

Student Manual

pGLO Transformation

Lesson 1 Introduction to Transformation

In this lab you will perform a procedure known as a genetic transformation. Remember that a gene is a piece of DNA which provides the instructions for making (coding for) a protein which gives an organism a particular trait. Genetic transformation literally means "change caused by genes" and it involves the insertion of a gene (s) into an organism in order to change the organism's trait (s). 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 bio-remediation, 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 gene involved in their disease.

You will use a procedure to transform bacteria with a gene that codes for a Green Fluorescent Protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*. The gene codes for a Green Fluorescent Protein which 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 "rise" of bacterial resistance to antibiotics is due to the transmission of plasmids.

Bio-Rad's unique pGLO plasmid encodes the gene for the Green Fluorescent Protein (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 the Green Fluorescent Protein 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.

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.

Pglo Transformation Student Manual

**Warren Dolphin, David Vleck, Linda
Westgate, James Colbert**



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