Transduction and Plaque Assay Protocols

Transduction is a method of genetic recombination in bacteria, in which DNA is transferred between bacteria via bacteriophages. In this process, DNA of a bacterial cell is transferred into another bacterial cell with the help of a bacteriophage. Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome.

The ability of a virus (bacteriophage) to carry bacterial DNA between bacteria It was first discovered by N. Zinder and J. Lederberg in 1952, in *Salmonella typhimurium* , amouse typhoid bacterium and named as transduction. The scientists found that, when a donor cell is lysed by P1 (bacteriophage), the bacterial chromosome is broken up into small pieces and sometimes the forming phage particles mistakenly incorporate a piece of the bacterial DNA into a phage head in place of phage DNA.

Based on their interactions with the bacterial cell, bacteriophages are classified into two types:
1-Virulent phages: These phages are replicated by host immediately after entry, when there is enough proportion of phages, they cause the host to lyse, so that they can be released and infect new host cells. The process is called lytic cycle.

2-Temperate phages: These phages enter host and instead of replicating, insert their genomes into bacterial chromosome. Once inserted, the viral genome is called prophage and it is passively replicated. The process is called lysogenic cycle and the bacteria that have been lysogenized are called lysogens.

Temperate phages can remain dormant in their host cells for thousands of generations, and replicate like any other segment of the host chromosome.If the lysogen is induced (by UV light for example), the phage genome is excised from the bacterial chromosome and initiates the lytic cycle ,which culminates in lysis of the cell and the release of phage particles.

Note: Before 1953, all viruses were considered lytic. In 1953, American microbiologist *Dr. Esther Lederberg* published her discovery of the lambda bacteriophage and lysogenic replication. Lambda phage is now used ubiquitously in molecular biology to study gene regulation and recombination.

There are two types of transduction:
1- Generalized Transduction
2- Specialized Transduction

1- Generalized Transduction: During this process any part of bacterial gene may be transferred to another bacterium via a bacteriophage and carries only bacterial DNA and no viral DNA.

2- Specialized Transduction : In this process specific part of bacterial genes that are near the bacteriophage genome may be transferred to another bacterium via a bacteriophage.

Note: *In this lab the process of transduction is demonstrated where the chloramphenicol resistant gene is transferred from the donor bacterial cell to the ampicillin resistant recipient through a bacteriphage.*

Duration of Experiment (Protocol: 5 days)

Day 1: Preparation of media and revival of strains
Day 2: Infection of donor by bacteriophage and preparation of phage lysate
Day 3: Infection of recipient
Day 4: Phage titration
Day 5: Observation and Interpretation

Procedure:

Day 1: Preparation of media and revival of strains

1. Streak a loopful of Donor culture (*E. coli strain containing the genetic marker as chloramphenicol resistant gene to be transduced*) on LB C20 plate, Recipient(*E. coli strain containing the genetic marker as ampicillin resistant gene)* on LB A100 plate and susceptible host on LB plate.
2. Along with streaking, inoculate loopful of culture in 5ml LB broth with respective antibiotics.
3. Incubate the plates overnight at 37˚C and Culture tubes at 37˚C shaker for overnight with a speed of 300 rpm.

Day 2: Infection of donor by bacteriophage and preparation of phage lysate

1. Store the 5 ml culture tubes at 4˚C for inoculation on Day 3.
2. Inoculate 10-15 colonies from revived donor plate into 5 ml LB C20 and label as Donor tube.
3. Incubate at 30˚C in shaker for 2 hours.
4. Keep a 5ml aliquot of sterile LB broth in water bath, set at 60-65˚C.
5. Add 100 µl of given phage lysate to above labeled donor tube, continue incubation for 30 min. at 30˚C.
6. Add 2 ml of preheated sterile LB broth to donor tube mix well and incubate this tube at 42˚C for another 20 minutes.
7. Transfer this tube to 37˚C and incubate for 3 hours.
8. After incubation, spin this culture at 5000 rpm for 10 min. Take the supernatant, filter it through 0.45 µm filter, label as Phage lysate 2.Store at 4˚C for further steps.
9. Inoculate single colony from recipient plate in 5 ml of LB broth with ampicillin (100 µg/ml). This is recipient tube; incubate this tube overnight at 37˚C shaker.

