Goals of this Assignment

This lesson attempts to teach middle school students some basic concepts in synthetic biology.

1. This lesson discusses plasmids and some ways they can affect phenotype. From this section, students should gain a better understanding of how DNA contains genes and how these genes can alter cell traits.
2. Students will be shown that there are factors controlling the expression of a gene with a basic introduction to promoters.
3. Students will be introduced to the concept of transcription factors, with an emphasis on repressors. They should finish the lesson with an understanding that phenotype expressed by some genes can be controlled by environmental signals.
4. This lesson seeks to introduce more complex circuit design with the introduction of multiple transcription factors in one circuit. Students should leave with an understanding that multiple environmental signals can allow for more control over phenotype.

The lesson seeks to do this by integrating short descriptions with simple lab protocols. There are five sections in total, most containing evaluation questions to assess the student's understanding and to encourage further thinking.

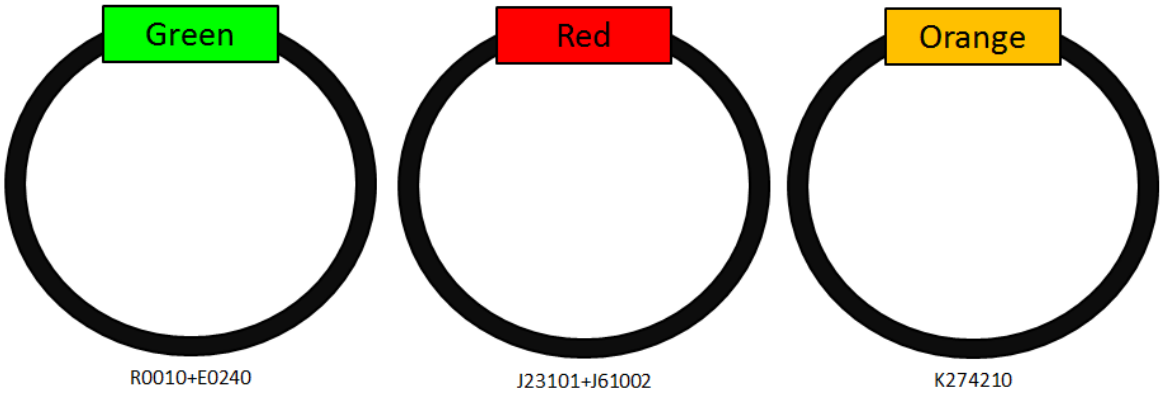
Key assumptions:

1. The students will have already been exposed to the concept of DNA as a chemical compound in the cell that encodes information about what an organism looks like.
2. The students should have a very basic understanding of the difference between plasmids and chromosomal DNA, although this lesson should help clarify.
3. The student is not expected to know about the processes of transcription or translation at this point.
4. Students should be somewhat familiar with the concept of antibiotics.
5. Students should be familiar with certain units of volume and concentration.

Part 1

Plasmid DNA: Changing cell appearance

DNA encodes all of the traits that you see in life around you, from the color of your hair to the number of petals on a flower. These characteristics and traits of life can be described as the **phenotype** of an organism. For scientists that work with bacteria, an easy way to change the phenotype/appearance of a cell is through the insertion of circular DNA called **plasmids**.



These plasmids contain genes that turn bacterial cells different colors. Notice that each one has a different label containing numbers and letters. Researchers have a lot of genes to choose from and use these labels to keep track of different ones.

Exercise:

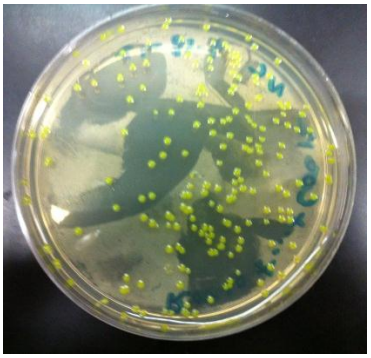
Through a process known as **transformation**, you can put these plasmids into cells and observe how they change the cell's appearance.

