Student Manual

pGLO Transformation

Lesson 1 Introduction to Transformation

In this lab you will perform a procedure known as genetic transformation. Remember that a gene is a piece of DNA which provides the instructions for making (codes for) a protein. This protein gives an organism a particular trait. Genetic transformation literally means "change caused by genes," and involves the insertion of a gene into an organism in order to change the organism's trait. Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be genetically transformed into plants. In bioremediation, bacteria can be genetically transformed with genes enabling them to digest oil spills. In medicine, diseases caused by defective genes are beginning to be treated by gene therapy; that is, by genetically transforming a sick person's cells with healthy copies of the defective gene that causes the disease.

You will use a procedure to transform bacteria with a gene that codes for Green Fluorescent Protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*. Green Fluorescent Protein causes the jellyfish to fluoresce and glow in the dark. Following the transformation procedure, the bacteria express their newly acquired jellyfish gene and produce the fluorescent protein, which causes them to glow a brilliant green color under ultraviolet light.

In this activity, you will learn about the process of moving genes from one organism to another with the aid of a plasmid. In addition to one large chromosome, bacteria naturally contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for one or more traits that may be beneficial to bacterial survival. In nature, bacteria can transfer plasmids back and forth allowing them to share these beneficial genes. This natural mechanism allows bacteria to adapt to new environments. The recent occurrence of bacterial resistance to antibiotics is due to the transmission of plasmids.

Bio-Rad's unique pGLO plasmid encodes the gene for GFP and a gene for resistance to the antibiotic ampicillin. pGLO also incorporates a special gene regulation system, which can be used to control expression of the fluorescent protein in transformed cells. The gene for GFP can be switched on in transformed cells by adding the sugar arabinose to the cells' nutrient medium. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on ampillicin plates. Transformed cells will appear white (wild-type phenotype) on plates not containing arabinose, and fluorescent green under UV light when arabinose is included in the nutrient agar medium.

You will be provided with the tools and a protocol for performing genetic transformation.

Your task will be to:

1. Do the genetic transformation.
2. Determine the degree of success in your efforts to genetically alter an organism.

Lesson 1 Focus Questions

There are many considerations that need to be thought through in the process of planning a scientific laboratory investigation. Below are a few for you to ponder as you take on the challenge of doing a genetic transformation.

Since scientific laboratory investigations are designed to get information about a question, our first step might be to formulate a question for this investigation.

Consideration 1: Can I Genetically Transform an Organism? Which Organism?

1. To genetically transform an entire organism, you must insert the new gene into every cell in the organism. Which organism is better suited for total genetic transformation—one composed of many cells, or one composed of a single cell?

2. Scientists often want to know if the genetically transformed organism can pass its new traits on to its offspring and future generations. To get this information, which would be a better candidate for your investigation, an organism in which each new generation develops and reproduces quickly, or one which does this more slowly?

3. Safety is another important consideration in choosing an experimental organism. What traits or characteristics should the organism have (or not have) to be sure it will not harm you or the environment?

4. Based on the above considerations, which would be the best choice for a genetic transformation: a bacterium, earthworm, fish, or mouse? Describe your reasoning.

Consideration 2: How Can I Tell if Cells Have Been Genetically Transformed?

Recall that the goal of genetic transformation is to change an organism's traits, also known as their phenotype. Before any change in the phenotype of an organism can be detected, a thorough examination of its natural (pre-transformation) phenotype must be made. Look at the colonies of *E. coli* on your starter plates. List all observable traits or characteristics that can be described:

The following pre-transformation observations of *E. coli* might provide baseline data to make reference to when attempting to determine if any genetic transformation has occurred.

