Purification of GST-fused proteins

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GST Fusion

Gene should be cloned in-frame with an N- or C- terminal GST-tag, using the pGEX4T-1 vector.

Growth and Protein Induction

Prepare a 10 ml culture overnight of BL21 (DE3) containing your plasmid of interest. Use the appropriate antibiotic selection: carb/cam.

After O/N growth at 37 °C, inoculate 100ml of fresh LB+carb/cam with 2ml of overnight culture. Grow culture to an OD_{600} of ~0.2 at 37 °C (~1hr), and then transfer to 25 °C and grow to OD_{600} of ~0.6 (~2hrs). (Remove 1ml of culture to be run as "un-induced" sample.)

Induce culture with 0.2mM IPTG. Use fresh IPTG or thaw only once from frozen 100mM stocks. Continue growth at 25 °C for 4hrs. (Remove 1ml culture after induction to be run as "induced" sample.)

Spin culture at 6000x g for 10mins to pellet the cells. Decant LB and store pellets O/N at -80 °C.

Solubilization

Make GST buffer, pH 8.0 (pH is extremely important!)

12.5ml	1M Tris-HCl pH ~ 8
15ml	5M NaCl
0.386g	DTT

pH to 8.0 and adjust up to 500ml.
Store at 4 °C.

Just before use, add:
PMSF to final 1.0 mM from 500mM PMSF stock in isopropanol
protease inhibitor cocktail tablets: 1 tablet per15-20ml of buffer
extra DTT if buffer has been stored >1 day

ALWAYS keep samples and buffers on ice for all the next steps to prevent denaturation of proteins.
Resuspend cell pellets in 5-10ml GST buffer. Make sure the pellets are completely suspended by pipetting up and down until there are no cell clumps left (this is important: clumps can clog the French press!) Keep the suspension on ice.

Use a French press to disrupt cells.

IMMEDIATELY spin out the lysate at 14700 x g for 35min (4°C) and separate the supernatant (sup) and pellet (pel) and save both: supernatant on ice, pellet at -20°C

Run a 15% SDS-PAGE gel. Run the un-induced, induced, and pellet and supernatant.
Run at 110V. Stain with Coomassie Brilliant Blue.

If it looks like there is induced protein in the supernatant, at the right size, proceed with the GST purification. (I normally load the supernatant on the resin while I run this gel for Coomassie staining.)

Purification

Do batch purification in a 1.5ml eppendorf tube.
First pipette 500ul of resin slurry (this slurry is 50% resin, 50% storage buffer, Glutathione Uniflow Resin from Clontech) into the tube and let it settle. Draw off the storage solution and wash the resin with cold dH_2O for 5 min, putting the tube on a nutator at 4 °C. Let the resin settle 5 mins and then equilibrate the resin by washing 3x 5 mins in 1ml of GST buffer.

Add 1.25 ml of spun cell lysate (supernatant**) to the equilibrated resin and incubate at 4°C on the nutator for 4 hrs.
Let the resin settle and draw off the sup after incubating. This is the "flow-thru" sample.
Wash the resin 3x with 1ml GST buffer, 5min each. Save each wash.
While washing, prep elution buffer:

Elution buffer (make FRESH):
10mM (final) L-Glutathione, reduced
50mM 1M Tris-HCl pH~8
Adjust up to 50ml, pH to 8.0 (if you're only doing one sample, you only need to make 5-10ml).
ALWAYS add protease inhibitor cocktail tablets before use.

Elute protein using 500ul of elution buffer three times: 5min for the first elution, then 10min for elution 2, then 15min for elution 3. **Most of the protein should come off in the first elution.**

Lastly, purge the resin by washing it for 20min in cold dH_2O. Save the purge and store the used resin at 4°C in 20% EtOH. (The resin can be reused; see the manufacturer instructions for how to reconstitute the resin. Only use a used resin to purify the same protein.)

Check purification by running samples on an SDS-PAGE gel. Silver staining is recommended so that you can determine the presence of impurities.

Sample MUST also be checked on a western blot with the appropriate anti-GST antibody.

Purified proteins (elutions) can also be stored at -80 °C in 25% glycerol aliquoted into PCR tubes (I aliquot 25-50ul per tube).

** The extra supernatant can be stored at -80 °C in 25% glycerol, aliquoted into 1.5ml tubes. This frozen supernatant can be thawed and loaded onto a resin for purification of fresh protein.