Unit 7

Recombination in Bacteria and Viruses:

The genetic information of bacteria is stored in —

① A single main chromosome, carrying a few thousand genes.

② From zero to several minichromosomes called episomes and plasmid.

Each bacterial chromosome characterized to date contains a single circular ~~model~~ molecule of DNA that exists in a highly condensed conformation in vivo.

Recombination is important in the evolution of bacteria just as it is in the evolution of eukaryotes. Three different processes have involved that mediate transfer of genetic materials from one bacterium to another making possible the subsequent recombin events.

The most obvious differences between these three processes is the mode of transfer of DNA from one cell to another —

① Transformation involves the uptake of naked DNA molecules from one bacterium (the donor cell) by another bacterium (the recipient)

② Transduction occurs when bacterial genes are carried from a donor cell to a recipient cell by a bacteriophage.

③ Conjugation is the process during which DNA from a donor or male cell is transferred to a recipient or female cell through a specialized sex pilus or conjugation tube.

The three modes of recombination in bacteria can be distinguished by two simple criteria

① Sensitivity to the presence of deoxyribonuclease (DNase)

② Dependance of cell contact.

These two criteria can be easily tested

experimentally.

⇒ The first criteria can be tested by simply adding DNase to the medium containing the bacteria involved in recombination. If recombination occurs in the absence of DNase, but not in its presence, then the DNase-sensitive recombination process, transformation, is occuring.

⇒ The protein coat of the bacteriophage vector and the cell wall of membrane enclosing the conjugation tube protect the donor DNA from degradation by DNase in the medium during transfer to the recepient cell during transduction and conjugation, respectively whether cell contact is required is tested by carring out a so called U-tube experiment, in which bacteria of two different genotypes are placed in opposite arms of a U-shaped culture tube.

Transformation :

Transformation was first discovered in pathogenic strains of Diplococcus pneumoniae by Griffith in 1928. The details of Avery, MacLeod and McCarty's (1944) ~~Proof~~ proof that the 'transforming principle' is DNA ~~were also~~.

The process of transformation can be divided into several stages —

(1) Reversible binding of double-stranded DNA molecules to receptor sites on the cell surface.

(2) Irreversible uptake of the donor DNA (at which time the donor DNA becomes resistant to DNase in the medium).

(3) Conversion of the double-stranded donor DNA molecules to single stranded molecules by nucleolytic degradation of one strand.

(4) Integration (covalent insertion) of all or part of the single strand of donor DNA into the chromosome of the recipient.

(5) The segregation and phenotypic expression of the integrated donor gene or genes in the

recombinant cells.

The U-tube experiment used to determine wheather cell contanct is required for recombination in bacteria. The two arms of the Davis U-tube are separated by a glass filter containing pores of a size that permit the passage of DNA molecules and viruses, but not cells.

Bacteria of one genotype (e.g., $a^+ b^-$) are placed in one arm of the Davis U-tube, those of another genotype (e.g., $a^- b^+$) are placed in the other arm.

Alternating suction and pressure are applied to one (arm) of the tube during incubation to mix the medium and any small particles suspended in it.

However, the glass filter prevents cell from passing from one arm of the tube to the other.

