Transduction of an Antibiotic Resistance Gene

Teacher's Manual

1. Infection of plasmid-containing host cell

2. Second infection: Transfer of Plasmid DNA (this lab)
This activity demonstrates the transmission of a bacterial antibiotic resistance gene by a bacterial virus. It is technically easy enough for ninth-grade students to perform and, using the more sophisticated discussion and optional modifications in the introduction that follows, can be incorporated into college-level microbiology, virology, and genetics courses. This activity was published in Recombinant DNA and Biotechnology: A Teacher’s Guide (Kreuzer and Massey, 2001, ASM Press, Washington, DC; Carolina Biological Supply Company catalog #RN-21-2218). The introduction in this Teacher’s Manual is adapted from that work with permission.

Introduction

Transduction is the process in which a bacterial virus, or bacteriophage, carries bacterial genes from one cell to another. Many different bacteriophages are capable of transduction. The details of transduction by any one of them depend upon that phage’s life cycle.

Virus Life Cycles

Viruses recognize their host cells through molecular interactions between the surface of the virus and the surface of the host cell. The proper interaction triggers changes that cause the viral genetic material to be injected into the host cell. Once the viral genetic material enters the cell, one of two essentially different types of infection may take place, depending on the specific virus.

If the virus is lytic, it takes over the cellular machinery. Normal cellular metabolism slows or stops. Cellular enzymes are diverted to make many new copies of the viral genetic material and many viral proteins. As the cell is filled with viral components, new virus particles assemble. Finally, the host cell dies, releasing the progeny virus into the environment. Sometimes this release of progeny is a gradual process; in other cases, the infected cell bursts (lyses), releasing all of the new virus particles at once.

The course of a latent infection is very different. After a latent virus injects its genetic material into the host cell, the virus does not hijack the cellular metabolism. Instead, a few viral proteins that direct the incorporation of viral DNA into the host chromosomes are produced. If the host cell is a bacterium, the latently infected host is called a lysogen. (Bacteriophages that set up latent infections are called lysogenic bacteriophages; lambda is the best known of this group.) The viral DNA lies dormant in the host.
chromosome until a signal directs it to begin an active (often lytic) infection cycle. In most cases, the nature of that signal is unknown. During the active infection cycle, viral genetic material is reproduced and viral proteins are made. New virus particles are assembled and released.

There are variations on the lytic- and latent-infection themes. For example, the varicella virus infects its human host and causes the disease, chicken pox. During the disease, the virus goes through active infection cycles. However, when the patient recovers, the virus is not gone. Copies of the viral DNA remain integrated into the chromosomes of certain cells as a latent infection. This viral DNA may remain dormant for the rest of the patient’s life, or it may reactivate, causing a second disease known as shingles.

**Transduction**

In general, transduction is the result of an error in bacteriophage reproduction. As bacteriophage reproduce, they replicate their genetic material and also produce new virus coats. The coats themselves (properly called capsids) are assemblies of viral proteins. At some point in the construction of the new virus, the newly replicated viral genetic material must be packaged into the capsids to create a virus particle. Each bacteriophage has a mechanism for packaging its genome into a capsid. Some bacteriophages occasionally make an error and package a piece of the host cell’s DNA instead. This event is usually random, so any bacterial gene could end up inside a virus particle.

Virus particles that contain bacterial DNA instead of viral DNA are completely capable of attaching to a new host cell and injecting DNA (those functions are carried out by the protein capsid and are independent of its contents). Once inside the new cell, the bacterial DNA can recombine with the resident genome and can be expressed and transmitted to future generations. When this happens, transduction has occurred.

Sometimes bacterial genes actually become part of a bacteriophage chromosome. This happens in lysogenic infections in which the initial infection was latent and the phage genetic material resides in the host genome for a time. Apparently, when the phage DNA pops back out of the host chromosome to reproduce and package itself, it occasionally brings a piece of host DNA with it. This host DNA then acts like part of the viral genome and is replicated and transmitted along with it. Newly infected cells receive that particular fragment of bacterial DNA instead of the random fragments described above.
Medical Importance of Transduction

Is transduction important to people other than scientists? Yes. Several transduction events have great medical significance. The ones that we know about involve the second form of transduction described previously, in which a bacterial gene has apparently become part of a bacteriophage chromosome and is transferred to other hosts by the phage.

One example of a phage-borne disease is the usually fatal food poisoning called botulism. This disease is associated with the bacterium *Clostridium botulinum*, but the disease itself is caused by only one particular protein made by that organism. This protein is the botulism toxin (a toxin is any protein that has a poisonous effect on an organism). The gene for the botulism toxin is carried by a bacteriophage that infects *C. botulinum* and is thought to have been transduced from another bacterium. Without the phage, there would be no botulism.

Lysogenic bacteriophages have also been implicated in several other severe diseases, including *Staphylococcus aureus* food poisoning, diphtheria, and cholera. As in botulism, the major symptoms of all these diseases are caused by single toxin proteins produced by the infecting bacterium: *S. aureus*, *Corynebacterium diphtheriae*, and *Vibrio cholera*, respectively. The genes for the diphtheria toxin, the cholera toxin, and the most common *S. aureus* food poisoning toxin are all encoded within the chromosomes of lysogenic bacteriophages. Strains of each of these bacteria that do not contain the lysogenic phages can be isolated, and they do not cause the diseases.

