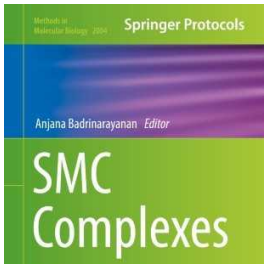
Sep 22, 2021

Cell Lysis and Sonication



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DOI
dx.doi.org/10.17504/protocols.io.bn3pmgmn

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DOI: https://dx.doi.org/10.17504/protocols.io.bn3pmgmn

External link: https://link.springer.com/protocol/10.1007/978-1-4939-9520-2_10

Protocol Citation: Vasso Makrantoni, Daniel Robertson, Adele L. Marston 2021. Cell Lysis and Sonication. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.bn3pmgmn**

Manuscript citation:
Makrantoni V., Robertson D., Marston A.L. (2019) Analysis of the Chromosomal Localization of Yeast SMC Complexes by Chromatin Immunoprecipitation. In: Badrinarayanan A. (eds) SMC Complexes. Methods in Molecular Biology, vol 2004. Humana, New York, NY. https://doi.org/10.1007/978-1-4939-9520-2_10

Protocol status: Working

Created: October 28, 2020

Last Modified: September 22, 2021

Protocol Integer ID: 43855

Keywords: Chromatin immunoprecipitation, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Cohesin, Condensin, Mitosis, Meiosis, Scc1, Rec8, Brn1 , seq from yeast cell, chromatin immunoprecipitation, native chromatin environment, chromatin environment, chromatin, chip assay, library preparation for chip, entire genome, gene transcription, dna recombination, genome, dna interaction, yeast cell, investigating protein, dna replication, dna, interest across the entire genome, step protocol for chip, chip, chromosome segregation, biological processes like gene transcription, cell lysi, protein, gene, yeast, cell, immunoprecipitation,

Abstract

A plethora of biological processes like gene transcription, DNA replication, DNA recombination, and chromosome segregation are mediated through protein–DNA interactions. A powerful method for investigating proteins within a native chromatin environment in the cell is chromatin immunoprecipitation (ChIP). Combined with the recent technological advancement in next generation sequencing, the ChIP assay can map the exact binding sites of a protein of interest across the entire genome. Here we describe a-step-by step protocol for ChIP followed by library preparation for ChIP-seq from yeast cells.

Guidelines

Chromatin immunoprecipitation (ChIP) is broadly used to study chromatin dynamics. Changes in occupancy of chromosomal proteins at specific loci within the genome can be measured by using ChIP-qPCR. However, this technique is costly and time consuming with high variability per experiment. Alternatively, ChIP-seq can be used to measure differences in a protein's occupancy genome wide. Finally, calibrated ChIP-seq is essential when measuring changes in occupancy between different experimental samples.

Here we describe an optimized ChIP protocol for yeast SMC proteins that can be completed within 3 days for samples analyzed by qPCR and 4 days for samples to be further processed by calibrated deep sequencing. The protocol encompasses five distinct steps: cross-linking and cell harvesting; cell lysis and sonication; immunoprecipitation, decross-linking and DNA extraction and finally determination of the size and DNA concentration of sonicated samples. These five steps are outlined here.

Materials

Yeast Strains and Growth Material:

1. Haploid *S. cerevisiae* strains of w303 background we have used include: (a) no tag control (AM1176), (b) *SCC1-6HA* (AM1145), (c) *BRN1-6HA* (AM5708), (d) *SCC2-6HIS-3FLAG* (AM6006), and (e) *SCC1-6HA pMET3-CDC20* (AM1105) as previously described [9,10,11,12].
2. For studies of protein occupancy during meiosis we have used diploid *S. cerevisiae* strains of SK1 background including (a) *REC8-3HA ndt80*Δ (AM4015), as previously described [13] and (b) *REC8-6HIS-3FLAG* (AM11000).
3. Haploid *S. pombe* strains used for calibration are: (a) *RAD21-3HA* (spAM76), (b) *RAD21-6HA* (spAM635), (c) *RAD21-6HIS-3FLAG* (spAM1863), or (d) *CND2-6HA* (spAM1862).
4. YPDA media: 1% yeast extract, 2% peptone, 2% glucose.
5. YPG agar plates: 1% yeast extract, 2% peptone, 2.5% glycerol, 2% agar.
6. YPDA4% agar plates: 1% yeast extract, 2% peptone, 4% glucose, 2% agar.
7. BYTA media: 1% yeast extract, 2% Bacto tryptone, 1% potassium acetate, 50 mM potassium phthalate.
8. SPO media: 0.3% potassium acetate, pH 7.0.
9. YES media: 0.5% yeast extract, 3% glucose, 225 mg/L supplements.