a) Number of colonies

b) Size of : 1) the largest colony
2) the smallest colony
3) the majority of colonies

c) Color of the colonies

d) Distribution of the colonies on the plate

e) Visible appearance when viewed with ultraviolet (UV) light

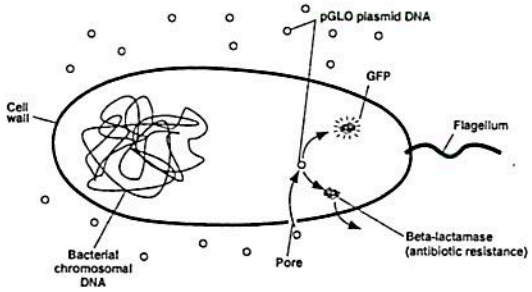
f) The ability of the cells to live and reproduce in the presence of an antibiotic such as ampicillin

1. Describe how you could use two LB/agar plates, some *E. coli* and some ampicillin to determine how *E. coli* cells are affected by ampicillin.

2. What would you expect your experimental results to indicate about the effect of ampicillin on the *E. coli* cells?

Consideration 3: The Genes

Genetic transformation involves the insertion of some new DNA into the *E. coli* cells. In addition to one large chromosome, bacteria often contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for more than one trait. Scientists use a process called genetic engineering to insert genes coding for new traits into a plasmid. In this case, the pGLO plasmid has been genetically engineered to carry the GFP gene which codes for the green fluorescent protein, GFP, and a gene (*bla*) that codes for a protein that gives the bacteria resistance to an antibiotic. The genetically engineered plasmid can then be used to genetically transform bacteria to give them this new trait.



Consideration 4: The Act of Transformation

This transformation procedure involves three main steps. These steps are intended to introduce the plasmid DNA into the *E. coli* cells and provide an environment for the cells to express their newly acquired genes.

To move the pGLO plasmid DNA through the cell membrane you will:

1. Use a transformation solution containing $CaCl_2$ (calcium chloride).
2. Carry out a procedure referred to as **heat shock**.

For transformed cells to grow in the presence of ampicillin you must:

3. Provide them with nutrients and a short incubation period to begin expressing their newly acquired genes.

Lesson 2 Transformation Laboratory

Workstation (✔) Checklist

Your workstation: Materials and supplies that should be present at your workstation prior to beginning this lab are listed below.

Student workstation

Material	Quantity	(✔)
E. coli starter plate	1	❑
Poured agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)	4	❑
Transformation solution	1	❑
LB nutrient broth	1	❑
Inoculation loops	7 (1 pk of 10)	❑
Pipets	5	❑
Foam microcentrifuge tube holder/float	1	❑
Container (such as foam cup) full of crushed ice (not cubed ice)	1	❑
Marking pen	1	❑
Copy of Quick Guide	1	❑
Microcentrifuge tubes	2	❑

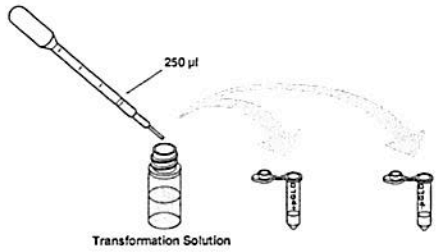
Common workstation. A list of materials, supplies, and equipment that should be present at a common location to be accessed by your team is also listed below.

Material	Quantity	
Rehydrated pGLO plasmid	1 vial	❑
42°C water bath and thermometer	1	❑
UV Light	1	❑
37°C incubator	1	❑
(optional, see General Laboratory Skills–Incubation)		
2–20 µl adjustable volume micropipets	1	❑
2–20 µl micropipet tips	1	❑

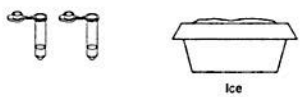
Transformation Procedure

1. Label one closed micro test tube **+pGLO** and another **-pGLO**. Label both tubes with your group's name. Place them in the foam tube rack.

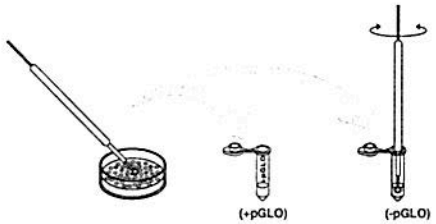
2. Open the tubes and, using a sterile transfer pipet, transfer 250 µl of transformation solution ($CaCl_2$) into each tube.



