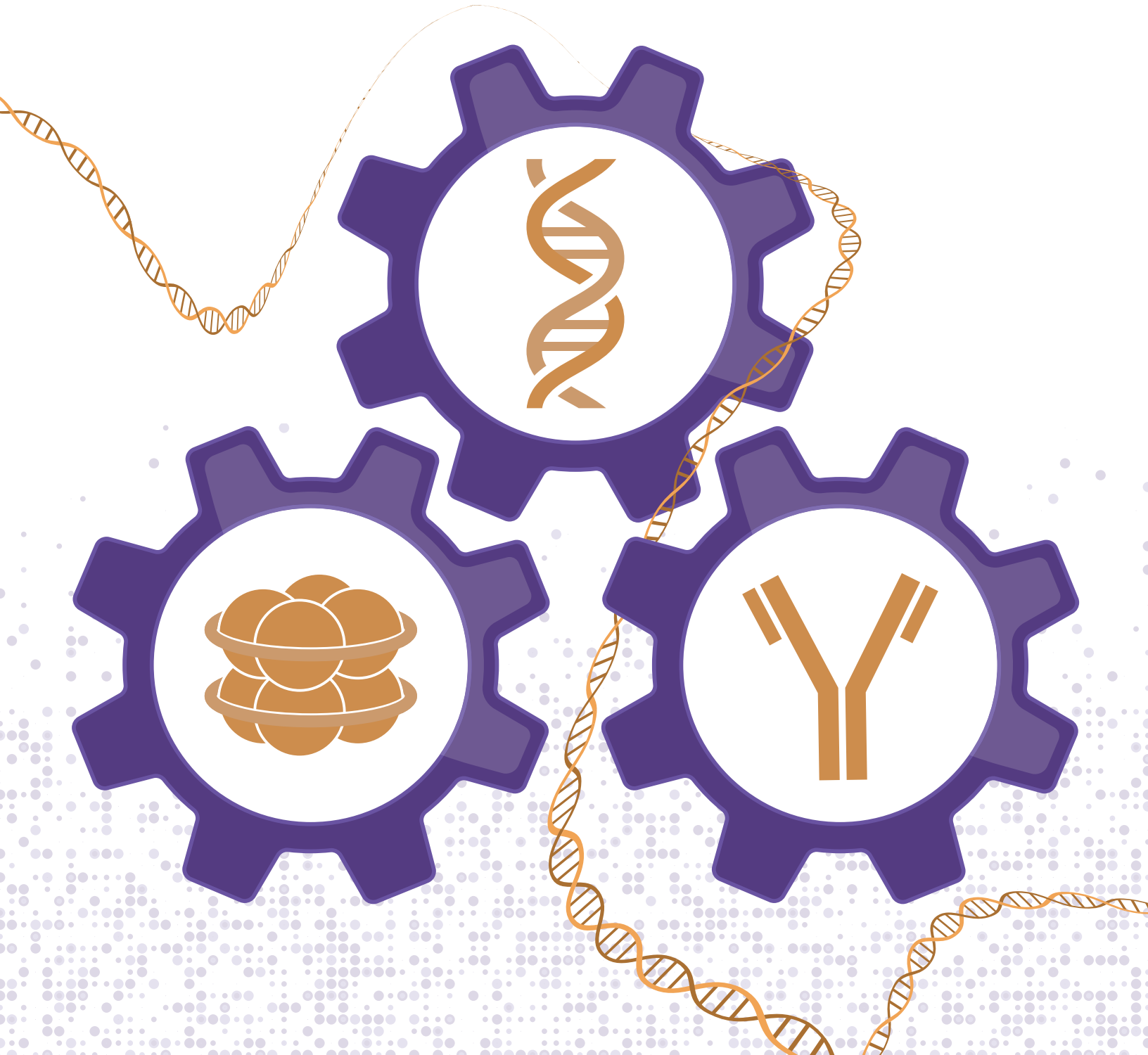
ACTIVE MOTIF®

Enabling Epigenetics Research

Custom Services

for Epigenetics and Gene Regulation Research



Active Motif Services

A Partner in Scientific Discovery

At Active Motif, we don't just run assays, we become a partner in your research. Every project begins with a one-on-one consultation to understand the goals of your experiment and how they connect to your broader research objectives, such as publications or grant proposals. We provide expert guidance on experimental design, helping you select the best cell type, number of replicates, and appropriate controls to ensure meaningful, publication-ready data.

Deep Expertise You Can Trust

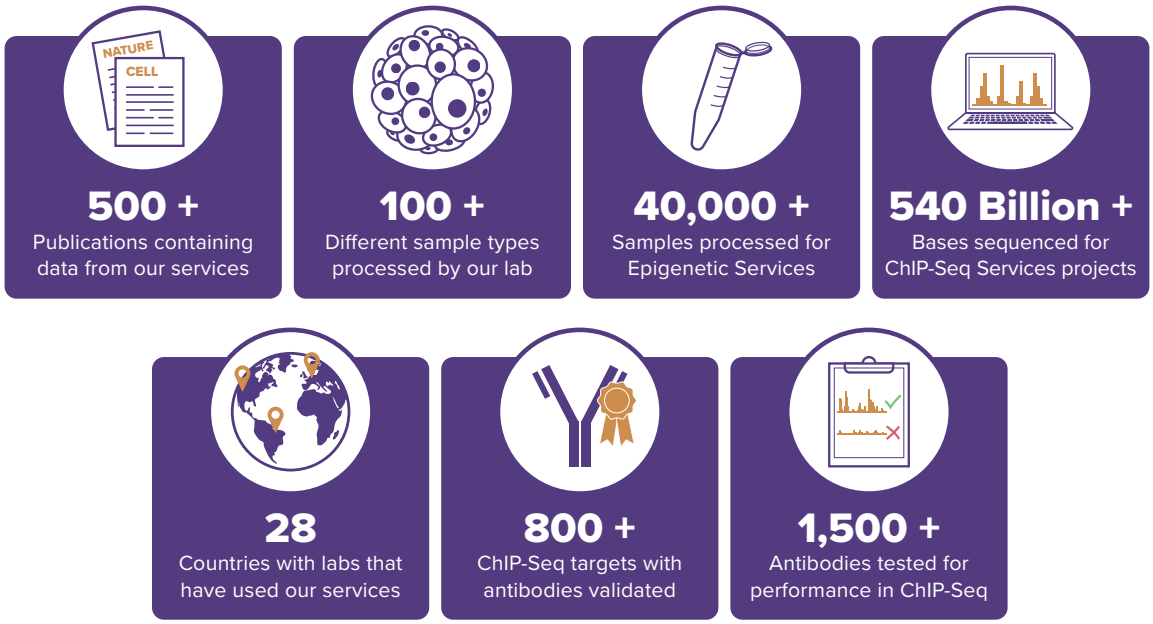
With our years of experience providing ChIP-Seq and other key epigenetic services, we've worked with a wide range of species, tissues, and targets. Our team has optimized protocols for even the most challenging workflows, helping researchers overcome technical hurdles like fixation, sonication, lysis conditions, and antibody selection. This depth of experience minimizes the time spent troubleshooting and accelerates your path to reproducible results.

Integrated Bioinformatics from Start to Finish

Our dedicated bioinformatics team is involved throughout the project: before, during, and after sample processing to ensure the data analysis is aligned with your experimental goals. Whether you need focused analysis on specific loci or motifs, or guidance on interpreting your results, we'll help you generate the insights and visualizations needed for impactful publications and successful funding applications.

Scalable Services That Grow with Your Research

We support not just individual experiments, but entire research programs. Many of our clients return for additional services on the same samples, such as RNA-Seq, ATAC-Seq, or DNA methylation profiling. Our integrated bioinformatics pipeline enables cross-platform data analysis for deeper biological insight and data integration. As your research evolves, our services scale with you to help to advance your discoveries and the field at large.



ChIP-Seq

End-to-End Services for genome-wide mapping of protein-DNA interactions

Chromatin immunoprecipitation (ChIP-Seq) combined with Next-Generation Sequencing is the most widely utilized technique to study protein-DNA interactions and histone modification localization across the genome. Given the importance of ChIP-Seq data sets for development and disease research, obtaining the highest quality data is crucial.

Choose the Global Leader in End-to-End ChIP Services

Active Motif offers the most diversified portfolio of ChIP Services. We bring over a decade of experience providing services, with over 10,000 samples processed, and the highest level of expertise of any service provider.

