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Regulation of Gene Expression Using *Lac Z* Reporter Gene: Expression of Gal 4-Activater Galactose Metabolism Genes with TATA/CATA Variant Elements at the Core Promoter in *Saccharomyces cerevisiae*

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Teaching the central dogma and gene expression to entry level Biology students is fundamental to Biology and Genetics courses. We introduced a laboratory exercise based on home-made yeast constructs where students study the role of promoters in gene expression using a *Lac Z* reporter system. Students measure the levels of b-galactosidase (β-gal) activity in four different *Saccharomyces cerevisiae* strains carrying plasmids with a Gal 4-inducible promoter fused to the prokaryotic *Lac Z* gene. Students then compare *LacZ* gene expression controlled by Gal4 promoter and four variants of the promoter consensus TATAAA sequence that drive variable levels of Lac Z expression. This laboratory exercise enforces student understanding of the components of gene expression and the importance of conserved promoter sequences in gene expression, provides them with hands-on experience in performing the galactosidase enzyme assay, and gives them the opportunity to work with the model eukaryotic organism, yeast.

Keywords: Gene expression, *LacZ* reporter gene, TATA promoter element, TATA Binding Proteins (TBP), Gal 4 regulon, *Saccharomyces cerevisiae, Genetics Laboratory exercise*

Introduction

Background: In teaching entry-level biology students, it is important to demonstrate the importance of gene regulation in controlling cellular processes and organismal development. The mechanism of eukaryotic gene expression based upon enhancers, promoters, and transcription factors has been widely taught (Burley and Roeder, 1996). The Stragell lab (Stewart and Stargell, 2001; Jennifer J. Stewart, Julie A. Fischbeck, Xu Chen, and Laurie A. Stargell, 2006) in the Department of Biochemistry and Molecular Biology at Colorado State University studies factors that affect the assembly of the transcription initiation complex at the core promoter near the transcription start site. The lab analyses the role of a key player, the TATA element at the core promoter in regulating gene expression as reported by Lac Z reporter gene. When the functional activity of two sequences: TATAAA (canonical) and CATAAA (noncanonical) were compared, it was found that TATAAA element can support high levels of transcription *in vivo*, whereas the CATAAA element was severely defective for this function (Stewart and Stargell, 2001; Stewart et al., 2006). The Stargell lab studied the functional activity of 14 different sequences of TATAA for their mechanistic differences in transcription initiation.

The Lab Exercise

We designed a lab exercise that utilizes four of the DNA constructs that were developed in the Stargel lab to study the factors affecting transcription initiation. The lab exercise uses yeast (*Saccaromyces cerevisiae*) strains that contain DNA constructs with either TATAAA or its variant elements CATAAA, TACAAA and TATAAG at the core promoter of a Galactose (GAL4) inducible regulon. The GAL promoters are fused to a prokaryotic *lac Z* reporter gene. Students measure TATAAA/CATAAA driven gene expression by assaying β-galactosidase (β-gal) activity of

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cells grown in Glucose or Raffinose (transcription repressed) and compare them to that of galactose-activated transcription. By comparing the levels of transcription, the students observe the induction of transcription by Galactose as well as the effect of varying the TATAAA sequence. The β-galactosidase assay uses ONPG as the substrate that is cleaved by β-galactosidase to produce a yellow product that is quantified using a spectrophotometer.

Students' results consistently show a dramatic decrease (ranging between 4 to 8 times less) in β-gal activity driven by CATA compared to that driven by TATA promoter when induced by galactose. Yeast containing the same gene constructs grown with glucose as the carbon source express minimal β-gal activity (0.1% of the activity seen with Galactose) demonstrating both repression and activation of transcription. Initial results with two additional variants of the TATA elements (TACAAA and TATAAG) reduced b-Gal activity to about 2.5 and 1 percent of that for TATAAA induced and driven transcription. This lab exercise demonstrates to the students the importance of the TATAAA consensus sequence in the assembly of the transcription initiation complex at the core promoter of genes. In addition, it demonstrates the effect of the activator proteins such as Gal4 in mediating the distal regulation of gene expression. *Lac Z* reporter gene and the simple eukaryotic yeast cells as research tools are additional learning outcomes.

