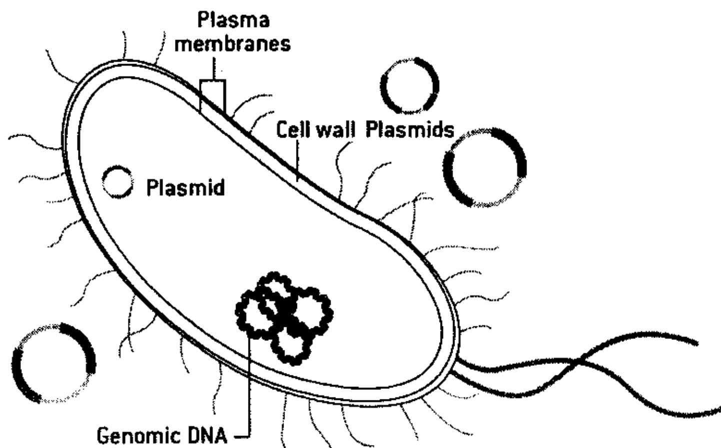
Transformation of *Escherichia coli* with pARA-R

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.

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.

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.

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 in which 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.

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. The plate 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 destroy 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 *rfp* gene. If a bacterium takes up pARA-R, arabinose helps the enzyme RNA polymerase, needed to transcribe the *rfp* gene, to align itself correctly on the plasmid. This relationship will be discussed in the next lab.

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

Materials

REAGENTS AND CULTURES	EQUIPMENT & SUPPLIES
E. coli (LMG) plate	1.5 mL microfuge tubes
Crushed ice	Sterile inoculating loop
10 µL pARA-R (10 ng/µL)	Disposable cell spreaders (2)
1 mL 50 mM $CaCl_2$	Microfuge tube rack
1 LB plate	Permanent marker
1 LB/amp plate	P-20 micropipette and tips
1 LB/amp/ara plate	P-200 micropipette and tips
	Beaker with disinfectant
	42° C water bath

Methods

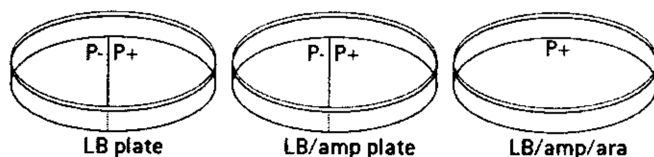
Preparing competent cells for transformation

1. Bacterial transformation requires **sterile** techniques. It is essential that directions be followed precisely.

2. Use the marker to label one of the 1.5 mL sterile microfuge tubes **P+** and the other tube mark **P-**. Plasmid DNA will be added **only** to the P+ tube. The P- tube will represent a negative plasmid control.

3. Pick up a tube containing $CaCl_2$. Use your P-200 pipette and a clean tip to transfer 250 µL of $CaCl_2$ to the P+ and P- tubes. **Hint:** Set the P-200 pipette to 125 µL and transfer two aliquots to each of the labeled tubes. The pipette should read "1-2-5" in the display window.

4. Your instructor will provide the class with a Petri plate containing colonies of *E. coli* cells. Use a sterile inoculating loop to gently scrape two or three large bacteria colonies from the Petri plate and transfer them to the P+ tube. Knock the loop against the side of the tube to dislodge the colonies from the loop. Cap the tube and vigorously drag the tube across the surface of your microfuge tube rack to suspend the cells in the $CaCl_2$. Continue to do this until you can no longer see any visible clumps of bacteria. Place this tube into your crushed ice.



5. Repeat this procedure using the same inoculating loop, but transfer the colony and suspend the cells in the P- tube. Place this tube in the crushed ice. Place the inoculating loop in disinfectant or cell waste bag for proper disposal.

6. Transfer 10 µL of plasmid (pARA-R) **directly into the cell suspension in the P+ tube.** Briefly finger vortex the mixture by gently flicking the bottom of the microfuge tube with your index finger. Avoid splashing the mixture on the sidewall of the transformation tube. Return the P+ tube to the crushed ice.

7. Incubate both tubes in crushed ice for 15 minutes. Be certain the tubes are in contact with the ice. It is important that the cells get very cold.

8. Obtain one each of the following plates: LB, LB/amp and LB/amp/ara.

9. Using a marker and straight edge, draw a line down the center of the LB and LB/amp plates, but **not** the LB/amp/ara plate. Make this division on the **bottom gel side** of the two plates. Label a "P-" and a "P+" on each half of LB and LB/amp plate bottoms and a "+" on the LB/amp/ara plate bottom.