Day 3: Infection of recipient

1. Inoculate 100 µl of overnight grown recipient culture in 5 ml of fresh LB broth with ampicillin (100 µg/ml), incubate on shaker at 37˚C for 2 hours.
2. After incubation take 50 µl of this culture in 2 ml collection tube, add 50 µl 0.1M $CaCl_2$ along with 250 µl of Phage lysate 2 obtained and stored at 4˚C on Day 2.
3. Mix well and incubate further at 30˚C for 2 hours.(Do Not keep on Shaker.)
4. After 2 hours, take each 50 µl of this culture and plate on LB C20, LB A100 and LB C20 A100 plates.
5. Along with it , take 50µl of overnight grown cultures of recipient strain and donor strain which are revived on Day 1, plate on LB C20, LB A100 and LB C20 A100 plates.
6. Incubate all plates at 37˚C for overnight.
7. On next day store these plates at 4˚C for observation and results.

Day 4: Phage titration

1. Inoculate 20-25 colonies from revived plate of susceptible host (Day 1) in 15 ml of fresh LB broth.
2. Incubate this culture on shaker at 37˚C for 2 hours.
3. After incubation spin down 1.5 ml of this culture at 8000rpm for 10 min. In 7 different collection tubes (2ml) at room temperature. Then resuspend each pellet in 100 µl of fresh sterile LB broth. Use this as plating cells for titration.
4. Before starting Titration protocol, keep 5 ml of LB broth at 60˚C.
5. To confirm presence of phages in lysogenized colonies of infected recipient culture, inoculate 10-15 Colonies from LB C20 A100 plate (of recipient infected with phage) in 5 ml LB C20 A100 broth. Label this as Lysogenized culture.
6. Incubate this tube at 30˚C for 3 hours. After incubation add 2 ml of hot LB broth (kept at 60˚C) to this lysogenized culture tube.
7. Further incubate this tube at 42˚C for 20 min., again transfer this tube to 37˚C and incubate for 2 hours.
8. After 2 hours of incubation centrifuge the culture tube at 5000 rpm for 10 min., filter the supernatant through 0.45µm filter and label this as concentrated lysate.

Phage Titration:

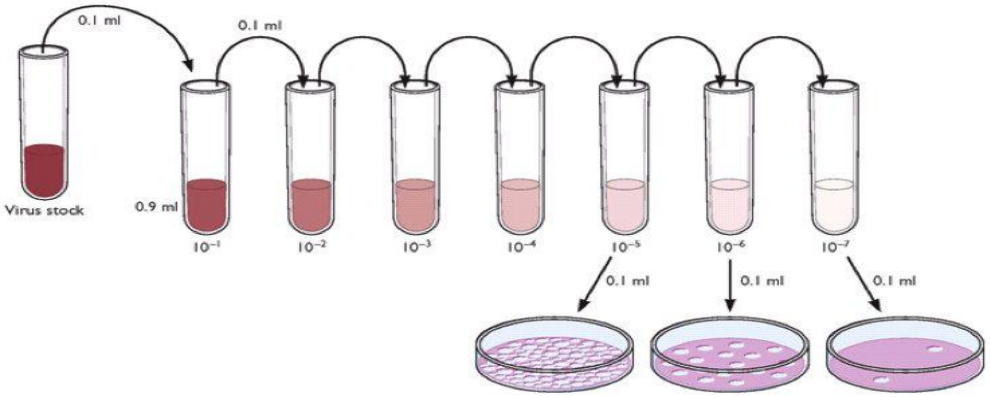
Theory

Aliquots of diluted bacteriophage are mixed with host bacterial cells in several milliliters of soft agar, which are then spread onto agar plates containing media. The use of soft agar allows the phage to easily diffuse through the medium giving more consistent plaque formation. It also eliminates the problem of uneven absorption of the bacterial-phage solution into the hard agar that often caused uneven plaque formation on the plate.

The bacteriophage adsorb onto the host bacterial cells, infect and lyse the cells, and then begin the process anew with other bacterial cells in the vicinity. After 6 – 24 hours, zones of clearing, plaques, are observable within the lawn of bacterial growth on the plate.

