

Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the *lac* Operon

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Abstract

The ability to construct and reason through a biological system is an important skill to develop in science education. This level of understanding forces students to recognize the interrelatedness of components within a biological process and appreciate the process as a dynamic system. To develop modeling and computation skills in undergraduate students, we developed a modeling-focused lesson in the context of the *lac* operon. The *lac* operon is a common topic in introductory biology, genetics, and molecular biology curricula. The *lac* operon is a great system for novice modelers as it exemplifies important biological concepts, including the central dogma of molecular biology and gene regulation. The lesson utilizes an online modeling platform called Cell Collective Learn. In our lesson, students explore the important structural and regulatory aspects of the *lac* operon by constructing a computational model of the system, validating their model through simulations, and then manipulating their model to further explore the system dynamics. The lesson contains all needed resources and question prompts, minimizing the need for instructor guidance.

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Supporting Materials: S1: Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the *lac* Operon activity packet

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Learning Goal(s)

Students will:

- experience how computational modeling can be used to represent systems.
- evaluate how computational modeling and simulations can be used to observe and semi-quantitatively measure the dynamics of gene expression and regulation.
- value how the regulation of gene expression is influenced by external and internal molecular signals.*
- evaluate how prokaryotic cells maintain homeostasis.**
- evaluate how mutations affect genes and their corresponding mRNA and protein products.***

*adapted from CourseSource Microbiology learning framework

**adapted from CourseSource Biochemistry and Molecular Biology learning frameworks

***adapted from CourseSource Genetics learning framework

Learning Objective(s)

Students will be able to:

- model how the components of the *lac* operon contribute to gene regulation and expression.
- generate and test predictions using computational modeling and simulations.
- interpret and record graphs displaying simulation results.
- relate simulation results to cellular events.
- describe how changes in environmental glucose and lactose levels impact regulation of the *lac* operon.
- predict, test, and explain how mutations in specific elements in the *lac* operon affect their protein product and other elements within the operon.

INTRODUCTION

Biological systems are complex networks of biological entities that work together to carry out specific functions. A hallmark of biological systems is the transfer, storage, and utilization of information (e.g., the flow of genetic information from DNA to protein). As such, it is important that undergraduate biology students understand and trace the flow of information through biological systems (1); students commonly demonstrate weak understanding of ways in which biological pathways exchange and store information, especially at a genetic level (2-5). Students struggle with concepts related to the central dogma of molecular biology, including recognizing the relationship between genes, their protein products, and phenotypic expression (2,4,6-10), as well as the regulation of gene expression (2,10).

The *lac* operon is a classic example of prokaryotic gene regulation. Showcasing core biological concepts such as the central dogma of molecular biology (10-12), it is commonly taught in undergraduate biology courses ranging from introductory biology and genetics to upper-level molecular biology. Since understanding regulation of the *lac* operon requires knowing the connection between external stimuli and changes to the intracellular environment, it is a useful system to teach regulatory pathways and dynamic systems behavior. Mastering the mechanisms of the *lac* operon provides a foundation to tackle more difficult and complex regulatory mechanisms in eukaryotic organisms. A basic challenge for students in understanding gene regulation systems, involves understanding how components in the system are connected (12).

Introducing computational models in the classroom shows promise for improving student learning (13-18). Computational models and simulations allow students to directly interact with and manipulate complex biological systems in order to understand overall biological processes. This approach has been successfully implemented in a variety of science courses, including physics, chemistry, and biology (13,16-18). Classrooms utilizing computational simulations in science education have observed improvements in student comprehension and problem-solving skills (13-18). While there are model-based (19) and interactive, computer-based (20,21) teaching resources on the *lac* operon available for undergraduate instructors, few resources exist that combine both interactive computer technology and modeling in an accessible classroom activity.

To teach students about core concepts while practicing core competencies in science, we developed a teaching approach that uses a user-friendly education platform, Cell Collective Learn (<https://learn.cellcollective.org>, [14,22-26]). In this approach, students use freely available lessons to develop their understanding of a biological system by creating a computational model themselves or by investigating a provided computational model of the system through simulations. Students engage in authentic yet accessible computational modeling by relating system components (nodes) with direct relationships (arrows/edges); connecting nodes and edges, students mimic behaviors demonstrated in real biological systems, such as activation and inhibition. Students can then observe, in real-time, the behavior of their computational

model through simulations. Students explore complex biological systems by predicting cellular mechanisms, testing their prediction with simulations, reporting their observations, and providing accurate cellular mechanisms based on their simulation results. The lessons developed with this approach are grounded in theories of learning and implement evidence-based best practices specific to modeling and using simulations to support learning science (13,23,27,28). We have previously shown that lessons using the simulation-based portion of this approach helped students learn relevant content and skills (14,22,24,25). Specifically, these lessons were shown to help develop students' systems thinking skills and conceptual understanding of biological systems in the context of cellular respiration and the *trp* operon (14,22,24,25).

