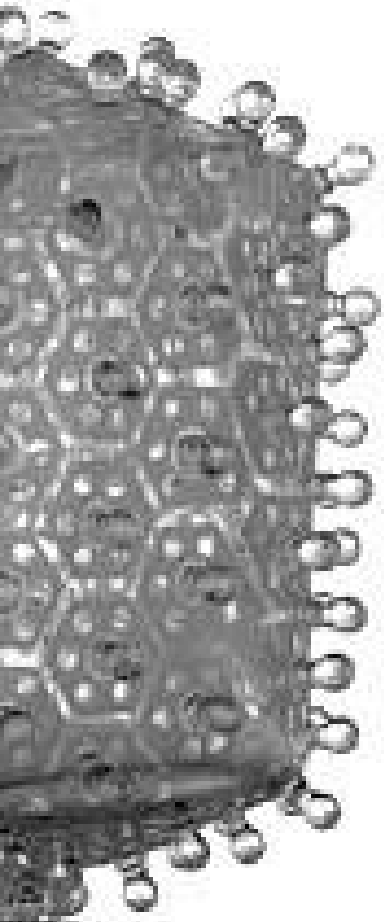
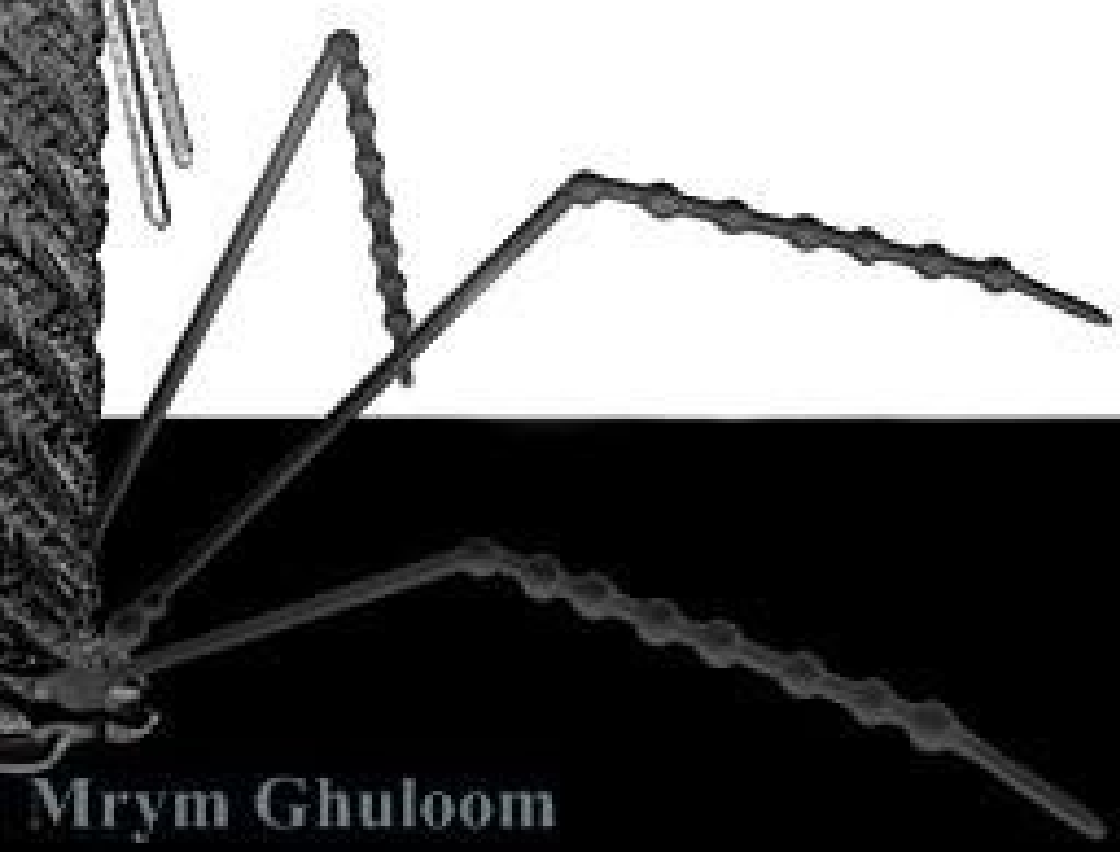


