HiChIP: Protein-centric chromatin conformation

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Method Article

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Abstract

Genome conformation is central to gene control but challenging to interrogate. Here we present HiChIP, a protein-centric chromatin conformation method. HiChIP improves the yield of conformation-informative reads by over 10-fold and lowers input requirement over 100-fold relative to ChIA-PET. HiChIP of cohesin reveals multi-scale genome architecture with greater signal to background than in situ Hi-C. Thus, HiChIP adds to the toolbox of 3D genome structure and regulation for diverse biomedical applications.

Introduction

See figure in Figures section.

Reagents

Hi-C Lysis Buffer: 10 mM Tris-HCl pH 7.5, 10 mM NaCl, 0.2% NP-40, 1X Roche protease inhibitors - 11697498001 Nuclear Lysis Buffer: 50 mM Tris-HCl pH 7.5, 10 mM EDTA, 1% SDS, 1X Roche protease inhibitors - 11697498001 ChIP Dilution Buffer: 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 7.5, 167 mM NaCl Low Salt Wash Buffer: 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 7.5, 150 mM NaCl High Salt Wash Buffer: 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 7.5, 500 mM NaCl LiCl Wash Buffer: 10 mM Tris-HCl pH 7.5, 250 mM LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, make fresh DNA Elution Buffer: 50 mM sodium bicarbonate pH 8.0, 1% SDS, make fresh Tween Wash Buffer: 5 mM Tris-HCl pH 7.5, 0.5 mM EDTA, 1 M NaCl, 0.05% Tween-20 Biotin Binding Buffer \(2X): 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 2M NaCl TD Buffer \(2X): 20 mM Tris-HCl pH 7.5, 10 mM magnesium chloride, 20% dimethylformamide

Procedure

Cell Crosslinking: 1. Pellet detached adherent or suspension cells and resuspend in freshly made 1% formaldehyde \(methanol free) at a volume of 1 mL of formaldehyde for every one million cells. 2. Incubate cells at room temperature for 10 minutes with rotation. 3. Add glycine to a final concentration of 125 mM to quench the formaldehyde, and then incubate at room temperature for 5 minutes with rotation. 4. Pellet cells, wash in PBS, pellet again, and then store in -80 or proceed into the HiChIP protocol. Lysis and Restriction Digest: 1. Resuspend up to 15 million crosslinked cells in 500 µL of ice-cold Hi-C Lysis Buffer and rotate at 4 for 30 minutes. For cell amounts greater than 15 million, split the pellet in half for contact generation and then recombine for the sonication. 2. Spin down at 2500 rcf for 5 minutes and discard the supernatant. 3. Wash pelleted nuclei once with 500 µL of ice-cold Hi-C Lysis Buffer. 4. Remove the supernatant and resuspend pellet in 100 µL of 0.5% SDS. 5. Incubate at 62 for 10 minutes and then add 285 µL of H2O and 50 µL of 10% Triton X-100 to quench the SDS. 6. Mix well and incubate at 37 for 15 minutes. 7. Add 50 µL of 10X NEB Buffer 2 and 375 U of MboI restriction enzyme \(NEB, R0147), and digest chromatin for 2 hours at 37 with rotation. For lower starting material, less restriction enzyme was used: 15 µL was used for 10-15 million cells, 8 µL for 5 million cells, and 4 µL for 1 million cells. 8. Heat

