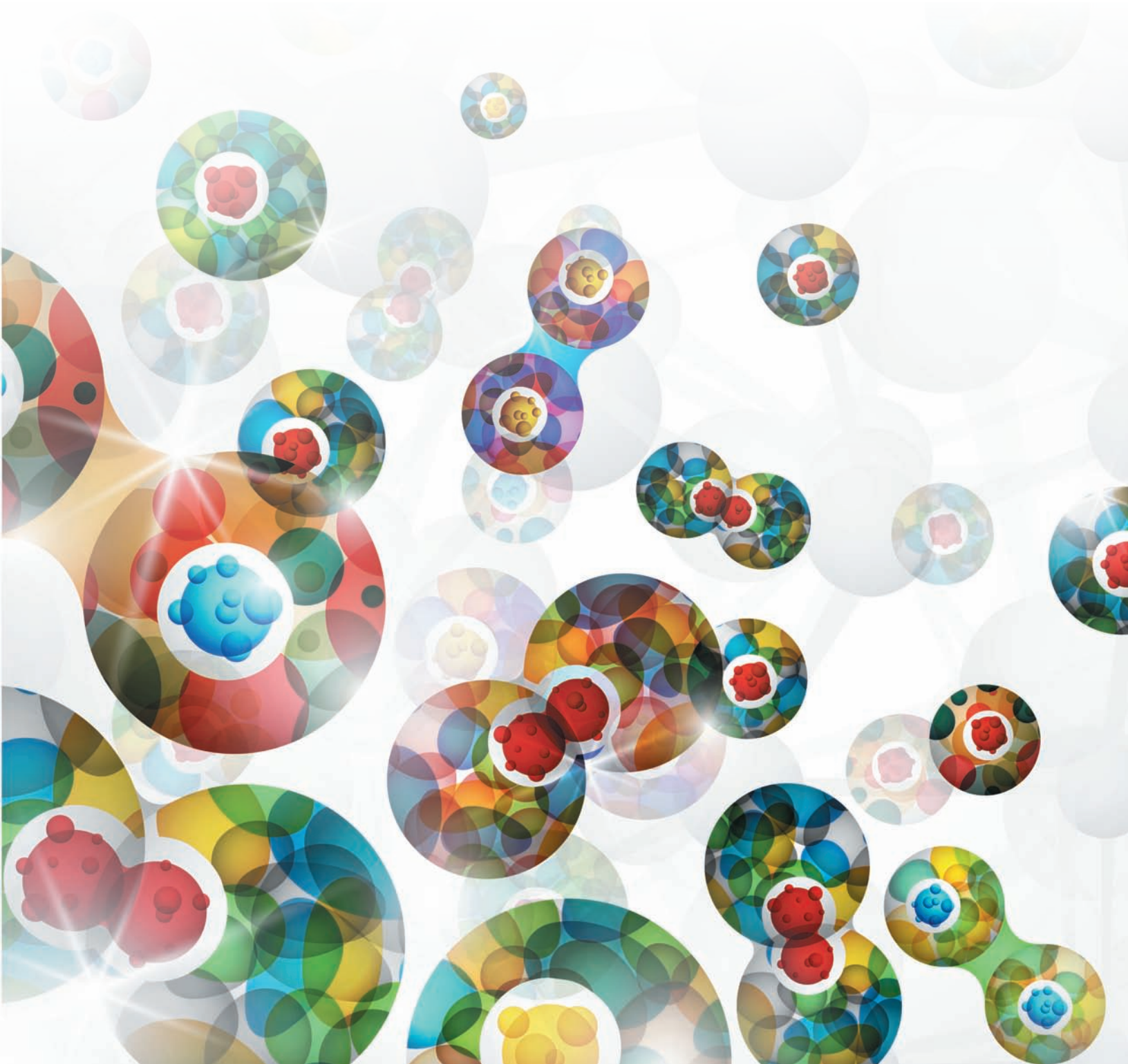
ACTIVE MOTIF®

TOOLS FOR DISEASE RESEARCH

TARGETING EPIGENETICS & GENE REGULATION

- Oncology
- Inflammation
- Immunology
- Neuroscience



DISEASE RESEARCH TOOLBOX

antibodies, assays & services to accelerate your research

As more and more diseases are linked to epigenetic abnormalities, there has been an exponential growth in research focused on the association between epigenetics and disease. As such, laboratories new to the areas of epigenetics and gene regulation research are experiencing a growing need for tools to facilitate the initiation of research into these areas.

Active Motif is the industry leader in developing and delivering innovative tools to enable epigenetics and gene regulation research. We provide the highest quality products and services to support advancements in disease research. Our staff of highly qualified scientists work in close collaboration with world-class researchers and opinion leaders to continually develop new and innovative tools to study chromatin, histones, DNA methylation, non-coding RNAs and transcription biology. Whether you are an expert in the field of epigenetics or a researcher interested in integrating epigenetics research into your studies, Active Motif makes it easy to find the right tools by providing the most comprehensive product portfolio to enable all aspects of the epigenetics workflow.

Antibodies & Chromatin

- Antibodies for Epigenetic & Disease Targets
- Chromatin Immunoprecipitation Overview
- ChIP for FFPE
- ChIP for Difficult-to-lyse Cells (ChIP PBMC)

Histones & DNA Methylation

- Multiplex Epigenetic Assays
- Global DNA Methylation Assays
- Histone Modification ELISAs
- DNA Methylation Analysis
- Tools for Neuroepigenetic Research
- Genome-wide Services

Transcription & miRNAs

- Tools for Inflammation Research
- Transcription Factor Activity Assays
- Multiplex AP-1 and NFκB Activity Assays
- Reporter Assays to Study Gene Regulation
- Cell-based Pathway Screening Assays
- Reporter Assays to Study miRNA

For a complete list of available products, please visit us at www.activemotif.com.

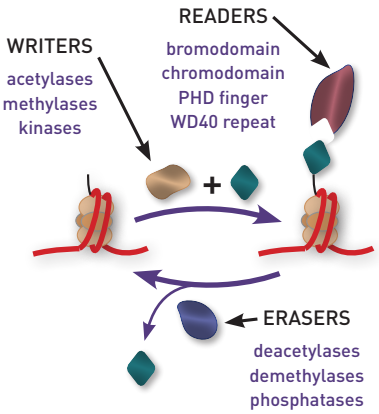
Epigenetics & Disease

The epigenetic state of the cell is controlled by the activity of proteins that add and remove small chemical modifications to histones and to DNA. Aberrant epigenetic regulation can lead to changes in gene expression and human disease (Tables 1 & 2). The link between epigenetics and disease has been substantiated through the identification of mutations in, or altered expression of, epigenetic regulator proteins that are associated with a multitude of pathological states and poor disease prognosis.

	Histone modification	DNA methylation	Noncoding RNA
Learning and memory	√	√	√
Addiction	√	√	√
Autism spectrum	?	?	?
Epilepsy	√	√	√
PTSD	√	√	
Alzheimer's disease	√	√	
Stress	√	√	√
Schizophrenia	√	√	
Bipolar disorder	√	√	√
Adult neurogenesis	√	√	

Table 1: Examples of neural processes and disorders associated with epigenetic modifications.

These mutations have been identified in all three major classes of epigenetic proteins: the *Writers* (enzymes that deposit modifications), the *Erasers* (enzymes that remove modifications) and the *Readers* (proteins that recognize and bind epigenetic modifications). Recent drug development strategies that target these enzymes have proved highly successful, resulting in four FDA approved cancer drugs, with many more promising leads on the horizon.



	Histone H3					Histone H4				
Tumor Type	Multi-Kac	K4me1-3	K9ac	K9me1-3	K18ac	Multi-Kac	R3me2	K12ac	K16ac	K20me3
Breast		√	√		√		√	√	√	√
Colorectal									√	√
Esophageal					√		√			
Hematological									√	√
Kidney	√	√		√	√	√				
Lung		√			√					
Pancreatic		√		√	√					
Prostate		√	√*	√	√	√				√

Table 2: Histone modifications exhibiting altered levels in neoplastic tissue.

The epigenetic tool kit

The development of methods to perform chromatin immunoprecipitation (ChIP) and analyses of DNA methylation, histones and gene regulation, along with Next-Generation sequencing tools, is allowing researchers to get a better picture of the genome-wide changes associated with cancer and other diseases. Active Motif provides products that utilize familiar techniques and reagents, or simplify technologies, to enable even the novice to perform epigenetic studies. These include:

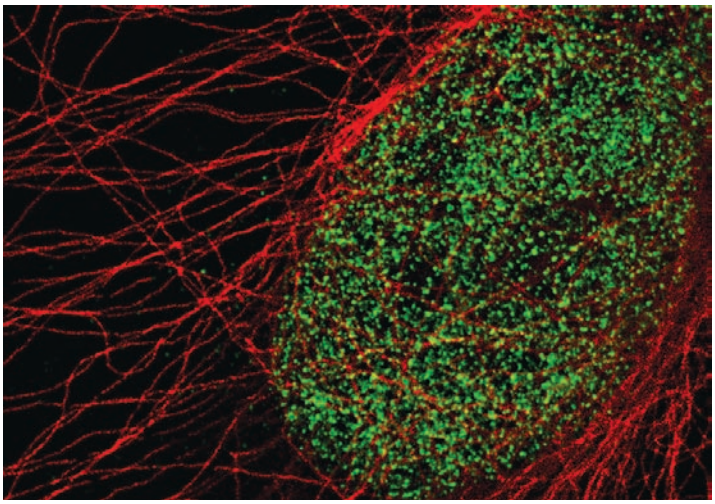
- *Antibodies* to discriminate between the multiple post-translational modifications on the histone tails and to detect methylated DNA
- *ChIP and ChIP-Seq* techniques to enable scientists to link specific states of chromatin to individual gene loci in a cell to understand how genes are regulated
- *ELISA-based methods* for analysis of global changes in levels of intercellular markers, such as histone modifications, DNA methylation and transcription factor binding
- *Luciferase reporter assays* to study the impact of promoter regulation and miRNA-3´UTR interactions on the transcriptional and post-transcriptional regulation of genes.

