Protein Overexpression and SDS-PAGE

Overexpression:

1. Set up a 200 mL overnight culture of the bacteria with 200 µL of the appropriate antibiotic and incubate at 37ºC for ~16 hours.
2. Pellet cells at 5000 g for 7 minutes using one 50 mL plastic falcon tube.
3. Remove supernatant and resuspend the pellet in 10 mL fresh LB.
4. Check the OD_{600} of the mixture (a 1000 fold dilution is needed for the UV spectrophotometer linear range 0.1-1).
5. Inoculate the expression flasks (2L flasks each containing 500 mL LB) to an OD_{600} of 0.1 with the overnight mixture.
6. Add the 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 concentration 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. Centrifuge cells in the JA-14 rotor at 5000 g (5700 rpm) for 10 minutes.
11. Discard supernatant in the bacterial waste and transfer the cells into a clean sterile 50 mL falcon tube and wash remaining cells out of the centrifuge tubes using LB. The LB must be poured off as supernatant after centrifugation at 5000 g for 10 minutes.
12. Record the cell pellet weight and freeze with liquid nitrogen. Store the pellet in a -80ºC freezer until needed for protein purification.

SDS-PAGE:

13. Open each of the T_0-T_3 samples with 100 µL of cell opening buffer and incubate at 95°C. Add 10 µL of SDS and pellet the debris by microcentrifugation at maximum speed for 1.5 minutes.

14. Prior to loading on the SDS gel, add the appropriate dye and 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. Better destaining can be achieved by including a folded wad of paper towel in the destaining vessel to soak up the dye.