pGLO™ Bacterial Transformation

Introduction to Transformation

In this lab, you will perform a procedure known as genetic transformation. Genetic transformation literally means "change caused by genes", and occurs when the cell incorporates and expresses a new piece of genetic material – DNA derived from another organism. Transformation involves the insertion of a gene into an organism in order to alter the recipient organism's expression. 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 and breakdown pollutants such as oil spills or heavy metals contamination In medicine, disorders caused by defective genes are being treated by gene therapy; that is, by genetically transforming a sick person's cells with healthy copies of the defective gene.

Your Protein of Interest - The Green Fluorescent Protein, GFP

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*. Following the transformation procedure, the bacteria will express their newly acquired gene and produce GFP, which causes them to glow a brilliant green color under ultraviolet or blue light.

The Host Cell - Escherichia coli (*E. coli*)

The bacterium, *E. coli*, is the ideal host for transformation because it is a small, single-celled organism that reproduces quickly, so its transformation will be seen rather quickly. Also, the strain of *E. coli* being used is nonpathogenic, does not make people or animals sick, and it does not survive outside the laboratory environment. Although it is safe, it requires the use of Standard Microbiological Practices, as directed by your instructor.

The Plasmid Vector - A Means of Gene Delivery

A plasmid will be used to transfer the GFP gene into the bacteria. A plasmid is a small circular piece of DNA that is capable of self-replicating. In addition to one large chromosome, many bacteria naturally contain one or more 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 bacterial transmission of plasmids.

In this activity, the pGLO plasmid will be used. The pGLO plasmid is unique because not only does it encode the gene for GFP, but it also encodes a gene for resistance to the antibiotic ampicillin, and a special gene regulation system, which is used to control expression of GFP in the transformed bacteria.

Antibiotic Selection

The gene, which codes for antibiotic resistance, produces the protein beta-lactamase. Normally bacteria cannot survive in the presence of antibiotics, such as ampicillin. However, the beta-lactamase protein inactivates the ampicillin present in the agar environment of the bacteria

allowing it to survive. Only transformed bacteria that contain the pGLO plasmid and express beta-lactamase can survive on agar plates containing ampicillin. You will observe that a small percentage of bacterial cells take up the plasmid DNA and are transformed. As untransformed cells cannot grow in the presence of ampicillin, we will use this as a selection method to calculate transformation efficiencies and determine the extent to which the *E. coli* cells were transformed.

Gene Regulation

As previously mentioned, the pGLO plasmid codes for a special gene regulation system which controls the expression of GFP in transformed bacteria. The gene regulation system is called the arabinose operon. In nature, this operon contains the machinery and three genes that code for three digestive enzymes involved in the breakdown of the plant sugar, arabinose, which is a food source for the bacteria. When arabinose is present, the genes are expressed to digest the sugar; when arabinose is not present, the bacteria do not express these digestive enzymes because they are not necessary. This design allows the bacteria to quickly adapt to its environment and to use its resources wisely.

In this activity, the pGLO plasmid has been designed with a modified arabinose operon. The three genes for the digestive enzymes have been replaced with the gfp gene which produces GFP. Therefore, in the presence of arabinose, the bacterial cells which have been transformed by the pGLO plasmid will fluoresce (when exposed to UV or blue light) because of the production of GFP. When GFP is not made, the bacterial colonies will appear whitish.

OBJECTIVES

In this experiment, you will

- Use a plasmid vector to transform bacteria with genes for Green Fluorescent Protein (GFP) and antibiotic resistance in a controlled experiment.
- Use the heat shock method of transforming *E. coli*.
- Regulate the expression of the GFP gene using arabinose.
- Describe the biological process involved in transforming bacterial cells.
- Calculate your transformation efficiency.
- Learn basic molecular biology techniques.

MATERIALS

E. coli starter plate	5 sterile DPTP pipets
4 agar plates (1LB, 2 LB/amp, 1 LB/amp/ara)	7 inoculation loops
transformation solution	foam microtube holder/float
LB nutrient broth	37ºC incubator
rehydrated pGLO plasmid DNA	42ºC water bath and thermometer
UV lamp, handheld or BlueView™ Transilluminator	cup of crushed ice
2 microcentrifuge tubes	marking pen

PRE-LAB QUESTIONS

1. Define bacterial transformation.

2. In this experiment, what particular type of DNA vector is used to transfer the GFP gene into *E. coli*?

3. How is GFP expression induced or "switched on" during the transformation?

4. To genetically transform an entire organism, you must insert the new gene(s) into every cell in the organism. Which organism is better suited for total genetic transformation: a single-cell or multi-cell organism? Explain your answer.