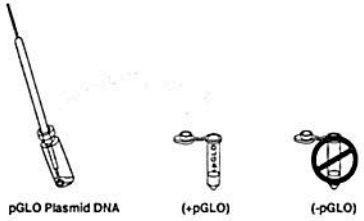
3. Place the tubes on ice.



4. Use a sterile loop to pick up **2–4 large colonies of bacteria** from your starter plate. Select starter colonies that are "fat" (ie: 1–2 mm in diameter). It is important to take individual colonies (not a swab of bacteria from the dense portion of the plate), since the bacteria must be actively growing to achieve high transforation efficiency. Choose only bacterial colonies that are uniformly circular with smooth edges. Pick up the **+pGLO** tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the **-pGLO** tube.



5. Examine the pGLO DNA solution with the UV lamp. Note your observations. Immerse a **new sterile loop** into the pGLO plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the **+pGLO** tube. Optionally, pipet 10 µl of pGLO plasmid into the +pGLO tube & mix. **Do not** add plasmid DNA to the **-pGLO** tube. Close both the **+pGLO** and **-pGLO** tubes and return them to the rack on ice.



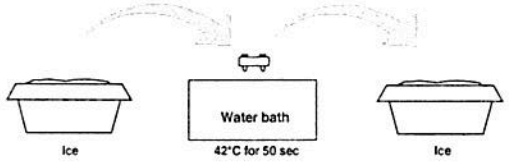
6. Incubate the tubes on ice for 10 min. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.

7. While the tubes are sitting on ice, label your four LB nutrient agar plates on the bottom (not the lid) as follows:

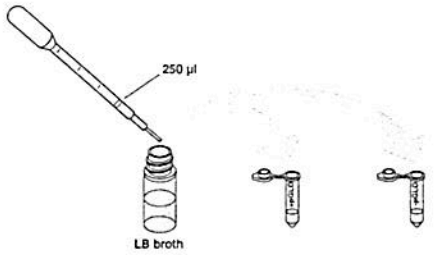
- Label one **LB/amp** plate: **+ pGLO**
- Label the **LB/amp/ara** plate: **+ pGLO**
- Label the other **LB/amp** plate: **- pGLO**
- Label the **LB** plate: **- pGLO**

8. **Heat shock.** Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42°C, **for exactly 50 sec.** Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. Double-check the temperature of the water bath with two thermometers to ensure accuracy.

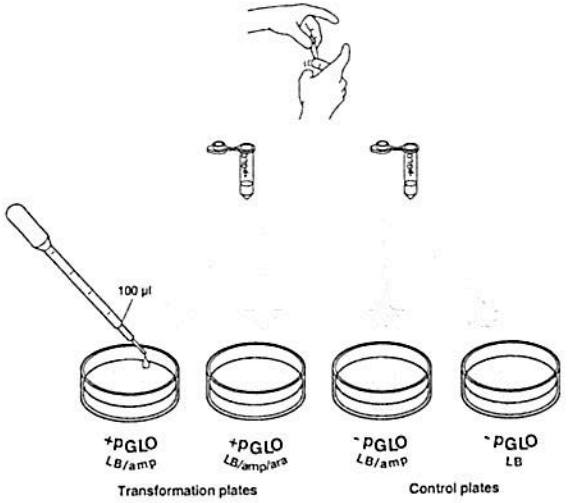
   When the 50 sec are done, place both tubes back on ice. For the best transformation results, the transfer from the ice (0°C) to 42°C and then back to the ice must be rapid. Incubate tubes on ice for 2 min.



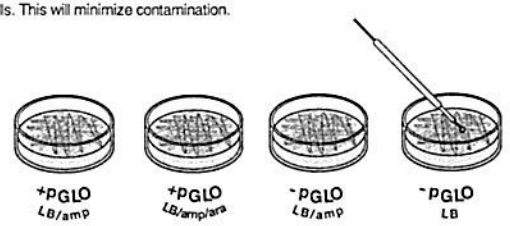
9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 min at room temperature.