Here we describe a lesson, using a computational modeling and simulation approach, on the regulation of gene expression called *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon*. This lesson aims to enhance understanding of the *lac* operon system, information flow within biological systems, and mechanisms of gene regulation, as well as to develop important skills of modeling, using simulations, and applying the process of science. During the lesson, students first build a computational model of the *lac* operon system, and then explore the regulatory elements of the *lac* operon by investigating the impacts of perturbations on the *lac* operon. We call these exercises Investigations. The lesson is versatile and can be completed individually or within small groups. Students receive all the needed instructions and prompts within the lesson itself and can progress through the lesson without additional help.

Intended Audience

This lesson was designed for undergraduate students at the introductory biology level. The lesson could be implemented in higher-level courses such as genetics by setting higher expectations for responses. The lesson could also be used in higher-level genetics and biochemistry courses as a refresher of basic gene regulation knowledge before students learn about more complicated gene regulatory systems.

Required Learning Time

The *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* activity packet (Supporting File S1: Prokaryotic Gene Regulation- Activity packet) could be implemented in-class or as homework. The activity packet (model building and Investigations) should take students about 90 minutes to complete.

Pre-requisite Student Knowledge

Before starting the lesson, students should have basic knowledge about genetics and genetic material (i.e. DNA contains protein-coding regions called genes, definitions of mutation, transcription, translation) and be able to define and distinguish between prokaryotes and eukaryotes. These topics are commonly discussed in introductory biology courses prior to the regulation of gene expression unit. Also, students should have basic computer and internet skills needed to navigate a web browser, type in text responses when prompted, and find and maneuver “play” and “pause” symbols.

While the lesson provides instructions on how to navigate Cell Collective Learn, it may be beneficial for students to first familiarize themselves with the platform in a low-stakes environment. To make students more comfortable with the platform, have them work through the Cell Collective Learn training lesson called *Cell Collective Training Module: Factors Influencing Exam Scores* (accessible from the home page of Cell Collective Learn at <https://learn.cellcollective.org>). This training lesson is designed to familiarize new users to the Cell Collective Learn platform by connecting model building and simulation to a relatable context: studying for an exam.

Pre-requisite Teacher Knowledge

Before implementing the lesson in your class, you should have a thorough understanding of the content and skills described above for students. In addition, you should have an understanding of operons and specifically the *lac* operon, including the biological role of operons (i.e. to coordinate the expression of related genes), the biological role of the *lac* operon (i.e. to make the enzymes needed for lactose import and breakdown), and the mechanisms regulating *lac* operon expression (e.g., *allo*lactose binds to and inhibits the *lac* repressor, allowing RNA polymerase to bind and transcribe the *lac* operon). This information is provided in the background reading section of the *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* activity packet (Supporting File S1: Prokaryotic Gene Regulation- Activity packet). In addition to the biological background, you should be familiar with Cell Collective Learn. You are encouraged to work through the training module, *Cell Collective Training Module: Factors Influencing Exam Scores*, in order to better familiarize yourself with the modeling platform. You should also complete the presented lesson activity packet prior to classroom implementation to ensure you are able to provide guidance to your students when needed. Instructors will need

to create a Cell Collective Learn account in order to complete the *Cell Collective Training Module: Factors Influencing Exam Scores* lesson and *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* lesson.

SCIENTIFIC TEACHING THEMES

Active learning

Students are active participants throughout the entirety of the lesson. As active participants, students will: 1) read about prokaryotic gene regulation and the *lac* operon in the provided background material, 2) answer short-answer questions aimed to stimulate reading comprehension, 3) describe how the regulatory components of the *lac* operon are connected, 4) diagram these relationships in order to flesh out their mental model of the *lac* operon system, and 5) construct, validate, and investigate a computational model of the *lac* operon. Students are responsible for completing their own work (e.g., reading the background material, building their model, running simulations) and are encouraged to discuss in small groups. Due to the novelty of this type of learning activity (i.e. computational modeling) and type of question probes (i.e. systems thinking), student discussion in this group dynamic is frequently persistent throughout the entire lesson, even though individual student roles are not specified within each group. This type of group structure, apart from ensuring that each student experiences the full value of the lesson, allows technologically-savvy group members to assist students that are struggling with the modeling software, while not domineering the entire lesson.