Demonstration of Random Transduction

The exercise in this kit demonstrates transmission of a gene for ampicillin resistance to *Escherichia coli* by the bacteriophage known as T4. Random transduction actually involves two infection steps: in the first infection, fragments of the host genome are packaged by mistake; in the second infection, new host cells receive the bacterial genes. In this exercise, however, you and your students will perform only the second step. The bacteriophage lysate (a suspension of phage particles) included in the kit already contains virus particles with packaged bacterial DNA. The suspension of virus particles is called a lysate because the phages are harvested from an infected culture of *E. coli* after the cells lyse (break open).

The transducing lysate is produced by growing a special strain (see below) of bacteriophage T4 on host cells that contained the plasmid pKK061 (Figure 1). This plasmid contains a gene for resistance to ampicillin but also has (in addition to its normal replication origin) an origin of replication recognized by bacteriophage T4 DNA replication proteins.
When T4 infects the host cells containing pKK061, the viral proteins replicate the plasmid along with the T4 DNA. The result is a lot of plasmid DNA available in the host cell for T4 to package by mistake. For every 10,000 virus particles produced in that infection, we observe one transduction event involving the ampicillin-resistance gene from the plasmid.

How will transduction be detected? Transfer of the plasmid DNA to a new host will render that host resistant to ampicillin, so transductants (cells that have been successfully transduced) can be selected by plating potentially transduced cells on ampicillin-containing media. The transducing particles represent only 0.01% of the viruses in the lysate. What about the other bacteriophage particles, which normally infect and kill E. coli? These normal viruses would usually make it difficult to detect the transductants. To avoid this problem, a special strain of T4 is used to make the transducing lysate. This T4 strain does not kill the indicator bacteria. However, the ampicillin in the selection plates kills all the indicator bacteria except the transductants, so that only those cells grow and form colonies.

In this activity, your class will use two different bacterial strains. The first is BE, the ampicillin-sensitive indicator strain used for detecting transductants. Recall that the transducing T4 does not reproduce in BE. In procedures A and C, students will infect BE with T4 and plate to detect ampicillin-resistant transductants. The second bacterial strain in the kit is CR63. The transducing T4 does reproduce in CR63 and kills the cells. This E. coli strain is included so that your students can observe cell lysis by bacteriophage T4. In procedures B and D, your students will infect CR63 and plate the infected bacteria on antibiotic-free medium to observe cell lysis.

The reason the indicator bacteria are not killed by the transducing T4 strain is discussed in the reading at the end of this manual. It makes an excellent discussion for higher-level students.
Follow-Up

This activity can be followed by a preparation of plasmid DNA from the transductants using the Plasmid DNA Isolation Reagent System #RN-21-1310. Have your students design restriction digestion tests (predicting fragment sizes) to confirm that the plasmid is pKK061. A procedure for plasmid DNA isolation can be found in DNA Science: A First Course in Recombinant DNA Technology by Micklos and Freyer (catalog #RN-21-2209) or Laboratory DNA Science by Bloom, Micklos, and Freyer (Benjamin/Cummings, Menlo Park, CA).

Materials

The materials in this kit are sufficient for six setups of the experiment and are designed for this experiment only. The bacterial virus T4 does not infect (and therefore is not harmful to) humans.

Included in the kit

- vial transducing lysate (200 µL)
- *E. coli* B₄ slant culture
- *E. coli* CR63 slant culture
- 3 50-mL bottles LB
- 4 bottles LB agar
- 4 sterile inoculating loops
- 3 25-pack sterile culture tubes
- 2 sterile 10-mL pipets
- 50 sterile petri plates
- 2 vials ampicillin
- glass beads
- 24 sterile transfer pipets
- Teacher’s Manual
- 6 Student Guides
- 4 sterile 1-mL pipets

Needed, but not supplied

- micropipets (to measure 10 µL and 50 µL)
- microcentrifuge tubes
- large beakers
- boiling water bath or microwave oven (to melt agar)
- test tube racks
Optional

37°C incubator
60°C water bath

Note: Store E. coli slant cultures and T4 lysate in the refrigerator. The bacterial cultures will keep for 3 months and the lysate is good for 1 year. Do not freeze these, however. Ampicillin solution will keep for 1 month in the refrigerator; 6 months to a year if frozen. All other materials may be stored at room temperature.