Questions Asked

How Does varying the TATAAA promoter consensus sequence affect Gene expression?

Will Galactose induce expression of genes downstream of Gal 4 driven promoter?

Hypothesis

High β-Galactosidase activity is produced in TATAAA driven expression compared to attenuated expression driven by TATA sequence variants.

Student Outline

Objectives

- Learn the components of gene expression that control the initiation of transcription: enhancers, regulator and activator proteins, and TATA consensus elements at the core promoter.
- Compare the effect of TATAAA element variants on gene expression.
- Use Gal 4 activator protein to affect the expression of galactose metabolism genes in response to galactose as a carbon source.
- Understand the *E. coli lacZ* reporter gene system by assaying for β-galactosidase activity and spectrophotometrically measure the expression of a gene.
- Work with yeast: a simple, single cell eukaryotic organism and an important model system.

Introduction

A. Regulation of Gene Expression

As you have studied in your genetics course, although cells in an organism contain the same genetic structure (genome), only a subset of genes are selectively expressed at any given time and space. Genes are turned on or off depending on the **type of the differentiated cell** as well as cell response to **environmental and biochemical cues**. Gene expression in cells is therefore tightly regulated and achieved at **three main levels**:

1. **Gene control regions:** include proximal promoters[1] such as RNA polymerase binding site and distal promoters such enhancers (Figure 1).
2. **Gene regulatory proteins,** and transcriptional activator proteins that bind specifically to the enhancers above to control the assembly of the RNA initiation complex at the proximal promoter of a gene.
3. **DNA Packaging proteins** that pack DNA into high order chromatin structures to regulate access of the RNA transcription machinery to the DNA.

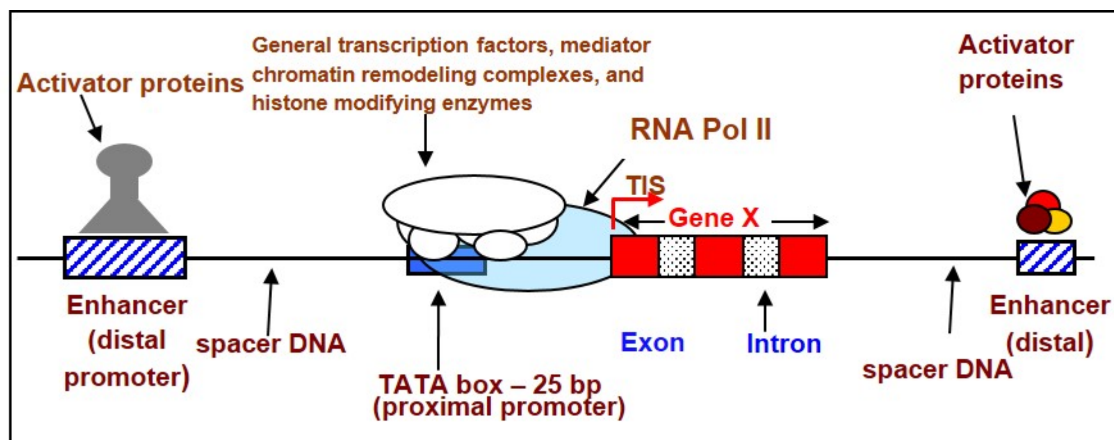


Figure 1. The gene control region, gene regulatory (activator) proteins and RNA initiation complex of a typical eukaryotic gene starting at TIS: Transcription Initiation Site.

