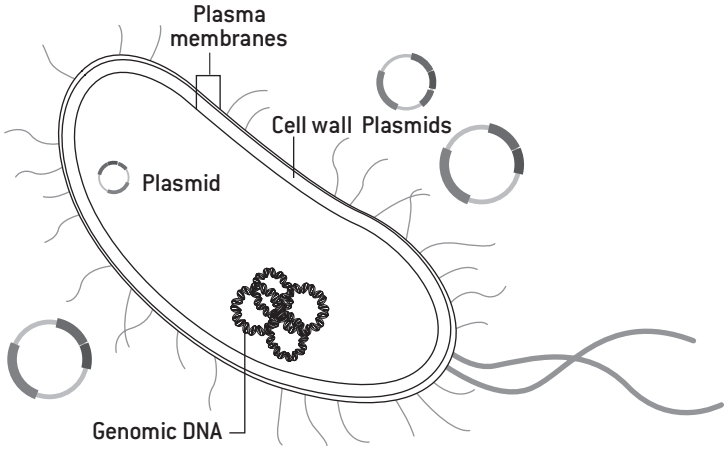
Transformation of *Escherichia coli* with pARA-R

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

100 µL of competent *E. coli* (LMG194)
Crushed ice
12 µL pARA-R (10 ng/µL)
350 µL of sterile LB broth
1 LB plate (one stripe)
1 LB/amp plate (two stripes)
1 LB/amp/ara plate (three stripes)

EQUIPMENT & SUPPLIES

1.5 mL microfuge tubes
42° C water bath
Disposable cell spreaders (2)
Microfuge tube rack
Permanent marker
P-20 micropipette and tips
P-200 micropipette and tips
Beaker with disinfectant

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 a sterile 1.5 mL microfuge tubes **P+** and another sterile 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 Styrofoam cup with packed crushed ice and place one tube containing 100 µL of competent cells into the ice. It is **important** that these cells remain at 0°C. Also, place your labeled P+ and P- tubes into the ice.

4 Set the **P-200** pipette to 50 µL (set to "0-5-0") and place a clean tip onto its barrel. Very carefully re-suspend the cells by **gently** pumping the cells in and out two times. Hold the tube by the upper rim to avoid warming the cells with your fingers. Aliquot 50 µL of the re-suspended cells into the pre-chilled P+ and P- microfuge tubes. Immediately return these tubes, containing the cells, to the wet ice.

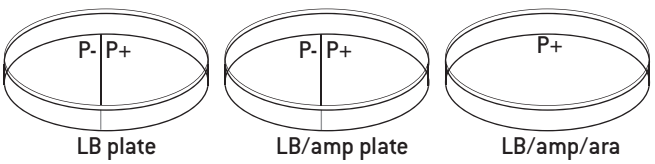
5 Pick up a tube of pARA-R plasmid and a tube of LB broth from your teacher.

6 Using your **P-20** pipette, 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. The cup should be solidly packed with ice. 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 a straight edge, draw a line down the ***center*** of the LB (one stripe) and the LB/amp (two stripe) plates, but not the LB/amp/ara (three stripe) plate. Draw the lines on the ***bottom*** of the two plates. Place a "**P-**" and a "**P+**" on each half of the LB and LB/amp plates and a "**P+**" on the LB/amp/ara plate.



Transforming *E. coli* with pARA-R

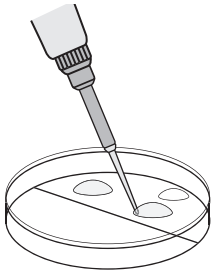
1 Following the 15 minute chilling in ice, heat shock the cells in both tubes using the following procedures:

- **Take the ice container containing your cells** to the 42° C water bath. It's important that the bacteria receive a distinctly abrupt change in temperature, so keep the tubes in the ice until you can place the tubes in the hot water bath.
- Hold both tubes into the water bath for **exactly 45 seconds.**
- Immediately return both tubes to the crushed ice for at least one minute

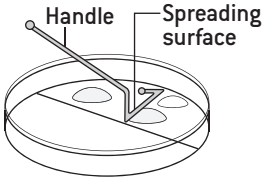
2 Following the one-minute cool down, use the **P-200** pipette to add 150 μL (set to "1-5-0") of LB broth to both the P- and P+ tubes. Cap the tube and gently flick the lower portion of the tube two or three times to mix. If time permits, allow the cells to incubate at room temperature or at 37° C for 15-20 minutes.

Spreading transformed cells on agar plates

1 Place the three plates in the following order: LB, LB/amp, LB/amp/ara.



2 Set the **P-200** pipette to 50μL. The pipette will read "0-5-0." Holding the P- tube (control) between your thumb and index finger, **gently** flick the bottom of the tube to re-suspend the cells. Deposit 50 μL of these cells onto the "P-" half of the LB and LB/amp plates. ***Do not* deposit these cells on the LB/amp/ara plate.**



3 Open the package of sterile cell spreaders **at the end closest to the spreader handles.** You will share this package with another group. Remove only one spreader, keeping the others sterile. Hold the spreader by the handle and do not allow the bent end to touch any surface as this will contaminate the spreader. Close the package to avoid contaminating any of the other spreaders.

4 Open the lid to the ***LB* plate**, like a clam shell, and gently using a light, gliding motion spread the cells across the surface of the agar keeping the cells on the "P-" side of the plate. Try to spread them evenly and along the sides of the plate as well.

5 Using the same spreader, repeat this spreading procedure for the LB/amp plate.

6 Discard the cell spreader in disinfectant or the cell waste bag following its use on the LB/amp plate.

7 After **re-suspending** the cells in the "P+" tube, use a new tip and deposit 50 μL of cells to the "P+" side of the LB **and** LB/amp plates; deposit **100 μL of cells to the LB/amp/ara plate.**

8 Using a new cell spreader, repeat steps 4 and 5 spreading the cells on the "P+" side of the LB and LB/amp plates and over the **entire** surface of the LB/amp/ara plate.

9 Discard this cell spreader in disinfectant or the cell waste bag.

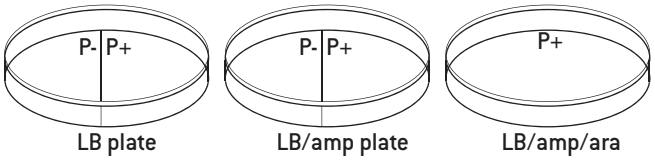
10 Use masking tape to keep your plates together. Place your name on the tape so that you can locate your plates later. Place the plates upside down into a 37° C incubator.

11 Incubate the plates for 24 hours at 37° C.

Conclusions

Answer questions 1-3 *before* seeing the results of your transformation.

1 Predict the growth, if any, on the following plates. Remember the cells from the P+ culture were given the plasmid while those from the P– were not. Use a "+" if you expect growth and a "–" if you expect no growth.



2a What do all the cells growing on the LB/amp and LB/amp/ara plates have in common?

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2b What fragment of the pARA-R plasmid allows these cells to grow on these plates?

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2c What is the size, in base pairs, of this fragment?

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3 On which plate(s) would you expect the cells to express the *rfp* gene?

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Answer these questions *after* viewing the results of your transformation.

4a Use the following table to compare how your actual transformation results differed from your predicted results.

Plate	Predicted results	Actual results
LB		
LB/Amp		
LB/Amp/Ara		

4b If your actual results differed from your expected, propose some reasons that might explain these differences.

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5a How many "red" colonies were present on your LB/amp/ara plate?

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5b Why did the red colonies only appear on this plate and not the LB/amp plate?

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