Bacterial Genetics

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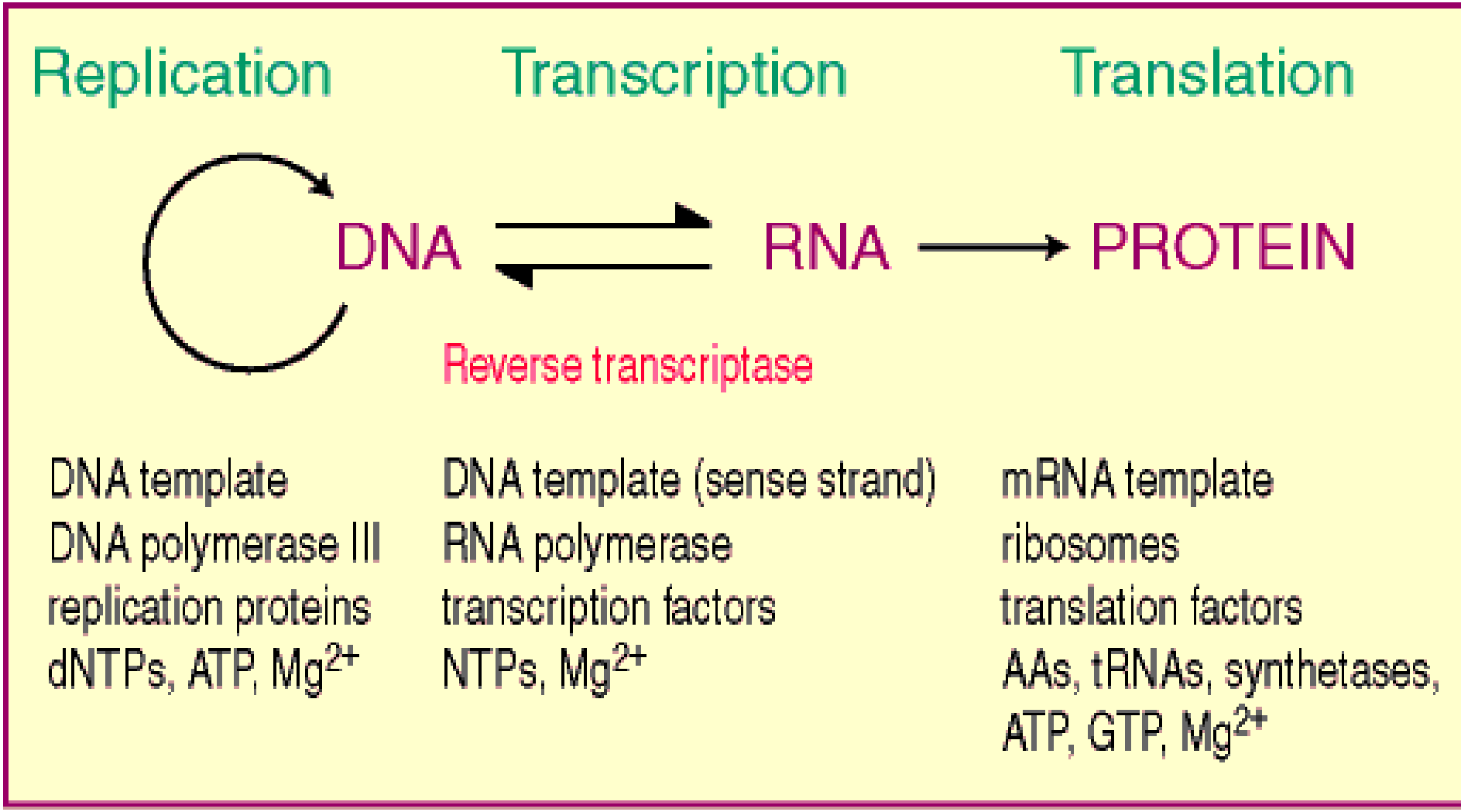
Bacterial Genes-1

- All patterns of growth, metabolism, essential cellular structures, biological characteristics of bacteria are controlled by DNA encoded & expressed genes. **Chromosome structure/sequence of nucleotides**
- **Bacterial Genome**: **Chromosome**.. single circular double-stranded DNA.. 1300 um long contains **2-5 x 10^6** nucleotide bases, enough DNA to encode **1- 4** thousand different genes.. According bacteria types.
- **Genetic information** is encoded in **DNA**, transcribed into **mRNA**.. translated on **Ribosomes** through **tRNA** into various protein structures..enzymes..Functions