You will need:

- Competent cells
- DNA template (it may look like just water, but there is DNA in there!)
    - R0010+E0240 (Green)
    - J23101+J61002 (Red)
    - K274210 (Orange)
- Luria Broth (LB)
- LB+Carbenicillin plates
- 15 mL conical tubes

Steps:

1. Thaw competent cells on ice. Be sure to keep them cold and be gentle!
2. Transfer 50 µL of competent cells to 15 mL conical tube.
3. Add 1 µL of DNA template to cells.



4. Incubate cells on ice for 30 minutes.
5. Heat shock in 42 °C water bath for 90 seconds.
6. Immediately place back on ice for 2 minutes.
7. Add 800 µL of LB to each tube.
8. Incubate at 37 °C for 1 hour.
9. Place 200 µL of the transformed cells on LB+Carbinicllin plates.
10. Incubate overnight at 37 °C.
11. Colonies should appear on plates. If no color is present, give the cells more time to produce it.

Evaluation:

Plasmids can contain genes for more than just color. They can provide antibiotic resistance that allows cells to survive on antibiotic plates, encode proteins that produce smells, and much more!

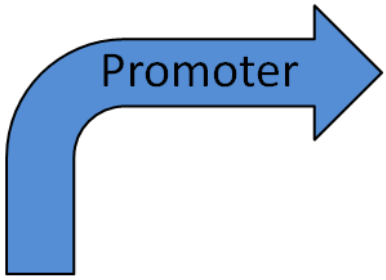
1. Fill out the table below with the phenotype you expect to see on the specified plate. Do you expect cells to grow in each situation?

Genes	Plate	Result/Phenotype
Green color gene	No antibiotic	
Green color gene Carbenicillin resistance	Carbenicillin antibiotic	
Banana smell gene Yellow color gene Kanamycin resistance	Kanamycin antibiotic	
Red color gene	Kanamycin antibiotic	
Green color gene Kanamycin resistance	Carbenicillin antibiotic	

2. Why might some researchers use plasmids instead of changing the genome of the cell?

Part 2

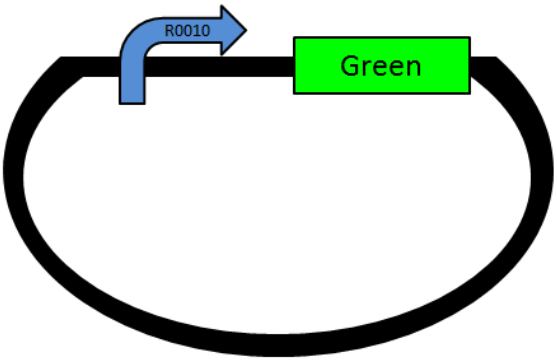
Promoters: Turning genes on



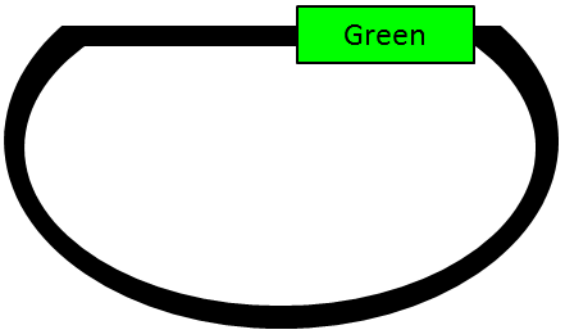
Just like the lights in your house, the genes above can be turned on or off. Instead of a light switch, scientists use a piece of DNA known as a **promoter** to switch genes on. All of the genes above contained a promoter that was on, but what would happen if the promoter was not there?

Exercise

Transform the two parts below by following the same steps from Part 1. Do you expect to see a difference between the two?



