

Identifying Promoter Activators and Repressors Using *lacZ* Transgene Expression in *Saccharomyces cerevisiae*

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Gene expression is an essential concept to biology majors. In this two-week guided inquiry laboratory, students are led to discover the role of promoters in gene expression using colorimetric assays measurable by spectrophotometry. Additionally, the two-week lab module aims to introduce students to elements of research including iterative skills, data analysis and data interpretation by comparison of their data to assigned figures in primary research papers. Students use a straight-forward protocol to perform the beta-galactosidase assay to analyze four *Saccharomyces cerevisiae* strains carrying the *lacZ* reporter transgene driven by a eukaryotic wild-type or mutant promoter.

Keywords: Gene expression, *lacZ*, beta-galactosidase, activator element, repressor element, *Saccharomyces cerevisiae*

Introduction

The **objectives** of this laboratory are to:

1. Provide students with a hands-on laboratory opportunity to investigate gene expression in a common, single-celled model organism.
2. Provide students with an opportunity to gain experience using a micropipette.
3. Use these terms appropriately and purposefully: promoter, reporter gene, *lacZ*, ONPG, beta-galactosidase, enzyme, substrate, reactants, and products.
4. Explain how reporter genes work and why they are used.
5. Learn and practice basic laboratory techniques, such as using micropipettes and spectrophotometers.

6. Interpret the data in a figure from the scientific primary literature.
7. Evaluate the results of experiments to determine the effects of oxidative stress and deletion of promoter elements on *lacZ* expression.

The two-lab sequence in Spring 2014 and Fall 2014 described herein has been used in a molecular biology lab for second-year biology and pharmaceutical science majors at North Carolina Central University (NCCU). The lab is derived from one developed at Webster University for Genetics students based on a research publication (Schroeder *et al.*, 1994). At Webster University, the lab is three hours long and involves eight yeast strains. Since the biology laboratories at NCCU are 1 hour and 50 minutes, the protocol described here uses only three yeast strains (from S. Schroeder). At NCCU, the molecular

biology lab starts by integrating course content on the central dogma of molecular biology (replication, transcription, and translation) and incorporates regulation of gene expression through changes in levels of the protein beta-galactosidase (beta-gal) in yeast. Given the substrate ONPG (*o*-nitrophenyl-beta-D-galactopyranoside), beta-gal can produce a yellow-colored product that is measureable by spectrophotometry. The first lab acclimates students to the *lacZ* reporter assay, asking them to measure the effects of a promoter and an oxidative stressor on expression of this gene. The second lab utilizes two yeast strains with different deletions in the promoter region upstream of *lacZ*, and students are to experimentally identify which strain has a deleted activator element and which strain has a deleted repressor element. Students are asked to examine data from the primary literature both before and after completing these labs. The articles used are referenced in the Literature Cited section as well as in the Student Outline.

After running this lab with approximately 60 students, we uncovered a common misconception they held. When students see that deleting a promoter element causes expression of *lacZ* to increase, they conclude that the deleted element was an activator; however, if removal of the element causes expression to go up, then the element was in reality a repressor. Not only are these labs an excellent opportunity for students to gain hands on wet lab experience, but it is also a chance for them to begin correctly interpreting data from genetic manipulations. Further course topics on mutations, protein function, and genetic studies using “knockout” model organisms can build upon the foundation created by these labs.

Alternatively, the lab could be expanded to allow students the opportunity to design experiments to test the effect of other stressors on *lacZ* gene expression.

For preparation, all reagents can be made ahead of time and stored at room temperature, -20°C freezer or refrigerator. Solutions can be prepared within a day by the instructor or lab technician (as at NCCU) or students can prepare them in a third lab session (as at Webster University). Yeast strains must be freshly streaked on plates and freshly expanded in liquid culture each week of the lab for reliable yeast growth. The night before the lab, yeast cultures are set up and times in order to achieve log phase one hour before the lab session begins. Lab setup takes about one hour to aliquot samples and set up a *lacZ* station from which students may retrieve their reagents. Students can complete the experimental protocols for each of these labs within a 1 hour and 50 minute class period; especially when there is an eight student: one instructor ratio for assistance with micropipetting (please see ‘Notes for the Instructor’ for other suggestions for inexperienced students). The majority of faculty (about 70%) reviewing this workshop, at Boston University 2015, suggested that it was most appropriate for freshman or sophomore students. Additionally, about 75% of faculty agreed this would be a good lab to use with their students to reinforce the concept of gene regulation in their curriculum. Furthermore, faculty are interested in how these modules lead into use of these strains in a semester-long research project. The authors plan to publish the course curriculum in a journal such as *Course Source* and/or *CBE Life Sciences*.

Student Outline

Week 1: Guided Inquiry on Gene Expression Using *lacZ* Yeast Strains

The investigation question is as follows: Does a stressor affect gene expression?

Learning Objectives

- Define reporter gene, *lacZ*, ONPG, and beta-galactosidase.
- Explain how reporter genes work and why they are used.
- In the chemical reaction taking place during the assay, identify: enzyme, substrate, reactants, and products.
- Gain hands-on experience detecting gene expression through the colorimetric beta-galactosidase assay.
- Measuring spectrophotometry readings of the beta-galactosidase yellow reaction product.
- Define ‘promoter’ and explain its role in gene expression.
- Discuss the difference between the two strains of yeast used in the assay and how this difference impacts that result of the assay.
- Study a figure from scientific primary literature; interpreting the data in that paper.
- Deciding whether the stressor, H₂O₂, has affected the *lacZ* reporter gene expression in our strain of yeast.

Background: What Is a “Reporter Gene?”

The reporter gene is a gene whose product protein can be easily detected in a colorimetric or fluorescent assay as a read-out of a biological process: gene expression (transcription followed by translation). In other words, this gene reports to us that gene expression, transcription into mRNA and also translation into a protein, has occurred inside the cell. We can easily see that gene expression because the reporter will either be colorful itself or it will be an enzyme that can make a colorful product. It is key that a reporter protein is not expressed naturally in the organism or broken down quickly, and can be easily assayed. We will be using a reporter gene to detect gene expression in yeast.

The reporter gene we will use is one that is not naturally found in yeast (eukaryotes), but rather found naturally in bacteria (prokaryotes): the *lacZ* gene. This gene produces an enzyme called beta-galactosidase. Bacteria need beta-galactosidase to metabolize a specific disaccharide sugar, lactose by breaking the beta-glycosidic bond holding the two monosaccharides that make up lactose: galactose and glucose. The *lacZ* gene is normally expressed in bacterial cells that live in your intestines or in your cup of yogurt. This is one of the ways in which bacteria help you digest food, since humans do not produce enough of the lactase enzyme (from the human *LCT* gene) to break down all the lactose-laden dairy products we consume (<http://ghr.nlm.nih.gov/gene/LCT>).

In order to use beta-galactosidase in a reporter gene system outside of bacteria, the *lacZ* gene has been taken out of bacteria and placed into the yeast genome using recombinant DNA techniques. Since yeast do not need to metabolize lactose to survive, we can use the *lacZ* gene product for another purpose: to detect when a yeast gene is being expressed. As more *lacZ* gene is transcribed and translated, the more beta-galactosidase will be present in the yeast cells. Rather than digesting lactose, the enzyme will be used to act on a substrate to convert it into a product that has a yellow color (Figure 1). We can detect the yellow color in the spectrophotometer. A higher the level of yellow product will yield a higher spec reading, meaning that the *lacZ* gene was expressed as functional beta-galactosidase and is catalyzing the conversion of the ONPG substrate into a yellow product.

Why Do We Care?

The *lacZ* reporter system has revolutionized our understanding of eukaryotic and prokaryotic gene regulation. Scientists now have an easy way to understand when, where and how certain genes are transcribed and translated: gene expression. Finding out how genes are regulated helps us understand how infectious diseases make us sick and why loss of cell cycle regulation can cause cancer. In the long run, using the gene reporter approach has given scientists a tool to understand mechanisms of gene regulation, which helps medical doctors treat a variety of diseases.