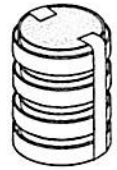
10. Gently flick the closed tubes with your finger to mix and resuspend the bacteria. Using a new sterile pipet for each tube, pipet 100 µl of the transformation and control suspensions onto the appropriate nutrient agar plates.



11. **Use a new sterile loop for each plate.** Spread the suspensions evenly around the surface of the LB nutrient agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface. DO NOT PRESS TOO DEEP INTO THE AGAR. Uncover one plate at a time and re-cover immediately after spreading the suspension of cells. This will minimize contamination.



12. Stack up your plates and tape them together. Put your group name and class period on the bottom of the stack and place the stack of plates **upside down** in the 37°C incubator until the next day. The plates are inverted to prevent condensation on the lid which may drip onto the culture and interfere with your results.



before day 2

Lesson 2 Review Questions Name _____

Before collecting data and analyzing your results answer the following questions.

1. On which of the plates would you expect to find bacteria most like the original non-transformed *E. coli* colonies you initially observed? Explain your predictions.

2. If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Explain your predictions.

3. Which plates should be compared to determine if any genetic transformation has occurred? Why?

4. What is meant by a control plate? What purpose does a control serve?

Lesson 3 Data Collection and Analysis

A. Data Collection

Observe the results you obtained from the transformation lab under normal room lighting. Then turn out the lights and hold the ultraviolet light over the plates. Alternatively the protocol can incorporate digital documentation of the plates with Vernier's Blue Digital BioImaging System (Appendix E).

1. Carefully observe and draw what you see on each of the four plates. Put your drawings in the data table below. Record your data to allow you to compare observations of the "**+ pGLO**" cells with your observations for the non-transformed *E. coli*. Write down the following observations for each plate.

2. How much bacterial growth do you see on each plate, relatively speaking?

3. What color are the bacteria?

4. How many bacterial colonies are on each plate (count the spots you see).

		Observations
Transformation plates	+pGLO LB/amp	
	+pGLO LB/amp/ara	
Control plates	-pGLO LB/amp	
	-pGLO LB	

B. Analysis of Results

The goal of data analysis for this investigation is to determine if genetic transformation has occurred.

1. Which of the traits that you originally observed for *E. coli* did not seem to become altered? In the space below list these untransformed traits and how you arrived at this analysis for each trait listed.

Original trait	Analysis of observations

2. Of the *E. coli* traits you originally noted, which seem now to be significantly different after performing the transformation procedure? List those traits below and describe the changes that you observed.

New trait	Observed change

3. If the genetically transformed cells have acquired the ability to live in the presence of the antibiotic ampicillin, then what might be inferred about the other genes on the plasmid that you used in your transformation procedure?

4. From the results that you obtained, how could you prove that the changes that occurred were due to the procedure that you performed?

Lesson 3 Review Questions Name _____

What's Glowing?

If a fluorescent green color is observed in the *E. coli* colonies then a new question might well be raised, "What are the two possible sources of fluorescence within the colonies when exposed to UV light?"

Explain:

1. Recall what you observed when you shined the UV light onto a sample of original pGLO plasmid DNA and describe your observations.

2. Which of the two possible sources of the fluorescence can now be eliminated?

3. What does this observation indicate about the source of the fluorescence?

4. Describe the evidence that indicates whether your attempt at performing a genetic transformation was successful or not successful.

STUDENT MANUAL
LESSON 3

Lesson 3 Review Questions Name _____

The Interaction between Genes and Environment

Look again at your four plates. Do you observe some *E. coli* growing on the LB plate that does not contain ampicillin or arabinose?

1. From your results, can you tell if these bacteria are ampicillin resistant by looking at them on the LB plate? Explain your answer.

2. How would you change the bacteria's environment—the plate they are growing on—to best tell if they are ampicillin resistant?

