Research Square

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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 MgCl2, 1mM CaCl2, and freshly complete with 0.5mM spermidine, 1mM -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 MgCl2 • 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

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