Antibodies

broad portfolio of disease-related targets

Active Motif develops and provides highly validated antibodies specific for epigenetic targets and proteins involved in gene regulation. Our antibodies have been referenced in hundreds of publications in top scientific journals. However, we are more than just an antibody supplier. We identify, produce and rigorously test each antibody in our collection to ensure that we offer only the highest quality product. We perform thorough specificity testing, including screening antibody specificity to histones and their post-translational modifications with our MODified™ Histone Peptide Array. We also collaborate with top scientists that help us identify the most relevant targets and validate our antibodies for the most desired applications.

We monitor every step from immunogen design and specificity screening to application validation. This thorough process ensures our antibodies meet the high standards for consistency and quality that you require to help you achieve the goals of your disease research.



OVER 700 DNA METHYLATION, HISTONE AND TRANSCRIPTION FACTOR TARGETS

EPIGENETICS

- H2A, H2B, H3 & H4 PTMs
- DNA methylation
- Bromodomains
- Methyltransferases
- Demethylases
- Acetyltransferases
- Deacetylases

TRANSCRIPTION FACTORS

- Hormone receptors
- NFκB family
- STAT family
- IRF family
- AP-1 family
- p53
- c-Myc

- Rigorous application testing
- Assay-compatible formulations
- Large lot sizes
- Monoclonals to histone PTMs
- Large selection of ChIP & ChIP-Seq validated antibodies

ANTIBODIES FOR RELEVANT DISEASE AREAS

CANCER

- BRD4
- CARM1
- ER
- EZH2
- DOT1L
- p300

INFLAMMATION

- AP-1
- ATF-2
- NFκB
- MAPK
- NFATc1
- STAT

NEURODEGENERATIVE

- 5-hmC
- APOBEC2
- MeCP2
- N-Myc
- NRF2
- TET

IMMUNOLOGY

- HDAC1
- ELK
- IFNA1
- IRF
- Nrf2
- TNF

For more information and a complete list of available antibodies, please visit us at www.activemotif.com/abs.

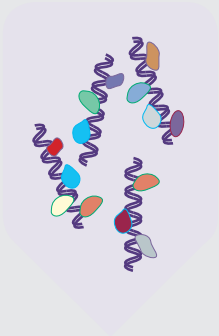
Map Disease-Specific Epigenetic Variances

simplified chromatin analysis tailored to the disease researcher

Chromatin immunoprecipitation, or ChIP, is widely used to study changes in chromatin modifications and interactions. Common array and whole-genome sequencing (WGS) methods to analyze epigenetic dysregulation in human disease have a number of shortcomings. Microarrays lack the qualitative information offered by sequencing-based approaches. However, more comprehensive and qualitative methods like WGS are often cost-prohibitive for disease researchers who are typically working with large numbers of samples. ChIP-Seq is a highly powerful tool in epigenetics research because it offers a simple, genome-wide method to enable mapping of chromatin states to specific regulatory loci.

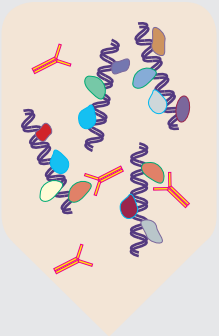
Previously, ChIP analysis has been challenging for disease researchers who are unfamiliar with these difficult-to-perform techniques or who principally work with human primary tissues that present a challenge for sample preparation. At Active Motif, we are continually expanding our portfolio of ChIP products and services to provide innovative solutions to address these challenges and meet the needs of disease research.

Chromatin Preparation



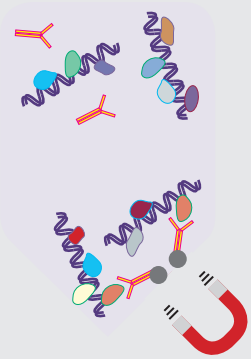
A wide selection of tools for sample preparation including homogenizers, sonicators, enzymatic shearing kits and pre-made chromatin.

ChIP Antibodies



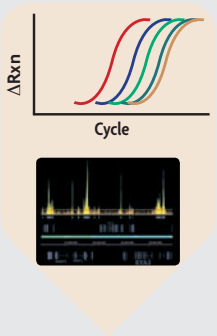
A large offering of ChIP and ChIP-Seq validated antibodies for histone modifications, chromatin remodeling proteins and transcription factors (see opposite page).

Chromatin IP



ChIP kits and accessories for a variety of cell and tissue types and downstream applications (see pages 6-7).

Downstream Analysis



A comprehensive selection of qPCR and ChIP analysis tools and a wide variety of Custom Epigenetic Services for genome-wide ChIP and DNA methylation analysis (see pages 12-13).

For more information on ChIP and ChIP-related products, please visit us at www.activemotif.com/chip.

Uncover Epigenetic Variances in Archived Tissue

perform ChIP on FFPE primary tissue samples with ChIP-IT® FFPE

Large collections of archived formalin-fixed, paraffin-embedded (FFPE) tissue are available that harbor invaluable information about the variances between normal and diseased tissue states. However, performing ChIP has not been possible on FFPE samples because of the difficulty in extracting high-quality chromatin from very limited and degraded material. Active Motif's ChIP-IT FFPE assay is the first-of-its-kind to enable extraction and ChIP analysis of chromatin from FFPE samples for Next-Generation sequencing.

Active Motif's ChIP-IT® FFPE products

The ChIP-IT FFPE Chromatin Preparation Kit is optimized to extract high-quality ChIP-grade chromatin from preserved human, mouse or rat FFPE material. The kit has been successfully used to extract chromatin from FFPE blocks that were stored over 10 years in less than ideal conditions (Table 1).

For ChIP and ChIP-Seq analysis, the ChIP-IT FFPE Kit, designed for use in conjunction with our ChIP-IT FFPE Chromatin Preparation Kit, is the only ChIP kit available that can enrich for ChIP DNA using FFPE-extracted chromatin. The assay produces ChIP-enriched DNA in sufficient amounts and quality for analysis by qPCR (Figure 2) or Next-Generation sequencing (Figure 3).

FFPE sample	Sample used per chromatin prep
Human colon	Tissue block – five 20 µm sections
Human kidney	Tissue block – twenty-five 20 µm sections
Human lung	Tissue block – two 20 µm sections
Rat whole brain	5 slides – two 5 µm sections per slide
Rat hippocampus	25 slides – two 5 µm sections per slide

Table 1: Examples of the sample material we have successfully validated with the ChIP-IT FFPE Chromatin Preparation method.

ChIP analysis of FFPE tissue enables insight into the cancer epigenome

The ability to perform ChIP on FFPE samples enables validation in primary tissue of results obtained using cell lines as model systems. In a recent study, the laboratory of Manuel Perucho* detected DNA hypomethylation of the CpG island associated with the promoter of a gene (Gene X) that is upregulated in colon cancer. Figures 1-3 below show correlative bisulfite sequencing and H3K4me3 and H3K27me3 ChIP and ChIP-Seq data obtained with the use of the ChIP-IT FFPE Chromatin Preparation and ChIP kits from normal and tumor samples from two colon carcinoma patients. These results indicate that upregulation of Gene X is associated with DNA hypomethylation as well as increased H3K4me3 and decreased H3K27me3 levels at the promoter region in tumors versus normal tissue.

*Alonso *et al.*, in preparation.

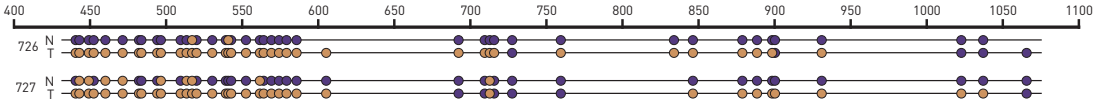


Figure 1: Bisulfite sequencing of the promoter-associated CpG islands in Gene X from normal (N) and tumor (T) samples in colon cancer cases 726 and 727. Purple and copper circles indicate methylated and unmethylated cytosines, respectively.

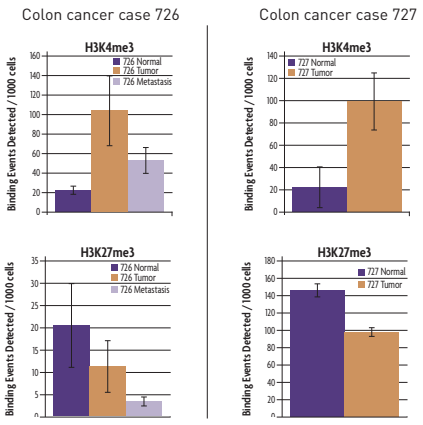


Figure 2: qPCR analysis of ChIP-IT FFPE ChIP DNA shows H3K4me3 and H3K27me3 enrichment at the Gene X locus in human colon FFPE samples.

