# HiPer® Bacterial Conjugation Teaching Kit

## Product Code: HTM004E

## Number of experiments that can be performed: 5/20

## Duration of Experiment: 4 Days

Day 1: Preparation of media and revival of strains

Day 2: Inoculation of strains

Day 3: Protocol

Day 4: Observation and Interpretation

## Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Streptomycin and Tetracycline 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

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## Aim:

To study the process of bacterial conjugation through transfer of genes coding for antibiotic resistance.

## Introduction:

Bacteria possess several methods of gene transfer for transmission of genes between individual cells. These mechanisms not only generate new gene assortments, they also help to move genes throughout populations and from species to species. The methods include transformation, transduction and conjugation. These methods occur by lateral gene transfer which is a potent evolutionary force that can create diversity within bacterial species. Conjugation is a recombination process where two live bacteria come together, and the donor cell transfers genetic material to the recipient cell. This process was first observed in 1946 by Joshua Lederberg and Edward Tatum in a series of experiments with *E. coli*.

## Principle:

Conjugation is the mode of gene transfer in many species of bacteria. In 1950 William Hayes, Francis Jacob and Elie L. Wollman established that conjugating bacteria are of two mating types. Certain "male" types (designated as $F^+$) donate their DNA and other "female types" (designated as $F^-$) receive the DNA as shown in Figure 1. $F^-$ cells become $F^+$ when they acquire a small amount of DNA. Hence the F factor is called as the Fertility factor. In contemporary microbiology, the donor's F factors are known to be plasmids which are the extrachromosomal elements. The factors (plasmids) contain about 20-30 genes, most of which are associated with conjugation. These genes encode enzymes that replicate DNA during conjugation and structural proteins needed to synthesize special pili at the cell surface. Known as F pili or sex pili, these hair like fibres contact the recipient bacteria, and then retract so that the surfaces of donor and recipient are very close or touching one another. At the area of contact, a channel or conjugation bridge is formed. Once contact via sex pili has been made, the F factor (plasmid) begins replicating by the rolling circle mechanism. A single strand of the factor then passes over through the channel to the recipient. When it arrives, enzymes synthesize a complementary strand, and a double helix is formed. The double helix bends to a loop and reforms an F factor (plasmid), thereby completing the conversion of recipient from $F^-$ cell to $F^+$ cell. Meanwhile, back in the donor cell a new strand of DNA forms, to complement the leftover strand of the F plasmid. The transfer of F factors involves no activity of the bacterial chromosome; therefore, the recipient does not acquire new genes other than those on the F factor.

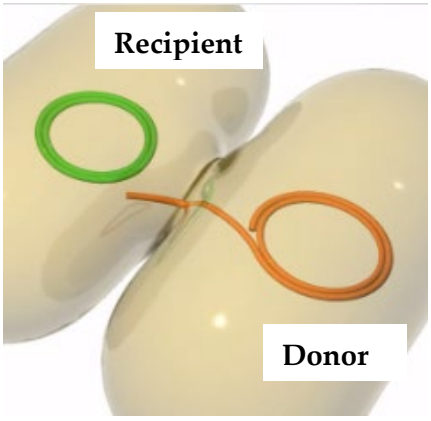



**Fig 1: In bacterial conjugation genetic material is transferred from the donor strain to the recipient strain**

On rare occasions an F-plasmid may become integrated in the chromosome of its bacterial host, generating what is known as an Hfr (high frequency of recombination) cell. Such a cell can also direct the synthesis of a sex pilus. As the chromosome of the Hfr cell replicates it may begin to cross the pilus so that plasmid and chromosomal DNA transfers to the recipient cell. Such DNA may recombine with that of its new host,

introducing new gene variants. Plasmids encoding antibiotic-resistance genes are passed throughout populations of bacteria, and between multiple species of bacteria by conjugation.

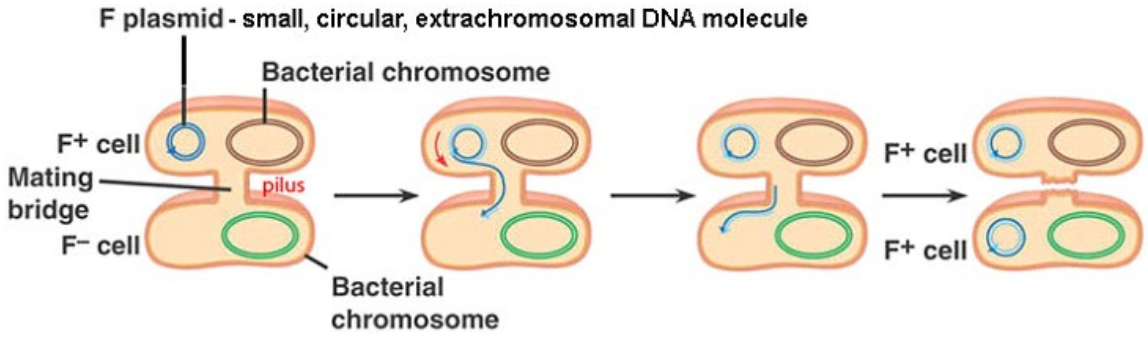



**Fig 2: Conjugation and transfer of an F plasmid from an $F^+$ donor to an $F^-$ recipient**

## Kit Contents:

The kit can be used to perform procedure of conjugation and to study the antibiotic resistance of the cells.