inactivate MboI at 62 for 20 minutes. Incorporation and Proximity Ligation: 1. To fill in the restriction fragment overhangs and mark the DNA ends with biotin, add 52 µL of fill-in master mix: 37.5 µL 0.4 mM biotin-dATP, 1.5 µL 10 mM dCTP, 1.5 µL 10 mM dGTP, 1.5 µL 10 mM dTTP 10 µL 5U/µL DNA Polymerase I, Large \(Klenow) Fragment \(NEB, M0210). 2. Mix and incubate at 37 for 1 hour with rotation. 3. Add 948 µL of ligation master mix: 150 µL 10X NEB T4 DNA ligase buffer with 10 mM ATP \(NEB, B0202), 125 µL 10% Triton X-100, 3 µL 50 mg/mL BSA, 10 µL 400 U/µL T4 DNA Ligase \(NEB, M0202), 660 µL Water. 4. Incubate at room temperature for 4 hours with rotation. 5. Pellet nuclei at 2500 rcf for 5 minutes and remove supernatant. Sonication: 1. Bring pellet up to 880 µL in Nuclear Lysis Buffer. 2. Transfer samples to a Covaris millitube and shear \(we use a Covaris E220) using the following parameters: Fill Level = 10, Duty Cycle = 5, PIP = 140, Cycles/Burst = 200, Time = 4 minutes. We kept the sonication constant at 4 minutes for different amounts of cell starting material, although sonication time may need to be adjusted for different cell types. The ideal sonication time will be as short as possible to allow for efficient ChIP signal over background. Too long of a sonication can lead to separation of the protein factor from the biotin contact, which will increase the likelihood of a DNA fragment not making it through both enrichments and a loss in sample complexity. Preclearing, Immunoprecipitation, IP Bead Capture, Washes: 1. Clarify sample for 15 minutes at 16100 rcf at 4 degrees. 2. Add 2X volume of ChIP Dilution Buffer \(split into two tubes of ~400 µL each and add 750 µL Dilution Buffer). For the Smc1a antibody \(Bethyl A300-055A), we dilute 1:2 in ChIP Dilution Buffer to achieve a SDS concentration of 0.33%. However, for other antibodies a lower SDS amount may be necessary, and lysate can be diluted up to 1:9 to achieve a SDS concentration of 0.1%. 3. Wash 60 µL of Protein A beads for every 10m cells in ChIP Dilution Buffer. Amounts of beads \(for preclearing and capture) and antibody should be adjusted linearly for different amounts of cell starting material. The amounts given are for Smc1a HiChIP; other factors may require different amounts or conditions. 4. Resuspend Protein A beads in 50 µL of Dilution Buffer per tube \(100 µL per HiChIP), add to sample and rotate at 4 for 1 hour. 5. Put samples on magnet and transfer supernatant into new tubes. 6. Add 7.5 ug of antibody for every 10m cells and incubate at 4 overnight with rotation. 7. Wash 60 µL of Protein A beads for every 10m cells in ChIP Dilution Buffer. 8. Resuspend Protein A beads in 50 µL of Dilution Buffer \(100 µL per HiChIP), add to sample and rotate at 4 for 2 hours. 9. Wash beads three times each with Low Salt Wash Buffer, High Salt Wash Buffer, and LiCl Wash Buffer. Washing was performed at room temperature on a magnet by adding 500 µL of a wash buffer, swishing the beads back and forth twice by moving the sample relative to the magnet, and then removing the supernatant. ChIP DNA Elution: 1. Resuspend ChIP sample beads in 100 µL of DNA Elution Buffer \(make fresh). 2. Incubate at RT for 10 minutes with rotation, followed by 3 minutes at 37 shaking. 3. For ChIP samples, place on magnet and remove supernatant to a fresh tube. Add another 100 µL of DNA Elution Buffer to ChIP samples and repeat incubations. 4. Remove ChIP samples supernatant again to the new tube. There should now be 200 µL of ChIP sample. 5. Add 10 µL of Proteinase K to each sample and incubate at 55 for 45 minutes with shaking. 6. Increase temperature to 67 and incubate for at least 1.5 hours with shaking. 7. Zymo purify the samples and elute in 10 µL of water. 8. Quantify post-ChIP DNA to estimate the amount of Tn5 needed to generate libraries at the correct size distribution. This is assuming contact libraries were generated properly, samples were not oversonicated, and material will robustly capture on streptavidin beads. For Smc1a HiChIP with 10m cells an expected yield of post-ChIP

