Protein Purification (GST tag):

Day 1:

-Setup overnight culture: 50 ml LB + Ampicilin + 1 colony. Let shake at 250 rpm overnight in 37^{o}C shaker.

Day 2:

-Dilute 10 ml of o/n culture into 1 liter LB

-Grow at 37^{o}C to OD of 0.6-0.8 (~2-3 hrs)

-Choose 'Basic'

-Choose 'Absorbance'

-600 nm

-Fill baseline one with LB

-Fill with bacteria LB

-Move to 16^{o}C incubator. Give another 10-15 min. to get to 16^{o}C.

-Take "uninduced sample:" 1 ml of bacteria. Spin down max speed 1 min. Remove supernatant, and resuspend in 75 ul dH_2O + 25 ul of 4X sample buffer.

-Induction: Add IPTG (0.5mM). If needed add $ZnCl_2$. Leave overnight 16^{o}C shaking.

Day 3:

-Take "induced sample:" 250 ul. Spin down max speed 1 min. Remove supernatant, and resuspend in 75 ul dH_2O + 25 ul of 4X sample buffer.

-Spin down cultures in bottles for 30 min. at 4^{o}C at 4000 rpm.

-Pour off supernatant.

[Option of stopping by putting pellet in -80^{o}C]

Protein Purification Begins:

-Add appropriate lysis buffer to pellet (30 ml/liter of culture) (Kept at 4^{o} C)

[50 mM Tris pH7.7 + 150 mM KCl + 0.1 % Triton X100] + 1 mM DTT (30ul) when ready to use

-Vortex to resuspend cells

-Add 1 mg/ml lysozyme (Stock 100 mg/mL; 100X)

-Pour into glass beaker on ice.

-Incubate on ice 30 min.

Now always keep on ice

-Sonicate: -6 min. w/ 45% output, 10 sec on/10 sec off for a 30 ml sample. Increase length of time for larger samples.

-Take a "whole-cell lysate sample" of:

7.5 ul + 67.5 ul dH_20 + 25 ul 4X sample buffer

-Spin down at 11,500 rpm, 4^{o}C, 60 min.

[While that's going on, wash your glutathione sepharose beads twice in GST Wash buffer, and prepare the protein assay buffer: Dilute protein assay buffer 1:5. Put 200 ul into several 1.5ml tubes]

-Keep supernatant. Take a "soluble sample:"
7.5 ul + 67.5 ul dH_2O + 25 ul 4X sample buffer
-Filter thru 0.45 micron filter(s)
-Pour into 50 ml conical tube(s)
-Add beads (0.5 ml/liter of culture, *tentatively*) Incubate 1 hr, 4^{o}C, rotating.
-Pour into plastic column
-Wash beads with ~100 ml of wash buffer (same ingredients as lysis buffer, minus the lysozyme)
-Note: You don't want the beads to dry out
-Note: Wash until protein assay buffer no long turns blue when a drop of column solution is added.

Cleaving off the GST Tag:

Add 3 ml protein storage buffer:
50 mM tris pH 7.7
100 mM KCl
10% glycerol
1 mM DDT (added fresh)
Take 30 ul sample into 10 ul 4X sample buffer of what's in the column. Shake the column to mix up beads and solution.
Add 75 ul precision protease to column. Cap it.
-Incubate overnight 4^{o}C, rotating, in column.

Day 4:
Collect fluid.
Take 30 ul sample into 10 ul 4X sample buffer (Both of beads and what's collected). (3ml protein storage buffer when collecting beads).
Add 6 more ml of protein storage buffer to beads. Collect second elution separate. Take 30 uL sample + 10 uL 4X sample buffer.
Run all the samples on a protein gel. After gel is done running, put it into a 1000 ml beaker and cover with ethanol stain. Microwave until just boiling. Let the gel stain on rocker for ~15 min. Destain (same procedure).
Liquid-nitrogen snap freeze the good collections and store at -80^{o}C

Eluting(Non-cleaving) Method:

Add elution buffer to beads in ~1ml increments.
Collect the fractions into 1.5 ml tubes. (Check from time to time w/ diluted protein assay buffer. When it no longer turns blue you are finished with elutions).

Combine 10 ul of your protein with 10 ul of 2 x sample buffer. Boil 5 minutes, Spin down 5 minutes. Run 15 ul on gel. Stain gel as described above.

Figure out which collections are good. Pool good fractions. Snap freeze in liquid nitrogen and store in -80 ^{o}C.