

# Green Fluorescent Protein (GFP): Genetic Transformation, Synthesis and Purification of the Recombinant Protein

## INTRODUCTION

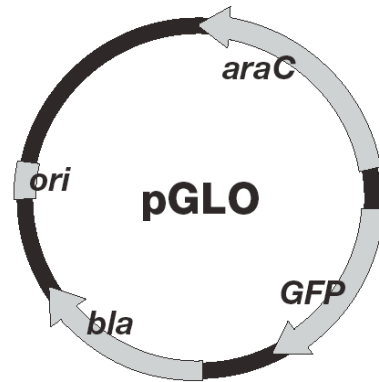
Green Fluorescent Protein (GFP) is a novel protein produced by the bioluminescent jellyfish *Aequorea victoria*. Green Fluorescent Protein causes the jellyfish to fluoresce and glow in the dark. Over the course of 3 weeks you will investigate GFP, beginning with the manipulation of its gene and ending by an analysis of the protein itself. Remember that a gene is a piece of DNA that provides the instructions for making (codes for) a protein. The protein encoded by the gene gives an organism a particular trait. In this lab you will start with a procedure known as genetic transformation. 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.

In the first week you will use a procedure to genetically transform bacteria with the gene that codes for Green Fluorescent Protein. 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. During the second week you will purify the protein away from all of the other proteins produced by the bacteria using chromatography, and during the third week you will evaluate the success of the purification procedure and estimate the size (molecular weight) of GFP by using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Fundamental to the process of genetic transformation of bacteria are plasmids. 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 in large part to the transmission of plasmids.

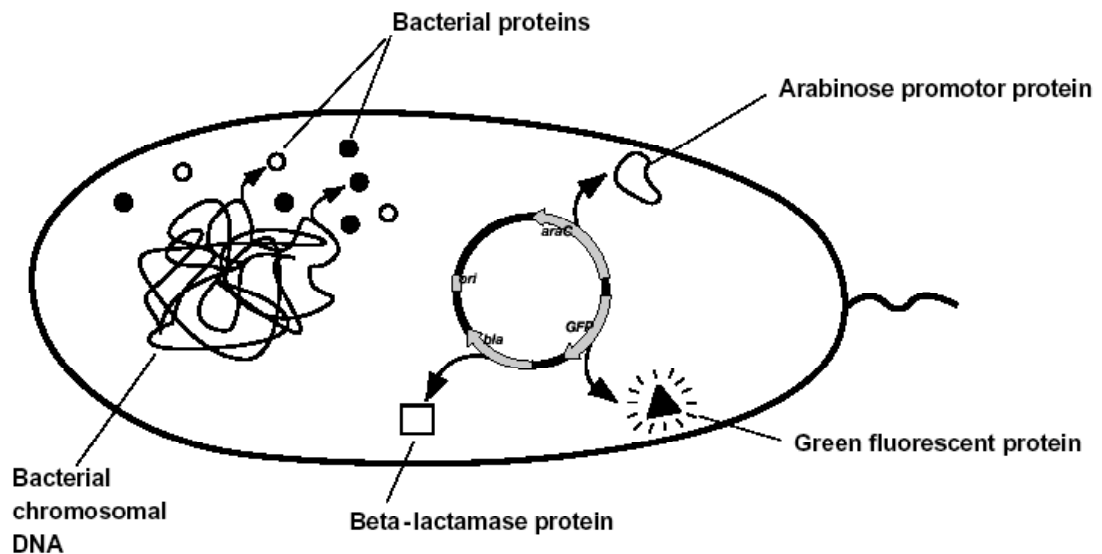
The pGLO plasmid (Fig. 1, not a naturally occurring plasmid) contains the gene for GFP, and also contains the gene for the enzyme  $\beta$ -lactamase (*bla*), which provides resistance to the antibiotic ampicillin. The  $\beta$ -lactamase protein is produced and secreted by bacteria that contain the plasmid.  $\beta$ -Lactamase inactivates the ampicillin present in the LB nutrient agar, allowing bacterial growth. Only transformed bacteria that contain the plasmid and express  $\beta$ -lactamase can survive on plates that contain ampicillin. Only a very small percentage of the cells take up the plasmid DNA and are transformed. Untransformed cells cannot grow on the ampicillin selection plates. 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.



