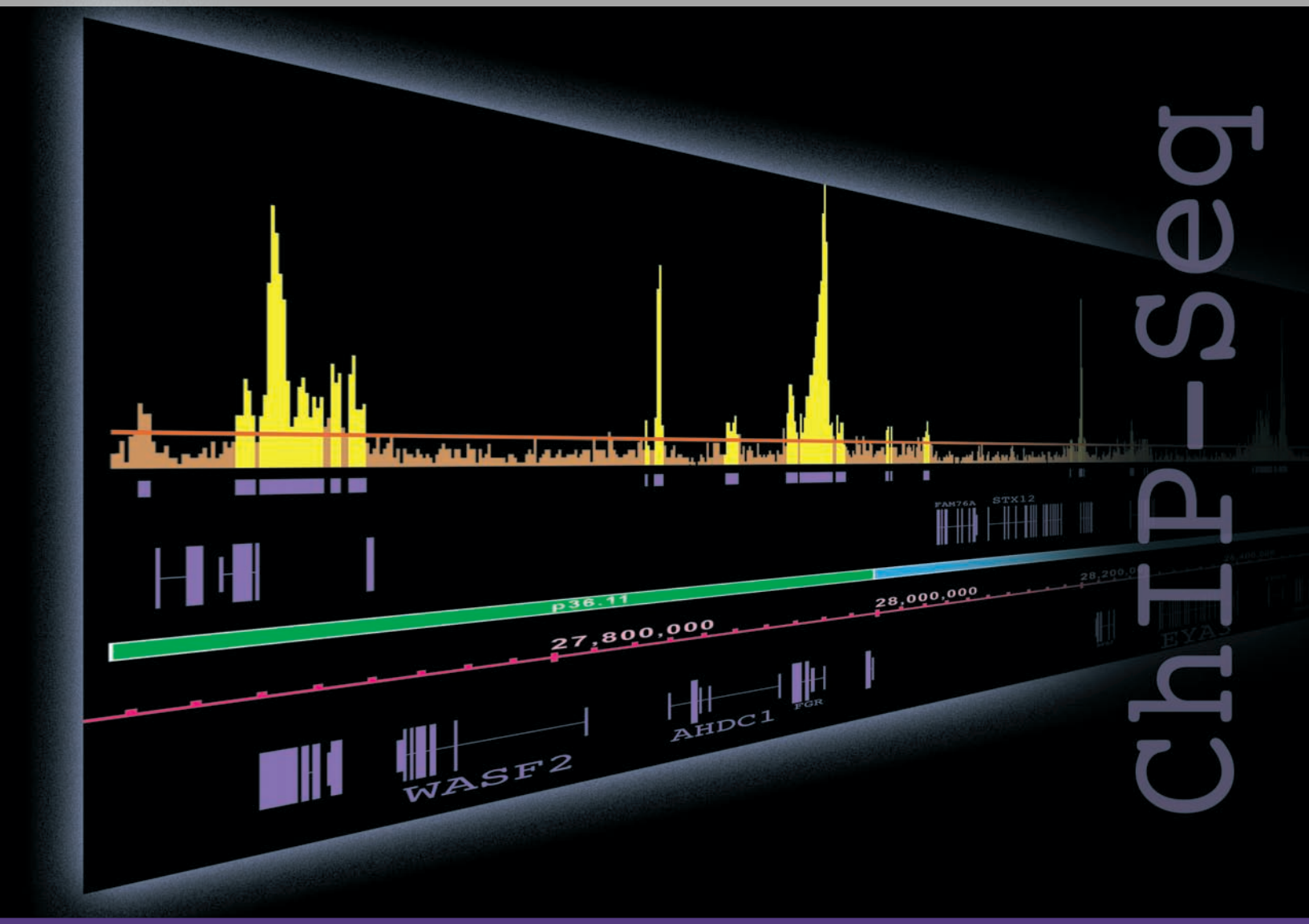


Epigenetic Services

get results that accelerate your research

ChIP-Seq

MeDIP-Seq

hMeDIP-Seq

ChIP-qPCR

ChIP Antibody Validation

RNA-Seq

ChIP-on-chip

Bisulfite Sequencing

Active Motif, the leading supplier of kits and reagents for the study of chromatin biology and epigenetics, also offers genome-wide data generation and analysis services that will help to expand your research capabilities in the areas of transcription biology, histone modifications and DNA methylation.

