Student Manual
pGLO Transformation

Lesson 1 Introduction to Transformation

In this lab you will perform a procedure known as genetic transformation. Genetic transformation literally means “change caused by genes,” and involves the insertion of a gene into an organismin 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 medicine, diseases caused by defective genes are beginning to be treated by gene therapy; that is, by geneticallytransforming 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 successful 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 occurrence of bacterial resistance to antibiotics is due to the transmission of plasmids.

The pGLO plasmid you will use in this experiment encodes the gene for GFP and a gene for resistance to the antibiotic ampicillin. pGLO also incorporates a special gene regulation system, which canbe 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’ environment. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on ampicillin plates. Bacteria that are not transformed will be killed by the ampicillin. 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.

Your task will be to:

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

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 takeon 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? Why?

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? Why?

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* on your starter plate will provide baseline data to make reference to when attempting to determine if any genetic transformation has occurred.

a) Number of colonies:

b) Color of the colonies:

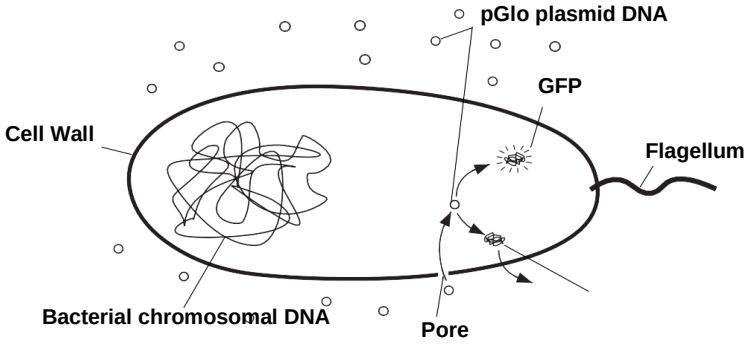
c) Visible appearance when viewed with ultraviolet (UV) light

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 <u>results</u> to show 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 this case, the pGLO plasmid has been genetically engineered to carry the GFP gene which codes for the green fluorescent protein and a gene (*bla*) that codes for a protein that gives the bacteria resistance to the antibiotic ampicillin.The genetically engineered plasmid can then be used to 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 present at your work station

Student workstation

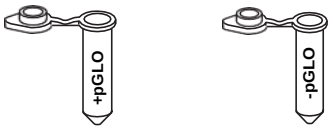
Material	Quantity
E. coli starter plate	1
Poured agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)	4
Transformation solution	1
LB nutrient broth	1
Inoculation loops	7 (1 pk of 10)
Pipets	5
Foam microcentrifuge tube holder/float	1
Container (such as foam cup) full of crushed ice (not cubed ice)	1
Marking pen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

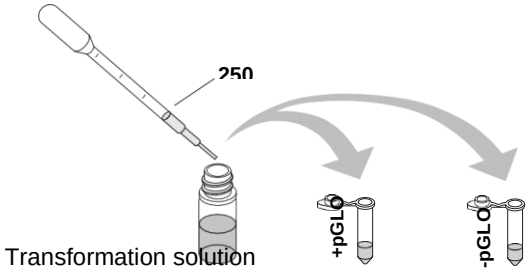
Material	Quantity
Rehydrated pGLO plasmid	1 vial
42°C water bath and thermometer	1
UV Light	1
37°C incubator	1
(optional, see General Laboratory Skills–Incubation)	
2–20 µl adjustable volume micropipets	1
2–20 µl micropipet tips	1

Transformation Procedure

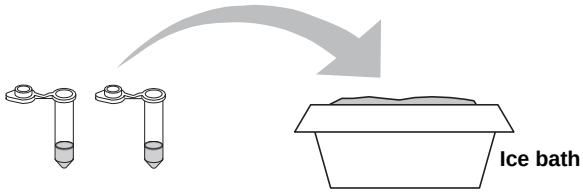
1. Label one closed micro test tube **+pGLO** and another **-pGLO**. Label both tubes withyour group's initials. Place them in the foam tube rack.



