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## Genome-Wide Mapping of Nucleosome Positions in Yeast Using High-Resolution MNase ChIP-Seq

Megha Wal and B. Franklin Pugh

Center for Eukaryotic Gene Regulation, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania, USA

B. Franklin Pugh: bfp2@psu.edu

### Abstract

Eukaryotic DNA is packaged into chromatin where nucleosomes form the basic building unit. Knowing the precise positions of nucleosomes is important because they determine the accessibility of underlying regulatory DNA sequences. Here we describe a detailed method to map on a genomic scale the locations of nucleosomes with very high resolution. Micrococcal nuclease (MNase) digestion followed by chromatin immunoprecipitation and facilitated library construction for deep sequencing provides a simple and accurate map of nucleosome positions.

### 1. Introduction

Nucleosomes form the building blocks of chromatin, consisting of ~ 147 base-pair (bp) DNA that is tightly wrapped around a histone octamer core (Richmond & Davey, 2003). This wrapping requires substantial bending of DNA. At some nucleosome positions, DNA sequences such as the AA/TT dinucleotides may be enriched every 10 bp and along with regions of higher GC content near the nucleosome dyad (midpoint) collaborate to favor nucleosome formation (Albert et al., 2007; Satchwell, Drew, & Travers, 1986; Segal & Widom, 2009; Tillo & Hughes, 2009). In contrast, Poly dA:dT tracts tend to be intrinsically rigid and hence disfavor nucleosome formation (Anderson & Widom, 2001; Bao, White, & Luger, 2006; Iyer & Struhl, 1995).

In addition to the role of DNA sequence, adenosine triphosphate (ATP)-dependent chromatin remodeling enzymes use the energy of ATP hydrolysis to alter histone–DNA contacts and control nucleosome positions. *In vivo*, the action of these enzymes may lead to diverse outcomes, ranging from shifting nucleosome positions to partial and/or complete eviction of nucleosomes and histone replacement (Hartley & Madhani, 2009; Langst & Becker, 2001; Whitehouse, Rando, Delrow, & Tsukiyama, 2007). Recent work has suggested specific roles of distinct chromatin remodelers. Isw2 functions adjacent to promoters, where it repositions nucleosomes at the interface between genic and intergenic regions (Whitehouse et al., 2007). The RSC complex is responsible for maintaining nucleosome-free promoter regions and its flanking nucleosomes (Hartley & Madhani, 2009). Isw1, Isw2, and Chd1 remodelers might work together in maintaining genome-wide nucleosome organization (Gkikopoulos et al.,

2011). *In vitro* studies have also indicated the role of ATP-dependent activities in reconstituting proper nucleosome positioning across the genome (Zhang et al., 2011).

Nucleosome locations affect every cellular process requiring access to DNA, from influencing evolution to regulating gene expression, development, aging, and human health (Zhang & Pugh, 2011). Thus, determining precise nucleosome positions is of importance and an area of active research. Changes in nucleosome positions, even by a few base pairs, can have significant outcomes.

In this chapter, we describe the experimental details of mapping nucleosome positions across the yeast genome with high precision. In brief, standard chromatin immunoprecipitation (ChIP) methods are initially employed, whereby cell cultures are first treated with formaldehyde, which cross-links proteins to DNA (Fragoso & Hager, 1997). Cells are then harvested, and the chromatin was isolated and fragmented by micrococcal nuclease (MNase) digestion. Histone H3 is immunopurified under semidenaturing conditions to ensure that isolated nucleosomal DNA is cross-linked to histone H3 *in vivo*, as cross-linking can be quite inefficient. Importantly, if such a requirement is not imposed, then there remains a possibility that at least some nucleosomes, particularly mutants, may become repositioned during the chromatin isolation and MNase digestion. A key novel feature of the protocol described here is the construction of the nucleosomal sequence library while it is present within the immunoprecipitate. This facilitates subsequent sample clean up.

## 2. Methodology

### 2.1. Harvesting

We use  $\sim 10^7$ – $10^8$  yeast cells as the starting material for mapping nucleosomes. Yeast cells are grown in YPD growth medium and utilize dextrose as the carbon source. We autoclave dextrose for 20 min and have not encountered any issues with crystallization of the media.

