HiPer® Transduction Teaching Kit

Product Code: HTM005E
Number of experiments that can be performed: 5/20
Duration of Experiment

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

Storage Instructions

- The kit is stable for 6 months from the date of manufacture
- Store Phage Lysate, 1M $CaCl_2$ Solution, Ampicillin and Chloramphenicol at 2- 8°C
- Other kit contents can be stored at room temperature (15-25°C)

ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

The information contained herein is believed to be accurate and complete. However no warranty or guarantee whatsoever is made or is to be implied with respect to such information or with respect to any product, method or apparatus referred to herein

Index

Sr. No.	Contents	Page No.
1	Aim	3
2	Introduction	3
3	Principle	3
4	Kit Contents	5
5	Materials Required But Not Provided	5
6	Storage	5
7	Important Instructions	6
8	Procedure	6
9	Observation and Result	8
10	Interpretation	9
11	Troubleshooting Guide	10

Aim:

To learn the process of transduction which is genetic transfer of a particular gene from one *E. coli* host to another through a bacteriophage.

Introduction:

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.

Principle:

Bacteria can exchange or transfer DNA between other bacteria in three different ways:

(1) Transformation: This process involves acquisition of DNA from the environment and susceptible to DNase.

(2) Conjugation: During this process DNA is directly acquired from another bacterium and cell-to-cell contact is required.

(3) Transduction: In this process DNA is incorporated to a bacterial cell via a bacteriophage intermediate. It does not require cell-to-cell contact and it is DNase resistant.

In all these cases the source cells of the DNA are called 'donors' and the cells that receive the DNA are called the 'recipients. In each case the donor DNA is incorporated into the recipient's cell's DNA by recombination exchange (as shown in Fig. 1). If the exchange involves an allele of the recipient's gene, the recipient's genome and phenotype will change.

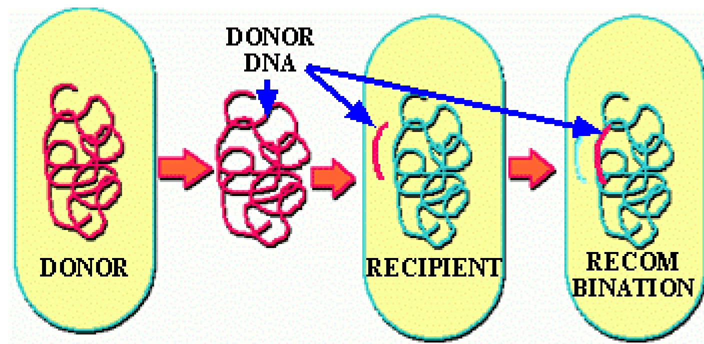

Fig 1: Exchange of genetic material between two bacterial cells

The ability of a virus (bacteriophage) to carry bacterial DNA between bacteria was discovered in 1952 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. The bacteriophage goes through either the lytic cycle or the lysogenic cycle. During the lysogenic cycle the phage chromosome is integrated into the bacterial chromosome and can remain dormant for several generations. If the lysogen is induced 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. The lytic cycle leads to the production of new phage particles which are released by lysis of the host.

The entire process of transduction involves the following steps:

1. First, the phage infects a susceptible bacterium (donor) and injects its DNA into the host.

2. The phage DNA utilizes the host's machinery and synthesizes phage components including phage DNA. During this process parts of bacterial chromosome is integrated into the phage DNA.
3. As a final step in the phage life-cycle, all the phage components in the cytoplasm are assembled into complete phage and the cell is lysed to release the newly made phage particles.
4. When this newly made virus particle infects the 'recipient' bacteria, the phage DNA (containing parts of 'donor's DNA) is injected into it and the transduced bacterial genes can be incorporated by recombination.

The transduction process is different from the usual gene recombination process. The most striking difference is the transfer of genetic material from cell to cell by viruses. The second feature is the fact that only a small part of the total genetic material of one bacterial cell is carried by the bacteriophage.