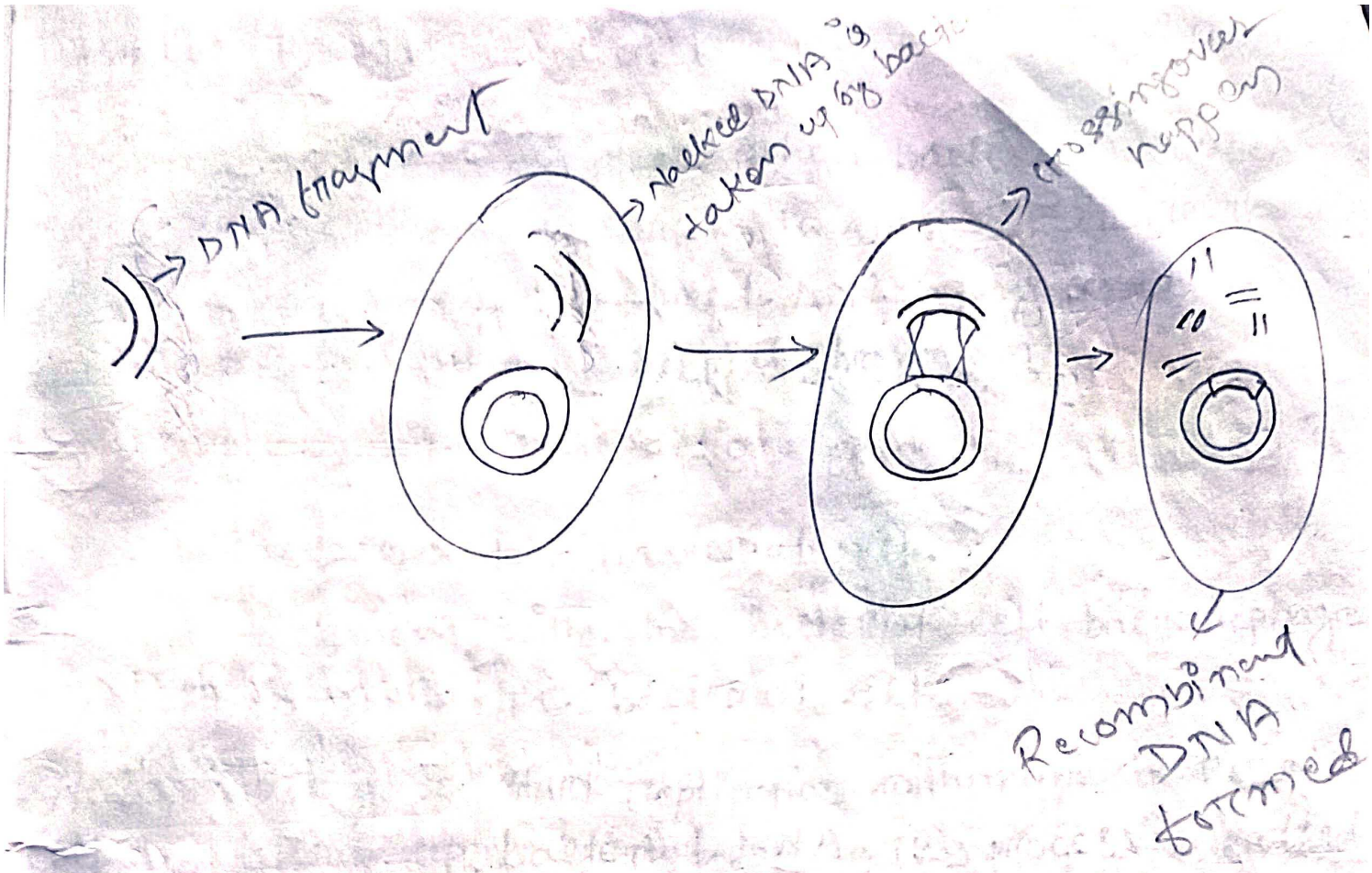

Fig: Transformation.

Transduction:

Transduction takes place when bacterial viruses carry DNA from one bacterium to another. Inside the bacterium, the newly introduced DNA may undergo recombination with the bacterial chromosome.

Steps involved in the transduction process:-

1) A virus attaches to a bacterial cell.

2) After attachment with the bacterial cell bacteriophage injects its DNA to the bacterial cell.

3) Now viral gene start replication within the bacterial cell by taking up bacterial DNA. This process is called lysogeny.

4) The viral DNA start to produce their body parts like, protein cote, ~~DNA and~~ ~~finally~~ neck, tail etc and ~~this~~ finally the viral gene produced new virus ~~which~~ and they are come out by destroying the bacterial cell.