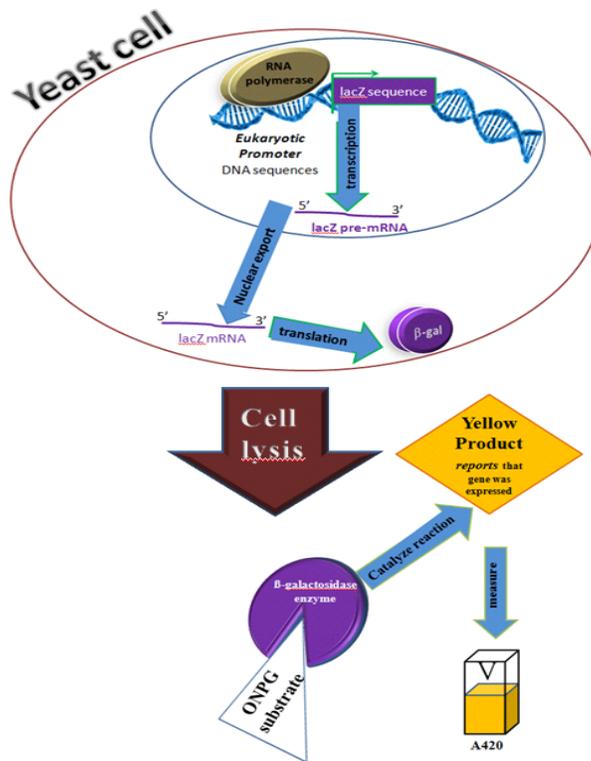


Figure 1. The beta-galactosidase assay. The *lacZ* reporter gene has been placed in the yeast genome attached to the promoter of the *TBP* gene. When the cell is signalled to transcribe the yeast gene, instead of expressing a yeast gene product (the TBP protein), the bacterial *lacZ* gene produces the enzyme, β -galactosidase. We will lyse the yeast cells so we can analyze the amount of enzyme present. We can visibly see that the gene was expressed because the enzyme converts the ONPG substrate to a measurable colored product. The amount of yellow product corresponds to the amount of gene expression.

Today’s Experiment

Today we are going to use the *lacZ* reporter system to look at gene expression in yeast. We will do this by running a beta-galactosidase assay with two strains of yeast:

1. One yeast strain is called “**Empty LacZ**” or “**E**” for short. The E strain contains all genes and proteins naturally found in yeast; it also has the *lacZ* reporter gene inserted into its genome. However, it does NOT contain a promoter to turn on expression of the *lacZ* gene.
2. The second yeast strain is called “**TBP-LacZ**”, or “**P**” for short. The P strain is identical to the “E” strain, but in front of the *lacZ* bacterial gene is a regulatory DNA sequence called a **promoter**. The specific promoter normally controls expression of the **TBP (TATA-binding protein) eukaryotic gene**. The protein product of the TBP gene is an essential protein needed for transcription of all yeast protein-encoding genes.

Using these strains, we will test how the presence of a promoter affects gene expression of *lacZ*. Also, we will test if a chemical **stressor, hydrogen peroxide (H₂O₂)** will affect the expression of the *lacZ* protein.

Beta-Galactosidase Assay Summary

- When lactose is presented to bacteria in the growth environment, the beta-galactosidase enzyme breaks down lactose into monosaccharides for energy production.
- During a beta-gal assay, lactose is replaced by a more stable **substrate**, a compound called ONPG (*o*-nitrophenyl-beta-D-galactopyranoside). Beta-galactosidase cleaves ONPG to produce the compound *o*-nitrophenol, which appears yellow and its absorbance (A420) can be measured by a spectrophotometer.

Pre-lab Questions

1. What is a reporter gene? Explain why we use them (what advantage does it offer researchers?).
2. What reporter gene will we use in this lab?
3. What gene product is expressed from the reporter gene we are using?
4. Looking at the assigned primary research paper (Zhang *et al.*, 2011):
 - a. What two dependent variables are shown in Figure 1 for H₂O₂?
 - b. What are the three independent variables shown in Figure 1?
 - c. How were the independent variables changed during the experiment?
5. In our experiment, what are the two independent variables?
6. What is our dependent variable?
7. State a hypothesis for the experiment using an “if (independent variable)...then (dependent variable)” statement.

Procedure Overview

Students will work together as a group of 3-4.

- Each student will have three tubes:
 - One for E strain
 - A second tube for the P strain.
 - A third tube for the P strain plus the hydrogen peroxide (H₂O₂) stressor.
- In the beginning of the lab, we will tell you the concentration of these cells. This will be in optical density (O.D.) as measured by a spectrophotometer at the wavelength of 600 nm: this is known as your absorbance 600 or A600 reading.

For the reaction to work correctly, it is important to add the solutions in the proper order below.

Procedure

1. Record the A600 reading written on the whiteboard into your Data Table 1 below.
2. Each student should label three microcentrifuge tubes:
 - a. E
 - b. P
 - c. P + H
3. Vortex the cultures to mix the cells for ~30 seconds. Stop when the yeast cells are evenly distributed in the tube.
4. Using a p200 micropipettor set at 200, pipette 200 μ L of the vortexed yeast cells into each labeled tube.
5. To each of the three tubes, add 800 μ L of Z buffer.
6. Then add 50 μ L of chloroform to each tube.
7. Add 20 μ L of 0.1% SDS to each tube.
8. Vortex each tube for no more than 10 seconds.
9. Add 200 μ L of ONPG and vortex.
10. Once you vortex, place all the tubes into a rack in the 37°C incubator.
11. Start your stopwatch to time the beta-gal reaction for 15 minutes.
12. While the reaction is incubating, prepare the cuvettes:
 - a. DO NOT TOUCH THE LOWER HALF OF THE CUVETTE WITH YOUR BARE HANDS.
 - b. Your group will need one cuvette labeled ‘BLANK’ – one student should volunteer to label this cuvette.
 - c. Each student will need to label three cuvettes for their samples. To do this, assign yourselves a number one through four. Then label your cuvettes as in the picture below (Figure 2):

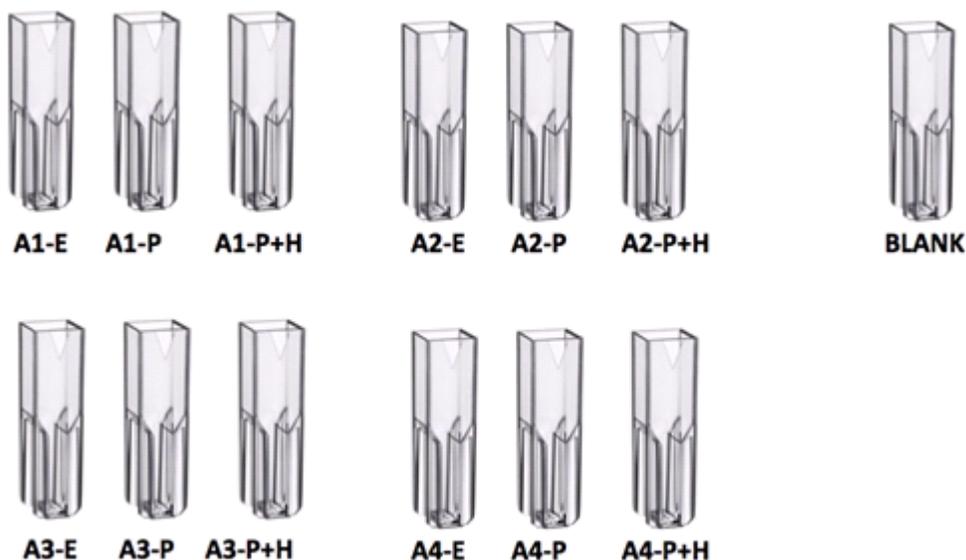


Figure 2. Example of cuvette labels for a group of four students. If, for example, ‘Group A’ consists of four students, then each student in the group is numbered one, two, three, or four. Use E for empty-*lacZ*, P for promoter-*lacZ* and P+H for promoter-*lacZ* plus the hydrogen peroxide stressor. Each group of students will only need one BLANK cuvette.

1. After 15 minutes, remove the rack of tubes from the incubator.
2. Stop the reaction by adding 500 μL of Stop Solution (Na₂CO₃) to each tube.
3. Centrifuge tubes at 10,000 g for five minutes.
4. There will be an aqueous portion at the top of the tube. Carefully remove 1,000 μL of the aqueous supernatant solution from each tube and transfer it into the correctly labeled cuvette. The bottom layer of chloroform should NOT be removed.
5. Set a spectrophotometer to 420 nm.
6. Place the BLANK cuvette into the spectrophotometer and use the ‘Zero’ button to set the A420 to zero.
7. Measure the absorbance of all your samples at A420 and record each reading in Data Table 1 below.

Clean-up Instructions

1. Put all used tips, tubes and cuvettes in a biohazard bag.
2. Return micropipettes to drawers.
3. Clean up all spills using paper towels and throw them away in the trash.
4. Please leave your bench cleaner than you found it.

Data Analysis

1. Calculate **Activity units** of each spectrophotometer reading using the equation below. Show your work in your lab notebook.

$$\text{Activity units} = \frac{\text{Absorbance at 420 nm} \times 1000}{\text{A600} \times 15 \text{ minutes} \times 0.2 \text{ mL}} = \text{_____ velocity/mL}$$

Figure 3. Formula for calculating the beta-gal activity from each strain of yeast.

