B. Sc. Biotechnology Part 2

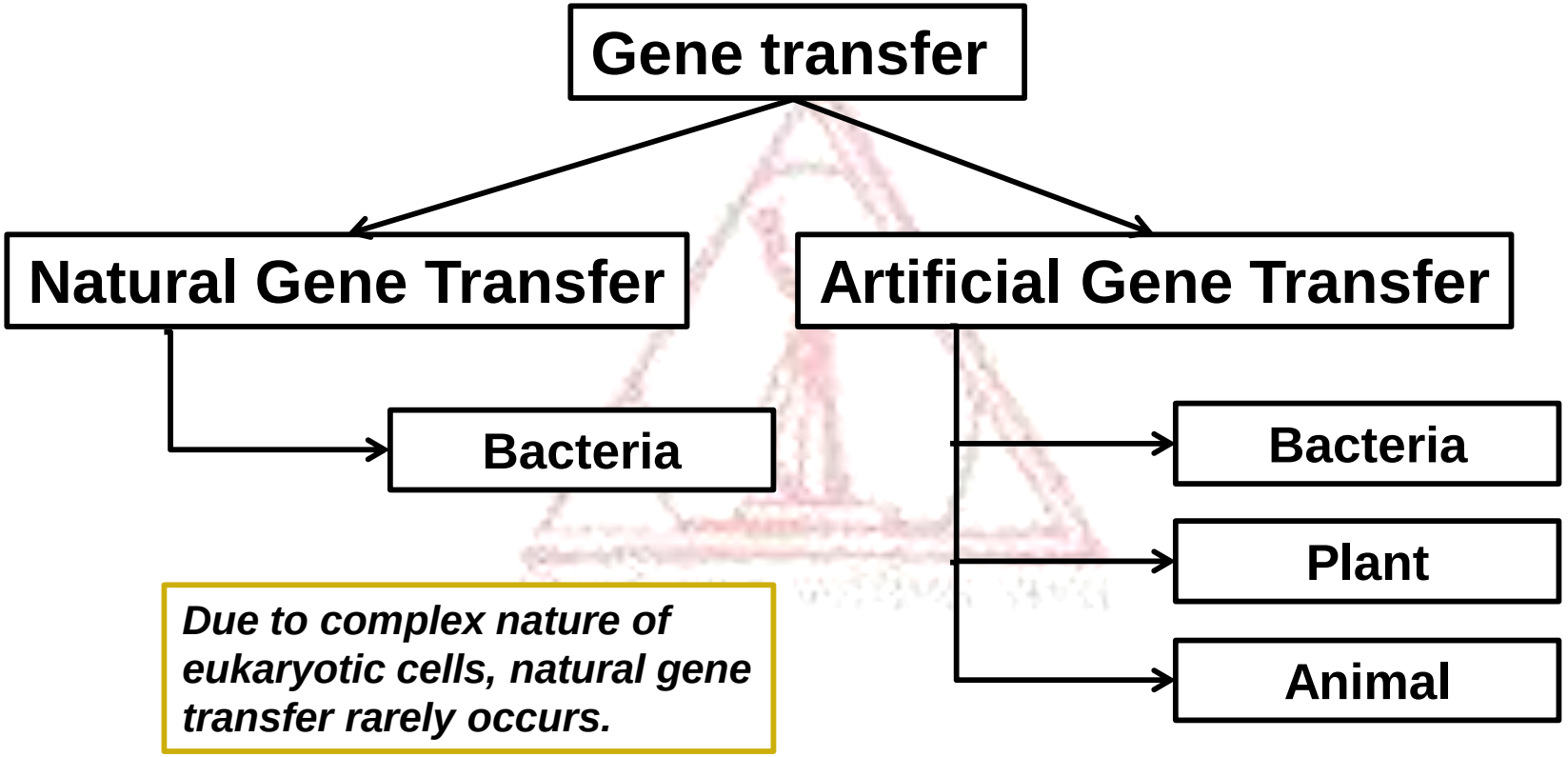
INTRODUCTION OF DNA INTO LIVING CELLS

Praveen Deepak
Assistant Professor of Zoology
S. S. College, Jehanabad

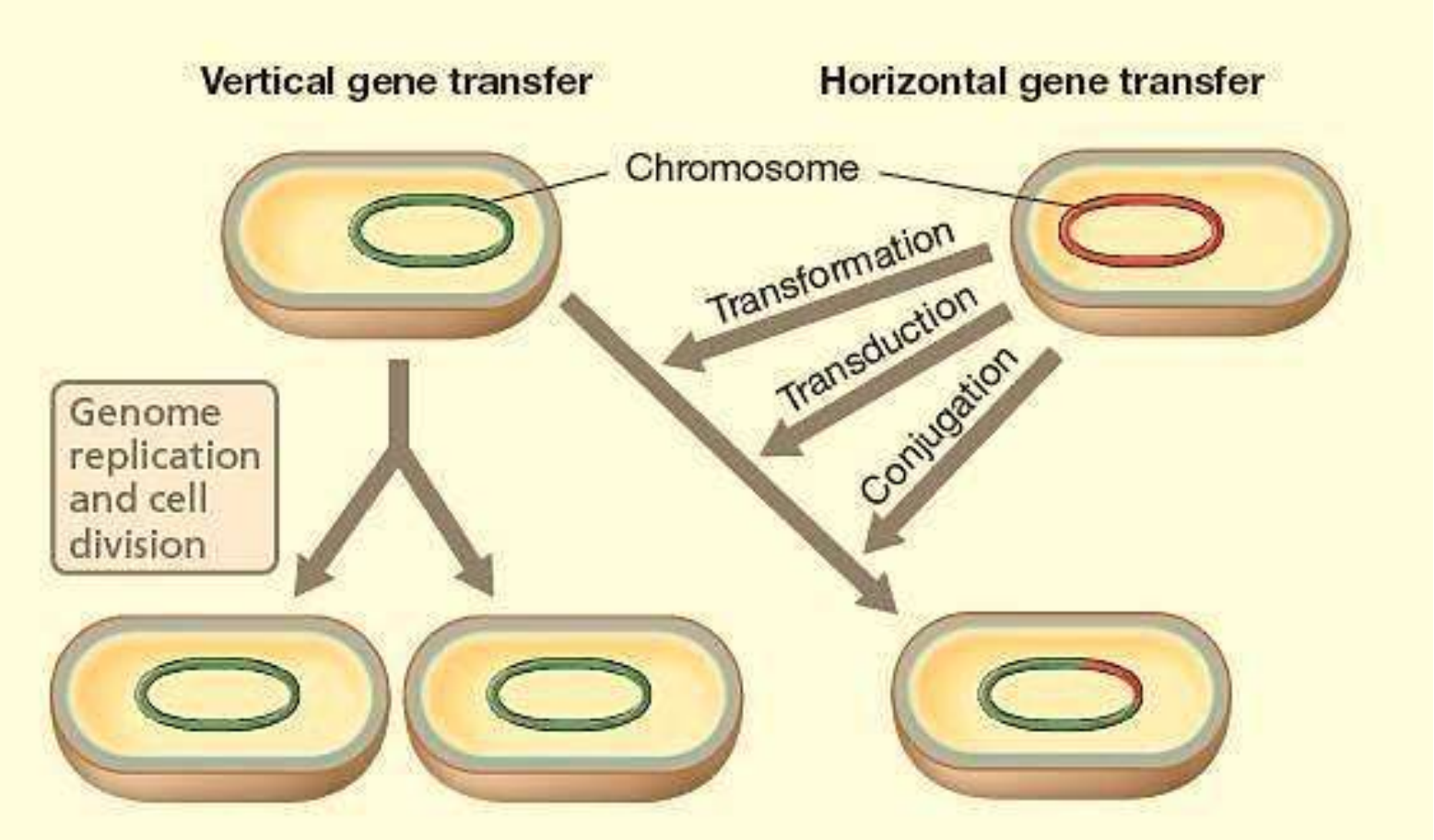
Introduction

- Specific DNA or gene of interest is introduced into living cells by various physical or chemical or other gene delivery method.
- It allows specific gene of interest to be expressed in the host cells.
- In bacteria, the process of genetic uptake is called transformation.
- Transformation requires the gene to be incorporated or recombined into host's genome.
- It can also occur naturally in bacteria through horizontal gene transfer or vertical gene transfer.
- The transformation leads to the expression of specific characters in host cells.

Gene transfer in bacteria

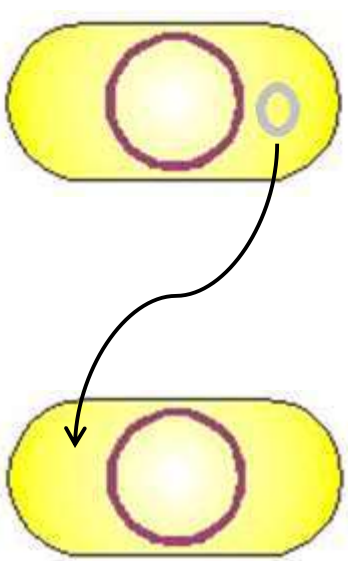


Gene transfer in bacteria



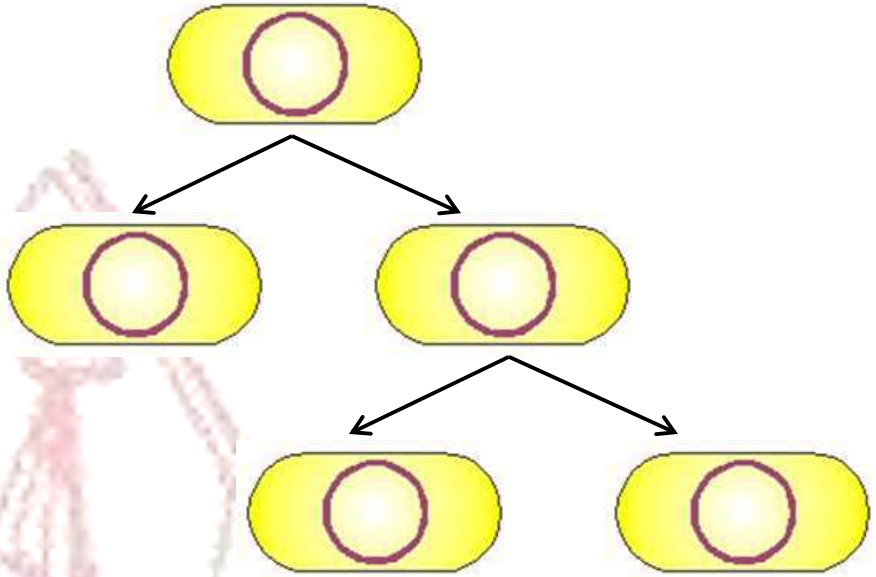
Jayashantha E. 2015, DOI:10.13140/RG.2.1.4382.9281

