**pGLO Bacterial Transformation**

AP Biology Lab #8 Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Per\_\_\_\_\_\_

# Introduction:

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 can give 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.

A second gene will also be given to the bacteria in this procedure. This gene will code for a protein that will allow the bacteria to live and grow in the presence of the antibiotic ampicillin.

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.

The pGLO plasmid DNA that we will use encodes the gene for GFP and a gene for resistance to the antibiotic ampicillin. This plasmid is identical to the model plasmid that you constructed out of paper, with the possible exception of the antibiotic resistance gene.

### The Genes:

### Genetic transformation involves the insertion of some new DNA into the *E. coli* cells. In addition to one large chromosome, bacteria often contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for more than one trait. Scientists can use a process called genetic engineering to insert genes coding for new traits into a plasmid. In this case, the pGLO plasmid carries the GFP gene that codes for the green fluorescent protein and a gene (*bla*) that codes for a protein that gives the bacteria resistance to an antibiotic. The genetically engineered plasmid can then be used to genetically transform bacteria to give them this new trait.



### The Act of Transformation:

This transformation procedure involves two main steps. These steps are intended to introduce the plasmid DNA into the *E. coli* cells and provide an environment for the cells to express their newly acquired genes.

**To move the pGLO plasmid DNA through the cell membrane you will:**

1. Use a transformation solution of CaCl2 (calcium chloride).
2. Carry out a procedure referred to as heat shock.

**For transformed cells to grow in the presence of ampicillin, you must:**

1. Provide them with nutrients and a short incubation period to begin expressing their newly acquired genes.

**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.



1. Adjust the pipette to read 250. Open the tubes and, using a pipette and a tip, transfer 250 µl of transformation solution (CaCl2) into each tube.



1. Place the tubes on ice.



1. 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). Close the tube and continue flicking to mix. Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the **–pGLO** tube. It is critical that the bacteria are thoroughly mixed.



1. Immerse a **new sterile loop** into the pGLO plasmid DNA stock tube from Mr. Erlenbeck. 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. Flick the tubes to thoroughly mix.



1. 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.
2. 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/ara** plate: +pGLO
* Label one **LB/amp** plate: +pGLO
* Label the other **LB/amp** plate: -pGLO
* Label the **LB** plate: -pGLO
1. **Heat shock.** Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42ºC, 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, immediately place both tubes back on ice. For the best transformation results, the transfer from the ice (0ºC) to 42ºC and then back to the ice must be rapid. Incubate tubes on ice for 2 minutes.

1. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a micropipette and a tip, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipette tip for the other tube. Flick the closed tubes to mix. Incubate the tubes for 10 minutes at room temperature.



1. Tap the closed tubes with your finger to mix. Adjust the micropipetter to read 100. Using a new pipette tip for each tube, pipette 100 µl of the transformation and control suspensions onto the appropriate nutrient agar plates.



1. **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 for a full 60 seconds. **DO NOT PRESS TOO DEEP INTO THE AGAR**.



1. 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** (lid side down) in the 37ºC incubator until the next day.



# Gene Regulation:

In addition to an ampicillin resistance gene and the GFP gene, the pGLO plasmid also contains a special **gene regulation** system which can be used to control the transcription of the green fluorescent protein gene in transformed bacteria. The gene for GFP can be “switched on” or activated by adding the sugar **arabinose** to the cells’ nutrient plate. **Selection** for cells that have been transformed with pGLO DNA is accomplished by growth on antibiotic plates. Transformed cells will only appear white or normal on plates not containing arabinose, and fluorescent green when arabinose is included in the agar plate.

Our cells 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 an estimated 19,000 - 20,000 genes in the human genome. Each gene codes for a unique protein: one gene, one protein.

Organisms need to **regulate** **expression** of their genes and ultimately the amounts and kinds of proteins present within their cells. Gene regulation allows for adaptation to differing conditions and prevents wasteful overproduction of unneeded proteins, which would be a waste of valuable ATP and amino acids. The proteins involved in the digestion 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 in bacteria. *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.

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**.

**Data Table:**

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| --- | --- | --- |
| Plate | Observations | Plate Sketch |
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1. What color are cells with arabinose present? What color are cells without arabinose present?
2. What is meant by saying regulation of gene expression?
3. Bacteria are able to turn on and off genes for digestive proteins. How is this beneficial for the bacteria?
4. How do arabinose and araC act as a molecular switch to turn on the GFP gene?
5. Suppose there was a mutation in the gene that coded for the protein araC making the cells unable to produce it. How would this affect the cells ability to glow and regulation of glowing?
6. Clearly molecular switches are beneficial to organisms. Why would a researcher create transformed bacteria with a switch in addition to the gene of interest?



Refer to the above figure

* The three genes (*araB, araA and araD*) that code for three digestive enzymes involved in the breakdown of arabinose are clustered together.
* These three proteins are dependent on initiation of transcription from a single promoter, PBAD.
* 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.
* a*raC* 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.



Refer to the above figure

* The DNA code of the pGLO plasmid has been engineered to incorporate aspects of the arabinose operon. Both the promoter (PBAD) and the *ara*C 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.