

ChIP-seq Library preparation

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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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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.

ChIP-seq Library preparation

1

There are commercially available kits for generating DNA libraries but it is relatively straightforward and cost effective to create libraries using standard molecular biology reagents and custom oligonucleotides. This protocol can be completed within 1 day, and it comprises five distinct steps: blunting reaction; dA-Tailing to the 3' end of the DNA fragments; adapter ligation to the DNA fragments; PCR for enrichment of adapter modified DNA fragments; and library size selection. Finally, given the high cost of ChIP-seq runs and the time-intensive bioinformatics analysis and data validation, it is essential that the quality and the concentration of the libraries is validated by an Agilent Bioanalyzer prior to sequencing.

DNA End-Repair

2



Perform the blunting reaction using the following recipe: if making multiple libraries prepare a master mix of buffer, dNTPs, and enzyme, and then aliquot to the required ChIP purified DNA. The final volume should be 50 µl.

- (a) **1 ng – 20 ng ChIP DNA** (ideally 2 ng)
- (b) **5 µl 10× blunting buffer**
- (c) **5 µl 1 mM dNTPs**
- (d) **1 µl blunting enzyme**

For library preparation we recommend to use only filter-tips and 1.5 ml DNA LoBind tubes.

3



45m

Incubate at **Room temperature** for **00:45:00**.

4



Perform a 1.6:1 AMPure XP selection by adding **80 µl AMPure beads** to the **50 µl blunting reaction** blunting reaction (see protocol "AMPure Purification Protocol").

5







Elute in **30 µl ddH₂O** and take **27.7 µl** to a new DNA LoBind Eppendorf.

"A"-Tailing Reaction

6 

Use end-repaired DNA (from step 5) to perform "A"-tailing reaction using the following recipe: if making multiple libraries prepare a master mix of buffer, dATP, and enzyme, and then aliquot to the required end-repaired DNA. The final volume should be 30 μ l.

- (a)  **27.7 μ l end-repaired DNA**
- (b)  **3.3 μ l 10 \times NEB buffer 2**
- (c)  **1 μ l 10 mM dATP**
- (d)  **1 μ l Klenow 3' to 5' exo minus (5 U/ μ l)**



7 

30m

Incubate at  **37 $^{\circ}$ C** for  **00:30:00**.

8 

5m

Heat-inactivate Klenow enzyme at  **75 $^{\circ}$ C** for  **00:05:00**.

9 





5m

Place reaction  **On ice** for  **00:05:00**.

10 Proceed immediately to adapter ligation reaction (next step).

Adapter Ligation Reaction

11 Use dA-tailed DNA (from previous step) to perform the adapter ligation reaction using the following recipe: the final volume should be 70 μ l. Use different barcoded adapters for each Input and IP sample.

- (a)  **33 μ l "A"-tailed DNA**
- (b)  **35 μ l 2 \times Quick Ligase buffer**
- (c)  **1 μ l 0.5 μ M Adapters** [15]
- (d)  **1 μ l Quick T4 DNA ligase (2000 U/ μ l)**


Always keep adapters on ice. The quantity of adapters stated here is recommended for 2 ng of DNA; however, the amount of adapters should be proportional to the amount of DNA used. Optimal concentration of adapters used is essential. High concentration can lead to adapter contamination in the final library, which can be visualized on the Bioanalyzer (Fig. 2b). We use for this protocol the NEXTflex DNA Barcodes—12 (Bioo Scientific; #NOVA-514102). The NEXTflex DNA Barcodes utilize an indexed adapter containing a 6 nt unique sequence. Details can be found in manufacturer's manual.

12 

25m

Incubate at  **Room temperature** for  **00:25:00**.

13 

Perform a 1:1 AMPure selection by adding  **70 µl AMPure XP beads** to the **70 µl adapter ligation reaction** (see protocol "AMPure Purification Protocol").

14 

Elute in  **52 µl ddH₂O**. Transfer  **50 µl** to a new DNA LoBind Eppendorf.



DNA fragments over 100 bp will bind to beads and be eluted.

15 Perform another 1:1 AMPure selection by adding  **50 µl AMPure beads** to the **50 µl elution fraction** from the previous step.

16 Elute in  **33 µl ddH₂O** and take up  **30 µl** to a new DNA LoBind Eppendorf.

DNA fragments above 100/200 bp will bind to beads and be eluted.

PCR Amplification Reaction

17 Transfer  **10 µl supernatant** per sample (previous step) to a 0.2 ml PCR tube and set up the following PCR reaction  **On ice** in a final volume of 50 µl.

- (a)  **10 µl adapter-ligated DNA**
- (b)  **10 µl 5× Phusion HF buffer**
- (c)  **4 µl 2.5 mM dNTPs**
- (d)  **2 µl 12.5 µM Primer mix**
- (e)  **1.5 µl DMSO**
- (f)  **0.5 µl Phusion polymerase**
- (g)  **22 µl ddH₂O**

18 

Amplify DNA with the following PCR program:

Temperature	Time	Cycles
98 °C	30 sec	12 -18 cycles
98 °C	10 sec	
65 °C	30 sec	
72 °C	30 sec	
72 °C	5 min	

When PCR for library amplification is performed, minimal cycling is desirable. The fewer number of PCR cycles used to amplify libraries, the less biased the resulting libraries will be for the products that are more efficiently amplified. Overamplification can result in daisy-chains of fragments that can be visualized as a higher molecular weight peak on the Bioanalyzer. If the library amplification fails, more DNA template can be used. For IP samples use up to 20 µl of template DNA.