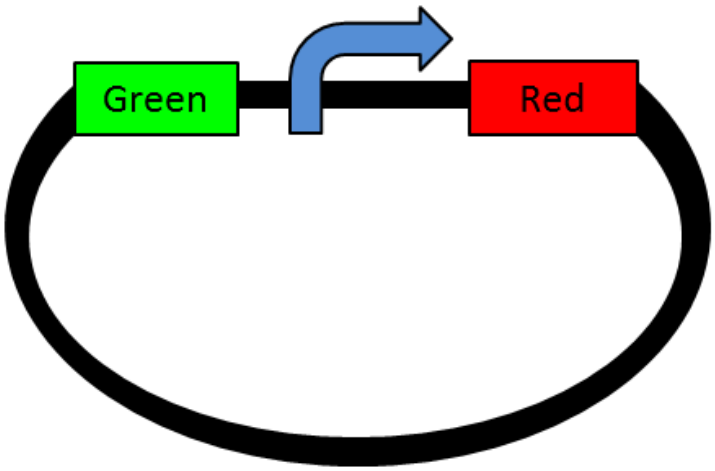
R0010+E0240



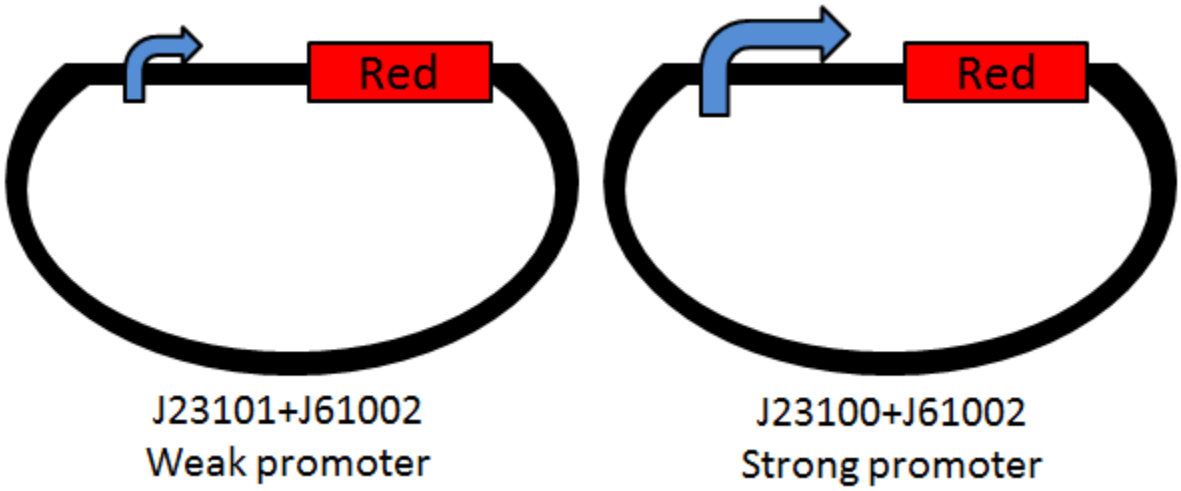
E0240

Evaluation

1. What would be the phenotype of a cell containing the plasmid below?



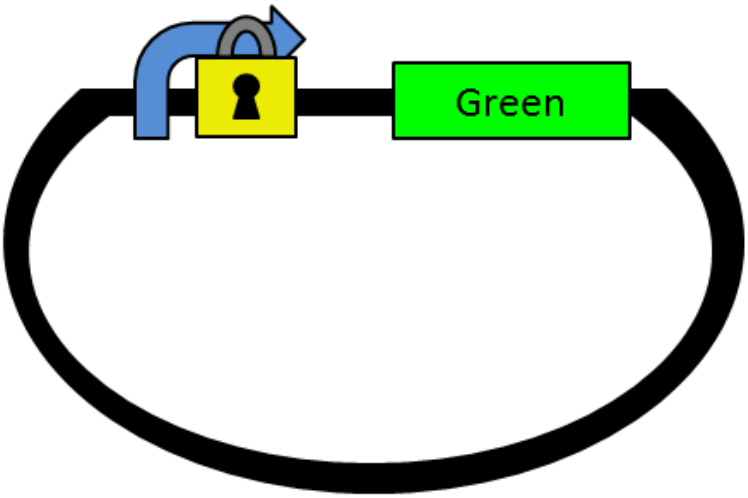
2. Different promoters can have different strengths. This can be used to produce more or less of a particular gene. What difference would you expect to see between the two circuits below?