2. Calculate the average activity units for each sample in your group and record it in Data Table 1.
3. Report the average A420 and Activity units on the whiteboard and in Data Table 2.
4. RECORD the data from all groups recorded on the whiteboard in Data Table 2.
5. *Graph #1:* Create a *bar graph* showing the data from just your group.
 - a. Use graph paper from Blackboard and draw the graph by hand.
 - b. Include an appropriate title that reflects the variables in the graph.
 - c. Label the axes clearly (including units).

6. For the class data: Calculate the average and the standard deviation for each yeast strain in your lab notebook showing all of the following (see *Appendix E Calculation of Mean and Standard Deviation*):
7. Sample size (N)
8. Class mean
9. Sum of the squared deviations
10. Variance
11. Standard deviation
12. *Graph #2*: Create another bar graph showing the class data. This graph should show means and standard deviations. To graph standard deviation:
 - a. Subtract the standard deviation from your mean
 - b. Then in a separate equation, add the standard deviation to your mean.
 - c. Graph each of these values on top of your bar graph using brackets. See example below.
 - i. Use graph paper from Blackboard and *draw* the graph by hand.
 - ii. Include an appropriate title that reflects the variables in the graph.
 - iii. *Label* the axes clearly (including units).

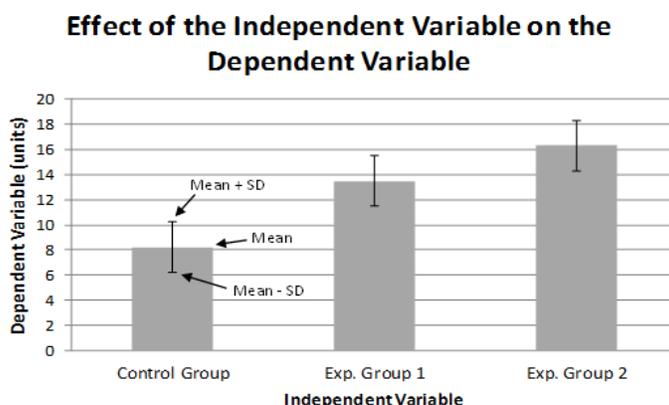


Figure 4. Example of a bar graph. The dependent variable (A420) should be graphed on the Y-axis and the independent variable (the yeast strain conditions) should be graphed along the X-axis as shown. The bars represent the mean (average) A420 result; while the lines with flat endings represent how much each reading varied (deviated) from the mean. We call the variance the Standard Deviation (SD). Please see Appendix E for instructions on how to calculate the SD. Please note that labels are required in the graph: a Title for the graph (up top), a title for the Y-axis ‘Dependent Variable (units)’ where you insert the appropriate name for the dependent variable and finally, the title for the X-axis which is the independent variable of your experiment.

Show ALL calculations in your lab notebook!

Use graph paper to make your graphs from each data table and then staple or otherwise attach the graphs into your lab notebook in the Data section.

Data Tables

Table 1. Group data on yeast with promoters and hydrogen peroxide exposure.

Strain/Condition (each student in group is numbered)	A600 of cells (whiteboard beginning of class)	Volume of yeast cells	Time (minutes)	A420 (your reading from the spec)	Activity Units	Average Activity Units for your group
Empty-LacZ (E) #1		200 µL	15			
Empty-LacZ (E) #2		200 µL	15			
Empty-LacZ (E) #3		200 µL	15			
Empty-LacZ (E) #4		200 µL	15			
TBP-LacZ (P) #1		200 µL	15			
TBP-LacZ (P) #2		200 µL	15			
TBP-LacZ (P) #3		200 µL	15			
TBP-LacZ (P) #4		200 µL	15			
P + H ₂ O ₂ #1		200 µL	15			
P + H ₂ O ₂ #2		200 µL	15			
P + H ₂ O ₂ #3		200 µL	15			
P + H ₂ O ₂ #4		200 µL	15			

Table 2. Class data on yeast with promoters and hydrogen peroxide exposure.

Yeast Strain/condition	Student Group						Mean	Standard Deviation
	A	B	C	D	E	F		
E								
P								
P + H ₂ O ₂								

Conclusions and Future Directions (in Lab Notebook)

1. *Decide/Interpret.* Based on the data you and the class collected, should you accept or reject your hypothesis?
 - a. Explain your reasoning clearly. Hint: start by reminding the reader what the hypothesis was.
 - b. Refer back to data and specifically point out what about the data either supports your hypothesis or what does not support your hypothesis.
 - c. Look at the assigned primary research paper that goes with this lab (Zhang *et al.*, 2011). In Figure 1, describe what happens to the beta-galactosidase assay as increasing amounts of hydrogen peroxide (H₂O₂) are added.
 - d. Relate your results with hydrogen peroxide to those in the paper. In the paper, they are using a yeast strain that is sensitive to genotoxins (stressors that affect gene expression). Did your yeast strain have similar or different results? Explain.
2. *What next?* Recall the scientific method.
 - a. Should you repeat the experiment as is, or perhaps with modifications?
 - b. Discuss any trouble you had, things that might need to be adjusted, errors that were made, etc.
 - c. If you were not able to reject or accept the hypothesis, discuss how to make sure you can next time.
3. *Extensions.* After thinking about the results you obtained today, think of a related experiment you would like to do next to find out more about what you did today.
4. *Applications.* Imagine you were using a different reporter gene, such as Green Fluorescent Protein. Describe how you would do a similar experiment but using *GFP* instead of *LacZ*.

Week 2: Guided Inquiry – Promoter Bashing

Introduction

The *Central Dogma* states that DNA is transcribed into RNA, which is then translated into protein. But how does the DNA know how much RNA to make and when to make it? In other words, how is *gene expression* regulated?

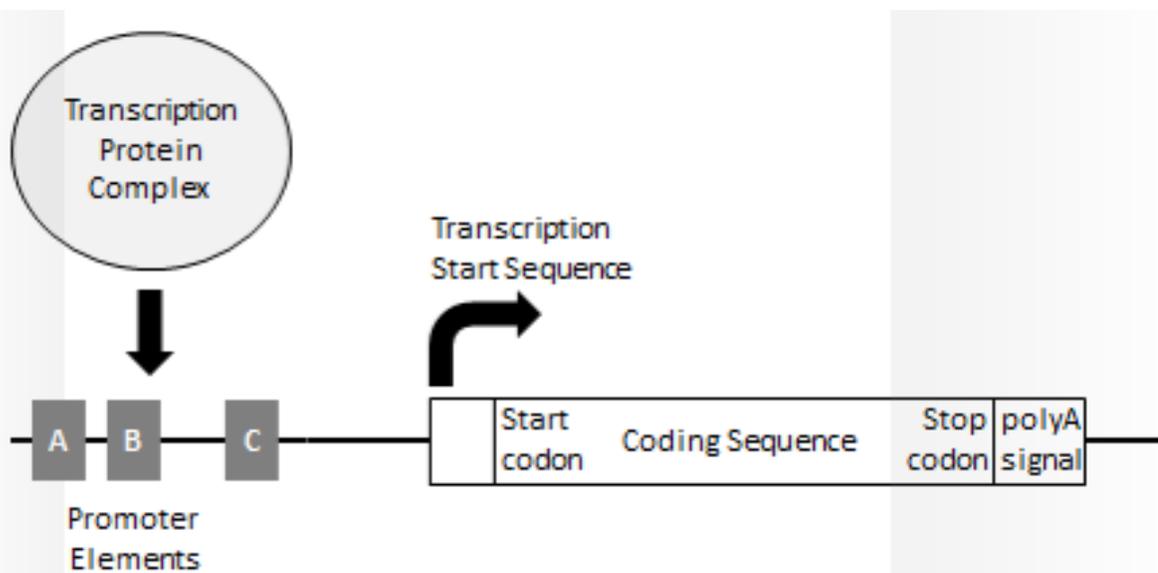


Figure 5. Diagram of a gene undergoing transcription.

RNA is synthesized by RNA polymerase, which is part of a large complex of many proteins. These proteins know which genes to bind to based on specific sequences of DNA in the *promoter region*. The general transcription factors bind to DNA at promoter sequences, and then they recruit other proteins and RNA polymerase to bind. Once the transcription complex has bound to the promoter, it will scan the DNA for the transcription start sequence, which is the DNA site where transcription and RNA synthesis is initiated. After transcribing the coding sequence and the untranslated DNA sequence that signals polyA tail addition during the RNA processing step, the transcription machinery will separate from the DNA and release the newly-made RNA.

