Green Fluorescent Protein II: Bacterial Transformation

Objective

To get some experience with recombinant DNA techniques and to genetically alter a bacterial strain to produce a particular protein.

Background

Green Fluorescent Protein (GFP) is a protein produced by the jellyfish *Aequoria victoria*. The protein fluoresces green when exposed to ultraviolet (UV) light. The goal of today's lab is to change the genes of a bacterium so that it now makes GFP and will fluoresce in UV light.

In today's lab, you will add a small DNA molecule called a plasmid to a bacterium called *E. coli*. These bacteria are the workhorse of modern recombinant DNA technology. The bacteria you are starting with cannot produce GFP (since the gene is normally only found in the jellyfish) and are killed by (aka "sensitive to") the antibiotic ampicillin

The plasmid you will be adding is called "pGLO" and it contains two genes:

- *Ampicillin Resistance* – this is a gene that confers the dominant phenotype of resistance to the antibiotic ampicillin.
- *GFP* – this is a gene that produces the GFP protein in bacteria.

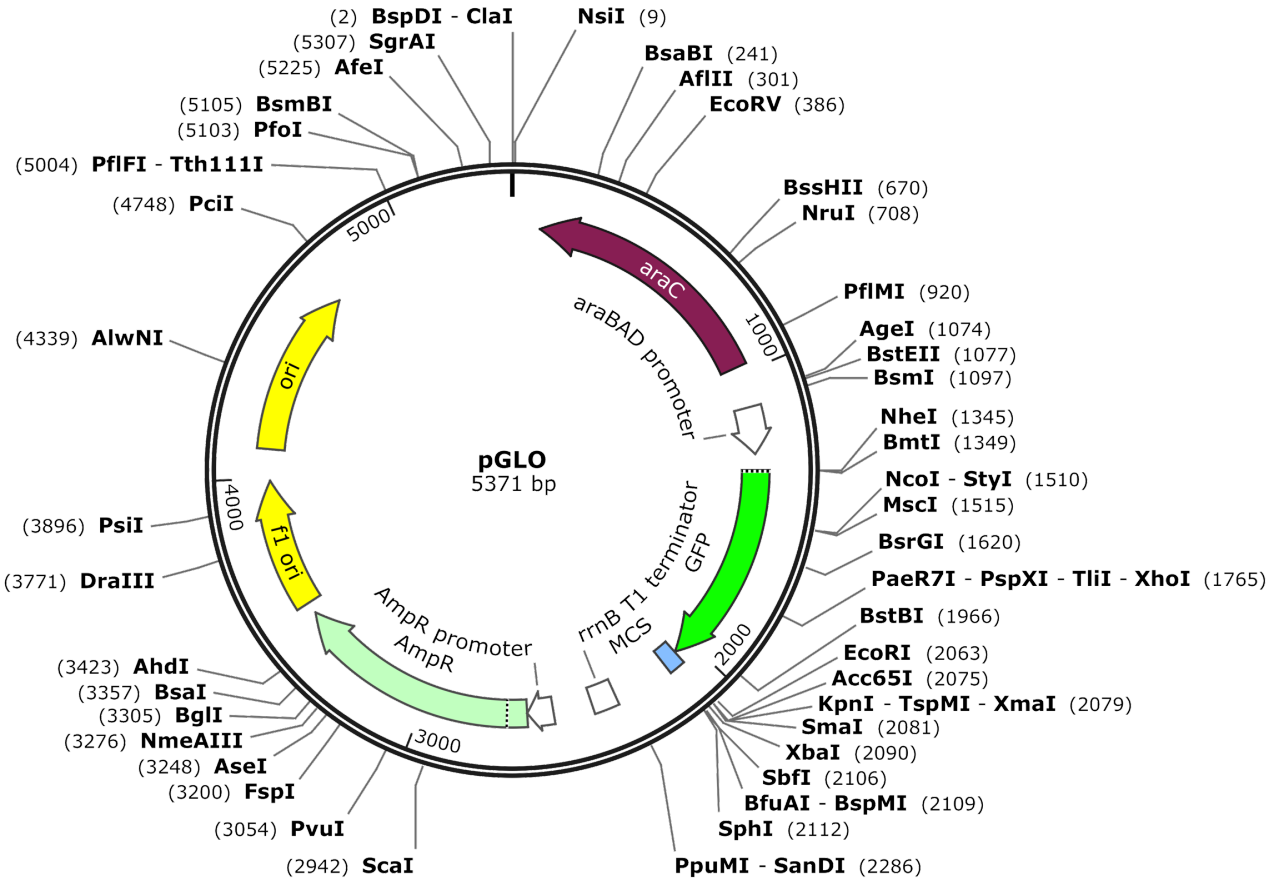
You will add the pGLO DNA to bacteria that have been treated to make them "competent" – that is, ready to take up DNA from the environment. However, only a very small fraction (fewer than 1 in 1,000,000) of the bacteria will take up the DNA.

