**Nuc extract Protocol**

See http://cshprotocols.cshlp.org/content/2009/12/pdb.prot5330.long

**Buffer A** (hypotonic cell lysis buffer)

10 mM HEPES-KOH pH 7.9

1.5 mM MgCL2

10 mM KCL

0.5 mM DTT

0.5 mM PMSF

1X PIC

**Buffer C** (nuclear lysis buffer)

20 mM HEPES-KOH pH 7.9

25% Glycerol

420 mM KCL

1.5 mM MgCL2

0.2 mM EDTA

0.5 mM DTT

0.5 mM PMSF

1X PIC

**Buffer D-** (Dialysis buffer)

20 mM HEPES-KOH pH 7.9

20% Glycerol

100mM KCl

0.2 mM EDTA

0.2 mM DTT

Buffer D 2 liters:

80ml HEPES 0.5M

800mL 50% glycerol

800ul EDTA 0.5M

100mL 2M KCL

1020mL H20

**Nuclear extract preparation:**

TSCs, XEN cells: 100ul of PCV per 15cm plate

1. Remove media, scrape cells in 10ml of PBS plus PMSF per plate
2. Spin, consolidate cells in 15ml conical with PBS and spin out
3. R/S in 2 PCV of Buffer A plus PI
4. Check under scope, add 0.1% Np40, wait 5 minutes
5. B-Dounce cells 10x with 2 PCV of Buffer A
6. Check under scope
7. Spin 4 mins at 2000g at 4C
8. Extract for 30 minutes with 1.5 PCV of buffer C plus PI
9. Spin down 5 min at 5k
10. Re-extract for 30 more minutes with 1.5 PCV of buffer C plus PI
11. During reextraction, fully clear extract from step 9 by spinning at top speed for 15 mins in cold room
12. Spin nuclei/buffC for 15 mins at top speed
13. Dialyze extract 2x 2hours at 4C in 50x of Buffer D (7K MW cutoff)
14. Clear supernatnant with high speed centrifugation step (14K for 15 min at 4C)
15. Aliquot and snap freeze in 250-500ul aliquots with liq N2 and store at -80C. Nuclear extracts will keep for years at -80C.