6.1: Genetic Transformation (using bacteria and the pGLO plasmid)

Genetic transformation is the process by which an organism acquires and expresses a new gene. Genetic engineering is the directed transfer of a gene, or piece of DNA, into a cell (typically a bacteria). Typically the intent is to force the cell to express (produce) the protein that the newly introduced piece of DNA codes for (known as heterologous expression). The organism commonly used for genetic transformation and heterologous expression of human genes/proteins is the single celled bacteria known as *Escherichia coli* (E. coli). This organism has several traits of importance in the laboratory:

- Single cell organism
- Doubling time is **20 minutes** (in rich media) to 1 hour (minimal media)
- Naturally lives in human gut (part of normal intestinal flora). Thus, **normal growth temperature is 37°C** (human body temperature)
- *E. coli* has a **single chromosome** that is a **circular DNA** molecule
- *E. coli* is **gram negative** - has a inner cytosolic membrane, periplasmic space, and outer cell membrane

![E. Coli genome](https://bio.libretexts.org/Bookshelves/Biochemistry/Supplemental_Modules_(Biochemistry)/6._Lab_Notes_Part_2/6.1%3A_Ge…)  

*Figure 6.1.1: E. Coli genome*

Foreign DNA can be introduced into the E. coli as a second "chromosome", or as another circular DNA molecule that
can replicate autonomously due to having its own origin of replication. Such "autonomously replicating elements" are referred to as "plasmids" or "vectors".

![Figure 6.1.2: Plasmid in E. Coli](image)

In order to "stably retain" the plasmid, there needs to be some type of metabolic reason for the *E. coli* to keep the plasmid around. If the plasmid contains a gene that codes for a protein that protects against antibiotics, then, only cells that have the plasmid will survive in the presence of that antibiotic. Drug resistance can therefore form the basis of a "selectable marker" for the presence of the plasmid in a sample of *E. coli*. Ampicillin is an antibiotic for gram negative cells such as *E. coli* (it is part of the penicillin family of antibiotics):

- Production of the outer cell membrane/proteoglycan structure is inhibited in the presence of ampicillin (a lethal event for the bacteria)
- Ampicillin, as a molecule, contains a **b-lactam ring** structure. This ring can be cleaved (and the ampicillin destroyed) by the enzyme **b-lactamase**
- **b-lactamase enzyme** is the product of the *bla* gene (genes are typically lower case and italicized)
- If a plasmid contains the *bla* gene, it will confer resistance to ampicillin to the host *E. coli*
- Such *E. coli* grown in the presence of ampicillin will be **selected for**. Under these conditions, any wild type *E. coli* in the sample will be **selected against**
- Thus, ampicillin resistance is a **selectable marker** for the **plasmid**

![Figure 6.1.3: Ampicillin resistance plasmid](image)

Other genes (that express other proteins) can now be introduced into the plasmid, and the host *E. coli* forced to express the protein of interest

**pGLO**

Plasmids are typically abbreviated with an acronym that begins with the lower case "p", and the name can provide some
information regarding the person that designed the plasmid, or the contents of the plasmid. The pGLO plasmid contains an origin or replication, a selectable marker, and the gene for Green Fluorescent Protein (GFP). The plasmid also contains a gene for the arabinose C protein, which is a protein that regulates expression from the arabinose BAD promoter (P\textsubscript{BAD}). Promoters are usually indicated with an acronym that begins with an upper case "P".

![Diagram of pGLO Plasmid]

**Figure 6.1.4:** pGLO Plasmid. The ori is the origin of replication for the pGLO plasmid, bla is the gene that codes for b-lactamase, and is the selectable drug-resistant marker for the plasmid, GFP is the GFP gene and araC is the gene coding for the arabinose C protein

A "promoter" is a region of DNA that signals RNA polymerase to initiate transcription (for production of mRNA). Promoters are typically located at the start (5' end) of a gene that codes for a protein (since mRNA production proceeds 5'->3'). The bla gene includes a promoter at the 5' end of the gene. This is a weak constitutive promoter (always "on" at a low-level). It will instruct RNA polymerase to continually make a low-level of mRNA for this gene. The mRNA will be translated to produce low-levels of the b-lactamase protein. The GFP is transcribed by the arabinose P\textsubscript{BAD} promoter. This is a **strong** promoter and will cause RNA polymerase to make a large number of copies of mRNA from this gene (and therefore, a lot of GFP protein). However, the arabinose P\textsubscript{BAD} promoter is regulated by the protein coded for by the araC gene (which has its own promoter, much like the bla gene):

- In the **absence** of the sugar arabinose, the araC protein binds to the PBAD promoter and **prevents transcription** (of GFP)
- In the **presence** of arabinose in solution, the araC protein binds the arabinose, and this results in a conformational change to the araC protein; the result of which is that it now instructs RNA polymerase to make **many copies** of the GFP mRNA (and thus, a lot of GFP protein)

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**How is the pGLO plasmid introduced into the E. coli cell?**

The general process by which foreign DNA is introduced into a cell is called **transformation**. There are several ways to transform DNA into an E. coli cell, but the most common way is by making the cells competent. "Competent" cells have the ability to take up DNA molecules from the environment. Normal E. coli are **not** competent, however, if they are treated with a solution of calcium chloride their cell membranes become competent. Actually, only a small fraction of the cells treated with CaCl\textsubscript{2} are able to take up foreign DNA, however, since the number of cells in a sample is large, the low efficiency of transformation is not much of a problem. The selectable markers ensure that only the cells that did take
up the foreign DNA (i.e. plasmid) will survive and grow.

![Diagram of transformation of E. Coli](image)

**Figure 6.1.5: Transformation of E. Coli**

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**Expression of GFP**

Addition of arabinose sugar to the growth media will cause RNA polymerase to start transcribing the GFP gene (i.e. making mRNA molecules). The cellular machinery (e.g. ribosomes) will translate this mRNA into corresponding GFP protein.

- GFP will fluoresce green under UV light
- The presence of green light from E. coli cells indicates that the transformation (and drug resistance selection) has been successful
- GFP is a gene from a jelly fish and is the reason that some jelly fish glow green. It is not a normal gene for E. coli, however, if introduced into E. coli, it will make the GFP protein (and fluoresce green)