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 that 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**. It 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 tolerance, pest resistance or protection from spoilage 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. In this application an ill person's cells are transformed replacing copies of the disease gene with a healthy version.

You will use a procedure to transform bacteria with a gene that codes for Green Fluorescent Protein (GPF). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*. GFP causes the jellyfish to fluoresce and glow in the dark. Following the transformation process, the bacteria will 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 especially important for the bacterium to survive.

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 is due to the transmission of plasmids

Bio-Rad's 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. The gene for GFP can be switched on in transformed cells by adding the sugar arabinose to the cells' nutrient medium. Cells that have been transformed with pGLO DNA can grow on Petri plates that contain ampicillin.

Transformed cells colonies will appear white when grown on plates not containing arabinose and will fluoresce green under UV light when grown on plates containing arabinose.

General Laboratory Skills

Sterile technique

Working with and culturing bacteria requires procedures that eliminate the possibility of introducing contaminating bacteria into the experiment. Bacteria are found everywhere. The round circle at the end of the inoculation loop, the tip of the pipet, and the surface of the Petri plate should not be touched or placed onto contaminating surfaces.

Use of the pipet

Before beginning the lab, look at the graduations on the pipette. Both the 100 and 250 microliter as well as the 1 milliliter marks will be used as units of measurement throughout the lab.

Working with *E. coli*

The host organism in this kit, an *E. coli* K-12 strain, the vector containing recombinant GFP, and the subsequent transformants created by their combination are not pathogenic organisms like the *E. coli* 0157:H7 strain that has been in the news. However, handing requires the use of Standard Microbiological Practices. These practices include:

- decontaminating surfaces once a day and after any spill
- decontaminating liquid or solid wastes before disposal
- hand-washing after handing materials containing recombinant DNA materials and before leaving the laboratory
- wearing protective eyewear and gloves
- using mechanical pipetting only
- prohibition of eating, drinking, smoking or applying cosmetics in work area.

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

Decontamination and disposal

Loops, pipets and petri plates should be placed in the biohazard bag to be autoclaved.

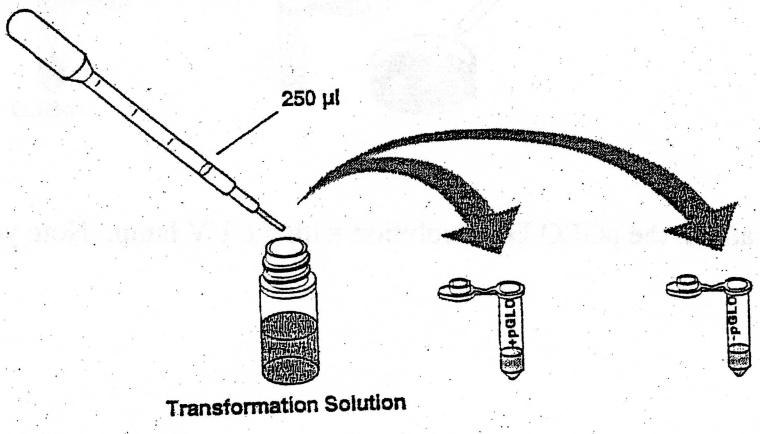
Decontaminate the tables by washing with disinfectant or soap and water. Wash your hands before leaving the classroom.

The Act of Transformation

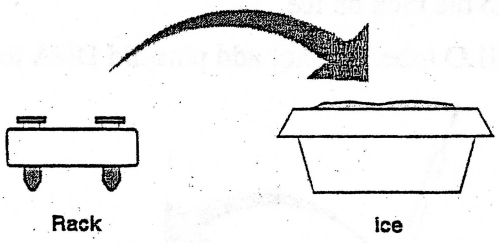
The two main steps are moving the pGLO plasmid through the cell membrane and providing the transformed cells with nutrients and an incubation period so they can express their new genes.

1. Label one closed micro test tube +pGLO and another –pGLO. Label both tubes with your name. Place them in the foam rack.

