Isolation of nuclei from cells.

Reagents

**Buffer A**
20 mM Tris pH 7.5–8.0
100 mM NaCl
300 mM sucrose
3 mM MgCl₂
*Buffer A contains sucrose and should be kept frozen at -20°C.*

**Buffer B**
20 mM Tris pH 8.0
100 mM NaCl
2 mM EDTA pH 8.0
*Keep at 4°C.*

**Buffer C**
20 mM Tris pH 8.0
100 mM NaCl
2 mM EDTA pH 8.0
2% SDS
*Keep at room temperature.*

Method

1. Prepare 1 ml of buffer A with added cocktail of usual protease inhibitors from frozen stock and store on ice.
2. Add 500 µl buffer A per large petri dish on ice and scrape thoroughly. Leave on ice for 10 min.
3. Centrifuge at 4°C at 3,000 rpm for 10 min.
4. Remove supernatant and keep. This will contain everything except nuclei.
5. On ice resuspend the pellet in 374 µl if buffer B and add 26 µl of 4.6 M NaCl to give 300 mM NaCl (high salt helps lyse membranes and forces DNA into solution).
6. Homogenize with 20 full strokes in Dounce or glass homogenizer on ice.
7. Leave on ice for 30 min.
8. Centrifuge at 24,000 x g for 20 min at 4°C.
9. Aliquot supernatant, remove 10 µl for protein quantification (ab102536) and store at -70°C.