

# Student Manual

## pGLO Transformation

### Lesson 1 Introduction to Transformation

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 (codes for) 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.

Bio-Rad's unique 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 in transformed cells. The gene for GFP can be switched on in transformed cells by adding the sugar arabinose to the cells' nutrient medium. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on antibiotic plates. 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.

You will be provided with the tools and a protocol for performing genetic transformation.

Your task will be:

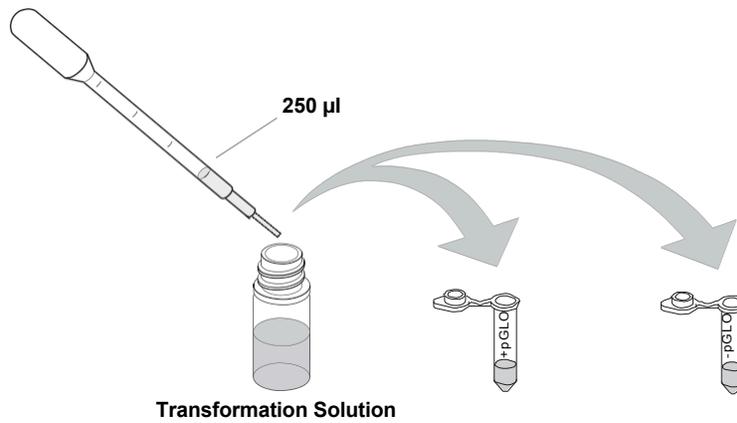
1. To do the genetic transformation.
2. To determine the degree of success in your efforts to genetically alter an organism.

### Transformation Procedure

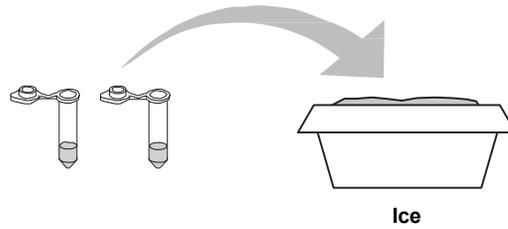
1. Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.



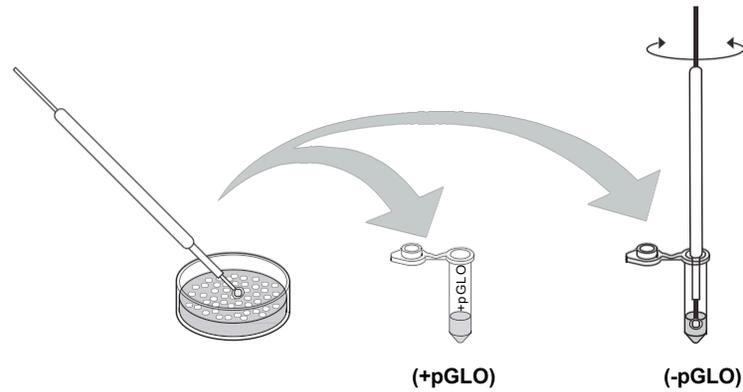
2. Open the tubes and, using a sterile transfer pipet, transfer 250  $\mu$ l of transformation solution ( $\text{CaCl}_2$ ) into each tube.



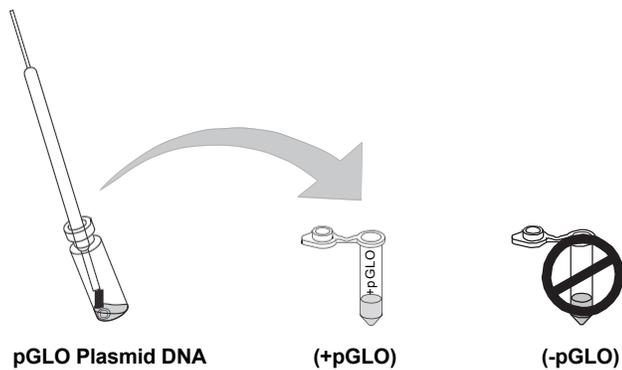
3. Place the tubes on ice.