Gene transfer in bacteria



Horizontal gene transfer

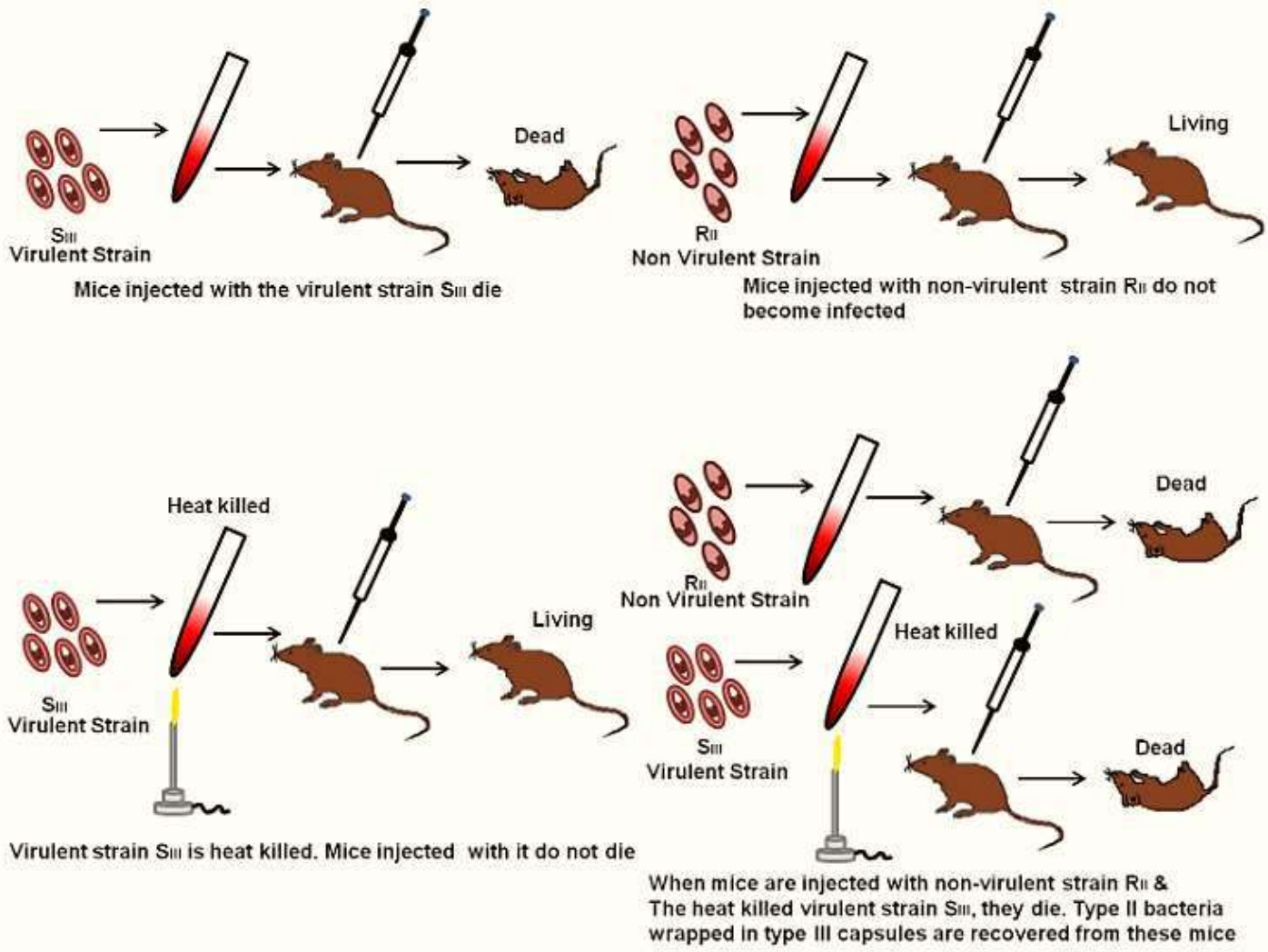
- The natural transfer of genetic material from one organism to another
- Also referred to as lateral gene transfer.
- Foreign DNA either inserted or recombines.



Vertical gene transfer

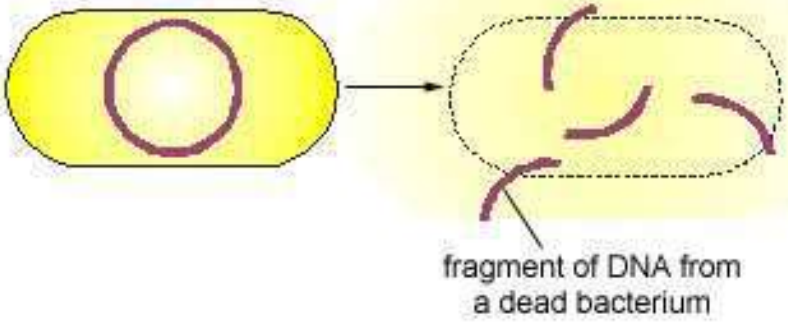
- genetic material is transferred from the parents to the offspring through sexual reproduction.

Discovery of Transformation – Frederick Griffith

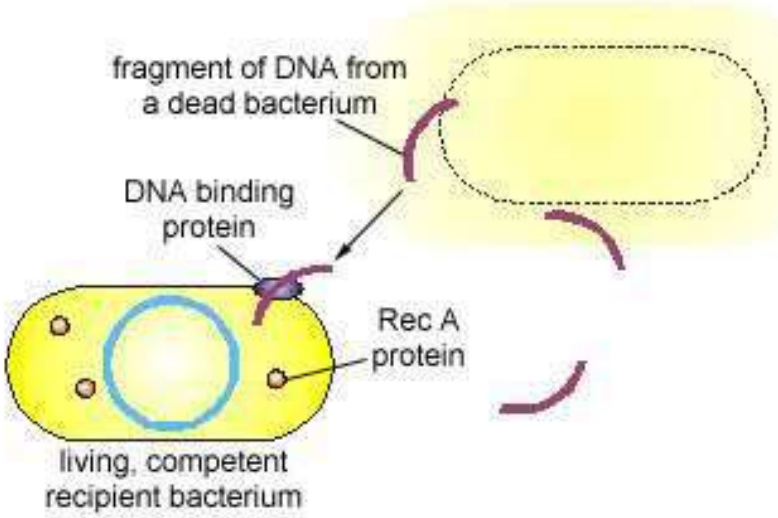


The 4 steps in Transformation

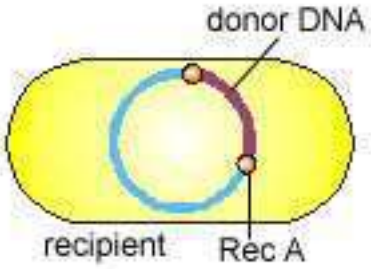
Frederick Griffith in 1928



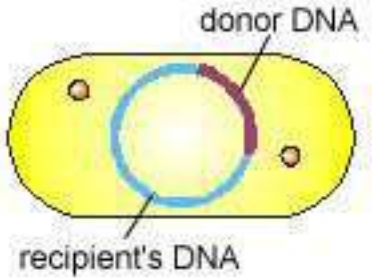
1. A donor bacterium dies and is degraded



2. A fragment of DNA from the dead donor bacterium binds to DNA binding proteins on the cell wall of a competent, living recipient bacterium



3. The Rec A protein promotes genetic exchange between a fragment of the donor's DNA and the recipient's DNA

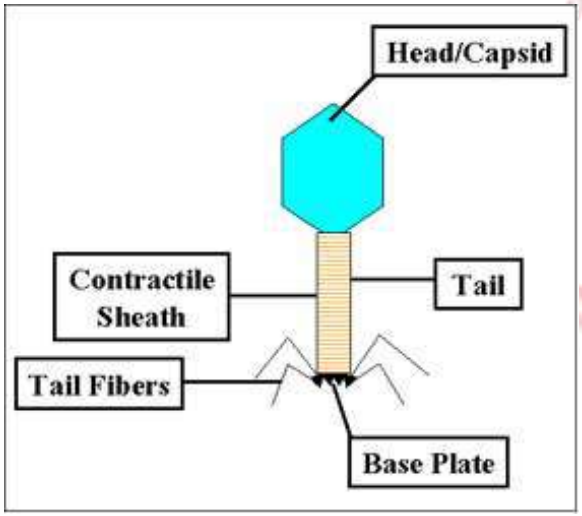


4. Exchange is complete

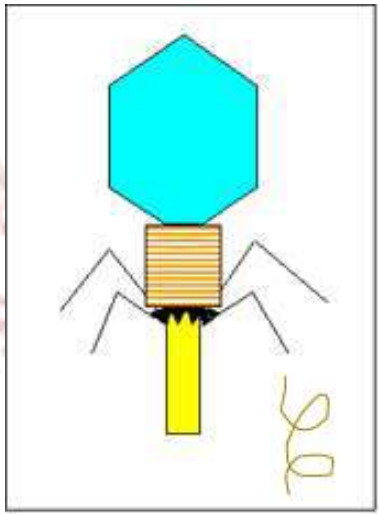
Gene transfer in bacteria

Transduction

- A process by which foreign DNA is introduced into a cell by a virus or viral vector. It results in genetic recombination.



Structure of T4 bacteriophage



Contraction of the tail sheath of T4

Gene transfer in bacteria

Transduction

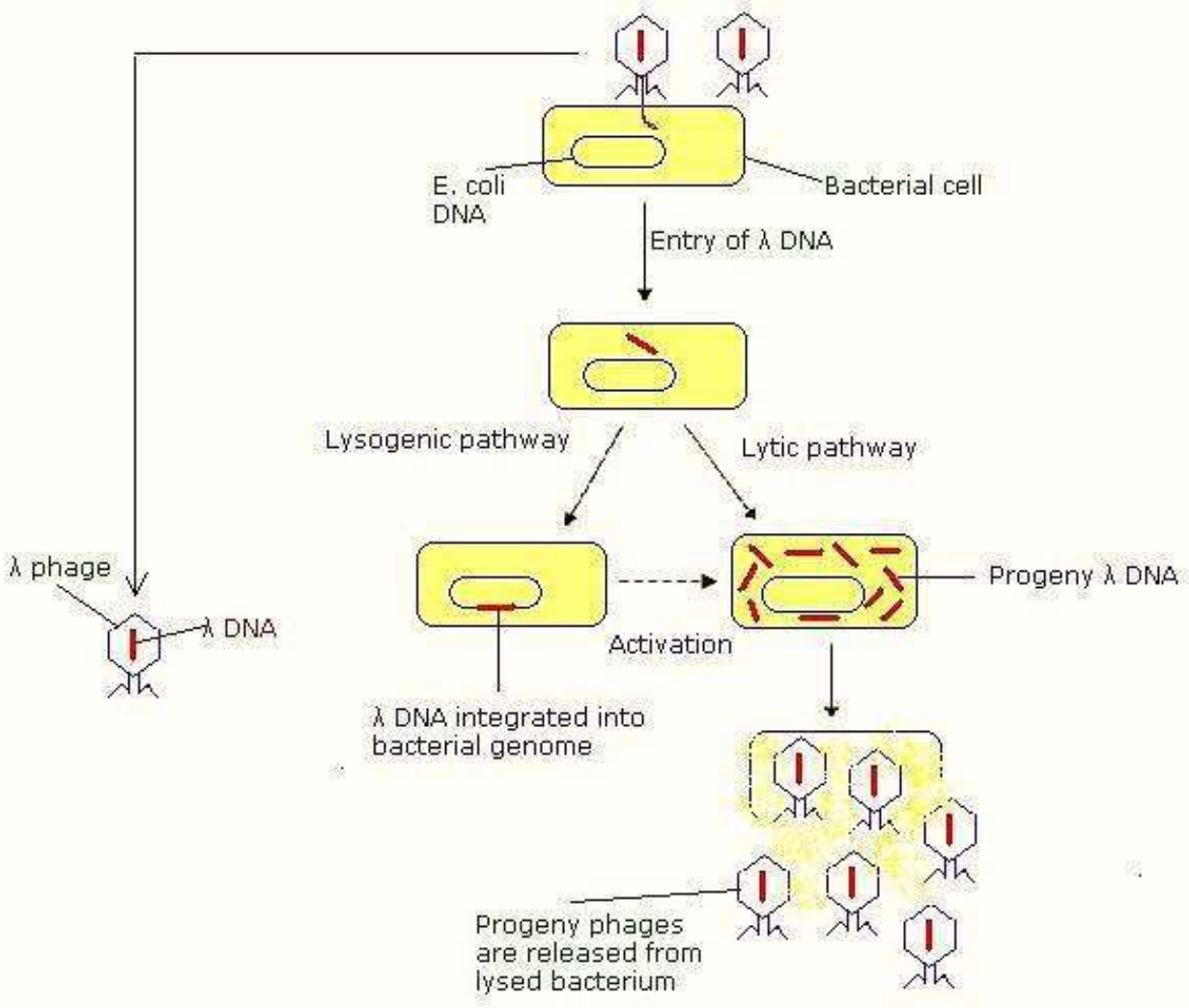
Two types

Generalized transduction: *A DNA fragment is transferred from one bacterium to another by a lytic bacteriophage that is now carrying donor bacterial DNA due to an error in maturation during the lytic life cycle.*

Specialized transduction: *A DNA fragment is transferred from one bacterium to another by a temperate bacteriophage that is now carrying donor bacterial DNA due to an error in spontaneous induction during the lysogenic life cycle.*

