pGLO Transformation

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 or the correct wavelength of blue 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 blue 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.

Consideration 1: 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 and answer the following questions in your lab notebooks:

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

b) Color of the colonies

c) Distribution of the colonies on the plate

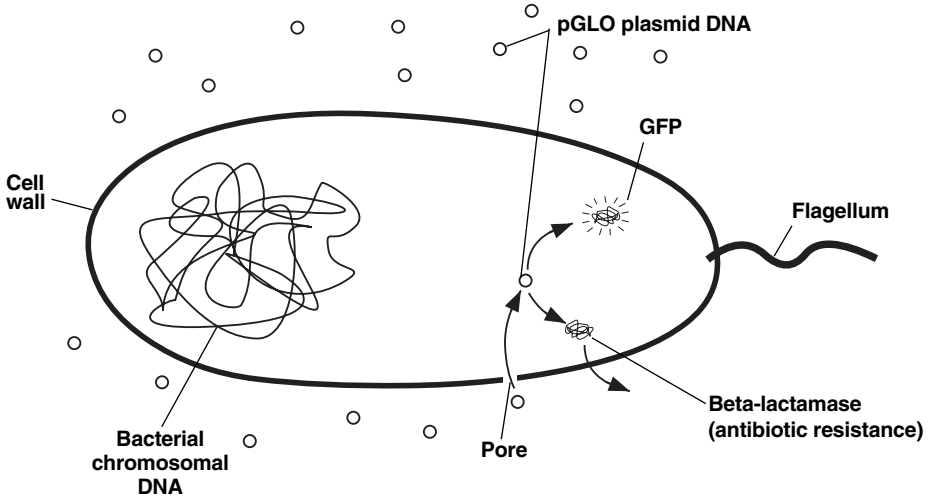
d) Visible appearance when viewed with blue light box

1. Describe in your notebooks 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 2: 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 3: 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**.

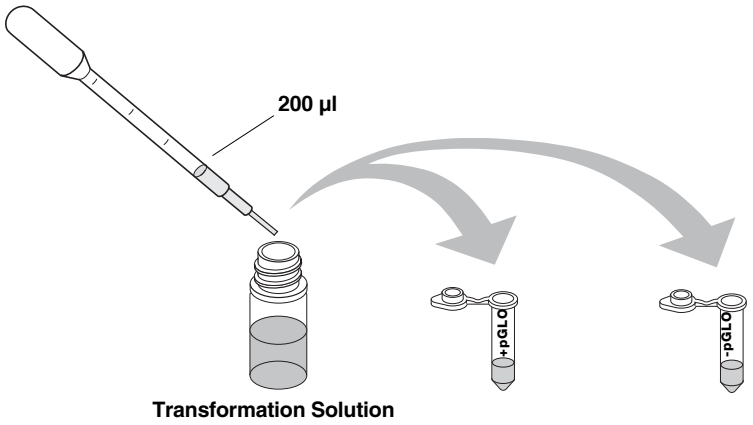
For transformed cells to grow in the presence of ampicillin you must:

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

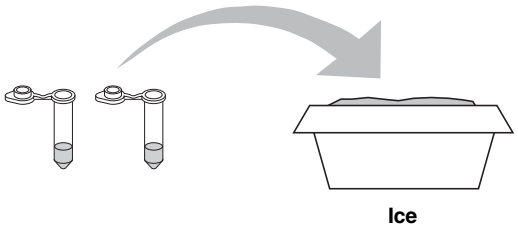
Transformation Procedure

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

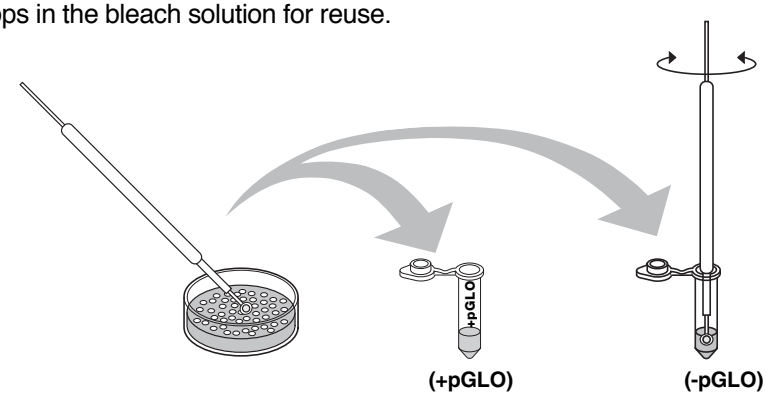
2. Open the tubes and transfer 200 µl of transformation solution (CaCl2) into each tube, making sure to use sterile tips.



