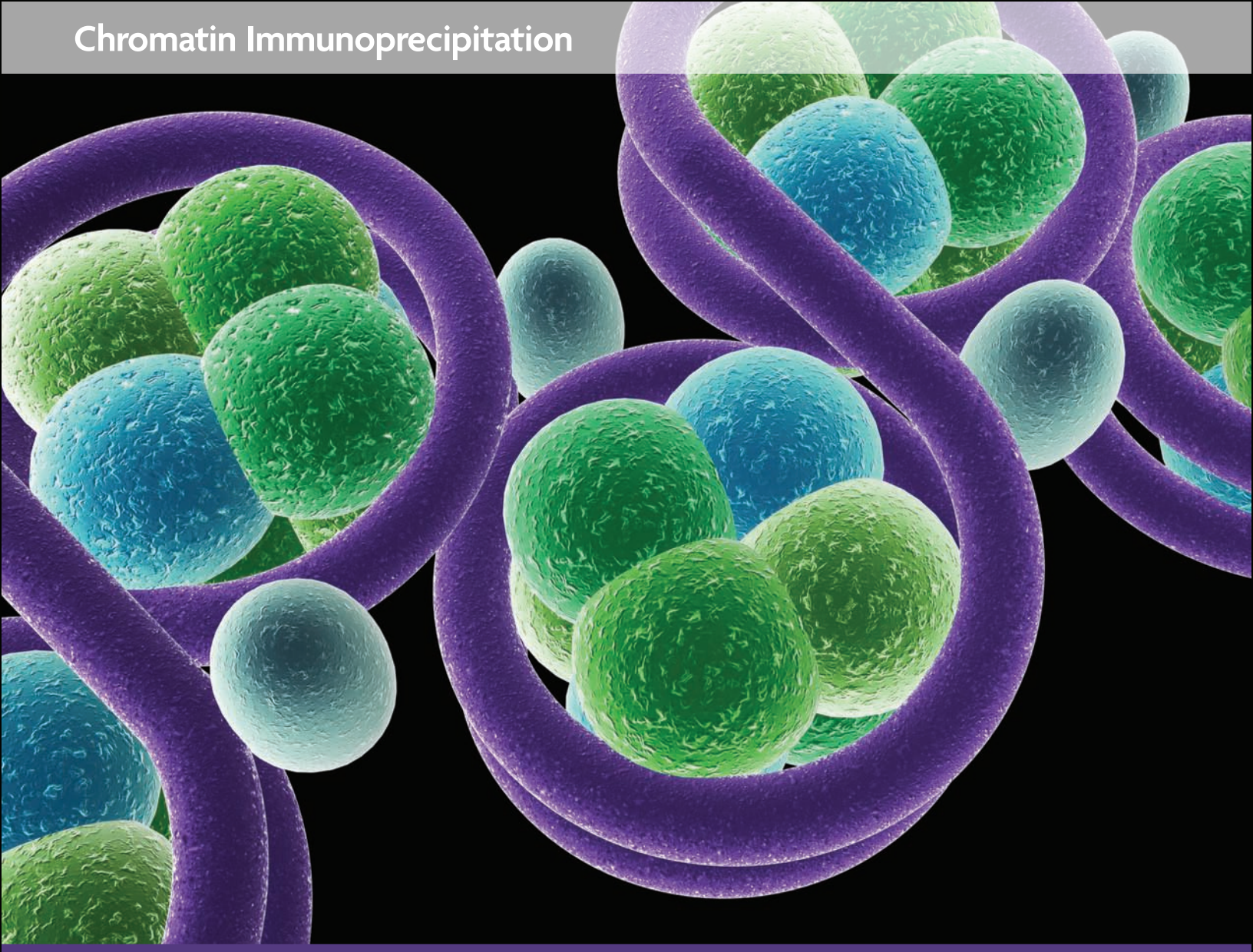
Chromatin Immunoprecipitation



ChIP-IT® Express

the innovation leader in ChIP products

RNA ChIP

Sequential ChIP

High-throughput ChIP

ChIP-validated Antibodies

ChIP Controls & qPCR Primers

EpiShear™ ChIP Sonicators

ChIP DNA Purification

ChIP-Seq Services

Active Motif's extensive line of ChIP-IT® Express Kits and reagents utilize the many innovations we have developed to make ChIP faster, simpler and more reproducible.

We also offer large, rapidly growing collections of ChIP-validated antibodies, qPCR primer sets and controls. Additionally, our Epigenetics Services can perform the latest ChIP techniques for you.

ACTIVE MOTIF®

Enabling Epigenetics Research

The Innovation Leader in ChIP products

Active Motif has consistently been the leader in introducing innovations that make ChIP faster, simpler and more reproducible. We were the first to introduce magnetic beads into a ChIP kit, the first to offer enzymatic chromatin shearing and, building on these improvements, the first with kits for performing sequential, high-throughput and RNA ChIP. We continue to expand our ChIP product line by focusing on providing a complete selection of proven reagents and equipment for ChIP, including the largest collection of antibodies validated for ChIP, ChIP-Seq and ChIP-chip. In addition, we recognize that as ChIP has transitioned to a genome-wide technology, not every lab has the equipment or know-how required to perform the latest techniques. To enable our customers to keep up in this rapidly evolving field, we offer end-to-end Epigenetic Services such as ChIP-Seq, MeDIP-Seq and ChIP antibody validation. Customers can send samples to Active Motif and weeks later receive fully analyzed data.