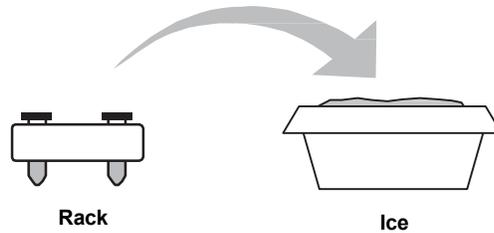
4. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (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 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. 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. Close the tube and return it to the rack on ice. Also close the -pGLO tube. Do not add plasmid DNA to the -pGLO tube. Why not?



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.



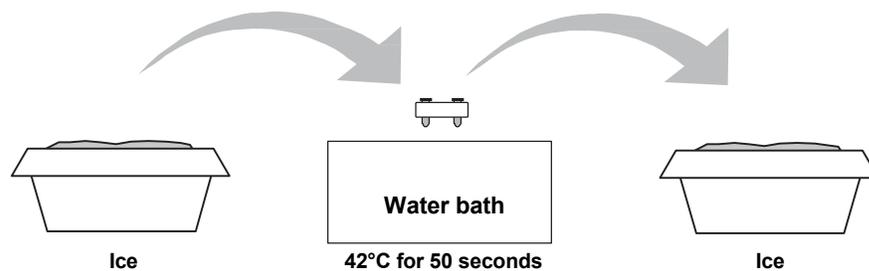
7. While the tubes are sitting on ice, label your four LB nutrient agar plates on the bottom (not the lid) as follows:

- 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

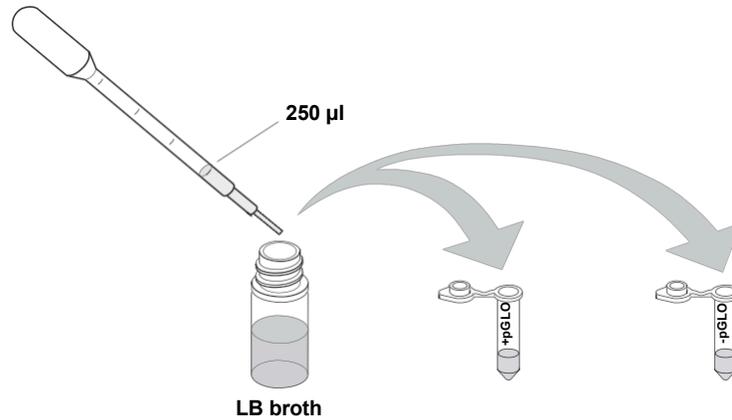


8. Heat shock. Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42°C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water.

