Chromatin Isolation by small-scale biochemical Fractionation

Mendez, J., and B. Stillman. 2000. Mol. Cell. Biol. **20:**8602-8612.

Buffer A
10 mM HEPES, pH 7.9
10 mM KCl
1.5 mM MgCl2
0.34 M Sucrose
10 % Glycerol
1 mM DTT
Protease inhibitor cocktail

Buffer B
3 mM EDTA
0.2 mM EGTA
1 mM DTT
Protease inhibitor cocktail

1. Harvest 1 x 10^7 – 2 x 10^7 cells by using a cell scraper; spin down at 1000 rpm for 2 min, discard supernatant

2. Wash cell pellet with PBS, spin down at 1000 rpm for 2 min.

3. Repeat second step.

4. Resuspend cell pellet in 200 μl of Buffer A.

5. Add Triton X-100 to a final concentration of 0.1%.

6. Incubate cells on ice for 8 min.

7. Centrifuge at 1,300 x g, 4 °C, for 5 min; separate supernatant = fraction **S1** from pellet (nuclei) = fraction **P1**.

8. Clarify **S1** by high-speed centrifugation at 20,000 x g, 4 °C, for 5 min; collect supernatant= fraction **S2** (discard P2)

9. Wash **P1** once with Buffer A and lyse it for 30 min in Buffer B (100 μl).

10. Centrifuge at 1,700 x g, 4 °C, for 5 min; separate supernatant = fraction **S3** from pellet (chromatin) = fraction **P3**.

11. Wash **P3** once with Buffer B and resuspend it either in SDS sample buffer (then boil for 10 min at 70 °C and analyse chromatin associated proteins by SDS PAGE/Western Blot) or in nuclease digestion buffer.