Assessment

You can assess student understanding throughout the lesson in several ways. For an outline of how you can use our lesson to assess student mastery of the outlined learning objectives, refer to Figure 1. During the building portion of the activity

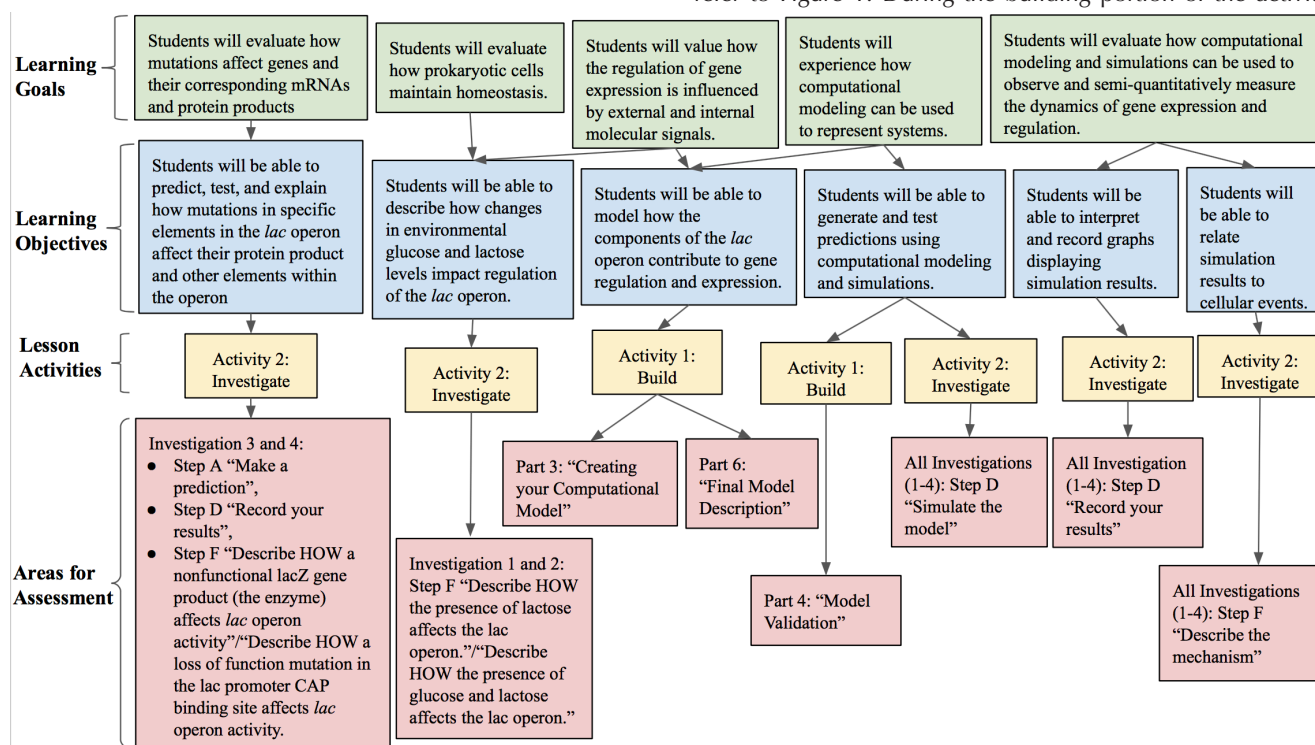


Figure 1: Alignment of Learning Goals. An alignment of learning goals, learning objectives, lesson activities, and assessments.

packet (Supporting File S1: Prokaryotic Gene Regulation-Activity packet), students construct and validate a model of the *lac* operon system. You can evaluate the diagrams students create (Part 6 of Supporting File S1: Prokaryotic Gene Regulation- Activity packet) after model validation in order to evaluate student understanding of the system. Additionally, you can have students create screenshots and submit images of their computational models in order to confirm that they correctly modeled the *lac* operon. During the investigate portion (pages 8-12 of Supporting File S1: Prokaryotic Gene Regulation- Activity packet), students predict the dynamics of the *lac* operon model, test their predictions with the simulation, report their observations, and interpret the results. You can use student responses to formatively assess knowledge by evaluating correctness. During the investigate portion, students are prompted to self-assess the correctness of their prediction after running their simulation. During our implementation of the lesson, we formatively assessed the model students built of the *lac* operon, their observations, and their interpretation of their simulation results.

Inclusive teaching

During the lesson students can work in small groups of two to four students. Having students work in groups encourages the exchange of ideas among students and offers feedback from peers when individual instructor feedback is not readily available. Working in groups has been shown to be beneficial for fostering student gains in underrepresented student groups, such as first-generation college and black students (29). The lesson also utilizes computational modeling and simulations that are not typically used at the introductory level. This computer-based learning approach may appeal to a variety of students, including those who are otherwise struggling with traditional teaching and/or group work.

LESSON PLAN

An overview and timeline for the lesson plan is provided in Table 1. The learning objectives for this lesson are in part informed by the core concepts for undergraduate biology (1) and specifically introductory biology defined by Khodor, et al. 2004 (30). The lesson is modular, providing flexibility to adapt for different classroom needs. The modules include background information and images about operons and specifically the *lac* operon, reading comprehension questions, an introduction to Cell Collective Learn, modeling the *lac* operon, and Investigations of the *lac* operon. All modules are grouped together in the *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* activity packet (Supporting File S1: Prokaryotic Gene Regulation- Activity packet).

