

Growth Conditions for SMC Proteins

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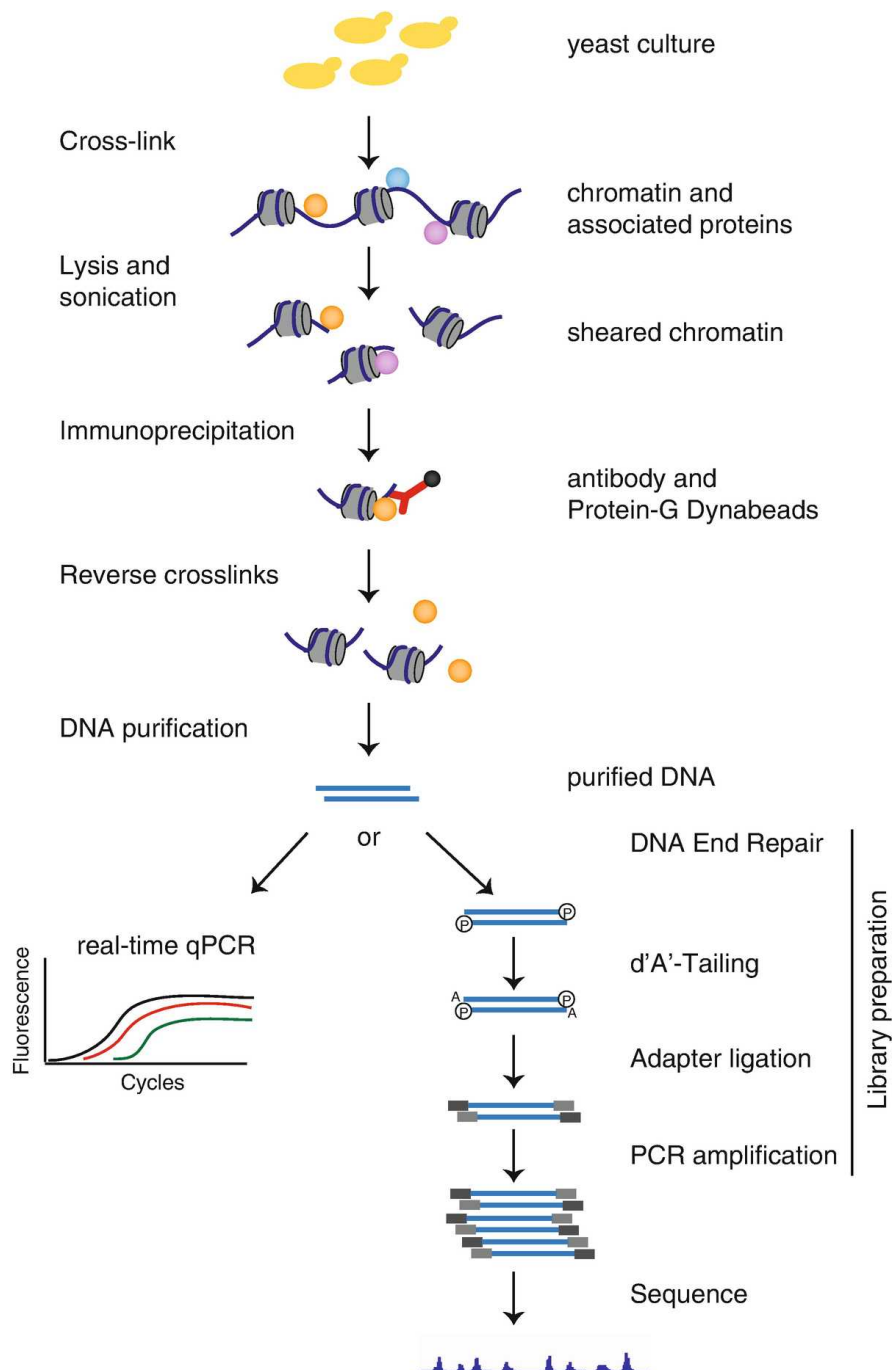
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ABSTRACT

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.