- Histone Mark ChIP-Seq
- Transcription Factor ChIP-Seq
- RNA Polymerase II ChIP-Seq
- ChIP Antibody Validation
- ChIP-Seq Spike-In Normalization
- Super-enhancer Profiling

Comprehensive ChIP-Seq services

1. Customer fixes cell lines or freezes tissue samples
2. Active Motif prepares chromatin and sonicates
3. Active Motif performs the ChIP reactions
4. Active Motif constructs ChIP-Seq libraries
5. Libraries sequenced using Illumina platform
6. Analysis and data delivery

Quality control steps to ensure success

- Chromatin preparation is quantified and sized
- Antibody is ChIP qualified prior to ChIP-Seq ChIP rxns
- Library is run on TapeStation to confirm fragment sizes
- Library yield is quantified via Qubit
- Sequencing to yield ~30 million tags

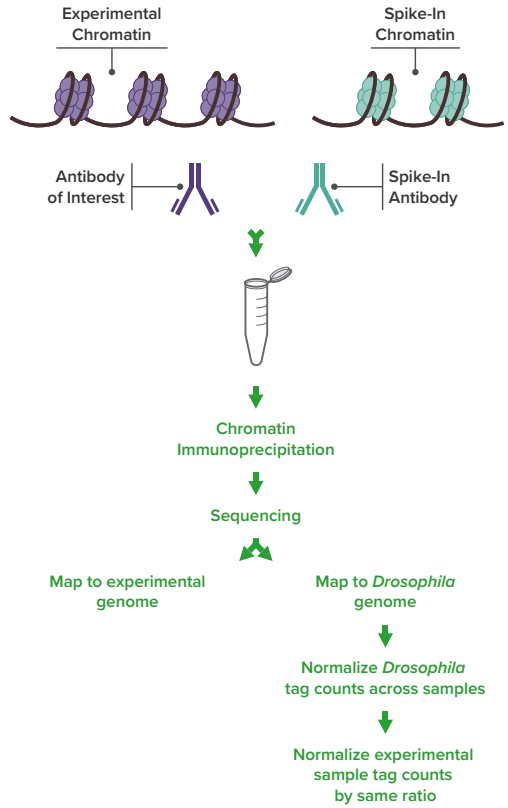
To learn more, visit **activemotif.com/services-chipseq**

ChIP-Seq Spike-In Normalization

A novel ChIP-Seq normalization strategy to reveal hidden biological effects

Active Motif's standard normalization method for ChIP-Seq data uses background signal for normalization, allowing for differences in peak signals to easily be observed. However, standard ChIP-Seq normalization may not be effective in some circumstances, for example when there are overall differences in sample signals due to experimental effects. In these cases, using our spike-in chromatin and antibody is recommended for normalization.

How Does It Work?



Why?

Without Spike-In normalization (-), uneven amplification of the ChIP DNA during preparation of Next-Gen sequencing libraries led to loss of differences between samples. With Spike-In normalization (+) the bias in PCR amplification was corrected and the difference between samples is clearly visible.

Uncover effects masked by large differences in overall levels of Immunoprecipitation

Monitor consistency between samples

Reduce sample bias

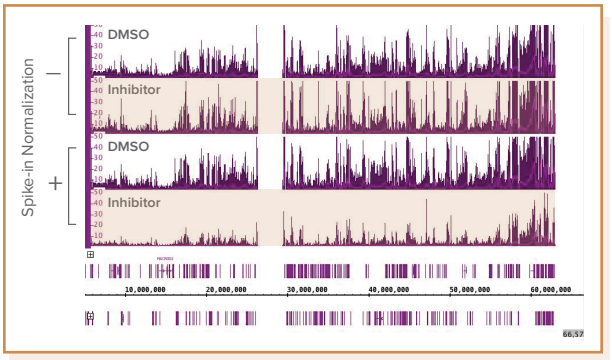


Figure 1.

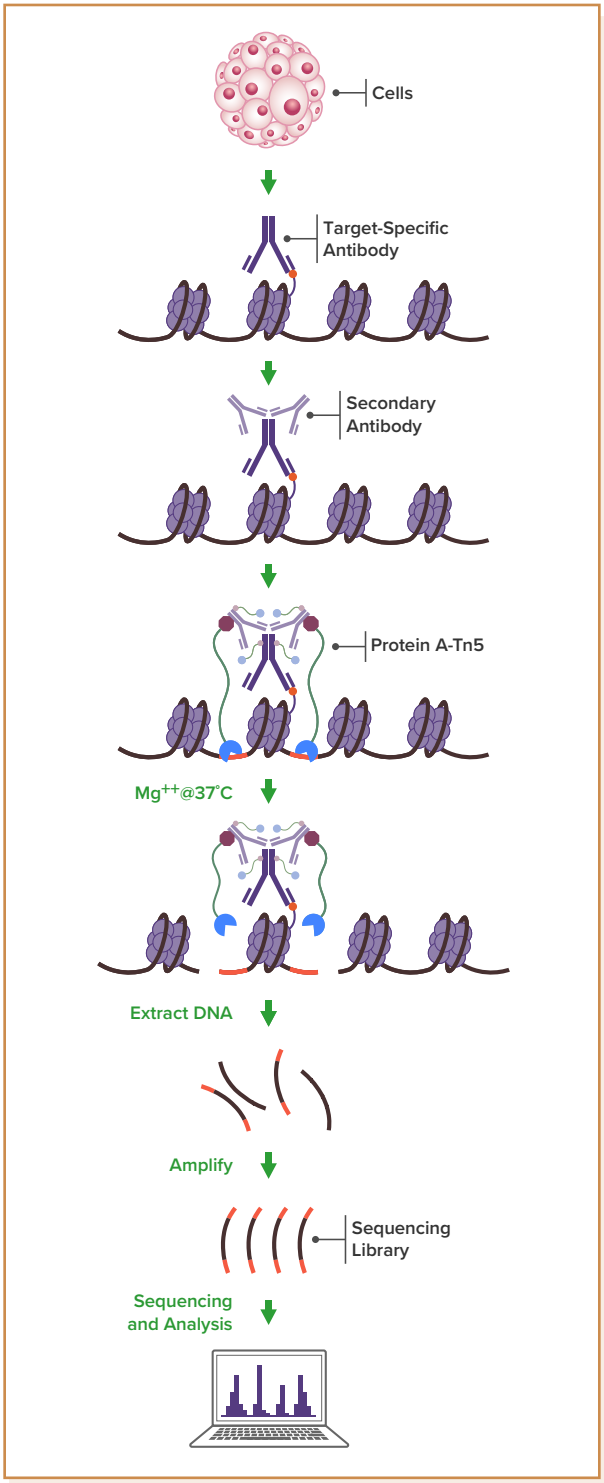
Cells treated with a small molecule inhibitor of EZH2 methyltransferase have dramatic reductions in global H3K27me3 levels. However, H3K27me3 ChIP-Seq using standard ChIP-Seq protocols (-) does not detect these differences. Incorporation of Active Motif's ChIP-Seq Spike-In Strategy (+) reveals the expected decrease in H3K27me3 ChIP-Seq signal.

To learn more, visit
activemotif.com/services-normalize

* Egan, B. et al. An alternative approach to ChIP-Seq normalization enables detection of genome-wide changes in histone H3 lysine 27 trimethylation upon EZH2 Inhibition. PLoS One. 11:e0166438

CUT&Tag-IT®* Service

Tn5 Transposase assisted chromatin profiling



Cleavage Under Targets and Tagmentation (CUT&Tag) is a method to map genomic localization of histone modifications that reveals interactions between proteins and DNA or identifies DNA binding sites for proteins of interest. CUT&Tag utilizes antibody directed Tn5 Transposase tagmentation* to target specific histone modifications to create genome-wide maps. Tn5 tagmentation sharpens resolution and decreases the sequencing depth requirement compared to ChIP-Seq.

- Best for histone modification targets
- Only low sequencing depth required
- Compatible with low starting material
- Low background signal

Figure 2.

