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

Irina Panteleeva, Catherine D'Andréa, Gilles Ansay, Dominique Poncelet

Diagenode sa, CHU, Tour GIGA B34, 3ème étage 1 Avenue de l'Hôpital, 4000 Liège, Sart-Tilman, Belgium

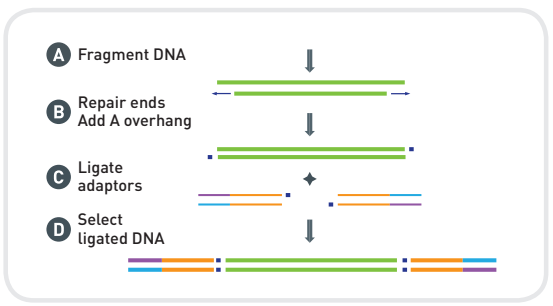
Abstract

The rapid adoption of next-generation, direct, and single-molecule sequencing technologies has made DNA shearing a critically important step in library template preparation. DNA sonication 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 and protocols using the Bioruptor® sonicator to make genomic DNA libraries for the Illumina®, HeliScope™, SOLIDT™ 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 adaptors for PCR or direct sequencing on each of the commercially-available "next-gen" sequencing platforms. As 12-24 samples can be sheared simultaneously, sonication time per sample is 2.5-5 min per sample (100-800 bp; 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 NG sequencing, ChIP or ChIP-Seq.

Methods

Samples: Prior to sonication, human DNA samples were dissolved in a sonication buffer of TE (10 mM Tris, 1mM EDTA), pH 8 with a DNA concentration of 0.01 µg/µl and a final volume of 100 µl. Costar® 0.65 ml Low Binding Microcentrifuge Tube (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 to fit those recommended by the manufacturers of each of the commercial sequencing platforms (Illumina, HeliScope, 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 only the duration of sonication (ie, the number of cyles), and leaving the buffer concentrations, power and other parameters identical between runs.

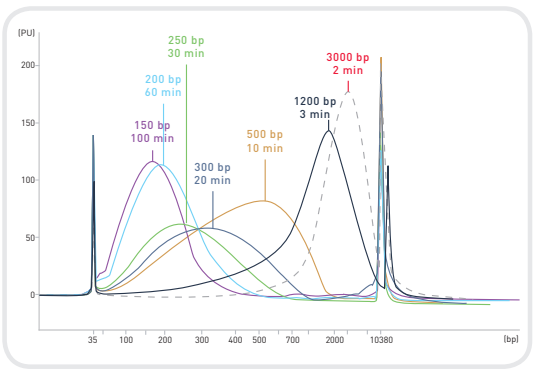
	Roche Genome Sequencer-FLX	Illumina Genome Analyzer	Life Technologies SOLiD™
Required fragment size post sonication	100–800 bp	100–500 bp	100–300 bp
Small genomes (e.g. Bacteria)	5–10 cycles	20–30 cycles	60–80 cycles
Large genomes (e.g. Human)	5–10 cycles	30–40 cycles	80–100 cycles

Table 1. Preferred size distributions and sonication duration for sequencing library generation

Sonication conditions: Low power, 1 min sonication cycles (30 sec ON, 30 sec OFF), and number of cycles based on desired DNA size. The temperature was kept at 4°C (using the Diagenode Water cooler, Cat. No. BioAcc-Cool) for optimal shearing results. For the larger fragments (3 kb curves), the Bioruptor was set on a special power setting and samples were kept at room temperature. Please contact us for more information.

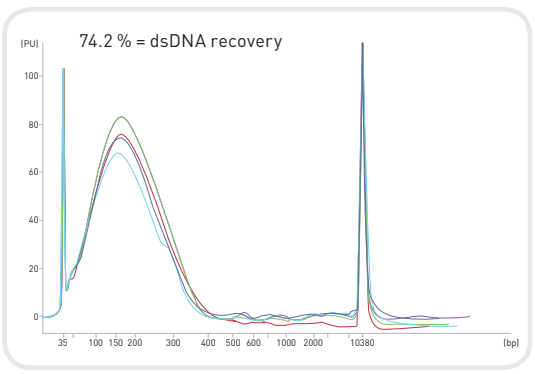
Results

A. Sonicated DNA Size Distributions



Fragment Size (mean +/- SD)	Time (mins)
150 +/- 13 bp	100
200 +/- 16 bp	60
250 +/- 19 bp	30
300 +/- 21 bp	20
500 +/- 27 bp	10
1500 +/- 39 bp	4
3000 +/- 52.5 bp	2

B. Reproducibility and High dsDNA Yields



Shearing Time (min)	Mean, %dsDNA recovery by Qubit	SD
10	88.4	2.9
20	79.3	2.7
30	76,7	3.4
40	75	1.1
50	68.2	4.5
60	67.8	8.2
80	65.1	3.1
100	50.7	4.4

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 55% dsDNA post-sonication (Table B2). 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.

