

Introductory Bacterial Conjugation Kit

In this experiment you are introduced to a naturally occurring mechanism called conjugation, by which DNA from one cell is transferred to another cell to produce a new recombinant cell. Sometimes the DNA that is transferred codes for antibiotic resistance. The intercellular transfer of this bacterial DNA coding for resistance to antibiotics enables the new recombinant bacterial cell to express resistance to an antibiotic to which it was formerly sensitive. This transfer is also considered a type of genetic recombination.

While bacterial chromosomes normally carry all the genes necessary for growth and reproduction, bacteria also contain genes carried on extra chromosomal DNA, called plasmids. Plasmids are double-stranded circular pieces of DNA that may carry anywhere from 3–25 genes. Numerous plasmids have been described in a variety of bacteria. Plasmids contain specialized genes, can replicate independently of the bacterial chromosome, can move from one bacterial cell to another, and may even be exchanged between cells of different bacterial species.

One of the first plasmids to be described was originally called the “F (fertility) Factor.” This plasmid, found in the common colon bacterium *Escherichia coli*, contains about 25 genes, most of which regulate the formation of pili, elongated appendages that extend from the surface of the cell. These pili can function as a bridge between two bacteria cells, thus allowing the transfer of DNA from the donor to the recipient. This process is called conjugation.

In 1959, it was shown that resistance to antibiotics can be transferred between bacteria during conjugation, and that this transfer involves plasmids. Plasmid-mediated drug resistance has created numerous problems for physicians and patients, because bacteria are able to transfer these “resistance genes” very rapidly. Under optimal conditions, the rate at which a conjugative plasmid can spread through a population can be exponential, showing resemblance to a bacterial growth curve.

In preparation for Lab Day 1, pure cultures of *E. coli* strains I (resistant to streptomycin; Str^r) and II (resistant to ampicillin; Amp^r) are grown one or more days in LB broth, an enriched culture medium lacking antibiotics. On Lab Day 1, samples of each strain are transferred to LB agar plates containing the enriched growth medium and the antibiotics to which each is known to be resistant. This exercise confirms resistance or sensitivity of these strains to the antibiotics. On Lab Day 2, after confirmation of an appropriate growth response for each strain, samples of the two strains are mixed together on an LB agar “mating” plate where conjugation between strains I and II is expected to occur. This should result in the transfer of DNA and the formation of recombinant cells.

After overnight growth, the cells on the “mating” plate are transferred to three different antibiotic-containing plates to test for their growth response to the same antibiotics previously tested. The results from these plates should demonstrate that DNA (coding for antibiotic resistance) can be transferred between two genetically different bacterial cells.

Lab Day 1

Confirming Antibiotic Resistance in Each Bacterial Strain

1. On the bottom surface, draw a line down the middle of each of the four different agar plates as shown in Figure 2. Write “I” on one side of the line, and “II” on the other side.
2. Obtain the *E. coli* I and II broth cultures that will be shared between your group and one other lab group. Strain I has a gene for streptomycin resistance (Str^r) in the chromosome. Strain II has a gene for ampicillin resistance (Amp^r) on a plasmid (see Figure 1).

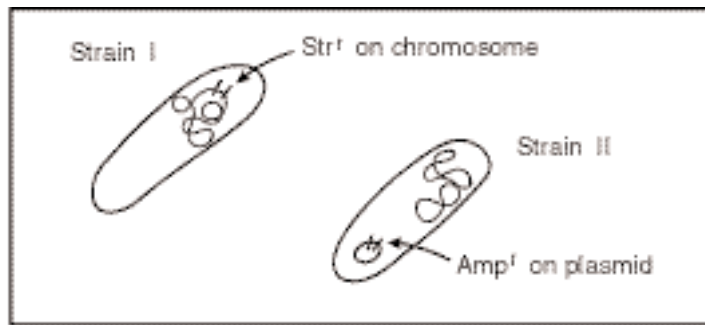


Figure 1. Location of genes for antibiotic resistance in *E. coli* strains I and II

- Open the handle end of a loop's wrapper and remove the loop, being careful not to touch anything. Using the sterile technique described by your teacher, remove and hold the cap (open surface down) from the Strain I broth culture bottle. Dip the sterile loop into the Strain I culture while holding the cap slightly away from and not touching anything. Withdraw the loop and replace the cap immediately. Be careful not to touch the loop to anything before placing it into the bottle or after withdrawing it from the bottle. If you suspect something has been touched, discard the loop and obtain a fresh one. (One person may want to maneuver the cap or steady the bottle for another person so that the work may be accomplished quickly.) Touch the loop lightly to the agar in the middle of each section marked "I" on each of the four plates (see Figure 2).