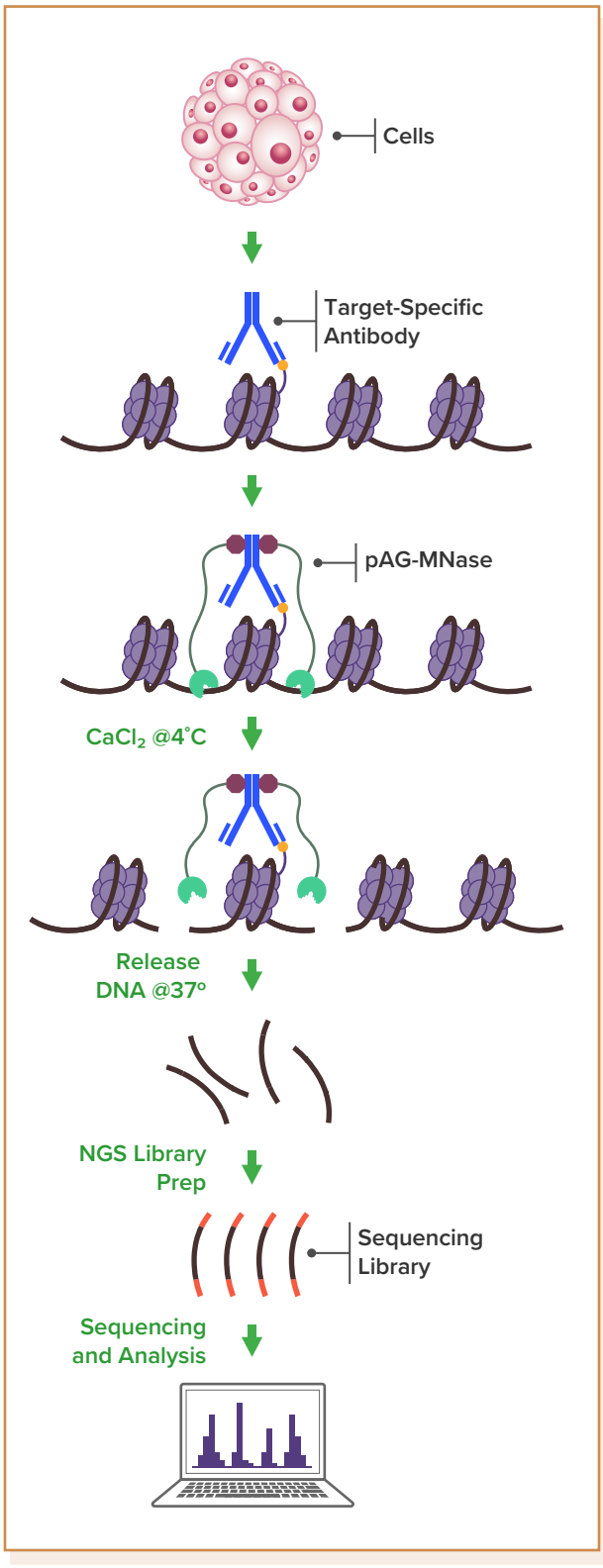
Our CUT&Tag- IT®* Service is based on the same principles as ChIP-Seq, but with several improvements advantageous for mapping histone marks. In CUT&Tag, unfixed cells are bound to concanavalin A beads and the antibody incubation is performed with cells in their native state. Directly following antibody binding, the chromatin is digested and NGS libraries are prepared in a single step by tagmentation using the protein A-Tn5 (pA-Tn5) transposase enzyme that has been pre-loaded with sequencing adapters.

To learn more, visit
activemotif.com/services-cut-and-tag

* Our Tn5 Transposase mediated chromatin tagmentation methods are covered by these patents: US9938524, US10689643B2, EP2783001B1, EP2999784B1.

ChIC/CUT&RUN Service

Native mapping of transcription factors and histone marks

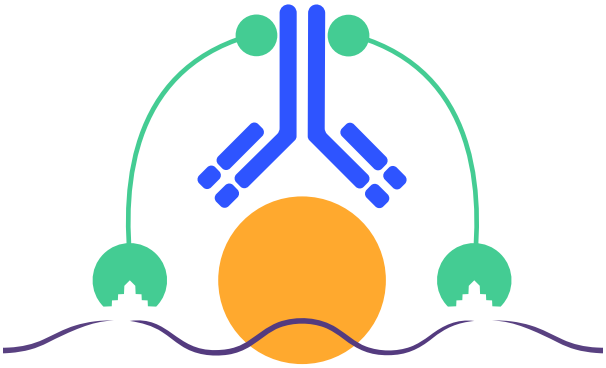


Cleavage Under Targets & Release Using Nuclease (CUT&RUN) is an epigenetic method used to investigate the genome-wide distribution of various chromatin-associated proteins and their modifications. CUT&RUN is a derivative of chromatin immunocleavage (ChIC). CUT&RUN is similar to chromatin immunoprecipitation (ChIP), in that it utilizes an antibody to target chromatin associated marks and proteins, but requires less sample material and sequencing depths than ChIP.

- Compatible with both histone mark and transcription factor targets
- Requires fewer cells than ChIP
- Low background signal

Figure 3.

In CUT&RUN, a protein of interest is tagged with an antibody and bound to chromatin in intact cells. Then, a micrococcal nuclease (MNase) is used to cleave the DNA specifically at the binding sites of the protein of interest. The released fragments are purified, sequenced, and mapped to the reference genome to determine the protein's binding sites. Unlike ChIP, CUT&RUN does not require crosslinking of the protein to the DNA, which can introduce artifacts.



To learn more, visit
activemotif.com/services-cut-run

ATAC-Seq Services

Genome-wide identification of open chromatin regions

Assay for Transposase Accessible Chromatin Sequencing (ATAC-Seq) is designed to study open chromatin, which is known to contain active gene regulatory elements including promoters, enhancers, and insulators. This assay provides data to enable identification of accessible chromatin regions across the genome that are distinct to individual cell types. ATAC-Seq is a perfect first step for those exploring the role of epigenetics in cell systems or disease models for which little information is available on mechanisms of gene regulation.

Determine if Epigenetic Mechanisms Are at Work

- Gain mechanistic insight into gene regulation in response to treatment
- Identify which transcription factors are driving disease or response
- Generate genome-wide profiles from patient samples (cells or tissues)
- Only 50,000 cells required

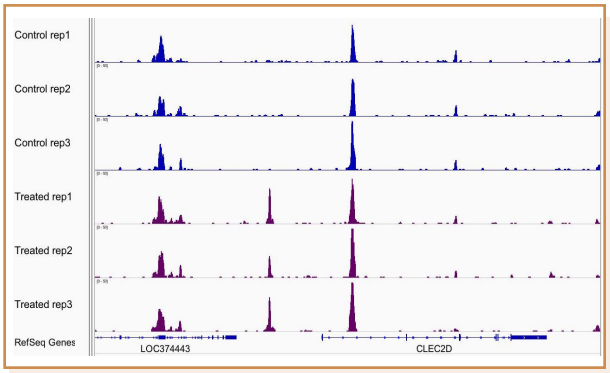
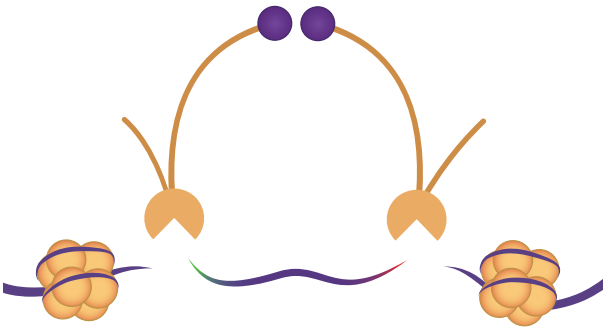


Figure 4.

Active Motif's ATAC-Seq assay was performed on control and treated cells, each in triplicate. Hundreds of differential peaks were detected. The one depicted is in an intergenic region.



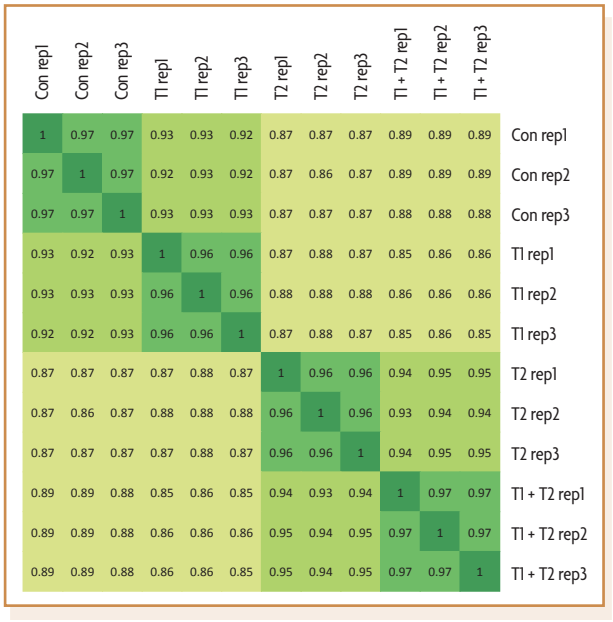


Figure 5.

Active Motif's ATAC-Seq assay was performed under four different cellular conditions, each condition in triplicate. The Pearson correlation coefficients were generated and graphed for each pair-wise comparison. The data demonstrates the assay is highly reproducible with correlation coefficients near 1 for replicates. Four separate groups are clearly visible in the heat map, showing that triplicates are more similar to each other than to other samples and indicating differences between sample types.

To learn more, visit
activemotif.com/services-atacseq

RRBS Services

Reduced Representation Bisulfite Sequencing

DNA methylation patterns are cell-type specific, and alterations in these patterns can be indicative of disease. RRBS is a bisulfite dependent method that provides single base pair resolution of cytosine methylation at millions of locations, allowing for sample-to-sample comparisons of DNA methylation patterns. Comparing DNA methylation profiles from normal and diseased patient samples can facilitate novel biomarker discovery.

Why is RRBS the Right Choice?