Gene transfer in bacteria

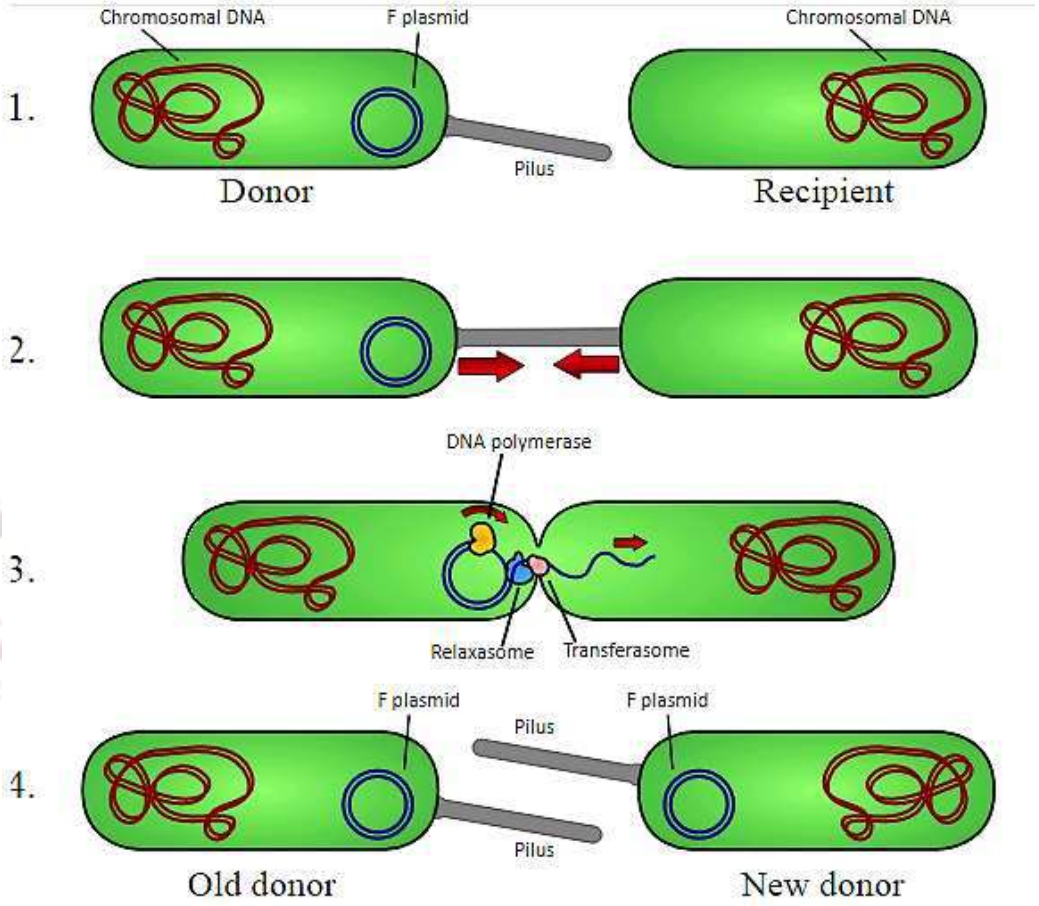
Transduction



Gene transfer in bacteria

Conjugation

- One bacterium transfers genetic material to another through direct contact.
- One bacterium serves as the donor and the other serves as the recipient.
- The donor bacterium carries a DNA sequence called the fertility factor or F-factor which allows the donor to produce a thin, tubelike structure called a pilus.



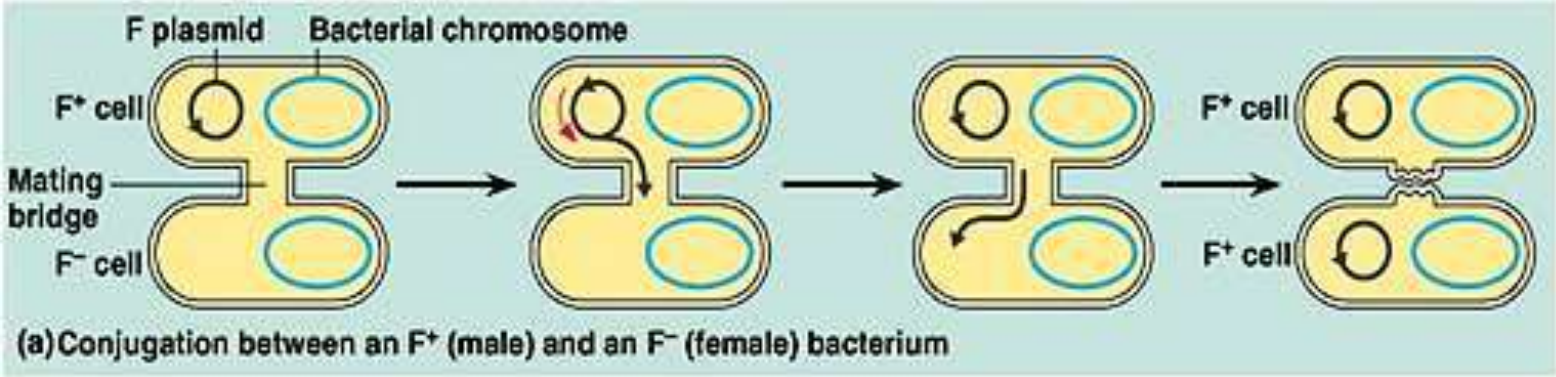
https://upload.wikimedia.org/wikipedia/commons/3/3e/Conjugation.svg

Gene transfer in bacteria

Conjugation

It is of three kinds:

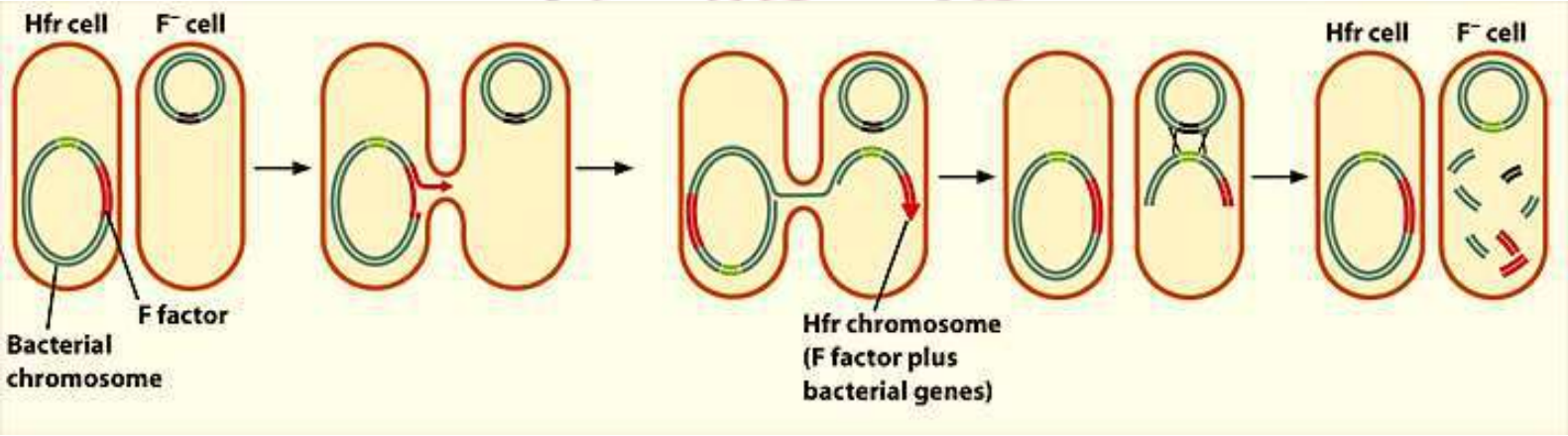
1. **F^+ conjugation:** It is basically a genetic recombination in which there is a transfer of an F^+ plasmid (coding for only sex pilus) but not genomic DNA. However other plasmids, such as antibiotic resistance plasmids may also be transferred.



Gene transfer in bacteria

Conjugation

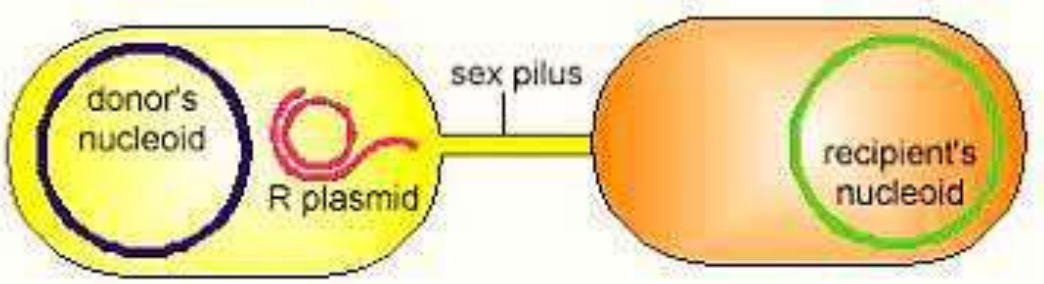
2. **Hfr Conjugation:** When the F factor is integrated into the bacterial genome, still acting as the donor in a conjugation process. These integrated strains are called Hfr, because of the high frequency of recombination occurring during the mating with F-bacteria. It is used to map the bacterial genome.