Equipment and Reagents:

1. 37% formaldehyde solution for molecular biology.
2. 2.5 M glycine: Dissolve 93.8 g glycine in ddH_2O (may require gentle heating) and bring up to 500 ml with ddH_2O.
3. Diluent buffer: 0.143 M NaCl, 1.43 mM EDTA, 71.43 mM Hepes–KOH pH 7.5.
4. TBS buffer: 20 mM Tris–HCl pH 7.5, 150 mM NaCl.
5. 2× FA lysis buffer: 100 mM Hepes–KOH pH 7.5, 300 mM NaCl, 2 mM EDTA, 2% Triton X-100, 0.2% Na-deoxycholate.
6. FastPrep screw-cap tubes.
7. 100 mM PMSF.
8. Protease inhibitor tablets Complete EDTA free.
9. Zirconia/Silica beads 0.5 mm diameter.
10. FastPrep-24 5G Homogenizer.
11. Bioruptor Twin.
12. Dynabeads Protein G.
13. Magnetic rack.
14. ChIP Wash buffer 1—low salt: 1× FA lysis buffer, 0.1%SDS, 275 mM NaCl.
15. ChIP Wash buffer 2—high salt: 1× FA lysis buffer, 0.1%SDS, 500 mM NaCl.
16. ChIP Wash buffer 3: 10 mM Tris–HCl pH 8.0, 0.25 M LiCl, 1 mM EDTA, 0.5% NP-40. 0.5% Na-deoxycholate.
17. ChIP Wash buffer 4 (TE): 10 mM Tris–HCl pH 8.0, 1 mM EDTA.
18. Chelex 100 Resin.
19. 10 mg/ml Proteinase K
20. TES buffer: 50 mM Tris–HCl pH 7.5, 10 mM EDTA, 1% SDS.
21. Nuclease-free molecular biology grade water.
22. Filter tips.
23. Luna Universal Probe qPCR Master Mix.

24. LightCycler 480 Multiwell Plate 96.
25. LightCycler real-time PCR.
26. Qiagen purification kit.
27. LoBind DNA microcentrifuge tubes.
28. Quick blunting kit.
29. AMPure XP beads.
30. Klenow 3′ to 5′ exo minus.
31. Quick ligation kit (T4 DNA ligase).
32. NEXTflex DNA Barcodes—12 (Bioo Scientific; #NOVA-514102).
33. Phusion High-Fidelity DNA polymerase.
34. DynaMag-PCR magnet.
35. WizardSV Gel and PCR cleanup system.
36. Qubit dsDNA-HS Assay kit (Invitrogen).
37. Qubit Fluorometric Quantitation machine.
38. Agilent 2100 Bioanalyzer system.
39. High Sensitivity DNA Reagents kit (Agilent Technologies).
40. High Sensitivity DNA Chips (Agilent Technologies).
41. MiniSeq High throughput Reagent Kit (150-cycle) (Illumina).
42. Illumina Mini-seq.

Troubleshooting

Safety warnings

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Formaldehyde and PMSF are toxic if inhaled, ingested or absorbed through the skin. Always wear a lab coat and gloves, and work in a chemical hood.

Cell Lysis and Sonication

32m 30s

1 Thaw cells On ice . Add 1 volume (0.3 mL – 0.5 mL ice-cold 1× FA lysis buffer) supplemented with 0.5% SDS, [M] 1 millimolar (mM) PMSF and protease inhibitors (Roche Complete EDTA-free tablet).