### Time Requirements

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Time Required</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Several days before lab</td>
<td>75–90 minutes</td>
<td>Prepare agar plates</td>
</tr>
<tr>
<td>1–2 days before lab</td>
<td>15 minutes</td>
<td>Start bacterial cultures</td>
</tr>
<tr>
<td>Day of lab</td>
<td>10 minutes</td>
<td>Dilute phage lysate</td>
</tr>
<tr>
<td>Day after lab</td>
<td>20 minutes</td>
<td>Set up work stations</td>
</tr>
<tr>
<td></td>
<td>45 minutes</td>
<td>Transduction experiment</td>
</tr>
<tr>
<td></td>
<td>30 minutes</td>
<td>Results and discussion</td>
</tr>
</tbody>
</table>

### Handling and Disposal of E. Coli

*E.coli* is a normal inhabitant of the digestive tract. Laboratory strains of *E. coli* are not considered dangerous or pathogenic. They are not the same strains as those that have been implicated in disease outbreaks. However, if *E. coli* is introduced into an open wound or the eye, it may cause infection. Follow these rules when working with the organism:

1. Always re-flame the inoculating loop or cell spreader before putting it down on the lab bench.
2. When pipetting a suspension culture, keep your nose and mouth away from the tip of the pipet to avoid inhaling any aerosol that might be created.
3. Avoid over-incubating the plates. Over-incubation may permit any contaminating organisms or fungi to grow. Refrigerate the plates after 24 hours at 37°C or after 48 hours at room temperature.
4. Wipe down the laboratory bench with 10% bleach, soapy water, or a disinfectant at the end of the lab period.
5. Wash your hands before leaving the lab.
6. Do not eat, drink, or apply cosmetics in the lab.
7. Properly dispose of all materials that contacted the bacteria. Options include the following:

- Soak all contaminated materials in a 10% bleach solution. Plates should be flooded. Let everything stand submerged in the bleach for at least 15 minutes (overnight is fine, too). Then drain the excess bleach, seal the materials in a plastic bag, and put the bag in the regular garbage.

- Autoclave all contaminated materials at 121°C for 15 minutes. Tape stacks of plates together and cap the tubes. Collect the materials in an autoclave bag for autoclaving. Dispose of the autoclaved bag and its contents in the regular trash.

Procedure

Part 1: Preparing Agar Plates

Four or five days before the lab, prepare LB agar plates and LB agar plates with ampicillin. You will need 4 plates of each type per student group, so a minimum of 24 plates of each type is required for six groups. Use good aseptic technique and, if possible, work in an area that does not have strong air currents or heavy activity.

1. Loosen the caps and place the bottles of LB agar in a boiling water bath or in a microwave oven until the agar has completely melted.

2. Allow the agar to cool to about 60°C. If you have a water bath, set it to 60°C and place the hot bottles in it to equilibrate. Antibiotics are inactivated by hot temperatures; the agar must be at 55–60°C before the ampicillin is added. At that temperature, the bottle will feel warm to the touch, but it will not burn. Label 2 bottles “with amp” to designate them for the addition of ampicillin.

3. Carefully cut the top of the sleeve containing the sterile petri plates and save the sleeve for storage of the poured plates. Remove the plates from the sleeve, being careful to keep the lids in place. Label the bottoms of 25 plates “LB” and the bottoms of 25 other plates “LB/amp.” Place the closed plates in a row near the edge of a table or lab bench away from drafts.

4. When the agar has cooled, aseptically add 1 vial of ampicillin each to the two bottles marked “with amp.” Work quickly. Do not put the caps down on the lab bench. Immediately replace the lids onto the agar bottles. Swirl the agar to mix the ampicillin.

5. Pour the melted agar from each bottle into the appropriately labeled plates. Lift the lid of each plate to pour enough agar to cover the plate
bottom (about 3 mm deep). Replace the lid. If you are using a 60°C water bath, leave the other bottles of agar in the bath while you work.

6. Allow the agar to solidify. Leave the plates out overnight, then store them topside up in the plate sleeves in a refrigerator. Leaving the plates at room temperature overnight allows you to detect contaminated plates and lets excess moisture evaporate.

Part 2: Starting *E. Coli* Cultures

On the day of the lab, you will need freshly grown cultures of *E. coli* strain BE and *E. coli* strain CR63. Each student team will use slightly over 2 mL (allowing for pipetting imprecision) of each culture. You can either grow one large culture of each strain, then aliquot the cells for student use on the day of the lab, or start 1 small culture of each strain for each student team. Start your cultures the day before the lab if you incubate them at 37°C. Start them 2 days before the lab if you incubate them at room temperature.

For the large-volume cultures, use 1 sterile disposable inoculating loop to introduce cells from the BE slant into one of the bottles of sterile LB (label the bottle). Use a fresh loop to introduce cells from the CR63 slant into a second bottle of sterile LB (label the bottle). Keep the third bottle of LB sealed until the day of the lab. You will use some of that broth from the third bottle to dilute the phage lysate. To make individual cultures, use a sterile 10-mL pipet to aliquot 5 mL of sterile broth into 14 sterile culture tubes. (This will require more than 1 bottle of LB.) Label 7 of the tubes “CR63” and 7 others “BE.” Use a sterile loop to introduce CR63 cells from the slant into the 7 designated tubes. You may reuse the loop as long as you do not touch anything but the slant and the sterile broth. Use a fresh loop to inoculate BE cells into the other 7 tubes. Incubate the tubes at 37°C overnight with shaking, if possible. If you do not have a shaker, simply shake the cultures yourself when you think about it. Grow the cells for 2 days if you are growing them at room temperature.

Part 3: Diluting Phage Lysate

1. On the day of the lab, use one of the sterile 1-mL pipets in the kit to aseptically add 0.45 mL (450 µL) of sterile LB to one of the sterile culture tubes. Label this tube “1 to 10.”

