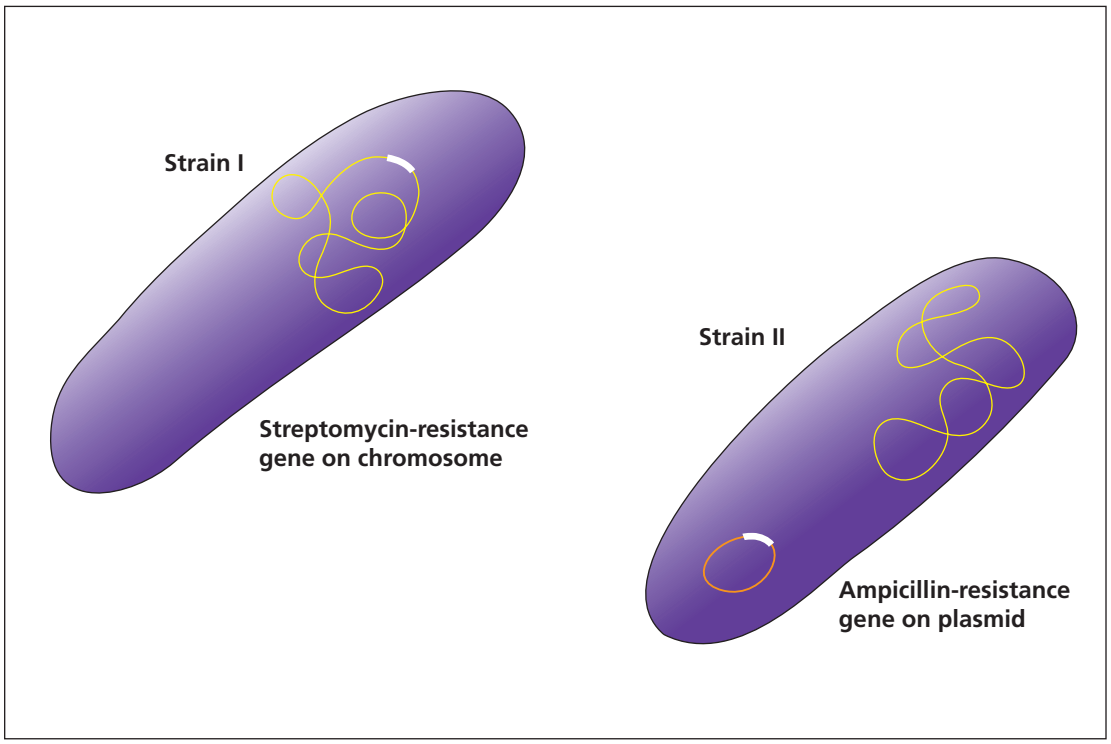
Introductory Bacterial Conjugation

TEACHER'S MANUAL AND STUDENT GUIDE



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Introductory Bacterial Conjugation

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TEACHER'S MANUAL

STUDENT GUIDE*

*Photocopy the Student Guide as needed for use in your classroom.

This kit was developed in cooperation with Dr. Robert Thomson, formerly of the Department of Biology, Marquette University, Milwaukee, Wisconsin. Some of the information and illustrations included are used with permission from *The American Biology Teacher*, February 1988 and April 1989.

Correlation to the Next Generation Science Standards

	Student Background	Student Procedure
Science and Engineering Practices		
Analyzing and Interpreting Data		◆
Constructing Explanations and Designing Solutions		◆
Engaging in Argument from Evidence		◆
Obtaining, Evaluating, and Communicating Information		◆
Crosscutting Concepts		
Patterns		◆
Cause and Effect: Mechanism and Prediction	◆	◆
Core Ideas: Life Sciences		
HS LS2.D: Social Interactions and Group Behavior	◆	◆
HS LS3.A: Inheritance of Traits	◆	◆
HS LS3.B: Variation of Traits	◆	◆
HS LS4.B: Natural Selection	◆	◆
HS LS4.C: Adaptation	◆	◆

To view additional national and local standards met by this kit, visit **www.carolina.com/correlations**.

Source: National Research Council. (2012). *A Framework for K–12 Science Education: Practices, Crosscutting Concepts, and Core Ideas*. Committee on a Conceptual Framework for New K–12 Science Education Standards. Board on Science Education, Division of Behavioral and Social Sciences and Education. Washington, DC: The National Academies Press.

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Introductory Bacterial Conjugation Kit

Overview

This kit introduces students to bacterial conjugation, a mechanism by which DNA from one bacterial cell is transferred to another to produce a new recombinant cell. This transfer is considered a type of genetic recombination, and the new genes acquired by a bacterium may increase its adaptability to its environment. In this lab, students observe the results of the transfer of a gene coding for antibiotic resistance from one *E. coli* strain to another. Thus, in addition to learning about bacterial conjugation, students learn about one of the mechanisms contributing to the problem of bacterial antibiotic resistance.

On the first day, students confirm the antibiotic resistance status of the two strains used in the experiment. Next, they set up a mating plate in which the two strains are mixed together to facilitate the transfer of DNA. On the last day, students transfer the mated bacteria to plates containing different antibiotics to ascertain whether DNA has been transferred from one strain to the other. The lab can be completed in four 50-minute lab periods. The kit is designed for eight work groups of two to four students.

Objectives

Students will

- learn about bacterial conjugation, a naturally occurring mechanism of genetic recombination.
- be introduced to one way in which bacterial antibiotic resistance is spread.
- practice interpreting experimental results and providing an explanation for observed patterns.
- acquire hands-on experience working with bacteria.

Time Requirements

Preparation

One to 10 days before starting liquid cultures, prepare agar plates. ..... 2 hours

One day before starting liquid cultures,
create streaked cultures (optional) ..... 15 minutes

One to 4 days before Lab Day 1, start liquid cultures. .....15 minutes

Laboratory

Day 1

Brief students about lab. .....20 minutes

Prepare confirmation plate. .....30 minutes

Day 2

Observe confirmation plates and set up mating plates. .....30 minutes

Day 3

Observe mating plate and test for recombination. .....30 minutes

Day 4

Observe and discuss results.....20 minutes

Because 12- to 24-hour incubations at 37°C or 2- to 4-day incubations at room temperature are required between each lab day, this lab requires at least four separate lab periods. Four consecutive days are best, but a weekend that falls at the end of any day's procedure may be accommodated by refrigerating the plates after the incubation times.

