

# Conjugation

Bacterial conjugation or "mating" is one method by which bacteria can transfer information from one organism to another. The transfer involves the formation of a conjugation pilus (sex pilus) which connects the donor and recipient cells. During conjugation a conjugal or mobile genetic element (plasmid) is replicated (or copied) while one copy of the genetic element is transferred from the donor to the recipient cell. Bacterial conjugation is an important process in studying bacterial genetics and molecular biology.

Conjugation is also used in biotechnology to transfer genetic information from one strain to another. It is important to realize, however, that this same process occurs naturally in the environment and is not limited to a procedure conducted in the lab. Many different types of genetic elements can be transferred during conjugation. These elements can carry information for bacterial fertility, encoding special enzymes, such as biodegradation enzymes, heavy metal or disinfectant resistance, and antibiotic resistance. Transfer of this information from one organism to another is a serious problem because a new (and perhaps more pathogenic) strains can suddenly acquire resistance for several antibiotics by producing enzymes that degrade the antibiotic. Overuse of antibiotics and failing to follow-through with a complete antibiotic regimen can also select for bacterial strains resistant to one or more antibiotics. Hospital environments, where several different pathogenic organisms carrying varying antibiotic resistance can conjugate, may accelerate the development of multiple-resistant bacterial strains.

This laboratory exercise will demonstrate the process of conjugation using two different *Escherichia coli* (*E. coli*) strains, each resistant to a separate antibiotic, either ampicillin or streptomycin. The conjugation will be done by mixing the donor and recipient strains on a membrane filter. Membrane filtration is often used to sterilize solutions that cannot withstand the heat of autoclaving. Membrane filtration is also used in some cases to count microorganisms.

## Equipment and supplies (per group of 4 students)

Vacuum or vacuum pump set up (pump, trap, valve, tubing side-arm filter flask)

Filter forceps

Sterile membrane filters (individually sterilized, 47 mm diameter, 0.45  $\mu$ m pore size with or without grid)

Bunsen burner and ethanol to sterilize forceps

Autoclave bag for the disposal of wastes

2ml of overnight *E. coli* - Donor strain - (Ampicillin<sup>+</sup> Streptomycin<sup>-</sup>)

2ml of overnight *E. coli* - Recipient strain - (Ampicillin<sup>-</sup> Streptomycin<sup>+</sup>)

LB agar plates

2 LB agar plate (without any antibiotic)

1 LB agar plate with ampicillin (100  $\mu$ g/ml)

1 LB agar plate with streptomycin (200  $\mu$ g/ml)

1 LB agar plate with ampicillin (100  $\mu$ g/ml) and streptomycin (200  $\mu$ g/ml)

Sterile membrane filter holder unit: base, funnel and clamp

3 tubes with 6 ml sterile LB broth

Sterile swabs

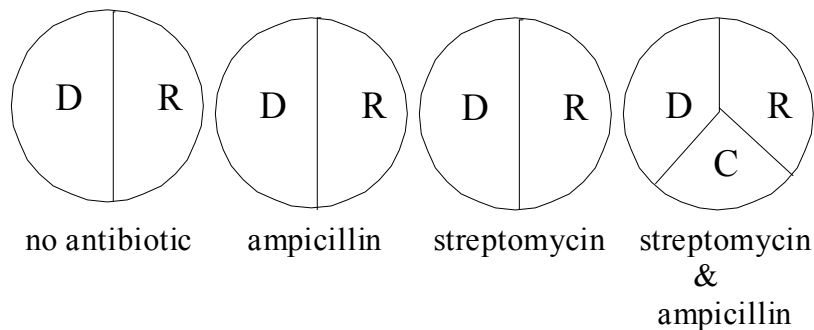
## Procedure

Disinfect each working surface with a Amphyl saturated sponge and wash your hands with germicidal soap thoroughly before and after each laboratory session. Amphyl is a bacterial disinfectant used to kill bacteria on solid surfaces. Be sure to wear and fasten your lab coats while doing this exercise. The following is to be conducted by each group of 4 students.