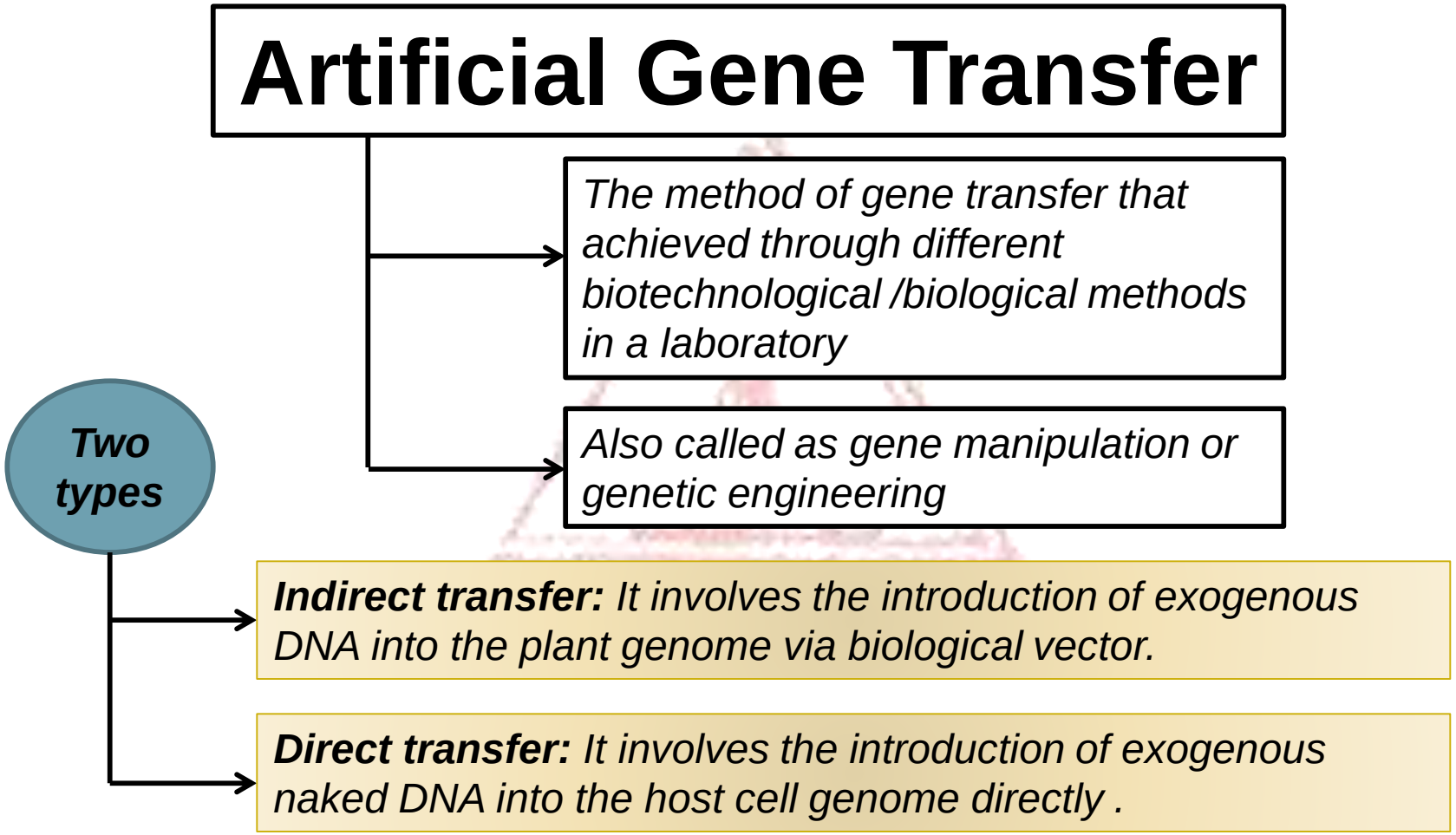
Gene transfer in bacteria

Conjugation

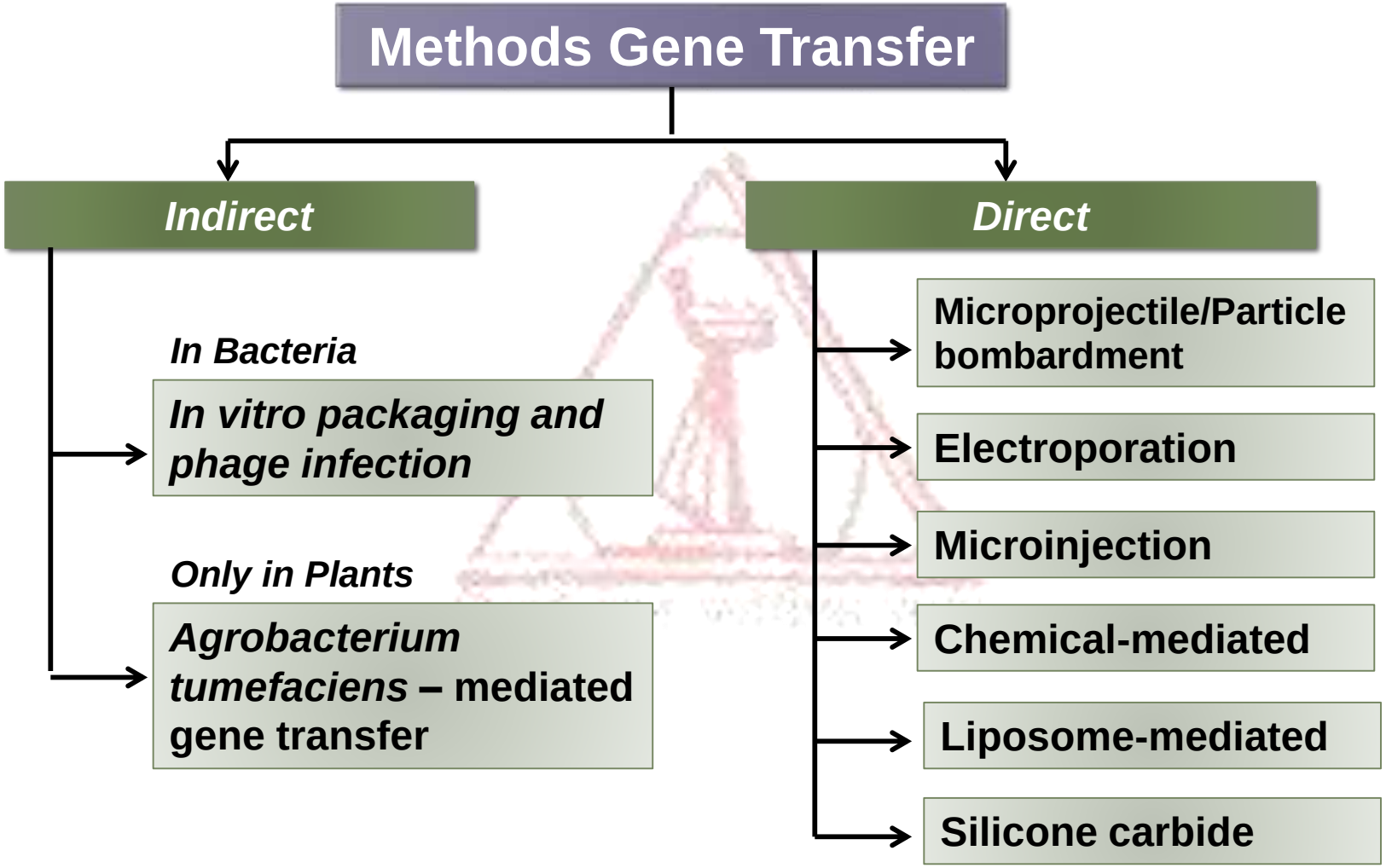
3. **Resistance plasmid conjugation:** Antibiotic sensitivity and resistance are mostly controlled by genes located on a plasmid which is a termed as drug-resistance (R) plasmids. The R plasmids also lead the formation of sex pilus during conjugation. Conjugation confers transfer of a copy of the plasmid from the resistant organism to one which may previously have been drug-sensitive.



Gene transfer in bacteria



Gene transfer in bacteria



Gene transfer in bacteria

Vectors

Cloning vectors: Cloning vectors are DNAs which can carry target genes, transfer them into the recipient cells.

Cloning vector classes

- **Plasmid DNA**
- **Phage DNA**
- **Virus DNA**

As for the expression vectors, they can make the proteins which are coded by the target gene expressed in the host cell

Gene transfer in bacteria

Vectors

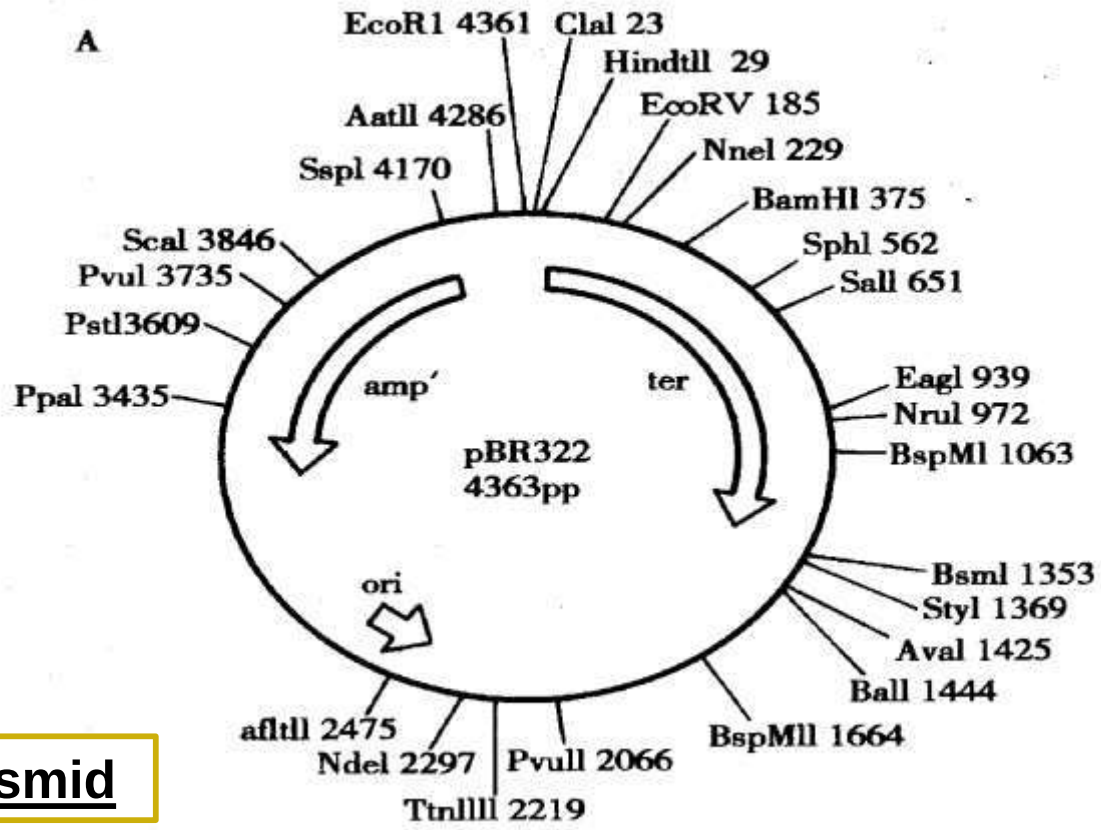
Plasmids

- Small (less than 10kb), Circular, duplex molecules of DNA
- Exist at low or high copies within the bacteria, but useful plasmid present in multiple copies
- Replicate independently from the bacterial cell
- Contain selectable markers, eg: the antibiotic resistance capability conferred to bacterium.
- Possess at least one DNA sequence that act as an origin of replication
- Multiple RE sites (multiple cloning sites, MCS)

Gene transfer in bacteria

Vectors

Plasmids



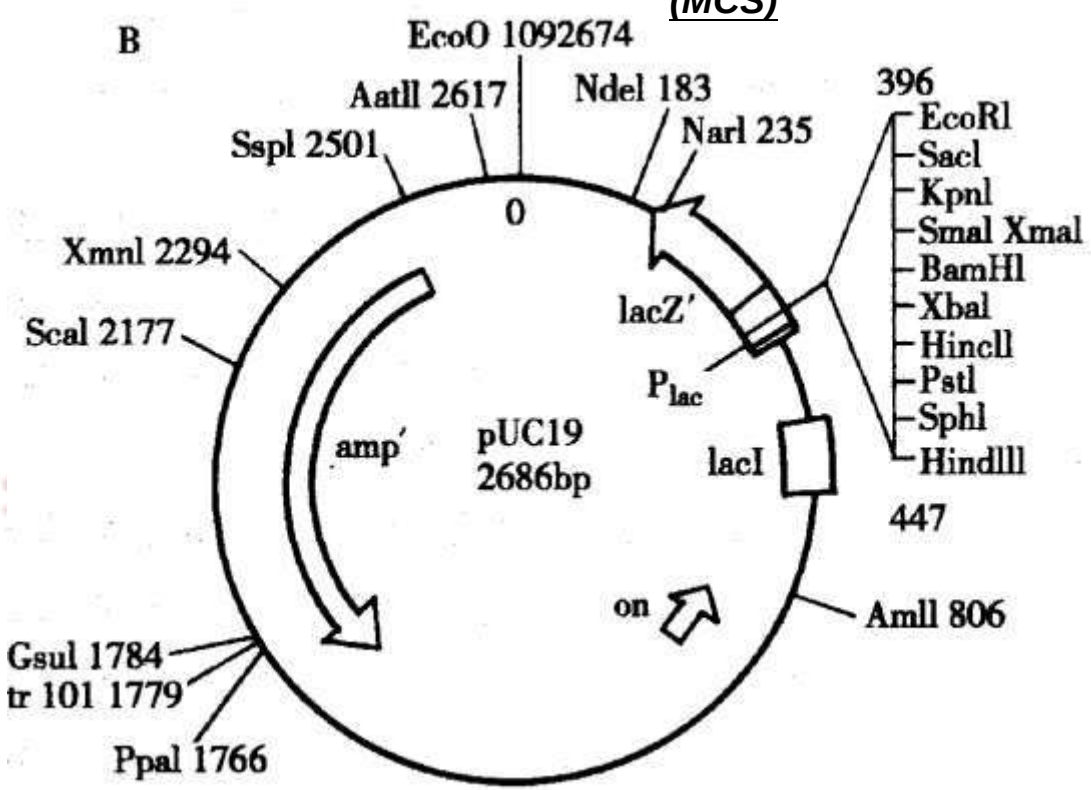
pBR322 Plasmid

Gene transfer in bacteria

Vectors

Plasmids

Multiple cloning site (MCS)



pUC19 Plasmid

Gene transfer in bacteria

Vectors

Bacteriophages

- Bacteriophages, or phages are viruses that specifically infect bacteria.
- Simple in structure, merely of a DNA (or occasionally RNA) carrying genes, including several for replication of the phage, surrounded by a protective coat or capsid made up of protein.

Pattern of infection

- Attaches to the outside of the bacterium and injects its DNA chromosome into the cell.
- The phage DNA is replicated, usually by specific phage enzymes coded by genes on the phage chromosome.
- Other phage genes direct synthesis of the protein components of the capsid, new phage particles are assembled and released.