The bacteria that do take up the pGLO DNA now have the genes on pGLO added to their genome. They have been "transformed". These bacteria are now resistant to ampicillin and produce GFP.

We next select for the transformed bacteria by growing the cells in the presence of ampicillin. Un-transformed cells are sensitive to ampicillin and are killed. However, those that were transformed with pGLO are resistant to ampicillin and will grow.

We will be growing our cells on solid medium, so a single surviving cell will give rise to a "colony" of 10^8 cells – a small pile of cells, all descendants of that original transformed cell. All the cells in the colony will carry pGLO and thus be ampicillin resistant and make GFP.

The genetic map of pGLO is shown below:



You can see the genes for ampicillin resistance (AmpR) and GFP; each of these genes has a promoter. We will talk more about plasmids and how they work in lecture.

Procedure I: Transformation

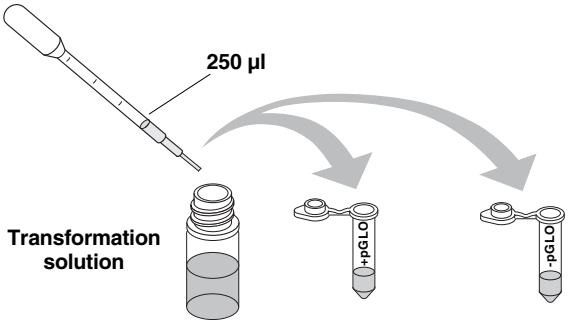
WARNINGS:

1. In general, the lab is unforgiving of mistakes like using the wrong solution or taking the wrong amount. The construction folks at "This Old House", say "Measure twice; cut once". We'll adapt this to "*Check twice; pipette once*".
2. Although the *E. coli* strain we use is non-pathogenic (it is not known to cause disease in healthy individuals), you should be careful with it. *Always wear gloves, don't eat or drink in lab, and wash your hands thoroughly when you are all done*.
3. **Sterile Technique**: Contamination is a big problem – it's a dirty world we live in. You should assume that all surfaces are crawling with nasty microbes. Never let any of the sterile picks, pipettes, etc. touch anything except the tube, plate, solution, or colony. If you even *think* that you've touched something you shouldn't, discard the loop, pipette, etc and get a clean one.

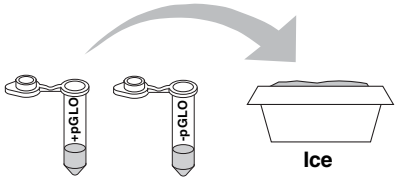
Note that while the figures show plastic transfer pipettes, we will be using pipetmen.

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.

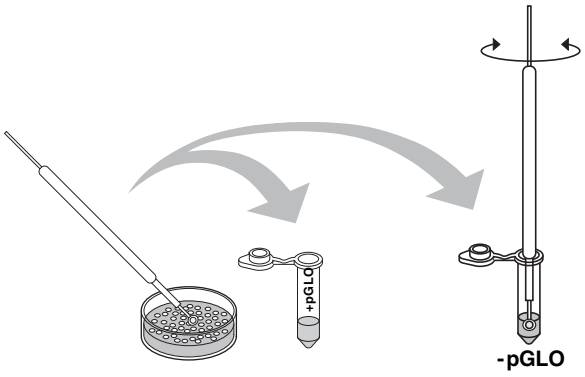
2) Open the tubes and using a transfer pipette, transfer 250µl of transformation solution ($CaCl_2$) into each tube.