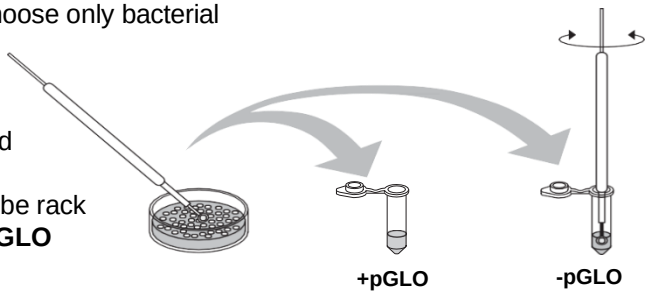
2. Open the tubes and, using a sterile transfer pipet, transfer 250 µl of transformationsolution ($CaCl_2$) into each tube.



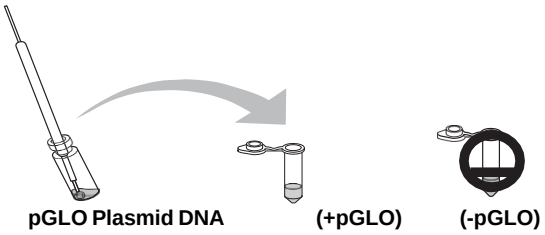
3. Place the tubes on ice.



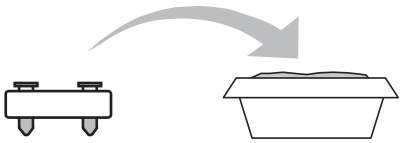
4. Use a sterile loop to pick up **2–4 large colonies of bacteria** from your starter plate. Select starter colonies that are "fat". It is important to take individual colonies, since the bacteria must be actively growing to achieve high transformation efficiency. Choose only bacterial colonies that 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. Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the **-pGLO** tube.



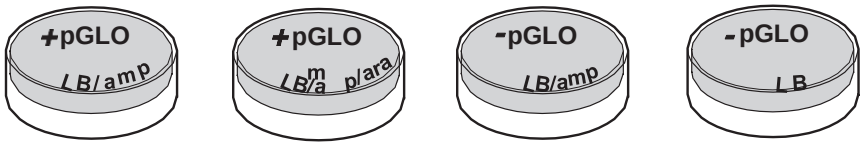
5. Examine the pGLO DNA solution with the UV lamp. Note your observations. Immerse a **new sterile loop** into the pGLO plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. Mix the loopful into the cell suspension of the **+pGLO** tube. **Do not** add plasmid DNA to the **-pGLO** tube. Close both the **+pGLO** and **-pGLO** tubes and return them to the rack on ice.



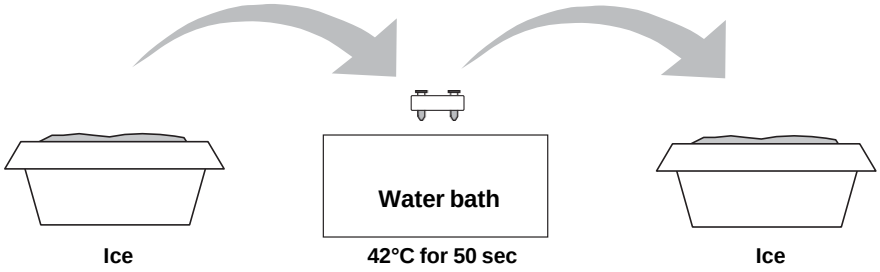
6. Incubate the tubes on ice for 10 min. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.



