

CHIP-IT™ Express

Magnetic Chromatin Immunoprecipitation Kits

(version D2)

Catalog Nos. 53008 & 53009

Active Motif North America

1914 Palomar Oaks Way, Suite 150

Carlsbad, California 92008, USA

Toll free: 877 222 9543

Telephone: 760 431 1263

Fax: 760 431 1351

Active Motif Europe

104 Avenue Franklin Roosevelt

B-1330 Rixensart, Belgium

UK Free Phone: 0800 169 31 47

France Free Phone: 0800 90 99 79

Germany Free Phone: 0800 181 99 10

Telephone: +32 (0)2 653 0001

Fax: +32 (0)2 653 0050

Active Motif Japan

Azuma Bldg, 7th Floor

2-21 Ageba-Cho, Shinjuku-Ku

Tokyo, 162-0824, Japan

Telephone: +81 3 5225 3638

Fax: +81 3 5261 8733

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Overview

Chromatin Immunoprecipitation (ChIP) is a powerful tool for studying protein/DNA interactions^{1,2}. In this method, intact cells are fixed using formaldehyde, which cross-links and preserves protein/DNA interactions. The DNA is then sheared into small, uniform fragments using either sonication or enzymatic digestion and specific protein/DNA complexes are immunoprecipitated using an antibody directed against the DNA-binding protein of interest. Following immunoprecipitation, cross-linking is reversed, the proteins are removed by treatment with Proteinase K and the DNA is recovered. The DNA is then analyzed to determine which DNA fragments were bound by the protein of interest (see Figure 1 on page 2).

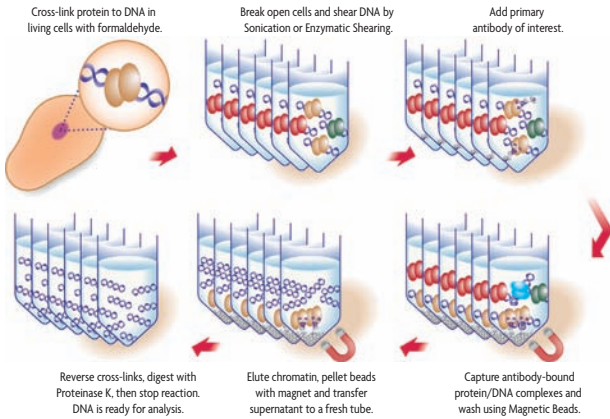
ChIP is extremely useful for the study of chromatin biology and transcriptional regulation because it enables localization of chromatin proteins, modified histones and transcription factors that are bound to specific DNA regions in specific cells. Furthermore, because protein/DNA interactions are fixed while in an endogenous, chromosomal context, ChIP results reflect the influence of chromosomal topology and the effects of cellular regulatory proteins^{3,4,5}.

ChIP can be technically demanding. The method requires high-quality antibodies to recognize the fixed, target-bound proteins of interest, and an efficient reagent (usually protein A or G agarose beads) to precipitate the antibody/chromatin complex. In addition, specialized buffers, inhibitor cocktails and blocking reagents are required to minimize non-specific enrichment and reduce protein degradation.

Active Motif's ChIP-IT™ Express Kits contain proven ChIP reagents that provide a complete solution for convenient, accurate monitoring of protein/DNA interactions. The ChIP-IT Express Kits utilize protein G-coated magnetic beads, which have made it possible to greatly simplify and streamline the ChIP protocol. A number of steps have been reduced in length or completely eliminated (see page 3 for details), making ChIP-IT Express an extremely rapid and efficient way to perform ChIP.

product	format	catalog no.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014

Flow Chart of Process & Example



GAPDH Primers

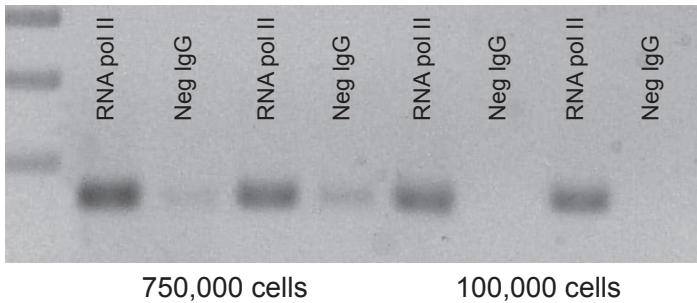


Figure 1: Improved Chromatin immunoprecipitation using ChIP-IT Express.

In ChIP-IT Express, cells are treated with formaldehyde to fix protein/DNA interactions and then the fixed chromatin is sheared by either sonication or enzymatic digestion. The sheared chromatin is incubated with an antibody directed against a protein of interest, and antibody-bound protein/DNA complexes are precipitated through use of magnetic Protein G-coated beads. The captured chromatin is then eluted and the cross-links are reversed so that the released DNA is ready for PCR analysis to determine which DNA regions were bound by the protein of interest.

A PCR analysis of immunoprecipitated DNA is shown. This figure was selected to demonstrate the efficiency of the ChIP-IT Express Kit. Typically ChIP requires between 1-3 million cells per reaction. However, we have been able to reduce the amount of starting material, scale down the volumes used to prepare the chromatin and then observe positive ChIP data from 100,000 cells or less.

HeLa cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared by sonication shearing (5 pulses). ChIP was performed in duplicate on chromatin isolated from 100,000 and 750,000 cells using a Negative Control IgG and an RNA pol II antibody. The DNA isolated through these ChIP reactions was then analyzed by 36 cycles of PCR using GAPDH positive control primers. (These antibodies and primers are available as the ChIP-IT Control Kit – Human. Kits for mouse and rat are also available; see Related Products.) Ten μ l of each PCR was separated on a 1% agarose gel and visualized by UV-illumination following ethidium bromide staining. PCR using the GAPDH primers on DNA isolated with the RNA pol II antibody reproducibly generated more product than similar reactions performed on DNA isolated using the Negative Control IgG. These results demonstrate that ChIP performed with RNA pol II antibody greatly enriched for GAPDH promoter DNA, while ChIP performed with negative IgG did not.

ChIP-IT Express Advantages

Complete kits for rapid and efficient ChIP

- Shorter protocol and dramatically reduced hands-on time
- The possibility to perform numerous ChIP experiments simultaneously
- Compatible with multi-channel pipetting

The ChIP-IT Express and ChIP-IT Express Enzymatic Kits provide reagents and protocols to simplify all aspects of the chromatin immunoprecipitation procedure. The kits can be used to prepare chromatin, determine optimal conditions for shearing chromatin and perform ChIP reactions.

ChIP-IT Express Kits contain components to make 15 chromatin preparations, and quantities of all other components to perform 25 ChIP reactions. The included protein-G coated magnetic beads are provided ready to use. These beads have a high binding capacity for IgG and low non-specific binding. As a result, these magnetic beads require fewer washing steps than agarose beads and it is not necessary to pre-clear the chromatin prior to ChIP. An added advantage is that the magnetic beads pellet much more quickly than standard agarose beads, which must be pelleted by centrifugation steps. In addition, magnetic stands (and the included bar magnet) are designed to pellet the beads onto the side of the tube. This makes it easier to remove buffers without disturbing the beads, so washing can be performed using multi-channel pipettors. This dramatically reduces hands-on time and ensures sample-to-sample consistency. **The provided siliconized microcentrifuge tubes (1.7 ml) simplify wash steps and ensure a minimal loss of Protein G beads.**

Other steps in the ChIP protocol have also been optimized. The specialized Elution Buffer, coupled with a reagent to inactivate Proteinase K, eliminates the need for DNA purification after the ChIP is complete. This saves time, minimizes manipulations and eliminates the DNA loss that can occur during purification.

These improvements greatly reduce hands-on time and 8-, 16- or 24-ChIP experiments can easily be performed at the same time. This is not possible with traditional ChIP methods, which are time- and labor-intensive. For even higher throughput ChIP, Active Motif offers ChIP-IT Express HT (Catalog No. 53018), which enables you to perform 96 ChIPs simultaneously.

Shearing options – ChIP-IT Express (Sonication) and ChIP-IT Express Enzymatic (Enzymatic)

The ChIP-IT Express Kit provides reagents sufficient to prepare 15 sonication-sheared chromatin preparations. Using this protocol, each preparation of sheared chromatin requires one 15 cm tissue culture plate of cells and yields chromatin sufficient for up to 14 ChIP-IT Express reactions (one ChIP reaction is considered to be the incubation of one sample of chromatin with one antibody). However, the chromatin preparation protocols can be scaled up or down depending on how many cells you would like to work with (see Appendix – Section D of this manual). If you want to prepare additional samples using sonication, the ChIP-IT Shearing Kit is sold separately (Catalog No. 53002).

The ChIP-IT Express Enzymatic Kit contains similar components to the ChIP-IT Express Kit (see Kit Contents), with the addition of a proprietary Enzymatic Shearing Cocktail and Digestion Buffer. Enzymatic shearing is easily controlled by time and temperature to yield fragments ideal for use in ChIP. The Enzymatic Shearing Kit replaces sonication shearing, thus eliminating problems due to variability in sonication power as well as complications arising from the emulsification of chromatin during sonication. The ChIP-IT Express Enzymatic Kit provides sufficient reagents to perform 15 shearing reactions. Each shear is based on using one 15 cm plate of cells and yields chromatin sufficient for up to 14 ChIP reactions. However, the chromatin preparation protocols can be scaled up or down depending on how many cells you would like to work with (see Appendix – Section D of this manual). If you want to prepare additional samples using enzymatic digestion, the Enzymatic Shearing Kit is sold separately (Catalog No. 53005).

Related Products

Please visit our website for complete information on the items below; catalog nos. of these and other useful products are listed in the Related Products section in the Appendix of this manual:

The **ChIP-IT Express HT** was designed for users who have many ChIPs to perform. It enables true high-throughput ChIP by providing you with the reagents and protocols needed to adapt the magnetic bead-based ChIP-IT Express Kit method to a format that makes possible 96-well ChIP.

Because appropriate controls make ChIP interpretation and troubleshooting easier, Active Motif sells **ChIP-IT Control Kits** for human, mouse and rat samples. These useful kits contain species-specific positive & negative control antibodies, appropriate positive control PCR primers, PCR buffer and loading dye.

Active Motif's **Ready-to-ChIP HeLa Chromatin** is another useful reagent that will save you time. This high-quality chromatin from HeLa cells has been sheared by sonication and is ready for use in ChIP. Please check our website for the availability of chromatin from other cell lines.

For users who need to make more chromatin than is possible with the reagents included in the ChIP-IT Kits, the **ChIP-IT Protein G Magnetic Beads** and the **ChIP-IT Shearing Kits** are also available separately.