Materials

Your kit includes a digital Teacher's Manual and Student Guide. See the Digital Resource Instruction Card for more information.

Included in the kit:

Order Form* for the following perishable items (if not shipped with the order):
- *E. coli* Strain I (Str^r)
- *E. coli* Strain II (Amp^r and Nal^r)†
- 2 4.0-mL vials of ampicillin solution, 1%
- 2 4.0-mL vials of streptomycin solution, 2%

8 10-mL bottles of LB broth

4 400-mL bottles of LB agar

70 sterile petri plates

100 sterile inoculating loops

Teacher's Manual and reproducible Student Guide

Digital Resource Instruction Card

Needed, but not supplied:

tape, labeling or masking

8 permanent markers

microwave oven or boiling water bath to melt LB agar

autoclave or 10% solution of household bleach for decontamination before disposal

spray bottle(s) of 10% household bleach solution, 70% isopropanol or ethanol, or another disinfectant for cleaning lab surfaces

37°C incubator (optional)

Parafilm® or plastic wrap

8 containers for bacterial contaminated waste

Note: In a warm climate, a warm windowsill with a light-blocking cover may be sufficient for incubation. In cooler climates, cultures show satisfactory growth after 2–4 days at room temperature.

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Upon receipt of this kit, store the antibiotic solutions at 4°C for up to 1 month or in a freezer for up to 3 months. Slants are to be stored at 4°C and are best used within 2 weeks, but they may be used up to 1 month after receipt. The LB agar and LB broth may be stored at room temperature and used up to 1 year from receipt.

*If the kit with perishables is ordered, there will be no Order Form—the perishables will be shipped at the same time as the other kit materials. Otherwise, follow the instructions on the Order Form for prepaid delivery of the perishable items. The perishable items also constitute the kit refill (211125A).

† The nalidixic acid resistance of this culture is not utilized in this lab activity.

Safety

Use this kit only in accordance with established laboratory safety practices, including wearing appropriate personal protective equipment (PPE) such as gloves, chemical splash goggles, and lab coats or aprons. Ensure that students understand and adhere to these practices. Know and follow all federal, state, and local regulations as well as school district guidelines for the disposal of laboratory wastes. Students should not eat, drink, or chew gum in the lab and should wash their hands after entering and before exiting the lab.

Handling and Disposal of *E. coli*

Escherichia coli is a normal part of the bacterial microflora of the human intestines. Although some varieties of *E. coli* are pathogenic, the laboratory strains included with this kit are not considered pathogenic and are not associated with illnesses under normal conditions. Adherence to the following guidelines for the handling and disposal of *E. coli* makes working with it safe and helps prevent the inadvertent transfer of antibiotic resistance to other types of bacteria.

1. If you use a wire loop instead of the disposables, always reflame the inoculating loop one final time before placing it on the lab bench.
2. When using disposable sterile inoculating loops, open the package at the end farthest from the inoculating portion and hold only the upper end of the handle. Do not touch any part of the loop to any surface other than the inoculants being gathered or the medium on or in which the inoculants are placed. Immediately after use, put these loops into a disposal cup or biohazard bag. If no disposal cup is available, replace the used loop into its paper cover and, as soon as possible, put it in a biohazard bag.
3. To avoid inhaling any aerosol that might be created when working with bacterial cultures, keep your nose and mouth away from the inoculating loop or any other tool being used to manipulate the bacteria.
4. Avoid over-incubating the plates. *E. coli* is generally the only organism that will appear on plates incubated for 12–24 hours at 37°C. However, with longer incubation, contaminating bacteria and slower-growing fungi may

arise. If students will not be able to observe the plates within 24 hours after the initial incubation at 37°C, refrigerate the plates to retard the growth of contaminants.

5. At the end of each lab, wipe down the lab bench with 10% bleach solution, 70% ethanol or isopropanol, soapy water, or other disinfectant.
6. Wash your hands before leaving the laboratory.
7. Treat all microorganisms as potential pathogens. Sterilize or disinfect equipment, materials, and work areas before and after use. Collect for treatment the bacterial cultures, disposal cups, inoculating loops, and any other materials that have come into contact with the bacteria. Disinfect these materials as soon as possible after use. Contaminants, sometimes pathogenic, can be cultured over a period of several days at room temperature. Disinfect bacteria-contaminated materials in one of two ways:
    a. Treat them with a 10% bleach solution (1 part household bleach to 9 parts water). Immerse contaminated materials in a sink or tub of bleach solution. Let the materials stand in the bleach solution for at least 2 hours. Then drain the excess bleach solution, seal the materials in a plastic bag, and dispose of the bag in the regular garbage.
    b. Autoclave the materials at 121˚C for 15 minutes. Collect the bacteria-contaminated materials in an autoclavable, disposable bag. Seal the bag **loosely** (so that steam can still enter it), autoclave it, and dispose of it in the regular garbage.

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Background

Below is a summary of the lab. See the Background of the Student Guide for more information.

In this lab, pure cultures of *E. coli* Strain I (streptomycin resistant, Str^r) and Strain II (ampicillin resistant, Amp^r) are grown overnight in Luria–Bertani (LB) broth, an enriched culture medium lacking antibiotics. Samples of each strain are then transferred to agar plates containing LB medium and the antibiotics to which each strain is known to be resistant. Growth or lack of growth on these plates confirms resistance or sensitivity of the strains to the antibiotics. After confirmation of antibiotic resistance or sensitivity, 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. Conjugation results in the transfer of DNA and the formation of recombinant cells.

After overnight growth, the cells grown on the mating plate are transferred to separate antibiotic-containing plates and tested for their growth response to the same antibiotics previously tested. The growth on these plates demonstrates that DNA (coding for antibiotic resistance) can be transferred between two genetically different bacterial cells.