In addition, we offer customized assay development and specific services to optimize ChIP-based procedures, including antibody qualification and preparation of ChIP-ready samples.

ACTIVE MOTIF®

Enabling Epigenetics Research

Epigenetic Services – get results that accelerate your research

Reproducibly generating high-quality, interpretable data from chromatin immunoprecipitation (ChIP) experiments can be challenging as it requires prior knowledge of working antibodies, optimized protocols for various cell types and knowledge of cell type-specific binding sites. Add in the technical and bioinformatics challenges associated with generating whole-genome data sets, and ChIP-Seq may literally be beyond your reach. Active Motif is committed to being a leader in the field of epigenetics and to making whole-genome epigenetic data sets accessible to everyone. That's why, in addition to our wide range of kits and antibodies for chromatin, DNA methylation and transcription research, our Epigenetic Services team has over 8 years of success providing a wide variety of ChIP services. This makes it possible for you to utilize our expertise and research tools without having to be an expert in the techniques yourself. All of our services are designed to be end-to-end assays. Send us your samples and we'll send you high-quality data that will propel your research forward.

Quality control – the steps to success

The extremely high project success rate at Active Motif Epigenetic Services can be attributed to the inclusion of quality control experiments at every critical step. The data generated in each of these QC experiment enables us to determine if the results from that step are "in the normal range." If our criteria are not met, we will repeat or will optimize the experiment to try and achieve a sample that is acceptable to move forward to the next step. An outline of the critical steps and associated quality controls is provided in Figure 1.

At Active Motif Epigenetic Services we perform thousands of ChIP reactions every year. Each experiment is different containing different amounts of chromatin, different antibodies, different primers with varying efficiencies and different final volumes depending on the downstream application. In an effort to make all of our experiments comparable and establish criteria for "normal", we devised a normalization strategy that takes all of these variables into account and presents the data as Binding Events Detected / 1000 cells (Figure 2). This unique normalization strategy allows us to directly compare every experiment we perform. Signal intensity and background levels consistently fall within a expected range that enable us to determine if ChIP reactions are successful, and identify outliers that may need to be repeated.

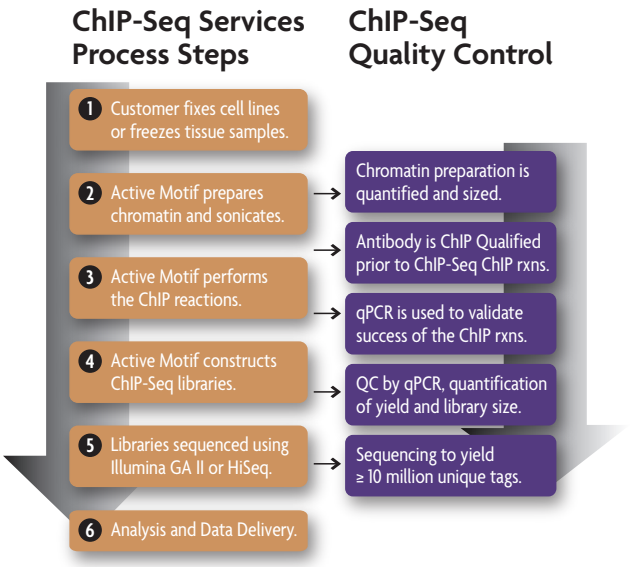


Figure 1: Quality Control Steps.
Active Motif's Epigenetic Services are design to be a complete genome-wide solution. Customers submit frozen cell pellets or animal tissues, and Active Motif performs every step of the assay including data analysis. Quality control steps are incorporated after chromatin preparation, ChIP, and library generation to ensure that sequencing will yield high-quality data.

