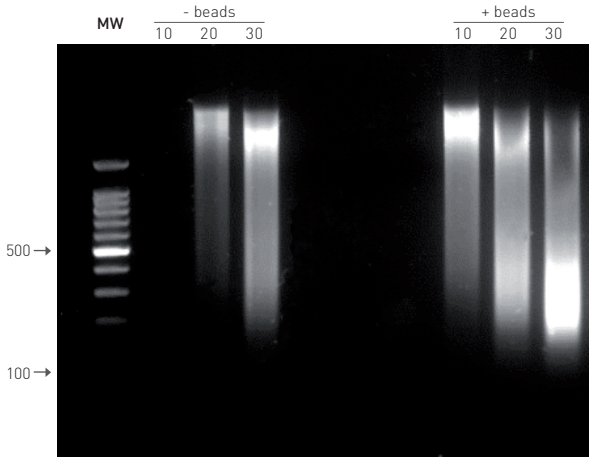
PROTOCOL

Consistent and highly reproducible chromatin shearing with the Bioruptor® Pico 500 µl - 2 ml

Chromatin shearing from HeLa cells is performed according to Diagenode's Chromatin Shearing Optimization kit – Low SDS protocol (Cat. No, AA-001-0100). Nuclei are resuspended in buffer iS1 at a final concentration of 1x10e6 per 100 µl.

2 ml nuclei aliquots are sonicated with the Bioruptor® Pico in 15 ml Bioruptor® Tubes (Cat. No. C01020031) with or without sonication beads (sonication beads are part of Cat. No. C01020031) for 10, 20 or 30 cycles (30 sec ON/30 sec OFF). Samples are vortexed every 10-cycle round.



For shearing in a final volume of 2 ml, the 15 ml Bioruptor® Tubes are filled with sonication beads up to the third line of the graduation scale (0.3 ml), corresponding to around 800 mg of beads.

Following shearing, 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.

Prior to use, beads are washed by vortexing in 3 volumes of PBS. All buffer is removed before adding the samples.

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.

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