Finally, one difficult aspect of ChIP is finding an antibody that recognizes the target protein when it is bound to DNA and fixed by formaldehyde. Antibodies that perform well in Western, Supershift and other applications may not work well in ChIP. For this reason, Active Motif offers an ever-increasing number of **ChIP-validated antibodies** that have been verified to work in ChIP. See Appendix – Section G of this manual or go to www.activemotif.com/chipabs to generate an up-to-date list of antibodies that will help make your ChIP successful.

Kit Components and Storage – ChIP-IT Express Kit

Please store each component at the temperature indicated in the table below. Do not re-freeze the Protein G Magnetic Beads.

Reagents	Quantity	Storage / Stability
RNase A (10 µg/µl)	40 µl	-20°C for 6 months
5 M NaCl	200 µl	-20°C for 6 months
100 mM PMSF	350 µl	-20°C for 6 months
Proteinase K (0.5 µg/µl)	250 µl	-20°C for 6 months
Proteinase K Stop Solution	80 µl	-20°C for 6 months
Protease Inhibitor Cocktail (PIC)	100 µl	-20°C for 6 months
1X Lysis Buffer	12 ml	-20°C for 6 months
10X Glycine	33 ml	-20°C for 6 months
10X PBS	120 ml	-20°C for 6 months
ChIP Buffer 1	70 ml	-20°C for 6 months
ChIP Buffer 2	70 ml	-20°C for 6 months
Elution Buffer AM2	1.3 ml	-20°C for 6 months
Reverse Cross-linking Buffer	1.3 ml	-20°C for 6 months
Protein G Magnetic Beads*	650 µl	4°C for 6 months
Shearing Buffer	10 ml	-20°C for 6 months
Siliconized 1.7 ml microcentrifuge tubes	25	Room temperature
Bar Magnet	1	Room temperature
Mini Glue Dots	1 sheet	Room temperature

* The Protein G Magnetic Beads are shipped on dry ice, but **should not be re-frozen** by the customer. Upon receipt of this kit, the beads should be stored at 4°C.

Kit Components and Storage – ChIP-IT Express Enzymatic Kit

Please store each component at the temperature indicated in the table below. Do not re-freeze the Protein G Magnetic Beads.

Reagents	Quantity	Storage / Stability
RNase A (10 µg/µl)	40 µl	-20°C for 6 months
5 M NaCl	200 µl	-20°C for 6 months
100 mM PMSF	350 µl	-20°C for 6 months
Proteinase K (0.5 µg/µl)	250 µl	-20°C for 6 months
Proteinase K Stop Solution	80 µl	-20°C for 6 months
Protease Inhibitor Cocktail (PIC)	100 µl	-20°C for 6 months
1X Lysis Buffer	12 ml	-20°C for 6 months
10X Glycine	33 ml	-20°C for 6 months
10X PBS	120 ml	-20°C for 6 months
ChIP Buffer 1	70 ml	-20°C for 6 months
ChIP Buffer 2	70 ml	-20°C for 6 months
Elution Buffer AM2	1.3 ml	-20°C for 6 months
Reverse Cross-linking Buffer	1.3 ml	-20°C for 6 months
Protein G Magnetic Beads*	650 µl	4°C for 6 months
Digestion Buffer	11 ml	-20°C for 6 months
Enzymatic Shearing Cocktail	6 µl	-20°C for 6 months
0.5 M EDTA	280 µl	-20°C for 6 months
Siliconized 1.7 ml microcentrifuge tubes	25	Room temperature
Bar Magnet	1	Room temperature
Mini Glue Dots	1 sheet	Room temperature

* The Protein G Magnetic Beads are shipped on dry ice, but **should not be re-frozen** by the customer. Upon receipt of this kit, the beads should be stored at 4°C.

Additional materials required

- A ChIP-validated antibody directed against the protein of interest
- Dounce homogenizer with a small clearance pestle (e.g. Wheaton part no. 357542 or Kimble-Kontes part no. 885302-002 with the tight-fitting “B” pestle). Use of a homogenizer is strongly recommended for shearing chromatin by sonication and required for enzymatic shearing. Dounce homogenization greatly improves your chances for successful ChIP.
- Magnetic stand. You can assemble a magnetic stand using the provided bar magnet (see Appendix – Section E) or use commercially available stands (e.g. the Promega MagneSphere® Technology twelve-position Magnetic Separation Stand or Ambion 6 Tube Magnetic Stand).
- 37% Formaldehyde (Fixation)
- 50% Glycerol in dH₂O (Enzymatic shearing)
- Phenol/chloroform (Purification of Input DNA and purification of sheared DNA prior to checking concentration by spectrophotometry or gel electrophoresis)
- 3 M Sodium Acetate pH 5.2 (Purification of Input DNA and purification of sheared DNA prior to checking concentration by spectrophotometry or gel electrophoresis)
- 100% ethanol (Purification of Input DNA and purification of sheared DNA prior to checking concentration by spectrophotometry or gel electrophoresis)
- 70% ethanol (Purification of Input DNA and purification of sheared DNA prior to checking concentration by spectrophotometry or gel electrophoresis)
- DNase-free H₂O (Purification of Input DNA)
- Rocking platform for culture plates
- Apparatus to rotate tubes end-to-end at 4°C (e.g. a Labquake from Barnstead/ThermoLyne)
- Microcentrifuge and microcentrifuge tubes
- Spectrophotometer
- Pipettors and tips (filter tips are recommended)
- Sonicator, for sonication only (Recommend Sonics Vibracell VC 130 with 3 mm stepped microtip)
- Agarose gel electrophoresis apparatus
- Minimal cell culture media
- Cell scraper (rubber policeman)
- 10 ml pipette, aspirator and 100 ml graduated cylinder

Optional materials

- 8-well PCR strips (e.g. Thermo Fisher part no. AB-0451).
- 500 µl siliconized Eppendorf tubes (e.g. Thermo Fisher part no. 02-681-460).
- PCR purification columns (e.g. Qiagen part no. 28104).

ChIP-IT Express Experimental Design

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Points to consider:

- **Cell growth and chromatin preparation.** ChIP-IT Express Kits used according to our standard protocols provide reagents sufficient to make fifteen preparations of sheared chromatin for ChIP. Each chromatin preparation uses cells grown in one 15 cm tissue culture plate (approximately 1.5×10^7 cells) and yields enough material to perform up to 14 ChIP reactions (see Troubleshooting in Appendix – Section F for a discussion of chromatin yield and the amount of chromatin to use per ChIP, as well as Section D for a protocol to scale up or down chromatin preparation). The kits contain Protein G-coated magnetic beads and other reagents sufficient to perform a total of 25 ChIP reactions once you have prepared the chromatin. Before starting an experiment, calculate the number of chromatin preparations you require and determine the number of ChIP reactions you plan to perform on each chromatin preparation. Be sure to include the appropriate control ChIP reactions in your calculations. Also, note that if you wish to analyze the effect of particular compounds or culturing conditions on transcription factor/DNA interactions, you should prepare chromatin from control (untreated) cells as a reference sample.
- **Formaldehyde fixation (1% solution).** In standard protocols, chromatin is fixed for 10 minutes prior to shearing by sonication or enzymatic digestion. While these are typical fixation conditions, some antibody/chromatin combinations may work better with shorter fixation. Fixation for two minutes with 1% formaldehyde has been very effective in some cases. When performing experiments to optimize ChIP, it is often useful to test reducing the time of fixation.
- **Shearing by Sonication.** Chromatin sheared to a size of 200-1500 bp is usually used for ChIP experiments. You may wish to optimize shearing conditions using chromatin that is not intended for use in ChIP. In general, shearing efficiency is improved through the use of a small shearing volume and a V-bottom tube rather than a round-bottom tube. Also, note that shearing is inefficient if the chromatin sample becomes emulsified. This can be avoided by using lower shearing power and by turning the power on gradually. If a shearing reaction is allowed to emulsify, discontinue shearing and centrifuge the sample at 4°C for 4 minutes at 8000 rpm in a microcentrifuge to remove trapped air. Finally, to prevent overheating and denaturation of chromatin, samples should be kept on ice as much as possible during shearing, and shearing should be performed discontinuously (*i.e.* sonicate for 15 seconds, then place on ice for 30 seconds, sonicate again for 15 seconds, *etc.*).
- **Shearing by Enzymatic Digestion.** While sonication is the most common method used to shear chromatin for ChIP, in some cases (*e.g.* when the sample is limited, when nucleosome ChIP is to be performed, when a sonicator is not available, or for high-throughput applications) enzymatic shearing is an excellent alternative. To provide a robust and user-friendly enzymatic shearing method, Active Motif offers the ChIP-IT Express Enzymatic Kit, which uses a proprietary Enzymatic Shearing Cocktail to quickly digest the chromatin DNA to 200-1500 bp fragments. Enzymatic activity can be easily controlled by time and temperature.

- **Protein G-coated magnetic beads.** The supplied magnetic beads are ready to use following complete resuspension to a homogeneous slurry. There is no need to pre-block the beads or pre-clear the sample. For best results, gently shake and roll the tube. The beads settle quickly, so should be resuspended just before pipetting. **Protein G Magnetic Beads are shipped on dry ice, but should not be re-frozen by the customer. Upon receipt, the beads should be stored at 4°C.** The ChIP-IT Protein G Magnetic Beads are also sold separately (Catalog No. 53014).
- **Antibodies must be suitable for ChIP.** ChIP antibodies must recognize fixed, native protein that is bound to DNA and/or complexed with other proteins. Many antibodies that perform well in other applications do not perform in ChIP. Thus, ChIP performed with an unproven antibody must include appropriate controls (such as Active Motif's RNA pol II antibody, Catalog No. 39097) to demonstrate that the antibody and the prepared chromatin are appropriate for ChIP. For your convenience, Active Motif sells ChIP-IT Control Kits for human, mouse and rat samples; these kits contain positive and negative control antibodies, appropriate positive PCR primers, PCR buffer and loading dye (see Related Products in Appendix).
- **Perform ChIP-IT Express in the provided siliconized 1.7 ml microcentrifuge tubes or in 8-well PCR strips.** The provided bar magnet can be used with either of these formats (see Appendix – Section E for detailed instructions). Commercially available side-pulling magnetic stands (e.g. Promega MagneSphere® Technology twelve-position Magnetic Separation Stand – 1.7 ml microcentrifuge tube format, or the Ambion 6 Tube Magnetic Stand) can also be used with the standard 1.5 or 1.7 ml microcentrifuge tubes.