2. Use a fresh sterile 1-mL pipet to add 1 mL to a second tube. Label this tube “1 to 100.”

3. Pulse the T4 lysate tube in a microcentrifuge or tap it on the counter to collect all the lysate in the bottom.

4. Using a micropipet with a sterile tip, add 50 µL of the undiluted T4 lysate to the tube marked “1 to 10.” Thump the tube gently to mix.
5. Use a sterile tip to add 10 µL of the lysate to the tube marked “1 to 100.” Thump to mix.

6. Store the phage dilutions on ice or in the refrigerator. Do not make them until the day of the lab.

*Dilution Math*

1 to 10 dilution: add 50 µL phage to 450 µL broth
Initial volume of phage: 50 µL
Final volume of phage: 50 µL + 450 µL broth = 500 µL
Ratio of initial/final volume = 50 µL/500 µL = 1/10

1 to 100 dilution: add 10 µL phage to 1000 µL
Initial volume of phage: 10 µL
Final volume of phage: 10 µL + 1000 µL = 1010 µL
Ratio of initial/final volume = 10 µL/1010 µL = 1/101 µL, or about 1/100
(For an exact 1/100 dilution, add 10 µL of phage to 990 µL of broth.)

**Part 4: Setting Up Student Workstations**

Prepare 6 student workstations with the following materials:
- container of 10% bleach or other disinfectant for biological waste
- labeled microcentrifuge tube containing 20 µL non-dilute phage lysate
- labeled microcentrifuge tube containing 25 µL 1 to 10 phage dilution
- labeled microcentrifuge tube containing 25 µL 1 to 100 phage dilution
- 4 LB plates
- 4 LB/amp plates
- 8 sterile culture tubes in a rack
- 4 sterile transfer pipets
- micropipet or other device to measure 10 µL
- sterile tips
- marking pen
- *E. coli* strain B6 liquid culture (at least 2 mL)
- *E. coli* strain CR63 liquid culture (at least 2 mL)

Students will share:
- glass beads for spreading

**Note:** If the phage solutions will be stored before they are used, keep them on ice or in a refrigerator.
Student Laboratory Briefing

Background
Discuss the process of transduction with your class. Be sure they understand how infecting *E. coli* with the bacteriophage T4 lysate could leave some cells ampicillin-resistant. If you are teaching an advanced class, discuss the information in the back of this manual about amber mutations and amber suppressors. This is an excellent topic for reinforcing discussions of the genetic code, protein synthesis, and mutations.

This lab exercise also provides a good opportunity to discuss the purpose of making dilutions and dilution mathematics. Ask students to predict the results for the undiluted lysate, the 1 to 10 lysate, and the 1 to 100 lysate. They should understand that since tenfold fewer phages are being added to each subsequent dilution, they should expect tenfold fewer colonies (representing transducing events) on the corresponding amp plate.

Review sterile technique.

Fine Points of Lab Procedure
Students should not place materials, such as pipet tips that have contacted bacterial cells, on their lab benches. Contaminated materials should be placed directly into the provided containers of disinfectant.

Be sure the phage lysate and dilutions are introduced directly into the cells. The point of the pipet tip should be in the cell suspension when the phage suspension is expelled. If the phages are on the side of the tube, they cannot infect the bacterial cells.

The objective of spreading the cells is to separate them so the transductants among them can form isolated colonies. The cells should be spread soon after they are transferred to the plate, or they will tend to soak into the agar in one spot. To spread the cells, shake 4 or 5 glass beads from the bead jar onto each plate and use a back-and-forth motion (not swirling round and round) to move the glass beads across the entire surface of the plate. This should evenly spread the cell suspension over the agar surface. To remove the glass beads, hold each plate vertically over a container, open the plates slightly (like a clam shell) and tap the beads into the container.
As supplied, the glass beads are sufficiently sterile such that, if the recommended incubation times are not exceeded, they can be used as-is. If you reuse the beads or are especially concerned with sterility, the beads can be sterilized by autoclaving, boiling for 10 minutes, or by washing in ethanol or a 10% bleach solution for 10 minutes (followed by a thorough rinsing in sterile water) and allowed to dry in a sterile container.

**Student Guide Procedures**

**Preparation**

1. Label 4 sterile culture tubes “BE.” Label 4 other sterile culture tubes “CR63.” These are the names of the strains of *E. coli* you will use to detect transduction and cell lysis by bacteriophage T4.

2. On one of the BE tubes and one of the CR63 tubes, write “non-dilute.” On a second BE tube and a second CR63 tube, write “1 to 10.” On a third BE tube and a third CR63 tube, write “1 to 100.” On the fourth tube of each set, write “no phage.”

**Procedures**

*Procedure A: Transduction of E. coli Strain BE*

1. Use a sterile transfer pipet (Figure 2) to place 0.5 mL of *E. coli* BE culture into the 4 labeled BE tubes. (Use the waste container provided to dispose of any materials that contact the cells.)

2. Take the tube of BE cells labeled “non-dilute” and add 10 µL non-dilute phage lysate to it, using a sterile micropipet tip. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phages are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phage and cells.

