





## Bioruptor® for next generation sequencing

Diagenode is committed to providing powerful DNA shearing and library preparation solutions for the next wave of sequencing technologies. Next generation sequencing technologies have emerged to advance genome sequencing at unprecedented speeds, transforming biological research with a number of novel applications. Researchers have used these new massively parallel sequencing methods to map global protein-DNA interactions (as in ChIP-Seq) or study epigenetic modifications such as DNAmethylation at both genome-wide levels and single-base precision.

Critical to the success of sequencing results, however, is the preparation of high-quality material prior to the sequencing run, and each sample preparation step, including DNA shearing, must be as efficient as possible to maximize yields, quality, and cost effectiveness. Researchers now recognize that the efficiency of DNA shearing and preserving DNA integrity is highly dependent on the quality of sonication devices. Diagenode's Bioruptor® Sonicator is proven as the shearing device of choice and provides optimal sample yield, fragment size, and consistency, which are all essential to next-generation sequencing workflows.

### High-throughput sequencing—Bioruptor® is critical for optimal library construction

In order to create high-quality genomic libraries for the success of next-generation sequencing results, large genomic DNA (gDNA) or double-stranded complementary DNA (dsDNA) must first be sheared into fragments of 200-600 bp on average (see Figure 1 for a workflow diagram of library preparation). The Bioruptor® Sonicator is critical to this shearing step and allows researchers to achieve:

- **Optimal size range and consistency** of sheared DNA (200-600 bp). See Figure 2.
- **Compatibility** with downstream workflow steps (e.g. optimal linker/adaptor additions, elimination of glycerol and buffer exchange steps).
- **High quality and yields of dsDNA**, providing unbiased libraries.
- **Control over DNA fragment size range** as distinct fragment size ranges may be required for specific downstream applications (e.g. bridge amplification) for sequencing. The Bioruptor® can easily be controlled by modifying sonication duration for the desired fragmentation range.
- **Sample multiplexing using standard laboratory plastic tubes** (e.g. microfuge or conical tubes) with a flexible range of adaptors suitable for various sample volumes, types, and formats.
- **A range of models, including high-throughput systems**, which are automatable for processing 24 or 48 samples.
- **Compatibility and validation** with several high-throughput sequencing platforms—SOLiD, Roche/454, Illumina, and others.

## Advantages of Bioruptor® sonication versus other methods

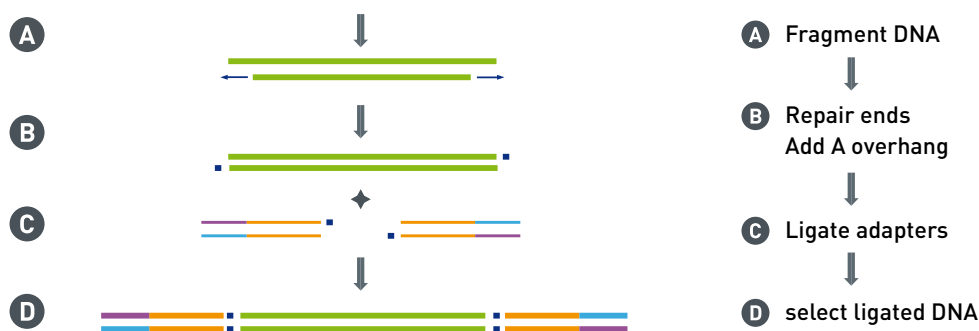
Many methods can be used to fragment DNA including enzymatic digestion, nebulization, probe sonication, or ultrasound sonication with the Bioruptor®. Even though some of these methods have been used in library construction, many have limitations which are successfully overcome by the Bioruptor®. Specifically, endonuclease digestion is known to introduce sequence-specific cleavage bias and nebulization can create too broad of a DNA fragment size distribution. Other devices provide limiting point-source sonication, and therefore require costly glass sample vials, provide no multiplexing capacity, and lack automation for unattended operation. For these reasons, the Bioruptor® is the leading shearing system for epigenetics and high-throughput sequencing.