BACTERIAL GENETICS

I. CENTRAL DOGMA



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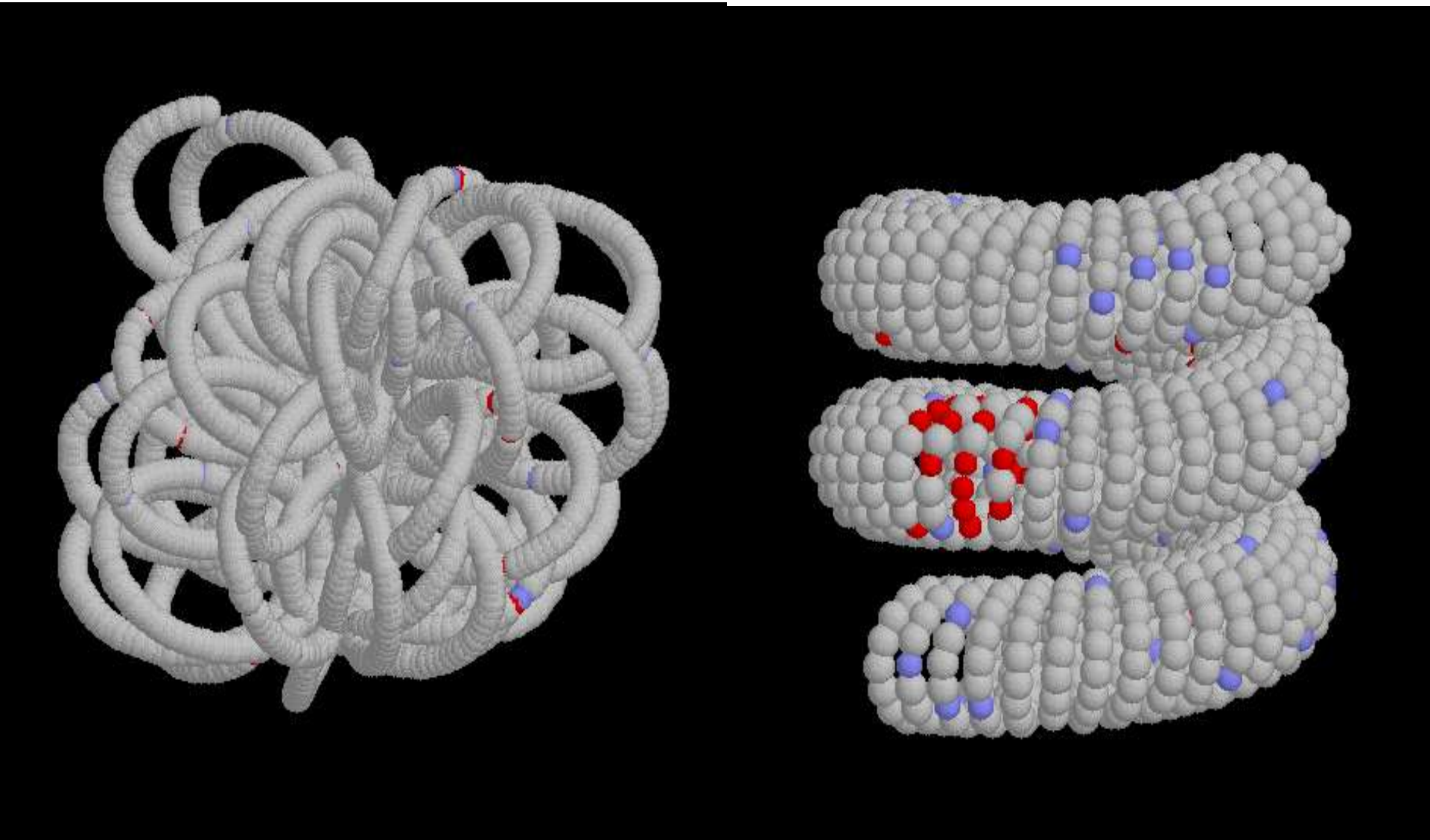
- **Gene** : A segment of DNA specifies production of a particular polypeptide chain function ..Enzyme, Protein
- Bacteria with similar organization and location of essential genes are grouped within the same Family- **Genus-Species-strains.**
- The sequence analysis of bacterial genomes has confirmed that **genetic change / mutation** in bacteria occurs both by alteration of the DNA base sequence, **gain or loss** of small/larger DNA segments containing genes.
- Bacterial genome includes Chromosome & DNA Plasmid, DNA / RNA Bacteriophage

Mutation in Bacterial Chromosome



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 observed characteristics of the of the individual bacteria species/strain.. Expressed by physical & biochemical properties.. Growth patterns, Fermentation products, Antibiotic resistance, Toxins production. .etc.
- Genetic manipulation by bioengineering allow to introduce new genes or cut genes to produce new clones of bacteria/strain with new properties.