3. Take the tube of BE cells labeled “1 to 10” and add 10 µL of the 1 to 10 phage dilution to it, using a sterile micropipet. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phages are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phage and cells.
4. Take the tube of BE cells labeled “1 to 100” and add 10 µL of the 1 to 100 phage dilution to it, using a sterile micropipet tip. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phages are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phages and cells.

5. Let all the tubes stand at room temperature for 5 to 15 minutes. During this time, the T4 phages attach to the cells and inject their genetic material. While the cells and phage mixtures incubate, go on to Procedure B.

Procedure B: Cell Lysis by Bacteriophage T4

1. Use a sterile transfer pipet (Figure 2) to place 0.5 mL of E. coli CR63 culture into the 4 labeled CR63 tubes. (Use the waste container provided to dispose of any materials that contact the cells.)

2. Take the tube of CR63 cells labeled “non-dilute” and add 10 µL non-dilute phage lysate to it, using a sterile micropipet tip. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phages are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phage and cells.

3. Take the tube of CR63 cells labeled “1 to 10” and add 10 µL of the 1 to 10 phage dilution to it, using a sterile micropipet. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phages are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phage and cells.

4. Take the tube of CR63 cells labeled “1 to 100” and add 10 µL of the 1 to 100 phage dilution to it, using a sterile micropipet tip. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phage are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phages and cells.

5. Let all the CR63 tubes stand at room temperature for 5 to 15 minutes for attachment of the phages to the cells. While they incubate, proceed with Procedure C, plating the phage/cell mixtures in the BE tubes.
**Procedure C: Plating to Detect Transductants**

1. Take your 4 LB/amp plates and label one (on the bottom) “no phage,” one “non-dilute,” one “1 to 10,” and one “1 to 100.”

2. One at a time, slightly open each plate and shake 4 or 5 beads onto the agar surface.

3. Take the B_E tube labeled “no phage” and pour its contents onto the appropriately labeled plate. Replace the plate lid.

4. Use a back-and-forth motion (not swirling round and round) to move the glass beads across the entire surface of the plate. This should evenly spread the cell/phage suspension over the entire agar surface.

5. To remove the glass beads, hold the plate vertically over a container. Open the plate slightly (like a clamshell) and tap the beads into the container.

6. Repeat steps 3–5 with the “non-dilute,” “1 to 10,” and “1 to 100” tubes and plates.

7. After the plates have sat for a few minutes at room temperature and the liquid is absorbed into the agar, invert the plates. Incubate them at 37°C overnight or at room temperature for 1–2 days.

8. When you have finished plating the B_E cell/phage mixtures, immediately plate the CR63 cell/phage mixtures. Follow Procedure D.

**Procedure D: Plating to Detect Cell Lysis**

1. Take your 4 LB plates and label one (on the bottom) “no phage,” one “non-dilute,” one “1 to 10,” and one “1 to 100.”

2. One at a time, slightly open each plate and shake 4 or 5 beads onto the agar surface.

3. Take the CR63 tube labeled “no phage” and pour its contents onto the appropriately labeled plate. Replace the plate lid.

4. Use a back-and-forth motion (not swirling round and round) to move the glass beads across the entire surface of the plate. This should evenly spread the cell/phage suspension over the entire agar surface.

5. To remove the glass beads, hold the plate vertically over a container. Open the plate slightly (like a clamshell) and tap the beads into the container.

6. Repeat steps 3–5 with the “non-dilute,” “1 to 10,” and “1 to 100” tubes and plates.
7. After the plates have sat for a few minutes at room temperature and the liquid is absorbed into the agar, invert the plates. Incubate them at 37°C overnight or at room temperature for 1–2 days.

What do you expect to see on the “no phage” plates with BE? With CR63?

Results

Part A: Transduction
Record the number of colonies on all 4 plates. If there is a reasonable number on a plate, count them and record the number. If there is a very large number of colonies on a plate, divide the plate evenly into quadrants by making a large + on the back of the plate, and count the colonies in 1 quadrant. Estimate the total number of colonies on the plate by multiplying the number in the quadrant by 4.

Part B: Cell Lysis
Record your results by describing the appearance of each plate. Drawing them may be helpful.

Questions

Part A: Transduction
1. Why was the phage suspension diluted?
   The phage suspension was diluted so that a reasonable number of transductants could be detected on one or two plates. The starting suspension might have been too concentrated and would then have given too many transductants to count.

2. Did you see colonies on the “no phage” plate? Was this what you expected? What was the purpose of this plate? (If you think it was a control, be sure to say what sort of control it was. In other words, what would it show you?)
   There should be no colonies on the “no phage” plate. The agar has ampicillin in it, and the “no phage” tube had only E. coli BE cells in it. This E. coli strain is sensitive to ampicillin and should not grow on the plate. If you see isolated colonies, there has been contamination or a mix-up. Either the phage were accidentally added, giving some ampicillin-resistant transductants, or the culture was contaminated with ampicillin-resistant organisms, or the wrong tube was emptied onto the plate. The “no phage” plate was intended to act as a control.
to verify that without the addition of transducing phage, there are no ampicillin-resistant cells in the BE culture. If the “no phage” plate fails to show colonies, we can conclude that the ampicillin-resistant colonies on the other plates are there because of the phage.