DNA can be anywhere from 15 ng – 50 ng depending on the cell type. 9. For libraries with greater than 150 ng of post-ChIP DNA, set aside material and take a maximum of 150 ng into the biotin capture step. Biotin Pull-Down and Preparation for Illumina Sequencing: 1. Prepare for biotin pull-down by washing 5 µL of Streptavidin C-1 beads with Tween Wash Buffer. 2. Resuspend the beads in 10 µL of 2X Biotin Binding Buffer and add to the samples. Incubate at room temperature for 15 minutes with rotation. 3. Separate on a magnet and discard the supernatant. 4. Wash the beads twice by adding 500 µL of Tween Wash Buffer and incubating at 55 for 2 minutes shaking. 5. Wash the beads in 100 µL of 1X \(from 2X) TD Buffer 6. Resuspend beads in 25 µL of 2X TD Buffer, the appropriate amount of Tn5 for your material amount \(2.5 µL for 50 ng of post-ChIP DNA), and water to 50 µL. Adjust Tn5 amount linearly for different amounts of post-ChIP DNA, with a maximum amount of 4 µL of Tn5. For example for 25 ng of DNA transpose using 1.25 µL of Tn5, while for 125 ng of DNA transpose with 4 µL of Tn5. Using the correct amount of Tn5 is critical to the HiChIP protocol to achieve an ideal size distribution. An overtransposed sample will have shorter fragments and will exhibit lower alignment rates \(when the junction is close to a fragment end). An undertransposed sample will have fragments that are too large to cluster properly on an Illumina sequencer. A maximum amount of Tn5 is used in order to save on Tn5 costs, and considering that a library with this much material will be amplified in 5 cycles and have enough complexity to be sequenced deeply regardless of how fully transposed the library is to achieve an ideal size distribution. 7. Incubate at 55 with interval shaking for 10 minutes. 8. Place samples on magnet and remove supernatant. 9. Add 50 mM EDTA to samples and incubate at 50 for 30 minutes, then quickly place on magnet and remove supernatant. 10. Wash samples twice with 50 mM EDTA at 50 for 3 minutes, removing quickly on magnet. 11. Wash samples twice in Tween Wash Buffer at 55 for 2 minutes, removing quickly on magnet. 12. Wash samples in 10 mM Tris. PCR and Post-PCR Size Selection: 1. Resuspend beads in 50 µL of PCR master mix: 25 µL Phusion HF 2X, 1 µL Nextera Ad1.1 \(Universal) 12.5 uM, 1 µL Nextera Ad2.x \(Barcoded) 12.5 uM, 23 µL Water. 2. Run the following PCR program: 5 minutes at 72C, 1 minute at 98C, then cycle 15 seconds at 98C, 30 seconds at 63C, and 1 minute at 72C. Cycle number can be estimated using one of two different methods: \(1) First run 5 cycles on a regular PCR and then remove from beads. Add 0.25X SYBR green and then run on a qPCR and pull out samples at the beginning of exponential amplification. \(2) Run reactions on a PCR and estimate cycle number based on the amount of material from the post-ChIP Qubit \(greater than 50 ng was ran in five cycles, while approximately 50 ng was ran in six, 25 ng was ran in seven, 12.5 ng was ran in eight, etc.). 3. Zymo purify and elute in the same 10 µL of water. PAGE purify libraries \(we use 6%) from a size range of 300 – 700. Note that if the bulk of the material is smaller those sizes can be included but the paired-end libraries will have a lower alignment rate. In the future Tn5 amount should be adjusted accordingly. 4. Instead of PAGE, a two-sided size selection with Ampure XP beads can be performed. After PCR place libraries on a magnet and elute into new tubes. Then add 25 µL of Ampure XP beads and keep the supernatant to capture fragments less than 700 bp. Transfer supernatant to a new tube and add 15 µL of fresh beads to capture fragments greater than 300 bp. 5. Final elution \(from either PAGE purification or Ampure XP) is in 10 µL of water. Quantify libraries with qPCR against Illumina primers and/or Bioanalyzer.

