TAM-ChIP Assay Reagents

(version A2)

Catalog No. 53128

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
Avenue Reine Astrid, 92
B-1310 La Hulpe, 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

Active Motif China
787 Kangqiao Road
Building 10, Suite 202, Pudong District
Shanghai, 201315, China
Telephone: (86)-21-20926090
Hotline: 400-018-8123

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Overview

Chromatin Immunoprecipitation (ChIP) is a powerful tool for studying protein/DNA interactions, including transcription factors, co-regulatory proteins, modified histones, chromatin-modifying enzymes and polymerases because it enables identification of the localization patterns of proteins bound to specific DNA loci. When used in combination with whole-genome sequencing, insights into gene regulation, gene expression, mechanisms of chromatin modification and pathway analysis are possible.

Traditional methods to prepare ChIP-Seq libraries involve a series of processing steps to end repair the DNA into phosphorylated blunt ends, followed by addition of an 'A' base to the 3´end of the DNA fragments for ligation to adapters containing single 'T' base overhangs. Libraries are then PCR amplified and size-selected prior to Next-generation sequencing (NGS). In addition to being time consuming, one of the biggest challenges associated with traditional library preparation is the sample loss that can occur throughout the multi-step process. To overcome this obstacle, Active Motif has developed TAM-ChIP*, for simultaneous genomic targeting and library preparation.

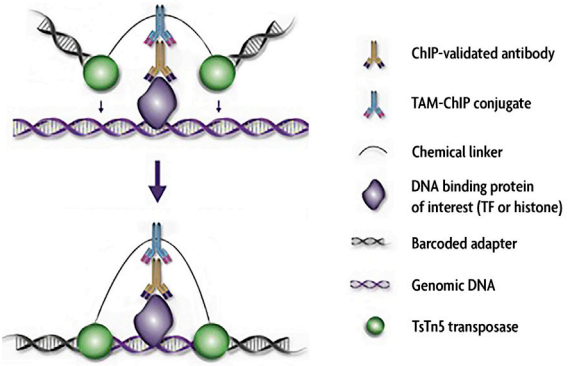
Active Motif's TAM-ChIP uses antibody/oligonucleotide conjugates to target a unique TsTn5 transposase to genomic regions containing a particular mark of interest (histone or transcription factor). Upon antibody binding, the transposase cuts the nearby DNA and pastes the antibody associated oligonucleotides into the DNA sequence. These oligonucleotides contain the necessary adapters for library amplification and NGS using Illumina® platforms, thereby eliminating multiple end-repair and ligation steps associated with traditional library preparation. Additionally, molecular identifiers (8 bp random N sequence) are included in the adapter sequences to easily remove PCR duplicates to avoid PCR bias. Another benefit of TAM-ChIP is that the combination of chromatin fragmentation and transposase tagmentation creates shorter DNA fragments for higher resolution of protein-DNA binding sites.

TAM-ChIP Assay Reagents includes the necessary buffers and PCR amplification reagents needed to perform 16 TAM-ChIP reactions. Additional reagents that are required, but not included are a ChIP-Seq validated antibody for the target protein of interest and the appropriate species-specific TAM-ChIP antibody conjugate.

product	format	catalog no.
TAM-ChIP Assay Reagents	16 rxns	53128
TAM-ChIP anti-rabbit conjugate	16 rxns	53126
TAM-ChIP anti-mouse conjugate	16 rxns	53127

* Patent pending

Overview of TAM-ChIP



Overview of the TAM-ChIP process.

TAM-ChIP is designed to perform antibody-based genomic targeting and NGS library preparation at once. First, intact cells are fixed with formaldehyde, which cross-links and preserves protein/DNA interactions. DNA is then sheared into small fragments using sonication and incubated with a ChIP-validated antibody directed against the DNA-binding protein of interest. Then, a species-specific TAM-ChIP antibody conjugate containing Illumina-compatible sequencing adapters and random molecular identifier is added to bind the ChIP antibody. The antibody-bound protein/DNA complexes are immunoprecipitated with Protein G agarose beads and washed to remove non-specific DNA. Activation of the TsTn5 transposase cuts the nearby DNA surrounding the genomic region of interest and pastes the antibody associated barcoded adapters into the DNA sequences. Following immunoprecipitation, cross-links are reversed, the proteins are removed by Proteinase K, and the DNA is recovered and purified. ChIP enriched DNA is now ready for library amplification and sequencing.

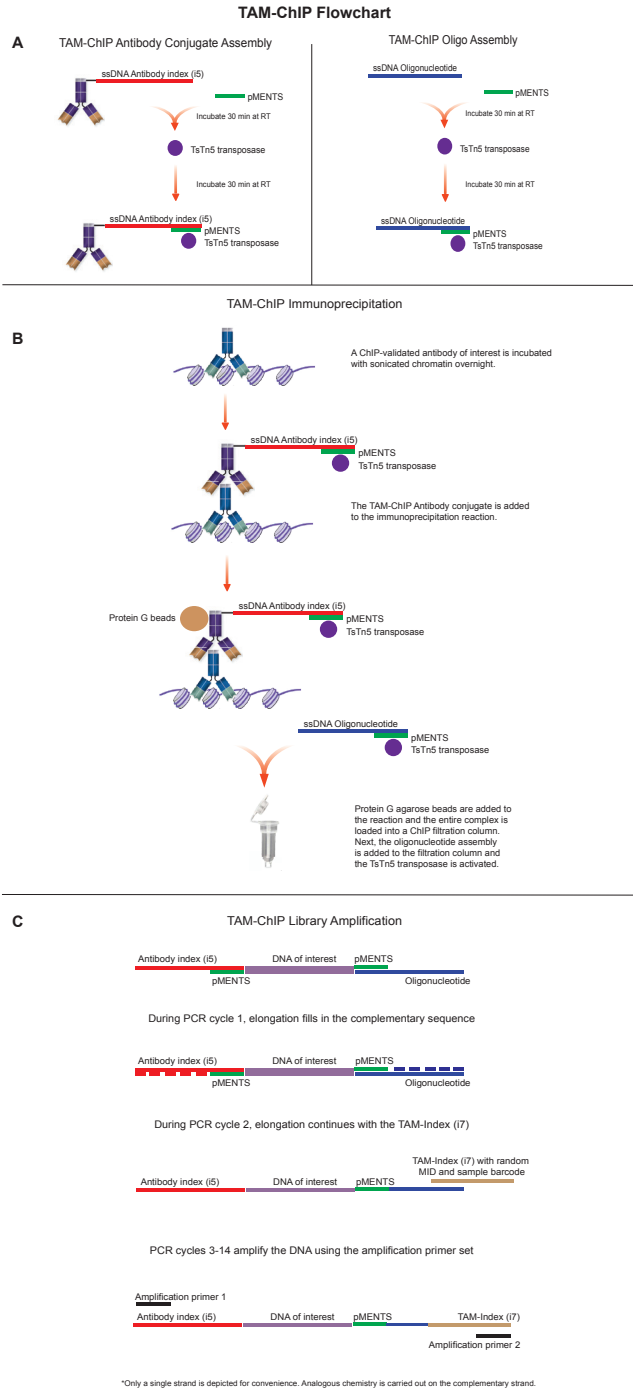
TAM-ChIP Flowchart (opposite page; also available on www.activemotif.com/tamchip and click the Methods tab)

TAM-ChIP is a multi-step process that involves assembling the unique TsTn5 transposase to the secondary antibody conjugate and an oligonucleotide prior to immunoprecipitation (**A**). The TAM-ChIP anti-species antibody conjugate contains a single-stranded oligonucleotide comprised of the i5 antibody index for Next-generation sequencing. Each anti-species antibody has its own i5 index to enable multiplexing of both the anti-rabbit and anti-mouse conjugates within the same sequencing reaction. When the antibody conjugate is combined with a single-stranded pMENTS oligonucleotide, the complementary sequences anneal to create a double-stranded recognition sequence for the TsTn5 transposase to assemble. This process loads inactivated transposase onto the antibody conjugate. At the same time, a single-stranded oligonucleotide is also annealed to pMENTS and loaded with inactive TsTn5.