Chromatin immunoprecipitation (ChIP) is a powerful method for assaying protein–DNA binding *in vivo* and is broadly used to estimate the density of DNA-bound proteins at specific sites in the genome. ChIP is a multistep assay and every step needs to be optimized for consistent results. Briefly, protein–DNA associations are immobilized by cross-linking with formaldehyde [1,2,3] before shearing the chromatin, either mechanically [4] or by enzymatic digestion [5] into DNA fragments of average size 200–500 bp. Specific cross-linked protein–DNA complexes are then isolated by immunoprecipitation using an antibody to the protein of interest. Finally, the cross-links are reversed, and the retrieved DNA is analyzed to determine the sequences bound by the protein. ChIP followed by quantitative real-time PCR (ChIP-qPCR), using specific primers, can be used to measure protein association and relative abundance at a particular genomic locus. Alternatively, ChIP can be combined with next generation sequencing (ChIP-seq) to provide a genome-wide view of protein occupancy. While ChIP-seq allows for relative protein abundance at distinct chromosomal addresses to be compared within a sample, differences between samples cannot be quantified without introducing a method to normalize. Typically, this involves “spike in” of a known amount of DNA or cross-linked cells from a different species, with sufficient sequence divergence from the organism of interest to allow sequencing reads to be confidently distinguished bioinformatically [6,7,8]. This technique, referred to as calibrated ChIP-seq, makes it possible to quantitate genome-wide changes in the distribution of an epitope tagged protein and allows for quantification of differences in occupancy between experimental samples [8]. Calibrated ChIP-seq requires that both calibration and experimental organisms carry the same epitope tag and can be immunoprecipitated by the same protocol. For this protocol we use *S. pombe* to calibrate *S. cerevisiae*, a combination that also allows us to invert the roles, that is, calibrate *S. pombe* with *S. cerevisiae*.

The ChIP method described here has been optimized for use with chromatin from two species of yeast, *S. cerevisiae* and *S. pombe*; however, it should be easy to adapt it for use with other chromatin sources. To demonstrate the robustness of our ChIP and library preparation protocols we performed ChIP against the Scc1 subunit of the cohesin multiprotein complex, tagged with the 6HA epitope [9,10,11]. We have also used a similar protocol for the condensin subunit Brn1 [12] and for the meiotic counterpart of cohesin, Rec8 [13]. Here, we outline in detail an optimized protocol for cross-linking and harvesting cells, fragmenting chromatin, immunoprecipitating the desired protein–DNA complexes, and preparing the library for sequencing on the Illumina MiniSeq platform. A schematic stepwise representation of the method is illustrated in Fig. 1.



References:

1. Solomon MJ, Varshavsky A (1985) Formaldehyde-mediated DNA-protein crosslinking: a probe for in vivo chromatin structures. *Proc Natl Acad Sci U S A* 82(19):6470–6474
2. Solomon MJ, Larsen PL, Varshavsky A (1988) Mapping protein-DNA interactions in vivo with formaldehyde: evidence that histone H4 is retained on a highly transcribed gene. *Cell* 53(6):937–947
3. Gilmour DS, Lis JT (1984) Detecting protein-DNA interactions in vivo: distribution of RNA polymerase on specific bacterial genes. *Proc Natl Acad Sci U S A* 81(14):4275–4279
4. Kuo MH, Allis CD (1999) In vivo cross-linking and immunoprecipitation for studying dynamic protein:DNA associations in a chromatin environment. *Methods* 19(3):425–433. <https://doi.org/10.1006/meth.1999.0879>
5. Thorne AW, Myers FA, Hebbes TR (2004) Native chromatin immunoprecipitation. *Methods Mol Biol* 287:21–44. <https://doi.org/10.1385/1-59259-828-5:021>
6. Bonhoure N, Bounova G, Bernasconi D, Praz V, Lammers F, Canella D, Willis IM, Herr W, Hernandez N, Delorenzi M, Cyli XC (2014) Quantifying ChIP-seq data: a spiking method providing an internal reference for sample-to-