So while the coding sequence of DNA is responsible for what a protein looks like and how it functions, the promoter region determines how much RNA is produced. Depending on the cell's needs, promoters can also regulate when the levels of RNA for a gene need to be increased or decreased.

Changes in the promoter region can cause aberrant gene expression – it can either increase or decrease compared to normal levels of expression. Promoters in eukaryotic cells are often complex, containing several different parts known as “elements.” It is useful to know which promoter elements are crucial for regulating gene expression (which really need to be there in order for the RNA to be transcribed). You can determine this by creating your own *DNA constructs*, using recombinant DNA techniques. A DNA construct simply means that by using recombinant DNA technology, you can put together different pieces of DNA inside a test tube by using enzymes that create phosphodiester bonds. In other words, you construct the DNA yourself, making a novel DNA sequence never seen before in nature. Your construct would contain the gene you are working on, but you would delete or mutate the promoter element you wish to study. Then you would put the DNA into yeast cells and measure gene expression. This type of experimenting is colloquially called *promoter bashing*.

In order to experimentally determine the effects of different promoter elements on gene expression, we need a way to measure gene expression quantitatively. One way to do this is to attach our promoters to the *lacZ* reporter gene. As we learned in the previous lab, the *lacZ* gene produces the enzyme beta-galactosidase (aka beta-gal). Beta-gal catalyzes the reaction to hydrolyze the substrate ONPG into the products galactose and *ortho*-nitrophenol (Figure 2). ONPG and galactose are both colorless while *ortho*-nitrophenol has a yellow color. So if there is beta-gal enzyme present in a solution, adding ONPG will turn the colorless liquid yellow as the ONPG is degraded. Using the beta-gal reaction in this way makes it a **reporter assay**. This reaction is going to “report” to us how much beta-gal is produced by our cells depending on what promoter elements are driving protein expression.

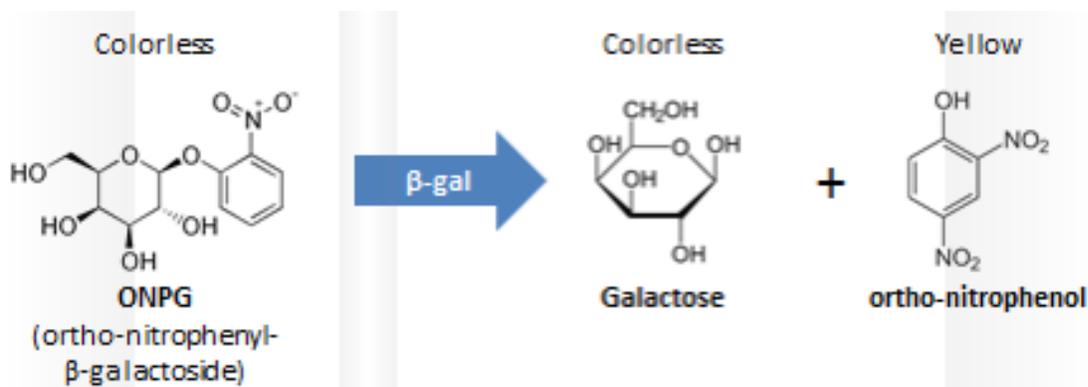


Figure 6. Beta-gal-catalyzed reaction used as a reporter assay.

Background on Yeast Strains

Yeast strain “E” contains the *lacZ* gene, but no promoter. Yeast strain “P” contains the *lacZ* gene under control of a wild-type (“normal”) promoter. Yeast strain “A” has the *lacZ* gene under control of a mutated promoter, and yeast strain “B” has a different mutation in the promoter. The mutations are thought to prevent the binding of transcription factors and *RNA polymerase* to the promoter. The four yeast strains are organized in Data Table 1 below.

Purpose

This week in lab, your purpose is to answer the following question: how does mutation (specifically, deletion) of promoter elements upstream of the *lacZ* gene affect expression of the *lacZ* protein beta-galactosidase? You will determine whether elements A and B are activator elements (that turn up transcription levels) or repressor elements (that turn down transcription levels).

Pre-lab Questions

1. What is the function of the beta-galactosidase protein?
2. Write a reaction to show the substrates (reactants) that beta-gal utilizes, and the products it produces.
 - a. Indicate if any of these compounds have a particular color
3. What were your major findings from the first *lacZ* experiment?
4. What is the independent variable for this lab?
5. What is the dependent variable?
6. Write an “if...then” hypothesis to answer the question for this experiment.
7. What is/are the control groups for this experiment?
8. What are the experimental groups?
9. List 5 standardized variables that should be held constant.
10. Looking at Figure 2 in the assigned paper,
 - a. What do you observe happens to the beta-galactosidase activity with the – construct which contains the full-length TBP-promoter compared to the *lacZ* construct labeled ‘vector alone’?
 - b. Do the results of these two lines look similar to your results from week 1? Specifically explain the similarities or differences in terms of the mean of the beta-galactosidase units of activity and the standard deviation.

Procedure

Your group will set up this experiment together, testing each sample in duplicate. You will use 4 different strains of yeast (listed in Table 1). Follow the same procedure used in the last lab, using additional tubes when necessary.

Data Tables

Table 3. Group data on beta-gal reporter assay on yeast strains with promoter mutations.

Yeast Strain		A600 (nm)	Volume (mL)	Time (minutes)	A420 (nm)	Activity Units (velocity/mL)	Average Activity Units
E	Student 1		0.2	15			
	Student 2		0.2	15			
	Student 3		0.2	15			
	Student 4		0.2	15			
P	Student 1		0.2	15			
	Student 2		0.2	15			
	Student 3		0.2	15			
	Student 4		0.2	15			
A	Student 1		0.2	15			
	Student 2		0.2	15			
	Student 3		0.2	15			
	Student 4		0.2	15			
B	Student 1		0.2	15			
	Student 2		0.2	15			
	Student 3		0.2	15			
	Student 4		0.2	15			

Table 4. Class data of beta-gal reporter assay on yeast strains with promoter mutations.

Yeast Strain	AVERAGE Activity						Mean	Standard Deviation
	Group A	Group B	Group C	Group D	Group E	Group F		
Strain E								
Strain P								
Strain A								
Strain B								

Data Analysis

1. Use Data Table 2 to calculate the *mean* and *standard deviation* of the class data for each yeast strain.
2. Using Data Table 2 *draw a bar graph* to summarize the *mean activity units* for each yeast strain.
 - a. Include a title, axis labels, units and figure legend like you see in the example assigned primary research article.
 - b. Show the *standard deviation* by drawing error bars on each bar of the graph (see first *lacZ* lab for instructions).

Conclusions

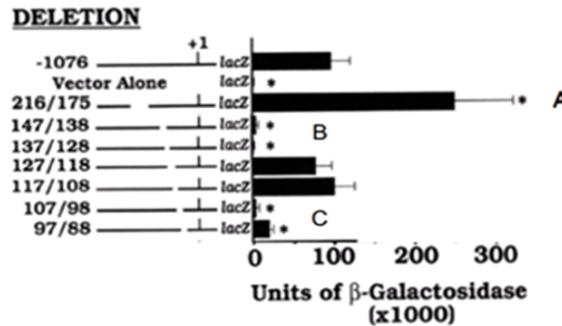


Figure 7. The yeast strains contained either the ‘normal’ promoter that extended 1076 base pairs upstream of the transcriptional start site (+1) in front of the *lacZ* gene or a deletion mutation of different promoter regions. The deletion mutations are shown as number (example 216/175) that correspond to the promoter region missing. The missing region is also depicted as a break in the line upstream of +1. Approximately 1×10^6 cells (0.1 OC600) were used in each assay. The average (mean beta-gal activity from 15 independent assays is shown. Error bars indicate standard deviation. Asterisks indicate that the data are significantly different than the empty-*lacZ* strain by the Student’s t-test (Schroeder *et al.*, 1994). *Figure 2 from Schroeder 1994 publication illustrating the actual lacZ strain results published in the Journal of Biochemistry. Title of Figure 2: Mapping the TATA-Binding Protein (TBP) Promoter Using beta-galactosidase assays.*

1. In paragraph form and using complete sentences, summarize your overall results.
2. Based on the data you and the rest of the class collected, should you accept or reject your hypothesis?
3. What do these results mean for expression of the *lacZ* gene?
4. Studying the Figure 2 excerpt from the assigned research paper (Schroeder shown above, answer the following:
 - a. From the above figure, which element (A or B) would represent a repressor element? And which would be the activator element? Why? Explain your answer in terms of beta-galactosidase activity.
 - b. Comparing your graphed results and the class results to Figure 2 above, how did your data compare. Did you come up with the same conclusion?
 - c. Analyzing the beta-galactosidase data in Figure 2, what type of element would you classify the “C” element shown?
5. Should you repeat this experiment, perhaps with modifications? Discuss any problems you had along with recommendations for how to correct them.

