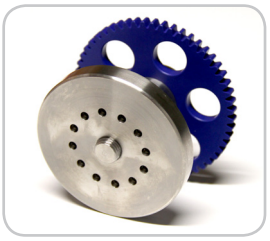
PROTOCOL

Small Volume DNA Shearing for Bioruptor® NGS

NOTICE: For DNA shearing we highly recommend to use the tube holder for 0.1 ml tubes (Cat. No. UCD-pack 0.1) and the corresponding 0.1 ml Microtubes for DNA Shearing (Cat. No. WA-006-0120).



0.1 ml tube holder
(Cat. No. UCD-pack 0.1)

0.1 ml Microtubes for DNA Shearing
(Cat. No. WA-006-0120)

To use the tube holder, remove the lower part by turning counterclockwise. Then place microtubes into the unit. Attach the lower part to the upper part of the adaptor. To guarantee homogeneity of DNA shearing, the tube holders should always be completely filled with tubes. Never leave empty spaces in the tube holder. Fill the empty spaces with tubes containing the same volume of distilled water.

Standard operating conditions

Sample volume: 10 µl

Tubes: 0.1 ml Microtubes for DNA Shearing (Cat. No. WA-006-0120)

Tube holder: 0.1 ml tube holder (Cat. No. UCD-pack 0.1) for 12 x 0.1 ml tubes

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

DNA concentration: 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 - Water cooler (Cat. No. BioAcc-Cool) & Single Cycle Valve for Water cooler (Cat. No. VB-100-0001)

Power setting: H position (High)

Sonication cycle and 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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Sonication table for small volume DNA Shearing

Target size	Cycle conditions (On/Off time)	Cycle number
200 bp	30"/30"	20 cycles
250 bp	30"/30"	15 cycles*
300 bp	30"/30"	13 cycles**
550 - 600 bp	15"/90"	7 cycles***
750 - 800 bp	15"/90"	6 cycles***

** Short centrifugation after 5 and 10 cycles added.*

*** Short centrifugation after 4 and 8 cycles added.*

**** Short centrifugation after 3 cycles added.*

- 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/or a greater concentration than the recommended range may require a different time course to ensure homogenous shearing results.
- Adding short centrifugation step(s) during the sonication round (e.g. after half of the cycle numbers) can significantly improve the results.
- Protocols for other size ranges are available on request.

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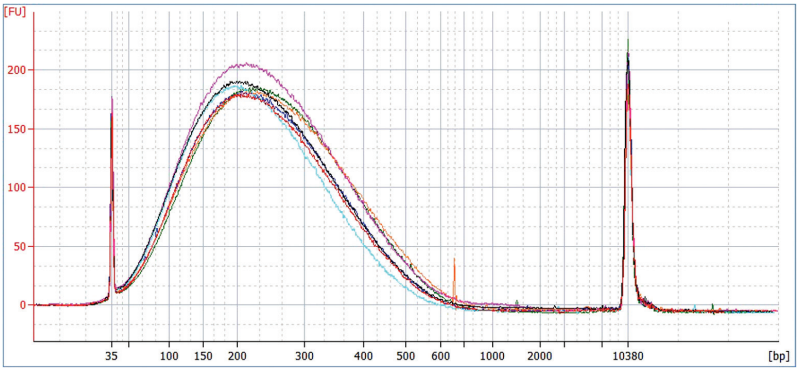
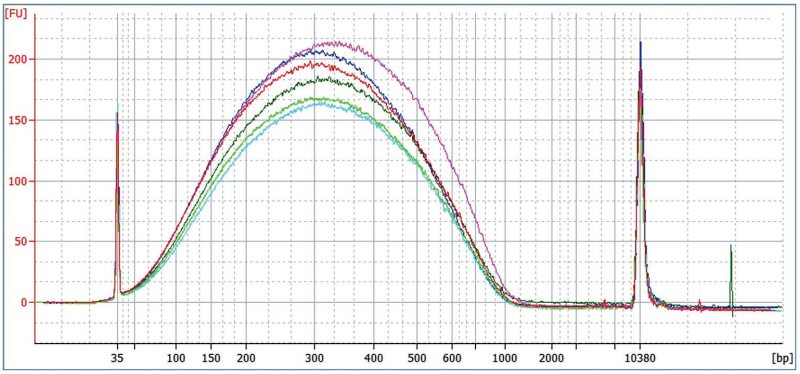
Small Volume DNA Shearing results

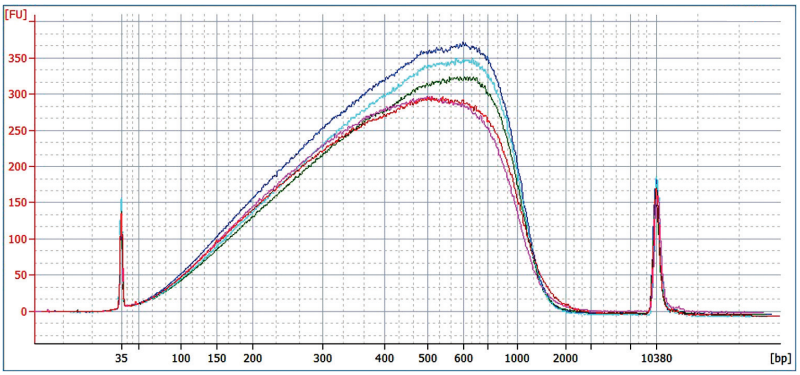
A.



B.



C.



Programmable DNA size distribution and high reproducibility with Bioruptor® NGS using 0.1 ml tubes

Panel A, B and C show DNA size distributions of sheared human genomic DNA using standard operating conditions.

Panel A: 200 bp after 20 cycles (30 sec ON/OFF). (Average size: 215 bp; CV%: 6.6%).

Panel B: 300 bp after 13 cycles (30 sec ON/OFF). Short centrifugation after 4 and 8 cycles added. (Average size: 316 bp; CV%: 4.5%).

Panel C: 550 – 600 bp after 7 cycles (15 sec ON/90 sec OFF). Short centrifugation after 3 cycles added. (Average size: 576 bp; CV%: 8.5%).

Panel A, B and C: peak electropherogram view.

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Important comments about DNA shearing

The Diagenode **ACT** (**A**daptative **C**avitation **T**ransfer 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 0.1 ml Microtubes for DNA Shearing (Cat No. WA-006-0120). Pay attention not to damage the cap when closing the tubes since this could alter sonication results.
- **Sample volume:** The recommended volume of the 0.1 ml Microtubes for DNA Shearing (Cat. No. WA-006-0120) is 10 µl. When using smaller volumes (e.g. < 10 µl; not smaller than 5 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 a DNA concentration of 10 ng/ µl. Using larger or smaller concentrations may result in 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.
- **Temperature control:** Optimal temperature for sonication is 4°C. Sample temperature should not exceed 10°C. The use of the Water cooler (Cat. No. BioAcc-Cool) is highly recommended to guarantee the automatic and precise temperature control of the water bath during the entire sonication process.

Note: The permanent installation of the sonication device in a cold room is possible, although not sufficient to avoid the temperature increase due to sonication. This location would only replace the "pre-cooling" step described above.

- **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.

- **Sonication time:** Minor adjustments in cycle number may be made to optimize results for various sample types and concentrations. The table above listing the cycle parameters and numbers 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.
- **Water bath:** The sonication water bath is a critical component of the 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 (at least once per week).
    2. **Water bath maintenance:** The water bath metal surface is fragile and requires a careful maintenance. Use only soft sponge and distilled water 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.