RRBS is significantly less expensive than Whole Genome Bisulfite Sequencing, while still providing the methylation status of up to 5 million CpGs at biologically relevant positions such as promoters and CpG islands. RRBS can be performed on cells, tissue, or purified DNA samples.

- Low starting material requirements
- Data provided on millions of CpGs
- Data from biologically relevant regions
  - Promoters
  - CpG Islands

Services Include

- Single base resolution
- Quantitation at each base
- Data at millions of locations across the genome
- Data enriched at promoters and CpG islands
- Dramatically less expensive than whole Genome Bisulfite Sequencing

RRBS Service

Customers submit DNA, cell pellets or frozen tissue then we perform:

1. DNA purification
2. DNA digestion
3. Sequencing adaptor ligation
4. Bisulfite conversion
5. PCR amplification
6. Sequencing on Illumina platform
7. Bioinformatic analysis

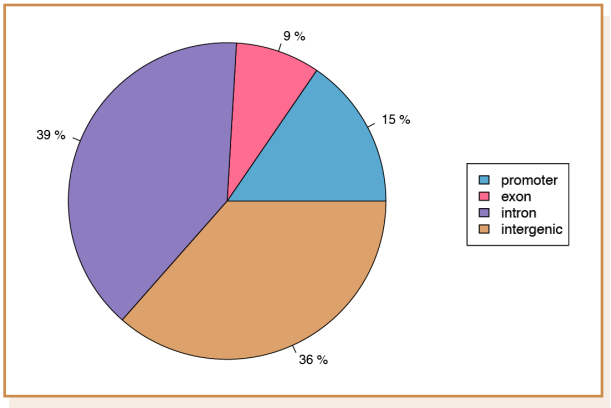


Figure 6. RRBS Data from Human Samples

Pie chart showing the assignment of differentially methylated cytosines to genic features.

A differential methylation analysis was performed on a per CpG basis, and identified sites were then aligned to annotated gene features such as promoters and exons.

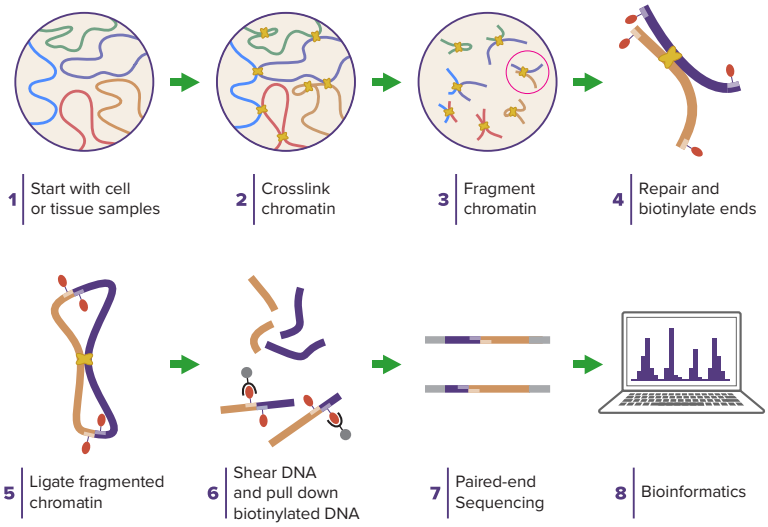
To learn more, visit
activemotif.com/services-rrbs

Hi-C Services

Map Genome-wide chromatin-chromatin interactions using our Hi-C Service

Functional elements such as enhancers can influence gene expression by interacting directly with promoters and other loci that may be thousands of kilobases away. Use our End-to-End Hi-C service to map these interactions and get a 3D view of genome organization. Elements detected include A/B compartments, topologically associated domains (TADs), and chromatin loops.

Schematic of Hi-C Workflow



- Identify changes in chromatin organization in response to treatment or stimuli
- Identify novel chromatin organization corresponding to different stages of development

Elements Detected Include:

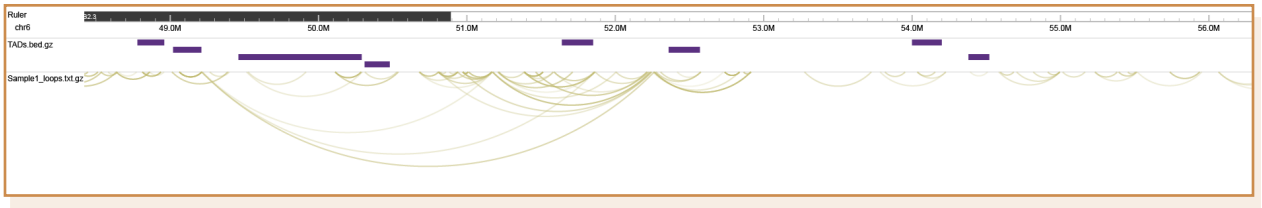


Figure 7. Hi-C enables identification of complex chromatin interactions like chromatin looping and Topological Associating Domains (TADs)

Hi-C was performed using mouse uterine tissue. Snapshot of Wash U Epigenome Browser looking at a 6.8 Mb region of chromosome 6. Topologically Associating Domains (TADs) are represented as purple bars. Chromatin loops are indicated by brown arcs.

To learn more, visit
activemotif.com/services-hi-c

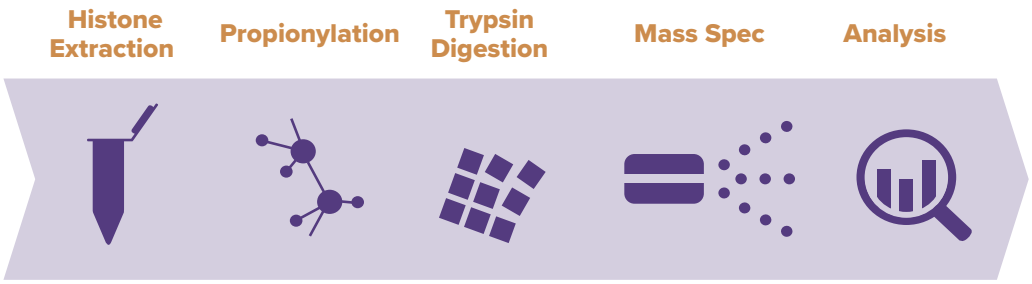
Mod Spec® Services

Histone Modification Detection Service

Total nuclear levels of histone post-translational modifications (PTM) may differ under varying conditions – disease vs normal, DMSO vs inhibitor, or WT vs KO. Active Motif's Mod Spec® service can verify expected differences, and more importantly, identify unexpected changes in histone PTM levels. This service uses mass spectrometry for relative quantitation of over 60 histone states.

- Detect over 60 different histone states
- Measure acetylation, methylation, ubiquitination, and unmodified peptides
- Analyze histone modifications on H1, H2, H3.1, H3.3, and H4
- More quantitative and comprehensive than western blots or ELISA

How Does Mod Spec® Work?



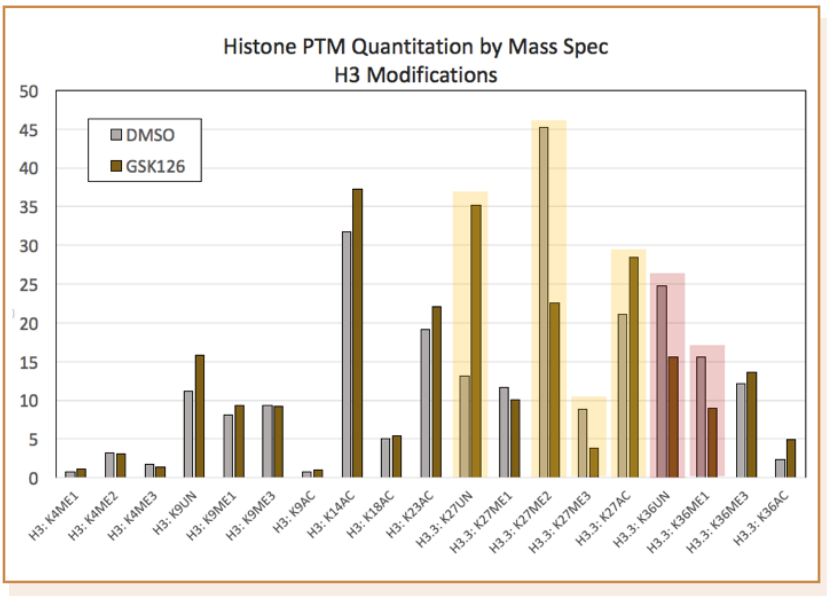


Figure 8. Mod Spec reveals the effects of GSK-126 treatment on HeLa cells.

GSK-126 is an inhibitor that blocks the methyltransferase activity of EZH2, resulting in global decreases in H3K27me2 and H3K27me3. A selection of H3 modifications are shown that confirm significant decreases in H3K27 methylation with concomitant increases in acetylated and unmodified H3K27 (highlighted in yellow). Changes in H3K36 are highlighted in red.