Future Directions/Application Questions

Answer the following questions with detailed, thoughtful responses.

1. After the results of this lab, what new questions do you have that you would want to answer experimentally?
2. Why do you think this type of experiment is called promoter bashing?
3. Imagine a different strain of yeast that you have not worked with before, strain U. When strain U is placed an environment with a high concentration of sugar, it increases expression of enzymes that break down sugar. Breaking down sugar is necessary in order to carry out cellular respiration. Strain X is identical to strain U, but a mutation has occurred somewhere in the DNA of strain X that prevents the cells from increasing expression of enzymes that break down sugar.
 - a. Predict what will happen to Strain X in a high-sugar environment compared to Strain U.
 - b. Explain the reasoning behind your prediction – in other words, what makes you think it will occur?
 - c. Explain how you will measure whether your prediction is correct or not. What approach would you be using (cytology, biochemistry, genetics, model organisms, etc).

Materials

Equipment

For Students in the Lab

- 37°C incubator or water bath
 - **REMEMBER: Incubators in room need to be set at 37°C night before lab.**
- one spectrophotometer (GENESYS™ 20 (Fisher Scientific 14-385-445) model or equivalent) per bench
- one vortex per bench
- p20, p200 and p1000 micropipettors

For Preparation of the Yeasts

- 250 mL sterile Erlenmeyer flasks (two flasks for week #1 yeast cultures; three flasks for week #2 yeast cultures)
- 30°C shaking incubator (water or dry)

Safety Precautions

Students need to wear gloves as Z buffer has beta-mercaptoethanol and they are using chloroform.

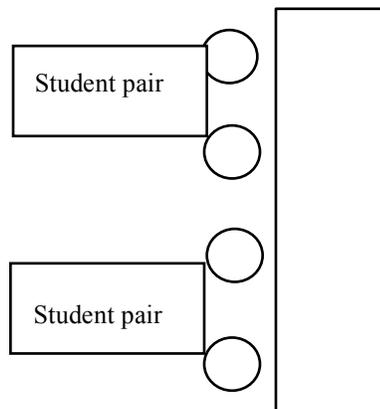
Material per Lab Bench

The following set up is for a 24-25 student classroom, set up for students working in groups of two students (objective: to increase number of times each student handles micropipette).

Each PAIR of Students Needs:

- one marker
- one waste bucket
- one timer
- pipet tips, p20, p200 and p1000
- one microfuge tube rack labeled A-F
- one 15-mL rack labeled A-F
- 15 empty cuvettes
- 15 empty white **2-mL** microfuge tubes (total volume of beta-gal assay is 1.8 mL)
- assay solutions
 - Z buffer, 10 mL
 - Chloroform, 1 mL
 - 0.1%SDS, 1 mL
 - ONPG substrate, 3 mL
 - Na₂CO₃ stop solution, 6 mL
- a tube of blank solution, 1 mL
- a tube of Yeast strain “E” which is really EmptyLacZ → 1.5mL
- a tube of Yeast strain “P” which is really TBPLacZ → 1.5mL
- a tube of Yeast strain “P + H” which is really TBPLacZ + H₂O₂ → 1.5mL

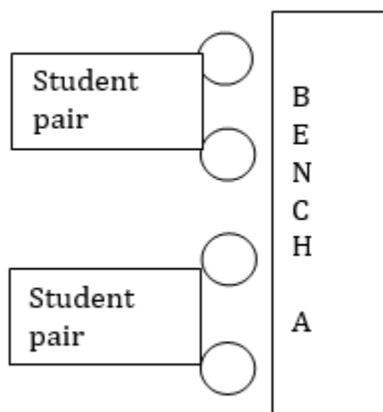
NOTE: For students, the Empty LacZ should be labeled “Yeast Strain E” and TBPLacZ should be labeled “Yeast Strain P” and the stressor tube should be “Yeast Strain P+ H”



Week #2 Set-Up

For every student pair (two student pairs per bench; Example Group A given below):

- one waste bucket
- one marker
- one timer
- one set of micropipettors – p20, p200, p1000
- pipet tips, p20, p200 and p1000 (remember p20s don't work with those yellow tips...)
- one microfuge tube rack labeled A-F
- one 15-mL rack labeled A-F
- 10 empty cuvettes
- 10 empty white **2-mL** microfuge tubes
- assay Solutions
 - Z buffer, 10-15 mL tube with orange cap
 - Chloroform, 1 mL – green microcentrifuge tube (fresh or day before stored at 4°C to reduce evaporation)
 - 0.1% SDS, 1 mL – blue microcentrifuge tube
 - ONPG, 3 mL – 15-mL tube with orange cap wrapped in aluminum foil
 - Na₂CO₃, 6 mL – 15-mL tube with blue cap
- a tube of Blank Solution, 1 mL
- a tube of Yeast strain “E” which is really EmptyLacZ → 1.0 mL
- a tube of Yeast strain “P” which is really TBPLacZ → 1.0 mL
- a tube of Yeast strain “A” which is really deltaALacZ → 1.0 mL
- a tube of Yeast strain “B” which is really deltaBLacZ → 1.0 mL



Other Notes on Preparation

- Store aliquots of chloroform in fridge or freezer to prevent evaporation.
- For each solution above, make enough aliquots for 18 groups instead of 12, in case extra is needed.
- Set up racks for all 12 groups ahead of time.
- Benches should be completely set up as listed above no later than 10 minutes before lab starts.

Notes for the Instructor

Introduction

Second semester freshman or first semester sophomore students have already learned the central dogma of molecular biology in lecture prior to starting this two-week laboratory. Students are required to turn in the pre-lab questions in order to enter the lab.

Since most students in our BIOL2200 course have not had extensive experience using **micropipettors**, we introduce how to use the instruments in a laboratory session on week two of the semester. Approaches we have used include:

1. Using an online video instruction and pre-lab questions.
2. Giving the students in class/supervised instruction on proper use of micropipettors.
 - a. Due to a generous grant from HHMI, we have one instructor per eight students to facilitate in supervising micropipettor use.
 - b. In the absence of funding or T.A.'s, the class can be introduced to the micropipettors in a simple activity where each student group of two can take turns quizzing each other of the proper setting. The instructor checks off students for each micropipettor as part of the lab: Signature line for points.

3. Using a one-day lab on measuring the accuracy of micropipetting as determined by spectrophotometry of food coloring. Incorporating ideas from online literature:
4. bio.classes.ucsc.edu/bio20L/MANUAL/Lab%201.pdf
5. www.rsc.org/learn-chemistry/resource/.../pdf

Preparation Notes

For preparation, all reagents can be made ahead of time and stored at room temperature, -20°C freezer or refrigerator. Solutions can be prepared within a day by the instructor or lab technician (as at NCCU) or students can prepare them in a third lab session (as at Webster University). Yeast strains must be freshly streaked on plates and freshly expanded in liquid culture each week of the lab for reliable yeast growth. The night before the lab, yeast cultures are set up and times in order to achieve log phase (A600 between 0.4 and 0.8) 1 hour before the lab session begins.

Day of Lab

Lab setup takes about one hour to aliquot samples and set up a *lacZ* station from which students may retrieve their reagents. Detailed instructions for set up are in the **Materials** section.

Literature Cited

- Schroeder S.C., C.K. Wang, and P.A. Weil. 1994. Identification of the *cis*-acting DNA sequence elements regulating the transcription of the *Saccharomyces cerevisiae* gene encoding TBP, the TATA box binding protein. *Journal of Biological Chemistry*, 269(45):28335-46.
- Zhang M., C. Zhang, J. Li, M. Hanna, X. Zhang, H. Dia and W. Xiao. 2011. Inactivation of YAP1 Enhances Sensitivity of the Yeast RNR3-lacZ Genotoxicity Testing System to a Broad Range of DNA-Damaging. *Toxicological Sciences*, 120(2): 310-321.

Acknowledgments

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About the Authors

Dr. David A McDonald is an assistant director for Graduate Services in the Duke University Career Center. He completed his B.S. in biochemistry from the University of Florida, and then received his Ph.D. in Genetics and Genomics from Duke University. As a postdoctoral fellow for the Center for Science, Math, and Technology Education at North Carolina Central University, he taught undergraduate labs for Principles of Biology: Molecules and Cells (BIOL 1202) as well as Molecular Biology of Cells (BIOL 2200).