Microbiology

Lecture No: 2-Bacterial Genetics

Dr Name: Asem Shehabi

Sheet ☐ Slide ■



Mrym Ghuloom

Bacterial Genetics

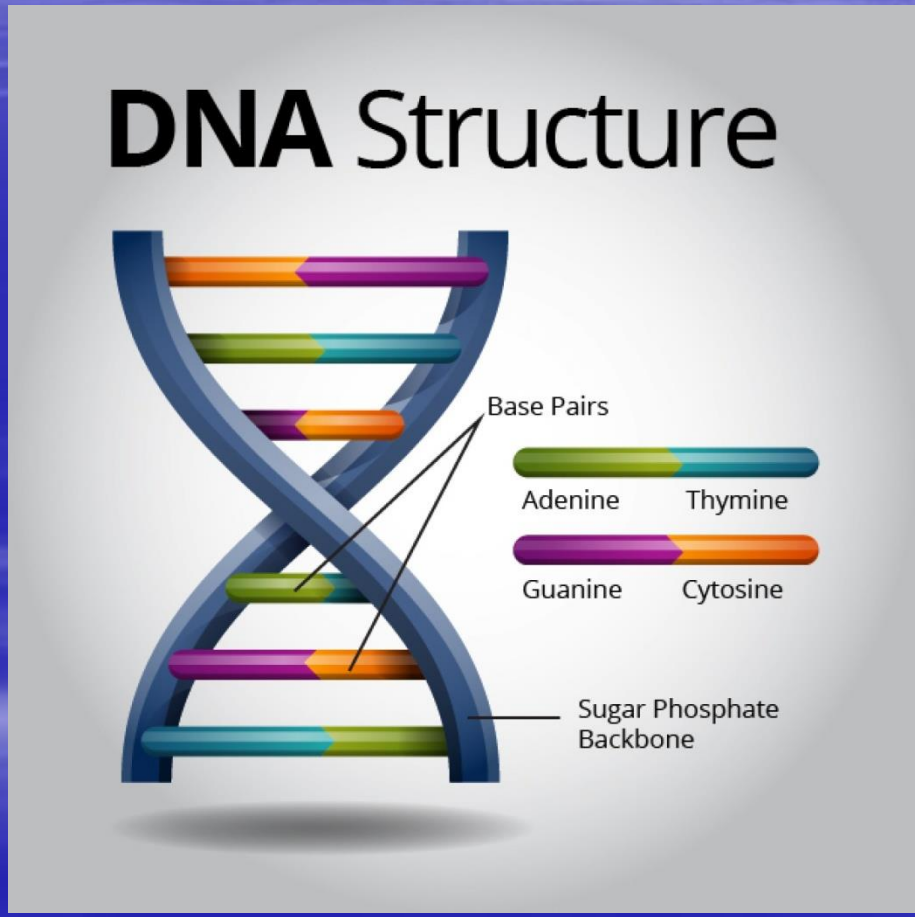
By

Prof. Dr. Asem Shehabi and Dr. Suzan Matar

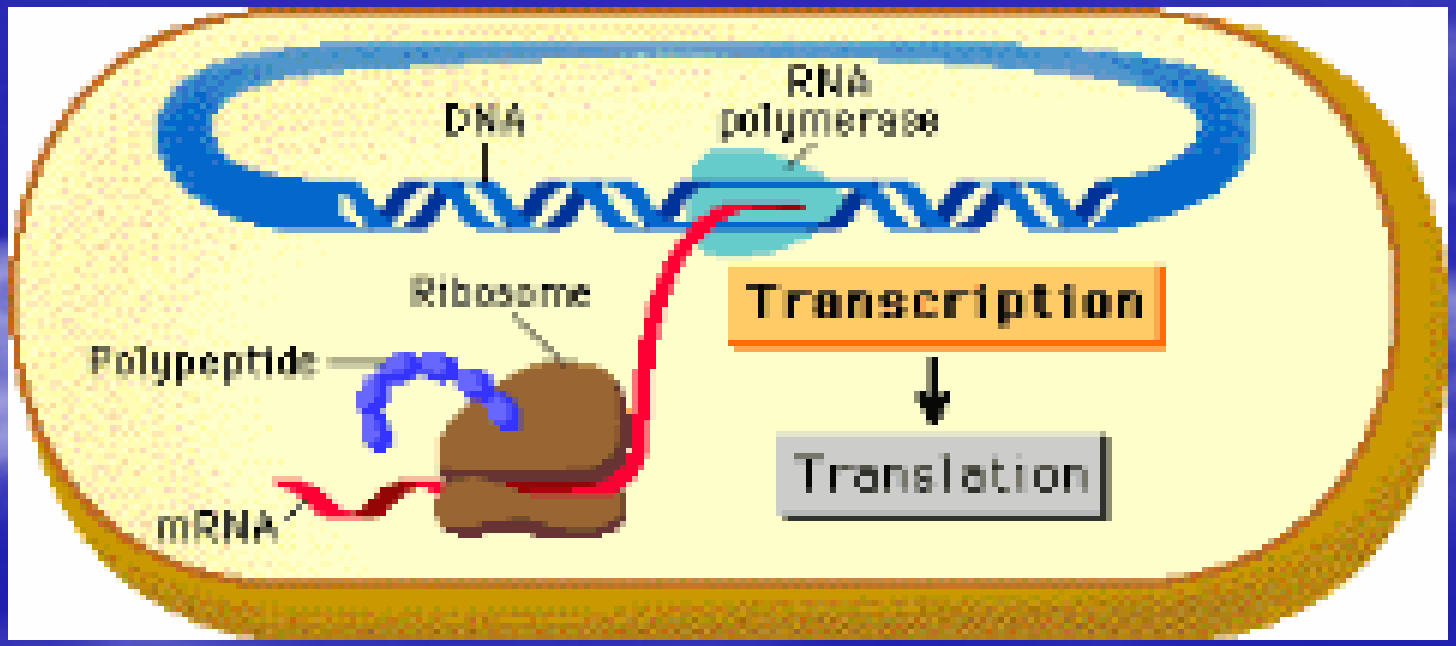
Bacterial Genes-1

- All patterns of growth, metabolism, essential cellular structures, biological characteristics of bacteria are controlled by **DNA encoded & expressed genes.**

- **Bacterial Genome**: **Chromosome,** single circular double-stranded DNA.
- 1300 um long contains **2-5 x 10^6** nucleotide bases, enough DNA to encode **1- 3** thousand different genes. According to bacteria types.

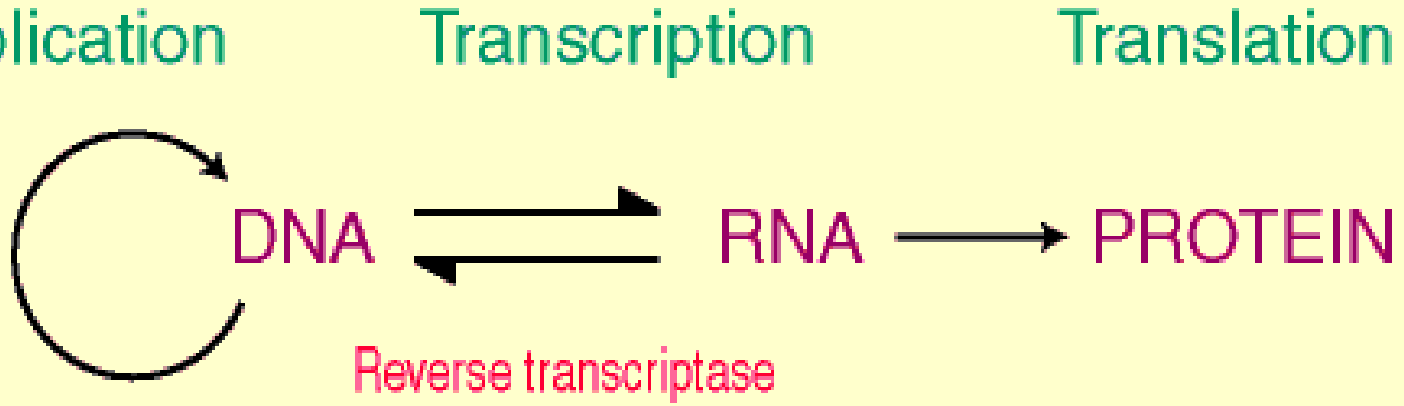


- **Genetic information** is encoded in **DNA**, transcribed into **mRNA,** translated on **Ribosomes** through **tRNA** into various protein polypeptides/structures and enzymes with diverse functions



BACTERIAL GENETICS

I. CENTRAL DOGMA



Replication	Transcription	Translation
DNA template	DNA template (sense strand)	mRNA template
DNA polymerase III	RNA polymerase	ribosomes
replication proteins	transcription factors	translation factors
dNTPs, ATP, Mg^{2+}	NTPs, Mg^{2+}	AAs, tRNAs, synthetases, ATP, GTP, Mg^{2+}

- **Gene**: A segment of DNA specifies production of a particular amino acid, polypeptide chain function (Enzyme, Protein)

- Bacteria with similar organization and location of essential genes are grouped within the same Family-**Genus-Species- strains.**

- The sequence analysis of bacterial genomes has confirmed that **genetic change / mutation** in bacteria occurs both by alteration of the DNA base sequence, **gain, loss** or **substitution** of one base pairs or more..Small/larger DNA segments containing genes.
- Bacterial genome includes Chromosome & DNA Plasmid, DNA / RNA Bacteriophage

