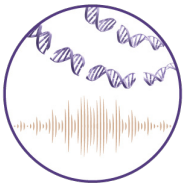


ACTIVE MOTIF®

Enabling Epigenetics Research

TECHNICAL NOTE

Chromatin Sonication Recommendations for ChIP

SUMMARY

Chromatin immunoprecipitation (ChIP) is an assay used to identify sites in the genome where transcription factors bind or histones have undergone post-translational modifications. An important step in the ChIP assay workflow is fragmentation of the chromatin, as fragment length determines the resolution of site identification. This technical note describes points to consider when optimizing sonication of your sample prior to performing ChIP.

FIXATION

Formaldehyde: Methanol-Buffered or Methanol-Free?

Formaldehyde is the most widely-used fixative to fix chromatin for ChIP assays, and we at Active Motif highly recommend it. Most sonicators are compatible with methanol-buffered formaldehyde. However, some multi-sample sonicators require methanol-free formaldehyde. Please see the table on the following page for more details.

Formaldehyde Alternatives & Additives

Active Motif's ChIP kits have been optimized for fixation with formaldehyde but there are some modifications to the standard formaldehyde fixation protocol that can provide advantages for certain applications. For instance, when performing ChIP for histone modifications, you can choose not to fix at all (native ChIP). For weak, transient, or indirect binding of proteins to DNA, you may consider adding another crosslinker to formaldehyde to extend the crosslinking distance, such as dimethyl adipimidate (DMA), disuccinimidyl glutarate (DSG), or ethylene glycolbis(succinimidyl succinate) (EGS).

The Dangers of Overfixing

Chromatin in overfixed samples is difficult to solubilize, which can lead to poor results when used in ChIP assays. Pay close attention to the sample concentration, the final formaldehyde concentration, and the length of time your sample is fixed. Using 1% formaldehyde for 10-15 minutes is the most common fixation condition for cells, cell lines, and tissues.

SONICATION

Variables that Affect Sonication

Many different factors can affect how well your sample is sonicated: the sample type, amount, and volume, crosslinking conditions, sonicator and tube type, and buffer composition, to name a few. Therefore, it is important to re-optimize your sonication protocol if any variables change.

Fragmentation Sizes

ChIP usually requires chromatin to be sheared to a size of 200-1200 bp, with a majority of ChIP applications further limiting that range to 200-600 bp (e.g. ChIP-Seq). It is important to optimize your shearing to produce fragments of optimal size for your application.

Sonicate and Chill

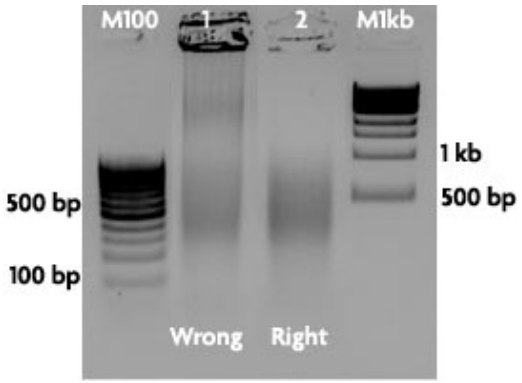
Sonication heats samples, and overheating can cause irreversible damage to the DNA or protein targets which would significantly decrease the quality of your ChIP data. It is important to keep samples cold by sonicating on a chilled sonication platform or resting on ice between sonication cycles.

Enzymatic Shearing

Enzymatic shearing is an option for researchers without access to a physical shearing instrument or performing native ChIP. However, enzymatic shearing is plagued by bias, and may not shear chromatin from difficult cell lines, primary cells, or tissues. We at Active Motif consider physical shearing with a sonicator the best practice, and only recommend enzymatic shearing when sonication is not an option.

VERIFY FRAGMENT SIZE

After shearing, we recommend treating a small aliquot of your sheared chromatin with RNase A and Proteinase K, reversing the crosslinking, and purifying the DNA to run on a 1.5% agarose gel for verification of the sonication efficiency. DNA should appear as a smear anywhere between 200 – 1200 bp. In the image to the right, Sample 1 omitted denaturing the sample with 500 mM NaCl (Step 14 of Section B in the ChIP-IT High Sensitivity® Manual) and does not look like well-sonicated chromatin. Sample 2 shows the expected fragmentation between 200-1200 bp.



Agarose Gel of Sheared Chromatin. 500 ng of chromatin from MCF-7 cells were fixed, sonicated, and run on a 1.5% agarose gel, according to the ChIP-IT High Sensitivity® Protocol.

WHERE TO BEGIN?

The table below lists recommendations for where to begin your sonication optimization. These parameters come from the peer-reviewed publications referenced in the column on the right. Remember, these are just starting points and it's still a good idea to optimize for your specific conditions! For more information on performing ChIP with Active Motif reagents, please visit our website.

Sonicator	Fixative	Shearing Conditions	References (PMIDs)
Active Motif PIXUL™ Multi-Sample Sonicator	Methanol-buffered or methanol-free formaldehyde	Pulse: 50 2 MHz cycles/pulse Pulse Repetition Frequency: 1 kHz Process Time: 36 minutes for 1-96 samples Burst Rate: 20 Hz ***NOTE:*** *We recommend diluting the sheared chromatin 5-fold in Active Motif ChIP buffer prior to performing ChIP.*	30927002
Active Motif EpiShear™ Probe Sonicator	Methanol-buffered formaldehyde	Amplitude: 25% Pulse: 30 seconds ON, 30 seconds OFF Cycles: 20	27460500 28455377
Covaris® Focused-Ultrasonicator Models: M220, E220, S2, S220	Methanol-free formaldehyde	Shearing Buffer: Buffer D3 Peak Power: 75 watts Duty Factor: 10.0 Cycles/Burst: 200 Time: 600 sec ***NOTE:*** *We recommend diluting the sheared chromatin 5-fold in Active Motif ChIP buffer prior to performing ChIP.*	28399410 24466341 29093577 28729413
Diagenode Bioruptor® Models: Pico, Plus, UCD-200	Methanol-buffered or methanol-free formaldehyde	High Power Pulse: 30 seconds ON, 30 seconds OFF Cycles: 7-12	29317594 28030801 25241747 30139998