First-time users of magnetic beads in the PCR tube format should familiarize themselves with the manipulations before performing a ChIP (see Appendix – Section E). Some commercially available magnets for 96-well plates are not ideal for use with 8-well PCR strips.

- **PCR analysis of immunoprecipitated DNA.** A successful ChIP results in an enrichment of chromatin fragments that are bound by the protein of interest, not complete purification. Thus, DNA isolated by ChIP is unavoidably contaminated with non-specifically captured DNA. For this reason, Real-Time Quantitative PCR analysis is preferred. If this method is not available, PCR of ChIP DNA should be performed such that cycling is stopped while the reaction is still in the linear stage of amplification. Hot-start PCR methods are recommended to ensure consistent PCR amplification results.

If you intend to analyze the binding of a known protein to a known binding site, design the PCR primers so that they flank the binding site and generate a 100-250 bp amplicon. Alternatively, if you hope to identify a protein binding site within a region of DNA, it may be best to design several primer pairs so that the DNA region in question can be systematically

analyzed. In this case, design a series of primer pairs that can be used to generate amplicons that overlap one another and span the region of interest. To facilitate this, the amplicons can be 250-400 bp in length. After these primer pairs have been used to roughly localize the binding site, design a more focused set of primers. Use of PCR design programs can be helpful in selecting good primer pairs. Also, as PCR analysis is extremely sensitive, precautions against contamination should be taken throughout the entire ChIP protocol.

- **PCR primers.** PCR primers should efficiently and specifically amplify the desired target. This should be proven on a relevant template, such as genomic or Input DNA. In addition, negative control primers can be useful to control for DNA shearing efficiency and to map putative protein binding sites. Ideally, PCR primers for ChIP should be 24 nt in length, have a GC content of 50% and a T_m of 60°C. See Troubleshooting for discussion.
- **Siliconized tubes.** The siliconized 1.7 ml tubes are provided only for performing the ChIP reactions. Do not use them for preparation of chromatin or for isolation of Input DNA.
- **Resuspend solutions completely.** Thaw the PMSF and the Proteinase K Stop Solution at room temperature until fully dissolved. Vortex gently and spin down briefly before use.
- **Maximum volume of chromatin.** Chromatin shearing (sonication) buffers usually contain detergents (*e.g.* 0.1% SDS and 0.5% sodium deoxycholate is typical). If you plan to use more than 60 µl sonicated chromatin in a ChIP reaction, use the 200 µl reaction volume from Table 1 (page 14). This will ensure that the detergent in the shearing buffer does not interfere with antibody activity. This is not a concern with chromatin prepared by the Active Motif Enzymatic Shearing Kit because the relevant buffers contain little or no detergent.
- **Quantity of antibody.** Best results are typically obtained by use of 1-3 µg antibody. However, this will vary according to the activity of the antibody and the quality of the chromatin, and you may need to use more of a particular antibody.
- **Stopping points in the protocol.** Convenient stopping points are mentioned in the Troubleshooting section in Appendix – Section F.
- **Safety precautions.** Formaldehyde, and PMSF are highly toxic chemicals. Appropriate safety precautions (*i.e.* safety glasses, gloves and lab coat) should be used. Also, formaldehyde is highly toxic by inhalation and should be used only in a ventilated hood. Finally, chromatin sonication shearing should be performed in a hood if the cells might contain something that could infect human cells.
- **Washing of magnetic beads.** See also Appendix – Section E. In the below protocols, bead washing is performed as follows:
 - a. Place tubes in the appropriate magnetic stand and allow beads to pellet on tube side.
 - b. Carefully remove the supernatant.
 - c. Add the appropriate wash buffer and resuspend pellet completely by pipetting up and down 2-3 times. Ensure the beads are not clinging to the pipette tips after pipetting. You may need to move the tubes away from the magnetic field before resuspending.
 - d. Repeat steps a-c for the desired number of washes.

Protocols – Preparation of Sheared Chromatin

A. Cell Fixation

Please read the Experimental Design section that begins on page 8 before starting. The protocol below describes fixation of cells from one 15 cm plate (1.5×10^7 cells). Appendix– Section D includes information on scaling the protocol for use with other amounts of cells. The fixed cells will be used in either protocol B1 to prepare chromatin using sonication shearing, or in protocol B2 to prepare chromatin using enzymatic shearing. Protocols B1 & B2 assume that you have already optimized shearing conditions for your cell line and treatment; if you have not, you should not use this protocol now as it does not prepare enough chromatin to test for the optimal conditions. Instead, begin with the protocols that begin in Appendix – Section A to generate sufficient chromatin to test and determine the optimized shearing conditions for your cell line and treatment.

Note: Several of the buffers used below require addition of PMSF and protease inhibitors (PIC). Thaw these reagents before starting the chromatin preparation and add to the buffers immediately before use.

1. Grow cells to 70-80% confluency in one 15 cm plate. Treat cells as desired to influence the pathway of interest.
2. When cells are ready to harvest, freshly prepare the following solutions. The below volumes assume you are working with one 15 cm plate:
 - a. Fixation Solution: Add 0.54 ml 37% formaldehyde to 20 ml minimal cell culture medium and mix thoroughly. Leave at room temperature.
 - b. 1X PBS: Add 2.33 ml 10X PBS to 21 ml dH_2O , mix and place on ice.
 - c. Glycine Stop-Fix Solution: Combine 1 ml 10X Glycine Buffer, 1 ml 10X PBS and 8 ml dH_2O . Mix well and leave at room temperature.
 - d. Cell Scraping Solution: Add 200 μl 10X PBS to 1.8 ml dH_2O , mix and place on ice. Just before using (in step 7 below) add 10 μl 100 mM PMSF.
3. Pour medium off the cells and add 20 ml Fixation Solution to each plate. Incubate on a shaking platform for 10 minutes at room temperature.
4. Pour Fixation Solution off and wash by adding 10 ml ice-cold 1X PBS to each plate. Rock the plate for 5 seconds, then pour off the PBS.
5. Stop the fixation reaction by adding 10 ml Glycine Stop-Fix solution to each of the plates. Swirl to cover and then rock at room temperature for 5 minutes.
6. Wash each plate by pouring off the Glycine Stop-Fix solution, then adding 10 ml ice-cold 1X PBS. Rock the plate for 5 seconds, then pour off the PBS.
7. Just before use, add PMSF to Cell Scraping Solution, as described in 2d above. Add 2 ml of this ice-cold Cell Scraping Solution to each plate and scrape cells with a rubber policeman. Hold the plate at an angle and scrape cells down to collect them at the bottom edge of the plate. Use a 1 ml pipette to transfer the cells to a 15 ml conical tube on ice.

8. Pellet the cells from step 7 by centrifugation for 10 minutes at 2,500 rpm (720 RCF) at 4°C.
9. Remove the supernatant and discard. At this point the protocol can be continued or the pellet can be frozen. If freezing the pellet, add 1 µl 100 mM PMSF and 1 µl PIC and freeze at -80°C. When you are ready, continue with either section B1 or B2 below.

B1. Shearing by Sonication

The section below describes the isolation and preparation of chromatin using sonication shearing.

1. Thaw pellet (if necessary) and resuspend cells in 1 ml ice-cold Lysis Buffer supplemented with 5 µl PIC + 5 µl PMSF. Incubate on ice for 30 minutes.
2. Transfer the cells to an ice-cold dounce homogenizer. Gently dounce on ice with 10 strokes to aid in nuclei release. Transfer cells to a 1.7 ml microcentrifuge tube and centrifuge at 5,000 rpm (2,400 RCF) for 10 minutes at 4°C to pellet the nuclei.
3. Carefully remove the supernatant and discard. Resuspend the nuclei pellet in 350 µl Shearing Buffer (supplemented with 1.75 µl PIC) and place the samples on ice.
4. Shear the DNA using your optimized conditions (that were previously determined in Appendix – Sections A & B1).
5. Centrifuge the sheared chromatin samples at 15,000 rpm (18,000 RCF) in a 4°C microcentrifuge for 10 minutes. Carefully transfer supernatant to a fresh 1.7 ml microcentrifuge tube. This is the sheared chromatin. It can be used right away or stored at -80°C. Before freezing, remove 50 µl so that the DNA shearing efficiency and DNA concentration can be checked. The remaining chromatin (approximately 350 µl) is sufficient for up to 14 ChIP reactions and should be aliquoted before freezing to minimize freeze-thaw cycles.

Note: To use the reserved 50 µl sample to determine DNA concentration and shearing efficiency, use the DNA Clean Up protocol described in Appendix – Section C.

This protocol is continued with Section C on page 14.

B2. Enzymatic Shearing

The section below describes the isolation and preparation of chromatin using enzymatic shearing.

1. Thaw pellet (if necessary) and resuspend cells in 1 ml ice-cold Lysis Buffer supplemented with 5 μ l PIC + 5 μ l PMSF. Pipette gently and vortex briefly to resuspend. Incubate on ice for 30 minutes.

During this incubation, prepare a working stock of Enzymatic Shearing Cocktail (200 U/ml) by diluting the supplied Enzymatic Shearing Cocktail (2×10^4 U/ml) 1:100 with 50% glycerol in dH₂O (not provided). The 200 U/ml working stock will be used in step 6 below and is stable at 4°C for 1-2 weeks.

Reagent	1-2 rxns	3-5 rxns	6-10 rxns
Stock Enzymatic Shearing Cocktail (2×10^4 U/ml)	0.5 μ l	1 μ l	2 μ l
50% glycerol	49.5 μ l	99 μ l	198 μ l

2. Transfer the cells to an ice-cold dounce homogenizer. Gently dounce on ice with 10 strokes to aid in nuclei release. Transfer cells to a 1.7 ml microcentrifuge tube and centrifuge at 5,000 rpm (2,400 RCF) for 10 minutes at 4°C to pellet the nuclei.
3. Carefully remove the supernatant and discard. Resuspend the nuclei pellet in 350 μ l Digestion Buffer (supplemented with 1.75 μ l PIC + 1.75 μ l PMSF) and incubate this solution at 37°C for 5 minutes.
4. Add 17 μ l of the working stock of Enzymatic Shearing Cocktail (200 U/ml) to the pre-warmed nuclei and vortex to mix.
5. Incubate the tube at 37°C for the amount of time that you determined to be optimal for your cell line (previously determined in Appendix – Sections A & B2). Flick or vortex the tube periodically during the incubation to ensure the chromatin is evenly sheared.
6. Stop the reaction by adding 7 μ l ice-cold 0.5 M EDTA to each tube; chill on ice 10 minutes.
7. Centrifuge the sheared chromatin samples at 15,000 rpm (18,000 RCF) in a 4°C microcentrifuge for 10 minutes. Collect the supernatant. This contains the sheared chromatin. It can be used right away or stored at -80°C. Before freezing, remove 50 μ l for use in checking the DNA shearing efficiency and DNA concentration. The remaining chromatin (approximately 350 μ l) should be sufficient for up to 14 CHIP reactions and should be aliquoted before freezing to minimize freeze-thaw cycles.

