MNase Digestion for Nucleosome Mapping in *Neurospora*

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[Abstract] Digestion of chromatin by micrococcal nuclease MNase followed by high throughput sequencing allows us to determine the location and occupancy of nucleosomes on the genome. Here in this protocol we have described optimized conditions of MNase digestion of filamentous fungus *Neurospora crassa* chromatin without a requirement of a nuclear fractionation step.

Materials and Reagents

1. Filter paper (VWR International, catalog number: 1001-090)
2. D-(+)-glucose monohydrate (Sigma-Aldrich, catalog number: 49159)
3. L-arginine-$^{13}C_6$,$^{15}N_4$ hydrochloride (Sigma-Aldrich, catalog number: 608033)
4. 1x Vogel's salt (Vogel, 1956; Vogel, 1964)
5. D-biotin (Thermo Fisher Scientific, Molecular Probes™, catalog number: B1595)
6. Sucrose (Sigma-Aldrich, catalog number: S0389)
7. KCl (Thermo Fisher Scientific, Ambion™, catalog number: AM9640G)
8. NaCl (Thermo Fisher Scientific, Invitrogen™, catalog number: 24740011)
9. Tris hydrochloride (Tris-HCl) (Sigma-Aldrich, catalog number: 10812846001)
10. $MgCl_2$ (Thermo Fisher Scientific, Ambion™, catalog number: AM9530G)
11. Calcium chloride ($CaCl_2$) (Sigma-Aldrich, catalog number: 793639-500G)
12. NP-40 (Thermo Fisher Scientific, Thermo Scientific™, catalog number: 85124)
13. Dithiothreitol (DTT) (Thermo Fisher Scientific, Thermo Fisher™, catalog number: 20290)
14. Spermidine (Sigma-Aldrich, catalog number: S0266)
15. HEPES (Sigma-Aldrich, catalog number: H4034)
16. EGTA (Sigma-Aldrich, catalog number: E3889)
17. Sodium dodecyl sulfate (SDS) (Sigma-Aldrich, catalog number: L3771)
18. EDTA (Thermo Fisher Scientific, Thermo Fisher™, catalog number: 17892)
19. Glycerol (Sigma-Aldrich, catalog number: G6279)
20. Glycogen, molecular biology grade (Thermo Fisher Scientific, Thermo Fisher™,

catalog number: R0561)

21. Ethanol
22. Paraformaldehyde (Sigma-Aldrich, catalog number: P6148)
23. Glycine (Thermo Fisher Scientific, Invitrogen™, catalog number: 15527013)
24. Nuclease free H_2O (Thermo Fisher Scientific, Thermo Fisher™, catalog number: R0581)
25. Liquid nitrogen
26. Agarose (Thermo Fisher Scientific, Fisher Scientific™, catalog number: BP1356100)
27. MNase (Sigma-Aldrich, catalog number: N3755-500)
28. RNase cocktail enzyme mix (Thermo Fisher Scientific, Invitrogen™, catalog number: AM2286)
29. Proteinase K solution (Thermo Fisher Scientific, Invitrogen™, catalog number: AM2548)
30. Wizard® SV gel and PCR clean-up system (Promega Corporation, catalog number: A9281)
31. EDTA-free protease inhibitor tablets (Roche Diagnostics, catalog number: 05892791001)
32. $C_6H_8O_7$
33. $ZnSO_4 \cdot 7H_2O$
34. $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$
35. $CuSO_4 \cdot 5H_2O$
36. $MnSO_4 \cdot 1H_2O$
37. H_3BO_3 (anhydrous)
38. $Na_2MoO_4 \cdot 2H_2O$
39. Chloroform
40. MilliQ water
41. $Na_3C_6H_5O_7$
42. KH_2PO_4
43. NH_4NO_3
44. $MgSO_4 \cdot 7H_2O$
45. $CaCl_2 \cdot 2H_2O$
46. Liquid medium (see Recipes)
47. Trace element solution (see Recipes)
48. 50x Vogel's salt (see Recipes)
49. Lysis buffer (see Recipes)
50. MNase resuspension buffer (see Recipes)
51. Stop buffer (see Recipes)
52. TE buffer (see Recipes)

Equipment

1. Centrifuge
2. Vortex
3. Mortar and pestle
4. Thermo-mixer (Eppendorf AG, model: Thermo-mixer R)
5. Buhner funnel (Thermo Fisher Scientific, Fisher Scientific™, catalog number: FB966B)
6. Shaking incubator
7. Agarose gel electrophoresis

Procedure

1. Inoculate ~ 2 x 10^8 conidia of *Neurospora crassa* to 200 ml liquid medium in a 500 ml flask.
2. Grow the culture with 100 rpm shaking under desired light and temperature conditions. Representative cultures were grown for three days at 24 °C and at 80 µE light intensity.
3. In order to crosslink, add paraformaldehyde to the culture to a final concentration of 1% and incubate for 10 min shaking at 24 °C.
4. Quench paraformaldehyde for 5 min with glycine at final concentration of 125 mM.
5. Filter liquid medium through Buhner funnel and rinse mycelia with 500 ml of water (room temperature). Squeeze mycelia between tissue paper to get rid of all water and then place the mycelia in Eppendorf tube and immediately freeze in liquid nitrogen. At this step mycelia can be stored at -80 °C.
6. Cool mortar and pestle by submerging in liquid nitrogen for 5 min. Take the mortar and pestle out and then place frozen mycelia immediately into mortar and grind mycelia with pestle until it is finely ground powder. At this step one should avoid warming mycelia/ground mycelia powder.
7. Resuspend ~400 mg ground mycelia in 3.75 ml of lysis buffer with freshly added 0.5 mM DTT, 0.5 mM spermidine and EDTA-free protease inhibitor tablets.
8. Mix the suspension well by vortexing and incubate on ice for 5 min.
9. Distribute the suspension into five 1.5 ml Eppendorf tubes (750 µl per tube).
10. Resuspend the MNase powder in 850 µl of MNase resuspension buffer (estimated concentration=0.58 U/µl). Aliquots can be stored at -20 °C. Avoid freeze-and-thaw cycles.
11. Add respectively 0 [control], 0.75, 1.5, 3, 6 Units of MNase to each tubes. Incubate all samples at 25 °C for 1 h while shaking at 400 rpm in a Thermo-mixer.
12. Add 85 µl of stop buffer to each sample to stop digestion and centrifuge at 20,000 *x g* for 20 min to pellet the cell debris. Transfer supernatants to new tubes

