SEED 2009

Day 8: Beta-galactosidase Assay

Learning Objective: In this lab you will become familiar with kinetic assays for determining enzyme activity. You will relate the differences in activity to the different conditions for each sample.

Background: In synthetic biology, a ***reporter*** is a molecule which produces a ***signal*** which can be measured to gain information about the system. So far in this class, you have cloned a beta-galactosidase gene with various ***promoter*** and ***ribosome binding site (RBS)*** sequences which affect the rate of synthesis of the enzyme. Now, for the remaining two class periods, you will characterize how efficiently the promoters and RBSs cumulatively lead to translation. To do this, you will use the activity of beta-galactosidase as a reporter.

In order to do this, we need to understand about how an enzyme works. Enzymes catalyze the reaction of one or more ***substrates*** into one or more ***products***. A critical feature of enzymes is that they are not altered by this process. The rate at which an enzyme catalyzes this reaction can depend on many things, but most importantly the concentration of substrate and enzyme. In this situation beta-galactosidase will catalyze the cleavage of o-nitrophenyl-beta-D-Galactoside (ONPG) into o-nitrophenol and galactose. Galactose is a simple carbohydrate which can be metabolized by the cell, but the o-nitrophenol is a yellow compound which remains intact.

If the concentration of enzyme is much less than the concentration of the substrate, than the enzyme works at a relatively constant rate. In this case, you can observe the procession of a reaction overtime and observe a linear accumulation of product, the slope of which is proportional to the amount of enzyme present.

The exercise: The instructors have started cultures of your various promoter-RBS-reporter constructs. You will use a portion of these cultures to determine the beta-galactosidase activity in each of these samples and thus infer the relative rates of enzyme synthesis in each construct.

Materials: 4 sample culture
1 negative control sample
11 spectrophotometer cuvettes
Spectrophotometer
Permeabilization solution
Substrate solution
Lab timer or stopwatch

Protocol

1. For each sample, pipette 1 mL of culture into a cuvette.

2. Take an absorbance readings at 600 nm and record it in your lab notebook.

3. For each sample, transfer 20 uL of sample into another cuvette.

4. Add 80 uL of permeabilization solution to the 20 uL sample.

5. Place the cuvette with permeabilization solution in the incubator at 30C.

6. Timing is important on the next steps; use your lab timer. Add 600 uL of the substrate solution to a cuvette with 100 uL of water and start the timer.

7. At an even time interval, add 600 uL of substrate solution to a sample and note the time and sample in your lab notebook.

8. Repeat step 7 until all samples have substrate solution.

9. When the timer reaches 10 minutes (or longer if needed), read the absorbance of each sample at 420 and 550 nm. Blank the spectrophotometer with the water cuvette. Record the exact time each sample is measured (Hint: try to use the same order and time interval over which substrate solution was added to have consistent durations across all samples).

10. Repeat the measurements every 10 minutes until absorbance at 420 nm exceeds 3.