Small Scale Expression Screening (His Tag or GST tag fusion proteins).

STEP	TIME
1. **Start with a single colony** picked from a plate not more than 3 weeks old***. USE THE (DE3) containing strains for pET clones***. XL or BL21 strains ONLY. Rossetta or other strains can be used if these do not work. • Alternatively, you can start with a glycerol stock and streak on a plate to obtain an isolated colony. Incubate the glycerol stock overnight to obtain a colony.	10 min - overnigh t
2. **Starter Culture -** Transfer the colony to a 5 ml culture (shaking) for 3 to 5 hours. • Use room temp LB with antibiotic. • Observe the OD600 at the beginning. • Incubate the culture with agitation at 37oC until OD600 0.6-0.8. DO NOT LET GET BEYOND this absorbance. *If the bacteria grow too dense, the cells will inhibit the protein expression and your time will be totally wasted.* • Once the culture has reached the correct OD, either place the culture at 4^{o}C (for overnight – no longer) or continue with the procedure.	3-5 hours.
3. **Expand the culture** by adding 1 ml of starter culture to 4 ml of LB with antibiotic (room temp) and incubate for 1-4 hours until culture density reaches 0.6-0.8 OD600. Continue to the next step. We have not tested to see if an overnight hold at 4^{o}C will alter expression.	1-4 hrs
4. **Induce Expression** – Induce expression by adding IPTG (1 or 0.5 M stock). IPTG is a frozen solution in the -20^{o}C freezer. Included an un-induced sample as reference. Refreeze unused IPTG. Culture with shaking at 37^{o}C for 3-4 hours.	3-4 hrs
5. **Collect Cells** – Centrifuge the cells in the clinical or swinging bucket rotor (next to the -80^{o}C freezer) at 5,000 x g for 10 min. Remove the supernatant and freeze pellet for later processing.	20 min.
6. **Lyse Cells and Collect Lysate** – • Thaw cells and add lyse in 1.0 ml of lysis buffer. Include protease inhibitors. • Transfer to a microfuge tube. • Vortex and use a pipettor to suspend the cells and incubate with rotation or rocking for 30 min. • Sonnicate with 3 pulses (while the sample is on ice) for 10-30 sec. • Centrifuge the samples at 16,000 x g for 30 min. • Save the supernatant (lysate) and discard the pellet (membranes and inclusion bodies). • Run on a column chromatography and elute with just a few fractions to determine the yield and purification. Refer to the Qiagen or GE Health Science handouts AND our protocols for conditions for a small column.	~ 2 hrs
7. **Assessing Protein Expression** – • Run a gel of the MW standards, lysate, flow through and main elution fractions (determined by quick bradford assay) and conduct a SDS PAGE analysis. Determine the molecular weight of your protein and find the appropriate sized band on your gel. The biggest band may NOT be your protein. • Pool the fractions with protein, measure the total volume and determine • Measure protein concentration using a Bradford assay for each sample. • Determine the total mass of protein and the concentration of protein in the sample. • Use purity (as measured by SDS PAGE) and yield to determine the best expression condition in a small scale. • Now repeat using a larger scale to see how your best condition scales to 100-2000 ml culture and purification.	3-4 hrs

Culture conditions – There are several factors to change if needed. Induce with a range of IPTG from 0.1 to 1.0 mM. Temperature of induction 37^{o}C for 2-4 hours; room temp for overnight; 16^{o}C for 24-48 hours. Sometimes waiting until cells are more dense to induce can help increase expression levels. A complete measurement might include different concentrations of IPTG at different temps. If the expression is high but purification is low (determined by lots of protein in lysis but much less than expected in the elution or flow through fractions), you may have expression in the inclusion bodies. Test by isolating inclusion bodies and decide if you want to refold proteins. Alternatives include including glucose in early cultures to limit protein expression until cell density is higher. There are several papers linked on our web page that troubleshoot you through the process. Using a specialty bacterial strain may also need to be determined.