Nucleosome mapping — MNase (with modifications noted for DNaseI)

This protocol is a modification of that of J. Hirschorn and F. Winston, (*Genes and Development,* 1992). Yields enough DNA for ~10 indirect end labeling experiments.

1. Grow 1 L cells in YPD to OD660 = 0.7
2. Spin down/wash in ~300 mL of H20, spin down.
3. Wash in 30 mL of cold DTT soln (10 mM DTT, 20 mM potassium PO4 pH 7.0, 1 M sorbitol) and spin down.
4. Resuspend pellet in 4 mL/g cell weight S buffer (1.1 M sorbitol, 20 mM potassium PO4 7.0, 0.5 mM CaCl2, 5 mg/ml Zymolase 20T, 0.5 mM PMSF). Nutate at 30 deg for 35'.
5. All subsequent steps until digestion at 4 degrees. Spin down and wash in 15 mL cold SPC (1M sorbitol, 20 mM PIPES 6.3, 0.1 mM CaCl2, 1 mM PMSF, + protease inhibitors). Spin down again.
6. Resuspend in 0.5 mL SPC and add gently down side of flask to 25 mL of slowly stirring Ficoll solution (9% Ficoll 400, 20 mM PIPES 6.3, 0.1 mM CaCl2, 1 mM PMSF, + protease inhibitors). Pour back into same tube and let solids settle for 10'.
7. Pipet supernatant to new tube and spin @13,200 for 20 min.
8. Resuspend pellet in 17 mL SPC and spin @ 10,100 for 10'.
9. Resuspend pellet in 4 mL/gm wet cell weight SPC. Transfer 1 mL aliquots to microfuge tubes
10. Add 0, 1.5, 5, 15, 50, 150, or 500 U MNase. 37 degrees for 10'. In general, only the 0, 50, and 150 digests are useful for indirect end labeling.
11. Stop digestion by adding 50 μL 0.5 M EDTA, 50 μL 20% SDS.
12. RNase 1 hr 42 deg.
13. Proteinase K (25 μL of 10 mg/mL) for at least 3 hr.
14. Phenol/Chloroform extract twice (at least), butanol extract once. EtOH ppt, wash 2X w/ 80% ethanol, resuspend in 100 μL TE.
15. Secondary restriction digest and do southern for indirect end labling. Use ~10 μL per digest.

For DNaseI:

i. include 3mM $MgCL_2$ in SPC buffer
ii. digest with 0, 1, 2, 4, or 8 units at 37 degrees for 20 minutes.

Note: there is also a quicker way to digest chromatin, but it yields less DNA for subsequent indirect end labeling experiments. The alternative method can be found by following the "Quick in vivo nucleosome mapping" link. The original method designed by Nick Kent can be found at http://www2.bioch.ox.ac.uk/~nakent/method.html