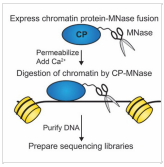


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# Chromatin Endogenous Cleavage and high-throughput sequencing (ChEC-seq) in *S. cerevisiae*

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bgthjwj6

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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

### The free MNase control

The essential negative control for any ChEC-seq experiment is MNase tagged with a nuclear localization signal and epitope tag and not fused to any specific factor ('free MNase'). Free MNase should be driven by a promoter of equal or greater strength to that of the protein to be mapped. A vector expressing free MNase can be integrated into the genome or maintained episomally with proper selection. MNase-tagged factor and free MNase protein levels should be assessed by western blotting prior to ChEC.

MATERIALS

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



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MATERIALS TEXT

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

BEFORE STARTING

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

Prepare a 'stop' 1.5 mL microfuge tube for each sample to be collected. 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 x 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.



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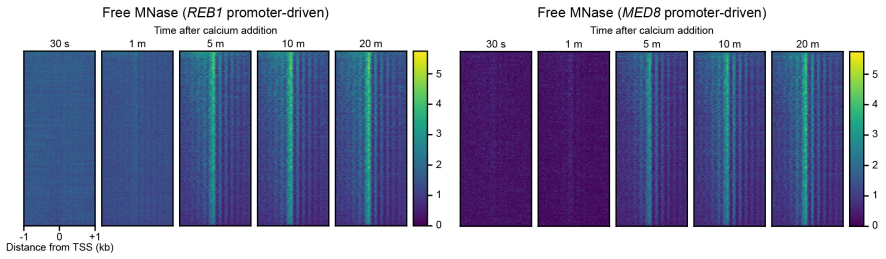
6 Add 1.1 µL 1 M $CaCl_2$ (~2 mM final), mix, and incubate at 30°C.

A recent study mapping the genome-wide distribution of components of the SAGA and TFIID coactivator complexes (PMID 31913117) with ChEC used a 10-fold lower calcium concentration with a 5 min digestion time in order to minimize background genomic digestion and overdigestion of target sites.

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

We have performed multiple time course ChEC experiments for both MNase-tagged factors and free MNase in distinct genetic backgrounds. For free MNase, we have observed that relatively long digestion times (≥ 2.5 min) yield cleavage of promoter nucleosome-depleted regions and nucleosome linkers due to their accessibility. It is therefore difficult to determine if enrichment of a particular factor is specific at these later time points. However, free MNase signal is quite low at short time points, allowing clear distinction between MNase-tagged factor and free MNase signal. We therefore recommend that, once an initial time course to determine the digestion kinetics of the factor under study and its corresponding free MNase control is performed, short time points be used for further experiments. We have found that a ≤ 1 min digestion is sufficient for all tested factors using the digestion conditions provided here.




**Free MNase signal increases substantially over time.** Heatmaps of CPM-normalized free MNase signal across a 40-fold range of digestion times in two distinct genetic backgrounds with free MNase expression driven by two different promoters. Heatmaps are centered around the TSSs of 4,857 genes encoding verified ORFs. Each heatmap is independently sorted in descending order by the average signal for each 2 kb window.

## 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.



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11 Wash pellet 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.

## Data processing and analysis

23 We align paired-end Illumina fastq files to the sacCer3 genome assembly with Bowtie 2 using additional parameters (-I 10 -X 700 --no-unal --no-mixed --no-discordant --dovetail). We then convert alignment SAM files to BAM format with



