**Low salt chromatin isolation**

Mendez. Chromatin Association of Human Origin Recognition Complex, Cdc6, and Minichromosome Maintenance Proteins during the Cell Cycle: Assembly of Prereplication Complexes in Late Mitosis. 2000. MCB. 8602-8612.

Wysocka. Loss of HCF-1 chromatin association precedes temperature induced growth arrest of BN67 cells. 2001. MCB. 3820-3829.

**Buffer A:**

10 mM HEPES pH 7.9 (K-salt, so pH with KOH)

1.5 mM MgCl2

10 mM KCL

340 mM Sucrose

10% Glycerol

Before use add:

0.1% Triton X-100

1 mM DTT

1 mM PMSF

Protease Inhibitor Cocktail

**Buffer B:**

3 mM EDTA: 60 uL of 0.5 M

0.2 mM EGTA: 20 uL of 0.1 M

1 mM DTT: 10 uL of 1M

1 mM PMSF: 100 uL of 100 mM

Protease Inhibitor Cocktail: 100 uL of 100X

To 10 mL with 9.7 mL H2O

**Protocol:**

1. Resuspend cells in ice cold Buffer A w/0.1% Triton (mendez/wysocka protocol at 4x10^7 cells/ml)
2. Rotate in cold room for 8 mins
3. Centrifuge at 1300g for 5 mins at 4C
4. Remove supernatant >**S1 or soluble cytoplasmic fraction**.
5. Clarify S1 fraction by centrifuging at 14K rpm for 10min at 4C
6. Wash nuclei (pellet from above) with buffer A (**w/o Triton**) by pipeting up and down with p1000.
7. Centrifuge nuclei at 1300 rpm for 10 min at 4C.
8. Resuspend nuclei in Buffer B and rotate for 30 min at 4C.
9. The solution should have a white fluffy appearance like freshly extracted DNA.
10. Centrifuge @1700g or ~5000 rpm for 10 min at 4C
11. Remove supernatant >**S2 or soluble nuclear fraction**.
12. Wash pellet >**P3 or insoluble/chromatin fraction** with 500uL of Buffer B.
13. Centrifuge @1700g or ~5000 rpm for 10 min at 4C
14. Resuspend chromatin pellet (or in 200 uL of 1X SDS-PAGE sample buffer and sonicate for 10s at 10% amplitude twice on ice. Once may be enough to reduce viscosity. Just keep the solution cold and amplitude low.
15. Add 100uL of 2X SDS sample buffer to 100uL of S1 and S2 above.
16. Run SDS-PAGE gel.