Preparation of whole body *Caenorhabditis elegans* extracts for chromatin immunoprecipitation using the Covaris® S220 Focused-ultrasonicator

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INTRODUCTION

Caenorhabditis elegans is a non-parasitic transparent nematode, approximately 1 mm in length, found in garden soil rich in nutrients. Ever since its introduction by Sydney Brenner half a century ago, it has been widely used in research laboratories and has arisen as a powerful model organism to investigate various aspects of eukaryotic biology, owing to its genetic tractability, affordability, rapid generation time, ease of propagation, a well-defined cell lineage map, and a fully sequenced genome that contains a large number of human orthologues.

Recent breakthroughs in genome-wide techniques, including chromatin immunoprecipitation ChIP followed by deep sequencing (ChIP-seq), have considerably expanded the usefulness of this small organism in basic research, including investigations into how proteins interact with DNA to regulate gene expression and on the role of histone post-translational modifications. To successfully sequence ChIP libraries, it is important to ensure that the sample fragments are within the acceptable size distribution range (100-800 bp [1]). In this application note, we provide a step-by-step sample preparation protocol using the Covaris S220 that can be used by investigators looking to process whole body *C. elegans* extracts for ChIP-based applications.

MATERIALS AND METHODS

Formaldehyde Fixation

Approximately 100,000 to 500,000 synchronized larval or adult worms were collected from agar plates and washed twice with M9 buffer (22 mM KH_2PO_4, 34 mM K_2HPO_4, 86 mM NaCl, and 1 mM $MgSO_4$). Fixation was performed for 30 min at 20 °C in cross-linking solution (2% paraformaldehyde in M9 buffer), and excess formaldehyde was quenched with 0.1 M Tris-HCl pH 7.5. The pellet was then washed twice with M9 buffer at 4 °C and either frozen (-80 °C) or processed immediately.

Chromatin Shearing

The pellet was reconstituted in 1 mL of ice-cold RIPA buffer (10 mM Tris-HCl pH 8.0, 0.1% SDS, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM EDTA, 0.15 M NaCl) supplemented with Halt™ Protease Inhibitor Cocktail (Thermo Fisher Scientific). The suspension was then transferred to a Covaris milliTUBE 1 mL with AFA® fiber and shearing (range 100 to 800 bp [1]) was performed using the following settings in SonoLab7: Peak Incident Power (PIP) = 240W; Duty Factor = 20%; Cycles Per Burst (CPB) = 200; time = 480 seconds. After shearing, the crude extract was spun for 10 min at 10,000 x *g* and the supernatant was collected.

Immunoprecipitation and Sequencing

Protein concentration was determined by the Bradford method. Extract corresponding to 1 to 2.5 mg protein was diluted to 500 µL with ice-cold RIPA buffer supplemented with Halt™ Protease Inhibitor Cocktail (Thermo Fisher Scientific). 25 to 50 µL was removed for preparation of input DNA. To the rest, an appropriate amount of antibody specific for the protein of interest (typically 1 to 5 µg) was added and incubated at 4 °C overnight. 50 µl of Dynabeads™ Protein G suspension (Thermo Fisher Scientific) were added and the mixture was incubated at 4 °C for 1-3 h on rotation. Beads were recovered with a Dynal® Magnetic Particle Concentrator (Invitrogen) and washed at 4 °C with: 0.5 mL buffer TSE-150 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl), 2 X 0.5 mL buffer TSE-500 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 500 mM NaCl), 0.5 mL buffer TSE-1M (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 1 M NaCl), 0.5 mL buffer LiCl (250 mM LiCl, 1% NP40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH 8.1), 2 X 0.5 mL buffer TE (10 mM Tris pH 8.0, 1 mM EDTA). Antibody-bound chromatin was recovered in 2 X 0.2 mL elution buffer (1% SDS, 0.1 M $NaHCO_3$) by shaking at room temperature for 15 min. Elution buffer was added to input samples to a total volume of 0.4 mL. Input and ChIP chromatin samples were subsequently processed in parallel. After 20 µl of 5 M NaCl was added to each sample, cross-links were reversed overnight at 65 °C. 10 µl of 0.5 M EDTA, 20 µl of 1 M Tris-HCl pH 6.5 and 0.5 µl of 20 mg/mL Proteinase K (Thermo Fisher Scientific) were added and each sample was incubated for 1 h at 42 °C. DNA was recovered using the QIAquick PCR Purification Kit (Qiagen) in accordance with the manufacturer's instructions. Input and immunoprecipitated samples were subjected to library preparation with unique adapters using the TruSeq ChIP Library Preparation Kit (Illumina), pooled with samples corresponding to other independent ChIP-seq experiments, and run on a NextSeq 500 (Illumina) at the Boston University Medical Campus Microarray and Sequencing Resource facility on high output mode (75 cycles, ~400M reads/lane).