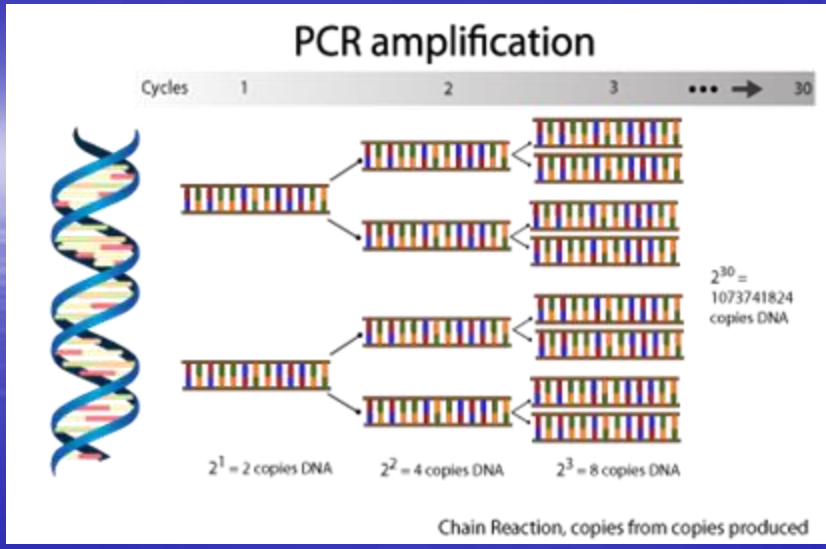
Bacterial Genes-2

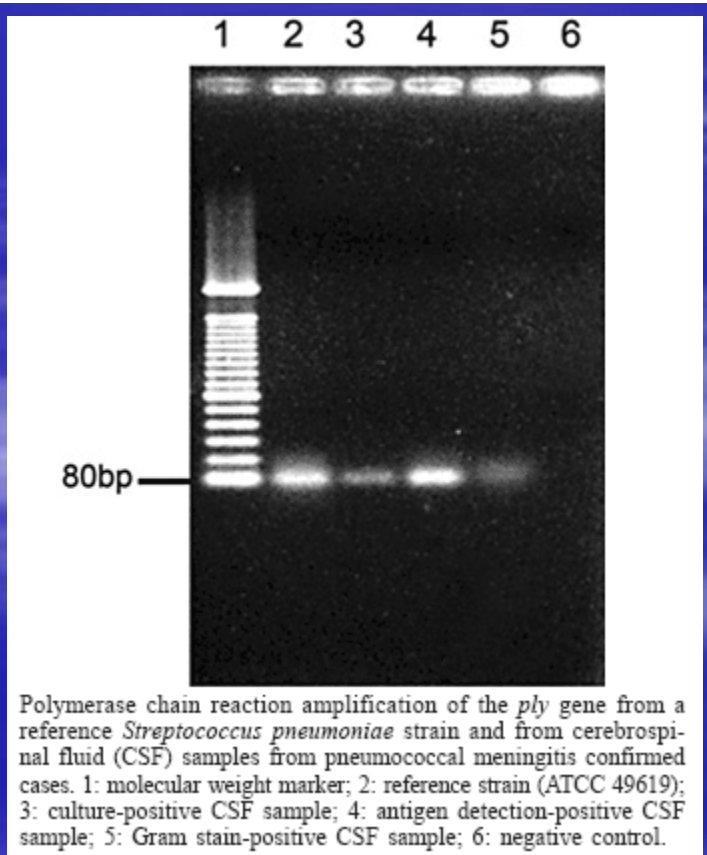
- The distinction between **genotype & phenotype** is fundamental to the understanding of heredity and evolution of microorganisms.
- **Genotype / Wild Type :** Represents all potential genes of bacteria cell.. Its genome.. All Inherited essential biological features & growth patterns.
- **Phenotype**: The expressed genes..The observed characteristics of the of the individual bacteria species/strain. Expressed by physical & biochemical properties. Growth patterns, Fermentation products, Antibiotic resistance, Toxins production. .etc.
- **Bacterial bio-engineering** has made important contributions to medicine, food agriculture & industry, medical drugs like Insulin ,Interferon, Vaccines

- **Lab diagnosis of pathogens:**

- **Polymerase Chain Reaction (PCR technique) ..** allows amplification of specific region of DNA to detect few number of microorganism/ cell DNA in clinical specimens.. Blood, Urine.. identify cause of Disease .. Bacteria, Viruses & others

16S ribosomal RNA gene (16srRNA) is highly stable in most bacterial types





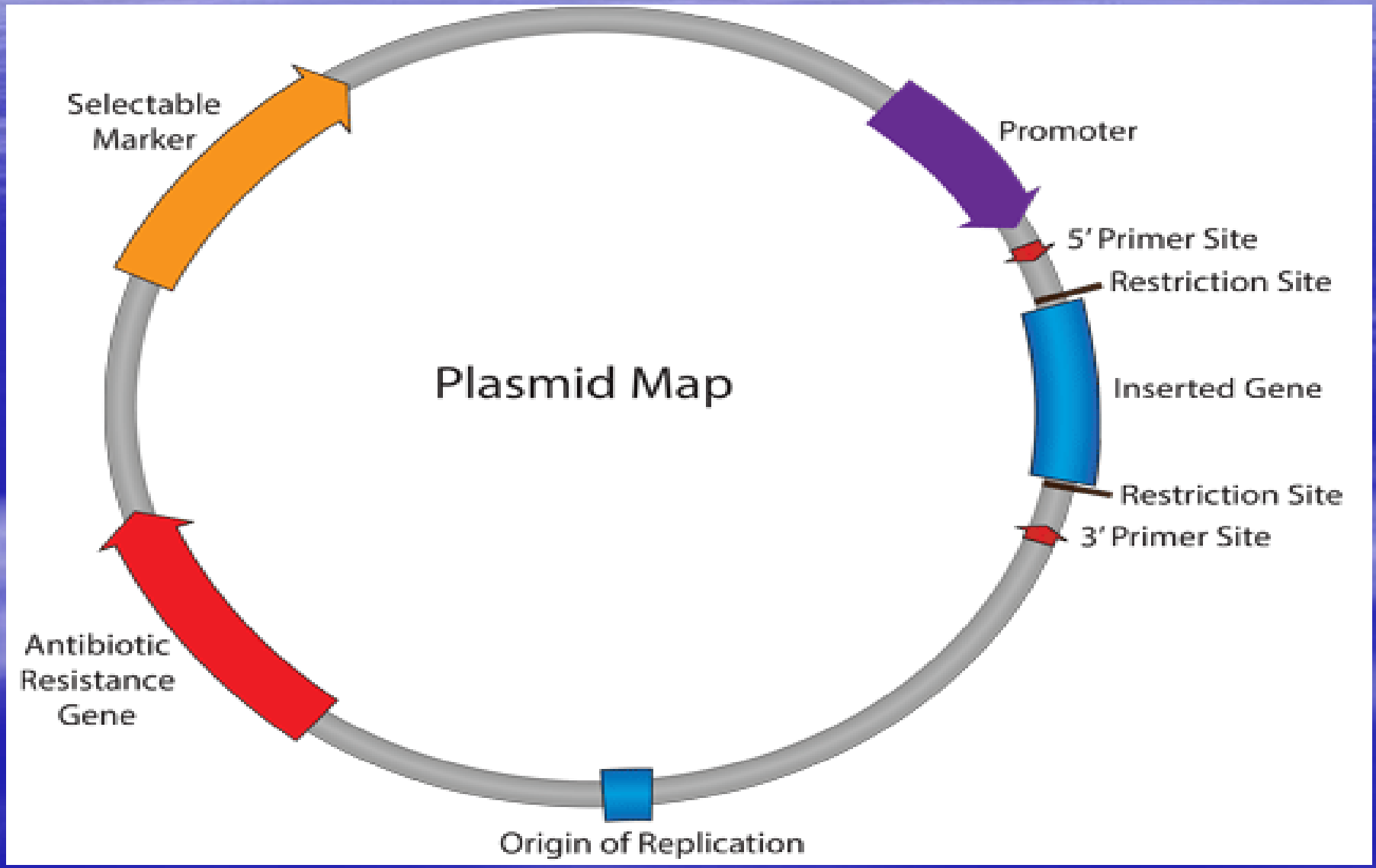
Polymerase chain reaction amplification of the *ply* gene from a reference *Streptococcus pneumoniae* strain and from cerebrospinal fluid (CSF) samples from pneumococcal meningitis confirmed cases. 1: molecular weight marker; 2: reference strain (ATCC 49619); 3: culture-positive CSF sample; 4: antigen detection-positive CSF sample; 5: Gram stain-positive CSF sample; 6: negative control.

- **Plasmid:**

- Extra-chromosomal piece of circular double-stranded autonomous DNA
- Replicate by itself
- It often carries **nonessential genes** such as resistance to antibiotics, virulence factors (bacteriocin, enterotoxin, adhesion factor).

- Plasmids vary in size (1 to over 1,000 kilobase - pairs (kbp), copy number and host range (1-1000).. Each contains **5-100 genes**.. Bacterial cell contains1-10 plasmids.