During immunoprecipitation (**B**), the ChIP-validated antibody of interest is combined with the sonicated chromatin and incubated overnight. The next day, the assembled TAM-ChIP conjugate is added to the immunoprecipitation reaction. Following an incubation, protein G agarose beads are added to capture the chromatin fragments of interest. Each ChIP reaction is loaded onto a ChIP filtration column and the oligonucleotide assembled with transposase is added to the column. The TsTn5 is activated to insert the i5 index and oligonucleotide sequence into the genomic region surrounding the protein of interest. Following elution and DNA purification, the ChIP library is ready for amplification.

The TAM-ChIP library is amplified by PCR prior to sequencing (**C**). During PCR, the second strand of the DNA template is extended. Then, the i7 TAM-ChIP Index containing the 3 bp sample barcode and 8 bp random molecular identifier (MID) for PCR de-duplication is incorporated into the template. Additional PCR cycles amplify the entire template using the amplification primer mix. Libraries are size-selected and sequenced using Illumina platforms.

Flowchart



*Only a single strand is depicted for convenience. Analogous chemistry is carried out on the complementary strand.

Kit Performance and Benefits

TAM-ChIP Advantages:

- Tagmentation of the DNA using the TsTn5 transposase aids in chromatin fragmentation and provides higher resolution of protein binding sites
- Avoid sample loss common with traditional library preparation steps
- Molecular identifiers (8 bp random barcodes) enable distinction of PCR duplicates from biological replicates during analysis to increase the number of unique alignments 3-fold
- Highly robust procedure has been validated across multiple sample types using both transcription factor and histone marks

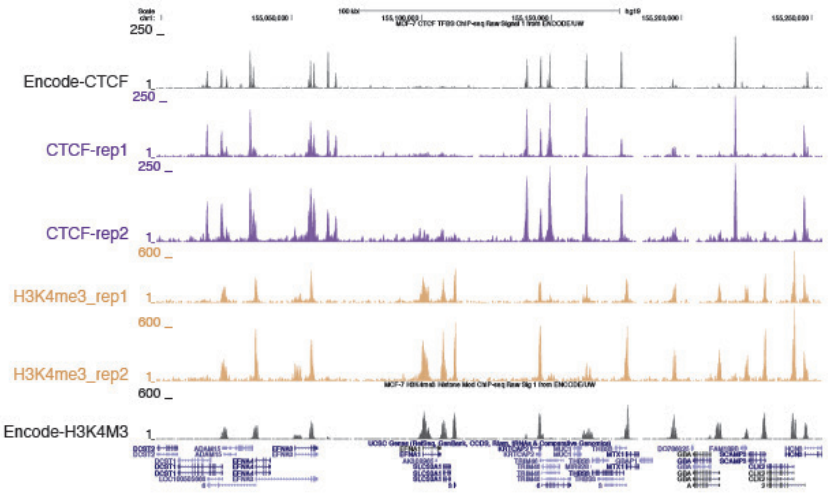
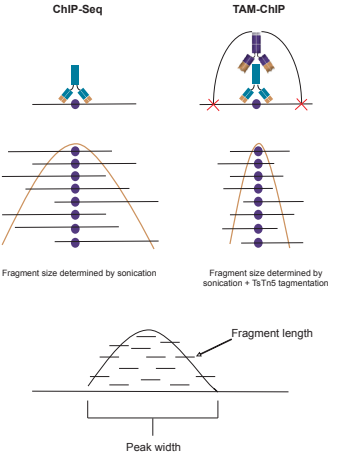


Figure 1: Sequencing data comparing TAM-ChIP replicates to traditional ChIP-Seq peaks.

TAM-ChIP was performed using 10 µg chromatin from MCF-7 cells with antibodies to CTCF and Histone H3K4me3. Results show reproducibility between replicates and peaks that are consistent with published ENCODE data sets.



	ChIP-Seq	TAM-ChIP
Fragment Length (nt)	250	125
Peak Width (nt)	1245	170

Figure 2: TAM-ChIP provides greater binding site resolution.

TAM-ChIP utilizes a combination of sonication and TsTn5 tagmentation to cut the DNA surrounding the genomic region of interest. The addition of tagmentation produces smaller DNA fragments than traditional ChIP-Seq, which improves the resolution of protein-DNA binding sites.

Protocol Overview and Time Table

	Required Time
Cell or Tissue Fixation and Lysis	1.5 hours
Chromatin Sonication	15 minutes per sample
Assessment of Chromatin Size*	4.5 hours for cell culture Overnight for tissue
Immunoprecipitation	Overnight
Assembly of Antibody Conjugate	1.5 hours
Binding of TAM-ChIP Conjugate	2 hours
Binding to Protein G agarose Beads	2 hours
Wash Immune Complexes	30 minutes
Activate TsTn5 transposase	1 hour
Reversal of Cross-links	2 hours
DNA Purification	30 minutes
Library Amplification & Size Selection	2 hours

* The protocol varies between cell culture and tissue samples.

Kit Components and Storage

Please store each component at the temperature indicated in the table below.

Reagents	Quantity	Storage
RNase A (10 µg/µl)	40 µl	-20°C
Proteinase K (10 µg/µl)	200 µl	-20°C
Blocker	100 µl	-20°C
100 mM PMSF	500 µl	-20°C
Protease Inhibitor Cocktail (PIC)	500 µl	-20°C
Precipitation Buffer	1.5 ml	-20°C
Carrier	35 µl	-20°C
10X PBS	120 ml	-20°C
1 M DTT	80 µl	-20°C
10X Elution Buffer AM2	250 µl	-20°C
Q5 High-Fidelity Polymerase (2 U/µl)	10 µl	-20°C
5X Q5 Reaction Buffer	2 x 130 µl	-20°C
dNTPs (10 mM)	40 µl	-20°C
Fixation Buffer	2 x 1.5 ml	4°C
Protein G Agarose Beads*	500 µl	4°C
TE pH 8.0	3 x 1.5 ml	RT
5 M NaCl	400 µl	RT
Detergent	25 ml	RT
Stop Solution	20 ml	RT
Chromatin Prep Buffer	85 ml	RT
ChIP Filtration Columns	16 ea	RT
Caps and plugs for columns	50 ea	RT
ChIP Buffer	35 ml	RT
ChIP Buffer without SDS	10 ml	RT
Wash Buffer AM1	100 ml	RT
Elution Buffer AM4	2 x 1.5 ml	RT
DNA Purification Elution Buffer	5 ml	RT

* The Protein G Agarose Beads are shipped on dry ice and can be stored frozen until their first use. Once thawed, the Protein G beads **should not be re-frozen**, but stored at 4°C.

