NE prep. Cells from Cell Culture Center- 20L/ prep, (request early passage cells and first am delivery)
(numbers in red are from an actual prep and could change depending on your cell volume)

Buffer A = 10 mM Tris pH7.9 (4 deg C), 10 mM KCl, 1.5 MgCl2, 0.5 mM DTT (add before using) => Made 1L, autoclaved.

Buffer B = 0.3 M Tris pH7.9, 1.4 M KCl, 0.03 M MgCl2 => Made 200 ml, autoclaved.

Buffer C = 20 mM Tris pH7.9, 0.02 M KCl, 1.5 mM MgCl2, 25% glycerol, 0.5 mM DTT & 0.2 mM PMSF (add before using) => Made 200 ml, autoclaved.

Buffer D = 20 mM Tris pH7.9, 1.2 M KCl, 1.5 mM MgCl2, 25% glycerol, 0.5 mM DTT & 0.2 mM PMSF (add before using) => Made 200 ml, autoclaved.

Buffer E = 20 mM Tris pH7.9, 0.1 M KCl, 0.2 mM EDTA, 20% glycerol, 0.5 mM DTT (add before using) => Made 4 L, autoclaved.

Stocks - 1M Tris pH7.3 at RT, 2M KCl, 1 M MgCl2, 0.5 M EDTA, 1M DTT, 7 mg/ml PMSF (40 mM). All my stocks, filtered with 0.45 um filter. Tris came from adding 1M Tris-base to 1M Tris-HCl.

1. Resuspend cells in 5x packed cell volume Buffer A in four 50mL Falcon tubes (remember to add DTT).
   20 mL = packed cell volume 100mL =Buffer A added It took a while to resuspend the cells- there were still a very few chunks that wouldn't resuspend.
2. Centrifuge at 3K for 5 min. in clinical centrifuge at 4 deg C. Discard the supernatant.
3. Resuspend in 2x packed cell volume and swell for 10 min. on ice.
   40mL =Buffer A added
4. Break cells with ~95 strokes in large (40 ml) glass Dounce homogenizer- "tight" type pestle. Keep the dounces in ice while doing the strokes. Check cells under microscope for 80-90% lysis. Stain cells with trypan blue. Nuclei of lysed cells tend to clump together. Checked cells at 50 and 80 dounce strokes.
5. Spin at 4K for 15 min. in two 50mL Falcon tubes. The loose pellet is the nucleoplasmic fraction. The supernatant is the cytoplasmic fraction.
6. For S-100 prep, take the above supernatant, measure it's volume and add 0.11 volumes of Buffer B. Centrifuge this for 1 hr. at 42K in 55Ti-sw rotor. Dialyze the supernatant against Buffer E. (Instead, we added the Buffer B and froze the supernatant in a 50 mL Falcon tube at –80 C in case someone actually wants it in the future)
7. Take the nuclear pellet and measure its volume. Add 0.5 volumes of Buffer C. Gently resuspend the pellet with a pipette.
   14mL =nuclear pellet volume 7mL =Buffer C added
8. Put this with a small stir bar in a 100 ml (we used 250ml- should have used smaller one) beaker surrounded by ice. While stirring, add dropwise 0.5 volumes of nuclear pellet of Buffer D (same amount as buffer C). Adding this too quickly will cause stuff to goo up, we didn't see too much of this. Turns very snotty due to the DNA being released.
   10.5mL =Buffer D added (should have been 7 mL)
9. Stir for 30 min.
10. Spin at 10K for 30 min. in two Oakridge tubes in pre-chilled JA-20 rotor.
11. Dialyze the supernatant in 50 volumes or more of Buffer E for 2.5 hours using two 3.5 Kd MW cutoff (should have been 10 Kd cutoff?) Pierce Slide-A-Lyzer (3-15mL volume cassette)-10ml in each, although we lost maybe a ml each when we injected into the cassette-make sure you can see the needle on the other side! Change buffer and go another 2.5 hours. We saw a lot of precipitate in the cassettes. I checked the pH of Buffer A and E- Cold, it was ~7.7. I made it up at pH7.3 at room temp, expecting it to be at pH7.9 at 4 deg C as per lab NE prep protocol.
    20 mL =Supernatant volume
    1.5L =Buffer E volume for first dialysis 1.5L =Buffer E volume for second dialysis
12. Spin at 10K for 20 min. in Oakridge tubes in pre-chilled JA-20 rotor.
13. Aliquot in 100 and 200ul volumes using the multi-pipettor. Freeze on dry ice and store at –80 deg C. We ended up with ____x200ul (II), and ____x100ul (I).