B. Reporter Genes for Studying Gene Expression

Variations in gene expression in cells are studied by monitoring gene activities or the **levels of mRNA** produced**.** Because many genes do not have a measurable gene activity, a convenient method for studying the expression of these genes is by splicing (fusing) the promoter of the gene whose expression is to be monitored to the coding sequence of a

[1] Conserved DNA regulatory sequences

chosen **"reporter" gene**. One favorite reporter gene is the ***lacZ* gene** of the E. coli lac operon that encodes the enzyme beta-galactosidase (Figure 2). β-galactosidase is very simple to detect biochemically by using one of the many substrates that produce a colored product upon cleavage by the β-galactosidase enzyme. This system therefore provides a convenient way to monitor the expression level of a gene specified by its control region. ***lacZ*** serves as a reporter gene since it "reports" the activity of a gene control region. Other popular reporter genes are: **luciferase,** an enzyme which produces **luminescence**, and the more recently discovered **Green fluorescent protein**. The latter reporter protein has been very useful in detecting expression of genes in live cells, and thus has provided researchers an invaluable tool for tracking **when** and **where** a gene is expressed in the **living** organism.

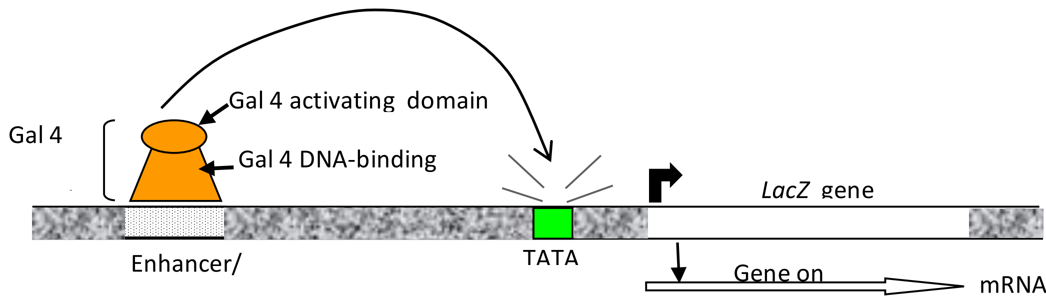


Figure 2. Bacterial *lacZ* expression reporter system showing the normal activation of gene transcription produced by the Gal4 regulatory protein.

C. The DNA Constructs and Conditions Used in this Experiment

In this experiment, you will be studying gene expression using gene constructs that were engineered by the Stargell Lab (Stewart et al., 2006) at the Department of Biochemistry at Colorado State University to study transcription inititation factors. Yeast upstream activating sequences, (UAS; control region) for the yeast galactose metabolism genes that are regulated by Gal4 protein, were fused to the *E. coli lacZ* gene (Figure 2 and 3). Gal4 is a key regulatory protein in yeast that is responsible for activating the transcription of yeast genes responsible for galactose metabolism, and the conversion of galactose to glucose when galactose instead of glucose is used as a carbon source. Also engineered directly upstream of the *E. coli lacZ* mRNA initiation site is the promoter sequence of TATA elements (Figure 3).

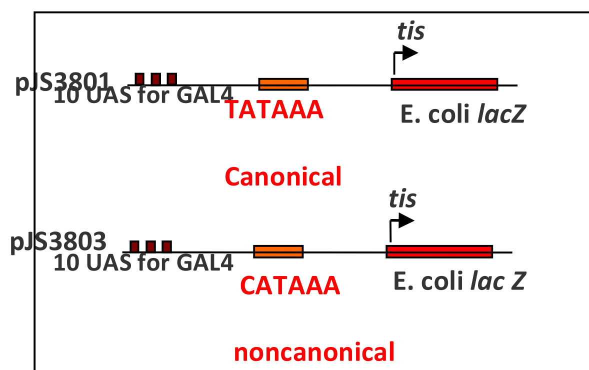


Figure 3. Reporter system to study the effects of variant sequences of TATA elements on transcription efficiency. UAS: Upstream Activating Sequences, tis: transcription initiation site. Figure is courtesy of graduate student Xu Chen, Department of Biochemistry, Colorado State University.

