particles minimize DNA loss and sample variation, enabling ChIP to be performed on as few as 100,000 cells. With over 500 citations in peer reviewed publications, this is the method of choice for routine ChIP (Figure 1).

What if I don't have a sonicator?

The kit comes in two formats, the original ChIP-IT Express sonication protocol and ChIP-IT® Express Enzymatic, the first kit that enables shearing of chromatin by enzymatic digestion rather than sonication for users who do not routinely perform ChIP and either do not own a sonicator or are not proficient in its use.

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

What if I have limited starting material?

Standard ChIP-Seq protocols require at least 10 million cells to perform ChIP-Seq, a number that is unattainable for many cell types. As a result, one of the major obstacles preventing researchers from generating quality ChIP-Seq data is the limited cell numbers available for their experiments. Active Motif's Epigenetic Services group

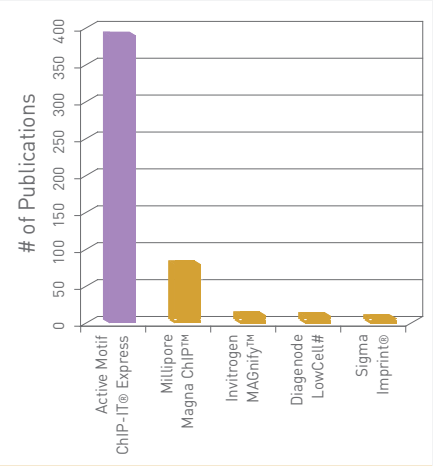


Figure 1: **ChIP-IT Express is the most highly published commercial ChIP kit on the market.** Comparison of ChIP-IT Express citations vs. competitor kits.

has made significant improvements to our ChIP-Seq protocols and pipeline and, as a result, reduced our starting material requirements by 1,000 fold. Our Low Cell Number ChIP-Seq Service enables generation of genome-wide binding profiles from as few as 10K cells.

For more information, please visit us at www.activemotif.com/lowcellcs.

Analyzing low abundance targets (transcription factors)

Performing ChIP-Seq on transcription factors is often problematic due to the relative low abundance of these proteins and quality of available antibodies. To address this, scientists at Active Motif have developed the ChIP-IT® High Sensitivity Kit, the most sensitive ChIP kit available on the market. The kit utilizes specially formulated reagents for preparation of high-quality chromatin from minimal amounts of cells or tissue (1000 cells for abundant targets and 50,000 cells for low abundance transcription factors per reaction; the chromatin preparation module is also available separately as the High Sensitivity Chromatin Preparation kit). During the immunoprecipitation (IP) reaction, low-background protein G agarose beads and an antibody blocker reduce non-specific binding. Specialized ChIP buffers enhance enrichment and reduce non-specific DNA interactions. ChIP filtration gravity flow columns are used for faster, simpler and more consistent capture and wash steps. The result is a kit that is capable of delivering both higher signals and reduced background levels when compared to other commercially available ChIP kits. To learn more about ChIP-IT High Sensitivity, visit www.activemotif.com/chipiths.

Choosing the correct antibody

Another challenge for epigenetics research is the lack of antibodies that have been validated for use in ChIP and ChIP-Seq. The problem is compounded by antibody suppliers who do not manufacture or test the antibodies they sell, and who sell them to one another and then to researchers. At Active Motif, we manufacture and rigorously test our antibodies in-house to ensure their quality and performance. Our ChIP antibodies are validated according to the guidelines of the ENCODE* Consortium and run through the same internal validation program used by our ChIP-Seq Services group.

For a complete, up-to-date list of available antibodies, please visit us at www.activemotif.com/chipabs.

*For ENCODE Consortium guidelines for antibody validation, please refer to Landt S.G. et al. (2012) Genome Res. 22, 1813–1831.

What if I don't have a good antibody?

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 (Figure 2).

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. To learn more, visit www.activemotif.com/tagchip.

Increasing DNA binding site resolution

For researchers working with transcription factors, another common issue encountered with ChIP, aside from low abundance of protein, is the poor resolution of binding sites. Traditional ChIP-Seq methods only

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

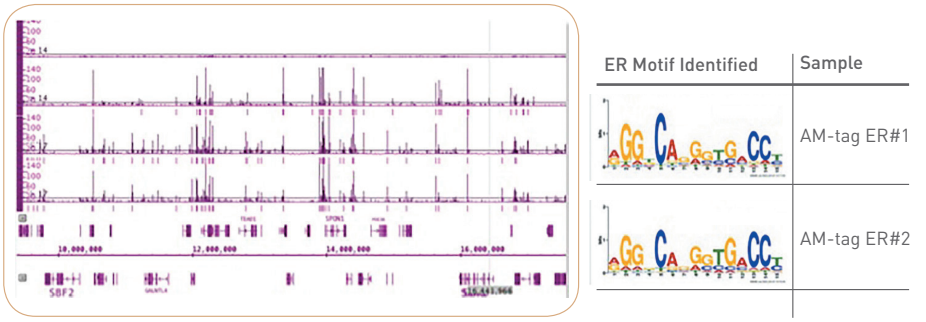


Figure 2: ER cDNA was cloned into pAM_1C Empty Vector and transiently transfected into cells. Cells were induced with estradiol and chromatin was harvested and Tag-ChIP performed using the Tag-ChIP-IT Kit. Following cross-link reversal, enriched DNA was submitted for next-generation sequencing. Data was compared to published anti-ER antibody ChIP-Seq results using 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.