#### Reagents

Prepare ahead of time.

1 × YPD

10 g Yeast extract

20 g Bacto-Peptide

20 g Dextrose

In 1 l water.

Autoclave at 121 °C for 20 min.

ST buffer

mM Tris–Cl, pH 7.5

mM NaCl

Filter 0.22 μm, store at 4 °C.

## Aliquot stocks

Complete, Mini, EDTA-free Protease Inhibitor Cocktail (CPI) tablets—Roche, catalog #04693159001

2.5 M Glycine

37% Formaldehyde

## Other

Liquid nitrogen

## Day 1

1. Make a primary inoculum by inoculating 5–10 ml of YPD with cells of interest.
2. Grow in shaker at 25 °C, 250–300 rpm until cultures have grown sufficiently to start large cultures at desired OD<sub>600 nm</sub>. This will take usually at least 6 h or overnight. The doubling time of wild-type yeast when grown at 25 °C is around 2 h. Alternatively, you can also grow yeast at 30 °C with a doubling time of 90 min.

## Day 2

3. Check and record the OD<sub>600 nm</sub> of starter cultures.
4. Inoculate 500 ml YPD with enough of starter culture to bring the culture to the desired starting OD<sub>600 nm</sub>.  
Calculation: (desired large culture OD)/(starter culture OD) × volume of large culture = ml's to inoculate.
5. Grow in shaker at 25 °C, 250–300 rpm.

## Day 3

6. Monitor growth carefully until OD<sub>600 nm</sub> reaches ~0.8. Do not go higher than OD of 1.0.
7. Add 37% formaldehyde to a final concentration of ~ 1.0%.
8. Return flasks to a room-temperature shaker for 15 min to cross-link.
9. Quench cross-linked culture by adding 2.5 M glycine to a final concentration of 125 mM. Incubate for 5 min at room temperature with constant shaking.
10. Transfer the contents of the flask to GS3 tubes or equivalent (maximum of 500 ml per tube).
11. Centrifuge in a Sorvall RC6 + centrifuge at 4 °C for 3 min at 4000 rpm or ~2800 ×g.
12. Resuspend one cell pellet with 1 ml ice-cold ST buffer to which fresh protease inhibitor cocktail is added.
13. Resuspend the second pellet with this cell lysate. Continue to repeat until all pellets are combined and resuspended. All the above steps are done on ice to keep the cells cold.

- 14 Divide the resuspended cells into microcentrifuge tubes (USA Scientific, catalog #1420-8700) to get the equivalent of ~ 100 ml of cells per tube.
- 15 Spin the tubes in bench-top microcentrifuge at 4 °C for 2 min at ~9300×*g*.
- 16 Resuspend each cell pellet in 0.5 ml of ST buffer.
- 17 Spin the tubes at 4 °C for 2 min at ~9300×*g*. Aspirate off the supernatant and freeze the cells in liquid nitrogen.

## 2.2. Cell lysis, MNase digestion

We use mechanical force (by agitating ceramic zirconia beads at high speed) to break yeast cell walls, when isolating chromatin. Alternatively, one can use glass beads coupled to a vortexer, or enzymatic treatment (zymolase or other enzymes) for cell wall digestion. Efficiency of cell disruption can be monitored by observing the cells under a microscope. The isolated chromatin is subsequently fragmented using MNase treatment. MNase is a unique endo–exonuclease, which results in cleavage at nucleosome linker regions. We include an additional step of brief sonication to completely solubilize the fragmented chromatin from the pellet into the supernatant. This sonication does not further fragment the nucleosomal DNA. A portion of the digestion chromatin is then extracted and visualized by agarose gel electrophoresis to obtain an MNase ladder corresponding to multiples of the nucleosomes core with the linker DNA.

### Reagents

Prepare ahead of time.

#### FA lysis buffer

- 50 mM HEPES/KOH, pH 8.0
- 150 mM NaCl
- 2.0 mM EDTA
- 1.0% (v/v) Triton X-100
- 0.1% (w/v) Sodium deoxycholate

Filter 0.22 μm, store at 4 °C.

