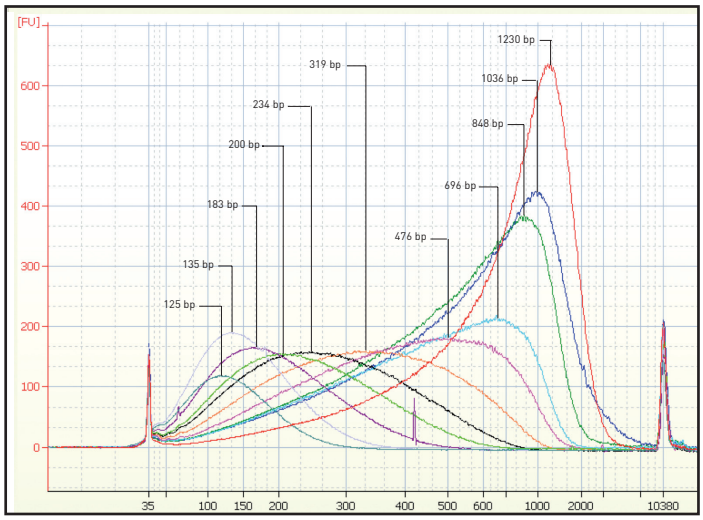
PROTOCOL

DNA Shearing for Bioruptor® NGS



Programmable DNA size distributions, excellent reproducibility, and high dsDNA yields with Bioruptor® NGS

Figure shows different DNA size distributions of sheared genomic DNA produced by varying the duration of sonication using power setting high (H). The different curves depict a specific Bioruptor® NGS run, optimized to produce specific mean sizes and size ranges for Next-Generation sequencing.
All samples were analyzed on Bioanalyzer 2100 using DNA High Sensitivity chip.

Standard operating conditions

Sample volume: 100 µl

Tubes: Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat. No. WA-005-0500)

Tube holder: 0.5/0.65 ml tube holder (Cat. No. UCD-pack 0.5) for 12 x 0.65 ml tubes

Sonication buffer: TE (10 mM Tris, 1mM EDTA, pH 7.5 - 8.0)

DNA concentration: 1-20 ng/µl (10 ng/µl recommended)

Samples are vortexed (10-15 sec) and centrifuged (10 sec) before shearing.

For optimal results samples should be stored on ice during 10-15 minutes prior to sonication.

Temperature: 4°C – Bioruptor® Water Cooler (Cat. No. BioAcc-Cool) & Single cycle valve for Water Cooler (Cat. No. VB-100-0001)

Power setting: H position (High)

Sonication cycle & total sonication time: varies depending on desired DNA size (see table)

Note: Recommended protocols are subject to change without notice. Additional protocols are available on demand.

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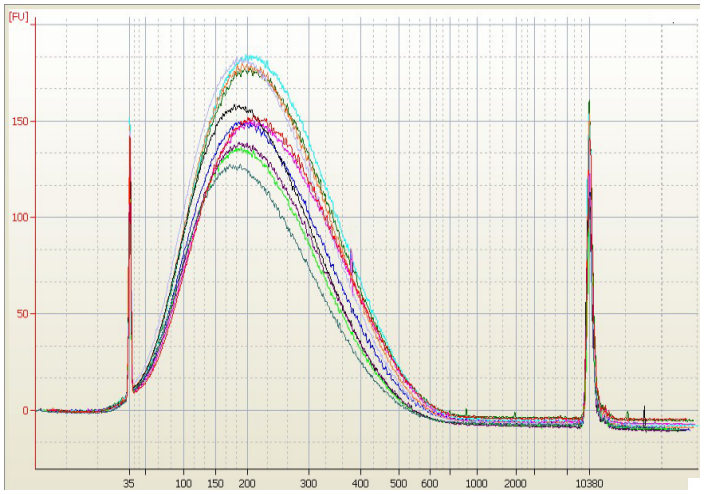
Target size	Cycle condition (On/Off cycle time)	Cycle number
150 bp	30''/30''	30
200 bp	30''/30''	13
400 bp*	15''/90''	7 - 8
1000 bp*	5''/90''	7 - 8

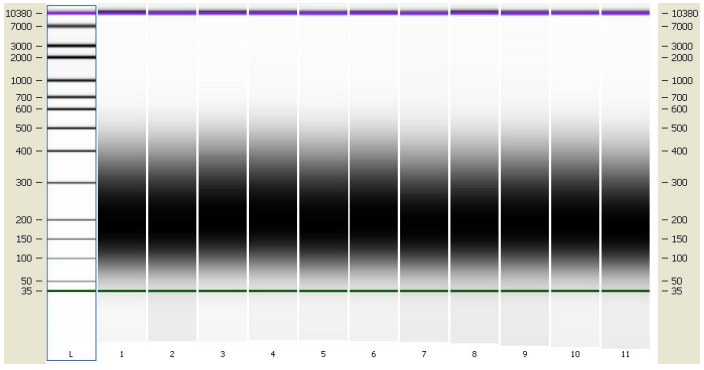
* For longer fragments (400 up to 1000 bp), a short centrifugation step after half of the cycle numbers can significantly improve the results. Protocols for other size ranges (incl. longer fragments up to 1300 bp) are available on request.

The protocol settings listed above are recommended guidelines and actual results may vary depending on the type and amount of starting material, purity level, concentration and/or sample viscosity. It is highly recommended that a time course response experiment be carried out (e.g. varying the time of "on" and "off" durations as well as the number of cycles) to determine the appropriate treatment for your specific sample. Starting material with a smaller sample volume and a greater concentration than the recommended range may require a different time course to ensure homogenous shearing results.