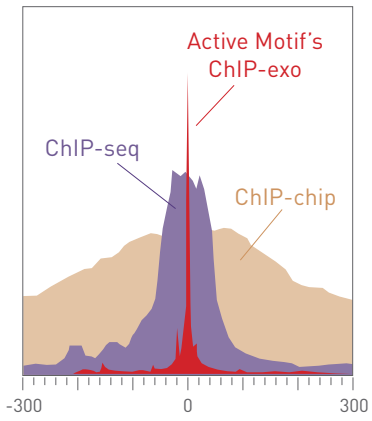


Figure 3: ChIP-exo achieves significantly higher resolution of DNA binding sites than traditional ChIP methods.

resolve DNA binding sites within ≥200 bp when the average DNA binding site length of individual transcription factors tends to be in the region of 6-10 bp. To address this, Active Motif offers the ChIP-exonuclease (ChIP-exo) Kit containing an optimized and streamlined protocol for high-resolution genome-wide mapping of transcription factor binding sites. ChIP-exo is a modified ChIP-Seq approach that enables researchers to identify protein-DNA binding sites at a resolution of 20-95 base pairs, refining the ability to identify DNA motifs more precisely and distill genome-wide protein binding profiles for studies of mutational or SNP effects on transcription factor binding sites (Figure 3). For more information, go to www.activemotif.com/chipexo.

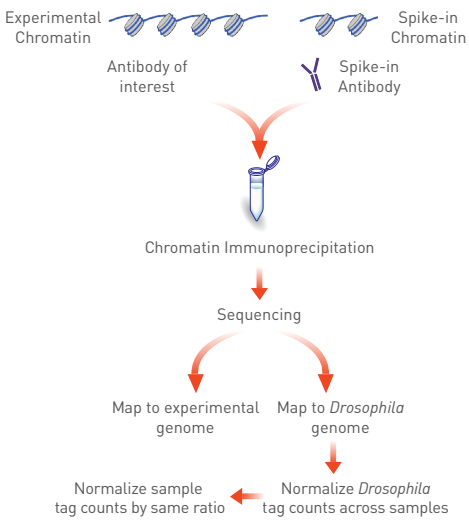
How do I normalize for sample bias?

ChIP-Seq is a multi-step process in which disparities caused by sample loss during immunoprecipitation and library preparation, uneven sequencing read depth or technical variation can lead to results that are difficult to interpret. To overcome this challenge, Active Motif has developed a ChIP Normalization Spike-in strategy for normalization of ChIP qPCR and ChIP-Seq data to reduce the effects of technical variation and sample processing bias. This strategy can be applied across samples and antibodies to eliminate bias and reveal any real or obscured biological differences. ChIP normalization can easily be implemented by integrating our Spike-in reagents into your existing ChIP protocol. For more complete information, go to www.activemotif.com/spikein. To learn about custom Spike-in Services, visit www.activemotif.com/services-normalize.

What interactions are occurring at my promoter of interest?

When performing ChIP experiments, it is often useful to analyze co-occupancy of proteins or epigenetic modifications at a given genomic locus. Active Motif's Re-ChIP-IT® Kit is a Sequential ChIP (Seq-ChIP, Re-ChIP) procedure in which ChIP samples are subjected to sequential rounds of immunoprecipitations using two different antibodies to determine whether proteins or epigenetic modifications co-occupy the same genomic region. The Active Motif Re-ChIP-IT Kit utilizes the same magnetic bead-based protocol used in our ChIP-IT Express Kits to provide a streamlined Re-ChIP protocol for sequential ChIP analysis. Unlike other Seq-ChIP methods, the Re-ChIP-IT method eliminates the need for direct antibody conjugates or an immunoselection matrix and includes specialized buffers that prevent carryover of primary-to-secondary immunoprecipitation reactions. To learn more, visit www.activemotif.com/rechip.

ChIP-Seq Normalization Workflow



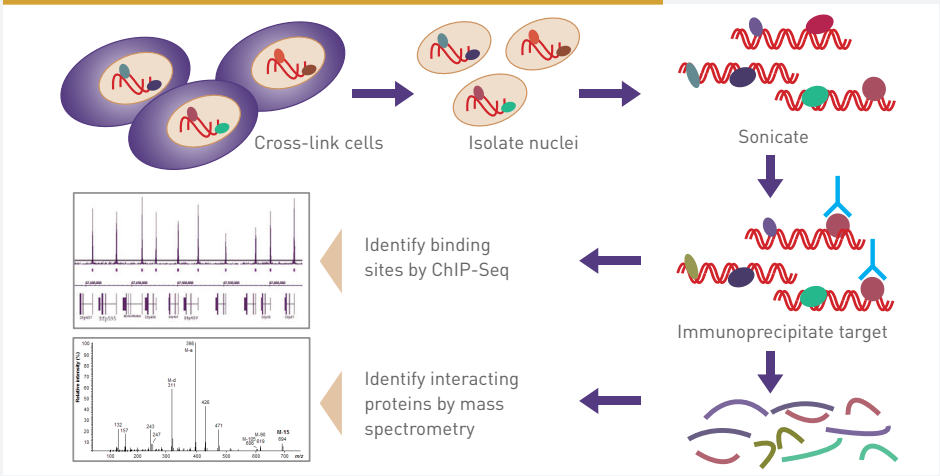
Active Motif's recent addition to it's suite of custom services is RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins). RIME resolves the complex process of gene regulation by enabling the capture and identification of interactomes, or the associated protein networks, of an endogenous protein of interest. Gene regulation research is often over-simplified by solely focusing on one regulatory factor. In reality, differential gene expression is the result of the highly orchestrated and complex interactions of chromatin modifiers, epigenetic modifications, coactivators and repressors, and other regulatory elements with chromatin. The RIME service includes immunoprecipitation of a target protein from cross-linked cell extracts followed by ChIP-Seq, mass spectrometry and data analysis.