#### NP-S buffer

- 0.5 mM Spermidine
- 0.075% (v/v) IGEPAL
- 50 mM NaCl
- 10 mM Tris–Cl, pH 7.5
- 5 mM MgCl<sub>2</sub>
- 1 mM CaCl<sub>2</sub>

Filter 0.22 μm, store at 4 °C

2 × Proteinase K buffer

40 mM of 1 M Tris–Cl, pH 7.5

40 mM EDTA

2% (w/v) SDS

Filter 0.22 μm, store at 4 °C

TE buffer

10 mM Tris–Cl, pH 8

1 mM EDTA

Filter 0.22 μm, store at 4 °C

Enzyme stocks

- 40 U/μl MNase—Worthington Biochemical.  
MNase is supplied as a lyophilized powder, which is resuspended in NP-S buffer containing 30% glycerol to a final concentration of 40 U/μl and stored at –20 °C.
- Proteinase K—Roche
- DNase-free RNase—Roche

Aliquot stocks

- 20% (w/v) SDS
- 14.3 M β-Mercaptoethanol (BME)
- 0.1 M Phenylmethylsulfonyl fluoride (PMSF)
- 0.5 M EDTA
- Phenol:chloroform:iso-amyl alcohol (PCIA).  
PCIA aliquots should be kept at 4 °C, stored in aluminum foil wrapped tubes as it is light sensitive. PCIA should be handled with care as it can cause skin burns.
- 20 mg/ml Glycogen
- Isopropanol, stored at room temperature
- 70% (v/v) Ethanol, stored at room temperature
- 6 × Xylene cyanol DNA-loading dye.  
30% (v/v) Glycerol.  
0.02% (w/v) Xylene cyanol
- CPI stock

Other

- Zirconia/silica beads—BioSpec Products, catalog #11079105z
- Agarose—OmniPur, EMD Chemicals, catalog #2125

### 2.2.1 Day1: Bead-beating lysis

1. Briefly (around 2 min) thaw 100-ml cell pellet aliquots of cross-linked cells. Vortex and spin the cells before use.
2. Resuspend the cell pellet in 1.0 ml of FA lysis buffer to which fresh protease inhibitor cocktail and PMSF (to a final concentration of 0.2 mM) are added.
3. Add 1.0 ml of zirconia beads.
4. To lyse the cells, use the Mini-Beadbeater-96 machine (BioSpec Products, catalog #1001) for three cycles of 3 min each with a gap of 5 min in between the cycles. (Keep the Eppendorf holder at  $-20\text{ }^{\circ}\text{C}$  for 10 min before lysis and on an iced block for the 5-min intervals.) Alternatively, one can use glass beads and vortex at maximum speed for 2 h at  $4\text{ }^{\circ}\text{C}$ .
5. Pierce the top and bottom of the tube with a hot 22-gauge needle and spin the extracts into a collection tube using a bench-top clinical centrifuge for 5 s at  $\sim 3500\times g$ . Mix and transfer into 1.5-ml tube.
6. Spin the tubes in bench-top centrifuge at  $4\text{ }^{\circ}\text{C}$  for 10 min at  $\sim 16000\times g$ . Discard the supernatant.

### 2.2.2 MNase digestion of lysed cells

7. Resuspend the chromatin pellet in 600  $\mu\text{l}$  NP-S buffer by pipetting (or vortex 30 s at maximum speed). Spin down the chromatin at  $4\text{ }^{\circ}\text{C}$  for 5 min at  $16,000\times g$  and discard the supernatant.
8. Add BME to NP-S buffer to a final concentration of 1 mM. BME is not stable in solution, so it must be added to the buffer on the day of experiment.
9. Resuspend the chromatin pellet in 300  $\mu\text{l}$  NP-S buffer to which BME is added. Mix thoroughly.
10. Remove 5 ml cell equivalent of the sample as “input” to MNase digestion for visualizing on the gel.
11. For each sample, titrate the amount of MNase added to determine the optimum MNase concentration where  $\sim 80\%$  of the material is in mononucleosomal form.
12. Incubate for 20 min at  $37\text{ }^{\circ}\text{C}$  in thermomixer with shaking. Alternatively, one can use a heat-block or thermocycler set at a particular temperature and mix by pipetting every 5 min.
13. Halt digestion by transferring samples to  $4\text{ }^{\circ}\text{C}$  and adding 0.5 MEDTA to a final concentration of 10 mM. Incubate the samples on ice for 10 min.
14. Spin at  $4\text{ }^{\circ}\text{C}$  for 10 min at  $16,000\times g$ . Collect the supernatant in a fresh tube and label as supe1.

