IPTG Induction of recombinant protein expression in Bacteria

Protocol:

1) At the end of the day before the lab day, pick a colony and grow a 5ml overnight culture at 30/37 °C in LB plus the right antibiotic in a snap cap test tube, in a rotator or shaker. Note: This step can be performed by the TAs if none of your group member is available to do it.

2) In the next morning, check the absorbance of the culture at 600 nm. Inoculate a new 5 ml culture with the overnight culture. Use a dilution factor that will make the new culture start from OD_{600}=0.15. It will help if you incubate the medium in a test tube for the new culture in the same incubator so that it will be at the right temperature when you inoculate it.

3) Start checking the OD of the new culture 2 hours after the inoculation. When the OD_{600} reaches 0.6 (it would be fine up the OD_{600}=1.2), remove 0.5 ml culture from the tube and place it 1.7ml tubes. Spin the tube at 5000 rpm for 1 min. Remove the supernatant and add 100 μl a 2X SDS sample buffer. Boil it for 5 min. and freeze it for later use. THIS IS THE UNINDUCED CONTROL.

4) Add 4 μl of a 1000x IPTG stock to the culture to start the induction of the protein expression.

5) After 3 hours (it would be fine up to 7 hours), transfer 0,5 ml of the culture to a 1.7 ml tubes and follow the same procedure in step 3 to prepare the INDUCED SAMPLE. Store the samples in the -20C° freezer for the next lab session. Note: for our class this may mean that some of your group members will need to come and harvest the cells at the end of the lab day.

Lab day 2. Electrophoresis:

6) Follow the protocols in another handout to prepare an SDS-gel.
7) Load your samples (the TAs will show you how) in the wells and run it