RESULTS

To establish a ChIP-Seq sample preparation workflow, we prepared whole body *C. elegans* extracts using the Covaris S220 Focused-ultrasonicator (***Figure 1***). After optimizing the shearing conditions, we prepared libraries for ChIP-qPCR and ChIP-Seq. Prior to sequencing, the size of the library was evaluated with the Agilent Bioanalyzer 2100 using the high sensitivity chip (***Figure 2***). As an initial evaluation, we used ChIP-qPCR to determine the relative abundance of H3 and the H3K79me2 levels in the *egl-30* and *pdk-1* gene promoter regions. Our previous work has shown that DOT-1.1 (the predominant H3K79 methyltransferase in *C. elegans*), together with zinc finger protein ZFP-1, have a global role in negatively modulating the level of polymerase II transcription on essential widely expressed genes [2]. The *pdk-1* and *egl-30* promoter regions are targeted by ZFP-1/DOT-1.1. Consistent with the predicted loss of histone methyltransferase activity of DOT-1.1, we show that the levels of H3K79me2 are decreased at the promoter regions upstream of *pdk-1* and *egl-30* in the *dot-1.1(lof)* strain compared with the wild-type strain (***Figure 3A***), whereas the total levels of H3 do not change (***Figure 3B***). Next, we evaluated the genome-wide distribution of LEM-2 by ChIP-seq. LEM-2 is a nuclear envelope protein that has previously been described to be involved in the anchoring of chromosome arms to the nuclear membrane [3]. In agreement, in our hands, visualization of the input-normalized coverage track of LEM-2 along the *C. elegans* chromosomes is indicative of its enrichment in chromosome arms, thereby reinforcing the idea that chromosome arms are anchored to the nuclear membrane via discontinuous association with LEM-2 (***Figure 4***).

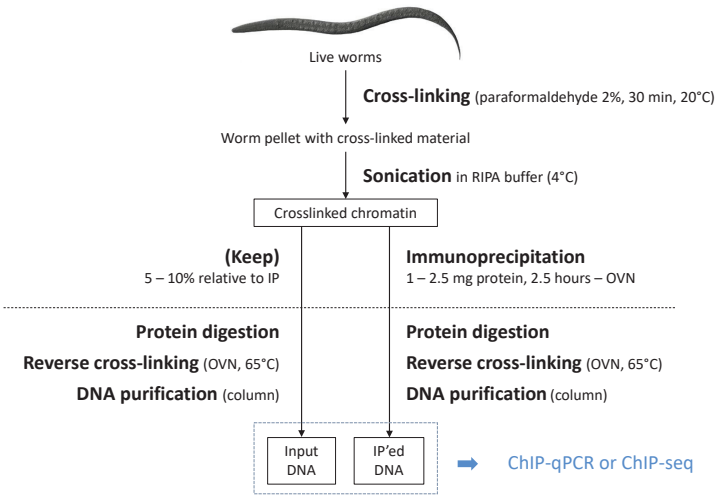

Figure 1. Overview of the *C. elegans* user-developed ChIP sample preparation workflow. An overview of the sample preparation workflow using the Covaris S220 for downstream sequencing applications such as ChIP-qPCR and ChIP-Seq is outlined here.

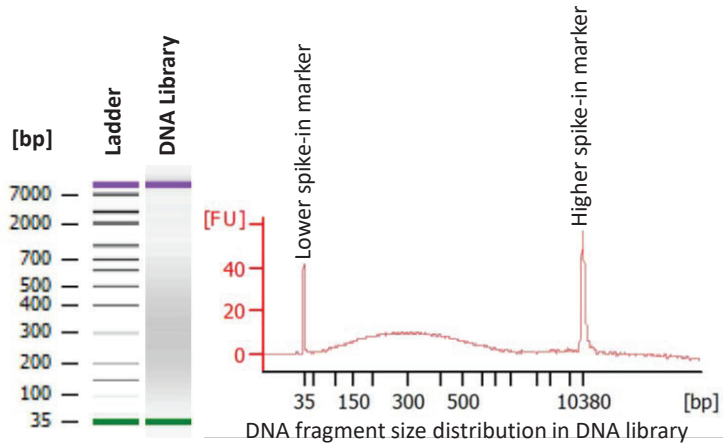

Figure 2. Analysis of fragment sizes in the DNA library. DNA fragment size analysis was measured by the Agilent 2100 Bioanalyzer using the High Sensitivity DNA Kit, as per manufacturer's protocol.

