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Micro-C XL

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Abstract

We present an improved method for analysis of chromosome folding at mononucleosome resolution, Micro-C XL, using long crosslinkers and isolation of insoluble chromatin to greatly increase signal to noise. Micro-C XL maps of budding and fission yeast genomes capture both short-range chromosome fiber features such as chromosomally-interacting domains, and higher-order features such as centromere clustering. Micro-C XL provides a single assay to interrogate chromosome folding at length scales from the nucleosome to the full genome.

Reagents

- YPD: yeast extract/peptone/dextrose
- 37% Formaldehyde (Sigma Aldrich # 252549)
- DSG (disuccinimidyl glutarate) (ThermoFisher #20593)
- EGS (ethylene glycol bis(succinimidyl succinate)) (ThermoFisher #21565)
- 2.5M Glycine (Sigma Aldrich #G7126)
- Buffer Z: 1M sorbitol, 50mM Tris pH 7.4
- 14.3M 2-Mercaptoethanol (Sigma Aldrich # M6250)
- Zymolyase solution: 10mg/ml in Buffer Z; lasts up to 2 weeks at 4°C (Sunrise Science #N0766555)
- MBuffer#1: 50mM NaCl, 10mM Tris-HCl pH 7.5, 5mM $MgCl_2$, 1mM $CaCl_2$, and freshly complete with 0.5mM spermidine, 1mM β-ME, and NP-40 (percentage is determined by titrating the ratio of pellet / sup).
- Micrococcal Nuclease (Worthington Biochem): resuspended from lyophilized powder at 20 U/µl in Tris pH 7.4. Aliquot into tubes upon first use and freeze at –80°C.
- 0.5M EDTA (Life technology # AM9261)
- MBuffer#2 (NEBuffer#2): 50mM NaCl, 10mM Tris-HCl pH 7.5, 10mM $MgCl_2$
- Shrimp Alkaline Phosphatase (rSAP) (New England Biolabs # M0371)
- T4 DNA Polymerase (New England Biolabs # M0203)
- T4 Polynucleotide Kinase (New England Biolabs # M0201)
- Biotin-14-dCTP (Life Technologies # 19518018)

• Biotin-14-dATP (Life Technologies # 19524016)

• T4 DNA Ligase (New England Biolabs # M0202)

• Exonuclease III (E. coli) (New England Biolabs # M0206)

• 20× Proteinase K solution: TE with 20 mg/ml proteinase K and 50% glycerol. Store in -20°C.

• Elution buffer (EB): 10 mM Tris-HCl pH 7.5

• TE buffer: 10 mM Tris-HCl pH 8.0, 1 mM EDTA

• End-It DNA End-Repair Kit (EpiCentre BioTechnologies # ER81050)

• Exo-Minus Klenow DNA Polymerase (EpiCentre BioTechnologies # KL111)

• Fast Link DNA Ligation Kit (EpiCentre BioTechnologies # lk6201)

• Dynabeads® MyOne Streptavidin C1 (Life Technologies # 65001)

• KAPA HiFi HotStart ReadyMix (KAPA Biosystems # KK2601)

Procedure

Micro-C XL Protocol

I. First Crosslinking

1. Culture 100 mL of yeast to the midlog stage, OD=0.55 o/n.
2. Add 37% formaldehyde directly to the culture to 3% of a final concentration.
3. Shake the culture at 210 rpm for 15 min at 30˚C (FA only) or 10 min at 30˚C (Dual crosslinking).
4. Quench the crosslinking by adding 10 mL of 2.5M Glycine.
5. Incubate for 5 min at room temperature.
6. Centrifuge the cells at 4000 rpm for 5 min at 4˚C.
7. Pour off the medium and wash the cells in 50 mL of sterile water by vortexing.
8. Centrifuge the cells at 4000 rpm for 5 min at 4˚C.
9. Pour off the water.

II. Permeabilize the cell wall

1. Resuspend the cell pellet in 10 mL of Buffer Z and add 7 µL of 2-Mercaptoethanol

(final 10mM).

2. Add 250 µL Zymolyase solution (final 250 µg/mL).
3. Shake the tube at 210 rpm for 40 min at 30˚C.
4. Centrifuge the cells at 4000 rpm for 10 min at 4˚C.
5. Aspirate the supernatant with a vacuum suction.
6. Rinse the permeabilized cells by 5mL cold 1× PBS.
7. Centrifuge the cells at 4000 rpm for 2 min at 4˚C.
8. Aspirate the supernatant with a vacuum suction.