2. Open the tubes and, using a sterile transfer pipet, transfer 250 microliters of transformation solution ($CaCl_2$) into each tube. $CaCl_2$ neutralizes the negative charges of the phosphate backbone of DNA and the negative charge of the phospholipids in the bacterial cell's membrane allowing the DNA to enter.



3. Place the tubes on ice for storage.



4. Transferring the bacteria

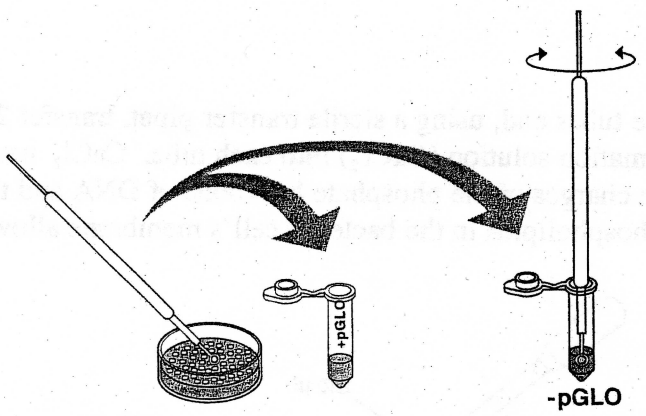
   a. Use a sterile loop to pick up a single colony of bacteria (it's about 1 mm in diameter and contains millions of bacterial cells. Note the appearance of the growth on this plate.

   b. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube.

   c. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution with no floating chunks.

   d. Place the tube back in the foam rack and in the ice for storage.

   e. Using a new sterile loop, repeat for the –pGLO tube.

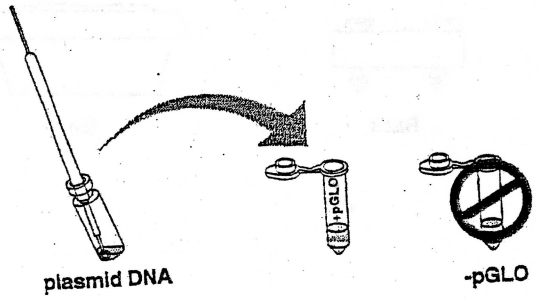


5. Examine the pGLO DNA solution with the UV lamp. Note your observations.

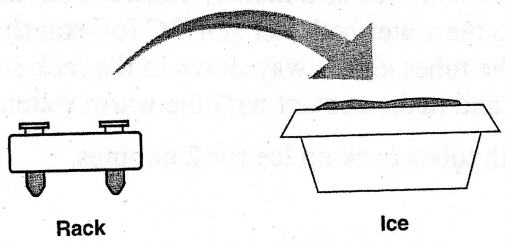
6. Immerse a new sterile loop into the pGLO plasmid DNA stock. Withdraw a loopful. There should be a film of plasmid solution across the ring similar to a soapy film across a ring for blowing soap bubbles.

7. Mix the loopful into the cell suspension of the +pGLO tube. Close the tube and return it to the rack on ice.

8. Close the –pGLO tube. Do not add plasmid DNA to the –pGLO tube. Why not?

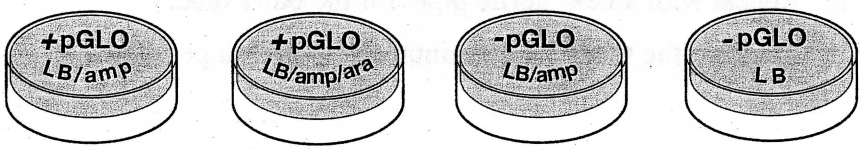


9. Incubate the tubes on ice for 10 minutes. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.



10. While the tubes are sitting on ice, label your four LB nutrient agar plates on the **bottom** as follows: Be careful to obtain the correct plates and label them correctly!

