Name ______________________________ Date ____________ Period ___

AP Lab: *pGLO* Bacterial Transformation

Introduction

In this laboratory, you will perform a procedure known as ***genetic transformation***. Genetic transformation occurs when a cell takes up (takes inside) and expresses a piece of genetic material—DNA. This new genetic information often provides the organism with a new trait, which is identified after transformation. Genetic transformation involves the insertion of one or more genes into an organism in order to change the organism's traits.

Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or drought 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.

Genes can be cut out of human, animal, or plant DNA and placed inside bacteria. For example, a healthy human gene for the hormone, insulin, can be put into bacteria. Under the right conditions, these bacteria can make authentic human insulin. This insulin can be used to treat patients with the genetic disease, diabetes, because their insulin genes do not function properly.

This ***pGLO transformation*** procedure will transform bacteria with a gene that codes for ***Green Fluorescent Protein (GFP)***. The real source of this gene is the bioluminescent jellyfish *Aequorea victoria*, and GFP causes the jellyfish to fluoresce and glow in the dark. After transformation, the bacteria should glow a brilliant green color under ultraviolet light. You will be moving the GFP gene with the aid of a ***plasmid***. In addition to one large chromosome, bacteria contain one or more small circular pieces of DNA called plasmids.

The pGLO plasmid also has a gene for resistance to the antibiotic Ampicillin. Bacteria that have not been transformed should not be able to grow in the presence of Ampicillin. The sugar ***arabinose (ara)*** is an energy source and source of carbon for bacteria. It initiates transcription of genes to make enzymes to break down arabinose for food. In the genetically engineered pGLO plasmid DNA, some of the genes involved in the breakdown of arabinose will be replaced by the jellyfish gene that codes for GFP. When bacteria that have been transformed are grown in the presence of arabinose, the bacteria will glow under ultraviolet light.

Objectives:

- To perform a procedure known as genetic transformation.
- To understand how plasmids are used in bacterial transformation.
- To understand the relationship between genes and proteins (DNA→RNA→Proteins→Traits).
- To understand method of using selection plates to determine if transformation has occurred.

Materials (per group of 4 students)

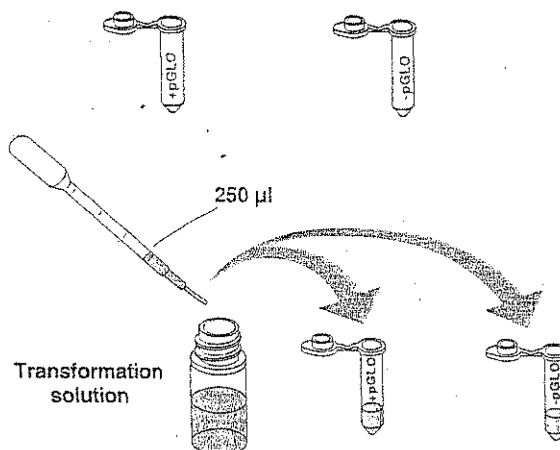
E.coli starter plate (LB)
1 transformation solution
7 inoculation loops
1 foam microtube holder/float
marking pens

4 agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)
1 LB nutrient broth
5 pipets
1 foam cup full of crushed ice
masking tape

Procedure (see the quick guide below)

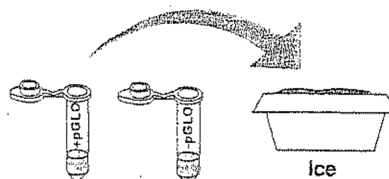
Transformation Kit—Quick Guide

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.

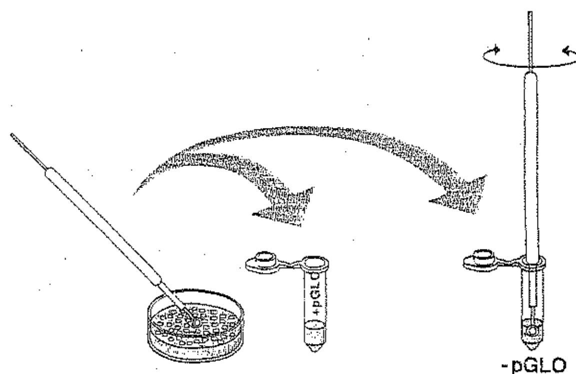


2. Open the tubes and using a sterile transfer pipet, transfer 250 µl of transformation solution ($CaCl_2$) into each tube.