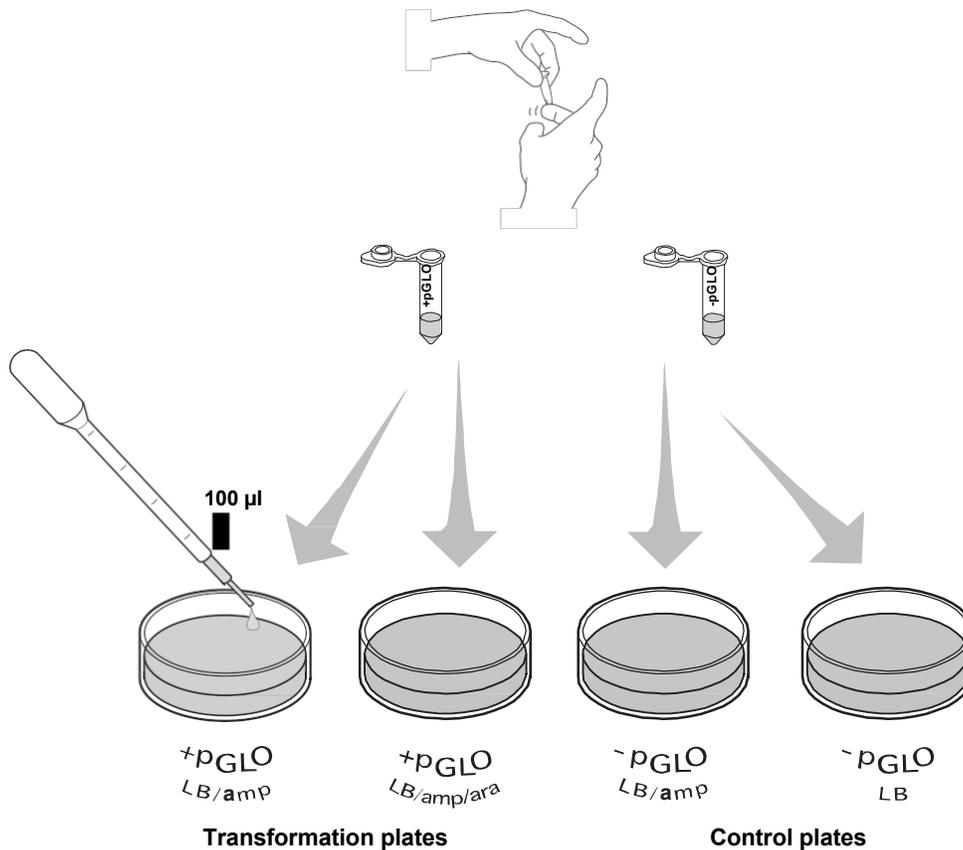
When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the transfer from the ice (0°C) to 42°C and then back to the ice must be rapid. Incubate tubes on ice for 2 minutes.



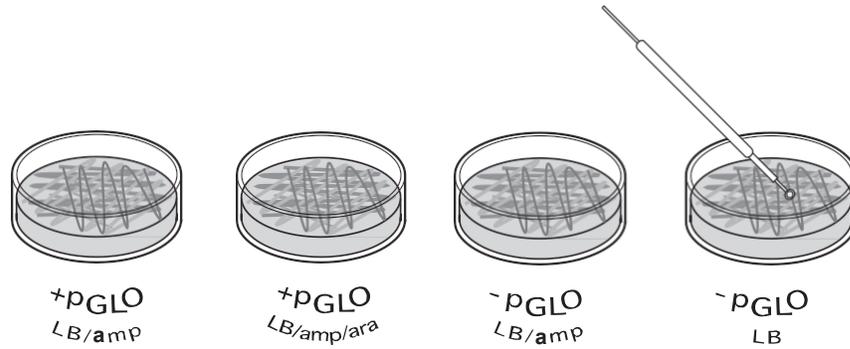
9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250  $\mu$ l of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 minutes at room temperature.



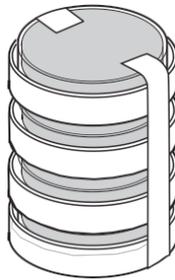
10. Tap the closed tubes with your finger to mix. Using a new sterile pipet for each tube, pipet 100  $\mu$ l of the transformation and control suspensions onto the appropriate nutrient agar plates.



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.



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 of plates upside down in the 37°C incubator until the next day.



## Appendix D Gene Regulation

Our bodies contain thousands of different proteins which perform many different jobs. Digestive enzymes are proteins; some of the hormone signals that run through our bodies and the antibodies protecting us from disease are proteins. The information for assembling a protein is carried in our DNA. The section of DNA which contains the code for making a protein is called a gene. There are over 30,000–100,000 genes in the human genome. Each gene codes for a unique protein: one gene, one protein. The gene that codes for a digestive enzyme in your mouth is different from one that codes for an antibody or the pigment that colors your eyes.

Organisms regulate expression of their genes and ultimately the amounts and kinds of proteins present within their cells for a myriad of reasons, including developmental changes, cellular specialization, and adaptation to the environment. Gene regulation not only allows for adaptation to differing conditions, but also prevents wasteful overproduction of unneeded proteins which would put the organism at a competitive disadvantage. The genes involved in the transport and breakdown (catabolism) of food are good examples of highly regulated genes. For example, the sugar arabinose is both a source of energy and a source of carbon. *E. coli* bacteria produce three enzymes (proteins) needed to digest arabinose as a food source. The genes which code for these enzymes are not expressed when arabinose is absent, but they are expressed when arabinose is present in their environment. How is this so?

