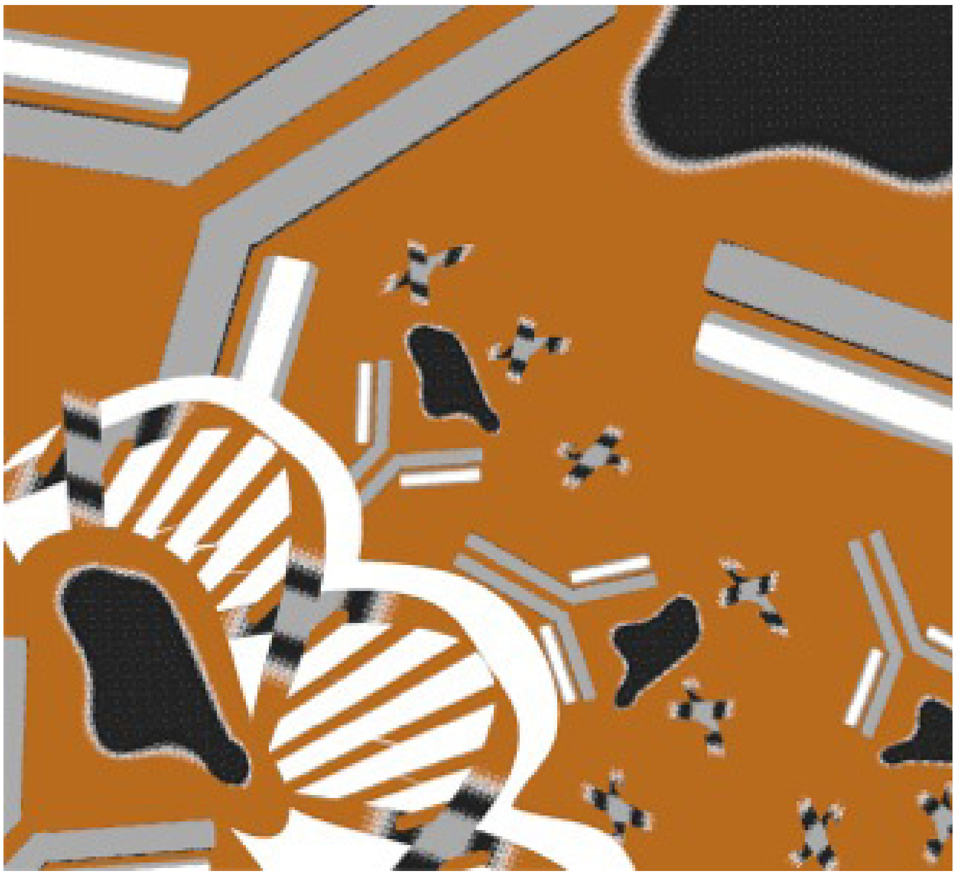
EpiGenie Guide: Chromatin Shearing



Chromatin Immunoprecipitation Sequencing (ChIP-Seq) has transformed the field of epigenetics, providing unprecedented resolution and coverage for surveying protein-DNA interactions.

Powerful, high-throughput sequencing is credited with most of the benefits, but there's a lot more that goes into ChIP-Seq than sequencing. Chromatin shearing is one of the first critical steps in the ChIP-Seq workflow. Depending on your approach, you will either faithfully represent the biological scenario you wanted to study, or disrupt epitopes and introduce all sorts of bias so you won't actually know what's relevant downstream. Our first hint: the former is better.

In this guide…

- Popular chromatin shearing approaches,
- Discuss when you might opt for one vs. another
- Share considerations for other aspects upstream that can impact

Chromatin Shearing Techniques

As with any scientific approach, there's multiple ways to get from A to B. Chromatin shearing is no different. Two general approaches are still widely used today: enzymatic digestion and mechanical shearing.

Despite sharing a similar end goal, these methods couldn't be more different in their approach; one uses a naturally occurring enzyme, micrococcal nuclease (MNase), to digest DNA, while the other uses acoustic energy to shear DNA into smaller fragments. Let's take a closer look at each of these.

MNase Digestion

For years, micrococcal nuclease (MNase) digestion of chromatin has helped researchers in nucleosome mapping experiments, but it's utility doesn't necessarily end there. This enzyme, which generally cuts linker DNA connecting between nucleosomes can be used in other chromatin-related analyses as well.

Advantages of MNase Digestion

Researchers often opt to use MNase digestion in their ChIP experiments for a number of reasons.

- It doesn't require any expensive equipment
- It can create high resolution maps since it generally digests to the ends of nucleosome-bound DNA
- The reaction is isothermal and can be milder than sonication approaches, resulting in minimal damage to epitopes of interest
- It is essential when working with native, or non-crosslinked chromatin (e.g. histone modification studies) where sonication would disrupt protein-DNA complexes

Drawbacks of MNase Digestion

Despite its' advantages, enzymatic digestion is far from perfect. There are a number of limitations that may be more or less meaningful depending on your experience level and experimental scenario.