To learn more, visit
activemotif.com/modspec

Gene Expression Services

RNA-Seq for steady state mRNA levels
RNA Pol II ChIP-Seq for transcription rate measurements

Active Motif transcriptome analysis services include RNA-Seq for identification and quantitation of RNA transcripts and RNA Pol II ChIP-Seq for quantitation of transcription rates to enable rapid profiling of changes in gene expression associated with transcription factor (TF) and histone modification occupancy.

RNA-Seq Services

Simply submit RNA, cells, or tissue samples. Order RNA-Seq alone or combine with ChIP-Seq data to uncover contextual information about:

- Differential gene expression
- Changes in gene structure or splicing patterns
- Effects of TF binding on gene expression

RNA Pol II ChIP-Seq Services

Analysis of RNA Pol II occupancy as a proxy measurement of transcription rates offers the advantage of enabling you to:

- Measure transcription without the influence of RNA half-life
- Identify genes poised for transcriptional activation
- Generate gene expression data from cells used for ChIP-Seq
- Measure changes at early time points post treatment

1. Prepare RNA from cells or tissues
2. Generate cDNA
3. Construct directional libraries
4. Perform Next-Generation Sequencing
5. Perform data analysis

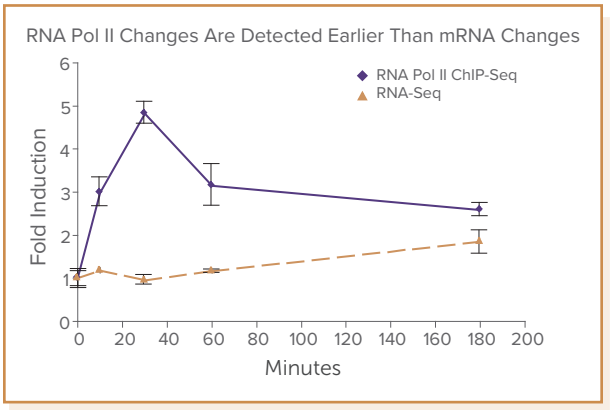


Figure 9: Gene expression profiles vary depending on the analysis method.

Data for Igf1r was extracted from RNA–Seq and RNA Pol II ChIP-Seq data sets. Cell treatment resulted in induced gene expression that was measured at various time points. The cumulative data show that transcription, as measured by RNA Pol II ChIP-Seq, is induced immediately, while mRNA levels only accumulate over time.

To learn more, visit
activemotif.com/rna-seq

Interactome Profiling (RIME)

Mass Spectrometry identifies co-factor recruitment into transcriptional complexes

RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins) sheds light on the complex process of gene regulation by enabling capture and identification of chromatin associated proteins that interact with an endogenous protein of interest.

Why RIME?

Gene regulation is often oversimplified when the focus is on one particular transcription factor in any given cell model. In reality, differential gene expression is greatly influenced by co-factors and other protein interactions within chromatin. RIME clarifies this complexity by providing a means to identify the protein interactions that are important for gene regulation.

- Targets DNA/chromatin associated proteins
- Enables capture of low affinity interactions
- Allows more stringent wash conditions resulting in less non-specific interactions

Experimental Design

- Antibody validation is performed on a single sample to show that the target protein is detected
- IP-mass spec using the target antibody is performed in duplicate
- IP-mass spec using anti-IgG is performed in duplicate
- IgG interactions are removed from the target antibody specific interaction list

How Does RIME Work?

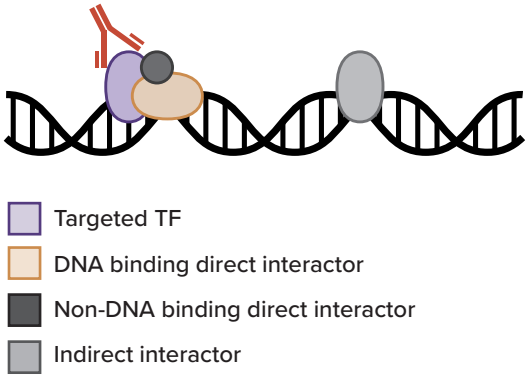


Figure 10. Example data from RIME

Different Estrogen Receptor binding profiles have been observed depending on the ligand used to stimulate binding. Differences are known to occur through ligand induced conformational changes in the receptor that influence cofactor recruitment. Our RIME data shows differential recruitment of cofactors to DNA bound estrogen receptor after treatment with ligand 1 and ligand 2. Light purple indicates recruited proteins with similar rank order for both ligands. Tan indicates common protein detected but with different rank order. Dark Purple indicates unique protein recruitment.

Ligand 1	Ligand 2
Estrogen Receptor	Estrogen Receptor
Nuclear receptor co-activator 3	Vang-like protein 1
Nuclear receptor interacting protein 1	Pericentriolar material 1 protein
Pericentriolar material 1 protein	Centrosomal protein of 131 kDa
Centrosomal protein of 131 kDa	Protein GREB1
CREB-binding protein	E3 ubiquitin-protein ligase TRIM33
E3 ubiquitin-protein ligase TRIM33	Nuclear receptor interacting protein 1

To learn more, visit **activemotif.com/rime**

Single-Cell RNA-Seq Services

Measure gene expression in heterogeneous populations at single-cell resolution

Single-Cell RNA-Seq enables transcriptome analysis at the single-cell level. scRNA-Seq can be used to identify cell subpopulations with different transcriptome profiles within complex samples, eliminating the need for isolation strategies like FACS or magnetic sorting that could alter the biology of the sample due to sample manipulation.

To learn more, visit **activemotif.com/services-scrna-seq**

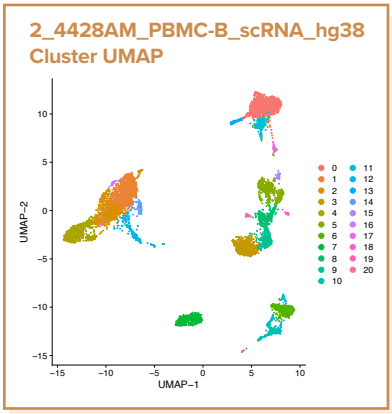


Figure 11. Identify unique subpopulations of cells within a single sample.

Single-Cell RNA-Seq data generated from human PBMCs. Each color-coded cluster on the UMAP plot represents populations of cells that have the same gene expression profile. 20 refined clusters were identified.

Active Motif's End-to-End scRNA-Seq service includes:

- Cell preparation
- Sample processed using 10X Genomics Chromium platform
- Library generation
- Sequencing
- Bioinformatic analysis

Single-Cell ATAC-Seq Services

End-to-End service to identify open chromatin regions at single-cell resolution

Active Motif's scATAC-Seq service enables examination of genome-wide chromatin accessibility of thousands of cells in parallel, allowing examination of subpopulations of cells within a heterogeneous population that would otherwise be lost in standard bulk ATAC-Seq.

To learn more, visit **activemotif.com/services-scmultiome**

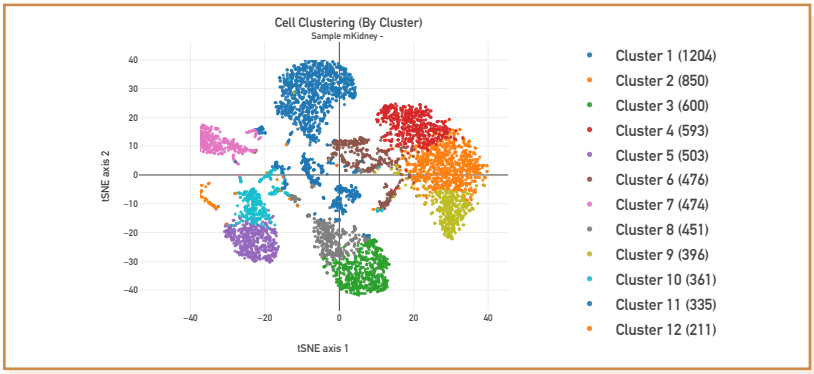


Figure 12. Identify variations in chromatin accessibility across different cell populations within a single sample.

Active Motif's End-to-End scATAC-Seq service includes:

- Cell preparation
- Transposase reaction
- Sample processed using 10X Genomics Chromium platform
- Library generation
- Sequencing
- Bioinformatic analysis

Single-Cell Multiome Service

End-to-End service to measure gene expression and open chromatin states from the same cell

10X GENOMICS Certified Service Provider

Single-Cell Multiome allows for both transcriptome analysis and genome-wide detection of open chromatin at the single cell level. Understanding both the gene expression profile and the chromatin state at single-cell resolution can help identify how epigenetic changes instruct gene expression in distinct cell populations.