5. To learn if a genetically transformed organism can pass its new traits to its offspring and future generations, which would be a better candidate for your investigation: an organism in which each generation quickly develops and reproduces or one that does so slowly?

PROCEDURE

The pGLO Bacterial Transformation Kit—Quick Guide	
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 crushed ice.	
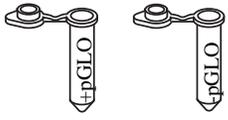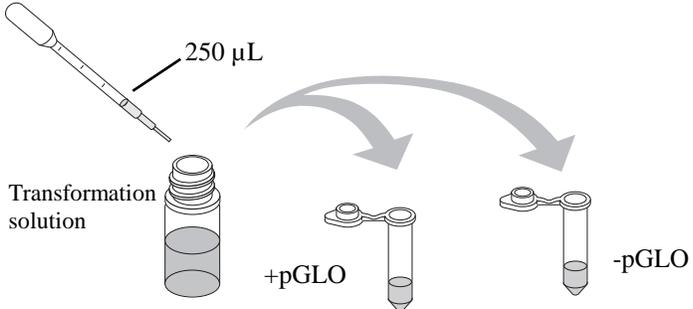
4. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (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.	 +pGLO -pGLO
5. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the 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 DNA. Close the tube and return it to the face on ice. Also close the -pGLO tube. Do not add plasmid DNA to the -pGLO tube. Why not?	 Plasmid DNA +pGLO -pGLO
6. Incubate the tubes on ice for 10 minutes. 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.	 Rack Ice
7. While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as indicated on the diagram.	 +pGLO +pGLO -pGLO -pGLO LB/amp LB/amp/ara LB/amp LB
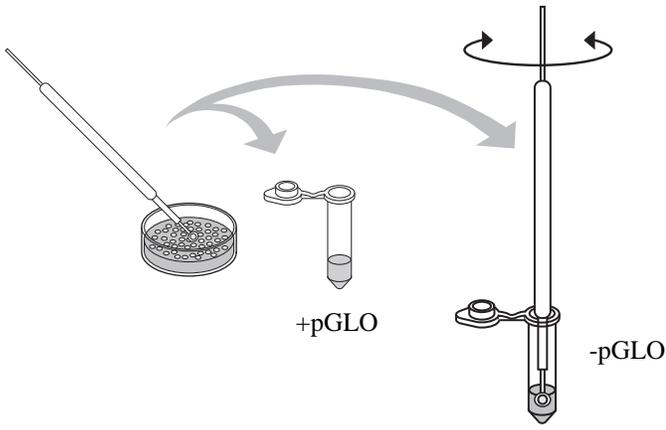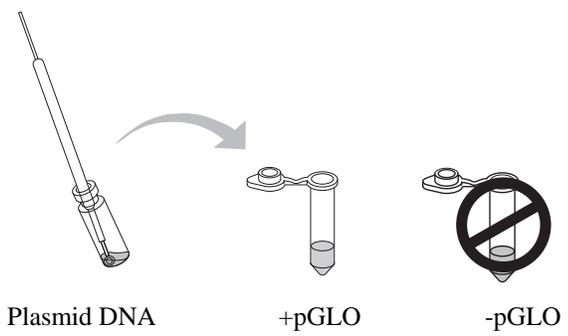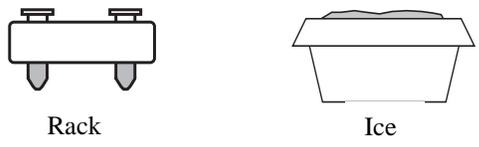
8. Heat shock. Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42°C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds are up, place both tubes back on ice. For the best transformation results, the change from the ice (0°C) to 42°C and then back to the ice must be rapid. Incubate tubes on ice for two minutes.	Water bath Ice 42°C for 50 seconds Ice
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 close it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 min at room temperature.	250 µL LB-Broth +pGLO -pGLO
10. Tap the closed tubes with your finger to mix. Use a new sterile pipet for each tube and pipet 100 µL of the transformation and control suspensions onto the appropriate plates.	+pGLO LB/amp +pGLO LB/amp/ara -pGLO LB/amp -pGLO LB
11. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.	
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 upside down in the 37°C incubator until the next day.	
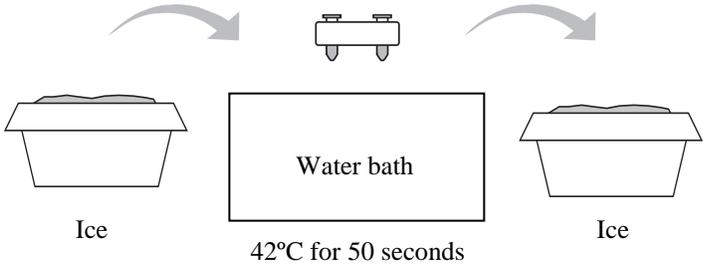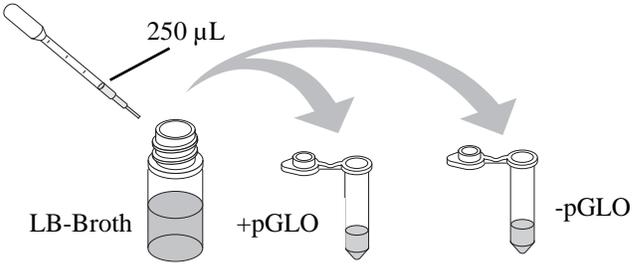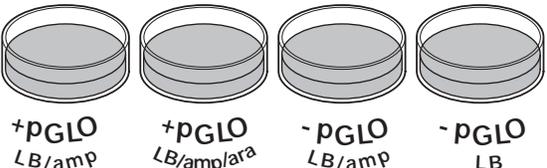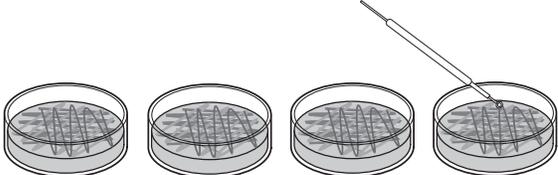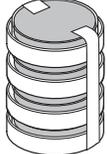
ANALYSIS

