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Bioinformatics Analysis

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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

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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.

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MATERIALS TEXT

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

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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.

Bioinformatics Analysis

1

Carry out all data processing on an Ubuntu 16.04 (xenial) operating system. Perform basecalls using Illumina Real-Time Analysis (RTA2) software on the MiniSeq System. Use FastQC to assess the quality of the raw sequence data (fastq reads), with fastq-screen used to detect any unwanted contamination.

2 Aggregate all quality control reports with MultiQC [17]. Trim ChIP-seq paired end reads with cutadapt, remove any adapter sequence from the 3' end of reads using standard Illumina adapter sequences. Also, perform quality trimming from the 3' end using a user-defined cutoff (phred-33 quality 10).

3

After adapter and quality trimming, remove any read less than the defined minimum length (30 bp). Map reads to both *S. pombe* calibration genome and *S. cerevisiae* w303 experimental genome, retaining only those reads that map to each reference.

4

To obtain reads mapping only to SacCer W303; trimmed fastq reads should first be mapped with the MiniMap2 alignment tool [18] ("-ax sr" short genomic reads) to reference *S. pombe*, whereas unmapped *S. pombe* reads should be selected using SAMtools [19] (include SAM Flag -F 4) and convert back into fastq format (interleaved), those unmapped *S. pombe* reads can then be mapped to SacCer W303.

5 Here, any unmapped reads can be filtered out using samtools (exclude SAM Flag -F 4) and remove rDNA regions from the section of chromosome XII which corresponds to the repetitive rDNA using BEDtools intersect [20], as this region is saturated with reads.

6

To obtain reads mapping only to *S. pombe* the above process should be performed in reverse. The original trimmed reads should also be mapped to SacCer w303, select unmapped SacCer w303 reads using SAMtools, map those unmapped SacCer w303 reads to *S. pombe*, and filter unmapped reads out using SAMtools. Exclude mitochondrial DNA using SAMtools for both genomes.

7

In order to visualize mapped reads, create bedGraphs from the aligned Binary Alignment Map (BAM) files using BEDtools genomeCoverageBed with reads per millions (RPM) normalization (calculated with custom script using SAMtools flagstat output) & use UCSC wigToBigWig to convert these into BigWigs.

8 For meiotic samples, where SK1 strains were used, perform mapping to the SK1 genome, rather than SacCer3 as described above.

9

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To generate the calibrated ChIP bigWigs; use SAMtools flagstat to count reads mapping to SacCer3 w303 and *S. pombe* only for each sample, these values can then be used to calculate the Occupancy Ratio (OR) value as previously described [8]; Wc*IPx/Wx*IPc (W = Input; IP = chIP; c = calibration genome (*S. pombe*); x = experimental genome (sacCer w303)).

10

Use each OR value to calibrate ChIP bedgraphs using BEDtools genomeCoverageBed and convert to bigWig with UCSC wigToBigWig. These bigWigs are viewable in a genome browser such as Integrative Genomics Viewer (IGV) [21] or the ensembl genome browser.

All bigWigs from our published analyses are submitted to the Genome Expression Omnibus (GEO) archive and raw reads to the Sequence Read Archive (SRA).

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