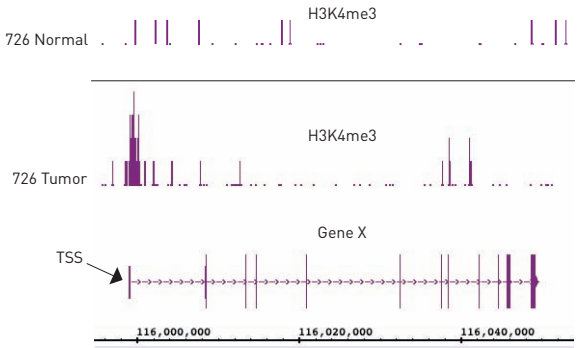


Figure 3: ChIP-Seq data profiles generated using ChIP-IT FFPE ChIP'd DNA from human colon cancer case 726 show H3K4me3 enrichment at Gene X.

For more complete information on ChIP-IT FFPE products, please visit us at www.activemotif.com/ffpe-chip.

Evaluate Chromatin Changes in Primary Cells

ChIP-IT® PBMC for difficult-to-lyse T and B cells

Active Motif's ChIP-IT PBMC Kit is the only ChIP kit available to enable ChIP to be performed on difficult-to-lyse peripheral blood mononuclear cells, or PBMCs, including lymphocytes (T cells, B cells & NK cells) and monocytes.

Because of the importance of PBMCs in cancer and immunology / infectious disease research, a robust method to extract quality chromatin from these cells for ChIP and ChIP-Seq analysis is highly warranted. PBMCs make it difficult to extract quality chromatin because they are highly resistant to lysis under conditions normally suitable for other cell types. Active Motif scientists have developed an optimized chromatin preparation method that yields high-quality chromatin from primary cells for ChIP analysis and Next-Generation sequencing.

ChIP-IT® FFPE & PBMC ADVANTAGES

- Obtain ChIP quality chromatin from primary cells & tissue
- Sensitive enrichment of DNA from nanogram quantities of chromatin
- Optimized reagents improve signal and minimize background
- Filtration based washes offer a faster, easier solution with better consistency

Identify epigenomic features regulating gene expression during an immune response

Lymphocytes undergo highly dynamic changes in gene expression programs during development and as a result of clonal selection and expansion. ChIP-Seq analysis enables researchers to study the molecular basis of gene regulation during these processes by revealing the protein interactions and epigenetic modifications at regulatory loci within the genome that are important for modulating gene expression.

Figure 1 demonstrates how ChIP-Seq data obtained using the ChIP-IT PBMC Kit reveals a correlation between a loss in H3K27 acetylation in the enhancer region upstream of the Cfc1 gene in mouse T cells in a mouse model harboring a deletion in a lymphoma-related gene. These results are consistent with the characteristic repression of the T cell expression program observed in many T cell lymphomas.

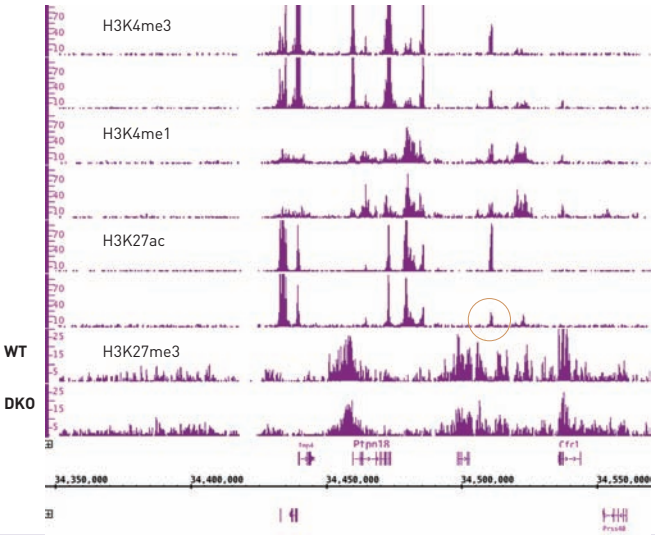


Figure 1: ChIP performed using the ChIP-IT PBMC Kit with antibodies against H3K4me3, H3K4me1, H3K27ac and H3K27me3 histone modifications and chromatin from primary mouse T cells. Epigenetic profiling was performed in a mouse model harboring a knockout (DKO) of a lymphoma related gene. Within the region of the genome shown, there is a clear loss of the enhancer associated H3K27ac modification at a location upstream of the Cfc1 gene (copper circle).

PRODUCTS COMPATIBLE WITH ChIP-IT® FFPE & PBMC KITS

- ChIP-IT® qPCR Analysis Kit – simplifies qPCR data analysis and enables normalization for comparison of samples across experiments
- qPCR Primer Sets – for a multitude of gene targets and model organisms
- ChIP-Seq Antibodies – internal validation program for guaranteed success (see page 4)

Product	Catalog No.
ChIP-IT® FFPE Chromatin Preparation Kit	53030
ChIP-IT® FFPE	53045
ChIP-IT® PBMC	53042
ChIP-IT® qPCR Analysis Kit	53029

For more complete information on ChIP-IT FFPE products, please visit us at www.activemotif.com/ffpe-chip.

Multiplex Epigenetic Screening Assays

assay specific and off-target effects in a single well

Active Motif has developed the Histone H3 PTM Multiplex Assay, the first ever multiplex epigenetic assay that enables interrogation of multiple post-translational modifications (PTMs) in a single reaction (Figure 1). Designed for use with Luminex instruments, this high-throughput / high-content assay uses only nanogram quantities of sample per well and can be completed in 3 hours. Get more data from less input, in less time and at a lower cost.

Profile changes in histone modifications in clinical and compound-treated samples in multiplex

Profiling of variances in clinical or compound-treated samples often involves large sample numbers in scarce supply. Traditional methods such as Westerns and ELISAs are limited by low throughput, large input requirements and the inability to multiplex the analysis of multiple targets. In contrast, the Histone H3 PTM Multiplex Assay provides a rapid, highly sensitive plate-based method that allows profiling of changes in histone modification levels in multiplex. The assay's ability to multiplex analysis of specific and off-target effects, and its minimal cell number requirement of 500 - 2000 cells per microplate well, makes it ideally suited for screening and profiling of clinical or compound-treated samples (Figure 2).

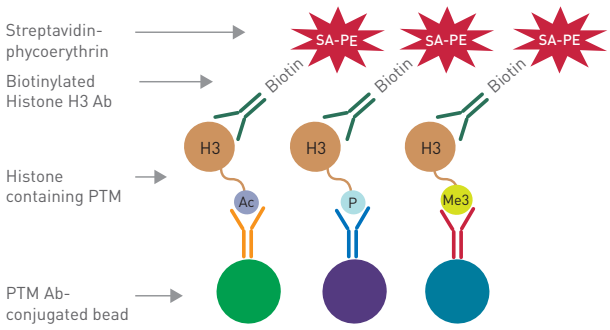


Figure 1: Schematic of the Histone H3 PTM Multiplex Assay.
The assay works as a solution-based sandwich ELISA to evaluate histone H3 PTM levels.

ASSAY FEATURES

- Multiplex – the only kit available to perform multiplexed histone modification analysis
- Efficient – use less input & less time than WB to assay multiple histone PTMs
- Sensitive – requires only nanogram amounts of crude acid extracts or purified histones
- High content – simultaneously assay specific and off-target effects in a single reaction

Multiplex Analysis of Specific and Off-target Effects

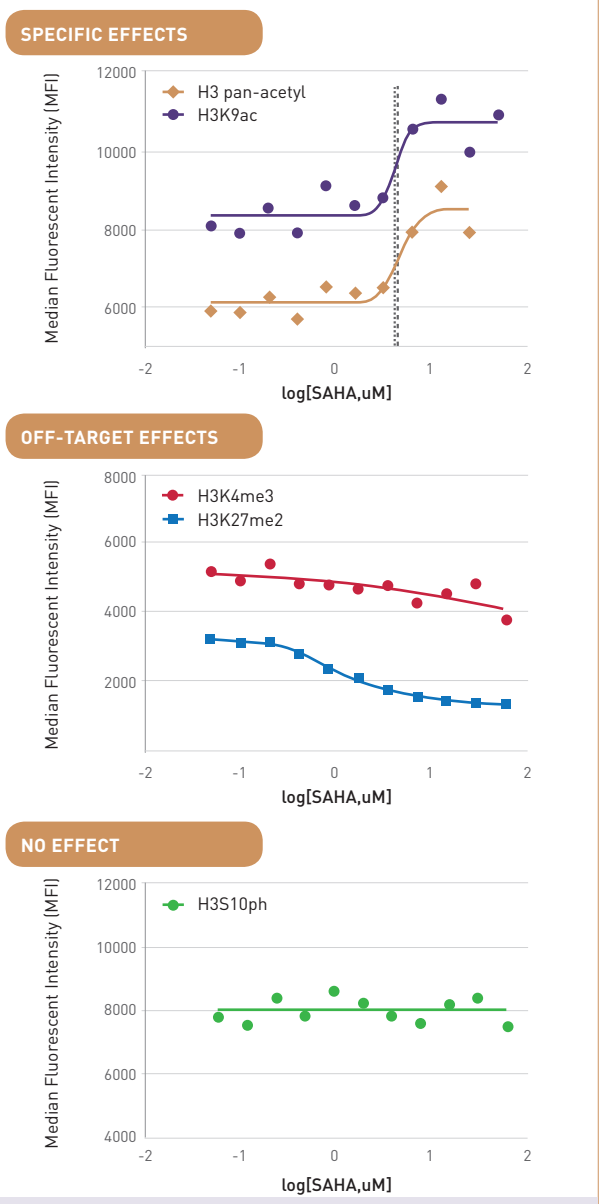


Figure 2: The Histone H3 PTM Multiplex Assay shows increased histone acetylation in response to SAHA-mediated HDAC inhibition.
HeLa cells treated with increasing concentrations of the HDAC inhibitor SAHA were evaluated in a multiplex of H3 pan-acetyl, H3S10ph, H3K9ac, H3K4me3, H3K27me2 & H3 Total Ab-conjugated beads using the Histone H3 PTM Multiplex Assay. The results demonstrate the ability of the assay to simultaneously assess specific and off-target effects of the treatment on histone modification levels. The dashed lines represent IC_{50} values, 4.0 µM and 4.6 µM, determined for pan-acetyl and H3K9ac, respectively.

