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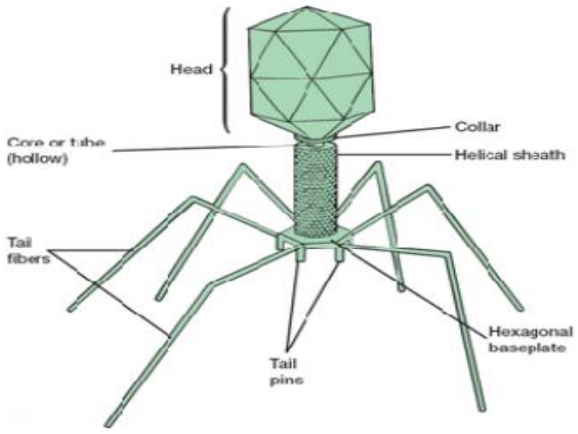
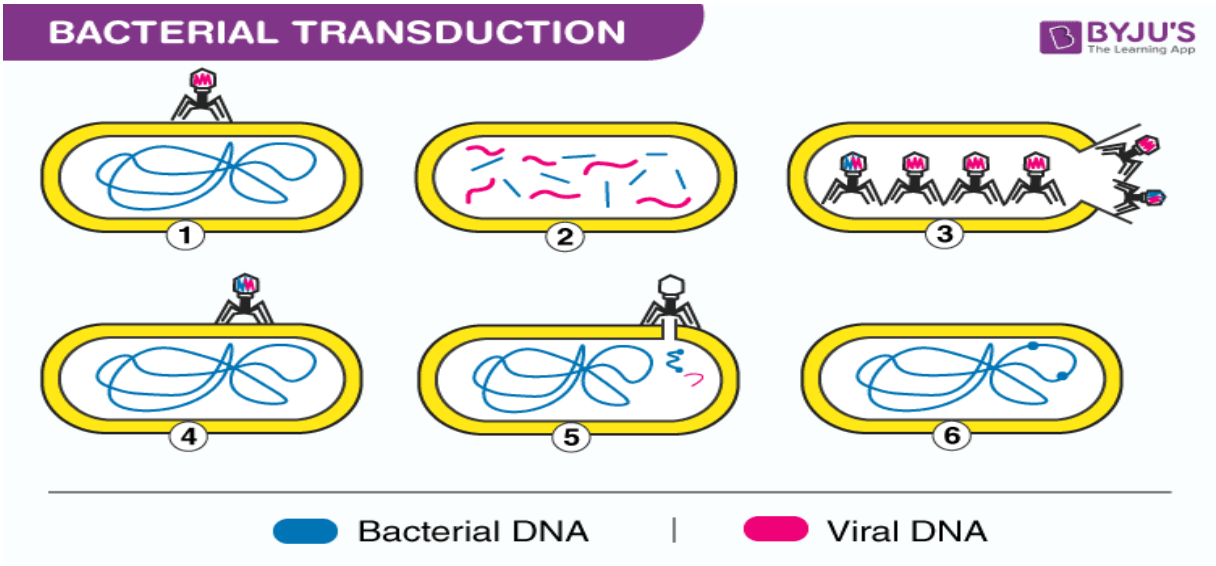
Lab 5 :Transduction in Bacteria

Transduction in bacteria is a process by which genetic material from a bacterium is transferred and incorporated into the genome of another bacterium by a **bacteriophage** (bacterial virus). Bacteriophages, also known as phages, infect and replicate in bacterial cells. They transfer genetic information during their infection cycles as they use bacterial cells as hosts to make more virus copies. The term transduction is also used to refer to the process where by a viral vector introduces foreign DNA into another cell. Transduction is an example of horizontal gene transfer, the movement of genetic information between genomes that is non-sexual in nature, and does not involve transfer between parent and offspring. Donor and recipient bacteria do not need to be in physical contact for transduction to occur. Due to the nature of the transduction process, molecular biologists use it in many applications to stably introduce foreign genetic material into the genome of desired cells.



Bacterial Transduction Steps

In transduction, the transfer of bacterial DNA depends on viral infection. The steps involve:



<u>bacteriophages are classified into two types: -</u>

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. 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.

Types of Transduction

Transduction is common in both virulent and temperate phages, i.e. by lytic or lysogenic cycle. Transduction is of two types:

- Generalized Transduction – In this, the phage can carry any part of DNA.
- Specialized Transduction – In this, the phage carries only the specific part of DNA.

Application of Transduction

Transduction is one of the most important tools for genetic engineering.

- Transduction is used to insert the genes of choices in animals and plant cells to modify the genetic constituents and get the desired characteristics.
- It can be used for gene therapy.
- It is an important tool in genetics and molecular biology research.

Transduction protocol

Making the bacteriophages

1. Inoculate 5 ml nutrient broth with colony of donor cell.
2. Grow at 37C to O.D 600 = 0.3-0.4 (barely turbid).
3. Add 500 µl of 50 mM CaCl2.
4. Add 100 µl bacteriophages.
5. Grow at 37 C until lysis occurs.
6. Add 500 µl chloroform.
7. Shake for 5 min.
8. Spin for 10 min at high speed.

9. Add 100 µl chloroform and store the supernatant at 4 C° and in dark.

The bacteriophages transduction

1. Grow overnight of recipient cell.

2. Add 100 µl of 50 mM CaCl2 to Eppendorf tube.

3. Samples are as follow:

- 200 µl recipient cell with –phage.
- 200 µl recipient cell with 50 µl phage.
- - Recipient cell with 500 µl phage.

4. Incubate for 20 min in 37C water bath (This allows adsorption of phage and injection of DNA).

5. Add 100 µl Na-citrate (which will chelate the Ca2+ and Mg2+ required for phage adsorption) and 700 µl nutrient broth.

6. Incubate for 40 min.

7. Plate 50-100 µl on selective media