GST Purification in Bacterial Cells

Part 2.

1. Cool down centrifuge.
    a. Set to 4°C, make sure lid is fastened tightly, and hit “Fast Temp”.
2. Find 400ml centrifuge bottles containing bacterial pellet being used for protein purification in freezer.
3. Make PBS buffer in a 50ml conical tube.
    a. 25 mls of PBS
    b. 6 mls of EDTA (0.5M)
    c. 300 µl PMSF (TOXIC protease inhibitor, pipette quickly and don’t breathe or can pipette in hood)
    d. 200 µl Triton X-100 (very thick solution so pipette VERY slowly to avoid bubbles)
        i. Use pipette to mix buffer by aspirating up and down
        ii. Label the tube as “PBS + Triton”
4. Transfer the bacteria to 80ml centrifuge tubes from Cinzia’s shelf
    a. Pipette 10ml of the Buffer into each of the 400ml centrifuge bottles
    b. Pipette up and down to fully resuspend the pellet and transfer solution into a labeled 80ml tube (use thick sharpie to label so can be removed with ethanol)
    c. Use another 5 ml of the PBS+Triton to wash the 400ml bottle and transfer to the 80ml tubes.
5. Sonicate 3 times for 10 seconds each (about 5 seconds in between) <u>keeping the tubes in ice</u>
    a. Clean the needle with a wet napkin
    b. Move some ice and the centrifuge tube into small beaker so sonicated <u>on ICE</u>
6. Balance tubes and centrifuge
    a. To equalize the weights in your tubes, go to the Balance and ‘Zero’ the scale using the heavier tube. Add the Buffer to the solution until the scale reads ‘0.0’ or add PBS if the Buffer runs out.
    b. Place balanced tubes in centrifuge to spin at 4°C for 30 minutes at 12,000 rpm.
7. Prepare the beads in the meantime.
    a. Obtain Protease Inhibitors from the Godzilla freezer and leave on bench to thaw.
    b. Pipette 300 µl of Glutathione agarose beads from Cinzia’s refrigerator (can vortex bottle to resuspend beads from bottom but should still pipette slowly) into each labeled 1.5ml Eppendorf tube.
    c. Place tubes in microcentrifuge for 30 sec and dispose of supernatant using small pipette tip.
    d. Wash the beads to remove the ethanol 2 times.

i. Add 700 µl of PBS, vortex, momentary spin with microcentrifuge, dispose of supernatant.
ii. Add 300 µl of PBS to each tube and transfer the solution to 50ml Falcon tubes. Use another 30 ml to rinse the Eppendorf tubes and transfer.

e. Add 15 µl of Protease Inhibitors to each tube and return it to Freezer.

8. When centrifuge is done, pour the supernatant into the Falcon tubes.
9. Parafilm the tubes and take to the Cold Room (4°C) and place on rocker overnight.
    a. Cleaning:
        i. 80ml Centrifuge Tubes: ethanol outside, antibacterial Hand Soap on inside and clean finally with distilled water to remove minerals.
10. Next Day: Spin the tubes in the TC Room centrifuge for 3 minutes at 3,000 rpm and remove supernatant.
11. Move beads into Eppendorf tubes
    a. Use 1 ml of PBS to wash out beads and transfer into the labeled 1.5 ml Eppendorf tubes.
    b. Spin down in microcentrifuge for about 20 seconds and remove supernatant without touching the beads (Make sure to switch pipette tips at every step).
12. Washes (4 or more)
    a. Pipette 1 ml of PBS into tube, vortex to resuspend the beads, centrifuge for 20 seconds, and remove the supernatant.
13. Store the beads
    a. After the last wash, add 300 µl of PBS to the tubes with 5 µl of the Protease Inhibitors, and store in refrigerator or freezer until use.
    b. Can be followed by Elution Step depending on procedure.
14. Elution Buffer
    a. 50 mM Tris, pH 8.0 containing 10 mM reduced glutathione:

    Elution Buffer

    125 ul Tris pH8 (0.4 Molar stock)
    40 ul Gluthatione (250mM stock)
    835 ul H2O

    1 ml final

    After at least one hour elution, proceed to tag digestion with thrombin or other enzymes:

80 ul protein (1.5-2 ug/ul concentrated)
10 ul buffer 10x
2ul of enzyme
8 ul H20
100 ul final

if the protein is more concentrated, use less volume.
The digestion is performed at 4 degrees over night.
The day after add 40 ul of streptavidin agarose to each sample and let it bind for 30 minutes at room temperature on the rocker.
Add 100 ul of PBS prewashed beads of Nickel resin or GST resin to each sample and let it attach for 1-2 hours.
Recover the supernatant.
Check the resulting concentration of each sample.
Run a gel to see hoe the protein look and stain it over night.