diagenode
Innovating Epigenetic Solutions

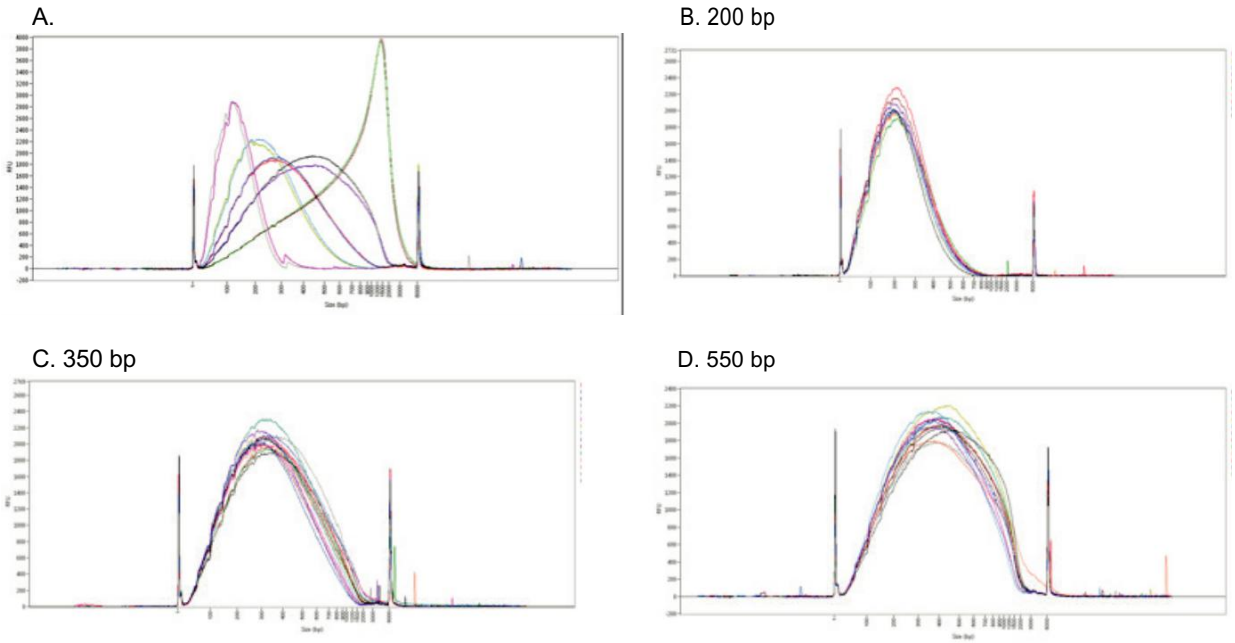
PROTOCOL

DNA shearing for Next-Generation Sequencing with the Bioruptor® Pico

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.

DNA shearing using the Bioruptor Pico

- allows a simultaneous shearing from 6 up to 16 samples
- compatible with a volume range from 20 µl up to 300 µl per sample
- compatible with a broad range of sample concentration
- provides unbiased shearing and high yield of dsDNA



Programmable DNA size distributions, excellent reproducibility, and high dsDNA yields with 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. Figures B-D show the excellent reproducibility in DNA shearing compatible with NGS libraries from Illumina, IonTorrent and exom capture protocols from Agilent and Roche NimbleGen requirements. All DNA samples were analysed on Fragment Analyzer™ (Agilent).

Tube Holders and Tubes for Bioruptor Pico recommended for DNA shearing

The holder is made up of a tube holder and a specific dock (see the table below). Each tube holder accommodates specific microtubes allowing simultaneous processing of multiple samples with variable sample volume as indicated in the table below.

Tube Holder	Tubes	Sample volume	# of samples per run
Tube holder for 0.65 ml tubes (B01201143)	0.65 ml microtubes for Bioruptor Pico (C30010011)	100 µl	12
Tube holder for 0.2 ml tubes (B01201144)	0.2 ml microtubes for Bioruptor Pico (C30010020)	50 µl	16
Tube holder for 1.5 ml tubes (B01201140)	1.5 ml microtubes for Bioruptor Pico (C30010016)	300 µl	6

The sonication settings listed below are recommended guidelines for the shearing of genomic DNA of high molecular weight. To ensure reproducible and consistent shearing, use pure RNA-free genomic DNA. Actual results may vary depending on starting material, purity level etc. Some additional optimization may be required to determine the appropriate treatment for your specific sample. Please note also that referred sizes can differ depending on the analytical systems used for the size assessment.

