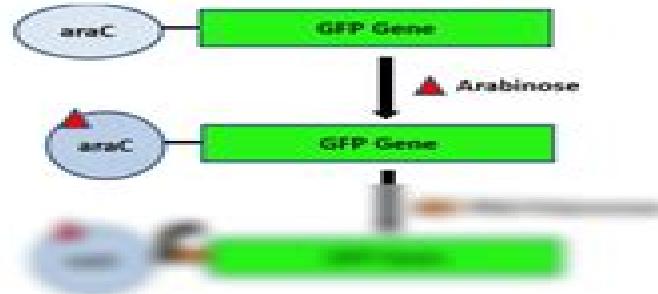


In this lab activity, you will be inserting pGLO into non-pathogenic *E. coli* bacteria. The procedure is never 100% efficient and only a few of your *E. coli* bacteria will successfully "take up" the pGLO. How will you know which cells contain the plasmid? pGLO contains a gene that codes for a protein 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



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