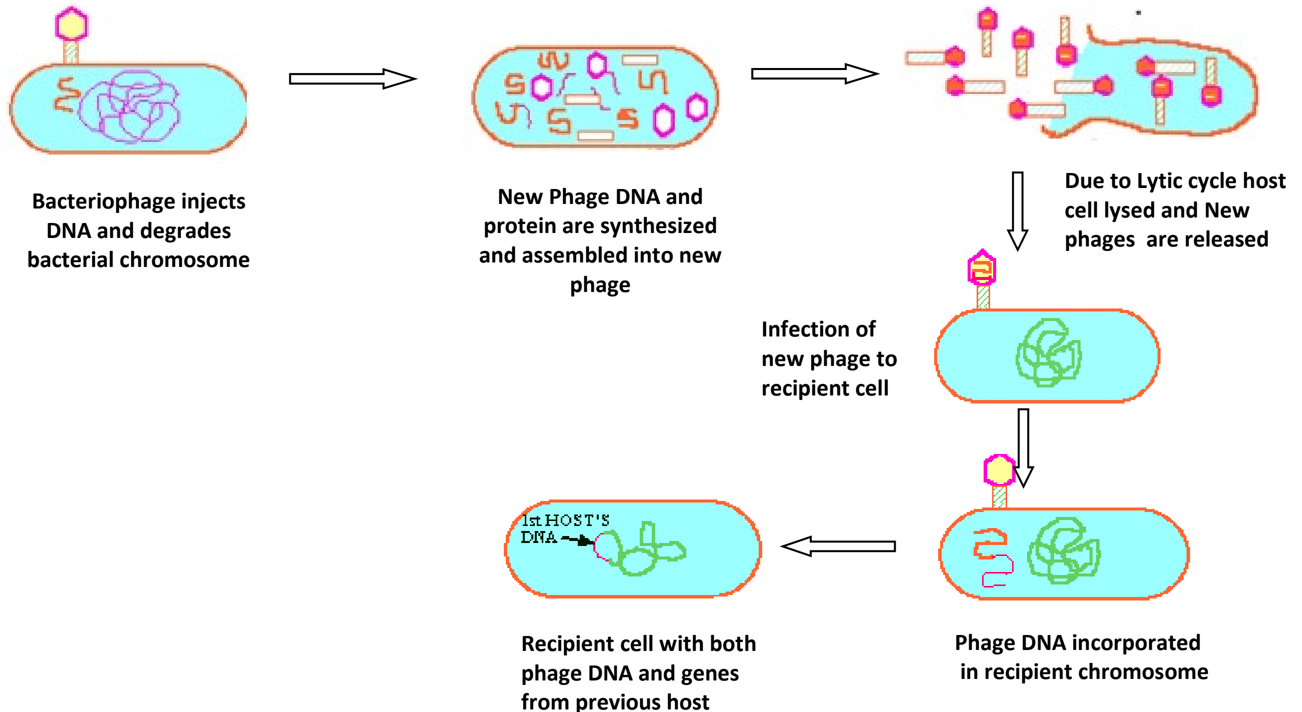

Fig 2: During transduction the donor's genetic material is transferred to the recipient cell through a bacteriophage

There are two types of transduction:

i) **Generalized Transduction -** During this process any part of bacterial gene may be transferred to another bacterium via a bacteriophage and it carries only bacterial DNA and no viral DNA. If bacteriophages undertake the lytic cycle of infection upon entering a bacterium, the virus will take control of the cell's machinery for use in replicating its own viral DNA. If by chance bacterial chromosomal DNA is inserted into the viral capsid which is usually used to encapsulate the viral DNA, the mistake will lead to generalized transduction.

ii) **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. The genes that get transferred always depend on where the phage genome is located on the chromosome. Specialized transduction leads to three possible outcomes:

1. DNA can be absorbed and recycled

2. The bacterial DNA can match up with a homologous DNA in the recipient cell and exchange it. The recipient cell has DNA from both itself and the other bacterial cell.
3. DNA can insert itself into the genome of the recipient cell like a virus resulting in a double copy of the bacterial genes.

In this kit 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 bacteriophage.

Kit Contents:

This kit can be used to study the process of transduction where an antibiotic resistant gene is transferred from donor bacterial cell to the recipient.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity		Storage
			5 expts	20 expts	
1	TKC365	Phage Lysate	0.060 ml	0.240 ml	2-8°C
2	MB104	Ampicillin	0.1 g	0.4 g	2-8°C
3	MB286	Chloramphenicol	0.02 g	0.08 g	2-8°C
4	M1245	LB Broth	130 g	520 g	RT
5	MB053	Agar powder, Bacteriological	45 g	180 g	RT
6	PW1139	Collection Tubes, Polypropylene (2.0 ml)	40 Nos.	160 Nos.	RT
7	TKC065	1M Calcium Chloride (sterile)	0.05	0.2 ml	2-8°C
8	TKC367	1M Magnesium Chloride (sterile)	3 ml	12 ml	RT

Materials Required But Not Provided:

Culture requirement: Donor, Recipient and Susceptible host strains
Glass wares: Conical flask, Measuring cylinder, Sterile tubes (15 and 50 ml), Petri plates
Reagents: Distilled water, Ethanol
Other requirements: Centrifuge, Incubator, Shaker, water bath (set at 60°C), Micropipettes, Tips, Sterile loops and spreaders, 0.45µ filters.

