Just 4 steps to foolproofing fragmentation for ChIP

1. Use a reliable thermo-controlled sonication device with ACT technology such as Diagenode's Bioruptor® Pico
2. Optimize the cross-linking and chromatin preparation steps
3. Master the fragmentation process using smart and gentle sonication
4. Control the correct fragment size and quality of your sheared chromatin before each ChIP experiment

1. Use a reliable fragmentation/sonication device

→ Use a reputable sonication device for chances of highest success. More than 1000 peer-reviewed scientific papers cite the Bioruptor®.

2. Optimize your crosslinking and chromatin preparation steps

→ **Use FRESH formaldehyde.** The use of high quality and fresh formaldehyde is crucial while using methanol-free reagents. Replace your stock every month. This will ensure high inter-assay reproducibility between ChIP experiments.

→ **Adopt the fixation strategy that is best suited to studying your particular target.** Some epigenetic marks may be more elusive than others. When studying weak or rare protein-DNA binding events fixation should be done promptly and thus directly in medium. -- When studing histones marks, trypsinised cells might be fixed.

→ **Always carry out a fixation time course for your cell line** to empirically determine the optimal fixation time for your cell line and epitope of interest. Cell lines and epitopes differ widely in their fixation efficiency and sensitivity to fixation. Generally, TFs require longer fixation than histones (eg. > 8 min fixation).

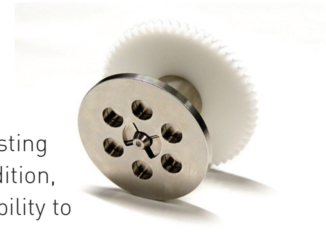
→ **Be precise with the fixation time.** Whether you use 8, 10 or 15 minutes (depending on your specific target and cell types) make sure that the time is consistent.

→ **Be precise with the fixation temperature.** The formaldehyde fixation is a temperature –dependent process. Whether you use RT or 37°C make sure that the temparuture is consistent.

→ Select the best buffers and kits for cell lysis and chromatin shearing. Every cell type is different and researchers can spend hours comparing numerous protocols. We recommend using optimized, commercially-available kits to simplify this process. Diagenode has not only carefully researched numerous published protocols but also has performed hands-on validation with actual ChIP experiments to produce optimal cell lysis and chromatin shearing optimization kits. These carefully tested buffers ensure guaranteed performance with any mammalian cell type, even with the most demanding applications such as ChIP-sequencing.

Europe Diagenode sa / LIEGE SCIENCE PARK // Rue du Bois Saint-Jean, 3 // 4102 Seraing (Ougrée) // Belgium // Phone: (+32) 4 364 20 50 // E-mail: info@diagenode.com
USA Diagenode Inc. / 400 Morris Avenue, Suite 101 // Denville, NJ 07834 // USA // Phone: (+1) 862 209-4680 // Mail: info.na@diagenode.com

3. Master the fragmentation process



→ **ACT beats AFA ultrasonication 12 – 1**

ACT is smart and gentle by design. The Bioruptor can accomodate up to 12 samples at a time. Testing more samples in one round is far more reliable than repeating individual sonication rounds. In addition, the optimization process will be easier, faster and more reproducible using a Bioruptor given this ability to simultaneously sonicate samples.

With ACT, the 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 range of fragment lengths. The Bioruptor® Pico delivers high performance ultrasounds in a very precise and uniform manner allowing improved performance when using difficult to lyse cells such as PBMC or primary cells.

→ **During shearing, keep the temperature low to preserve the quality of your chromatin**

The Bioruptor's water bath ensures optimal energy transfer, reducing the chance of overheating the sample. If using a Bioruptor® Pico, the built-in cooling system additionally ensures high precision temperature control during the entire sonication process, resulting in higher quality samples.

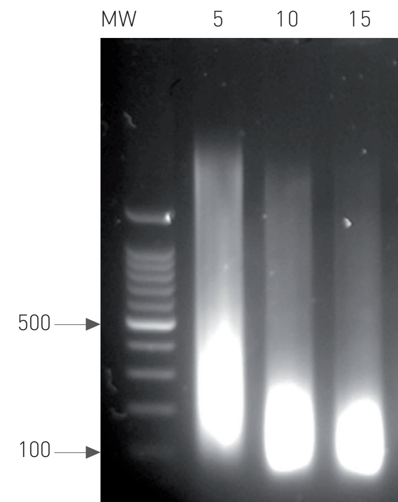
→ **Be wary of enzymatic fragmentation methods**

Enzymatic (eg. MNase, enzyme coktail) digestion is limited in nature : Enzymes exhibit sequence-specific cutting, introducing potential bias. Nuclear accessibility to nucleases can vary in cell types, so efficient and reproducible shearing is more difficult to achieve. Finally, different lots of enzyme can have varying levels of activity, so re-optimizing the digestion conditions is often necessary to qualify new lots. Reaching complete lysis may be problematic using enzymes since they might not gain access to the nucleus, thus resulting in incomplete digestion.

4. Control the fragment size and quality of your sheared chromatin

- **Assure the correct size of your chromatin fragments before each ChIP experiment.**
  Verify the each input chromatin batch even if you are carrying out the same process repeatedly. It is crucial to check the quality of your chromatin before each experiment.

Just follow these four steps for shearing chromatin, and you'll be well on your way to excellent ChIP results without the troubleshooting headache.



Chromatin samples are sheared for 5, 10 and 15 cycles of 30 sec ON/30 sec OFF with the Bioruptor® Pico using 1.5 ml Bioruptor Microtubes (Cat. No. C30010016).