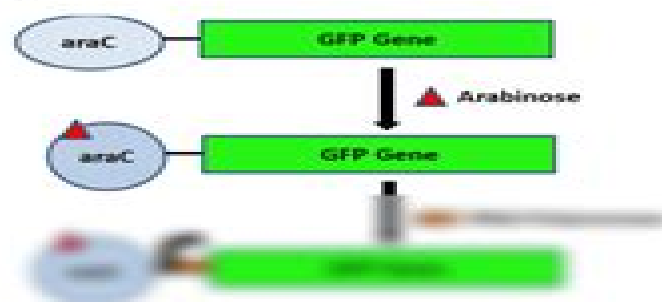


In this lab activity, you will be inserting pGLO into non-pathogenic *E. coli* bacteria. The procedure is never 100% efficient and only a few of your *E. coli* bacteria will successfully “take up” the pGLO. How will you know which cells contain the plasmid? pGLO contains a gene that codes for a protein that protects the cell against the toxic effects of antibiotics. This means that only cells that have the plasmid will survive in the presence of antibiotics. In this procedure, we use **ampicillin**, an antibiotic very similar to penicillin. This step is called **antibiotic selection**, and it allows you to select only the cells that have been transformed. The beta-lactamase gene in pGLO codes for a protein that breaks down ampicillin. Expression of the beta-lactamase gene in cells that have been successfully transformed allows them to grow in the presence of ampicillin. Non-transformed cells will die.

Your transformed cells will grow on a plate with ampicillin, but they will not fluoresce green until the GFP gene is turned “on”. Here’s where the idea of gene regulation comes into play. Transformed cells will grow on plates not containing arabinose, but will only fluoresce green under UV light when arabinose is included in the nutrient agar. Therefore, **arabinose**, a sugar that bacteria consume for energy, is the critical ingredient for making your bacteria glow.

What’s so special about arabinose? It teams up with the *araC*, the regulatory protein that is expressed by pGLO. Regulatory proteins control the timing and location of many cellular processes. Specifically, *araC* is a transcription factor which, as described previously, functions to turn genes on and off. But it can’t turn GFP on by itself – it needs the help of arabinose. Together, they work to bring in RNA polymerase, the enzyme that makes RNA, and only then can the glowing, green protein be made. It’s a finely orchestrated dance, and all the right players have to be in place for success.

**Figure 5**  
Gene regulation of  
GFP in pGLO



The diagram illustrates the process of gene regulation of GFP in pGLO. It shows a plasmid with the *araC* gene and the GFP gene. In the first state, the *araC* gene is transcribed into mRNA (indicated by a red triangle) and translated into the AraC protein (indicated by a blue circle). In the second state, the AraC protein binds to the GFP gene, and the GFP gene is transcribed into mRNA (indicated by a red triangle) and translated into the GFP protein (indicated by a green circle). The GFP protein then fluoresces green (indicated by a green circle).

#### The Transformation Procedure

The transformation procedure involves several steps. First, the plasmid is extracted from a donor cell. Then, the plasmid is mixed with a competent cell (a cell that is able to take up foreign DNA). The mixture is then subjected to heat shock, which allows the plasmid to enter the cell. Finally, the cell is allowed to recover, and the plasmid is expressed. The resulting cells are then grown on a plate containing ampicillin, which selects for cells that have successfully taken up the plasmid. The cells are then grown on a plate containing arabinose, which induces the expression of the GFP gene. The resulting cells are then grown on a plate containing both ampicillin and arabinose, which selects for cells that have successfully taken up the plasmid and are expressing the GFP gene. The resulting cells are then grown on a plate containing both ampicillin and arabinose, which selects for cells that have successfully taken up the plasmid and are expressing the GFP gene.

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**Rosina Ehmann**



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