## *Name \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Block \_\_\_\_\_\_\_# \_\_\_\_\_\_\_\_*

## *lac* Operon Simulation

 <https://phet.colorado.edu/en/simulation/gene-machine-lac-operon>

Today you will work with a simulation[[1]](#footnote-1) regarding the *lac* operon. You may use resources provided (PPt, book, videos, etc.) or the operon POGIL as notes for help.

### Part 1: Lactose Regulation

On the screen you should see two floating RNA polymerase (RNAP) in blue and the incomplete operon beneath them. You can turn on the legend in the lower right and that will help you identify the molecules. Click the “show legend” box in the lower right.

You can start by assembling your operon. You will need to match each part at the bottom to its specific place.

1. Drag the *lacZ* gene into place. What happens?
2. Drag the *lacI* gene into place. What happens?
3. Drag the *lac* operator into place. What happens?
4. **What is the role of the promoter in the *lac* operon?**
5. Drag the promoter into place. **What happens?**
6. Inject a lactose molecule. **What is the role of the enzyme *lacZ*?**
7. **Explain whether it is efficient for the *lacZ* enzyme to be made by the bacteria when there is no lactose around. How might the bacterium control the enzyme production?**

Operons are controlled at the transcription level. A regulator molecule can be transcribed and translated to block the operator so that the *lacZ* gene protein is only made when lactose is present in the environment.

1. Drag the *lacI* promoter into place. **What happens? (be patient)**

Inject 5 lactose molecules into the environment.

1. **Describe the interaction between the following:**
	1. **Lactose and operator**
	2. ***LacI* and the operator (no lactose present)**
2. **Explain how lactose (allotacose) acts as an effector in controlling the *lac* operon. (use back of sheet, if needed)**

### Part 2: Lactose Transport

The simulation environment has become more complicated now. Click on the “show legend” button to help you keep track of everything.

You now have a cell membrane which separates the inside and outside of the cell. There are also 2 genes present in the operon (*lacZ* and *lacY*) and the regulatory gene *lacI*.

Drag the 3 genes into place, as well as the operator. Drag the *lacL* promoter into place so we can “turn off” the *lac* operon. Drag the promoter into place.

Pump 5 molecules of glucose.

1. **Why can’t the lactose enter the bacterium?**
2. **Based on your legend, which molecule looks like a transporter? What gene is it associated with?**

Drag the *lacL* promoter and the *lacZ* genes off of the DNA for now. This will allow you to express the *lacY* operon transporter gene.

1. **What happens to the permeability of lactose once you express *lacY*?**
2. **What happens to lactose once it enters? What does this mean for the energy production of the bacterium?**

Drag the *lacZ* gene back into place.

1. **How do these two genes (*lacZ* and *lacY* ) back to back work to control the intake and hydrolysis of lactose?**
2. **If these genes were far apart on the bacterial chromosome, what would that mean for overall efficiency of breaking down lactose?**

### Part 3: Mutants

Reset your operon model. You are going to do a series of mutations, testing one at a time. You will have to design each trial so that only one mutation variable is manipulated. For each mutation, we will effectively “break” that part of the operon. Test each one by pumping in 5 lactose molecules.

|  |  |  |
| --- | --- | --- |
| **Mutation** | **Immediate Effect** | **Effect on Operon** |
| ***lacL* Gene (always OFF)** |  |  |
| ***lacL* Gene (always ON)** |  |  |
| ***lacZ* Gene (OFF)** |  |  |
| ***lacY* Gene (OFF)** |  |  |
| ***lac* Operator (OFF)** |  |  |
| ***lac* Promoter (OFF)** |  |  |

### Reflection Questions

1. Explain the evolutionary advantage of having multiple operons for various sugars.
2. How detrimental to the cell would it be to not have a backup metabolic pathway (lac operon) for when glucose is not present?
3. Are there any drawbacks to only regulating at the transcription level and not the translational level or protein level?
4. If scientists wanted to engineer a bacterium that could triple the digestion of lactose in the operon, which parts might the scientist add/subtract?
5. In the laboratory you grow your bacteria on a medium that contains X-gal (a lactose analog that turns blue when broken down by b-galactosidase (*lacZ*). X-gal cannot induce the *lac* operon like lactose does (meaning it cannot bond to the repressor on the operator switching the operon “on”). You add another chemical called IPTG that is an inducer of the *lac* operon (meaning it can bind to the regulator, turning the operon “on”). However IPTG cannot be digested by b-galactosidase.[[2]](#footnote-2)
	1. Which of the following would you predict would bind to b-galactosidase?

 Lactose (or allolactose) X-gal IPTG

* 1. Which of the following would you expect to bind to the lac repressor?

Lactose (or allolactose) X-gal IPTG

1. pHET Interactive Simulations - Gene machine: The Lac Operon 2017. <https://phet.colorado.edu/en/simulation/gene-machine-lac-operon> [↑](#footnote-ref-1)
2. MIT Fundamentals of Biology 2011 - <https://goo.gl/wkifQn> [↑](#footnote-ref-2)