ChIP-IT® Express – chromatin immunoprecipitation made easy

Chromatin immunoprecipitation (ChIP) is a powerful tool for studying protein/DNA interactions. It is the most-widely cited epigenetic technique and can be used to generate both single-gene and genome-wide data. However, ChIP can be technically challenging and yield results that are difficult to interpret. In order to minimize the challenges associated with ChIP and help ensure your success, Active Motif offers its ChIP-IT® Express Kits. Each kit features a streamlined protocol that reduces the amount of sample required and minimizes the background signal obtained. Consequently, ChIP-IT Express Kits are the most widely published ChIP kits available (Figure 1).

In the ChIP-IT Express method, intact cells are fixed using formaldehyde, which cross-links and therefore preserves protein/DNA interactions. The DNA is sheared into small fragments using either sonication or enzymatic digestion. The protein/DNA complexes are then immunoprecipitated using antibodies directed against DNA-binding proteins or histone modifications. Following immunoprecipitation, the cross-linking is reversed and the enriched DNA fragments are analyzed by qPCR or in whole-genome applications such as ChIP-Seq (Diagram 1).

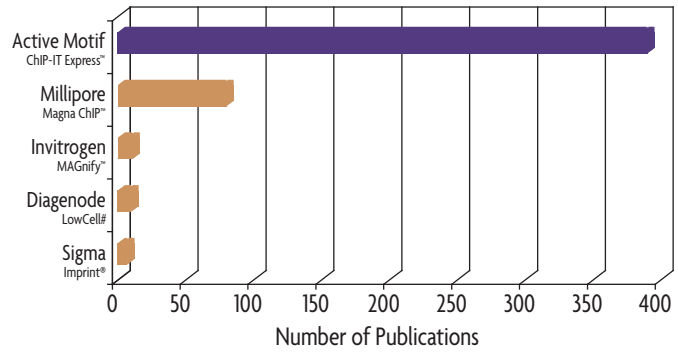


Figure 1: ChIP-IT Express Kits are cited in more publications than any other manufacturer's ChIP kit.
Trade name searches were performed on HighWire Press (http://highwire.stanford.edu/) for ChIP kit journal citations.

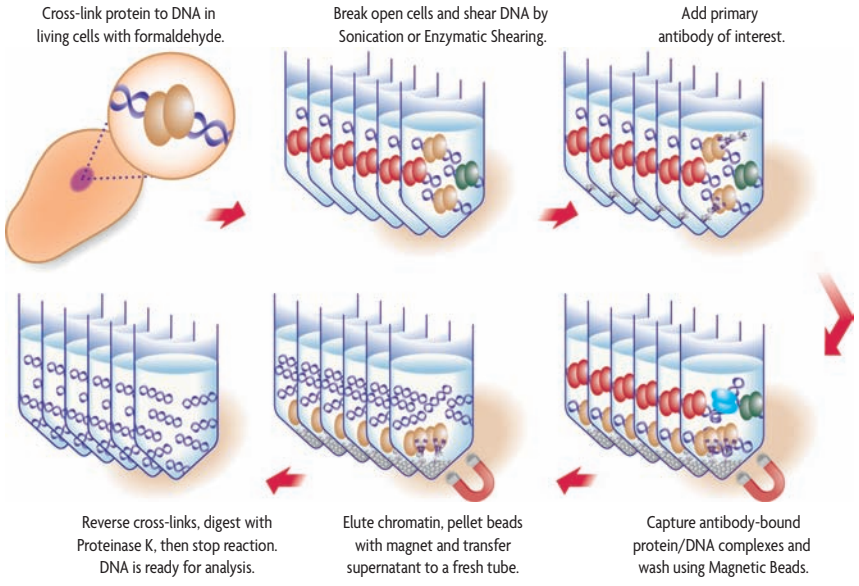


Diagram 1: Schematic of chromatin immunoprecipitation using ChIP-IT Express.
In ChIP, protein/DNA interactions are fixed, and the DNA is sheared and precipitated using an antibody. After cross-link reversal, the DNA is analyzed, usually by qPCR, to determine which genes were bound by the protein of interest.

ChIP-IT Express is a faster, more streamlined method for successful ChIP

ChIP-IT Express Kits improve on traditional ChIP by reducing or eliminating several time-consuming steps. Our kits were the first to utilize magnetic beads, which have 4much lower background binding than traditional agarose beads. This reduced background has made it possible to eliminate some of the steps required in more conventional protocols like pre-clearing and blocking. Washing is much easier because the spin steps have been replaced by rapid magnetic pull-down. Collectively, all of the improvements included in ChIP-IT Express give you the capability to perform your reactions in PCR tubes with a multi-channel pipettor, which reduces your effort and improves consistency (Figure 2).