	Bioruptor® Sonicator	Tip/Probe Sonicators	Nebulization	Enzymatic methods
Desired size range of DNA for sequencing	✓	✓		
Buffer exchanges and glycerol steps eliminated	✓	✓		
High yields of unbiased dsDNA	✓			
Multiplexing capability	✓			
Reduced contamination risk	✓			
Fits into existing lab workflows	✓		✓	✓
High-throughput	✓	+/-		
Simple operation	✓	+/-		✓
Consistency	✓	+/-		

## High-throughput sequencing—Bioruptor® in action

The procedure for library construction is illustrated in Figure 1. The workflow includes DNA fragmentation, followed by DNA repair and end polishing (blunt end or A overhang) and, finally, platform-specific adaptor ligation. The Bioruptor® is a powerful tool for providing optimal fragmentation length, quality, and yield critical for the downstream steps of sequencing.

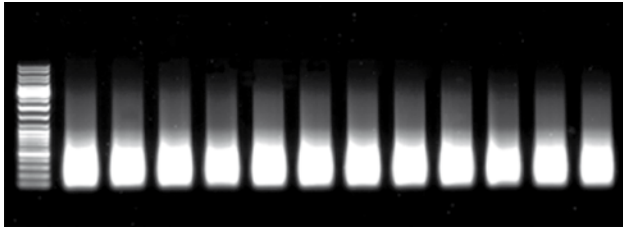
### Workflow for next-generation sequencing library preparation following generation of proper-sized DNA molecules using the Bioruptor®.



**Figure 1. Bioruptor® shears gDNA or dscDNA into appropriately-sized DNA molecules for optimal next-generation sequencing library preparation (~200-600 bp)**

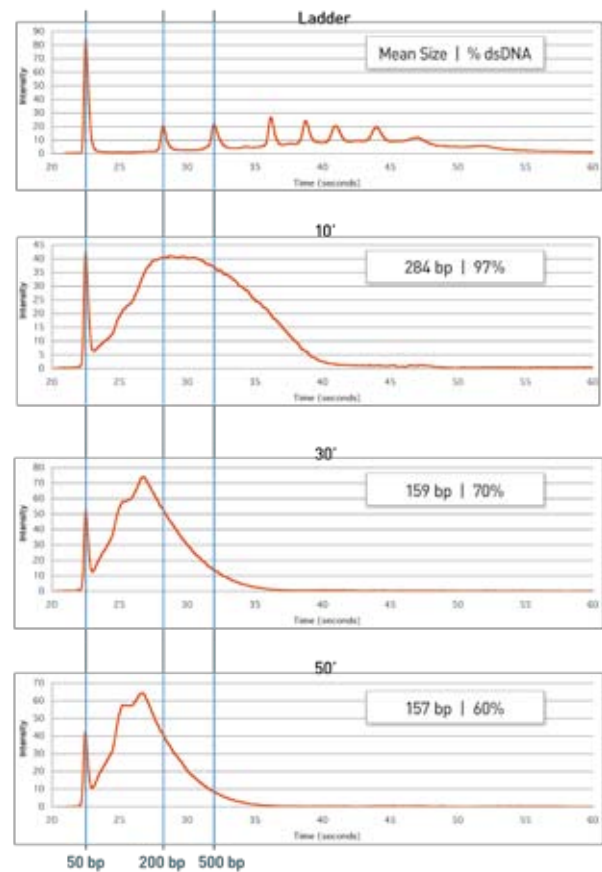
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**Bioruptor® Sonicator shows excellent reproducibility for high-throughput sequencing library generation.**



**Figure 2.** The Bioruptor® achieves highly consistent fragmentation in shearing chromatin-DNA complexes.

**Figure 3.** (Right) Bioruptor® Sonicator achieves optimal size distributions and yields for high-throughput sequencing. By manipulating the duration of sonication, the Bioruptor® ensures that most DNA fragments lie within the narrow size range required for applications such as ChIP or sequencing. Here, an automated shearing time of 80 minutes is best to maintain yield and complexity for optimal library construction for next generation sequencing, as shown by the narrow size distribution (compared to the DNA ladder) and high dsDNA yields (measured by Qubit; data not shown here).