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Preparation

1. If your kit included an Order Form for delivery of perishable items, follow its instructions to ensure that the items arrive when you need them.
2. Review the SDS provided with this kit. Make certain you have appropriate personal protective equipment for every student in your class.
3. Review the content of the Teacher's Manual and the Student Guide. Familiarize yourself with the activity instructions, required materials, and assessments. Review the classroom management procedures and the timing of the materials setup steps described below.
4. Photocopy the Student Guide for each student or student group.
5. Gather the materials that are needed but not supplied.
6. **Three to 10 days before Lab Day 1**, pour the plates. Prepare 64 plates as follows: 16 LB, 16 LB+amp*, 16 LB+str*, and 16 LB+str+amp. Use good aseptic technique and work in an area away from drafts and heavy activity. Before pouring the plates, clean the bench top with 10% bleach, 70% ethanol, or another disinfectant. If you are working alone, consider melting only two bottles at a time; agar sets quickly, and remelting the agar destroys any antibiotic that has been added. It is best to prepare the plates at least 1 day prior to when they are needed to allow time for any excess moisture that collects on the plates during pouring to evaporate.

   * Pour 17 plates (+amp and +str) if you prepare a streaked plate of each culture from which to start the liquid culture. For more information, see the following section on preparing the liquid cultures.

   a. **Loosen the cap** on the bottle of agar and melt the agar, either in a boiling water bath or in a microwave oven. Heat the agar until it is completely melted (with no lumps left). When heating agar, be sure to use gloves designed for handling hot items.

      When using a boiling water bath, make sure that the water in the bath or beaker remains at or above the level of the agar in the bottle, and that the water remains at a full boil the entire time. Once the agar has been placed in the boiling water, it melts in 45–55 minutes. Schedule additional time for initially bringing the water to a boil.

      When using a microwave, be aware that the time required for melting agar may vary greatly, depending upon the power of the microwave. While the agar is melting, stop the microwave and swirl the bottle every minute or two to prevent the agar from boiling over. Allow at least an hour for melting all four bottles.

   b. Allow the agar to cool until the bottle can be held comfortably in a bare hand (20 to 30 minutes). Swirl every 5–7 minutes to ensure even cooling. Since antibiotics are inactivated by high temperature, the agar must be cooled to approximately 55°C before the antibiotic solutions are added. While the agar is cooling, label one of the four bottles "+amp" to designate it for the addition of ampicillin. Label one of the four bottles

"+str" to designate that a vial of streptomycin solution will be added to this bottle. The third bottle should be labeled "+str/amp" because streptomycin and ampicillin will be added to it. The fourth bottle, labeled "LB," will not have anything added.

c. Carefully cut the top of the plastic sleeves containing the sterile petri dishes and remove the dishes from the sleeves, being careful to keep the lids in place. Save the sleeves for storing the poured plates. Divide the petri dishes into four stacks of 16 each. Note: If you prepare streaked plates of the two cultures (from which to start the liquid cultures), pour 17 LB+amp plates and 17 LB+str plates.

d. Label the bottoms of the petri dishes in each stack as described below. Label along the outside edge of the bottom.
Stack 1: "LB"
Stack 2: "LB+str"
Stack 3: "LB+amp"
Stack 4: "LB+str+amp"

e. Place the closed plates topside-up in a row on the edge of the lab bench away from drafts.

f. When the agar has cooled to "comfortably warm" (not too hot for the bottle to be held in your hand for a short time), aseptically add one 4-mL vial of ampicillin solution to the agar bottle that you labeled "+amp." Add one 4-mL vial of streptomycin solution to the "+str" bottle. Add one vial each of ampicillin and streptomycin to the bottle labeled "+str+amp." Work quickly, and do not place open caps down on the lab bench. Immediately after the addition of the antibiotic solution, replace the cap on the agar bottle and swirl the agar to thoroughly mix in the antibiotics.
Note: You may find it easier to add the antibiotic to and pour plates from one bottle at a time.

g. Pour the melted agar from each bottle into the appropriately labeled culture plates. Lift the lid of each plate and pour just enough agar to cover the bottom about 3 mm deep. Replace the lid immediately after pouring.

h. Allow the agar to solidify. If there is much condensation on the lids, allow the plates to dry overnight. Return the plates to the storage sleeve or wrap them with plastic wrap and store them topside-down in a refrigerator at 4˚C until use.

7. **One to 4 days before lab**, prepare the liquid cultures. When preparing the liquid cultures, plan your steps, have all materials ready for use, and work quickly. A sterile loop should be considered contaminated whenever it comes into contact with anything (other than the intended culture or culture medium) in the environment—lab bench, hands, or clothing. When contamination is suspected, discard the loop and start with a fresh one. Have the procedures clearly visualized in your mind so that you can work quickly.

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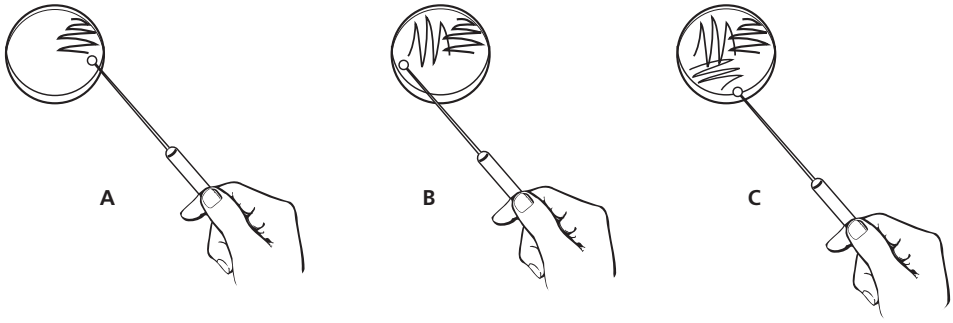
Streaking a plate from which to start the liquid cultures (optional):
Ideally, the liquid culture should be made from one colony to ensure purity. If there is not enough time for this, satisfactory cultures can be made from the slant provided in the kit.
If there is enough time, streak a fresh plate of each culture the day before you want to make the liquid culture. Below are instructions on how to streak a plate to obtain individual colonies.
Note: The sterile technique to follow in opening and handling the loops and vials is described fully in the following section, "Starting the liquid culture."