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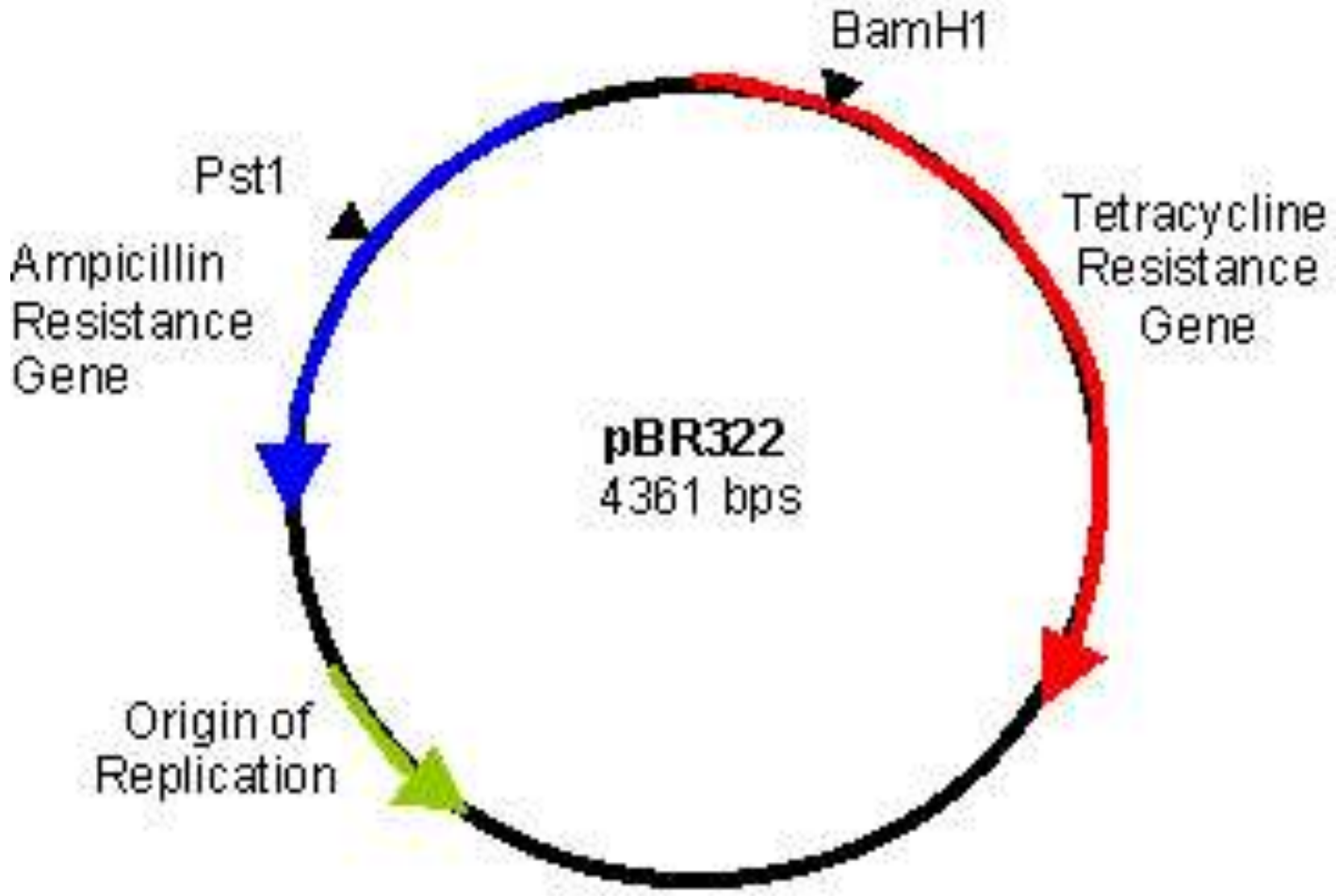
- **Lab diagnosis of pathogens:**

- 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.
- **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, copy number and host range.. contain **5-100 genes**.. Bacterial cell contains1-10 plasmids.. saml & large.

Fig.1- A simple Plasmid



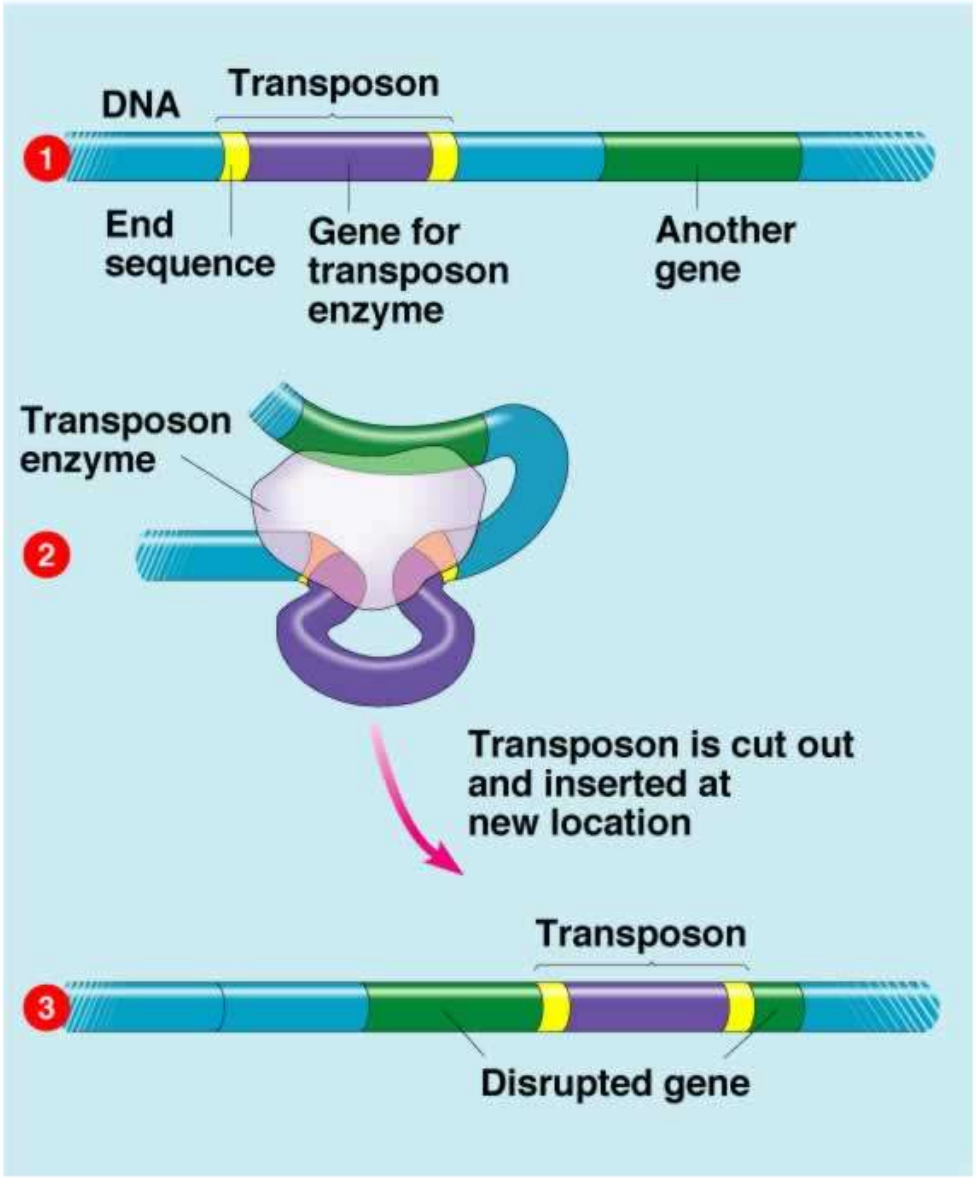
A simplified map of pBR322, one of the most common *E. coli* cloning vectors.