Procedure:

1.Take 7 collection tubes (2 ml) label them as concentrated lysate(virus stock), 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} (as shown in the following figure).



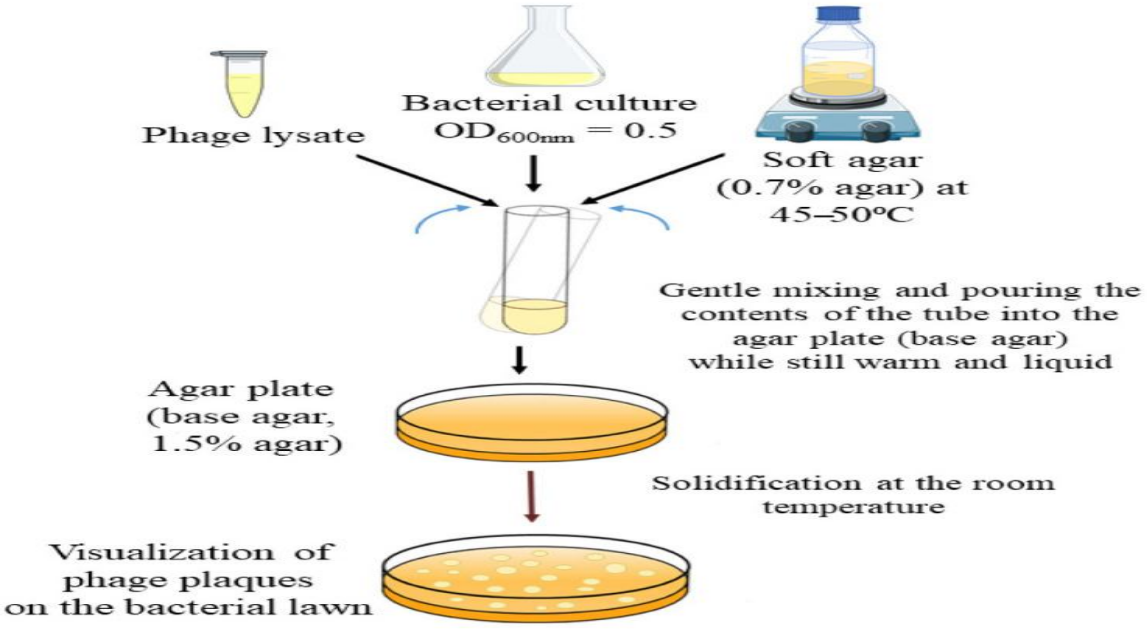
2. Put 900µl of sterile LB broth in each tube (from 10^{-1} to 10^{-6}); add 100µl of concentrated lysate to 10^{-1} tube.

Perform serial dilution up to 10^{-6} tube. Change tip every time while preparing dilution.

3. In 100 µl of plating cell tubes add 20 µl of concentrated and 20 µl phage lysate dilutions respectively And incubate all tubes at 37˚C for 15 min.

4. Meanwhile, melt the soft agar; dispense 5 ml of melted soft agar in 15 ml tubes. Add 0.1ml of 10% Dextrose, 0.125 ml of 1M MgCl2 and 0.025ml of 1M CaCl2. Mix well and keep at 45˚C.

5. Pipette out the mixture of plating cells and concentrated lysate in Soft agar tube mix well and immediately pour on labeled LB hard agar plate (Concentrated lysate) Let the agar solidify.



6. Repeat step 5 for phage dilutions from 10^{-1} to 10^{-6}.
7. Close the lids of Petri plates and incubate these plates at 37˚C.
8. Note down the results and observation next day.

Day 5: Observation and Interpretation

A- Screening of Transductants (from Day 3) : Look for transductant colonies which are growing on LB plates containing ampicillin and chloramphenicol. Note down the observations in the following table. (Indicate bacterial growth with positive symbol and absence of growth with negative symbol).