**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 | MB287 | Streptomycin sulphate | 0.15 g | 4 X 0.15 g | 2-8°C |
| 2 | MB178 | Tetracycline hydrochloride | 0.045 g | 4 X 0.045 g | 2-8°C |
| 3 | M1245 | Luria Bertani Broth | 50 g | 200 g | RT |
| 4 | MB053 | Agar Powder, Bacteriological | 23 g | 92 g | RT |

## Materials Required But Not Provided:

**Culture requirement:** Donor, Recipient and Susceptible host strains
**Glass wares:** Conical flask, Measuring cylinder, Sterile test tubes, Petri plates
**Reagents:** Distilled water
**Other requirements:** Incubator, Shaker, Spectrophotometer, Micropipettes, Tips, Sterile loops and spreaders

## Storage:

HiPer® Bacterial Conjugation Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store Tetracycline hydrochloride and Streptomycin sulphate 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. **Tetracycline solution preparation:** Dissolve 45 mg of tetracycline in 1.5 ml of 70% ethanol, mix by gentle pipetting to give a final concentration of 30mg/ml. Cover with aluminum foil and store in refrigerator. Use this solution within a month.
3. **Streptomycin solution preparation:** Dissolve 150 mg of streptomycin in 1.5 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 2.5 g of Luria Bertani broth in 100 ml of distilled water and autoclave.
5. **Preparation of LB (Luria Bertani) agar plates with Streptomycin (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. Allow the media to cool down to 40-45°C. Add 100 µl of streptomycin in 100 ml of autoclaved LB agar media and pour on sterile petri plates.
6. **Preparation of LB (Luria Bertani) agar plates with Tetracycline (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. Allow the media to cool down to 40-45°C. Add 100 µl of tetracycline to 100 ml of autoclaved LB agar media and pour on sterile petri plates.
7. **Preparation of LB agar plates with Tetracycline + Streptomycin (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. Allow the media to cool down to 40-45°C. Add 100 µl of tetracycline and 100 µl of Streptomycin to 100 ml of autoclaved LB agar media and pour on sterile petri plates.

## Procedure:

### Day 1:

1. Pick up a loopful of Donor culture and streak onto LB agar plates with Tetracycline (30 µg/ml).
2. Pick up a loopful of Recipient culture and streak onto LB agar plates with Streptomycin (100 µg/ml).
3. Incubate overnight at 37°C.

### Day 2:

1. Pick up a single colony from Donor and Recipient Strain grown overnight on LB agar plates and inoculate a single colony in 6 ml of LB broth adding 6µl of respective antibiotics.
2. Incubate the test tubes overnight at 37°C.

### Day 3:

1. Take 25 ml of LB broth and add 25 µl of tetracycline into it and inoculate 1 ml of overnight grown culture into it. Incubate at 37°C in a shaker.
2. Take 25 ml of LB broth and add 25µl of streptomycin into it and inoculate 3 ml of overnight grown culture in it. Incubate at 37°C in a shaker.

3. Grow the cultures till O.D of the donor culture reaches 0.8-0.9 at $A_{600}$.
4. Add 0.2 ml of each donor and recipient cultures in a sterile test tube labeled as conjugated sample. Mix by gentle pipetting and incubate at 37°C for 1-1.5 hours.
5. Take 2 sterile test tubes and label them as donor and recipient. Add 0.2 ml of respective cultures to the test tubes and incubate at 37°C for 1-1.5 hours.

   NOTE: Do not place the tubes in shaker for conjugation and further incubation period.
6. Add 2 ml of LB broth into each tube after incubation. Incubate the tubes at 37°C for 1.5 hours.
7. Plate 0.1 ml of each culture on the antibiotic plates as indicated in Table 2.
8. Incubate the plates overnight at 37°C overnight.

**Table 2: Samples to be spread on respective plates as follows:**

| | **LB + Streptomycin** | **LB + Tetracycline** | **LB + Streptomycin, Tetracycline** |
|---|---|---|---|
| **Donor Strain A** | 0.1 ml | 0.1 ml | 0.1 ml |
| **Recipient Strain B** | 0.1 ml | 0.1 ml | 0.1 ml |
| **Conjugated Sample** | 0.1 ml | 0.1 ml | 0.1 ml |

## Observation and Result:

Note down the observations in the following table. Indicate bacterial growth with positive symbol and absence of growth with negative symbol.

| | **LB + (Streptomycin)** | **LB + (Tetracycline)** | **LB + (Streptomycin, Tetracycline)** |
|---|---|---|---|
| **Donor Strain A** | | | |
| **Recipient Strain B** | | | |
| **Conjugated Sample** | | | |

## Interpretation:

On observing colonies on different plates, the following interpretation can be made:

1. Donor strains will grow only on tetracycline plates, similarly recipient strains will grow only on streptomycin plates.
2. Donor strain is sensitive to streptomycin and recipient strain is sensitive to tetracycline, hence no growth will be seen in these plates.
3. The conjugated sample will grow on tetracycline and streptomycin plate. The reason being, transfer of gene has occurred by means of conjugation.
4. The donor and recipient strain will not grow on tetracycline + streptomycin plate since each of the strain is sensitive to one antibiotic in the plate.

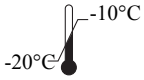
## Troubleshooting Guide:

| Sr. No. | Problem | Possible Cause | Solution |
|---|---|---|---|
| 1 | Improper growth seen on respective plates | Pouring media at high temperature | Ensure that the respective antibiotic is added to the LB media at 40-45°C before and then pour the plates. Adding antibiotics at higher temperature will deactivate its activity |
| | | Plates stored for long period of time | Use plates within 1 month of preparation |
| | | Pipetting error | Always add the respective samples as mentioned in Table 1. Use fresh and autoclaved tip for each sample while spreading on plates |

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

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