Purification of GST-fusion protein expressed in E.coli ver. 4

Screen of GST-Fusion Protein Expression
(pGEX system by Amersham: for check clones for expression of the desired fusion protein prior to large-scale purification)

1. Pick several colonies of *E.coli* transformed with the pGEX recombinants into separate tubes containing 2 ml of 2 x YTA medium.
   -Note: For comparison, it is advisable to inoculate a control tube with bacteria transformed with the parental pGEX plasmid.
2. Grow liquid cultures to an A_{600} of 0.6-0.8 (3~5 h) with vigorous agitation at 20~37˚C.
3. Incubate fusion protein expression by adding 2 µl of 100 mM IPTG (final concentration 0.1 mM).
4. Continue incubation for an additional 1~2 h
5. Transfer 1.5 ml of the liquid cultures to labeled 1.5 ml microcentrifuge tubes.
6. Centrifuge in a microcentrifuge for 5 sec and discard the supernatant.
7. Resuspend each pellet in 300 µl of ice-cold 1 x PBS, remove 10 µl of these resuspend cells into labeled tubes (for later use in SDS-PAGE analysis).
   -Note: Except where noted, keep all samples and tubes on ice.
8. Lyse the cells using the sonicator equipped with an appropriate probe.
   -Note: Lysis is complete when the cloudy cell suspension becomes translucent. The frequency and intensity of sonication should be adjusted such that complete lysis occurs in 10 sec, without frothing (it may denature proteins).
   -Note: Crude sonicates can be screened for the relative level of expression of GST fugion proteins using the GST substrates CDNB (1-chloro-2,4-dinitrobenzene).
9. Centrifuge in a microcentrifuge for 5 min to remove insoluble materials. Save a10 µl aliquots of the insoluble material for analysis by SDS-PAGE. Transfer the supernatants to fresh tubes,
10. Add 20 µl of a 50% slurry of Glutathione Sepharose 4B (prepared as described above) to each supernatant and mix gently for 5 min at r.t..
11. Add 100 µl of 1 x PBS, vortex briefly, and centrifuge for 5 sec to sediment the Sepharose beads.
12. Discard the supernatant, repeat this 1 x PBS wash twice
13. Elute the fusion protein by adding 10 µl of Glutathione Elution Buffer. Suspend the Sepharose beads and incubate for 5 min at r.t.
14. Centrifuge in a microcentrifuge for 5 min to sediment the Sepharose beads, then transfer the supernatant to fresh tubes.

*Glutathione elution buffer: 10 mM reduced glutathione in 50 mM Tris-HCl (pH 8.0).

Dispense in 1-10 ml aliquots and store at –20C until needed. Avoid more than five freeze/thaw cycles.

Preparation of Glutathione Sepharose 4B

(for bulk matrix for batch purification)

1. Gently shake the bottle of Glutathione Sepharose 4B to resuspend the matrix.

2. Use a pipet to remove sufficient slurry for use and transfer to an appropriate container/tube. (Glutathione Sepharose 4B as supplies is approximately a 75% slurry. The following procedure results in a 50 % slurry; Based on the bed volume requirement, dispense 1.33 ml of the original Glutathione Sepharose 4B slurry per ml of bed volume required).

3. Sediment the matrix by centrifugation at 500 xg for 5 min, carefully decant the supernatant.

4. Wash the Glutathione Sepharose 4B by adding 10 mol of cold (4˚C) 1 x PBS per 1.33 ml of the originally slurry of Glutathione Sepharose 4B dispensed, Invert to mix.
   -Note: Glutathione Sepharose 4B must be thoroughly washed with 1 x PBS to remove the 20% ethanol storage solution. Residual ethanol may interfere with subsequent procedures.

5. Sediment the matrix by centrifugation at 500 x g for 5 min. Decant the supernatant.

6. For each 1.33 ml of the original slurry of Glutathione Sepharose 4B, and 1 ml of 1 x PBS. This results in a 50% slurry. Mix well prior to subsequent pipetting steps.
   -Note: Glutathione Sepharose 4B equilibrated with 1 x PBS may be stored at 4˚C for up to 1 month.

Bulk purification of protein expressed in E.coli

1. Pick several colonies of *E.coli* transformed with the pGEX recombinants into separate tubes containing 2 ml of 2 x YTA medium.
2. Grow o/n at 20~37˚C.
3. Add 2 ml culture into 100 ml 2 x YTA medium.
4. Grow liquid cultures to an A_{600} of 0.6-0.8 (about 2 h) with vigorous agitation at 20~37˚C.
5. Incubate fusion protein expression by adding 50 µl of 100 mM IPTG (final concentration 0.1 mM).
6. Continue incubation for an additional 1~2 h
7. Transfer the liquid cultures to 50 ml tubes.

8. Centrifuge at 3000 rpm for 10 min at 4˚C and discard the supernatant.
9. Resuspend each pellet in 5 ml of ice-cold 1 x PBS.
10. Sonicate on ice with three to five brief (10 sec) pulse.
11. Transfer to new microcentrifuge tubes (1 ml, each) and centrifuge for 10 min at 10000 rpm at 4˚C to remove insoluble materials. Transfer the supernatants to fresh tubes.
12. Add 50 µl of a 50% slurry of Glutathione Sepharose 4B to each supernatant and rock gently for 10 min at 4˚C.
13. Add 1 ml of 1 x PBS to each tube, vortex briefly, and centrifuge 10000 rpm at for 5 sec to sediment the Sepharose beads.
14. Discard sup, repeat 1 x PBS wash twice.
15. Elute the fusion protein by adding 100 µl of Glutathione Elution Buffer. Suspend the Sepharose beads and incubate for 10 min at 4˚C.
16. Centrifuge in a microcentrifuge for 5 min to sediment the Sepharose beads, then transfer the supernatant to fresh tubes.