

Bioruptor® power-up allows for sonicating primary human T-cells

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Introduction

Diagenode's Bioruptor® Sonicator uses state-of-the-art ultrasound technology to disrupt, disperse, or shear a variety of sample types for biological, chemical, pharmaceutical, and industrial applications. The Bioruptor® Sonicator is widely used in biological settings and has proven success for efficient, reproducible sonication required for applications such as DNA/chromatin shearing, cell lysis, and protein extraction. Researchers have confirmed the Bioruptor® Sonicator as an optimal shearing system, surpassing industry standards with high yields of superior quality material, as exemplified by over 200 publications.

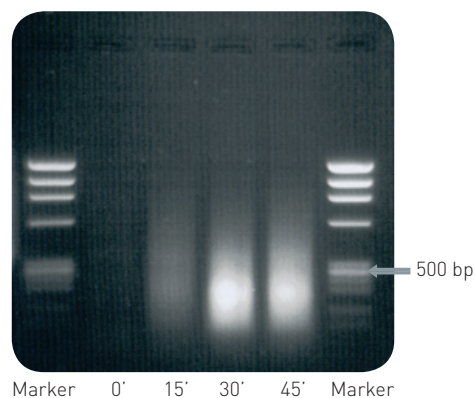
The Bioruptor® Sonicator range (standard power settings) provides excellent chromatin shearing results for 90% of the chromatin samples providing from different cell types. However, in some cases (very dense and compact chromatin, e.g. lymphocytes, mouse embryonic fibroblasts) the standard Bioruptor® might not be adapted. Therefore, Diagenode has developed a new Bioruptor model, called Bioruptor® power-up to allow for shearing of this specific type of chromatin. This new version takes the robust platform of the UCD-200/300 and delivers a higher power to the sample to allow for an optimal and highly consistent fragmentation.

Methods

1.3 million lymphocyte T (CD4+) cells are cross-linked for 10 min at room temperature with 1% formaldehyde. Neutralised with 125 mM glycine and centrifuged at 1000 g for 5 min. Supernatant is discarded and the pellet resuspended in 1.3 ml buffer 1. Four aliquots of 300 µl are prepared. Therefore each aliquot contains approximately 3x10e5 crosslinked CD4+ cells.

Ensure the Bioruptor® has been filled with ice for 1 hour before use to cool it. Just before use, remove the ice and add water with a thin layer of ice. Place the samples (300 µl) into the 1.5 ml tube holder and ensure each position is filled, using blank balanced eppendorf tubes as appropriate.

Set the Bioruptor® at full setting [high] and place the dial at 30 sec on/off. Perform different time points: 0 min, 15 min, 30 min and 45 min. Adding a new layer of ice every 5 min. When all aliquots are completed, RNase A is added and NaCl to a final concentration of 250 mM. Reverse the cross-link by incubating at 65°C for 4 hours and add proteinase K to the samples for 1 hour at 37°C. Perform a phenol:chloroform:isoamyl clean up and precipitate with NaCl and isopropanol at -80°C overnight. 40 µg of glycogen has been added previously to each sample as a carrier. The following day the precipitated samples are washed with ice-cold 70% ethanol, pellets are air-dried and resuspended in 18 µl of water. Prepare a 1.5% TAE agarose gel and run out all of the samples (see figure below).



Conclusion

The 30 min and 45 min samples appear to have been successfully sheared to the appropriate size required for ChIP.

Buffer 1:

- 140mM NaCl
- 10mM Tris pH7.5
- 1mM EDTA
- 0.5mM EGTA
- 1% TX-100
- 0.01% SDS
- 0.1% sodium deoxycholate