To learn more about our RIME Services, visit www.activemotif.com/rime.

How can ChIP aid in genome editing?

CRISPR/Cas9 genome editing has gained prominence in genetic research and has expanded its capabilities in so many ways, providing a simple method to potentially genetically modify plants, eradicate viruses, and identify cancer genes. However, there are unintended consequences of this technology, mainly issues with Cas9 nuclease specificity in creating double-stranded breaks leading to off-target effects. To enable you to assess Cas9 specificity, Active Motif's enChIP Kit (Engineered DNA-binding molecule-mediated Chromatin Immunoprecipitation) offers a modified ChIP assay that utilizes the CRISPR/Cas9 system to target a specific DNA locus for immunoprecipitation. Using the enChIP Kit for your genome editing

RIME Service Workflow for Identification of Endogenous Interactomes



experiments enables you to biologically validate each target sequence for specificity. Sequences demonstrating off-target effects can be excluded, saving valuable time and resources.

To learn more, go to page 10 or visit us at www.activemotif.com/enchip.

Can I also interrogate the DNA methylation status of my ChIP DNA?

For those interested in DNA methylation profiles of their genomic region of interest, Active Motif's ChIP-Bisulfite-Sequencing (ChIP-Bis-Seq) Kit offers a method to directly interrogate both chromatin and DNA methylation status within the same sample. ChIP-Bis-Seq combines a ChIP procedure for target-specific enrichment of protein-bound or modified chromatin with bisulfite conversion and sequencing (Bis-Seq) to allow single nucleotide resolution DNA methylation profiles of ChIP-enriched chromatin. This approach is ideal for genome-wide studies of the interactions between DNA methylation and chromatin features, such as in assessing allele-specific DNA methylation variances (*e.g.* imprinting, X-inactivation), or in evaluating global gene regulatory effects. To learn more, visit www.activemotif.com/chip-bis-seq.

Difficult-to-ChIP clinical samples

In disease research, when attempting to harvest valuable biological information from clinical samples, many of the common clinical sample types are primary tissues that present a challenge either because of the difficulty in extracting high-quality chromatin from difficult-to-lyse cells, very limited amounts of sample, or highly degraded material. Active Motif provides the ChIP-IT® FFPE and ChIP-IT® PBMC assays to finally enable researchers to perform ChIP analysis of primary tissue samples. These ChIP assays are the only commercially available assays specifically developed and optimized to extract high-quality chromatin from primary cells for ChIP analysis and next-generation sequencing. For more information on the ChIP-IT FFPE and PBMC kits, please visit us at www.activemotif.com/ffpe or www.activemotif.com/pbmc.

Analyzing chromatin-associated RNA

For researchers interested in the role of RNA in regulation of the genome, Active Motif has adapted the magnetic bead-based method used in our ChIP-IT Express Kits for the development of the first-of-its-kind kit for RNA-ChIP. The RNA ChIP-IT® Kit is specifically designed to study RNA-protein interactions in a chromatin context. RNA ChIP-IT's innovative fixation method is superior to existing RIP technologies that use native nuclear extracts because fixation preserves chromatin interactions, taking into account the dynamic chromatin environment of the RNA-protein complexes of interest. For more, visit www.activemotif.com/rnachip.

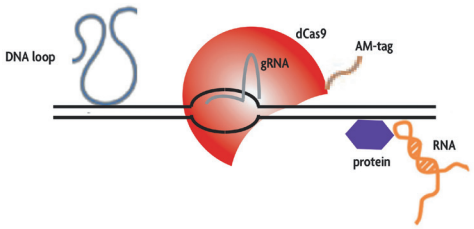
For a complete list of available ChIP products & services, please visit us at www.activemotif.com/chip

Product	Format	Catalog No.
ChIP-IT® Express Kit	25 rxns	53008
ChIP-IT® Express Enzymatic Kit	25 rxns	53009
ChIP-IT® High Sensitivity Kit	16 rxns	53040
High Sensitivity Chromatin Preparation Kit	16 rxns	53046
Tag-ChIP-IT® Kit	16 rxns	53022
ChIP-exo Kit	12 rxns	53043
Spike-in Antibody	50 µg	61686
Spike-in Chromatin	10 µg	53083
Re-ChIP-IT® Kit	25 rxns	53016
ChIP-Bis-Seq Kit	10 libraries	53048
ChIP-IT® FFPE Kit	16 rxns	53045
ChIP-IT® PBMC Kit	16 rxns	53042
RNA ChIP-IT® Kit	25 rxns	53024

NEW

Determine CRISPR/Cas9 Specificity Using enChIP

enChIP* is a modified chromatin immunoprecipitation (ChIP) assay that utilizes the CRISPR/Cas9 system to target a specific DNA locus for immunoprecipitation. Using the enChIP Kit enables you to biologically validate each target sequence for specificity. Sequences demonstrating off-target effects can be excluded from genome editing experiments, thereby saving valuable time and resources.