Collect the following items:

6 sterile inoculating loops
Str^r slant culture (I)
Amp^r slant culture (II)
LB+amp plate
LB+str plate
Bunsen burner
lighter
spray bottle of 10% bleach, 70% ethanol, or other disinfectant
permanent marker

a. Disinfect your work surface.
b. Label an LB+str plate "Strain I" and an LB+amp plate "Strain II."
c. Open the slant for Strain I and touch a sterile loop to the culture. Reclose the slant.
d. While opening the plate as little as possible, glide the loop containing the bacteria back and forth across one quadrant of the LB+str plate (Figure A).
e. Use a fresh loop to create a second streak. Begin the streak by drawing the loop tip through the primary streak as shown in (Figure B).
f. Use a fresh loop to create a third streak. Begin the streak by drawing the loop tip through the secondary streak as shown in (Figure C).



g. Repeat steps c–f using a fresh loop, the slant for Strain II, and the LB+amp plate.
h. Incubate the plates overnight at 37°C or for 24–48 hours at room temperature.

Starting the liquid culture:

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Collect the following items:

- Str^r slant culture (I)
- Amp^r slant culture (II)
- 8 sterile inoculating loops
- permanent lab marker
- 8 bottles of LB broth (10 mL)
- labeling tape
- Bunsen burner
- lighter
- spray bottle of 10% bleach, 70% ethanol, or other disinfectant

Note: If you have created streaked plates, start the liquid cultures by using a loop to pick a single colony from the appropriate plate instead of from the slant cultures.

a. Cleanse and disinfect the lab bench. Using tape (for easy cleanup), label four bottles of LB broth as "I" and four bottles as "II."

b. Unscrew the cap of each bottle, but leave the cap sitting loosely on top. Loosen the caps on the slant culture vials only enough to make it easy to unscrew the caps when you are inoculating the cultures.

c. Light the Bunsen burner.

d. Open the package containing a sterile loop from the handle end (the end opposite the circle). Withdraw the loop carefully and quickly, without touching the lower half to anything.

e. While holding the Strain I slant culture vial in one hand, use the pinkie finger of the other hand (the hand holding the loop) to grasp and remove the cap from the vial. **Note:** Do not place the cap on the lab bench or touch the inner surface of the cap.

f. Quickly pass the neck of the slant culture vial through the flame before inserting the sterile loop.

g. Pick up a small cell mass (about 10% of the area of the loop) and remove it from the vial.

h. Quickly reflame the opening of the vial and replace the cap. Lay the vial down.

i. With the pinkie finger of the hand holding the inoculated loop, grip the cap of a bottle of LB broth labeled "Strain I" and remove the cap carefully (twist the bottle slowly if necessary). Continue holding the cap with your finger.

j. Pass the neck of the bottle quickly through the flame.

k. Immerse the tip of the loop into the broth and agitate it to dislodge the cell mass. Break the cell mass up with the loop, if possible. Discard the loop in a disposal container for contaminated waste or replace it into its wrapper for disposal later.

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l. Reflame the neck of the bottle and replace the cap tightly.

m. Repeat steps d–l for the other three bottles of LB labeled "I."

n. Repeat steps d–l using the slant culture vial containing Strain II and the four bottles of LB broth labeled "II."

o. Incubate the liquid cultures for 24 hours in a shaking water bath prewarmed to 37°C, or on a shaking platform in an incubator prewarmed to 37°C. If no mechanical shaker is available, incubate 2–3 days at 37°C, or at room temperature for 4 days. If the cultures are not on a shaker, shake them occasionally by hand. After use on Lab Day 1, the cultures may be refrigerated for as long as 1 week if need be. See the Procedure for more details.

8. Just prior to lab on Lab Day 1, set up eight workstations, each stocked with these materials:

LB plate	2 sterile inoculating loops
LB+str plate	permanent marker
LB+amp plate	container for bacterial contaminated waste
LB+amp+str plate	

Make labeling tape available to the class and set up the following to be shared between two groups:

E. coli Strain I liquid culture
E. coli Strain II liquid culture

9. Just prior to Lab Day 2, set up eight workstations, each stocked with these materials:

LB plate	3 sterile inoculating loops
permanent marker	container for bacterial contaminated waste

Set up the following to be shared between two groups:

E. coli Strain I liquid culture
E. coli Strain II liquid culture

10. Just prior to Lab Day 3, set up eight workstations, each stocked with these materials:

LB+amp plate	3 sterile inoculating loops
LB+str plate	container for bacterial contaminated waste
LB+str+amp plate	labeling tape
permanent marker	

Procedure

1. Distribute the Student Guide to each student. Have students read the Background information and answer the Pre-laboratory Questions.
2. If they are not already familiar with it, instruct students in sterile technique.
3. Have them conduct the lab according to the instructions in the Student Guide. Monitor the class to ensure that students are using safe laboratory techniques and wearing appropriate PPE.

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4. If you have an extended break immediately after Lab Day 1, the cultures may be stored in the refrigerator for as long as a week. Leave the caps loose on the bottles and place the bottles in a tray or bin to prevent an accidental spill contaminating the refrigerator.
5. If at any time during the lab, the inoculum placed on the plates has not soaked in before the end of a lab period or shortly thereafter, place the dishes in the incubator topside-up.
6. At the beginning of Lab Day 2, 3, and 4, either distribute to students their plates from the previous lab day or have students retrieve them from the incubator.
7. If you keep plates beyond their incubation time, wrap them in plastic wrap or Parafilm to keep them from drying, and place them in the refrigerator.
8. After students have completed the lab activities, have them answer the Post-laboratory Questions in the Student Guide.

Answers to Questions in the Student Guide

Table 1. Confirmation of Antibiotic Resistance of Strains I and II

Type of Plate	Strain I		Strain II	
	Expected Results	Observed Results	Expected Results	Observed Results
LB		+		+
LB+str		+		–
LB+amp		–		+
LB+str+amp		–		–

Students' "Expected Results" will vary depending upon their level of understanding prior to completing the lab.