Note: To use the reserved 50 μ l sample to determine DNA concentration and shearing efficiency, use the DNA Clean Up protocol described in Appendix – Section C.

This protocol is continued with Section C on page 14.

Protocols – Chromatin Immunoprecipitation

C. Immunoprecipitation

1. Thaw chromatin (if necessary). Transfer 10 μl to a microcentrifuge tube; this tube is the “Input DNA” that will be processed in Step E6. It will then be used as a control in PCR analysis. Store this reserved chromatin at 4°C if it will be used within 6 hours; otherwise, store at -20°C.
2. Set up the ChIP reactions by adding the components shown in Table 1 below to the provided siliconized 1.7 ml microcentrifuge tubes or to PCR tubes. Before pipetting the magnetic beads, they should be fully resuspended by inverting and/or vortexing the bottle. **The antibody should be the final component added to the reaction.**

Table 1

Reagent	One reaction (if using less than 60 μl of chromatin)	One reaction (if using more than 60 μl of chromatin)
Protein G Magnetic Beads	25 μl	25 μl
ChIP Buffer 1	10 μl	20 μl
Sheared Chromatin (-7 μg)*	20-60 μl	61-100 μl
Protease Inhibitor Cocktail (PIC)	1 μl	1 μl
dH ₂ O	Add enough so that the final reaction volume will be 100 μl	Add enough so that the final reaction volume will be 200 μl
Antibody (added last)	1-3 μg	1-3 μg
Total Volume	100 μl	200 μl

***Note:** If you followed our protocols and not quantified the chromatin, 25 μl will contain -7 μg of chromatin from a 15 cm plate. Depending on the application, ChIP can be performed using anywhere from 1-50 μg of chromatin. An important factor is the volume of the chromatin being added, especially if the chromatin was prepared using sonication, as the detergents used during sonication will impact ChIP. Use the 200 μl ChIP in the right column above if the volume of chromatin will be greater than 60 μl . See the Appendix for discussions on the amount of chromatin to use and for methods to quantify DNA in chromatin.

3. Cap tubes and mix thoroughly.
4. Incubate on an end-to-end rotator for 4 hours at 4°C (e.g. a Labquake from Barnstead/ThermoLyne with a tube holder for 1.7 ml microcentrifuge tubes). In some cases, sensitivity may be improved if the incubation is performed overnight.
5. Spin tube briefly to collect liquid from the inside of the cap.
6. Place tube on magnetic stand to pellet beads on the tube side.
7. Carefully remove and discard supernatant.

D. Wash Magnetic Beads

For suggestions regarding bead washing methods, see the Experimental Design section that begins on page 8, as well as the information in Appendix – Section E.

For 1.7 ml microcentrifuge tubes:

1. Wash beads one time with 800 μ l ChIP Buffer 1.
2. Wash beads two times with 800 μ l ChIP Buffer 2.
3. After the final wash, remove as much supernatant as possible without disturbing the beads. Use a 200 μ l pipette if necessary.

For 8-well PCR strips:

1. Wash beads three times with 200 μ l ChIP Buffer 1.
2. Wash beads two times with 200 μ l ChIP Buffer 2. After the final wash, remove as much supernatant as possible without disturbing the beads.

E. Elute Chromatin, Reverse Cross-links and Treat with Proteinase K

1. Resuspend washed beads with 50 μ l Elution Buffer AM2.
2. Incubate 15 minutes at room temperature on an end-to-end rotator.
3. Briefly spin tubes to collect liquid from caps.
4. Add 50 μ l of the Reverse Cross-linking Buffer to eluted chromatin and immediately place tubes in magnetic stand; allow beads to pellet to sides of tubes.
5. Quickly transfer the supernatant, which contains the chromatin, to a fresh tube.
6. "Input DNA" sample: take the 10 μ l Input DNA aliquot (that was reserved in Step C1 above) from the ice. Add 88 μ l ChIP Buffer 2 and 2 μ l 5M NaCl to the Input DNA sample only, so that its final volume is 100 μ l.
7. Incubate the ChIP and Input DNA samples at 95°C for 15 minutes in a thermocycler.
Note: If you are using larger microcentrifuge tubes, it may be easier to perform a 2.5 hour incubation at 65°C.
8. Return tubes to room temperature, spin tubes briefly if liquid has collected on the inside of the caps, then add 2 μ l Proteinase K.
9. Cap tubes, mix well and incubate at 37°C for 1 hour.
10. Return the tubes to room temperature and add 2 μ l Proteinase K Stop Solution. Briefly centrifuge the tubes to collect liquid from the caps. DNA can be used immediately in PCR or stored at -20°C.

F. PCR Analysis

The protocol below is a guideline for optimizing PCR analysis of DNA collected through ChIP. Accurate PCR analysis of ChIP DNA requires that the PCR be stopped during linear amplification. The appropriate number of PCR cycles must be determined empirically. PCR is performed on four DNA templates: DNA from ChIP with the positive control RNA pol II antibody and Negative Control IgG (from the ChIP-IT Control Kit – Human), the Input DNA and DNA from ChIP with the test antibody. A water-only control is performed to ensure the PCR reagents are not contaminated.

In the example below, PCR reactions are set up using 2 different PCR cocktails, which contain positive & negative PCR primer sets. If you are using a positive control antibody from one of Active Motif's ChIP-IT Control Kits, only positive control PCR primers are used because the positive control antibodies included in those kits can bind many regions along a chromosome, making it difficult to design "negative control" primers that function with all cell types and shearing conditions. For PCR analysis of ChIP performed with other antibodies, we recommend that you use both positive and negative PCR primer sets that are appropriate for your antibody. See the Experimental Design section that begins on page 8 and Troubleshooting in the Appendix for details.

Note: PCR is extremely sensitive and all precautions should be taken to guard against contamination. Gloves should be worn and filter-tip pipettes should be used.

1. Program the thermocycler. The program should start with a initial melt step at 94°C for 3 minutes, then 36 cycles of [94°C for 20 seconds, 59°C for 30 seconds and 72°C for 30 seconds], then a hold cycle at 10°C. The total volume of each PCR will be 25 µl. You may need to optimize the number of cycles for your specific system.
2. Dilute the Input DNA 1:10 by adding 20 µl Input DNA to 180 µl dH₂O.
3. Use the table below to label PCR tubes and add the PCR templates and water-only control, keeping the tubes on ice. Add the PCR cocktails you will make in Step 4:

Reaction No.	PCR Template (5 µl each)	PCR cocktail (20 µl each)
1	ChIP DNA – Positive control antibody	Positive PCR cocktail
2	ChIP DNA – Negative control IgG	Positive PCR cocktail
3	Input DNA (diluted 1:10)	Positive PCR cocktail
4	ChIP DNA – Test antibody	Positive PCR cocktail
5	H ₂ O (no DNA control)	Positive PCR cocktail
6	ChIP DNA – Positive control antibody	Negative PCR cocktail
7	ChIP DNA – Negative control IgG	Negative PCR cocktail
8	Input DNA (diluted 1:10)	Negative PCR cocktail
9	ChIP DNA – Test antibody	Negative PCR cocktail
10	H ₂ O (control)	Negative PCR cocktail

- Set up the Positive PCR cocktail and the Negative PCR cocktail on ice according to the tables below. Add the dH₂O first and the *Taq* polymerase last. Mix thoroughly and keep on ice. This ensures that the reaction mixture is inactive until the cycling is started. As discussed above, if you are using the positive control antibody and Negative control IgG from one of the ChIP-IT Control Kits, only positive control PCR primers are provided (as a mixture of forward and reverse primers). However, for your own test antibody we recommend that you design and test both positive and negative PCR primer sets.

Positive PCR cocktail:

Reagent	1 reaction	5 reactions
DEPC H ₂ O	12.3 µl	61.5 µl
Positive Forward primer (5 pmol/µl)	2.0 µl	10 µl
Positive Reverse primer (5 pmol/µl)	2.0 µl	10 µl
dNTP mixture (5 mM each dNTP)	1.0 µl	5.0 µl
10X PCR Buffer	2.5 µl	12.5 µl
<i>Taq</i> (5 U/µl)	0.2 µl	1.0 µl
Total Volume (Not including DNA template)	20 µl	100 µl

Negative PCR cocktail:

Reagent	1 reaction	5 reactions
DEPC H ₂ O	12.3 µl	61.5 µl
Negative Forward primer (5 pmol/µl)	2.0 µl	10 µl
Negative Reverse primer (5 pmol/µl)	2.0 µl	10 µl
dNTP mixture (5 mM each dNTP)	1.0 µl	5.0 µl
10X PCR Buffer	2.5 µl	12.5 µl
<i>Taq</i> (5 U/µl)	0.2 µl	1.0 µl
Total Volume (Not including DNA template)	20 µl	100 µl

Note: We recommend that PCR analysis be performed using the 10X PCR Buffer included with the ChIP-IT Control Kits. The composition of the 10X PCR Buffer is 750 mM Tris-Cl (pH 8.8), 200 mM (NH₄)₂SO₄, 0.1% Tween 20 and 25 mM MgCl₂.

- Add the appropriate PCR cocktail to each of the PCR tubes (on ice) prepared in Step 3. Cap PCR tubes carefully and ensure that each reaction mixture is in the bottom of the tube.
- Place PCR tubes in thermocycler and start the PCR program described in Step 1. After 36 cycles, remove tubes 1-10 and place on ice.
- These PCR reactions can be immediately analyzed as described below or stored at -20°C.

G. Analysis of PCR Products

1. Run ~8 μ l of each PCR product on a 3% agarose gel. Save remaining PCR product in case additional gels must be run. Use gel combs with 2.5 mm-wide wells.
2. PCR products obtained with the GAPDH positive control primers are 166 bp. Use either a 50 or 100 bp ladder as the migration standard. Run the gel until PCR amplification products are well separated from PCR primers and primer dimers. Stain gel and analyze.