3. Very often an organism's traits are caused by a combination of its genes and its environment. Think about the green color you saw in the genetically transformed bacteria:

   a. What two factors must be present in the bacteria's environment for you to see the green color? (Hint: one factor is in the plate and the other factor is in how you look at the bacteria).

   b. What do you think each of the two environmental factors you listed above are doing to cause the genetically transformed bacteria to turn green?

   c. What advantage would there be for an organism to be able to turn on or off particular genes in response to certain conditions?

extra credit

Lesson 4 Extension Activity: Calculate Transformation Efficiency

Your next task in this investigation will be to learn how to determine the extent to which you genetically transformed *E. coli* cells. This quantitative measurement is referred to as the transformation efficiency.

In many experiments, it is important to genetically transform as many cells as possible. For example, in some types of gene therapy, cells are collected from the patient, transformed in the laboratory, and then put back into the patient. The more cells that are transformed to produce the needed protein, the more likely that the therapy will work. The transformation efficiency is calculated to help scientists determine how well the transformation is working.

The Task

You are about to calculate the transformation efficiency, which gives you an indication of how effective you were in getting DNA molecules into bacterial cells. Transformation efficiency is a number. It represents the total number of bacterial cells that express the green protein, divided by the amount of DNA used in the experiment. (It tells us the total number of bacterial cells transformed by one microgram of DNA.) The transformation efficiency is calculated using the following formula:

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the agar plate}}{\text{Amount of DNA spread on the agar plate (in µg)}}$$

Therefore, before you can calculate the efficiency of your transformation, you will need two pieces of information:

(1) The total number of green fluorescent colonies growing on your LB/amp/ara plate.

(2) The total amount of pGLO plasmid DNA in the bacterial cells spread on the LB/amp/ara plate.

STUDENT MANUAL
LESSON 4

1. Determining the Total Number of Green Fluorescent Cells

Place your LB/amp/ara plate near a UV light. Each colony on the plate can be assumed to be derived from a single cell. As individual cells reproduce, more and more cells are formed and develop into what is termed a colony. The most direct way to determine the **total number of bacteria that were transformed with the pGLO plasmid** is to count the colonies on the plate.

Enter that number here ➜

Total number of colonies = _____

2. Determining the Amount of pGLO DNA in the Bacterial Cells Spread on the LB/amp/ara Plate

We need two pieces of information to find out the amount of pGLO DNA in the bacterial cells spread on the LB/amp/ara plate in this experiment. (a) What was the total amount of DNA we began the experiment with, and (b) What fraction of the DNA (in the bacteria) actually got spread onto the LB/amp/ara plates.

Once you calculate this data, you will multiply the **total amount of pGLO DNA** used in this experiment by the **fraction of DNA** you spread on the LB/amp/ara plate. This will tell you the amount of pGLO DNA in the bacterial cells that were spread on the LB/amp/ara plate.

a. Determining the Total Amount of pGLO plasmid DNA

The total amount of DNA we began with is equal to the product of the concentration and the total volume used, or

(DNA in µg) = (concentration of DNA in µg/µl) x (volume of DNA in µl)

In this experiment you used 10 µl of pGLO at concentration of 0.08 µg/µl. This means that each microliter of solution contained 0.08 µg of pGLO DNA. Calculate the **total amount of DNA** used in this experiment.

Enter that number here ➜

Total amount of pGLO DNA (µg) used in this experiment = _____

How will you use this piece of information?

b. Determining the fraction of pGLO plasmid DNA (in the bacteria) that actually got spread onto the LB/amp/ara plate: Since not all the DNA you added to the bacterial cells will be transferred to the agar plate, you need to find out what fraction of the DNA was actually spread onto the LB/amp/ara plate. To do this, divide the volume of DNA you spread on the LB/amp/ara plate by the total volume of liquid in the test tube containing the DNA. A formula for this statement is

$$\text{Fraction of DNA used} = \frac{\text{Volume spread on LB/amp plate (µl)}}{\text{Total sample volume in test tube (µl)}}$$

You spread 100 µl of cells containing DNA from a test tube containing a total volume of 510 µl of solution. Do you remember why there is 510 µl total solution? Look in the laboratory procedure and locate all the steps where you added liquid to the reaction tube. Add the volumes.

