

## Protocol

Before you start any of the experiments today, you must first determine how many cells you have in each of your samples. Transfer 0.5 ml to plastic cuvettes and measure the OD600 of each, blanking the spectrophotometer with water or M9, then convert the optical density to a concentration using the approximation that 1 OD600 is  $\sim 1 \times 10^9$  cells/ml.

| Sample            | OD600 | cells/ml |
|-------------------|-------|----------|
| no IPTG           |       |          |
| plus IPTG         |       |          |
| plus IPTG plus aa |       |          |

### Part 1: Protein Gel (SDS-PAGE)

There are some important differences in protein and DNA gel electrophoresis. One thing you'll notice right away is that the gel itself is different. DNA molecules are typically separated through an agarose matrix where as acrylamide is used for proteins. Both are porous sieves that retard molecules based on their length, with smaller molecules moving through the matrix faster than longer molecules. Agarose gels are run horizontally and acrylamide gels are set in the tank vertically but gravity has nothing to do with either separation. Electrical poles draw the charged molecules through the matrix. Unlike DNA, proteins do not have a uniform charge so before electrophoresis they are coated with a charged molecule (called SDS) to add negative charge proportional to their length. You could expect proteins of identical length but folded into different shapes to separate differently (not the desired outcome) so proteins are also unfolded before they are loaded on a protein gel. This is done by boiling them in the presence of a reducing agent, breaking disulfide bridges and denaturing the protein. The last notable difference in DNA and protein electrophoresis is the visualization techniques used to find the molecules once they've passed through the gel. Recall that DNA was visualized with Ethidium Bromide, an intercalating dye that changes its fluorescence when bound to DNA. Proteins can be detected with Coomassie stain, which detects abundant proteins in the gel, turning them blue. Other more sensitive techniques are available for staining protein gels (for example silver stain). The proteins can also be transferred from the gel to a membrane and probed with an antibody specific to a protein of interest. This last technique is called a Western blot.

1. Move 1 ml of each culture to an eppendorf tube.
2. Spin the samples in microfuge for 1 minute at 13,000 RPM.
3. Aspirate the supernatant, put on gloves and resuspend the pellet in 100  $\mu$ l of "sample buffer" for each 1 OD600 of cells (for example, 64  $\mu$ l sample buffer added to 0.64 OD of cells). Sample Buffer contains glycerol to help your samples

- sink into the wells of the gel, SDS to coat amino acids with negative charge, BME to reduce disulfide bonds, and bromophenol blue to track the migration of the smallest proteins through the gel.
4. Boil the eppendorf tubes with lid locks for 5 minutes. You have been given a 10  $\mu$ l aliquot of a molecular weight marker. Don't forget to boil it too!
  5. Time to load the gels. Two groups will share each gel. Put on gloves, then load 5  $\mu$ l of each sample in the order below. Once you have loaded a sample from one tube, move it to a different row in your eppendorf rack. This will help you keep track of which samples you have loaded.
  6. Once all the samples are loaded, turn on the power and run the gel at 200 V. The molecular weight standards are pre-stained and will separate as the gel runs. The gel should take approximately one hour to run. During that hour, you should perform the beta-galactosidase assays described below.
  7. When the gel has run, an instructor will show you how to disassemble the apparatus, and move the gel to Coomassie stain. The gels will be stained for at least one hour and then destained (to remove random blue stain from the pores of the gel) overnight.

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## **Part 2: Beta-galactosidase assay**

With this assay you will determine the amount of beta-galactosidase activity associated with each sample of cells. You will perform assays of each sample in triplicate to gain some confidence in the values you measure. A table is included here to help you organize your assay, but you can make one of your own if you prefer.

1. Make 1 ml of a 1:10 dilution of each cell sample, using Zbuffer-BME as the diluent, then use 100  $\mu$ l of this dilution to make another 1:10, for a final concentration of 1:100.
2. Add 400  $\mu$ l of Zbuffer-BME to 13 eppendorf tubes labeled 0-12.
3. Add 100  $\mu$ l of the appropriate cell dilution to each tube (1:100 of no IPTG in tubes 1-3 etc). See chart below for guidance. Add 100  $\mu$ l of Zbuffer-BME to tube 0, to serve as your blank.
4. Next you will lyse the cells by add 20  $\mu$ l of 0.1% SDS to each eppendorf.
5. To better lyse the cells, you should also add 30  $\mu$ l of chloroform ( $\text{CHCl}_3$ ) to each tube. Do this in the hood since chloroform is volatile and toxic. You will need to hold the pipet tip close to the eppendorf as you move between the chloroform stock bottle and your eppendorfs since chloroform has a low surface tension and will drip from you pipetmen. Be sure to dispose of your pipet tips in the chloroform waste container located on the right side of the hood.
6. To really really lyse the cells, vortex the tubes for 10 seconds each. You should time these precisely since you want the replicates to be treated as identically as possible.
7. Start the reactions by adding 100  $\mu$ l of ONPG to each tube at 10 second intervals, including your blank.

8. Stop the reactions by adding 250  $\mu$ l of  $\text{Na}_2\text{CO}_3$  to each tube at 10 second intervals once sufficient yellow color has developed. "Sufficient" is defined as yellow enough to give a reliable reading in the spectrophotometer, best between 0.3 and 1.0. Be sure to note the time you are stopping the reactions. Also be sure to remember that adding the  $\text{Na}_2\text{CO}_3$  makes the reactions more yellow.
9. When all your samples have been stopped, add 250  $\mu$ l of  $\text{Na}_2\text{CO}_3$  to the blank and spin all the tubes in the microfuge for 1 minute at 13,000 RPM to pellet any cell debris.
10. Move 0.5 ml of each reaction to plastic cuvettes and read the absorbance at 420nm. These values reflect the amount of yellow color in each tube.
11. Read the absorbance of each at 550 nm. These values reflect the amount of cell debris and differences in the plastic cuvettes themselves.
12. Calculate the beta-galactosidase activity in each sample according to the following formula:
  - o Beta-gal Units = Abs at 420 minus (1.75 times Abs at 550nm) all divided by the product of time in minutes, volume of cells (from original culture) in ml and OD600 (of the original culture), then all times 1000.
13. Dispose of your samples properly. Place all cuvettes and tubes in the chloroform waste container in the hood.

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### **Part 3: Harvest Cells for Protein Purification**

Next time you will purify the beta-galactosidase from your induced samples. Store these cells by moving 1 OD600 from the "plus IPTG" sample and 1 OD600 from the "plus IPTG plus aa" sample to eppendorf tubes. Microfuge these tubes for 1 minute, remove the supernatant and freeze the pellets at  $-20^\circ\text{C}$  until next time.