Storage:

HiPer® Transduction Teaching Kit is stable for 6 months from the date of manufacture without showing any reduction in performance. On receipt, store Chloramphenicol, Ampicillin, 1M $CaCl_2$, Phage lysate at 2-8°C. Other kit contents can be stored at room temperature (15-25°C).

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 petri plates.
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 petri plates.
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 petri plates.
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 petri plates.
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 and store it at 2-8°C.
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.

Procedure:

Day 1:

1. Pick up a loopful of Donor culture and streak onto LB agar plates with Chloramphenicol (20mg/ml) and label as LB C_{20}
2. Pick up a loopful of Recipient culture and streak onto LB plates with Ampicillin (100mg/ ml) and label as LB A_{100}
3. Pick up a loopful of Susceptible Host culture and streak onto LB agar plates.
4. Incubate overnight at 37°C.

Day 2:

1. Inoculate 10-15 colonies from revived donor plate into 5 ml of LB C_{20} and label as Donor Tube.

2. Incubate at 30°C in shaker with a speed of 250 rpm for 4 hours.
3. Keep a 5ml aliquot of sterile LB broth in water bath set at 60°C.
4. Add 10 µl of given phage lysate to above labeled donor tube, continue incubation for 30 minutes at 30°C in static mode.
5. Add 5 ml of preheated sterile LB broth to donor tube mix well and incubate this tube at 42°C for another 20 minutes.
6. Transfer this tube to 37°C and incubate for 3 hours in static mode.
7. After incubation, spin this culture at 5000 rpm for 10 minutes. Take the supernatant, filter it through 0.45 micron filter, label it as **Phage lysate 2**. Store at 4°C for further steps.
8. Inoculate single colony from recipient plate in 5 ml of LB broth with ampicillin (100 mg/ml) label it as recipient tube. Incubate this tube at 37°C shaker for overnight (for 16-18 hours) at 250 rpm.
9. Inoculate single colony from donor plate in 5 ml of LB broth with chloramphenicol (20 mg/ml) and label it as donor tube. Incubate this tube at 37°C shaker for overnight (for 16-18 hours) at 250 rpm.

Day 3:

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 shaker for 3 hours at 250 rpm.
2. After incubation take 50 µl of this culture in 2 ml collection tube, add 50 µl 0.1M $CaCl_2$ along with 50 µl of Phage lysate 2 obtained at Day 2 step 7.
3. Mix well and incubate further at 30°C for 2 hours in static mode.
4. After 2 hours, plate 50 µl of this culture on LB-C_{20}, LB-A_{100} and LB-C_{20} A_{100} plates.
5. Along with it take 50µl of overnight grown cultures of recipient strain and donor strain (from Day 2 step 8 and 9) and plate on LB C_{20}, LB A_{100} and LB C_{20} A_{100} plates.
6. Incubate all the plates at 37°C for overnight.
7. On the next day store these plates at 4^0C for observation and results. Proceed with positive lysogen obtained on LB-C_{20} A_{100} plate for further confirmation by induction.
8.

Day 4:

NOTE: Inoculation for Preparation of plating cells and induction of the lysogens have to be started simultaneously.

I. Preparation of Plating Cells of Susceptible Host:

1. Inoculate 10-15 colonies from revived plate of susceptible host (Day 1) in 15 ml of fresh LB broth.
2. Incubate this culture at 37°C for 4 hours in static mode.
3. After incubation, take 1.5 ml of this culture in 4 different 2.0ml collection tube and spin down at 6000rpm for 10 minutes at RT. Resuspend each of the pellet in 100 µl of fresh sterile LB broth. Use this as plating cells for titration.
4. Before starting Titration protocol, keep 5ml of LB broth at 60°C.

II. To confirm the presence of phages in lysogenized colonies of infected recipient culture:

1. Inoculate 10-15 Colonies from LB C_{20} A_{100} plate (of recipient infected with phage) in 5 ml LB C_{20} A_{100} broth. Label this as 'Lysogenized culture'.
2. Incubate this tube at 30^0C for 4 hours in static mode. After incubation add 5 ml of hot LB broth (kept at 60°C) to this lysogenized culture tube.
3. Further incubate this tube at 42°C for 20 minutes, again transfer this tube to 37°C and incubate for 4 hours under static condition.