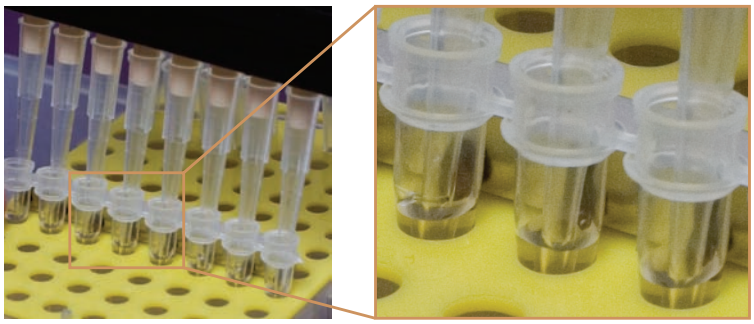


Figure 2: Multiple sample ChIP using ChIP-IT Express.
Washing the magnetic beads is fast and easy because the pellet forms against the side of the tube above the level of the supernatant. This speeds the procedure, eliminates sample loss and enables you to ChIP multiple samples in 8-well PCR tubes using a multi-channel pipettor.

(A bar magnet is included with all ChIP-IT Express Kits, but you can also use commercially available magnetic stands.) For more complete information on ChIP-IT Express Kits, please give us a call or visit our website at www.activemotif.com/chip.

Efficient ChIP enables you to use fewer cells

ChIP is an enrichment technique, not a purification method. Most marks of interest exist throughout the genome, but in different relative proportions. Consequently, meaningful results require efficient enrichment. Conventional ChIP requires at least two million cells as starting material, which can be problematic with some cell lines, and labor-intensive even in the best case. ChIP-IT Express Kits have been optimized to provide superior target gene enrichment, resulting in unmatched sensitivity. The kits can routinely produce excellent results with chromatin from as few as 100,000 cells (Figure 3).

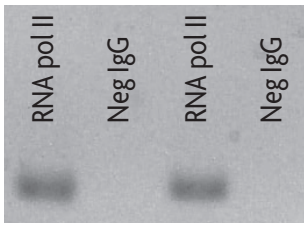


Figure 3: ChIP-IT Express works with 100,000 cells.
ChIP-IT Express was performed in duplicate on sonicated HeLa cell chromatin (1.0 x 10^5 cell equivalents per ChIP). Two µg of RNA pol II and Neg IgG antibody was used for IP. GAPDH PCR primers were used to analyze the immunoprecipitated DNA. DNA isolated with RNA pol II antibody generated more product than the negative control IgG. These results demonstrate that positive ChIP data was obtained from 100,000 cells.

ChIP-IT Express advantages

- **Reliability** – ChIP-IT Express Kits are the most widely cited ChIP kits
- **Faster procedure** – magnetic capture is much faster than centrifugation
- **Simplified wash steps** – improved consistency and dramatically reduced hands-on-time
- **No pre-blocking needed** – magnetic beads are inert and exhibit low binding of proteins
- **Less effort required** – our kits are compatible with multi-channel pipettors to streamline wash steps

Shear chromatin with your choice of sonication or enzymatic digestion

Chromatin shearing is most commonly done by sonication. However, sonication can be difficult to optimize due to emulsification and overheating of the sample. Because of this, or if you don't have a sonicator, Active Motif has developed a robust, user-friendly method to shear chromatin by enzymatic digestion (Figure 4). Because Active Motif offers both ChIP-IT Express and ChIP-IT Express Enzymatic Kits, you can choose whichever shearing method you prefer. (Stand-alone shearing kits are also available.)

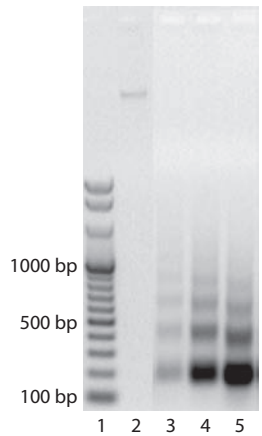


Figure 4: Analysis of enzymatically sheared DNA.
HeLa cells were fixed for 10 minutes with formaldehyde and then chromatin was prepared using the Enzymatic Shearing Kit protocol & reagents. Chromatin was sheared with the Enzymatic Shearing Cocktail for 5, 10 & 15 minutes before the reaction was stopped.

Lane 1: 100 to 1000 bp ladder.
Lane 2: Unsheared HeLa DNA.
Lane 3: HeLa DNA treated for 5 minutes.
Lane 4: HeLa DNA treated for 10 minutes.
Lane 5: HeLa DNA treated for 15 minutes.