Gene transfer in bacteria

Vectors

Bacteriophages

Result of infection

- Lytic cycle: With some phage types the entire infection cycle is completed very quickly, possibly in less than 20 min. This type of rapid infection is called lytic cycle.
- Lysogenic cycle: Characterized by retention of the phage DNA molecule in the host bacterium, possibly for many thousands of cell divisions

Gene transfer in bacteria

Vectors

Bacteriophages

Common phages used as vectors: Bacteriophage λ

- A linear dsDNA approximately 49 Kb in length
- After infection it forms circular structures
- The phage DNA is inserted into the bacterial genome
- The first two classes of vector to be produced were λ insertion (λgt phages) and λ replacement (EMBL phages).

Common phages used as vectors: Bacteriophage M13

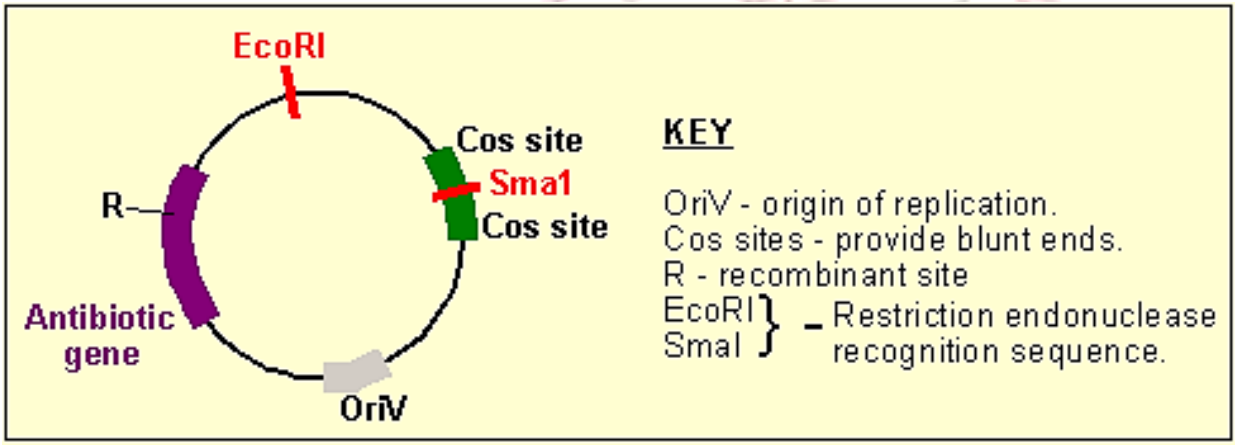
- A circular ssDNA, and has been used for sequencing of a cloned target DNA fragment

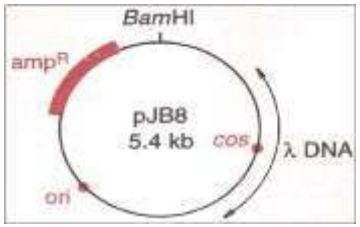
Gene transfer in bacteria

Vectors

Other vectors – Cosmids

- Cosmic vectors are hybrids between plasmid and phage λ vectors. The classic example of cosmid vector is c2RB, which carries an origin of replication and a cloning site and has antibiotic-resistant genes.
- It is formed by joining ends of a linearized plasmid DNA with cos-site of lambda DNA. Thus, It is a derived vector.





Gene transfer in bacteria

Vectors

<u>Other vectors</u> – <u>Viruses & artificial chromosome</u>

- **Bacterial artificial chromosome (BAC) and yeast chromosome**
- **Viruses are used as vectors, e.g. Retrovirus, adenovirus, adeno-associated virus, etc.**

Gene transfer in bacteria

Enzymes of Gene Transfer

- **Nucleases (e.g. endonuleases):** cut, shorten or degrade nucleic acid molecules
- **Ligases:** join nucleic acid molecules together
- **Polymerase:** make copies of molecules
- **Modifying enzymes:** remove or add chemical groups
- **Topoisomerases:** introduce or remove supercoils from covalently closed-circular DNA

Gene transfer in bacteria

Enzymes of Gene Transfer

Endonuleases

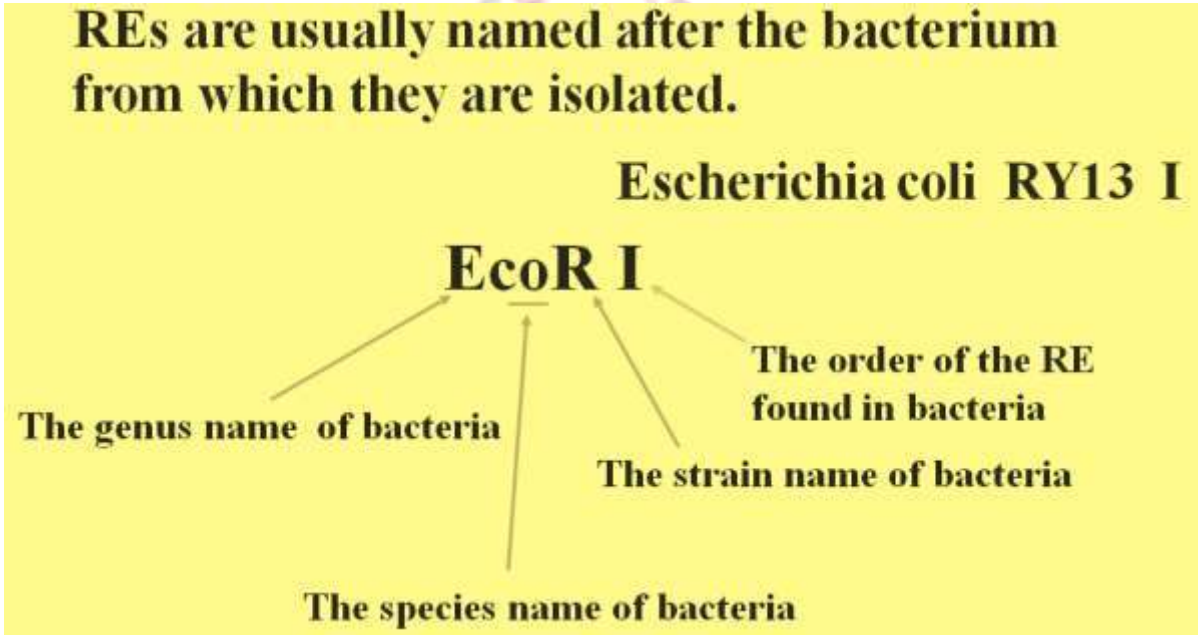
- The initial observation that led to the eventual discovery of restriction endonucleases (RE) was made in the early 1950s
- Restriction occurs because the bacterium produces an enzyme (called restriction endonucleases) that degrades the phage DNA
- Three different classes of RE are recognized, but the most important one is RE II which is used in DNA manipulation
- The discovery of these enzymes led to Nobel prizes for W. Arber, H. Smith and D. Nathans in 1978.
- Type II restriction endonucleases (RE) cut DNA at specific nucleotide sequences
  - Generally, 4~8 bases be found, mostly 6 bases, a few of 8~10 bases.
  - The sequences discriminated usually are palindrome structure.
  - To cut the double strands of DNA at special sites and to yield two kinds of ends: blunt ends and sticky ends

Gene transfer in bacteria

Enzymes of Gene Transfer

Endonuleases

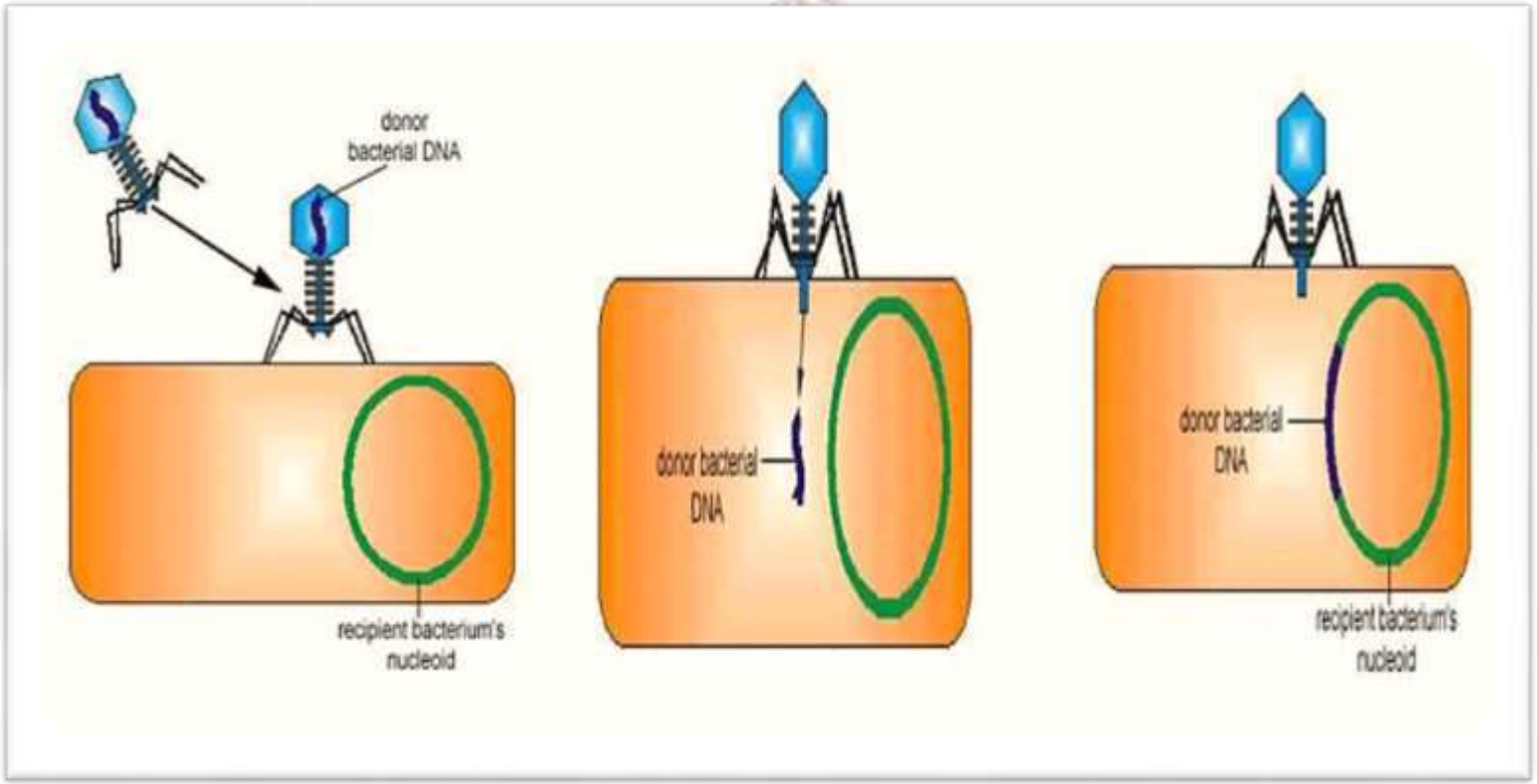
Naming of Restriction Endonucleases



Gene transfer in bacteria

Indirect method

In vitro packaging and phage infection



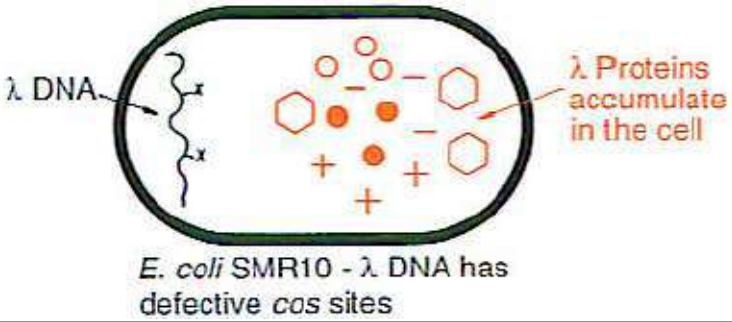
Gene transfer in bacteria

Indirect method

In vitro packaging and phage infection

- It involves packaging of λ cloning vectors – the recombinant λ molecules are packed into their λ head-and-tail structure in the test tube.
- Packaging requires a number of different proteins coded by the λ genome, but these can be prepared from cells infected with defective λ phage strains.
- Synthesis of λ capsid proteins by *E. coli* strain SMR10, which carries a λ phage that has defective *cos* sites not recognized by the endonuclease that cleaves the λ catenanes during phage replication.

