

# Quick and Easy Nuclear Extracts

## Notes

- i) The method appears to work best with fresh (not frozen) cells
- ii) This will yield a splicing-competent extract *in theory*; in practice it might take you a few goes to obtain an extract that works
- iii) The volumes depend on your initial packed cell volume. I've given examples for a 1 ml pellet, which I obtained from five T175s of sub-confluent (i.e. rapidly growing) HEK293 cells.
- iv) Add 1 mM DTT, PMSF, leupeptin/'Complete' etc. ('goodies') to HLB and NEX just before use.

## Reagents

Make these 'RNase-free', but avoid DEPC (because friends don't let friends use DEPC).

Hypotonic Lysis Buffer (HLB):

- 1.5 mM MgCl<sub>2</sub>
- 10 mM KCl
- 10 mM HEPES-KOH pH 7.9

Extraction Buffer (NEX):

- 1.5 mM MgCl<sub>2</sub>
- 0.42 M NaCl
- 0.2 mM EDTA
- 25% v/v glycerol
- 20 mM HEPES-KOH pH 7.9

10% IGEPAL (NP-40) in water

Buffer D:

- 100 mM KCl
- 0.2 mM EDTA
- 20% glycerol
- 20 mM HEPES-KOH pH 7.9
- 1 mM DTT, PMSF added fresh

## Method

1. Harvest cells with trypsin in the usual way and wash twice with PBS (use 5 minute, 400 x g spins). Estimate the volume of the packed pellet.
2. Gently resuspend the cell pellet in 4 volumes cold HLB+ goodies (1 ml cells+ 4 ml HLB). Sit on ice for 15 minutes. Check under microscope that cells have swollen.
3. Add IGEPAL to 0.6% final (300 µl of 10% stock), mix and incubate on ice, 5 minutes.
4. Centrifuge for 5 minutes at 400 x g, 4°C.
5. Resuspend the pellet (nuclei) in 4 volumes (4 ml) NEX + goodies. Mix at 4°C for 15 minutes.
6. Pellet debris by centrifugation at 10k x g for 30 minutes at 4°C.
7. Dialyse against 0.5 l appropriate buffer overnight (e.g. Buffer D for splicing extract), and against fresh buffer for 4 hours, all at 4°C.

8. Centrifuge for 20 minutes to remove any cloudiness.
9. Freeze drop-wise directly into liquid nitrogen and store at  $-80^{\circ}\text{C}$ .