Preparation of Nuclear Envelopes from Somatic Nuclei

This protocol has been adapted from the procedure of Dwyer and Blobel (1976; J. Cell Biol. 70, 581-591). This procedure entails only one digestion step. "Classical" protocols include two digestion steps.

Working Solutions
Prepare the following solutions before starting isolating or washing nuclei. Alternatively, thaw out these solutions out of the freezer:

All solutions should contain 1 mM PMSF (10 μl/ml solution) and 1 mM DTT (1 μl/ml solution), added to an aliquot just prior to use. Solutions should be kept on ice at all times.

1- TKM buffer (for 50 ml):	Tris-HCl, pH 7.5	50 mM	2.5 ml of 1 M stock
	KCl	25 mM	0.4 ml of 3 M stock
	$MgCl_2$	5 mM	0.25 ml of 1 M stock
	H_2O		47 ml

2- $MgCl_2$ solution: 0.1 mM (1 μl of 1 M $MgCl_2$ stock into 10 ml H_2O)

3- Digestion solution (50 ml):

- 10% sucrose	7.8 ml of 2 M stock (NB: 2 M sucrose = 64%)
- 20 mM Tris, pH 7.5	1 ml of 1 M stock of Tris-HCl, pH 7.5
- H_2O	41.2 ml

4- DNAse/RNase mix:

- DNAse I: 2 mg/ml in H_2O (100x stock solution). 100 μl aliquots, freeze.
- RNAse A: make a 1 mg/ml stock and dilute to 20 g/ml in H_2O (100x stock solution); aliquot into 100 μl and freeze. NB: Problem with RNAse A is possible contamination with proteases. Boil the 1 mg/ml stock solution for 15 min prior to feezing.

5- sucrose cushion (50 ml):

- 25 % sucrose	19.5 ml of 2 M stock
- 20 mM Tris, pH 7.5	1 ml of 1 M stock
- H_2O	29.5 ml

6- Tris-NaCl solution (50 ml):

- 0.5 M NaCl	5 ml of 5 M stock
- 20 mM Tris, pH 7.5	1 ml of 1 M stock
- H_2O	44 ml

7- Sucrose-NaCl cushion (to be used only when NEs are extracted with NaCl) (50ml):

- 25 % sucrose	19.5 ml of 2 M stock
- 0.25 M NaCl	2.5 ml of 5 M stock
- 20 mM Tris, pH 7.5	1 ml of 1 M stock
- H_2O	27 ml

Procedure

- start with 1-2 ml of packed purified nuclei
- wash nuclei in 5 ml of TKM buffer at 1,000 rpm (200 g), 10 min, 4°C
- decant supernatant
- while vortexing, add 4 ml of 0.1 mM $MgCl_2$ solution. It is important to vortex or else nuclei are impossible to resuspend
- add 16 ml of Digestion Solution and mix by inversion
- add 100 μl of DNAse/RNAse mix (or 50 μl each of DNase and RNase) and mix well
- incubate 30 min at RT on a shaker. The nuclear suspension becomes first very viscous, but clarifies over time as the DNA is digested. Prolong incubation time if necessary until solution turns clear.
- transfer solution into two 15 ml Corex glass tubes
- underlay solution with 3 ml sucrose cushion
- centrifuge at 10,000 g in Sorvall or Beckman centrifuge for 15 min. The NEs pellet at the bottom of the tube
- remove the supernatant, wash the walls of the tube as per protocol and removel all supernatant
- resuspend the pelleted NEs into 500 μl TKM buffer
- keep on ice until use or freeze in TKM buffer
- Alternatively, NEs can be washed with 0.5 M NaCl at this stage if necessary (see below).

Note: if significantly smaller volumes of NEs are dealt with initially, adjust the volumes accordingly and work when possible in 1.5 ml Eppendorf tubes. Use a table-top centrifuge if possible with a swing-out rotor.

Wash of nuclear envelopes with 0.5 M NaCl

This removes some nucleoporins but does not extract the lamina.

- resuspend the pellet of NEs into a minimum volume of TKM (~100 μl); transfer into a 1.5 ml tube
- add drop-wise 1 ml of Tris-NaCl solution while vortexing
- incubate 10 min on ice
- underlay with 0.5 M sucrose-NaCl cushion
- centrifuge at 10,000 g for 10 min in a swingout rotor at 4°C
- remove the supernatant after washing the tube wall
- resuspend the pellet in TKM buffer for further use or freezing