

## Expression and Purification of Glutathione S-transferase (GST) Fusion Protein

### Day 1

1. Transform plasmid into E. coli BL21 (DE3) and plate out.

Transformation:

- 1) Thaw 50ul competent cells on ice
- 2) Add 1-3ul (1-10ng) of plasmid to 50ul comp cells
- 3) Tap to mix
- 4) Leave on ice 30 min
- 5) Heat shock at 37°C for 20sec
- 6) Ice 2min
- 7) Add 950ul LB (no AMP) and shake at 37°C for 30 min - 1hr
- 8) Plate on LB AMP plate

### Day 2

1. Pick colony and inoculate 2ml LB AMP overnight culture (37°C)

### Day 3

1. Add 2ml culture to 200ml 2xYT broth in 1L erlenmeyer
2. Grow ~3hrs at 27°C until ~0.600 absorbance at 595nm
3. Add IPTG to 0.4mM and continue growing 2-4 more hrs. at 27°C
4. Pellet bacteria by cent. 5min at 5000rpm in Sorvall GSA 600
5. Store pellet at -80°C

### Day 4 (note: remember to pre-chill centrifuge rotor)

1. Resuspend pellet in 1/5 original culture vol. of lysis buffer -40ml for a 200ml culture- (50mM tris 8.0, 5mM EDTA, 10% glycerol, 0.5% NP40 (NP40 now called IGEPAL by sigma), 50mM NaCl)
2. Add PMSF to 1mM, DTT to 1mM (also add aprotin & leupeptin to 1X if available)
3. Incubate 15min at room temp w/ occasional shaking
4. Sonicate 3x 20sec on ice w/ 1min interval
5. Centrifuge sonication lysate in SA600 for 10min at 10k rpm
6. Save SUPERNATANT in 50ml tube
7. In a 50 ml tube, pre-wash sigma #4510 GT agarose beads (1/100 of starting culture volume) twice, each w/ 5ml lysis buffer (w/ DTT and PMSF) in 15ml tube. Spin down each wash in 4°C table top cent., 1000rpm for 2min.
8. Add supernatant to pre-washed GT agarose beads in 50ml tube and incubate at 4°C 4hrs- overnight w/ gentle rocking.

### Day 5 (if step 8 is done overnight)

1. Spin down beads 2min at 1000rpm
2. Wash beads w/ 20ml cold PBS
3. Transfer bead slurry to BioRad column (empty glass tube w/ yellow parts on ends)
4. Elute bound GST-fusion protein w/ 5ml FRESH 5mM glutathione (dissolve 0.023g glutathione powder in 15ml of 50 mM Tris pH 8.0). Collect one ml fractions. (protein usually comes off in first two)- aliquot and store at -80°C

Lysis Buffer for protein expression:

For 1L:

-50 ml 1M Tris pH 8.0

-10ml 0.5 M EDTA

-2.92g NaCl

-5 ml NP40 (now called IGEPAL by Sigma)

-100ml glycerol

-water to 1L