Additional materials required but not included

- A ChIP-Seq validated antibody directed against the protein of interest
- TAM-ChIP antibody conjugate appropriate for the ChIP-seq validated antibody species used for immunoprecipitation (Catalog Nos. 53126 and 53127)
- Dounce homogenizer with a small clearance pestle (*e.g.* Active Motif Catalog Nos. 40401 & 40415) with the tight-fitting "A" pestle). Use of a homogenizer is necessary to prepare cells for chromatin shearing.
- 37% formaldehyde solution with 10-15% methyl alcohol to prevent polymerization (*e.g.* Sigma Aldrich Catalog No. 252549). Do not use paraformaldehyde.
- Phenol, saturated (DNA Purification, Molecular Biology Grade, Amresco Catalog No. 0945)
- Chloroform/isoamyl alcohol (24:1) (DNA Purification, Molecular Biology Grade)
- Phase Lock Gel™ Heavy tubes (VWR, Catalog No. 10847-802)
- 100% ethanol (absolute)
- 80% ethanol
- Agencourt AMPure XP Beads (Beckman Coulter, A63880)
- DNase-free H_2O
- Rocking platform for culture plates
- Apparatus to rotate tubes end-to-end at 4°C (*e.g.* a Labquake from Barnstead/Thermolyne with a tube holder for 1.5 ml microcentrifuge tubes)
- Microcentrifuge (table top centrifuge 4°C) and microcentrifuge tubes
- 250 µl PCR tubes
- Thermocycler
- 15 and 50 ml conical tubes
- DNA quantitation apparatus
- Pipettors and tips (filter tips are recommended)
- Sonicator (*e.g.* Active Motif's EpiShear™ Sonicator with a 1/8" probe (Catalog No. 53051) with the EpiShear™ Cooled Sonication Platform (Catalog No. 53080))
- Agarose gel electrophoresis apparatus or method for visualizing fragmentation
- Razor blades (for tissue preparations)
- Hand-held homogenizer for tissue preparations (*e.g.* Biospec Products Tissue-Tearor)
- Cell scraper (rubber policeman)

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Cell Growth Recommendations

When planning an experiment, calculate the number of chromatin preparations you will require and determine the number of ChIP reactions you plan to perform on each chromatin preparation. Be sure to include the appropriate positive and negative 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. The minimum recommended number of cells that should be used for the preparation of chromatin is 100,000 cells.

	6-well plate	60 mm dish	100 mm dish	150 mm dish
Seeding Density	0.3 x 10^6	0.8 x 10^6	2.2 x 10^6	5.0 x 10^6
Cells at 70-80% Confluency*	0.9 x 10^6	2.4 x 10^6	6.6 x 10^6	15.0 x 10^6
Growth Medium Volume	3 ml	5 ml	10 ml	20 ml
Cell Fixative Solution	300 µl	500 µl	1 ml	2 ml
Stop Solution	165 µl	275 µl	550 µl	1.1 ml
PBS Wash Buffer (used per wash)	1 ml	2 ml	5 ml	10 ml
Chromatin Prep Buffer	1 ml	2 ml	5 ml	5 ml
ChIP Buffer	500 µl	500 µl	500 µl	500 µl

* The number of cells on a confluent plate or dish will vary with cell type. For this table, HeLa cells were used. Please adjust as needed based on your particular cell type.

**Please refer to the descriptions below for complete details on buffer preparations

Buffer Preparation

Complete Cell Fixation Solution

Buffer should be prepared fresh before each experiment. For every 20 ml of cell growth medium used, prepare 2.5 ml of Complete Cell Fixation Solution by adding 180 µl Fixation Buffer to 1.57 ml sterile water in a 15 ml conical tube. Using appropriate precautions (*i.e.* safety glasses, gloves and lab coat), add 750 µl 37% formaldehyde to the tube and vortex to mix. Use 1/10 growth medium volume per plate. Complete cell fixation solution can be added to the growth medium in the presence or absence of serum.

Complete Tissue Fixation Solution

Buffer should be prepared fresh before each experiment. Prepare 10 ml of Tissue Fixation Solution for each tissue sample to be processed by adding 1 ml 10X PBS to 8.7 ml sterile water in a

15 ml conical tube. Using appropriate precautions (*i.e.* safety glasses, gloves and lab coat), add 280 µl 37% formaldehyde to the tube and vortex to mix.

Stop Solution

Is provided ready to use. Use 1/20 media volume per cell culture plate or 515 µl per 10 ml Complete Tissue Fixation Solution.

PBS Wash Buffer

Prepare 25 ml PBS Wash Buffer for every 15 cm plate or tissue sample. To a 50 ml conical tube add 21.25 ml sterile water, 2.5 ml 10X PBS and 1.25 ml Detergent. Mix by inverting. Place PBS Wash Buffer on ice to chill. PBS Wash Buffer can be prepared in large quantities and stored at 4°C for 6 months.

100 mM PMSF and Protease Inhibitor Cocktail (PIC)

Thaw the PMSF and the PIC at room temperature until fully dissolved, which takes about 30 minutes. Vortex gently and spin down briefly before use, then add to the buffers immediately before use.

1 M DTT

Prepare a fresh 70 mM DTT working stock for each TAM-ChIP experiment. Take 5 µl 1M DTT and add to 66 µl sterile water. Vortex to mix. This makes a 70 mM working solution. Store any unused solution at -20°C. Discard at the end of the day.

Chromatin Prep Buffer

Is supplied ready to use.

ChIP Buffer without SDS

Is supplied ready to use.

ChIP Buffer

Is supplied ready to use.

Protein G Agarose Beads

The supplied agarose beads require washing prior to use. Follow the instructions in the manual to wash the beads for use in the ChIP reactions. There is no need to pre-block the beads or pre-clear the sample. For best results, gently shake and invert the tube to resuspend the agarose beads. The beads settle quickly, and therefore should be resuspended just before pipetting. We recommend cutting 2 mm from the end of a pipet tip prior to pipetting to prevent the tip from becoming clogged. **Protein G Agarose Beads are shipped on dry ice and can be stored frozen until their first use. Once thawed, beads should not be re-frozen by the customer. Protein G Agarose Beads should be stored at 4°C.**

Recommendations

ChIP-validated Antibody

We recommend using 4 µg antibody per ChIP reaction. However, this will vary according to the affinity of the antibody and the quality of the chromatin; you may need to use more of a particular antibody. 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 antibody that has not been ChIP-validated must include appropriate controls. To see a list of available ChIP-validated antibodies available from Active Motif, please visit www.activemotif.com/chipabs.

Chromatin Shearing Tips

We suggest using a probe sonicator (*i.e.* Active Motif's EpiShear Probe Sonicator) which employs a direct sonication method to prepare chromatin for use in TAM-ChIP. Indirect sonication systems may require longer sonication times to achieve optimal chromatin shearing. ChIP experiments usually require chromatin that has been sheared to a size of 200-1000 bp. 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 with air bubbles. To determine the appropriate shearing level for your sample, set up a "practice" tube containing only ChIP Buffer. Slowly increase the sonication amplitude until foaming starts to occur. Reduce the amplitude setting down slightly and mark this as the highest possible intensity to use without foaming. If a chromatin preparation becomes emulsified inadvertently, discontinue shearing and centrifuge the sample at maximum speed for 4 minutes at 4°C 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 20 seconds, then place on ice/water for 30 seconds, sonicate again for 20 seconds, *etc.*). If possible, shear while on ice or use Active Motif's EpiShear™ Cooled Sonication Platform (Catalog No. 53080) to help regulate sample temperature.

Chromatin Quantity

It is recommended to use 5-10 µg chromatin per IP reaction (0.75-1.5 million cell equivalents).

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 should be performed in a biosafety hood if the chromatin is extracted from biohazardous or infectious materials.

Section A: Cell Fixation Starting with Cultured Cells

This protocol describes cell fixation and chromatin preparation from one 15 cm plate (approximately 1.5 x 10^7 cells). We recommend using 20 ml growth medium per 15 cm plate. Please refer to page 7 for information on scaling the protocol for use with other amounts of cells. The minimum cell number to be used for the preparation of chromatin is 100,000 cells.