References

1. Solomon, M.J. *et al.* (1988) *Cell* 53(6): 937-47.
2. Solomon, M.J. and Varshavsky A. (1985) *PNAS USA* 82(19): 6470-4.
3. Kuo, M.H. and Allis, C.D. (1999) *Methods* 19(3): 425-33.
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Appendix

Optimizing the Shearing Conditions

Chromatin shearing conditions can vary significantly depending on the cell type and (occasionally) the cell culturing conditions and treatments used to induce specific pathways. However, after shearing has been optimized for a given cell type, those conditions usually give consistent results. For this reason, we recommend use of the protocols below the first time you make chromatin from a cell line to determine its optimal shearing conditions. While optimization requires you to grow and work with three times more cells than are normally needed for ChIP, you will not need to grow as many cells in subsequent experiments. You will simply use your optimized conditions.

Section A. Cell Fixation to Optimize Shearing Conditions

In the protocols below, chromatin is prepared from cells grown in three 15 cm plates and the chromatin is sheared using multiple conditions. Generally at least one of these conditions yields chromatin suitable for use in ChIP. Because only a fraction of the sheared chromatin is used for shearing analysis, the remaining sample (usually ~300 μ l for each shearing condition) can be used for approximately 12 ChIP experiments, provided that PIC and PMSF are included in the buffers.

Note: Several of the buffers used below require addition of PMSF and protease inhibitor cocktail (PIC). Thaw these reagents before starting the protocol and add to the buffers immediately before use. If you are performing optimization simply to identify shearing parameters, then you do not need to add PIC and PMSF. This will conserve these reagents for use in other ChIP experiments.

1. Grow cells to 70-80% confluency on three 15 cm plates. If applicable, treat all three plates equally to influence the pathway of interest.

2. When cells are ready to harvest prepare fresh Fixation Solution, ice-cold 1X PBS, Glycine Stop-Fix Solution and Cell Scraping Solution as follows:
 - a. Fixation Solution: Add 1.62 ml of 37% formaldehyde to 60 ml minimal cell culture medium and mix thoroughly. Leave at room temperature.
 - b. 1X PBS: Add 7 ml 10X PBS to 63 ml dH₂O, mix and place on ice.
 - c. Glycine Stop-Fix Solution: Combine 3 ml 10X Glycine Buffer, 3 ml 10X PBS and 24 ml dH₂O. Mix well and leave at room temperature.
 - d. Cell Scraping Solution: Add 600 µl 10X PBS to 5.4 ml dH₂O, mix and place on ice. Just before use (in step 7 below) add 30 µl 100 mM PMSF.
3. Pour medium off the three plates and add 20 ml Fixation Solution to each plate. Incubate on a shaking platform for 10 minutes at room temperature.
4. Pour Fixation Solution off the plates and wash by adding 1X 10 ml ice-cold PBS to each plate. Rock the plate for 5 seconds, then pour off the PBS.
5. Stop the fixation reaction by adding 10 ml Glycine Stop-Fix solution to each of the plates, swirling to cover and then rocking at room temperature for 5 minutes.
6. Wash each plate by pouring off the Glycine Stop-Fix solution, then adding 10 ml ice-cold 1X PBS. Rock the plate for 5 seconds, then pour off the PBS.
7. Just before use, add PMSF to Cell Scraping Solution, as described in 2d above. Add 2 ml of this ice-cold Cell Scraping Solution to each plate and scrape cells with a rubber policeman. Hold the plate at an angle and scrape cells down to collect them at the bottom edge of the plate. Use a 1 ml pipette to transfer the cells to a 15 ml conical tube on ice. Do the same for the other two plates, pooling the cells from all three plates in one tube.
8. Pellet the pooled cells by centrifugation for 10 minutes at 2,500 rpm (720 RCF) at 4°C.
9. Remove the supernatant and discard. At this point the protocol can be continued or the pellet can be frozen. If freezing the pellet, add 1 µl 100 mM PMSF and 1 µl PIC and freeze at -80°C. When you are ready, continue with either section B1 or B2 below.

Section B1. Optimization of Chromatin Shearing by Sonication

Please read Experimental Design before starting. In the protocol below, chromatin is sheared for 5, 10, and 15 pulses. Generally at least one of these conditions yields chromatin suitable for use in ChIP. Because only a fraction of each preparation is used for shearing analysis, all remaining optimal preparation(s) can be used for ChIP experiments, provided that PIC and PMSF are used in the buffers below, and those above for cell fixation.

Our sonication optimization protocol was developed using the Sonics Vibracell VC 130 probe-type sonicator (with a 3 mm stepped microtip) at 25% power in a volume of approximately 300 µl.

1. Thaw pellet (if necessary) and resuspend cells in 1 ml ice-cold Lysis Buffer (supplemented with 5 µl PIC + 5 µl PMSF). Incubate on ice for 30 minutes.

2. Transfer the cells to an ice-cold dounce homogenizer. Gently dounce on ice with 10 strokes to aid in nuclei release. Transfer cells to a 1.7 ml microcentrifuge tube and centrifuge at 5,000 rpm (2,400 RCF) for 10 minutes at 4°C to pellet the nuclei.
3. Carefully remove the supernatant and discard. Resuspend the nuclei pellet in 1.0 ml Shearing Buffer (supplemented with 5 µl PIC), aliquot into equal volumes into three 1.7 ml microcentrifuge tubes, then place on ice. Each aliquot should be approximately 350 µl.
4. Shear the three aliquots of fixed chromatin at 25% power using three different conditions:
 - a. Five pulses of 20 seconds each, with a 30-second rest on ice between each pulse.
 - b. Ten pulses of 20 seconds each, with a 30-second rest on ice between each pulse.
 - c. Twenty pulses of 20 seconds each, with a 30-second rest on ice between each pulse.
5. Centrifuge the sheared chromatin samples at 15,000 rpm (18,000 RCF) in a 4°C microcentrifuge for 10 minutes. Transfer the supernatants to fresh tubes and save 50 µl aliquots from each, which will be used to determine shearing efficiency. The sheared chromatin and the aliquots can be stored at -80°C. Or, use the 50 µl aliquots immediately in Section C below to reverse cross-links and purify the chromatin prior to gel analysis.

Section B2. Optimization of Enzymatic Shearing

Please read Experimental Design before starting. Note that although 1 ml of chromatin is prepared, only 200 µl is used to analyze shearing efficiency. If PIC and PMSF are included, the unused 800 µl of chromatin can be stored frozen while optimal shearing conditions are identified. This chromatin can then be thawed, sheared according to the optimal conditions, and used in ChIP.

1. Thaw pellet (if necessary) and resuspend cells in 1 ml ice-cold Lysis Buffer (supplemented with 5 µl PIC + 5 µl PMSF). Incubate on ice for 30 minutes.
2. During this incubation, prepare a working stock of Enzymatic Shearing Cocktail (200 U/ml) by diluting the supplied Enzymatic Shearing Cocktail (2×10^4 U/ml) 1:100 with 50% glycerol in dH₂O (not provided). The 200 U/ml working stock will be used in step 15 below and is stable at 4°C for 1-2 weeks.

Reagent	µl for 5 rxns
Stock Enzymatic Shearing Cocktail (2×10^4 U/ml)	0.5 µl
50% glycerol	49.5 µl

3. Transfer the cells to an ice-cold dounce homogenizer. Gently dounce on ice with 10 strokes to aid in nuclei release. Transfer cells to a 1.7 ml microcentrifuge tube and centrifuge at 5,000 rpm (2,400 RCF) for 10 minutes at 4°C to pellet the nuclei.

Note: To ensure adequate cell lysis, examine ~10 µl of sample under a microscope before and after homogenization. See Troubleshooting in the Appendix for details.

4. Carefully remove the supernatant and discard. Resuspend the pellet in 1 ml Digestion Buffer supplemented with 1.75 μl PIC + 1.75 μl PMSF (and other inhibitors, if appropriate).
5. Transfer 50 μl of the chromatin in Digestion Buffer to each of 4 fresh microcentrifuge tubes and incubate the tubes at 37°C for 2 minutes. Freeze the remaining chromatin at -80°C.
6. To optimize shearing conditions using Enzymatic Digestion, set up 4 reactions as indicated below. Vortex the tubes on a low setting to mix components.
 - a. 50 μl chromatin plus 2.5 μl dH₂O (No Enzyme) – incubate for 10 minutes at 37°C
 - b. 50 μl chromatin plus 2.5 μl working stock Enzyme – incubate for 5 minutes at 37°C
 - c. 50 μl chromatin plus 2.5 μl working stock Enzyme – incubate for 10 minutes at 37°C
 - d. 50 μl chromatin plus 2.5 μl working stock Enzyme – incubate for 15 minutes at 37°C
7. Stop the reactions by adding 1 μl ice-cold 0.5 M EDTA to each tube. Chill on ice 10 minutes.
8. Centrifuge the sheared chromatin samples at 15,000 rpm (18,000 RCF) in a 4°C microcentrifuge for 10 minutes. Collect the supernatant. This sheared chromatin can be stored at -80°C. Or, continue immediately with Section C below to reverse cross-links and purify the chromatin prior to gel analysis.

Section C. DNA Clean Up

1. If necessary, thaw the 50 μl aliquots of each sheared chromatin sample.
2. Add 150 μl dH₂O, then 10 μl 5 M NaCl to each tube.
3. Heat all samples at 95°C in a water bath or a thermocycler for 15 minutes to reverse the cross-links, taking care to prevent the lids from popping open if you use a water bath.
4. Add 1 μl RNase A to each sample and incubate at 37°C for 15 minutes.
5. Add 1 μl Proteinase K to each sample and incubate at 67°C for 15 minutes

Note: If you intend to use a spectrophotometer to determine the DNA concentration, the DNA should first be column purified or phenol/chloroform extracted and precipitated. This can be performed as follows:

- a. Add 200 μl phenol/chloroform to the sample, vortex to mix completely and centrifuge for 5 minutes at maximum speed in a microcentrifuge.
- b. Transfer supernatant to a fresh microcentrifuge tube, then add 20 μl 3 M Sodium Acetate pH 5.2 and 500 μl 100% ethanol. Vortex to mix completely and place at -80°C for at least 1 hour. Alternatively, the sample can be left at -20°C overnight.
- c. Centrifuge at maximum speed for 10 minutes in a microcentrifuge at 4°C.
- d. Carefully remove and discard supernatant. Do not disturb the pellet.
- e. Add 500 μl 70% ethanol to the pellet and spin 5 minutes at 4°C in microcentrifuge at maximum speed.
- f. Carefully remove and discard supernatant. Do not disturb pellet. Allow pellet to air-dry.

- g. Resuspend pellet in 30 μ l dH₂O and use a spectrophotometer to measure the OD at 260 nm to determine the DNA concentration (1.0 A₂₆₀ unit = 50 μ g/ml).

Alternatively, purify the DNA in a QIAquick MINelute column (Qiagen part no. 28104). Elute in 10 μ l dH₂O and measure the concentration using the NanoDrop from Thermo.

