Biology 196 Laboratory
Gene Expression

Laboratory Objectives
After completing this lab you should be able to:

- Describe and explain the need for each of the controls in the two gene expression experiments.
- Draw a representation of a plasmid and annotate necessary components.
- Perform the heat-shock $CaCl_2$ method of bacterial transformation, and understand the role and function of the cell membrane in the process of plasmid insertion.
- Describe all of the *lac* operon's components, and predict the expression of the *β-galactosidase* enzyme based on the carbohydrates present in the *E. coli* growth medium.

pGLO Transformation[1]

Background

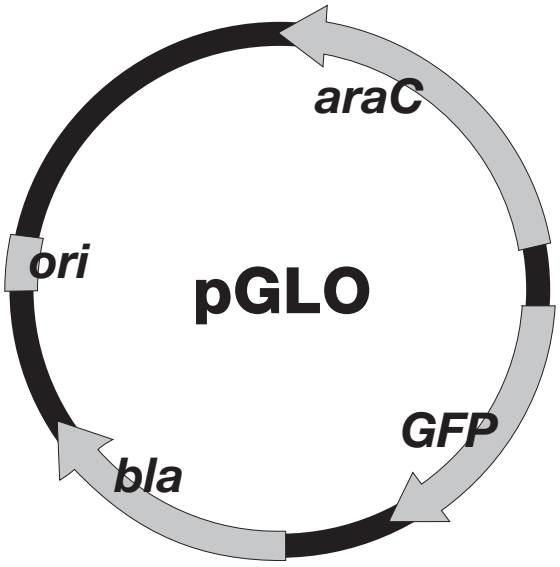
In this lab you will perform a procedure known as genetic **transformation**. Remember that a **gene** is a piece of DNA which provides the instructions for making (codes for) a protein. This protein gives an organism a particular trait. Genetic transformation involves the insertion of a gene into an 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 (*bla*). 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 **ampillicin** plates. Transformed cells will appear white (wild-type phenotype) on plates not containing arabinose, and fluorescent green under UV light when arabinose is included in the nutrient agar medium.

[1] Reproduced for classroom use only from BIO-RAD - Catalog #166-0003EDU



The important segments of pGLO are:

ori: origin of replication site that allows the plasmid to be replicated within the E. coli.

bla: ampicillin resistant site that allows the E. coli to grow in the presence of the drug ampicillin. Ampicillin will normally kill any bacteria without this plasmid.

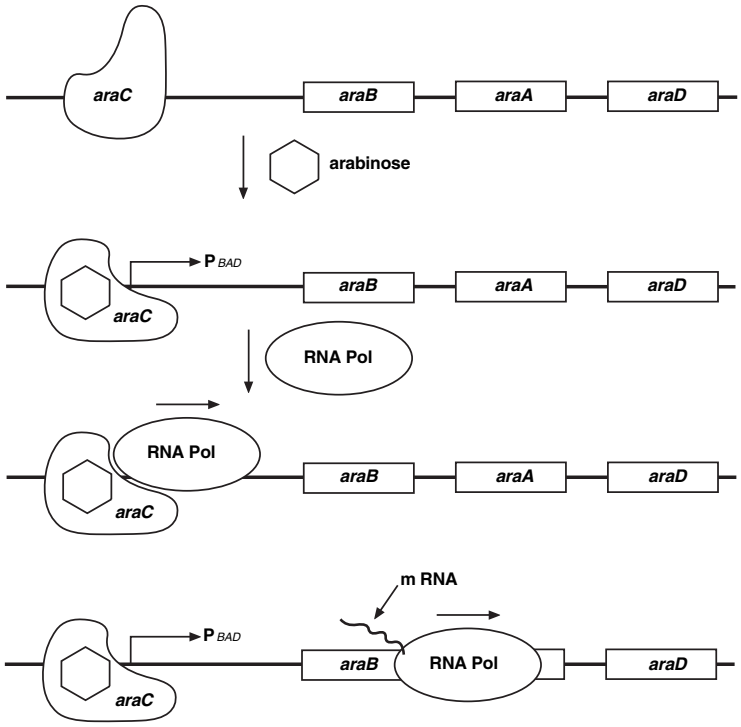
GFP: gene sequence coding for the fluorescent jellyfish protein. It was isolated from jellyfish chromosomal DNA.

araC: segment of DNA coding for the production of the regulatory protein araC.

Although pGLO contains the GFP gene, its protein product is not automatically produced. In order to see the fluorescent protein the GFP DNA must be transcribed into RNA and then that RNA must be translated into the protein. A special promoter placed in front of the GFP gene allows this process to be turned on and off at will (see figure below).

The promoter is taken from the arabinose operon. The arabinose operon is involved in the production of three proteins (*araB*, *araA*, and *araD*) that are required to break down the carbohydrate arabinose to provide energy for the *E. coli*.