5) The newly replicated virus infects a new bacteria.

6) The newly replicated viral DNA carrying some parts of bacterial DNA.

⇒ A crossing over happen in the recipient cell leads to the creation of a recombinant chromosome.

Transduction are of two types—

① Generalized transduction.

② Specialized transduction.

Generalized transduction:

- In generalized transduction, a random or newly random segment of bacterial DNA is "wrapped up" during phage maturation in place of.
- Therefore, generalized transducing particles contains only bacterial DNA
- Generalized transduction is mediated by some virulent bacteriophages and by certain temperate bacteriophages whose chromosomes are not integrated at specific attachment sites on the host chromosome

Specialized transduction:

- specialized transduction is mediated by temperate bacteriophages whose chromosomes are able to integrates at one or a few specific attachment

sites on the host chromosome.

The excision process is usually very precise in cutting out the phage chromosome in exactly the form in which it existed prior to its integration. Occasionally, however, the excision even occurs at a site other than the original attachment site. When this happens, a portion of the phage chromosome is left in the host chromosome and a portion of the bacterial chromosome is excised with the phage DNA. Such, mistakes during prophage excision are responsible for the formation of specialized transducing particle.

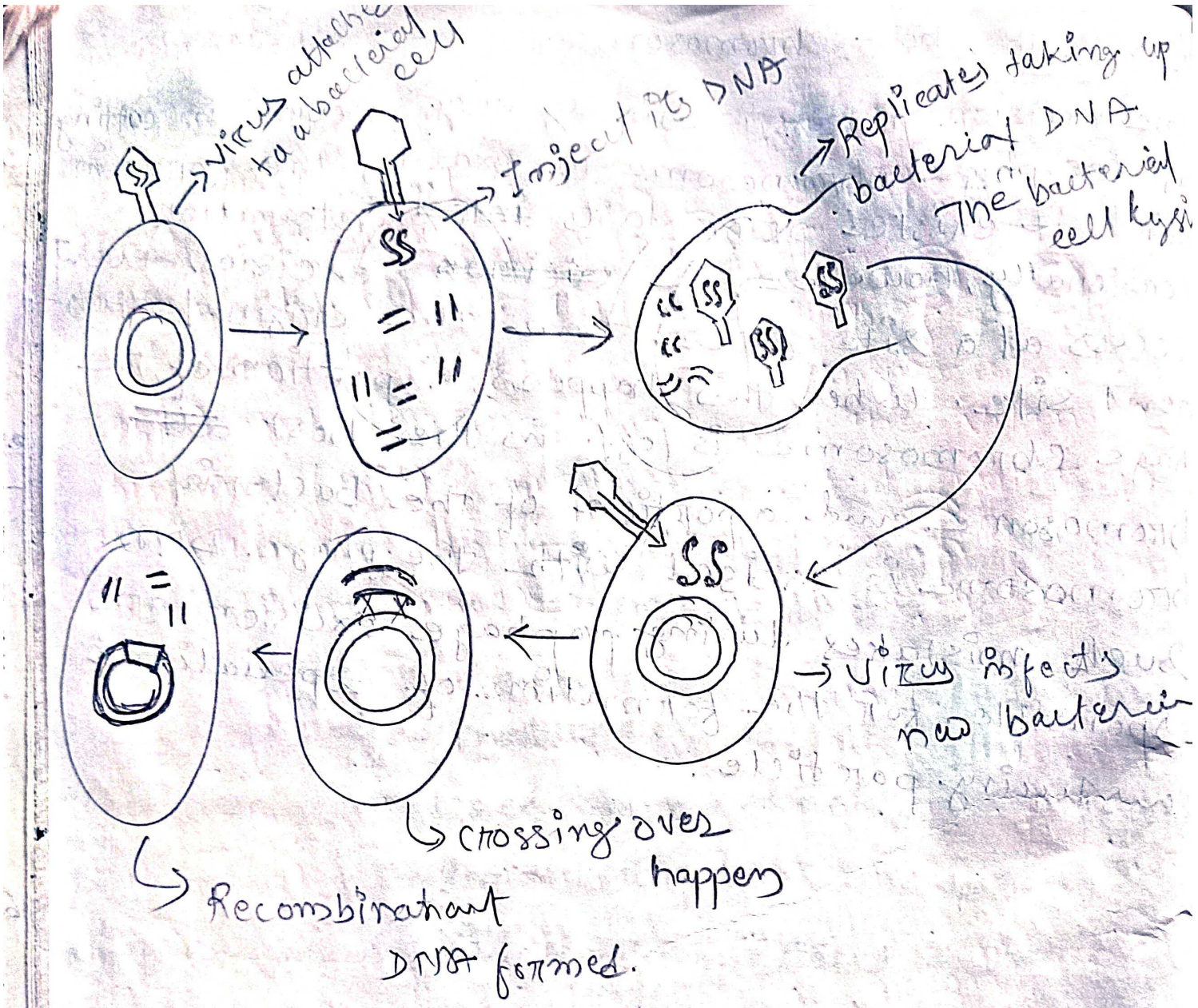

Fig: Transduction.

Conjugation:

- In 1946, Joshua Lederberg and Edward Tatum demonstrated that bacteria can transfer and recombine genetic information.