A single-strain packaging system – *E. coli* SMR10

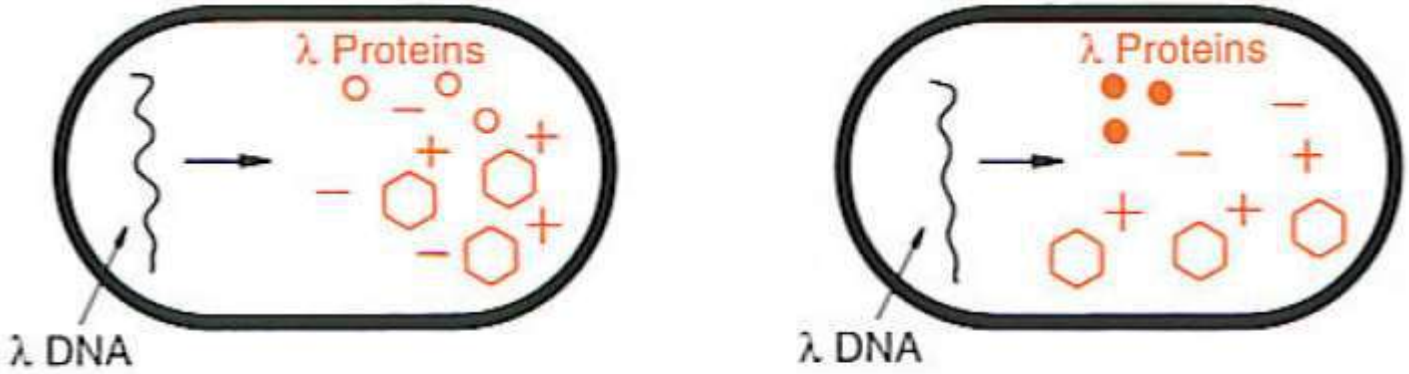


Gene transfer in bacteria

Indirect method

In vitro packaging and phage infection

A single-strain packaging system – *E. coli* strain BHB2688 and BHB2690



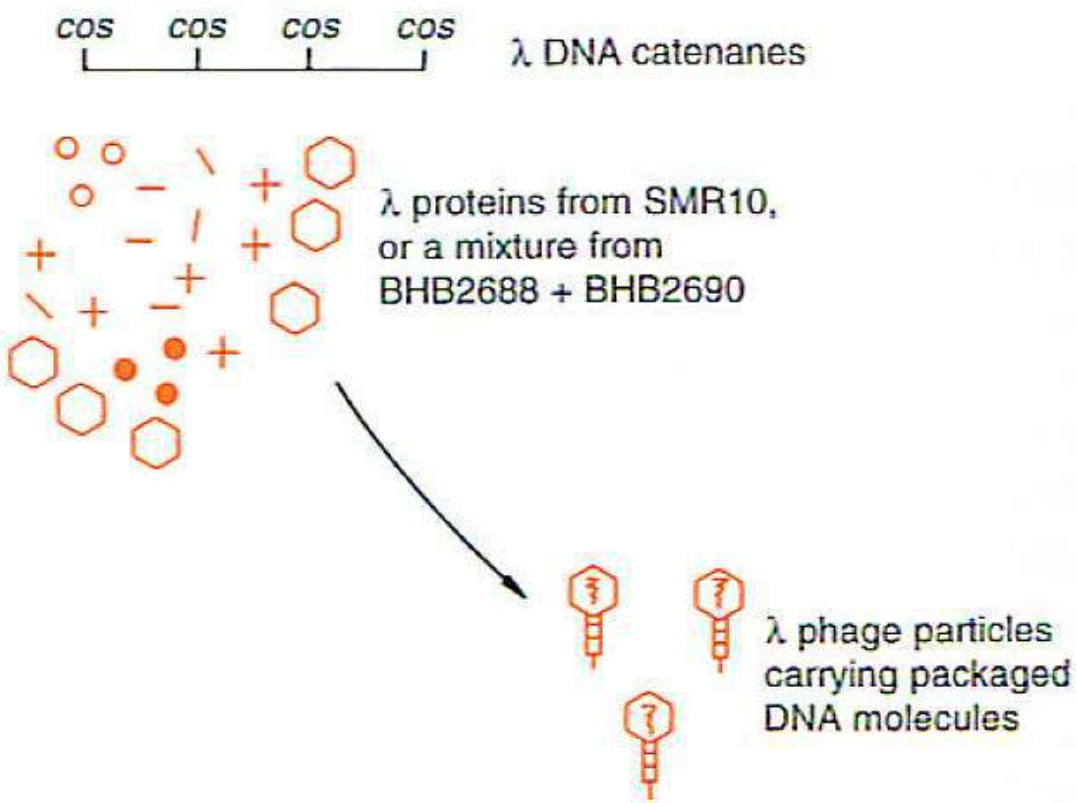
E. coli BHB2688 – λ defective for synthesis of protein E (●)

E. coli BHB2690 – λ defective for synthesis of protein D (○)

Gene transfer in bacteria

Indirect method

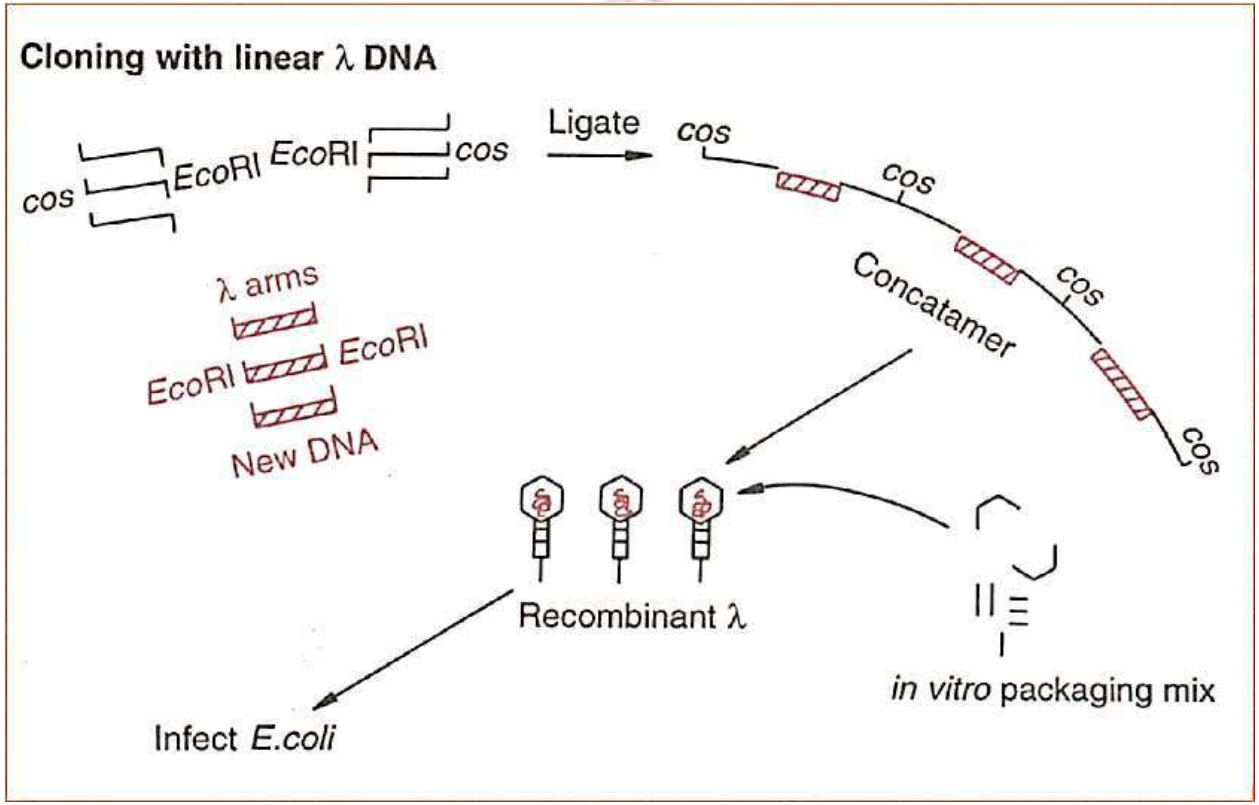
In vitro packaging and phage infection



Gene transfer in bacteria

Indirect method

In vitro packaging and phage infection



Gene transfer in bacteria

Indirect method

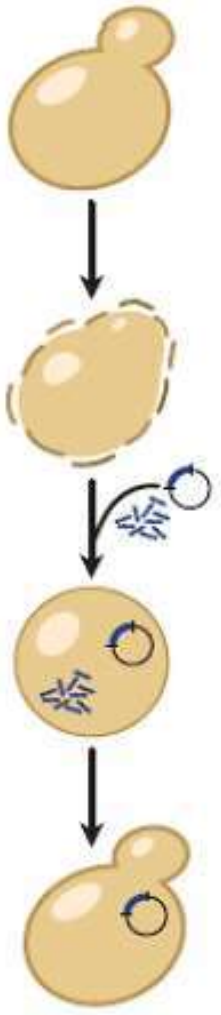
Introduction of DNA into *Saccharomyces cerevisiae*

1. Grow yeast to mid-log phase

2. Treat cells with Lithium/Cesium Acetate Solution

3. Introduce plasmid DNA, carrier DNA, histamine, PEG, and TE/CationMIXX

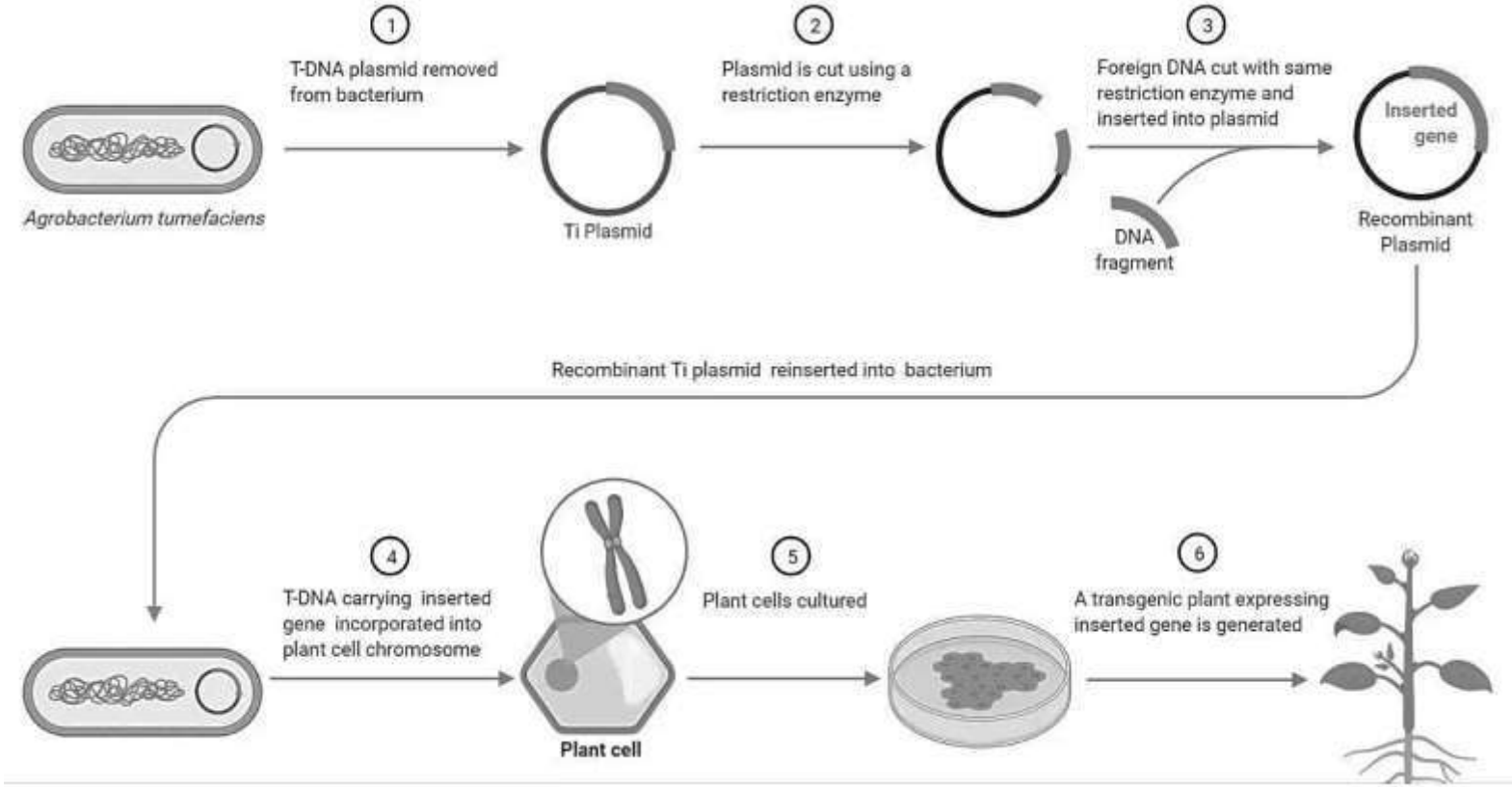
4. Heat shock and plate on regeneration media to allow cell wall reconstruction and growth of transformants