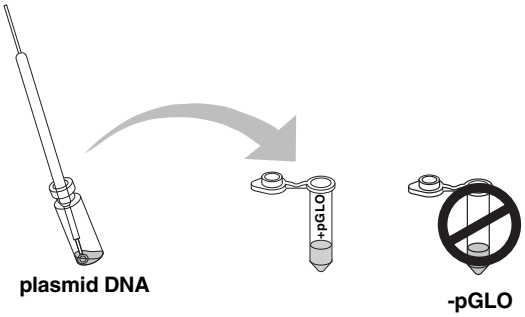
3) Place the tubes on crushed ice. Do not use cubed ice.



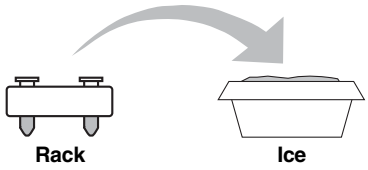
4) Use a sterile loop to pickup a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (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.



5) Your TA will give you a sample of pGLO plasmid DNA solution. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Using a pipettor, put 10µl of pGLO plasmid into the +pGLO tube and mix. Close the +pGLO tube and return it to the rack on ice. Do not add plasmid DNA to the -pGLO tube. Why not? Close the -pGLGO tube and return it to the rack on ice.

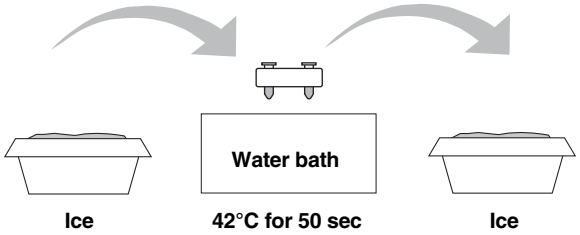


6) Incubate the tubes on ice for 10 min. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.

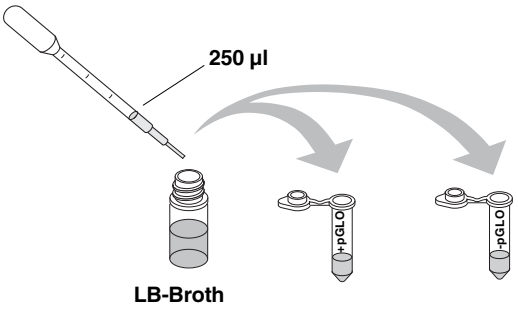


7) While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as shown on the diagram.

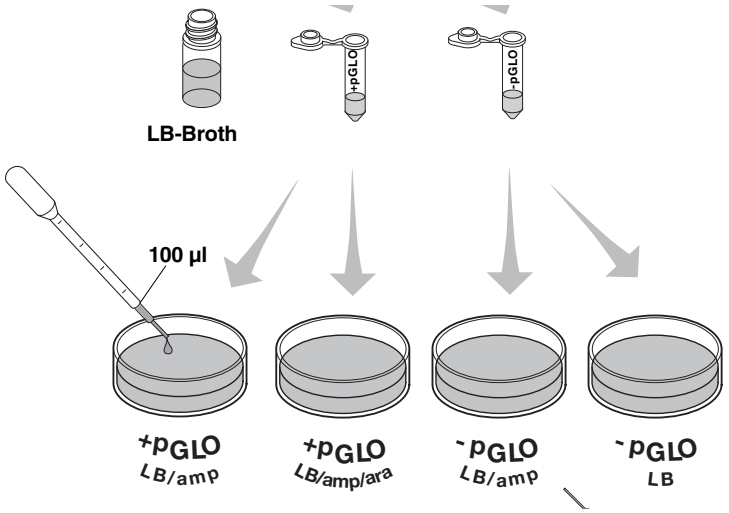
8) Heat shock. Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42°C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds have passed, place both tubes back on ice. For the best transformation results, the change from the ice (0°C) to 42°C and then back to the ice must be rapid. Incubate tubes on ice for 2 min.



