diagenode
A Hologic Company

Evaluation of Bioruptor (Diagenode) in Shotgun Library Preparation for Massively Parallel Sequencing on Illumina Genome Analyzer II

Contributed by Choli Lee
Shendure Lab - Department of Genome Sciences - University of Washington - Seattle, WA

The Illumina Genome Analyzer II (GA-II) performs massively parallel short-read DNA sequencing, with the potential to generate up to 10 gigabases in a single run. The technology provides researchers with a significantly cheaper alternative to conventional sequencing by capillary electrophoresis. A consistent and reliable method of DNA fragmentation is essential in Illumina library construction. We have evaluated and compared two types of fragmentation methods: nebulization and shearing using the Bioruptor.

The Illumina library construction protocol is outlined below. A key determinant of library quality and complexity relates to the fragmentation step.

Illumina Library Construction Protocol

1. Shear 1-5 micrograms (µg) DNA to fragments ranging from 200 to 500bp
2. End-repair DNA fragment library
3. Ligate Illumina adapters on both ends
4. Size-selection to select desired size range and eliminate adapter dimers
5. PCR of complex shotgun library by primer pair corresponding to adaptors
6. Sequencing on Illumina GA-II

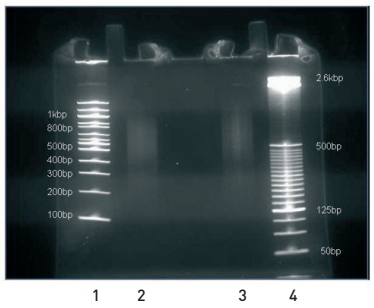
Shearing Criteria for Bridge amplification

Different size ranges are frequently used for bridge amplification on the Ilumina platform. The ideal fragmentation method ensures most DNA fragments lie within this desired size range to maintain library yield and complexity.

- 200-300bp- The standard size for efficient amplification of a single-end sequencing library.
- 300-500bp- This range may be used for paired-end libraries, depending on the desired separation for mate-paired reads.

Standard procedure - Nebulization

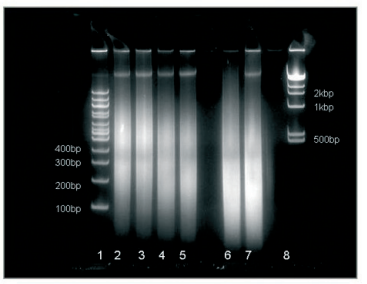
1. Mix 2-5 µg DNA with glycerol and EB to total 750 µl
2. Add to nebulizer
3. Bleed air line for 15 seconds. Then nebulize on ice with 25psi for 6 minutes
4. Centrifuge for 5 seconds to collect solution
5. Purify DNA with QiaQuick column



1. 100 bp ladder
2. Nebulized-25psi 6 minutes
3. Nebulized-25psi 6 minutes
4. 25 bp ladder

Standard procedure - Bioruptor

1. Mix 2-5 µg DNA up to 300 µl total volume with TE
2. Place in the Bioruptor chamber
3. Set the machine to output "high", 30 seconds for both On and Off and run for 15 minutes
4. Pump out warm water and add the cold water to the Bioruptor after initial 15 minutes
5. Repeat step 3.
6. Purify DNA using QiaQuick column



1. 100 bp ladder
2. High-30 minutes
3. High-30 minutes
4. High-60 minutes
5. High-60 minutes
6. High-120 minutes
7. High-120 minutes
8. 1 kb ladder

Europe - Diagenode s.a. // orders@diagenode.com / techsupport@diagenode.com / Phone: +32 4 364 20 50 / Fax +32 4 364 20 51
North America - Diagenode Inc. // orders.na@diagenode.com / techsupport.na@diagenode.com / Phone: +1 862 209 4680 / Fax: +1 862 209 4681

Comparison

Bioruptor	Nebulizer
Shears more of DNA mass to the desired size range	Most DNA mass outside of desired size-range
Up to 6 samples	No equipment required
Easier to operate	No cold room or other cooling device is required
Less chance of contamination	Risk of aerosol formation
Consistent performance	

Conclusions

We have tested both nebulization and Bioruptor methods of DNA fragmentation. Our experiments indicate that the Bioruptor provides a wider and more uniform size range compared to nebulization. Additionally, more of the DNA mass is sheared to the relevant size-range. This increases the flexibility of size selection and decreases the amount of DNA lost in the process. The Bioruptor is also capable of processing up to 6 samples in parallel, while nebulization is performed one-at-a-time, with a single sample per device.

In conclusion, in our experience DNA shearing with the Bioruptor is easy and fast to perform, and is less prone to aerosol contamination. Our lab now largely depends on the Bioruptor and has incorporated it in our standard Illumina library construction protocol.

Europe - Diagenode s.a. // orders@diagenode.com / techsupport@diagenode.com / Phone: +32 4 364 20 50 / Fax +32 4 364 20 51
North America - Diagenode Inc. // orders.na@diagenode.com / techsupport.na@diagenode.com / Phone: +1 862 209 4680 / Fax: +1 862 209 4681