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Budding yeast ChEC V.2

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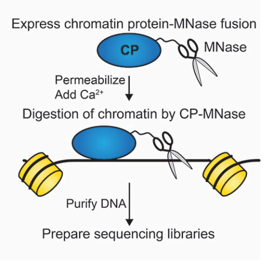

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Yeast Protocols, Tools, a...

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External link: https://www.jove.com/video/55836/genome-wide-mapping-protein-dna-interactions-with-chec-seq

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We use this protocol and it's working

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Abstract

Genome-wide mapping of protein-DNA interactions is critical for understanding gene regulation, chromatin remodeling, and other chromatin-resident processes. Formaldehyde crosslinking followed by chromatin immunoprecipitation and high-throughput sequencing (X-ChIP-seq) has been used to gain many valuable insights into genome biology. However, X-ChIP-seq has notable limitations linked to crosslinking and sonication. Native ChIP avoids these drawbacks by omitting crosslinking, but often results in poor recovery of chromatin-bound proteins. In addition, all ChIP-based methods are subject to antibody quality considerations. Enzymatic methods for mapping protein-DNA interactions, which involve fusion of a protein of interest to a DNA-modifying enzyme, have also been used to map protein-DNA interactions. We recently combined one such method, chromatin endogenous cleavage (ChEC), with high-throughput sequencing as ChEC-seq. ChEC-seq relies on fusion of a chromatin-associated protein of interest to micrococcal nuclease (MNase) to generate targeted DNA cleavage in the presence of calcium in living cells. ChEC-seq is not based on immunoprecipitation and so circumvents potential concerns with crosslinking, sonication, chromatin solubilization, and antibody quality while providing high resolution mapping with minimal background signal. We envision that ChEC-seq will be a powerful counterpart to ChIP, providing an independent means by which to both validate ChIP-seq findings and discover new insights into genomic regulation.

Guidelines

This is a condensed, bench-friendly version of the detailed protocol in the linked JoVE article.

Materials

MATERIALS

- EGTA **Merck MilliporeSigma (Sigma-Aldrich)**
- RNase A **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R4642-10MG
- Spermidine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**85558
- Buffered Phenol Chloroform Isoamyl alcohol (P:C:I) ((25:24:1, saturated with 10 mM Tris, pH 8.0 and 1 mM EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Sigma P2069
- Proteinase K **Thermo Fisher Scientific Catalog #**EO0491
- Sodium Dodecyl Sulfate (SDS) **Fisher Scientific Catalog #**BP166-500
- Roche Complete Protease Inhibitor EDTA-Free tablets **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**5056489001
- Agencourt Ampure XP **Beckman Coulter Catalog #**A63AA0
- Potassium Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9541
- Calcium Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C4904
- EDTA **Invitrogen - Thermo Fisher Catalog #**AM9261
- Digitonin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**300410
- Linear acrylamide **Thermofisher Catalog #**AM9520
- Spermine **Fisher Scientific Catalog #**AC132750010

Protease inhibitors should not contain EDTA so as not to interfere with MNase cutting.

SPRI beads can also be made in-house using the protocol found here: https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf

Troubleshooting

Before start

Prepare and store the following stock solutions prior to beginning. In addition to these solutions, you will need 1 M $CaCl_2$, 10% SDS, and 75% ethanol.

2% digitonin
Add 20 mg high-purity digitonin to 1 mL DMSO. Vortex for ~30 sec to dissolve and store 100 µL aliquots at -20°C.

Buffer A (100 mL)
1.5 mL 1 M Tris, pH 7.5 (15 mM final)
8 mL 1 M KCl (80 mM final)
50 µL 0.2 M EGTA (0.1 mM final)
H_2O to 100 mL
Prior to use, add:
Protease inhibitors to 1X
1 µL 200 mM spermine/1 mL buffer A (0.2 mM final)
0.5 µL 1 M spermidine/1 mL buffer A (0.5 mM final)
Make 4 mL complete buffer A/sample

2X Stop buffer (100 mL)
8 mL 5 M NaCl (400 mM final)
4 mL 0.5 M EDTA (20 mM EDTA)
2 mL 0.2 M EGTA (4 mM EGTA)
H_2O to 100 mL

For each strain, prepare 6 microfuge tubes for collecting time points. To each tube, add 90 µL 2X stop buffer and 10 µL 10% SDS

Yeast culture and harvest

1 The day before the experiment, inoculate 3 mL YPD or SC medium with a single colony. Grow overnight at 30°C.

2 In the morning, dilute the overnight culture to OD600 = 0.2-0.3 in 50 mL YPD or SC medium in a 300 mL flask. Grow 50 mL culture at 30°C until OD600 = 0.5-0.7.

3 Harvest cells in a 50 mL conical tube at 1,500 x g for 1 min.

4 Wash cells 3 × 1 mL Buffer A. Transfer cells to a 1.5 mL tube with the first wash and spin as above between washes.

ChEC

5 Permeabilize cells. Resuspend pellet in 600 µL Buffer A + 0.1% digitonin (add 30 µL 2% digitonin in DMSO to 570 µL Buffer A) and incubate at 30°C for 5 min. Remove 100 µL as zero timepoint prior to step 6.

6 Add 1.1 µL 1 M CaCl2 (~2 mM final), mix, and incubate at 30°C.

7 At each desired time point, remove a 100 µL aliquot of the digestion to a tube containing 90 µL stop solution and 10 µL 10% SDS and vortex to mix.

> **Note**
>
> ChEC experiments with factors that rapidly cleave DNA at 30°C may be performed at lower temperatures to slow MNase cleavage kinetics.

DNA extraction

8 Add 2 µL 20 mg/mL proteinase K. Digest protein at 55°C for 20 min.

9 Extract nucleic acids. Add 200 µL phenol/chloroform/isoamyl alcohol, mix well, and spin at max. speed for 5 min in a microfuge. Transfer aqueous phases to new tubes, add 2 µL (10 µg) linear acrylamide and 500 µL 100% ethanol, mix, and precipitate at -80°C for ≥30 min.

10 Spin at max speed and 4°C for 10 min.

11 Wash pellets with 1 mL 75% ethanol and aspirate ethanol.

12 Briefly air-dry pellets and resuspend in 29 µL Qiagen EB or comparable buffer + 1 µL 10 mg/mL RNase A and incubate at 37°C for 10 min.

13 Run 5 µL RNase-treated DNA on a 1.5% agarose gel to check DNA fragmentation if desired.

Size selection

14 Dilute RNase-treated DNA to 200 µL with Qiagen EB or comparable buffer.

15 Add 160 µL Ampure beads (0.8:1 beads:sample ratio) and pipet up and down 10X to mix. Incubate at room temperature for 5 min.

16 Collect beads on magnetic rack for 2 min.

17 Remove the supernantant (~400 µL) to a new tube containing 16 µL 5 M NaCl (~200 mM final).

18 Extract DNA from the unbound fraction. Add 400 µL phenol/chloroform/isoamyl alcohol, mix well, and spin at max. speed for 5 min in a microfuge.

19 Transfer aqueous phases to new tubes, add 2 µL (10 µg) linear acrylamide and 1 mL 100% ethanol, mix, and precipitate at -80C for ≥30 min.

20 Spin at max speed and 4°C for 10 min.

21 Wash pellets with 1 mL 75% ethanol and remove ethanol with vacuum.

22 Briefly air-dry pellets and resuspend in 25 µL Qiagen EB or comparable buffer. Recovered DNA can be quantified by Qubit and the size distribution analyzed via TapeStation using a high-sensitivity tape. Sequencing libraries can be prepared using any standard ChIP-seq-style method.