1. On the bottom of each of the following three plates, draw a line dividing the plate into two halves. The bottom of a petri dish is that part that of the dish that holds the LB agar. Label one half D for "Donor". Label the other side of each plate R for "Recipient":

LB agar without any antibiotic  
LB agar with streptomycin (200 ug/ml)  
LB agar with ampicillin (100 ug/ml)

On the bottom of the fourth plate (LB agar with ampicillin and streptomycin) draw a three-way division. Label one third D, one third R, and the final third C for "conjugates".



2. Label each plate with your group name and the date.

3. Using a sterile swab streak the suspended cells from the recipient *E. coli* tube onto the R side of each plate labeled above. Between streaks sample the recipient cell suspension. Keep the remainder of the suspended recipient cells for later use. Dispose the cotton swab in the beaker labeled Amphyl.

4. Using a sterile swab streak the suspended cells from the donor *E. coli* tube onto the D side of each plate labeled above. Between streaks sample the recipient cell suspension. Keep the remainder of the suspended donor cells for later use. Dispose the cotton swab in the beaker labeled Amphyl.

5. Open the upper chamber of the membrane filtration unit. Add 2 ml of sterile LB broth to the upper chamber. Turn on the vacuum and close the valve. The LB solution should pass through the membrane filter. This step is to remove any wetting agents or other chemicals which might adversely affect the bacteria. Release the vacuum with the valve or by shutting the vacuum off.

6. With the vacuum off, add another 2 ml volume of sterile LB to the upper filter of the membrane filtration unit. (This step is to help ensure uniform distribution of the donor and recipient cells.)

7. Add the remainder of the suspended Recipient (R) *E. coli* cells to the upper chamber. Then add the remainder of the suspended Donor (D) *E. coli* cells to the upper chamber. Once both cells have been added, turn on the vacuum. The cells should be retained on the membrane filter while the solution passing through should be clear. Release the vacuum once all the liquid has passed through.

8. Add 2 ml of sterile LB broth to the filtration funnel and apply the vacuum. Release the vacuum when all the liquid has passed through the filter. (This step is to remove any residual antibiotics from the donor and recipient cells)

9. Pry apart the top of the filtration unit from the bottom, Using sterile filter forceps remove the top membrane filter (with the printed grid) and place it grid side up on a fresh LB plate containing no antibiotics (cell side up). Incubate the properly labeled plate with the filter in the 35°C or 37°C incubator for at least 45 minutes.

10. Below the membrane filter is a porous fiber filter which is only used to support the membrane filter and ensure uniform filtration. This bottom filter can be discarded along with the rest of the filtration unit.

11. Having allowed the donor and recipient some "privacy" in the incubator for conjugation, remove the LB plate with the filter just before the end of the laboratory. Using a wet sterile cotton swab (swab dipped in sterile LB broth without bacteria) wipe the surface of the membrane filter several times. Streak the Conjugated cells onto the "Conjugated" section of the ampicillin + streptomycin plate.

12. Tape the plates for your group together with masking tape. Incubate them inverted in the 35°C incubator until the next class period.

### Scoring conjugation experiment

Examine your plates and score whether or not bacteria grew on each section:

	<b>Recipient</b>	<b>Donor</b>	<b>Conjugates</b>
<b>no antibiotics</b>			
<b>ampicillin</b>			
<b>streptomycin</b>			
<b>ampicillin + streptomycin</b>			

Discard all plates in a discard bag for autoclaving. Disinfect all working surfaces and scrub your hands well with germicidal soap at the end of *each lab period*.

Compare your results to those of other teams. Make a note if your results are not in line

with everyone else's.

- Explain what happened when the conjugated Donor and Recipient strain were on LB plates containing both antibiotics?
- Explain the growth or non-growth observed in each section of the ampicillin + streptomycin plate.
- In what other natural environment could bacterial conjugation occur?
- Why did we incubate the conjugation filter on LB without antibiotics?