Types of Plasmids

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

  F-factor Plasmid ..Fertility.. F^+, F^-, Produces Pilus,
- **Nonconjugative plasmid**: Carried &Transmitted by a conjugative plasmid between bacterial cells.
- **Transposones/ Integrons**: Nonessential small genetic elements which can exist in two ways in the bacterial cell .
- Both can be integrated into the bacterial chromosome or attached to Plasmid & carried by plasmids during growth of bacteria.

Simple transposon



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- **Transposons:** are gene sequences that can move from one location to another within the bacterial cell's DNA (chromosome and plasmid).. genes.. Transfer resistance markers(genes).
- The simplest form of a transposon is an **insertion sequence (IS)** containing only few genes required for transposition.
- **Integrons:** Like transposons.. smaller in Size..carry R-gene cassettes.. transfer resistance markers among related and unrelated bacterial populations.. Normally attached to Plasmid/Chromosome .
- **Insertion sequences** (IS): are small segment of DNA units that can insert themselves into Plasmid/ Integrons.. and later attached to Chromosome.. Both are not capable of autonomous replication.

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- **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* or very closely related bacteria species.
- **Donor** bacterial cell that donates some of its DNA to another cell**..**
- **Recipient** bacterial cell that receives DNA from the donor cell.
- Gene Transfer is common in most Bacteria.. Result in emerge of new property.. pathogenic strains.. Antibiotic R-strains, Toxic/Virulent strains etc.

Genetic Change in Bacteria

- **Genetic changes/Mutation** .. A major mechanism for the appearance of new pathogens. 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** may result in new bacterial strains additive bacterial antigens/virulence factors, increased pathogenic potential due to capsule ,toxins, R-factors
- Genetic change accounts for the evolution of bacterial new pathogens.. Complicate Treatment of Infections.

Bacterial Mutation

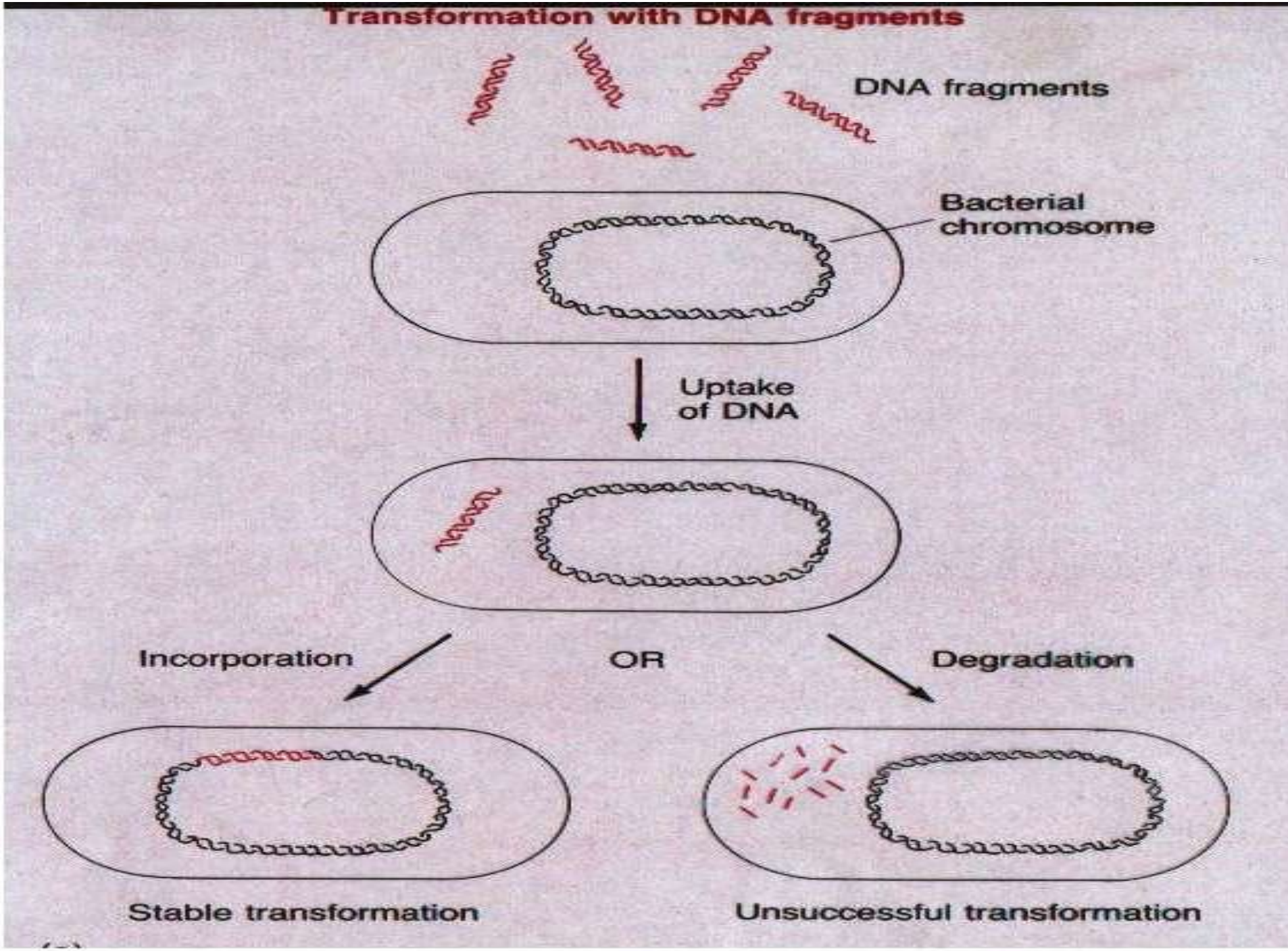
- There are two basic mechanisms that produce genetic change in bacterial cells: **Natural and Induced**.
- Mutation of existing DNA is expressed in nucleotide sequence changes (insertions, deletions, DNA rearrangements like inversions, duplications, transpositions) occur mostly spontaneously at a low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