**Figure 1.** A diagram of the pGLO plasmid. Arrows indicate specific genes. *GFP*, the gene coding for green fluorescent protein; *bla*, the gene coding for  $\beta$ -lactamase; *araC*, the gene coding for AraC; *ori*, the origin of replication. *Ori* is not a gene, but a specific sequence of the DNA that the replication enzymes of *E. coli* recognize as the site to begin replication.

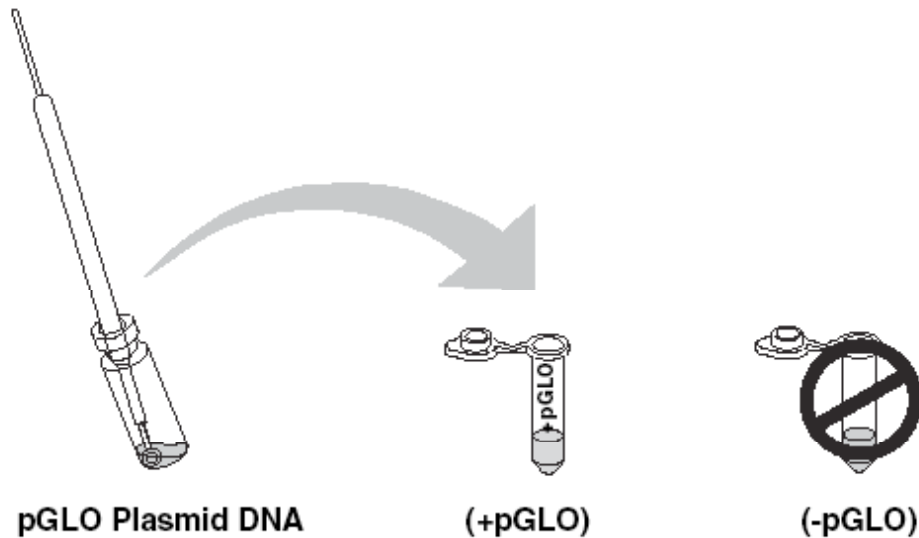
The transformation procedure involves three main steps. These steps are intended to introduce the plasmid DNA into the *E. coli* cells and provide an environment for the cells to express their newly acquired genes. 1) Cells are first treated with a solution of  $\text{CaCl}_2$ , which is thought to neutralize the repulsive negative charges of the phosphate backbone of DNA and the phospholipids of the cell membrane, allowing the DNA to adhere to the cells. This is referred to making the cells ‘competent’ (*i.e.* the cells are capable, or competent, to take-in exogenous DNA). 2) Cells are then subjected to a heat shock, which is thought to increase the permeability of the cell membrane, allowing the cells to take-in the plasmid DNA. 3) Cells are allowed to grow in a ‘recovery’ phase in the absence of ampicillin. During this time the cells begin to produce the  $\beta$ -lactamase protein, which will allow them to survive when they are subsequently placed on agar plates containing ampicillin.



**Figure 2.** Schematic of a bacterial cell that contains the pGLO plasmid.

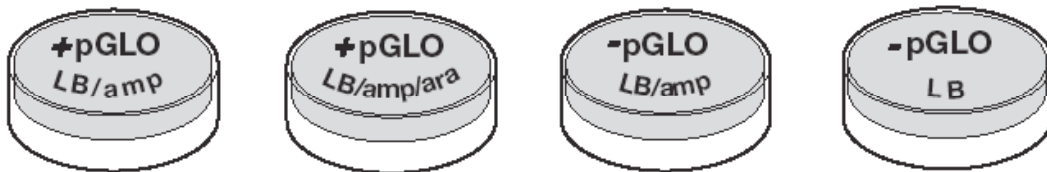
## EXPERIMENTAL PROCEDURE

1. Obtain two tubes of frozen competent *E. coli* cells and place on ice. Label one tube +pGLO and the other -pGLO. Label both tubes with your group's name. Place them back in ice.
2. Examine the pGLO DNA solution with the UV lamp. Note your observations. Withdraw 1  $\mu$ L of the plasmid solution with a clean, sterile pipet tip and place it in the +pGLO tube. Close the tube and mix the DNA into the cell suspension by gently flicking the side of the tube with your finger. Return the tube to the ice. Do not add plasmid DNA to the -pGLO tube. (Why not?)