Gene transfer in bacteria

Indirect method

Agrobacterium tumefaciens – mediated gene transfer

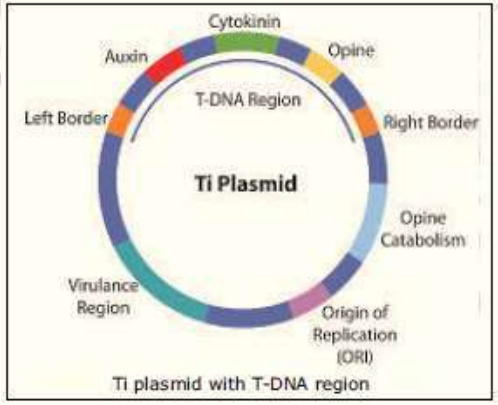


Gene transfer in bacteria

Indirect method

Agrobacterium (a soil bacteria) **– such as *A. radiobacter, A. vitis, A. rhizogenes, A. rubi* and *A. tumefaciens.***

- It is a phytopathogen that infects plants through wound sites causing crown gall disease.
- It utilizes its bacterial type IV secretion system for the transfer of its transferred (T)-DNA into the host
- Infectivity depends on different bacterial as well as plant factors. Bacterial factors include virulence genes and T-DNA oncogenes, whereas the plant factors include genes required for transformation and tumor formation.



Ti plasmid with T-DNA region


Crown gall disease caused by *Agrobactrium* on rose stem

Gene transfer in bacteria

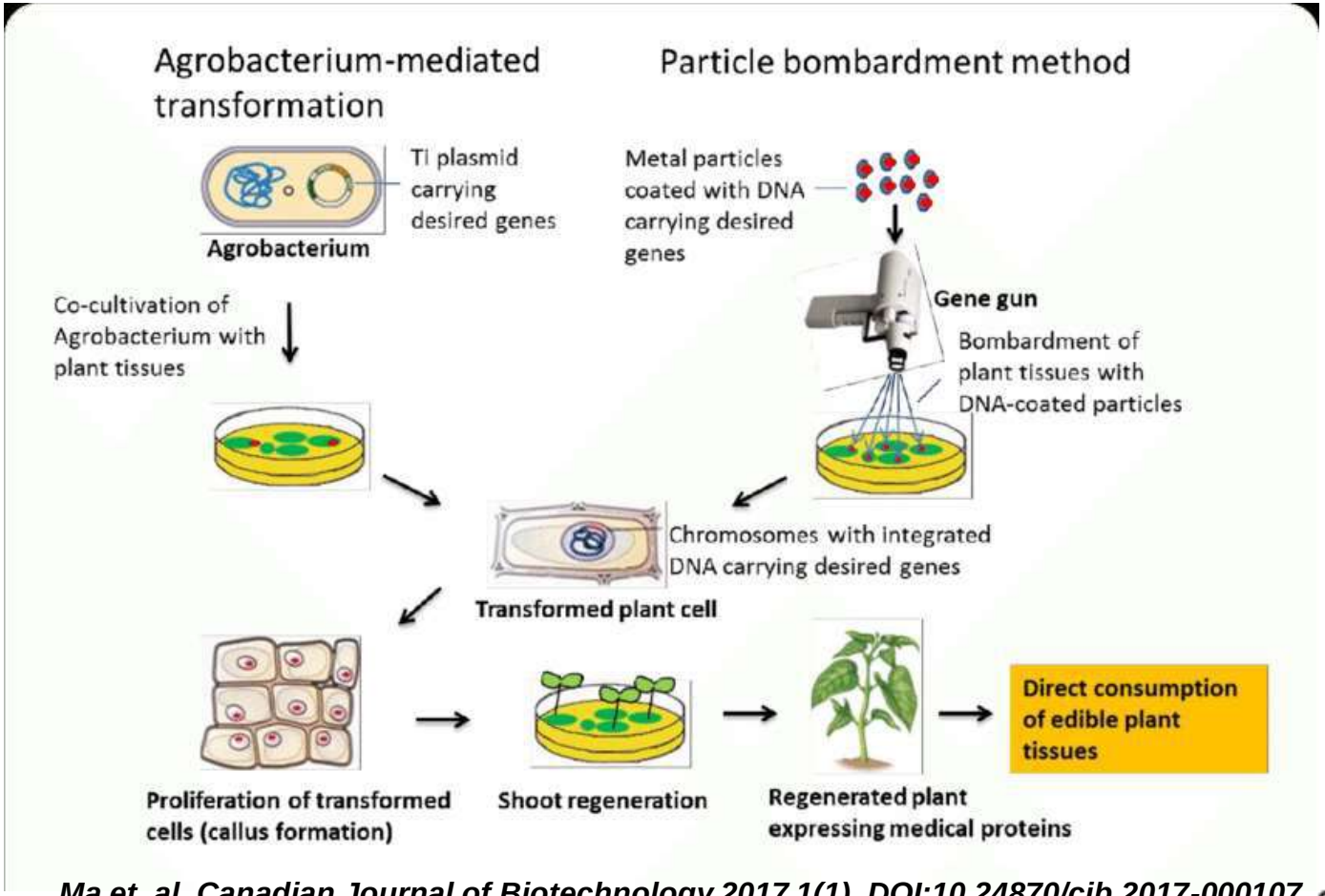
Direct method

Gene Gun Mediated Gene Transfer

- Gene gun was introduced in 1987 for DNA-coated particle bombardment.
- The method of transferring the gene into the target cell is performed by GNPs (Gold Nano Particle) coated with DNA under pressurized inert gas such as helium or by high-voltage electronic discharge.
- Besides gold, tungsten is also used in the gene gun.
- The metal particles are 0.45 – 1.5 μm in diameter. They punch holes in and pass through the cell wall and enter the plant cells due to very high velocity, leaving the cargo inside the cells.
- Once the DNA cargo diffuses from the surface of the metal carrier, it influences the intracellular genetic process.

Gene transfer in bacteria

Direct method



Gene Gun Mediated Gene Transfer

Ma et. al. Canadian Journal of Biotechnology 2017 1(1), DOI:10.24870/cjb.2017-000107

Gene transfer in bacteria

Direct method

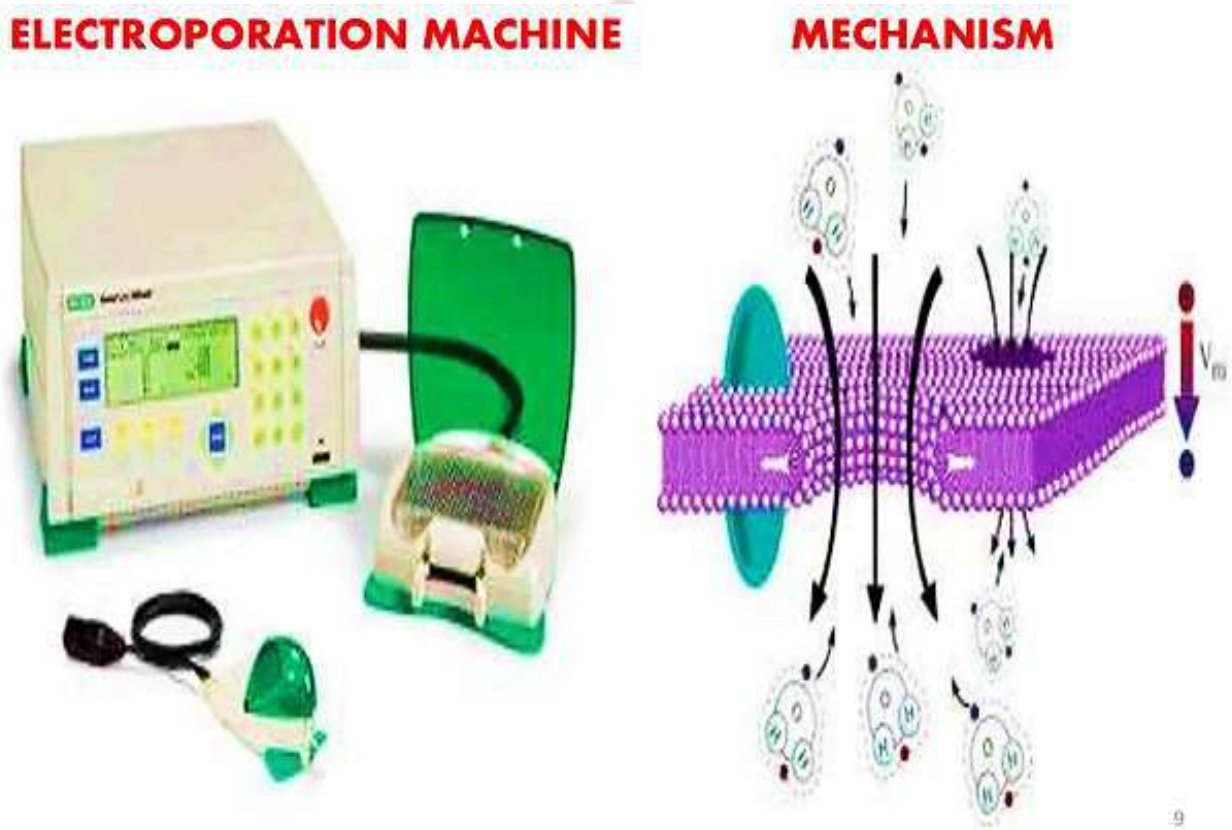
Gene Transfer by Electroporation

- It has been used extensively to transfer DNA to bacteria, yeast, and mammalian cells in culture for the past 30 years.
- It uses electrical fields to transiently destabilize the membrane allowing the entry of normally impermeable macromolecules into the cytoplasm.
- Electroporation can lead to between 100 and 1000-fold increases in gene delivery and expression.
- Almost any tissue can be targeted with electroporation, including muscle, skin, heart, liver, lung, and vasculature.