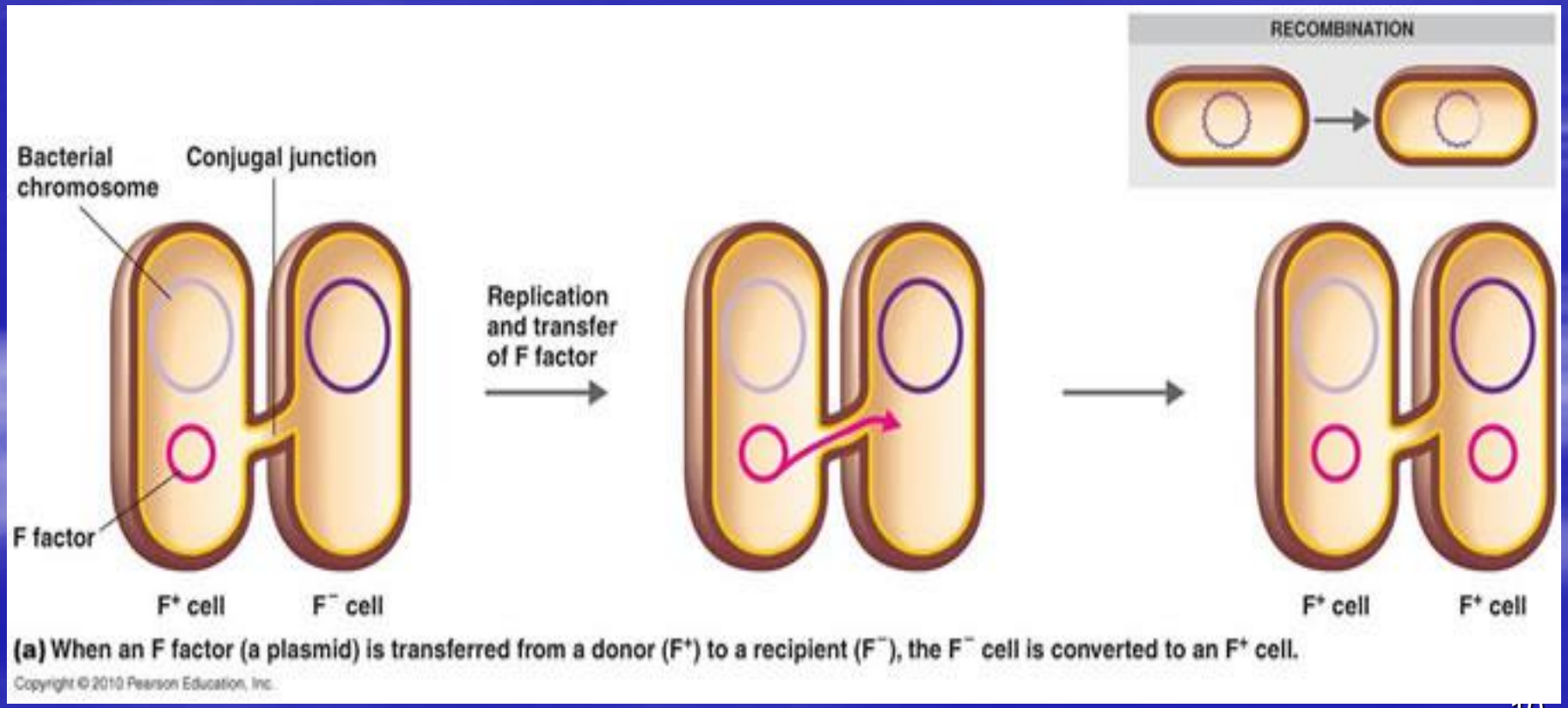
Simple Plasmid



Types of Plasmids

1. **Conjugative plasmid**: A plasmid capable of transmitting itself between bacteria.

   F-plasmid: *F-factor Plasmid* Fertility. F^+, F^-, Produces Pilus.



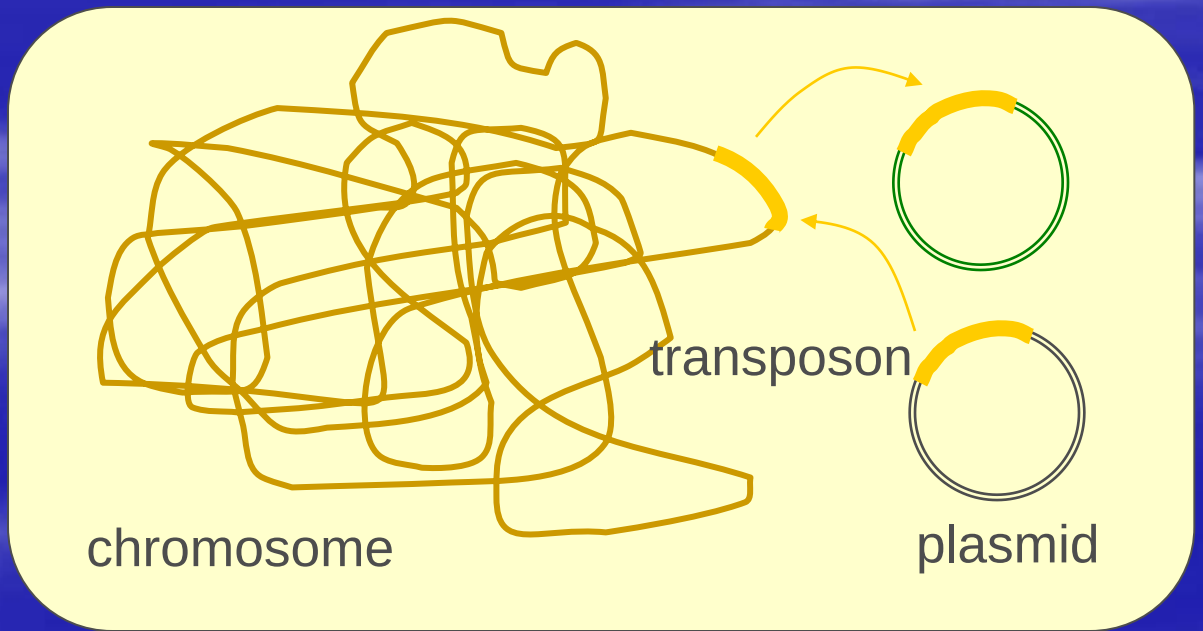
(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted to an F^+ cell.

2. Nonconjugative plasmid:

Carried & transmitted by a conjugative plasmid between bacterial cells.

3. Transposones / integrons: (jumping genes)

Nonessential small genetic elements that can exist in two ways in the bacterial cell: Both can be integrated into the bacterial chromosome or attached to plasmid in the cytoplasm



- **Broad Host Range Plasmid**:

- Capable of replication in many unrelated bacteria.. different genera.. Species.. *E.coli ,Salmonella-Pseudomonas*.. etc. contribute to spread antibiotic resistance within short time.

- **Narrow Host Range Plasmid**: Only capable of replication in a single bacteria species. ***E.coli , Staphylococcus* species** or very closely related bacteria species.
- **Donor** bacterial cell (**F^+)** that donates some of its DNA to another cell**.. F^+ Cell**
- **Recipient** bacterial cell (**F^-)**that receives DNA from the donor cell.
- Gene Transfer is common in most Bacteria.. Emerge of new pathogenic strains.. R-strains, Toxic/virulent strains etc.

Genetic Change in Bacteria

- **Genetic changes/Mutation** .. A major mechanism for the appearance of new pathogens/toxigenic strains. development of antimicrobial resistance.. can occur and become widespread over a short period of time

- **Mutation** affects the epidemiology & virulence of a pathogen.. contribute to changes in the nature and prevalence of certain important infections.

