From the BioRad pGLO instruction manual

Introduction to Transformation

In this lab, your students will perform a procedure known as genetic transformation. Genetic transformation occurs when a cell takes up (takes inside) and expresses a new piece of genetic material—DNA. This new genetic information often provides the organism with a new trait which is identifiable after transformation. Genetic transformation literally means change caused by genes and involves the insertion of one or more gene(s) 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 their 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 then be used to treat patients with the genetic disease, diabetes, because their insulin genes do not function normally.

The pGLO System

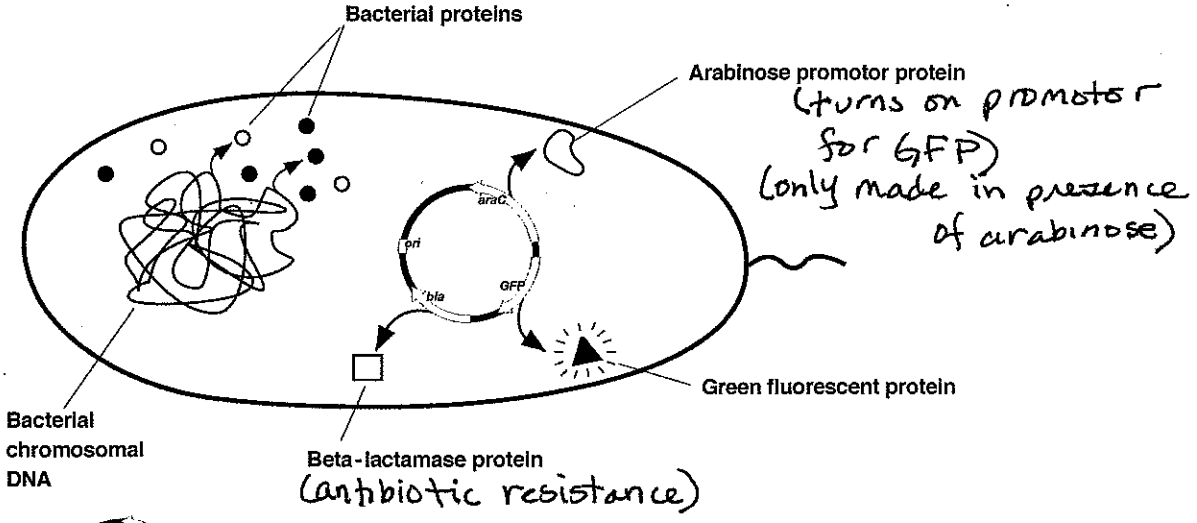
With the pGLO transformation kit, students use a simple procedure to transform bacteria with a gene that codes for Green Fluorescent Protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*, and GFP causes the jellyfish to fluoresce and glow in the dark. Following 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.

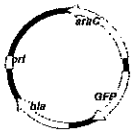
In this activity, students will learn about the process of moving genes from one organism to another with the aid of a plasmid. In addition to one large chromosome, bacteria naturally contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for one or more 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 contains the gene for GFP and a gene for resistance to the antibiotic ampicillin. pGLO also incorporates a special gene regulation system that can be used to control expression of the fluorescent protein in transformed cells. The gene for GFP can be switched on in transformed cells simply by adding the sugar arabinose to the cell's 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. The unique construction of pGLO allows educators and students, for the very first time, to easily explore mechanisms of gene regulation (Appendix D) and genetic selection. And, the entire process is observable with an inexpensive long-wave UV lamp.

Antibiotic Selection

The pGLO plasmid which contains the GFP gene also contains the gene for beta-lactamase, a protein that provides bacteria with resistance to the antibiotic, ampicillin. The beta-lactamase protein is produced and secreted by bacteria which contain the plasmid. The secreted beta-lactamase inactivates ampicillin, allowing only transformed cells to grow in its presence. Only transformed bacteria which contain the pGLO plasmid, and produce beta-lactamase can survive in the presence of ampicillin. (See schematic below.)





Genetically engineered plasmid used to insert new genes into bacteria.

"GFP"	Gene which codes for the Green Fluorescent Protein.	
"*bla*"	Gene which codes for beta-lactamase, a protein which gives bacteria resistance to the antibiotic, ampicillin.	
"*araC*"	Gene which codes for AraC, a protein which regulates production of the Green Fluorescent Protein.	

The plasmid