Nuclear Protein Extraction (small scale) large scale protocol on pg 107.

need ~2mL/sample of Buffer A
~.5mL/sample of Buffer B

Buffer A	10cc
10mM Hepes 7.9	100λ of 1M
10mM KCl	100λ of 1M
1.5mM $MgCl_2$	15λ of 1M
	+ 9.785cc H_2O

Buffer B	10cc
20mM Hepes 7.9	200λ of 1M
20% glycerol	2mL
420mM NaCl	840λ of 5M
1.5mM $MgCl_2$	15λ of 1M
.2mM EDTA	4λ of .5M
	+ 6.941mL H_2O

Just before use, add to A&B:

5mM DTT	50λ / 10mL of 1M
.5mM PMSF	50λ / 10mL of 100mM
1:1000 Aprotinin (10mg/mL)	10λ / 10mL of (10mg/mL)

Spin down cells @ 1000rpm X ~5-10', 4°C — aspirate super.
Resuspend pellets in ~10mL PBS in 15cc tube
Spin @ 1000rpm X 5-10', 4°C & aspirate supernatant
Resuspend in 4 vol (relative to pellet) of buffer A
Incubate on ice X 1 hour.
Dounce homogenize (type B pestle) 20 strokes
Transfer suspension to an eppendorf
Spin @ 2000rpm, X5', 4°C — aspirate
resuspend in ~1mL buffer A
Spin @ 2000rpm X5', 4°C — aspirate
Resuspend in 3 vol buffer B
incubate on ice X 30'
Spin 20' high speed
Transfer supernatant to new tube
✓ protein with Bradford assay (1:100 dil, 595 nm)
Snap freeze → -70°C

→ 5λ → 800λ + 200λ bradford