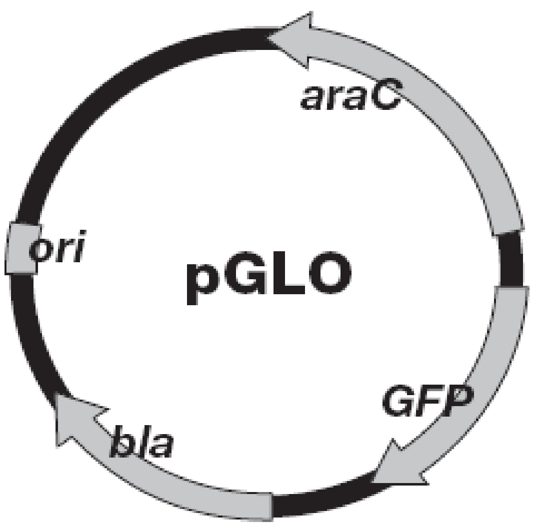
**Name:**

# Transformation

Bacterial Transformation and Green Fluorescent Protein

e. 2010 www.mdbiolab.org • info@mdbiolab.org
Adapted for use on the MdBioLab from *pGLO Bacterial Transformation Kit*, Bio-Rad #166-0003EDU

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, 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.




**pGLO plasmid map**

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.

The plasmid used is Bio-Rad's unique pGLO plasmid that 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 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 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 medium.

You will be provided with the tools and a protocol for performing genetic transformation.

Your task will be:

- To do the genetic transformation
- To determine the degree of success in your efforts to genetically alter an organism by calculating the transformation efficiency

## LESSON 1 – FOCUS QUESTIONS

There are many considerations that need to be thought through in the process of planning a scientific laboratory investigation. Below are a few for you to ponder as you take on the challenge of doing a genetic transformation. Since scientific laboratory investigations are designed to get information about a question, our first step might be to formulate a question for this investigation.

**Consideration 1: Can I Genetically Transform an Organism? Which Organism?**

1. To genetically transform an entire organism, you must insert the new gene into every cell in the organism. Which organism is better suited for total genetic transformation— one composed of many cells, or one composed of a single cell?

2. Scientists often want to know if the genetically transformed organism can pass its new traits on to its offspring and future generations. To get this information, which would be a better candidate for your investigation, an organism in which each new generation develops and reproduces quickly, or one that does this more slowly?

3. Safety is another important consideration in choosing an experimental organism. What traits or characteristics should the organism have (or not have) to be sure it would not harm you or the environment?

4. Based on the above considerations, which would be the best choice for a genetic transformation: a bacterium, earthworm, fish, or mouse? Describe your reasoning.

## Consideration 2: How Can I Tell if Cells Have Been Genetically Transformed?

Recall that the goal of genetic transformation is to change an organism's traits (phenotype). Before any change in the phenotype of an organism can be detected, a thorough examination of its natural (pre-transformation) phenotype must be made. Look at the colonies of *E. coli* on your starter plates. List all observable traits or characteristics that can be described:

The following pre-transformation observations of *E. coli* might provide baseline data to make reference to when attempting to determine if any genetic transformation has occurred.

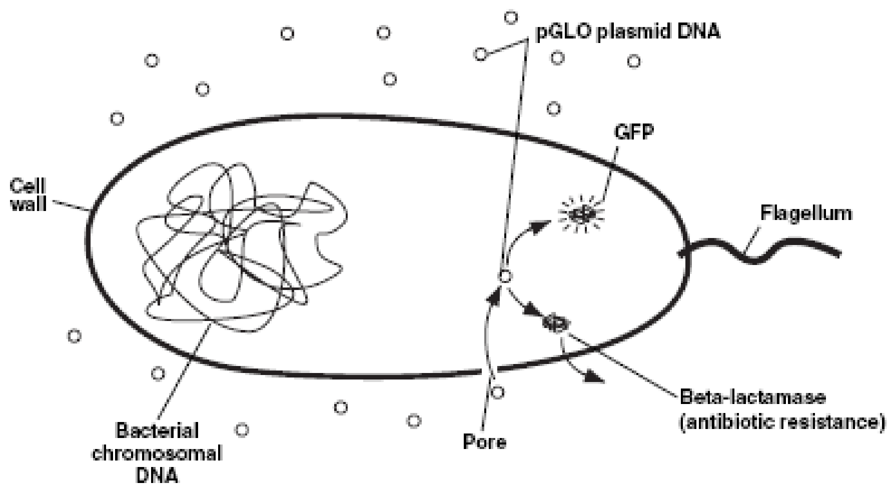
- Number of colonies
- Size of
    - Largest colony
    - Smallest colony
    - Majority of colonies
- Color of the colonies
- Distribution of the colonies on the plate
- Visible appearance when viewed with ultraviolet (UV) light
- The ability of the cells to live and reproduce in the presence of an antibiotic such as ampicillin

1. Describe how you could use two LB/agar plates, some *E. coli* and some ampicillin to determine how *E. coli* cells are affected by ampicillin.