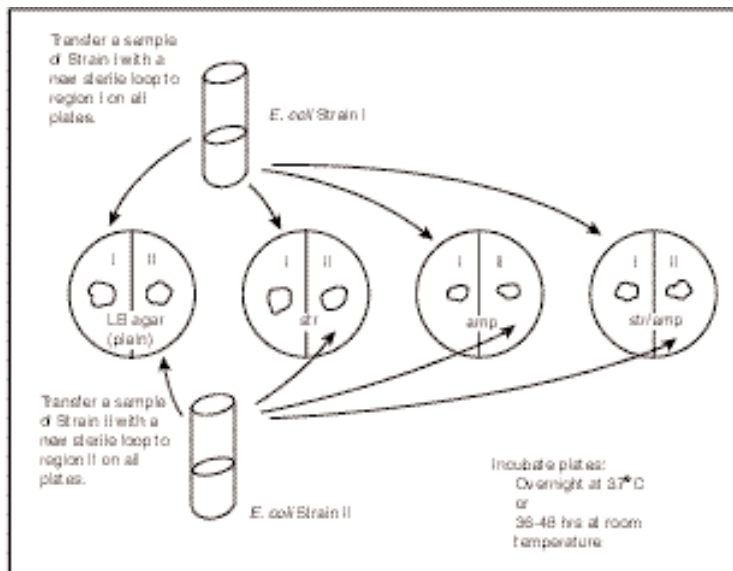


Figure 2. Preparation of "confirmation" plates to confirm the resistance (growth) or sensitivity (lack of growth) of *E. coli* strains I and II to streptomycin (str) and ampicillin (amp)

- Using a new loop, repeat Step 3 with the second culture to inoculate Region II in each plate. Discard the loop into a disposal cup or biohazard bag or replace it into its wrapper for disposal later. Allow the plates to sit briefly so the liquid can soak in before the plates are inverted for incubation. Tape the four plates together and mark them clearly with your group's initials. Incubate the plates overnight at 37°C, or for 36–48 hours at room temperature.
- In Table 1, list the results you "expect" to obtain following incubation. Use (+) for bacterial growth (i.e., resistance to the antibiotic) and (–) for lack of growth (i.e., sensitivity to the antibiotic). In the space below, give your reasons for your "expected" results.

***E. coli* Strain and Results**

Contents of Petri Plates	I		II	
	Expected	Observed	Expected	Observed
LB agar				
LB agar+str				
LB agar+amp				
LB agar+str+amp				

Table 1. Expected and observed results of the growth of *E. coli* strains I and II on plain LB agar and the antibiotic "confirmation" plates containing LB agar plus streptomycin (str), ampicillin (amp), and streptomycin plus ampicillin (str+amp)

Lab Day 2

Observation of "Confirmation" Plates and Preparation of "Mating Plates"

1. Examine the "confirmation" plates prepared during the first lab session and record the "observed" results in Table 1. Compare the "expected" and "observed" results and account for any differences between these results and those of other students and with those provided by the instructor.
2. Prepare a "mating" plate as shown in Figure 3. Remember that this plate is used to bring the cells of strains I and II into close proximity so that conjugation (mating) and the transfer of DNA between cells can occur. Use a sterile loop to place a Strain I sample on the mating plate as shown in Figure 3. Use a new sterile loop to place a Strain II sample on the mating plate as shown in Figure 3. Use a new sterile loop to mix Strains I and II as shown in Figure 3. Open the lid of the plate just enough to slip the loops under, being careful not to touch anything. Do not lay the lid down on the lab bench. The loops should be disposed of in a disposal cup or biohazard bag or replaced into their wrappers for disposal later.

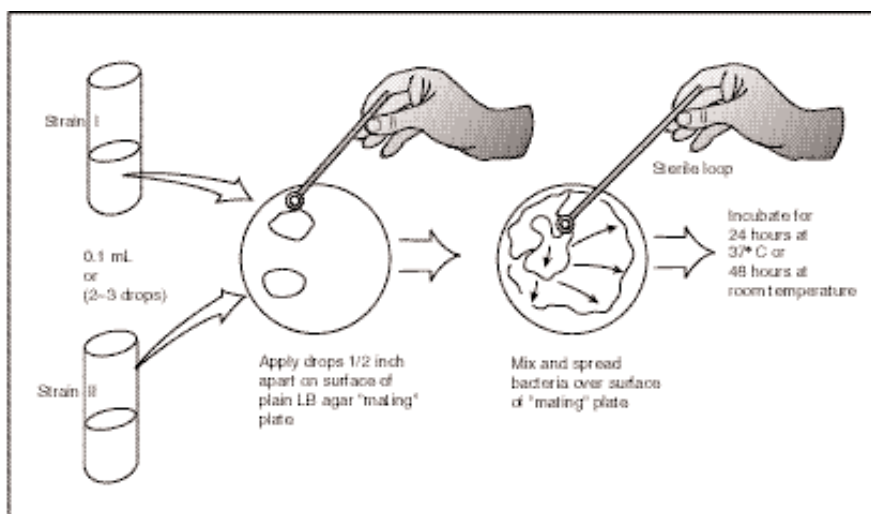


Figure 3. Cells of strains I and II are mixed and spread on a plain agar plate to allow for conjugation