- sample normalization. *Genome Res* 24(7):1157–1168. <https://doi.org/10.1101/gr.168260.113>
7. Orlando DA, Chen MW, Brown VE, Solanki S, Choi YJ, Olson ER, Fritz CC, Bradner JE, Guenther MG (2014) Quantitative ChIP-Seq normalization reveals global modulation of the epigenome. *Cell Rep* 9(3):1163–1170. <https://doi.org/10.1016/j.celrep.2014.10.018>
 8. Hu B, Petela N, Kurze A, Chan KL, Chapard C, Nasmyth K (2015) Biological chromodynamics: a general method for measuring protein occupancy across the genome by calibrating ChIP-seq. *Nucleic Acids Res* 43(20):e132. <https://doi.org/10.1093/nar/gkv670>
 9. Fernius J, Marston AL (2009) Establishment of cohesion at the pericentromere by the Ctf19 kinetochore subcomplex and the replication fork-associated factor, Csm3. *PLoS Genet* 5(9):e1000629. <https://doi.org/10.1371/journal.pgen.1000629>
 10. Fernius J, Nerusheva OO, Galander S, Alves Fde L, Rappsilber J, Marston AL (2013) Cohesin-dependent association of scc2/4 with the centromere initiates pericentromeric cohesion establishment. *Curr Biol* 23(7):599–606. <https://doi.org/10.1016/j.cub.2013.02.022>
 11. Hinshaw SM, Makrantonis V, Kerr A, Marston AL, Harrison SC (2015) Structural evidence for Scc4-dependent localization of cohesin loading. *elife* 4:e06057. <https://doi.org/10.7554/eLife.06057>
 12. Verzijlbergen KF, Nerusheva OO, Kelly D, Kerr A, Clift D, de Lima Alves F, Rappsilber J, Marston AL (2014) Shugoshin biases chromosomes for biorientation through condensin recruitment to the pericentromere. *elife* 3:e01374. <https://doi.org/10.7554/eLife.01374>
 13. Vincenten N, Kuhl LM, Lam I, Oke A, Kerr AR, Hochwagen A, Fung J, Keeney S, Vader G, Marston AL (2015) The kinetochore prevents centromere-proximal crossover recombination during meiosis. *elife* 4. <https://doi.org/10.7554/eLife.10850>
 14. Nelson JD, Denisenko O, Bomsztyk K (2006) Protocol for the fast chromatin immunoprecipitation (ChIP) method. *Nat Protoc* 1(1):179–185. <https://doi.org/10.1038/nprot.2006.27>
 15. Cockram CA, Filatenkova M, Danos V, El Karoui M, Leach DR (2015) Quantitative genomic analysis of RecA protein binding during DNA double-strand break repair reveals RecBCD action in vivo. *PNAS* Aug 25;112(34):E4735–42.
 16. DeAngelis MM, Wang DG, Hawkins TL (1995) Solid-phase reversible immobilization for the isolation of PCR products. *Nucleic Acids Res* 23(22):4742–4743
 17. Ewels P, Magnusson M, Lundin S, Kaller M (2016) MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 32(19):3047–3048. <https://doi.org/10.1093/bioinformatics/btw354>
 18. Li H (2018) Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* 34(18):3094–3100. <https://doi.org/10.1093/bioinformatics/bty191>
 19. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* 25(16):2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
 20. Quinlan AR, Hall IM (2010) BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26(6):841–842. <https://doi.org/10.1093/bioinformatics/btq033>
 21. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP (2011) Integrative genomics viewer. *Nat Biotechnol* 29(1):24–26. <https://doi.org/10.1038/nbt.1754>
 22. Tian B, Yang J, Brasier AR (2012) Two-step cross-linking for analysis of protein-chromatin interactions. *Methods Mol Biol* 809:105–120. https://doi.org/10.1007/978-1-61779-376-9_7
 23. Craig DW, Pearson JV, Szeling S, Sekar A, Redman M, Corneveaux JJ, Pawlowski TL, Laub T, Nunn G, Stephan DA, Homer N, Huentelman MJ (2008) Identification of genetic variants using bar-coded multiplexed sequencing. *Nat Methods* 5(10):887–893. <https://doi.org/10.1038/nmeth.1251>
 24. Ford E, Nikopoulou C, Kokkalis A, Thanos D (2014) A method for generating highly multiplexed ChIP-seq libraries. *BMC Res Notes* 7:312. <https://doi.org/10.1186/1756-0500-7-312>

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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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PARENT PROTOCOLS

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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.