The Arabinose Operon



Since E. coli works in a very efficient manner, these proteins are only produced when arabinose is present in the environment. *araC* binds to the promoter region of the arabinose operon. If arabinose is present it binds to the *araC* changing the conformation of *araC*, and allowing RNA polymerase to bind to the promoter and turn on transcription.

For pGLO, the promoter for the arabinose operon was inserted just upstream of the GFP sequence. It will work in the same way just described, except that instead of leading to the production of *araB*, *araA*, and *araD* proteins, the GFP will be produced.

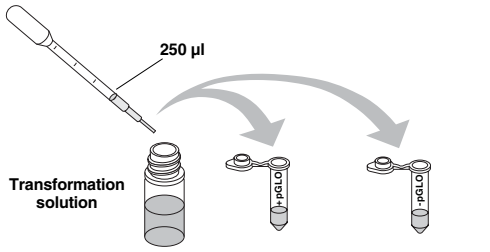
Activity #1: Transformation of pGLO into *E. coli*

Materials:

- 4 Poured agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)
- Transformation solution
- LB nutrient broth
- Inoculation loops
- Pipets and pipet tips
- microcentrifuge tubes
- rehydrated pGLO plasmid (from your instructor)
- timer

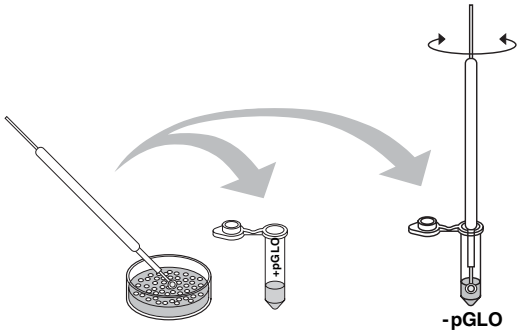
Procedure:

1. Obtain two 1.5 ml tubes and label one **+** and one **-**. Using a plastic pipette add 250 μl $CaCl_2$ (also called the transformation solution) to each tube.



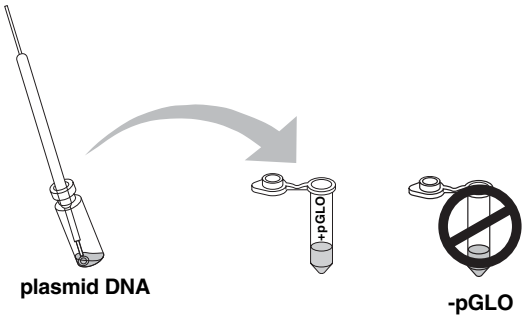
Be sure that a tub of ice water is set up in the lab for the ice/ water bath and that a second water bath is set at 42ºC.

2. From a LB agarose plate containing *E. coli* colonies, pick 2-3colonies with a sterile loop. Add the colony to the + tube. Follow the same procedure for the – tube, using the same loop.

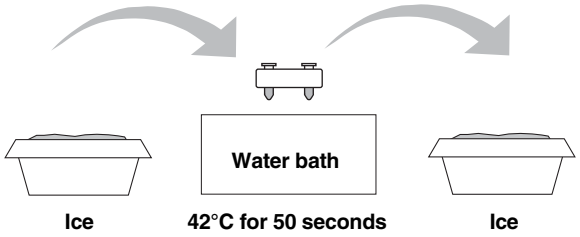


3. Add pGLO DNA to the + sample only. Accomplish this task by twirling a fresh, sterile loop within the pGLO container and then quickly transferring the loop into the 1.5 ml tube marked +.

4. Keep both the + and the – tubes on ice for 10 min.



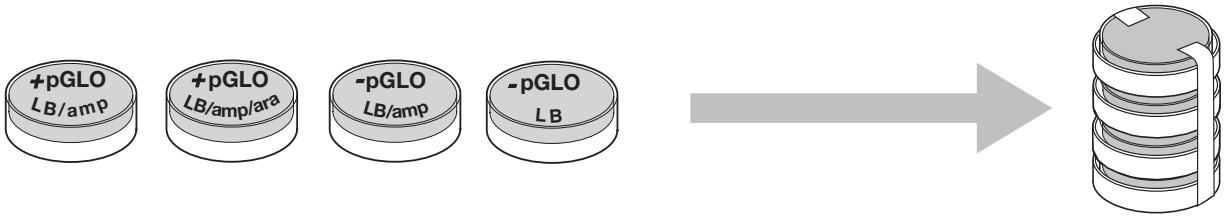
5. Heat shock both tubes for 50 sec at 42ºC in a water bath. Return the tubes to the ice immediately for 2 min. This is an extremely important step! The heat shock causes the Ca^{2+} treated E. coli to "open" their plasma membrane, allowing pGLO to enter into the bacteria.