- 15 To completely solubilize chromatin from the pellet, wash the pellet with 300  $\mu$ l of NP-S buffer containing 0.2% SDS and then sonicate for four cycles (30-s pulse) at medium strength.
- 16 Spin at 4  $^{\circ}$ C for 15 min at 16,000  $\times g$  and add this second supernatant to supe1.
- 17 Remove 5 ml cell equivalent of the pellet and supernatant sample for visualization on gel.
- 18 Store the remaining supernatant and the pellet sample at  $-80^{\circ}$ C. The pellet sample can be discarded after DNA extraction once you establish that it does not have any significant amount of insoluble DNA.

**2.2.3 Reversing cross-links for sample checks**—Exposure to high temperature reverses the protein–DNA cross-links. Proteinase K treatment digests all DNA-bound proteins.

- 19 Take 5 ml cell equivalent of the input, pellet and supernatant sample.
- 20 Make up the volume of all samples to 200  $\mu$ l by adding NP-S buffer.
- 21 Add equal volume of 2 $\times$  proteinase K buffer.
- 22 Add 3  $\mu$ l of 20 mg/ml proteinase K enzyme to each sample.
- 23 Incubate the samples at 65  $^{\circ}$ C overnight for proteinase K treatment.

#### **2.2.4 Day 2: DNA extraction for sample checks**

- 24 Extract the samples with 450  $\mu$ l of PCIA. Vortex the samples for 20 s.
- 25 Spin in a bench-top microcentrifuge at 16,000  $\times g$  for 6 min at room temperature.
- 26 Carefully remove the upper aqueous layer and transfer to fresh Eppendorf tube.
- 27 Repeat “PCIA” extraction once more.
- 28 Add 1  $\mu$ l of 20 mg/ml glycogen.
- 29 Precipitate DNA from aqueous layer with 0.6 volume of isopropanol (room temperature). Mix well and then incubate for 30 min at room temperature.
- 30 Pellet by microcentrifugation for 30 min, 16,000  $\times g$  at room temperature. Check for the presence of pellet and then decant the supernatant into the sink.
- 31 Wash the pellet with 500  $\mu$ l of 70% ethanol (room temperature). Mix by pipetting. Spin for 10 min, 16000  $\times g$  at room temperature.
- 32 Repeat 70% ethanol wash once more.
- 33 Decant the supernatant. Dry the pellet for  $\sim$  15 min (or until dry) in a speed vac (medium temperature setting).
- 34 Add 30  $\mu$ l TE buffer containing 50  $\mu$ g/ml RNase to each sample.
- 35 Incubate the samples at 37  $^{\circ}$ C for 2 h (minimum of 30 min).

- 36 Dissolve the samples in  $6 \times$  xylene cyanol loading dye and run on 2% agarose gel at 135 V for 35 min.
- 37 Visualizing DNA from the supernatant sample provides a determination of whether optimum MNase digestion had occurred—see Fig. 10.1A. Any DNA present in the pellet is an indication of unsolubilized DNA— see Fig. 10.1B.
- 38 Proceed with the best titration sample for immunoprecipitation.

### 2.3. ChIP and library preparation

We enrich for nucleosomal DNA by immunoprecipitation (ChIP) with anti-H3 antibody and select using magnetic Protein A beads. We use 0.05% SDS during IP to remove uncross-linked DNA fragments. Enriched ChIP DNA is then processed for sequencing library preparation while on the magnetic beads. Here we describe library preparation when using Illumina as the sequencing platform, but this procedure can be modified according to the sequencing platform used.

#### Reagents

Prepare ahead of time.

FA lysis buffer, see above

FA high-salt wash buffer

50 mMHEPES-KOH, pH 7.5

1 MNaCl

1.0% (v/v) Triton X-100

1.0% (w/v) Sodium deoxycholate

2 mMEDTA, pH 8.0

Filter 0.22  $\mu$ m. Store at 4 °C.