*Patent number: WO2014/125668

How does enChIP work?
Active Motif's enChIP Kit (Engineered DNA-binding molecule-mediated Chromatin Immunoprecipitation) utilizes the CRISPR/Cas9 system to direct a guide RNA (gRNA) to a specific genomic locus for immunoprecipitation. The gRNA is co-expressed with an enzymatically inactive form of the *Streptococcus pyogenes* Cas9 endonuclease (dCas9). The dCas9 protein contains Active Motif's unique AM-tag sequence that was designed specifically for high sensitivity enrichment in ChIP. Following transfection of the gRNA and AM-tagged dCas9, cells are formaldehyde fixed, lysed, and the chromatin is fragmented by sonication. A monoclonal antibody directed against the AM-tag is used to enrich for genomic sequences bound by the gRNA/dCas9 complex. Following immunoprecipitation, purified DNA can be analyzed by qPCR or NGS to evaluate binding specificity or chromosomal looping.

For more information, please visit us at www.activemotif.com/enchip.

enChIP Kit Validates Specificity

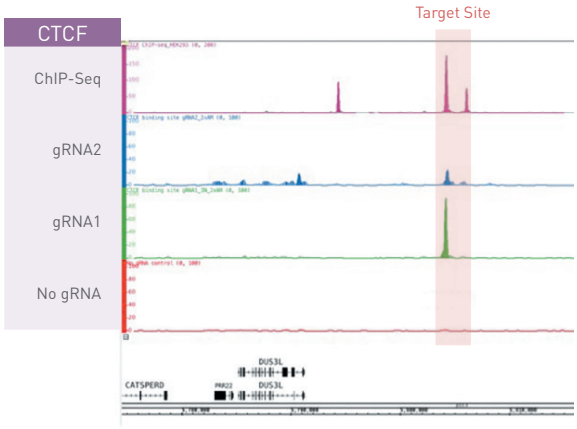


Figure 1: Two gRNAs were designed targeting different 20 nt sequences within a 500bp region of a CTCF binding site on chromosome 19. Each gRNA sequence was cloned into the pAM_dCas9 vector and transfected into HEK293T cells. A 'No gRNA' negative control was also performed. Chromatin was prepared and immunoprecipitated according to the enChIP Kit. Enriched DNA was analyzed by NGS and background was subtracted using the 'No gRNA' control. Data was compared to ChIP-Seq data for CTCF in the same cell line. Results show gRNA1 had a strong peak and specific binding at the target location. Data for gRNA2 revealed a large number of off-target binding events outside of the target location. This confirms that enChIP provides biological validation for CRISPR/Cas9 specificity.

enChIP Kit Highlights

- Identify off-target binding events
- Evaluate chromosomal looping
- Versatility to use your own gRNA constructs or clone into one of Active Motif's vectors
- Utilizes AM-tag specifically designed for use in ChIP
- High sensitivity assay detects sequences present at only 1 or 2 genomic locations

What's in the box?
The enChIP Kit contains chromatin preparation reagents, enChIP buffers, AM-tag monoclonal antibody, protein G magnetic beads and DNA purification reagents for 16 enChIP reactions. The gRNA and dCas9 expression vectors are sold separately.

Product	Format	Catalog No.
enChIP Kit	16 rxns	53125
pAM_gRNA Vector	10 µg	53121
pAM_dCas9 Vector	10 µg	53122
pAM_gRNA_CTCF Vector (Positive control)	10 µg	53123
pAM_dCas9_CTCF Vector (Positive control)	10 µg	53124
FuGENE® HD Transfection Reagent	0.2 ml	32042

Quantify Global Changes in 5-mC & 5-hmC in Normal and Diseased Samples

The cancer epigenome is marked by alterations in a myriad of epigenetic features, including DNA methylation, nucleosome positioning and histone post-translational modifications. These changes influence gene regulation and can serve as signatures of cancer. To better understand how changes in DNA modifications influence normal and diseased states, Active Motif offers simple, easy-to-use assays to perform comparative studies of global changes in 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC).

Global DNA methylation & disease
In mammals, DNA methylation is characterized by the covalent addition of a methyl group within the cytosine of a CpG dinucleotide. These CpG sites are often found within repetitive regions and are normally methylated to repress transcription. In cancer, hypomethylation of these repetitive elements is observed. Likewise, the DNA methylation variant 5-hmC is known to play a role in transcriptional regulation and embryonic development and may also serve as a prognostic indicator in certain cancers and neurodegenerative disorders. Hydroxymethylcytosine results from the oxidation of 5-mC by the Ten Eleven Translocation (TET) enzymes. Because of their importance in biological processes and disease, methods to accurately quantify global 5-mC and 5-hmC are highly warranted.

Quantify changes in global 5-mC levels
Active Motif's simple, plate-based Global DNA Methylation – LINE-1 Kit uses a consensus sequence within the human repetitive Long Interspersed Nucleotide Element 1 (LINE-1) as a surrogate readout for global 5-mC levels. First, genomic DNA is enzymatically digested to create LINE-1 fragments. The DNA is then hybridized with a LINE-1 consensus probe. Following capture onto a 96-well plate, a 5-mC antibody and colorimetric detection reagents are used to generate a signal that is easily measured with a microplate reader. The assay includes DNA standards for 5-mC quantification and can detect as little as 0.5% methylcytosine (Figure 1).

