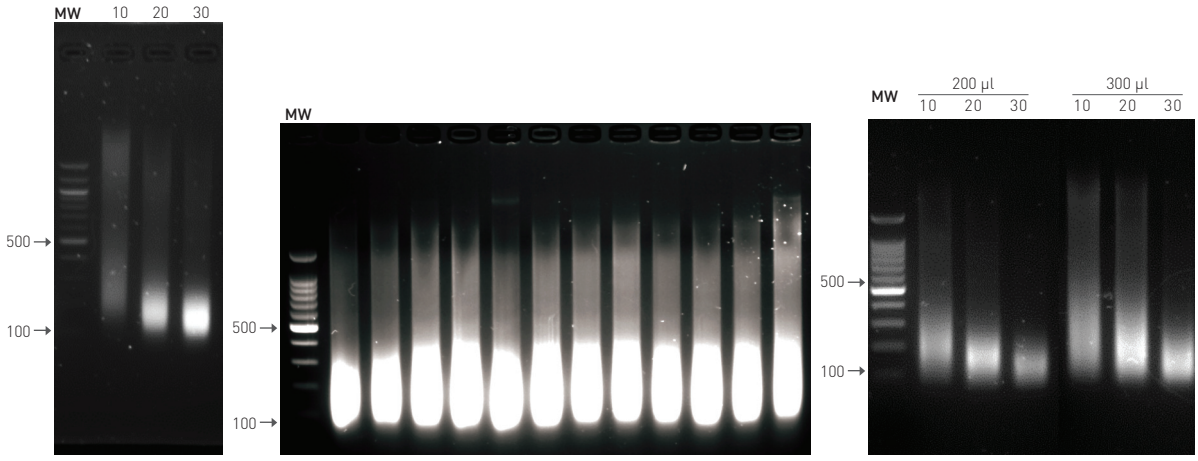
PROTOCOL

Consistent and highly reproducible chromatin shearing with the Bioruptor® Pico 10 µl - 300 µl

HeLa cells are fixed with 1% molecular grade formaldehyde (for 8 min at room temperature). Nucleus isolation is performed using buffers and reagents of Diagenode's Chromatin Shearing Optimization kit - Low SDS (Cat. No. AA-001-0100) 1x10e6 cells are then resuspended in 100 µl Shearing Buffer prior to chromatin shearing.



A: 10 µl volume B: 100 µl volume C: 200 & 300 µl volume

Panel A, 10 µl volume: Chromatin samples are sheared for 10, 20 and 30 cycles of 30 sec ON/30 sec OFF with the Bioruptor® Pico using 0.1 ml Bioruptor® Microtubes (Cat. No. C30010015).

Panel B, 100 µl volume: Chromatin samples are sheared for 10 cycles of 30 sec ON/30 sec OFF with the Bioruptor® Pico using 0.65 ml Bioruptor® Microtubes (Cat. No. WA-005-0500).

Panel C, 200 & 300 µl volume: Chromatin samples are sheared for 10, 20 and 30 cycles of 30 sec ON/30 sec OFF with the Bioruptor® Pico using using 1.5 ml Bioruptor® Microtubes (Cat. No. C30010016).

Prior to de-crosslinking, samples are treated with RNase cocktail mixture at 37°C during 1 hour. The sheared chromatin is then de-crosslinked overnight and phenol/chloroform purified as described in the kit manual. 10 µl of DNA (equivalent of 500, 000 cells) are analyzed on a 2% agarose gel (MW corresponds to the 100 bp DNA molecular weight marker).

(MW corresponds to the 100 bp DNA molecular weight marker)

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