

Regulation of GFP Expression

Background¹:

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 ability of bacteria to become resistant to antibiotics is due to their ability to accept and donate plasmids coding for antibiotic resistance from other bacterium.

Scientists have engineered plasmids to contain various restriction enzyme sites, so that DNA cut with the same restriction enzyme as the plasmid can be cloned into that plasmid to form recombinant DNA molecules. This is the basis for cloning human or other genes of interest into a carrier, or vector, DNA molecule for further use. For example, a human gene for insulin can be cloned into a bacterial plasmid and then put into bacteria in a process called **bacterial transformation**. Under the right conditions, these bacteria can then make human insulin, which can be purified and used to treat diabetes.

In this lab you will be transforming bacteria with a plasmid vector containing the gene for Green Fluorescent Protein (GFP), which comes from bioluminescent jellyfish *Aequorea victoria*. The gene codes for a protein that causes the jellyfish to glow and fluoresce green in the dark or under ultraviolet light. The biotechnology company Bio-Rad has developed this plasmid vector, called pGLO (figure 1), into which the GFP gene is cloned.

In addition to the GFP gene, pGLO contains the gene that encodes the enzyme for beta-lactamase, *bla*, which breaks down the antibiotic ampicillin (figure 1). Cells that do not incorporate the pGLO plasmid cannot grow in the presence of ampicillin. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on ampicillin-containing plates. Maintaining the transformants on plates containing ampicillin also ensures that they will continue to incorporate and pass on the pGLO plasmid to the next generation of bacteria as they divide. If the transformants are not continuously grown on ampicillin, they will eject the plasmid and fail to express the GFP gene.

pGLO also includes a positive gene regulation system, which can be used to control expression of the green fluorescent protein in transformed cells. In this system, the sugar arabinose turns the GFP gene on. The GFP gene is cloned behind the promoter, P_{BAD} (figure 1). Also contained in the pGLO plasmid is the *araC* gene (figure 1), which encodes a DNA binding protein that helps RNA polymerase bind to the P_{BAD} promoter. When arabinose is present in the nutrient medium, bacteria will take it up. Once inside, the arabinose interacts directly with *araC* protein, which is bound to the DNA. The interaction causes the *araC* protein to change shape, which in turn helps RNA polymerase to bind the P_{BAD} promoter and transcribe the GFP gene (figure 2b). Therefore, in the presence of arabinose, *araC* protein promotes the binding of RNA polymerase, and GFP is produced (figure 2b). Transformed cells will fluoresce green under ultraviolet light when arabinose is added to the nutrient agar but will remain white on plates not containing arabinose.