TATA elements influence the selection of **start** sites and **initiation** of transcription. Therefore, *lacZ* gene expression is regulated by both: the activation of expression by Gal4's binding to UAS and the TATA elements. The TATA sequence in eukaryotic promoters is highly conserved, it binds to the transcription factor TFIID (transcription factor regulating RNA polymerase II) to effect initiation of transcription. Any change in the TATA consensus sequence

affects the transcription efficiency. In this experiment a change of the TATA sequence into one of these variant sequences CATAAA, TACAAA and TATAAG is made by substituting a Cytosine for a Thymidine nitrogen base or others in the nucleotides. This allows for the study of the importance of the TATA consensus sequence in controlling transcription and thus the rate and amount of gene expression using the *LacZ* reporter gene system.

D. The Yeast Model System

You will be using yeast *Saccharomyces cerevisiae* (baker's yeast) as the experimental model system for studying regulation of gene expression. Yeast is an ideal eukaryotic microorganism for biological and genetic studies because, like bacteria, is a simple single cell yet is a eukaryotic microorganism that share many biological and evolutionary properties with higher eukaryotic organisms including humans, has rapid and simple reproduction, its genome has been sequenced and many mutations and gene libraries are available through its extensively researched gene data base. Yeast strain yJS156 and plasmid constructs pJS3801-14 are described in Stewart and Stargell (2001).

Methods and Data Collection

Part A: Selecting the Yeast Strains and Growing the Yeast Cultures:

You will be divided into groups of four students. Each group will choose a yeast strain that contains a variant of the TATAAA element construct. You will be guided through growing and inducing the expression of the Lac Z gene. You will induce the *LacZ* expression by growing them in Galactose as a carbon source and compare the Beta galactosidase enzyme catalyzed activity to those cultures grown in Glucose as a carbon source. You will also study the effect of your TATAA element variant on expression (Figure 4)

Part B: β-Galactosidase (β-Gal) Assay

You will assay for *LacZ* expression of the β-galactosidase enzyme by assaying its activity. The enzyme's function in the cell is to cleave lactose (substrate) to glucose and galactose so that they can be used as carbon/energy sources. In this assay, you will be using the synthetic substrate o-nitrophenyl-β-D-galactoside (ONPG). ONPG will be cleaved to yield galactose and o-nitrophenol which has a yellow color as in the enzyme catlayzed reaction below.

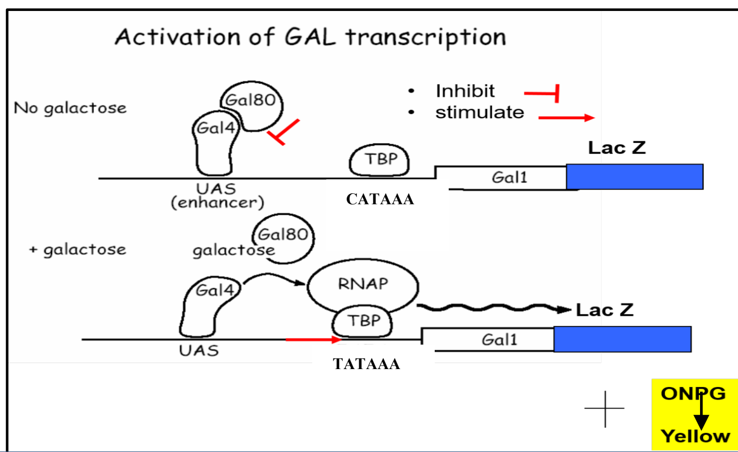
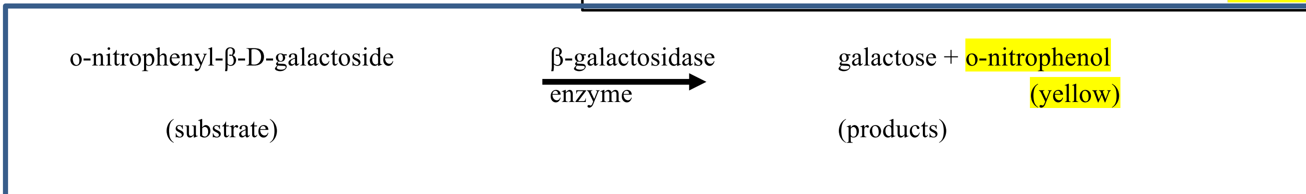


