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Cross-linked Yeast Chromatin Shearing on the Covaris Focused-ultrasonicator

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ABSTRACT

The Broad Institute has developed a robust, cross-linked chromatin shearing protocol for yeast samples, to be used prior to immunoprecipitation. The protocol makes use of the Covaris E210 Focused-ultrasonicator that is high throughput and decreases the probability of contamination relative to the Branson Digital Sonifier.

INTRODUCTION

The distribution of sheared chromatin is critical to next generation library construction, as sample fragment size must be optimal for the sequencing technology being employed. For the current Illumina sequencing system, standard library construction sample sizes range from 200 to 700 base pairs. Many standard yeast ChIP protocols include instructions for using the Digitial Branson Sonifier to shear cross-linked yeast chromatin, after cell walls have been lysed. The Branson Digital Sonifier can only process one sample at a time. It utilizes a single probe that requires manual cleaning in between samples and can lead to sample contamination.

To increase throughput, decrease contamination risks, and improve shearing size distribution, a shearing time test was conducted on the Covaris E210 Focused-ultrasonicator. This instrument uses focused, acoustic bursts of ultrasonic energy to shear DNA in a sample vessel immersed in a water bath.

Formaldehyde fixation time was also a tested variable (methods), as formaldehyde treatment time can impact shearing size distribution. Over-fixing cells can lead to incomplete chromatin shearing and incomplete fixation can decrease the probability that the target protein will be cross- linked to the DNA. Figures 2 to 4 demonstrate that shearing and formaldehyde fixation time both affect chromatin fragmentation. Post immunoprecipitation, samples were assessed via quantitative qPCR, with primer sequences for the *S. cerevisiae* house keeping gene ACT1 as well as known Gal4 binding sites (Harbison et al., 2004). Each sample was assessed for fold enrichment relative to input, (Arocho, Chen, Ladanyi, & Pan, 2006).

MATERIALS AND METHODS

Formaldehyde fixation and Shearing time titration testing

ChIP protocol was performed as described by Lefrencois et al. in "The Guide to Yeast Genetics" (Weissman, Guthrie, & Fink, 2010, pages 81-86) with modifications to the cross-linked chromatin shearing. Briefly, 500 ml *S. cerevisiae* myc-tagged Gal4 cultures were grown to optical densities of 600 nm wavelength (OD600) between 0.6 and 0.8; media contained galactose to induce over expression of Gal4. Cells were then subject to a formaldehyde fixation and shearing time course study. Samples were fixed with formaldehyde for either 0, 5, 10 or 15 minutes with occasional shaking every 5 minutes. Cells were washed with 1 ml water and divided equally into two, 2 ml screw cap tubes. Cells were pelleted and supernatant was removed. 1 ml of 0.5 mm zirconia beads (BioSpec products) were added to the cell pellet. 500 µl sample lysis / IP buffer (50 mM Hepes / KOH [pH 7.5], 140 mM NaCL, 1 mM EDTA, 1% Triton X-100 and 0.1% sodium deoxycholate) with 1 mM PMSF (Fluka) and protease inhibitors (one tablet of Roche Complete protease inhibitor cocktail / 50 ml lysis / IP buffer) was added to cell pellet and glass beads. Samples were lysed in the FastPrep machine (MP Biomedical) five times at 60 second intervals in a cold room (samples sat on ice for 1 minute in between each treatment), at a speed of 6.0 m/s. Lysates were recovered in a 5 ml snap cap tube by piercing the 2 ml screw cap with a sterile, hot needle and centrifuging at 1500 rpm of 3 minutes. 500 µ of lysis /IP buffer was added to each screw cap tube and centrifuged again. Lysates from each of the two, 2 ml screw caps/sample were then combined in one 5 ml tube. The total volume in each 5 ml tube was brought to 4 ml with lysis / IP buffer 1 ml of each sample was aliquoted into four, 12x12, 1 ml glass tubes containing a fiber (Covaris, cat. number 520080; compatible tube rack, Covaris, cat. number 500276). Samples were then sheared on the Covaris E210 Focused-ultrasonicator for 8, 12, or 16 minutes using the following settings: Intensity = 8, Duty Factor = 20%, and Cycles per Burst = 200. For shearing settings on the S220 and E220 Focused-ultrasonicators, see Appendix A on the last page.

Post Shearing QC

Traditional post chromatin shearing quality control (QC) via agarose gel suggested in this protocol and others is not sufficient to accurately assess shifts in shearing size distributions that can greatly affect downstream processing of ChIP samples (Figure 9). To mitigate this, 40 µ of each sample lysate was reverse cross-linked, proteinase k treated and cleaned via Qiagen PCR minelute column. Samples were then run on DNA High sensitivity Bioanalyzer Chip (cat. no 5067-4626, Agilent); this greatly increased our ability to resolve sample size distribution and chose parameters that led to the majority of sample fragments between 200 to 700 bp (fixation time 15 minutes, shearing time 16 minutes or fixation time 10 minutes, shearing time 16 minutes).