FA wash buffer 3

10 mMTris-Cl, pH 8.0

25 mMLiCl

1.0% (v/v) IGEPAL

1.0% (w/v) Sodium deoxycholate

2 mMEDTA, pH 8.0

Filter 0.22  $\mu$ m. Store at 4 °C.

ChIP buffers

- FA lysis buffer+0.2% (w/v) SDS
- 10 mMTris-HCl, pH 7.5

Antibody



- Anti-histone H3 antibody—Abcam, catalog #ab1791

#### Enzymes

- T4 DNA polymerase (NEB)
- T4 DNA ligase (NEB)
- Phi29 DNA polymerase (NEB)
- Klenow fragment exo- (NEB)
- Taq DNA polymerase (NEB)

#### Enzyme buffers

- 10 × NEBuffer 2 (NEB)
- 10 × T4 DNA ligase buffer (NEB)
- 10 × Phi29 polymerase buffer (NEB)
- 10 × Taq polymerase buffer (NEB)

#### Aliquot stocks

- 20% (w/v) SDS
- 3 mM dNTPs
- 25 mM dNTPs
- 3 mM dATP
- 1 × BSA (1 mg/ml)
- CPI stock

#### Adaptors and primers

- Sequencing adaptors (15  $\mu$ M)
- PCR primers (20  $\mu$ M)
- Index (20  $\mu$ M)

#### Other

- Magna ChIP Protein A Magnetic Beads (Millipore)
- DynaMag—15 magnet—Invitrogen, catalog #123-01D
- DynaMag—2 magnet—Invitrogen, catalog #123-21D
- Agarose, see above
- Qiagen QIAquick Gel Extraction Kit

### 2.3.1 Day 1: Antibody-histone attachment

1. Thaw the MNase-digested supernatant from — 80 °C storage. Make sure samples go on ice as soon as thawing is complete.

2. Adjust the sample with NP-S buffer to have a minimal volume of 500  $\mu$ l. Then add 20% SDS for a final concentration of 0.05%. Add 1.25  $\mu$ l of 20% SDS to a 500- $\mu$ l sample without SDS. Dilute the sample with NP-S buffer if it contains an SDS concentration higher than 0.05%.
3. Thaw one aliquot of anti-H3 antibody for each sample. Each antibody aliquot should contain 10  $\mu$ g.
4. Add a 10- $\mu$ g aliquot of antibody to each sample and incubate the samples on inverting rotator, 4 °C overnight (12–16h). Check after  $\sim$  10 min to make sure samples are not leaking.

### 2.3.2 Day 2: ChIP—Notes

- Do not let the beads remain “dry” for longer than about 30 s. If necessary, aspirate a subset of tubes, then add buffer.
  - Only spin the samples down before and during enzymatic reactions.
  - Follow these instructions for all washes (not just the ChIP washes).
  - Wash buffers should be kept on ice, do not set buffers out at room temperature.
1. Briefly resuspend Protein A Magna beads, then immediately add 50  $\mu$ l Magna beads to each sample in a 15-ml Falcon tube.
  2. Mix the sample by inverting the tube.
  3. Incubate the reactions for 1.5 h at 4 °C on rotator (slowly rotating).
  4. After incubation, spin down the samples briefly.
  5. Place in a DynaMag—15 magnet (which can hold 15-ml Falcon tubes) for 1 min.
  6. Using P1000 pipette, carefully transfer the supernatant (flow-through) to new tubes labeled “FT.”
  7. Store flow-through at  $-80$  °C until the resulting data set is validated, then it may be discarded.

### 2.3.3 ChIP wash series

1. Wash the beads with FA lysis buffer+0.025% SDS.
  - a. Add 0.8 ml FA lysis buffer+0.025% SDS+CPI (4 °C) to each sample.
  - b. Mix by pipetting up and down and transfer to a new 1.5-ml tube (including beads).
  - c. Place in a DynaMag—2 magnet (magnetic rack) for  $\sim$  1 min.
  - d. Aspirate off the supernatant (Wash 1).
  - e. Add 1.4 ml FA lysis buffer+0.025% SDS+CPI.
  - f. Mix gently by repeated inversions.



- c. Place on magnetic rack for ~ 1 min.
  - d. Carefully remove the supernatant with P1000 pipette (Wash 9).
6. Wash the beads with double-distilled water+CPI.
- a. Add 0.5 ml double-distilled water+CPI.
  - b. Mix gently by repeated inversions, place on magnetic rack for ~ 1 min.
  - c. Carefully remove the supernatant with P1000 pipette.

