Lab 3 – Transformation of Bacteria with pGLO

Introduction

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 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.

The **pGLO plasmid** encodes the gene for GFP **and** a gene called ***bla*** 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. Arabinose initiates transcription of these genes by promoting the binding of RNA polymerase. In the genetically engineered pGLO plasmid DNA, some of the genes involved in the breakdown of arabinose have been replaced by the jellyfish gene that codes for GFP. When bacteria that have been transformed with pGLO plasmid DNA are grown in the presence of arabinose, the GFP gene is turned on and the bacteria glow brilliant green when exposed to UV light. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on antibiotic plates containing ampicillin. Transformed cells will appear white (wild-type phenotype) on plates not containing arabinose, and fluorescent green when arabinose is included in the nutrient agar medium.

Purpose:
To learn and understand the implications of genetic transformation.

Materials

- *E. coli* starter plate (2 per class)
- Agar plates (1 LB, 2 LB/Amp, & 1 LB/Amp/ara)
- 50 mM $CaCl_2$ (calcium chloride) transformation solution
- pGlo plasmid (at teacher's desk)
- LB nutrient broth
- **Inoculation loop**
- Ethanol for sterilization
- Sterile Eppendorf tubes from instructor
- Micropipettes
- Micropipette tips
- Ice
- 42^oC heat block
- 37^oC incubator
- Bunsen burner and striker

Procedure – Week 1 – Transformation

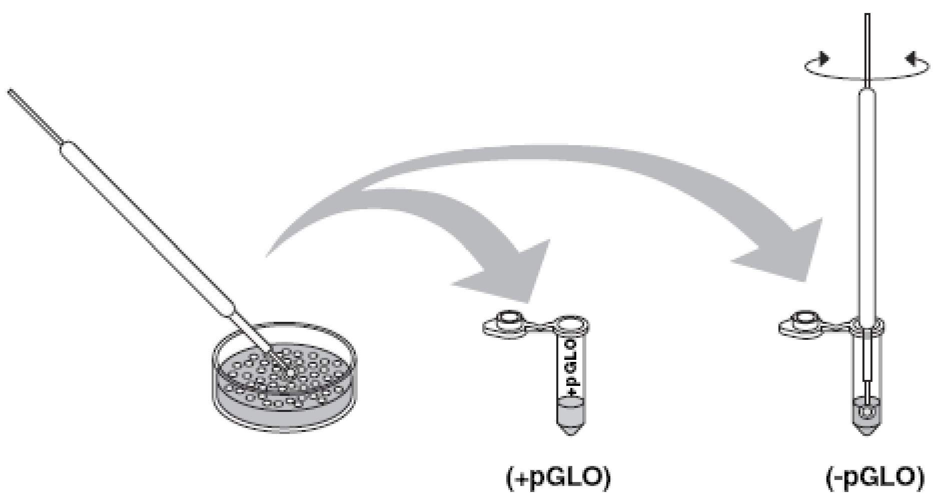
1. Divide into groups of no more than 4 people.

2. Label one sterile **closed** Eppendor tube **+pGLO** and another **-pGLO**. Label both tubes with your group's name. Place them in the Eppendorf tube rack.

3. Open the tubes and transfer 250 μL of the $CaCl_2$ transformation solution into each tube.

4. Place the tubes on ice.

5. Sterilize the inoculation loop as demonstrated by the instructor. Use this 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.



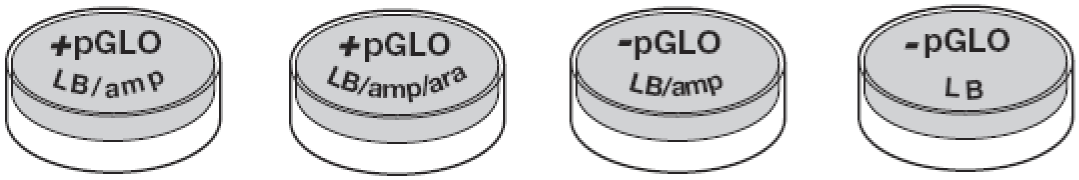
6. Sterilize the inoculation loop as demonstrated by the instructor and repeat Step #4 for the **-pGLO** tube.

7. Using a sterile pipette tip, add 1 μL of the pGLO plasmid DNA to the cell suspension of the **+pGLO** tube. Close the tube and return it to the ice. **Do not add plasmid DNA to the -pGLO tube**. Leave the closed **-pGLO** tube on ice.