3. What are the colonies growing on the amp plates to which you added cells mixed with phages? Are there more of them on the “non-dilute” plate than on the “1 to 10” plate or the “1 to 100” plate? Why or why not?

*The colonies growing on the plates to which phage/cell mixtures were added are transductants (unless there were colonies on the “no phage” plate; in that case, we cannot be sure what any of the colonies are). These colonies contain plasmid DNA transduced by the phages.*

There should be approximately 10 times more colonies on the “non-dilute” plate than on the “1 to 10” plate because the non-dilute phage suspension should have 10 times more phages in it than the 1 to 10 dilution. Likewise, the “1 to 10” plate should have 10 times more colonies than the “1 to 100” plate.

4. Suppose you looked first at the plate to which you added the cells containing 10 µL of the 1 to 10 dilution and counted 50 colonies. How many would you expect to see on the plate with the cells to which you added 10 µL of the non-dilute lysate? On the plate with the cells to which you added 10 µL of the 1 to 100 dilution?

*Five hundred colonies would be expected on the “non-dilute” plate because 10 times more phage suspension was added to it. Five colonies would be expected on the “1 to 100” plate (see answer to Question 3 above).*

5. (Optional) You have 100 µL of a phage suspension which you need to dilute 1 to 10 with LB broth. Describe how you could do this, specifying what volumes of phage suspension and broth you would use.

*Mix 100 µL of the phage suspension with 900 µL Luria broth. Any answer in which the final volume of the phage + broth is exactly 10 times the initial volume of phage in the mixture is correct. For example, 50 µL phage + 450 µL broth = 500 µL final volume; 50 µL phage / 500 µL final volume = 1/10.*

**Part B: Cell Lysis**

1. Do the plates to which phages were added look the same as the “no phage” plate? If they are different from the “no phage” plate, what is causing the difference? If they are the same, why do you think they are?

*The “phage” plates should be different from the “no phage” plates. The difference is that the phage lyses CR63 and new phage may then*
infect neighbor cells. Perhaps no cells at all will be growing on the “phage” plates because of the spread of reproducing phages. On the other hand, some growth may be visible, depending on the efficiency of spreading. Some growth will give the plates a littered or splotchy appearance.

2. What was the purpose of the “no phage” plate? (If you think it was a control, be sure to say what sort of control it was. In other words, what would it show you?)

The purpose of the “no phage” plate was to let you see what a normal plate of CR63 would look like so that you could compare it to the plates to which phages were added. The differences in the “no phage” and “phage” plates (which were treated identically except for the phage) are attributable to the phages. The “no phage” plate is a control.

Addendum: Amber Mutations and Amber Suppressors

The transducing T4 strain carries mutations in two different genes that change an amino acid codon to the stop codon UAG. This particular stop codon is called an amber codon. Mutations converting an amino acid codon to an amber codon are called amber mutations.

Because they create a stop codon, amber mutations terminate protein synthesis and usually result in loss of function of the protein in question. (If the amber mutation is very near the end of the protein-encoding sequence, it may not have much effect.) In the transducing T4 strain, the two amber mutations block production of two essential proteins and are, therefore, lethal.

Amber mutations in bacteriophages are useful to scientists because in combination with special host strains, they provide an “on-off” switch for phage growth. Here is how the on-off system works. Amber codons (and the other two stop codons) stop translation because no transfer RNAs (tRNAs) have matching anticodons. When an amber codon in messenger RNA reaches the ribosome, no new amino acid is added to the growing peptide chain; instead, protein synthesis is terminated. There is nothing magic about the base sequence of the terminator codons except that they have no matching tRNAs that will add amino acids to the peptide chain in response to them.

However, tRNA molecules are also encoded by DNA (to make tRNA, the DNA base sequence is simply transcribed into RNA by a special RNA polymerase). The base sequence of any tRNA can be changed by mutations in the gene encoding it. In some bacterial strains, mutations
change the anticodon of a tRNA so that the tRNA can recognize what used to be one of the stop codons. In these strains, that codon is no longer a stop codon, but instead encodes whatever amino acid the mutant tRNA carries (serine, for instance). If the mutant tRNA recognizes the amber stop codon and inserts a serine at its position, an amber codon no longer terminates protein synthesis. A bacterial strain that makes a tRNA that recognizes the amber codon is called an amber suppressor.

In amber suppressor strains, amber mutations are essentially erased. The effect of an amber mutation comes from its ability to terminate protein synthesis; in amber-suppressing strains, protein synthesis is not terminated. Since the mutant tRNA does not usually insert the original amino acid at the site of the amber mutation, the protein may be impaired in function, but often it can still perform adequately. So, bacteriophages carrying normally lethal amber mutations may be able to reproduce in bacterial hosts that suppress the amber mutations through a mutant tRNA.

Our transducing T4 strain fits this description. In bacterial hosts with normal tRNAs, the phage is “dead” (unable to produce new virus particles) as a result of its amber mutations. However, in strains that make a mutant tRNA that suppresses the amber stop codon, the phage can reproduce itself quite well. Thus, laboratory personnel can control whether the phage reproduces through choice of the host bacterial strain. The amber mutations in our bacteriophage T4 are an example of conditional lethal mutations—under some conditions, they are lethal; under others, they are not.