To learn more about our Luminex assays, visit us at www.activemotif.com/luminex.

Measure Global Changes in the Epigenome

generate DNA methylation & histone profiles of your cell models

Active Motif offers a variety of high-throughput, quantitative, simple ELISA-based assays to enable you to accurately and efficiently measure global changes in epigenetic DNA and histone modifications in response to compounds, stimulus, or disease-related alterations in cell signaling and regulation.

Quantify changes in global DNA methylation levels

Because changes in global methylation are a hallmark of many human diseases, including cancer, simpler methods than HPLC or bisulfite sequencing are warranted for correlative studies that analyze variances in genome-wide DNA methylation status.

Active Motif's Global DNA Methylation – LINE-1 Assay uses a unique hybridization approach that quantitates 5-methylcytosine (5-mC) levels at LINE-1 repeats as a surrogate measure of global methylation. This unique hybridization approach offers better specificity and reproducibility than other available methods that utilize non-specific passive adsorption (Figure 1).

Screen the effects of compounds and other variables on histone modification levels

The addition or removal of modifications such as phospho-, methyl- and acetyl- functional groups to histones have a profound effect on the regulation of transcription, chromosome packaging and DNA damage repair. Screening extracts for specific histone modifications is a simple way to assess cell health and compound effects.

Active Motif's comprehensive selection of Histone Modification ELISAs provides a simple solution for screening changes in histone H3 modification levels from purified core histones (see Product Ordering Information for a list of available Histone Purification Kits) or histones isolated by acid extraction.

HISTONE MODIFICATION ELISAs FOR:

- Total Histone
- H3K4me1
- H3K4me2
- H3K4me3
- H3K9me2
- H3K9me3
- H3K27me1
- H3K27me3
- H3K9ac
- H3K14ac
- H3S10ph
- H3S28ph

Global DNA Methylation – LINE-1

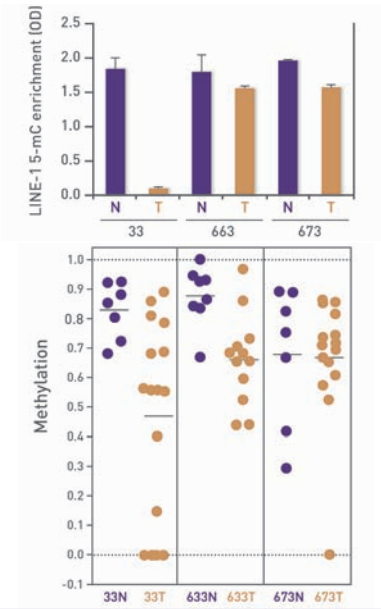


Figure 1: Comparative data obtained using the Global DNA Methylation – LINE 1 Assay (above) and Bisulfite Sequencing (below, each clone represents one single LINE-1 transposable element ORFI and II, accession number X52235) to analyze methylation of LINE-1 repetitive elements of normal (N, purple) and tumor (T, copper) colon samples. Data was provided courtesy of Dr. Johanna Samuelsson and Dr. Manuel Perucho. Samuelsson *et al.*, in preparation.

Product	Catalog No.
Global DNA Methylation - LINE-1 Kit	55017
Total Histone H3 ELISA	53110
Histone H3 monomethyl Lys4 ELISA	53101
Histone H3 dimethyl Lys4 ELISA	53112
Histone H3 trimethyl Lys4 ELISA	53113
Histone H3 acetyl Lys9 ELISA	53114
Histone H3 dimethyl Lys9 ELISA	53108
Histone H3 trimethyl Lys9 ELISA	53109
Histone H3 phospho Ser10 ELISA	53111
Histone H3 acetyl Lys14 ELISA	53115
Histone H3 monomethyl Lys27 ELISA	53104
Histone H3 trimethyl Lys27 ELISA	53106
Histone H3 phospho Ser28 ELISA	53100
Histone Purification Kit	40025
Histone Purification Mini Kit	40026
Histone Purification Microplate Kit	40027

For more complete information on the Global DNA Methylation - LINE-1 Assay, visit www.activemotif.com/gdm.
To learn more about our histone analysis products, visit www.activemotif.com/hismodinfo.

Profile Methylation States Associated with Disease

tools for DNA methylation analysis

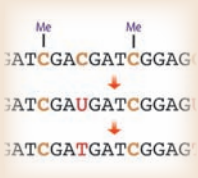
DNA methylation, generated by the transfer of a methyl group to the 5'-position of cytosine to form 5-methylcytosine, or 5-mC, is an important epigenetic regulator of gene expression and genomic organization. Aberrant DNA methylation is responsible for local silencing of genes through promoter hypermethylation, a process implicated in tumorigenesis and other abnormalities, and the ubiquitous global hypomethylation commonly observed in human cancers. There are also several methylation variants thought to have their own unique epigenetic functions. One of these, 5-hmC, is highly expressed in the brain during development and is thought to have a unique biological role in the Central Nervous System. However, the role of this and other methyl variants is still not fully understood.

The development of methods such as DNA methylation enrichment techniques and bisulfite conversion, along with Next-Generation sequencing tools, is allowing researchers to get a better picture of the genome-wide and gene-targeted changes that occur in DNA methylation and its association with disease. Active Motif offers a number of tools and services to simplify and streamline DNA methylation analysis.

Antibodies & Enzymes

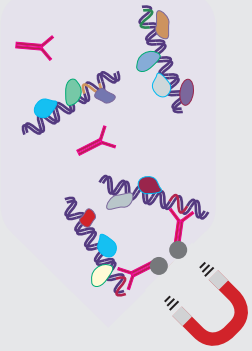
A comprehensive portfolio of antibodies (see page 4) and proteins for 5-mC and DNA Methylation variant (5-hmC, 5-fC, 5-caC & 3-mC) analysis.

Bisulfite Conversion



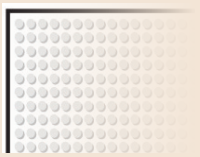
Bisulfite Conversion Kits and Sequencing Services to enable analysis of methylation patterns to differentiate between methylated and unmethylated sequences (see opposite page).

Methylated DNA Enrichment



A broad selection of kits for enrichment of methylated DNA variants (5-methyl-cytosine, or 5-mC and 5-hydroxy-methylcytosine, or 5-hmC) (see opposite page).

Quantitative Assays



Simple, high-throughput ELISA-based assays for measuring changes in global DNA methylation levels (see page 9) or DNMT activity.

For more information on DNA methylation-related products, please visit us at www.activemotif.com/dna-methylation.

Tools for Neuroepigenetic Research

products to study DNA methylcytosine variants

5-Hydroxymethylcytosine (5-hmC) is highly abundant in the CNS where it regulates various processes, including development, aging and neuroplasticity (Figure 1). 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 suggesting 5-hmC is involved in cognitive function and psychiatric disorders such as stress-induced PTSD. Also, aberrant 5-hmC distribution is observed in various neurodegenerative disorders, including Alzheimer's and Huntington's disease.

Profile changes in 5-hmC status at specific loci

Active Motif offers two methods for hydroxymethylated DNA enrichment for downstream 5-hmC analysis by PCR or sequencing. Both utilize magnetic beads for faster processing.

- hMeDIP Kit – an antibody-based technique that utilizes a 5-hmC antibody to immunoprecipitate DNA fragments containing 5-hmC from genomic DNA. The assay works with both single-stranded and double-stranded DNA (Figures 1 and 2).
- Hydroxymethyl Collector™ Kits – utilize a β-Glucosyltransferase enzyme to label 5-hmC residues within double-stranded DNA fragments with a modified glucose moiety to enable distinction from other cytosine modifications during enrichment. This sensitive technique can enrich DNA fragments containing as little as two 5-hmC residues.
- Methylation variant profiling services – Active Motif's Custom Epigenetic Services include genome-wide hydroxymethylcytosine (hmC), formylcytosine (fC) and carboxylcytosine (caC) profiling. For more complete information, go to page 13 or visit www.activemotif.com/meth-variant-svc.

Differentiate between methylated & unmethylated sequences

Active Motif has a selection of kits for bisulfite conversion analysis and determination of DNA methylation status:

- Bisulfite Conversion Kit – provides single nucleotide resolution information of the methylation status of your enriched DNA. See page 13 for related custom services.
- ChIP-Bis-Seq Kit – takes it one step further by combining bisulfite conversion with ChIP to enable you to obtain DNA methylation profiles focused on your specific genomic region(s) of interest.

