# Lab 4.1: Regulation of Gene Expression by the *lac* Repressor

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# Introduction – prokaryotic operons

Some genes are expressed *constitutively* – all the time. The products of constitutively-expressed genes are needed all the time, *e.g.* enzymes for glycolysis, or the RNAs that are the structural components of the ribosome. Other gene products are not needed (or wanted) at all times – the expression of these genes is *regulated*. You have heard about some regulated genes in lecture, *e.g.* those involved in cell differentiation.

The *Escherichia coli lac* operon was one of the first gene regulation system to be studied. It led Jacob and Monod to enunciate the principles of *operon* organization and regulation[[1]](#footnote-1), for which they were awarded the Nobel Prize in 1965[[2]](#footnote-2). Examples of these original ideas, essentially unchanged, are found in all cells.

In prokaryotes, genes are often arranged into a functional group called an operon (Figure 1). Operons consist of:

* a **promoter** – the binding site on the DNA for RNA polymerase
* an **operator** – the binding site for the repressor protein, in this case LacI. When LacI binds to the operator (*lacO*), RNA polymerase cannot carry out transcription. Note that genes and DNA elements are written in italics whereas the protein is written in standard script.
* **structural genes** that are co-transcribed as a single mRNA molecule, coding for proteins that function together. In the case of the *lac* operon, these genes code for
	+ β-galactosidase (LacZ), which breaks the disaccharide lactose into galactose and glucose
	+ Lac permease (LacY), a pore protein that facilitates the transport of lactose across the inner membrane
	+ β-galactoside transacetylase (LacA).

The **regulatory gene**, *lacI*, encodes the LacI **repressor protein**. The binding of certain sugars to LacI causes it to dissociate from the operator site, which then allows transcription of the structural genes (Figure 1).



Figure 1. A schematic representation of the *lac* operon

After lactose is taken up by the cell, β-galactosidase catalyzes its isomerization to allolactose (structure in Figure 2). Allolactose binds to Lac I, which lowers its affinity for DNA. The dissociation of LacI allows RNA polymerase to begin transcription.

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| 200px-Allolactose**Figure 2.** The disaccharide allolactose. | *E. coli* makes more efficient use of glucose than any other carbon (and energy) source, partly because glucose is the first substrate in glycolysis. So, the availability of glucose can inhibit lactose utilization. This is mediated through cAMP (cyclic adenosine monophosphate), an inverse indicator of glucose availability, *i.e.* the smaller the glucose concentration, the greater the concentration of cAMP. cAMP binds to cAMP-dependent catabolite activator protein (CAP). The cAMP-CAP complex binds to the promoter region increasing the affinity of binding of RNA polymerase. Therefore, glucose starvation should increase *lac* structural gene transcription. |

#### Detecting *lac* operon activity

A significant experimental tool in studies of gene regulation is the *reporter* gene, which produces a signal in proportion to transcriptional activity, when it is placed downstream of the promoter under investigation.

In this lab, we will use an *E. coli* strain that harbors an *expression plasmid*, a genetically-engineered DNA element in which the operator/promoter region has been optimized for protein expression. The plasmid drives the expression of green fluorescent protein (GFP) under control of LacI and *lacO*. GFP is fluorescent so it can be excited by blue light and emits green light.

#### Lactose analogues

Lactose is a disaccharide, that is, a two-part sugar consisting of galactose linked to glucose with glycosidic bond. Analogs of lactose, *i.e.* chemicals that are similar, but not identical to lactose, can also bind to and deactivate the repressor. Most analogs are galactosides with another chemical group in place of the glucosyl group of lactose. (See Figure 3.) We will use isopropyl β-D-1-thiogalactopyranoside (IPTG) in this lab. IPTG can bind to the repressor protein, but it is not digested by β-galactosidase. Thus, IPTG is not consumed and it is not a food source.

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| IPTG | mfcd00063273 |  | galactose | mfcd00151230 mfcd00063774 | glucose |
| Lactose(disaccharide of galactose and glucose) | mfcd00151251 |  | Maltose (disaccharide of glucose) | mfcd00135877 |  |

Figure 3. Chemical structures of sugars and sugar analogs available in this lab. All images from Sigma Aldrich Chemical Co. (sigmaaldrich.com).

**Part 1: Qualitative analysis of gene regulation**

During the first part of this lab, we will observe and then measure the fluorescence from GFP produced in *E. coli* under control of the *lac* operator under different growth media conditions with different sugars added (see Materials for Part I below). You seek ‘yes/no’ answers to the following questions:

* Which molecules induce expression, which do not?
* How closely related are the structures of these molecules?
* Do any of the sugars actively inhibit the action of the inducer?

*To answer these questions, you and your partner will select from cultures of E. coli grown with a variety of sugar or sugar-analogues the ones that you hope will address these questions. The chemical structures of these sugars are illustrated in Figure 3.*

### Materials for Part I

* Actively growing cultures of bacteria harboring the *gfp* gene under the control of the *lac* operator and the *lac* repressor protein, grown in LB with (BTW, note the concentrations):
	1. No additives
	2. Lactose (0.25 M)
	3. IPTG (0.1 mM)
	4. Glucose (0.25 M)
	5. Galactose (0.25 M)
	6. Maltose (0.25)
	7. IPTG (0.1 mM) + glucose (0.25 M)
	8. IPTG (0.1 mM) + maltose (0.125 M)
	9. Lactose (0.25 M) + glucose (0.25 M)
	10. Lactose (0.25 M) + maltose (0.125 M)
	11. Galactose (0.25 M) + glucose (0.25 M)
	12. Galactose (0.25 M) + maltose (0.125 M)
* A handheld blue light and a suitable filter to observe GFP fluorescence.