Figures

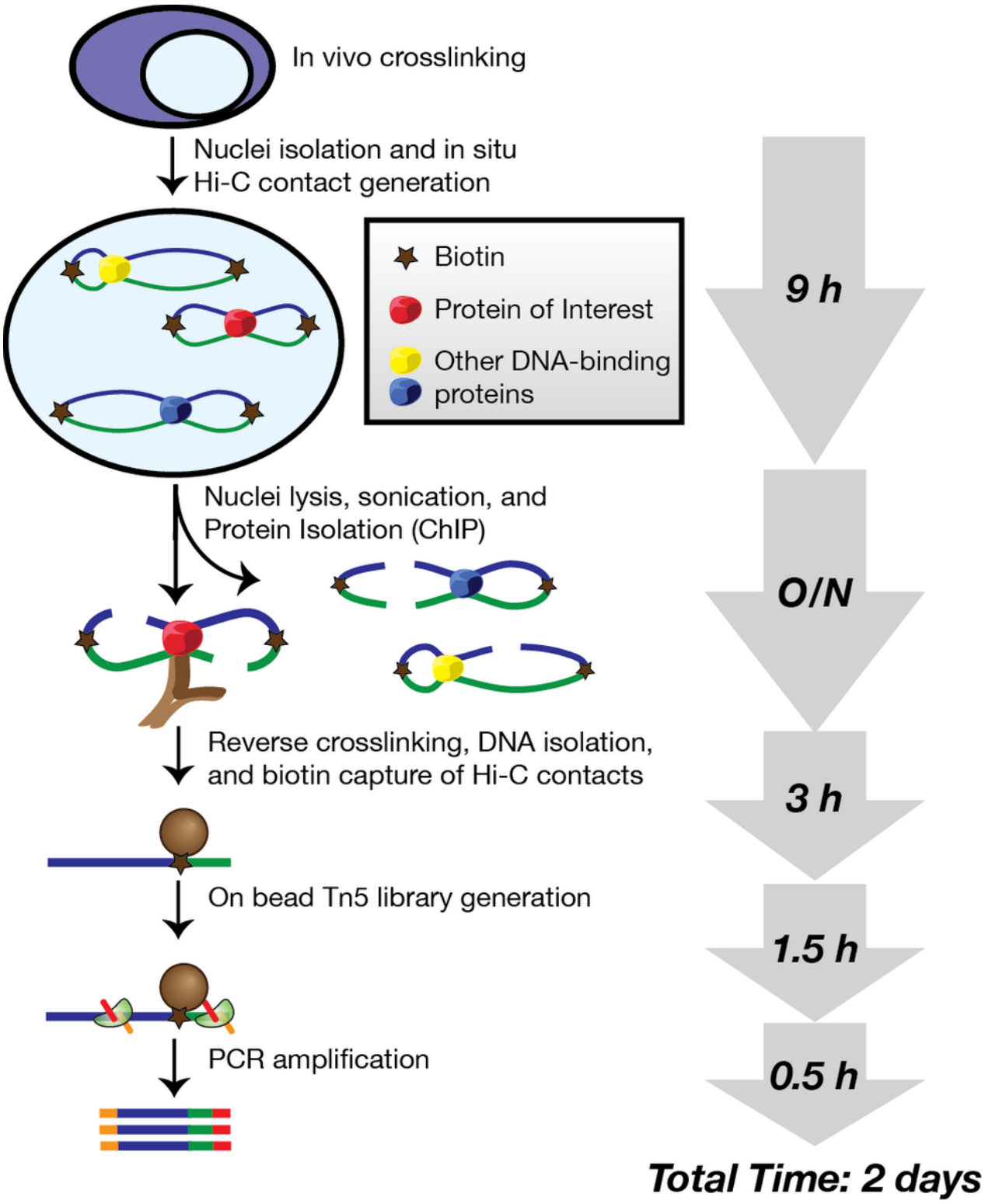

Figure 1

HiChIP Method HiChIP Method

Supplementary Files

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- supplement0.xlsx