- They studied auxotrophic strains of E. coli. The K10 strain required the amino acid threonine (thr⁻) and leucine (leu⁻) and the vitamin thiamine (thi⁻) for growth but did not require the vitamine biotin (bio⁺) or the amino acids phenylalanine (phe⁺) and cysteine (cys⁺).
- To study Bernard Davis constructed a U-shape tube that was divided into two compartments by a filter having fine pores. This filter allowed liquid medium to pass from one side of the tube to the other, but the pores of the filter were too small to allow the passage of bacteria.
- Two auxotrophic strains of bacteria were placed on opposite sites of the filter, and suction was applied alternatively to the ends of the U-tube, causing the medium to flow back and forth between the two compartments.

- Despite hours of incubation in the U-tube bacteria plated out on minimal medium did not grow; there had been no genetic exchange between the strains.
- The exchange of bacterial genes clearly required direct contact or conjugation between the bacterial cells.

Mechanism of ~~cell~~ conjugation:-

Mechanism of conjugation involve following steps —

1) Two bacterial cells - one is donor cell and other is recipient cell require physical contact.
2) The donor cell and recipient cell ~~produce pili~~ forms cytoplasmic bridge called pili.
3) Now one strand of the ~~DNA~~ donor cell DNA start replication and transfer from donor to recipient cell through conjugation tube.
4) ~~There after~~ Now the donor cell ssDNA start crossing-over with recipient DNA.
5) The crossing-over leads to the creation of a recombinant chromosome.