Customer  
Feedback

"Although many options exist, we routinely generate high quality libraries [with sonication]. The Bioruptor® is lower in cost, while achieving higher energy transfer efficiency and more reproducible performance than standard probe sonicators. In addition, multiple samples can be processed simultaneously in a uniform manner."

Hodges et al. (Nature Protocols 4, - 960 - 974, 2009) describing preparation of DNA for high-throughput sequencing

#### Customer Feedback

"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."

Shendure lab, University of Washington USA

## Summary

The Bioruptor® Sonicator is highly effective in creating unbiased libraries with even distribution and representation of the genome. While other sonication methods have been used in genomic library construction, the Bioruptor® has proven the most reliable and reproducible instrument of choice providing:

- Unsurpassed quality, consistency, and efficiency
  - Closed tube format prevents cross-contamination and aerosol formation
  - Variable power range efficiently and evenly disrupts samples
  - Unique cooling system maintains integrity of biological complexes
  - Gentle ultrasound method preserves samples
  - Sample rotation in water bath ensures shearing and lysis consistency
- Compatibility with existing lab workflows: Uses standard microfuge and conical tubes.
- Ease of use: Easy set-up, operation, and maintenance ensures success.
- High-throughput capability: Enables parallel processing of up to 48 samples.
- Scaling ability: Interchangeable sample holders allow for microliter to milliliter quantities.
- High yields: Efficient ultrasound technology enables high yields and reproducibility.

### **Bioruptor® Resources: Protocols for high-throughput sequencing**

The Diagenode Bioruptor® has a unique ability to shear DNA in an unbiased fashion. Below, we present three protocols which were developed for three different purposes—optimal dsDNA recovery (Protocol 1) and ease of library construction efficiency in human and Arabidopsis, respectively (Protocols 2 and 3). We recommend that you use these protocols as a starting point to optimize template preparation for your custom next generation sequencing applications.



# Bioruptor® for next generation sequencing

## Protocol 1

The following protocol was optimized to achieve a high percentage of the sheared DNA in double-stranded (dsDNA) form.

1. Bring the sample concentration to 0.01 µg/µl in TE buffer. Vortex and centrifuge the sample.
2. Using the Bioruptor® on low power, perform a sonication time course to determine suitable fragmentation. We recommend evaluating increments of 10 minutes, ranging between 30 and 60 minutes for sequencing applications.
3. Use cycle durations of 30 seconds, for both “ON” and “OFF” cycles.
4. Approximate yields for E. Coli DNA, were as follows:

Shearing Time	% Recovery of dsDNA (Qubit)
10 min	97%
20 min	80%
30 min	70%
40 min	65%
50 min	63%
60 min	60%
80 min	55%
100 min	45%
120 min	40%

The following additional protocols have been validated in our customers’ laboratories and can be adapted for your own library construction requirements.

## Protocol 2—Illumina library construction protocol

Provided by: Shendure Lab—Department of Genome Sciences—University of Washington—Seattle, WA

1. Shear 1–5 micrograms (µg) DNA to fragments ranging from 200 to 500bp\*, using the Bioruptor®.
2. Perform end-repair for DNA fragment library.
3. Ligate Illumina adapters on both ends.
4. Size-select for desired size range and eliminate adapter dimers.
5. PCR complex shotgun library by primer pair corresponding to adaptors.
6. Sequence on Illumina GA-II.

\*Note on shearing criteria for bridge amplification: Different size ranges are frequently used for bridge amplification on the Illumina 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 for paired-end libraries, depending on the desired separation for mate-paired reads.

### Protocol 3

#### Bioruptor® cycle recommendations for shearing gDNA or dscDNA

Provided by: Dr. Brian D. Gregory —Salk Institute —La Jolla, CA

This Bioruptor®-specific protocol was used in shearing larger DNA molecules from *Arabidopsis thaliana* into properly sized molecules to make high quality libraries. The protocol, with minor modifications, can be adapted to shear gDNA and dscDNA from any organism.