Table 2. Recombinant Cell Growth on Antibiotic Plates

Type of Plate	Expected Results	Observed Results
LB+str		+
LB+amp		+
LB+str+amp		+

Students' "Expected Results" will vary depending upon their level of understanding prior to completing the lab.

Pre-laboratory Questions

1. What is a plasmid?

   Plasmids are double-stranded, circular pieces of DNA that exist independently from the chromosome. They are present in many bacteria, can carry anywhere from a few to more than 20 genes, and can be passed from one bacterium to another.

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2. What is bacterial conjugation, and what is the significance of plasmids with respect to conjugation?

   Conjugation is the process by which DNA from one bacterial cell is transferred to another cell to create a new recombinant cell. Bacteria exchange plasmids through conjugation.

Post-Laboratory Questions

1. In the confirmation tests, why did Strain I not grow on the LB+amp plate?

   Strain I has a gene for resistance to streptomycin but not to ampicillin.

2. In the confirmation test, why did Strain II not grow on the LB+str plate?

   Strain II has a gene for resistance to ampicillin but not to streptomycin.

3. Why did neither strain grow on the confirmation medium containing streptomycin and ampicillin?

   Each strain was sensitive to one of the antibiotics in the plate and would not grow in the presence of that antibiotic.

4. Based on the growth response to the various antibiotics, have conjugation and transfer of DNA (recombination) occurred? Explain.

   Yes. Before mating, neither Strain I nor Strain II was resistant to both ampicillin and streptomycin. After mating, at least some of the bacteria taken from the mating plate are resistant to both antibiotics. This suggests that one strain acquired an antibiotic-resistance gene from the other strain.

5. Can you tell whether the growth on the mating plate consists of recombinant cells only or whether it merely contains cells of strains I and II? Explain.

   Since no antibiotics are present in the medium of the mating plate, growth may consist of Strain I and Strain II cells that had not conjugated, of recombinant cells, or of all three types. In order to identify the cells, the bacteria on the mating plate have to be tested for resistance or sensitivity to the antibiotics previously tested.

6. On the basis of 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 in Strain II was transferred into Strain I? Explain.

   No. Simply from observing the recombinant growth, it cannot be determined which strain was the "donor" and which was the "recipient." In order to determine which strain acquired the antibiotic-resistance gene from the other, you would have to devise a way to distinguish the two strains aside from their resistance to streptomycin or ampicillin.

Extension Activities

1. It is now known that other DNA elements (such as some transposons) are transferred from one bacterium to another via conjugation. Have your students research what these DNA elements are.
2. Conjugation involves multiple steps. Challenge your students to think about and then research what is required for conjugation to occur. For example, the transfer of a plasmid during conjugation usually involves transfer of just one strand of the plasmid. For that to occur, the plasmid must first be "nicked," so that it is free to unwind and separate.
3. Biofilms are populations of bacteria living together in a thin layer on a surface. The plaque that forms on teeth is one example of a biofilm. Biofilms may be beneficial or detrimental and are very important ecologically and industrially. Medically, they are a source of concern because when a biofilm forms in an atypical place in the human body, the infection can be difficult to treat. Have your students research bacterial conjugation in the context of biofilms.

Resources

Carolina's Teacher Resources. ©Carolina Biological Supply Company.

Carolina has a huge collection of educational videos, activities, articles, and instructional materials available free online.

http://www.carolina.com

Carolina Science Online®. ©Carolina Biological Supply Company.

Carolina's online science education portal provides free and for-fee access to interactive resources for every topic, grade level, and learning style.

http://www.carolinascienceonline.com

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NAME _____

DATE _____

Introductory Bacterial Conjugation

Background

In this experiment, you are introduced to bacterial conjugation, a natural mechanism by which DNA is transferred from one bacterial cell to another. This transfer, a type of genetic recombination, is a mechanism through which bacteria increase their adaptability to their environment. For example, genes coding for antibiotic resistance may be transferred. Such a transfer enables the new recombinant bacterial cell to be resistant to an antibiotic or antibiotics to which the cell was formerly sensitive. The resistance gene may then be passed to the cell's offspring.

While bacterial chromosomes generally carry all the genes necessary for growth and reproduction, bacteria also contain genes carried on extrachromosomal pieces of DNA called plasmids. Plasmids are double-stranded circular pieces of DNA that may carry anywhere from just a few to more than 20 genes. Numerous plasmids have been described in a variety of bacteria. Plasmids contain specialized genes (e.g., genes coding for a toxin or for a protein that confers antibiotic resistance), 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 Factor," for "fertility factor." This plasmid, found in the common colon bacterium *Escherichia coli*, contains multiple genes, many of which regulate conjugation.

The multiple steps involved in bacterial conjugation are carried out by multiple protein complexes. One of these steps is the formation of pili. Pili are elongated appendages that extend from the surface of a bacterial cell. While the details of pili's role in facilitating bacterial conjugation are still being studied and analyzed, it is known that a pilus functions as a bridge between two bacterial cells, retracting to bring the cells together and thus facilitating the transfer of DNA from the donor to the recipient. Another step necessary for single-stranded bacterial conjugation, like that carried out by the bacteria in this lab, is the separation of the two strands of DNA in the plasmid, so that a single strand can be transferred from one bacterium to the other. While many bacteria and archaea use the same mechanism of conjugation as the *E. coli* you will work with in this lab, there are other mechanisms through which conjugation occurs in both groups of organisms.

In 1959, it was first shown that resistance to antibiotics can be transferred between bacteria during conjugation and that this transfer involves plasmids. It has since been discovered that other DNA elements can also spread antibiotic resistance through conjugation. Conjugation-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, a conjugative plasmid can spread through a bacterial population at an exponential rate.