Dr. Sarah E. Council is a postdoctoral fellow for the Center for Center for Science, Math and Technology Education at North Carolina Central University and in the Genomics and Microbiology laboratory at the North Carolina Museum of Natural Sciences. She received her B.S. in biological sciences from North Carolina State University and her Ph.D. in oral biology from University of North Carolina at Chapel Hill. She helped design and implement Research Infused laboratories for Principles of Biology I: Organisms and Diversity (BIOL 1201) and Cell and Molecular Biology (BIOL2200) lab at North Carolina Central University. Her primary research focus is the skin microbiome in humans and other primates at the North Carolina Museum of Natural Sciences.

Dr. Stephanie C. Schroeder is an associate professor and chair of the Department of Biological Sciences at Webster University. She received her B.S. in biochemistry at Purdue University and her Ph.D. in molecular physiology and biophysics at Vanderbilt University. She teaches undergraduate Biochemistry I and II lecture and labs (CHEM 3100/3101, CHEM3110/3111), Gene Expression (BIOL4050), Virology (BIOL 4500) and Research Methods (BIOL 4400) as well as graduate Genetics of Anesthesia (BIOL5780). She mentors undergraduate students in BIOL 4420 (B.A. senior thesis) and BIOL4430 (B.S. senior thesis). Her research interests have focused recently on the effects of volatile anesthetics on gene expression in *S. cerevisiae* and *D. melanogaster*. Stephanie developed the yeast *lacZ* labs when she started at Webster University in 2003, using plasmids constructed for her Ph.D. thesis in the laboratory of Dr. Tony Weil at Vanderbilt in 1994.

Dr. Ruth Phillips is a visiting assistant professor in the Department of Biology at North Carolina Central University. Dr. Phillips earned a B.S. from the University of Michigan and her Ph.D. in pharmacology and cancer biology from Duke University. Her post-doctoral research at the National Institute of Environmental Health Sciences was devoted to post-translational gene regulation and nucleo-cytoplasmic shuttling of RNA regulatory proteins. Dr. Phillips teaches a variety of undergraduate courses including General Biology for Non-Majors (Biology 1300) General Biology I (Biology 1202), Cell and Molecular Biology (Biology 2200). In addition, she

developed the laboratory component of General Biology for Non-Majors (Biology 1100 and Biology 1300), Cell and Molecular Biology (Biology 2200), Undergraduate Genetics (Biology 3100) and Graduate Genetics (BIOLG5120). Dr. Phillips' research interests include the following: *i*) infusing research modules in the undergraduate science curriculum, *ii*) implementing the "flipped" instructional strategy into undergraduate courses, and *iii*) developing a service-learning based STEM outreach program in the community.

Sandra Utile was the laboratory technician, Center for Science, Math, and Technology Education. She prepared reagents and set up the laboratories for all of the NCCU-HHMI research-infused laboratories on a weekly basis. She would also trouble shoot laboratory protocols as deemed necessary. She received her B.S. in biology from Meredith College and her M.S. in biology from NCCU. Sandra has now secured a position at the US Environmental Protection Agency in Research Triangle Park, NC.

Dr. Gail P. Hollowell is an associate professor in the Department of Biology at North Carolina Central University. She received her B.S. in biology from North Carolina Central University, and both her M.S. in microbiology and Ph.D. in molecular biology from Howard University. She also completed a postdoctoral fellowship at the National Eye Institute, National Institutes of Health on eukaryotic gene expression. Dr. Hollowell teaches Principles of Biology: Molecular Biology of Cell (Biology 1202) and Cancer Biology (Biology 4350). Dr. Hollowell's research interests include the following: *i*) infusing research modules in the undergraduate science curriculum, *ii*) the impact of technology in the science classroom, and *iii*) studying what motivates students to learn science. In addition to her scholarly work, Dr. Hollowell has been recognized for her teaching as a recipient of the Outstanding Faculty Teaching Award, Department of Biology (2006) and the NCCU Award for Teaching Excellence (2007). Dr. Hollowell currently serves as the co-program director for the HHMI grant at NCCU.

Dr. S. Catherine Silver Key is an associate professor of biology at North Carolina Central University. She received her B.S. in biology from the University of Missouri at St. Louis and her Ph.D. in microbiology and immunology from University of North Carolina at Chapel Hill and completed a post-doctoral fellowship in developmental genetics at UNC-CH through the Seeding Post-doctoral Innovators in Research and Education (SPIRE). She currently mentors SPIRE post-doctoral scholars in teaching and also mentors Duke Preparing Future Faculty (PFF) graduate students and post-doctoral fellows. She teaches undergraduate Genetics (BIOL3100), Cell and Molecular Biology (BIOL2200), Introduction to Research (BIOL4400), Inquiries in Developmental Biology (BIOL4100), and Graduate Genetics

(BIOD8020/BIOG5120). She has also mentored undergraduates in BIOL4400 and from various training grants (RISE, MARC, FUTURES, S-STEM) and graduate students at the NCCU Master's Program in her *Drosophila*-based research lab focusing on the effect of the CRL4^{Cdt2} ubiquitin-mediating complex during development, silver-nanoparticle exposure effect on *Drosophila* development and *Drosophila* genome annotation through the Genomics Education Partnership

(GEP, Dr. Sarah Elgin, P.I.) where she met Stephanie Schroeder. This being her third major ABLE workshop, Dr. Silver Key is an enthusiastic ABLE participant and she began implementing the yeast *lacZ* labs in Spring 2014 as part of a program to transform introductory biology labs into HHMI-funded Curriculum Undergraduate Research Experience (CURE) labs.

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Appendix A Assay Reagents

Table A1. Checklist for Sequential Addition of *lacZ* Assay Reagents.

Purpose of Reagent	Reagent to be added to 2 mL in sequence:	Volume	Check when added
Potentially contains the bacterial <i>lacZ</i> gene product: beta-galactosidase	1. Vortexed log phase yeast cells	200 µL	
Ideal salt concentration and pH for optimal beta-galactosidase activity	2. Z buffer	800 µL	
Together these lyse the cells , but allow beta-galactosidase to be in right conformation. <ul style="list-style-type: none"> • The SDS (sodium docecyl sulfate) is a detergent found in most human hygiene products. It solubilizes the membrane by mildly disrupting lipid and protein interactions. • Chloroform is one of the organic solvents that can permeabilize (create canals in) membranes. 	3. Chloroform	50 µL	
	4. 0.1% SDS	20 µL	
<i>o</i>-nitrophenyl-beta-galactoside (ONPG) is the colorless substrate for the beta-galactosidase enzyme. It is similar in structure to the lactose disaccharide and beta-galactosidase cleaves it into 2 products: galactose and <i>ortho</i>-nitrophenol which is yellow (A420)!	5. (<i>After vortexing 10 second</i>) ONPG added	200 µL	
	<i>Vortex and incubate as per protocol; prepare cuvettes</i>		
Inhibits beta-galactosidase activity (stops ONPG hydrolysis)	6. Stop Solution (Na ₂ CO ₃)	500 µL	
	<i>Continue to follow procedure</i>		

Appendix B Solution Recipes

1:100 dilution of H₂O₂

1. You will be working with a very high concentration of H₂O₂ which will eat through anything. Therefore, put all your protective gear on and make the dilution under a fume hood.
2. Get a **sterile** 250-mL bottle and label it 97 mM H₂O₂ with your initials and date.
3. Place 99 ml of sterile distilled H₂O in it.
4. Then add 1 ml of 30% H₂O₂.

2 Liters of Z Buffer

Start with 1 L deionized water. Using stir bar add all of the dry ingredients and allow to completely dissolve. Bring up to almost 2 L (leaving 5 mL of room for addition of beta-mercaptoethanol). After autoclaving, place a label on the bottle(s) that says 'Z Buffer' (also your initials and the date) and a little box followed by beta-MeOH. Once the solution has cooled and you have added the beta-MeOH, then check the box. After aliquoting, the aliquots can be stored in the freezer at -20°C. Make sure you allow one hour in order for the aliquots to thaw when needed. Alternatively, can leave at 4°C for up to a month.

Component	MW (g/mole)	Desired Final Concentration	Quantity needed for 1 Liter	Quantity needed for 2 liters
Na ₂ HPO ₄	141.96	60 mM	8.518 g	17.036 g
NaH ₂ PO ₄ · H ₂ O	137.99	40 mM	5.520 g	11.04 g
KCl	74.56	10 mM	0.746 g	1.490 g
MgSO ₄	120.37	1 mM	0.120 g	0.240 g
beta-MeOH	14 M	37.8 mM	2.7 mL	5.4 mL

50 ml ONPG

After adding the ONPG to the Z-buffer, put it on the nutator for an hour. After an hour, check to see if all the ONPG is dissolved. If not, leave it on the nutator for another hour. **DO NOT autoclave. This solution is LIGHT SENSITIVE; therefore, wrap in foil or keep in an opaque storage box.** Aliquot the ONPG into 1.5 mL tubes and freeze at -20°C.

