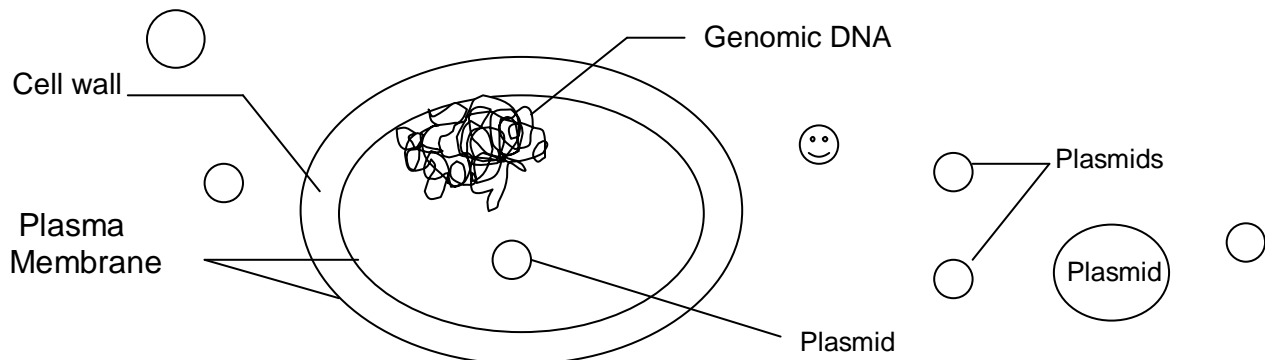


Introduction to Transformation: (more complete explanation for pGLO lab)

Thus far, you've produced ligated, recombinant plasmids. Hopefully, some of these DNA recombinants will have the small pGRN fragment, the one representing the gene for green fluorescent protein, ligated into the pDRK plasmid in the correct orientation. This plasmid is referred to as rpGLO ("r" referring to *recombinant*). Now, we want to get these recombinant plasmids into bacterial cells so that we can get the cells to express this gene and make green fluorescent protein.

The process of taking up foreign pieces of DNA, like a plasmid, into a bacterial cell is called *transformation*. Transformation is a process that occurs in nature although it is probably somewhat rare. A British medical officer, Frederick Griffith first studied the process, in 1928. Bacteria usually pass on extra chromosomal genetic material, like plasmids, during conjugation (bacterial sex) rather than relying on luck. But taking up plasmids can provide bacteria with certain genes that confer selective advantage, for example antibiotic resistance. Under experimental conditions, however, it is possible to prepare cells so that about one cell in a thousand will take in a plasmid from the surrounding environment.

There are several factors that determine transformation efficiency. Two of these are related directly to the plasmid used for transformation. The larger the plasmid, the less likely it will be taken up by the bacterium. Remember, in order for the bacterium to take in foreign DNA, the plasmid must pass through bacteria's plasma membrane and cell wall.



Therefore, small plasmids are more likely to pass through the bacterium's plasma membranes (*E. coli* has two) and its cell wall than large plasmids.

Plasmids can assume different shapes. The supercoiled form is the easiest to get into the cell while the nicked-circle or the multimer, two or more plasmids linked together, are more difficult. The ligation tube, containing the recombinant plasmids you prepared, does not contain any supercoiled plasmids. This form requires an enzyme that is found only in the bacterial cell. The recombinant plasmids you prepared are primarily nicked-circled but there is a wide variation in sizes.

In nature, transformation is a relatively rare event. To increase our chances of getting our recombinant plasmids into bacterial cells we will use "competent" cells. When cells are "competent," it means that they are ready to receive plasmids. For the most part, you don't find competent cells in nature; instead, cells have to be made competent in the laboratory. One common way this is done is by soaking the cells in calcium chloride.

Although the *E. coli* strain that you are using in these labs, HB101, is relatively benign, it is important that you use proper techniques when handling them.

Remember that DNA is negatively charged. Do you remember why? The plasma membranes surrounding the bacterial cell also contain phosphate groups and are negatively charged. The problem of trying to get negatively charged DNA past a negatively charged membrane is that *like* electrical charges tend to *repel* each other. When cells are made competent, they are suspended in a solution of calcium chloride because calcium ions (positively charged atoms of calcium, Ca^{++}) help to neutralize the negative electrical charges of the plasma membrane and the plasmid. With these repulsive charges neutralized by the calcium ions, the plasmid DNA has an easier time passing by the plasma membrane of the bacterial cell. The cells have already been made competent for you and your teacher will give you an aliquot. You will, however, need to do the next step.

Now that we have the negative charges on the DNA and the plasma membranes neutralized, we need to create a bit of a pressure difference between the inside and the outside of the bacterial cell. This is done by first getting the bacteria really cold and then quickly putting them into warm water. This is called “heat shock” and it creates a situation where the pressure outside the cell is a tiny bit higher than inside the cell. This pressure gradient will help to move the plasmid DNA from the outside to the inside of the bacterial cell. Following this brutal treatment, we’ll need to feed our bacteria and let recover for a few minutes before we spread them onto agar plates.

Once the cells have recovered, you’ll take samples of these cells and spread them on a series of sterile agar plates. One of these plates will contain only bacterial food; it contains no antibiotic. This plate is marked “**LB.**” A second plate contains LB and ampicillin; this plate is marked “**amp.**” The third plate contains LB, ampicillin and a simple sugar called arabinose; this plate is marked “**ara.**”

Ampicillin is an antibiotic that prevents bacteria from fully forming its cell wall. Cells that are not ampicillin resistant cannot grow in its presence; the new cells simply rupture or lyse. If a cell receives an ampicillin resistant gene, *amp^r*, it will produce a protein that will chemically decompose ampicillin and, therefore, will be able to grow with ampicillin in its environment.

Arabinose, a simple sugar, is needed by the bacterium to express the green fluorescent protein gene. If a bacterium takes up rpGLO, arabinose helps the enzyme needed to transcribe the *gfp* gene to align itself correctly on the gene. This relationship will be discussed in the next lab.