Figure 4. Galactose Regulon: Gal-4-regulated promoter system in yeast with *Lac Z* reporter gene



By measuring the intensity of the yellow colored product and keeping track of the time it takes for the color to develop, you quantify the level of activity of the o-nitrophenol production per unit time. This level is proportional to the concentration of β-Galactosidase enzyme; thus, the production of yellow color can be used to determine enzyme levels of *LacZ* gene expression. In this system, the β-Galactosidase concentration in the cell is a read-out for Gal4/TATAAA or Gal4/CATAAA promoter activity.

Beta Galactosidase Assay Procedure

- Grow yeast to midlog stage OD_{600}=0.8-1. in strain-specific medium containing either 2% galactose or raffinose / glucose
- Pellet cells, replace media with Z buffer + B-meracaptoethanol
- Permeabilize cells with 10 uL of 0.1% SDS and 20 uL Chloroform
- Vortex vigorously for 30 sec
- Incubate at 30° C for 10 minutes
- Add warm ONPG 4 mg/mL (*O*-Nitrophenyl-*β*—D-galactopyranoside)
- Watch for yellow color, stop with 100 uL of Na_2CO_3, record time
- Spin cell debris and measure supernatant at OD at 420 nm

- Calculate the amount of β-Galactosidase activity in "Miller Unit" as described below (JMiller, 1972)

> - **1 Miller Unit = $\mathbf{A_{420}}$*1000**
> - **$\mathbf{A_{600}}$* *t*(min)**v* (ml)**

- Plot your data as a bar graphs

Data Analysis

Results: Values of **β** -gal activity of TATAAA strain grown in galactose were set to 100%. Figures were averages of three samples each representing a different biological replicate for each treatment. One figure represents one experiment although the data was reproducible by students over several years.

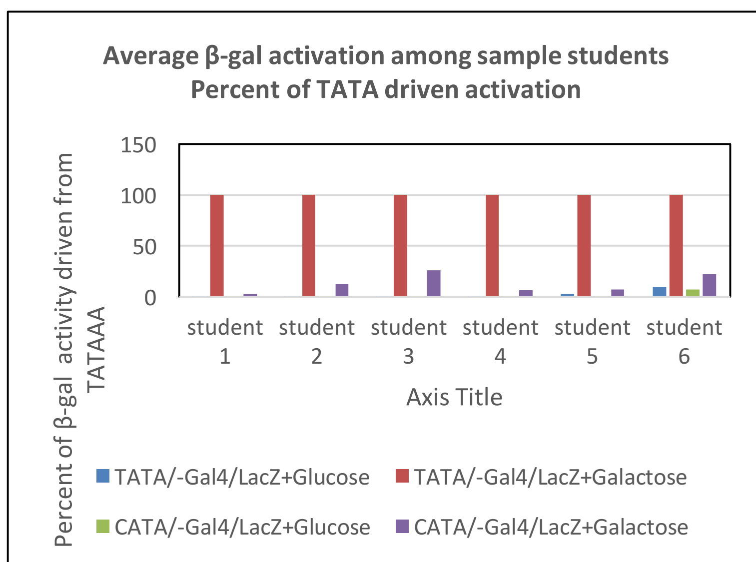


Figure 5. Representative Students' data: β-galactosidase Activity of TATAAA/*Lac Z* and CATAAA/*Lac Z* in Glucose and Galactose

