

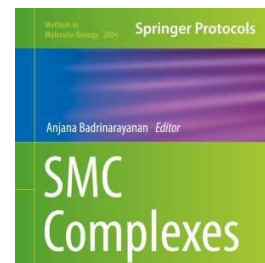
Sep 22, 2021

AMPure Purification Protocol

 In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.bn39mgr6



Vasso Makrantonis¹, Daniel Robertson¹, Adele L. Marston¹

¹The Wellcome Centre for Cell Biology, Institute of Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK

Springer Nature Books



Satyavati Kharde

Springer Nature

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bn39mgr6>

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



Protocol Citation: Vasso Makrantonis, Daniel Robertson, Adele L. Marston 2021. AMPure Purification Protocol. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bn39mgr6>

Manuscript citation:

Makrantonis 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

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: October 28, 2020

Last Modified: September 22, 2021

Protocol Integer ID: 43873

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, chip assay, chromatin, library preparation for chip, dna recombination, gene transcription, step protocol for chip, entire genome, purification, dna interaction, dna replication, chip, genome, investigating protein, chromosome segregation, dna, interest across the entire genome, yeast cell, protein, biological processes like gene transcription, gene, immunoprecipitation, yeast,

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 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. YPGA media: 1% yeast extract, 2% peptone, 2% glucose.
5. YPG agar plates: 1% yeast extract, 2% peptone, 2.5% glycerol, 2% agar.
6. YPGA4% 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.

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.

AMPure Purification Protocol

56m


1

Note

AMPure purification relies on the principle of solid-phase reversible immobilization (SPRI) as previously described [16]. SPRI beads are paramagnetic and coated with carboxyl molecules, which reversibly bind DNA in the presence of polyethylene glycol (PEG) and salt (20% PEG, 2.5 M NaCl mix). PEG causes the negatively charged DNA to bind on the bead surface. This DNA immobilization is dependent on the concentration of PEG and salt in the reaction, and the volumetric ratio of SPRI beads to DNA is critical. Equal volume of beads to DNA will give an SPRI–DNA ratio of one. As this ratio is changed the length of fragments binding and/or left in solution also changes. A lower SPRI–DNA ratio results in larger fragments at elution.

- 2 Equilibrate an aliquot for all purifications needed of AMPure XP beads at Room temperature for 00:30:00 before use. Vortex to resuspend.
- 3 Pipet carefully the indicated amounts so that no extra beads adhere to the outside of the tip.
- 4 Add the AMPure XP beads to DNA in solution and immediately mix thoroughly by repeated pipetting.
- 5 Incubate at Room temperature for 00:10:00 to allow binding of DNA to beads. 10m
- 6 Place on a magnetic rack for 00:05:00 . 5m
- 7 Remove and discard the supernatant taking great care not to take any beads.
- 8 Keep sample on magnetic rack and add 250 μ L freshly prepared 80% ethanol without disturbing the beads.
- 9 Incubate for 00:00:30 . Remove and discard all supernatant. 30s






- 10 Repeat the previous 2 steps once more: Keep sample on magnetic rack and add  250 μ L freshly prepared 80% ethanol without disturbing the beads.



- 11 Incubate for  00:00:30 . Remove and discard all supernatant.

30s



- 12 Let the beads air-dry for  00:02:00 –  00:03:00 at  Room temperature .



5m

Note

Overdrying the AMPure beads after the washing step will negatively impact on the DNA recovery. Beads are dry enough as soon as they lose their sheen.

- 13 Add the recommended amount of elution buffer (EB from Qiagen kit or ultrapure ddH₂O) and resuspend the beads by pipetting.



- 14 Incubate at  Room temperature for  00:03:00 .

3m



- 15 Place in magnetic rack for  00:02:00 .

2m

- 16 Transfer the supernatant to a new DNA LoBind Eppendorf tube.



Note

If, for example, eluting in 30 μ L, remove 28 μ L very slowly, being careful to prevent bead carryover. If beads are accidentally removed, pipet the sample back into the tube and allow the beads to bind.