Part 3

Inducible Promoters: Turning genes on and off

Many promoters are simple and always turned on, while others are more complicated. Some promoters can be turned off by proteins called **repressors**. Think of these as locks that will not open unless you have a key. If the promoter is locked, the gene cannot be expressed. The keys that unlock these promoters come from environmental signals and can vary from things like different chemicals or light. Each lock had a different and unique key which scientists call an **inducer**. Similarly, the promoters affected by these inducers are called **inducible promoters**. In the following exercise, you will be working with a chemical inducer called arabinose.



Promoter with Arabinose Repressor

Arabinose

Exercise

By making a **liquid culture** you can grow cells in solution. Liquid culture the part above and add different amounts of inducer.

You will need:

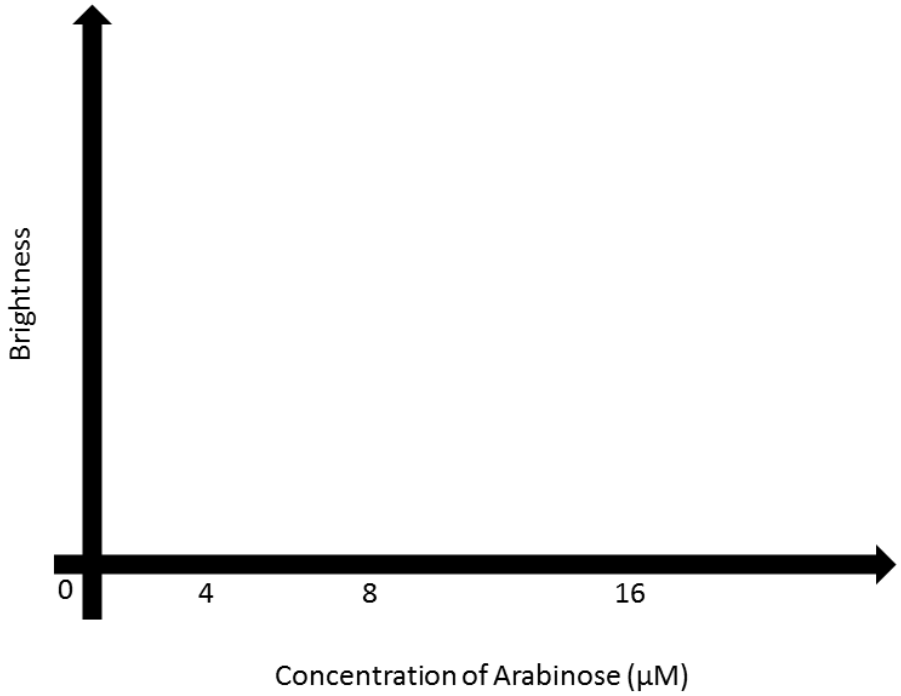
- Luria Broth (LB)
- Cells containing the above part (I13453+E0240)
- Carbenicillin
- 15 mL conical tubes
- Arabinose

Steps:

1. Take four 15 mL conical tubes and fill them each with 5 mL of LB.
2. Add 0.25 mg of carbenicillin to each tube. (Making a stock solution beforehand is recommended).
3. Add a colony of cells with the part to each tube.

4. To adjust the arabinose concentration, add 0, 1.5 µL, 3 µL, and 6 µL of a stock solution of made from 0.1 g of arabinose in 50 mL of water.
5. Let cultures grow overnight.
6. Centrifuge cells and observe pellet brightness.

While waiting for the cells to grow, make a plot of how you expect the brightness of green to change with different concentrations. After observing the pellets, how did what you saw compare to what you expected?