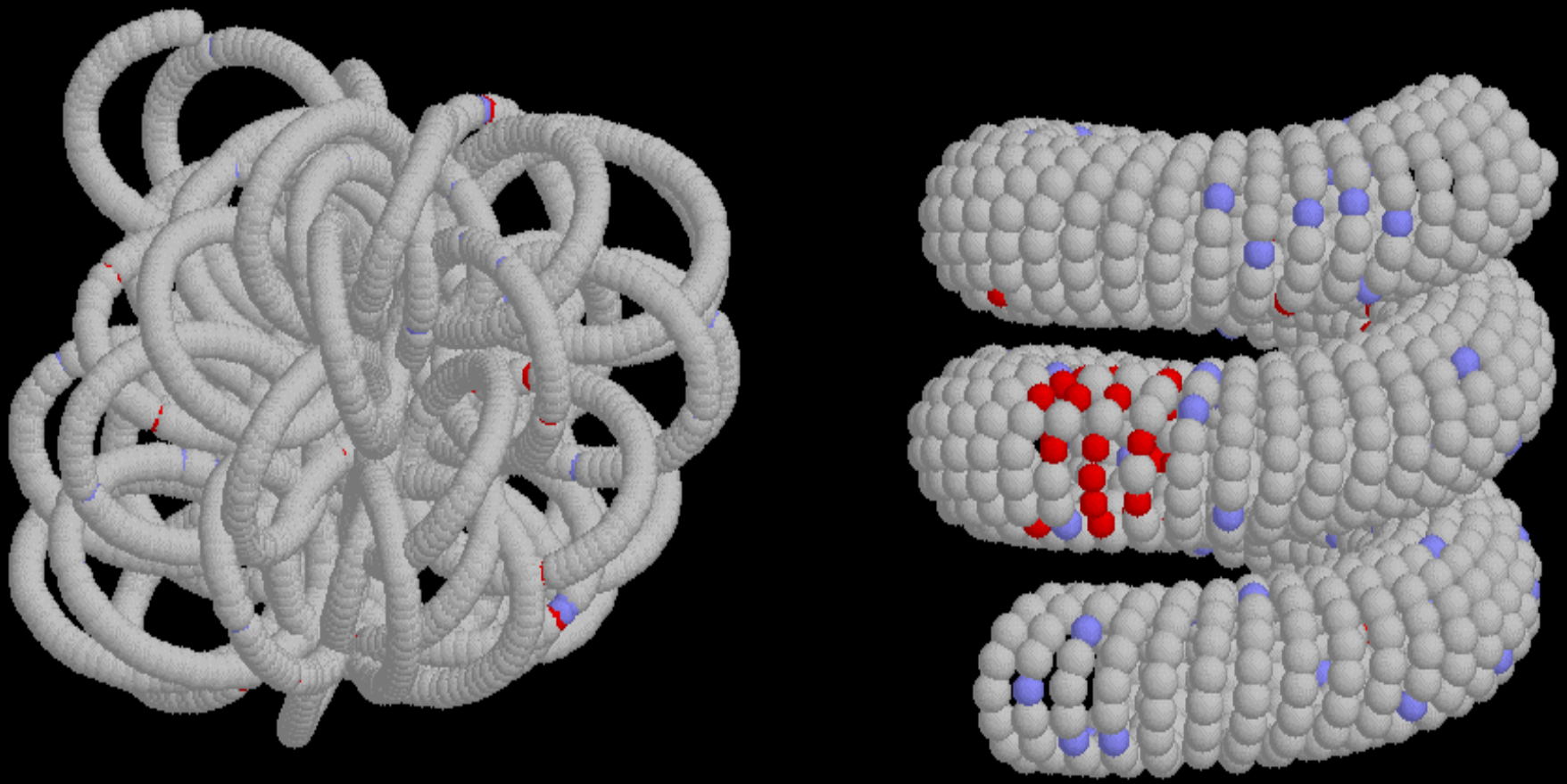
- **Genetic variation** in bacterial antigens (capsule, toxins) of some pathogens can seriously complicate the development of vaccines against those organisms.

- Genetic change accounts for the evolution of bacterial pathogens.. Complicate Treatment of Infections.

Bacterial Mutation

- There are two basic mechanisms that produce genetic change in bacterial cells: **Natural and Induced**.
- Mutation of existing DNA is expressed in nucleotide sequence changes (insertions, deletions, DNA rearrangements like inversions, duplications, transpositions) occur mostly spontaneously at a low frequency of **10^{-3} to 10^{-10}** per bacterial cell.. bacterial Strain
- **Induced mutation** followed mostly used chemical agents or radiation.. A slow genetic process can develop in vivo & vitro..**natural /induced conditions.**
- This genetic exchange process can produce dramatic changes in the **phenotypic properties** of an bacterial strain.. Development of Resistance.. Toxigenic Strains

Mutation in Bacterial Chromosome



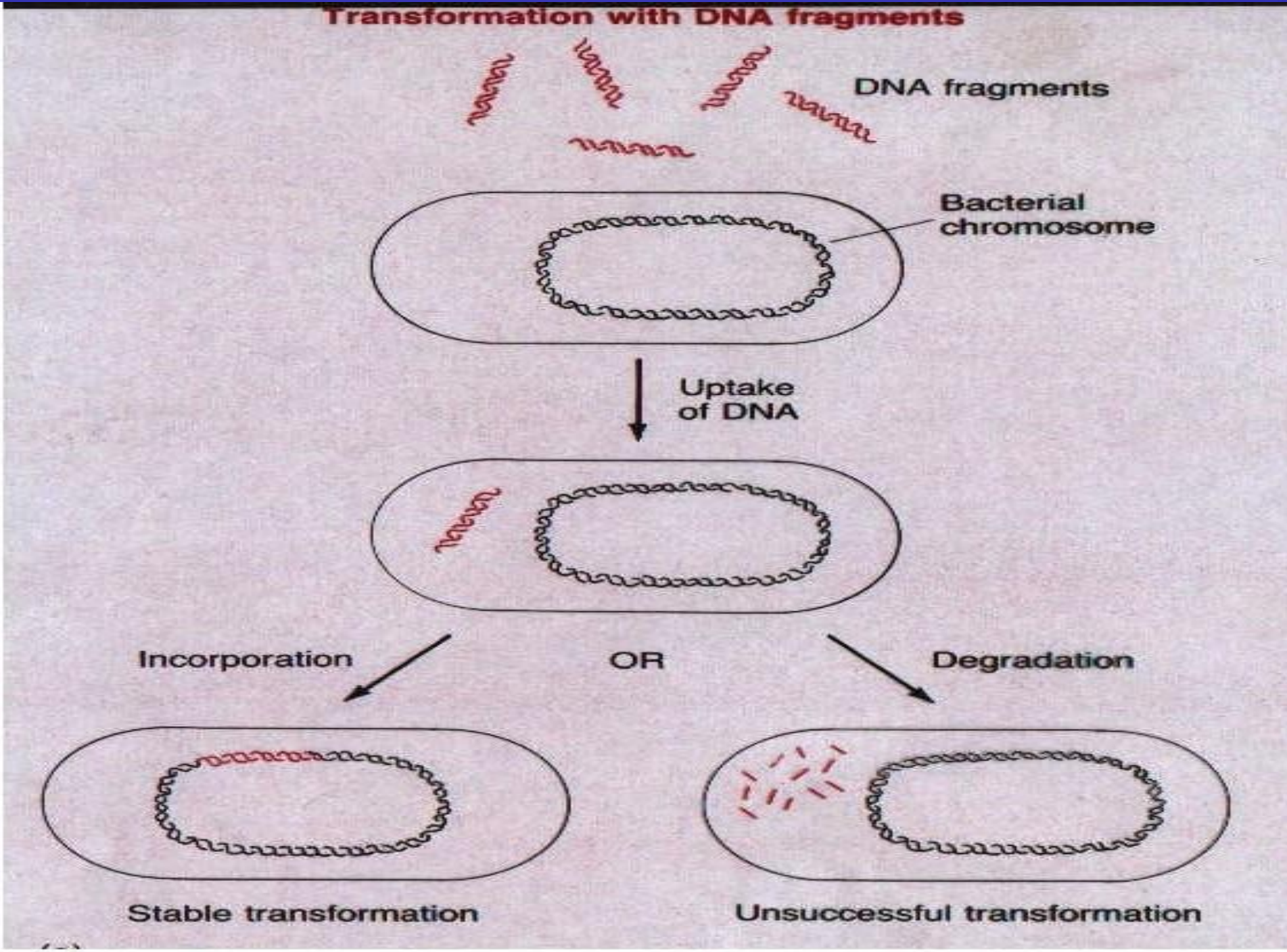
Mechanism of gene transfer between bacteria

1-Transformation: the process of genetic exchange .. free linear DNA released by dying bacteria .. taken up by other bacterial cells and incorporated into the chromosome/ plasmid by homologous recombination.