Below is a list of materials that are necessary and/or useful for teaching the lesson, all of which are provided as supporting materials, links to websites, or by request from the authors.

- *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* activity packet (Supporting File S1: Prokaryotic Gene Regulation- Activity packet)
- *Lac Operon Construction* model (accessible at <https://learn.cellcollective.org>)
- *Cell Collective Training Module: Factors Influencing Exam Scores* (accessible at <https://learn.cellcollective.org>)

The *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* activity packet answer key is available by request by contacting the authors.

Before Class

Inform students of the upcoming computer-based activity

You should inform students that they will need a computer for the upcoming class and encourage students to bring their own; tablets and cell phones are currently not supported by Cell Collective Learn. It is ideal for every student to have his or her own computer. However, because the lesson can be done in groups, students without a computer can work with other members of the group who do. Alternatively, you could schedule the activity in a computer lab, if available for your class size and institution.

Review in-class materials

In preparation for the lesson, go through the activity packet (Supporting File S1: Prokaryotic Gene Regulation- Activity packet) as if you were a student. This experience will provide you with a deeper understanding of the lesson content, develop your skills for navigating Cell Collective Learn, and help you prepare for potential issues and questions from your students. All of the necessary materials (Supporting File S1: Prokaryotic Gene Regulation- Activity packet) are provided in this article and are also publicly available on Cell Collective Learn. An answer key is available by request for instructors to prevent easy access by students. For further information, contact Dr. Tomáš Helikar at thelihar2@unl.edu.

Print in-class materials

Each student will also need a copy of the activity packet (Supporting File S1: Prokaryotic Gene Regulation- Activity packet), which includes all background, instructions, and question prompts. You can provide copies for each student or require that students print their own copy and bring it to class.

In Class

Facilitate the *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* in-class activities

First, make sure that each student has a copy of the *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* activity packet (Supporting File S1: Prokaryotic Gene Regulation-Activity packet). This activity packet provides students with all of the background information, directions, and question prompts they need to complete the activity successfully.

Exercise 1. Background reading and reading comprehension questions

Have students read the background information on pages 1-2 and answer the provided reading comprehension questions on page 3 of the activity packet. By reading the background information, students will receive a concise introduction to the

structure and function of operons, structure and function of the *lac* operon, and the basic mechanisms of gene regulation. The accompanying questions on page 3 of the activity packet ensure that students complete the reading and are prepared for the computer-centered portion of the lesson. Reading the background information and answering the questions on page 3 of the activity packet should take students approximately 10 minutes.

Exercise 2. Describe the components and interactions of the *lac* operon

In the first part of the activity, found on page 4 of the activity packet, students are instructed to identify the positive and negative regulators for each component of the *lac* operon system and describe the biology behind that relationship (e.g., the *lac* enzymes are responsible for breaking down lactose). After describing how the different components in the *lac* operon system impact one another, students translate this information into an on-paper model of the system, using inhibitory arrows (\dashv) and activating arrows (\longrightarrow) to denote relationships between components. This on-paper model serves as a draft for the model that students will construct in Cell Collective Learn. In this exercise students are expected to start using their group members as a valuable resource by discussing how each component of the *lac* operon system plays a role in regulating the *lac* operon expression and how that translates into a two-dimensional model. Each group should reach a consensus before moving forward. This need to reach a consensus prompts students to conduct thoughtful discussions about the *lac* operon system. Instructors should allocate 20 minutes for students to complete this portion of the lesson.

Exercise 3. Access Cell Collective Learn

In the second part of the activity, students use their computers to access Cell Collective Learn at <https://learn.cellcollective.org>, create or log into their account, and open the *Lac Operon Construction* model from the home page. Instructions on navigating to Cell Collective Learn and selecting the *Lac Operon Construction* model are provided on page 5 of the activity packet.

Students who need to register for a Cell Collective Learn account will need to select the “Create Account” icon and provide their full name along with their email and their institution of study. Students who already have an account will select the “Login” icon and input their selected email and password. A Cell Collective Learn account is free for all users. Students are able to explore Cell Collective Learn as a guest by selecting the “Explore All Learning Modules” icon, which allows students to simulate pre-built models and download any provided files associated with each lesson. However, students will not be able to utilize all of the features of Cell Collective Learn as a guest, including the ability to create and save new models as well as any other lesson-related work that requires the saving of data on the Cell Collective Learn servers. Thus, students need to have a Cell Collective Learn account in order to complete the *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* lesson.