Gene Ontology of Top 2500 5-hMeDIP Genes

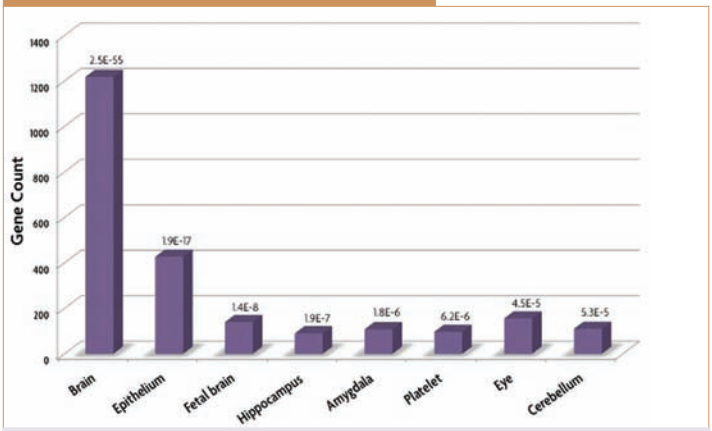


Figure 1: Gene Ontology analysis of the 2,500 genes selected with the highest hMeDIP signals.
The analysis shows that the top selected genes were biased toward brain expression. In addition, the other most statistically significant observed ontologies were also subsets of the brain. These results show that 5-hmc enrichment correlates well with expected gene expression.

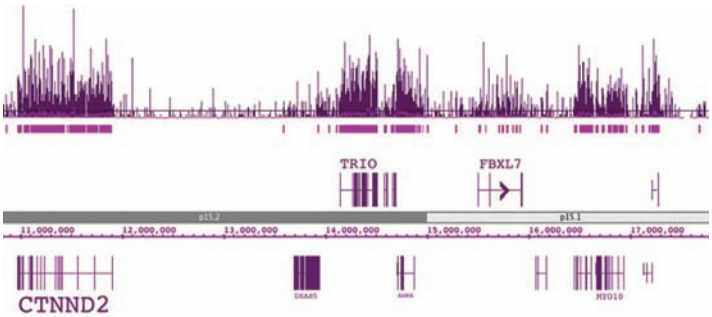


Figure 2: hMeDIP-Seq performed on human brain.
hMeDIP was performed with 5-Hydroxymethylcytosine (5-hmC) antibody (Catalog No. 39999) and human brain DNA to identify regions enriched for 5-hmC. The image above shows an 8 Mb region on chromosome 5 with 5-hmC enrichment across several gene bodies.

Product	Catalog No.
5-Hydroxymethylcytosine (5-hmC) antibody	39769
5-Methylcytosine (5-mC) antibody	61225
hMeDIP Kit	55010
MeDIP Kit	55009
Hydroxymethyl Collector™	55013
Hydroxymethyl Collector™-Seq	55019
MethylCollector™ Ultra	55005
HypoMethylCollector™	55004
Bisulfite Conversion Kit	55016
ChIP-Bis-Seq Kit	53108

To learn more about products for 5-hmC analysis, please visit us at www.activemotif.com/hmc.

Custom ChIP and ChIP-Seq Services

let the ChIP experts do the work for you

Whole genome analysis using Next-Generation sequencing has significantly broadened the ability of researchers to understand how epigenetic events influence disease. However, the technical and bioinformatics challenges associated with generating whole-genome data may make ChIP-Seq beyond your reach.

Active Motif's Epigenetic Services team provides a wide variety of ChIP-Seq Services to make it possible for you to utilize our expertise and research tools without having to be an expert in the techniques yourself. As the only end-to-end ChIP-Seq provider, we have performed services using state-of-the-art techniques for over 10 years to help accelerate the research of scientists in academic laboratories, government institutions, biotechnology and pharmaceutical companies.

Map genome-wide chromatin interactions and modifications associated with disease

ChIP-Seq services enable you to perform whole genome analysis of chromatin interactions and/or modifications influencing disease propensity, outcome or responsiveness to therapies. Figure 1 shows ChIP-Seq data revealing details about SRC3 genomic interactions that potentially influence its ability to cause resistance to hormone therapy in breast cancer.

OBTAIN ChIP-SEQ EPIGENETIC PROFILES OF:

- Epigenetic inhibitor treated cells
- Primary human tumors
- Xenograft models

ACTIVE MOTIF EPIGENETIC SERVICES

- Experience – thousands of genome-wide data sets generated
- Quality – QC steps ensure high-quality data
- Support – all services include bioinformatics analysis

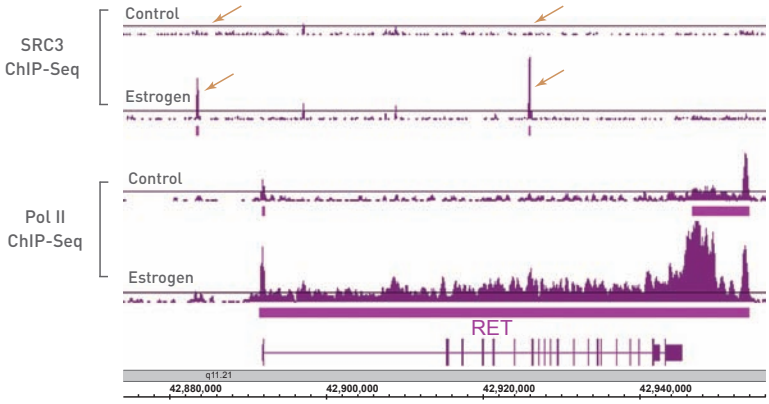
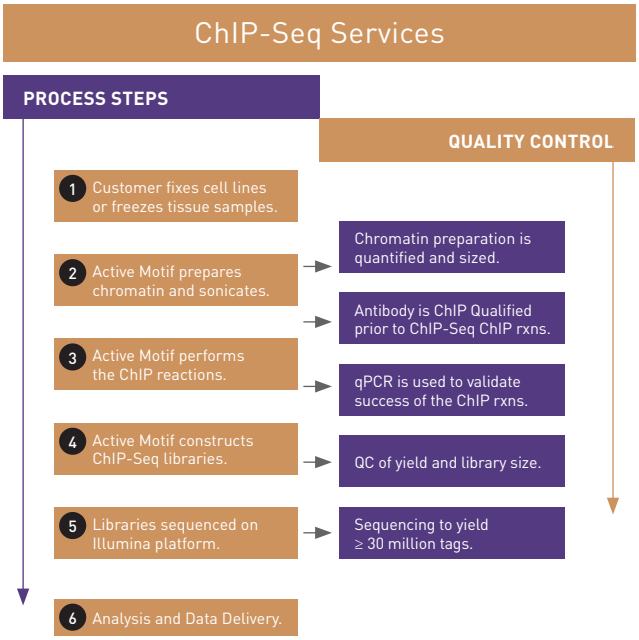


Figure 1: ChIP-Seq analysis of breast cancer cells reveals link between SRC3 activity and resistance to endocrine therapy. ChIP-Seq was performed using chromatin from control and estrogen-treated MCF-7 cells using antibodies against RNA pol II and SRC3, a protein that is upregulated in breast cancer and associated with resistance to endocrine therapy. The estrogen-induced SRC3 binding observed at the promoter and gene body of the RET gene (top panel) correlates with induced transcription of the RET gene as measured by RNA pol II occupancy (bottom panel). RET is overexpressed in some ER-positive breast cancers and is known to influence the ER-mediated response to endocrine therapy.



ACTIVE MOTIF ChIP-SEQ SERVICES

- FactorPath™ ChIP-Seq – discover, identify and quantitate transcription factor and cofactor binding sites
- HistonePath™ ChIP-Seq – map histone modifications or histone modifying enzymes across the genome
- TranscriptionPath™ ChIP-Seq – measure transcription rates globally as a function of RNA pol II occupancy

End-to-end DNA methylation Services

send us your samples, we send you the data

ACTIVE MOTIF DNA METHYLATION SERVICES

- **MeDIP-Seq** – enrichment of methylated DNA with a highly specific 5-methylcytosine antibody
- **MethylCollector™ Ultra-Seq** – based on the patented MIRA (Methylated CpG Island Recovery Assay) technology
- **hMeDIP-Seq** – enrichment using the most specific, highly cited 5-hydroxymethylcytosine antibody
- **fC & caC Genome-wide Profiling** – enrichment using a 5-formylcytosine or a 5-carboxylcytosine antibody, followed by Next-Generation sequencing
- **Bisulfite Sequencing** – detects the methylation status of select genomic regions at single-base-pair resolution

Profile DNA methylation variances to identify biomarkers and gain insight into disease and drug mechanisms

Active Motif offers a comprehensive selection of end-to-end DNA Methylation Services, including methylated DNA enrichment, methyl variant profiling and bisulfite sequencing, for genome-wide DNA methylation analysis.

DNA methylation occurs mainly at CpG sites. CpG islands are high CpG density regions commonly found within the promoters of mammalian genes. Most variance in DNA methylation observed between tissue types and in normal versus diseased tissue occurs a short distance from the CpG islands at "CpG shores" (Figure 1). Profiling variances in these regions at a genome-wide scale is crucial to understanding disease epidemiology and to identifying potential targets for therapeutic intervention. Our Epigenetic Services group makes it easy by doing the work for you from sample preparation all the way to bioinformatics analysis of data.

