Purification of GST-fusion proteins produced in the BL21(DE3) *E. coli* strain.
(also check purification of His-tag protei)

Production of GST-fusion proteins

1. BL21(DE3) cells transformed with a pGEX derivative.
    *No heat shock when transformed.
    *Do not leave colonies at 4˚C. Use fresh colonies.
2. Inoculate a single colony in 7.5/2.5 ml LB+amp, culture @25˚C O/N
3. Dilute in 750ml/250ml (**s ml**) LB+amp
4. Cultivate at 30˚C until an OD660 of approx. 0.8 (you can start induction at lower OD, at least 0.4)
    *Take 1ml culture for an SDS-PAGE sample (**pre-induction sample**). (Screw top tube)
    *Spin down cells. Remove supernatant.
    *Add 1xSDS-PAGE loading buffer (OD x 300 ul) to the pellet.
    *Suspend cells by vortex and boil for 2min
    *Vortex again briefly and boil another 3min
    *Chill on ice, freeze at -20˚C
5. Add IPTG to the culture (final 0.67 mM IPTG) (0.1 ~ 1.0 mM depending on you proteins)
6. Cultivate @30˚C for 4h
    *Measure OD, and prepare SDS-PAGE sample as described below (**post-induction sample**)
    *Take 1ml culture for SDS-PAGE sample. (Screw top tube)
    *Spin down cells. Remove supernatant.
    *Add 1xSDS-PAGE loading buffer (OD x 300 ul = **t**) to the pellet.
    *Suspend cells by vortex and boil for 2min
    *Vortex again briefly and boil another 3min
    *Chill on ice, freeze at -20˚C
7. Collect cells in 500 ml centrifuge tube (**NOT be filled to 75% of total capacity**)
    (4,200 rpm/up to 4,800g, 30min, 4˚C, 375 ml twice)
8. Suspend cells in 40ml of TBS and transfer into the 50ml conical tube.
9. Collect cells (3,000 rpm, 20min, 4˚C)
10. Wash cells with TBS (suspend cells in 40 ml TBS again, collect cells, discard TBS)
10. Store pellet @-80˚C

Purification of GST-fusion protein

All the procedures must be performed at 4˚C or on ice unless otherwise specified

1. Add 10/5 ml (**w ml**) of ice-cold lysis buffer to cell pellet (4˚C)

2. Sonicate cell (4˚C)
    With Branson Digital Sonifier: amplitude 20%, processing time 30 sec, ON time 1 sec, OFF time 3 sec. Need to repeat 3 ~ 5 times.
    Keep the tube on ice while sonicating.
    *Cell suspension changes its color from white to brown
    *Avoid foaming. Put down the sonicator tip well below the buffer surface during sonication.

3. Transfer cell lysase to a 30ml centrifuge tube (4˚C)

4. Clarify lysate by centrifugation (JA-17, 15krpm, 30min, 4˚C) (**use adaptor if necessary**)

5. During centrifugation, pre-wash glutathione sepharose beads
    a. Take 400/200 ul (50% slurry, 200/100 ul bed volume) of beads in 15 ml tube
    b. Spin down to collect beads, 1000 rpm, 1sec, 4˚C (remove supernatant)
    c. Add 1 ml of lysis buffer, mix gently/thoroughly

d. repeat steps: b, c, b, c and b

6. After centrifugation, save (**w x 1/s) ml** of supernatant for SDS-PAGE sample (**sup sample, 4˚C**)

7. Filtrate supernatant though 0.45um filter into a 15ml conical tube containing pre-washed glutathione sepharose beads (50% slurry in lysis buffer) (4˚C) (**Do not make foam by pushing syringe to the very end**): Do not vortex. Do not pipet up and down. Mix by inversion.