1. Prepare 15 cm plates for each cell line to be tested. Grow the cells to 70-80% confluency. Stimulate cells as desired to activate the pathway of interest.
2. Freshly prepare Complete Cell Fixation Solution for each 15 cm plate. The volumes listed below are enough to process one 15 cm plate. Please refer to the chart on page 8 to scale the solution volumes.
3. To fix cells, add 1/10 growth medium volume of freshly prepared Complete Cell Fixative Solution to the existing culture media for the cells (*e.g.* 20 ml growth medium would get 2 ml Complete Cell Fixation Solution). Shake gently at room temperature for 15 minutes.
4. Stop the fixation reaction by adding 1/20 media volume of Stop Solution to the existing culture media for the cells (*e.g.* 20 ml growth medium would get 1.1 ml Stop Solution). Swirl to mix and incubate at room temperature for 5 minutes.
5. Following the incubation, hold the plate at an angle and using a rubber policeman scrape cells down to collect them at the bottom edge of the plate. Use a pipette to transfer the cells to a 50 ml conical tube on ice.
6. Pellet the cells from step 5 by centrifugation for 3 minutes at 1,250 x g at 4°C.
7. Remove the supernatant and discard. Resuspend the pellet(s) in 10 ml ice-cold PBS Wash Buffer by pipetting up and down. Keep samples ice-cold for the remainder of the procedure.
8. Centrifuge for 3 minutes at 1,250 x g at 4°C. Remove the supernatant and discard. Wash the pellet(s) a second time in 10 ml ice-cold PBS Wash Buffer by pipetting up and down. Centrifuge for 3 minutes at 1,250 x g at 4°C. Remove the supernatant and discard. Cell pellets may be stored at -80°C at this stage.
9. Resuspend each pellet(s) in 5 ml Chromatin Prep Buffer supplemented with 5 µl PIC and 5 µl 100 mM PMSF. Pipet up and down to mix.
10. Incubate on ice for 10 minutes.
11. Transfer the resuspended pellets individually to a chilled dounce homogenizer on ice. Use the tight fitting pestle (Type A) to homogenize the sample for 30 strokes. Transfer the contents to a new 15 ml conical tube and centrifuge for 3 minutes at 1,250 x g at 4°C.

    Monitor Cell Lysis: To ensure cell lysis, take 10 µl of the cell lysate from the dounce and look at it under a phase contrast microscope using a hemocytometer to verify that the nuclei have been released. It is often helpful to look at the cells before and after the lysis step as this makes it easier to identify the nuclei versus whole cells. Intact cells should have a dark central region (nucleus) surrounded by a halo of less dense cytoplasm. In lysed cells, the

nuclei will appear as dots surrounded by asymmetric debris. If the cells are not lysed, then dounce on ice with an additional 10 strokes, or until the cells are lysed.

12. Remove the supernatant and discard. Resuspend each pellet in 500 µl ChIP Buffer supplemented with 5 µl PIC and 5 µl 100 mM PMSF. Transfer the contents to a new 2 ml microcentrifuge tube.

13. Incubate on ice for 10 minutes. Proceed to Step B: Chromatin Sonication of Cultured Cells.

Section B. Chromatin Sonication of Cultured Cells

The section below describes the fragmentation of chromatin using sonication. Sonication results may vary depending on cell type and sonication device being used. This protocol has been validated using Active Motif's EpiShear™ Probe Sonicator in combination with an EpiShear™ Cooled Sonication Platform to maintain probe height and temperature consistency between samples. We do not recommend sonication of samples containing less than 100,000 cells and/or 350 µl volume.

The ChIP Buffer has been optimized for immunoprecipitation performance, however, due to its unique composition optimization of sonication conditions may be required. To maintain the high sensitivity of the assay, we recommend using our buffer system and altering the sonication time and/or amplitude of your sonication system to achieve the desired fragmentation (*e.g.* some systems may require as much as a three-fold increase in sonication time to improve chromatin shearing). Please pay particular attention to our protocol regarding the processing of input chromatin for agarose gel analysis prior to the chromatin immunoprecipitation reaction as many steps may differ from traditional ChIP protocols and failure to follow the outlined procedure may lead to artifacts in the gel images as shown in Figure 3 on page 14.

1. Place the 2 ml microcentrifuge tube containing the chromatin into the tube cooler or packed ice. Open cap and submerge the microtip into the liquid until the microtip is approximately 5 mm from the bottom of the tube. Sonicate according to optimized settings for the cell type being used (see Recommendations on page 10). A recommended starting range for cultured cells is: 25% amplitude, pulse for 30 seconds on and 30 seconds off for a total sonication "on" time of 10 minutes (or 20 minutes elapsed time).

2. Spin tubes at 4°C in a microcentrifuge at maximum speed for 2 minutes to pellet the cellular debris.

3. Transfer 25 µl of each chromatin preparation into a 250 µl PCR tube for analysis of shearing efficiency and chromatin quantification.

4. Aliquot the remainder of each chromatin preparation into 1.5 ml microcentrifuge tubes. We recommend making aliquots of 150 µl volume and storing at -80°C.

Note: The size of the chromatin sonication should be verified before proceeding to the immunoprecipitation step.

Chromatin Quantification

5. To each 25 µl chromatin preparation from Step 3 above, add 175 µl TE pH 8.0 and 1 µl RNAse A. Cap the PCR tubes and vortex to mix
6. Incubate in a thermocycler at 37°C for 30 minutes.
7. Add 2 µl Proteinase K to each tube and vortex. Incubate tubes in a thermocycler at 55°C for 30 minutes and then increase the temperature to 80°C for 2 hours.
8. Transfer each chromatin input to a 1.5 ml microcentrifuge tube. Add 83 µl Precipitation Buffer, 2 µl Carrier and 750 µl absolute ethanol. Vortex to mix and chill at -80°C for 30 minutes to overnight.
9. Spin tubes at 4°C in a microcentrifuge at maximum speed for 15 minutes.
10. Carefully remove the supernatant taking care not to disturb the pellet. Wash the pellet with 500 µl 70% ethanol and spin at 4°C in a microcentrifuge at maximum speed for 5 minutes.
11. Carefully remove the supernatant taking care not to disturb the pellet. Remove residual ethanol with a pipet tip. Leave the tubes uncapped and air dry for 10-15 minutes.
12. When the pellets are dry, add 25 µl DNA Purification Elution Buffer to each tube. Incubate at room temperature for 10 minutes. Then vortex to ensure the pellet is completely resuspended. This solution contains your DNA for quantification.
13. Read the absorbance of each sample on a NanoDrop or other spectrophotometer at 260 nm to determine the DNA concentration of each chromatin preparation. Set aside 500 ng of DNA for analysis as described in Step 14. Store the remaining DNA at -20°C.
14. Analyze each chromatin preparation on an agarose gel by following the instructions below.
    a. Prepare 500 mM NaCl by adding 2 µl 5M NaCl to 18 µl sterile water. Vortex to mix.
    b. Transfer 500 ng of Input DNA to a 250 µl PCR tube and add 1 µl 500 mM NaCl. Adjust the final volume to 10 µl with sterile water if needed.
    c. Heat samples in a thermocycler at 100°C for 20 minutes followed by ramping the temperature down to 50°C.
    d. Remove tubes from the thermocycler and incubate at room temperature for 5 minutes.
    e. Add gel loading buffer to each sample and run on a 1.5% agarose gel. Include 100 bp and 1 kb DNA ladders to analyze chromatin size. DNA should appear as a smear anywhere between 200-1000 bp.

    Note: Chromatin may look different on an agarose gel compared to chromatin prepared using traditional ChIP methods. However, this will not affect the sensitivity of the assay or increase background signal. Please follow the protocol as listed above for preparing Input DNA. Use of an alternative reverse cross-linking method or omitting the 20 minute incubation at 100°C in NaCl is not recommended as this will cause artifacts that make the DNA appear larger. As long as the chromatin falls within the recommended 200-1000 bp range, proceed with the ChIP reaction. If fragments do not fall within this range sonication conditions should be further optimized.

15. If chromatin preparations were successful, the aliquots stored at -80°C from Section B, Step 4 can be used to perform the ChIP reactions in Section E.

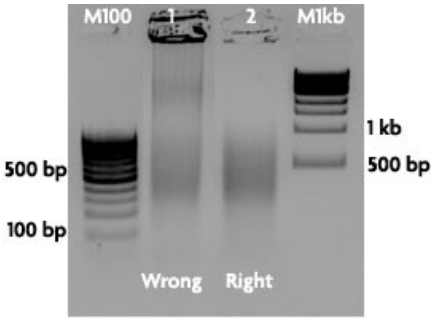


Figure 3: Validation of chromatin shearing efficiency following reversal of cross-links at 80°C for 2 hours.