Exercise 4. Creating a computational model

Students next use Cell Collective Learn to construct a computational model of the *lac* operon. Students are responsible for adding components (e.g., *lac* repressor) and the relationship between those components (e.g., allolactose inhibits the *lac* repressor) in order to correctly model behavior of the *lac* operon. Instructions on how to construct a computational model in Cell Collective Learn are provided on page 5 of the *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* activity packet (Supporting file S1: Prokaryotic Gene Regulation- Activity packet). Instructors should allocate approximately 20 minutes for students to construct their model of the *lac* operon. In this exercise, students should assist their fellow group members in navigating the modeling functions and confirming that their constructed computational model accurately reflects the biological relationships between components in the *lac* operon.

Exercise 5. Model validation and revision

After constructing their model, students must check that the model “behaves” in accordance to known literature on the *lac* operon. Students are given the literature-supported expression of the *lac* operon (provided on page 6, Table 2 of the activity packet) based on environmental conditions (e.g., if glucose and lactose are present in the environment, the *lac* operon will be inactive). Students simulate their model under different environmental conditions (all combinations of presence and absence of glucose and lactose) and compare their simulation results to the expected biological behaviors. If their simulation results do not match the expected behaviors, students are prompted to revisit their model and make revisions as necessary. Students record their revisions on page 7 of the activity packet. This validation and revision step parallels ways in which researchers validate their models before further exploring the system (31,32). Students proceed through the remaining exercises only after achieving the correct model behavior for the *lac* operon.

Students set the parameters of the simulation by altering the levels of external components. While the quantitative values of external components (e.g., environmental glucose set to 100%) do not directly correspond to a specific, measurable biological property (such as concentration), students can interpret values (e.g., 100% activity) of external components (e.g., environmental glucose) semi-quantitatively (23). For example, environmental glucose set to 80-100% can be interpreted as “high amounts of environmental glucose” and 0-10% as “low amounts of environmental glucose.”

When students simulate the computational model, they observe internal components (e.g., *lac* operon) as lines on a graph with the x and y axes representing time steps and activity level (ranging from 0 - 100), respectively. The activity level is determined by the regulatory mechanisms of other directly interacting components and also provides a semi-quantitative measure as a probability of a component being active at a given time point. This measure is used to describe the relative activity of a particular model component in response to environmental signals and/or perturbations in the model (23,33,34). Simulation settings have been optimized to

provide clear visuals of the system. During this exercise, group discussions should center around simulation interpretation, and well as revisiting discussions from exercise 4 as necessary, such as relating their computational model to real biological interactions.

The time required for students to validate and revise their models varies greatly per group and depends on 1) the amount of revisions required to reach the correct model and 2) student comfort level with Cell Collective Learn. We suggest approximately 10 minutes for students to validate and revise their model.

Exercise 6. Investigate the impact of changes to the *lac* operon

After confirming the validity of their computational model, students will further explore the *lac* operon system through simulation activities called Investigations on pages 8-12 of the activity packet. During these Investigations students setup simulations to monitor the outputs and regulatory elements of the *lac* operon computational model. Because the computational model of the *lac* operon is based on probabilistic logical modeling (further details on the mathematics can be found in 23, 33, 34 and 35), students should focus on whether and how the activity of a given component is affected positively or negatively (as opposed to specific values) in response to mechanistic changes in the modeled system (this is similar to the interpretation of the output of many biological experiments, including Western Blots, differential gene expression analysis, etc.). Instructors should allocate 30 minutes for students to complete the Investigations.

In Investigations 1 and 2, students explore the mechanism behind *lac* operon activity in response to the availability of nutrients (i.e., lactose and glucose). These Investigations expand upon the simulations performed during the model validation exercise; now students are responsible for monitoring not only *lac* operon activity but the activity of the regulatory components within the system. In Investigation 1 students explore how the presence of only lactose in the environment impacts the *lac* operon system. In Investigation 2, students examine how the presence of lactose and glucose in the environment impacts *lac* operon system. In Investigations 3 and 4, students will explore the impact of mutations on *lac* operon regulation. Our goal for these two Investigations is to have students recognize how DNA mutations influence gene regulation by influencing protein function. In Investigation 3 and 4, students explore how a loss-of-function mutation in the CAP binding site and *LacZ* (gene that codes for an enzyme that breaks down lactose) affects *lac* operon regulation and expression.

During each Investigation, students engage in the process of science with the following steps: A) making predictions, B) developing a mechanistic explanation to support the prediction, C) testing the prediction using simulations, D) recording the simulation results, E) evaluating the prediction based on simulation results and retesting as necessary, and F) providing an accurate mechanistic explanation supported by simulation results. For example, in Investigation 1 Step A (page 8), students are asked to predict how the presence of environmental lactose in the absence of environmental glucose will affect the activity of the *lac* operon. In Step B,

students support their prediction with a mechanism based on the provided computational model. In Step C, students test their prediction through simulation. In Step D, students report their simulation results. In Step E, students use the simulation results to evaluate the correctness of their prediction. If their simulation results do not align with their prediction, students are prompted to repeat Steps A-E until both their prediction and their results match. Finally, in Step F, students attempt to describe the mechanism responsible for the biological phenomena represented by the simulation results. This organization for the Investigations has previously been shown to be beneficial for student learning of complex biological systems (14,22,24,25).