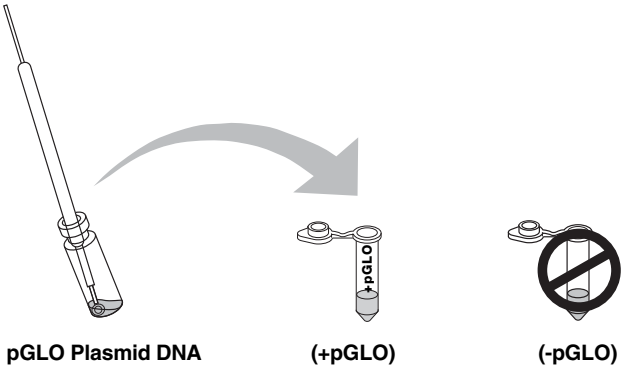
3. Place the tubes on ice.



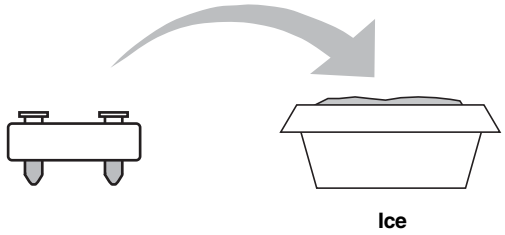
4. Use a sterile loop to pick up **2–4 large colonies of bacteria** from your starter plate. Select starter colonies that are "fat" (ie: 1–2 mm in diameter). It is important to take individual colonies (not a swab of bacteria from the dense portion of the plate), since the bacteria must be actively growing 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 and close the tube. Place used loops in the bleach solution for reuse.



5. Examine the pGLO DNA solution with the blue light imaging stations. Note your observations. Pipet 7 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 ice so the bottom of the tubes make significant contact with the ice.

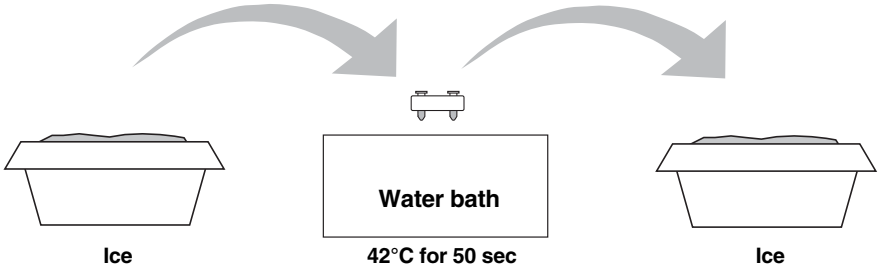


7. While the tubes are sitting on ice, label your four LB nutrient agar plates on the bottom edges (not the lid) as follows:

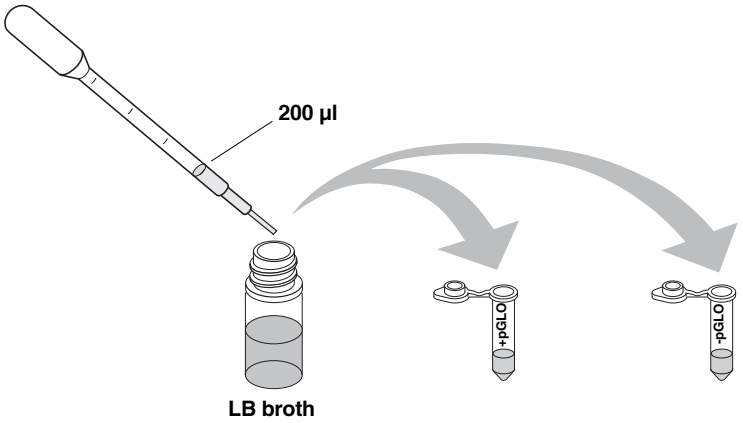
- Label one **LB/amp** plate: **+ pGLO**
- Label the **LB/amp/ara** plate: **+ pGLO**
- Label the other **LB/amp** plate: **- pGLO**
- Label the **LB** plate: **- pGLO**

8. **Heat shock.** Bring your tubes **on ice** to the water bath. Transfer both the (+) pGLO and (-) pGLO tubes into the water bath set at 42ºC, **for exactly 50 sec**.

