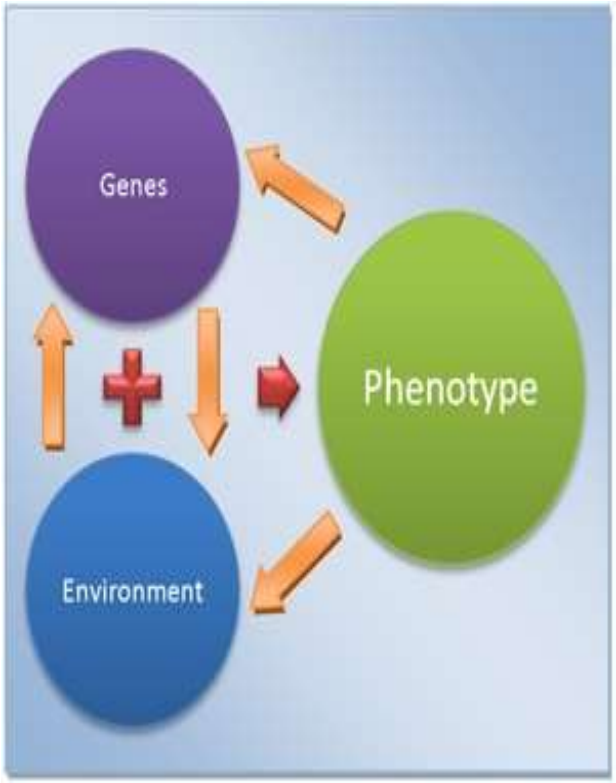
# Bacterial genetics

**Genetics** is the science that defines & analyzes heredity & variation. It aims to understanding the structure & functions of microbial genome, its gene products & their role in infection & disease. The unit of heredity is gene.

The **gene** is a segment or portion of DNA that carries in its nucleotide sequence information for a specific biochemical or physiological property, through coding for a single polypeptide sequence.

**Phenotype** refers to the observable properties (or characters) of an organism which are produced by interaction of genotype with the environment, i.e. the effect of both genes & environment. These include the structural & physiological properties of a cell or an organism (e.g. the eye color in human, resistance to an antibiotic in bacterium).

**Genotype** refers to the genetic constitution of an organism.



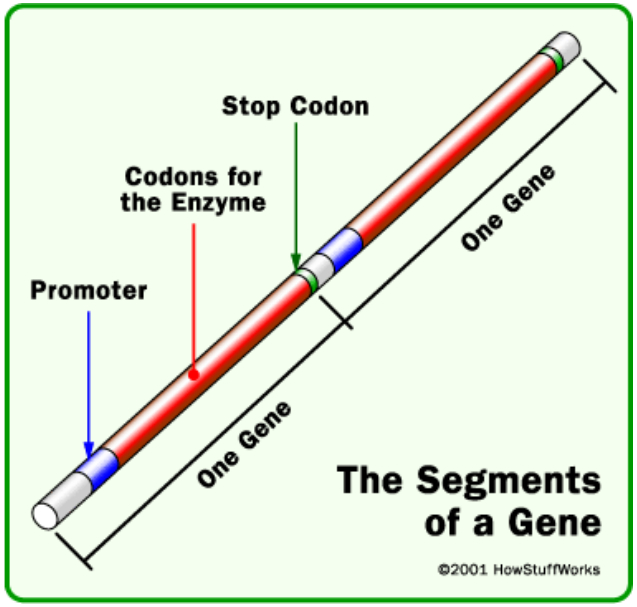
# Bacterial genetics

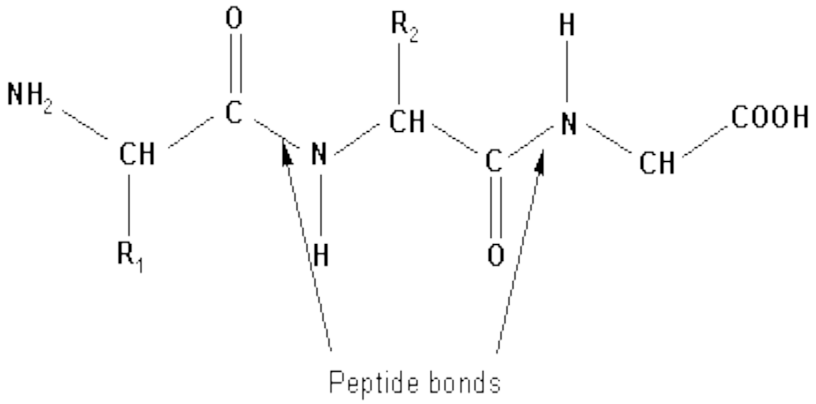
**Genome** is the sum of the genes of an organism, or the totality of genetic information in an organism.