We designed Steps A - F based on the recommended best practices of using simulations to facilitate science learning (28,36,37) specifically to have students

- explicitly make their conceptions as predictions (Step A) (37),
- provide reasons for their predictions (Step B) (37),
- test their prediction by setting up computer simulations (Step C)
- confront alternative conceptions with simulation results (Step D) (28,36),
- evaluate the consistency of their predictions with their results (Step E) (37), and
- use evidence (from simulation results) to support findings (Step F).

Ways to Facilitate the *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon Activity Packet*

While instructor guidance is not required for this lesson, we encourage you to be engaged with your students and be available as they navigate this lesson. When building and simulating their model, encourage students to thoroughly read the provided instructions. All steps needed to build and investigate the model are included, along with additional tips on navigating the Cell Collective Learn platform. You can verify that students are applying mechanistic explanations by reading over their answers to Steps B and F of each Investigation. To do so, look for descriptions that 1) include two or more components, 2) describe the interactions between the components, and 3) provide an explanation of how the phenomena described in the prompt occurs. Be aware that students favor why answers instead of how answers (38). To mediate this kind of thinking, we suggest first checking in with one student within a group and then encouraging group discussion. This strategy provides some feedback to all students in the group, even if indirectly, and promotes peer instruction.

Common areas of difficulty and solutions

Many times, students skip the instructions and become confused or frustrated when the software does not respond as they expected. This behavior is consistent for both the model building and investigation activity. If you suspect frustration from a student, have him or her go back to carefully read and apply the instructions. Retracing steps commonly provides clarity and productive engagement with the software. Additionally, encourage students to ask their peers for help first. If one student skipped an important step in the instruction,

their group member might recognize that and guide them to the appropriate action.

During the model building portion of the lesson, the most frequent student issue is setting the appropriate dominance on the *lac* operon. Dominance refers to the hierarchy of signals regulating a component's behavior (e.g., *lac* operon). When both the positive regulator (e.g., CAP) and negative regulator (e.g., *lac* repressor) for the *lac* operon are active at the same time, you need to decide and tell the model which (activating or inhibiting) signal will determine *lac* operon activity (i.e., which regulator is dominant). In the instructions, students are shown how to evaluate and set the dominance, but oftentimes students will forget to do this step and become confused when their model does not simulate correctly. This is likely the problem if a student approaches you claiming that they are sure their model is built correctly but is not simulating properly.

Students may haphazardly make changes to the simulation settings which may lead to incorrect simulation results. While students are welcome to explore the model and the modeling platform, correctly working through the lesson requires that students run their simulation with the parameters outlined in the instructions. Running the simulation under different parameters, especially when students are not aware of how those parameters influence the simulation output, may prevent students from confronting their alternative conceptions with correct simulation results. Therefore, it is essential for you to mediate this activity by verifying that students are adjusting the simulation settings correctly. To minimize the need for your intervention, remind students to regularly compare and discuss their simulation results to those of their group mates. Encouraging students to explain to each other why their settings gave observed results builds argumentation skills. This type of discussion will help students detect and resolve any settings errors. Additionally, you can prompt students to focus on simpler predictions about model behavior, such as “if lactose is present, should the *lac* repressor be on or off?” If these simpler predictions do not match their simulation results, guide them to check the simulation settings.

TEACHING DISCUSSION

Lesson Implementation

We have implemented this lesson five times between 2015-2017 in an introductory biology course and are continuing to do so once per semester (Fall, Spring, and Summer). The lesson was provided to introductory biology students during a discussion period (i.e. dry laboratory) that lasted up to three hours. These discussion periods serve to reinforce student knowledge of relevant material in an interactive manner. Students completed the lesson in groups of three to four. All data collected were from consenting students with institutional review board approval.

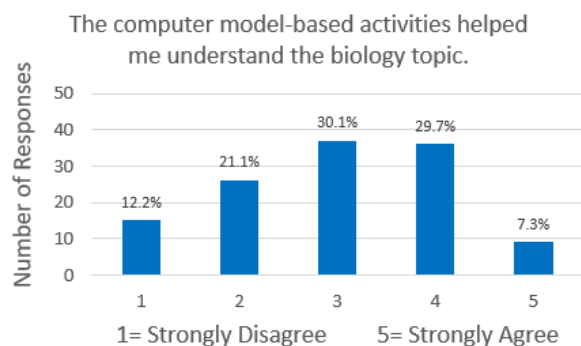
Student Reaction to the Lesson

During some of the implementations, students who completed the lesson were given an optional survey. Students reported their experience by rating their level of agreement to statements about the lesson using a 1-5 Likert scale.