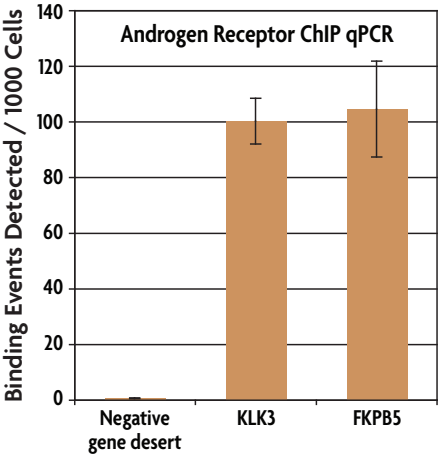


Figure 2: ChIP qPCR normalization.
Active Motif has developed a unique ChIP-qPCR normalization strategy that takes into account the starting amount of chromatin, final ChIP volume, and primer efficiency. This approach allows all ChIP reactions to be presented on the same scale; negative control primers typically give a signal below 1; enrichment of specific sites can be between 10 and 500.

FactorPath™ – mapping transcription factor binding sites across the genome with ChIP-Seq

Chromatin immunoprecipitation is a powerful tool for studying protein/DNA interactions. When combined with Next-Gen sequencing platforms such as the Illumina GA II or HiSeq, mapping all of the binding sites for a given transcription factor across the entire genome is possible. Active Motif's FactorPath™ ChIP-Seq Services have been used to:

- Map the binding of novel transcription factors that have never before been targeted in ChIP.
- Identify the consensus binding motifs of poorly characterized transcription factors.
- Map the binding of transcription factors:
  - In response to drug treatments.
  - In immune cells challenged with various pathogens.
  - In WT and KO models of important genes.

HistonePath™ – use Histone ChIP-Seq to localize specific histone modifications

The genome-wide study of histone modifications has expanded significantly in the past 10 years. There are now extensive histone maps of numerous modifications in multiple species. In addition, many of the enzymes responsible for adding and removing these modifications are now known, and some have been implicated in diseases such as cancer. Data from ChIP experiments have contributed greatly to our current understanding in this field. Advances in ChIP technology, such as ChIP-Seq, will continue to provide data sets that will ultimately help us understand mechanistically how histone modifications contribute to disease states, development and normal cellular responsiveness. Active Motif is a leading provider of histone modification-specific antibodies and our HistonePath™ Services have provided Histone ChIP-Seq data to leading academic labs and pharmaceutical companies. Our expertise in this field can bring a whole new layer of epigenetic understanding to your field of interest.

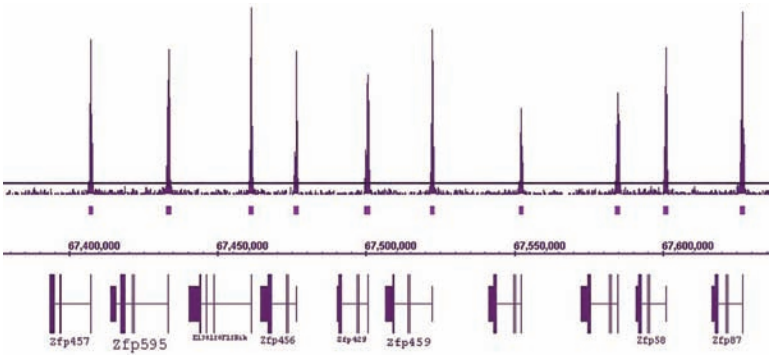


Figure 3: Histone ChIP-Seq.
ChIP–Seq was performed using chromatin from mouse livers and an antibody against H3K4me3 (Catalog No. 39159). Sequencing was performed on the Illumina GA II using 36 bp single-end reads. Genome alignment was performed with ELAND and peak calling was performed with MACS. The image above focuses on a 3 Mb window containing a Zfp gene cluster on chromosome 13. H3K4me3 peaks are present at the start site of all Zfp genes. Gene annotations run from right to left, therefore TSSs are on the right side of each gene annotation.