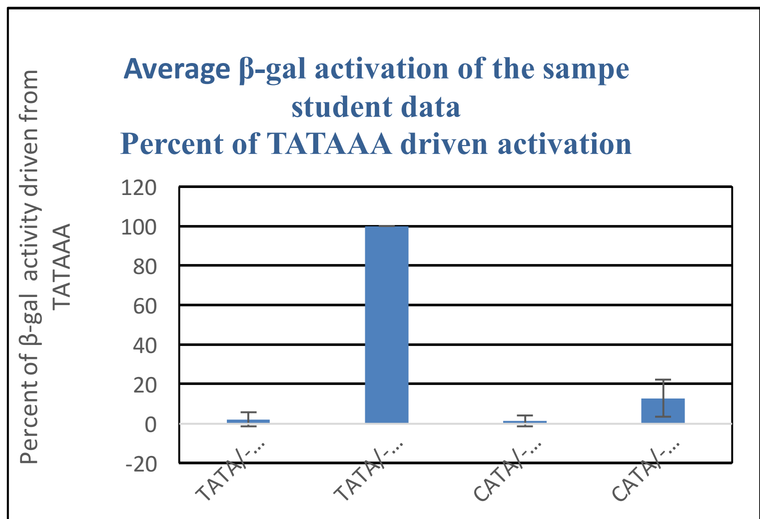


Figure 6. Average β-galactosidase Activity of TATAAA/*Lac Z* and CATAAA/*Lac Z* in Glucose and Galactose. Average of about 6 students' assays

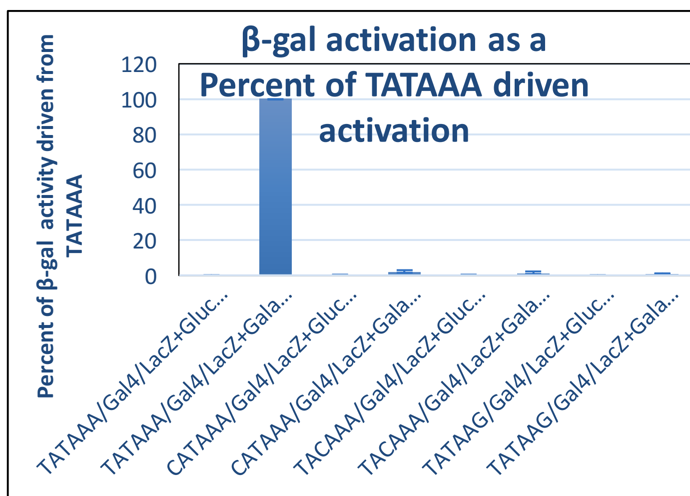


Figure 7. Average β-galactosidase Activity of TATAAA variants: CATAAA, TACAAA and TATAAG in glucose and galactose. Average of about 3 replicates, one experiment students assays

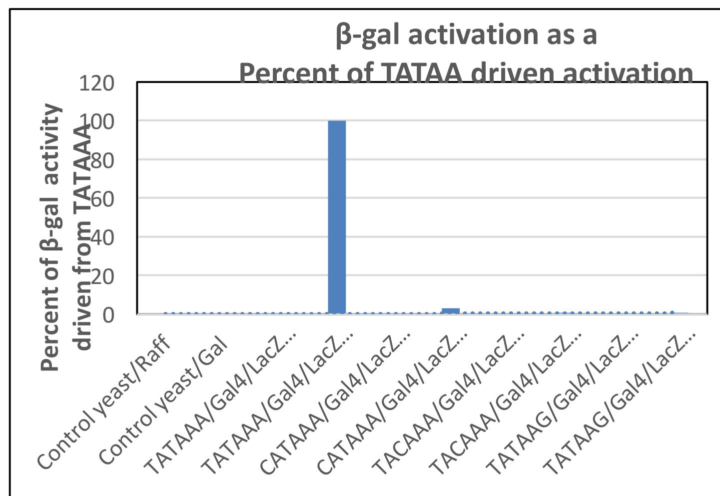


Figure 8. Average β-galactosidase Activity of TATAAA variants: CATAAA, TACAAA and TATAAG raffinose and galactose. Average of about 3 replicates, one experiment students assays