Use the above formula to calculate the **fraction of pGLO plasmid DNA** you spread on the LB/amp/ara plate.

Enter that number here ➜

Fraction of DNA = _____

- How will you use this piece of information?

So, how many micrograms of pGLO DNA did you spread on the LB/amp/ara plates?

To answer this question, you will need to multiply the **total amount of pGLO DNA used** in this experiment by the **fraction of pGLO DNA** you spread on the LB/amp/ara plate.

pGLO DNA spread in µg = Total amount of DNA used in µg x fraction of DNA used

Enter that number here ➜

pGLO DNA spread (µg) = _____

- What will this number tell you?

Look at all your calculations above. Decide which of the numbers you calculated belong in the table below. Fill in the following table.

Number of colonies on LB/amp/ara plate =	
Micrograms of pGLO DNA spread on the plates	

Now use the data in the table to calculate the efficiency of the pGLO transformation

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the agar plate}}{\text{Amount of DNA spread on the agar plate (in µg)}}$$

Enter that number here →

Transformation efficiency = _____ transformants/µg

Analysis

Transformation efficiency calculations result in very large numbers. Scientists often use a mathematical shorthand referred to as scientific notation. For example, if the calculated transformation efficiency is 1,000 bacteria/µg of DNA, they often report this number as:

10^3 transformants/µg (10^3 is another way of saying 10 x 10 x 10 or 1,000)

- How would scientists report 10,000 transformants/µg in scientific notation?

Carrying this idea a little farther, suppose scientists calculated an efficiency of 5,000 bacteria/µg of DNA. This would be reported as:

5×10^3 transformants/µg (5 x 1,000)

- How would scientists report 40,000 transformants/µg in scientific notation?

One final example: If 2,600 transformants/µg were calculated, then the scientific notation for this number would be:

2.6×10^3 transformants/µg (2.6 x 1,000)

Similarly:

5,600 = 5.6×10^3 271,000 = 2.71×10^5 2,420,000 = 2.42×10^6

- How would scientists report 960,000 transformants/µg in scientific notation?

- Report your calculated transformation efficiency in scientific notation.

- Use a sentence or two to explain what your calculation of transformation efficiency means.

Biotechnologists are in general agreement that the transformation protocol that you have just completed generally has a transformation efficiency of between 8.0×10^2 and 7.0×10^3 transformants per microgram of DNA.

- How does your transformation efficiency compare with the above?

- In the table below, report the transformation efficiency of several of the teams in the class.

Team	Efficiency

- How does your transformation efficiency compare with theirs?

- Calculate the transformation efficiency of the following experiment using the information and the results listed below.

DNA plasmid concentration: 0.08 µg/µl

250 µl $CaCl_2$ transformation solution

10 µl pGLO plasmid solution

250 µl LB broth

100 µl cells spread on agar

227 colonies of transformants

Fill in the following chart and show your calculations to your teacher:

Number of colonies on LB/amp/ara plate =
Micrograms of DNA spread on the plates =
Transformation efficiency =

- Extra Credit Challenge:

If a particular experiment were known to have a transformation efficiency of 3×10^3 bacteria/µg of DNA, how many transformant colonies would be expected to grow on the LB/amp/ara plate? You can assume that the concentration of DNA and fraction of cells spread on the LB agar are the same as that of the pGLO laboratory.

Appendix D
Gene Regulation

Our bodies contain thousands of different proteins which perform many different jobs. Digestive enzymes are proteins; some of the hormone signals that run through our bodies and the antibodies protecting us from disease are proteins. The information for assembling a protein is carried in our DNA. The section of DNA which contains the code for making a protein is called a gene. There are over 30,000–100,000 genes in the human genome. Each gene codes for a unique protein: one gene, one protein. The gene that codes for a digestive enzyme in your mouth is different from one that codes for an antibody or the pigment that colors your eyes.