Introductory biology students from the Spring 2016 implementation (n=124) stated that the lesson helped them understand the biology topic (i.e. the *lac* operon) (Figure 2A) and helped them learn something new about the *lac* operon system (Figure 2B).

- “[T]hat one [lesson] taught them how to solve problems in creative ways” (TA 1).
- “[S]tudents had to understand the models at a more fundamental level and keep track of those things in mind when they assembled those elements together and work around the software, which may sometimes be not easily understandable by students as well. [...] I think, despite the second one [model building exercise] being troublesome, students probably learned a bit more” (TA 1).
- “[T]his is a very structurally sound recitation. It starts from very basic all the way to very end. But if anyone were impatient and they jumped straight into the parts where you get to toggle stuff and it looks fun, you will not be able to solve the problems that you’re on because you did not have

A.



B.

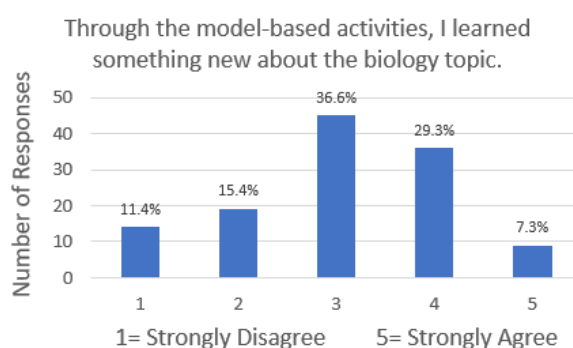


Figure 2. Student Response To The Lesson. Student responses from the introductory biology Spring 2016 implementation on a survey. Students were asked to A) rate whether the computer model-based activities helped them understand the biology topic. B) rate whether they learned something new about the biology topic through the model-based activities. The y-axis represents the number of student responses. The percentages on the columns represent the percentage of students that selected each response.

the fundamental basics there” (TA 1).

- “I love these simulations, I think that they allow you to see the pathways in ways that you don’t when they’re on paper” (TA 2).
- “I think being able sit and play with the levels and manipulate it [the system] really gives you an insight into it that you don’t have in a traditional lecture when you’re staring at an image of it” (TA 2).

Possible Adaptations

Our lesson is highly flexible and can be adapted to fit a variety of different classroom needs. Students can complete the *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* activity packet individually, and outside of class should timing not allow for an in-class implementation. Alternatively, the background reading and reading comprehension questions could be implemented as homework in order to prime students for the other parts of the lesson. Instructors may wish to split the activity packet into two separate assignments: model building/validation (pages 1-7) and Investigations (pages 8-12). Splitting the lesson this way prevents students from using Figure 8 in the activity packet, which is a pathway diagram of the *lac* operon system, to replicate this model in Cell Collective Learn during the building exercise. While students with access to this figure did not appear to A) realize this resource existed until after building and validating their model and B) have enough motivation to change their models after-the-fact based on this image, instructors that are grading students on the correctness of their model may wish to separate these two portions of the lesson.

If timing is limited in your class, we have a simulate-only version of this lesson called *Regulation of the Lac Operon*. This version of the lesson provides students with a pre-constructed model of the *lac* operon so that students are only responsible for completing the *lac* operon (simulation) Investigations. The model and corresponding lesson materials are provided publicly on Cell Collective Learn (<https://learn.cellcollective.org>).

Because providing mechanistic explanations is novel for many students, we suggest having students practice creating a simple mechanistic explanation with feedback from you and their peers before attempting the *lac* operon Investigations. This type of feedback is essential for supporting student learning (39) and can provide instructors with valuable information on student understanding of mechanisms and on writing mechanistic explanations.

Some instructors and students may prefer teaching this material along with other concepts. For example this lesson could be linked to readings and/or lectures on gene regulation. Additionally, you could couple this lesson with discussions about cellular respiration in order to connect how bacterial cells use the glucose generated from lactose breakdown.

For advanced courses, instructors can challenge students to consider the limitations of the *lac* operon model and to provide possible ways to improve this model to better represent the biology. For example, students could suggest expanding the model by adding beta-galactosidase, the enzyme responsible

for lactose breakdown, or RNA polymerase to the *lac* operon model. This type of thinking requires strong modeling skills and mastery of the biology. While a potentially valuable experience, we caution instructors teaching introductory courses that students at this level may not have the skills needed to benefit from this type of task. Furthermore, we are currently developing a new lesson designed specifically for senior-level biochemistry students, which will be available in the near future on Cell Collective Learn. Finally, you could add a summative post-assessment after the lesson to gauge what information students retained from the lesson. This post-assessment could focus on content related to the *lac* operon, gene regulation, and/or mechanistic descriptions. For example, the post-assessment can ask about *lac* operon expression if the *lac* repressor gene has a gain- or loss-of-function mutation.