6. It is recommended to load each shearing sample on the gel in two different amounts to avoid over- or under-loading. Add 4 μ l of a 6X Loading Buffer to 16 μ l of sample, then load 5 μ l & 10 μ l of each sample on a 1% TAE agarose gel. Run the gel at 100V for 45 minutes to 1 hour until the loading dye reaches 3/4 of the way to the end of the gel rack.
7. Optimal sonication shearing should result in a 200-1500 bp smear similar to that shown in lanes 2 or 3 of Figure 2 below. Optimal enzymatic shearing should produce a 200-1500 bp ladder-like smear similar to Lane 4 of Figure 3 below:
8. If PIC and PMSF were used during the optimization experiment, the remaining chromatin of appropriate length can be used in ChIP experiments. For shearing by sonication, simply use the preparation(s) that produced chromatin sheared to the appropriate length.

For enzymatic shearing, the ~800 μ l of chromatin that was stored at -80°C can be sheared according to the optimal conditions. The digest volumes should be proportionate to the digest used in step 6 of Section B2 above. For example, 400 μ l unsheread chromatin will require 20 μ l working stock Enzyme. Incubate for the time that yielded suitable chromatin. Stop the reaction with 16 μ l ice-cold 0.5 M EDTA to each tube. Chill on ice 10 minutes. Centrifuge the sheared chromatin samples at 15,000 rpm (18,000 RCF) in a 4°C microcentrifuge for 10 minutes. Collect the supernatant. This sheared chromatin can be stored at -80°C or used immediately in ChIP reactions.

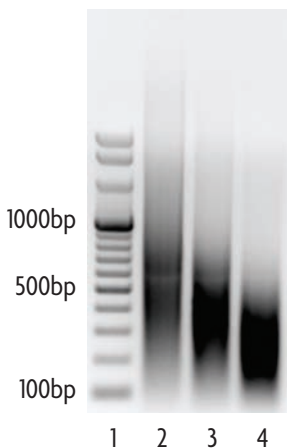


Figure 2: Gel analysis of sonication shearing (ChIP-IT Express).

HeLa cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared using the ChIP-IT Express Kit. Chromatin was sheared with 5, 10 and 20 pulses at 25% power using a Sonics Vibracell VC 130 sonicator with a 3 mm stepped microtip in a volume of approximately 300 μ l. Each pulse consisted of a 20-second sonication followed by a 30-second rest on ice. The sheared and unsheread chromatin samples were subjected to cross-link reversal, treated with Proteinase K, phenol/chloroform extracted and precipitated as described. Samples were separated by electrophoresis through a 1% agarose gel. Optimally sheared chromatin will yield a smear between 200-1500 bp.

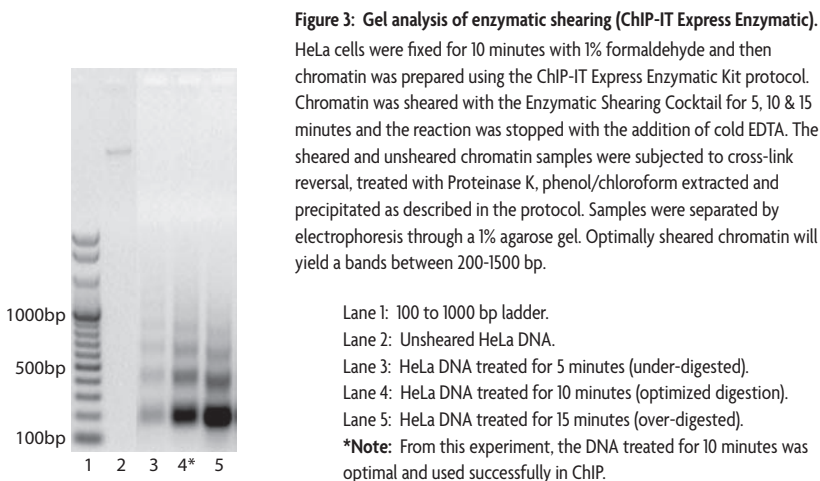
Lane 1: 100 to 1000 bp ladder.

Lane 2: HeLa DNA sheared for 5 pulses (optimized).

Lane 3: HeLa DNA sheared for 10 pulses (optimized).

Lane 4: HeLa DNA sheared for 20 pulses (over-sheared).

Note: From this experiment, the DNA sonicated for both 5 and 10 pulses are suitable to be used successfully in ChIP.



Section D. Scale Up/Down of Chromatin Preparation

Our standard chromatin preparation protocols use cells grown in one 15 cm tissue culture plate (approximately 1.5×10^7 cells) and yield enough material to perform up to 14 ChIP reactions. Depending on your experiments, you may wish to work with different volumes of cells. The comments and table below are designed to help adapt our protocols for using other amounts of cells.

- It is not recommended to use less than 500 μ l or more than 2 ml in dounce homogenization.
- If you intend to compare ChIP results from various samples, try to treat the samples equally. For example, grow induced and uninduced cells in the same size plate and to the same density, then use equal volumes and shearing conditions. This will help ensure that the chromatin preparations are equivalent in terms of cell concentration, DNA shearing efficiency, *etc.* You may also quantify the chromatin DNA (by following the DNA Clean Up protocol above) prior to the ChIP to ensure the use of equal amounts of chromatin for the IP.
- Human diploid cells contain 6.6 picograms of DNA. This can be used to estimate DNA in a chromatin preparation if the number of cells in the starting material is known. We estimate DNA recovery of chromatin shearing to be about 70%, a little less from smaller samples.
- When using a sonicator with a 3 mm (or similar) probe, shearing should be performed in a 1.5 or 1.7 ml Eppendorf in 300-400 μ l. Expect to lose 50 μ l of chromatin (due to aerosolized sample). Smaller volumes (50-100 μ l) will require a 1 mm probe for effective shearing.
- Perform sonication shearing so that the resulting chromatin is an appropriate concentration for ChIP. ChIP reactions should be performed in a small volume (ideally 100 μ l, and not more than 200 μ l total volume). Because a 200 μ l ChIP reaction contains 25 μ l beads, 20 μ l ChIP 1 Buffer, and a few μ l of antibody and PIC, the maximum volume of chromatin that can be added to a ChIP reaction is likely to be about 150 μ l. And, this amount is more than should be used because the detergent in the chromatin preparation will negatively impact the ChIP. Try to keep the volume of chromatin used for ChIP to no more than 100 μ l.

	1 well of a 24-well plate	10 cm plate	15 cm plate	3 x 15 cm plates
Number of Cells	130,000	0.66×10^7	1.5×10^7	4.5×10^7
Fixation Solution	2 ml	10 ml	20 ml	60 ml (20 ml/plate)
Glycine Stop-Fix	1 ml	5 ml	10 ml	30 ml (10 ml/plate)
1X PBS	1 ml	5 ml	10 ml	30 ml (10 ml/plate)
Cell Scraping Solution + PMSF	500 μ l + 2.5 μ l PMSF	1 ml + 5 μ l PMSF	2 ml + 10 μ l PMSF	6 ml + 30 μ l PMSF (2 ml + 10 μ l/plate) Pool the 3 plates
Lysis Buffer + PIC + PMSF	200 μ l + 1 μ l PIC + 1 μ l PMSF	500 μ l + 2.5 μ l PIC + 2.5 μ l PMSF	1 ml + 5 μ l PIC + 5 μ l PMSF	1.5 ml + 7.5 μ l PIC + 7.5 μ l PMSF
Shearing Buffer + PIC (sonication only)	50-100 μ l + 0.25-0.5 μ l PIC	300 μ l + 1.5 μ l PIC	350 μ l + 1.75 μ l PIC	1000 μ l + 5 μ l PIC
Digestion Buffer + PIC + PMSF (enzymatic only)	50 μ l + 0.25 μ l PIC + 0.25 μ l PMSF	175 μ l + 0.875 μ l PIC + 0.875 μ l PMSF	350 μ l + 1.75 μ l PIC + 1.75 μ l PMSF	1000 μ l + 5 μ l PIC + 5 μ l PMSF
Enzymatic Shearing Cocktail, diluted (enzymatic only)	2.5 μ l	8 μ l	17 μ l	50 μ l
0.5 M EDTA (enzymatic only)	1 μ l	3.5 μ l	7 μ l	20 μ l

Section E. Use of Magnetic Beads and Included Bar Magnet

- The magnet should be stored in the provided tube.
- Be careful when working near metal objects or surfaces. A free magnet will jump great distances onto nearby metal surfaces with surprising speed. This can break the magnet.
- Use the provided Mini Glue Dots to attach the bar magnet to an empty pipette tip box to create an effective magnet stand for use with either PCR strips or microcentrifuge tubes.
- If the magnet becomes attached to a flat metal surface, it should be removed by sliding it off the edge of the surface. The magnet may be broken if you attempt to pull one end away from the metal.

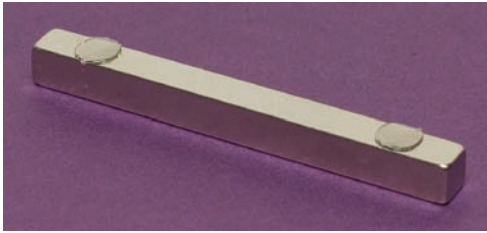
Caution: The included neodymium bar magnet is extremely powerful and is easily broken if handled incorrectly.

Creating a magnetic stand for 8-well PCR strips:

Note: 8-well strip tubes for use with standard 96-well PCR cyclers are recommended (e.g. Thermo Fisher AB-0451).

1. Place a strip of PCR tubes in the wells of an empty tip box (200 μ l tips) and place the magnet directly against the tubes. This is the way the magnet will be positioned when the glue dots are used to affix it to the box.

- Remove the covering tape from one side of two glue dots and attach the glue dots on the bar magnet (the uncovered face of the dot is placed on the magnet) as shown below.

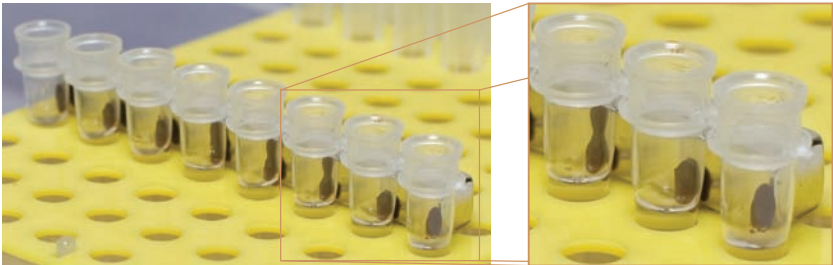


- Remove the covering tape from the exposed side of the glue dots. Fix the magnet to the tip box so that it is against the PCR tubes. The magnetic stand is now ready for use.