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



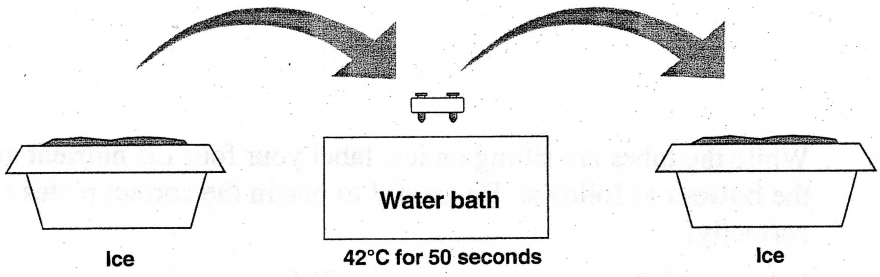
LB refers to Luria and Bertani, two important genetic researchers. The nutrients in the plate are composed of yeast, meat byproducts (containing carbohydrates, amino acids, nucleotides, salt, and vitamins) suspended in agar which is a seaweed gel.

11. Heat shock

For the best transformation results, the transfer from the ice (0 degrees C) to 42 degrees C and back to the ice must be rapid.

a. 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.

b. Place both tubes back on ice for 2 minutes.

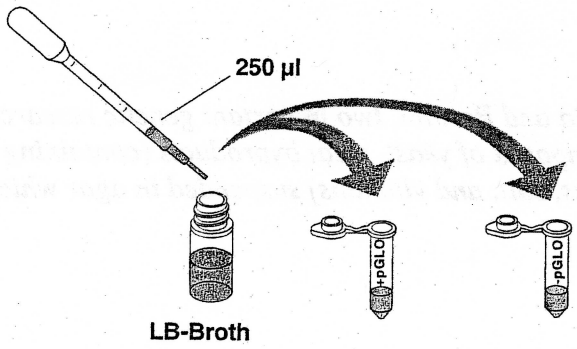


12. Remove the rack containing the tubes from the ice and place on the bench top.

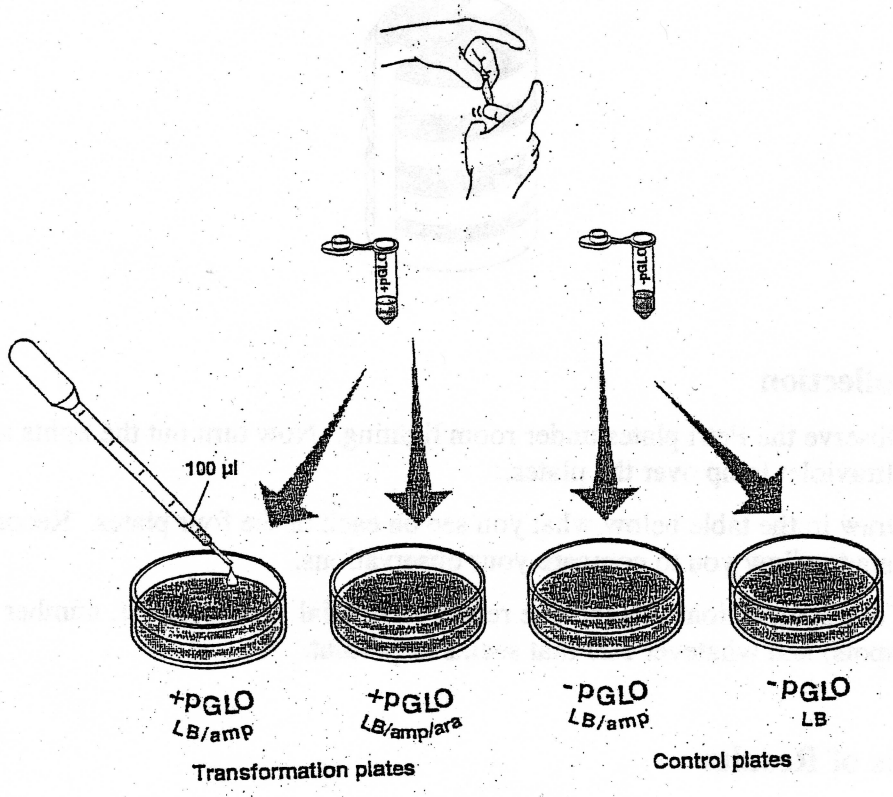
a. Open a tube and, using a new sterile pipet, add 250 microliters of LB nutrient broth to the tube and close it.

b. Repeat with a new sterile pipet for the other tube.