Data analysis – view and understand data faster

Generating data is only half the battle in ChIP-Seq. When the sequencing is complete, tens of millions of short sequence tags must be mapped back to the genome followed by peak calling. Peak calling is complicated by the fact that different algorithms are required for accurate peak calling depending on the antibody used. Thousands to tens of thousands of binding sites must then be exported into a meaningful output that relates the data to genes and allows for multiple samples to be compared to one another. Challenges continue with clustering, heat maps and graphical representations of genome-wide localization patterns. This type of in-depth bioinformatics analysis is beyond the capabilities of most labs. That is why data analysis is part of the standard package for all of our ChIP-Seq Service projects.

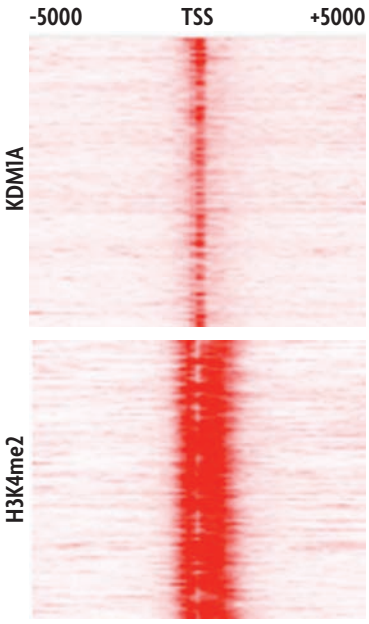


Figure 4: Compilation of all genomic binding sites relative to gene transcription start sites.
ChIP-Seq was performed with antibodies against the histone demethylase KDM1A and one of its targets H3K4me2. Thousands of binding sites were identified and the positions of all binding sites, as they relate to gene annotations, were compiled. **Left.** A heat map was created using all genes bound by H3K4me2 and KDM1A and the occupancy of these factors around the transcription start site (TSS) is depicted. **Top.** The same data depicted a different way shows KDM1A occupancy between the two peaks of H3K4me2 occupancy.

TranscriptionPath™ – change the way you look at gene expression with RNA Pol II ChIP

ChIP is routinely used to look at changes in transcription factor binding and histone modifications. While these changes are expected to correlate with changes in gene expression, they often do not due to the need for corroborating modifications and/or additional co-factor recruitment. Therefore, to determine the biological significance of changes detected by ChIP, gene expression studies should be performed in parallel. While it is common to compare RNA expression microarray data with ChIP data, it is not an ideal approach because the data is not derived from the exact same sample. In addition, information on alternative transcription start sites is not available from RNA microarray data, making the association of transcription factor binding sites to specific genes error prone. Lastly, mechanisms of post-transcriptional regulation influence RNA in ways that may not accurately reflect the changes in transcription.

As an alternate approach to RNA-based methods to measure gene expression, Active Motif's TranscriptionPath™ Service utilizes RNA Pol II ChIP. Because occupancy of RNA polymerase correlates strongly with gene transcription rates, the transcription rates of all genes can be measured in a single ChIP-Seq experiment. RNA Pol II ChIP-Seq is a valuable addition to any transcription factor or histone ChIP experiment, enabling researchers to correlate binding of TFs or histones with gene transcription.

Advantages of RNA Pol II ChIP

- Measure transcription rates without the influence of RNA half-life.
- Ideal for measuring changes in gene expression at early time points (minutes).
- Detect alternate start sites.
- Identify genes that are poised for transcriptional activation.
- Perform in parallel with transcription factor-targeted ChIP to correlate TF binding with changes in transcription.