mechanism of gene transfer between bacteria

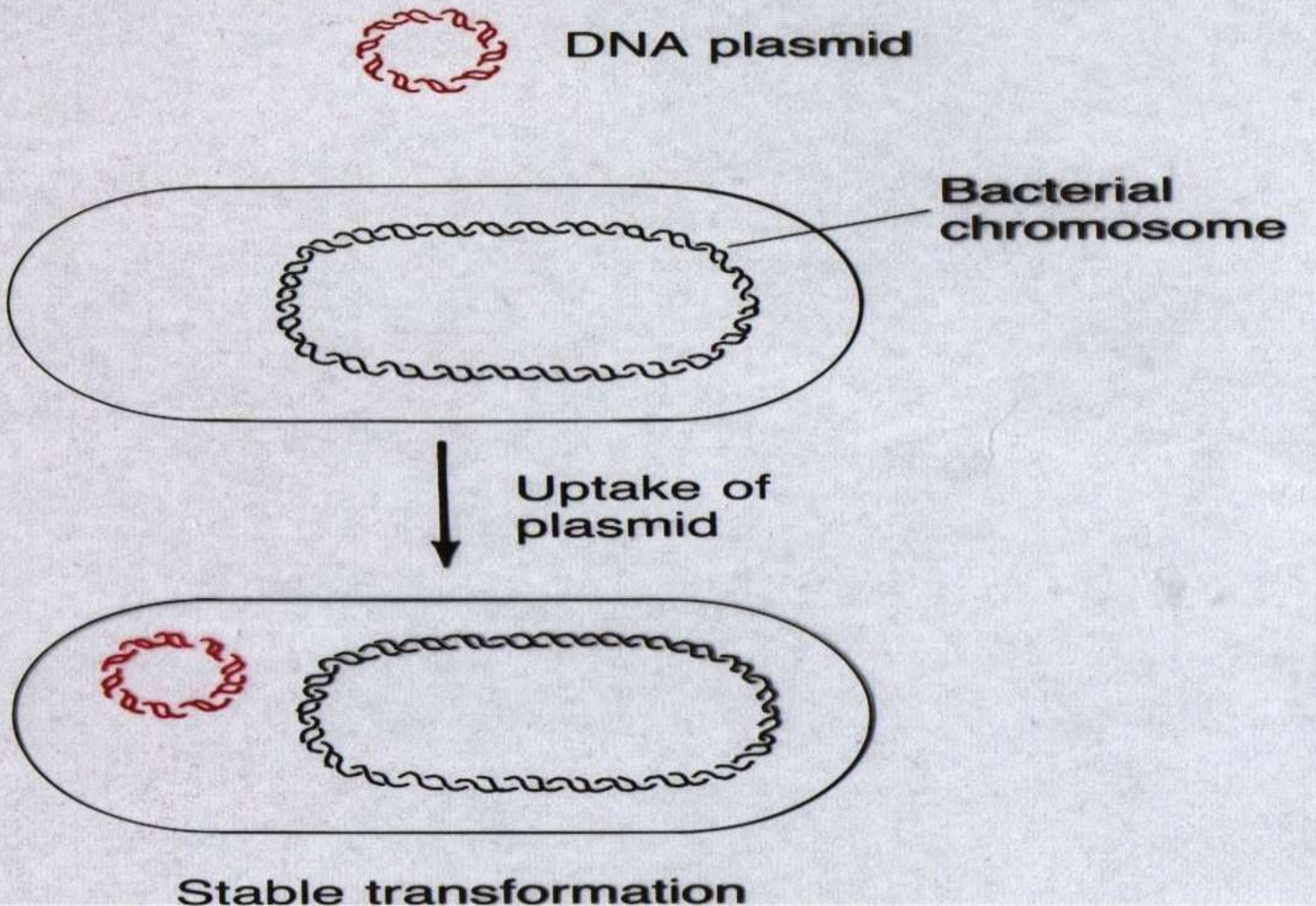
1-Transformation: This process occurs when free linear DNA released by dying bacterial cells .. taken up by other bacterial cells and incorporated into the chromosome/ plasmid by homologous recombination.