### Methods

* Check for the induction of GFP expression using the special blue flashlight (holding it as far away as necessary to see the green fluorescence) and the filter (or goggles).
* What do you observe? What do you think is happening based on the sugars added to the growth media?
* Record your observations in your notebook

**Part 2: Check your observations quantitatively**

You are now going to determine if your qualitative observations match with quantitative ones. To do this, you will use the plate reader to take OD595 and Fl488 measurements of your cultures. We have one 96-well plate, so you will need to share. One person from one table will get wells A1-A12, another person from another table B1-B12, etc. Rows A through F should be filled by one person from each of the 6 tables and so you will have this data in 6 replicates. Use row G to load four LB blanks.

* Load 250 uL of each of your samples into the provided 96 well plates.
* Run two programs: Bug\_OD and Bug\_Fluor
	+ If any value is 65000 for the fluorescence, see your instructor to correct the gain and re-run the samples.
* The data should be sent to Laura by email, who will then post it to Moodle for each group to analyze
	+ You have 6 replicates for this data. What do you think you should do with it?
* Do your qualitative and quantitative observations agree?
* Why does the OD595 matter?
* Do you need a blank for fluorescence?
* **Share your question and observations with your table. Formulate some ideas and hypotheses.**

### Part 3: Check your plan for your quantitative experiment

* In your notebook you should have formulated your question and the predictions. Now check to make sure you’re happy with your questions and predictions and calculations.

#### Solutions supplied to you for your experiment:

* 0.5 M sugar solutions (lactose, maltose, galactose, glucose dissolved in LB)
* 0.2 mM IPTG solution (in LB)
* medium (LB) to make dilutions

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# Experiment Planning

The qualitative and quantitative observations made in part I and II in Lab 4.1 will suggest an experiment that you and your partner will design to quantitatively test the specificity and potency of inducing and anti-inducing agents. After making qualitative and quantitative observations, you may ask questions such as the following, or whatever can be tested with our system:

* *What concentration of inducer (or inhibitor) is required to induce (or inhibit) expression?*
* *How does the potency of different inducers compare?*
* *What is the time course of induction (slower or faster than growth rate)?*
* *How many molecules of an inducer are required* ***per cell*** *to induce expression?*
* *How well does glucose work as an inhibitor?*
* *Does maltose work as an inhibitor? If so, how does it work as compared to glucose?*
* *Is galactose an inducer or inhibitor? If inducer, how does it compare to lactose? If inhibitor, how does it compare to glucose?*

To do this you and your lab partner will be given one row in a 96 well plate (12 wells), to conduct a small quantitative experiment. If you are interested in doing a more complex experiment, you can do that in the Unit 5 Project Lab.

Samples will be loaded into 96-well plates after you design your experiment. The data will be collected during and after the lab period. The results will be distributed digitally for analysis in Lab 4.2.

#### The experiments that you and your colleagues plan will measure:

* GFP fluorescence as a function of time.
* Cell concentration (OD595) as a function of time.

The plan should use the plate format effectively, including dilution series and control samples.

*Before you begin Part 4 of Lab 4.1,* you and your partner must receive approval for your planned experiment. You need to submit this plan for approval. The plan should include a plate layout and describe (1) the questions addressed, (2) the composition of each well, and (3) the methods of well preparation.

**Part 4: Set up your quantitative experiment**

The plates will be constructed in the following manner:

1. Assemble your ingredients into microcentrifuge tubes. **You should make a total of 400 uL (this is more than you’ll need). Use your planning document.**
2. You and your coworkers will load 200 uL of each solution into each of your wells. Put tape over your completed wells to prevent any accidental additions to them.
3. Pass the plate to the next group and they will do the same.
4. After all the groups have loaded the wells, the instructors will add 2 uL of active *E. coli* culture into each of the wells. ***This will not appreciatively change the total volume in the well, and can be effectively ignored.***
5. The instructors will load control samples into reserve wells. These controls will be serially diluted cells (without any added sugar) to produce a calibration curve and an on-plate blank.

#### Data collection

The plates containing the experimental solutions will be loaded into the plate readers, and a program will run which takes OD and fluorescence in rapid succession every 15 minutes for several hours.

### Clean up

Save unopened sugar solutions.

Any sugar solutions that have been opened should be disposed of down the drain, and the tubes thrown in the trash.

*E. coli* in plastic microfuge tubes should go straight into the red biohazard bin (for Thursday lab, for Tuesday we will save them)

All *E. coli* cultures in glass tubes or flasks should be killed with bleach, and then poured down the drain. Put flasks with dirty dishes, glass tubes in the glass trash. (for Tuesday lab, for Thursday we will save them)

1. Jacob, F, J Monod. 1961. Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.* **3,** 318-356. [↑](#footnote-ref-1)
2. Nobel Web, The Nobel Prize in Physiology or Medicine, 1965, available from <http://nobelprize.org/nobel_prizes/medicine/laureates/1965/>. [↑](#footnote-ref-2)