Gal11 Expression

Revised Protocol for the Expression and purification of Gal11-GST
1) Transformed cells are grown on LB-amp plates (0.1 mg/mL)

2) Pick best colony (if not distinguishable, just scrape a few colonies) and scrape into 50 mL of LB-amp (50 mL of 100 mg/mL) + chloramphenicol (170 mL of 10mg/mL dissolved in ethanol). Shake overnight at 37° and 275 rpm.

3) Place 50mL overnight culture (should be extremely cloudy) into 1L of LB-amp (1mL of 100mg/mL ampicillin) + chloramphenicol (3.4mL of 10mg/mL dissolved in ethanol) and shake at 37° and 275 rpm. Allow to shake until OD$_{600}$ is 0.6 - 0.8. This typically takes 2-3 hours; check optical density about every forty-five minutes after the second hour.

4) Allow the incubator to cool down before inducing the cells. To bring incubator temperature down to 16° it may be necessary to place 2L Erlenmeyer flasks of ice into shaker.

5) Once the temperature of the shaker has reached 16° add IPTG to the cells at a final concentration of 0.5 mM (.119 g of IPTG / L). Shake at 16° and 275 rpm for 6 hours. Alternatively the expression can be done at 24° for 3-5 hours. The higher the temperature, the less likely that clean Gal11 will be produced.

6) Centrifuge the cells at 6000 rpm for 10 minutes in a Beckman JLA 8.1000 rotor.

7) When removing the supernatant from the cells, make sure that no liquid remains.

8) The pellet should be frozen for at least 45 minutes at -80 °C to help gently break up the cell wall. Storing the pellet overnight in a 50mL falcon tube will further assist in this process.

The next few steps must be carried out very carefully since Gal11-GST is easily degraded. Make sure to keep everything on ice at all times.
9) Resuspend the pellet in 10 mL of Buffer A per Liter of cells grown. The pellet does not mix well in the buffer, but following sonication, a homogenous mixture should result.

   **Buffer A**: 100 mM PBS at pH 7.4, 1mM PMSF, 0.2% NP-40, 10% glycerol (v/v) 1mM DTT and protease inhibitor. The protease inhibitor and the DTT are added fresh before each use.

10) Sonicate the resuspended cells **on ice** to break open the cell walls. Five 10-second pulses with two-minute intervals should be sufficient to break open cells without excessive damage to the Gal11-GST. For 10 mL of Gal11 resuspension (1 Liter Culture) three 10-second pulses with two-minute intervals works well. Make sure the solution is homogenous following sonication. Use the upright sonicator in the Marsh lab, not the hand held.

11) Centrifuge the lysate at 9600 rpm and 4° for 30 minutes.

12) Add the lysate to pre-equilibrated glutathione beads. Use 1 mL of bead slurry for every liter of cells grown.

   **Equilibration of beads**: Mix the beads in the bottle. Remove 1mL of beads for every liter of cells grown and add to a 15 mL Falcon tube. Spin at 2000 rpm for 5 minutes and decant supernatant. Add 10 mL of cold PBS buffer and mix by inverting. Centrifuge again at 2000 rpm for 5 minutes and decant supernatant. Repeat washing procedure twice. Wash one time with 10 mL of Buffer A and mix by inverting. Spin at 2000 rpm for 5 minutes and decant supernatant. Add 500 mL of Buffer A for every mL of beads and store on ice.

13) Mix on a rotator for 30 minutes at 4°

14) Centrifuge at 2000 rpm at room temperature for 2 minutes and decant supernatant.

15) Wash beads three times with 10 mL of buffer A

16) Check the expressed protein by running 15mL of beads on an SDS gel.
17) The beads can be stored for extended periods of time (two months or more) at -20 °C if as much buffer is removed as possible.

When desired, the protein can be eluted from the beads with the following steps:

At this point one might not want to elute all of the protein from the beads, since it is much more stable on the beads then in solution. From past experience, each liter culture will produce about 1 mL of 1-2 mM Gal11-GST.

1) Add 1 mL of elution buffer to one mL of glutathione bound Gal11-GST and rotate at 4° overnight.
   **Elution Buffer:** (50 mM Tris, 0.015M glutathione, 0.1% NP-40, pH 8.0)

2) Centrifuge beads at 3300 rpm for 5 minutes and remove supernatant into a separate, clean 15 mL Falcon tube and store at 4°.

3) Add 1mL of elution buffer to beads and rotate at room temperature for 1 hour.

4) Following elution, once again centrifuge the beads at 3300 rpm for 5 minutes and the supernatant was combined with supernatant from step 2.

5) Repeat elution procedure (from steps 2,3) two more times for a total volume of 4 mL of Gal11-GST in elution buffer.

6) Concentrate 4mL of elution buffer mixture to 0.5 mL at 4°.

7) Dilute the solution to 2 mL with storage buffer and again concentrate to 0.5mL.
   **Storage Buffer** (0.01M PBS pH 7.4, 10% glycerol (v/v), 0.01% NP-40, 1mM DTT)

8) Repeat storage buffer dilution and concentration three more times and bring final volume up to 1mL with storage buffer.

At this point in time it is ideal to perform a Bradford assay to determine protein concentration. Since Gal11-GST is fairly fragile, it is important to only freeze once and thaw just prior to use. For this reason, the Gal11 should be aliquotted into
volumes that will be used entirely for each experiment. The procedure for the Bradford assay is as follows:

1) Dilute 1 mL of Bradford reagent 1:5 with water. Add one mL of this diluted solution to 20mL of Gal11-GST solution.

2) Allow the solution to incubate at room temperature for 20 minutes.

3) Using a quartz cuvette and the diluted dye as a blank, measure the absorbance of the Gal11 solution at 595.

4) One should also create a standard curve in the same fashion as the concentration determination for Gal11-GST.

A previously determined standard curve can be used in most cases, where

\[ Y=1.0495 \times X + 0.0468. \]

Where \( Y \) = absorbance and \( X \) = Concentration.

For a precise measurement of concentration, a new standard curve should be determined.

5) Following Bradford assay, one should calculate the concentration and aliquot into eppendorf tubes with appropriate volumes for experiments to be performed in the future.

6) Before storing the Gal11-GST at -80 °C place the eppendorf tubes into liquid nitrogen to quick freeze and prevent denaturation of the protein while freezing.

7) Store at -80 °C until ready to use.