4. After 4 hours of incubation centrifuge the culture tube at 5000 rpm for 10 mins at RT, filter the supernatant through 0.45µm filter and label this as **'concentrated lysate'.**

III. Phage Titration:

1. Take 3 collection tubes (2.0ml) and label them as 10^{-1}, 10^{-2}, 10^{-3} (as shown in the following figure).

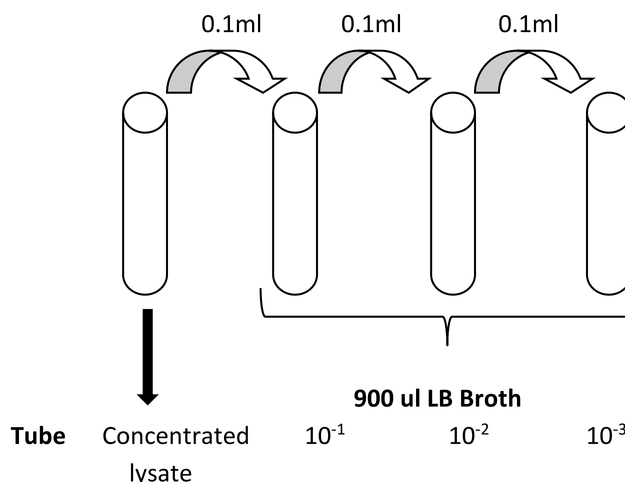

Fig 3: Diagrammatic representation of phage dilution

2. Take 900µl of sterile LB broth in all tubes (10^{-1} to 10^{-3}), add 100µl of concentrated lysate to 10^{-1} tube.
3. Perform serial dilution up to 10^{-3} tube. Change tip every time while preparing dilution.
4. Label the tubes containing "plating cells" as 1,2,3,4. To the First tube, add 20 µl of concentrated lysate and label it as **Concentrate** and add 20 µl phage lysate dilutions to tube No's 2, 3 and 4 respectively.
5. Incubate all four tubes at 37°C for 15 minutes under static condition.
6. Meanwhile, melt the soft agar; dispense 5 ml of melted soft agar in 15 ml tube and keep at 45°C.
7. Add 1000µl of 1M $MgCl_2$ and 200µl of 1M $CaCl_2$ & 800µl of 10% Dextrose to it.
8. Pipette out the mixture of plating cells/ concentrated lysate in Soft agar tube, mix well and immediately pour on LB agar plate labeled as No.1. Let the agar solidify.
   Note: Care should be taken to ensure that temperature of soft agar does not exceed 45^{0}C as the plating cells culture will die or should not fall below 40^{0}C as soft agar will solidify.
9. Repeat steps 5-6 for phage dilutions from 10^{-1} to 10^{-3}.
10. Close the lids of Petri plates and incubate these plates at 37^{0}C.
11. Note down the results and observation the next day.

Observation and Result:

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 Table 2:

Table 2:

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

B. **Phage Titration (from Day 4) -** Check the plates for clear and distinct plaques. Count the number of plaques for each dilution and note down the results as per Table 3:

Table 3: Results of the phage titration assay

Tube No.	Dilutions	Number of plaques	Phage titer value
1			
2			
3			
4			

Calculation of phage titer:

Phage titer (PFU/ml) = Plaque count / [dilution factor X aliquot volume (ml)]

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 chloramphenicol. 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.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Improper growth seen on respective plates	Induction is improper	Induction of lysogen should be done according to the protocol.
2.	Numbers of plaques do not correlate with the phage dilution	Dilution of the phage is not done properly	Vortex every tube thoroughly before pipetting. Make sure that the tip is changed for every dilution
3.	No clear plaques observed on the LB plates	Host cells have died before plating	Temperature of the soft agar should not be more than 45°C
4.	Distinct and clear plaques are not observed	The LB agar plates contain moisture	Make sure that the LB agar plates are completely dry before performing the experiment

Please refer disclaimer Overleaf.

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Storage temperature

Do not use if package is damaged

HiMedia Laboratories Private Limited,
Reg. Off: Plot No. C-40, Road No. 21Y,
MIDC, Wagle Industrial Area, Thane,
(West) 400604, Maharashtra, INDIA.
Web: www.himedialabs.com

01/2028

PIHTM005E_O/0125 HTM005E-07

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.