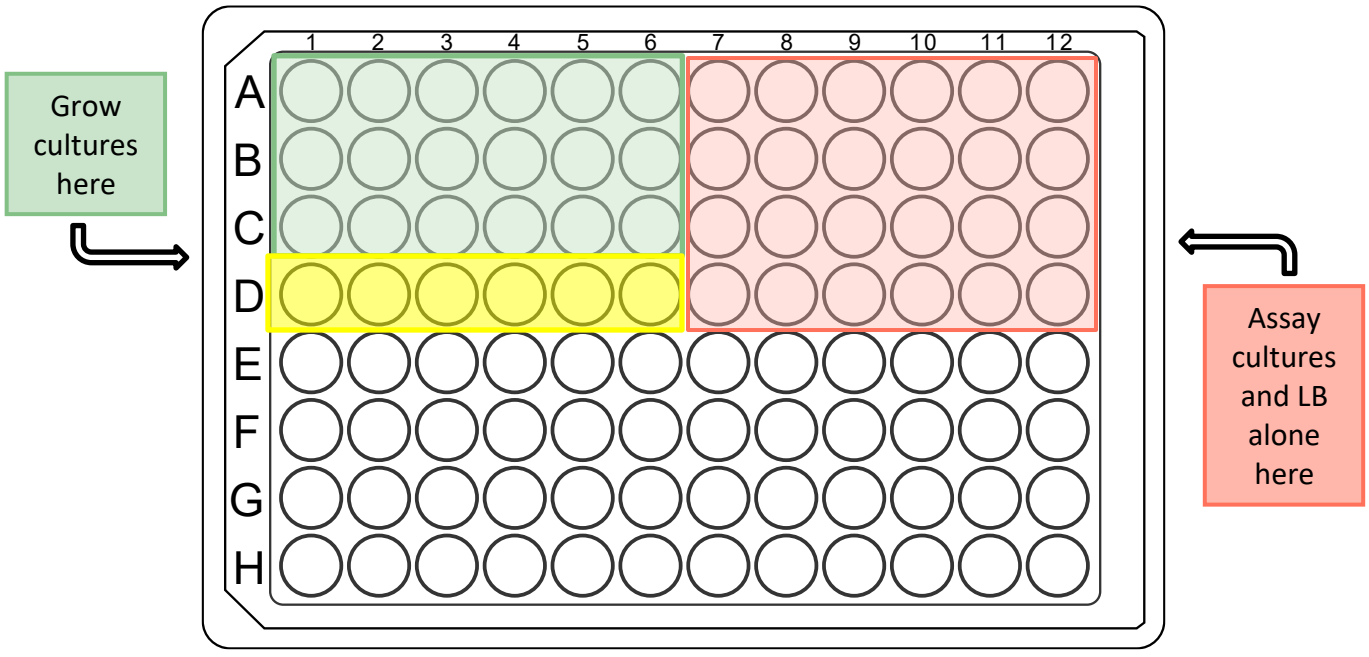
BioPharmaceutical Technology Center
I N S T I T U T E

Measuring β-Galactosidase Expression – GLUCOSE

In this laboratory, we will test the impact of various additives on expression levels of β-galactosidase in *E. coli* strain W3110. In our experiment, we will cleave a colorless β-galactosidase substrate (ONPG) to produce ortho nitrophenol, which is yellow in color. Due to this color change, we can employ spectrophotometry to measure how much β-galactosidase our bacteria have produced. We will test various additives (sugars and buffers) to see how they impact the bacteria's ability to produce β-galactosidase. Bacteria are used for a wide variety of bioindustrial manufacturing and understanding how bacteria can be induced to produce more of a given protein product is very useful.

Part I: Construction of Test Cultures

Students will work in groups of **two or four** to perform this lab. Each group of students will receive a clear 96-well plate. The top portion of the plate will be used for growth of cultures, and the other fourth will be used for the assay of these cultures, as shown below. In Rows A-C, columns 1 – 6 you will grow bacteria in the presence or absence of additives. In Rows A-C, columns 7-12 you will test your bacteria for expression of β-Gal enzyme. In Row D, columns 7-12, we will have LB broth alone as a control (no cells).



1. Each group will **test all of the additives in tubes 1-4** and will choose **two additional** test additives from the remaining options for a total of six additives. Use the microcentrifuge at your bench to spin down tubes all additive reagents. Note that additives are either sugars or combinations of sugars with buffers.