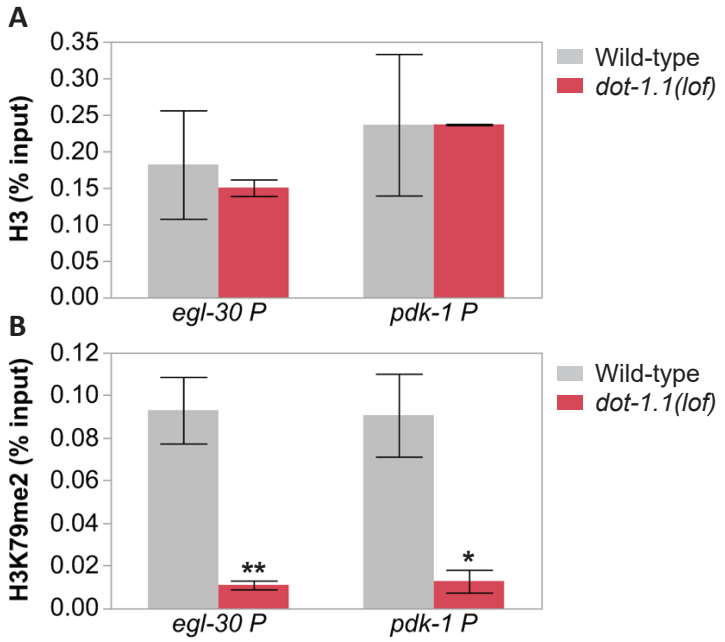

Figure 3. ChIP-qPCR analysis of abundance of H3 (A) and the H3K79me2 modification (B) in egl-30 and pdk-1 gene promoters. * and ** denote p-values less than 0.05 and 0.01, respectively (Student's t-test).

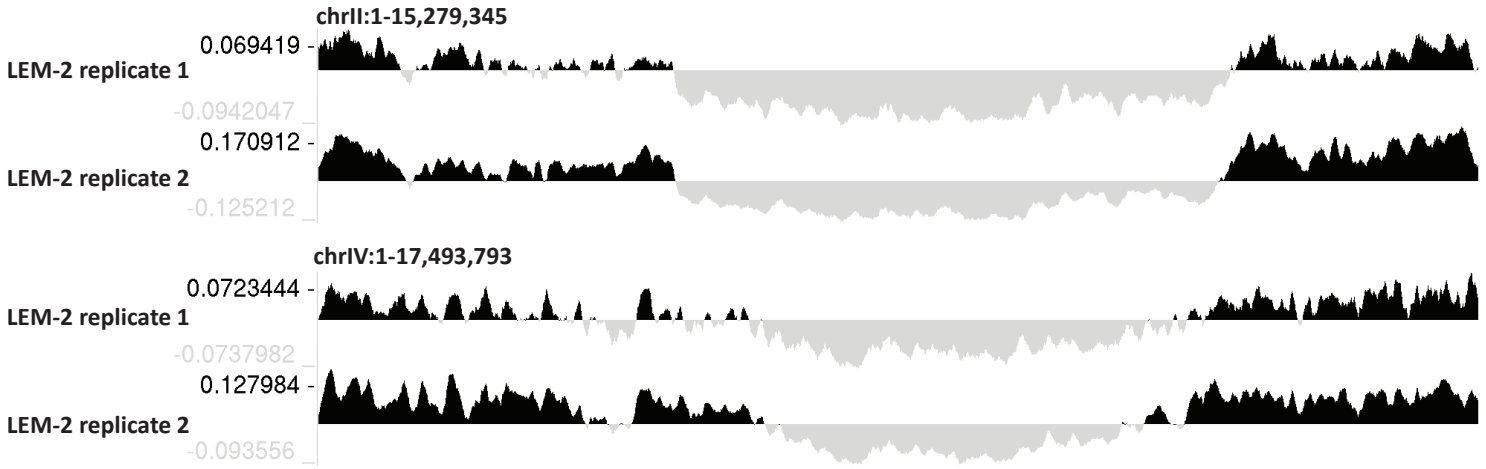

Figure 4. Genome browser snapshots of input-normalized coverage tracks for ChIP-seq of nuclear membrane protein LEM-2. 24 samples were multiplexed and approximately 20 million reads were obtained for each. LEM-2 is a nuclear envelope protein. ChIP-seq analysis in our lab is in agreement with a previous observation [3].

CONCLUSION

In summary, we have successfully developed a protocol for performing chromatin immunoprecipitation on whole body *C. elegans* extracts that can be employed using worms obtained at different developmental stages. The distribution of DNA fragment sizes in chromatin extracts obtained after ultrasonication, centrifugation to remove debris, and reverse cross-linking is similar to previously published workflows and considered acceptable for downstream immunoprecipitation and analysis by quantitative PCR or sequencing [1,4,5]. Importantly, chromatin shearing in our hands is consistent and yields reproducible results. Signal-to-noise ratio depends on the specificity of the application (*e.g.*, protein of interest and choice of antibody) and the contribution from the sonication step is considered minimal. We have transitioned from using a hand-held probe sonicator to the Covaris S220 Focused-ultrasonicator, which has greatly improved our workflow. Specifically, the key benefits of using this technology are: ease of use, fast operation, and consistent performance enabling more accurate and reproducible results.

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