Immunoprecipitation and Sequencing

Post shearing QC, samples were processed in accordance with protocol "Chromatin Immunoprecipitation" section 2.1 of the Guide to Yeast Genetics", steps 8 to 26. Samples underwent qPCR to evaluate fold enrichment of known Sfp1 binding sites and housekeeping genes, relative to "input" samples. Libraries were created which were compatible with Illumina's sequencing technology with the Broad's custom barcoded adapters. Samples were pooled in equal molar ratios and run on Illumina's Miseq. Peaks were the assessed via Integrated genomic viewer (IGV) (Robinson et al., 2011).

RESULTS

1. Formaldehyde fixation time course with 12 minute shearing

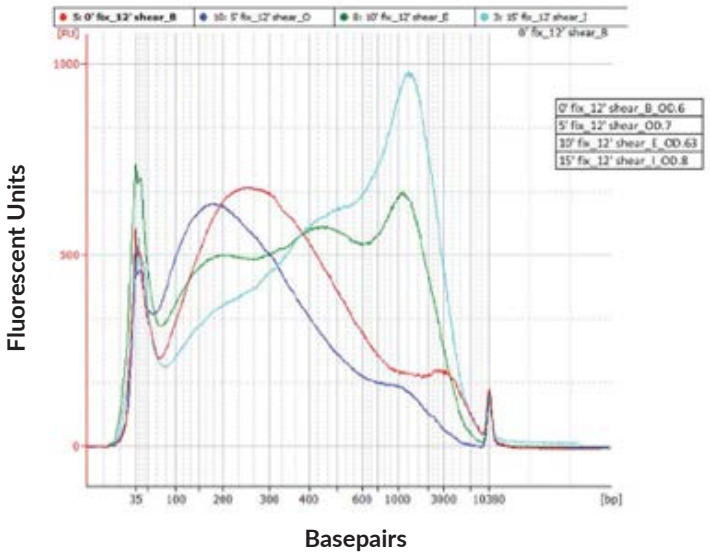

Figure 1. Sample shearing visualized via Agilent's Bioanalyzer. ChIP Samples were fixed for 0, 5, 10 or 15 minutes. All samples were then sheared via Covaris E210 (settings detailed in the Materials and Method section) for 12 minutes. The X-axis measures size in base pairs. The Y-axis represents Fluorescent Units, and this is quantitative measure of how much material is within a given size range. While Optical Density (OD) of each sample varied at the time of cell harvest, these electropherograms still provide relative quantities of fragments within a given distribution. It is clear that fixation time does play a role in sample size distribution that is independent of Covaris E210 shearing settings.

2. Formaldehyde fixation time course with 16 minute shearing

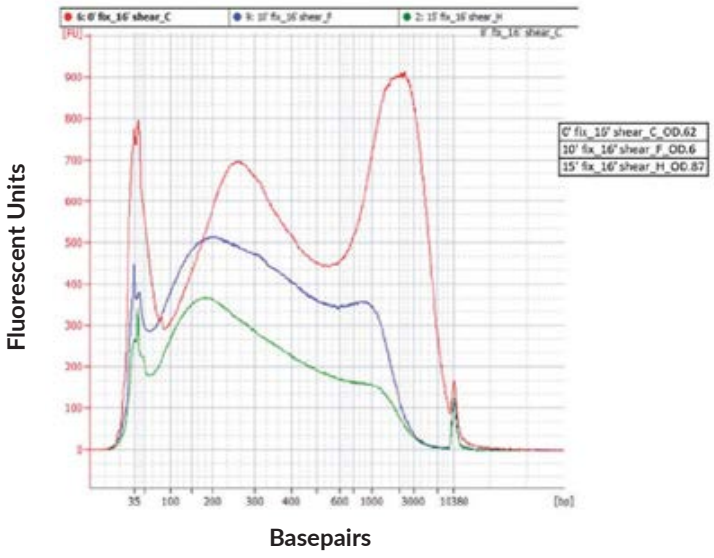

Figure 2. Varying formaldehyde treatment time & shearing for 16 minutes. Samples were fixed for 0, 10 or 15 minutes. All samples were then sheared on the Covaris E210 (settings detailed in Materials and Methods) for 16 minutes. The X-axis represents size in base pairs. The Y-axis represents fluorescent units (FU), and this is a quantitative measure of how much material is within a given size range. A shear time of 16 minutes and preferred formaldehyde fixation times of 10 or 15 minutes create optimal chromatin shearing distributions

