GST Fusion Protein Purification from Bacteria

***For protein induction*:** Grow O/N culture of 50mLs AMP^r culture of desired BL21 cells carrying pGex recombinant plasmid. In the morning, dilute culture 1:500 in 2Liter flask with 1L LB + fresh AMP and grow until O.D. = 0.7. Add 1:2000 dilution of IPTG stock and incubate shaking at Room Temperature for 3-4hours. Harvest cells, freeze at –80˚C.

1. Prepare PBS Buffer, Elution Buffer w/Glutathione:

   50mM Tris (pH 7.7)
   300mM KCl
   \+ 10mM Glutathione for GST Fusions

   pH Elution Buffer back to 7.7 after addition of
   ****glutathione****

2. Lyse Cell Pellet

   Add 20mLs PBS+1:100 dilution of Lysozyme/liter of BL21 cells harvested post 3hr induction, sit on ice for 30 minutes. Sonicate for 3 minutes w/50% duty cycle, output control at 5. Be sure that lysate is very liquid, not gelatin.

   *Take sample of lysate for SDS PAGE gels

3. Prepare Supernatant-

   Spin Lysate at top speed (~18,000 rpm) for 50 min.

   *Take sample of supernatant for SDS PAGE gels

4. Equilibrate 1.5 ml solid beads/Liter of BL21 cells by washing with PBS in large orange cap tube. Add beads to lysate, incubate in cold room, rotating, for 2 hours.

5. Start equilibrating QuickChange columns with protein storage buffer (need 5X flow through!!!!)

6. Wash beads 5X column volume with PBS

*Take sample of first Wash for SDS PAGE gels

8. Collect 2 Elutions with 2.5 ml elution buffer, Let each elution incubate with suspended beads for 5 minutes before collecting elution. Take third elution of 5 ml to elute any remaining protein.

*Take sample of Elutions for SDS PAGE gels

9. Use QuickChange column to change buffer on first two elutions to protein storage buffer if needed (see Sf9 protein purification.)

*Take samples of Quickchange column elutions

10. Run SDS PAGE gel on fractions to determine purification quality and quantity (LYSATE, SUPERNATANT, WASH, ELUTIONS)