Double-Sided AMPure Selection and Library Elution

15m

15m

19



Perform a 0.65×:1 AMPure selection. To the **50 µl PCR reaction** (previous step) add

31.85 µl AMPure XP beads, resuspend by pipetting and leave at **Room temperature** for **00:10:00** to bind.

Place 0.2 µl PCR tubes in a DynaMag-PCR magnet for **00:05:00**.

20



KEEP THE SUPERNATANT, this will contain fragments <300 bp. To **80 µl supernatant** add

50 µl Ampure beads (adjust bead volume for smaller supernatant volumes) and perform AMPure selection according to steps in protocol "AMPure Purification Protocol".

DNA fragments of 100–250 bp will bind to the beads.

21



Elute in **52 µl EB (Qiagen purification kit) or ultrapure ddH2O** and pipet **50 µl** to a new DNA LoBind Eppendorf.

22



Perform another 1:1 AMPure selection by adding **50 µl AMPure beads** to the **50 µl elution fraction** from the previous step.

DNA fragments above 100/200 bp will bind to the beads.

23 

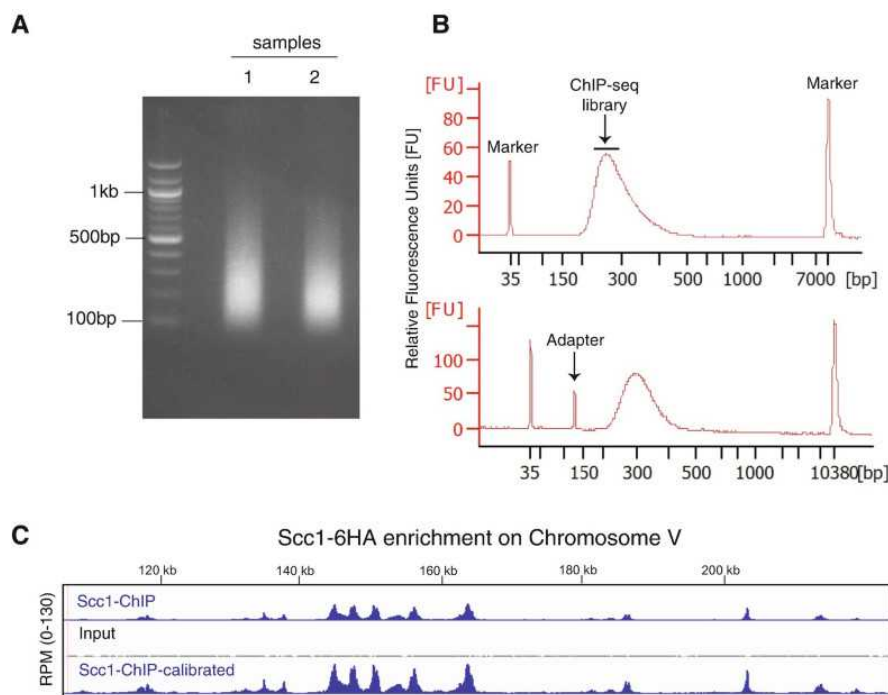
Elute in  33 μ l EB buffer (Qiagen purification kit) and transfer  30 μ l to a new LoBind Eppendorf.

24 

Determine DNA library concentration by Qubit HS kit (use  2 μ l).

25 

Run the library on a Bioanalyzer to determine average fragment size and general purity. Use a High Sensitivity DNA Kit (Agilent Technologies) as per manufacturer's instructions. Fragments of the sequencing library should have a size range of 150–300 bp (Fig. 2b, upper panel). If not pure, that is, adapter dimers are visible (Fig. 2b, lower panel), perform 1:1 AMPure purification to remove small adapter dimers.



(a) Representative image of mitotic yeast cells sonicated with a Bioruptor Twin (Diagenode) for a 30-min round (power setting: High, 30 s ON/30 s OFF). DNA from two different samples was loaded on a 2% agarose gel with a 100 bp marker ladder.
(b) Representative optimal BioAnalyzer trace (upper panel) and contaminated trace with adapter (bottom panel)
(c). Examples of profiles generated by chromatin immunoprecipitation followed by sequencing (ChIP-seq) of the cohesin subunit Scc1 in wild-type cells (IP shown in blue; Input shown in grey) and calibrated with *S. pombe* Scc1 distribution in representative chromosome V (IP shown in blue, bottom panel)

26 Libraries are now ready for sequencing using a sequencing platform of choice.

27 The final concentration of the library to load on the flow cell is 1.5 pM with an Input–IP ratio 15%:85%. Perform paired-end sequencing with 76 bp–76 bp for Read 1 and Read 2.

Several different next generation sequencers are available; for this protocol we use the Illumina platform. While platforms vary by target sequence length, accuracy and cost all give reproducibly comparable results. In order to perform experiments in a cost-effective manner, multiplexing can be used, that is, multiple ChIP-seq libraries, each carrying a different barcode to identify different samples can be sequenced together on a single flow cell of MiniSeq or lane of a HiSeq. The output of the Illumina MiniSeq is ~25 M clusters. We typically, sequence 8–10 barcoded uncalibrated samples in a single pooled library or 4–5 barcoded calibrated samples in a single pooled library. Typically one ChIP library generates six to ten million reads [23,24]. However, the above is subject on the level of enrichment of the protein of interest and the resolution required.