Component	MW (g/mole)	Desired Final Concentration	Grams needed for 25 mL	Grams needed 50 mL
2-nitrophenyl-beta-D-galactopyranoside (ONPG)	301.25	4 mg/mL	100 mg (0.1 g)	200 mg (0.2 g)

2 Liters of STOP SOLUTION

Start with 1 L deionized water. Using stir bar, add the 211.20 g Na₂CO₃. Bring it to 2 L. Pour into bottles, leaving room for boiling and autoclave.

Component	MW (g/mole)	Desired Final Concentration	Grams needed for 1 Liter	Grams needed for 2 Liter
Na ₂ CO ₃	105.6	1 M	105.6	211.20

1 L 0.1% SDS

Dissolve 1 gram SDS in 1 L of deionized water. Autoclave the SDS with other solutions above.

BLANK solution

Combine the following in a 50-mL conical tube, around 10 AM on day of lab. This can be made this ahead of time and just add the ONPG fresh the morning of lab before making aliquots.

component	amount
SD-URA (does not need to be sterile)	2 mL
Z buffer	8 mL
0.1% SDS	200 µL
ONPG	2 mL
Na ₂ CO ₃	5 mL

This makes a total of 17.2 mL.

Appendix C

Yeast Preparation

Yeast Week #1

1. Ahead of time, autoclave 2 flasks that can fit into the shaker. They need to be 50-mL flasks or larger.
2. **OVERNIGHT CULTURES.** Pick large (>3 mm) colonies of each yeast strain below into **8 mL** of SD-URA medium (in a 50-mL conical tube).
 - a. Empty-*LacZ*
 - b. **TBP-*LacZ***
3. Shake the above starter cultures about 24 hours at 30°C.
4. Take the OD's of the 8-mL overnight cultures. If they are less than OD=1, they need to grow longer. They can wait in fridge up to 48 hours until you are ready to use them to set up the overnight cultures to achieve log phase cells ready at the beginning of the teaching lab. We routinely seed 50 mL of SD-URA liquid media with yeast 8-mL overnight cultures as indicated below:
 - Use 1 mL of overnight culture of yeast with A600≈1.
 - Media – 50 mL of SD-URA (vortex 30 seconds and then verify A600 ~0.02)
 - Sterile flask size = 500 mL (plenty of surface area for liquid/air gas exchange)
 - Set up time: 4:00 PM day prior to experiment
 - Shaker incubator temperature: 30°C
 - Agitate in shaker incubator ~250 rpm
 - Remove at 9 AM next morning and verify the OD600 is between 0.4-0.9.
 - The assay actually is very robust at the higher ODs; students would be able to see the shade differences without the aid of a spectrophotometer.

Note: One 50-mL overnight culture provides enough yeast cells for two sections of twenty-four students! During the ABLE 2015 workshop, the presenting authors (Council and Silver Key) discovered that the log phase cells from the morning culture could be used successfully for the afternoon session. The cells were at about A600 of 0.9. Simply refrigerate the cultures or aliquots to be used later that day. Students will just need to vortex 30 seconds prior to use. Therefore, a 50-mL culture of each type of yeast strain can definitely cover 50 students over the course of the day. If more yeast is needed, scale up the above instructions as needed.

1. FOR WEEK 1 LAB: Label three 50-mL sterile flasks with the following labels:
 - a. 'E' = **Empty*LacZ***
 - b. 'P' = **TBP-*LacZ***
 - c. 'P + E' = **TBP-*LacZ* + H₂O₂**
2. Use the 8-mL cultures to set up **25-mL** cultures in the flasks, at OD600=**0.02**:
Use the equation below and dilute the 8-mL cultures to set up the **THREE 25-ml** cultures at OD600=**0.02**.

$$\text{OD600 of 8-mL culture (X mL)} = 0.02 (25 \text{ mL})$$
 For the amount of SD-URA: 25 mL – X mL (from above equation) = mL of sterile SD-URA added to each flask.
3. Using sterile technique, put the calculated amount of sterile SD-URA media into flask.
4. Then add the appropriate volume of the appropriate 8-mL culture to each labeled flask.
5. Add 39 uL of sterile H₂O to the Empty-*LacZ* and the TBP-*LacZ* flasks.
6. Add 39 uL of sterile H₂O₂ (1:100 dilution above; 97 mM) to the TBP + *LacZ*+ H₂O₂ flask.
7. Grow the 25-mL cultures about 12-15 hours (start about 6 PM night before lab).
8. Next morning at 8 AM, take the OD of each; it should be between 0.5-1.

Yeast Week #2

9. Pick large (>3 mm) colonies of deltaA, deltaB, TBP-*LacZ* and Empty*LacZ* yeast into STARTER CULTURES (**6 mL** each of SD-URA medium). Shake 24-48 hours at 30°C.
10. Take the OD's of the 10-mL cultures. If they are less than OD=1, they need to grow longer. They can wait in fridge until you are ready to use them.
11. Use the 10-mL cultures to set up WORKING CULTURES (**50 mL** in flasks, A600=**0.02**).
12. Grow the 50-mL cultures about 12-15 hours. Take the OD of each; it should be between 0.5-1.

	A₆₀₀ of starter culture (6 mL)	Volume of starter needed for seeding 50-mL culture	Volume of medium to QC to 50 mL	A₆₀₀ of working culture on Monday AM (before lab)
Empty <i>LacZ</i>	0.883	1.132 mL	48.868	
TBP	0.917	1.09 mL	48.91	
deltaA	0.758	1.319 mL	48.681	
deltaB	0.865	1.156 mL	48.844	

Appendix D

Sources and Suppliers for Materials

Supplies		Catalog Number	Company
Sodium Phosphate Dibasic Anhydrous (White Granules or Powder), Fisher BioReagents		BP332-500	Fisher Scientific
Fisherbrand Premium Microcentrifuge Tubes; Natural; 2.0 mL; O.D. x L: 11 x 40.6 mm; 5000/CS		05408138 (CS)	Fisher Scientific
Semi-micro Polystyrene disposable cuvette 1.5 mL (case of 500)		14-955-127	Fisher Scientific
Hydrogen Peroxide, 34-37% (Technical), Fisher Chemical		H327-500	Fisher Scientific
2-mercaptoethanol; extra pure; 99% Acros Organics		AC12547-2500	Fisher Scientific
2-Nitrophenyl β -D-galactopyranoside (ONPG) substrate		N1127-500MG	Sigma-Aldrich
Potassium Chloride (White Crystals), Fisher BioReagents		BP366-500	Fisher Scientific
124900010 magnesium sulfate heptahydrate; extra pure; $\geq 99\%$		AC424395000	Fisher Scientific
sodium carbonate; extra pure; 99.95%		AC124900010	Fisher Scientific
42439 5000 sodium phosphate, monobasic monohydrate		AC207765000	Fisher Scientific
Yeast media	15 mm petri dishes, case of 500	875713	Fisher Scientific
	MP Biochemicals SD-TRP powder, 10 X 0.5 L pouches	MP114812065	Fisher Scientific
	Fisher BioReagents Granulated Agar; 500 g	BP1423500	Fisher Scientific

Supplier Contact Information

1. Fisher Scientific

<https://www.fishersci.com/us/en/home.html>

General Customer Service

Phone: 1-800-766-7000

Fax: 1-800-926-1166

Mailing Address: 300 Industry Drive, Pittsburgh, PA 15275, USA

2. Sigma-Aldrich

<https://www.sigmaaldrich.com/>

Customer Support within the USA

Phone: 1-800-325-3010

Fax: 1-800-325-5052

Mailing Address: Sigma-Aldrich, Customer Support, PO Box 14508, St. Louis, MO, 63178, USA

Appendix E Calculating the MEAN and Standard Deviation (SD)