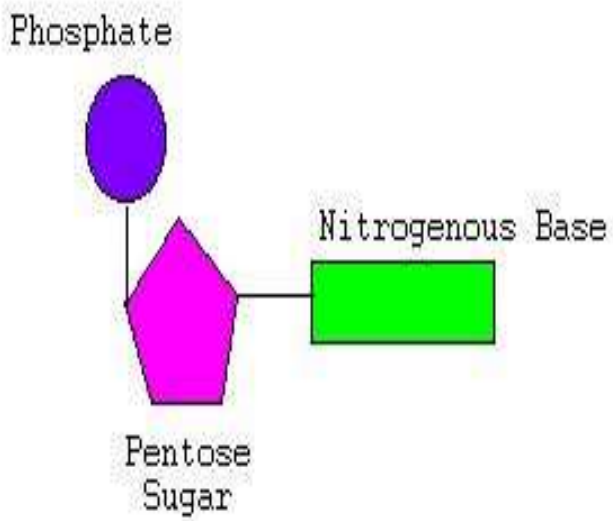
**Gene expression** refers to a gene product that can be observed under appropriate condition at the level of phenotype.

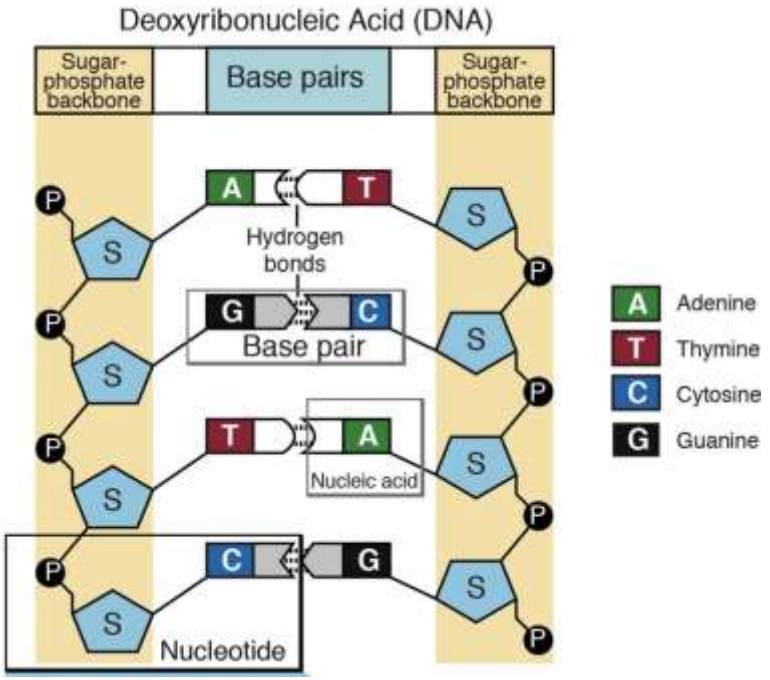
Much of the genetic information in bacteria is contained on a single chromosome located in the cytoplasm. Bacterial genomes differ in size, a feature which determines the bacterial characteristic traits or phenotype.

Properties of bacterial cell such as antimicrobial resistance & virulence are determined by bacterial genome. The genomic structure consists of 3 types of genetic information: **chromosome**, **plasmids** and **bacteriophages**, the later 2 structures provide additional genetic information & are transient in some instances.