In the lab you are about to perform, you will observe the effects of bacterial conjugation. The lab consists of four different sessions. On Lab Day 1, you will confirm the antibiotic resistance and sensitivity of two different strains of antibiotic-resistant *E. coli* by transferring them to plates containing enriched growth medium (LB) and antibiotics. In preparation for Lab Day 1, your instructor grew pure cultures of these *E. coli* strains in LB broth, an enriched culture medium lacking antibiotics. Strain I is streptomycin resistant (Str^r), and Strain II is ampicillin resistant (Amp^r). On Lab Day 2, after confirmation of the appropriate growth response for each strain, samples of Strain I and Strain II are mixed together on a plain LB "mating"

plate where conjugation between the two strains is expected to occur. After overnight growth, you will transfer the cells on the mating plate 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 conjugation has occurred and that DNA coding for antibiotic resistance can be transferred between two genetically different bacterial cells.

Pre-laboratory Questions

1. What is a plasmid?

2. What is bacterial conjugation, and what is the significance of plasmids with respect to conjugation?

Lab Day 1. Confirming Antibiotic Resistance of Strain I and Strain II

Materials

For each group:

- 2 sterile inoculating loops
- LB plate
- LB+str plate
- LB+amp plate
- LB+str+amp plate
- permanent marker
- labeling tape
- container for bacterial contaminated waste

Shared between two groups:

- liquid culture of Strain I
- liquid culture of Strain II

Shared (optional):

- 37°C incubator

Procedure

1. On the bottom surface of the petri dish, 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* Strain I and Strain II liquid 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).

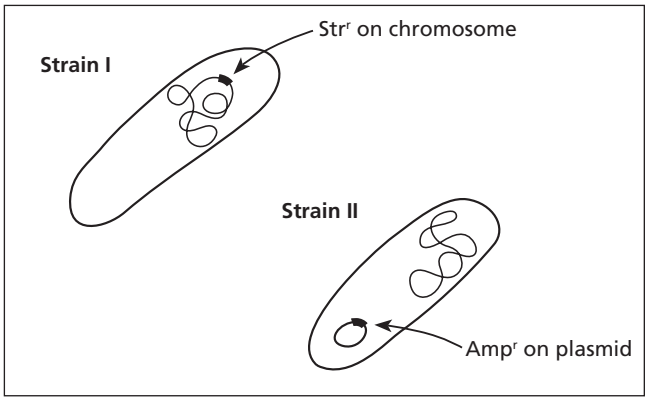


Figure 1. Location of genes for antibiotic resistance in *E. coli* strains I and II

3. According to the directions below, inoculate each of the four labeled plates with Strain I and Strain II. Mark the plates to identify them as belonging to your group.

   a. Open the wrapper at the handle end of an inoculating loop (the end opposite the circle) and remove the loop, being careful not to touch the lower half of the loop to anything.

   b. Using the sterile technique described by your teacher, remove and hold the cap (open surface down) from the Strain I broth culture bottle.

   c. Dip the sterile loop into the Strain I culture. While doing this, do not place the cap down or allow it to touch anything. Withdraw the loop and replace the cap immediately. Be careful not to touch the loop to anything before placing it into or withdrawing it from the bottle. If you suspect something has been touched, discard the loop and obtain a fresh one. **Note:** One person may want to maneuver the cap or steady the bottle for another person so that the work may be accomplished quickly.

   d. Touch the loop lightly to the agar in the middle of section I on each of the four plates (see Figure 2). To ensure plenty of bacteria for inoculation, flip the loop over after inoculating the first two plates and use the other side for inoculating the third and fourth plates. When inoculating the plates, open the lid of each plate slightly, just enough to perform the task, and close the plate immediately after inoculation. Do not place the lid on the bench. Also be careful not to touch the lower third of the loop to anything but the bacteria and the agar plate.

   e. Discard the loop into a disposal container or replace it into its wrapper for disposal later.

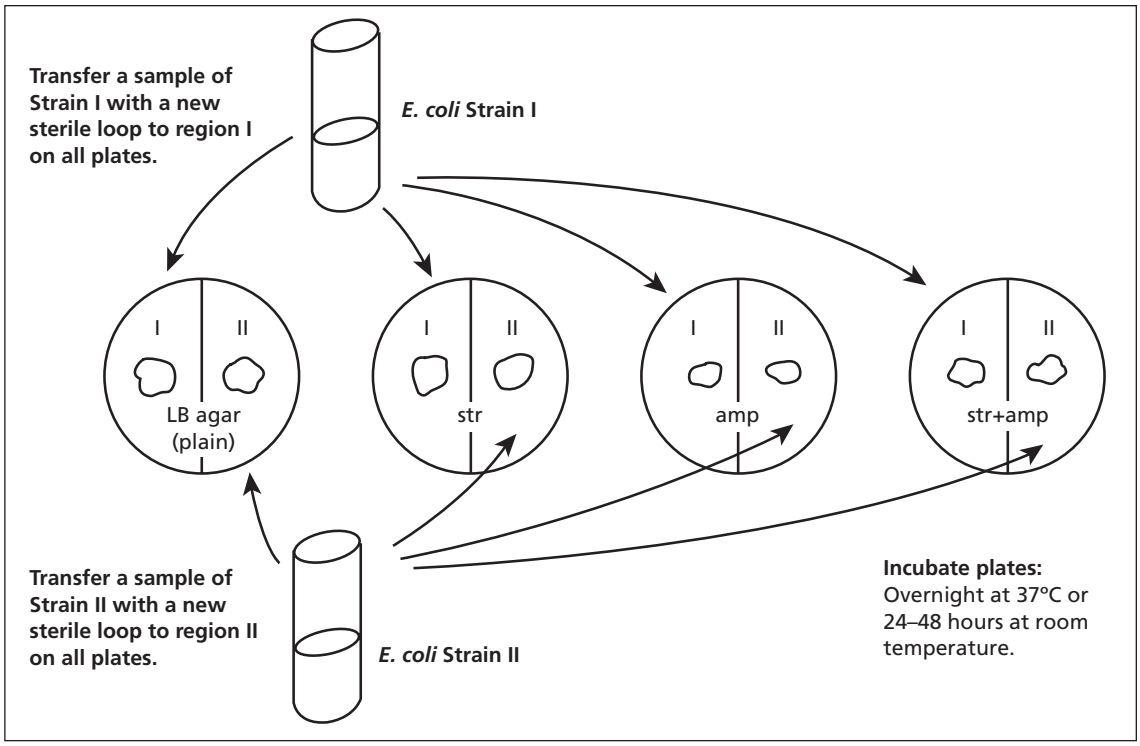


Figure 2. Preparation of "confirmation" plates to confirm the resistance (growth) or sensitivity (lack of growth) of *E. coli* strains I and II to streptomycin (str) and ampicillin (amp)

   f. Using a new loop, repeat steps a–e with the liquid culture for Strain II to inoculate the section II of each plate. Allow the plates to sit briefly so that the liquid can soak in before the plates are inverted for incubation. Tape the four plates together and incubate them, either overnight at 37°C or for 24–48 hours at room temperature.