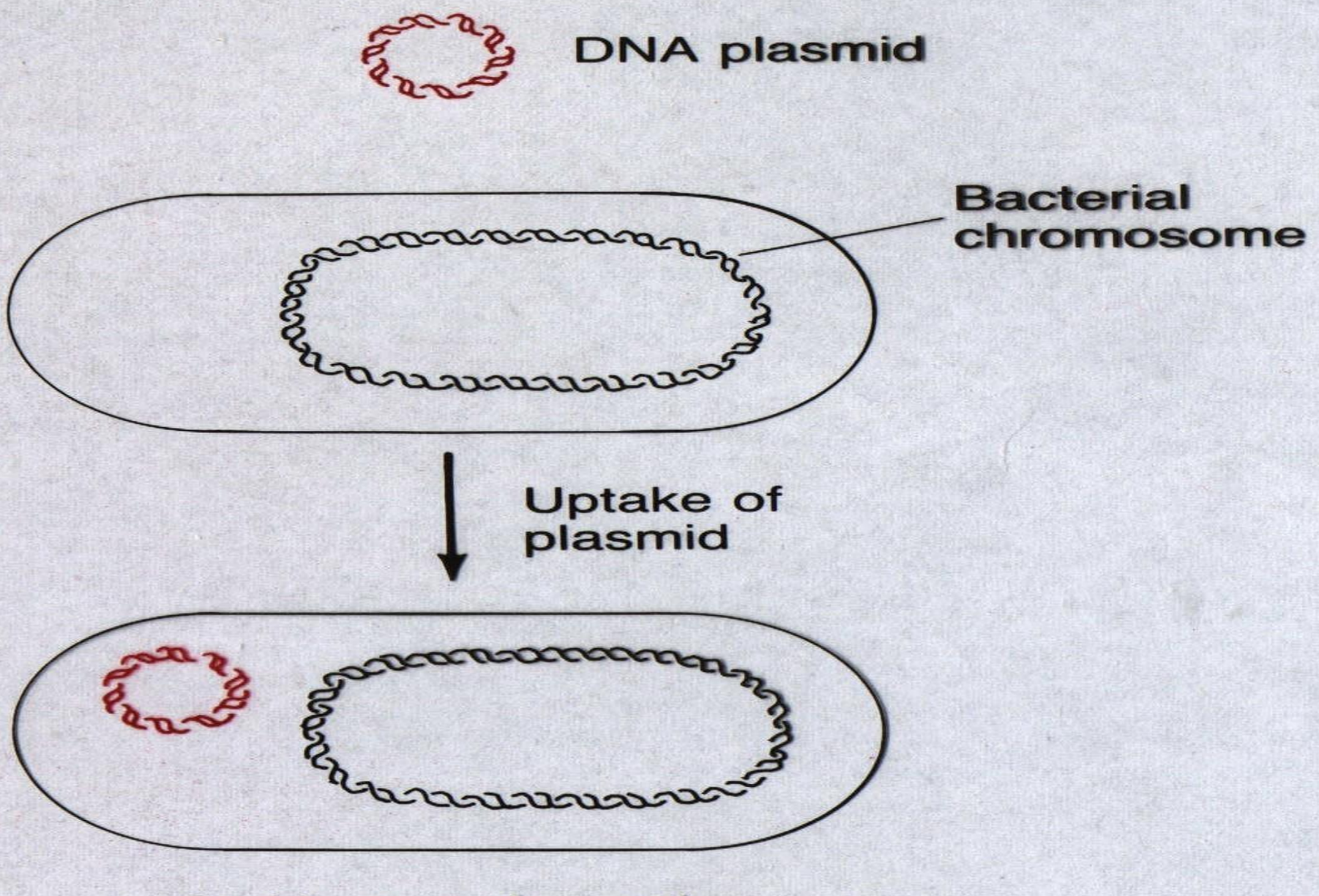
- Only certain pathogens (*S. pneumoniae*, N. *gonorrhoeae*) are capable of doing this process in vitro or vivo ..under natural condition.

2. Conjugation: It occurs mostly in Gram negative bacteria.. By presence Factor F (fertility factor)..

- F plasmid.. Contains F-factor is capable of replicating itself. It is also capable of transferring itself from host to host ..conjugative plasmid.

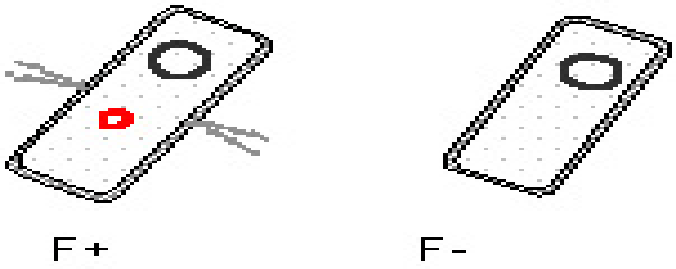

Transformation with DNA fragments
DNA fragments
Bacterial chromosome
Uptake of DNA
Incorporation
OR
Degradation
Stable transformation
Unsuccessful transformation

Transformation with a plasmid

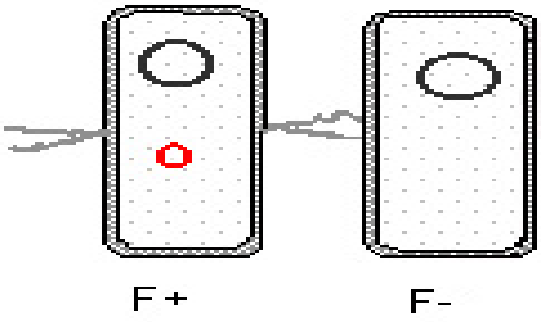


Conjugation

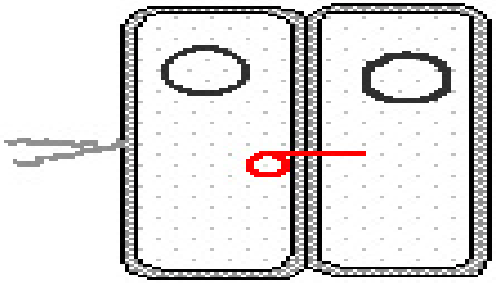
A cell containing the F factor is called an F^+ cell. Bacteria that carry F factor can produce sex pili. As was mentioned in Bacterial Structure, only a few sex pili are found per cell.



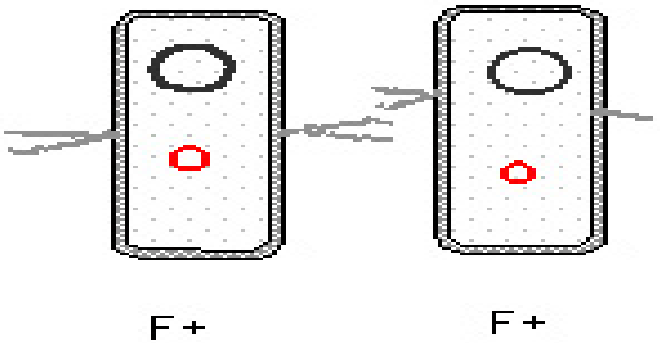
These structures reach out and anchor the F^+ cell (aka donor cell) to a recipient cell that does not contain the F factor (aka F^- cell).



Once contact is established between the F^+ and F^- cells, the F plasmid transfers a linear strand of its DNA to the recipient (F^-) cell and retains the circular strand.



For plasmid transfer, the contact between donor and recipient cells has to be maintained for only a couple of minutes. When the single-stranded, linear copy of F enters the recipient cell, it circularizes and replicates the missing strand. The single-stranded, circular copy of F retained by the donor cell also replicates the missing strand. The end result of conjugation is that both the donor and recipient cells now contain functional copies of the F plasmid. (So, they are both F^+ cells.)



Transduction

- **Bacteriophage:** A virus that infects bacteria.. Phage genomes consist of either RNA or DNA
- Each phage requires the presence of a particular receptor.. bacteria lacking specific receptor are immune to infection by that particular phage.
- **Transduction** is the process of moving bacterial DNA from one cell to another using a bacteriophage.