ChIP-validated antibodies – guaranteed to work in ChIP

Active Motif is committed to providing the highest quality antibodies for studying chromatin and the biology of the nucleus, especially antibodies validated for use in chromatin IP (Figure 5). We specialize in manufacturing antibodies against histones, histone modifications and chromatin proteins. Because ChIP is an extremely challenging technique, only antibodies of the highest quality that recognize the target protein in its native, chromatin-associated context will work. Unlike other antibody "suppliers", we manufacture and test our own antibodies, so we guarantee they will perform as specified. Simply let us know if you are not satisfied. For a complete listing of our ChIP-validated antibodies, please visit our website at www.activemotif.com/chipabs.

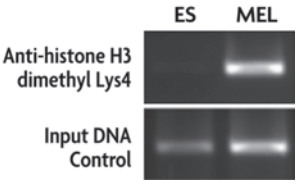


Figure 5: ChIP of methylated histone H3.
ChIP was performed using chromatin prepared from mouse embryonic stem cells (left lane) or mouse erythroleukemia cells (right lane) with H3K4me2 pAb (Cat. No. 39141). PCR primers specific for β-globin were used to amplify a 210 bp region of the promoter.

ChIP-IT Control Kits help ensure successful ChIP and simplify antibody validation

ChIP is a method of enrichment of DNA bound by a particular protein, not a complete purification of the protein-bound DNA. As a result, ChIP reactions are unavoidably contaminated with non-specifically captured chromatin. This can lead to false positive PCR products that make data interpretation difficult. To solve this problem, Active Motif offers species-specific (human, mouse and rat) ChIP-IT Control Kits for real-time or endpoint PCR. Incorporation of all of the controls into your ChIP experiment allows you to evaluate your success at multiple steps. The control kits help confirm the chromatin preparation and immunoprecipitation procedures worked properly and enable you to assess the quality of your test antibody and PCR reactions.

The ChIP-IT Control qPCR Kits include a positive control antibody, a bridging antibody to enhance binding affinity of mouse monoclonal antibodies, a negative control antibody to evaluate non-specific binding, and species- specific positive and negative control qPCR primers. The positive and negative control primers allow you to show that the ChIP worked and to establish background binding levels within the same ChIP. Negative primers can be a more relevant control than IgG as different antibodies will have different background binding capacities. (Figure 6).

Our original ChIP-IT Control Kits are designed for use in endpoint PCR. Each kit provides positive and negative control antibodies, a positive control PCR primer set as well as a PCR buffer/DNA loading dye that makes your PCR reactions ready to run on the gel straight out of the thermocycler. Please visit www.activemotif.com/chipcontrols for details.

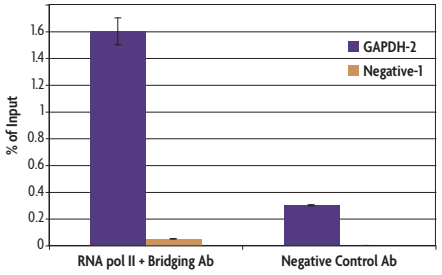


Figure 6: qPCR analysis of human myeloma LP1 ChIP.
ChIP was performed on 5 µg of chromatin from human LP1 cells using the ChIP-IT Express Kit and the positive & negative control antibodies and the positive & negative control qPCR primers that are supplied in the ChIP-IT Control qPCR Kit – Human.

Convenient collections of species-specific qPCR primers and Ready-to-ChIP Chromatin

As detailed above, ChIP data interpretation is highly dependent on the use of controls. But, identifying appropriate controls is a challenging undertaking. Accessing this information in the published literature is time consuming and may not be relevant to your cell system. To help, Active Motif Epigenetic Services has compiled data from thousands of ChIP experiments and developed human, mouse, rat, Zebrafish, *Drosophila* and yeast positive and negative control qPCR primer sets for many common ChIP targets, including histone modifications (Figure 7), histone modifying enzymes, RNA Pol II and methylated DNA. To see the full list of over 35 available ChIP Control qPCR Primer Sets and their targets, visit www.activemotif.com/chipprimers.

We also offer Ready-to-ChIP Chromatin from a number of ENCODE cell lines that are optimally sheared by sonication and ChIP-validated. The chromatin can be used with all ChIP-IT Express Kits and controls, so you can be certain that the only variable when validating a new ChIP antibody is the antibody itself. See www.activemotif.com/chipready.

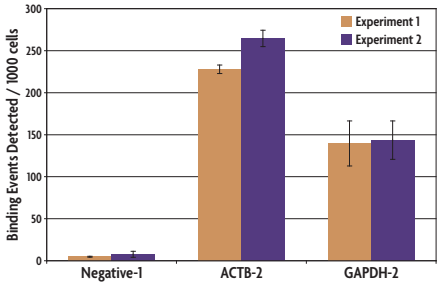


Figure 7: qPCR with positive & negative primer sets.
ChIP was performed in duplicate on HeLa chromatin with ChIP-IT Express and H3K4me3 pAb (Cat. No. 39159), then the Human Negative-1 (Cat. No. 71001), Human ACTB-2 (Cat. No. 71005) and GAPDH-2 (Cat. No. 71006) Positive Control Primer Sets were used for qPCR.

