GST-fusion protein expression and purification

1. Use a single colony of E. coli cells containing a recombinant pGEX plasmid to inoculate 10 ml of 2×YT medium (containing 10µL Ampicillin (50 mg/mL))

2. Incubate overnight at 37°C with vigorous shaking.

3. Transfer 2 mL of culture to a 20 mL 2xYT medium with 20 µL Ampicillin
(note: culture volumes can be scaled up or down as needed)

3. Incubate at 37ºC until A_{600} reaches 0.6- 0.8 (~1-2hrs).

4. Induce expression by adding 25µL 100mM IPTG to the culture. Incubate at 37ºC with shaking for 3 hours

5. Centrifuge at 3,000rpm for 5 mins at 4ºC and discard the supernatant. (pellet can be stored at -80oC for weeks)

6. Resuspend pellet with 1mL PBS on ice. Add 1x protease inhibitors and 10µL 100 mM PMSF.

7. Add 100µL Lysozyme (10mg/ml stock) and incubate samples on ice for 30min

Note: other means of cell lysis, like sonication, can also be performed

8. Add 5µl DNase and incubate samples at 30oC for 1hr

9. Add 5µl of Triton X-100 and incubate with gentle rocking at room temperature for 15mins

10. Centrifuge at 2,000rpm for 5 minutes at 4ºC and save the supernatant (can be stored at -80ºC).

Purification

1. Prepare a 50% slurry of Glutathione Sepharose 4B (Amersham Biosciences): Equilibrate slurry in PBS, spin for 2 mins at 2000rpm and discard wash. Add equal volume of PBS to generate 50% slurry.

2. Add 100-200µL Glutathione Sepharose 4B slurry to cell lysate and incubate with gentle rocking for 30 minutes at RT

3. Centrifuge at 2000rpm for 2 min.

4. Aspirate off supernatant without getting too close to the beads and wash 4 times with 1X PBS by inverting the tube until the beads are resuspended. Centrifuge for 2 mins at 2000rpm to pellet beads).

5. Purified proteins can be stored at -20oC

6. Recombinant protein can be eluted from GST beads for blot overlay assays. Aliquot protein and elute in equal volume of elution buffer (Elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0) by mixing for 2-3 mins at RT. Consecutive elutions can be performed (usually up to 3 times). Eluted proteins can be store at -20°C

7. Analyze proteins by SDS-PAGE and Coomassie staining

2xYT medium (1L):
16 g tryptone,
10 g yeast extract,
5 g NaCl
Sterilize by autoclaving