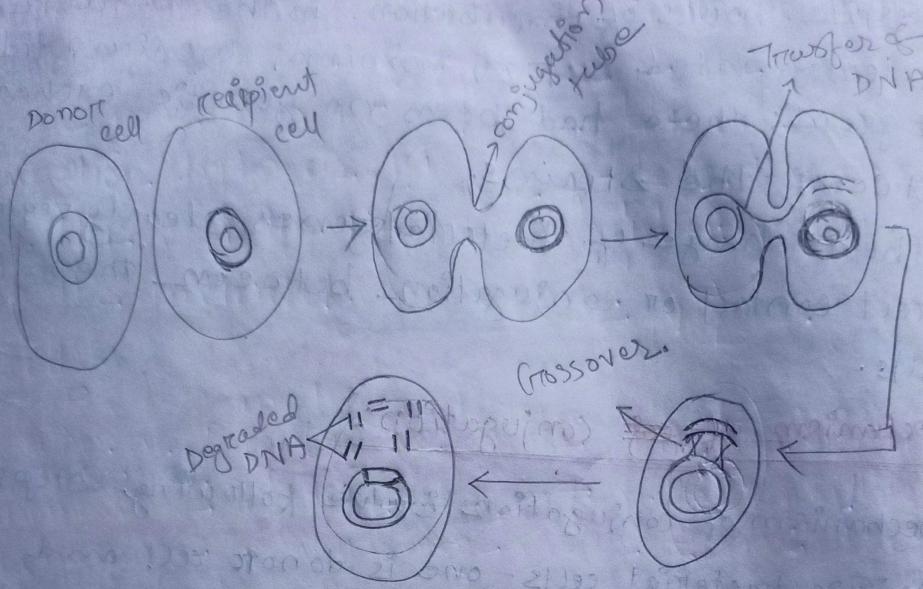

Fig: Conjugation.

F^+ and F^- cells:

In most bacteria, conjugation depends on a fertility (F) factor that is present in the donor cell and absent in the recipient cell. cells that contain F are refferred to as F^+ and cells lacking F are F^-.

DNA transferred from the F^+ cell to the F^- cell. Conjugation can take place only between a cell that possesses F and a cell that lacks F.

In most cases the only gene transferred during conjugation between ~~and~~ an F^+ and F^- cell are those on the F factor. Transfer is initiated when one of the DNA strands on the F factor is nicked at an origin (oriT)

If the entire F factor is transferred to the recipient F^- cell, that cell becomes F^+ cell.

Hfr cells: (Hfr = High frequency)

In Hfr strains, the F factor is integrated in to the bacterial chromosome. Hfr cells behave as F^+ cells, forming sex pili and undergoing conjugation with F^- cells.

In conjugation between Hfr and F⁻ cells, the integrated F factor is nicked and the end of the nicked strand moves into F⁻ cell, just as it does in conjugation between F⁺ and F⁻ cells. Because, in an Hfr cell, the F factor has been integrated into the bacterial chromosome.

F' cells:

When an F factor does excise from the bacterial chromosome, a small amount of bacterial chromosome may removed with it, and these chromosomal genes will then carried with F plasmid. cells containing an F plasmid with some bacterial genes are called F prime (F').

Complementation experiment:

Benzer's mapping experiment demonstrated that some rII mutations were very closely linked. ~~This~~ To determine whether different rII mutation belonged to different functional loci, Benzer used the complimentation test.

To carry out the complementation test in bacteriophage, Benzer infected cells of E. coli K with large numbers of two mutant strains of phage. So that cells would become doubly infected with both strains.

Consider two rII mutations: r_{101}^- and r_{104}^-. cells infected with both mutant were effectively heterozygous for phage genes with the mutations in the trans configuration.

$$\frac{r_{101}^- \quad r_{104}^-}{r_{101}^+ \quad r_{104}^+}$$

Step involves in complimentation test:

1) E. coli K cells are simultaneously infected by two different rII mutant (r_{101}^- and r_{104}^-).
2) Making the cells functionally heterozygous for the mutations.
3) If these two mutations belong to different cistron i.e $\frac{r_{101}^-}{r_{104}^+}$ and $\frac{r_{104}^-}{r_{104}^+}$.

There is complementation and functional

proteins are produced.

protein A produced from $\frac{r_{101}^-}{r_{101}^+}$ and protein B produced from $\frac{r_{104}^-}{r_{104}^+}$.

④ This causing the formation of plaques.

⑤ If the two mutations belong to the same cistron i.e. $\frac{r_{101}^- \; r_{104}^+}{r_{101}^+ \; r_{104}^-}$ it does not produced functional protein.

⑥ This leads to no plaques are formed.

Conclusion:

The complimentation test indicates whether two mutations are at the same locus or a different loci.