   "Quick spin" the tubes for 3 - 5 seconds, returning the tubes to the tube rack when done.
   Be sure that your tubes are BALANCED in the microcentrifuge!

2. Wells A-C, columns 1-6 of the 96-well plate contain W3110 bacterial cells. This is where you will pipette your additives, and where we will allow cells to manufacture the β-galactosidase enzyme before testing. Also pipette the additives into Wells D 1-6, which contains LB broth only as a control.

3. Add 50 µl of each "mandatory" additive, 1-4 to columns 1-4 in order (ex. column 1 = tube 1; column 2 = tube 2, etc.). Add 50 µl of each "choice" additive into columns 5 & 6. Record what these additives are here;

   Tube number: _____ Additive: _____ Column: 5 6 (circle one)

   Tube number: _____ Additive: _____ Column: 5 6 (circle one)

ADD ALL TUBES 1-4	CHOOSE 2 TUBES 5-8
1 (C) – DI Water	**5 (GH) –** 1.6% Glucose + HEPES
2 (i) – 1mM IPTG	**6 (GP) –** 1.6% Glucose + Phosphate Buffer
3 (G) – 1.6% Glucose	**7 (GY) –** 1.6% Glucose + 1.6% Glycerol
4 (Gi) – 1.6% Glucose + 1mM IPTG	**8 (GR) –** 1.6% Glucose + 1.6% Rhamnose

Note that we are setting up our experiment with three *replicates*. We will have three samples with the same conditions. This will allow us to perform more accurate analyses of our results.

4. Incubate the reactions at 37°C for 90 minutes.

Part II: Assay of Results

1. Place 5 mls of FastBreak™ Cell Lysis reagent into the plastic reagent reservoir on your bench. Using a multichannel pipette, add 10 µl of FastBreak™ Cell Lysis reagent to each of the wells in the assay portion of the plate (rows A-D, columns 7-12).

2. Using a multichannel pipette, transfer 100 µl of bacterial cells from the growth portion of the plate to the assay portion of the plate into their respective assay wells. You are moving cells from Rows A-C, columns 1-6 to Rows A-C, columns 7-12. Row 1 A-C will be moved to Row 7 A-C; Row 2 A-C will be moved to Row 8 A-C; so on and so forth. Do the same for the LB alone well (Row D 1-6 move to Row D 7-12).

3. Place 5 ml of ONPG (4 mg/ml ortho-Nitrophenyl-β-galactoside) + HEPES (100mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.5) into a plastic reagent reservoir. Using a multichannel pipette, add 100 µl of ONPG + HEPES to each of the wells in the assay portion of the plate (rows A-D, columns 7-12). This is the β-Galactosidase substrate with a buffer.

4. Note the time you complete step 3 above: _____

5. Incubate at room temperature for 15 minutes, watching for a yellow color to start to develop. Place 2-3 mls of the STOP reagent into a plastic reagent reservoir. At the 15-minute mark, use a multichannel pipette to move 25 µl of STOP reagent (500mM potassium hydroxide) to each of the wells in the assay portion of the plate (rows A-D, columns 7-12).

   CAUTION: The stop solution is caustic. Make sure you are wearing all your proper laboratory protective clothing – gloves, lab coat & safety glasses.

6. At the end of the incubation period, your instructor will measure the absorbance at 600nm to determine the cell concentration in each well (Rows A-C, 1-6 + Row D 1-6 control). The instructor will also measure the absorbance at 405nm to determine the enzyme activity (Rows A-C, 7-12 + Row D 7-12 control).

BioPharmaceutical Technology Center
I N S T I T U T E

Part III: Data Analysis

1. What is the significance of setting up our experiment in replicates (3 of each compound)?

2. Which wells (on average) had the highest cell density?
   Is that what you expected? Why or why not?

3. Which wells (on average) had the overall highest enzyme activity?
   Explain why this does or does not make sense based on your prediction.

4. Which wells (on average) had the highest **relative** enzyme activity?
   Explain why this does or does not make sense based on your prediction.

5. Describe the difference between overall highest enzyme activity and highest *relative* enzyme activity.

6. What possible sources of error or variability may have affected our results?

7. Out of your experiments, which protein expression method would you recommend to someone using bacteria to manufacture enzymes and why?