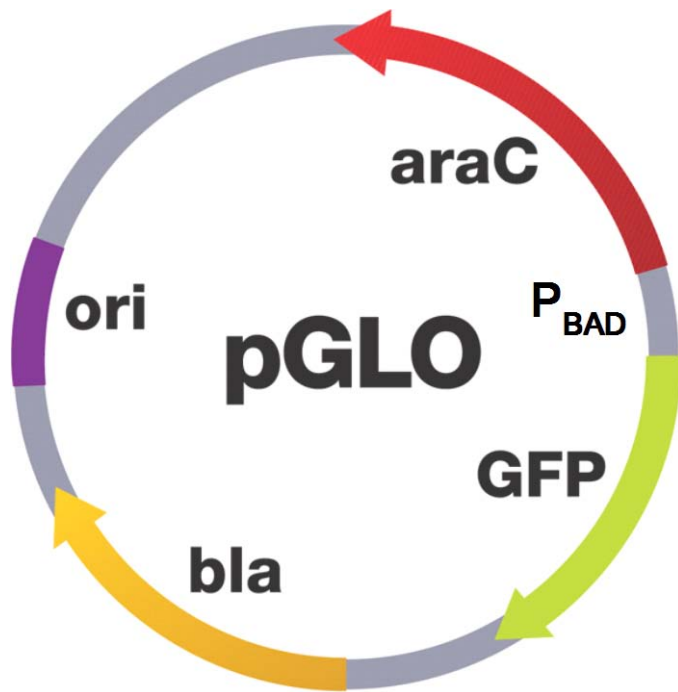
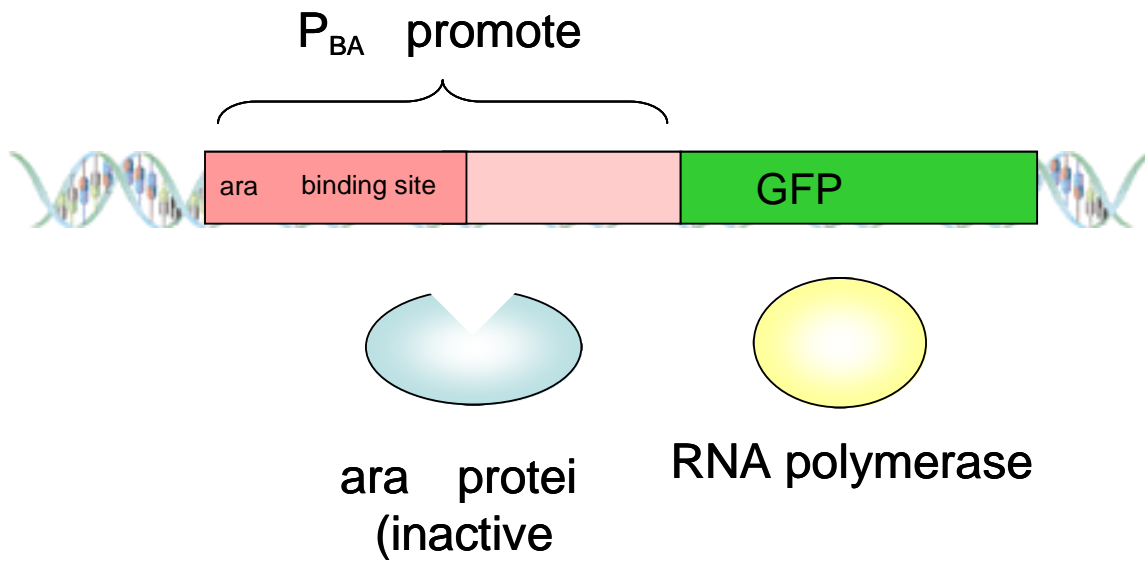
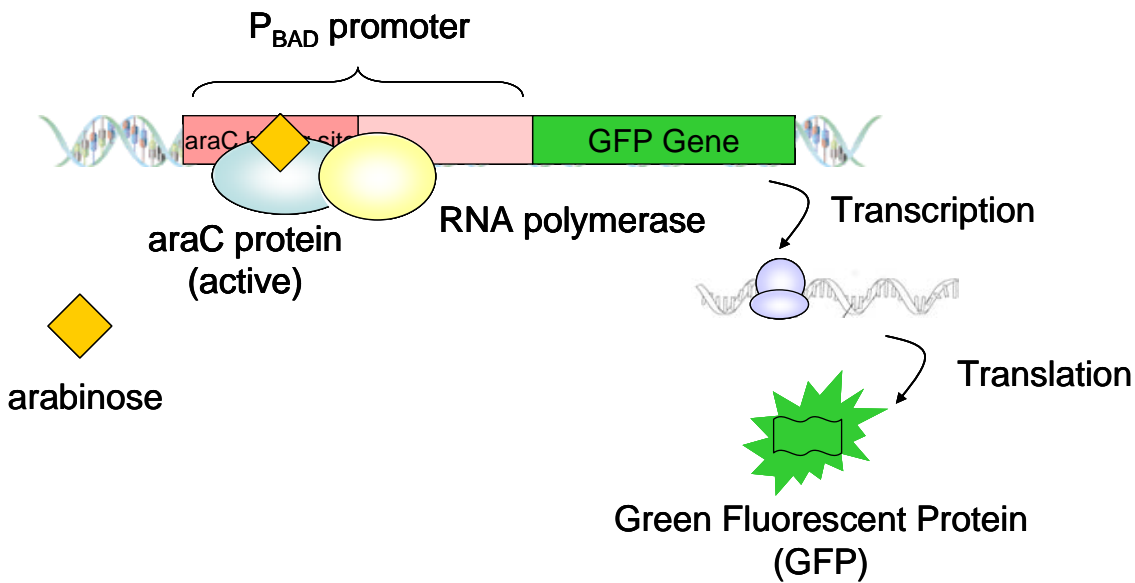


Figure 1. The bacterial plasmid pGLO containing the GFP gene cloned behind the PBAD promoter, beta-lactamase gene encoding ampicillin resistance, araC gene encoding the araC activator protein, and the bacterial origin of replication, ori.



Arabinose absent. In the absence of arabinose, araC is inactive. AraC does not help RNA polymerase to bind and transcribe the GFP gene.



Arabinose present. When the sugar arabinose is added to the agar medium it will bind to and activate araC. araC then binds to its binding site within the P_{BAD} promoter and facilitates the binding of RNA polymerase. Transcription and subsequent translation of the GFP gene leads to fluorescently green protein.

Figure 2. Positive regulation of GFP gene expression by arabinose and araC protein. (a) In the absence of arabinose. (b) In the presence of the sugar arabinose.