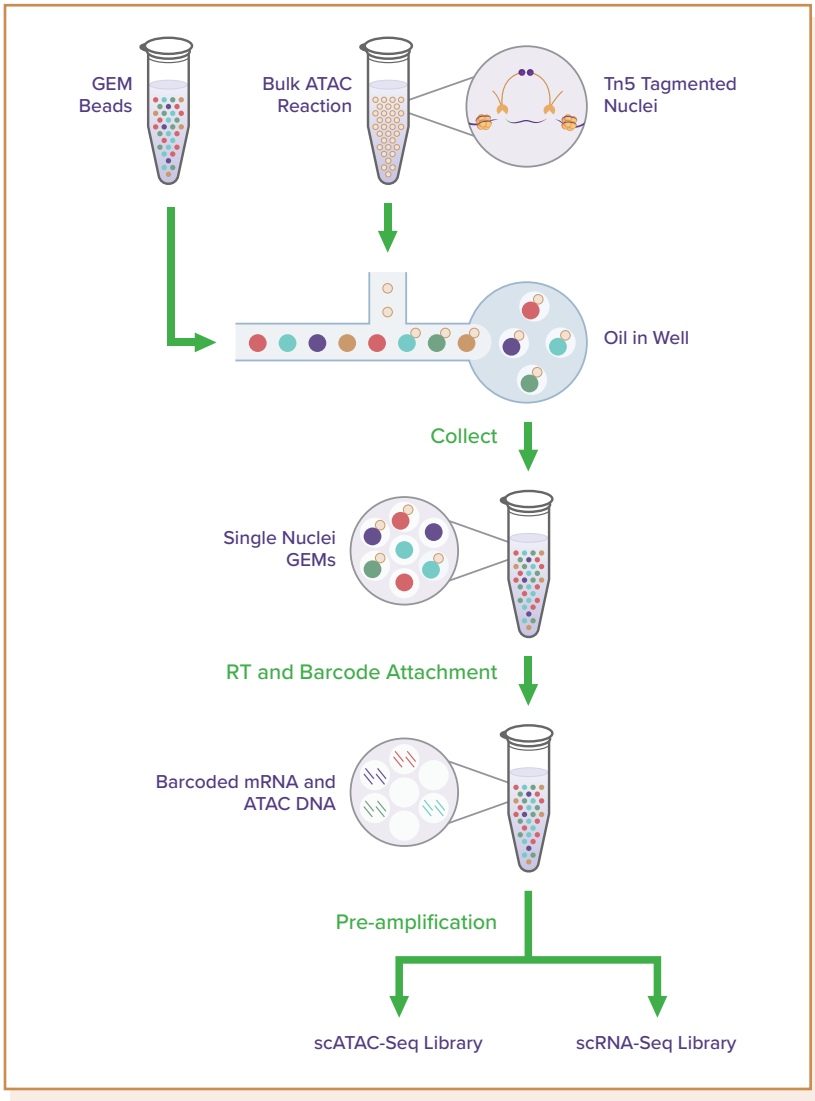


Figure 13.

Single-Cell Multiome measures gene expression and open chromatin from the same cell. Tn5 tagmentation is performed on nuclei, which are loaded onto the 10X Genomics Chromium Controller and met with GEMs (gel bead-in-emulsion) containing reverse transcriptase and sequencing adapters. Open chromatin fragments and cDNAs are barcoded, creating two unique libraries per cell.

What are the advantages of using Single-Cell Multiome?

Single-Cell Multiome can be used to identify cell subpopulations with different transcriptomal and epigenetic profiles within complex samples, eliminating the need for isolation strategies like FACS or magnetic sorting that could alter the biology due to sample manipulation.

For example:

- Identifying novel cell subpopulations that modulate response to drug treatments (e.g., responders vs. resistant cells)
- Identifying subpopulations of cells with variations in gene expression that can provide insight into developmental trajectories (e.g., brain development, T-helper cell development, B-cell differentiation)

Active Motif's End-to-End Multiome service includes:

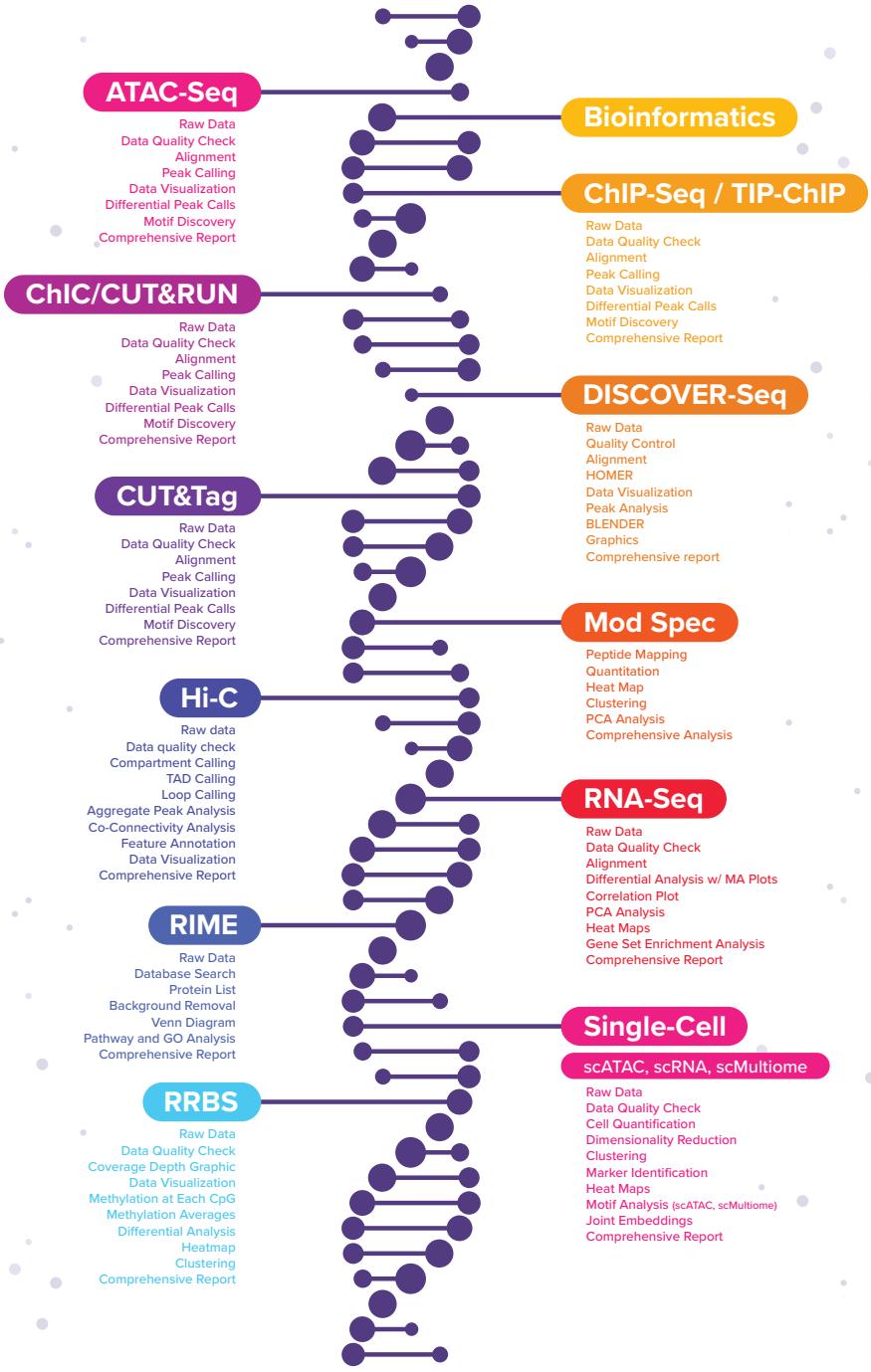
- Cell preparation
- Sample processed using 10X Genomics Chromium platform
- Library generation
- Sequencing
- Bioinformatic analysis

To learn more, visit **activemotif.com/services-scmultiome**

Bioinformatics

Comprehensive and customizable data analysis and support from our expert team of scientists

At Active Motif, our team of bioinformatic scientists has been providing premium bioinformatic analysis as part of our End-to-End Epigenetic Services for more than a decade. Now, we're offering this expertise for our customers who just require assistance with their bioinformatics. Perhaps you sent your samples away for sequencing and now don't know where to begin with your delivered sequencing files. Aren't sure how to move forward? We can help.