For this activity, the transducing lysate was produced by infection of an amber-suppressing strain carrying pKK061 (see Figure 1). The suppressing strain allowed the bacteriophage to reproduce. To detect transductants without interference from cell lysis, the lysate is allowed to infect *E. coli* strain B_E, which does not suppress amber codons. The T4 strain cannot grow in B_E, so no cell lysis is seen. However, any transducing particles that were made during the first infection can transduce B_E to ampicillin resistance. The B_E-T4 mixture is plated on ampicillin-containing medium, so that transductants can be detected in the absence of cell killing by the virus.
Transduction of an Antibiotic Resistance Gene

Background

Transduction is a natural method of gene transfer that occurs in bacteria. The key player in transduction is a bacterial virus, or bacteriophage ("phage" for short). Many different bacteriophages infect many different bacteria. You may have met one of these phages—lambda. Today, you will meet a different phage, one that also infects *Escherichia coli*. It is called T4.

In transduction, a bacteriophage transfers bacterial genes from one host cell to another. How does T4 transfer genetic material between *E. coli* cells? The answer is found in its life cycle. T4 infects *E. coli* by attaching to its outer membrane and injecting its DNA into the bacterial cell. Once inside the cell, the phage DNA takes over. The *E. coli* cell becomes a factory for producing many copies of the T4 DNA and new proteins, some of which are assembled into new T4 heads and tails. After many copies of the T4 genome have been made and many new heads and tails are floating around in the cytoplasm, still other T4 proteins begin to put together new virus particles. These proteins fill the empty phage heads with T4 DNA and then attach the tails. After many new viruses are assembled, the *E. coli* cell bursts, releasing the virus progeny.

What does this have to do with transferring *E. coli* genes? The critical step is the point at which the new virus particles are assembled. Once in a while, the T4 assembly proteins make a mistake. Instead of filling a phage head with T4 DNA, the proteins fill the phage head with a piece of the host cell’s bacterial DNA. The filled head gets a tail and becomes a virus particle fully capable of injecting DNA into a new bacterial cell. However, when it does so, the new host cell receives that bacterial DNA instead of the dangerous viral DNA. When the new host expresses the bacterial DNA it received, it is said to have been transduced. (Remember, no virus infection took place, since the virus particle was filled with harmless bacterial DNA instead of the viral genome.)

In this exercise, you will observe the transmission of an antibiotic resistance gene by phage T4. The T4 virus particles you will work with were grown on a plasmid-containing host strain, so some of the virus particles produced from that infection contain plasmid DNA. Your job is to detect some of these plasmid DNA-containing particles by their ability to transduce *E. coli* with the antibiotic resistance gene. Figure 1 illustrates the transduction process.

1. Infection of plasmid-containing host cell

![Diagram of T4 infection process]

2. Second infection: Transfer of Plasmid DNA (this lab)

![Diagram of second infection process]

*Figure 1*
How do you think you could detect transductants (*E. coli* that have received plasmid DNA)?

You will be infecting two different *E. coli* strains with the transducing T4 lysate. One is BE, the indicator strain for detecting transductants. The transducing T4 does not reproduce in BE, although the phage can deliver DNA to it and, thus, transduce it. In procedures A and C, you will infect BE and plate the infected bacteria to detect ampicillin-resistant transductants. The other bacterial strain in this lab is CR63. The transducing T4 does reproduce in CR63 and kills the cells. This *E. coli* strain is included so that you can observe cell lysis by bacteriophage T4. In procedures B and D, you will infect CR63 and plate the infected bacteria on antibiotic-free medium to observe cell lysis.

**Preparation**

1. Label 4 sterile culture tubes “BE.” Label 4 other sterile culture tubes “CR63.” These are the names of the strains of *E. coli* you will use to detect transduction and cell lysis by bacteriophage T4.

2. On one of the BE tubes and one of the CR63 tubes, write “non-dilute.” On a second BE tube and a second CR63 tube, write “1 to 10.” On a third BE tube and a third CR63 tube, write “1 to 100.” On the fourth tube of each set, write “no phage.”

**Procedures**

**Procedure A: Transduction of *E. coli* Strain BE**

1. Use a sterile transfer pipet (Figure 2) to place 0.5 mL of *E. coli* BE culture into the 4 labeled BE tubes. (Use the waste container provided to dispose of any materials that contact the cells.)

2. Take the tube of BE cells labeled “non-dilute” and add 10 µL non-dilute phage lysate to it, using a sterile micropipet tip. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phages are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phage and cells.

3. Take the tube of BE cells labeled “1 to 10” and add 10 µL of the 1 to 10 phage dilution to it, using a sterile micropipet. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phages are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phage and cells.

4. Take the tube of BE cells labeled “1 to 100” and add 10 µL of the 1 to 100 phage dilution to it, using a sterile micropipet tip. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phages are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phages and cells.