- MNase does exhibit sequence specific cutting, so there's an opportunity for bias
- Nuclear accessibility to MNase can vary in cell types and model systems, so optimization is often required
- Different preparations (lots) of enzyme can have varying levels of activity, so it is often necessary to qualify before using

Whenever you're using an enzyme as a tool, there's going to be variables to consider and adjust. MNase digestion is no different. Here's what some R&D leads familiar with the method had to share:

Michael Sturges, Sr. Product Manager at EMD Millipore expands, "Reaction conditions will vary depending upon amount of material (cell equivalents), concentration of enzyme, and time of digestion. Undigested material is often spun out of the sample following digestion since this is largely insoluble."

> "As with any enzymatic process, temperature, sample concentration, and salt content can greatly affect the reproducibility and robustness of the process. These parameters need to be tightly controlled with each sample to assure the shearing is reproducible."
>
> J.D. Herlihy, Product Manager at Covaris

Mechanical Shearing of Chromatin

If enzymatic digestion isn't a good fit given your experimental situation, or experience, then fear not; there are number of different derivatives of mechanical shearing using acoustical energy to choose from.

Chromatin Sonication

Ultrasonic energy has been used in the lab for years to disrupt everything from cell membranes to chromatin. Unlike, enzymatic digestion, sonication uses energy to shear chromatin into smaller fragments. In addition to wearing some stylish earphones, sonication brings several advantages to the table for chromatin shearing.

Advantages of Chromatin Sonication

Unlike MNase digestion, there are no enzymes involved here. Generally that means these approaches will be less susceptible to sample-to-sample variation and might see less variation in different types of species and tissues. Also, since sonication is mechanical in nature, there are no issues with sequence bias etc., that can sometimes be problematic with MNase digestion.

Dr. Kyle Hondorp, R&D Scientist at Active Motif shares more on this, "Sonication will perform better in difficult to lyse cells such as T cells. If complete lysis does not occur the enzymes may not gain access to the nucleus, thus resulting in incomplete digestion. Rigorous sonication will not only shear DNA but will also aid in the lysis of difficult to lyse cells, thus improving yields."

Another general benefit of sonication is that it is often easier to obtain a more homogenous sample population that can be "fine-tuned" to a certain length. This makes it useful when using different types of downstream applications, where you may desire a longer or shorter fragment size.

There are a number of types of sonication platforms in use today including direct probe sonicators, Cup Horn sonicators, and focused ultrasonicators to name a few. Let's take a closer look at each of these to see how they compare.

Direct Probe Sonicators

As the name suggests, these sonicators use a probe immersed directly into a sample tube. Probe sonicators have been used in the lab for years and are definitely useful. They deliver a whole lot of power to the sample, which makes for short sonication times. That said, this isn't always ideal for ChIP applications.

First of all, since you're using a probe that interacts directly with a sample, there's always a risk for cross contamination when sonicating multiple samples, which is already a bit laborious since you can only sonicate one sample at a time.

Additionally, the energy can agitate samples to the point where they foam. This can cause headaches, particularly when you're working with smaller volumes. Lastly, the heat form the direct energy can also introduce another variable to optimize as it can also degrade samples further.

If your lab is well into its ChIP-based studies, it might be time to move towards other options though that are better suited to accommodate more throughput.

Cup Horn Sonicators

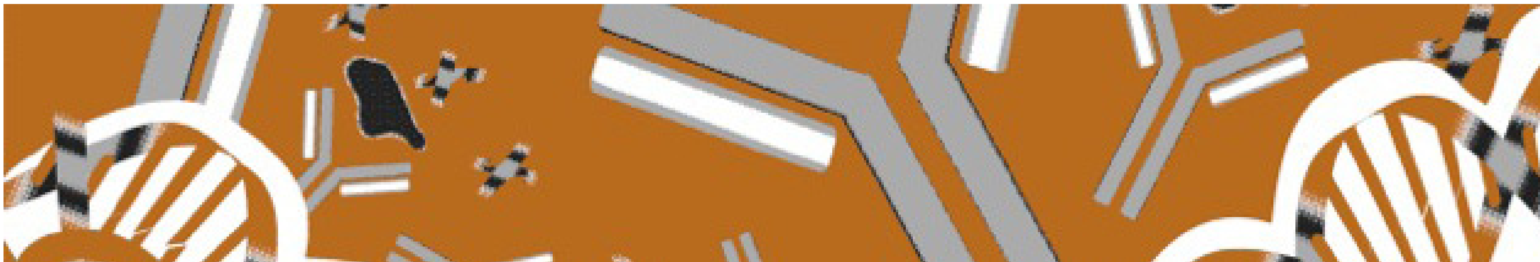
Instead of applying direct energy to samples, Cup Horn sonicators use a separate water bath to indirectly deliver energy to your sample. Since this type of a water bath approach is less intense than direct sonication, the duration of sonication required can be a bit longer. Unlike probe sonicators, Cup Horn sonicators can generally process multiple samples simultaneously and since samples remain in a sealed tube, there is minimal risk of cross contamination.