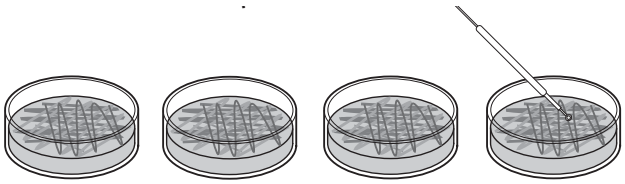
9) Remove the rack containing the tubes from the ice and place on the bench top. Open the pGLO+ tube and, using a new sterile pipet, add 250µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the pGLO- tube. Incubate the tubes for 30 min at room temperature.



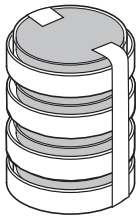
10) Gently flick the closed tubes with your finger to mix. **Using a new sterile pipet for each tube**, pipet 100ul from each of the tubes to the corresponding plates, as shown on the diagram onto the appropriate plates. Be sure to put the cells on the jello-like agar medium, not the plastic lid!



11) **Use a new sterile loop for each plate**. Spread the suspensions evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.



12) 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 upside down in the 37°C incubator until the next day.



Once your cells have grown, your TA will put them in the refrigerator so you can look at them during the last week of lab.

<u>Lab Report</u>

There is no lab report for this session. You will write up the results when you see the plates in a later lab.

Im9 III: Results

Purpose:
To explore protein structure and function by making novel mutations in a protein with known structure.

Procedure
Today, you will see the results of the functional assay of your mutant proteins.

Your TA will return your Im9 Worksheet to you and show you the results of applying Colicin E9 toxin to cells carrying the mutations you chose. You should complete the Im9 worksheet and discuss the results.

Cancer Cells

Purpose
To see some examples of what tumor cells look like and to connect this to what we've been talking about in lecture. This is the first year we've done this lab so we're interested to see what you can observe and find out.

Background
Several labs at UMB work on cancer in mice. Over the summer, one of the graduate students noticed a tumor in a mouse. We removed the tumor, preserved it, and sliced it into very thin sections. These sections were stained with a dye mixture called Hematoxylin-Eosin, a commonly-used tissue stain.

A commonly-used methods manual (https://pubmed.ncbi.nlm.nih.gov/21356829/) says the following about Hematoxylin-Eosin Staining:

> Hematoxylin and eosin (H&E) stains have been used for at least a century and are still essential for recognizing various tissue types and the morphologic changes that form the basis of contemporary cancer diagnosis. The stain has been unchanged for many years because it works well with a variety of fixatives and displays a broad range of cytoplasmic, nuclear, and extracellular matrix features. Hematoxylin has a deep blue-purple color and stains nucleic acids by a complex, incompletely understood reaction. Eosin is pink and stains proteins nonspecifically. In a typical tissue, nuclei are stained blue, whereas the cytoplasm and extracellular matrix have varying degrees of pink staining. Well-fixed cells show considerable intranuclear detail. Nuclei show varying cell-type- and cancer-type-specific patterns of condensation of heterochromatin (hematoxylin staining) that are diagnostically very important. Nucleoli stain with eosin. If abundant polyribosomes are present, the cytoplasm will have a distinct blue cast. The Golgi zone can be tentatively identified by the absence of staining in a region next to the nucleus. Thus, the stain discloses abundant structural information, with specific functional implications.

Today, you will look at the stained slides of the tumor. Try to determine which cells are normal and which are cancerous. What are the visible differences? Typically, cells in normal tissue are well-organized and similar in size while tumor cells are more disordered.

IGV Practice

Many students find it challenging to use the Integrative Genome Viewer (IGV) on the SPOC. In this part of the lab, your TA will help you to learn how to use the IGV by working through some of the SPOC IGV problems.