Lab Set-up:

In this lab you will verify the positive regulatory mechanism of GFP gene expression by the sugar arabinose. To do this you will transform bacteria with the plasmid containing the *Aequorea victoria* jellyfish Green Fluorescent Protein gene (pGLO) and growing the transformed bacteria in the presence and absence of arabinose. Those bacteria that have successfully incorporated pGLO and are grown in nutrient medium containing arabinose should fluoresce green under ultra-violet light. You will also determine how successful your transformation was by calculating the transformation efficiency.

MATERIALS:

Bacteria starter plate

pGLO DNA plasmid

microcentrifuge tubes

Luria Broth (LB) nutrient medium (liquid)

Ice

Water bath

CaCl₂ transformation solution

Luria Broth plate (LB) agar plates

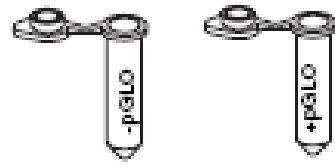
Luria Broth plus ampicillin (LB/amp) agar plates

Luria Broth plus ampicillin plus arabinose (LB/amp/ara) agar plates

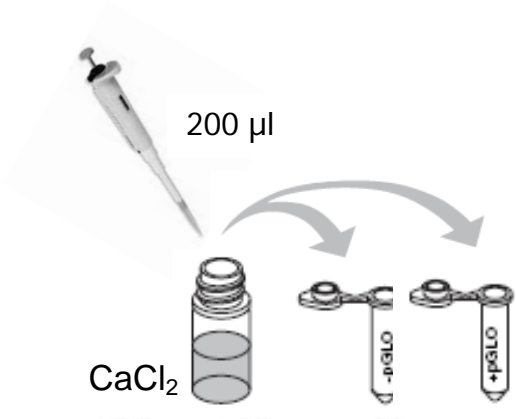
Micropipettors and sterile tips

METHODS¹:

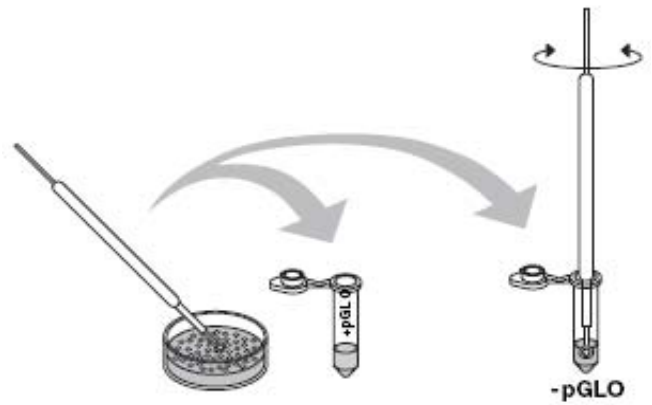
1. Along with your group's name, label one tube with - pGLO and another tube with + pGLO.



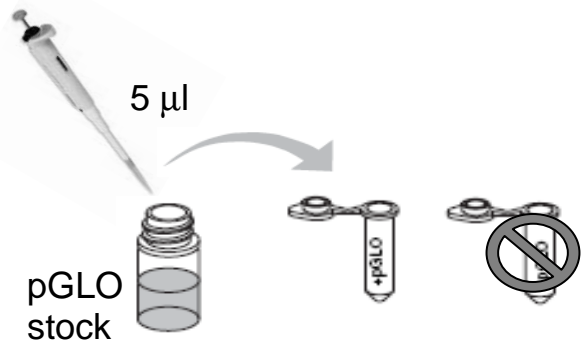
2. Using micropipettors and sterile tips, transfer 200 μ l of CaCl_2 (transformation solution) to each tube. Place the tubes on ice.



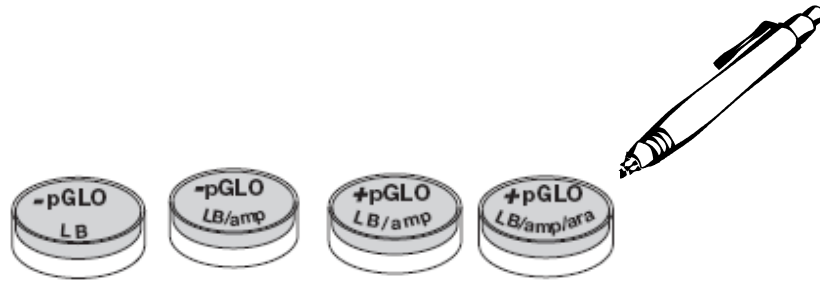
3. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution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



