Nuclei Isolation from Tissue Culture Cells

Nuclei Isolation

- Thaw frozen cell pellets, put on ice and let them come to ~4°C
  *if using frozen tissue, let thaw out a little bit. Cut into small cubes with a razor first and then proceed
- Add NIB-250+0.3% NP-40 to a final ratio of 10:1 (10mL of buffer for 1mL cells/tissue)
- Mix cells by pipetting gently and incubate on ice for 5 min
  *if using tissue, gently use a dounce homogenizer to more fully dissolve the tissue cubes in the NIB buffer. This should be a cloudy homogenous tissue milkshake when you are finished.
- Pellet the nuclei at 600 rcf for 5 min at 4°C (nuclei pellet should be smaller and much whiter in color than whole cells)
- Transfer sup (this is the cytoplasmic extract) to a different tube and keep aside for RNA extraction if wanted or just discard if not
- Resuspend the nuclei pellet gently with 10:1 NIB-250 (without NP-40 detergent) for washing
- Pellet nuclei at 600 rcf for 5 min at 4°C (discard supernant)
- Resuspend again with 10:1 NIB-250 and aliquot no more than 1mL of sample in 1.5mL tubes and keep remaining suspension
- Spin down all nuclei samples (again at 600 rcf for 5 min at 4°C), discard supernatant and either freeze collected nuclei in liquid nitrogen or use promptly for acid extraction of histones

NIB-250 (Nuclei Isolation Buffer) *****This general buffer can be pre-made and frozen, and below inhibitors added fresh each time

15mM Tris-HCL (pH 7.5)
60mM KCl
15mM NaCl
5mM $MgCl_2$
1mM $CaCl_2$
250mM Sucrose

Add reagents below to pre-made NIB buffer fresh each time:
1mM DTT (or 0.1% v/v β-mercaptoethanol)
2mM sodium vanadate (or any commercial phosphatase inhibitor cocktail tablet)
0.5% v/v MPI or AEBSF (or any commercial protease inhibitor cocktail tablet)
10mM sodium butyrate (histone deacetylase inhibitor)

Standard Acid Extraction of Histones Protocol

- To a gently vortexed pellet of isolated nuclei, slowly add while vortexing 0.4N H_2SO_4 at a 5:1 final ratio (add 5mL for a 1mL pellet)
- Let this sit on ice for at least 1 hour or as long as overnight, mixing intermittently. For a million cells use a 2 hour incubation, for lower populations of cells much longer incubations.
- Spin down in refrigerated centrifuge at 3400 g Transfer the sup to a 15mL conical tube
- Repeat first two steps to generate a second supernatant, and combine with the first
- To the supernatant, add 25% of the pooled sup volume of 100% TCA (vol. TCA= vol. sup/4) to give 20% TCA final
- Let this mixture precipitate for at least 1 hour or as long as overnight (for very dilute extracts)
- Spin down in refrigerated centrifuge at 3400 g and discard supernatant
- Re-suspend pellet in acetone w/ 0.1% HCl by vortexing and using a sealed glass pipette (also, only use glass to transfer acetone!!)
- Spin down in refrigerated centrifuge at 4000 g and discard sup
- Wash in 100% acetone 2 times
- Air dry final pellet, what is left is histone and can be re-suspended in ~50 µL of water
- Check purity and mount of histone by running 5-10% of extraction on a 15% SDS-PAGE gel and stain with Coomassie Blue.
- If possible, check concentration by protein assay and adjust volume to have a roughly 1 µg/µL concentration.