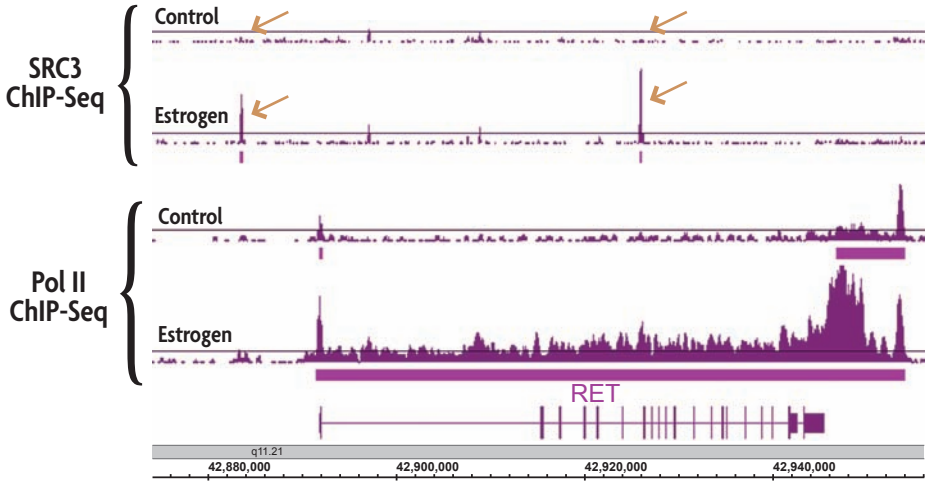


Figure 5: Changes in transcription factor binding correlate with changes in gene expression.
Performing ChIP-Seq using an antibody against RNA pol II produces a genome-wide profile of gene transcription rates. ChIP-Seq was performed using chromatin from control and estrogen treated MCF-7 cells using antibodies against RNA Pol II phosphoserine 2 and against the estrogen inducible transcription factor SRC3. Estrogen treatment induced the binding of SRC3 in the promoter and gene body of the RET gene (top 2 panels). Induced SRC3 binding correlates with induced transcription of the RET gene as measured by RNA Pol II occupancy (bottom 2 panels).

Bisulfite sequencing – determine DNA methylation status at base pair resolution

Bisulfite sequencing is a method used to reveal the methylation status of select genomic regions at base pair resolution. The method relies on sodium bisulfite treatment of double-stranded genomic DNA, which leads to deamination of unmethylated cytosines to uracils, while methylated cytosines remain unchanged. The DNA is amplified by PCR using primers that anneal to the converted DNA and the amplicons are then sequenced. This multistep method is laborious, time consuming and can be challenging, especially at the step of primer design and testing. Active Motif's Bisulfite Sequencing Service includes all steps from primer design and optimization, bisulfite conversion, PCR, cloning and sequencing to final analysis.

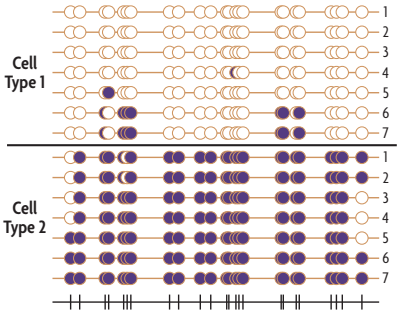


Figure 6: Bisulfite sequencing reveals differential methylation in two cell lines.
Active Motif performs custom Bisulfite sequencing on customer selected genomic locations. The assay includes primer design, bisulfite conversion, cloning, sequencing and data analysis. The data at left compares the methylation state (empty circle = unmethylated, purple circle = methylated) of a 400 bp region containing 24 CpGs in two different cell lines. Seven clones from each of the cell lines were sequenced.

ChIP antibody validation – is your antibody suitable for ChIP?

