Nuclear Protein Extraction (small scale) large scale protocol or p.107

Buffer A
10 mM HEPES 7.9
10 mM KCl
1.5 mM MgCl₂
+ 9.785 ml H₂O

Buffer B
20 mM HEPES 7.9
2% glycerol
420 mM NaCl
1.5 mM MgCl₂
2 M EDTA
+ 6.941 ml H₂O

Just before use, add to A&B:

| 5 mM DTT | 50X 10 ml of 1 M
| 1.5 mM PMSF | 50X 10 ml of 100X
| 1:1000 Apotinin (10 mg/ml) | 10X 1 ml of (10 mg/ml)

Spin down cells 10000 rpm, X × 10³, 4°C - aspirate supernatant
Resuspend pellets in ~10 ml PBS × 5 stroke
Spin 1000 rpm, X × 10³, 4°C - aspirate supernatant
Resuspend in 4 vol (relative to pellet) of buffer A
Incubate on ice X 1 hour
Bounce homogenize (type B pestle) 20 strokes
Transfer suspension to an eppendorf
Spin 10000 rpm, X × 10³, 4°C - aspirate
Resuspend in ~1 ml buffer A
Spin 10000 rpm, X × 10³, 4°C - aspirate
Resuspend in 3 vol buffer B
Incubate on ice X 30'
Spin 20' high speed
Transfer supernatant to new tube

Run protein with Bradford assay (1:100 dil, 595 nm)
Snap freeze → -70°C

P 5A + 800x + 2001 Bradford