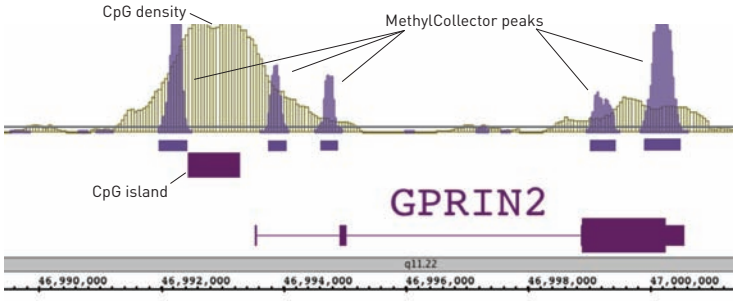


Figure 1: MethylCollector™ Ultra-Seq data shows DNA methylation at CpG shores in PBMC enriched methylated DNA. Next-Generation sequencing was performed on DNA enriched from human PBMC DNA using MethylCollector™ Ultra, and tags were mapped to generate a whole-genome DNA methylation profile. Data show DNA methylation is detected at CpG shores rather than in the CpG island itself.

Bisulfite Sequencing Services

Bisulfite Sequencing is the only method that enables detection of the methylation status of individual cytosines at single-base-pair resolution. Active Motif Epigenetic Services offers two Bisulfite Sequencing options:

- Targeted Next-Generation Bisulfite Sequencing
  - Multiplex many samples and multiple amplicons into a single Next-Generation sequencing reaction
  - 100X to 10,000X coverage of each amplicon
  - No cloning bias
  - Multiplexing makes large experiments cost-effective

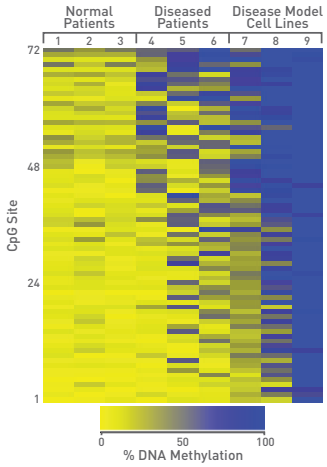


Figure 2: Targeted Next-Generation Bisulfite Sequencing was performed using 9 separate samples and 10 different primer pairs. All 90 amplicons were sequenced in a single Next-Generation sequencing run. This heat map shows methylation data from one of the ten primer pairs across the population of 9 samples.

- Sanger Bisulfite Sequencing

This traditional bisulfite approach requires cloning of bisulfite converted amplicons and sequencing of 8 to 16 clones.

- Service includes all steps from primer design to analysis
- Recommended for small-scale experiments of < 5 samples

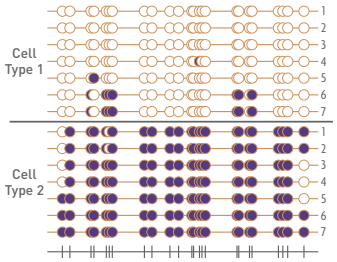


Figure 3: Bisulfite sequencing reveals differential methylation in two cell lines. Sanger bisulfite sequencing was performed on customer-selected genomic locations. This data compares the methylation state of a region with 24 CpGs in two different cell lines. Seven clones from each of the cell lines were sequenced.

To view the full range of services and for more information, please visit us at www.activemotif.com/services.

Tools for Inflammation Research

products to study immune function and inflammatory response

The inflammation / immune response is a highly orchestrated process that involves multiple cellular signaling pathways regulated by the NFκB, AP-1 and STAT transcription factor families, which in turn are regulated by numerous cytokine proteins including TNF-α and interferons. In a healthy human, it protects its host from foreign invasion, toxins, and tumor formation. However, unregulated inflammation and aberrant immune function can damage normal cells and tissues and can cause the onset of human disease, including autoimmune disorders and cancer.

Active Motif offers a large number of products and services that can help you study the regulation and function of inflammation proteins.

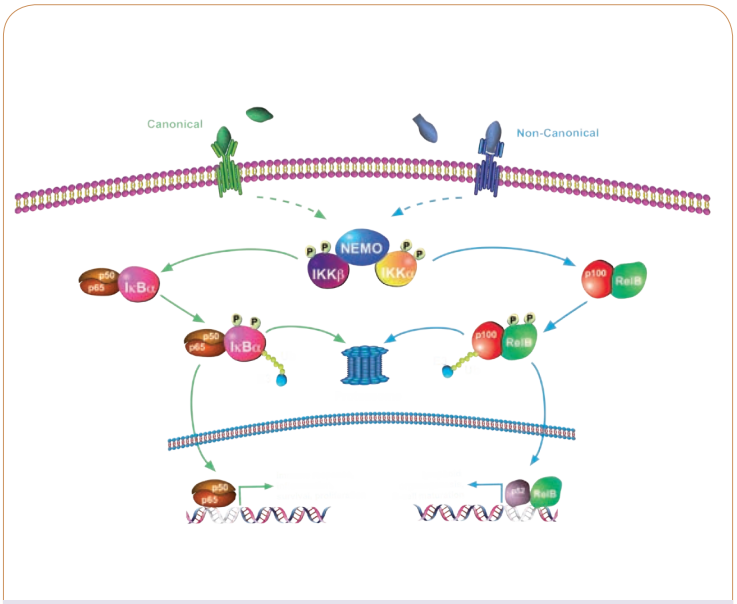


Figure 1: The canonical and non-canonical NFκB cell signaling pathways.

Measure activity of NFκB, STAT and other transcription factors regulating inflammation

Active Motif offers two methods for quantitative measurement of transcription factor-DNA binding activity of immunoresponsive transcription factors.

- TransAM® Transcription Factor Assays – a non-radioactive plate-based method that measures transcription factor-DNA binding activity in mammalian tissue and cell extracts. Assays are available for over 40 different targets including NFκB, STAT and MAPK (see opposite page).

- Transcription Factor Multiplex Assays – utilizes Luminex® xMAP® technology to enable multiplexing for comparative analysis of DNA binding between proteins of the same family. Kits are available for the study of AP-1 and NFκB transcription factor families (page 16).

Tools to study transcriptional and post-transcriptional regulation of immunoresponsive genes

Active Motif's LightSwitch™ Luciferase Assay System is a complete solution for performing gene regulation studies in living mammalian cells.

- LightSwitch™ Pathway Screening Assays – cell-based screening assays to measure the effects of compounds or treatments on the expression of genes associated with a number of disease-related biological pathways (see page 18).

- LightSwitch™ 3´UTR & miRNA Collection™ – pre-cloned luciferase 3´UTR reporter constructs and miRNA mimics & inhibitors for performing miRNA target validation studies of inflammation genes (Table 1 & page 19).

miRNA	Target(s)	Function
miR-125b	TNFα	Host immunity
miR146a	TRAF6, IRAK	Suppression of IL and TNFα expression
miR-142-3p	N-WASP, IRAK-1	Phagocytosis, TLR signaling
miR-128a	TGFβ	TGFβ signaling, cell survival
miR-99b	TNFα	Bacterial burden
miR-155	SHIP1	Enhanced TNFα activity
miR-466l	IL-10	Phagocytosis, TLR signaling
miR-124a	CDK2, MCP1	Proliferation, monocyte chemoattractant secretion

Table 1: miRNA protein targets and function in regulation of inflammation.

To learn more about our NFκB-related antibodies & assays, visit us at www.activemotif.com/nfkbtools.

Measure Transcription Factor Activity

quantitative ELISAs for transcription factor activation

TRANSAM® TRANSCRIPTION FACTOR ASSAYS

Active Motif's TransAM Transcription Factor Assays enable you to assess changes in transcription factor-DNA binding activity for over 40 different transcription factors important for inflammation, cancer, development, stem cell biology and other disease research areas.

Assays are available for:

- c-Myc
- NFκB
- IRF-3
- HIF-1
- STAT
- AP-1
- PPARγ
- MEF2
- ER
- ATF-2
- AP-1
- p53
- Nrf2
- MAPK
- Oct4
- and more...

TransAM ASSAY FLOW CHART

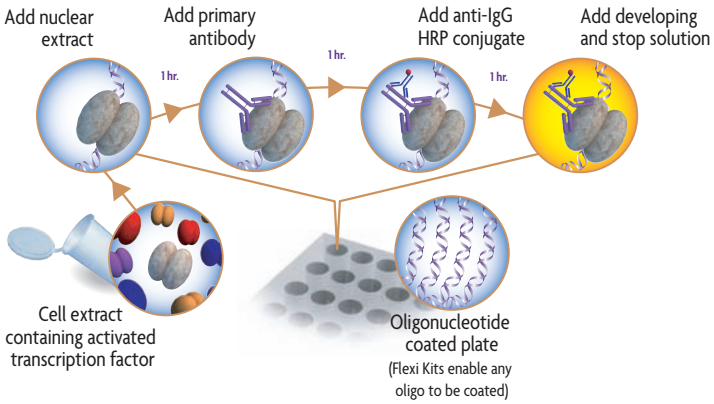


Figure 1: Flow chart of the TransAM process.
Activated transcription factor in the cell extract binds to the consensus-binding site on the oligo immobilized in the well. Incubation with the supplied primary and secondary antibodies specifically quantifies the amount of activated transcription factor.

TransAM Transcription Factor Assays are highly sensitive non-radioactive assays that facilitate the study of transcription factor-DNA binding activity in mammalian tissue and cell extracts. Small changes in transcription factor activity levels can have a significant impact on cellular function. Therefore, it is important to use a sensitive assay to measure these changes.

TransAM Kits combine a fast, user-friendly high-throughput assay for measuring transcription-factor DNA binding activity with supreme sensitivity and specificity (Figure 1). TransAM assays are up to 100-fold more sensitive than gelshift assays and also yield more quantitative results (Figure 2). Come see why we are the most cited kit on the market with over 900 citations, and counting.

