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Growth Conditions for SMC Proteins

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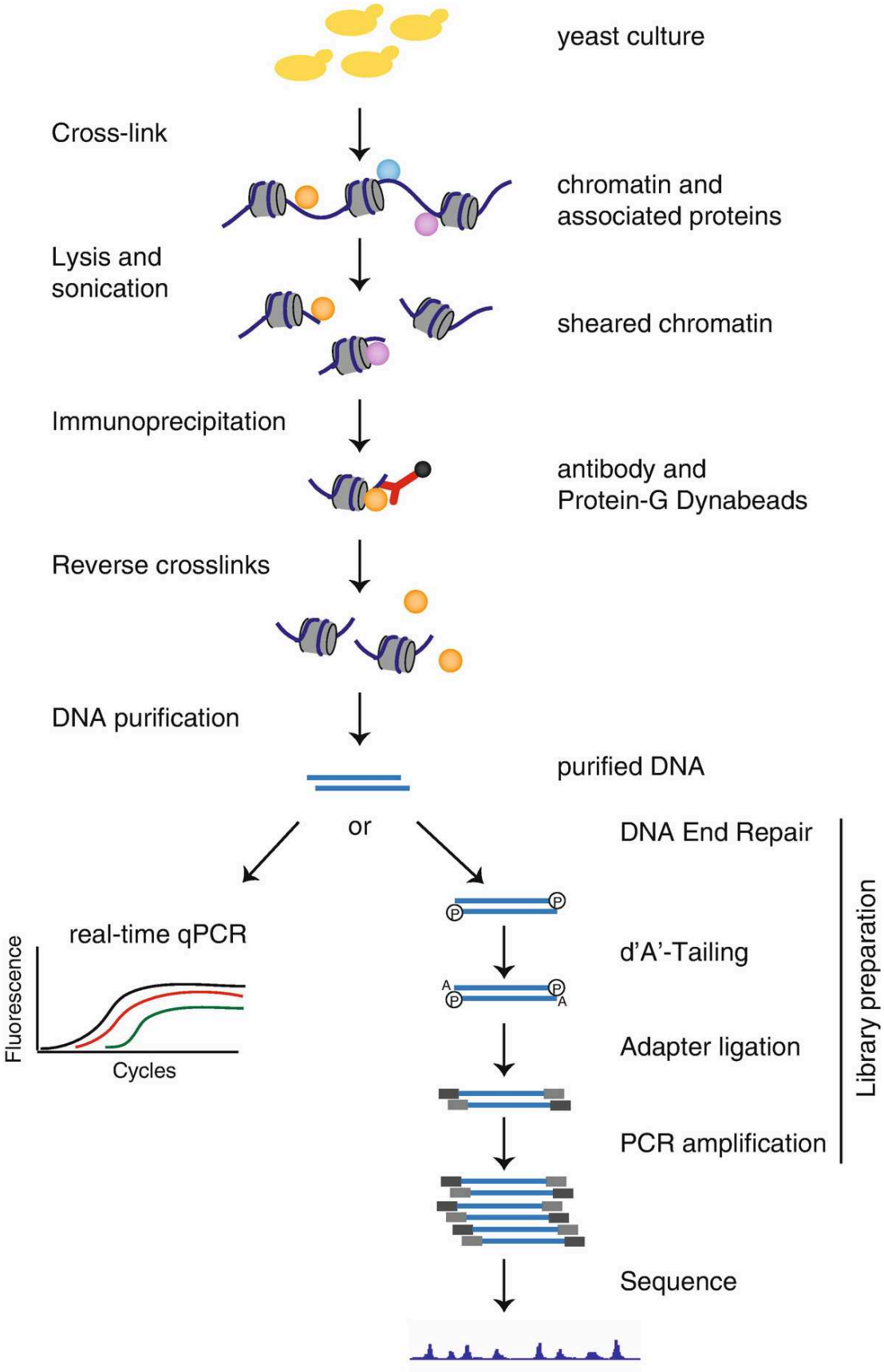
Abstract

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.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or
real-time qPCR
Fluorescence
Cycles
DNA End Repair
d'A'-Tailing
Adapter ligation
PCR amplification
Library preparation
Sequence

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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.

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

Note

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 Briefly for **Cdc20 depletion**, dilute an overnight culture to OD_{600} = 0.2 in minimal media lacking methionine and grow for 01:00:00 – 02:00:00 at 25 °C to OD_{600} = 0.3–0.4. 3h

1.2 Dilute culture back to OD_{600} = 0.2 in same media and arrest cells in G1 by adding [M] 5 microgram per milliliter (µg/mL) α-factor for 01:30:00 and [M] 2.5 microgram per milliliter (µg/mL) α-factor for an additional 01:30:00. 3h

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 Quickly resuspend cells in YPDA containing [M] 8.5 millimolar (mM) methionine and re-add methionine to [M] 4 millimolar (mM) every 00:45:00. 45m

1.5 Harvest cells after 02:00:00 – 02:30:00 in a metaphase arrest confirmed by microscopy. 4h 30m

2 For **nocodazole arrest**, follow these subsequent steps:

2.1 Dilute an overnight culture to OD_{600} = 0.2 in YPDA and grow for 01:00:00 – 02:00:00 at 25 °C to OD_{600} = 0.3–0.4. 3h

2.2 Dilute culture back to OD_{600} = 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 after 02:00:00 – 02:30:00 confirming metaphase arrest by microscopy. 5h 30m

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

Note

For studies of protein occupancy during meiosis we use diploid *S. cerevisiae* strains of SK1 background including (a)*REC8-3HA ndt80*Δ (AM4015), as previously described [13] and (b)*REC8-6HIS-3FLAG* (AM11000).

3.1 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 . 1d 18h

3.2 Inoculate cultures in liquid YPDA at 30 °C with shaking for ~ 24:00:00 , prior to inoculating into BYTA medium to OD_{600} = 0.3 Overnight . 1d

3.3 The next morning, spin cells down, wash with dH_2O and resuspend in SPO medium to OD_{600} = 1.8 and shake at 30 °C .

3.4 For prophase I arrest (*ndt80*Δ) for Rec8 cells, harvest 50 mL media 06:00:00 after resuspension in sporulation medium and confirm arrest by FACS. 6h

Note

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 .