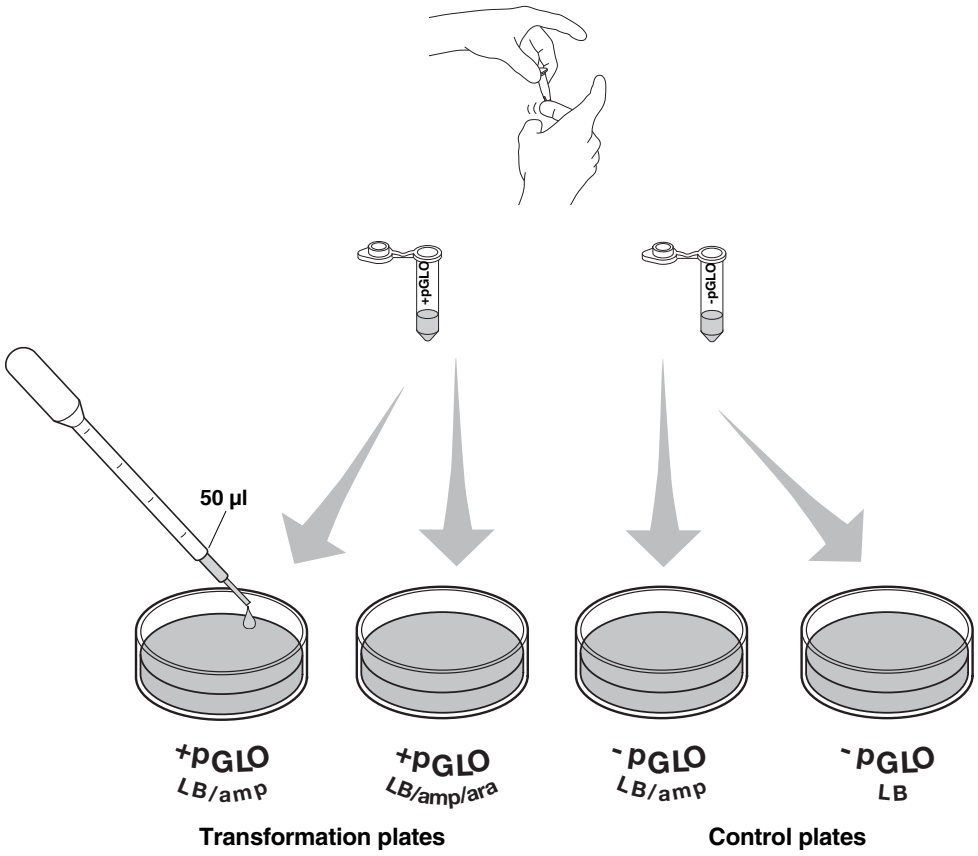
   When the 50 sec are done, place both tubes immediately back on ice. For the best transformation results, the transfer from the ice (0°C) to 42°C and then back to the ice must be rapid.
   Incubate tubes on ice for 2 min.



9. Remove the tubes from the ice and place in a rack on the bench top.
   Open a tube and, using a new sterile pipet tip, add 200 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet tip for the other tube. Incubate the tubes for 1 hour at 37º C.

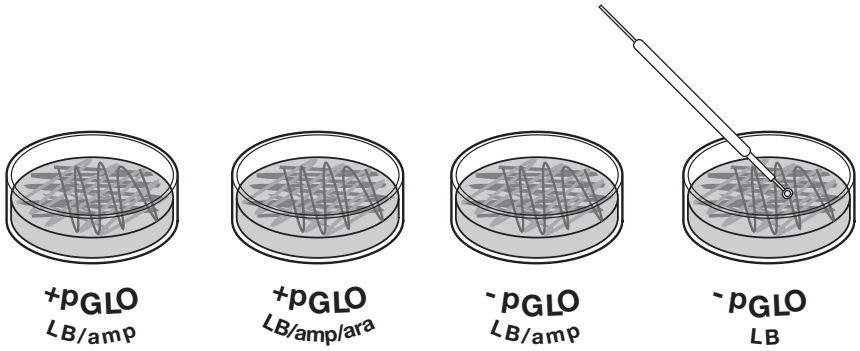


10. Gently flick the closed tubes with your finger to mix and resuspend the bacteria. Using a new sterile pipet tip for each tube, pipet 50 µl of the transformation and control suspensions onto the appropriate nutrient agar plates.

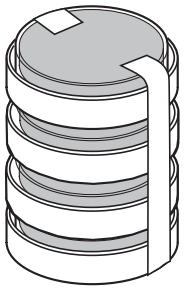


11. **Use a new sterile loop for each solution (**one loop for + pGLO and a different loop for - pGLO).

    Spread the suspensions evenly around thesurface 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. Allow the liquid to sink in for a few minutes before inverting and stacking.



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.



Review Questions

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

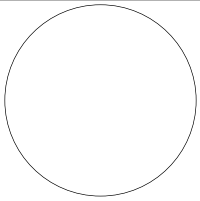
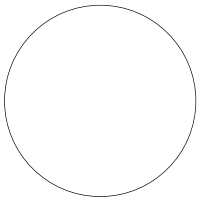
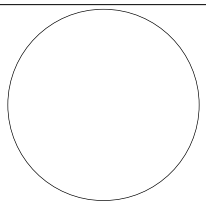
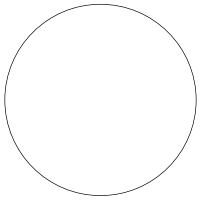
1. On which of the plates would you expect to find bacteria most like the original non-transformed *E. coli* colonies you initially observed? Explain your predictions.
2. If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Explain your predictions.
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?

Data Collection and Analysis

A. Data Collection

After one day, observe the results you obtained from the transformation lab first under normal room lighting. Then use the blue light imaging station and "dark room" box with your phones to view and take pictures of each of your plates.

1. Carefully observe and draw what you see on each of the four plates. Put your drawings in a data table like the one below in your lab notebooks. 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 in your notebooks.
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 or say "lawn" if the bacteria are growing continuously across the plate).

		Observations
Transformation plates	+pGLO LB/amp	
	+pGLO LB/amp/ara	
		Observations
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 your lab notebooks list these untransformed traits and how you arrived at this analysis for each trait listed.

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

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?

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 blue light?

1. Recall what you observed when you shined the blue 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.

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?