4. In the appropriate space in Table 1 below, record the results you expect to obtain after 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 the table, explain your reasoning.

Type of Plate	Strain I		Strain II	
	Expected Results	Observed Results	Expected Results	Observed Results
LB				
LB+str				
LB+amp				
LB+str+amp				

Table 1. Growth of *E. coli* strains I and II on plates used to confirm the strains' resistance or sensitivity to streptomycin and ampicillin.

Lab Day 2. Observing Confirmation Plates and Preparing "Mating" Plates

Materials

For each group:

3 sterile inoculating loops
LB plate
permanent marker
plates from Lab Day 1
container for bacterial contaminated waste

Shared between two groups:

E. coli Strain I liquid culture
E. coli Strain II liquid culture

Shared (optional):

37°C incubator

Procedure

1. Examine the confirmation plates prepared during Lab Day 1 and record the Observed Results in Table 1.
2. Compare the Expected Results and the Observed Results and account for any differences. Compare your results to those of your classmates and account for any differences between your results and theirs.
3. Using the directions below, prepare an LB agar "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. Label the mating plate to identify it as belonging to your group.
   a. Using the same sterile technique you used during Lab Day 1, dip a sterile inoculating loop into the liquid culture of Strain I.
   b. Place a drop of culture on the LB mating plate by touching the inoculated loop to the agar. Remember, open the lid of the plate just enough to slip the loop under and be careful not to touch the loop to anything but the agar. Do not lay the lid down on the lab bench.

c. Using a new loop and the liquid culture of Strain II, place a drop of Strain II ½ inch from the drop of Strain I on the mating plate.

d. Use a new sterile loop to mix strains I and II as shown in Figure 3.

e. The loops should be disposed of in a disposal container, or replaced in the wrapper for later disposal.

f. Allow the liquid on the plate to soak in completely before inverting the plate.

g. Incubate the plates overnight at 37°C, or for 24–48 hours at room temperature.

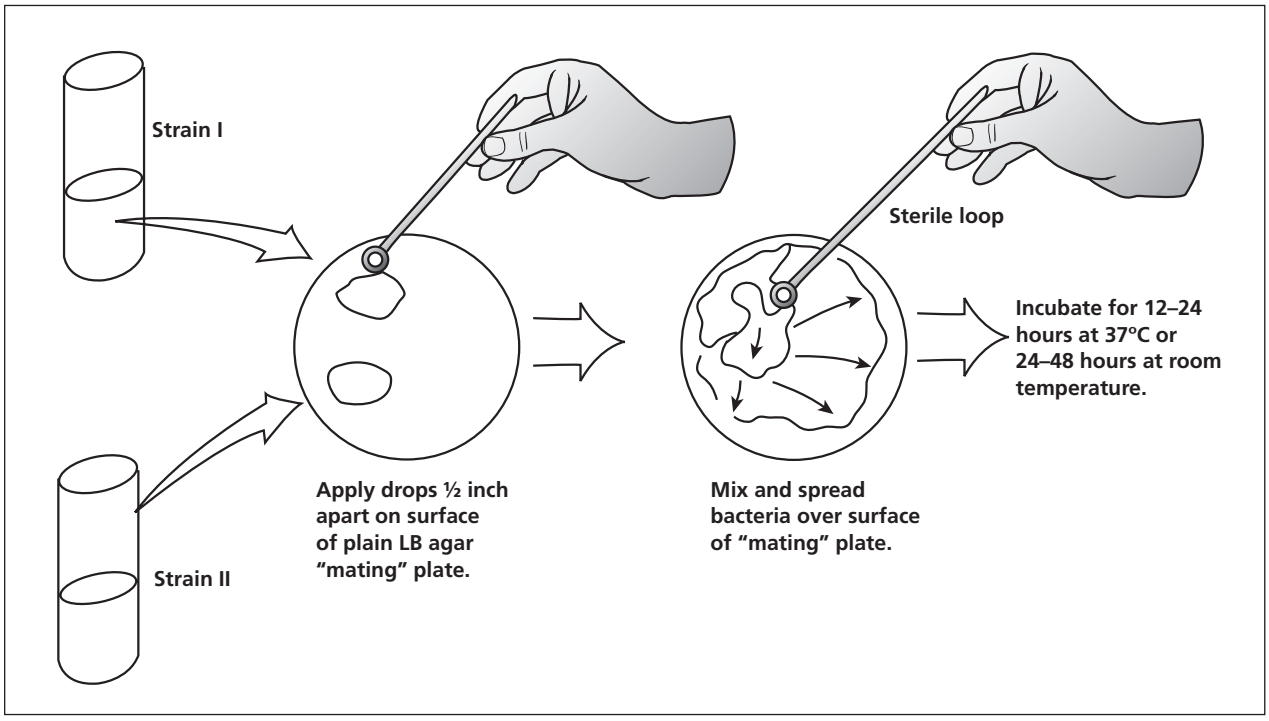


Figure 3. Cells of strains I and II are mixed and spread on an LB agar plate to allow for conjugation and the subsequent formation of recombinant cells.

