SEED 2009

Day 9: Beta-galactosidase Assay Part 2: Inducible Promoters

Learning Objective: In this lab you will observe the behavior of an inducible promoter and generate a transfer curve between the level of inducer input and reporter output.

Background: In biology, the control of the expression of genes is critical. The production of protein is an energy-intensive process, and the synthesis of unnecessary proteins is a waste of resources. Additionally, the behavior of the cell can change drastically depending on which genes are expressed; the expression of inappropriate proteins can have dire consequences.

Cells utilize different mechanism for controlling the expression of genes. Some genes are ***constitutively*** expressed while others are under ***inducible*** expression. The expression of genes behind inducible promoters depends on the conditions within the cell. These may be triggered by environmental factors (temperature, pH, salinity, etc.), protein and cellular interactions, or "small molecules" in the media.

The mechanism by which "small molecules" induce expression is complicated and can vary greatly. In general, proteins within the cell interact with the DNA molecule to either block or initiate transcription a gene. These proteins (or proteins that affect these proteins) also contain regions which bind the small molecule. The binding of the small molecule changes the configuration of the protein and in turn reverses the behavior of the transcription process. Through this cascading the effect, the amount of the small molecule in the media determines the amount of protein which is synthesized.

The exercise: The instructors have started cultures of the inducible construct F2620.E0435. 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 at each level of inducer concentration.

Materials:
8 sample cultures
1 negative control (blank) sample
9 spectrophotometer cuvettes
Spectrophotometer
Substrate solution
Lab timer or stopwatch

Protocol

1. For timing purposes, your instructors have already grown cultures with the appropriate levels of inducer. The concentrations of AHL in the cultures are: 0, 1E-5, 1E-6, 1E-7, 1E-8, 1E-9, 1E-10, and 1E-11 M. These samples have already been added to permeabilization solution and incubated for the required duration. The OD_{600} of these cultures has been provided for you.

2. For each sample, transfer 200 uL of sample (with permeabilization solution pre-added) into a cuvette. Make sure to label the cuvettes so that you know which concentration of AHL is associated with each cuvette.

3. Timing is important on the next steps; use your lab timer. Add 1200 uL of the substrate solution to a cuvette with 200 uL of blank sample and start the timer.

4. At an even time interval, add 1200 uL of substrate solution to a sample and note the time and sample in your lab notebook.

5. Repeat step 4 until all samples have substrate solution.

6. 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 blank sample 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).

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