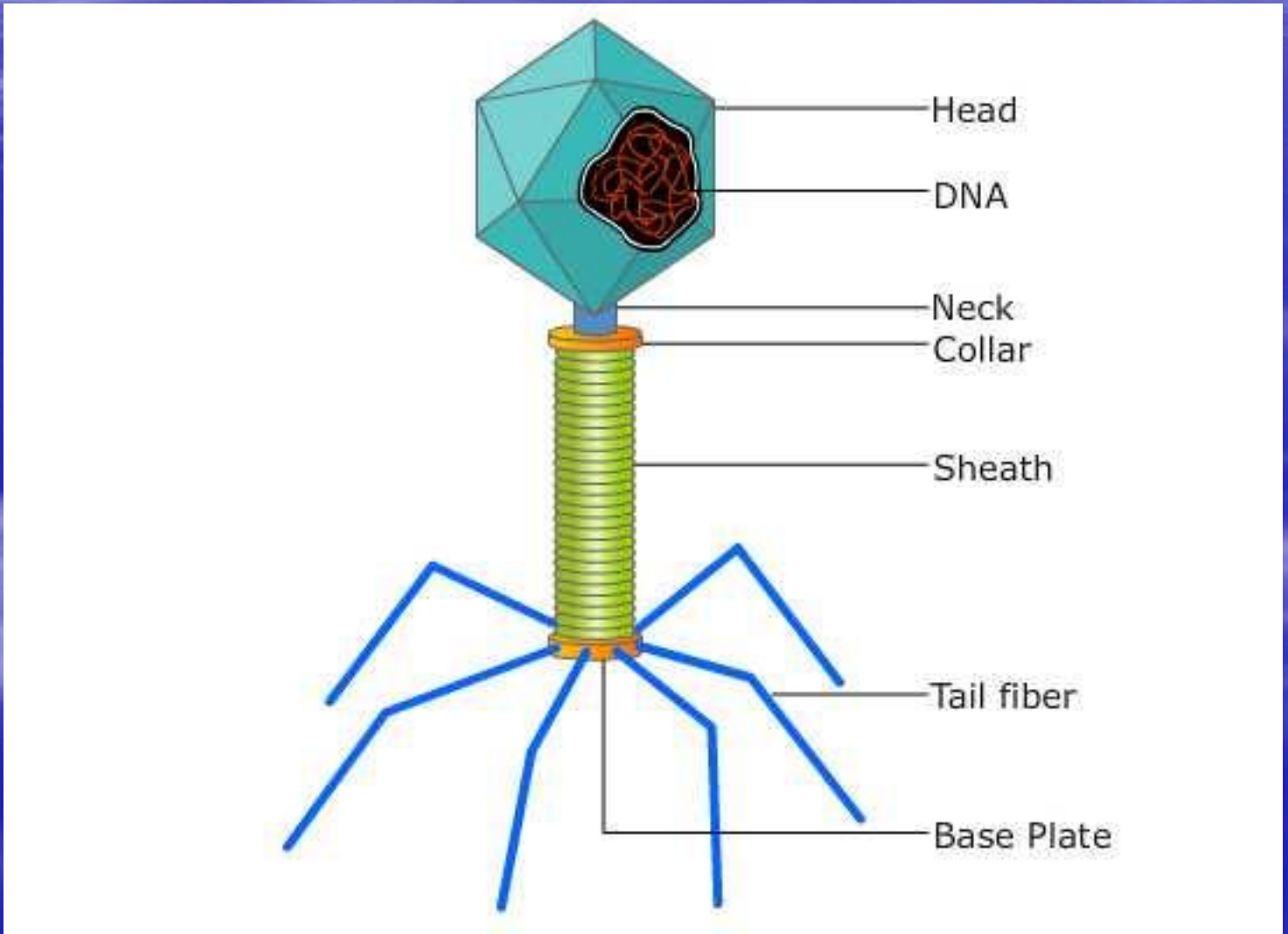
Bacteriophage or just "phage" are bacterial viruses. They consist of a small piece of DNA inside a protein coat. The protein coat binds to the bacterial surface, then injects the phage DNA. The phage DNA then takes over the cell's machinery and replicates many virus particles.

Two forms of transduction:

1. Generalized: any piece of the bacterial genome – can be transferred

2. specialized: only specific pieces of the chromosome can be transferred.