Note

For calibrated ChIP-seq use a 2:1 ratio of S. cerevisiae to S. pombe cells, as measured by OD600, mix pellets of different organisms in a single fastprep tube and lyse together as previously described [8]. Use the same batch of S. pombe in all samples of the same experiment. Perform each IP individually and pool samples together after the final wash step by combining beads from multiple IPs in the same 200 µl of TES elution buffer (see Step 4 in protocol "Immunoprecipitation, Decross-linking, and DNA Extraction"). Both calibration and experimental genomes need to express proteins tagged with the same epitope for immunoprecipitation and the calibration organism needs to be sufficiently similar that the ChIP protocol works for both.

2 Add an equal volume (200 µL – 250 µL 0.5-mm Zirconium Silicate beads) and lyse cells in a FastPrep-24 Homogenizer at 4 °C for 00:00:30 (homogenization speed 6.5). Leave On ice for 00:10:00 . 10m 30s

2.1 Repeat this twice more: Add an equal volume (200 µL – 250 µL 0.5-mm Zirconium Silicate beads) and lyse cells in a FastPrep-24 Homogenizer at 4 °C for 00:00:30 (homogenization speed 6.5). Leave On ice for 00:10:00 .
(1/2) 10m 30s

2.2 Add an equal volume (200 µL – 250 µL 0.5-mm Zirconium Silicate beads) and lyse cells in a FastPrep-24 Homogenizer at 4 °C for 00:00:30 (homogenization speed 6.5). Leave On ice for 00:10:00 .
(2/2) 10m 30s

3 Place samples On ice .

4 Dry the outside of the tubes, invert and puncture the tube bottom with a red flamed (under a Bunsen burner) 25G × 5/8″ needle.

5 Immediately place the fastprep tube within a chilled 15 ml conical Falcon tube already containing an empty fastprep tube and centrifuge at 1250 x g, 4°C, 00:03:00 .

6 Transfer the entire lysate (both pellet and supernatant) to a prechilled 1.5 ml Eppendorf tube.
Centrifuge at 16000 x g, 4°C, 00:15:00 .

7 Remove the supernatant by vacuum aspiration and resuspend the pellets thoroughly in 1 mL ice-cold 1× FA buffer supplemented with 0.1% SDS, [M] 1 millimolar (mM) PMSF and protease inhibitors.

8 Centrifuge at 16000 x g, 4°C, 00:15:00 . Discard supernatant.

Note

Notice the presence of a pellet with a glass-like layer. This is the cross-linked chromatin.

9 Resuspend washed chromatin pellets well in 0.3 mL ice-cold 1× FA buffer containing 0.1% SDS, [M] 1 millimolar (mM) PMSF and protease inhibitors.

10 Shear the chromatin by sonication, using a Bioruptor Twin with circulating water bath at a temperature of **less than** 5 °C and power settings: High, 00:00:30 ON / 00:00:30 OFF , 30 cycles. 1m

Note

Sonication conditions must be determined empirically for each cell type, and sonicator model; the optimal average DNA fragment size is below 0.5 kb. Overfragmentation of chromatin is not recommended as it can damage the protein epitope targeted by the antibody of choice. If the Diagenode water bath sonicator is not available, a probe sonicator or Covaris instrument can also be used. Sonication time and intensity will need to be optimized and DNA fragment size determined as in protocol "Determine the Size of Sonicated Samples and the DNA Concentration".

11 Centrifuge the sonicated mixture at 16000 x g, 4°C, 00:15:00 to remove cell debris and transfer the supernatant into a new cold Eppendorf tube containing

1 mL 1× FA lysis buffer with 0.1% SDS, [M] 1 millimolar (mM) PMSF, protease inhibitors.

12 Mix by inversion and centrifuge at 16000 x g, 4°C, 00:15:00.

13 Carefully transfer the supernatant into a new, cold Eppendorf tube. This chromatin preparation will be used for the immunoprecipitation in protocol "Immunoprecipitation, Decross-linking, and DNA Extraction".

Note

The amount of SDS in immunoprecipitation can interfere with antibody binding efficiency therefore, lower amount of SDS can also be used. Either omit SDS in 2xFA buffer or reduce SDS added afterward to a final concentration of 0.05% before chromatin immunoprecipitation.

14 Store 10 µL supernatant at -20 °C. This will be the “Input” sample.

15 Use 100 µL chromatin preparation (from step 13) to determine fragment size as determined in protocol "Determine the Size of Sonicated Samples and the DNA Concentration".