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

2. Conjugation: This process occurs mostly in Gram negative bacteria.. By presence Factor F (carry fertility factor genes).. F-plasmid/ conjugative plasmid.. is capable of replicating itself & transferring itself from bacterial donor cell (F^+) to recipient bacterial cell (F^-) by conjugation.


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

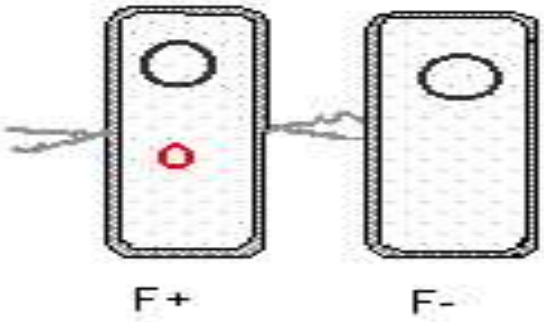
Transformation with a plasmid



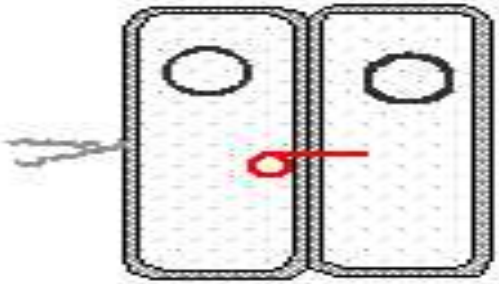
Conjugation

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.

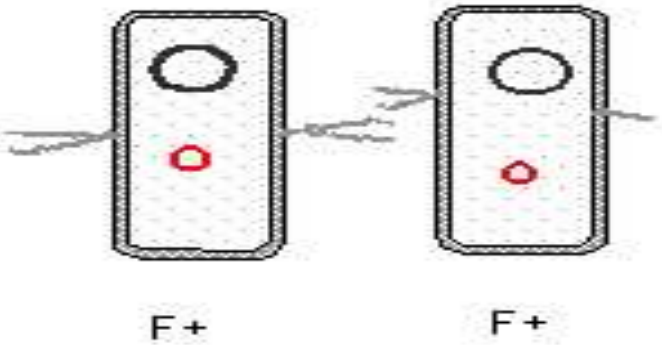
These structures reach out and anchor the F^+ cell (aka donor cell) to a recipient cell that does not contain the F factor (aka F^- cell).



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.



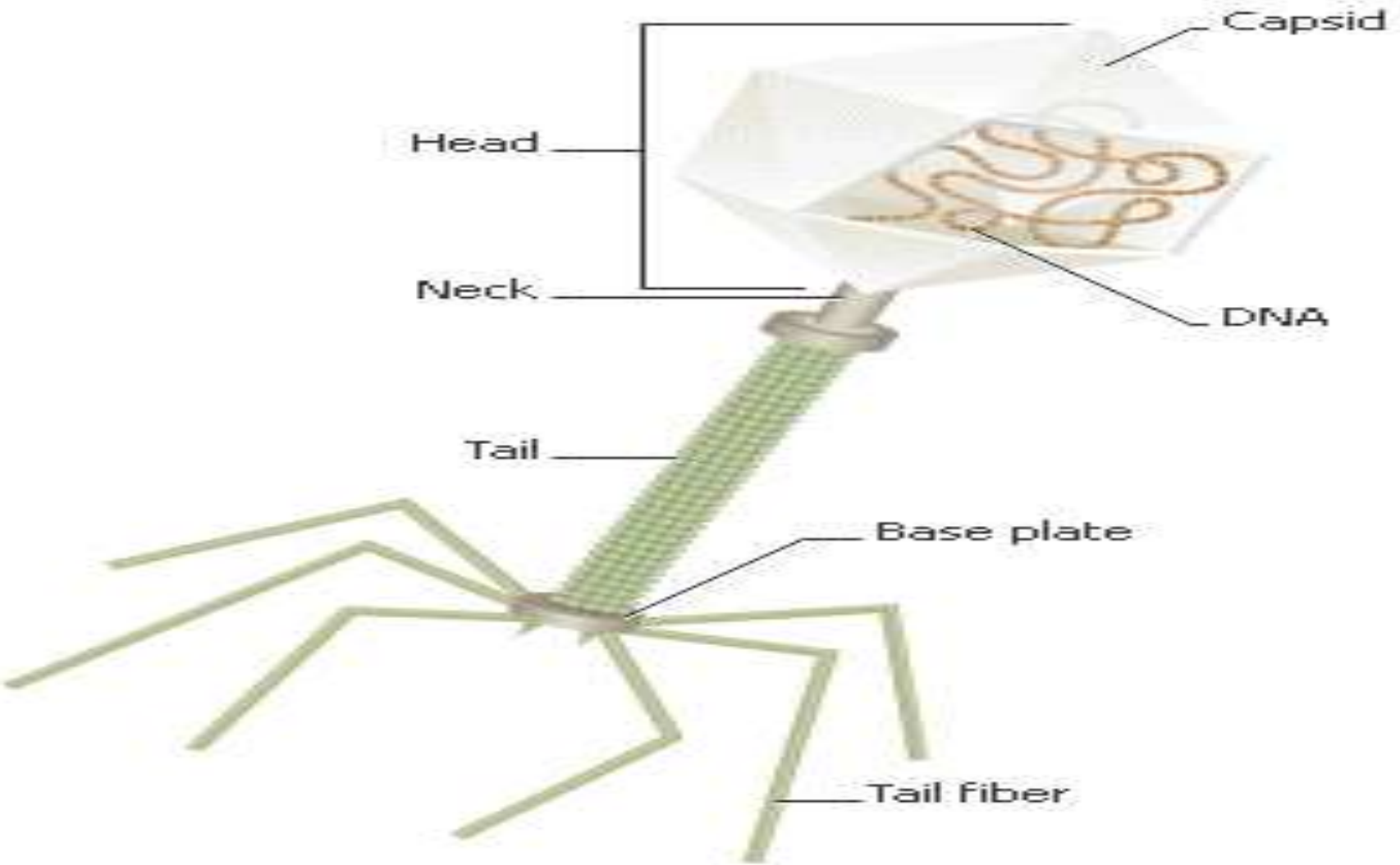
For plasmid transfer, the contact between donor and recipient cells has to be maintained for only a couple of minutes. When the single-stranded, linear copy of F enters the recipient cell, it circularizes and replicates the missing strand. The single-stranded, circular copy of F retained by the donor 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.)



