Protocol for FLAG or HA AP-MS in yeast using magnetic beads

2010/05/18 – updated by ACG
(adapted from Breitkreutz et al., Science, 2010)

Protocol type: Biological Material
Protocol name: Growth and gal induction for HTP yeast project

Plasmids encoding galactose-inducible GAL1-ORF-FLAG or GAL1-ORF-HA were transformed into the yMT2398 pep4delta strain, and a single colony was grown to saturation at 30 degrees C in 1 ml SC medium (SC; 0.2% amino acid mix, 0.5% ammonium sulfate, 0.17% yeast nitrogen base) lacking leucine, supplemented with 0.1% glucose and 2% raffinose. An aliquot of this culture was spiked into 340 ml SC -leu 0.1% glucose 2% raffinose, and cultured for 16 hours at 30 degrees C to reach an OD600 of 0.3-0.5. Bait expression was induced for 2 hours by adding galactose to a final concentration of 2%. Cultures were harvested by spinning at 5500 rpm at 4 degrees C for 2 min. The pellet was resuspended in 1.5 ml of cold harvest buffer (50 mM Hepes (pH 7.5), 150 mM NaCl, 5 mM EDTA). Cell pellets were snap-frozen in liquid nitrogen and stored at -80 degrees C (this yields 2 x 0.3 g pellets).

Protocol type: Affinity Purification
Protocol name: ProteinA-magnetic bead IP for HTP yeast project

Preparation of ProteinA-antibody beads:
Protein A magnetic bead slurry (16 ul for 4 IPs; Invitrogen cat no 100.01D) was washed 3 x 1 ml with PBS (no Mg and Ca). PBS (268 ul) and the appropriate antibody (32 ul; concentration of 1mg/ml for FLAG, Sigma F3165, 0.2mg/ml for HA, Santa-Cruz SC-7392), were added to the beads, and the mixture incubated with nutating at 4 degrees C for 90 min. Beads were then washed twice (1 ml) in lysis buffer, resuspended in 40 ul lysis buffer, and 10 ul used for each IP (equivalent to 4 ul initial slurry).

Immunoprecipation:
An equal volume of cold lysis buffer (50mM Hepes (pH 7.5), 150mM NaCl, 5mM EDTA, 0.1% NP-40, 5mM DTT and 1X Roche Complete protease inhibitors without EDTA (REF 11873580001)) was added to frozen cell pellets, and cells thawed slowly by warming tubes in hands. Once pellets were thawed, acid-washed glass beads were added to the tubes until the beads reached the level of the solution. Cells were lysed with a Retch agitator set at speed 30 1/s for 1 min cycles in cold blocks, putting tubes in iced water for 1 min between cycles (two cycles typically resulted in >75% lysis). The double-tube method was used to clean the lysate using a bench-top centrifuge at 2500 rpm for 2 min, at 4 degrees C. The supernatant was transferred to cold 1.5 ml tubes on ice and spun at 14000 rpm, 4 degrees C for 15 min. (Optional: Save 5 ul of lysate supernatant to check bait expression.) The supernatant was passed through a pre-wetted 0.45 um syringe filter and placed into fresh 1.5 ml tubes. Benzonase (250U/ul) was added at 2.5 ul/ml of lysate, and the sample incubated on ice for 15 min. 10 ul of ProteinA-bound beads were aliquotted into filtered lysate and the sample incubated on the nutator for 1 hour at 4 degrees C. Tubes were placed into a magnetic holder to pull beads out of solution, and the supernatant was decanted. The resin was washed once with 500 ul lysis buffer by pipetting up and down 10 times. Beads were transferred to a clean chilled eppendorf tube, re-magnetized and the supernatant decanted. Samples can be snap-frozen in liquid nitrogen and stored at -80 degrees C.