Finding an antibody that performs well in ChIP is one of the biggest hurdles to overcome when initiating a ChIP project. Many companies offer "ChIP-validated" antibodies that have not been internally tested. Furthermore, data published in journals can not always be reproduced due to variables such as differences in ChIP buffer composition, lack of controls, lot-to-lot variability and inappropriate normalizations. Traditional ChIP antibody validation using qPCR requires substantial time searching the literature for good binding sites in an appropriate cell type. In addition, a negative qPCR result does not mean that the antibody does not work in ChIP. It may simply mean that there is no binding at the genomic locations investigated. Active Motif Epigenetic Services takes the pain out of identifying a good ChIP antibody by providing a list of proven antibodies against more than 175 different targets. If your target of interest is on our list you can start your project immediately. If you want to ChIP validate your own internally developed antibody or if your target of interest is not on our validated list, we offer an antibody validation service that can give you an answer in as little as two weeks. Active Motif Epigenetic Services' antibody validation assay is a streamlined ChIP-chip protocol that quickly provides a yes or no answer (Figure 7).

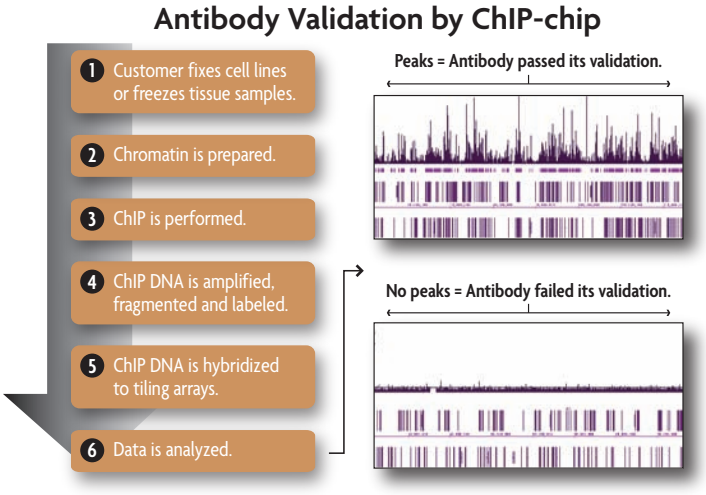


Figure 7: Antibody Validation.
Active Motif makes ChIP antibody validation simple and fast by offering a ChIP antibody validation service. Validation is performed using ChIP-chip on tiling arrays. Active Motif performs all steps of the experiment including data analysis and the performance of the antibody is assessed based on the identification of ChIP-chip peaks.

MeDIP-Seq – determine genome-wide DNA methylation patterns

The reliable identification of differential DNA methylation is important for researchers interested in biomarker identification as well as for those trying to understand the basis of disease, drug mechanism of action or environmental influences on epigenetics. To help speed your research, Active Motif now offers MeDIP-Seq as an end-to-end, genome-wide epigenetic service to identify differentially methylated regions. In MeDIP-Seq a highly specific antibody that recognizes 5-methylcytosine is used to immunoprecipitate sonicated genomic DNA, resulting in the enrichment of genomic regions that are methylated. Because 5-methylcytosine antibody binds only to methylated cytosines in the context of single-stranded DNA, the DNA must be denatured prior to immunoprecipitation. As a result of denaturation the enriched DNA can not be processed for Next-Gen sequencing using the typical sequencing library generation protocols, as these require adaptor ligation to double-stranded DNA. This problem is circumvented by ligating Next-Gen sequencing adaptors to genomic DNA prior to the immunoprecipitation. Following MeDIP, the enriched regions can be directly amplified with Next-Gen sequencing compatible primers. Unique alignment of the sequence tags across the genome reveals regions where DNA methylation resides.