Attributes of Different Shearing Methods

	Bioruptor Sonicator	Competitor C	Tip/Probe Sonicators	Nebulization	Enzymatic methods
Desirable fragment sizes for sequencing	●	●	●	●	Not applicable
No need for glycerol or buffer exchanges	●	●	●	○	○
High yields of dsDNA	●	●	●	○	Not applicable
Multiplexing capability	●	○	○	○	●
Reduced contamination risk	●	●	○	○	○
Fits into existing lab workflows	●	○	●	●	●
High-throughput	●	○	○	○	○
Simple operation	●	●	●	○	○
Consistency	●	●	○	○	○
Value for price	●	○	○	○	○
Maintenance	●	○	Not applicable	Not applicable	Not applicable

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

Using the Bioruptor, we developed workflows that produce the desired size range, and which are gentle enough to produce high yields of high quality dsDNA. The system has low risk of sample-to-sample contamination, since standard plastic laboratory tubes can be used. The system is high throughput and easy to operate, and provides the consistency required for day-to-day operation in the lab.

QC of Sonicated DNA

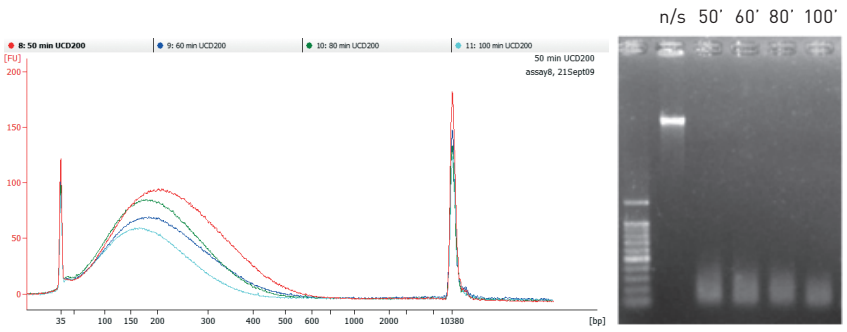


Figure 2. Shearing validation by Bioanalyzer achieves optimal QC.

We use the Bioanalyzer 2100 and its DNA High Sensitivity Chip as a standard QC procedure at Diagenode in order to validate results of genomic DNA shearing. Different sonication times courses (50', 60', 80', and 100') are shown, as run on the Bioanalyzer (panel A) and an agarose gel (panel B).

No Effect of DNA Sample Concentration

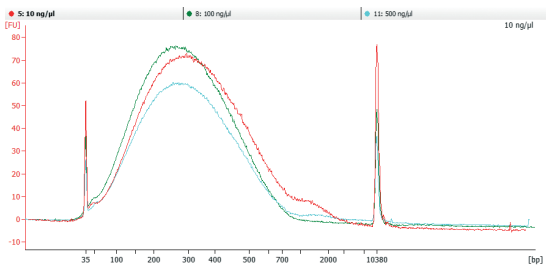


Figure 3. Sample concentration and shearing efficiency effects.

We sonicated increasing concentrations of genomic DNA (10ng/µl, 100 ng/µl, and 500 ng/µl)in duplicates 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 at Low power setting, 30' On/Off; Costar tubes 0.65ml, 100 µl final volume; E.coli gDNA).

Advantages of Parallel Processing: Substantial Time Savings

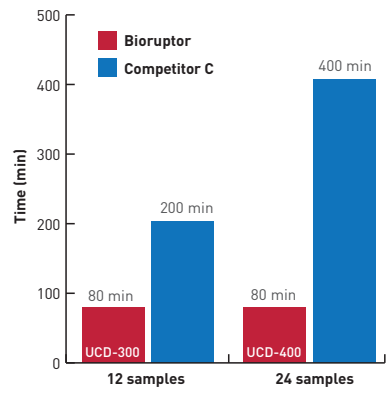


Figure 4. Bioruptor shearing adds efficiency to sequencing workflows.

The Bioruptor efficiently processes large numbers of samples simultaneously. The instrument can process 12, 24, or 48 samples at the same time, with unattended operation. Compared to another leading sonicator, the Bioruptor UCD-300 requires only 80 minutes to sonicate 12 samples, for a time savings of 2 hrs. Importantly, to process 24 samples, the UCD-400 requires 80 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 QC on the Agilent BioAnalyzer
- High yields and integrity of dsDNA content required for optimal library construction