GST Fusion Protein Purification

Day 1:

Transformations:

1. Obtain 30 ul of BL21 Competent cells.
2. Add 1 ul of plasmid to cells. Keep on ice for 30 minutes.
3. Heat shock for 30 seconds in 42°C water bath.
4. Put on ice for 2 minutes.
5. Add 250 ul of LB Broth and shake at 37°C for 45 minutes.
6. Plate 50 ul on antibiotic plates. Incubate at 37°C overnight.

Day 2:

1. Check to see colony growth on plate.
2. Inoculate a single colony in 10 ml of LB Broth with appropriate antibiotic in a flask. Shake overnight at 37°C.
3. Seal plate with parafilm and store at 4°C.

Day 3:

1. Keep 650 ul of the overnight culture with 350 ul of Glycerol Storage solution in -80°C as a glycerol stock.
2. Add 100 ml of fresh LB Broth with antibiotic to the culture flask. Incubate for 1 hour, shaking at 37°C.
3. Add IPTG to final concentration of 0.1 mM.
4. Incubate for 4 hours, shaking at 30°C.
5. Centrifuge at 5000 rpm for 5 minutes at 4°C and discard supernatant.
6. Resuspend pellet in 500 ul of ice-cold 1x PBS. Transfer to microcentrifuge tube. At this point, sample can be kept at -80°C.

Day 4:

1. Thaw sample, but keep on ice.
2. Sonicate sample at 30% amplitude using pulsations (0.5 s on, 0.5 s off) for 16 seconds. Make sure to keep sample on ice. Sonicate again using pulsations for 10 seconds.
3. Centrifuge at max speed for 10 minutes at 4°C.
4. During centrifugation, prepare Glutathion sepharose beads:
   Cut tip of pipette tip to collect beads. Wash 100 ul of beads once with 500 ul of ice-cold 1x PBS. Centrifuge and remove supernatant.
   Add the supernatant from the sample to the tube. Rock for 3-5 hours or overnight at 4°C.
5. Wash the rocked beads 3 times with 500 ul of ice-cold 1x PBS, with centrifugation in between each to remove PBS (max speed at 4°C for 1 minute).
6. Prepare fresh Gluthation Reduced Buffer 33mM by mixing Elution Buffer with Glutation Reduced (0.01 g of Gluthation Reduced per 1 ml of Elution Buffer).

7. Add 200 ul of Gluthation Reduced Buffer to the beads and rock for 2-3 hours or overnight at 4°C.
8. Centrifuge beads at max speed for 5 minutes at 4°C. Save the supernatant and keep at -80°C.
9. For gel preparation: Add 5 ul of 2x Loading Buffer to 5 ul of sample. Boil for 1 minute at the hot plate. Load 10 ul of each sample into gel. Run at 100 V for about 1 hour or until front reaches close to the end of gel.
10. When gel is ready for staining, use Coomassie stain for about 10 minutes, then destain with destaining solution for about 10 minutes. Optional: Rock with water overnight to further destain.
11. When gel is ready, dry by using cellophane foil. This may take 2-3 days.

Troubleshooting:

- If protein expression is not well achieved, add IPTG and induce overnight at room temperature.
- To increase binding of GST-fusion proteins to beads, add 5mM DTT to cell lysis buffer (making the lysis buffer become 1xPBS plus 5mM DTT)
- To increase the protein elution from beads, add non-ionic detergent (such as 0.05% Tween 20) to the elution buffer.
- To reduce basal expression levels, add 2% glucose to the growth media when adding IPTG.

Elution Buffer
100mM Tris HCl pH 8.0
120mM NaCl
30 mM Glutatione Reduced (add just prior to use)

Coomassie Blue Staining Solution
0.5 g Coomassie Blue
200 mL 100% methanol
Stir 1 hour, then add:
300 mL 0.3M acetic acid

Destaining Solution
30% methanol
10% glacial acetic acid
add water

5x Protein Loading Buffer
250mM Tris HCl pH 6.8
10% SDS
0.5% Bromophenol Blue
50% Glycerol
0.1M DTT

Glycerol Storage Solution
65% Glycerol
0.1M $MgSO_4$
25mM Tris HCl pH 8.0