4. What do you expect to happen in regard to growth? Give your answer and explain your reasoning in the space below.

Lab Day 3. Observing "Mating" Plate and Testing for Recombination

Materials

For each group:

3 sterile inoculating loops
LB+amp plate
LB+str plate
LB+str+amp plate
permanent marker
plates from Lab Day 2
tape
container for bacterial contaminated waste

Shared (optional):

37°C incubator

Procedure

To determine whether any growth observed on the mating plate represents "recombinant" cells (those in which conjugation has occurred) or is merely growth of the separate strains of *E. coli*, you must transfer samples of cells from the "mating" plate onto plates containing the antibiotics previously tested.

1. Examine the mating plate prepared during Lab Day 2. Confirm that you have growth.
2. Transfer some bacterial cells from the mating plate to LB+amp, LB+str, and LB+str+amp plates using the procedure below. Remember to label the plates to identify them as belonging to your group. Use a fresh loop for streaking each plate.
   a. Using the same sterile technique as during the two previous lab days, remove a sterile inoculating loop from the package by opening it from the handle end. Do not touch the lower half of the loop to anything.
   b. Slightly lift the lid of the mating plate and pick up some of the bacteria by touching the loop to some of the bacteria on the plate. Close the lid. Remember to not touch the lower half of the loop to anything but the bacteria and the agar.
   c. Streak some of the bacteria onto the LB+amp plate (see Figure 4). Open the LB+amp plate as little as possible as you do this.
   d. Discard the loop in the disposal container or replace it into its wrapper for disposal later.
   e. Using a fresh loop for each plate, repeat steps a–d to streak some of the mated bacteria onto the LB+str and the LB+amp+str plates.
3. Tape your plates together.
4. Incubate the plates overnight at 37°C or for 24–48 hours at room temperature.
5. In Table 2, record the "expected" growth results for the recombinant cells. Explain your reasoning in the space below the table.

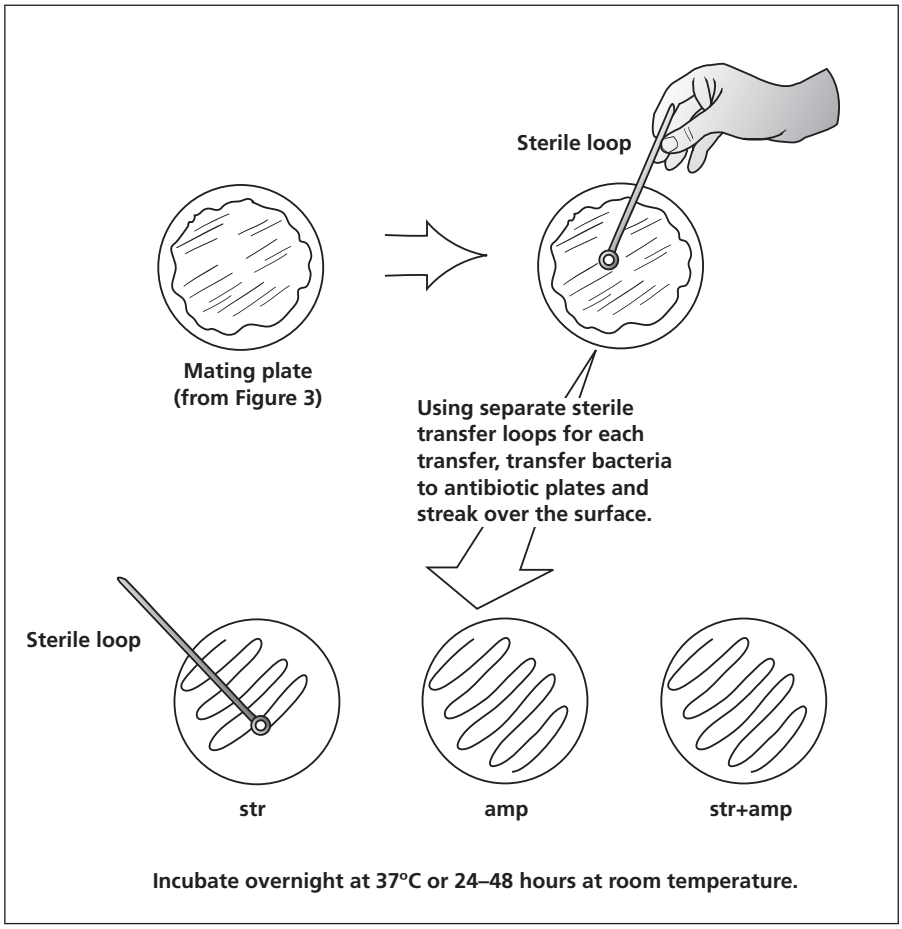


Figure 4. Testing for recombination on three plates: LB agar plus streptomycin (str), LB agar plus ampicillin (amp), and LB agar plus streptomycin plus ampicillin (str+amp).

Recombinant Cell Growth

Type of Plate	Expected Results	Observed Results
LB+str		
LB+amp		
LB+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).

Lab Day 4. Observing Recombinant Cell Growth

Materials

plates from Lab Day 3

Procedure

1. In Table 2, record the recombinant cells' growth on the various antibiotic plates.
2. Answer the Laboratory Question.

Post-laboratory Questions

Answer the following questions.

1. In the confirmation tests, why did Strain I not grow on the LB+amp plate?

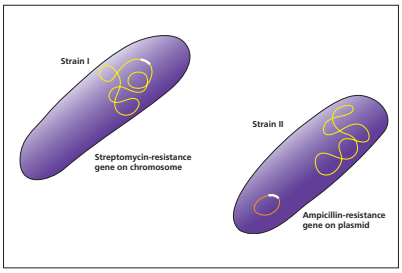
2. In the confirmation test, why did Strain II not grow on the LB+str plate?

3. Why did neither strain grow on the confirmation medium containing streptomycin and ampicillin?

4. Based on the growth response to the various antibiotics, have conjugation and transfer of DNA (recombination) occurred? Explain.

5. Can you tell whether the growth on the mating plate consists of recombinant cells only or whether it merely contains cells of strains I and II? Explain.

6. On the basis of 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 in Strain II was transferred into Strain I? Explain.



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Teacher's Manual and Student Guide

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