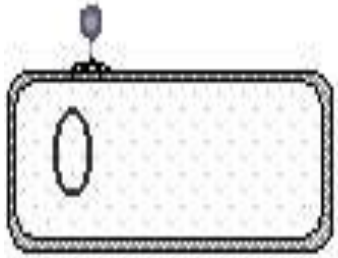
Transduction-1

- **Bacteriophage**: A virus that infects bacteria.. Phage genomes consist of either RNA or DNA.
- The phage nucleic acid is packaged with phage-encoded proteins that determine the phage structure, and interact with specific receptors on the bacterial cell surface to initiate infection.
- Each phage requires the presence of a particular receptor.. bacteria lacking specific receptor are immune to infection by that particular phage.

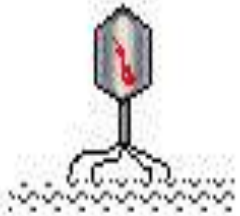
Bateriophage Structure



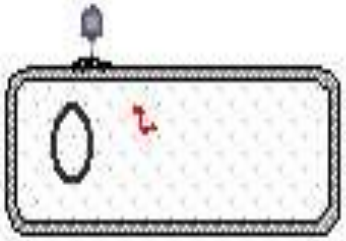
Lytic Infection



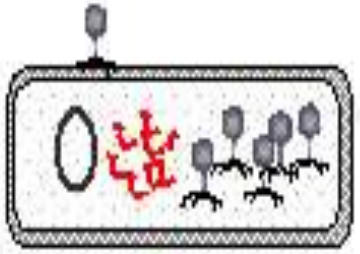
The bacteriophage attaches to a bacterium via a specific receptor.



The capsid stays on the outside of the host cell. The viral DNA is injected into the host cell.



Once in the host cell, the bacteriophage subverts the host's machinery.