ATAC-Seq
Raw Data
Data Quality Check
Alignment
Peak Calling
Data Visualization
Differential Peak Calls
Motif Discovery
Comprehensive Report

ChIC/CUT&RUN
Raw Data
Data Quality Check
Alignment
Peak Calling
Data Visualization
Differential Peak Calls
Motif Discovery
Comprehensive Report

CUT&Tag
Raw Data
Data Quality Check
Alignment
Peak Calling
Data Visualization
Differential Peak Calls
Motif Discovery
Comprehensive Report

Hi-C
Raw data
Data quality check
Compartment Calling
TAD Calling
Loop Calling
Aggregate Peak Analysis
Co-Connectivity Analysis
Feature Annotation
Data Visualization
Comprehensive Report

RIME
Raw Data
Database Search
Protein List
Background Removal
Venn Diagram
Pathway and GO Analysis
Comprehensive Report

RRBS
Raw Data
Data Quality Check
Coverage Depth Graphic
Data Visualization
Methylation at Each CpG
Methylation Averages
Differential Analysis
Heatmap
Clustering
Comprehensive Report

Bioinformatics

ChIP-Seq / TIP-ChIP
Raw Data
Data Quality Check
Alignment
Peak Calling
Data Visualization
Differential Peak Calls
Motif Discovery
Comprehensive Report

DISCOVER-Seq
Raw Data
Quality Control
Alignment
HOMER
Data Visualization
Peak Analysis
BLENDER
Graphics
Comprehensive report

Mod Spec
Peptide Mapping
Quantitation
Heat Map
Clustering
PCA Analysis
Comprehensive Analysis

RNA-Seq
Raw Data
Data Quality Check
Alignment
Differential Analysis w/ MA Plots
Correlation Plot
PCA Analysis
Heat Maps
Gene Set Enrichment Analysis
Comprehensive Report

Single-Cell
scATAC, scRNA, scMultiome

Raw Data
Data Quality Check
Cell Quantification
Dimensionality Reduction
Clustering
Marker Identification
Heat Maps
Motif Analysis (scATAC, scMultiome)
Joint Embeddings
Comprehensive Report

TIP-ChIP

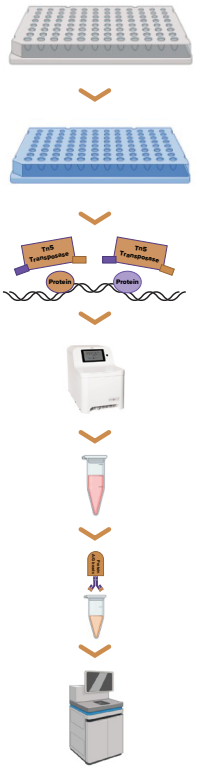
Ultra-fast, high-throughput ChIP

TIP-ChIP is a high-throughput ChIP-Seq method designed to process up to 96 samples efficiently. It reduces cell input needs, lowers variability, and offers faster turnaround and lower costs. The workflow involves tagmenting chromatin in a 96-well plate, pooling at least 12 samples, sonicating with the PIXUL® Multi-Sample Sonicator to release the tagmented protein/DNA complexes, and performing a single-tube immunoprecipitation to generate high-quality, consistent results.

TIP-ChIP Highlights

- Low cell input, recommended 300K to 1M cells per sample
- Consistency - Eliminate sample-to-sample technical variability
- High-throughput format reduces batch-to batch variation

How Does It Work?



Crosslink 96 different samples in a 96-well plate

Open chromatin, lyse cells and transfer 96 different nuclei pellets to a 96-well indexed Tn5 transposase plate

Fragment DNA and insert unique indexed adapters by Tn5 transposase tagmentation in each sample

Release protein-tagmented DNA complexes in 96 samples simultaneously by PIXUL

Combine all 96 samples into one pool for one ChIP reaction

Immunoprecipitation, reverse crosslinking, digest protein, and purify indexed DNA for library amplification

Sequencing analysis

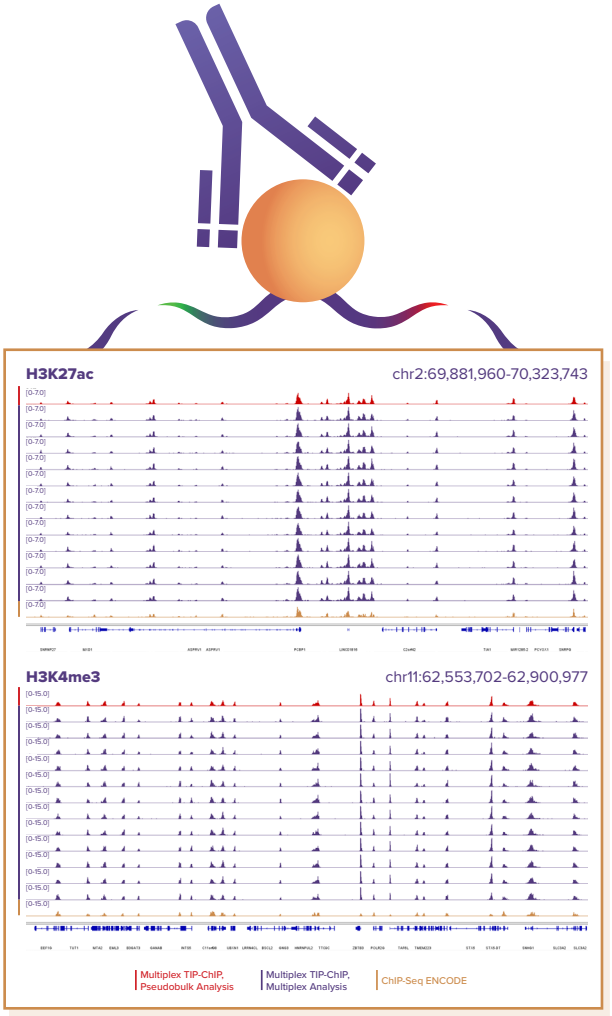


Figure 14. Comparison of TIP-ChIP and ChIP-Seq enrichment patterns.

TIP-ChIP and ChIP-Seq show similar enrichment patterns in K562 cells. TIP-ChIP was used to profile H3K27ac and H3K4me3 across 12 barcoded samples, which were pooled for a single TIP-ChIP reaction and later deconvoluted. Pseudobulk (red) and demultiplexed (purple) TIP-ChIP results are compared to ENCODE ChIP-Seq tracks (gold).

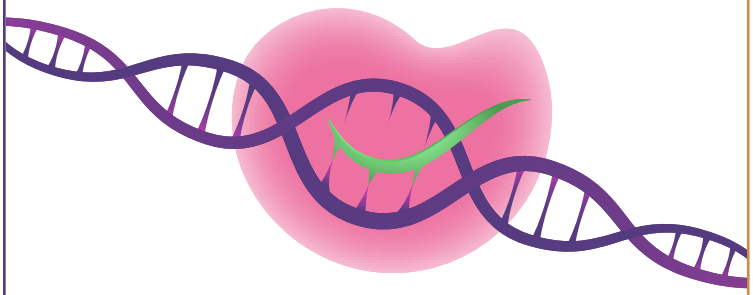
To learn more, visit
activemotif.com/services-tip-chip

DISCOVER-Seq

Off-target Discovery Using DISCOVER-Seq

DISCOVER-Seq (Discovery of In Situ Cas Off-targets and Verification by Sequencing) is an unbiased approach for identifying CRISPR-Cas off-target activity from both *in vitro* and *in vivo* samples. Using chromatin immunoprecipitation and next-generation sequencing (ChIP-Seq) it can precisely detect DNA double-stranded breaks, at single base resolution using MRE11, a protein that plays a key role in DNA double-strand break repair.

- Low numbers of false positives
- Can be used for CRISPR-Cas treated *in vitro* samples like cultured cells and *in vivo* samples such as patient derived iPS cells and animal models



DISCOVER-Seq Off Targets

PAM	DISCOVER Score	Mismatches	Coordinates
GACCCCCTCCACCCCGCCTCNGG	64	Target	chr6:43770822-43770841
ACA	45	4	chr9:100837364-100837383
GG	32	2	chr11:31795932-31795951
T CA	19	3	chr17:4455454-4455473
CTA	16	3	chr5:6714989-6715008
TC A	16	3	chr9:27338860-27338879
CCT CA	16	6	chr1:151059393-151059412
TG TC	15	4	chr17:41888501-41888520
ATT C	14	4	chr2:241275175-241275194
C A C T	11	4	chr3:140679942-140679961
CT C T	10	4	chr5:139648655-139648674
CTG C A	10	5	chrX:150764039-150764058
TA A C	9	4	chr8:143740775-143740794
C C	8	2	chr15:32993903-32993922
TG C C	8	4	chrX:129906648-129906667
CCA C C	8	5	chr2:169716824-169716843
TG C A	7	4	chr16:56929514-56929533
CCA C C	6	5	chr20:10933310-10933329
CG T C	5	4	chr10:133336427-133336446
CC C C	5	4	chr22:50446357-50446376
CC C C	5	4	chr2:128486625-128486644
C T C C	5	4	chr9:123375899-123375918
A G C C	5	4	chr9:137368990-137369009
C AC	3	3	chr18:23779592-23779611

Figure 15. Off-target discovery using DISCOVER-Seq

The table above shows off-target discovery using DISCOVER-Seq with rows showing nucleotide differences from the target sequence at the top. On the right are columns showing the following: DISCOVER Score which correlates with the number of off-targets, Mismatches which is the number of mismatched bases, and Coordinates which is the location of the sequence in the genome.