Capturing an Image of the Transformation

1. View the LB/amp and LB/amp/ara plates either with a handheld UV lamp or place them on the BlueView™ Transilluminator to view the fluorescent green colonies. Photograph them either with a camera or a Vernier ProScope.

Calculation of Transformation Efficiency

2. Based on your observations above, note the number of colonies and their color on each plate:

   a. LB (-pGLO):
   b. LB/amp (-pGLO):
   c. LB/amp (+pGLO):
   d. LB/amp/ara (+pGLO):

3. Count the total number of green fluorescent colonies and enter here: _____

4. Did you transform bacteria? State your evidence.

5. Calculate the transformation efficiency in the table below:

Calculating Transformation Efficiency			
Description	Formula	Sample calculation	Your calculations
Number of Transformed Colonies (transformants)	Count directly from LB/amp/ara plate	100	
Mass (µg) of plasmid used	Volume of plasmid (µl) x Concentration (µg/µl)	10 µl x 0.08 µg/µl = 0.8 µg	
Fraction of plasmid that was plated	Volume spread on plate (µl)/Total volume in microtube (µl)	100 µl/510 µl = 0.196	
Mass of plasmid plated (µg)	Mass of plasmid used (µg) x Fraction of plasmid plated	0.8 µg x 0.196 = 0.156 µg	
Transformation Efficiency (transformants/µg plasmid)	Number of transformants/µg of plasmid	100/0.156 µg = 641 transformants/µg plasmid	

QUESTIONS

1. In this experiment, which plates are control plates and what purpose does each control plate serve?

2. If the genetically transformed bacteria have acquired the ability to live in the presence of ampicillin, then what can be inferred about the other genes on the plasmid that were involved in the transformation?

3. Very often an organism's traits are determined 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 order for you to see the green color? Hint: one factor is in the plate, the other is in how you look at the bacteria.
   b. What is the advantage to having this type of gene regulation?

Vernier Lab Safety Instructions Disclaimer

This copy does not include:

- **Safety information**

- **Essential instructor background information**

- **Directions for preparing solutions**

- **Important tips for successfully doing these labs**

The complete *Advanced Biology with Vernier* lab manual includes 27 labs and essential teacher information. The full lab book is available for purchase at:
http://www.vernier.com/cmat/bioa.html