S.pombe – Simple Protein Extraction

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NOTE 1: As used for confirming TAP-tag integration – this protocol is NOT recommended if you want to look at chromatin components (particularly things like modified histones).

NOTE 2: All buffers are pre-chilled on ice unless stated otherwise.

NOTE 3: Major advantage of this protocol - all manipulations can be done in a single tube, increasing throughout.

1. Grow 10 ml culture to OD_{600} ≈ 0.5 (or even easier, collect cells from a freshly sectored plate). Harvest by centrifugation (pellet volume ≈ 50µl). Remove supernatant, resuspend cells in 1 ml ddH_2O (see **NOTE 2**) and transfer to eppendorf tube. Flash spin (5 sec, 12K RPM) to collect pellet and discard supernatant.

2. Add 200µl Modified TEG (40mM Tris-HCl pH 7.5, 1 mM EDTA, 10% Glycerol, 0.1% NP-40, 150 mM NaCl, 1 µg/mL leupeptin, 1µg/mL aprotinin, 1µg/mL antipain, 1µg/mL pepstatin A, 1 mM PMSF (see **NOTEs 4** & **5**) and resuspend by pipetting. Add acid-washed glass beads to level of the meniscus (about 200-250µl).

   NOTE 4: Leupeptin, aprotinin, antipain, pepstatin A stocks all 1000 x (1mg / ml) at -20°C.

   NOTE 5: PMSF is highly toxic (LD50 <500mg / kg) - take care when weighing it out (stock 100mM in MeOH). Add to working solutions just before use: unstable in aqueous: half-life 110 min at pH 7, 35 min at pH 8.

3. Vortex 5 - 6 cycles (1 min vortex, 1 min on ice; ≈ 90% cell breakage).

5. Microfuge 5 sec / 1K rpm (low speed spin to collect glass beads). Add 200 µl 2X reducing loading buffer (see below) and vortex 10 sec. Microfuge 5 sec / 1K rpm. Boil 5 min 95°C. Spin 12K / 5min / RT°C. Resolve 20-30 µl on an SDS-PAGE mini gel of the appropriate % resolving gel. It is not necessary to transfer the S/N to a new ependorff tube – just remove the supernatant above the glass beads / junk.

2 x reducing loading buffer -

60mM Tris pH 6.8
2% SDS
10% Glycerol
0.2% Bromophenol Blue
100mM DTT