## 2.4. Sequencing library preparation

**2.4.1 Kinase reaction**—This step is essential as MNase leaves a 3' phosphate, whereas a 5' phosphate is needed. T4 polynucleotide kinase (PNK) removes 3' phosphates and adds 5' phosphates in the presence of ATP.

1. Preheat thermomixer to 37 °C.
2. Prepare Master Mix with 1 × T4 DNA ligase buffer and 1.5 U T4 PNK to a total volume of 18 µl per reaction tube.  

Ligase buffer contains DTT, which can form a white precipitate when cold. Ensure all DTT is resuspended before use.
3. Add 18 µl Kinase Mix to 2 ml bead-bound DNA for total of 20 µl. Pipette up/down ~10 times gently.
4. Incubate the samples on thermomixer at 37 °C for 30 min.
5. Wash the beads with FA high-salt buffer.
  - a. Add 0.5 ml FA high-salt buffer.
  - b. Mix gently by repeated inversions, place on magnetic rack for ~ 1 min.
  - c. Aspirate off the supernatant.
6. Wash the beads with 10 mM Tris-HCl, pH 8.0.
  - a. Add 1 ml of 10 mM Tris-HCl, pH 8.0.
  - b. Mix gently by repeated inversions, place on magnetic rack for ~ 1 min.
  - c. Carefully remove the supernatant with P1000 pipette.

**2.4.2 A-tailing**—This step adds a single adenosine nucleotide to the 3' end of the DNA, which will increase the efficiency of ligation to the Illumina adapters (which have a T overhang). Other sequencing platforms may not use this strategy, in which case the A-tailing step can be skipped.

1. Prepare Master Mix with 1 × NEBuffer 2, 100 µM dATP, and 10 U Klenow fragment, exo- to a total volume of 28 µl per reaction tube.
2. Add 28 µl A-Tail Mix to 2 ml bead-bound DNA for total of 30 µl. Pipette up/down ~10 times gently.

3. Incubate the samples on shaking thermocycler at 37 °C for 30 min.
4. Wash the beads with FA high-salt buffer.
  - a. Add 0.5 ml FA high-salt buffer,
  - b. Mix gently by repeated inversions, place on magnetic rack for ~ 1 min.
  - c. Aspirate off the supernatant.
5. Wash the beads with 10 mM Tris–HCl, pH 8.0.
  - a. Add 1 ml of 10 mM Tris–HCl, pH 8.0.
  - b. Transfer the sample to fresh 1.7-ml LoBind tube.
  - c. Mix gently by repeated inversions, place on magnetic rack for ~ 1 min.
  - d. Carefully remove the supernatant with P1000 pipette.

**2.4.3 Adaptor ligation**—Here, it is necessary to ligate the sequencing adaptor that is appropriate for the sequencing instrument. If planning to multiplex, then each sample gets assigned a unique identity by ligating an individual index sequence for each sample.

1. Preheat thermomixer to 15 °C.
2. Prepare Master Mix with 15 pmol of each adaptor, 30 pmol index (if multiplexing), 1 × T4 DNA ligase buffer, and 1800 U T4 ligase to a total volume of 48 µl.

We use nonphosphorylated adapters to minimize primer dimer. This necessitates a later phi29 fill-in step.

3. Add 48 µl ligation mix to bead-bound DNA for a total of 50 µl. Pipette up/down ~10 times gently.
4. Incubate the samples on thermomixer at 15 °C overnight.

Day 3: Continuation of Section 2.4.3

5. Wash the beads with FA high-salt buffer.
  - a. Add 0.5 ml FA high-salt buffer.
  - b. Mix gently by repeated inversions, place on magnetic rack for ~ 1 min.
  - c. Aspirate off the supernatant.
6. Wash the beads with 10 mM Tris–HCl, pH 8.0.
  - a. Add 1 ml of 10 mM Tris–HCl, pH 8.0.
  - b. Transfer the sample to a fresh 1.7-ml LoBind tube.
  - c. Mix gently by repeated inversions, place on magnetic rack for ~ 1 min.
  - d. Carefully remove the supernatant with P1000 pipette.