Chromatin preparations of MCF-7 cells were fixed and sonicated using the EpiShear™ Probe Sonicator and EpiShear™ Cooled Sonication Platform from Active Motif. Input DNA was prepared in duplicate according to Section B, Steps 5-13 in the manual. In Sample 1, Step 14 was not performed and 500 ng of input DNA was loaded directly onto a 1.5% agarose gel without receiving the addition of NaCl and incubation at 100°C. The omission of Step 14 has caused a buffer artifact that makes the DNA appear larger on a gel. The duplicate sample, Sample 2, was processed according to the manual instructions and included the addition of NaCl and incubation at 100°C as stated in Step 14. Analysis of 500 ng of this input DNA on a 1.5% agarose gel shows the expected fragmentation between 200-1000 bp. The difference in DNA sizing on the gel between the two samples illustrates the importance of following the protocol recommendations regarding the processing of input chromatin for agarose gel analysis prior to chromatin immunoprecipitation. Omission of key steps can lead to inaccurate analysis of chromatin shearing efficiency. If the protocol steps were followed and the DNA fragments fall outside of the recommended range, sonication conditions should be further optimized.

Section C: Cell Fixation Starting with Fresh or Frozen Tissue

This protocol describes cell fixation and chromatin preparation from 100-400 mg fresh or frozen animal tissue. If performing chromatin preparation on multiple tissue samples, we recommend completing Steps 1-7 for each sample before processing the next sample.

1. For tissue fixation, transfer 10 ml Complete Tissue Fixation Solution (see Buffer Preparation on page 8) to a 60 mm petri dish. Place the dish on ice.
2. Add 100-400 mg fresh or frozen tissue sample to the petri dish and ensure that the sample is fully immersed. Cut the tissue sample into small pieces (approximately 1 mm cubes) using a razor blade.
3. Transfer the sample plus the Complete Tissue Fixation Solution to a 15 ml conical tube and rotate at room temperature for 15 minutes.
4. Stop the fixation reaction by adding 515 µl Stop Solution to the conical tube and rotate at room temperature for 5 minutes.
5. Place the conical tube on ice and homogenize the contents with a hand-held tissue homogenizer set at 30,000 rpm for 45 seconds.
6. Pellet the cells from Step 5 by centrifugation for 3 minutes at 1,250 x g at 4°C.
7. Remove the supernatant and discard. Resuspend the pellet in 10 ml ice-cold PBS Wash Buffer by pipetting up and down. Keep samples ice-cold for the remainder of the procedure.
8. Centrifuge for 3 minutes at 1,250 x g at 4°C. Remove the supernatant and discard. Wash the pellet(s) a second time in 10 ml ice-cold PBS Wash Buffer by pipetting up and down. Centrifuge for 3 minutes at 1,250 x g at 4°C. Remove the supernatant and discard. (Cell pellets may be stored at -80°C at this stage).
9. Resuspend each pellet in 5 ml Chromatin Prep Buffer supplemented with 5 µl PIC and 5 µl 100 mM PMSF.
10. Incubate on ice for 10 minutes.
11. Transfer the resuspended pellet(s) individually to a chilled dounce homogenizer on ice. Use the tight fitting pestle (Type A) to homogenize the sample for 30 strokes. Once finished, transfer the contents to a new 15 ml conical tube.

    Monitor Cell Lysis: To ensure cell lysis, take 10 µl of the cell lysate from the dounce and look at it under a phase contrast microscope using a hemocytometer to verify that the nuclei have been released. It is often helpful to look at the cells before and after the lysis step as this makes it easier to identify the nuclei versus whole cells. Intact cells should have a dark central region (nucleus) surrounded by a halo of less dense cytoplasm. In lysed cells, the nuclei will appear as dots surrounded by asymmetric debris. If the cells are not lysed, then dounce on ice with an additional 10 strokes, or until the cells are lysed.
12. Centrifuge for 3 minutes at 1,250 x g at 4°C.
13. Remove the supernatant and discard. Resuspend each pellet in 500 µl - 1 ml ChIP Buffer supplemented with PIC and 100 mM PMSF. (For 500 µl add 5 µl PIC and 5 µl PMSF. For 1 ml

add 10 µl PIC and 10 µl PMSF.) Transfer the contents to a new 2 ml microcentrifuge tube.

14. Incubate on ice for 10 minutes. Proceed to Section D: Chromatin Sonication of Tissue.

Section D. Chromatin Sonication of Tissue

The section below describes the fragmentation of chromatin using sonication. Due to the increased concentration of protein and cellular debris present in animal tissue, we recommend following this protocol for the preparation of chromatin and input DNA from tissue. Sonication results may vary depending on tissue type and sonication device being used. This protocol has been validated using Active Motifs EpiShear™ Probe Sonicator in combination with the EpiShear™ Cooled Sonication Platform to maintain probe height and temperature consistency between samples. We do not recommend sonication of samples containing less than 50 mg tissue and/or 350 µl volume.

The ChIP Buffer has been optimized for immunoprecipitation performance, however, due to its unique composition optimization of sonication conditions may be required. To maintain the high sensitivity of the assay, we recommend using our buffer system and altering the sonication time and/or amplitude of your sonication system to achieve the desired fragmentation (*e.g.* some systems may require as much as a three-fold increase in sonication time to improve chromatin shearing). Please pay particular attention to our protocol regarding the processing of input chromatin for agarose gel analysis prior to the chromatin immunoprecipitation reaction as many steps may differ from traditional ChIP protocols and failure to follow the outlined procedure may lead to artifacts in the gel images as shown in Figure 4 on page 18.

1. Place the 2 ml microcentrifuge tube containing the chromatin into the tube cooler or packed ice. Open cap and submerge the microtip into the liquid until the microtip is approximately 5 mm from the bottom of the tube. Sonicate according to optimized settings for the tissue type being used (see Recommendations on page 10). A recommended starting range for tissue samples is: 25% amplitude, pulse for 30 seconds on and 30 seconds off for a total sonication "on" time of 10 minutes (or 20 minutes elapsed time).
2. Spin tubes at 4°C in a microcentrifuge at maximum speed for 2 minutes to pellet the cellular debris.
3. Transfer 25 µl of each chromatin preparation into a 250 µl PCR tube for analysis of shearing efficiency and chromatin quantification.
4. Aliquot the remainder of each chromatin preparation into 1.5 ml microcentrifuge tubes. We recommend making aliquots of 150 µl volume and storing at -80°C.

   Note: The size of the chromatin sonication should be verified before proceeding to the immunoprecipitation step.

