

Cross-Linking and Cell Harvesting

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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.

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COLLECTIONS ⓘ



Analysis of the Chromosomal Localization of Yeast SMC Complexes by Chromatin Immunoprecipitation

KEYWORDS

Chromatin immunoprecipitation, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, Cohesin, Condensin, Mitosis, Meiosis, Scc1, Rec8, Brn1

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[Analysis of the Chromosomal Localization of Yeast SMC Complexes by Chromatin Immunoprecipitation](#)

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.

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 ndt80Δ* (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 ddH₂O (may require gentle heating) and bring up to 500 ml with ddH₂O.
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.

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.

BEFORE STARTING

1. For ChIP-qPCR:

- For cohesin subunit, **Scc1**, in cycling cells, harvest 50 ml yeast cells of density 0.3–0.6 OD₆₀₀ grown in YPDA media.

Alternatively, cells can be arrested in mitosis either by treatment with nocodazole or by depletion of Cdc20, as described in protocol "Growth Conditions for SMC Proteins".

- For the less abundant cohesin loader subunit **Scc2** and condensin subunit **Brn1** harvest 100 ml yeast cells of density 0.3–0.6 OD₆₀₀ grown in YPDA.
- For the meiotic counterpart of cohesin, **Rec8**, harvest 50 ml yeast cells of density 1.8 OD₆₀₀ grown in SPO media.

2. For ChIP-seq:

- Grow 2× the amount of cell culture of yeast cells of density 0.3–0.6 OD₆₀₀ in YPDA media (100 ml **Scc1**, 200 ml **Scc2** and **Brn1**) or 1.8 OD₆₀₀ in SPO media (100 ml **Rec1**) and process each 50 ml sample individually.

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.

Cross-Linking and Cell Harvesting

5m

1



Cross-link cells by adding **5 mL 11% formaldehyde in diluent buffer** to give a final concentration of 1% formaldehyde in the culture.

2

Gently rotate on an orbital shaker at **90 rpm, Room temperature, 00:30:00 with 1.8 cm orbit** for Scc1, Rec8, Scc2, and Brn1.

The cross-linking time and formaldehyde concentration can affect both the efficiency of chromatin shearing and of antigen precipitation. Shorter cross-linking times (5–10 min), lower formaldehyde concentrations (1%, wt/vol), or both, may improve shearing efficiency. However, for some proteins, especially those that do not directly bind DNA, this might reduce the efficiency of cross-linking and thus the yield of precipitated chromatin. It is advisable to perform a cross-linking time course to determine optimal fixation conditions.

In vivo cross-linking for ChIP is traditionally achieved with formaldehyde; however, formaldehyde is a short spacer arm cross-linker (2 Å), limiting its functionality. For higher order interactions, longer cross-linkers such as EGS (16.1 Å) or DSG (7.7 Å) or combination of cross-linkers can be used so to more efficiently trap larger protein

complexes with complex quaternary structure [22].

3 

To quench cross-linking, add glycine at a final concentration of **[M]125 Milimolar (mM)** .

Addition of glycine to quench the formaldehyde is particularly important when harvesting large volumes of cell culture as the harvesting process can be long and thereby can increase fixation time between samples.

4 

5m

Incubate with gentle shaking for **⌚00:05:00** at **🌡Room temperature** .

5 

Collect cells by centrifugation at **🌀1800 x g, 4°C** .

6 

Wash cells twice in 10 ml ice-cold TBS buffer and once in 10 ml ice-cold 1× FA lysis buffer supplemented with 0.1% SDS:

6.1 

Wash cells in **📄10 mL ice-cold TBS buffer** . (1/2)

6.2 

Wash cells in **📄10 mL ice-cold TBS buffer** . (2/2)

6.3 

Wash with **📄10 mL ice-cold 1× FA lysis buffer supplemented with 0.1% SDS** .

7 

Collect cells by centrifugation at **🌀1800 x g, 4°C** .

8



Carefully aspirate the supernatant and snap freeze pellets in liquid nitrogen in fastprep screw-cap tubes. Store the pellets at **-80 °C** until ready to use.