8. Incubate both tubes on ice for 10 minutes.

9. While the tubes are sitting on ice, label your four LB nutrient agar plates on the **bottom edge** (not the lid) as follows (do not remove the lid as you are risking contamination of your nutrient plates):

    - 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**



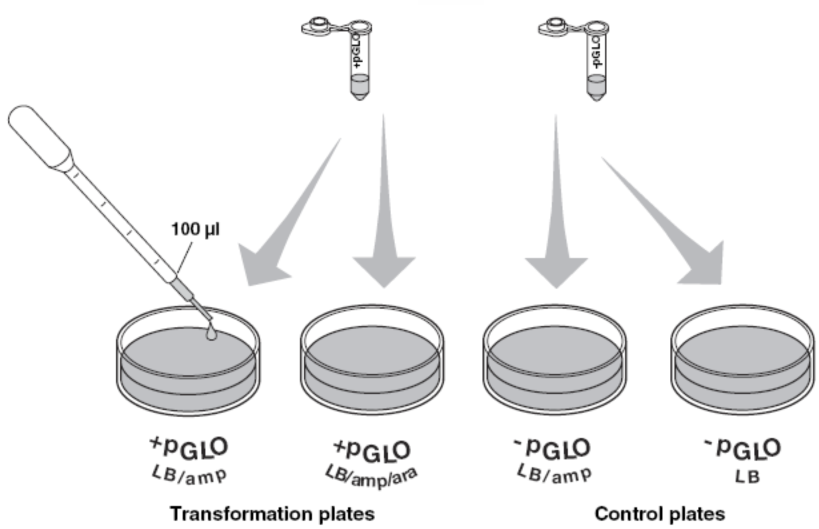
10. **Heat shock.** Transfer both the **+pGLO** and **-pGLO** tubes into the heat block set at 42°C for **exactly** 50 seconds.

11. When the 50 seconds 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 the tubes on ice for 2 minutes.

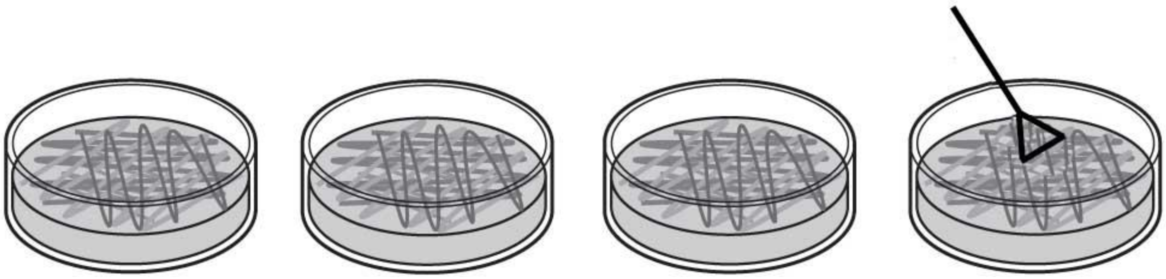
12. Transfer the tubes from the ice into an Eppendorf rack on the bench top. Open the **+pGLO** tube and add 250 µL of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the **-pGLO** tube. Incubate the tubes for 10 minutes at room temperature.

13. After the incubation at room temperature, tap the closed tubes with your finger to mix.

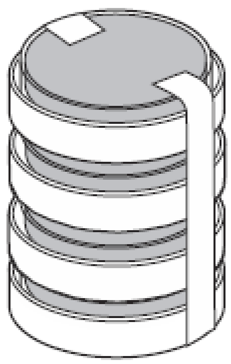
14. Using a new sterile pipette for each tube, pipette 100 µL of the transformation and control suspensions onto the appropriate nutrient agar plates.



15. Using a sterilized spreader that has been cooled in the agar on the side of the plate, spread the suspensions evenly around the surface of the LB nutrient agar by quickly skating the flat surface of the loop back and forth across the plate surface (see diagram below). **DO NOT PRESS TOO DEEP INTO THE AGAR.**



16. Stack up your plates and tape them together. Put your group name and class section on the bottom of the stack and place the stack of plates **upside down** in the 37°C incubator.



Procedure – Week 2 – Data Collection and Analysis

Data Collection

1. Your plates were sealed with Parafilm and stored at 4°C.

2. Obtain your plates from last class. Observe the results you obtained from the transformation lab under normal room lighting.

3. Then set your plates on the UV light box in the gel doc station. Carefully observe and draw what you see on each of the four plates in your lab notebook. Use the template below. Write down the following observations for each plate:

   - How much bacterial growth do you see on each plate, relatively speaking?
   - What color are the bacteria?
   - How many bacterial colonies are on each plate (count the spots you see).

Analysis of Results

The goal of data analysis for this investigation is to determine if genetic transformation has occurred. Answer the following questions in your lab notebook.

1. On which of your LB plates do you expect to find no bacterial colonies? What is this plate a control for?

2. If you **only** obtained colonies on the LB only –pGLO plate, what do you think might have gone wrong?

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

References

1. Biotechnology Explorer™ pGLO™ Bacterial Transformation Kit from Bio-Rad (Cat. No. 166-0003EDU)