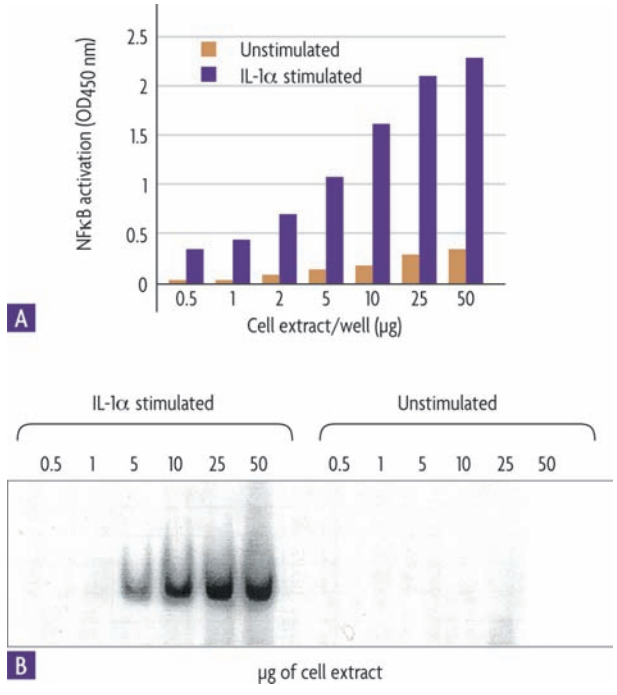


Figure 2: TransAM Kits are more sensitive than gelshift.
Human fibroblast WI-38 cells are stimulated with IL-1a for 30 minutes. Increasing amounts of cell extract are assayed using the TransAM NFκB p50 Kit (A) or gel retardation (B). The TransAM method is 10-fold more sensitive and provides more quantitative data than gelshift.

TransAM ADVANTAGES:

- Up to 100-fold greater sensitivity than gelshift assays
- Results in less than 5 hours
- Non-radioactive, colorimetric readout that is easily quantified by spectrophotometry
- No cloning or cell transfections required
- Ability to assay tissue samples
- 96-stripwell format compatible with high-throughput automation

For more information and an up-to-date list of available TransAM kits, please visit us at www.activemotif.com/transam.

Multiplex AP-1 & NFκB Assays

study activity of transcription factors regulating inflammation

Active Motif partnered with Luminex®, the industry leader for multiplexing, to develop Transcription Factor Multiplex Assays for studies of AP-1 and NFκB families.

Simultaneously identify responsive and non-responsive AP-1 and NFκB transcription factor targets

The AP-1 family of transcription factors plays a large role in the regulation of differentiation, proliferation and apoptosis, in particular in relation to inflammation and cancer. Likewise, the NFκB family of transcription factors is one of the most widely studied protein families in immunology and cancer research. However, comparative analysis of the relative activities or responses of members within the same transcription factor family have been encumbered by the lack of multiplexing capability of the currently available technologies to study them.

Traditional methods to assay transcription factor activity, such as Western blot and ELISA, are limited by the inability to quantify DNA binding events or to simultaneously compare activity of members of the same transcription factor family.

Active Motif's Transcription Factor Multiplex Assays overcome these limitations. These high-throughput / high-content assays are designed as pull-down reactions utilizing the revolutionary Luminex xMAP® technology to enable you to quantitatively measure the DNA binding activity of multiple AP-1 or NFκB family members in multiplex (Figures 1 and 2). The plate-based design makes it ideally suited for high-throughput large cohort studies and compound screens and enables you to simultaneously assess for responsive / non-responsive or functional / non-functional AP-1 and NFκB transcription factor targets in a single well.

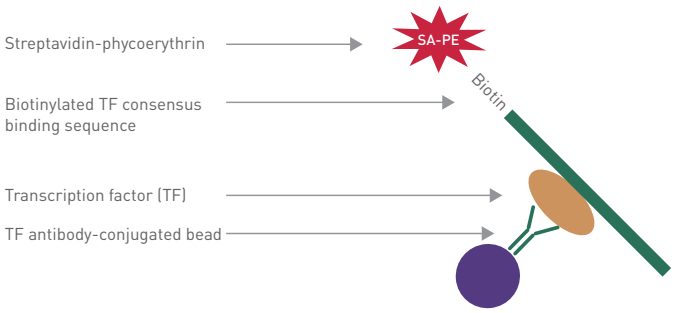


Figure 1: Bead-based Transcription Factor Multiplex Assay.
In the bead-based pull-down assay, lysates are combined with a biotinylated consensus binding sequence for the transcription factor (TF) family of interest. Fluorescent-labeled magnetic beads conjugated to antibodies specific for each TF, each with a unique signal, are added to capture the analyte(s) of interest. Streptavidin-phycoerythrin (SA-PE) is then added to bind biotin. Fluorescent signals from the beads and SA-PE are used to measure the levels of transcription factor-bound DNA with a Luminex instrument.

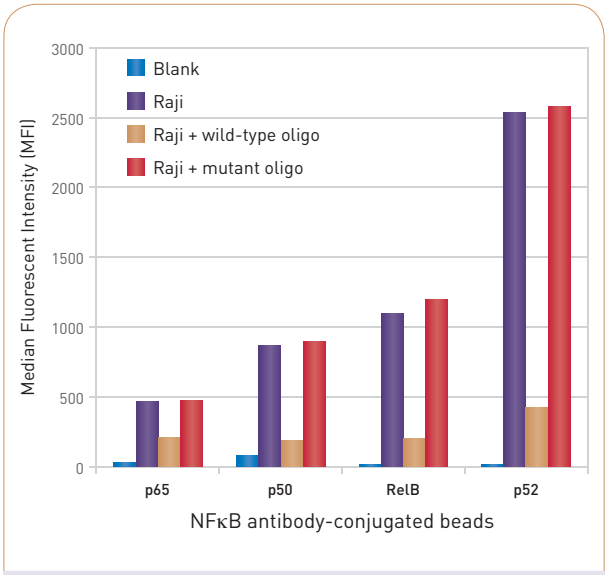


Figure 2: Multiplex analysis of NFκB DNA binding activity using the Transcription Factor Multiplex Kit – NFκB.
The graph shows a multiplex assay in which antibody-conjugated beads for multiple analytes were combined into a single well for simultaneous comparison of the activity of various NFκB proteins within the same sample.

Product	Catalog No.
Transcription Factor Multiplex Kit – AP-1	33100
c-Fos-conjugated beads	33101
FosB-conjugated beads	33102
JunB-conjugated beads	33104
JunD-conjugated beads	33105
Transcription Factor Multiplex Kit – NFκB	33110
RelB-conjugated beads	33111
NFκB p52-conjugated beads	33112
NFκB p65-conjugated beads	33113
NFκB p50-conjugated beads	33114

To learn more about our Luminex assays, visit us at www.activemotif.com/luminex.

Functional Validation of Molecular Interactions

reporter assays to study gene regulation

The LightSwitch™ Luciferase Assay System provides a quick and efficient method to measure the functional response of promoters to stimulus in nearly any cell-based system. The LightSwitch promoter reporter GoClone™ collection includes transfection-ready luciferase reporter constructs utilizing the novel RenSP luciferase technology for over 18,000 human promoters and highly optimized LightSwitch reagents.

- 18,000 pre-cloned human promoter reporters for screening expression changes of any human gene
- 100 transcription factor response element reporters for screening transcription factor activity
- Validated promoter reporters for measuring pathway activation are also available (see page 18)

Measure induction of select promoters in response to treatments or compounds

Much of disease research is focused on binding events, whether they are transcription factor studies, protein-protein and protein-DNA interactions, or analysis of the formation or disruption of these molecular interactions under various conditions or treatments. Active Motif's GoClone collections of transfection-ready human promoters, 3´UTRs and enhancer elements along with our LightSwitch luciferase Assay System provide a highly robust method to analyze the functional consequences of these interactions on a gene-by-gene basis (Figure 1).

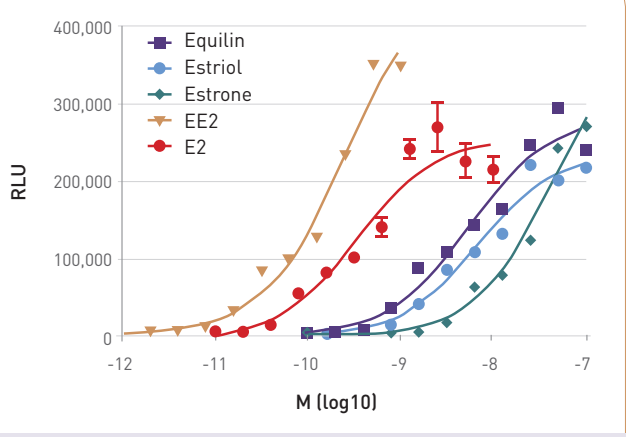
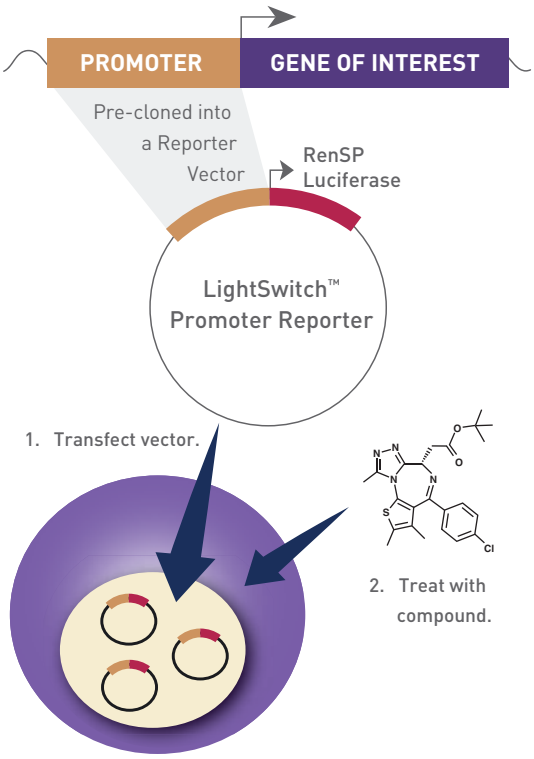
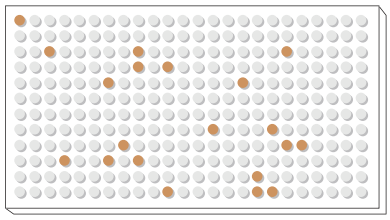


Figure 1: Dose response of LightSwitch SYT8 Promoter Reporter construct to estrogen compounds. HT1080 cells were co-transfected with a SYT8 Promoter GoClone™ (Product ID S714388) and an ER cDNA expression plasmid, then treated with five different estrogen compounds for 24 hours before the luminescence was measured using the LightSwitch Luciferase Assay Kit.