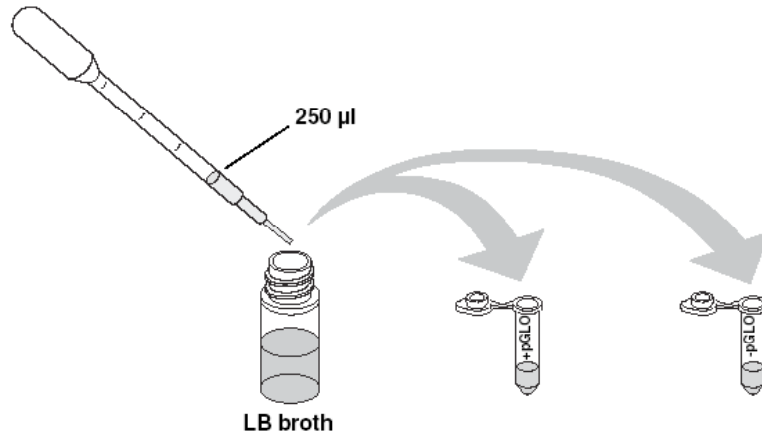
3. Incubate the tubes on ice for 10 minutes.
4. While the tubes are sitting on ice, label your four LB nutrient agar plates on the bottom (not the lid) as follows:

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

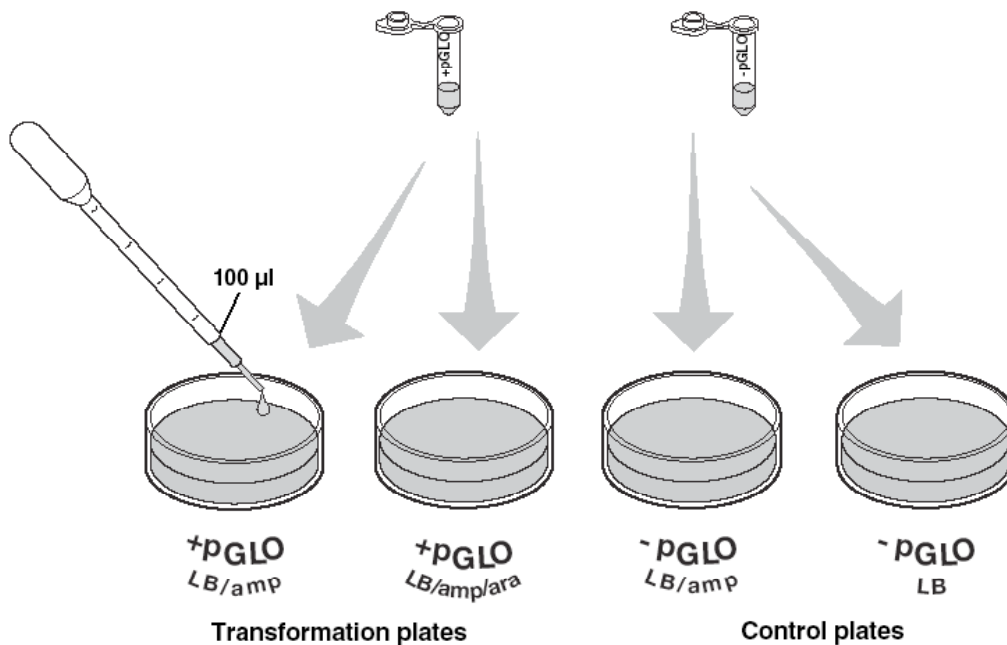


5. **Heat shock.** Using a microtube rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42 °C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the transfer from the ice (0 °C) to 42 °C and then back to the ice must be rapid. Incubate tubes on ice for 2 minutes.
6. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet tip, add 250  $\mu$ l of LB nutrient broth to the tube and reclose it. Repeat with