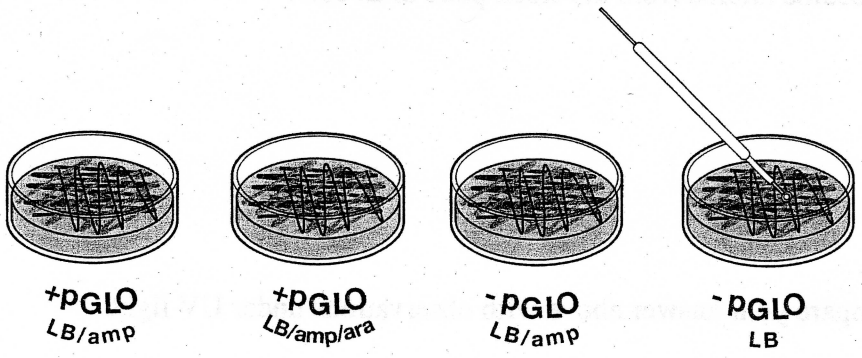
c. Incubate the tubes for 10 minutes at room temperature.



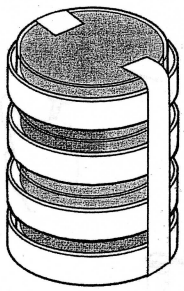
13. Tap the closed tubes with your finger to mix. Using a new sterile pipet for each tube, pipette 100 microliters of the transformation and control suspensions onto the appropriate nutrient agar plates. Be sure to match the +pGLO and –pGLO tubes and plates.



14. 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 deeply into the agar.**



15. Stack up your plates and tape them together. Put your name on the bottom of the stack and place them upside down in the 37 degree C incubator until the next day.



Data Collection

1. Observe the Petri plates under room lighting. Now turn out the lights and hold the ultraviolet lamp over the plates.

2. Draw in the table below what you see on each of the four plates. Record your data to allow you to compare your observations.

   Your observations can include relative bacterial growth, color, number of colonies (spots) and whatever else that seems important.

Analysis of Results

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

1. Which of the traits that you observed for *E. coli* under room lighting did not seem to become altered from the stock plate of *E. coli*?

2. Compare your answer above with observations under UV light.

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?

5. What's glowing?

   What are the two possible sources of fluorescence within the colonies when exposed to UV light?

   a.

   b.

Review Questions

1. What is a plasmid?

2. Why is it a useful biological tool?

3. Describe recombinant gene technology and give an example of a therapy that uses it.

Workstation Checklist

Student workstations. Materials and supplies that should be present at each student workstation prior to beginning the lab experiments are listed below. The components provided in this kit are sufficient for 8 complete student workstations.

Instructor's (common) workstation. A list of materials, supplies and equipment that should be present at a common location accessible by all student groups is also listed below. It is up to the discretion of the teacher as to whether students should access common buffer solutions and equipment, or whether the teacher should aliquot solutions in the microtubes provided.

Lesson 2 Transformation Lab

Student workstations	Number required	(✔)
E. coli starter plate (LB)	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 microtube holder/float	1	❑
Containers full of crushed ice (foam cup)	1	❑
Marking pen	1	❑
Instructor's (common) workstation		
Rehydrated pGLO plasmid DNA	1 vial	❑
42°C water bath and thermometer	1	❑
37°C incubator (optional, see General Laboratory Skills–Incubation)	1	❑