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

Protocol attempts to recover soluble and insoluble fractions
(some proteins will exclusively partition into one or the other)

NOTE 1: The choice of expression vector and *E.coli* strain (see ***E.coli* strains list**) is influenced by a number of factors. For example, consider whether the protein is toxic. If so it may be necessary to tag with an export sequence to transport it to the periplasmic space. Alternatively expression can be tightly regulated for a short induction. Note that recombinant protein expression is generally not done in a standard *E.coli* strain as used for cloning.

As used: To make HIS-Tfb1 and HIS-Tfb3 for polyclonal Rabbit-Ab production (Keogh *et al* (2002) *Mol Cell Biol* **22:**1288).

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) and grow to $OD_{600} \approx 0.6$ at 37°C. Chill the cultures 15 minutes on ice and induce with 0.1 - 0.5mM IPTG (determine empirically; 0.5mM if you can't be bothered) at RT° (3 hours to overnight: determine empirically).

NOTE 2: Growth at RT° significantly aids the yield of insoluble proteins, so is done as standard. Optimal conditions for production of HIS-tagged TFIIH subunits Ccl1, Tfb1 and Tfb3 have been published (Feaver *et al*, (1997) JBC **272:**19319). Note that these three proteins are predominantly insoluble. For Rtt103 (MCK notebook pH14.75) it was about 50:50, but the insoluble preparation was much cleaner.

2. Collect cells by centrifugation and transfer to 2 x falcon tubes (following volumes are for equivalent of a 4L culture) with ice-cold ddH_2O (can freeze pellets -80°C at this point). Resuspend in 50mls (2 x 25mls) *Soluble Lysis buffer* (4°C) and sonicate on ice (4 x 20s pulses 50% output, 40 duty cycle). Add Triton X-100 to 1%.

3. Collect insoluble material by centrifugation (10K, 15m, 4°C) and transfer the soluble supernatant to a falcon tube on ice. Resuspend pellet in 50ml (2 x 25ml) *Insoluble Lysis buffer* **(RT°)**. Centrifuge again (10K, 15m, 25°C) to remove any really insoluble cell wall components, etc. Discard the junk and transfer the insoluble supernatant to a fresh falcon tube (RT°).

4. Bind the two supernatants (soluble and insoluble) in batch to 3ml Ni-NTA agarose for 2hrs at 4°C or RT° (as appropriate). Wash columns with:

- 25ml appropriate lysis buffer (10mM imidazole)
- 25ml appropriate lysis buffer (25mM imidazole)
- 25ml appropriate lysis buffer (50mM imidazole) (be careful: could be a little high ?)

Elute with 10ml appropriate lysis buffer (500mM imidazole). Collect 1ml fractions.

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 to remove the imidazole. Store in aliquots at –20°C.

Buffers:

Soluble Lysis buffer	*Stock*	*1L*
10% Glycerol	1 M	100ml
20mM HEPES pH 7.6	1 M	20ml
500mM NaCL	5 M	100ml
0.2% Tween 20	100%	2ml
2mM Imidazole	5 M	400µl
5mM β-ME	14.25M	350µl
Protease inhibitors as standard		

Insoluble Lysis buffer	*Stock*	*1L*
8M Urea (MW60)		480g
10mM Tris (pH8)	1 M	10ml
100mM Phosphate (pH8):	1M Na_2HPO_4	93.2ml
	1M NaH_2PO_4	6.8ml
2mM Imidazole	5 M	400µl
5mM β-ME	14.25M	350µl
Protease inhibitors as standard		

Dialysis buffer **(example)**	*Stock*	*1L*
10mM Tris.Ac pH 7.5	2 M	5ml
150mM KAc (pH7.5)	5 M	30ml
1mM EDTA (pH8.0)	500mM	2ml
1mM DTT	1 M	1ml
1mM MgAc	1 M	1ml
Protease inhibitors as standard		