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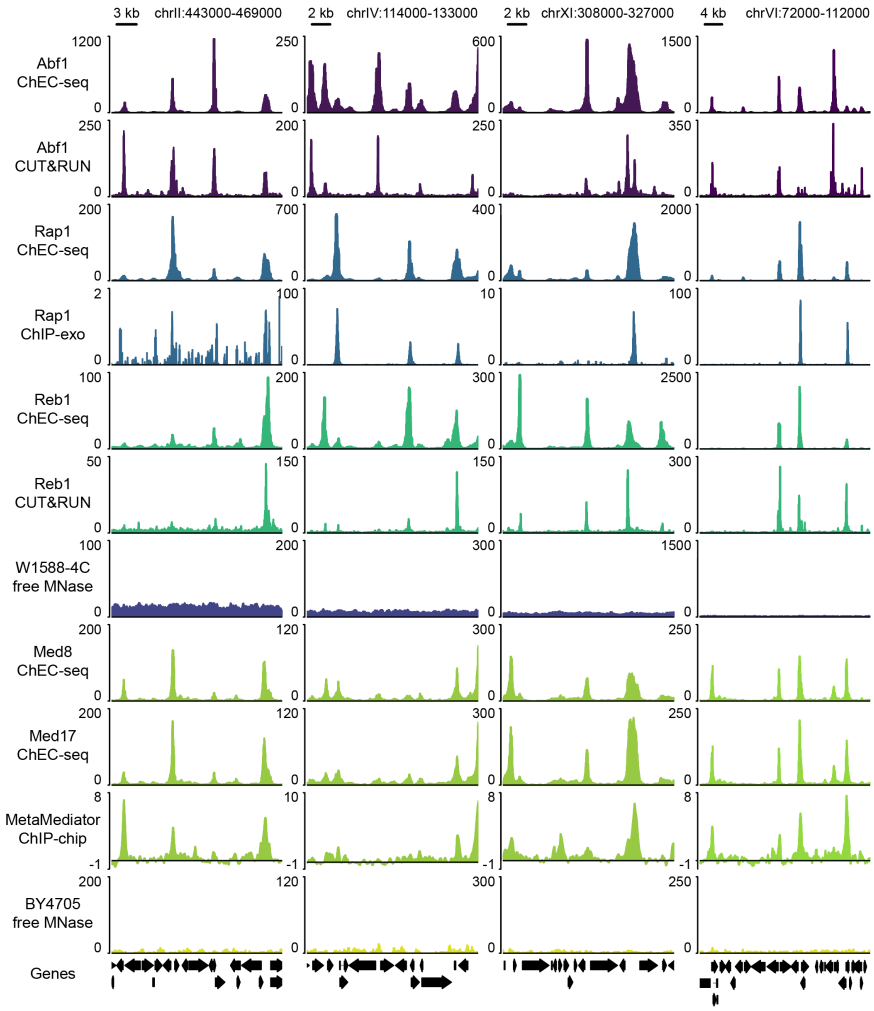
SAMtools and use deepTools *bamCoverage* to generate bigWig coverage tracks with single-base resolution (-bs 1), counts per million normalization (--normalizeUsing CPM), and extension of reads to their paired-end fragment length (-e). Further analysis can be done as for ChIP-seq (e.g. peak calling using MACS2 and motif discovery with MEME or HOMER).




**Short-duration ChEC-seq samples display high signal-to-noise ratios and are similar to datasets generated by other methods.** Genome browser-style tracks at four regions of the budding yeast genome showing ChEC-seq signal for the general regulatory factors (GRFs) Abf1, Rap1, and Reb1, the Mediator subunits Med8 and Med17, and their corresponding free MNase samples. CUT&RUN (PMID 28079019), ChIP-exo (PMID 29563167), and ChIP-chip (PMID 27773677) data are also displayed for comparison.

The following samples were combined following alignment with SAMtools *merge*:

1) GRF ChEC-seq and W1588-4C free MNase: 10 s, 20 s, 30 s, 40 s, 50 s, 60 s

2) Abf1 and Reb1 CUT&RUN: 1 s, 2 s, 4 s, 8 s, 16 s, 32 s, 64 s, 128 s

3) Mediator ChEC-seq and BY4705 free MNase: 30 s, 60 s

4) Rap1 ChIP-exo: four biological replicates



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