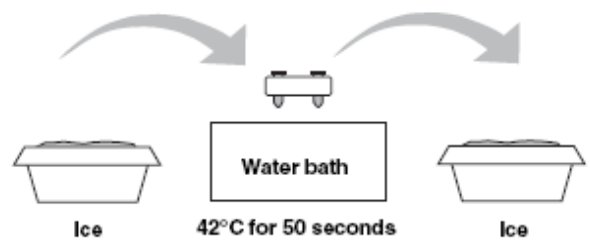
4. Add 5 μ l of pGLO from the pGLO DNA stock tube to your + pGLO reaction tube. Gently flick the tube to disperse the pGLO DNA. Do not add pGLO to the - pGLO tube (Why not?). Return both tubes to ice.



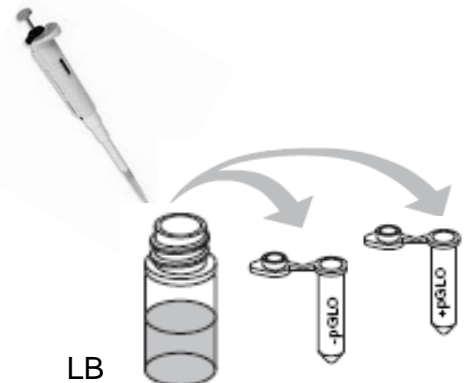
- Incubate the tubes on ice for 10 minutes. Make sure the bottoms of the tubes containing the solutions contact the ice.
- While the tubes are sitting on ice, label four agar plates on the bottoms (not the lid) as follows: LB plate: label it -pGLO. LB/amp plates: label one - pGLO and one + pGLO. LB/amp/ara plate: label it + pGLO.



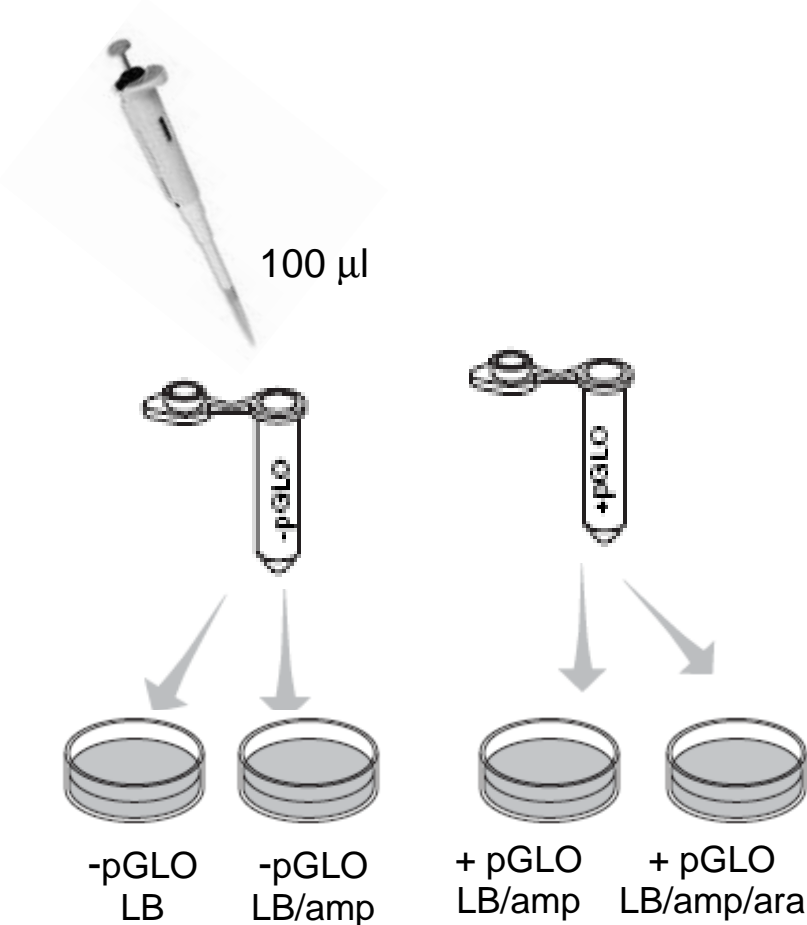
- Heat shock. Transfer the tubes to plastic holders in a water bath set at 42 °C for exactly 50 seconds. Make sure the bottom of the tubes contact the warm water. When the 50 seconds are done, place the tubes back on ice. For the best results, the change from the ice to 42 °C and then back to ice must be rapid. Incubate the tubes on ice for 2 minutes.