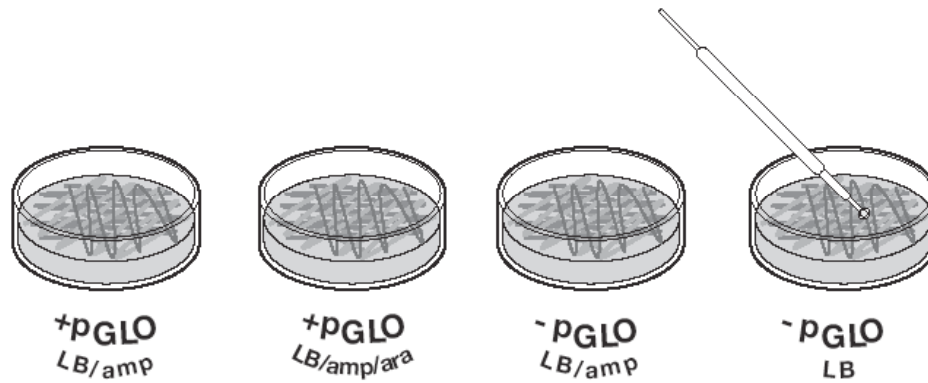
a new sterile pipet tip for the other tube. Incubate the tubes for 40 minutes at room temperature. While you are waiting for the cells to grow observe the untransformed *E. coli* cells on plates that your lab TA has prepared for the lab. For your lab write-up you will need to answer questions about these cells.



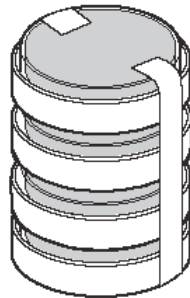
7. Tap the closed tubes with your finger to resuspend the cells evenly throughout the solution. Using a pipet transfer 10 µl of the transformation and control suspensions onto the appropriate nutrient agar plates. USE A NEW STERILE TIP FOR EACH TRANSFER.



8. 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. USE A NEW STERILE LOOP FOR EACH PLATE. DO NOT PRESS INTO THE AGAR.



9. 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** in the 37°C incubator until the next day.



## LABORATORY WRITE-UP

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 the plates provided by the TA. List all observable traits or characteristics that can be described:

- 1) 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.
  - a) Number of colonies
  - b) Size of:
    - i) the largest colony
    - ii) the smallest colony
    - iii) the majority of colonies
  - c) Color of the colonies
  - d) Distribution of the colonies on the plate
  - e) Visible appearance when viewed with ultraviolet (UV) light
  - f) The ability of the cells to live and reproduce in the presence of an antibiotic such as ampicillin
- 2) 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?

- 3) 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 which does this more slowly?
- 4) 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.
- 5) If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Explain your predictions.
- 6) Which plates should be compared to determine if any genetic transformation has occurred? Why?
- 7) What is meant by a control plate? What purpose does a control serve?

The following questions should be answered by observing the plates at least 24 hours after they have been plated (if you are unable to arrange a time to during the first week to view your plates you may do this during the second weeks experiment).

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.

- 8) Carefully observe what you see on each of the four plates. 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.
  - a. How much bacterial growth do you see on each plate, relatively speaking?
  - b. What color are the bacteria?
  - c. Which of the traits that you originally observed for *E. coli* did not seem to become altered? List these untransformed traits and how you arrived at this analysis for each trait listed.
  - d. Of the *E. coli* traits you originally noted, which seem now to be significantly different after performing the transformation procedure? List those traits and describe the changes that you observed.
- 9) 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?
- 10) Describe the evidence that indicates whether your attempt at performing a genetic transformation was successful or not successful.
- 11) Very often an organism’s traits are caused by a combination of its genes and its environment. Think about the green color you saw in the genetically transformed bacteria:
  - a. What two factors must be present in the bacteria’s environment for you to see the green color?
  - b. What do you think each of the two environmental factors you listed above are doing to cause the genetically transformed bacteria to turn green?
  - c. What advantage would there be for an organism to be able to turn on or off particular genes in response to certain conditions?