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APPLICATION NOTE

Comparison of the S. cerevisae chromatin shearing with Covaris™ S2 and Bioruptor® Pico

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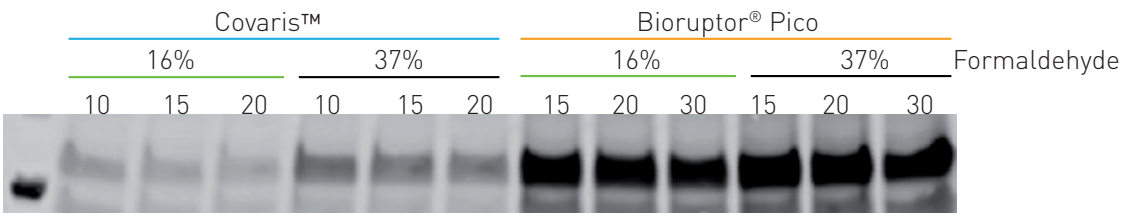
The chromatin shearing is very important step for all kind of ChIP analysis followed by next-generation sequencing. The efficiency, reproducibility and straightforwardness are the most important characteristics for shearing. The using of S. cerevisiae as a model organism is even more complicated because they have a cell wall which require special lysis and can effect shearing. In this protocol we compared the yeast chromatin shearing by Covaris™ S2 and Bioruptor® Pico, and the chromatin crosslinking by 16% methanol-free and 37% methanol-containing formaldehyde.

The cells which have RFA1 tagged with 9-myc tag were grown in YPD medium till density OD600 around 1OD/ml. 50 ml of cells were crosslinked with formaldehyde (final concentration 1%) during 10 min at room temperature with agitation. To find the best condition for our work we compare two types of formaldehydes – 37% (Sigma-Aldrich) which contain a methanol that can potentially effect crosslinking and 16% (PierceTM Life Technologies) methanol-free. We quenched reaction with glycine in PBS (final concentration is 125mM) during 5 min at room temperature with agitation. The cells were centrifuged at 4000 rpm for 5 min and the medium were discarded. We washed once with 25 ml ice-cold PBS, centrifuge as before. The pellet was re-suspended in 600 µl RIPA buffer containing Protease Inhibitor Cocktail (Sigma-Aldrich) and transferred to 2 ml tubes with 500 µl 0.5 mm Zirconia/silica beads (BioSpec). We lysed the cells with PRECELLYS® 24 machine, used condition number 5 and repeated it three times. All process was performed at 40C.

We transferred the samples to new tubes and adjusted volume to 1 ml with RIPA buffer contained Protease Inhibitor Cocktail. For Covaris™ S2 machine we used milliTUBE 1ml AFA Fiber with 20% Duty cycle, 8 intensity, 200 cycles per burst and treatment time – 10, 15 20 seconds. With the Bioruptor® Pico we used 15 ml Bioruptor® Tubes (Cat. No. C01020031) with sonication beads (sonication beads are part of Cat. No. C01020031) for 15, 20 or 30 cycles (30 sec ON/30 sec OFF). Samples are vortexed every 10-cycle round. From each conditions 30 µl of the samples were taken for reverse crosslinking and 100 µl of Elution buffer (50 mM Tris-HCl, pH8.0; 10 mM EDTA; 1% (w/v) SDS) was added. The samples were incubated at 650C overnight.

After incubation we added 120 µl of Tris EDTA, pH 8.0. Then we took 10 µl of each samples for immunoblotting analysis with c-Myc Antibody (9E10). To the rest we added 5 µl of 10 mg/ml RNAse and incubated 1h at 500C, after added 5 µl of Proteinase K from Tritirachium album (Sigma-Aldrich) and incubate 1h more at 500C. The fragmented DNA was purified with ChIP DNA Clean & Concentrator (Zymo Research), eluted in 50 µl. The size distribution was monitored on an Agilent Bioanalyzer 2100 with Agilent High Sensitivity DNA Kit and amount of DNA was checked with Qubit dsDNA HS Assay Kit.

A.



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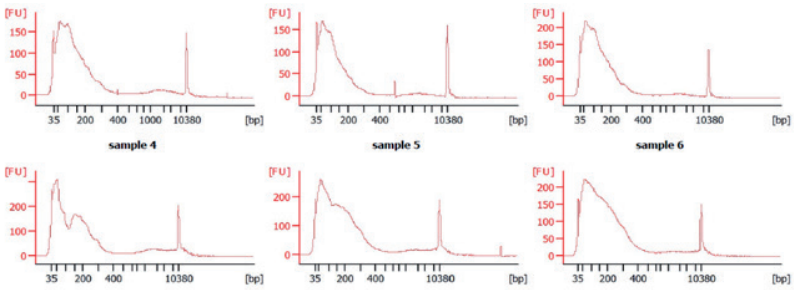
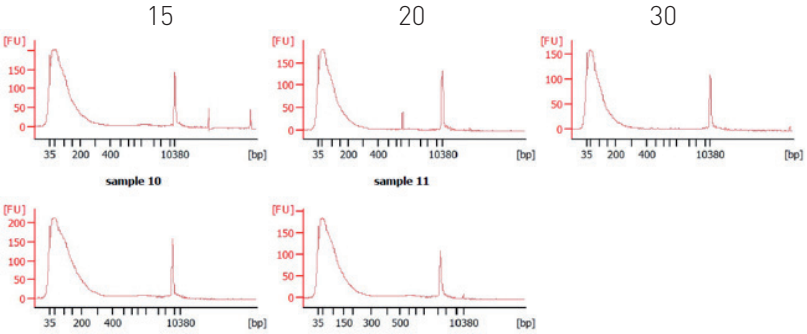
B.



C.



D.

	Formaldehyde	Conditions	ng/µl
Covaris™ 2S	16%	10	2.06
		15	2.1
		20	2.9
	37%	10	9.9
		15	9
		20	8.34
Bioruptor® Pico	16%	15	39.6
		20	37.8
		30	35.8
	37%	15	46.4
		20	41
		30	49.4

Figure 1. A. The immunoblotting with c-Myc Antibody (9E10). **B.** The resulting lengths of the sheared DNA using Covaris™ S2 machine with different sonication condition. **C.** The resulting lengths of the sheared DNA using Bioruptor® Pico machine with different sonication duration. **D.** The DNA concentration after reverse crosslinking.

Summary

With Bioruptor® Pico we stronger RFA-9myc signal in samples sheared with Bioruptor® Pico, also, we extracted more DNA in this condition. There was no difference in the Bioanalyser profile between samples treated with 16% and 37% of formaldehyde in case of Bioruptor® Pico. But in case of Covaris™ S2 we observe more smooth profile with 16% formaldehyde.