5. Let all the tubes stand at room temperature for 5 to 15 minutes. During this time, the T4 phages attach to the cells and inject their genetic material. While the cells and phage mixtures incubate, go on to Procedure B.
Procedure B: Cell Lysis by Bacteriophage T4

1. Use a sterile transfer pipet (Figure 2) to place 0.5 mL of *E. coli* CR63 culture into the 4 labeled CR63 tubes. (Use the waste container provided to dispose of any materials that contact the cells.)

2. Take the tube of CR63 cells labeled “non-dilute” and add 10 µL non-dilute phage lysate to it, using a sterile micropipet tip. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phages are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phages and cells.

3. Take the tube of CR63 cells labeled “1 to 10” and add 10 µL of the 1 to 10 phage dilution to it, using a sterile micropipet. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phage are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phages and cells.

4. Take the tube of CR63 cells labeled “1 to 100” and add 10 µL of the 1 to 100 phage dilution to it, using a sterile micropipet tip. Place the end of the tip into the cell culture when expelling the phage suspension to be certain the phage are directly introduced into the culture. Replace the cap of the culture tube. Dispose of the tip in the biological waste container, then thump the tube to mix the phages and cells.

5. Let all the CR63 tubes stand at room temperature for 5 to 15 minutes for attachment of the phages to the cells. While they incubate, proceed with Procedure C, plating the phage/cell mixtures in the BE tubes.

Procedure C: Plating to Detect Transductants

1. Take your 4 LB/amp plates and label one (on the bottom) “no phage,” one “non-dilute,” one “1 to 10,” and one “1 to 100.”

2. One at a time, slightly open each plate and shake 4 or 5 beads onto the agar surface.

3. Take the BE tube labeled “no phage” and pour its contents onto the appropriately labeled plate. Replace the plate lid.

4. Use a back-and-forth motion (not swirling round and round) to move the glass beads across the entire surface of the plate. This should evenly spread the cell/phage suspension over the entire agar surface.

5. To remove the glass beads, hold the plate vertically over a container. Open the plate slightly (like a clamshell) and tap the beads into the container.

6. Repeat steps 3–5 with the “non-dilute,” “1 to 10,” and “1 to 100” tubes and plates.

7. After the plates have sat for a few minutes at room temperature and the liquid is absorbed into the agar, invert the plates. Incubate them at 37°C overnight or at room temperature for 1–2 days.

8. When you have finished plating the BE cell/phage mixtures, immediately plate the CR63 cell/phage mixtures. Follow Procedure D.
Procedure D: Plating to Detect Cell Lysis

1. Take your 4 LB plates and label one (on the bottom) “no phage,” one “non-dilute,” one “1 to 10,” and one “1 to 100.”
2. One at a time, slightly open each plate and shake 4 or 5 beads onto the agar surface.
3. Take the CR63 tube labeled “no phage” and pour its contents onto the appropriately labeled plate. Replace the plate lid.
4. Use a back-and-forth motion (not swirling round and round) to move the glass beads across the entire surface of the plate. This should evenly spread the cell/phage suspension over the entire agar surface.
5. To remove the glass beads, hold the plate vertically over a container. Open the plate slightly (like a clamshell) and tap the beads into the container.
6. Repeat steps 3–5 with the “non-dilute,” “1 to 10,” and “1 to 100” tubes and plates.
7. After the plates have sat for a few minutes at room temperature and the liquid is absorbed into the agar, invert the plates. Incubate them at 37°C overnight or at room temperature for 1–2 days.

What do you expect to see on the “no phage” plates with Bφ? With CR63?

Results

Part A: Transduction

Record the number of colonies on all 4 plates. If there is a reasonable number on a plate, count them and record the number. If there is a very large number of colonies on a plate, divide the plate evenly into quadrants by making a large + on the back of the plate, and count the colonies in 1 quadrant. Estimate the total number of colonies on the plate by multiplying the number in the quadrant by 4.

Part B: Cell Lysis

Record your results by describing the appearance of each plate. Drawing them may be helpful.
Questions

Part A: Transduction

1. Why was the phage suspension diluted?

2. Did you see colonies on the "no phage" plate? Was this what you expected? What was the purpose of this plate? (If you think it was a control, be sure to say what sort of control it was. In other words, what would it show you?)

3. What are the colonies growing on the amp plates to which you added cells mixed with phages? Are there more of them on the "non-dilute" plate than on the "1 to 10" plate or the "1 to 100" plate? Why or why not?
4. Suppose you looked first at the plate to which you added the cells containing 10 µL of the 1 to 10 dilution and counted 50 colonies. How many would you expect to see on the plate with the cells to which you added 10 µL of the non-dilute lysate? On the plate with the cells to which you added 10 µL of the 1 to 100 dilution?

5. (Optional) You have 100 µL of a phage suspension which you need to dilute 1 to 10 with LB broth. Describe how you could do this, specifying what volumes of phage suspension and broth you would use.

Part B: Cell Lysis

1. Do the plates to which phages were added look the same as the “no phage” plate? If they are different from the “no phage” plate, what is causing the difference? If they are the same, why do you think they are?

2. What was the purpose of the “no phage” plate? (If you think it was a control, be sure to say what sort of control it was. In other words, what would it show you?)