2. What would you expect your experimental results to indicate about the effect of ampicillin on the *E. coli* cells?

## Consideration 3: 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.




## Consideration 4: The Act of Transformation

This transformation procedure involves three 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:**

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

## LESSON 2: TRANSFORMATION LABORATORY

**MATERIALS:**

- Rehydrated pGLO plasmid
- *E. coli* starter plate
- Tape
- LB nutrient broth
- Inoculation loops
- 42°C heat block
- Cold block/ice
- Poured agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)
- Marker
- Transformation Solution: Calcium chloride ($CaCl_2$)
- Micropipette and tips
- 37°C incubator

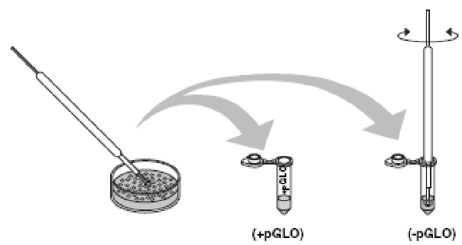
**LABORAOTRY PROCEDURE:**

☐ 1. Label one closed micro test tube **+pGLO** and another **-pGLO**. Label both tubes with your group's name. Place them in the cold block.

☐ 2. Open the tubes and transfer 250 µL of transformation solution (CaCl2) into each tube.

☐ 3. Place the tubes into the cold block.

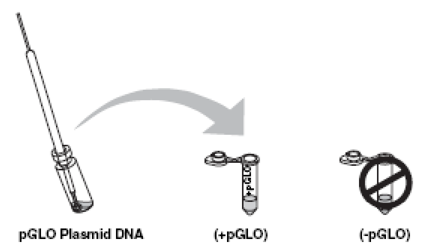
☐ 4. Use a sterile loop to pick up **a single colony of bacteria** from your starter plate. Pick up the **+pGLO** tube and immerse the loop into the $CaCl_2$ at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the cold block. Using a new sterile loop, repeat for the **-pGLO** tube.




☐ 5. Examine the pGLO DNA solution with the UV lamp. Note your observations:

Add 10µL of pGLO plasmid to the cell suspension in the **+pGLO** tube. Close the tube and return it to the ice block. Also close the **-pGLO** tube.

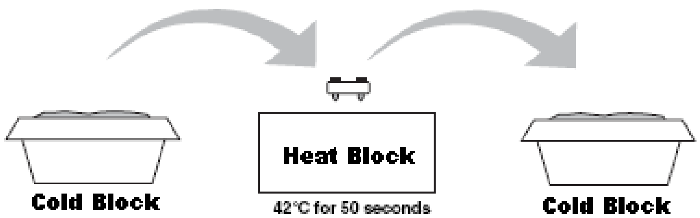
**DO NOT** add plasmid DNA to the **-pGLO** tube.




**QUICK CHECK:** Why was no plasmid added to the –pGLO tube?

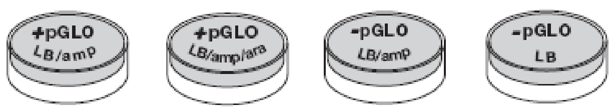
☐ 6. Incubate the tubes in the cold block for 5 minutes. Make sure to push the tubes all the way down into the cold block.

☐ 7. **Heat shock.** Transfer both the (+) pGLO and (-) pGLO tubes into the heat block, set at 42°C, for exactly 50 seconds. Make sure to push the tubes all the way down into the heat block. When the 50 seconds are done, place both tubes back into the ice block. For the best transformation results, the transfer from the cold block (0°C) to 42°C and then back to the cold block must be rapid. Incubate tubes in the cold block for 2 minutes.




☐ 8. While the tubes are sitting in the cold block, label your four LB nutrient agar plates on the bottom (not the lid) as follows:

- Label one **LB/amp** plate: **+ pGLO**
- Label the **LB/amp/ara** plate: **+ pGLO**
- Label the other **LB/amp** plate: **- pGLO**
- Label the **LB** plate: **- pGLO**

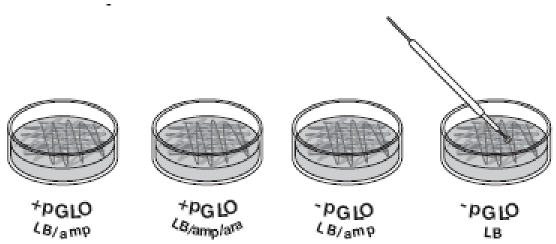



**QUICK CHECK:** Why would you label the bottom of the plate and not the lid?

☐ 9. Remove the tubes from the ice block and place on the bench top. Open a tube and, using a new sterile pipette, add 250 µL of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipette for the other tube. Incubate the tubes for 5 minutes at room temperature.