III. Second Crosslinking

1. Freshly prepare the long crosslinker stock and working solution as below:

Crosslinkers	MW	Spacer (Å)	Stock	Working
DSG	326.26	7.7	0.3M in DMSO	3mM in PBS
EGS	456.36	16.1	0.3M in DMSO	3mM in PBS

2. Resuspend the cells homogenously by 5 mL of working solution.
3. Rotate the tube for 40 min at 30˚C.
4. Quench the crosslinking by adding 1 mL of 2.5M Glycine.
5. Centrifuge the cells at 4000 rpm for 10 min at 4˚C.
6. Aspirate the supernatant with a vacuum suction.
7. Rinse the permeabilized cells by 5 mL cold 1× PBS.
8. Centrifuge the cells at 4000 rpm for 2 min at 4˚C.
9. Aspirate the supernatant with a vacuum suction.
10. The crosslinked pellet can be store at -80˚C for few months.

IV. Chromatin fragmentation

1. Resuspend the cell pellet in 200 µL of MBuffer#1 (freshly complete).
2. Add the appropriate amount of MNase to digest the chromatin to > 95%

mononucleosomes.

3. Incubate the tube for 20 min at 37˚C.

4. Add 2 mM EGTA and incubate the tube for 10 min at 65˚C to stop the MNase activity.

5. Here, you can continue processing the sample in multiple ways prior to Micro-C sequencing library generation, depending on the desired experimental design. Micro-C has been successfully carried out using three chromatin preps: 1) Total chromatin. 2) Supernatant. 3) Pellet. Any of these fractions can be subjected to the following Micro-C protocol, although we note that optimal signal-to-noise is achieved using relatively insoluble (Pellet) chromatin.

V. Chromatin cleaning and concentration

1) Total chromatin:

1. Transfer the whole sample into the 0.5 mL Amicon 10K spin column.
2. Concentrate the sample at 16000xg for at 4˚C until the volume goes down to ~ 50 µL.
3. Wash / pipette the sample by 450 µL MBuffer#2.
4. Repeat wash step 2 - 3.
5. Concentrate the sample at 16000xg for at 4˚C until the volume goes down to ~ 30 µL.
6. Add BSA to 1× final concentration. 2) Supernatant:

1. Centrifuge the tube at 16000xg for 5 min at 4˚C.
2. Collect the supernatant.
3. Concentrate the sample by the 0.5 mL Amicon 10K spin column at 16000xg for at 4˚C until the volume goes down to ~ 50 µL.
4. Wash / pipette the sample by 450 µL MBuffer#2.
5. Repeat wash step 2 - 3.
6. Concentrate the sample at 16000xg for at 4˚C until the volume goes down to ~ 30

µL.

7. Add BSA to 1× final concentration. 3) Pellet:

1. Centrifuge the tube at 16000xg for 5 min at 4˚C.

2. Collect the pellet.

3. Resuspend the pellet in 1 mL MBuffer#2.

4. Centrifuge the tube at 16000xg for 5 min at 4˚C.

5. Aspirate the buffer with a vacuum suction.

6. Repeat wash steps 3 - 5.

7. Resuspend the pellet to 30 µL of MBuffer#2 + final 1× BSA (or NEBuffer 2.1).

VI. Repair and label the end of chromatin fragments

1) De-phosphorylation

Total 32.5µL Final condition

Chromatin sample 30µL 50mM NaCl, 10mM Tris, 10mM MgCl2, 1X BSA

1U/µL r-Shrimp alkaline phosphatase 2.5 2.5U

 Incubate for 45min at 37°C.

 Inactivate for 5min at 65°C.

2) End-Chewing

Total 42.7µL Final condition

Chromatin sample from VI.1 32.5µL -

10X NEBuffer#2 3 70mM NaCl, 14mM Tris, 14mM MgCl2

100mM ATP 0.5 1mM ATP

200X BSA 0.2 1X

0.1M DTT 1 3mM

3U/µL T4 DNA polymerase 2.5 7.5U

10U/µL T4 PNK 3 30U

 Incubate at 37°C for 7min.

3) End-labeling

Total 100µL Final condition

Chromatin sample from VI.2 42.7µL -

0.4mM Biotin-dATP 25 100µM

0.4mM Biotin-dCTP 25 100µM

10mM dTTP + dGTP 1 100µM

10X T4 DNA ligase buffer 6 30mM NaCl, 35mM Tris, 12mM MgCl2, 7mM DTT

200× BSA 0.3 1× BSA

 PCR machine: Incubate for 25min at 25°C 15min at 12°C 4°C.