**2.4.4 Phi29 fill in**—Phi29 DNA polymerase activity will start at a nick and continue to the end of the DNA. It has a strand displacement activity.

1. Preheat thermomixer to 30 °C.
2. Prepare Master Mix with 200 µg/ml BSA, 1 × phi29 Buffer, 200 µM dNTPs, and 10 U phi29 polymerase to a total volume of 48 µl.
3. Add 48 µl Fill in Mix to 2 µl bead-bound DNA for total of 50 µl. Pipette up/down ~ 10 times gently.
4. Incubate the samples on thermomixer at 30 °C for 20 min.
5. Wash the beads with FA high-salt buffer.
  - a. Add 0.5 ml FA high-salt buffer.
  - b. Mix gently by repeated inversions, place on magnetic rack for ~ 1 min.
  - c. Aspirate off the supernatant.
6. Wash the beads with 10 mM Tris–HCl, pH 8.0.
  - a. Add 1 ml of 10 mM Tris–HCl, pH 8.0.
  - b. Mix gently by repeated inversions, place on magnetic rack for ~ 1 min.
  - c. Carefully remove the supernatant with P1000 pipette.
7. Resuspend the 2-ml bead-bound DNA in 48 µl double-distilled water+CPI.

#### 2.4.5 Ligation-mediated PCR

1. Prepare Master Mix with 1 × Taq buffer, 0.25 mM each dNTP, and 0.3 µM PCR primers to a total volume of 49 µl.
2. Add 49 µl PCR Mix to 50 µl bead-bound DNA for a total of 99 µl. Pipette up/down ~10 times gently.
3. Run the samples in thermocycler with the following program: “Hot start” the samples by adding 1 µl of Taq DNA polymerase to the reaction mix at the “forever” 95 °C step and then continue. Alternatively, a latent heat-activated DNA polymerase may be used.

Time	Temperature (°C)	Cycles
Forever	72	1
20 min	72	1
Forever	95	1
5 min	95	1
15 s	95	18
15 s	52	
1 min	72	
5 min	72	1

Forever 4 Hold

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It is not recommended to go above 18 cycles of PCR amplification, rather < 18 cycles is preferable if sufficient sample is present.

**2.4.6 Gel purification**—It is important to size select for appropriate size DNA and remove small molecular weight DNAs that preferentially get amplified.

1. Thoroughly clean and rinse an appropriate size gel box.
2. Pour 2% agarose gel with thick combs.
3. Mix 1/6th volume of 6× sequencing-grade xylene cyanol dye with each sample.
4. Load 7 µl of 1-kb ladder. Load the samples (region B) on agarose gel between 1-kb ladders (regions A and C) as shown in Fig. 10.2.
5. Excise Lanes A and C (dashed black lines) from the gel. Leave “region B” of the gel at your bench.
6. Visualize Lanes A and C on a short-wavelength UV transilluminator and mark the desired DNA fragment size on the DNA ladders encompassing the 200 and 400 bp markers (blue boxes) by cutting the gel in that area.
7. Reassemble the gel at your bench; using Lanes A and C as markers, excise the sections of agarose-containing DNA fragments of the desired size (red box).
8. Place each gel excision into a 1.7-ml LoBind tube
9. Record the weight of each excised piece. Write this weight directly onto the LoBind tube.
10. Take short-wavelength UV image of excised gel.

**2.4.7 Qiagen cleanup**—Notes

- All buffers and centrifuge spins should be at room temperature.
- Do not heat the gel to dissolve the gel slice as it may result in denaturation of the library. Instead, dissolve the gel slice by vortexing at room temperature until completely dissolved.

Use QIAquick Gel Extraction Kit to purify the DNA from the gel. Quantify DNA using bioanalyzer and/or qPCR and then send for deep sequencing. It is recommended that you make clear to the sequencing facility that your library has already been constructed, so that they do not attempt to construct a library with your sample. The sequencing facility should be able to map your DNA sequences to the reference genome that you specify. Those sequencing reads can then be clustered into peak calls using publicly available peak calling algorithms, and nucleosome positions and occupancy levels defined, as discussed elsewhere (Zhang & Pugh, 2011).