Epigenetic Services – ChIP-Seq, MeDIP-Seq and Antibody Validation Services

Genome-wide ChIP applications require extensive optimizations at multiple steps including chromatin preparation, antibody validation, background reduction and sequencing library construction. The investment in time and money is significant and the quality of the final data is not known until the entire project is completed. To take the guesswork out of generating high-quality data, Active Motif Epigenetic Services offers a variety of genome-wide data generation and analysis services such as MeDIP-Seq, ChIP-Seq (Figure 8), and ChIP antibody validation (Figure 9). Customers send cell pellets and receive fully analyzed ChIP-Seq data. Active Motif performs the chromatin preparation, antibody validation, ChIP, library generation, sequencing and data analysis. Quality control steps incorporated at multiple points in the process ensure high-quality data upon completion of the project. For more information on all of the services available, please visit www.activemotif.com/services.

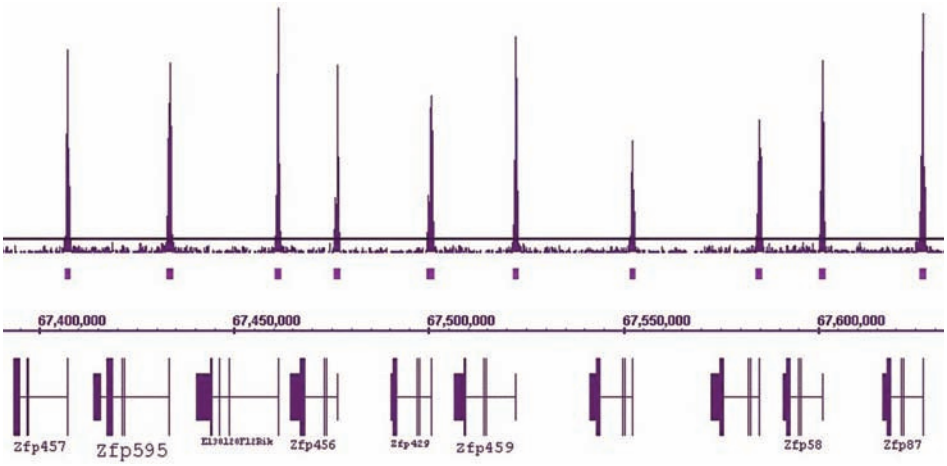


Figure 8: ChIP-Seq of a specific histone modification.
ChIP–Seq was performed using chromatin from mouse livers and an antibody against H3K4me3 (Cat. No. 39159). Sequencing was performed on the Illumina GA II using 36 bp single-end reads. Genome alignment was performed with ELAND and peak calling was 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.

Antibody validation for ChIP-Seq and ChIP-chip

The field of epigenetics has grown rapidly over the past 10 years. Chromatin immunoprecipitation has evolved from a technique used to explore transcription factor binding and epigenetic changes at single genes using PCR to a technique capable of profiling the entire genome. Active Motif believes that antibodies should be validated for the applications in which they will be used. In addition to the existing ChIP PCR and specificity testing we do on every new antibody we produce, we have undertaken an effort to validate all new antibodies in ChIP-Seq and/or ChIP-chip. We are also re-validating our existing histone modification and transcription factor antibodies in these applications. An updated list of our ChIP-Seq and ChIP-chip validated antibodies is available at www.activemotif.com/chipseqabs.

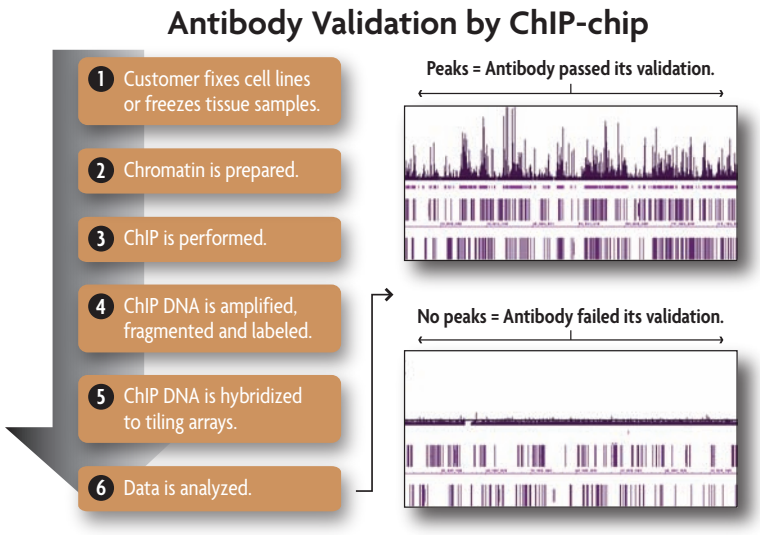


Figure 9: Flow chart of the steps in our ChIP-chip antibody validation process.
Active Motif makes ChIP antibody validation simple and fast by offering a ChIP antibody validation service, which we also employ on our own antibodies. Validation is performed using ChIP-chip on tiling arrays, as shown above, or by ChIP-Seq. 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.