Chromatin Quantification

5. To each 25 µl chromatin preparation from Step 3 above, add 175 µl TE pH 8.0 and 2 µl RNAse A. Cap the PCR tubes and vortex to mix.
6. Incubate in a thermocycler at 37°C for 1 hour.
7. Add 5 µl Proteinase K to each tube, vortex and incubate in a thermocycler at 37°C for 3 hours.
8. Add 10 µl 5 M NaCl, vortex and incubate at 65°C for 6-16 hours to reverse cross-links.
9. Remove tubes from the thermocycler and add 250 µl phenol and 125 µl chloroform:isoamyl alcohol (24:1). Vortex vigorously and spin tubes in a room temperature microcentrifuge at maximum speed for 2 minutes.
10. Transfer each upper aqueous layer to a new 1.5 ml microcentrifuge tube and add 250 µl chloroform:isoamyl alcohol (24:1). Vortex vigorously and spin tubes in a room temperature microcentrifuge at maximum speed for 2 minutes.
11. Transfer the upper aqueous layer to a new 1.5 ml microcentrifuge tube. Add 83 µl Precipitation Buffer, 2 µl Carrier and 900 µl absolute ethanol. Vortex to mix and chill at -80°C for 30 minutes to overnight.
12. Spin at 4°C in a microcentrifuge at maximum speed for 15 minutes.
13. Carefully remove the supernatant taking care not to disturb the pellet. Wash the pellet with 500 µl 70% ethanol and spin at 4°C in a microcentrifuge at maximum speed for 5 minutes.
14. Carefully remove the supernatant taking care not to disturb the pellet. Remove residual ethanol with a pipet tip. Leave the tubes uncapped and air dry for 10-15 minutes.
15. When the pellets are dry, add 25 µl DNA Purification Elution Buffer to each tube. Incubate at room temperature for 10 minutes. Then vortex to ensure the pellet is completely resuspended. This solution contains your DNA for quantification.
16. Read the absorbance of each sample on a NanoDrop or other spectrophotometer at 260 nm to determine the DNA concentration of each chromatin preparation. Set aside 500 ng of DNA for analysis as described in Step 17. Store the remaining DNA at -20°C.
17. Analyze each chromatin preparation on an agarose gel by following the instructions below.
    a. Prepare 500 mM NaCl by adding 2 µl 5M NaCl to 18 µl sterile water. Vortex to mix.
    b. Transfer 500 ng of Input DNA to a 250 µl PCR tube and add 1 µl 500 mM NaCl. Adjust the final volume to between 10 µl with sterile water if needed.
    c. Heat samples in a thermocycler at 100°C for 20 minutes followed by ramping the temperature down to 50°C.
    d. Remove tubes from the thermocycler and incubate at room temperature for 5 minutes.
    e. Add gel loading buffer to each sample and run on a 1.5% agarose gel. Include 100 bp and 1 kb DNA ladders to analyze chromatin size. DNA should appear as a smear anywhere between 200-1000 bp.

    Note: Chromatin may look different on an agarose gel than chromatin prepared using traditional ChIP methods. However, this will not affect the sensitivity of the as-

say or increase background signal. Please follow the protocol as listed above for preparing Input DNA. Use of an alternative reverse cross-linking method or omitting the 20 minute incubation at 100°C in NaCl is not recommended as this will cause artifacts that make the DNA appear larger. As long as the chromatin falls within the recommended 200-1000 bp range, proceed with the ChIP reaction. If fragments do not fall within this range sonication conditions should be further optimized.

18. If chromatin preparations were successful, the aliquots stored at -80°C from Section D, Step 4 can be used to perform the ChIP reactions in Section E.

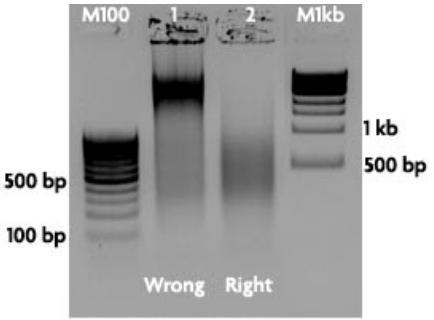


Figure 4: Validation of chromatin shearing efficiency following reversal of cross-links overnight at 65°C.

Chromatin preparations were fixed and sonicated using the EpiShear™ Probe Sonicator and EpiShear™ Cooled Sonication Platform from Active Motif. Input DNA was prepared in duplicate according to Section D, Steps 5-16 in the manual. In Sample 1, Step 17 was not performed and 500 ng of input DNA was loaded directly onto a 1.5% agarose gel without receiving the addition of NaCl and incubation at 100°C. The omission of Step 17 has caused a buffer artifact that makes the DNA appear larger on a gel. The duplicate sample, Sample 2, was processed according to the manual instructions and included the addition of NaCl and incubation at 100°C as stated in Step 17. Analysis of 500 ng of this input DNA on a 1.5% agarose gel shows the expected fragmentation between 200-1000 bp. The difference in DNA sizing on the gel between the two samples illustrates the importance of following the protocol recommendations regarding the processing of input chromatin for agarose gel analysis prior to chromatin immunoprecipitation. Omission of key steps can lead to inaccurate analysis of chromatin shearing efficiency. If the protocol steps were followed and the DNA fragments fall outside of the recommended range, sonication conditions should be further optimized.

Section E. Immunoprecipitation

Successful chromatin immunoprecipitation depends on the quality of the ChIP antibody and the abundance of the target protein. Completion of this section requires a TAM-ChIP anti-species secondary antibody conjugate (Catalog Nos. 53126 or 53127). Each TAM-ChIP antibody conjugate is provided with the necessary pMENTS, oligonucleotide, TsTn5 transposase and 5X Tagmentation buffer required to perform TAM-ChIP.

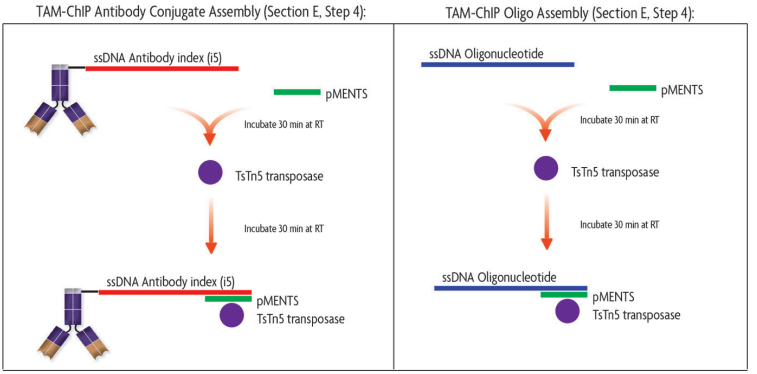
1. Thaw sonicated chromatin on ice. Spin chromatin at 4°C in a microcentrifuge at maximum speed for 2 minutes. Use the DNA concentration that was determined for your sonicated chromatin sample to calculate the volume to use. We recommend using 5-10 µg chromatin (0.75 - 1.5 million cell equivalents) per ChIP reaction.
2. Determine the number of ChIP reactions to perform. We strongly recommend setting up a ChIP reaction without primary antibody to serve as a tagmentation control. This control will show the effects of the transposase in the absence of genomic targeting and provide a background reference during sequencing. Set up the ChIP reactions using separate 1.5 ml microcentrifuge tubes for each ChIP antibody. Add the components in the order shown in Table 1 below:

Table 1

Reagent	1 reaction	Tagmentation Control
Blocker	5 µl	5 µl
ChIP-validated antibody	4 µg	–
Sheared Chromatin	5-10 µg	5-10 µg
Protease Inhibitor Cocktail (PIC)	5 µl	5 µl
ChIP Buffer without SDS	Adjust to 200 µl	Adjust to 200 µl

3. Cap tubes and incubate on an end-to-end rotator overnight at 4°C.

4. The next day, prepare the TAM-ChIP antibody conjugate and the oligo assembly in parallel. Label separate microcentrifuge tubes for antibody conjugate and oligo assembly. If preparing multiple ChIP reactions scale up the volumes accordingly. Refer to the lot-specific data sheet provided with the TAM-ChIP anti-species conjugate for details on the volumes to use.



TAM-ChIP Antibody Conjugate	
Reagent	**1 reaction**
Antibody conjugate	See lot-specific data sheet
pMENTs	See lot-specific data sheet
Incubate for 30 minutes at RT	
TsTn5 Transposase	See lot-specific data sheet
Incubate for 1 hour at RT	

Oligo Assembly	
Reagent	**1 reaction**
Oligonucleotide	See lot-specific data sheet
pMENTs	See lot-specific data sheet
Incubate for 30 minutes at RT	
TsTn5 Transposase	See lot-specific data sheet
Incubate for 1 hour at RT (Keep at RT until ready for use in Step 19)	

5. Remove the immunoprecipitation reaction from the rotator and quick spin to collect contents to the bottom of the tube. Add the assembled TAM-ChIP antibody conjugate to the immunoprecipitation reaction. Pipet up and down to mix.
6. Incubate for 1 hour at 4°C on an end-to-end rotator.
7. Add 1 µl of 70 mM DTT to each sample. Pipet up and down to mix.
8. Incubate samples at 37°C for 1 hour on an end-to-end rotator. During the incubation, prepare the Protein G agarose beads as described in Step 9 below.
9. The Protein G agarose beads require washing before use. Transfer 30 µl Protein G agarose beads for each immunoprecipitation reaction to a 1.5 ml microcentrifuge tube. Add an equal volume of TE, pH 8.0 and invert to mix. Spin at 1250 x g in a microcentrifuge for 1 minute. Remove the supernatant equivalent to the volume of TE added to the agarose beads.

