Periods 10/11 (Apr. 9 and 16)

Assay of β-galactosidase in the cells from Period 9

Background**:** See background for Period 8.

Materials:

1. Solution of *o*-nitrophenylgalactoside = ONPG (13.3 mM; 4 mg/ml) (10 ml/pair)
2. Z buffer (see period 8, page 8-4) (100 ml/pair)
3. Na_2CO_3 (1 M; 106 g/l) (50 ml/pair)
4. Chloroform (in the hoods) (10 ml/pair but it can all be put in four bottles)
5. 0.1% SDS (sodium dodecylsulfate) (10 ml/pair)
6. Glass tubes 50/pair
7. Eppendorf tubes (50/pair)
8. Eppendorf centrifuges
9. Pipettemen and tips of various sizes
10. Two water baths at 28°C for β-galactosidase assays
11. Stopwatches (1/pair)
12. Spectrophotometers and associated equipment
13. Graph paper (linear) (3 sheets/pair provided in manual)
14. Vortexers
15. Test tubes and test tube racks for taking samples

Methods:

1. For all samples treated with IPTG dilute 1/5, 1/10, 1/50, 1/100. To 1 ml of each dilution add 1 drop of 0.1% SDS and then 2 drops of chloroform in the hood. Vortex for 10 sec.
2. After allowing the chloroform to settle, add 0.1 ml of each dilution to 0.9 ml Z buffer and incubate at 28°C for 5 min. Include a sample with no cells (1.0 ml Z buffer) to use as a blank.
3. Then start the reactions by adding 0.2 ml ONPG and mixing. Return them to 28°C immediately and time them to the addition of Na_2CO_3 stop solution. Based on our pilot experiment, you should have good yellow color in 20 to 25 minutes for some of the samples. These are the ones you should use to calculate β-galactosidase activity. **You will have to write a protocol for these reactions.**
4. Permeabilize 1 ml of your uninduced sample and the fully grown *E. coli* culture, which will have very low β-galactosidase activity. Use 0.5 ml + 0.5 ml Z buffer. Run controls with 1 ml Z buffer alone (no cells) to correct for spontaneous (non-enzymatic) cleavage of ONPG. Stop with Na_2CO_3. If you do not see color, allow these tubes to incubate as long as possible and stop them just before you are ready to leave the lab. Remove the cells by centrifugation before reading the absorbance (O.D.) at 420 nm. If you do not remove the cells they will scatter light and interfere with your absorbance reading**. You will have to write a protocol for this.**
5. At home calculate β-galactosidase specific activity as follows:

$$\text{specific activity (Miller units)} = \frac{1000 \times \text{O.D.}_{420}}{t \times v \times \text{O.D.}_{650}}$$

This will be discussed in class.

6. Separately, plot β-galactosidase activity / ml culture (without dividing by $O.D._{650}$) as a function of the $O.D._{650}$ of the culture. That is, use β-galactosidase activity / ml culture as the ordinate (y-axis) and $O.D._{650}$ of the culture as the abscissa (x-axis). This, too, will be discussed in class. Use the slope of this plot to calculate the differential rate of synthesis of β-galactosidase. The units will be the same as those for the calculation of specific activity in part 6.
7. Finally, try plotting β-galactosidase activity / ml culture or β-galactosidase specific activity as a function of time.

Data from my lab:
The data below was obtained by my technician. Please plot and analyze this data in addition to your own. It will help you to do this BEFORE you perform the experiment. Plot the growth curve and determine the doubling time. Plot β-galactosidase activity / ml culture as a function of $O.D._{650}$ of the culture. Calculate the differential rate of synthesis of β-galactosidase after IPTG induction.

Time (min)	$O.D._{650}$	$\frac{\text{β-galactosidase activity}}{\text{ml culture}}$ $\left[\frac{1000 \times O.D._{420}}{t \times v}\right]$	β-galactosidase specific activity (Miller units) $\left[\frac{1000 \times O.D._{420}}{t \times v \times O.D._{650}}\right]$
0	0.027	—	
40	0.122	—	
80	0.246	6	
Add IPTG			
100	0.360	315	
120	0.569	2,220	
140	0.919	5,030	
160	1.400*	8,685	
180	1.714*	11,960	

* Cultures were diluted 1:2 with LB for O.D. reading. Actual readings were 0.700 and 0.857, respectively.

Questions:

1. What are the advantages of determining the differential rate of synthesis of β-galactosidase as opposed to its specific activity? What is the relationship between differential rate of synthesis and specific activity?
2. How does a plot of β-galactosidase activity as a function of $O.D._{650}$ allow us to determine the differential rate of synthesis of β-galactosidase? What conversion factors are needed and how would you use them?
3. What are some of the uses to which communal understanding of the lactose operon of *E. coli* have been put? What are the assumptions underlying these uses?