Protein Overexpression

1. Set up a 200 mL overnight culture of the bacteria with the appropriate antibiotic. And incubate at 37ºC for ~16 hours.
2. Pellet cells at 5000 g for 7 minutes using a 50 mL plastic falcon tube (multiple spins).
3. Remove supernatant and resuspend the pellet in 10 mL fresh LB.
4. Check the OD_{600} of the mixture (will need a 1000 fold dilution for the UV spectrophotometer linear range 0.1-1).
5. Inoculate the expression flasks (2L flasks 500 mL LB) to an OD_{600} of 0.1 with the overnight mixture.
6. Add appropriate antibiotic to the expression cultures and incubate at 37ºC with shaking.
7. Take OD_{600} readings from 1 flask every ½ hour to establish a growth curve.
8. When the expression cultures reach a 0.6 OD_{600} take a 1 OD_{600} sample (T_0) and induce the culture with IPTG to a final [IPTG] of 1 mM IPTG.
9. Take 1 OD_{600} samples every hour for 3 hours (T_1-T_3) and OD_{600} readings every ½ hour. Dilute samples appropriately when the OD_{600} becomes more than 1.
10. Harvest cells in the JA-14 rotor at 5000 g (5700 rpm) for 10 minutes using multiple spins.
11. Discard supernatant in the bacterial waste and scrape the cells into a clean sterile 50 mL falcon tube and rinse out the centrifuge tubes using LB to wash off remaining cells. Pour LB into the 50 mL falcon tube and centrifuge at 5000 g for 10 minutes.
12. Discard supernatant weigh cell pellet and freeze with liquid nitrogen. Store pellet in the -80ºC freezer until ready to purify protein.
13. Open the samples with 100 µL of cell opening buffer and heating to 95°C. Add 10 µL of SDS and pellet the debris by microcentrifugation at max speed for 1.5 minutes.
14. Prior to loading on the SDS gel heat the samples to 95°C for 5 minutes.
15. Load 10 µL of the sample on a 10-15% acrylamide gel and run at 200V for ~1 hour.
16. Stain the gel for 30 minutes with shaking using, SDS staining solution and then destain overnight with SDS destaining solution. Faster/better destaining can be achieved by including a folded wad of paper towel to the destaining vessel to soak up the dye.