Growth Conditions for SMC Proteins

3d 18h 45m

1

S. cerevisiae strains for mitotic studies are grown in YPDA at **25 °C**. The most consistent results, at least for cohesin, are obtained when cells are arrested in metaphase of mitosis prior to the ChIP procedure. This can be achieved either by depletion of the anaphase-promoting complex subunit, Cdc20, or treatment of the cells with the microtubule-depolymerizing drug nocodazole.

For depletion of Cdc20, use a construct where *CDC20* is under control of the methionine-repressible promoter, *pMET3* (*pMET3-CDC20*).

1.1 3h

Briefly for **Cdc20 depletion**, dilute an overnight culture to OD₆₀₀ = 0.2 in minimal media lacking methionine and grow for **01:00:00 – 02:00:00** at **25 °C** to OD₆₀₀ = 0.3–0.4.

1.2 3h

Dilute culture back to OD₆₀₀ = 0.2 in same media and arrest cells in G1 by adding **5 microgram per milliliter (µg/mL) α-factor** for **01:30:00** and **2.5 microgram per milliliter (µg/mL) α-factor** for an additional **01:30:00**.

1.3

Check microscopically that at least 90% of cells are arrested before collecting on a filter (Whatman ME25, 0.45 µm), and wash with 10 volumes of medium lacking sugar with the aid of a vacuum pump.

1.4 45m

Quickly resuspend cells in YPDA containing **8.5 Milimolar (mM) methionine** and re-add methionine to **4 Milimolar (mM)** every **00:45:00**.

1.5 4h 30m

Harvest cells after **02:00:00 – 02:30:00** in a metaphase arrest confirmed by microscopy.

2 For nocodazole arrest, follow these subsequent steps:

2.1 3h

Dilute an overnight culture to OD₆₀₀ = 0.2 in YPDA and grow for **01:00:00 – 02:00:00** at

↻ **25 °C** to OD₆₀₀ = 0.3–0.4.

2.2 Dilute culture back to OD₆₀₀ = 0.2 in YPDA media containing a mixture of

[M] **15 microgram per milliliter (µg/mL) nocodazole** and

[M] **30 microgram per milliliter (µg/mL) benomyl** .

2.3 Read nocodazole every ⌚ **01:00:00** at [M] **7.5 microgram per milliliter (µg/mL)** . Harvest cells^{5h 30m} after ⌚ **02:00:00** – ⌚ **02:30:00** confirming metaphase arrest by microscopy.

3 For **inducing meiosis**, follow these subsequent steps:

For studies of protein occupancy during meiosis we use diploid *S. cerevisiae* strains of SK1 background including (a) *REC8-3HA ndt80Δ* (AM4015), as previously described [13] and (b) *REC8-6HIS-3FLAG* (AM11000).

3.1  1d 18h

Recover SK1 strains from ↻ **-80 °C** on YPG agar plates ⌚ **Overnight** at ↻ **30 °C** , before transferring to YPDA4% agar plates for a further ⌚ **12:00:00** - ⌚ **30:00:00** at ↻ **30 °C** .


3.2  1d

Inoculate cultures in liquid YPDA at ↻ **30 °C** with shaking for ~ ⌚ **24:00:00** , prior to inoculating into BYTA medium to OD₆₀₀ = 0.3 ⌚ **Overnight** .

3.3 

The next morning, spin cells down, wash with dH₂O and resuspend in SPO medium to OD₆₀₀ = 1.8 and shake at ↻ **30 °C** .

3.4  6h

For prophase I arrest (*ndt80Δ*) for Rec8 cells, harvest  **50 mL media** ⌚ **06:00:00** after resuspension in sporulation medium and confirm arrest by FACS.

S. pombe strains used for calibration are listed in the materials under "Yeast Strains and Growth Material" and are grown in YES at ↻ **30 °C** .