NH2
CH
R1
C
O
N
H
R2
CH
C
O
H
N
CH
COOH
Peptide bonds

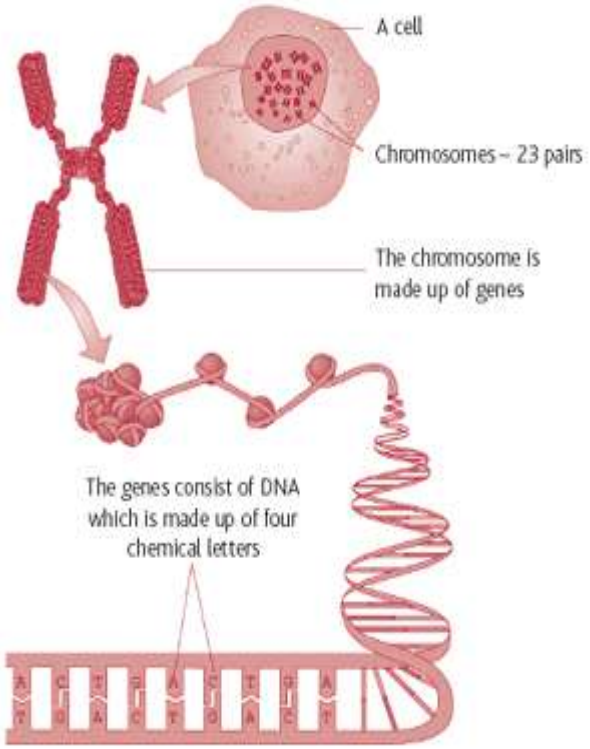

Phosphate
Nitrogenous Base
Pentose Sugar


Deoxyribonucleic Acid (DNA)
Sugar-phosphate backbone
Base pairs
Sugar-phosphate backbone
Hydrogen bonds
Base pair
Nucleic acid
Nucleotide
A Adenine
T Thymine
C Cytosine
G Guanine

# Bacterial genetics

Most bacteria are defined as haploid with a **single circular chromosome** consisting of double-stranded DNA. e.g. in *E.coli*, the chromosome is circular ds-DNA molecule of approximately **$4.6 \times 10^6$ base pairs**.

Although the chromosome exists free in the cytoplasm, it is compacted through super-coiling & looping of its structure. The central function of genetics consists of expression of a gene from its locus on the chromosome or on a plasmid through transcription (production of messenger RNA) & finally translation: decoding of mRNA to produce a polypeptide. The gene sequence & its subsequent expression through these biochemical pathways accounts for the phenotypic variation observed among bacteria.

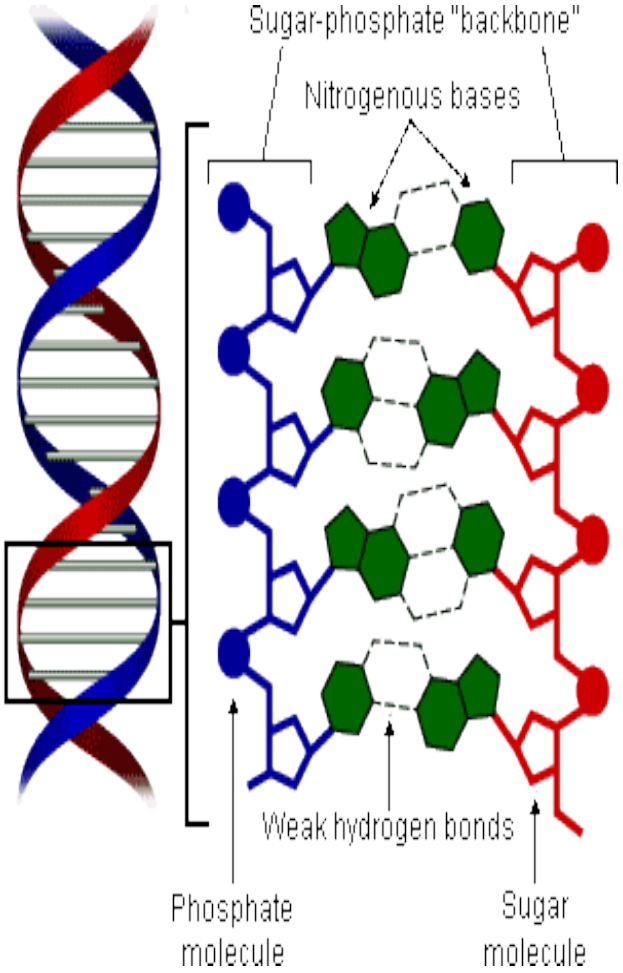


# Bacterial genetics

## Replication of bacterial DNA:

As bacteria replicate by binary fission, the daughter cells produced are usually indistinguishable genetically. Replication of chromosome in bacteria begins at a specific location called origin of replication. It proceed at a rate of 1000 uncleotide/second.