3. Shearing time affects chromatin size distribution when fixation time remains constant

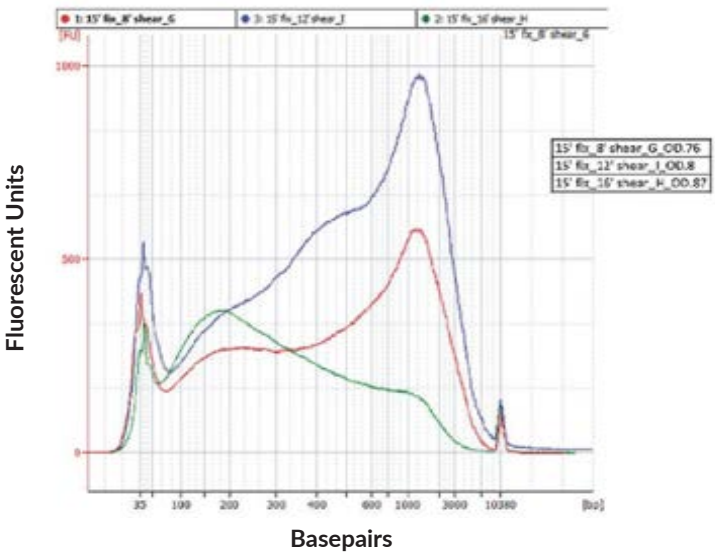

Figure 3. Total shearing time affects sample size distribution when cross-linking time remains constant. Samples were fixed for 15 minutes. Samples were then sheared via Covaris E210 (settings detailed in above) for 8, 12 or 16 minutes. The X-axis measures size in base pairs. The Y-axis represents Fluorescent Units, and this is quantitative measure of how much material is within a given size range.

4. Myc-GAL4 ChIP sample visualized in IGV

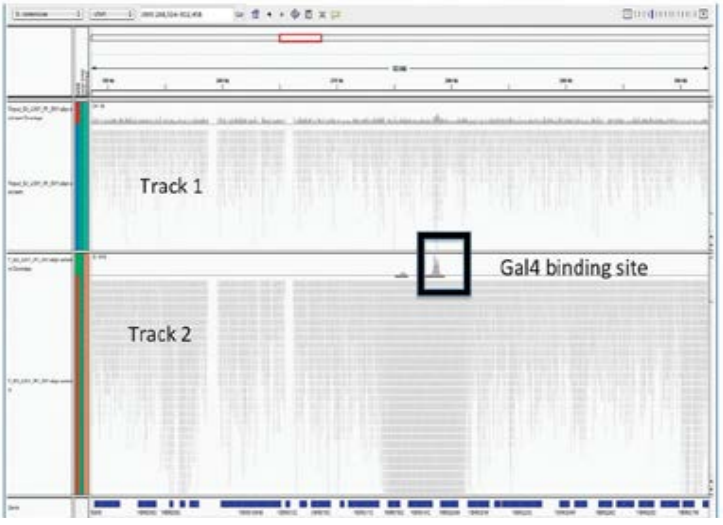

Figure 4. Myc-GAL4 ChIP sample visualized in IGV. Track 1 is "input" sample F ("input" is an aliquot of sample F was taken pre-antibody incubation). Track 2 is ChIP sample F (formaldehyde fixation time 10 minutes, shearing treatment time 16 minutes). There is extreme sequence enrichment at a chromosomal location, chrII:273,576..283,575, a predicted Myc-GAL4 binding site: (SGD, http://www. yeastgenome. org/cgi-bin/locus.fpl?locus=GAL4,]; accessed: 12/23/12) in the S. cerevisiae ChIP sample, relative to the "input" sample.

DISCUSSION

In conclusion, we've developed a reproducible and robust protocol for shearing cross-linked chromatin from yeast cells. The Covaris E210 is the only machine we've tested that has been able to consistently produce fragment sizes needed for library construction prior to Next Gen sequencing. The ability to shear 12 samples in one machine run versus one sample with the Branson Digitial Sonifier greatly enhances our ability to create ChIP Seq libraries. To obtain fragments between 200 to 500 bp, we've found that the optimal formaldehyde fixation time and shearing program/ treatment time is as follows: formaldehyde fixation time of 10 or 15 minutes with occasional shaking every 5 minutes. Shearing on the Covaris E210 instrument for 16 minutes (settings: 20% / 8 intensity / 200 cycles per burst / samples are sheared in 1 minute treatment time cycles) in 12 x 12, 1 ml glass tubes containing a fiber (Covaris, cat. number 520080) in compatible tube holder (Covaris, cat. number 500276).

ACKNOWLEDGEMENTS

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APPENDIX A

	S220 Focused-ultrasonicator	E220 Focused-ultrasonicator
Holder	Holder milliTUBE 1mL (PN: 500371)	Holder milliTUBE 1mL (PN: 500371)
Consumable	milliTUBE-1mL with AFA fiber (PN: 520130)	milliTUBE-1mL with AFA fiber (PN: 520130)
Sample Volume (mL)	1 mL	1 mL
PIP (w)	240w	240w
DF (%)	20%	20%
CPB	200	200
Temperature (c)	4	4
Treatment Time (min)	8 to 30	8 to 30

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