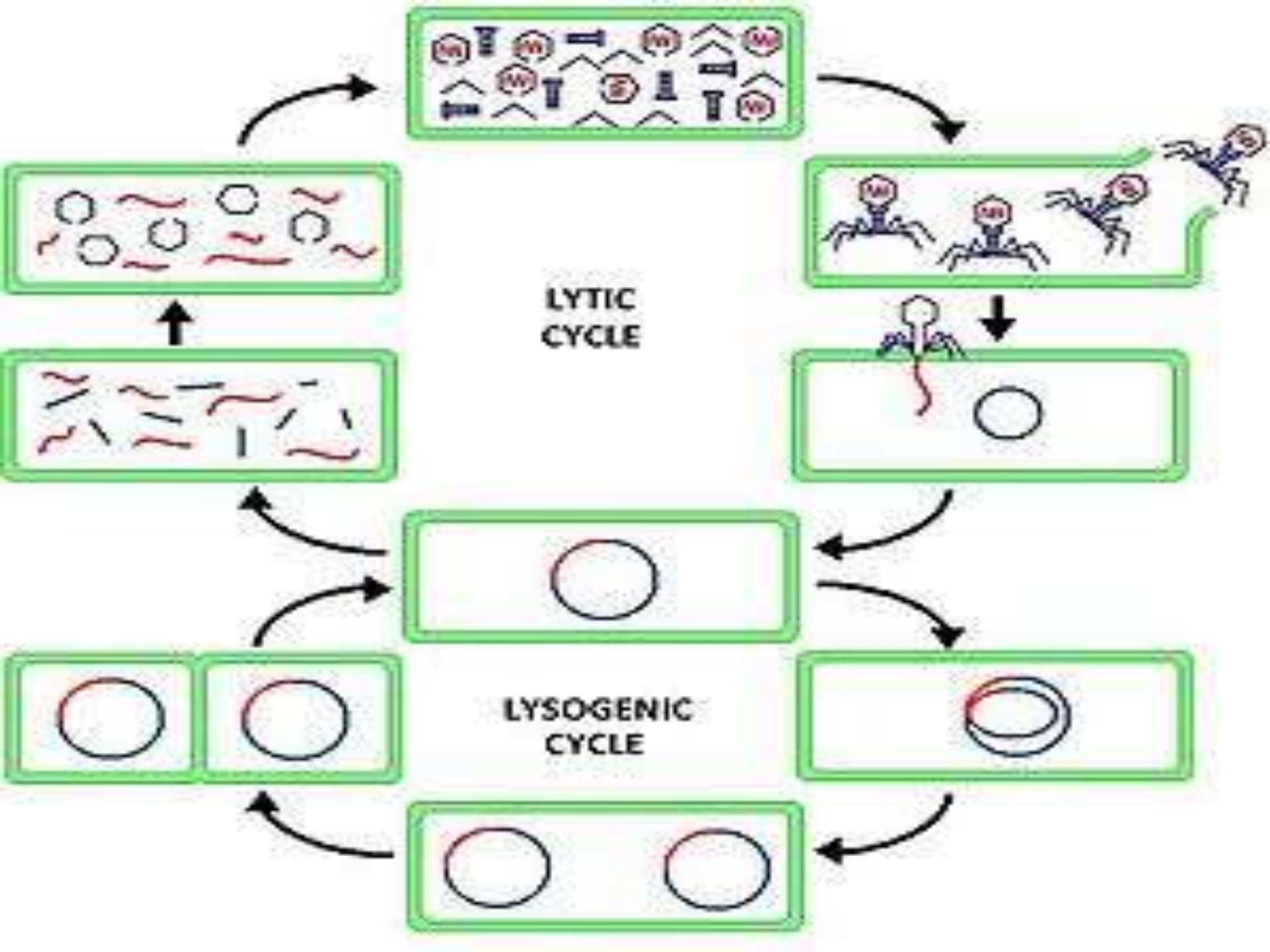


The virus goes through its replication cycle. Viral genomes are produced and packaged into capsids.

The host bacterium is lysed and the viral progeny are released.

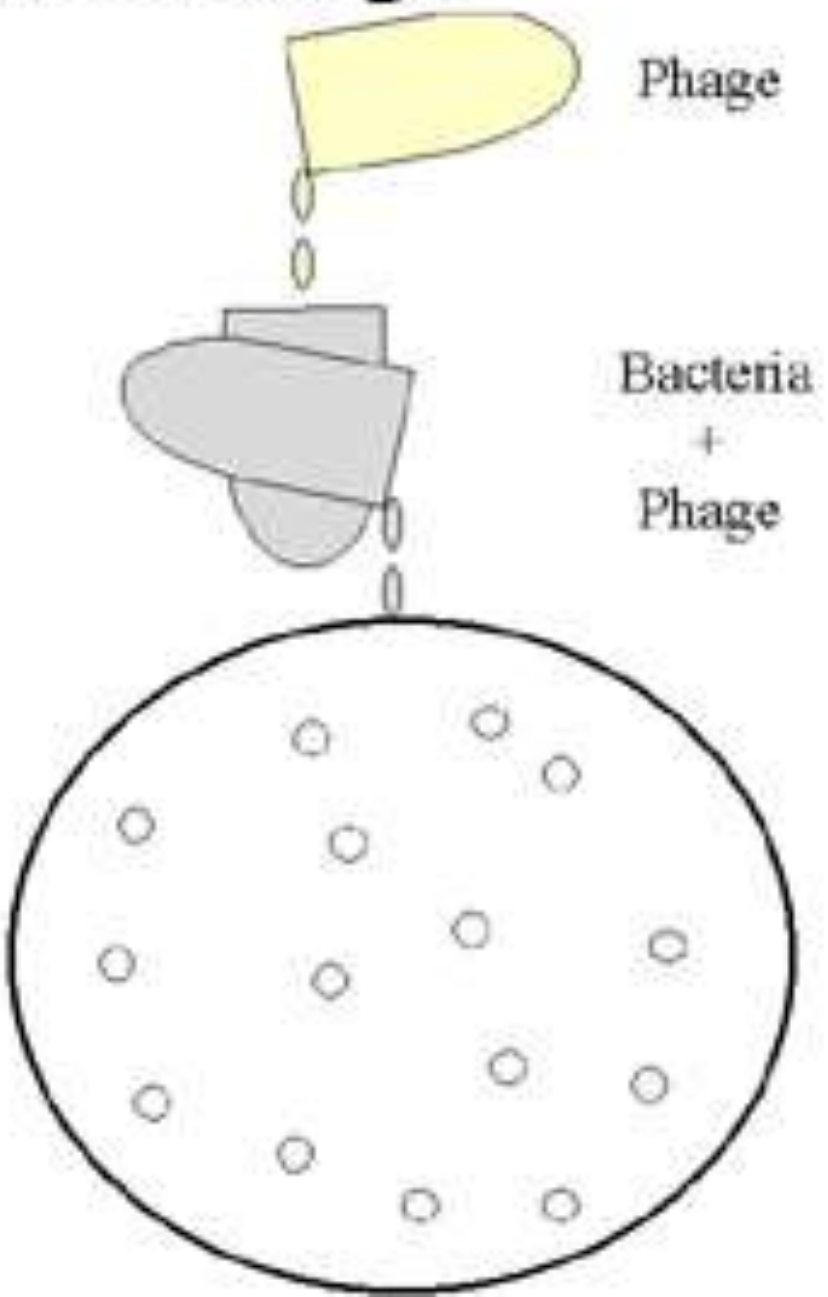
Transduction-2

- Two types of phage bacterial infections : **Lytic** & **Lysogenic infection.**
- **Lytic / Virulent phage**.. the phage produces progeny and lysis the host cell.. Generalized Transduction.. Phage attacks any part of bacterial chromosome
- **Lysogenic / Temperate phage**.. When a phage can insert a number of genes into bacterial chromosome.. **Prohage** ..the bacterial cell clone becomes in lysogenic stage/ 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


LYTIC
CYCLE
LYSOGENIC
CYCLE

Assay for Lytic Phage

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



Detection of Lytic Cells (Plaques)