Cup Horn sonicators generally require more energy as they are energizing the entire water bath in which the samples sit. This can sometimes lead to unwanted heat, so having a cooling mechanism in place will help control unwanted degradation.

Active Motif uses a Thermoelectric Chiller along with its EpiShear™ Multi-Sample Sonicator to obtain the best performance. "This platform is great for multi-sample processing but is also essential if sonication is to be done in low volumes (<100 µl)," added Active Motif's Kyle Hondorp.

> "To address some sources of variability in probe sonication, we developed a **Cooled Sonication Platform** that not only keeps samples at a consistently cool temperature but also includes a height counter and vertical alignment tool. These features allow the researchers to place the probe tip in the exact same position within the tube for each sample."

Kyle Hondorp, R&D Scientist with Active Motif.



Ultrasonicator Systems

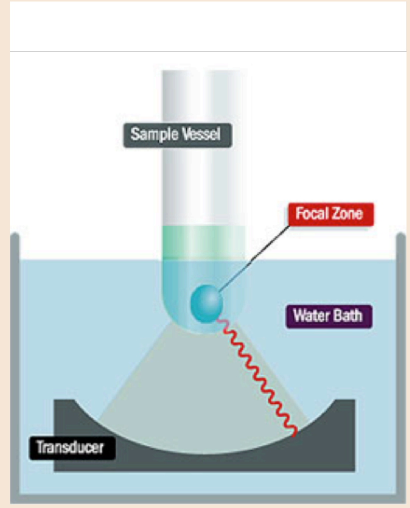
Acoustic systems can differ by more than the probe/sample interaction. There are also different levels of frequency and corresponding wavelength of energy used to shear chromatin.

Ultrasonication platforms such as Covaris' Adaptive Focused Acoustics™ (AFA)-powered platforms use a higher frequency (500kHz) when compared to other sonicators (20kHz). This higher frequency energy results in a smaller wavelength applied to the chromatin samples. Why should you care? Generally, the smaller the wavelength, the more precisely it can be targeted so less power needs to be applied to do the job. Less power means less heat beating up the protein-DNA interactions you may want to map.

Covaris' J.D. Herlihy explains, "Other mechanical shearing methods (sonication) generate heat during processing that is ineffectively dealt with by ice baths or cooling mechanisms. The excess heat damages proteins, DNA, and formaldehyde cross-links; decreasing sensitivity, reproducibility, and reliability of the assay."

The Shearing Breakdown

With chromatin shearing, there are many options and approaches. The benefits of each of these will vary depending on your sample, model, experience, and practical considerations like budget and available lab equipment. Most of these approaches will require some level of optimization and tweaking.



The AFA acoustic process

Additional Chromatin Shearing Tips and Recommendations

Here are some tips from some industry experts who've logged some serious hours optimizing enzymatic and mechanical chromatin shearing.

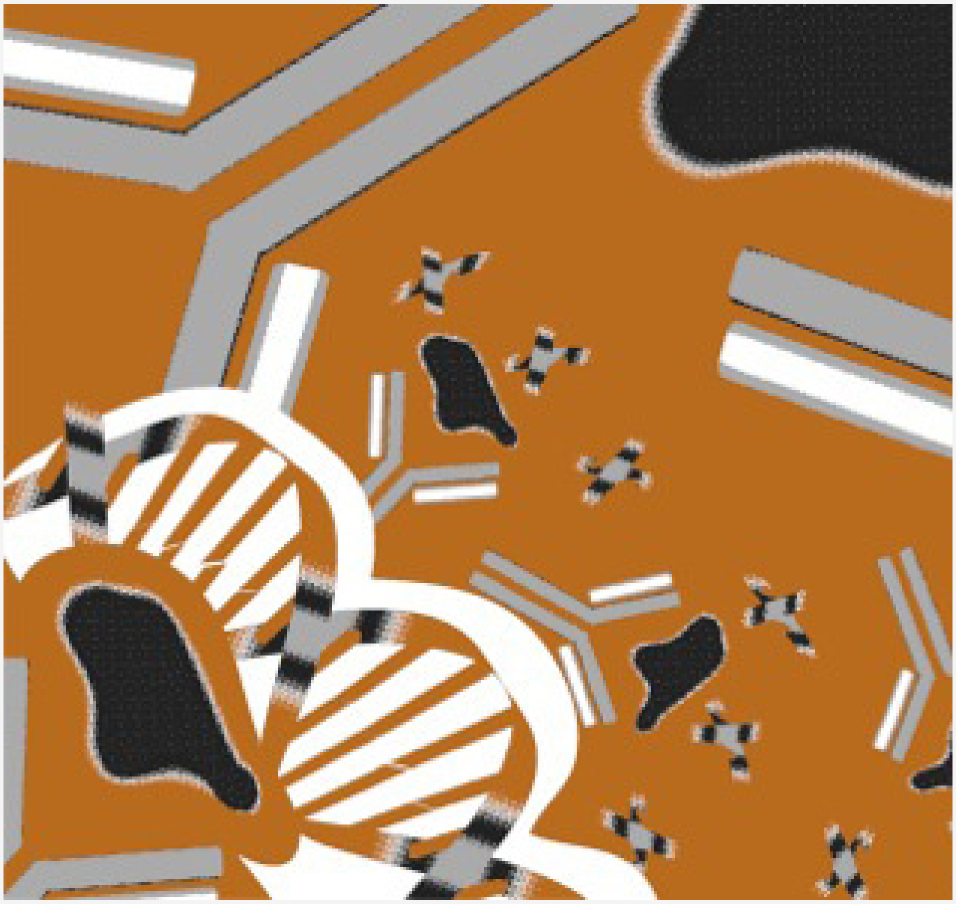
Chromatin Shearing Tips: Sonication: MNase Digestion

