GST Protein Prep. Ver.2

1) Grow 50ml of culture in LB or TB +antibiotic o/n at 37ºC shaker.

2) Dilute culture in LB or TB +antibiotic 1:10

3) Grow 3hrs at 37ºC.

4) Induce culture by adding 0.4 mM IPTG final concentration. (For 50 ml final culture add 20 µl of 1M IPTG).

5) Grow at 25ºC for 1 hr.

6) Spin 5 min at 5 K

7) Wash pellet with half volume of cold H_2O. (For 50 ml culture use 25 ml H_2O.)

8) Wash bacteria again.

9) Resuspend in 1 ml of resuspension buffer per 50 ml of culture.

Resuspension Buffer- NETN + protease inhibitors

20 mM Tris pH 8.0
100 mM NaCl
1 mM EDTA
0.5% NP-40 or Triton-X
1 µg/ml Aproteinin
1 µg/ml PMSF
1 µg/ml Benzaminide

Note: Tim has 100x stock of protease inhibitors.

10) Sonicate for 2x for 10 seconds in cold room.

11) Pellet debris by spinning at 4ºC (Easiest way is to put samples in multiple eppendorfs and spin in cold room at max for 2 min.

Binding of Fusion Protein to Beads

1) Pipette 400 µl of GST-Sepharose into Eppendorf

2) Spin beads 15 sec @ 8,000 RPM

3) Wash beads 2x with 4ºC NETN

4) Resuspend pellet in 320 µl of NETN (Final volume will be ~550 µl) and put into 4 eppendorfs.

5) Add 300 µl of resuspension buffer and 75 µl of E. Coli. GST Lysate to each tube.

6) Incubate for 30 min while rocking in cold room.

7) Spin 15 seconds at 8,000 rpm.

8) Wash 3x with ~600 µl of resuspension buffer

9) Remove supernatant and resuspend in appropriate volume resuspension buffer. (You can add this directly to SDS load buffer for running on a gel.)