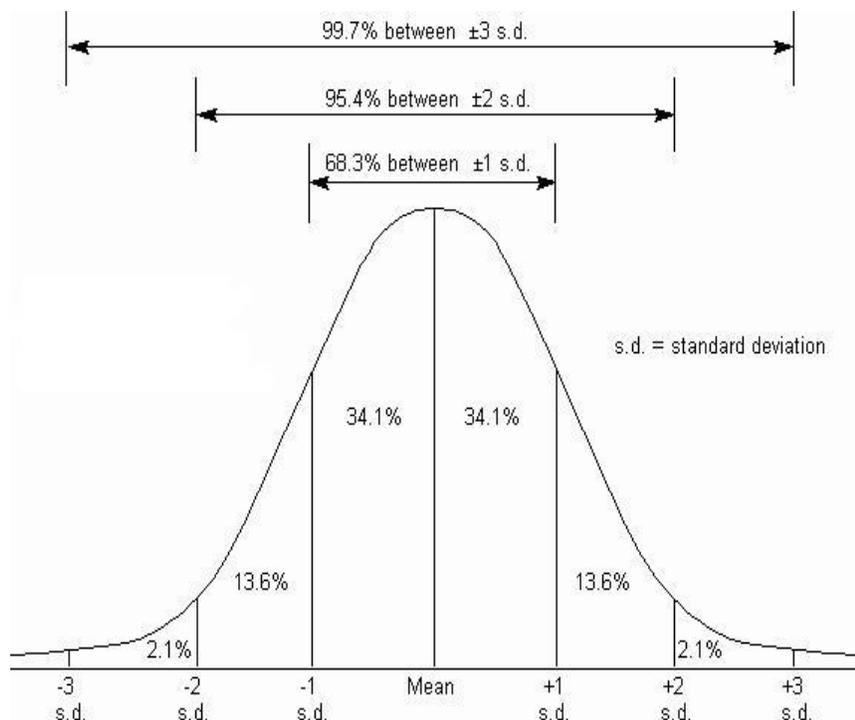


Figure 8. Standard Deviation (SD) is defined as the amount each sample deviates from the mean (average) of the sample population. The samples that are used to calculate the mean (average) measurement can all be clustered. Image credit: <http://www.biologyforlife.com/standard-deviation.html>

Example for calculating the Mean and Standard Deviation (SD): Measuring the average diameter of M&M candies!



Figure 9. Image of M&M candies that have diameter. Data set in millimeters (mm) = 3, 3.5, 3, 4, 2.5.

Goal: To calculate the average size of an M&M plus or minus the standard deviation in mm.

1. **First**, calculate the **mean** (average) in mm:

$$\text{Mean} = 3+3.5+3+4+2.5 = 3.2$$

2. **Second**, calculate how much each sample **deviates** from the mean:

Sample 1: $3-3.2 = -0.2$

Sample 2: $3.5-3.2 = 0.3$

Sample 3: $3-3.2 = -0.2$

Sample 4: $4-3.2 = 0.8$

Sample 5: $2.5-3.2 = -0.7$

3. Third, calculate the Square of each deviation:

Sample 1: $(-0.2)^2 = 0.04$

Sample 2: $(0.3)^2 = 0.09$

Sample 3: $(-0.2)^2 = 0.04$

Sample 4: $(0.8)^2 = 0.64$

Sample 5: $(-0.7)^2 = 0.49$

4. Third, calculate the Square of each deviation:

Sample 1: $(-0.2)^2 = 0.04$

Sample 2: $(0.3)^2 = 0.09$

Sample 3: $(-0.2)^2 = 0.04$

Sample 4: $(0.8)^2 = 0.64$

Sample 5: $(-0.7)^2 = 0.49$

5. Fifth, plug the answer obtained in the fourth step into the equation below to calculate **variance**.

Variance = sum of squared deviations/ (N-1) where N=the total # of samples.

$$\frac{1.3}{(5-1)} = 1.3 / 4 = 0.325$$

6. Sixth, calculate the **Standard Deviation (SD)** by calculating the square root of the variance as shown below:

$$SD = \sqrt{0.325} = 0.5701$$

****Conclusion:** Therefore, the average diameter of an M&M is 3.2 ± 0.5701 mm; where 3.2 is the **MEAN** and 0.5701 is the **Standard Deviation (SD)**.

Appendix F Survey Results

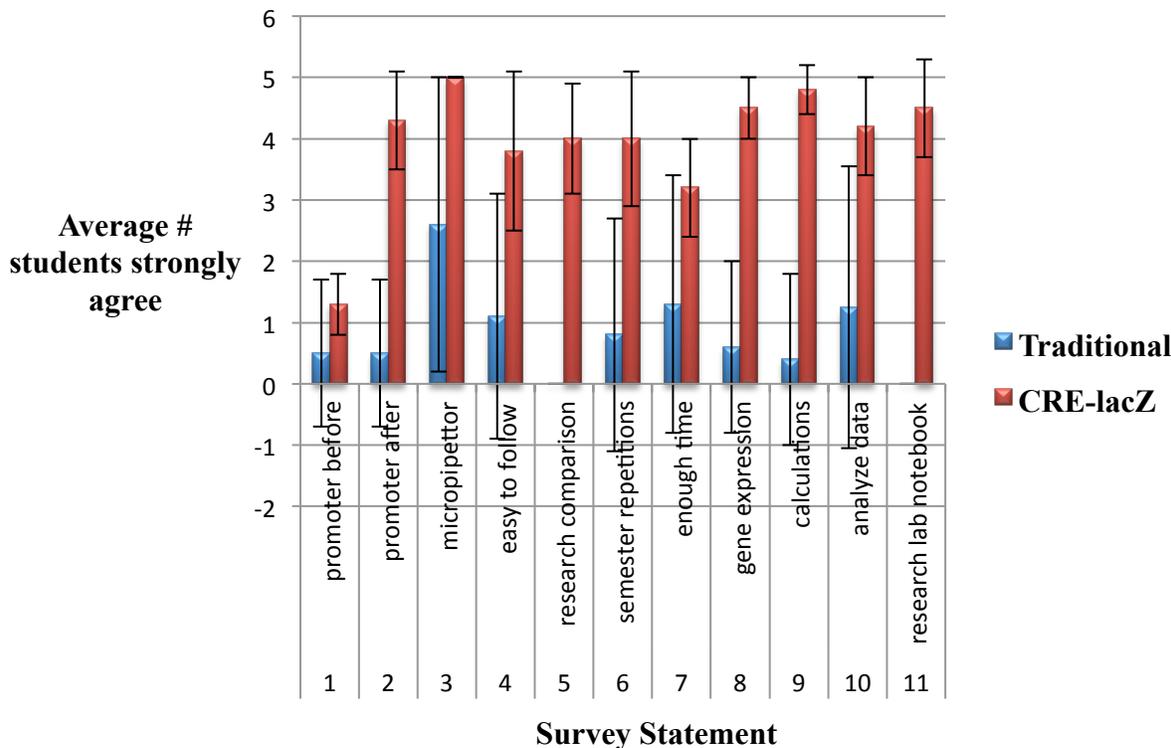


Figure 10. Post-*lacZ* Survey of Traditional vs CRE-*lacZ* teaching lab sections of NCCU BIOL2200 Molecular Biology of the Cell indicate that students felt they gained confidence and understanding in a subject while participating in a Course Research Experience (CRE) n. = 12 traditional teaching lab; n=6 Course Research Experience (CRE)-*lacZ* teaching lab.

Conclusions: Students especially agree that the *lacZ* hands on laboratory increased:

- a. their confidence in how promoters work (survey statement #2)
- b. their understanding of gene expression as measured by spectrophotometry
- c. research notebooks contributed to the research experience.

Student User Evaluation Survey

LacZ (beta-galactosidase assay) Lab

Student Name: _____

Read each statement in the table and circle the number that best characterizes your own response. If you do not think the question applies to your lab this semester, then circle not applicable (n/a).

General Observation	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	n/a
1. I felt confident in my knowledge about how a promoter works before completing the <i>lacZ</i> labs.	1	2	3	4	5	n/a
2. I feel confident in my knowledge about how a promoter works after completing the <i>lacZ</i> lab.	1	2	3	4	5	n/a
3. I had a lot of opportunity to use micropipettors in <i>lacZ</i> lab.	1	2	3	4	5	n/a
4. The <i>lacZ</i> lab procedure was easy to follow.	1	2	3	4	5	n/a
5. I had an opportunity to compare my graphed <i>lacZ</i> data to data from a research article.	1	2	3	4	5	n/a
6. The students worked together to collect many repetitions of	1	2	3	4	5	n/a

the *lacZ* data throughout the semester.

7.	I had enough time to finish the hands-on, wet-lab portion of the <i>lacZ</i> lab.	1	2	3	4	5	n/a
8.	I feel that after completing the <i>lacZ</i> lab, I have a significantly greater understanding of how genes are expressed and how to spectrophotometer measurements changes in response to gene expression.	1	2	3	4	5	n/a
9.	This <i>lacZ</i> lab emphasized calculating the mean/average data and standard deviation.	1	2	3	4	5	n/a
10.	In the <i>lacZ</i> labs, we had to analyze data to answer a question.	1	2	3	4	5	n/a
11.	I feel recording <i>lacZ</i> data in a lab notebook, contributed to having a research experience.	1	2	3	4	5	n/a