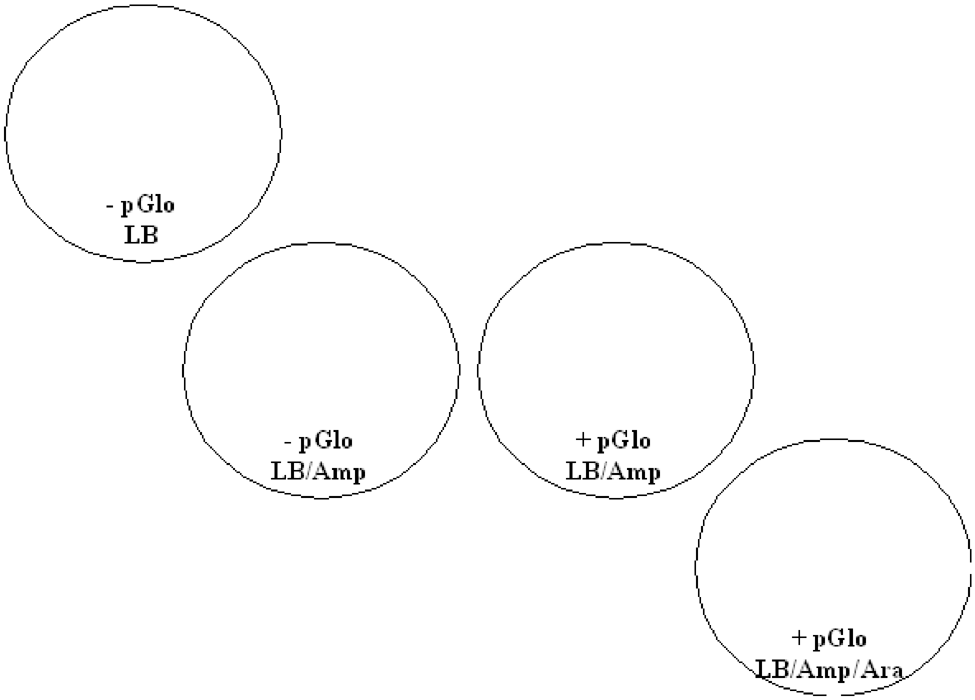
☐ 10. Tap the closed tubes with your finger to mix. Using a new sterile pipette for each tube, pipette 100 µL of the transformation and control suspensions (+/- tubes) onto the appropriate nutrient agar plates.

☐ 11. **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. DO NOT PRESS TOO DEEP INTO THE AGAR.




☐ 12. Stack up your plates with the +pGlo/LB/Amp/Ara plate on bottom and tape them together. Put your group name and class period on the bottom of the stack. After 15 minutes, place the stack of plates **upside down** in the 37°C incubator until the next day.

Draw your anticipated results. Draw bacterial colonies and label if they will glow in UV light.




**Lesson 2 Review Questions**

1. On which of the plates would you expect to find bacteria most like the original non-transformed *E. coli* colonies you initially observed? Explain your predictions.

2. If there were any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Explain your predictions.

3. Which plates should be compared to determine if any genetic transformation has occurred? Why?

4. What is a control plate? What purpose does a control serve?

## LESSON 3: DATA COLLECTION & ANALYSIS

**Data Collection: to be done after 24 hour incubation of plates**

Observe the results you obtained from the transformation lab under normal room lighting. Then turn out the lights and hold the ultraviolet light over the plates.

1. Carefully observe and draw what you see on each of the four plates. Put your drawings in the data table in the column on the right. Record your data to allow you to compare observations of the "**+ pGLO**" cells with your observations for the non-transformed *E. coli*. Write down the following observations for each plate.
2. How much bacterial growth do you see on each plate, relatively speaking?
3. What color are the bacteria?
4. How many bacterial colonies are on each plate (count the spots you see).

| | | Observations |
|---|---|---|
| Transformation Plates | **+pGLO LB/amp** | |
| | **+pGLO LB/amp/ara** | |
| Control Plates | **-pGLO LB/amp** | |
| | **-pGLO LB** | |

**Analysis of Results -** The goal of data analysis for this investigation is to determine if genetic transformation has occurred.

1. Which of the traits that you originally observed for *E. coli* did not seem to become altered? In the space below list these untransformed traits and how you arrived at this analysis for each trait listed.

| **Original trait** | **Analysis of observations** |
|---|---|

2. Of the *E. coli* traits you originally noted, which seem now to be significantly different after performing the transformation procedure? List those traits below and describe the changes that you observed.

| **New trait** | **Observed change** |
|---|---|

3. If the genetically transformed cells have acquired the ability to live in the presence of the antibiotic ampicillin, then what might be inferred about the other genes on the plasmid that you used in your transformation procedure?

4. From the results that you obtained, how could you prove that the changes that occurred were due to the procedure that you performed?