Consistent improvements have lead to the development of new ChIP methods

Active Motif has consistently been the leader in introducing improvements that make chromatin IP simpler and more reproducible. We were the first company to develop and introduce a ChIP kit employing magnetic beads. We were also the first to produce complete ChIP kits containing all critical components, including antibody and PCR primer controls. This lead to the release of more convenient species-specific control kits. We were also the first to introduce enzymatic chromatin shearing. All of these innovations have made ChIP faster, easier and more successful.

We have strengthened our position as the leader in developing innovative ChIP products by applying these improvements to the creation of kits for performing high-throughput ChIP, sequential ChIP and RNA ChIP. The methods provided in these kits will help you to expand the utility of the ChIP technique.

High-throughput ChIP a reality with ChIP-IT Express HT

If you need to perform many ChIP experiments at once, ChIP-IT Express HT is your solution. It combines the time-saving, magnetic bead-based protocol of ChIP-IT Express with a high-throughput, 96-well microplate-based format (Figure 10). With ChIP-IT Express HT you can efficiently process up to 96 ChIP reactions simultaneously (Figure 11). ChIP-IT Express HT is compatible with our enzymatic and sonication-based shearing kits, as well as with our control kits, qPCR primers and Ready-to-ChIP Chromatin. For information, please visit www.activemotif.com/htchip.

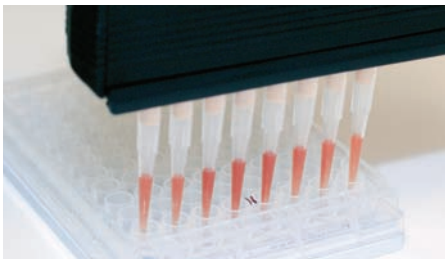


Figure 10: True high-throughput ChIP.
With magnetic beads, optimized buffers and the efficient plate-based protocol of ChIP-IT Express HT, you can process up to 96 ChIP reactions at a time.

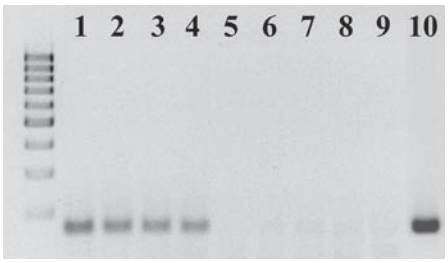


Figure 11: High-throughput ChIP performed on HeLa chromatin using ChIP-IT Express HT.
PCR carried out using GAPDH-specific primers. Lanes 1-4: ChIP using 2 µg RNA Pol II antibody. Lanes 5-8: ChIP using normal mouse IgG as a negative control. Lane 9: no DNA control. Lane 10: input DNA control.

Identify protein co-localization *in vivo* using sequential chromatin IP

When performing ChIP experiments to decipher the Histone Code, it is often useful to prove that two different proteins or histone modifications co-occupy the same site in the genome. Alternatively, you may want to determine if a protein coincides with a specific histone modification at the same regulatory element. Sequential ChIP (also called Re-ChIP) is a technique in which successive chromatin immunoprecipitation reactions are performed using two different antibodies, enabling you to assay for the simultaneous presence of two proteins or distinct histone modifications at the same genomic region of interest. Performing sequential chromatin IP was technically challenging and difficult, until now. Active Motif's Re-ChIP-IT Kit has been optimized for sequential ChIP (Figure 12), enabling you to perform this difficult technique as easily as our standard ChIP-IT Kits, and with the ability to use ChIP-IT controls. Please visit www.activemotif.com/rechipit.