3. Measure luciferase activity to determine functional effects.

CUSTOM SERVICES AVAILABLE

- Stable cell line generation
- Custom assay development
- Custom cloning of novel regulatory elements (or TF response elements)

For more on the LightSwitch Luciferase Assay System and products, visit us at www.activemotif.com/lightswitch.

Identify Biological Responses to Compound Treatments

cell-based pathway screening assays

To understand a compound's mechanism of action it is crucial to identify activation of both intended and unintended cellular pathways. LightSwitch™ Pathway Screening Assays help you characterize cellular responses to compound treatment and understand off-target effects.

- Analyze 15 pathways in an HTS environment
- Identify compound-mediated pathway activation
- Determine the mechanism of action (MOA) and off-target effects of your lead compounds
- Available as a product or a service

A SOLUTION FOR:

- Secondary screening, "Hits-to-Lead"
- Off-target analysis
- Lead compound validation / optimization
- Dose-response analysis

Measure the effects of your compounds on a combination of biologically relevant pathways

Our in-house experts use our unique collection of validated assays to measure gene expression changes associated with a number of disease-related biological pathways. Leverage our unique collection of validated promoter reporter vectors from various pathways: Tox/AhR, Androgen, CREB, Estrogen, Glucocorticoid, Heat Shock, Hypoxia, NFκB, STAT, SREBP, and p53 (Figure 1). Our standard LightSwitch Pathway Screening Assays are available as stable cell lines or transfection-ready plasmids. Custom pathway sets are also available.

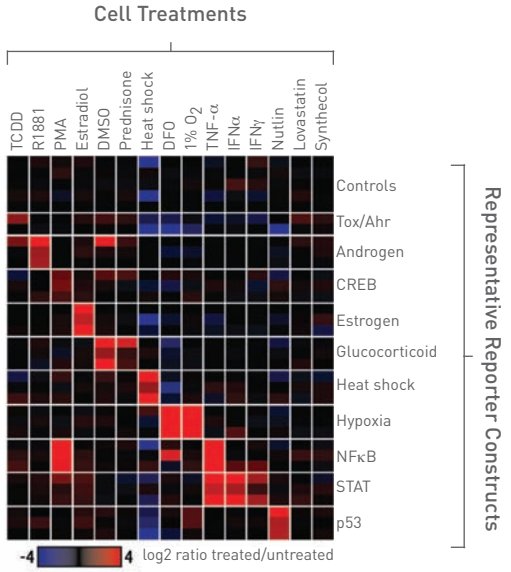


Figure 1: LightSwitch Pathway Screening Services can profile pathway responses to treatments. The heat map above shows the inducible activity of 29 different reporter constructs that represent 10 different pathways in response to 15 different treatments.

DISEASE PROFILING PANEL
48 promoters and controls

Pathway activity readout for:

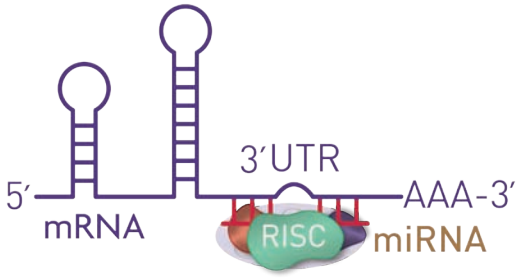
HIF-1α	Hypoxia
NFκB	Inflammation
CREB	cyclic AMP
HSF1	Heat shock
p53	DNA damage, apoptosis
STAT	Interferon
SREBP	Cholesterol biosynthesis
ER	Estrogen
AR	Androgen
GR	Glucocorticoid
AhR	Toxicity

To learn more about LightSwitch Pathway Screening Assays and Services, visit us at www.activemotif.com/lightswitch.

Measure miRNA Function and Validate miRNA Targets

functional reporter assays for miRNAs

MicroRNAs (miRNAs) are important regulators of gene expression and have been shown to play a role in numerous biological processes such as cellular signaling, development, and apoptosis. Mutations and improper regulation of miRNAs have been linked to a variety of physiological disorders such as cancer and heart disease. The LightSwitch™ 3´UTR Reporter Assay provides an HTS system that is ideally suited for screening the effects of compound treatments or other modifications on miRNA function.



- Measure the effects of small molecules or chemical modifications on miRNA activity
- Validate hundreds of miRNA targets in a single experiment
- Identify all miRNAs that regulate a gene of interest
- miRNA screening services also available

Measure RNA stability, translation efficiency and the functional impact of miRNAs on a gene-by-gene basis

Genome-wide transcript analysis can identify candidate target transcripts but cannot measure both the changes in a transcript's stability or translational efficiency attributable to miRNAs. With the LightSwitch 3´UTR Reporter GoClone™ Collection and Assay System, we have created a genome-wide library of human 3'UTR-luciferase reporter constructs to enable researchers to screen thousands of potential miRNA targets in high-throughput. Figures 1-3 demonstrate how, using this strategy, we were able to identify new targets of miR-122, an important regulator of cholesterol and fatty-acid metabolism in liver that has been implicated as a therapeutic target for metabolic disease.

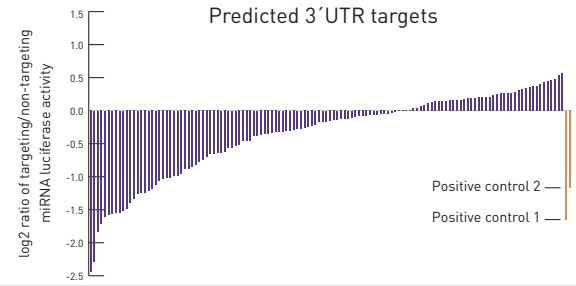


Figure 1: Results from functional screen of predicted miR-122 targets in HT-1080 cells. The log2 ratio observed for each tested UTR reporter in the presence of the miR-122 mimic over the luminescence when transfected with the non-targeting control shows 58/142 (40.8%) of the predicted targets were significantly altered in the mimic co-transfection (P<0.05, t-test). Furthermore, of those 3´UTRs with significantly altered luminescence, 25/58 (43.1%) were repressed 2-fold or more by the miR-122 mimic.

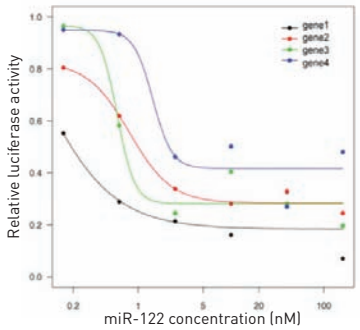


Figure 2: Knock-down of 3'UTR luciferase changes in a dose-dependent manner. Highly repressed targets from the above screen were tested at miRNA concentrations ranging from 0.0625 to 100 nM. The tested 3'UTR reporters responded to miR-122 mimic in a dose-dependent fashion, with EC_{50} at or below 1.5 nM.

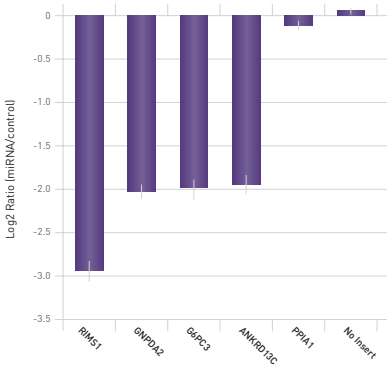


Figure 3: Interaction of miR-122 with 3´UTR targets. 3´UTR targets of miR-122 were cloned into the LightSwitch 3´UTR vector and co-transfected with either miR-122 mimic or a non-targeting control miRNA in K-562 cells. The graph shows the four strongest responders along with two non-responding controls.

PRODUCTS FOR miRNA FUNCTIONAL SCREENS

- 12,000 pre-cloned human 3´UTR reporters
- Synthetic target reporters containing sequences fully complementary to 1000 human miRNAs
- miRNA mimics and inhibitors
- Validated 3´UTR controls
- Complete screening service to validate 3´UTR targets for any miRNA

To learn more about available miRNA targets, mimics, inhibitors and services, visit us at www.activemotif.com/ls-3utr.

ACTIVE MOTIF®

Enabling Epigenetics Research

NORTH AMERICA

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