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APPLICATION NOTE

Application of the Bioruptor® Pico for shearing chromatin fragments for ChIP-seq from human tumor tissue

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Introduction

Over the last several years, chromatin immunoprecipitation followed by massive parallel sequencing (ChIP-seq) has helped to identify transcription factor function and epigenetic modifications on a genome-wide scale. Most of these studies have classically been performed using cell line material. In this application note, we illustrate that the Bioruptor® Pico (Diagenode) can process various human tumor tissue for ChIP-seq analyses of transcription factors as well as epigenetic marks.

Chromatin shearing of prostate tumor tissue

Chromatin must be optimally sheared prior to immunoprecipitation and subsequent library preparation for Illumina sequencing. Figure 1 demonstrates optimal chromatin shearing at 10 cycles of 30 seconds ON/OFF with chromatin fragments in the ideal range of 300-500 bp. The tumor specimen was over-sonicated at 20 and 25 cycles.

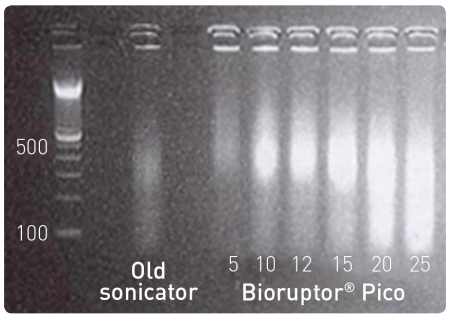


Figure 1: Around 25 µg of prostate tissue was fixed using 1% formaldehyde for 10 minutes and homogenized using a tissue grinder. Subsequently, nuclei were isolated as described before (Schmidt et al., 2009, Methods) lysed and split in two samples. One sample was sonicated using a probe sonicator as applied previously (Jansen et al., 2013 Cancer Research; "old sonicator"), the other on the Bioruptor® Pico. For the Bioruptor® Pico, after the indicated cycles a few µl of sample were set apart and later analyzed on a 2% agarose gel. 1 cycle = 30 sec ON/30 sec OFF.

Sample processing for ChIP-qPCR analyses on human breast tumor specimens

To test the Bioruptor® Pico for chromatin shearing followed by immunoprecipitation, we used a breast tumor specimen (15 cryosections of 30 µm thickness). Well-known binding sites for ERα were tested proximal to the RARA, GREB1 and PgR loci. As a negative control region, one of the introns of CCND1 was chosen, over which all other samples were normalized. For this specific specimen, enrichment for RARA and GREB1 was observed. In parallel, ChIP was performed from the same supernatant for the promoter mark H3K4me3. Here, strong enrichment was observed for RARA and PgR.

A.

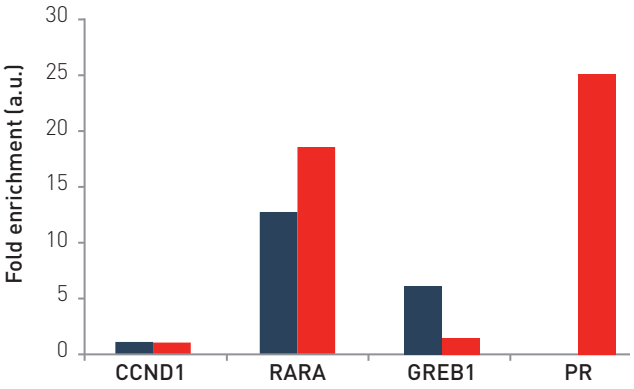
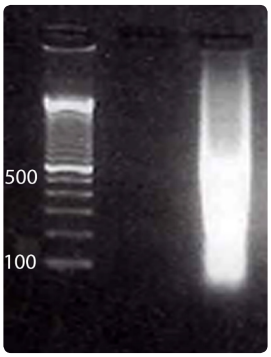


Figure 2: A breast tumor specimen was applied, starting with 15 cryosectioned slices of 30 µm thickness. Tissue was fixed using 1% formaldehyde, homogenized using a tissue grinder, lysed and sonicated using the Bioruptor® Pico (10 cycles of 30 sec ON/30 sec OFF) in 300 µl buffer (for protocol, see Schmidt et al., 2009 Methods). Six samples were processed in one run. After sonication, sampels were analyzed on a 2% agarose gel. Subsequently, overnight chromatin immunoprecipitation was performed for Estrogen Receptor alpha (ERα; HC-20, SC-543) and Histone 3 lysine 4 trimethylated (H3K4-3me; Ab8580). After reverse cross-linking (65 degrees, 6 hours) samples were processed for qPCR, see **Figure A**: qPCR data were normalized over CCND1 negative control region and over input. Error bars depict standard deviation from triplicate meassurements. CCND1 = cyclinD 1, negative control. RARA = Retinoic Acid Receptor alpha, GREB1 = Growth Regulation by Estrogen in Breast cancer 1, PR = Progesteron Receptor, ND = not determined.

ChIP-sequencing analyses for transcription factors and epigenetic modifications

To assess applicability of the Bioruptor® Pico for processing tumor specimens for ChIP-seq, frozen endometrial tumor specimens were analyzed. Tissue was processed as described above and immunoprecipitation was performed for ERα and enhancer histone mark H3K27Ac.

A.



B.

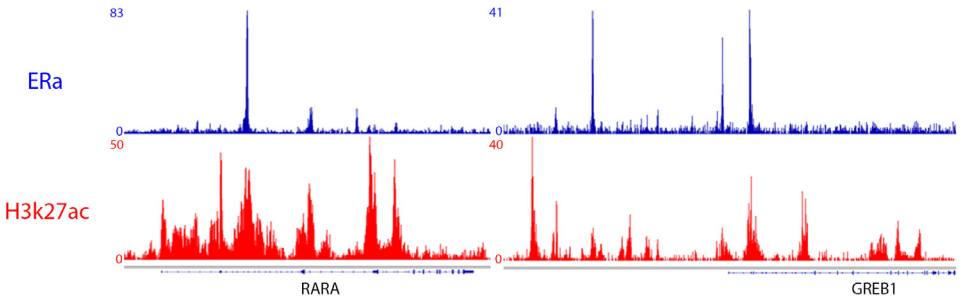


Figure 3: Fresh frozen endometrial tissue was analysed, using 10x 30 µm cryosections. Tissue was cross-linked using 1% formaldehyde for 20 minutes. After cross-linking, tissue was homogenized using a tissue grinder and lysed (for protocol, see Schmidt et al., 2009 Methods). Tissue was sonicated using the Bioruptor® Pico (10 cycles of 30 sec ON/30 sec OFF). After sonication, shearing was assessed using a 2% agarose gel (**Figure A**). Subsequently, lysate was used for chromatin immunoprecipitations for Estrogen Receptor alpha (HC-20; 10 µg antibody/100 µl protein A magnetic beads) & H3K27ac (6 µg antibody/60 µl protein A magnetic beads) **Figure B**.

Conclusion

For preparing tumor tissue specimens for ChIP-seq analyses the Bioruptor® Pico is a suitable and powerful machine. Tissue processing is fast, efficient and the quality of the ChIP-seq data provided is high. With this, we believe the Bioruptor® Pico is an ideal sonication device for human tumor specimens for ChIP-seq of transcription factors and epigenetic modifications.

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