The successful shearing of FFPE-derived DNA relies on the initial quality and size of DNA. If extracted DNA is of high molecular weight above 10 kb, the standard DNA shearing protocols should be applied. Smaller DNA fragments are more resistant to sonication and the total sonication time should be extended by additional cycles to reach the target size. Time course monitoring after 5, 10, 15 additional cycles is recommended. Please refer for more details on FFPE-derived DNA shearing to the application note https://www.diagenode.com/files/application_notes/AN-FFPE-07_2017.pdf .

Shearing of PCR amplicons of the size down to 500 bp can be achieved using the Bioruptor Pico. PCR fragments bigger than 700 bp are sheared according to the standard protocol, smaller PCR fragments from 500 - 700 bp are less susceptible to sonication. For Fragments of this size range the sonication time has to be increased by adding 3 to 5 additional cycles to the standard protocol.

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1. **Switch** on the Bioruptor Pico and **set** the temperature of the cooler at 4°C.
2. **Prepare** the DNA solution at concentration 2-100 ng/µl* using TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5 - 8.0) or Low TE buffer (10 mM Tris, 0.1 mM EDTA, pH 7.5 - 8.0).

   **NOTE: The smear of a sample with a concentration below 2 ng/µl will not be detected by most Qc instruments.*
3. **Transfer** a required volume of DNA solution to the appropriate sonication microtubes accordingly to volume specification.
4. Briefly **vortex** (5-10 sec) and **centrifuge** (10 sec) samples.
5. **Incubate** on ice for 10 min.
6. **Fill** the tube holder with sonication tubes.

   NOTE: To guarantee homogeneity of shearing, always completely fill the tube holder with tubes. Never leave empty spaces in the tube holder. Fill the empty spaces with tubes containing the same volume of distilled water.
7. **Sonicate** samples using the Bioruptor Pico. Sonication cycle & total sonication time varies depending on accessories and consumables, desired DNA size and initial sample volume. Please choose appropriate settings.

Sonication settings for 0.2 ml tubes Bioruptor Pico holder and 0.2 ml Bioruptor® Microtubes **50 µL** SAMPLE VOLUME

target size, bp *	Cycle number	Cycle conditions (On/Off time)
600	3	15/30
450	5	15/30
350	4	30/30
300	6	30/30
200	13	30/30
150	23	30/30

**-referred sizes have been assessed using the Fragment Analyzer™ from Agilent (smear analysis option)*

Sonication settings for 0.65 ml tubes Bioruptor Pico holder and 0.65 ml Bioruptor® Microtubes **100 µl** SAMPLE VOLUME

target size, bp *	Cycle number	Cycle conditions (On/Off time)
1000	1	30/30
600	2	30/30
400	5	30/30
350	6	30/30
200	13	30/30
180	25	30/30
150	30	30/30

**-referred sizes have been assessed using the Fragment Analyzer™ from Agilent (smear analysis option)*

Sonication settings for 0.2 ml tubes Bioruptor Pico holder and 0.2 ml Bioruptor® Microtubes **20 µL** SAMPLE VOLUME

Under validation

Sonication settings for 0.2 ml tubes Bioruptor Pico holder and 0.2 ml Bioruptor® Microtubes **100 µl** SAMPLE VOLUME

Under validation

Sonication settings for 1.5 ml tubes Bioruptor Pico holder and 1.5 ml Bioruptor® Microtubes **300 µL** SAMPLE VOLUME

target size, bp*	Cycle number	Cycle conditions (On/Off time)
200	15	30/30

**-referred sizes have been assessed using the Fragment Analyzer™ from Agilent (smear analysis option)*

Sonication settings to achieve other target sizes should be optimized by user.