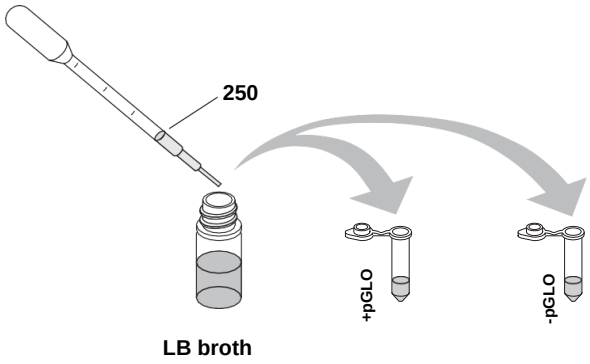
7. While the tubes are sitting on ice, label your four LB nutrient agar plates on the bottom(not the lid) as follows:
    - Label one **LB/amp** plate: **+ pGLO**
    - Label the **LB/amp/ara** plate: **+ pGLO**
    - Label the other **LB/amp** plate: **- pGLO**
    - Label the **LB** plate: **- pGLO**



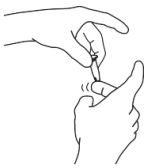
8. **Heat shock.** Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42°C, for **exactly** 50 sec. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. Double-check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

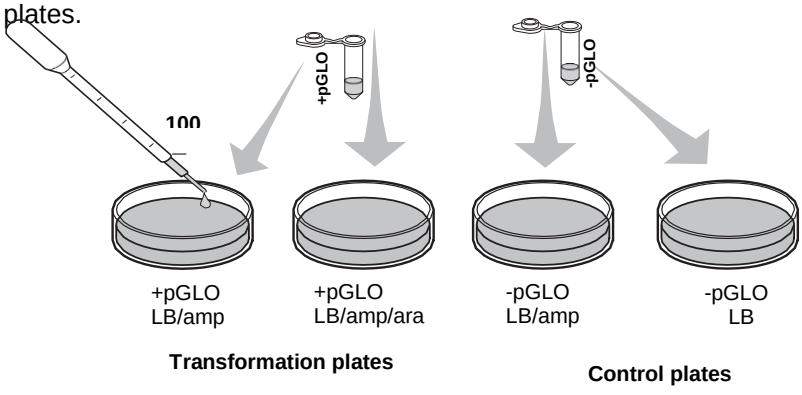


9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate thetubes for 10 min at room temperature.

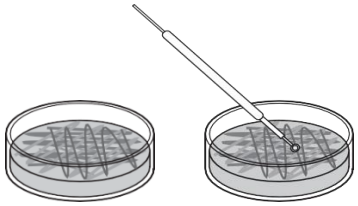


10. Gently flick the closed tubes with your finger to mix and resuspend the bacteria. Using a new sterile pipet for each tube, pipet 100 µl of the transformation and control suspensionsonto the appropriate nutrient agar plates.

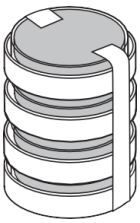




11. **Use a new sterile loop for each plate**. Spread the suspensions evenly around the surface of the LB nutrient agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface. DO NOT PRESS TOO DEEP INTO THE AGAR.Uncover one plate at a time and re-cover immediately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



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



Lesson 2 Questions

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, Analysis and Conclusion

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. Carefully observe and draw what you see on each of the four plates. Put your drawingsin 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 thefollowing observations for each plate.

1. How much bacterial growth do you see on each plate, relatively speaking?
2. What color are the bacteria?
3. 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	

Analyze

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

1. From the results that you obtained, describe the evidence that indicates whether your attempt at performing a genetic transformation was successful or not successful.

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

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

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

4. 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?

5. Transformation efficiency:
Your next task 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.

You will calculate the transformation efficiency, which gives you an indication of how effective you were in getting DNA molecules into bacterial cells. (It tells us the total number of bacterial cells transformed by one microgram of DNA.) The transformation efficiency is calculated using the following formula:

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

Do the calculation and report the results in scientific notation –

Total # of colonies on the LB/Amp/Ara plate	
Micrograms (µg) of pGlo DNA spread on the plate	0.16
Transformation efficiency:	

6. 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?

Conclude:

7. To genetically transform an entire organism, you must insert a new gene into every cell in the organism. Based on this, discuss what characteristics make the *E.coli* bacteria a good choice for genetic transformation compared with some other multicellular organism.

8. From your results in this experiment, how could you prove that the changes that occurred were due to the procedure you performed?

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