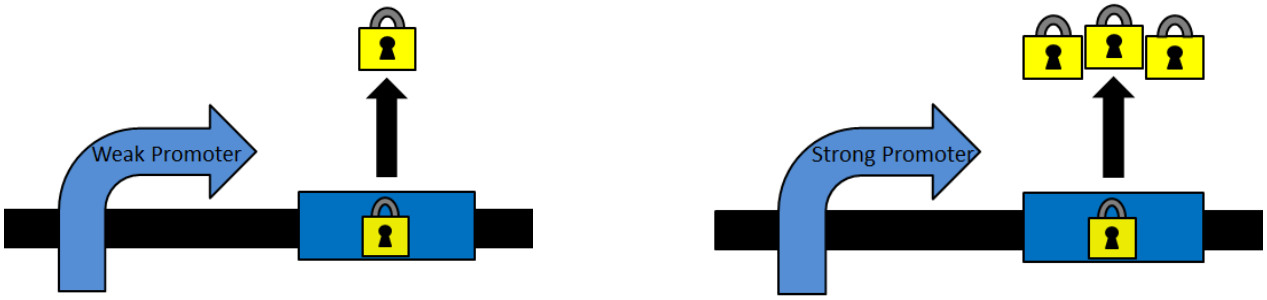
Evaluation

1. Expressing more or less of a certain gene can be very useful in some situations. In the previous section we saw this could be accomplished by using different strength promoters. Why might it be better to use an inducible promoter? Why might it be better to only use a promoter that does not respond to environmental signals?

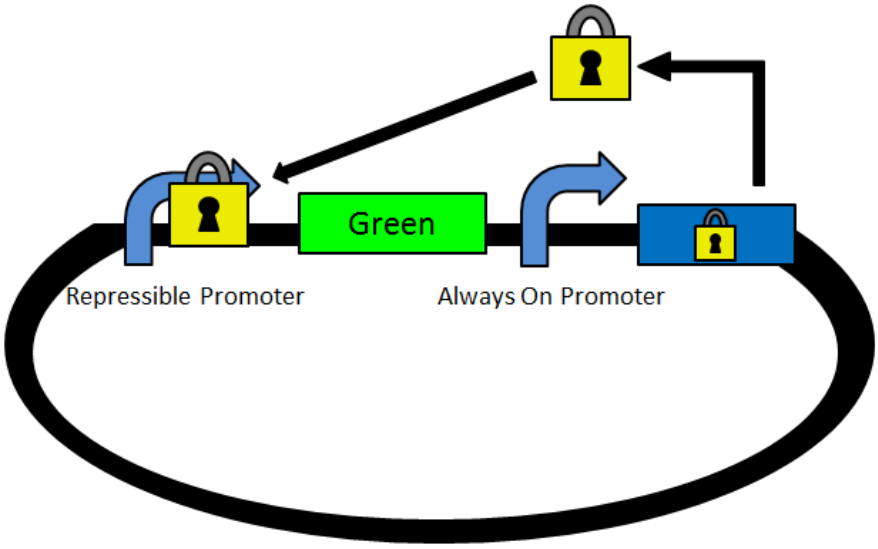
Part 4

Multiple Promoters: Complex systems allow fine tuning of expression

Plasmids can get a lot more complicated! The repressors (locks) mentioned in the last section can also be expressed on a plasmid. Remember, promoters can have different strengths, so this means that we can control the amount of repressors present to lock a promoter.

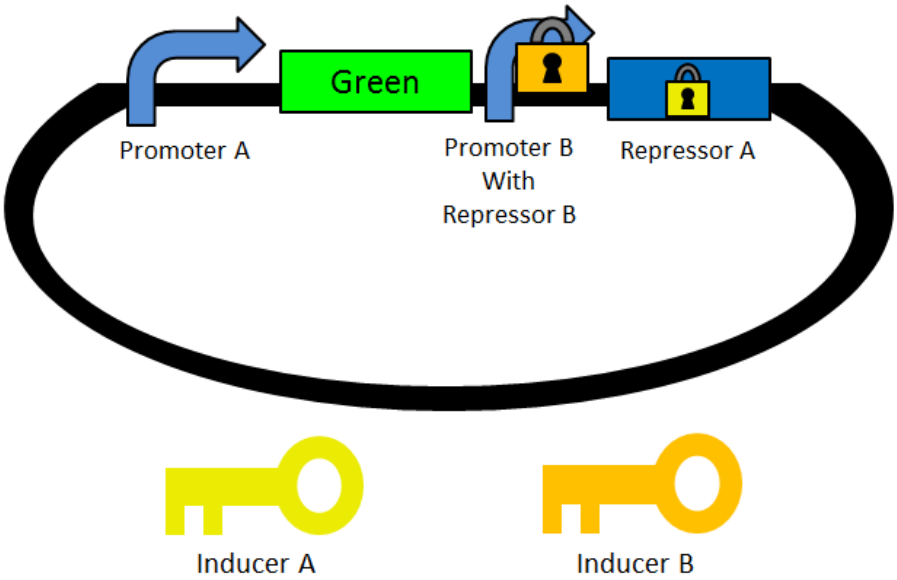


The plasmid below is exactly the same as the one in the previous exercise, except another promoter is producing the plasmid.