Organisms regulate expression of their genes and ultimately the amounts and kinds of proteins present within their cells for a myriad of reasons, including developmental changes, cellular specialization, and adaptation to the environment. Gene regulation not only allows for adaptation to differing conditions, but also prevents wasteful overproduction of unneeded proteins which would put the organism at a competitive disadvantage. The genes involved in the transport and breakdown (catabolism) of food are good examples of highly regulated genes. For example, the sugar arabinose is both a source of energy and a source of carbon. *E. coli* bacteria produce three enzymes (proteins) needed to digest arabinose as a food source. The genes which code for these enzymes are not expressed when arabinose is absent, but they are expressed when arabinose is present in their environment. How is this so?

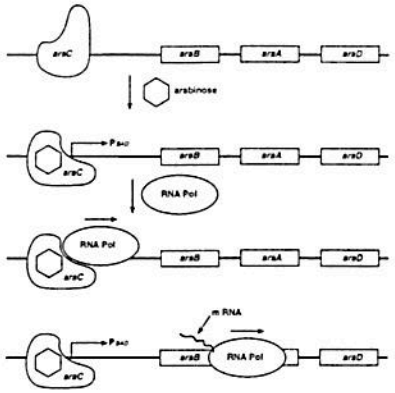
Regulation of the expression of proteins often occurs at the level of transcription from DNA into RNA. This regulation takes place at a very specific location on the DNA template, called a promoter, where RNA polymerase sits down on the DNA and begins transcription of the gene. In bacteria, groups of related genes are often clustered together and transcribed into RNA from one promoter. These clusters of genes controlled by a single promoter are called operons.

The three genes (*araB, araA* and *araD*) that code for three digestive enzymes involved in the breakdown of arabinose are clustered together in what is known as the arabinose operon.[3] These three proteins are dependent on initiation of transcription from a single promoter, P_{BAD}. Transcription of these three genes requires the simultaneous presence of the DNA template (promoter and operon), RNA polymerase, a DNA binding protein called *araC* and arabinose. *araC* binds to the DNA at the binding site for the RNA polymerase (the beginning of the arabinose operon). When arabinose is present in the environment, bacteria take it up. Once inside, the arabinose interacts directly with *araC* which is bound to the DNA. The interaction causes *araC* to change its shape which in turn promotes (actually helps) the binding of RNA polymerase and the three genes *araB, A* and *D*, are transcribed. Three enzymes are produced, they break down arabinose, and eventually the arabinose runs out. In the absence of arabinose the *araC* returns to its original shape and transcription is shut off.

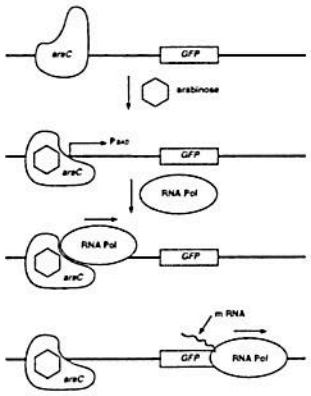
The DNA code of the pGLO plasmid has been engineered to incorporate aspects of the arabinose operon. Both the promoter (P_{BAD}) and the *araC* gene are present. However, the genes which code for arabinose catabolism, *araB, A* and *D*, have been replaced by the single gene which codes for GFP. Therefore, in the presence of arabinose, *araC* protein promotes the binding of RNA polymerase and GFP is produced. Cells fluoresce brilliant green as they produce more and more GFP. In the absence of arabinose, *araC* no longer facilitates the binding of RNA polymerase and the GFP gene is not transcribed. When GFP is not made, bacteria colonies will appear to have a wild-type (natural) phenotype—of white colonies with no fluorescence.

This is an excellent example of the central molecular framework of biology in action:
DNA→RNA→PROTEIN→TRAIT.

The Arabinose Operon



Expression of Green Fluorescent Protein



APPENDIX D