A.



B.



Programmable DNA size distribution and excellent reproducibility with Bioruptor® NGS

Panel A and B show DNA size distributions of 200 bp of sheared human genomic DNA after 13 cycles (30 sec ON/OFF) of sonication. Bioruptor® NGS 0.65 ml microtubes for DNA Shearing (WA-005-0500) were used.
All samples were analyzed on Bioanalyzer 2100 using DNA High Sensitivity chip.

Panel A : peak electropherogram view

Panel B: gel virtual view

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Important comments about DNA shearing

The Diagenode ACT (Adaptative Cavitation Transfer technology) process is highly reproducible. However, attention must be paid to the following treatment attributes to ensure best results:

- **Tubes:** At present, the recommended tube vessels are the Diagenode's Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat No. WA-005-0500). Pay attention not to damage the cap when closing the tubes since this could alter sonication results.
- **Sample volume:** The recommended volume of the Diagenode's Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat No. WA-005-0500) is 100 µl. When using lower volumes (e.g. ≤ 50 µl), less reproducible results may be observed due to an alteration of the ultrasonic waves distribution in the sample fluid; thus, reducing the efficiency of sonication which may result in broader size distribution or larger peaks.
- **Sample concentration:** Diagenode recommends using DNA concentration ranging between 1 and 20 ng/µl (10 ng/µl recommended). Using larger concentration (e.g. 50-100 ng/µl) may result in broader peaks or variable peak distribution.
- **Sample preparation:** Sample viscosity may have a major impact on sonication results. Careful resuspension of DNA sample is strongly recommended before sonication processing. Multiple pipetting and gentle vortexing followed by a short centrifugation to recover sample volume at the bottom of the tube is therefore strongly recommended. Storing DNA samples on ice during 10-15 minutes before sonication has also been shown to improve reproducibility.
- **DNA quality:** DNA quality and quantity must be considered carefully since bad quality and quantity DNA may have several impacts on sonication and next-gen sequencing downstream applications. First, DNA contamination (e.g. from superfluous nucleic acids such as RNA, small nucleic acid fragments, excess proteins, or other contaminating materials) may interfere with DNA measurement method leading to incorrect DNA quantitation thus. Also contaminating RNA in genomic DNA preparation might generate a biased fragment distribution profile on microfluidics-based platform (e.g. Agilent Bioanalyzer) or alter sonication effiency.

  Therefore it is highly recommended to use only high quality DNA when sonicating DNA for Next-Gen sequencing library preparation. The DNA sample to be processed should be highly pure, having an OD260/280 ratio of between 1.8 and 2.0, and should be as intact as possible. DNA extracted using standard techniques (e.g. Proteinase K digested, double phenol/chloform extraction, ethanol precipitated, treatment with RNase-DNase free enzymatic digestion to remove contaminant RNA) or commercial spin-column based kits are recommended.
- **Water temperature:** Propagation of ultrasound in a liquid unavoidably produces heat that can ultimately alter DNA sample (e.g. by thermal denaturation). To ensure the best preservation of the sample, it is recommended to start the sonication process with cold water in the water bath. During sonication, especially when doing long sonication runs, the temperature must also be controlled. This is obtained by the automatic temperature control.

Note: The permanent installation of the Bioruptor® in a cold room is possible, although not sufficient to avoid the temperature increase due to sonication.

- **Automatic temperature control:** A recirculating water cooler is used to guarantee the automatic temperature control of the water bath during the whole sonication process. This water cooler (Cat No. BioAcc-cool) produces a regular water flow with a constant water level in the tank. An additional regulating valve (Single cycle valve for Water Cooler, Cat. No. VB-100-0001) ensures

that water will only be replaced during the off cycle to avoid any interference between the water flow and the sonication process.

- **Sonication time:** Minor adjustments in cycle number may be made to optimize results for various sample types and concentrations. Cycle number listed above is a recommended guideline. Actual results may vary depending on the amount and type of starting material, concentration, viscosity and/or plastic tubes. Diagenode recommends setting up a time dose response experiment for determining appropriate cycle number. Larger length starting material (e.g. total genomic DNA) and higher concentration may require a longer dose to ensure a homogeneous shearing result.
- **Water bath:** The sonication water bath is a critical component of the Bioruptor® sonication system.

  1. **Water purity:** Contaminants such as algae and particules may alter the ultrasonic waves propagation, resulting in broader size distribution or larger peaks. Bath water should be pure distilled water, changed regularly.
  2. **Water bath maintenance:** The water bath metal surface is fragile and requires a careful maintenance. Use only soft sponge to remove traces. Never use scratch scrub sponge since this would alter the ultrasonic wave emitter surface.
  3. **Water type:** Distilled water

Supplementary Data:

Please note that there are three main sources of variation in both peak base-pair size and distribution:

1) The physical process of DNA fragmentation might not be entirely random in AT- or GC- rich regions.

2) The analytical process to determine fragment size has inherent variances (for example, gel electrophoresis and microfluidics-based platform). Therefore, fragment distributions and peak values, even from technical replicates, may not appear identical. If the sheared DNA sample will be resin or column purified or concentrated prior to analysis, please remember to take out an aliquot for use as control prior to that step. Column purification and concentration of the sheared DNA will generate a biased fragment distribution profile due to the inherent greater loss of the smaller DNA fragments.

3) RNA contamination in genomic DNA preparation should be carefully removed using RNase-DNase free enzymatic digestion since they might generate a biased fragment distribution profile on microfluidics-based platform (e.g. Agilent Bioanalyzer) or alter sonication effiency.