Production of GST-tagged recombinants in *E.coli*

Glutathione agarose is relatively cheap and it's an easy matter to elute the recombinant proteins by competition with glutathione

NOTE 1: As with other recombinant protocols, the choice of *E.coli* strain (see ***E.coli* strains list**) is influenced by a number of factors. Note that recombinant protein expression is generally not done in a standard *E.coli* strain as used for cloning. See strains list at http://mckeogh.googlepages.com/protocols.

As used: To make HIS-GST-CTD-HA_3: Keogh *et al* (2004) *MCB* **23:**7005.

NB. Is the expression vector Ampicillin or Kanamycin ? Double check

1. Grow 100ml culture *E.coli* (Strain: BL21/DE3 or equivalent) overnight at 37°C. Sub-innoculate next morning 1/100 (2-4L of culture: volumes given are for 4L, so scale accordingly) and grow to $OD_{600} \approx 0.6$ at 37°C. Chill cultures 15 minutes on ice and induce with 0.1 - 0.5mM IPTG (can be determined empirically) at RT° (3 hours to overnight): see **NOTES 2 & 3**.

   NOTE 2: Growth at RT° can significantly aid the yield of an insoluble protein. In general induction is always done at this temperature.

   NOTE 3: To determine optimal conditions induce a small scale culture and take 1ml samples at each time point (**including a pre-induction**). Collect cells by micro-centrifugation (6000rpm, 5min, RT°C) and discard supernatant. Resuspend pellet in 10µl *GST buffer* A (*0.1M NaCl)* and add 15µl *2 x Reducing loading buffer*. Vortex, 95°C, 5 min and micro-centrifuge (14000rpm, 5min, 4°C). Resolve supernatant by SDS-PAGE and stain gel with Coomassie: look for the appearance of new bands.

2. Collect cells by centrifugation and transfer to falcon tubes with ice-cold ddH_2O (can freeze pellets -80°C at this point). Resuspend in 50mls *GST buffer* A (*1M NaCl)* (4°C) and lyse on ice by sonication (4 x 20s pulses, 50% output, 40 duty cycle). Add Triton X-100 to 1%.

   NOTE 4: Increasing sonication increases background over yield – don't.

3. Collect insoluble material by centrifugation (10K, 20m, 4°C) and transfer the supernatant to a falcon tube on ice. Add 8mls preswollen washed *Glutathione Agarose* (**NOTE 5**). Place on rotator, RT°, 10mins to bind. Collect by centrifugation (benchtop: 1500rpm, 5m, 4°C) and batch wash:
   - 3 x 40ml *GST buffer* A (*1M NaCl)*
   - 3 x 40ml *GST buffer* A (*0.1M NaCl)*

   NOTE 5: Glutathione Agarose (**Sigma G4510**, 5ml, $129). Store at –20°C as powder, but 4°C when swollen: one gram powder to 14ml gel. Swell in ddH_2O but equilibrate into appropriate buffer before use. As required collect by centrifugation in a benchtop: 1500rpm, 5m. Do not exceed these speeds: the beads can't take it.

4. Pack into disposable column (ensure no bubbles or gaps) with *GST buffer* A (*0.1M NaCl)*. Elute with 10ml *GST buffer* A (*0.1M NaCl)* ***+ 10mM Glutathione*** (L-Glutathione, reduced: **Sigma G4251**, 10g $52.60: MW 307.32). Collect 1ml fractions. The bulk of the protein will generally elute in fractions #2 and #3 (see sample purification: **pH10.124**)

5. Analyze fractions by Biorad Dc and SDS-PAGE. Estimate the cleanliness of the prep (how many bands visible on SDS-PAGE ?) and yield per L. Pool positive fractions and dialyze into buffer of choice.

Buffers:

GST buffer A	*Stock*	*1L*
20mM Tris pH 7.4	1M	20ml
100mM – 1M NaCL	5M	20 – 200ml
0.2mM EDTA	0.5M	400µl
1mM DTT	1M	1ml

Protease inhibitors as standard
Leupeptin, pepstatin-A, aprotinin (all 1000x stocks at –20°C)
Add just before use (PMSF is unstable in aqueous)
1mM PMSF (Stock 100mM in MeOH)

10mM Glutathione as required (**step 4**)

2x reducing loading buffer

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