- Remove the tubes from ice and place them in a rack at room temperature. Using new sterile tips, add 200 μl of LB broth to each tube and reclose them. Incubate the tubes for 20 minutes at room temperature.



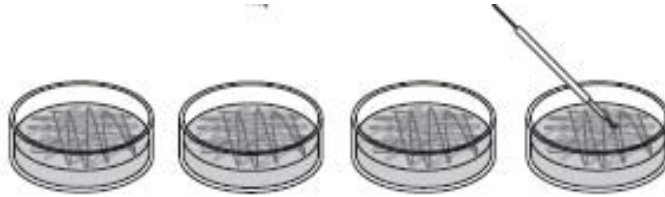
9. Gently flick the tubes to mix. Using fresh sterile tips for each tube, pipette 100 μ l of the transformation reactions onto the appropriate plates.



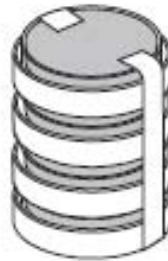
10. Using a sterile L-shaped spreader or loop, spread the transformation reactions around the surface of the agar by quickly skating the flat surface of the loop back and forth across the plate surface.



Use a fresh sterile spreader for each plate.



11. Stack up your plates in groups of 3 and 4 and tape them together. Put your group name and class section on the bottom of the stacks and place them upside down in the 37 °C incubator until the next day. (The prep lab personnel will remove them.)



Waste Disposal: When finished, dispose of any materials that contacted bacteria in the biohazard container. This includes tips, loops and microfuge tubes. Gloves and any remaining liquid LB and transformation solution can be disposed of in the regular trash. Save the starter bacteria plates and any remaining pGLO solution.

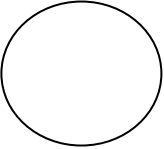
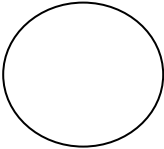
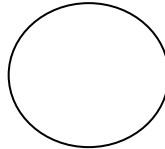
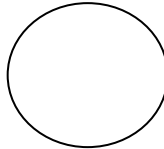
Name: _____

Section: _____

Bacterial Transformation & GFP Regulation Lab Worksheet

1. What is the purpose of the bacterial transformation lab?

2. **Data Collection:** Observe your plates under regular visible lighting and record the colony appearance by sketching a diagram of your colonies for each plate. Then observe your plates under UV light, and note the color of the colonies. Finally, count and record the number of colonies on the + GLO control LB/amp and + pGLO variable LB/amp plates.

| | | - pGLO LB | - pGLO LB/amp | + pGLO LB/amp | + pGLO LB/amp/ara |
|--------------------------------------|----------|--|---|--|--|
| Sketch of plate | |  |  |  |  |
| Number of Colonies | | | | | |
| Grow? | Expected | | | | |
| | Actual | | | | |
| Fluoresce green under UV light? | Expected | | | | |
| | Actual | | | | |
| Transformation Efficiency | | | | | |
| Average Transformation Efficiency | | | | | |

3. **Transformation Efficiency Calculation:** Bio-Rad reports that the average transformation efficiency, or how successful the bacteria were at incorporating the pGLO plasmid, for the procedure you followed is between 8.0×10^2 and 7.0×10^3 successful transformations per μg of plasmid DNA. The transformation efficiency is independent of arabinose in the nutrient agar. Calculate the transformation efficiency for the + pGLO LB/amp and + pGLO LB/amp/ara plates. Then, calculate the average transformation efficiency for these two plates. **Show all work and all units (μg , μl , etc.) for your calculations.**

a. From your pre-lab assignment, indicate the total amount, in μg , of pGLO DNA used:

Total μg pGLO = _____ μg

b. From your pre-lab assignment, indicate the total volume of your transformation reaction by adding the volumes of the reaction components:

Total Transformation Reaction Volume = _____ μl

c. From your pre-lab assignment, indicate the fraction of the transformation reaction spread:

Fraction of Transformation Reaction Spread = _____

d. From your pre-lab assignment, indicate the amount of pGLO DNA that went onto each plate:

Amount (μg) pGLO onto each plate = _____ μg

e. Calculate the transformation efficiency for the + pGLO LB/amp and + pGLO LB/amp/ara plates. **Show all work**, and write your answer in **scientific notation**. Scientific notation expresses numbers in powers of ten rather than multiple zeros. Review your notes on scientific notation from the Math and Chemistry Review in the Appendix if needed.

| |
|---|
| $\text{Transformation Efficiency} = \frac{\# \text{ colonies}}{\mu\text{g pGlo per plate}}$ |
|---|

f. Calculate the average transformation efficiency for the two plates above.

Average transformation efficiency = _____ colonies/ μg pGLO