- 1) Place sample (gDNA or dscDNA) into the Bioruptor®, ensuring that the entire sample is submerged below the water level surface in the Bioruptor® tank.
- 2) Set up the Bioruptor® to carry out 5 cycles, using settings of 30 second “ON” and 2 minute “OFF” cycles (i.e. 10 minutes, 30 seconds total sonication).
- 3) Upon completion of sonication, centrifuge the samples to collect all liquid to bottom of the sample tube, and then replace into Bioruptor® making sure that all of the DNA-containing liquid is below surface of water in Bioruptor® reservoir.
- 4) Repeat entire protocol twice, for a total of three sets of 5 cycles.

#### Selected References for Next Generation Sequencing library preparation

Lister R., O'Malley R.C., Tonti-Filippini J., Gregory B.D., Berry C.C., A. Harvey Millar C.A. and Ecker J.R. Highly Integrated Single-Base Resolution Maps of the Epigenome in *Arabidopsis*. *Cell* **133**, p523-536 (2008).

Gregory B.D., O'Malley R.C., Lister R., Urich M.A., Tonti-Filippini J., Chen H., Millar A.H. and Ecker J.R. A Link between RNA Metabolism and Silencing Affecting *Arabidopsis* Development. *Developmental Cell* **14**, 854-866 (2008).

Cokus S.J., Feng S., Zhang X., Chen Z., Merriman B., Haudenschild C.D., Pradhan S., Nelson S.F., Pellegrini M and Jacobsen S.E. Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature* **452**, 215-219 (2008).

**Application Note: Gregory B.D.** Next generation sequencing library preparation following preparation of proper-sized DNA molecules using the Bioruptor® (complete application note [http://www.diagenode.com/pages/support\\_appl\\_notes.html](http://www.diagenode.com/pages/support_appl_notes.html)).

## Ordering information

Description	Reference
<b>Bioruptor® Models</b>	
Bioruptor® Standard	UCD-200 TM (1.5 mL) UCD-200 TO (1.5 mL & 15 mL)
Bioruptor® Standard (new)	UCD-300 TM (1.5 mL) UCD-300 TO (1.5 mL & 15 mL)
Bioruptor® Twin	UCD-400 TM (1.5 mL) UCD-400 TO (1.5 mL & 15 mL)
Bioruptor® XL	XL TM (1.5 mL) XL TO (1.5 mL & 15 mL)
<b>Sample Tube Adaptor Kits</b>	
0.5 mL (12) Microfuge tube accessory kit	UCD-pack 0.5
1.5 mL (6) Microfuge tube accessory kit	UCD-pack 1.5
10 mL (6) tube accessory kit	UCD-pack 10
15 mL (6) tube accessory kit	UCD-pack 15
50 mL (3) tube accessory kit	UCD-pack 50
<b>Cooling System</b>	
Water Cooler	BioAcc-cool
Connectors Kit for Water Cooler	CONN-7D1
Peristaltic pump (including Connectors kit)	TWI-pump
<b>Valve Kits</b>	
Valve kit for UCD-300	VB-100-0001
Valve kit for UCD-400	VB-101-0001



## Diagenode Headquarters

### Diagenode s.a. BELGIUM | EUROPE

Avenue de l'hôpital, 1  
Tour GIGA, 3rd Floor  
4000 Liège - Belgium  
Tel: +32 4 364 20 50  
Fax: +32 4 364 20 51  
orders@diagenode.com  
info@diagenode.com

### Diagenode Inc. USA | NORTH AMERICA

376 Lafayette Road, Suite 202  
Sparta, NJ 07871 - USA  
Tel: +1 973-300-0976  
Fax: +1 973-300-1862  
orders.na@diagenode.com  
info.na@diagenode.com

## Regional Offices

### | FRANCE

Centre d'affaire Genopole  
Rue Henri Desbruères, 5  
Bât. Genavenir 8  
91030 Evry cedex, France  
Tel: +33 1 60 87 14 82

### | UK

Wellington House, East Road, Room 101  
CB1 1BH Cambridge, UK  
Tel: +44 1223 911396  
Fax: +44 1223 451100

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<http://www.diagenode.com/pages/distributors.html>  
For the rest of the world, please contact Diagenode s.a.