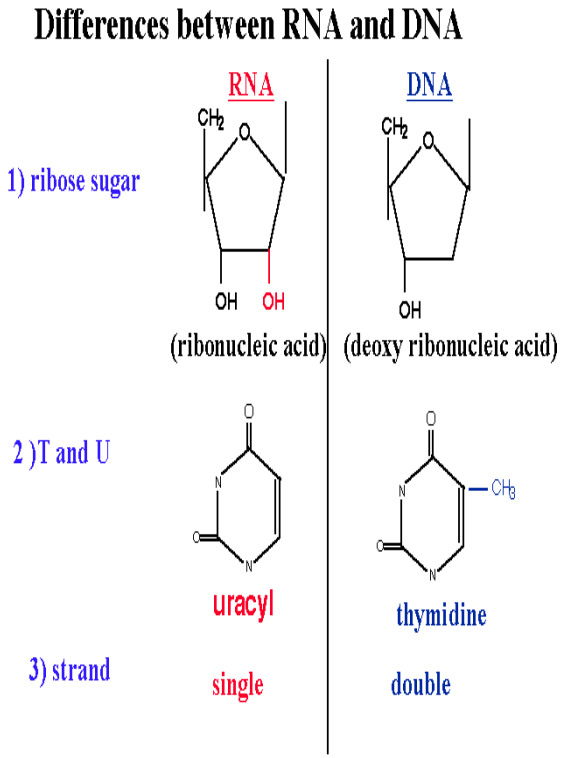
During replication, the purine [**adenine (A) & guanine (G)]** & pyrimidine [**thymine (T) & cytocine (C)** ] (**Each of the 4 bases is bound to phospho-deoxyribose to form a nucleotide. Adenine always pairs with thymine and the guanine pairs with cytocine by hydrogen bonding in the center of the molecule**) nucleotides in each DNA strand are accurately copied into 2 new ds daughter molecules. Each of these molecules is composed of a strand from the parent molecule & a newly synthesized complementary strand, a process termed semi-conservative replication.



# Bacterial genetics

As the 2 parent strands of the helical DNA unwind under the influence of the enzyme DNA helicase, each acts as a template for the synthesis of a complementary strand. In this manner, 2 identical helical DNA molecules are formed through the action of replicating enzyme DNA polymerase. The ends of the new fully formed strands are joined by DNA ligase.

RNA most frequently occurs in single stranded form. The base **uracil (U)** serves in RNA the hybridization function that thymine (T) serves in DNA. So the complementary bases that determine the structure of RNA are A-U and C-G.



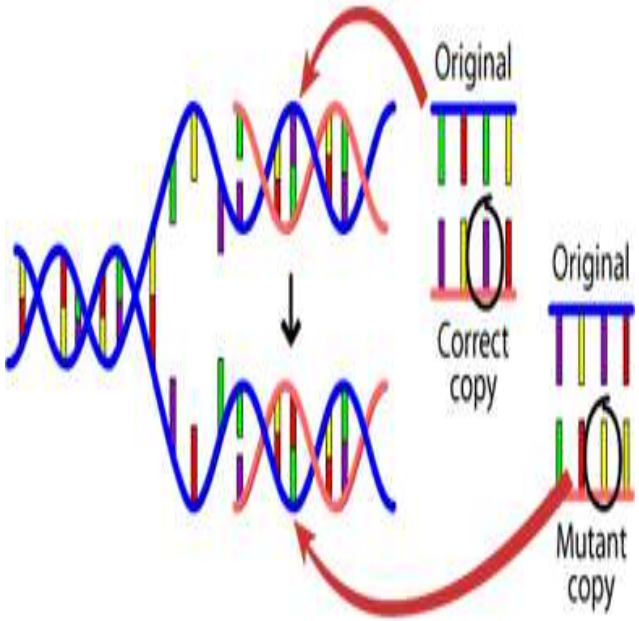
# Bacterial genetics

## Mechanisms of genetic variation:

The genotype of a cell determines its inheritable potential, while the phenotype represents those recognizable characteristics expressed by the cellular nucleic acid. Thus both the genotype of a bacterium & its environment can influence phenotypic expression.

**Mutations** are changes in the DNA sequence. **Spontaneous mutations** for a given gene generally occur with a frequency of $10^{-8}$ - $10^{-6}$ in a population derived from a single bacterium. The mutations include **base substitution, deletion, insertion and rearrangement**.

Base substitution can arise as a consequence of mispairing between complimentary bases during replication. Many base substitutions escape detection at the phenotypic level because they do not significantly disrupt the function of the gene product, for example substitution of one amino acid for another.