13. Add 2 µl of RNase cocktail enzyme mix to each supernatant and incubate at 37 °C for 45 min shaking at 400 rpm in a Thermo-mixer.
14. Add 15 µl of Proteinase K solution to each sample and incubate at 65 °C for 2 h shaking at 400 rpm in a Thermo-mixer.
15. Add 40 µg glycogen and 1,250 µl of 100 % EtOH to each sample and incubate at -20 °C for 30 min.
16. Perform centrifugation at max speed (~20,000 *x g*) for 30 min at 4 °C. Discard supernatant. Wash the DNA pellet with 700 µl 70 % ice-cold ethanol. Remove the ethanol slowly without disturbing the pellet. Drying the pellet is not required.
17. Dissolve the washed DNA pellet in 100 µl DNAse/RNAse free H_2O.
18. Further purify DNA samples by using PCR-clean up kit.
19. Load samples on 1.7% agarose gel (80 volts for 1 h) to analyze nucleosomal ladder (Figure1).

Representative data

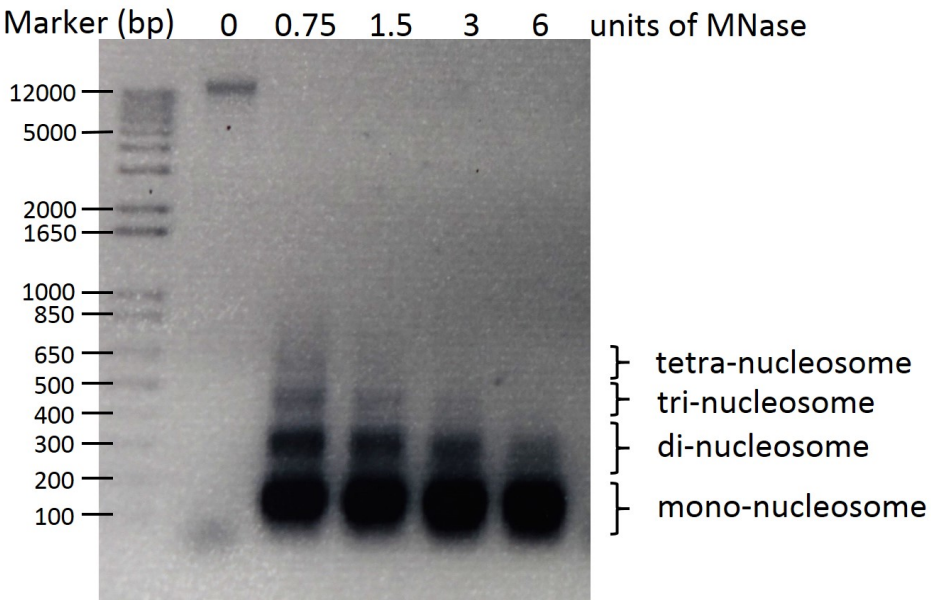

Figure 1. Representative image of MNase digested and purified *Neurospora* nucleosomal DNA loaded on 1.7% agarose gel

Recipes

1. Liquid medium (prepare freshly)
   2% (w/v) glucose monohydrate
   0.5 % (w/v) L-arginine
   1x Vogel's salt (Vogel, 1956; Vogel, 1964)

10 ng/ml biotin

2. Trace element solution (stored at RT)

   Dissolve indicated amount in 95 ml MilliQ water

   5 g $C_6H_8O_7$

   5 g $ZnSO_4 \cdot 7H_2O$

   1 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$

   0.25 g $CuSO_4 \cdot 5H_2O$

   0.05 g $MnSO_4 \cdot 1H_2O$

   0.05 g H_3BO_3 (anhydrous)

   0.05 g $Na_2MoO_4 \cdot 2H_2O$

   Add 1 ml chloroform as preservative

3. 1 L 50x Vogel's salt (stored at RT)

   Dissolve indicated amount in 755 ml MilliQ water

   125 g $Na_3C_6H_5O_7$

   250 g KH_2PO_4

   100 g NH_4NO_3

   10 g $MgSO_4 \cdot 7H_2O$

   5 g $CaCl_2 \cdot 2H_2O$

   5 ml of trace element solution

   250 µg biotin

   No pH adjustment is necessary

   Add 5 ml chloroform as preservative

4. Lysis buffer (stored at 4 °C)

   250 mM sucrose

   60 mM KCl

   15 mM NaCl

   15 mM Tris-HCl (pH 7.4)

   3 mM $MgCl_2$

   1 mM $CaCl_2$

   0.2% NP-40

5. MNase resuspension buffer (stored at 4 °C)

   10 mM HEPES-KOH (pH 7.6)

   50 mM KCl

   1.5 mM $MgCl_2$

   0.5 mM EGTA

   10% glycerol

6. Stop buffer (stored at RT)

   2% SDS

100 mM EDTA (pH 8)

7. TE buffer (stored at 4 °C)
   10 mM Tris-HCl (pH 8)
   1 mM EDTA

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References

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