Chromatin Preparation and Mnase Digestion (Nucleosome Mapping Assay)

NOTE 1: Somewhat similar at the early stages to the Yeast fractionation protocol

As used in: Kastaniotis *et al* (2000) *MCB* **20:**7088
Mennella *et al* (2003) *Euk Cell* **2:**1288

1. Grow small seed culture (~5mL) of cells harboring a **high copy** (required for sensitivity), episomal plasmid containing your sequence of interest at 30°C in appropriate selective media. Innoculate 400mls to desired OD_{600} so that cells reach ≈ 0.8 after overnight culture.

2. Collect cells by centrifugation: (5 min, 5000rpm, 4°C). Resuspend pellet in 76mls H_2O, 4mls 1M Tris-Cl (pH 7.5), 75µL βME (14.3M stock). Incubate at 30°C with shaking for 15 - 20 min. Collect cells by centrifugation: (5 min, 5000rpm, 4°C)

3. Resuspend completely in 4 mLs 1M Sorbitol$^+$ (see: **Media / Buffers** section) and transfer to a 50ml Falcon. Add 500µl Zymolase 20T (10mg/ml in Sorbitol, stock at 4°C) (**NB.** to flocculent cells add twice this amount). Remove 10µl and add to 1ml 1% SDS; determine $OD_{\lambda 600}$, blanking with 10µl Sorbitol$^+$ in 1ml 1% SDS. Incubate 30°C with rotation. Determine $OD_{\lambda 600}$ every 30min, continuing incubation until it drops > 80% (should take about one hour).

NOTE 2: Zymolase is relatively expensive. Plan accordingly.

NOTE 3: This is a simple way to track the digestion of the cell wall which usually protects yeast from exploding when they are dropped into 1% SDS. Some mutants may be more zymolase-resistant than WT: if digestion is suspiciously slow at one hour add more Zymolase 20T and continue incubation. Flocculent cells must also checked by microscopy.

4. Add 40 mLs of **COLD** Sorbitol$^+$ with 50mM Tris-Cl (pH 8). Collect cells by centrifugation: 5 min, 1500rpm, 4°C. Discard supernatant, Resuspend pellet gently with a glass pipette and repeat 40 mL Sorbitol$^+$/Tris wash. Again collect cells by centrifugation: 5 min, 1500rpm, 4°C. Remove as much supernatant as possible with a paper towel. Place tube on ice.

NOTE 4: At this point, samples can be treated as **Chromatin Samples** or **Naked Samples**. Chromatin samples will allow the detection of positioned nucleosomes as chromatin will be kept intact and micrococcal nuclease (MNase) digestion will be protected by nucleosomes. However, MNase does exhibit sequence preference (AT-rich regions) and digestion of naked, nucleosome-free DNA will give discrete bands. To identify these preferred sites, naked DNA must be digested as a control (see: Kastanitois *et al* and Mennella *et al*).

A. Chromatin Samples

A.1 Resuspend pellet in 2mL Lysis Buffer (+ protease inhibitors). Transfer to 13mL falcon tube. Add ~500µL of glass beads (Sigma G8772). 5 x cycles 30 sec vortex, 30 sec on ice. Collect

by centrifugation: 2min, 9500rpm, 4°C. Keep supernatant – discard debris pellet and glass beads. Use a small volume of supernatant to test pH with pH paper/strip.

A.2 Divide samples into 400µL aliquots in microfuge tubes. Incubate at 37°C for 5 mins. Add MNase. *If samples are at pH 7.5-8 then use 0 - 20 unit range of enzyme*. Incubate at 37°C for 10 min.

> **NOTE 5:** This MNase treatment should result in "one-hit kinetics" (one cleavage/molecule), with midrange digestion (~5 units MNase). On agarose gel analysis, undigested should show a single HMW band (genomic DNA); midrange should show a faint HMW genomic DNA band and a LMW smear; and complete digestion (~20 units MNase) should only show a LMW smear. Be sure to RNase sample prior to agarose anaylsis and the samples contain large amounts of RNA and this will obscure the LMW smear.