J.D. Herlihy – Covaris
As with any enzymatic process, temperature, sample concentration, and salt content can greatly affect the reproducibility and robustness of the process. These parameters need to be tightly controlled with each sample to assure the shearing is reproducible.

Kyle Hondorp – Active Motif
When optimizing cell lines for enzymatic digestion, the fixation time can be very important. Over-fixation can result in large cross-linked aggregates that are difficult for the enzyme to digest. This can be resolved by reducing the fixation time. Cell lysis is another critical aspect of enzymatic digestion. Without proper lysis the enzyme will not be able to access to the nucleus, therefore we recommend monitoring cell lysis under a microscope and using a dounce homogenizer.

Chromatin Shearing Tips: Sonication

Michael Sturges – EMD Millipore
If starting chromatin preparation for the first time, it's useful to use a cell line or tissue sample that is abundant so you can play with parameters on your instrument to learn about it's performance. Once you've selected an instrument's power setting and a buffer volume/chromatin equivalent density that has worked successfully, make minor tweaks to the protocol around on/off processing times and number of cycles. The new PureEpi Chromatin Preparation and Optimization Kit was designed to help researchers dial in all of these conditions.



J.D.Herlihy – Covaris
Conducting a two-step lysis process preparing nuclei before shearing. By lysing the plasma membrane first, followed by a nuclei preparation before chromatin shearing, the method can be more universal and you receive reproducible results with a wider range of cell types.

Optimizing fixation. Many protocols are based on older sonication technologies and use prolonged fixation times. Depending on the cell/tissue type and shearing methods employed, it is possible to obtain much better shearing, and therefore, much better ChIP results by optimizing fixation time for each cell/tissue type and each antibody employed.

Fixative. Fresh methanol-free formaldehyde is essential to obtaining reproducible results. Therefore, the use of fresh single-use ampules of formaldehyde is highly recommended. Methanol containing formaldehyde does not work well with ChIP as the methanol permeabolizes the cell membrane affecting fixation rate in an uncontrolled manner.

Kyle Hondorp – Active Motif
For the probe sonicators the best approach is to test the sonication setting with sonication buffer only. When using a microfuge tube, place the probe tip approximately 5 mm from the bottom of the tube and slowly increase the intensity until the sample begins to foam. Then dial back the intensity a notch or two and use these settings for the "real" sample.

We find that probe sonication shearing efficiency is improved through the use of a small shearing volume (< than 500 µl) and a V-bottom tube.

Product	Catalog Number
Active Motif	
ChIP-IT® Express Enzymatic Shearing Kit	53032
EpiShear™ Probe Sonicator	53051
EpiShear™ Cooled Sonication Platform	53073
EpiShear™ Multi-Sample Sonicator	53065
Covaris	
M220 Focused-ultrasonicator	500295
S220 Focused-ultrasonicator	500217
E220 Focused-ultrasonicator	500239
LE220 Focused-ultrasonicator	500219
truChIP Chromatin Shearing Kit High Cell Non-Ionic	520075
truChIP Chromatin Shearing Kit High Cell SDS	520076
truChIP Chromatin Shearing Kit Tissue SDS	520083
truChip Chromatin Shearing Kit Low Cell Non-ionic	520084
truChIP Chromatin Shearing Kit Low SDS	520085
EMD Millipore	
EZ-Zyme™ Chromatin Prep Kit	17-375
PureEpi Chromatin Preparation and Optimization Kit	17-10082