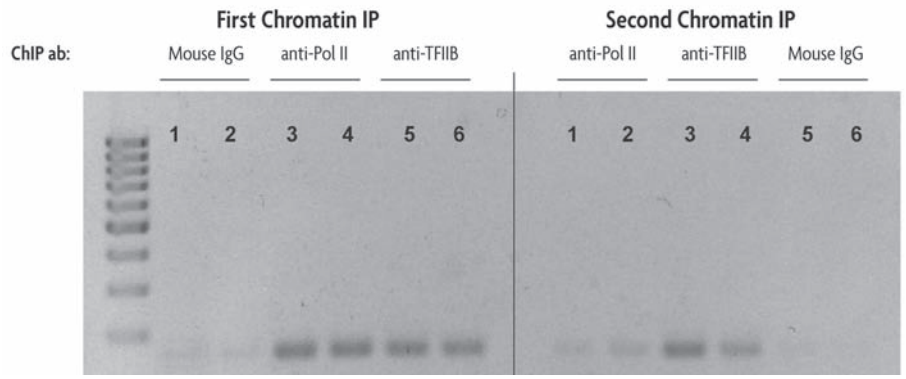


Figure 12: Sequential chromatin immunoprecipitation using Re-ChIP-IT.
The lane numbers are the same in each panel to indicate that the DNA is from the same chromatin sample. The left panel shows the results of PCR performed on an aliquot of DNA removed from the experiment after the first ChIP step; the right panel represents PCR results on DNA from chromatin samples after both ChIP steps. For example, chromatin samples subjected to first ChIP using Mouse IgG as a negative control (lanes 1 and 2 in the left panel) were then subjected to a second ChIP with an RNA Pol II antibody (lanes 1 and 2 in the right panel). Chromatin samples in which Mouse IgG was used as either the first antibody (lanes 1 and 2) or second antibody (lanes 5 and 6) show little amplification of GAPDH DNA in either the left (first ChIP) or right panel (first and second ChIP). Chromatin samples in which the first antibody used was anti-RNA Pol II and the second antibody was anti-TFIIB (lanes 3 and 4) show good amplification of GAPDH DNA after the second ChIP (right panel) indicating co-localization of RNA Pol II and TFIIB at the same region of the GAPDH promoter.

Optimized method to study RNA/protein interactions in a chromatin context

Evidence is building that RNA-directed processes play a critical role in orchestrating chromatin architecture and epigenetic memory. Nucleic acids purified from chromatin are 2-5% RNA; these RNAs are non-coding sequences that play important roles in chromatin structure and transcriptional silencing. However, characterizing these RNAs using conventional ChIP techniques is difficult due to the complexity of chromatin and the large amount of DNA present in chromatin.

To make the characterization of the role of RNA in genome regulation possible, Active Motif has leveraged its expertise in ChIP to develop the first of its kind kit for RNA-ChIP. The RNA ChIP-IT Kit was designed to study RNA-protein interactions in a chromatin context, and optimized to recover RNA for real-time RT-PCR analysis. The kit utilizes magnetic beads for the RNA capture, which improve results while reducing time and effort.

RNA ChIP-IT uses a modified ChIP protocol that has been optimized for RNA preservation and recovery. RNA-protein interactions are fixed with formaldehyde, and chromatin shearing is combined with DNase treatment to yield RNA/protein complexes that can be immunoprecipitated with antibodies to specific proteins. Cross-links are subsequently reversed; RNA is recovered and again treated with DNase to ensure the absence of DNA. The optimized method is quick and has been successfully used to study several non-coding RNAs in the chromatin context (Figure 13). For complete information, please visit us at www.activemotif.com/rnachip.

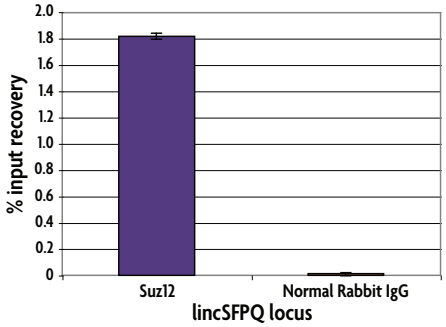


Figure 13: Percent recovery of RNA-ChIP samples. The RNA ChIP-IT Kit was used on 10 µg samples of DNase I-treated HeLa chromatin with 10 µl of Suz12 antibody (Cat. No. 39357) and 2 µg of Normal Rabbit IgG. Real-time RT-PCR was performed using primers for the lincRNA SFPQ locus, and the % input recoveries were then calculated and plotted above. Dividing the input recovery of the Suz12 antibody by that of the rabbit IgG indicates a 141-fold enrichment of the lincSFPQ region with Suz12 antibody. These results suggest that the SFPQ lincRNA is a structural component of the Suz12-containing complex, PRC2.

Rapid, simple purification of DNA from your ChIP experiments

The use of ChIP in combination with a variety of analysis techniques such as qPCR, ChIP-Seq and ChIP-chip can yield a tremendous amount of information regarding the distribution of transcription factors and histone modifications. But, data quality in these downstream applications is directly linked to the quality of the ChIP DNA.

While Phenol/chloroform extraction followed by ethanol precipitation is considered the gold standard in terms of DNA recovery and purity, the technique uses dangerous chemicals and is time consuming. Standard column-based DNA purification kits have been tried as an alternative, but are often not compatible with some components of ChIP elution buffers.

In contrast, Active Motif's Chromatin IP DNA Purification Kit is a column-based DNA purification method that is compatible with our ChIP-IT Express Kits and other ChIP protocols. Clean up is quick, easy, reliable and reproducible. The quality of the purified DNA is equivalent to that isolated by phenol/chloroform extraction and ethanol precipitation (Figure 14). In addition, the kit can be used to purify methylated DNA enriched using Active Motif's MethylCollector™, UnMethylCollector™, MeDIP and hMeDIP Kits. For more complete information, please visit www.activemotif.com/dnapure.