8. Rotate for 1h at 4˚C
9. During the step 7:
    *Suspend pellet in 10/5 ml of lysis buffer by sonication. Save (**w x 1/s = u) ml** for SDS-PAGE sample (**pellet sample, 4˚C**)
    *Prepare sup & pellet samples: sample must contains same concentration of proteins as pre- and post-induction sample
        *Add (**u x 0.5) ml** of 3XSDS-PAGE loading buffer.
        *Add (**t- 1.5u**) ul of 1XSDS-PAGE loading buffer (**total t ul**)
        *Suspend by vortex and boil for 2min
        *Vortex again briefly and boil another 3min

10. Wash beads with lysis buffer (5 ml x 3 times) (4˚C) (brief centrifuge at 1,000 rpm for 1sec)

11. Incubate beads in 10 ml of DnaK removing buffer at 30˚C for 30 min

12. Go to Step 13 for Option 1. OR go to Step 15 for Option 2

For "Option 1: Elution of GST fusion"
13. Wash beads with lysis buffer (5 ml x 2 times) (4˚C) (brief centrifuge at 1,000 rpm for 1sec)

14. Store beads as 50% slurry in lysis buffer, and follow the protocol for "Option 1"
    *Gently suspend and take 10 ul for SDS-PAGE sample (**beads sample**)
    *Add 50ul of 1XSDS-PAGE loading buffer
    *Suspend by vortex and boil for 2min
    *Vortex again briefly and boil another 3min

For "Option 2: Thrombin digestion"
15. Wash with cleavage buffer (5ml x 3 times) (4˚C)

16. Store beads as 50% slurry in cleavage buffer, and follow the protocol for "Option 1"
    *Gently suspend and take 10 ul for SDS-PAGE sample (**beads sample**)
    *Add 50ul of 1XSDS-PAGE loading buffer
    *Suspend by vortex and boil for 2min
    *Vortex again briefly and boil another 3min

option 1* Elution of GST fusion
1. Spin down briefly to collect beads (4˚C)
2. Remove supernatant
3. To the beads, add 200/100 ul of lysis buffer containing 10 mM reduced glutathione in (RT)
4. Vortex briefly and wait 1min (RT)
5. Centrifuge to collect beads (RT)
6. Save supernatant in a new tube (4˚C)
7. Repeat 2 – 6 twice; prepare an SDS-PAGE sample for each elution
8. Pool all supernatants (4˚C)
9. Pass through mini empty column (4˚C)

10. Store protein solution at 4˚C or -80˚C

option 2* Thrombin digestion
1. Centrifuge briefly, and discard supernatant
2. Add 1ml of cleavage buffer (4˚C)
   *For a test sample, add 300 ul of cleavage buffer to beads and transfer to a 1.5ml tube
3. Add thrombin (1/10 volume) (4˚C)
4. Incubate (shake) for 1.5h at 30˚C
5. Pass through mini empty column (RT)
   *For a test sample, centrifuge briefly. Supernatant is the protein solution
6. Store protein solution at 4˚C or -80˚C (snap freeze in liquid nitrogen)
   *Prepare SDS-PAGE sample using 10 ul of the protein + 50 ul of 1xSDS-PAGE *loading buffer.

Reagents

Lysis buffer (prepare the amount you need: pAPMSF is expensive)

	Stock soln	For 100ml		
50mM Tris-HCl pH7.5*	1M	5ml		
150mM NaCl	5M	3ml		
10% Glycerol	60%	16.7ml		
0.25% Tween 20	40%	0.625ml		
1mM DTT**	1M	0.1ml		
0.2mM pAPMSF**	0.2M	0.1ml		
Total		100ml		

*or 40mM HEPES-NaOH pH7.5
****add before use***
*** add 2.5mM $MgCl_2$ for G proteins

DnaK removing buffer
Lysis buffer + 2.5mM $MgCl_2$ and 2mM ATP

Cleavage buffer (prepare the amount you need)

	Stock soln	For 100ml		
25mM Tris-HCl pH7.5	1M	2.5ml		
150mM NaCl	5M	3ml		
10% Glycerol	60%	16.7ml		
2.5mM $CaCl_2$	1M	0.25ml		
1mM DTT**	1M	0.1ml		
Total		100ml		

****add before use***
*** add 2.5mM $MgCl_2$ for G proteins

Glutathione Sepharose 4B beads in 15ml tube
Wash in 5ml lysis buffer 3times

Thrombin stock soln. (10x)
5mg/ml thrombin (Roche)
(5 units/ml)

0.2M Reduced Glutathione (-20˚C)