A.3 Add 55µL of STOP solution. Incubate at 37°C for 2 hrs – overnight. Extract with 400µl Phenol-Chloroform-Isoamyl alcohol (25:24:1 PCI) and then 400µl chloroform. In each case, vortex well and microfuge 12000rpm (max speed), 10 min, RT°. Precipitate DNA by adding 1/10 vol 8M NH_4OAc and 2.5 vol 100% EtOH (-20°C). Incubate for ≥ 20min, -20°C. Microfuge max speed, 10 min, RT°.

A.4 Wash pellet with 70% EtOH. Air-dry and resuspend pellet in 100 µL TE. Run ~10uL of 0U samples to estimate relative yield.

B. Naked Protocol

B.1 After Sorbitol+ / Tris washes, resuspend cell pellet in 4mL 50mM EDTA / 0.3% SDS (*cells can be stored in freezer at -20°C for ~1 week as a cell pellet prior to resuspension)*

B.2 Vortex vigorously and incubate 65°C for 20 min. Chill samples on ice. Add 800µL of Solution III (from *E.coli* miniprep protocol; **3M KAc, 11.5% glacial acetic acid**). Incubate on ice for 2 hr. Collect by centrifugation: 5min, 5000rpm, 4°C.

B.3 Transfer supernatant to 15mL falcon. Add 2.4mL 95% EtOH (-20°C). Incubate 5min RT°. Collect by centrifugation: 5min, 5000rpm, 4°C.

> **NOTE 6:** In the pellet, there should be a white, obvious nucleic acid pellet under the cell debris mess.

B.4 Discard supernatant and wash with 8 mL of 70% EtOH. VORTEX extensively. Collect by centrifugation: 5min, 5000rpm, 4°C. Remove supernatant and lyopholyze pellet (*pellet is huge – air drying is usually insufficient for complete drying*). Resuspend pellet in 1.6mL TE. Can aid solubilization by 65°C for 20min.

B.5 Resuspend completely and divide into four 400µL aliquots. Extract with 400µl PCI and then 400µl chloroform. In each case, vortex well and microfuge 12000rpm (max speed), 10 min,

RT^{o}. Precipitate DNA by adding 1/10 vol 3M NaOAc and 2.5 vol 100% EtOH (-20^{o}C). Incubate for ≥ 20min, -20^{o}C. Microfuge max speed, 10 min, RT^{o}.

B.6 Wash pellet with 70% EtOH and air-dry. Resuspend in 400µL Lysis Buffer. Incubate at 37^{o}C for 5 min. Add MNase (*0 - 9 unit range; Enzyme is more efficient on naked DNA)*. Incubate 37^{o}C for 10 min. Add 55µL STOP solution. Incubate at 37^{o}C for 2 hr – overnight. Extract with 400µl PCI and then 400µl chloroform. In each case, vortex well and microfuge 12000rpm (max speed), 10 min, RT^{o}. Precipitate DNA by adding 1/10 vol 8M NH_4OAc and 2.5 vol 100% EtOH (-20^{o}C). Incubate for ≥ 20min, -20^{o}C. Microfuge max speed, 10 min, RT^{o}.

B.7 Wash pellet with 70% EtOH. Air-dry and resuspend pellet in 100 µL TE. Run ~10uL of 0U samples to estimate relative yield

> **NOTE 7:** Samples are typically analyzed by Southern blot. All samples are treated with restriction endonuclease(s) to isolate the region of interest and run on a 1.4% agarose gel. Blot is probed by indirect end-labelling.

Media / Buffers

Sorbitol^{+}	(per liter)
1M Sorbitol	182.2 g
YNB	6.7 g
Glucose	20 g

Add Tris as required

Lysis Buffer	(per 100mL)
15 mM Tris-HCl (pH 8)	1.5 mL of 1M
60 mM KCl	0.447 g
15 mM NaCl	0.088 g
5 mM $MgCl_2$	0.102 g
1 mM $CaCl_2$	1 mL of 0.1M
5mM DTT	50 uL of 1M
0.5mM spermadine	0.027 g
0.5% Triton X-100	0.5 mL

Filter Sterilize and store at 4^{o}C. Add protease inhibitors immediately before use.

STOP Solution	(per 1 mL)
500 uL H_2O	
250 uL Proteinase K	
250 uL 20% SDS	

Solution III (from *E.coli* miniprep protocol)
3M KAc
11.5% glacial acetic acid