Note: Before pipetting the Protein G agarose beads, they should be fully resuspended by inverting the tube. When pipetting the beads, cut 2 mm from the end of a pipet tip to prevent the tip from becoming clogged.

10. Wash the beads a second time with the same volume of TE, pH 8.0. Invert to mix. Spin at 1250 x g for 1 minute in a microcentrifuge. Remove the supernatant equivalent to the volume of TE added to the agarose beads. The beads are now ready to use.
11. Spin the ChIP reactions at 1250 x g for 1 minute to collect liquid from the inside of the cap.
12. Using a cut pipet tip, add 30 µl washed Protein G agarose beads to each immunoprecipitation reaction. Cap tubes and incubate on an end-to-end rotator at 4°C for 2 hours.
13. Label a ChIP Filtration Column for each ChIP reaction. Remove the tab from the bottom of the column and place in an empty 1 ml pipet tip box as a holder (see Figure 5 on page 22).
14. Remove ChIP reactions from rotator and spin at 1250 x g for 1 minute to collect liquid from inside of the cap.
15. Transfer the entire ChIP reaction (including the protein G agarose beads) to the labeled ChIP Filtration Column an allow flow-through to occur by gravity.
16. Dry the bottom of each column with a Kim wipe and add a yellow plug to the bottom of the ChIP filtration column to seal.
17. Add 800 µl ChIP Buffer to each column and incubate at room temperature for 5 minutes. Then, remove and discard the yellow plug. Allow flow-through to occur by gravity.
18. Once all the liquid has flowed through the column, blot the bottom of the column with a Kim wipe and add a new yellow plug to the bottom of the ChIP filtration column.
19. Prepare a Tagmentation Master Mix. Using the volumes required per reaction, calculate the volumes needed for the master mix. Combine the reagents in the order shown in Table 2 to a 2 ml (or larger) tube. Pipette up and down to mix.

Table 2: Tagmentation Master Mix

Reagent	1 reaction		Master Mix
ChIP Buffer without SDS	400 µl	X # rxns =	µl
Tagmentation Buffer	100 µl	X # rxns =	µl
Oligo Assembly (From Step E.4)	variable	X # rxns =	µl
70 mM DTT	2.5 µl	X # rxns =	µl
Total Volume	variable		

20. Calculate the single reaction total volume of tagmentation master mix needed. Activate the TsTn5 transposase by adding the calculated volume of tagmentation master mix to each labeled ChIP Filtration Column.

21. Add a top cap to the tubes. We also suggest to seal the cap and plug with Parafilm to prevent any loss of liquid. Incubate reactions on an end-to-end rotator at 37°C for 1 hour.
22. At the end of the incubation, prepare the Elution Buffer. For each ChIP reaction, combine 90 µl of Elution Buffer AM4 and 10 µl 10X Elution Buffer AM2 in a 1.5 ml microcentrifuge tube. Place at 37°C to pre-warm during the wash steps.
23. Remove ChIP reactions from rotator and discard the cap and plug. Allow flow-through to occur by gravity.
24. Wash each column with 900 µl Wash Buffer AM1.
25. Repeat Step 24 four more times for a total of 5 washes.
26. Transfer columns to a new 1.5 ml microcentrifuge tube and spin in a room temperature microcentrifuge at 1250 x g for 3 minutes to remove residual Wash Buffer.
27. Following the spin, transfer the ChIP Filtration Columns to new 1.5 ml microcentrifuge tubes. Add 50 µl 37°C Elution Buffer to each column. Incubate at room temperature for 5 minutes. Spin in a room temperature microcentrifuge at 1250 x g for 5 minutes.
28. With columns remaining in the same microcentrifuge tube, add another 50 µl 37°C Elution Buffer to each column. Incubate at room temperature for 5 minutes and spin in a room temperature microcentrifuge at 1250 x g for 5 minutes.
29. Discard the ChIP Filtration Columns. The flow-through (~100 µl volume) contains the ChIP DNA. Proceed to Section F: DNA purification.

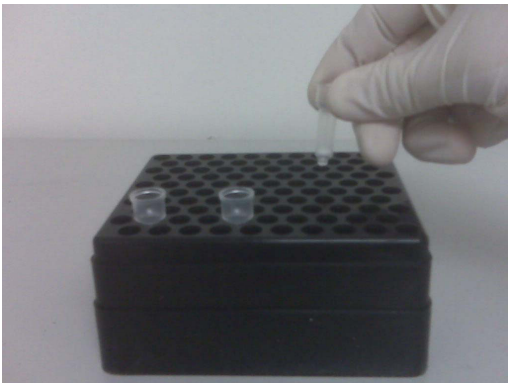


Figure 5: Using the ChIP Filtration Columns.

Remove the tab from the bottom of the ChIP Filtration Columns and place columns in an empty 1 ml pipet tip box to perform the wash steps.

Section F. Reversal of Cross-links and DNA Purification

1. Transfer each eluted ChIP DNA to a 250 µl PCR tube and add 2 µl Proteinase K. Vortex to mix and heat in a thermocycler at 55°C for 30 minutes and then increase the temperature to 80°C for 2 hours.
2. To each PCR tube, add 150 µl phenol.
3. Transfer reactions to Phase Lock Gel™ Heavy tubes (VWR, Catalog No. 10847-802).
4. Add 75 µl chloroform:isoamyl alcohol and mix.
5. Centrifuge for 5 minutes at maximum speed.
6. Purify the DNA using Qiagen's QIAquick PCR Purification Kit (Qiagen, Catalog No. 28104)

   Note: For column purification, add 5 volumes of the appropriate binding buffer to each Phase Lock tube. Transfer each supernatant to a new tube. Then proceed with the column purification protocol.

7. Elute in 25 µl DNA Purification Elution Buffer or Qiagen EB Buffer.
8. Purified DNA may be stored at -20°C for future use.

Section G. Library Amplification

This section is designed to amplify the ChIP library using the DNA oligonucleotides that were inserted into the chromatin by the TsTn5 transposase following genomic targeting. Completion of this section requires a TAM-ChIP Index and the Amplification primer mix provided with the anti-species TAM-ChIP conjugate (Catalog Nos. 53126 or 53127).

1. Place a new 0.2 ml PCR tube on ice and prepare the library PCR reaction by adding components in the order shown in Table 3 below:

Table 3

Reagent	1 reaction
ChIP DNA	20 µl
TAM-ChIP i7 Index* (0.5 µM)	5 µl
Amplification primer mix (10 µM ea)	5 µl
5X Q5 Reaction Buffer	10 µl
Q5 High-Fidelity Polymerase (2 U/µl)	0.5 µl
dNTPs (10 mM)	1 µl
Sterile H_2O	8.5 µl
Total volume	**50 µl**

Note: If combining multiple TAM-ChIP samples together for sequencing, make sure to use unique TAM-ChIP Index primers for each sample.

2. Place tubes in a real time PCR instrument and program as below:

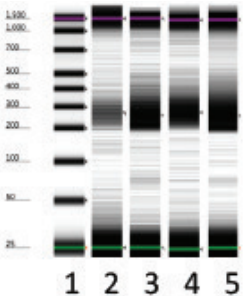
4. Place in the thermal cycler and run the library PCR thermal cycler program as described below:

Library PCR Thermal Cycler Program	98°C for 30 seconds 63°C for 3 minutes
	98°C for 10 seconds 63°C for 30 seconds x 14 cycles 72°C for 30 seconds
	4°C Hold
Proceed immediately to Library Size Selection	

Section H: Library Size Selection

This section is designed to clean up the Library PCR reaction using AMPure beads and freshly prepared 80% ethanol. Libraries should be between 200-500 bases.