Conclusion

The *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* lesson introduces students to basic concepts relating to gene regulation and the *lac* operon using a free, interactive, web-based modeling platform, Cell Collective Learn (<https://learn.cellcollective.org>). The model building and model simulation activities encourages student learning in an interactive manner while also exposing students to computational practices in biological system investigations. This approach is well received in the classroom, with both students and teaching assistants claiming that the lesson was beneficial to student learning. The lesson can be modified to fit a range of classroom needs including varying time constraints, student familiarity with computational modeling and specifically with Cell Collective Learn, and student familiarity with the *lac* operon.

SUPPORTING MATERIALS

- S1. *Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon* - Activity packet

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Table 1. Teaching Timeline

Activity	Description	Estimated Time	Notes
Preparation for Class			
Review in-class materials	<ol style="list-style-type: none"> 1. Familiarize yourself with the <i>lac</i> operon content. 2. Familiarize yourself with learn.cellcollective.org. 3. Prepare for questions students may have during class, such as questions about the <i>lac</i> operon and how to use the modeling platform. 4. Instruct the students to bring computers to the in-class portion of the lesson. Tablets and cell phones are not supported by Cell Collective Learn 	1-2.5 hours, depending on expertise on the <i>lac</i> operon content and modeling platform skills. First-time users of Cell Collective Learn will require closer to 2 hours for full mastery of the topic and software.	Go through the <i>Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon</i> activity packet provided in supporting file S1: Prokaryotic Gene Regulation- Activity packet.
In Class Activities			
Distribute the <i>Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon</i> lesson	Hand out the <i>Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon</i> activity packets to each student.	>5 minutes	<i>Discovering Prokaryotic Gene Regulation by Building and Investigating a Computational Model of the lac Operon</i> activity packet is provided in supporting file S1: Prokaryotic Gene Regulation- Activity packet.
Exercise 1. Background reading and reading comprehension questions	Instruct students to read the provided background material and answer the reading comprehension questions.	10 minutes	All needed content and question prompts for Exercise 1 are provided on pages 1-3 of supporting file S1: Prokaryotic Gene Regulation- Activity packet.
Exercise 2. Describe the components and interactions of the <i>lac</i> operon	<ol style="list-style-type: none"> 1. Have students identify the regulatory relationships (positive or negative) between components in the <i>lac</i> operon system. 2. Have students use these relationships to construct a model of the <i>lac</i> operon system on paper. 3. Instruct students to reach a consensus within their groups on the relationships between components and on their paper models before moving forward with the lesson. 	20 minutes	Instructions and needed resources for Exercise 2 are located on page 4 of supporting file S1: Prokaryotic Gene Regulation- Activity packet.
Exercise 3. Access Cell Collective Learn	<ol style="list-style-type: none"> 1. Instruct students to take out their computers and go to https://learn.cellcollective.org. 2. First-time users will need to register for a free Cell Collective Learn account. Students must have an account to complete the remainder of the lesson. 3. Have students open the <i>Lac Operon Construction</i> model. 	>5 minutes	Instructions for accessing Cell Collective Learn and creating a Cell Collective Learn account are located on page 5 of supporting file S1: Prokaryotic Gene Regulation- Activity packet.
Exercise 4. Creating a computational model	4. Have students use Cell Collective Learn to construct a computation model of the <i>lac</i> operon based off of their on-paper model.	20 minutes	Instructions for building a computational model in Cell Collective Learn are located on page 5 of supporting file S1: Prokaryotic Gene Regulation- Activity packet.
Continued on next page...			

Activity	Description	Estimated Time	Notes
Exercise 5. Model validation and revision	<ol style="list-style-type: none"> 1. Have students validate their model by comparing their simulation results with known expression patterns for the <i>lac</i> operon. 2. Have students revise their models if their simulation results do not match these known expression patterns. 3. When students have validated their model, have them draw an updated paper model of the <i>lac</i> operon system. 4. Students should consult with their group members for assistance when needed. 	10 minutes	Instructions and needed question prompts for Exercise 5 are provided on page 6 and 7 of supporting file S1: Prokaryotic Gene Regulation-Activity packet.
Exercise 6. Investigate the impact of changes to the <i>lac</i> operon	<ol style="list-style-type: none"> 1. Have students work through the guided simulation activities. 2. Students should consult with their group members for assistance when needed. 3. Collect student work once students have completed Exercise 6. 	30 minutes	Instructions and needed question prompts for Exercise 5 are provided on page 8-12 of supporting file S1: Prokaryotic Gene Regulation-Activity packet.