COMING SOON:
Global LINE-1 Kit for MURINE samples

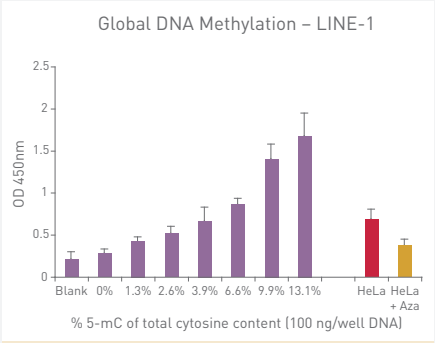


Figure 1: The Global DNA Methylation – LINE-1 Kit was used to compare the methylcytosine levels between two genomic DNA samples. Assay results show a decrease in 5-mC levels resulting from treatment of HeLa cells with 5-azacytidine (Aza), a DNA methyltransferase inhibitor. The % 5-mC is calculated using the included DNA standards. Methylcytosine levels of the DNA standards are displayed as a percentage of total cytosine content.

Quantify changes in global 5-hmC levels
Using Active Motif's Global 5-hmC Quantification Kit, hydroxymethylcytosine levels can be quantified from as little as 20-50 ng of genomic DNA. This easy-to-use plate-based assay offers higher throughput quantification using less input material than mass spectrometry and can detect as little as 0.02% 5-hmC with the included DNA standards (Figure 2).

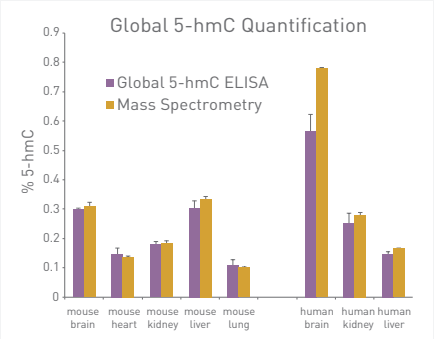


Figure 2: The Global 5-hmC Quantification Kit was used to determine the % 5-hmC in genomic DNA isolated from mouse and human tissues. Genomic DNA was tested at 20 ng/well for human brain and 50 ng/well for all other DNA samples. The % 5-hmC was determined using the included DNA standards. Results were compared to mass spectrometry data obtained from 500 ng of the same DNA samples. Results show the Global 5-hmC Kit provides equivalent quantification using only a fraction of the starting material.

Product	Format	Catalog No.
Global DNA Methylation – LINE-1 Kit	1 x 96 rxns	55017
Global 5-hmC Quantification Kit	1 x 96 rxns	55018

A Genome-wide 3'UTR Collection for High-throughput Functional Screening of miRNA Targets

To enable researchers to study microRNAs (miRNAs) using 3'UTR-reporter assays on a high-throughput scale, we have created a genome-wide collection of 12,000 human 3'UTR luciferase reporters in the highly-optimized LightSwitch™ Luciferase Assay System. The assay system is ideal for performing miRNA target validation and assessing the functional impact of miRNA-3'UTR interactions. Combined with our large collections of miRNA Mimics & Inhibitors, you have everything needed to study miRNA-3'UTR interactions, validate miRNA targets, and measure RNA stability and the functional impact of miRNAs on a gene-by-gene basis.

LightSwitch™ 3'UTR reporter collection and assay system

Identification and validation of miRNA targets can be a complicated task. There are several alternatives available, starting with numerous target prediction methods and continuing with different biological procedures that encompass reporter-based screenings, shotgun proteomics and Ago2-based immuno-precipitation methods. 3'UTR-reporter assays have become an important component of thorough miRNA target studies because they provide functional evidence for, and quantitate the effects of, specific miRNA-3'UTR interactions in a cell-based system. Thus, to enable researchers to leverage 3'UTR-reporter assays on a high-throughput scale, we have created a genome-wide collection of 12,000 human 3'UTR luciferase reporters in the highly optimized LightSwitch Luciferase Assay System (Figure 1).

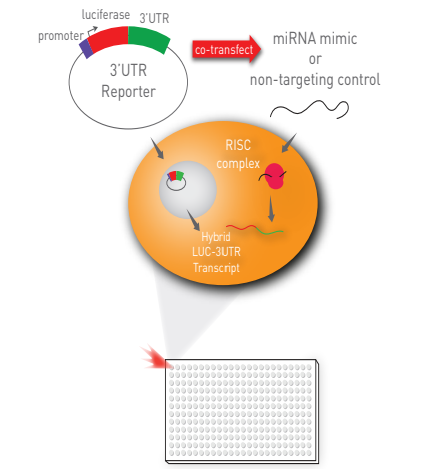


Figure 1: Experimental design for an miRNA target functional screen using the LightSwitch System.

