Rapid, Small-Scale Subcellular Fractionation of Yeast to Produce Nuclei and Nuclear Envelops

Yeast nuclei and NEs are prepared on a small scale using the methods below, which are modifications of previously described protocols, where detailed solution formulations can be found (Rout and Kilmartin, 1990; Rout and Kilmartin, 1994; Strambio-de-Castillia et al., 1995).

1. A 1-2 l YPD culture of the haploid protein A- tagged yeast strain was grown at 30°C to ~5 x 10^7 cells/ml. The cells were harvested in the appropriate number of 500-ml centrifuge pots (3000 x g for 5 min), washed in distilled water, resuspended to an approximate volume of 50 ml, placed in a 50 ml graduated Falcon tube and centrifuged in a swing-out rotor at ~1,500x g for 5 min.

2. The cell pellet was resuspended in 100 mM Tris-Cl, 10 mM DTT pH 9.4 and incubated for 10 min at 30°C.

3. The cells were washed in 1.1M sorbitol and resuspended in a volume of 1.1M sorbitol equal to the volume of the packed cell pellet. To this volume was added 0.1 volumes glusulase, 0.01 volumes 1% zymolyase 20T / 1% mutanase, and the mixture incubated at 30°C for 3-4 hr.

4. The resulting spheroplasts were then pelleted in a swing-out rotor at 1,500 x g for 10 min at 4°C, washed once in 1.1 M sorbitol, resuspended to a total of 20 ml with 1.1 M sorbitol, and overlayered onto a cushion of 7.5% Ficoll-400 in 1.1M sorbitol and centrifuged in a swing-out rotor at 10,500 x g for 15 min at 4°C.

5. Each spheroplast pellet was resuspended with a Polytron into 20 ml of ice-cold 8% PVP solution, 100 ml 1 M DTT, 200 ml Solution P, 50 ml 10% Triton X-100. Each tube was then gently underlayered with 10 ml of a 0.3 M sucrose-PVP solution, and centrifuged in a swing-out rotor at 16,500 x g for 20 min at 4°C.

6. This resulted in a cytosolic supernatant and a crude nuclei pellet; the latter was resuspended with 6 ml of cold 1.7 M sucrose-PVP containing 60 ml Solution P, adjusted to a refractive index of 1.425 with 2.30 M sucrose-PVP (making a total volume of ~12 ml), overlayered onto Beckman SW28 stepped gradients of 2.01 / 2.10 / 2.30 M sucrose-PVP, and centrifuged in an SW28 rotor at 28,000 rpm for 8 hr at 4°C.

7. ~50 OD_{260} units of nuclei were then mixed with 0.2 volumes of 8% PVP solution and 0.01 volumes of Solution P in a Sorvall 10 ml Ultra bottle and centrifuged in a Sorvall T-875 rotor, 40,000 rpm, 1 hr, 4°C.

8. The nuclei pellet was resuspend by vortexing into 0.5 ml of 10 mM bisTris-Cl pH 6.50, 10mM $CaCl_2$, 10mM $ZnCl_2$, plus 0.5 ml 2.0% DNase I and 5 ml Solution P. 2.0 ml of Sucrose / Nycodenz solution was mixed in after ~5 min at room temperature, and this was then placed in a Beckman SW55 centrifuge tube.

9. After sequentially overlaying with 1.5 ml of 2.25 M sucrose-bt containing 0.005 volumes of Solution P and 1.5 ml of 1.50 M sucrose-bt plus 0.005 volumes of Solution P, the tube was centrifuged in a Beckman SW55 rotor at 50,000 rpm for 24 hr at 4°C and the NEs recovered from the 1.50 M / 2.25 M interface.