Optimizing DNA Shearing for Next Generation Sequencing Library Preparation with the Bioruptor®

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Abstract
The rapid adoption of next-generation, direct, and single-molecule sequencing technologies has made DNA shearing a critically important step in library preparation. DNA shearing is the preferred method to prepare high quality, random, and size-appropriate sequencing libraries, and also ideal to fractionate chromatin for epigenetics studies. Here we present optimized sonication methods using the Bioruptor® sonicator to make genomic DNA libraries for the Illumina®, HeiScope®, SOLiD® and 454 platforms. We optimized the number of sonication cycles, cycle duration, power, buffers, and other parameters in order to produce DNA size distributions with mean fragment sizes of 150, 200, 250, 300, 500, 1500 and 3000 bp. We obtained 55.7%-74.2% double-stranded DNA content, demonstrating that the more gentle Bioruptor sonication technology results in high-quality fragments that are amenable to easy ligation with sequencing adapters for PCR or direct sequencing on each of the commercially-available “next-gen” sequencing platforms. As 12–26 samples can be sheared simultaneously, sonication time per sample is 2.5–5 min per sample (100–800 µg human genomic DNA) with highly reproducible fragment distributions. Since the Bioruptor uses standard plastic laboratory tubes, the entire process integrates into existing laboratory workflows for NGS sequencing, ChiP or ChiP-Seq.

Methods

Samples: Prior to sonication, human DNA samples were dissolvated in a sonication buffer of TE (10 mM-Tris, 1 mM EDTA), pH 8.0 with a DNA concentration of 0.01 µg/µl and a final volume of 100 µl. Costar® 0.65 ml Low Binding Microcentrifuge (Cat. No. COR-3206) and Tube holder for 12 x 0.5 ml tubes (Diagenode, Cat. No. UCD-pack 0.5). All samples were vortexed and centrifuged before shearing in the Bioruptor sonicator.

Sequencing Library Preparation: As shown in Table 1, sequencing libraries were prepared by sonicating genomic DNA to obtain optimal fragment size distributions for HiSeq those recommended by the manufacturers of each of the commercial sequencing platforms (Illumina, HeiScope, SOLiD and 454 platforms). We established a set of sonication conditions that could be used to generate each of the required size distributions, by varying the duration of sonication (i.e., the number of cycles), and leaving the buffer concentrations, power and other parameters identical between runs.

Results

A. Sonicated DNA Size Distributions

B. Reproducibility and High dsDNA Yields

Figure 1. Programmable DNA size distributions, excellent reproducibility, and high dsDNA yields with the Bioruptor

Panel A shows different DNA size distributions of sheared genomic DNA produced by varying the duration of sonication at low power. The different colored curves each depict a specific Bioruptor run, optimized to produce specific mean sizes and size ranges for next-generation sequencing. For example, a typical range for library generation of 150–300 bp can be obtained after just 50’ and produce an average yield of 50% dsDNA post-sonication (Table B). Such high yields of resulting dsDNA content can be critical or optimal library prep. We achieved greater than a 70% yield of dsDNA by shortening the sonication time, again demonstrating the flexibility of the Bioruptor for various sequencing platform needs.

Table 2. Attributes of the different shearing methods for DNA sequencing

GC of Sonicated DNA

Table 3. Fractile size distributions and sonication duration for sequencing library generation

Figure 3. Sample concentration and shearing efficiency effects.

We mixed increasing concentrations of genomic DNA (100 ng/µl, 200 ng/µl, and 500 ng/µl) to assess the impact of DNA concentration over a 50-fold range. Our results show that DNA concentration does not have a significant impact on performance as the shearing distribution and efficiency is similar across all concentrations. Therefore, it is not necessary to modify Bioruptor sonication parameters or protocols when changing template DNA concentrations, adding to the ease-of-use of the system. Sonication and sample parameters were as follows: 30 min anticipation period, 25’ high power setting, 35°C (on/off), Costar tubes 0.65 ml, 150 µl final volume, E.coli (DNA).

Advantages of Parallel Processing: Substantial Time Savings

The Bioruptor efficiently processes large numbers of samples simultaneously. The instrument can process 32, 24, or 68 samples at the same time, with unattended operation. Compared to another leading sonicator, the Bioruptor UCD-100 requires only 80 minutes to sonicate 12 samples, for a time savings of 2 hrs. Importantly, to process 24 samples, the UCD-400 requires 90 minutes, saving more than 5 hrs. These time savings are important in the lab, particularly as sequencing moves to high throughput mode.

Summary & Conclusions

We have developed optimized protocols for the Bioruptor for use in Next Generation Sequencing and demonstrate that it provides consistent, high-quality templates for sequencing library preparation. Our results show:

- Excellent shearing reproducibility from individual shearing experiments
- Superior shearing efficiency and time savings over competing systems
- Flexibility in shearing control for required DNA size distributions for any sequencing platform
- Assurance of a high quality Bioruptor system, backed by GC on the Agilent BioAnalyzer
- High yields and integrity of dsDNA content required for optimal library construction

Figure 2. Shearing validation by Bioanalyzer achieves optimal GC

We use the Bioanalyzer 2100 and its DNA High Sensitivity Chip as a standard GC procedure at Diagenode in order to validate results of genomic DNA shearing. Different sonication time-points (5’, 15’, 30’, and 1 hour) are shown, run on the Bioanalyzer (panel A) and an agarose gel (panel B).

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