Luciferase-based screening to identify miR-122 targets

The LightSwitch system represents a sensitive, specific and economical means to probe miRNA-UTR interactions and functionally validate miRNA targets. To demonstrate, we performed a high-throughput screen to identify new targets of miR-122. Target predictions were obtained from commonly used sources such as Miranda and Target Scan. Individual 3'UTR luciferase constructs (Catalog No. 32011) were employed in co-transfection experiments with either an miR-122 mimic (Catalog No. MIM0136) or a non-targeting control (Catalog No. MIM9001). Luminescence for 58/142 (40.8%) of the predicted targets was significantly different in the mimic co-transfection compared to the non-targeting control ($p<0.05$) (Figure 2). Thus, not only did this screen identify numerous novel targets of miR-122, but we also found several targets with more potent interactions with miR-122 than previously observed. These studies exemplify how our genome-wide library of human 3'UTR-luciferase reporter constructs enables researchers to screen thousands of potential miRNA targets and understand the roles of miRNAs in a single experiment. To learn more, please visit us at www.activemotif.com/ls-3utr.

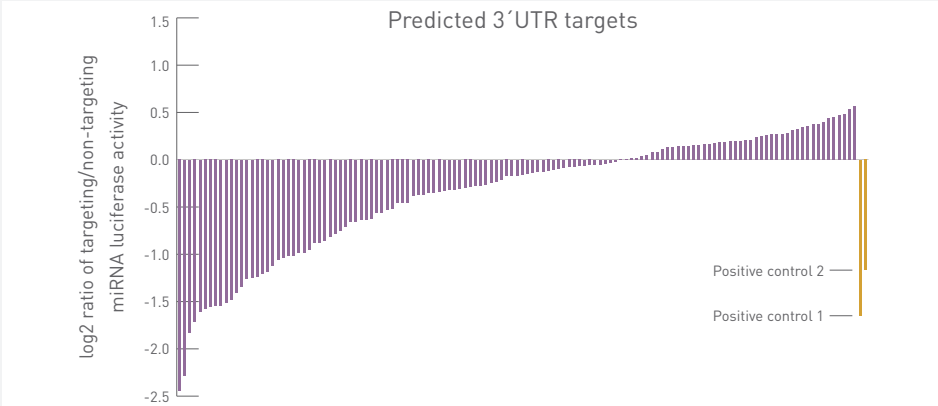


Figure 2: A functional screen of 142 human 3'UTRs with predicted miR-122 sites in HT-1080 cells. 44 of the targets decreased significantly by t-test ($p<0.05$) and 24 were significantly repressed >2-fold.

Validated Antibodies Reveal Histone-mediated Effects on miRNA in Non-small Cell Lung Cancer

Research has shown that dysregulation of microRNA (miRNA) function is associated with many cancers. Furthermore, epigenetic silencing via DNA methylation can alter miRNA expression. In a recent study led by the laboratory of Dr. Nobuya Ohishi at the University of Tokyo Hospital, researchers looked at miR-139, a known tumor suppressor in several cancers, to study its function in non-small cell lung cancer (NSCLC). Using chromatin immunoprecipitation (ChIP) and Active Motif antibodies to trimethylated marks on Histone H3, they suggest that H3K27me3-mediated silencing of miR-139 may represent a promising biomarker of lung cancer metastasis.

Histone methylation-mediated silencing of miR-139 enhances invasion of NSCLC

MiR-139-5p (denoted thereafter as miR-139) has recently been reported to function as a tumor suppressor in several types of human cancers, but its function in NSCLC and the mechanism of its suppression have not been studied in detail. In a recent publication, research conducted in the laboratory of Dr. Nobuya Ohishi reveals that miR-139 is epigenetically silenced by histone H3 lysine 27 trimethylation (H3K27me3) of its host gene PDE2A, and that transcriptional regulation is independent of promoter DNA methylation.

For more detailed information, go to www.ncbi.nlm.nih.gov/pubmed/26256448.

To support their findings, researchers first confirmed the suppression of the host gene PDE2A, and the association of expression levels with miR-139, by measuring their expression across several lung cancer cell lines, as well as NSCLC and adjacent normal lung tissue, using RT-PCR.

With coordinated expression between miR-139 and PDE2A established, they then turned to chromatin immunoprecipitation to look at histone methylation around the promotor of the PDE2A short transcript (Figure 1) to determine if H3K27me3 was silencing PDE2A. Bisulfite sequencing was also used to rule out any dependencies between histone and DNA methylation.

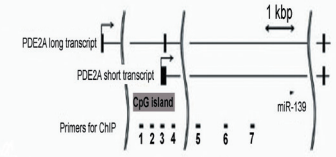


Figure 1: Structure of the PDE2A genomic locus. The locations of miR-139 and the ChIP primers are shown.

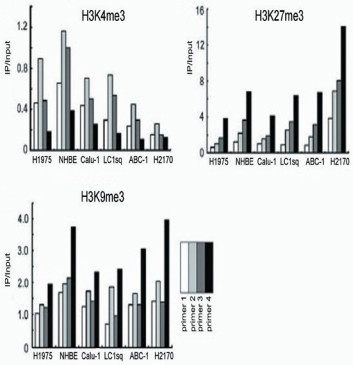


Figure 2: ChIP analysis of H3K4me3, H3K27me3, and H3K9me3 in five lung cancer cell lines and normal human bronchial epithelial cells. IP/Input values were normalized to a control locus (ACTB promoter).