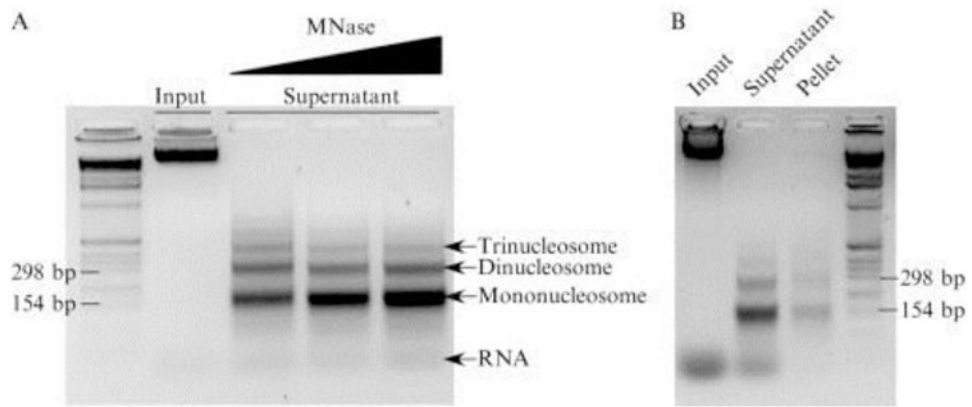
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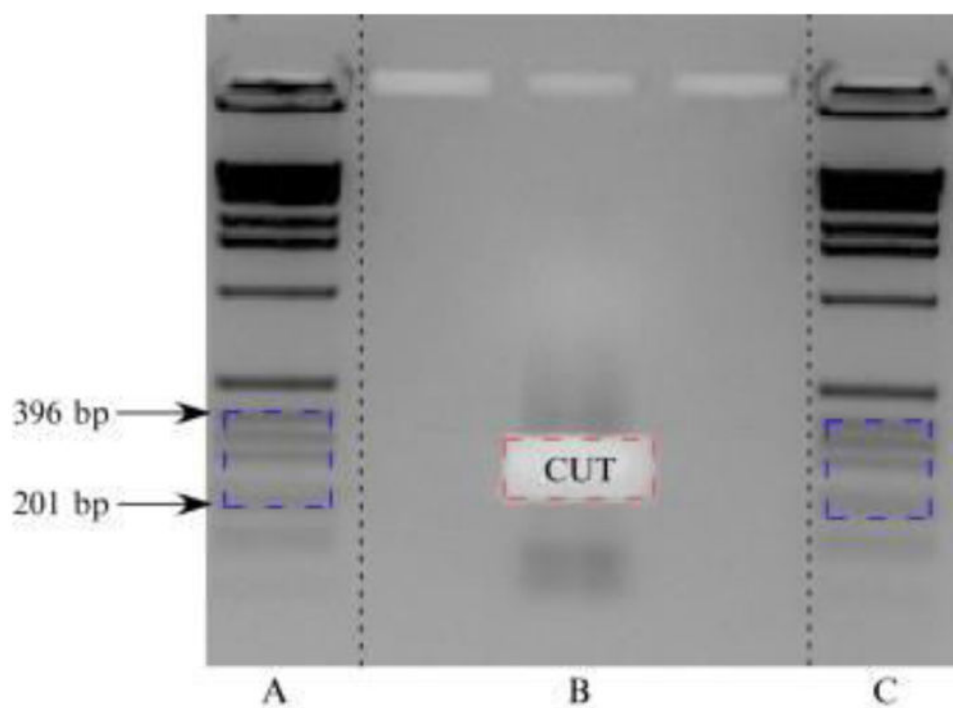
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**Figure 10.1.** MNase digestion results. The input lane indicates undigested chromatin prior to MNase treatment. (A) An MNase ladder is visible in all three MNase-treated supernatant lanes. As indicated in the gel, with increasing MNase concentration, there is an increase in mononucleosomal DNA and decrease in higher molecular weight bands corresponding to “polynucleosomes.” Left-most lane indicates a 1-kb ladder. (B) The pellet lane indicates a small amount of unsolubilized DNA trapped in the pellet prior to sonication.



**Figure 10.2.** Agarose gel purification. Regions A and C indicate 1-kb ladder. Region B: red box indicates the excised gel portion.