Record the results as shown in this table:

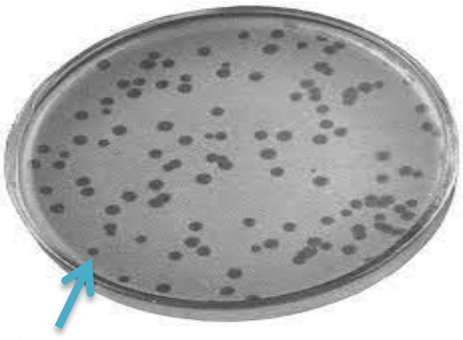
	LB + Chloramphenicol	**LB + ampicillin**	**LB + Chloramphenicol + Ampicillin**
Donor Strain			
Recipient Strain			
Transduced/Lysogenized			

B. Phage Titration (from Day 4) : Observe and count the number of plaques on those plates that have between 30 and 300 plaques. Students can work through the dilution calculations to determine the number of pfu's/ml in the original sample of phage. Count the number of plaques for each dilution and note down the results as a table.

Note: plaques are not necessarily round. Although many are, many others take a variety of shapes and sizes.

Table: Results of the phage titration assay

Tube No.	**Dilutions**	**Number of plaques**	**Phage titre value**
1			
2			
3			
4			
5			



Plaques

Interpretation:

A. Screening of Transductants: During this experiment the chloramphenicol resistance gene is transferred from donor bacterial cell to recipient through a bacteriophage by the process known as transduction as only the lysogenized or transduced bacterial cells grow on LB plates containing ampicillin and

chloroform. When bacteriophage infects the ampicillin sensitive and chloramphenicol resistant donor strain, the phage DNA enters the donor cell and integrates into the bacterial chromosome. Upon induction of lysogen (by heating), the phage DNA is excised from the bacterial chromosome and new phage particles are released by lysing the host cell. When these new phage particles infect the recipient strain lysogenization occurs and as a result the chloramphenicol resistant gene is transferred to the recipient strain which is indicated by the growth on LB plates containing ampicillin and chloramphenicol.

B. Phage Titration: When the bacteriophage is induced and titrated against the given susceptible host, the clear plaques confirm the presence of phage particles in the lysogenized recipient strain and clear plaques.

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.

2. **Chloramphenicol solution preparation:** Dissolve 20 mg of Chloramphenicol in 1 ml of 70% ethanol, mix by gentle pipetting to give a final concentration of 20 mg/ml. Cover with aluminum foil and store in refrigerator. Use this solution within a month.

3. **Ampicillin solution preparation:** Dissolve 100 mg of Ampicillin 1 ml of sterile distilled water to give a final concentration of 100mg/ml. Cover with aluminum foil and store in refrigerator. Use this solution within a month.

4. **Preparation of LB (Luria Bertani) broth (100 ml):** Dissolve 5 g of Luria Bertani broth in 200 ml of distilled water and autoclave.

5. **Preparation of LB (Luria Bertani) agar plates:** Dissolve 2.5 g of LB media and 1.5 g of agar in 100 ml of sterile distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 ˚C and pour on sterile petriplates.

6. **Preparation of LB (Luria Bertani) agar plates with Ampicillin (100 ml):** Dissolve 2.5 g of LB media and 1.5 g of agar in 100 ml of sterile distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 ˚C. Add 100 µl of Ampicillin in 100 ml of autoclaved LB agar media and pour on sterile petriplates.

7. **Preparation of LB (Luria Bertani) agar plates with Chloramphenicol (100 ml):** Dissolve 2.5 g of LB media and 1.5 g of agar in 100 ml of sterile distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 ˚C. Add 100µl of Chloramphenicol to 100 ml of autoclaved LB agar media and pour on sterile petriplates.

8. **Preparation of LB agar plates with Ampicillin + Chloramphenicol (100 ml):** Dissolve 2.5 g of LB media and 1.5 g of agar in 100 ml of sterile distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 ˚C. Add 100 µl of Ampicillin and 100 µl of Chloramphenicol to 100 ml of autoclaved LB agar media and pour on sterile petriplates.

9. **Preparation of 0.1M $CaCl_2$ (1 ml):** Mix 0.1ml of given 1 M $CaCl_2$ with 0.9 ml of sterile distilled water to get 0.1M $CaCl_2$ solution.

10. **Preparation of Soft agar:** To prepare soft agar, dissolve 2.5 g of LB Media and 0.8 g of Agar powder in 100 ml of sterile distilled water and autoclave.