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Materials

Yeast strains with the proper plasmid constructs
amino acid dropout media
Glucose, Raffinose or Galactose
Shake incubator
ONPG
Spectrophotometers/visible spectrum
Computers

Notes for the Instructor

The Gal4 gene expression /LacZ constructs that we used in these labs, successfully demonstrated for the students the importance of inducing the upstream gene specific promoters to induce gene expression. Students developed better understanding of the gene expression reporter systems, and the importance of the conserved TATAA promoter element sequences in initiation transcription. Substituting the Thiamine nucleotide for Cytosine in the TATAAA core promoter element significantly diminishes the functional activity of this promoter element in initiating transcription as indicated by the levels of transcription of *lac Z* reporter gene. These results are in line with the Stargell lab findings where changing the consensus sequence in 14 different variants rendered this promoter defective in supporting transcription

Nucleotide substitution is slightly dependent on the location of the substitution in supporting transcription. Substituting C for T at the first nucleotide is less detrimental than substitutions at other locations. The variant TATA promoter elements other than the CATAAA compromised the initiation of transcription significantly which made it difficult for the students to show clear B-Gal activity reduction in the graphs. We would like to either find mutations that show a milder reduction in expression or expose the yeast to environmental stresses such as ischemic stress or oxidation stress that moderately and controllably affect transcription.

This experiment is very easy to implement, the yeast is easy to grow, easy to induce expression, and the β-galactosidase assays always a success. The enzyme β-gal remains stable for days in the yeast cells. One can grow yeast cells, induce the expression and save for many students' projects or sections for the whole week. The Miller formula normalizes for the yeast culture density and volume, so the overgrowth or undergrowth of the yeast is forgiving.

Students also learn many concepts in gene expression machinery and regulation. They analyze their replicate data using excel and plot the error bars by calculating the standard deviation. Students fill a report with their data and graphs and answer critical thinking questions about the protocol and the data. We have about 140 students divided in 20 students per section. We run 2 concurrent sections with 2 GTAs and 2 undergraduate TAs per section.

Media preparation and recipes are easy to prepare and the yeast strains store well in glycerol stocks at -80° C. It would be an easy exercise to implement for an Inquiry based laboratory exercise.

Conclusions

We were successful in extrapolating the Stargell lab's research findings into a meaningful experiment that solidifies students' understanding of the mechanism through which the transcription of a gene is regulated. Concepts of repressible/inducible transcription, including core promoter elements like the TATA box and enhancer or UAS elements which bind transcriptional repressors and/or activators, are reinforced in the context of hands-on techniques using the model organism yeast and the beta galactosidase enzyme assays.

Future work may involve turning this lab into an inquiry based laboratory exercise where students choose the conditions that reduce or increase gene expression to enhance students' creativity and critical thinking skills.

Acknowledgments

I thank the Dr. Laurie Stargell, Dr. Xu Chen, and Dr. Cathy Radebaugh providing me with the yeast strains and the protocols for growing and maintaining them. I also thank the students and the teaching assistants for their comments that allowed me to improve this laboratory exercise over the years.

About the Author

Farida Safadi-Chamberlain has been an Instructor at the Department of Biochemistry and Molecular Biology at Colorado State University since 2005. She teaches Introductory Genetics and Cell Biology laboratory courses to mostly sophomore students in the Biochemistry, Neuroscience, and Biomedical Sciences majors.

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