

# 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 (GFP). 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* O157:H7 strain that has been in the news. However, handling 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 handling 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?

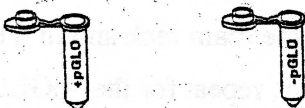
### **Decontamination and disposal**

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.

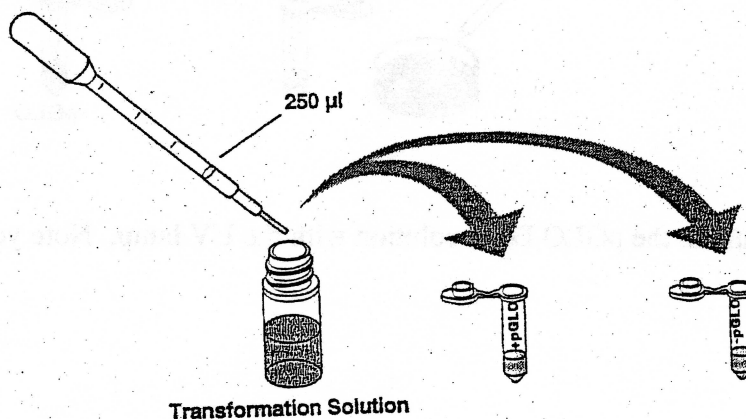
## The Act of Transformation

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. Place them in the foam rack.



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