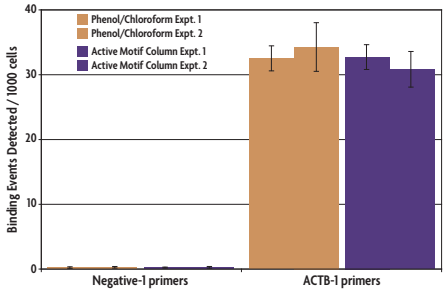


Figure 14: ChIP DNA Purification columns perform equivalent to phenol/chloroform extraction. ChIP was performed in duplicate using ChIP-IT Express, RNA pol II phospho Ser2 mAb (Cat. No. 61083) and chromatin from REC1 cells. After the cross-links were reversed, half of each reaction was purified by phenol/chloroform extraction followed by ethanol precipitation. The other half was purified using the Chromatin IP DNA Purification Kit. The purified ChIP DNA was then used in qPCR with negative primers that amplify a region in a gene desert (Cat. No. 71001) and positive control primers that amplify a region in the ACTB gene (Cat. No. 71003).

EpiShear™ sonication products make it easier to reproducibly shear chromatin for ChIP

Active Motif's EpiShear™ line of sonication products were designed to save you time and effort while ensuring that you obtain more reproducible results when shearing chromatin or DNA for use in ChIP, DNA methylation studies and Next-Gen sequencing.

The **EpiShear Multi-Sample Sonicator** (Figure 15) is a high-intensity cup horn sonicator that can process up to eight vials (20 µl - 1.2 ml sample per vial) simultaneously. It comes with a sound enclosure and, optionally, a thermoelectric chiller. For complete information, please visit us at www.activemotif.com/cuphorn.

While the **EpiShear Probe Sonicator** is a compact, economical unit (Figure 16), it is still a fully programmable unit with all the features needed to help ensure reproducible results. It is supplied with a 1/8" microtip probe that enables you to shear one sample at a time (500 µl to 15 ml). Other size probes are available that expand its range from 200 µl to 50 ml. For more information, please visit www.activemotif.com/probe.

Both sonicators are controlled by microprocessor units that offer both programmable and manual operation. Each has a keypad and digital display that make it easy to program the amplitude and set the total sonication time and duration of On and Off pulse cycles. Pulse intensity can be set from 20-100%, enabling you to optimize exact parameters for all of your cell types.

The **EpiShear Cooled Sonication Platform** improves sample-to-sample reproducibility when using any probe sonicator by positioning the tip of the probe at the same depth in the sample for each sonication. The platform has been machined out of stainless steel and aluminum, and includes a hand crank, a height counter and a vertical alignment tool. After determining your optimal settings, you can recreate them every time.

The platform can be used in a sound enclosure or on its base, and includes a Tube Cooler (available for microfuge, 15 ml and 50 ml tubes). These keep the sample cold during sonication, so you don't need to move the sample to and from an ice bath during sonication. With a programmable sonicator like the EpiShear Probe Sonicator, you can simply set the duration of the On and Off pulses, press Start, then walk away. For more information, visit www.activemotif.com/platform.

Figure 15: The EpiShear Multi-Sample Sonicator / Chiller.
The EpiShear Multi-Sample Sonicator / Chiller includes a powerful 750-watt generator with a keypad and digital display that make it easy to program and monitor sonication. The unit's cup horn sonicator is housed in a compact sound enclosure to reduce sonication noise. The cup horn can process up to 8 samples simultaneously, which rotate continuously to ensure all samples are processed equally. It is mounted in an acrylic bath that is filled with water. The bath and enclosure are plumbed with quick-connect hoses that attach to a thermoelectric chiller, which keeps the samples at 2-4°C during sonication.

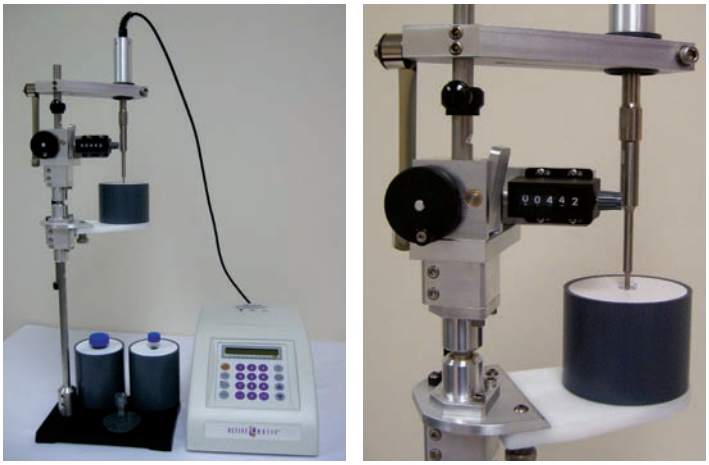


Figure 16: The EpiShear Probe Sonicator and the EpiShear Cooled Sonication Platform.
The EpiShear Probe Sonicator is a compact 120-watt unit that can process small or large samples. The EpiShear Cooled Sonication Platform, sold separately, precisely positions the probe within each sample, greatly enhancing sample-to-sample reproducibility.

Figure 17: Dounce homogenizer with two pestles.

Whether you shear your chromatin by sonication or enzymatically, the first step in preparing your sample is cell lysis. Active Motif's **Dounce Homogenizers** (Figure 17), which physically disrupt the cell membranes, are the best way to prepare lysates that are of the highest quality, yield and concentration.