To learn more, visit
activemotif.com/services-discover-seq

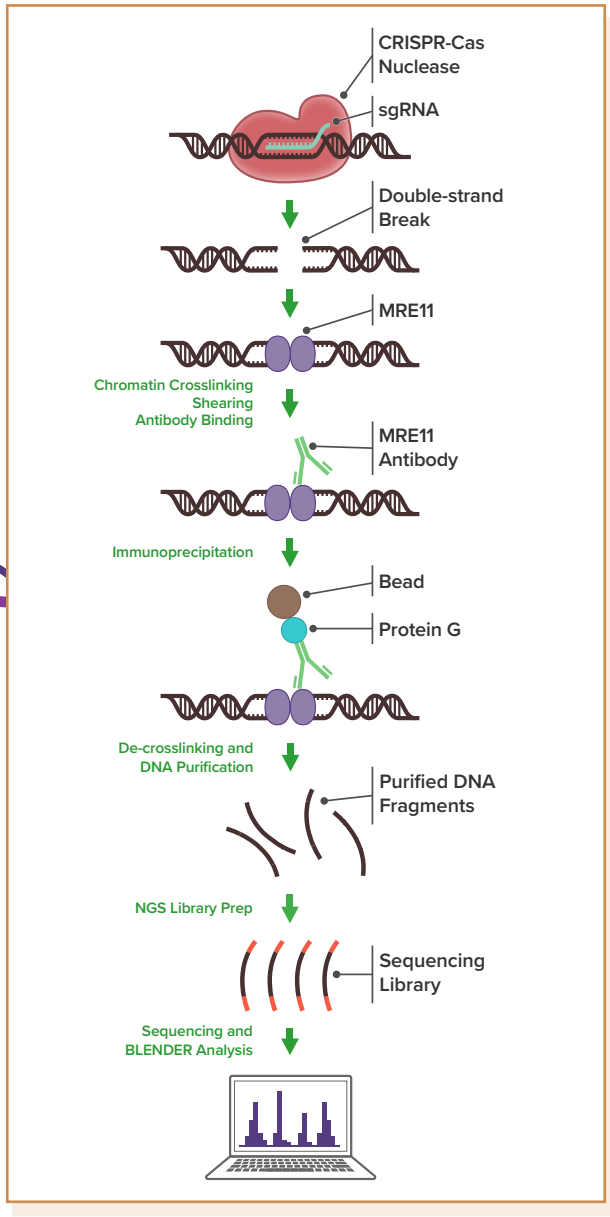


Figure 16.

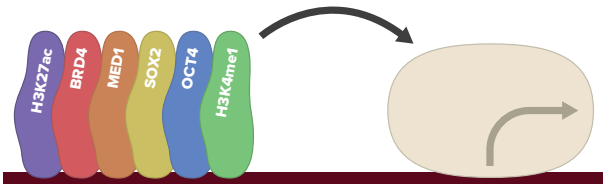
DISCOVER-Seq combines MRE11 ChIP-Seq with custom software (BLENDER – Blunt End Finder) within its analysis pipeline to identify off-target sequences across the genome.

Super Enhancer Profiling

Specialized ChIP-Seq data generation and analysis services for genome-wide super-enhancer profiling

Most genes that are considered master regulators are transcription factors. Super-enhancers are regulatory regions that control the expression of these master transcription factors. Active Motif offers a specialized ChIP-Seq service to identify super-enhancers which helps define the master regulators of any given cell type or disease sample.

There are many proteins that assemble into super-enhancers, however H3K27ac is a universal marker of super-enhancers. Active Motif can generate a super-enhancer profile from any sample by simply performing an H3K27ac ChIP-Seq experiment



- Identify master regulators of cell identity
- Find regulatory regions associated with disease
- Determine mechanisms of BRD4 inhibitors

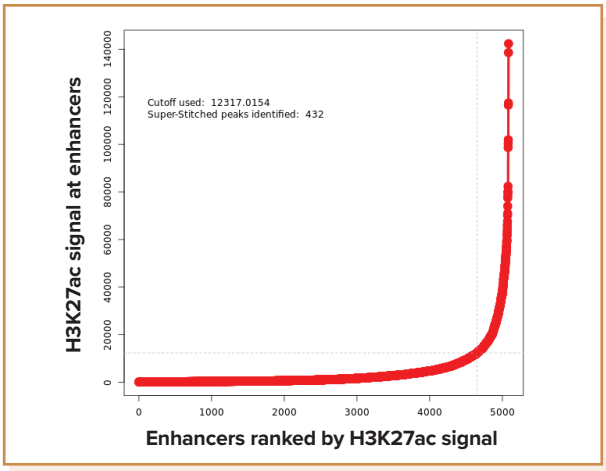


Figure 17. Identification of Super-Enhancers using Bioinformatic Analysis of ChIP-Seq Data

Enhancers are plotted in increasing order based on ChIP-Seq peak intensity. Super-enhancers are the group of enhancers above the inflection point of the curve.

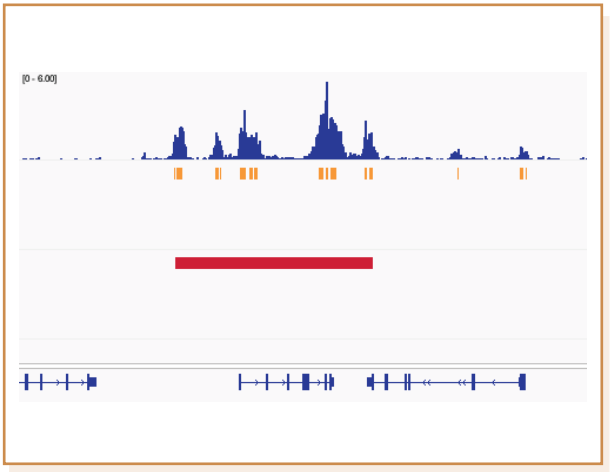


Figure 18. Super-Enhancer Profiling Using H3K27ac ChIP-Seq Data

Super-enhancers in the region of the genome shown are identified by the presence of high-intensity H3K27ac ChIP-Seq peaks. The super-enhancer is defined by the clustering of high-density peaks (indicated by red bar).

To learn more, visit
activemotif.com/services-superenhancer

ChIP Antibody Validation Services

Services to test the suitability of your antibody for ChIP applications

One of the greatest challenges in ChIP experiments is the lack of available antibodies that can recognize fixed, target-bound proteins and that function in immunoprecipitation. Active Motif's ChIP Antibody Validation Service makes this process simple, fast, and convenient.

Let the ChIP Experts do the work for you.

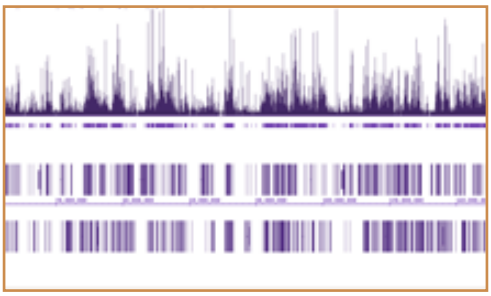
Only 30% of all antibodies work in ChIP-Seq. Therefore, identification of a good ChIP-Seq antibody presents a significant barrier to project initiation and completion. Our Epigenetic Services team has validated antibodies to over 800 targets. If your target of interest is on our list, we can start your project immediately. Otherwise, submit an antibody to us and our Antibody Validation Service can give you an answer in as little as 4 weeks.

- Hundreds of antibodies already validated
- "Yes" or "No" results for ChIP-Seq functionality
- Submit any antibody for testing

Antibody Validation by ChIP-Seq

1. Customer fixes cell lines or freezes tissue samples
2. Chromatin is prepared
3. ChIP is performed
4. ChIP-Seq libraries are constructed
5. Libraries are sequenced on an Illumina instrument
6. Data is analyzed

Peaks = Antibody passed validation



No Peaks = Antibody failed validation

To learn more, visit **activemotif.com/ab-val**

ACTIVE MOTIF

Custom Services

The Active Motif Custom Services team makes advanced research methodologies accessible to the wider life science community. We provide services for state-of-the-art epigenetics and gene regulation analysis techniques to accelerate your research.

ChIP-Seq	RIME
CUT&Tag	Single-cell ATAC-Seq
ChIC/CUT&RUN	Single-cell/nucleus RNA-Seq
ATAC-Seq	Single-cell Multiome
RRBS	Hi-C
RNA-Seq	Bioinformatics
Histone Modification Analysis	TIP-ChIP
IP-Mass Spec	DISCOVER-Seq

Learn more and get a quote at **activemotif.com/services**

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