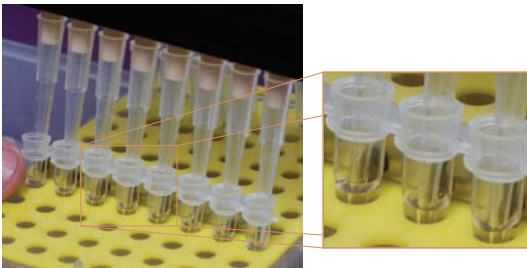
Note: Familiarize yourself with using the magnetic stand before performing with PCR tubes for the first time. Add 5 μl of magnetic beads to 100 μl ChIP Buffer 1 in one tube of an 8-well strip of PCR tubes. Use this tube with the assembled bar magnet stand to become familiar with use of the beads and magnet. It is difficult to re-suspend the beads if the tubes are directly adjacent to the magnet, so it is usually best to move the tubes away from the magnet for resuspension.

Washing should be performed as follows:

- Place the tubes in the rack against the magnet and allow the beads to be pinned to the side of the tube, as shown below.



- Remove supernatant with a 200 μl pipette or a 200 μl eight-channel pipette.



- Move the tube strip into a row that is not adjacent to the magnet.

- d. Add wash buffer and pipet up and down to fully re-suspend the beads. Ensure that a minimal amount of beads cling to the tips when the re-suspension is complete.
- e. Repeat steps a-d until desired washing steps are complete.

Centrifugation of 8-well PCR strip tubes:

When working with 8-well PCR strip tubes, it may be desirable to centrifuge the tubes to collect the liquid and beads from the inside the caps. This is easily accomplished in a centrifuge fitted with adaptors for spinning microtiter plates. Place a standard 96-well plate in the adaptor to hold the tubes in place. Be sure to balance the rotor (*i.e.* place a microtiter plate and tubes of appropriate mass in the rotor's opposing 96-well plate adaptor). Spin the plates briefly to let the rotor reach a speed of 1000 x *g* before allowing the rotor to stop.

Creating a magnetic stand for 1.7 ml microcentrifuge tubes:

1. Remove the covering tape from one side of two glue dots.
2. Place two 1.7 ml microcentrifuge tubes in the wells of an empty tip box (1000 μ l) and place the magnet directly against the tubes. This is the way the magnet will be positioned when the glue dots are used to affix it to the box.
3. Attach the glue dots on the bar magnet (the uncovered face of the dot is placed on the magnet) as shown above.
4. Remove the covering tape from the exposed side of the glue dot. Fix the magnet to the tip box so that it is against the tubes. The magnetic stand is now ready for use.

Note: 1.7 ml microcentrifuge tubes are held less securely in this assembled tube stand than in a typical commercial magnetic stand. This is not a problem if the below washing protocol is followed. Work with 1 tube at a time, and keep the tubes in the standard tube rack unless you are holding the tube next to the magnet.

Washing is best performed one tube at a time, and should be performed as follows:

1. Place the tube in a standard 1.7 ml microcentrifuge tube rack and open the cap.
2. Place the opened tube in the assembled magnetic stand. The beads will pellet more rapidly if the bottom of the tube is held against the magnet, as shown below, and then slowly lowered into the well. This will pellet the beads up onto the side of the tube.



3. Allow the beads to pellet completely and remove supernatant with a 1000 µl pipette. You can either leave the tube in the rack or pull it out when you remove the buffer. The beads will remain on the side of the tube, even when not next to the magnet.
4. Return the tube to the standard microcentrifuge tube rack, add 800 µl wash buffer and fully resuspend the beads by pipetting up and down.
5. Repeat steps 2-4 until desired washing steps are complete. After the final wash has been removed, the last traces of wash buffer should be removed with a 200 µl pipette.

Section F. Troubleshooting Guide

Problem/question	Recommendation
At what points in the protocol can I stop?	The protocol may be stopped and samples stored at the times and temperatures below: <ol style="list-style-type: none"> 1. After formaldehyde fixation and centrifugation (intact cell pellet), -80°C. 2. After chromatin shearing, -20°C. 3. After bead washing, -20°C. 4. After the cross-link reversal, -20°C. 5. After DNA clean up, -20°C.
How much sheared chromatin should I use for a ChIP reaction?	Best results are obtained when the amount of chromatin used in ChIP is from 1-3 x 10 ⁶ cells. If it is assumed that human diploid cells contain 6.6 picograms of DNA and recovery after cell fixation and shearing is 70%, then this is between 4.6 and 13.9 µg DNA. The minimum amount of sheared chromatin that can be used is 1 µg while the maximum is 50 µg.
Preparing a ChIP reaction with a large volume	It is better to set up several small ChIP reactions (200 µl each) and pool the samples at the end, rather than trying to ChIP a single large sample.
Poor enrichment with ChIP antibody	In some cases, use of an antibody in ChIP results in lower-than-expected enrichment of a target of interest. This is often because the antibody does not efficiently recognize fixed proteins, either because the epitope is destroyed by fixation or because the epitope is masked by other proteins in a larger complex. In this case, use more antibody when performing the ChIP. Alternatively, try to find an antibody that has been proven to work in ChIP, or that is known to recognize an epitope distinct from the one recognized by the unsatisfactory antibody.
There is no difference in band intensity between negative control and positive control.	See the recommendation on page 28 regarding increasing washing stringency.
	Decrease the number of PCR cycles (30, 32, 34 PCR cycles). The exponential phase of amplification occurs in PCR cycles where reaction components are still in excess and PCR products are accumulating at a constant rate. During this phase, each copy of DNA is being actively amplified, making it a better measure than endpoint PCR. In endpoint PCR, reagents such as nucleotides or primers may become exhausted. This can result in inefficient amplification, which can cause inaccurate quantification of the gene of interest. Thus, high background due to endpoint PCR can be decreased if the number of PCR cycles are reduced, so the results reflect exponential PCR. Real-time PCR can also be used in such cases.
	Shearing should produce DNA fragments that are small enough to exclude background from neighboring chromosomal sequences, but still large enough that there is a good possibility your amplicon remains intact. We recommend 200-1500 bp fragments. If the DNA fragments are too large, the background is increased. So, consider increasing the time of the enzymatic digestion, or the time and/or number of pulses for sonication.
	Confirm species specificity of your primers. You may need to redesign your primers.
	See the recommendation on page 28 regarding blocking the magnetic beads.

Problem/question	Recommendation
Strong PCR signal when using target PCR primers to amplify ChIP DNA that was isolated with a negative control (non-target) antibody.	In most cases, the washing procedure in the enclosed protocol is appropriate. However, when the background is high you can increase washing stringency in several ways: <ol style="list-style-type: none"> 1) After adding ChIP Buffer 1 and/or ChIP Buffer 2 during the wash steps, gently agitate the samples for several minutes before removing the buffer. 2) Perform additional washes. Sufficient ChIP Buffer 1 is provided for two "extra" washes per sample. Sufficient ChIP Buffer 2 is provided for one additional wash. 3) Add two washes using a high-salt buffer (20 mM Tris-Cl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 500 mM NaCl, pH 7.4), which is not provided. These additional washes should be performed after the washes with ChIP Buffer 1. Then, proceed with the ChIP Buffer 2 washes, as outlined in the protocol.
	Confirm the species specificity of your primers. You may need to redesign your primers.
	See the next recommendation regarding blocking the magnetic beads.
Is blocking of the magnetic beads ever required?	The beads provided are ready to use for most ChIPs. However, for applications highly sensitive to non-specific binding (such as when cloning ChIP DNA or using antibodies that require extra blocking), you may add blocking reagents to the ChIP reaction. In these cases, a combination of BSA (e.g. Sigma Cat. No. 4503) and either tRNA (e.g. Sigma Cat. No. R3629) or salmon sperm DNA (e.g. Sigma Cat. No. A-7888) can be added directly to the ChIP reaction. 2.5 µg/µl BSA and 1.25 µg/µl tRNA or 2.5 µg/µl Salmon sperm DNA (final concentrations) can be used as a starting point and more or less can be added as desired.
20 pulses of sonication does not result in adequately sheared DNA.	Sonicate for more pulses and sonicate for 30 seconds per pulse rather than 20 seconds. Take care to keep chromatin well-chilled on ice during these extended sonication pulses. It is also possible that the chromatin has been "overfixed" and is resistant to shearing. In these cases, it may be best to prepare new chromatin and to take care not to overfix. If you continue to experience difficulties with sonication shearing we suggest using our Enzymatic Shearing Kit, as detailed in the manual.
The nuclear pellets do not resuspend in Shearing or Digestion Buffers.	Repeat the dounce homogenization step being certain to use the B pestle of the Kimble-Kontes, Cat. No. 885302-002, which has a smaller clearance than the A pestle.
The gel for optimizing chromatin shearing has bands stuck in the wells, and smears from the top to bottom of the lane.	The sheared chromatin needs to have the cross-links reversed, protein removed (Proteinase K) and the RNA removed (RNase). Follow the DNA clean up protocol in Section C of the Appendix.
After sonication shearing and centrifugation, a viscous or cloudy layer is visible.	Depending upon the cell type, lipid or glycogen layers may be seen after centrifugation. For example, liver tissue may have a glycogen layer and a milky appearance, while fatty tissues can have a lipid layer. Avoid such layers when you remove the supernatant. However, if the whole supernatant is cloudy, it should not interfere with the IP reaction.
The PCR products are the correct size, but are very light.	Load more PCR product, and/or use smaller wells for the agarose gel. It should be noted that because the PCRs should be stopped while the reactions are in the linear phase of amplification, the yield of PCR product will be lower than in typical PCR amplifications, which are performed for maximum product yield. You can also perform more PCR cycles.
No PCR bands for Input DNA or ChIP'd samples.	In the presence of 0.8 mM total dNTP concentration, perform a MgCl ₂ titration series in 0.5 mM increments over a range of 1-4 mM. This will identify the magnesium ion concentration that produces the highest yield of a specific PCR product. When using Taq DNA Polymerase, too little free magnesium ion results in little or no PCR product, while excess free magnesium ion can cause unwanted products and promote misincorporation.
	Confirm species specificity of your primers. You may need to redesign your primers.
No PCR products with Real-time PCR.	The DNA may need to be purified before performing real-time PCR. We recommend the QIAquick PCR Purification Kit (Qiagen Cat. No. 28104). The column elutes in 50 µl and you use 2 µl for each reaction, giving you enough DNA for 25 PCR reactions.

