

### **Standard procedure for inducing and freezing BL21 expressing GST-fusion proteins:**

- 1) Grow 2 x 30mL of LB + 30 ug/mL chlor, 200 ug/mL amp (30 uL each of stocks) inoculated with transformed BL21(Rosetta) colonies from plate overnight at 37°C.
- 2) Prepare 6 L of autoclaved LB for next day.
- 3) In morning, add 10 mL of overnight culture per 1 L of LB+ 30 ug/mL chlor, 200 ug/mL amp (1 mL each of stocks), for 6 L total. Grow ~4 hrs at 37°C with shaking.
- 4) Double check OD<sub>600</sub> is between 0.5 and 1.0.
- 5) Reduce temperature to 18°C. Induce by adding 0.2 mL of 1M IPTG (final 0.2mM concentration) to each liter of culture. Spike amp by adding 0.5 mL of stock to 100 ug/mL amp per liter.
- 6) Shake cells overnight at 18°C.
- 7) In morning, harvest cells. Reduce shaker temperature to 4°C to cool cells meanwhile. Our centrifuge holds 6 x 0.5 L bottles. Fill and balance bottles. Spin down cells at 2700 g for 20 minutes.
- 8) Add a small amount of bleach to emptied flasks. Decant supernatant of spun cells into the bleach flasks. Let sit for ~2 minutes. Empty into sink and flush with lots of water.
- 9) Since our centrifuge bottles hold only 0.5 L, add the remaining 0.5 L of culture to the pelleted cells and repeat the spin. Decant supernatant as before.
- 10) Place bottles with cells on ice in square ice bucket. Add 9 mL Sonicator Buffer (1M NaCl, 25 mM Tris pH 8.0) per liter of cell culture, using spatula and pipettor.
- 11) Store resuspended cells by placing in 2 x 50mL tubes (labeled), submerge in liquid nitrogen, place in labeled box in -80 freezer.