To form a complete picture of transcriptional regulation, ChIP was performed on histone H3 trimethylated at lysine 4 (H3K4me3), a mark of active transcription, and lysine 9 (H3K9me3) which, along with H3K27me3, represent transcriptional repressors. Aided by Active Motif's ChIP validated antibodies to all 3 marks, ChIP analysis showed no real change for H3K9me3 across all cell lines and a positive correlation between H3K4me3 and PDE2A. However, H3K27me3 had a strong negative correlation, more so with NCI-H2170 cells with the lowest expression levels of PDE2A (Figure 2). To further demonstrate this correlation, siRNA inhibition of EZH2 H3K27me3 methyltransferase led to an increase in PDE2A in cancer cells, leaving the researchers to conclude, at least in part, that H3K27me3 represents a promising biomarker of lung cancer metastasis.

For a complete up-to-date list of available antibodies, please visit us at www.activemotif.com/abs.

This article is a summary of the research performed by Watanabe *et al.* at the University of Tokyo Hospital, Hongo, Bunkyo-ku, Tokyo, Japan, published in *Cancer Medicine* (2015) 4,1573–1582.

Product	Format	Catalog No.
Histone H3K4me3 antibody (pAb)	100 µg	39915
Histone H3K9me3 antibody (pAb)	100 µl	39161
Histone H3K27me3 antibody (pAb)	100 µg	39155

High Quality Bromodomain Proteins for Developing Robust Assays for Epigenetic Drug Discovery

Bromodomain proteins play an integral role in the regulation of transcription and chromatin remodeling. Because BET (Bromodomain and Extraterminal domain) proteins have been shown to also regulate transcription of certain oncogenes, they are promising therapeutic targets for cancer. As a result, there has been an increase in focus on bromodomain proteins in drug discovery research that has led to the discovery of a class of small molecule BET inhibitors. Early successes of BRD4 inhibition has peaked interest in the therapeutic potential of other BET inhibitors. To support research efforts, Active Motif offers a comprehensive collection of recombinant bromodomain proteins.

BET family members are promising therapeutic targets

The BET family of bromodomain proteins has been shown to play a key role in the development of several kinds of cancer. Drug discovery efforts have led to the identification of multiple small molecule inhibitors that target BET proteins, and several of these inhibitors currently have advanced to clinical trials.

BETs are a subclass of proteins that are characterized by two N-terminal bromodomains and one ET (Extra Terminal) domain. The ET domain functions as a protein binding motif and exerts atypical serine-kinase activity, while the two N-terminal bromodomains, also known as BD1 and BD2, recognize acetylated lysines on histones and function to recruit other BET proteins to chromatin. Because the recruitment and targeting of serine-kinase and other activities by BETs to chromatin involve the concerted interplay of several BET domains, it is crucial that we understand the effects of existing inhibitors on individual domain functions.

The quest for inhibitors of BET function

One of the most widely studied BET inhibitors is JQ1, a small molecule inhibitor of BRD4 with nanomolar affinity that shows promise across many BRD4 dependent cell lines. As an effective anti-proliferative agent, JQ1 acts as a competitive binder, displacing BRD4 from chromatin resulting in *MYC* downregulation. JQ1 has also been shown to target BRD2 in hematologic malignancies and inhibit expression of BRD3 and BRD4 in Tamoxifen resistant breast cancer cells, giving it the ability to perform as a pan-BET inhibitor.

I-BET151 and I-BET762 are similar to JQ1 in that they possess nanomolar affinity and show strong anti-proliferative effects. I-BET151 also functions as a pan-BET inhibitor showing anti-tumor activity in multiple myeloma, malignant brain tumor and other murine models. I-BET762 is considered a more specific inhibitor and first showed promise in NUT midline carcinoma (NMC). Phase I clinical trials for I-BET762 are ongoing for NMC as well as refractory hematologic malignancies.

Active Motif Bromodomain Proteins

- Over 40 reader domains available
- Specifically designed for epigenetic drug discovery
- Custom & bulk orders available
- Lot specific data, including:
  - Activity by HTRF
  - Purity by SDS-PAGE

Due to the preliminary success of these therapeutics in the treatment of cancer, there is much interest in manipulating specific domain interactions as a novel approach for identifying bromodomain targeted drug therapies. However, there is still much unknown regarding BET proteins and the individual BDs.

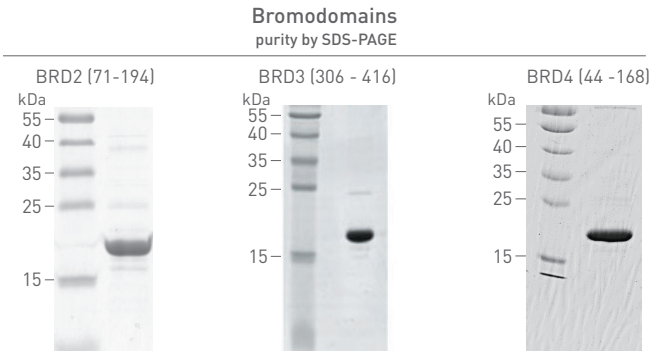
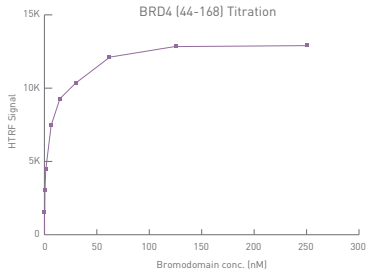


Figure 1: Purified recombinant proteins were run on an SDS-PAGE gel and stained with Coomassie blue. Coomassie stains reveal that the recovered proteins are intact with little or no detectable degradation. All proteins were determined to have ≥ 98% purity.