1. To each PCR reaction, add 50 µl ddH_2O.
2. Add 100 µl AMPure beads to each reaction. Pipet up and down repeatedly (~10 times) to ensure a homogenous mixture. Incubate at room temperature for 5 minutes.
3. Place the tubes on the magnet to separate. Let sit for 2 minutes. Remove and discard the supernatant.
4. Without removing the tubes from the magnet, add 200 µl 80% ethanol to wash the beads. Allow beads to sit in the ethanol wash for 30 seconds before removing and discarding the supernatant.
5. Repeat Step 4 one more time.
6. Without removing the tubes from the magnet, allow the beads to air dry for 5 minutes.
7. Remove the tubes from the magnet and add 20 µl DNA Purification Elution Buffer to the tube. Pipet up and down to mix.
8. Place the tubes on the magnetic stand to separate. Transfer the supernatant to a new PCR tube. This contains the eluted DNA library.
9. Assess the DNA size using a TapeStation or equivalent method to ensure it falls within the 200-500 base range.



10. The library can be quantified and is ready for sequencing on the Illumina platform.

Section I. Sequencing Guidelines

For sequencing, we recommend 30 million reads. For additional information regarding the run set-up, sample sheet preparation, data analysis and de-duplication of the MIDs, please visit Active Motif's website **www.activemotif.com/tamchip** and download the *Guideline for TAM-ChIP Molecular Identifier (MID) Analysis* from the Documents tab. Or contact Technical Support at tech_service@activemotif.com

Appendix

Section J. 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: 1. After formaldehyde fixation and centrifugation (intact cell pellet), -80°C. 2. After chromatin shearing, -80°C. 3. After DNA clean up, -20°C.
After sonication shearing and centrifugation, a viscous or cloudy layer is visible in the chromatin.	Depending upon the cell type, lipid or glycogen layers may be seen after centrifugation. For example, fatty tissues may 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.
Poor yield of sheared chromatin.	Insufficient cell numbers were used. Repeat using a larger number of cells.
	Nuclei not released. It is highly recommended to perform dounce homogenization, even when using sonication. Use a dounce homogenizer with a small clearance pestle (Active Motif Catalog Nos. 40401 & 40415). Monitor cell lysis under a microscope. Generally, the more cells that are lysed, the higher the sheared chromatin yield.
	Sonication samples were emulsified. Avoid emulsification by turning up the power of the sonicator gradually. If a chromatin preparation becomes emulsified inadvertently, discontinue shearing and centrifuge the sample for 4 minutes at 8,000 rpm in a 4°C microcentrifuge to remove trapped air.
	Use fresh formaldehyde when preparing Complete Cell Fixation Solution and Complete Tissue Fixation Solution.
	Buffers were not scaled proportionally to the size of the sample. Use the chart in Cell Growth Recommendations to scale up or down chromatin preparation.
Shearing efficiency is not clear from gel analysis.	Material is stuck in the wells, and smears or streaks are seen from the top to bottom of the lane. The sheared chromatin needs to have the cross-links reversed, protein removed (Proteinase K) and RNA removed (RNase), followed by DNA purification.
	High molecular weight products. Decrease the size of the fragments by re-sonicating the sample. If an alternative reverse cross-linking method was used, or the 20 minute incubation at 100°C in NaCl was omitted prior to running the agarose gel for analysis, please repeat the input chromatin preparation and follow the manual instructions.
Performing ChIP with a large volume of chromatin.	This is not recommended. It is better to set up several small ChIP reactions. Do not perform a single scaled-up reaction, as the capture efficiency is lower.
High background.	Antibody issue. Too much antibody relative to the amount of chromatin in the ChIP reaction. Excess antibody will result in more non-specific binding, which will be detected as increased background.
Poor or no enrichment with target antibody.	Too little chromatin. Generally, we recommend using 5 - 10 µg of chromatin per ChIP reaction. Be sure to quantitate the concentration of the sheared chromatin sample(s) being ChIP'd to ensure both that adequate chromatin is used per sample, and that equal mass quantities of chromatin are used in each ChIP.
	Antibody is not ChIP-Seq validated, or has low binding affinity. 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. To assist in ChIP-Seq validating an antibody, it is very useful to use a positive control antibody such as Histone H3K4me3 (Catalog No. 39915).
	Wrong anti-species TAM-ChIP antibody conjugate was used. Ensure that the TAM-ChIP conjugate will properly detect the species (rabbit or mouse) of your ChIP-Seq validated primary antibody.

Section K. Related Products

ChIP-IT® Kits	Format	Catalog No.
ChIP-IT® Express	25 rxns	53008
ChIP-IT® Express Enzymatic	25 rxns	53009
ChIP-IT® Express Shearing Kit	10 rxns	53032
ChIP-IT® Express Enzymatic Shearing Kit	10 rxns	53035
ChIP-IT High Sensitivity®	16 rxns	53040
ChIP-IT® qPCR Analysis Kit	10 rxns	53029
Next Gen DNA Library Kit	16 rxns	53216
Next Gen Indexing Kit	64 rxns	53264
Low Cell ChIP-Seq	16 rxns	53084
ChIP-IT® ChIP-Seq	10 libraries	53041
ChIP-IT® FFPE	16 rxns	53045
ChIP-IT® FFPE Chromatin Preparation Kit	5 rxns	53030
ChIP-IT® Express HT	96 rxns	53018
Re-ChIP-IT®	25 rxns	53016
RNA ChIP-IT®	25 rxns	53024
Chromatin IP DNA Purification Kit	50 rxns	58002
EpiShear™ Probe Sonicator	110 V	53051
EpiShear™ Cooled Sonication Platform, 1.5 ml	1 platform	53080
ChIP-IT® Protein G Magnetic Beads	25 rxns	53014
Protein G Agarose Columns	30 rxns	53039
Siliconized Tubes, 1.7 ml	25 tubes	53036
ChIP-IT® Control qPCR Kit – Human	5 rxns	53026
ChIP-IT® Control qPCR Kit – Mouse	5 rxns	53027
ChIP-IT® Control qPCR Kit – Rat	5 rxns	53028
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
Bridging Antibody for Mouse IgG	500 µg	53017
Dounce Homogenizer	1 ml	40401
Dounce Homogenizer	15 ml	40415

ChIP-validated Antibodies

For an up-to-date list of over 125 ChIP-validated antibodies, please visit **www.activemotif.com/chipabs**.

Whole Genome Amplification	Format	Catalog No.
GenoMatrix™ Whole Genome Amplification Kit	1 kit	58001

Co-Immunoprecipitation	Format	Catalog No.
Nuclear Complex Co-IP Kit	50 rnxs	54001
Universal Magnetic Co-IP Kit	25 rxns	54002

Modified Histones Array	Format	Catalog No.
MODified™ Histone Peptide Array	1 array	13001

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
E-mail: tech_service@activemotif.com

Toll Free: 877 222 9543
Direct: 760 431 1263
Fax: 760 431 1351

Active Motif Europe

Avenue Reine Astrid, 92
B-1310 La Hulpe, Belgium
E-mail: eurotech@activemotif.com
Direct: +32 (0)2 653 0001

Germany Free Phone: 0800 181 99 10
France Free Phone: 0800 90 99 79
UK Free Phone: 0800 169 31 47
Fax: +32 (0)2 653 0050

Active Motif Japan

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

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

Active Motif China

787 Kangqiao Road
Building 10, Suite 202
Pudong District
Shanghai, 201315, China

Direct: (86)-21-20926090
Hotline: 400-018-8123
E-mail: techchina@activemotif.com

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