P.T.O →

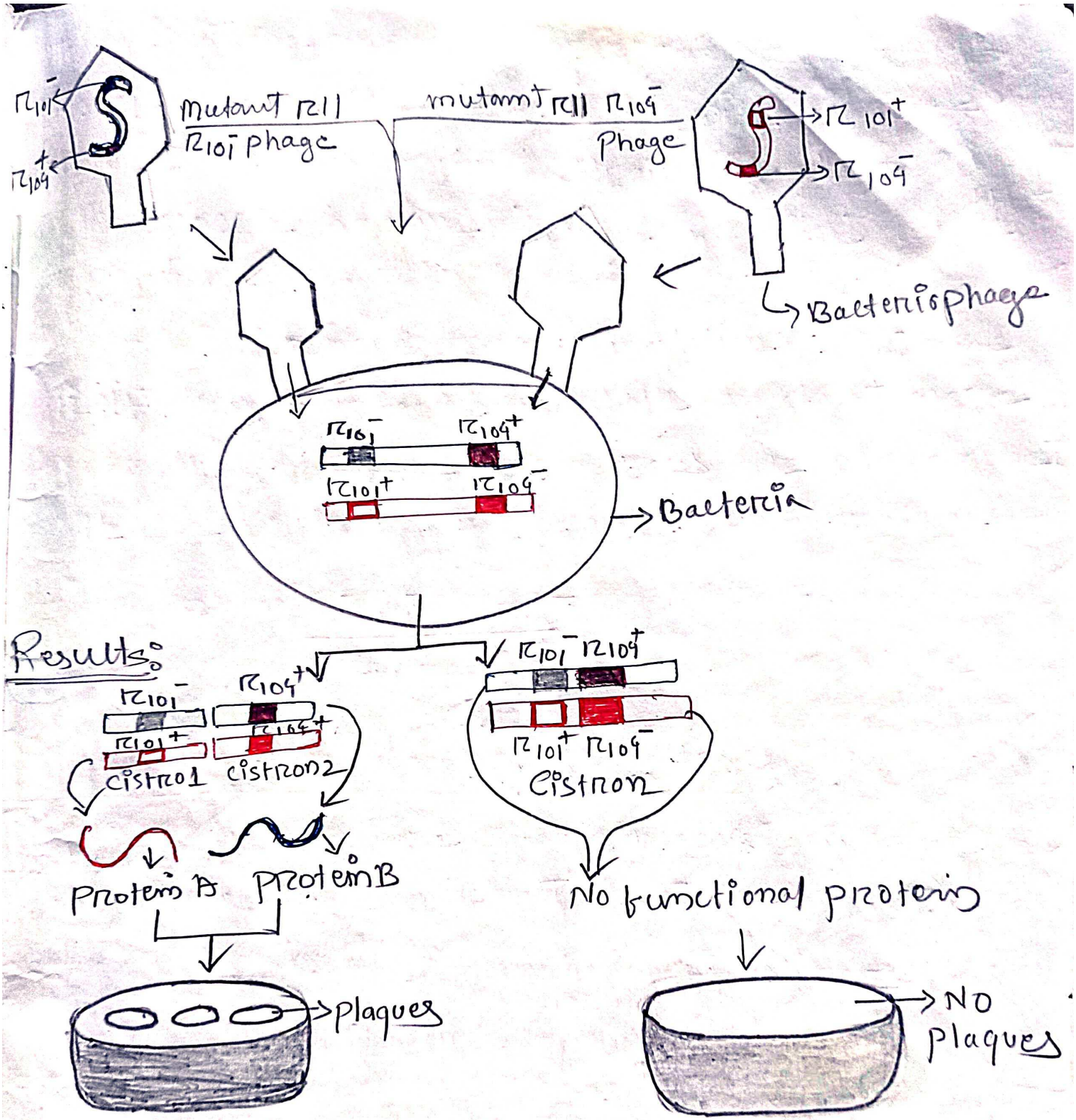

Fig: Complementation tests in bacteriophage.