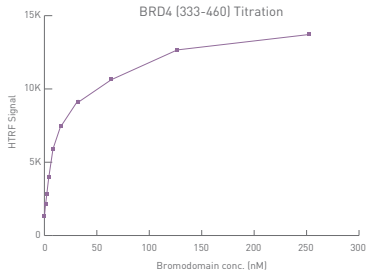


Figure 2: **BRD4 (44-168) (top panel), and BRD4 (333-460) bromodomains (bottom panel) tested by HTRF.** Assay conditions for bromodomain (BRD) activity were as follows: 3.3 uM histone peptide H4K5/8/12/16(tetra-acetyl) was incubated with the protein indicated in reaction buffer containing 50mM HEPES-NaOH pH 7.0, 0.1% BSA for 1 hour at room temperature. Anti-FLAG antibody was used to detect the reaction products.

Research and inhibitor screening has markedly increased in recent years suggesting a need for validated products to support these efforts.

Active Motif has responded to this need by providing the most comprehensive and validated collection of reader domain proteins for use in the development of robust assays for bromodomain drug discovery.

The most comprehensive reader domain collection available for drug discovery

As a global leader in providing new and innovative products and services to enable epigenetics research, Active Motif offers a comprehensive portfolio of recombinant epigenetic enzymes and reader domains for drug discovery research (Figure 3). Our recombinant proteins are manufactured to meet the highest standards of purity (Figure 1) and activity required for conventional drug discovery assays, such as HTRF (Figure 2), mass spectrometry, AlphaLISA and Alphascreen. These assays are routinely incorporated as part of our quality control process to ensure that our recombinant proteins and enzymes demonstrate the nM range activities needed for use in identification of potent inhibitors.

At Active Motif, we have optimized our protein production and testing to facilitate seamless integration of our recombinant proteins into drug discovery pipelines. We strive to instill confidence in our proteins by providing comprehensive application data that is relevant to drug discovery platforms and unparalleled customer support for our drug discovery partners.

For more information and an up-to-date list of available proteins, please visit www.activemotif.com/proteins.

Phylogenetic Tree Diagram of the Human Bromodomain Family

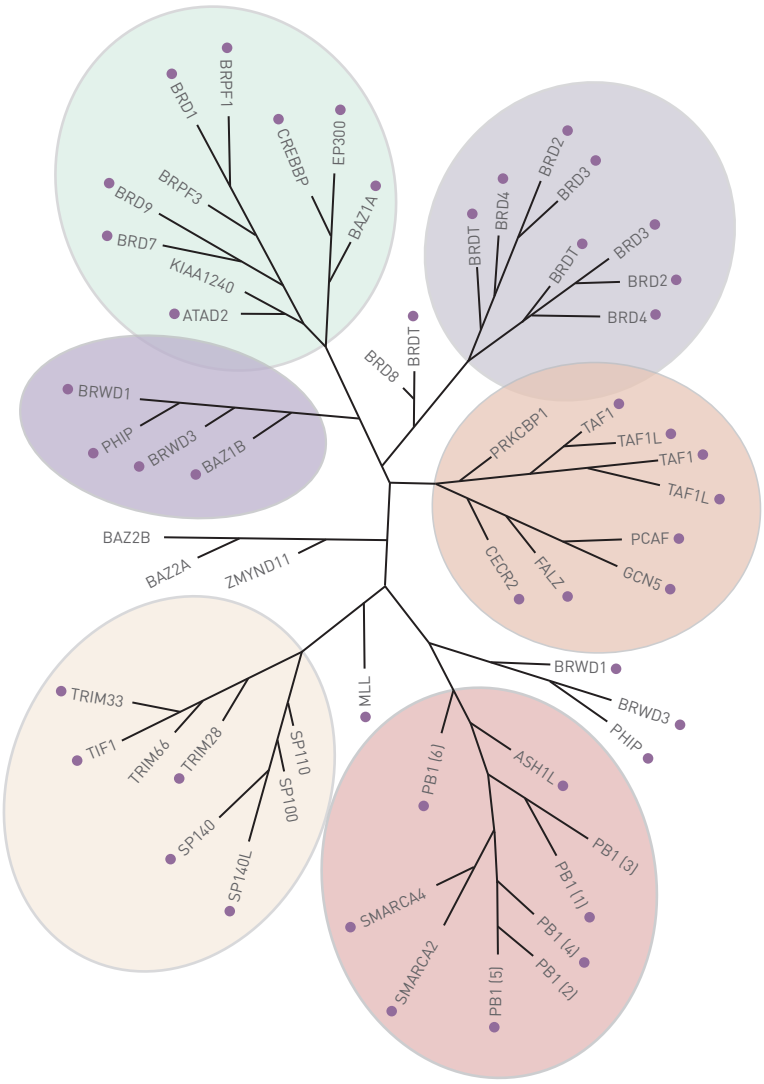


Figure 3: **Active Motif provides a comprehensive collection of high quality reader domain proteins for use in assay development in early drug discovery.** Bromodomain proteins, designated with purple dots, represent currently available bromodomains in our collection. In addition, as part of our integrative solutions for epigenetic drug discovery, Active Motif also offers custom and bulk orders, as well as antibodies and inhibitors to these proteins.

To inquire about Custom and Bulk Orders CALL: 877-222-9543

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