Bacteriophage Structure



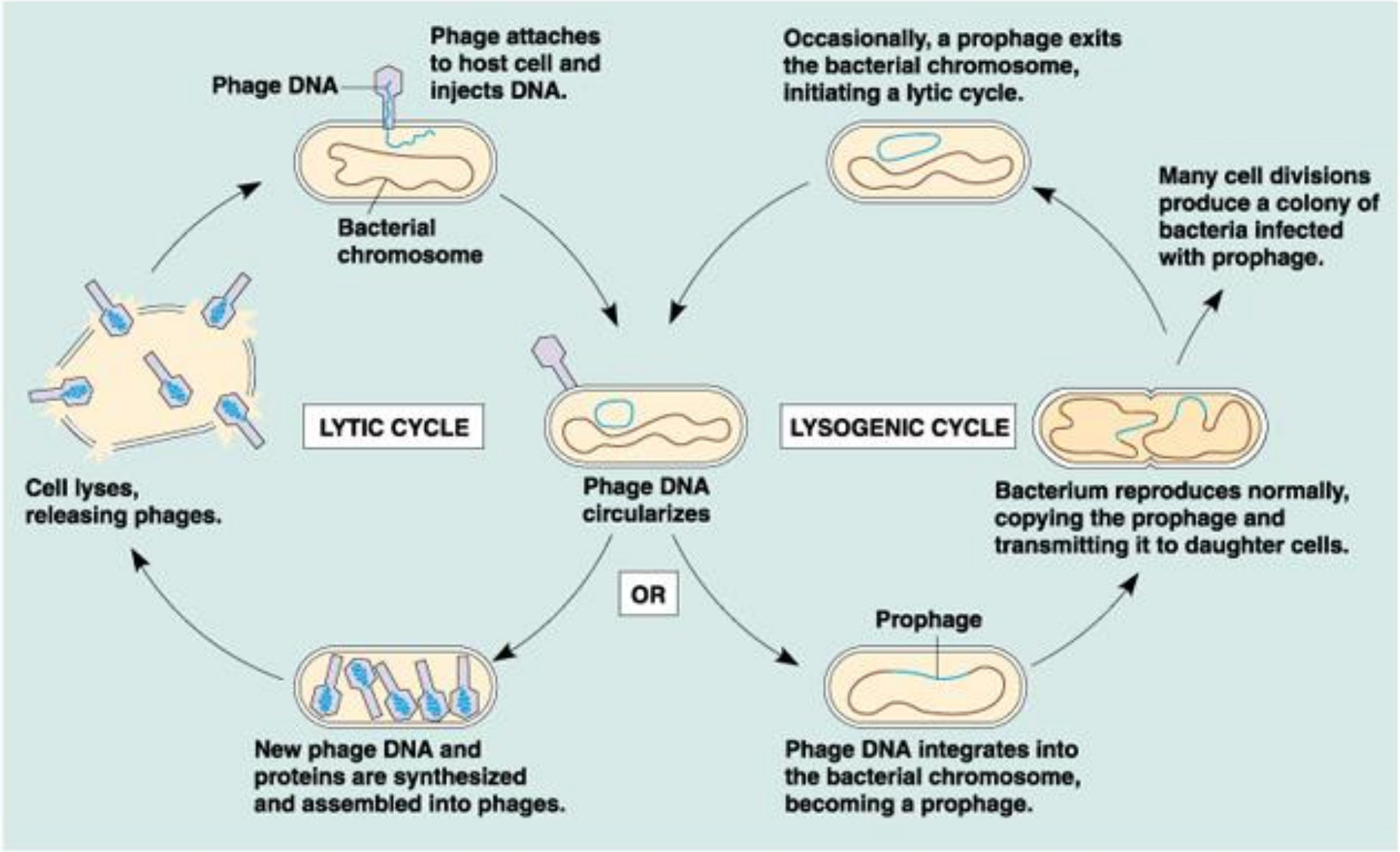
Transduction

- Two types of phage infections :
  Lytic and **Lysogenic infection.**

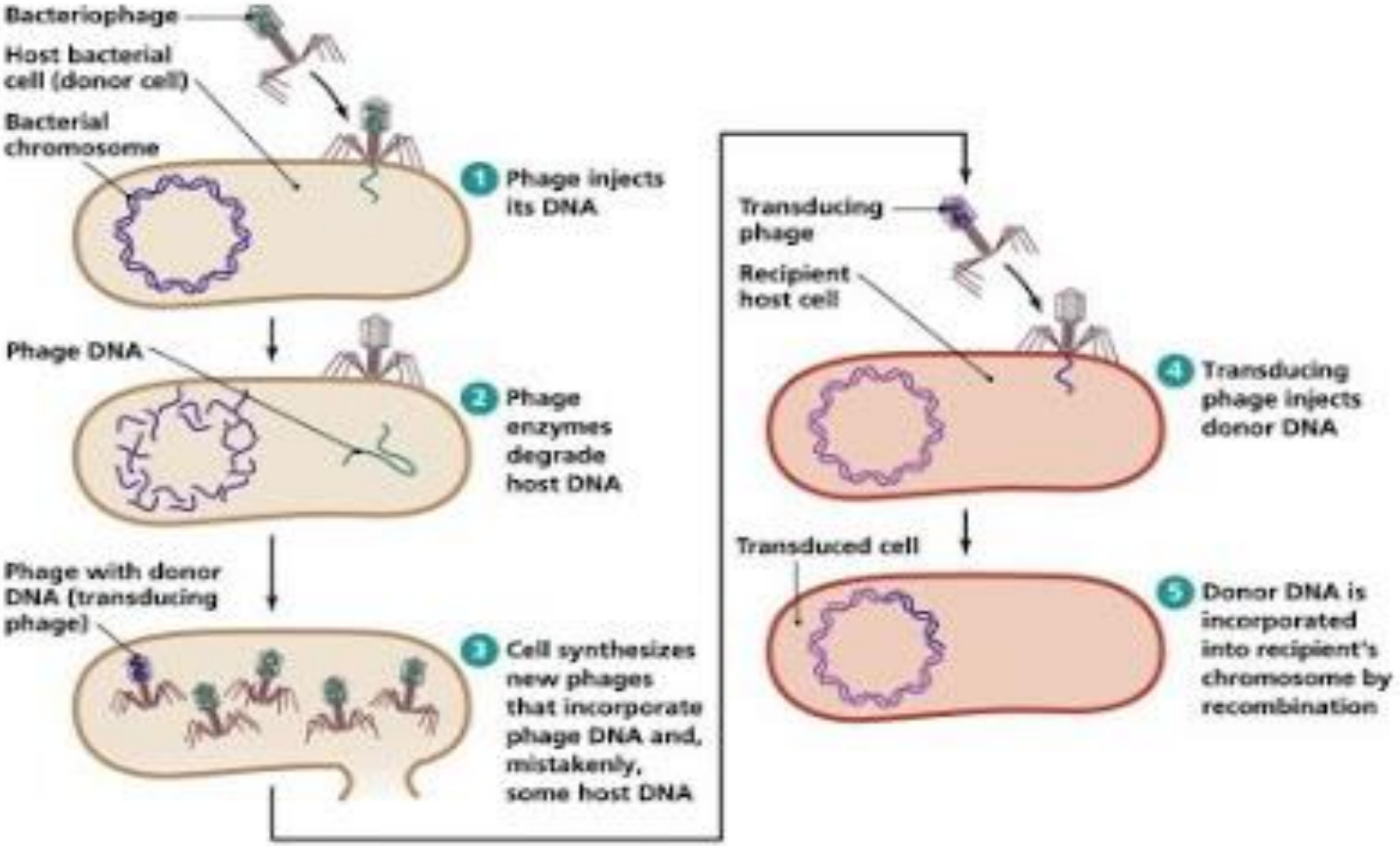
- **Lytic / Virulent phage**.. the phage produces progeny and lysis the host cell.. *Generalized Transduction..* Pick any part of bacterial chromosome

- **Lysogenic / Temperate phage**.. A phage that can enter into lysogeny with its host. insert *certain genes* into bacterial chromosome.. **Prohage** ..

- **lysogenic state / lysogeny**..
  Lysogenic conversion from nontoxigenic strain to toxogenic..
  C.diphtheria, *Beta-Hemolytic Streptococci* (Group A)..
  Staphyloccocus aureus.. production of toxins by specific bacteriophages.. increased virulence

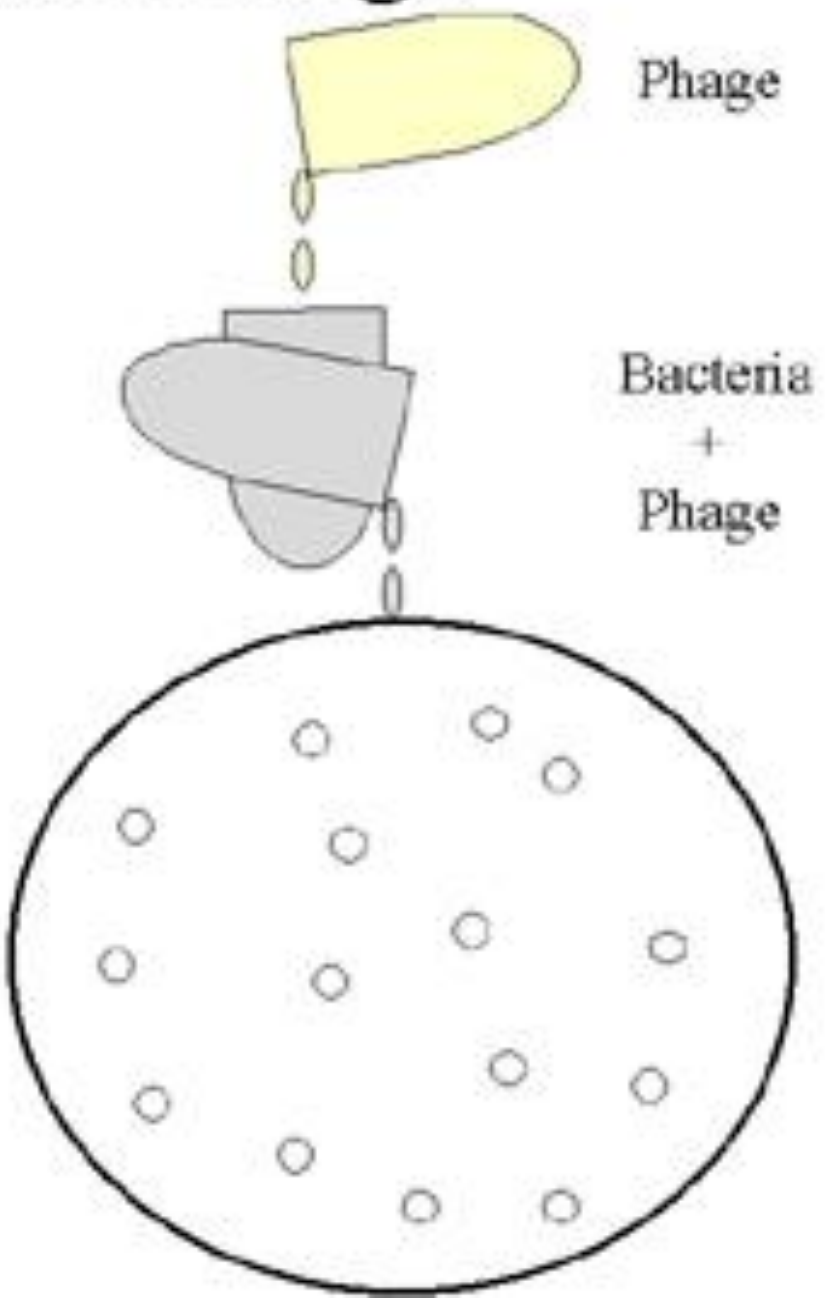


Generalized transduction



Assay for Lytic Phage

- Plaque assay
  - Method
  - Plaque forming unit (pfu)
  - Measures infectious particles



Detection of Lytic Cells (Plaques) in vitro-Petri dish