6. Using the plastic pipettes, add 250 μl LB to each tube, gently flick and sit on the bench for 2 minutes.

7. You need four agar plates, as shown in the figure below. Using the plastic pipettes, add 100 μl of each sample to the following plates:
(+) sample: add 100 ul each to a plate with LB/AMP and to a plate with LB/AMP/ara.
(-) sample: add 100 ul each to a plate with LB and a plate with LB/AMP.

Following addition of the mixture to the plates, carefully spread the solution across the plate with a sterile loop and swirling. Be careful not to puncture the agar. Use a clean, sterile loop for each plate. Leave the



lid off the plate to allow it to dry.

8. Grow plates overnight at 37ºC. Next week you will examine the results to see if colonies grew and which ones are fluorescent. Fluorescence will be determined with a hand held UV light source.

Activity #2: The *lac* Operon

Background

See your lecture text for a thorough review of the lac operon

You have just performed an experiment involving the arabinose operon. Now let's examine the lactose operon. This segment of DNA in bacteria is designed to produce three enzymes which will be utilized by the bacteria to break down lactose to provide energy. Since bacteria are efficient, they will only perform transcription and translation of this operon when lactose is present in the surrounding environment, and rarely when glucose is present. Today you will find out whether this statement is accurate.

Three media have been inoculated with *E. coli*. One medium contains glucose as the sole sugar source, one contains lactose as the sole sugar source, and one contains both glucose and lactose as sugar sources.

Materials:

- 3 borosilicate glass test tubes
- Marking pen
- E. coli cultures (in shaking incubator)
- ONPG solution (from instructor)

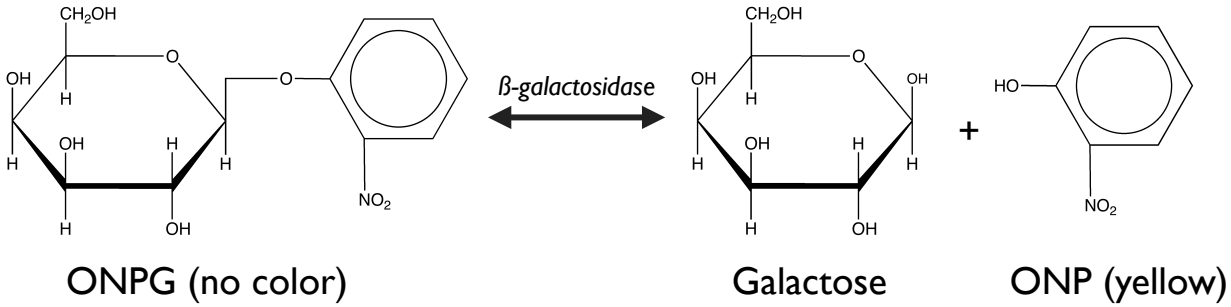
Procedure:

1. Obtain three glass test tubes and label them:

   A) Glucose (G)
   B) Lactose (L)
   C) Glucose + lactose (G +L)

2. Add 12 drops of the different *E. coli* cultures to each of the three tubes that corresponds to the correct carbohydrate (i.e. add 12 drops of the E. coli culture grown in glucose to the tube labeled "Glucose", etc.).

3. To each of the three tubes add 10 drops of the ONPG (O-Nitrophenyl-beta-D-galactopyranoside) solution from your instructor. ONPG is a chemical compound that can be hydrozyed by *beta-galactosidase* into two components, one of which is a yellow color (see below)



4. Incubate all the tubes at 37ºC. Check for yellow color changes at 10 minute intervals and score them in the tables below.

10 Minutes	No Color	Weak color	Strong color
Glucose			
Lactose			
Glucose + Lactose			

20 Minutes	No Color	Weak color	Strong color
Glucose			
Lactose			
Glucose + Lactose			

30 Minutes	No Color	Weak color	Strong color
Glucose			
Lactose			
Glucose + Lactose			

Study Checklist

To perform well on the quiz, you need to have a thorough knowledge of the following:

lac Operon experiment

- You should know and understand all of the terms which appear in **boldface** type.
- Use your textbook for a thorough review of the *lac* operon. What proteins and factors activate or inhibit expression of the structural genes?
- What product of the *lac* operon caused the yellow color in some of the E. coli cultures?
- Contrast the results of the 'lactose' culture vs. the 'glucose + lactose' culture. Were they different? Why or why not?

Transformation experiment

- How does chilling the bacteria at 4º in $CaCl_2$ promote transformation of the pGLO plasmid? What does the heat-shock at 42º do?
- Make predictions about growth of the E. coli on the three different plates (LB, LB + amp, LB + amp + arabinose) with and without the pGLO plasmid.
- Which plate(s) would you expect to fluoresce?