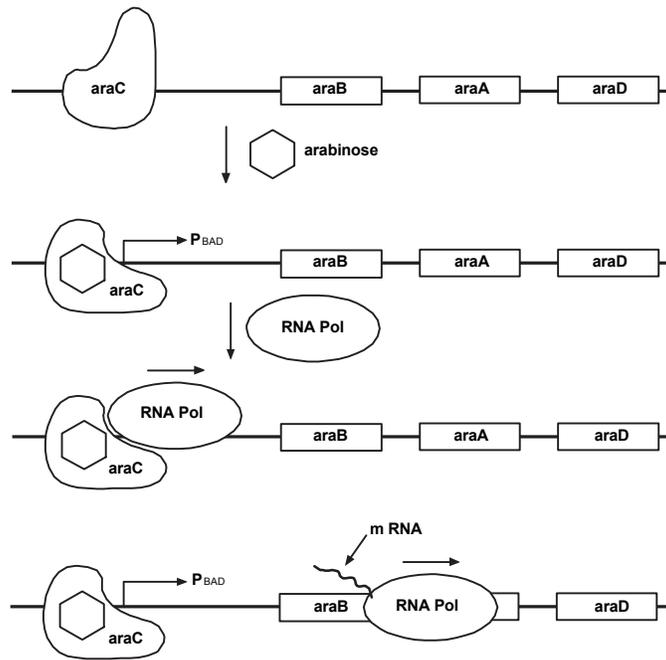
Regulation of the expression of proteins often occurs at the level of transcription from DNA into RNA. This regulation takes place at a very specific location on the DNA template, called a promoter, where RNA polymerase sits down on the DNA and begins transcription of the gene. In bacteria, groups of related genes are often clustered together and transcribed into RNA from one promoter. These clusters of genes controlled by a single promoter are called operons.

The three genes (*araB*, *araA* and *araD*) that code for three digestive enzymes involved in the breakdown of arabinose are clustered together in what is known as the arabinose operon.<sup>3</sup> These three proteins are dependent on initiation of transcription from a single promoter,  $P_{BAD}$ . Transcription of these three genes requires the simultaneous presence of the DNA template (promoter and operon), RNA polymerase, a DNA binding protein called *araC* and arabinose. *araC* binds to the DNA at the binding site for the RNA polymerase (the beginning of the arabinose operon). When arabinose is present in the environment, bacteria take it up. Once inside, the arabinose interacts directly with *araC* which is bound to the DNA. The interaction causes *araC* to change its shape which in turn promotes (actually helps) the binding of RNA polymerase and the three genes *araB*, *A* and *D*, are transcribed. Three enzymes are produced, they break down arabinose, and eventually the arabinose runs out. In the absence of arabinose the *araC* returns to its original shape and transcription is shut off.

The DNA code of the pGLO plasmid has been engineered to incorporate aspects of the arabinose operon. Both the promoter ( $P_{BAD}$ ) and the *araC* gene are present. However, the genes which code for arabinose catabolism, *araB*, *A* and *D*, have been replaced by the single gene which codes for GFP. Therefore, in the presence of arabinose, *araC* protein promotes the binding of RNA polymerase and GFP is produced. Cells fluoresce brilliant green as they produce more and more GFP. In the absence of arabinose, *araC* no longer facilitates the binding of RNA polymerase and the GFP gene is not transcribed. When GFP is not made, bacteria colonies will appear to have a wild-type (natural) phenotype—of white colonies with no fluorescence.

This is an excellent example of the central molecular framework of biology in action:  
DNA→RNA→PROTEIN→TRAIT.

## The Arabinose Operon



## Expression of Green Fluorescent Protein