Lab Day 3

Observation of Mating Plate and Testing for Recombination

Examine the “mating” plate prepared during the second lab session. Whether the growth observed represents “recombinant” cells (i.e., those in which conjugation has occurred) or merely growth of the separate strains of *E. coli* must be determined by transferring samples of cells from this “mating” plate onto plates containing the antibiotics previously tested. When doing this, lift the lids only enough to allow access for the transfer loops. Determine whether conjugation and the subsequent formation of recombinant cells have occurred, as follows:

1. Remove one sterile yellow transfer loop from the package using the handle end. Do not touch the loop to anything. Slightly lift the lid of the agar “mating” plate containing the “suspected” recombinant cells and pick up some bacteria. Close the lid. Streak some of these bacteria onto the LB/amp plate (see Figure 4). Discard the loop in the disposal cup or biohazard bag or replace it into its wrapper for disposal later. Repeat this process for the LB+str and LB+amp+str plates so that the LB+amp, LB+str, and LB+amp+str plates have each been innoculated with suspected “recombinant” cells from the mating plate. A new sterile loop should be used for each transfer. Incubate the plates overnight at 37°C or for 24–48 hours at room temperature.
2. Record the “expected” growth results for the recombinant cell growth only, in Table 2.

Recombinant Cell Growth

Contents of Petri Plate	Expected	Observed
LB agar+str		
LB agar+amp		
LB agar+str+amp		

Table 2. Growth response of recombinant cells to the antibiotics streptomycin (str), ampicillin (amp), and str+amp. Use (+) for growth (i.e., resistance to the antibiotic) and (–) for lack of growth (i.e., sensitivity to the antibiotic).

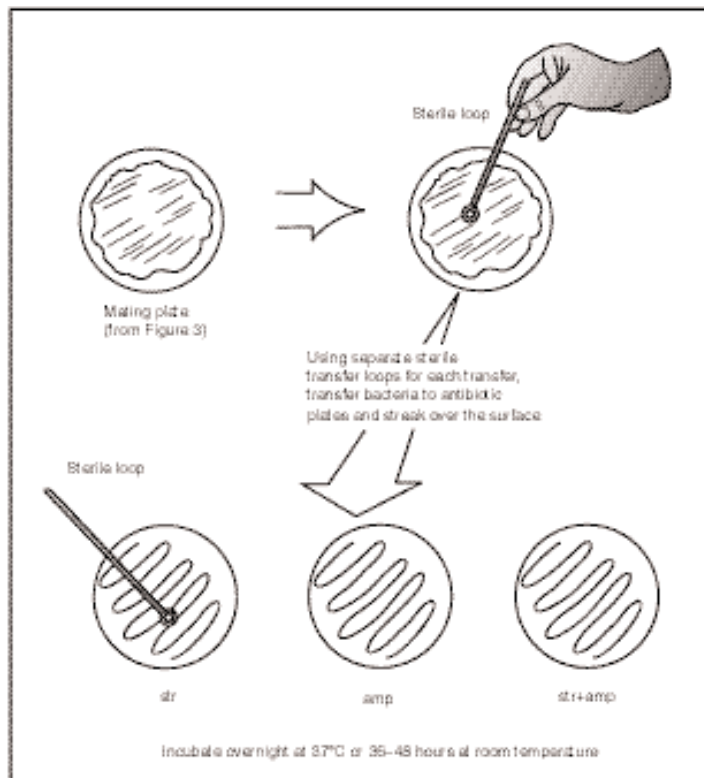


Figure 4. Testing for recombination. Plates contain LB agar plus streptomycin (str), ampicillin (amp), and streptomycin plus ampicillin (str+amp).

Lab Day 4

Observation of Recombinant Cell Growth

Record the “observed” results of the recombinant cell’s growth response on the various antibiotic plates in Table 2. Based on the growth responses to the various antibiotics, have conjugation and transfer of DNA (recombination) occurred? Explain.

Results and Discussion

1. In the confirmation tests, why did Strain I not grow on the LB+amp plate?
2. In the confirmation tests, why did Strain II not grow on the LB+str plate?
3. Why did neither strain grow on the confirmation plate containing streptomycin and ampicillin?
4. Can you tell whether the growth on the mating plate consists of recombinant cells only or merely the growth of strains I and II? Explain.

5. If growth is present on the plate containing ampicillin plus streptomycin (Figure 4), does this support the prediction of the recombination of antibiotic-resistant genes into a new strain? Explain.
6. Based on the results of this study, can one determine whether the Str^r gene from Strain I was transferred to Strain II or whether the Amp^r gene on the plasmid was transferred from Strain II into Strain I? Explain.