Cross-linked Yeast Chromatin shearing on the Covaris E210

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

Toni M. Delorey, Jenna M. Pfiffner, Dawn A. Thompson, Aviv Regev — Broad Institute, Cambridge, MA

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 200-700 base pairs. Many standard Yeast ChIP protocols include instructions for using the Digital 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 is utilizes a single probe that requires manual cleaning in between samples and thus 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 instrument. 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 2-4 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 Lefrancois 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, 2ml 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 ul 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 seconds 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 ul 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 ml with lysis/IP buffer. 1 ml of each sample was split 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 instrument for 8, 12, or 16 minutes (settings: 20% / 8 intensity/ 200 cycles per burst for 16 minutes; samples are sheared in one-minute cycles).

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 ul 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 200-700 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 8-26. 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).

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RESULTS

FIGURE 1: Formaldehyde fixation time course with 12 minute shearing

FIGURE 2: Formaldehyde fixation time course with 16 minute shearing

FIGURE 3: Shearing time affects chromatin size distribution when fixation time remains constant

FIGURE 4: Myc-GAL4 ChIP sample visualized in IGV.

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 Digital Sonifier greatly enhances our ability to create ChIP Seq libraries.

To obtain fragments between 200-500 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 12x12 , 1 ml glass tubes containing a fiber (Covaris, cat. number 520080) in compatible tube holder (Covaris, cat. number 500276).

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REFERENCES:


