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| **Nuclear extraction**  Last modified 11.01.2012 |
| **Materials** |
| * Phosphate buffered saline (PBS) * Buffer A (low salt): 10mM HEPES-KOH pH 8.0, 1.5 mM MgCl2, 10 mM NaCl * Buffer A\*: Buffer A + protease inhibitors + 0.15% NP40 * Buffer C (high salt): 20mM HEPES-KOH pH 8.0, 2 mM MgCl2, 420 mM NaCl, 20% (v/v) glycerol, 0.2 mM EDTA, 0.1% (v/v) NP40. **Add fresh before use: 0.5 mM DTT, complete protease inhibitors mini EDTA free (1 tablet/10 ml)** (Roche 11836170001) * Fresh cells (not frozen pellets) |
| **Equipment** |
| * Glass douncer with type B pestle (in 500 µl, 2ml, 7 ml and 15 ml volumes) * Cooled bench centrifuge (Heraeus Multifuge 3 S-R; for 15, 5 or 500 ml tubes) * Cooled tabletop centrifuge (Heraeus Fresco 21; for 1.5 or 2 ml vials) |

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| **Procedure** | **Comments** |
| **A) Harvesting cells (adherent cells)** | |
| 1. Wash cells with 10 ml PBS 2. Trypsinise with 1.5 ml trypsin/15 cm dish 3. Resuspend with 10 plates with 10 ml of medium 4. Pool in a 50 ml tube 5. Repeats steps 3-4 6. *Perform all subsequent steps at 4 °C/on ice* 7. Centrifuge cells 5 min, 400 g, 4 °C. Remove supernatant | * Before starting, pre-cool buffer A, buffer A\*, buffer C , PBS and the douncer on ice * For recommended douncer volumes, refer to step 24 comments * These steps may vary; in general, wash, trypsinise, neutralise, resuspend and pool |
| 1. Resuspend cells with 50 ml PBS 2. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant 3. Repeat steps 8-9 4. Resuspend cells with 8 ml PBS and transfer to the 15 ml tube 5. Rinse the 50 ml tube again with 5 ml PBS and transfer to the 15 ml tube 6. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant |  |
| **B) Harvesting cells (suspension cells)** | |
| 1. Centrifuge 500ml of cells in a 500ml tube for 5 min, 400 g, 4 °C. Remove supernatant 2. Resuspend cells with 50 ml PBS 3. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant 4. Repeat steps 14-15 5. Resuspend cells with 8 ml PBS and transfer to the 15 ml tube 6. Rinse the 50 ml tube again with 5 ml PBS and transfer to the 15 ml tube 7. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant |  |
| **Mechanical lysis of cells** | |
| 1. Determine cell pellet volume and resuspend cells with 5 volumes of buffer A 2. Incubate on ice for 15 min 3. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant | * **Cell pellet should swell after incubation** with buffer A. Increase in cell volume may vary depending on cell type. * While waiting, wash douncer with demi water and rinse with buffer A. |

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| 1. Determine cell pellet volume and resuspend in 2 volumes of **buffer A\*** 2. Apply 3 x 10 to 4 x 10 strokes. Wait 30s after every set of strokes 3. Transfer the cell suspension to a 15 ml tube 4. An aliquot of cell suspension can be checked under a microscope to see whether the cells have lysed from douncing. Perform during step 28. | * **Recommended douncers**:   + 0.1 ml douncer: >50 µl swelled pellet   + 2 ml douncer: 50-600 µl swelled pellet   + 7 ml douncer: 600-2500 µl swelled pellet * **The plunger must remain in the cell suspension** to avoid air bubbles * The 30s rests between sets of strokes prevent heating up of the cell suspension that may result in protein instability * Douncing opens up cells (but not nuclei) |
| 1. Centrifuge 15 min, 3200 g, 4 °C 2. Transfer or discard the supernatant (**cytoplasmic extract (CE)**) | * **To store the CE, add glycerol (10% final conc.) and NaCl (150 mM final conc.)**. If the nuclear extraction has priority perform after step 29 |
| **Lysis of nuclei** | |
| 1. Determine the volume of cell pellet (**crude nuclei**) 2. Wash the pellet with 10 volumes of PBS. **The nuclei are very fragile. Quickly add the PBS and invert. Do not pipette up and down.** 3. Centrifuge 5 min, 3200 g, 4 °C. Discard supernatant |  |
| 1. Determine the volume of cell pellet 2. Add 2 volumes of buffer C 3. **Cut the tip of a 1 ml pipette and resuspend as best as possible by pipetting up and down vigorously** 4. Transfer the suspension to 1.5 or 2 ml vials 5. Incubate 1 h, 4 °C on a rotating wheel | * After incubation, the suspension will become viscous and white clouds of chromatin should appear |
| 1. Centrifuge on a table-top centrifuge for 45 min, 20,800 g or max speed, 4 °C 2. Aliquot the supernatant (**nuclear extract (NE)**) and snap freeze with liquid nitrogen 3. Store at -80 °C | * An aliquot of NE can be analysed for protein conc. via Nanodrop. Expected conc.: ~5 mg/ml * The pellet contains chromatin |