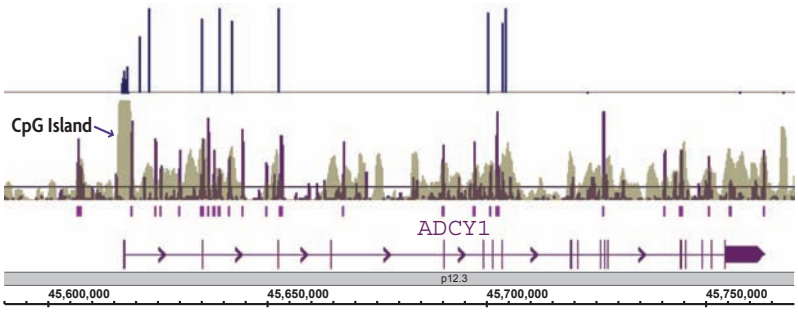


Figure 8: MeDIP-Seq data compared to CpG density and RRBS.
MeDIP-Seq was performed using 2 µg of DNA from human PBMCs. Methylation peaks were mapped across the genome and the image is zoomed in to look at the ADCY1 gene. The middle panel shows good correlation of MeDIP signal (purple bars) with CpG density (overlaid gray peaks) indicating that MeDIP covers most CpG sites across the ADCY1 gene. RRBS data is shown at the top as blue bars, and coverage is limited to only a few regions.

Several methodologies exist that detect DNA methylation on a genome-wide scale, including Reduced Representation Bisulfite Sequencing (RRBS). Although RRBS gives base pair resolution, it only interrogates ~10% of all CpGs, and is extremely biased toward heavily CpG-enriched regions. MeDIP-Seq, however, has the potential to interrogate all regions across the genome. Our MeDIP-Seq assays show high correlation with CpG density across the entire genome, indicating that high and low CpG densities are immunoprecipitated proportionately (Figure 8). The expanded genomic coverage of MeDIP-Seq compared to RRBS increases the likelihood of identifying differentially methylated regions in multi-sample studies.

Why do customers choose Active Motif Epigenetic Services?

Genome-wide analysis using Next-Gen sequencing has significantly broadened the ability of scientists to understand epigenetic events. However, antibody selection, sample preparation and data analysis can create significant barriers to incorporating these valuable techniques into your experimental repertoire. The goal of Active Motif Epigenetic Services is to make cutting-edge research accessible to the wider life science community. For over 8 years we have provided services for state-of-the-art techniques that have helped accelerate the research of scientists in academic laboratories, government institutions, biotechnology and pharmaceutical companies.

Experience – we are the only end-to-end ChIP service provider

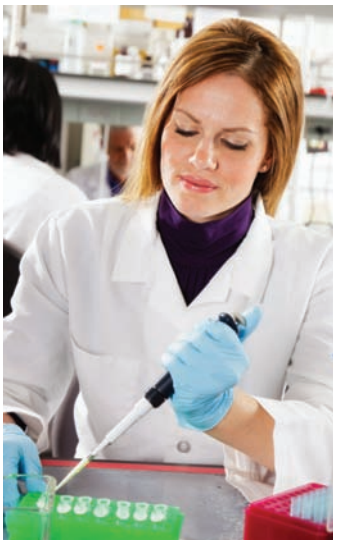
- 8 years of experience providing ChIP Services
- Over 10,000 ChIP samples processed
- Over 4,000 ChIP-on-chip tiling arrays processed
- Over 1,000 ChIP-Seq reactions processed

Expertise – don't spend weeks optimizing each step

- Antibody recommendations for 175 targets
- Chromatin prep protocols optimized for 9 species and over 25 tissues types
- Custom primer design and testing for each assay

Collaborative interaction – have control over your samples and data

- Direct access to our lab scientists
- Recommendations for experimental design and appropriate controls
- Suggestions/help with next steps



Security – protect your valuable samples, time and resources

- Active Motif Epigenetic Services qualifies all antibodies as a first step in all assays to ensure success and prevent wasting of precious samples
- Quality control criteria must be met at multiple critical steps
- Steps are repeated, optimized or assay stopped if QC is not met

High-quality data – know it is done right the first time

- ChIP is optimized for low background, high signal and consistency
- QC steps effectually ensure final ChIP-Seq data is of high quality

Bioinformatics support – answer important biological questions faster

- Data for all assays are delivered fully analyzed
- ChIP-Seq data analysis includes peak calling
- Data delivered in several visual formats and custom Excel output
- Quick gene list generation