 Add EDTA (final 30mM) and heat inactivation for 20 min at 65°C.

VII. Proximity ligation and Remove unligated ends

1) Ligation

Although in our test Micro-C in “pellet” can be scaled down to a 1 mL ligation reaction, we suggest

using at least 2.5 mL for routine experiments.

Total 2.5mL

(pellet) 10mL

(Total & Sup) Final condition

Chromatin sample from VI. 100L 100µL -

Water 2122 8809 -

10× T4 DNA ligase buffer w/ ATP 250 1000 1×

200× BSA 12.5 50 1×

1M MgCl2 3 3 Equal to the moles of EDTA from previous part.

400U/µL T4DNA ligase 12.5 38 1.5 – 2U/µL

 Incubate for 60min at room temperature.

 Pellet: Centrifuge the pellet by 16000xg for 10min at 4°C.

 Total/Sup: 15mL Amicon 30k concentrates sample by spin at 4000g for 40min at 4°C.

2) Remove the biotin-dNTP at unligated ends

Total 100µL

(pellet) 280µL

(Total & Sup) Final

Chromatin sample - 250 -

10× NEBuffer#1 10 28 1×

Water 89 - -

100U/µL Exonuclease Ⅲ 1 1 100U

 Incubate for 5 min at 37°C.

3) Reverse crosslinking

 Add 20X proteinase K to 1× final concentration.

 Incubate for overnight at 55°C.

VIII. Dinucleosomal DNA purification

1. Phenol:Chloroform:Isoamyl Alcohol extraction twice spin at 19800xg for 10min.
2. Ethanol precipitation: 0.1x volume of sodium acetate and 2.5× volume of 100% ethanol -80°C for > 1hr spin at 19800xg for 15min at 4°C wash pellet by 75% ethanol spin at 19800xg for 5 min at 4°C Air dry pellet for 10min.
3. Resuspend pellet in 50L of TE buffer (+ 1× RNase solution) and incubate for 30min at 37°C.
4. ZymoClean to purify DNA.
5. Run DNA samples on 3% Nusieve agarose DNA gel.
6. Size selection of the band between 250 – 350 bp.
7. ZymoGel purification and dissolve final product in 17µL of elution buffer.
8. Quantify the input DNA by Qubit.

IX. Library construction by “with-bead” method

1) End-it

Total 25µL

DNA 17

10× End-it buffer 2.5

10× ATP 2.5

10× dNTP 2.5

End-it enzyme mix 0.5

 Incubate for 45 min at room temperature.

 2× Ampure XP purification.

2) A-tailing

Total 25μL

DNA 16

10X Exo- Klenow buffer 2.5

1mM dATP 5

Exo- Klenow fragment enzyme 1.5

 Incubate for 30min at 37°C.

 2× Ampure XP purification. (PEG/NaCl solution: 20% PEG, 2.5M NaCl)

3) Adapter ligation

Total 15μL

In-line / Indexing Adapter Ratio of Adapter:Input DNA = 10:1 – 50:1

Water to total 15μL

10× Fast-link DNA ligase buffer 1.5

10× ATP 1.5

Fast-link DNA ligase 1

 Incubate for > 2 hr at room temperature.

 Add 10μL of EB to total volume 25 μL.

 2× Ampure XP purification and elute DNA in 150 μL of water.

4) Streptavidin beads purification

1. Wash 2.5 μL of beads per sample (100mL culture) by 1× TBW twice.

2. Resuspend the washed beads in 150 µL 2× BW.
3. Mix with 150 µL of adapter-ligated DNA sample.
4. Rotate for 15 min at room temperature.
5. Wash by 500 µL of 1× TBW twice.
6. Rinse by 200 µL of MBuffer#2.
7. Resuspend in 15 – 20 µL of EB buffer. 5) On-beads PCR

Total 10µL

(scalable)

Streptavidin-Biotin-DNA sample 1

Water 3.5

2× KAPA HiFi Hot Start Mix 5

10µM PE1 primer 0.25

10µM PE2 primer 0.25

Denaturation 98°C 45sec

8-12 cycles 98°C 15sec

60°C 30sec

72°C 30sec

Extension 72°C 1min

4°C Hold

 Check the size and quantity of library by DNA gel or Fragment analyzer.

 Size-selection of dimer size library by 3% Nusieve agarose DNA gel or 1:1 Ampure XP beads purification.

X. Deep sequencing by Illumina PE-50

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

Hsieh_Rando_2016_protocol.pdf