Gene transfer in bacteria

Direct method

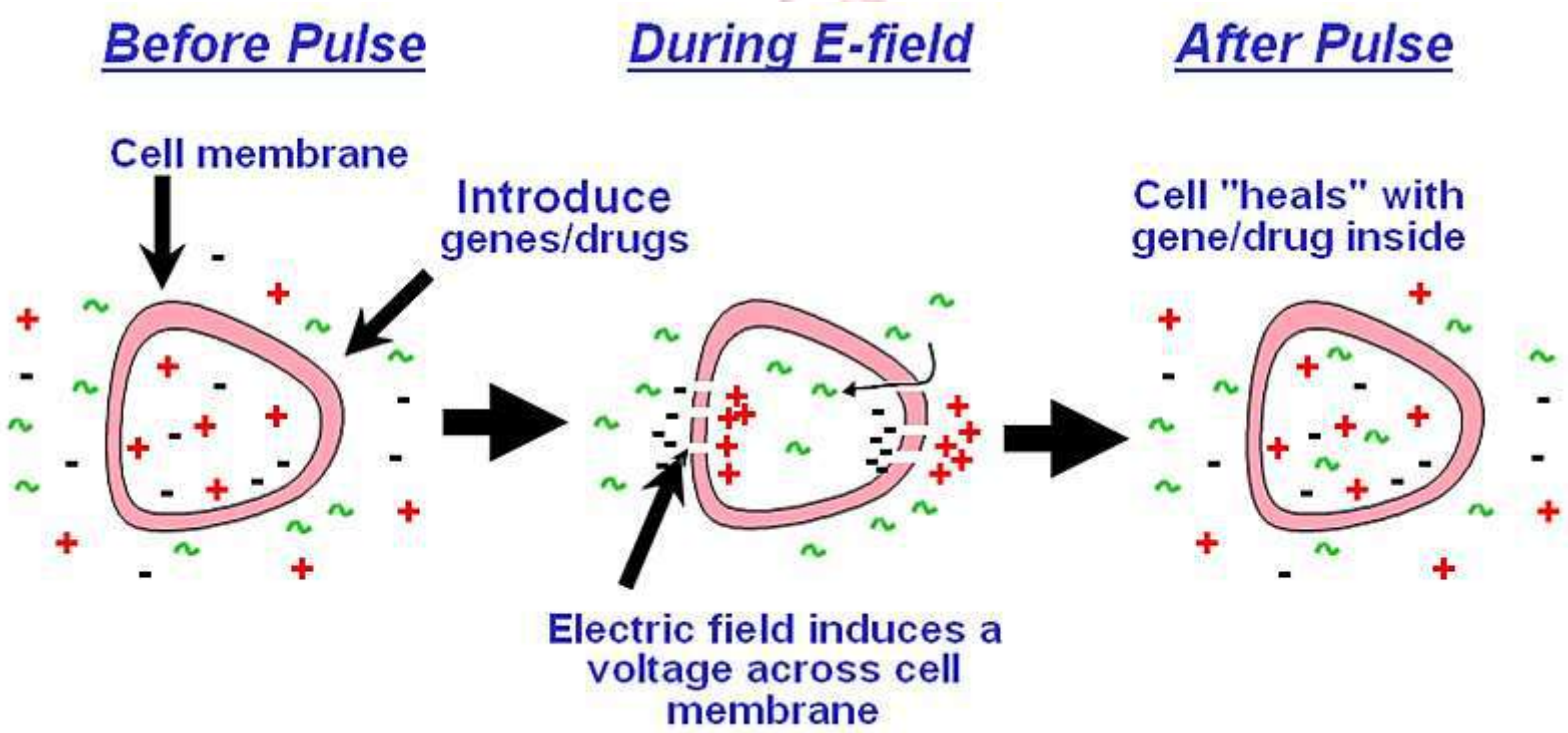
Gene Transfer by Electroporation



Gene transfer in bacteria

Direct method

Gene Transfer by Electroporation

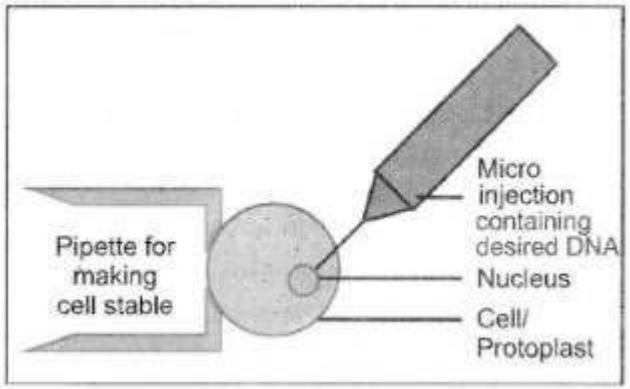


Gene transfer in bacteria

Direct method

Gene Transfer by Microinjection

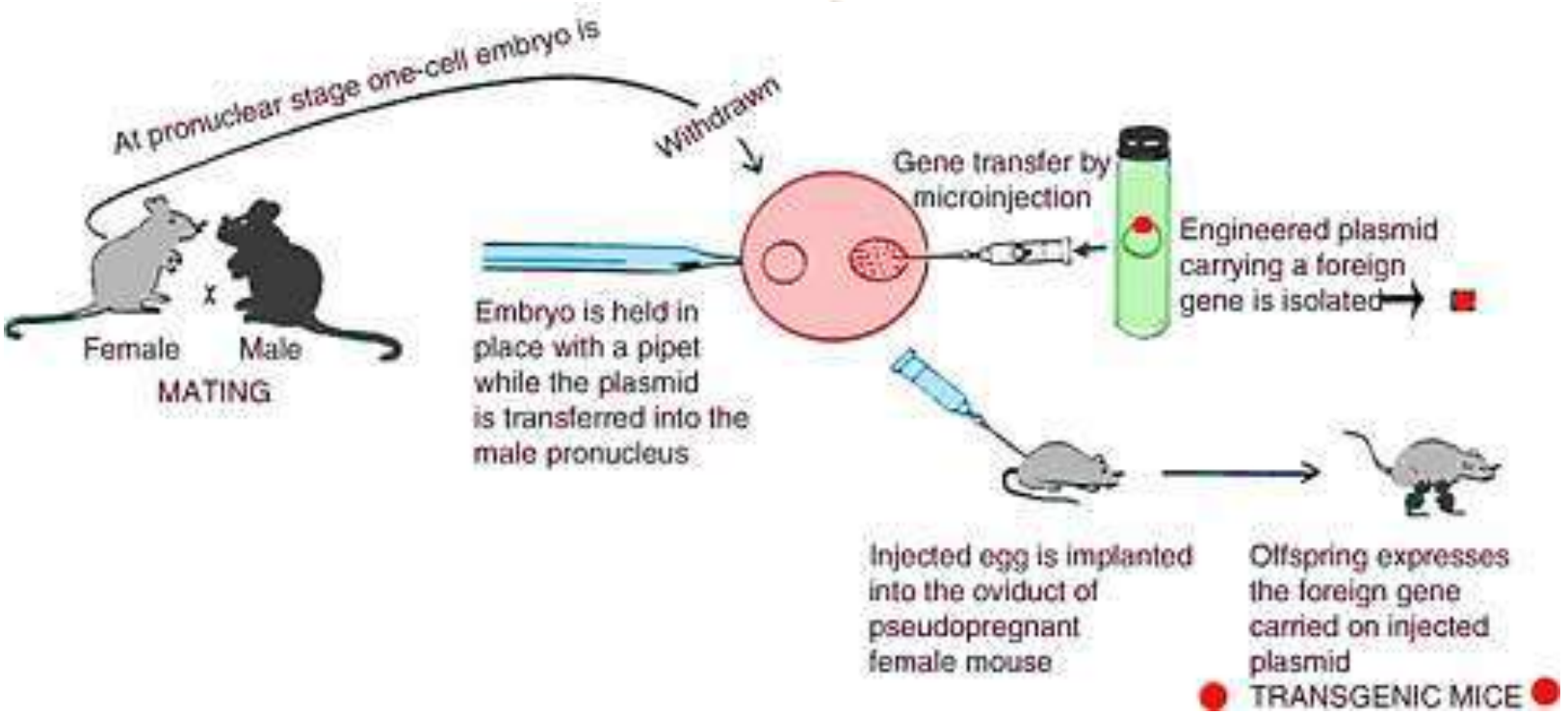
- Microinjection is the process of transferring genetic materials directly into a living cell nucleus using glass micropipettes or metal microinjection needles.
- Glass micropipettes can be of various sizes with tip diameters ranging from 0.1 to 10 µm.
- This method is extensively used in the development of transgenic animals.



Gene transfer in bacteria

Direct method

Gene Transfer by Microinjection



(2008) Gene Transfer by Microinjection. In: Encyclopedia of Genetics, Genomics, Proteomics and Informatics. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6754-9_6605

Gene transfer in bacteria

Direct method

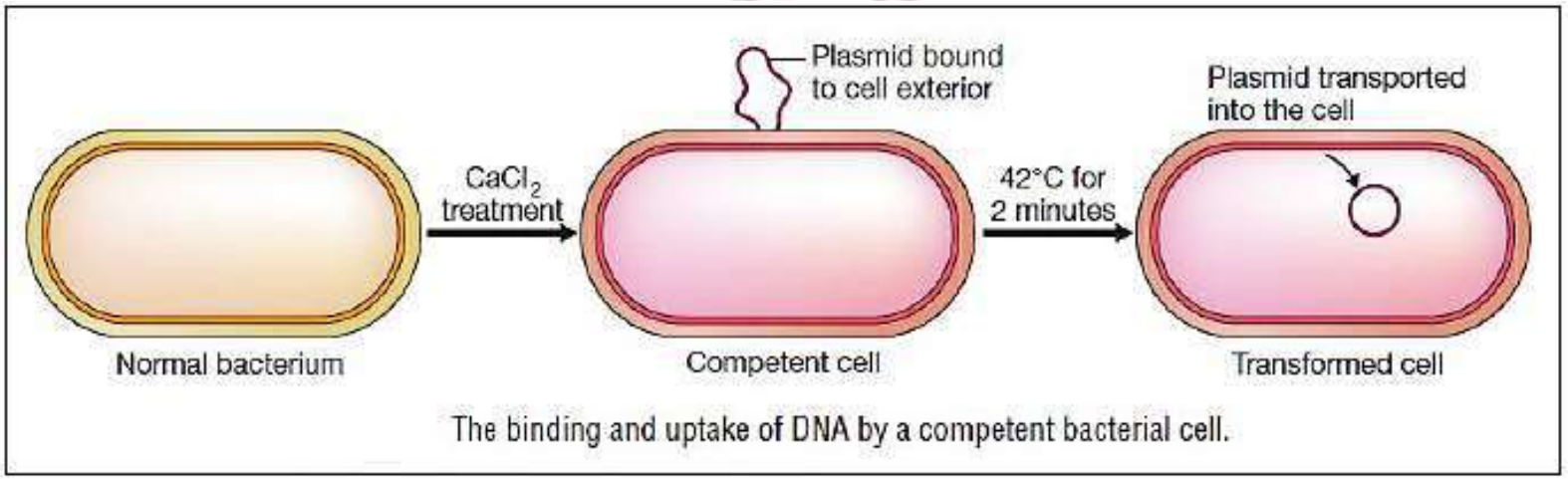
Gene Transfer by Chemical Means

METHOD	ORGANISM TRANSFORMED
CaCl2 / Heat shock	Bacteria, Yeast
Ca-DNA co-precipitation	Plants, Animals
Liposome packaging and fusion or Endocytosis	Plants, Animals
DEAE-dextran transfection	Plants, Animals
Poly cation-mediated DNA uptake	Plants, Animals

Gene transfer in bacteria

Direct method

<u>Gene Transfer by Chemical Means</u> – *$CaCl_2$ and Heat Shock*



The binding and uptake of DNA by a competent bacterial cell.

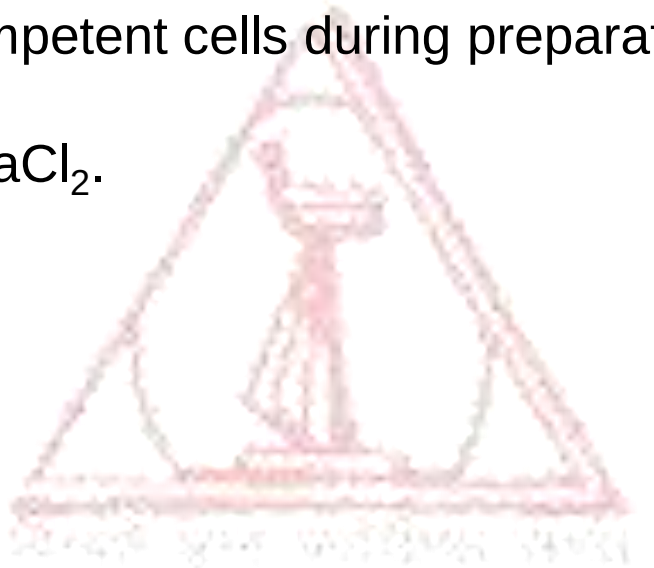
Transformation Efficiency

- Number of colony forming units (cfus) produced by 1 microgram of plasmid DNA.
- Transformation efficiency decreases with increase in the size of DNA molecule.

$$cfu = \frac{No.of\ bact.colonies\ \times dilution\ ratio\ \times Orig.transformation\ volume}{Plate\ volume}$$

Factors Affecting Transformation Efficiency

- Growth period.
- Temperature of competent cells during preparation, storage and transformation.
- Concentration of $CaCl_2$.
- Culture medium



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