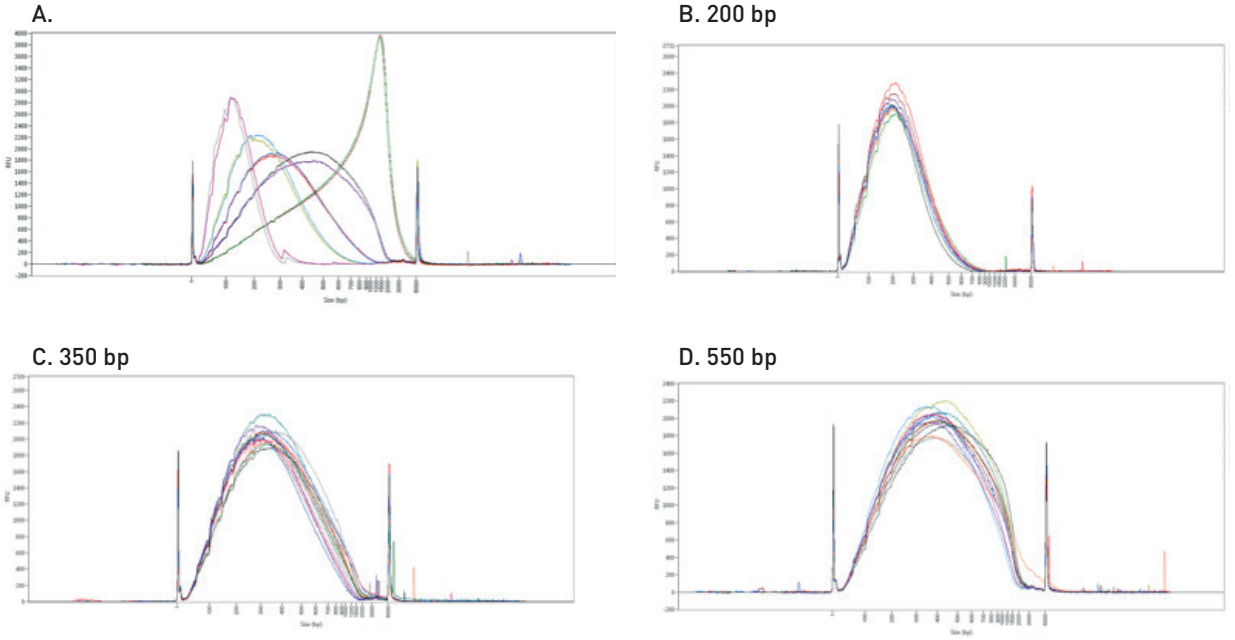
diagenode

Innovating Epigenetic Solutions

PROTOCOL

DNA shearing for Next-Generation Sequencing with the Bioruptor® pico, 0.2 ml Bioruptor® microtubes and the corresponding holder.

Optimal data generation using NGS platforms relies on a few sample-preparation prerequisites, among others the precise DNA fragmentation. The Diagenode Bioruptor® Pico ensures that the DNA is efficiently sheared to an appropriate and consistent fragment size. This first step is critical to generate high quality unbiaised libraries.



Programmable DNA size distributions, excellent reproducibility, and high dsDNA yields with the 0.2ml tube holder for Bioruptor® Pico. Figure A shows different DNA size distributions of sheared genomic DNA produced by varying the duration of sonication. The different curves depict a specific Bioruptor® Pico run, optimized to produce specific mean sizes and size ranges for NGS. Figure B-D show the excellent reproducibility in DNA shearing compatible with NGS librairies from Illumina, IonTorrent and exom capture protocols from Agilent and Roche NimbleGen requirements. All DNA samples (100ng-4ug/50ul) were analysed on Fragment Analyzer™ (Advanced Analytical).

STANDARD OPERATING CONDITIONS

DNA shearing using the new 0.2 ml microtubes for Bioruptor® Pico allows a simultaneous shearing of 16 samples and it is compatible with a volume range from 20 µl up to 100 µl. Different sonication settings should be used for sample volume 20-50 µl and 60-100 µl. Please refer to corresponding table below to find right settings.

Sample volume: 20-50 µl or 60-100 µl

Tubes: 0.2 ml microtubes for Bioruptor® Pico (Cat. No C30010020)

www.diagenode.com Please contact us for more information
Europe Diagenode sa / LIEGE SCIENCE PARK // Rue du Bois Saint-Jean, 3 // 4102 Seraing (Ougrée) // Belgium // Phone: (+32) 4 364 20 50 // E-mail: info@diagenode.com
USA Diagenode Inc. / 400 Morris Avenue, Suite 101 // Denville, NJ 07834 // USA // Phone: (+1) 862 209-4680 // Mail: info.na@diagenode.com

Tube holder: Tube Holder 0.2 ml for Bioruptor® Pico (Cat. No. B01200044) for 16 samples

Sonication buffer: TE (10 mM Tris, 1mM EDTA, pH 7.5 - 8.0) or Low TE (10 mM Tris, 0.1mM EDTA, pH 7.5 - 8.0)

DNA concentration: 1-50 ng/µl

DNA purity: RNA-free high molecular weight genomic DNA with ratio A260/280 of 1.8 - 2.0

Temperature: 4°C – Water Cooler (Cat. No. B02010002) & Single Cycle Valve (Cat. No. B02020004)

Samples are vortexed (5-10 sec) and centrifuged (10 sec) before shearing. For optimal results samples should be stored on ice during 5-10 minutes prior to sonication.

Sonication cycle & total sonication time: varies depending on desired DNA size and initial sample volume. Please check tables below.

SONICATION SETTINGS FOR A SAMPLE VOLUME OF 20-50 µL:

Target size, bp *	Cycles number	Cycles conditions (On/Off time)
650	3	15"/30"
500	5	15"/30"
350	5	30"/30"
250	10	30"/30"
200	15	30"/30"
150	30	30"/30"

SONICATION SETTINGS FOR A SAMPLE VOLUME OF 60-100 µL:

Target size, bp *	Cycles number	Cycles conditions (On/Off time)
1300	3	15"/30"
1000	5	15"/30"
600	5	30"/30"
400	10	30"/30"
300	15	30"/30"
250	20	30"/30"
200	30	30"/30"

Please note that referred sizes have been assessed using Fragment Analyzer™ from Advanced Analytical (smear analysis option). The target size can differ if another system is used for the size assessment.

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