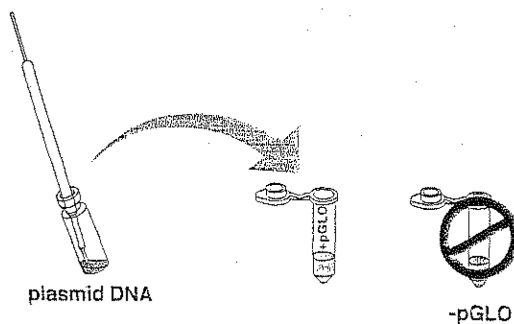
3. Place the tubes on crushed ice. Do not use cubed ice.



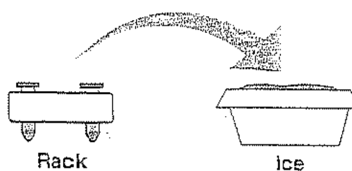
4. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (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.



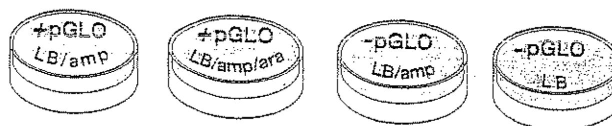
5. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the +pGLO tube. Optionally, pipet 10 µl of pGLO plasmid into the +pGLO tube and mix. Close the -pGLO tube and return it to the rack on ice. Do not add plasmid DNA to the -pGLO tube. Why not? Close the -pGLGO tube and return it to the rack on ice.



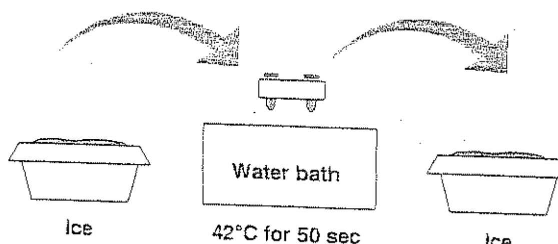
6. Incubate the tubes on ice for 10 min. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.



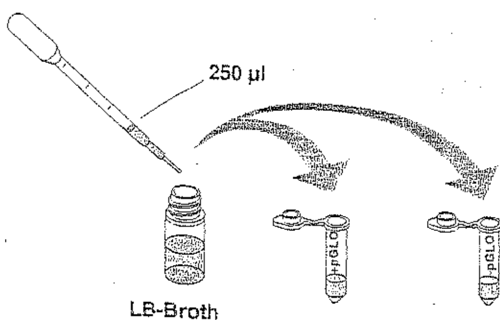
7. While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as shown on the diagram.



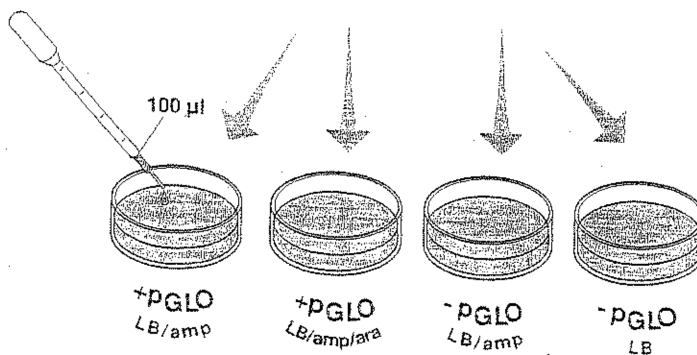
8. Heat shock. Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42°C, for **exactly** 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds have passed, 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 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 the tubes for 10 min at room temperature.



10. Gently flick the closed tubes with your finger to mix. Using a **new sterile pipet for each tube**, pipet 100 µl from each of the tubes to the corresponding plates, as shown on the diagram onto the appropriate plates.



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.

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AP Lab: pGLO Bacterial Transformation
~~Post~~ Lab Evaluation (20 points)
PRE

1. Discuss why bacteria are the best organisms to use for DNA transformation. (2 pts)

2. What do the designations amp and ara indicate and what were their functions in this experiment? (4 points)

3. What two genes does the pGLO plasmid contain that is required for this experiment? (2 points)

4. What was the purpose of the Heat Shock? (2 points)

5. What were the control plates? What plates were they compared to and why? (2 points)

6. On which plate did you not find any bacterial growth? Why? (2 points)

7. Compare your results on the +pGLO, LB/amp plate and the +pGLO, LB/amp/ara plate. Why did bacteria grow on both plates but only fluoresce on the +pGLO, LB/amp/ara plate? (3 points)

8. What is this type of biotechnology useful for commercially (give at least 3 uses)? (3 points)