Excercise

The Following part has two promoters that are controlled by two different repressor and inducer pairs. Add different amounts of each inducer and predict what will happen.



Liquid Culture the part above and add different amounts of each inducer.

Prepare five culture tubes with the following amounts:

- 0 g of inducer A (IPTG) and 0.001 g inducer B (arabinose)
- 0.006 g of inducer A (IPTG) and 0 g inducer B (arabinose)
- 0 g of inducer A (IPTG) and 0 g inducer B (arabinose)
- 0.006 g of inducer A (IPTG) and 0.001 g inducer B (arabinose)
- 0.004 g of inducer A (IPTG) and 0.001 g inducer B (arabinose)

What do you predict will happen in each case (high, small, middle amounts of green)?

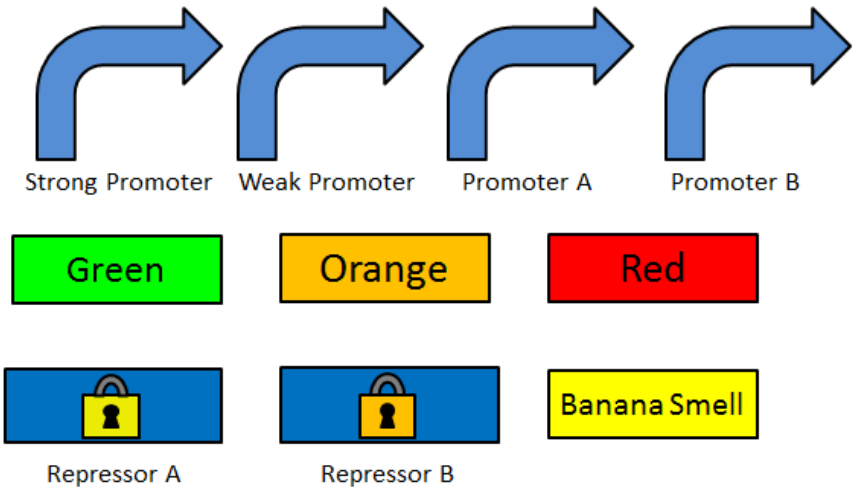
Inducer A	Inducer B	Phenotype (Amount of Green)
None	High Amount	
High Amount	None	
None	None	
High Amount	High Amount	
Middle Amount	High Amount	

Part 5

Make your own biological device

You have learned many new things about DNA and synthetic biology and have seen that plasmids can be both simple and complicated. To wrap up, try creating a part of your own!

Below is a bank of parts to choose from. After arranging your part, take a moment to describe and predict its function.



Part:

Description:

Researchers also have a bank with hundreds of parts to choose from called the registry of standard biological parts.

Vocabulary – Fill in the definitions.

Phenotype –

Plasmid –

Promoter –

Repressor –

Inducible Promoter –

Safety Guidelines

Please follow the guidelines below to ensure that your lab experience is safe and fun!

- Clear off work/desk area before starting.
- Wear gloves at all times when working with bacteria.
- Work near a flame when working with media or competent cells to prevent contamination.
- When disposing anything that has bacteria in it, you must put bleach in it before dumping it into the sink. Plates, falcon tubes and other trash go in a special bin afterwards to be autoclaved.
- Check with your instructor before dumping anything down the drain.
- Be sure to change the pipet tip each time you pipet anything.
- Do not leave a flame unattended! Make sure to turn off the Bunsen burner when finished.
- Make sure to tell your instructor if a spill occurs.
- Wipe down work area afterwards.
- No eating or drinking in lab.
- Make sure to wear close-toed shoes.
- Wash your hands before leaving lab.
- Be sure to identify locations of all eye washes and fire extinguishers.