Problem/question	Recommendation
Low yield when using enzymatic shearing.	It is critical to perform dounce homogenization when using enzymatic shearing, and is highly recommended when using sonication shearing. (Our recommended dounce is the Kimble-Kontes Cat. No. 885302-002, 2 ml, with the B pestle.) To ensure cell lysis, look at 10 µl of the sample before and after homogenization under a phase contrast microscope using a hemocytometer. Check that the nuclei have been released: Intact cells have a dark central region (nucleus) surrounded by a halo of the less dense cytoplasm. Comparing cells before and after lysis makes it easier to determine the extent of lysis, as this can be difficult looking only at homogenized cells. If the cells are not lysed, perform an additional 10 strokes of the dounce homogenizer until the cells are lysed.
	Cross-linking for longer periods of time tends to cause cells to form into a giant cross-linked aggregate that is not sheared efficiently. Decrease the incubation time of the formaldehyde fixation step.
Low yield when using sonication shearing.	It is highly recommended to perform dounce homogenization, even when using sonication shearing, as this will increase your yield and improve your ChIP.
	Cross-linking for longer periods of time tends to cause cells to form into a giant cross-linked aggregate that is not sheared efficiently. Decrease the incubation time of the formaldehyde fixation step.
How do I design PCR primers to analyze shearing efficiency and to map putative DNA-binding sites?	<p>Negative control PCR primers can be used to demonstrate that chromatin was sufficiently sheared. For example, negative control primers can be designed to amplify a DNA fragment that is 2 kb away from the "Target DNA" (the region bound by the protein of interest). Following ChIP reactions (performed with antibody against the protein of interest and with a negative IgG), PCR is performed with the negative control primers and with primers that amplify the Target DNA. The PCR should show that the anti-protein-of-interest ChIP enriches for Target DNA, but not for the negative control DNA. This result would support the conclusion that the enrichment was due to protein binding to (or near) the putative Target, and not due to binding elsewhere on a very large (poorly sheared) chromatin fragment.</p> <p>Similarly, appropriately designed PCR primers can be used to roughly map the DNA Target of the protein of interest. For example, primers can be designed to amplify short (approximately 100 bp) DNA fragments that are progressively closer to the putative Target DNA (e.g. within 1.5 kb, 1 kb, 500 bp, 250 bp). This type of analysis can help confirm the exact binding site of the protein of interest. For such higher-resolution mapping, the chromatin must be extensively sheared (DNA fragment size should be less than 500 bp).</p>

Section G. Related Products

ChIP-IT™ Kits	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express HT	96 rxns	53018
Re-ChIP-IT™	25 rxns	53016
ChIP-IT™	25 rxns	53001
ChIP-IT™ Enzymatic	25 rxns	53006
ChIP-IT™ Shearing Kit	10 rxns	53002
Enzymatic Shearing Kit	10 rxns	53005
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021

ChIP-validated Antibodies	Application	Format	Catalog No.
AP-2 pAb	ChIP, EMSA	17 rxns	39304
c-Jun pAb	ChIP, EMSA, IF	100 µg	39309
C/EBPα pAb	ChIP, EMSA, IF, WB	100 µg	39306
CTCF mAb	ChIP, WB	200 µg	39621
DNMT1 mAb	ChIP, IHC, IP, WB	100 µg	39204
DNMT3A mAb	ChIP, IF, IHC, WB	100 µg	39206
DNMT3B mAb	ChIP, IF, IP, WB	100 µg	39207
E2F-1 pAb	ChIP, EMSA	17 rxns	39313
E2F-6 mAb	ChIP, WB	100 µl	39509
EZH2 pAb	ChIP, IF, IP, WB	200 µl	39103
HBP-1 mAb	ChIP, IF, WB	100 µl	39511
HDAC1 mAb (Clone 10E2)	ChIP, IF, IHC, IP, WB	200 µl	39531
HDAC2 mAb (Clone 3F3)	ChIP, IF, IHC, IP, WB	200 µl	39533
HDAC3 pAb	ChIP, WB	100 µg	40968
HDAC4 pAb	ChIP, WB	100 µg	40969
HDAC5 pAb	ChIP, WB	100 µg	40970
HDAC6 pAb	ChIP, WB	100 µg	40971
Histone H2A pAb	ChIP, WB	200 µl	39235
Histone H2A phospho Ser129 pAb	ChIP, IF, IP, WB	200 µl	39271
Histone H2A.Z pAb	ChIP, WB	200 µl	39113
Histone H2B pAb	ChIP, WB	200 µl	39237
Histone H2B acetyl Lys5 pAb	ChIP, WB	200 µl	39123
Histone H2B acetyl Lys16 pAb	ChIP, WB	200 µl	39121
Histone H2B acetyl Lys46 pAb	ChIP, WB	200 µl	39571
Histone H3, C-terminal pAb	ChIP, WB	200 µl	39163
Histone H3 acetyl Lys4 pAb	ChIP, IF, WB	200 µl	39381
Histone H3 monomethyl Lys4 mAb	ChIP, WB	100 µg	39635
Histone H3 dimethyl Lys4 pAb	ChIP, WB	200 µl	39141
Histone H3 trimethyl Lys4 pAb	ChIP, WB	200 µl	39159
Histone H3 dimethyl Lys9 pAb	ChIP, IF, WB	200 µl	39239
Histone H3 trimethyl Lys9 pAb	ChIP, WB	200 µl	39161
Histone H3 acetyl Lys18 pAb	ChIP, IF, WB	200 µl	39587
Histone H3 acetyl Lys27 pAb	ChIP, IF, WB	200 µg	39133
Histone H3 acetyl Lys27 pAb	ChIP, WB	200 µl	39135
Histone H3 dimethyl Lys27 pAb	ChIP, IF, WB	200 µl	39245
Histone H3 trimethyl Lys27 mAb	ChIP, WB	200 µl	39535
Histone H3 trimethyl Lys27 pAb	ChIP, IF, WB	200 µg	39155
Histone H3 trimethyl Lys27 pAb	ChIP, WB	200 µl	39156
Histone H3 acetyl Lys36 pAb	ChIP, IF, WB	200 µl	39379
Histone H3 acetyl Lys56 pAb	ChIP, WB	200 µl	39281
Histone H3 acetyl Lys64 pAb	ChIP, IF, WB	200 µl	39545
Histone H3 acetyl Lys79 pAb	ChIP, WB	200 µl	39565
Histone H4 pan-acetyl pAb	ChIP, IF, WB	200 µl	39243
Histone H4 tetra-acetyl pAb	ChIP, WB	50 µl	39179
Histone H4 acetyl Lys5 pAb	ChIP, IF, WB	200 µl	39169
Histone H4 acetyl Lys5 pAb	ChIP, IF, WB	200 µl	39583
Histone H4 acetyl Lys12 pAb	ChIP, IF, WB	200 µl	39165
Histone H4 acetyl Lys16 pAb	ChIP, WB	200 µl	39167
Histone H4 monomethyl Lys20 pAb	ChIP, IF, WB	200 µl	39175
Histone H4 trimethyl Lys20 pAb	ChIP, IF, WB	200 µl	39180
IRF-3 pAb	ChIP, WB	100 µl	39033
JunB pAb	ChIP, EMSA	17 rxns	39326
JunD pAb	ChIP, EMSA	100 µl	39328
L3MBTL1 pAb	ChIP, IP, WB	200 µl	39182
p53 pAb	ChIP, EMSA	17 rxns	39334

ChIP-validated Antibodies (cont.)	Application	Format	Catalog No.
PP2A pAb	ChIP, IP, WB	200 µl	39192
RbAp46/48 pAb	ChIP, WB	200 µl	39198
RNA pol II mAb	ChIP, ELISA, IF, IP, WB	200 µl	39097
SNF2h mAb	ChIP, IF, IP, WB	200 µl	39543
Sp1 pAb	ChIP, WB	100 µl	39058
TRF2 Goat pAb	ChIP, IP, WB	100 µg	39223

Application Key: ChIP = Chromatin Immunoprecipitation; EMSA = Electrophoretic Mobility Shift Assay; IF = Immunofluorescence; IHC = Immunohistochemistry; IP = Immunoprecipitation; WB = Western blot; For an up-to-date list of ChIP-validated antibodies, please visit www.activemotif.com/chipabs

Histone Purification & Chromatin Assembly	Format	Catalog No.
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	10 rxns	40026
Chromatin Assembly Kit	10 rxns	53500
HeLa Core Histones	36 µg	53501

Histone Acetyltransferase and Deacetylase Activity	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

Methylated Histones	Format	Catalog No.
Recombinant Histone H3 (C110A)	50 µg	31207
Recombinant Histone H3 monomethyl Lys4	50 µg	31208
Recombinant Histone H3 dimethyl Lys4	50 µg	31209
Recombinant Histone H3 trimethyl Lys4	50 µg	31210
Recombinant Histone H3 monomethyl Lys9	50 µg	31211
Recombinant Histone H3 dimethyl Lys9	50 µg	31212
Recombinant Histone H3 trimethyl Lys9	50 µg	31213
Recombinant Histone H3 monomethyl Lys27	50 µg	31214
Recombinant Histone H3 dimethyl Lys27	50 µg	31215
Recombinant Histone H3 trimethyl Lys27	50 µg	31216
Recombinant Histone H3 monomethyl Lys36	50 µg	31217
Recombinant Histone H3 dimethyl Lys36	50 µg	31218
Recombinant Histone H3 trimethyl Lys36	50 µg	31219
Recombinant Histone H3 monomethyl Lys79	50 µg	31220
Recombinant Histone H3 dimethyl Lys79	50 µg	31221
Recombinant Histone H3 trimethyl Lys79	50 µg	31222
Recombinant Histone H4	50 µg	31223
Recombinant Histone H4 monomethyl Lys20	50 µg	31224
Recombinant Histone H4 dimethyl Lys20	50 µg	31225
Recombinant Histone H4 trimethyl Lys20	50 µg	31226

DNA Methylation	Format	Catalog No.
MethylDetector™	50 rxns	55001
MethylCollector™	25 rxns	55002
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55005
Fully Methylated Jurkat DNA	10 µg	55003

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

Active Motif North America

1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008

USA

Toll Free: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351
E-mail: tech_service@activemotif.com

Active Motif Europe

104 Avenue Franklin Roosevelt
B-1330 Rixensart, Belgium

UK Free Phone: 0800 169 31 47
France Free Phone: 0800 90 99 79
Germany Free Phone: 0800 181 99 10
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Active Motif Japan

Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan

Telephone: +81 3 5225 3638
Fax: +81 3 5261 8733
E-mail: japantech@activemotif.com

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