# Bacterial genetics

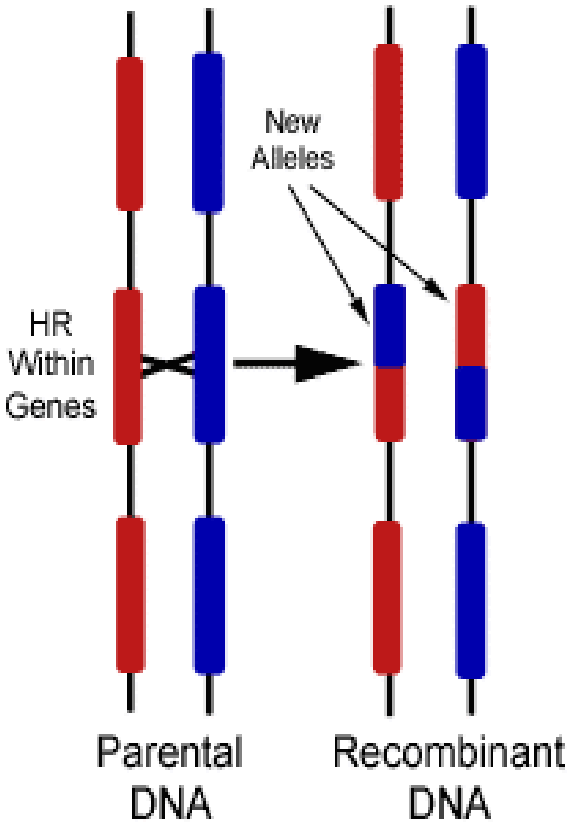
The consequences of deletion or insertion mutations are severe because they can drastically alter the amino acid sequence of gene products..

**Mutagenes:**

The frequency of mutation is greatly enhanced by exposure of cells to mutagens. **Physical mutagen**, e.g. ultraviolet light (UV) that damage DNA by linking neighboring thyamine bases to form diamer. **Chemical mutagens** may act by altering either the chemical or the physical structure of DNA. **Biological mutagens** refers to those pathogens that are able to induce alterations in the host DNA.

## Recombination:

Recombination occurs when sequences of DNA from 2 separate sources are integrated in bacteria, recombination includes an unexpected inheritable change due to introduction of new genetic material from a different cell. This genetic material can be introduce by: **Conjugation, Transduction or Transformation.**

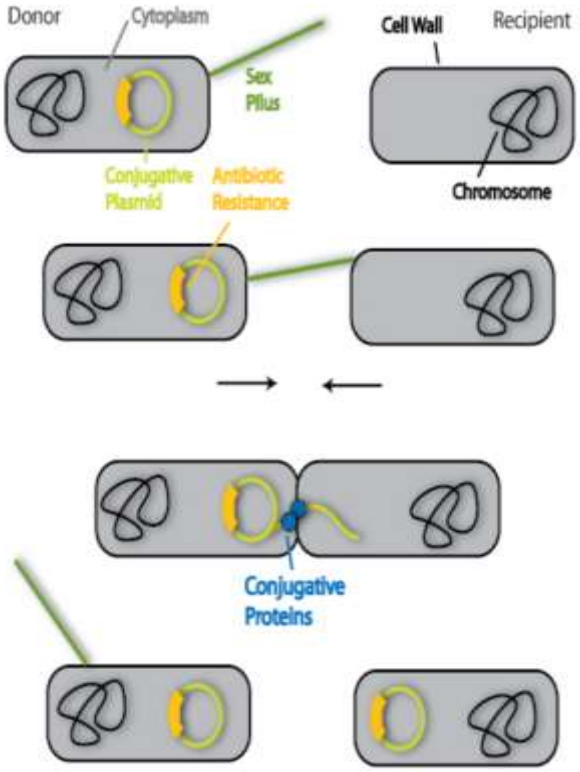


# Bacterial genetics

**1. Conjugation:**

In conjugation only one strand of DNA is transferred. The recipient cell completes the structure of ds-DNA by synthesizing the complementary stand. Plasmids are most frequently transferred by conjugation.

Genetic analysis of *E. coli* revealed the presence of **fertility factor** carried on plasmid called **($F^+$)**. Sex pilus; an extracellular protein extrusion that attaches donor cell to recipient cell lacking the fertility factor. A bridge between the cells is formed & allows a strand of $F^+$ factor plasmid to pass into the recipient, where the complementary strand is formed. The $F^+$ factor can integrate in the chromosome of donor cell creating **HFr** donors from which chromosomal DNA is transferred.

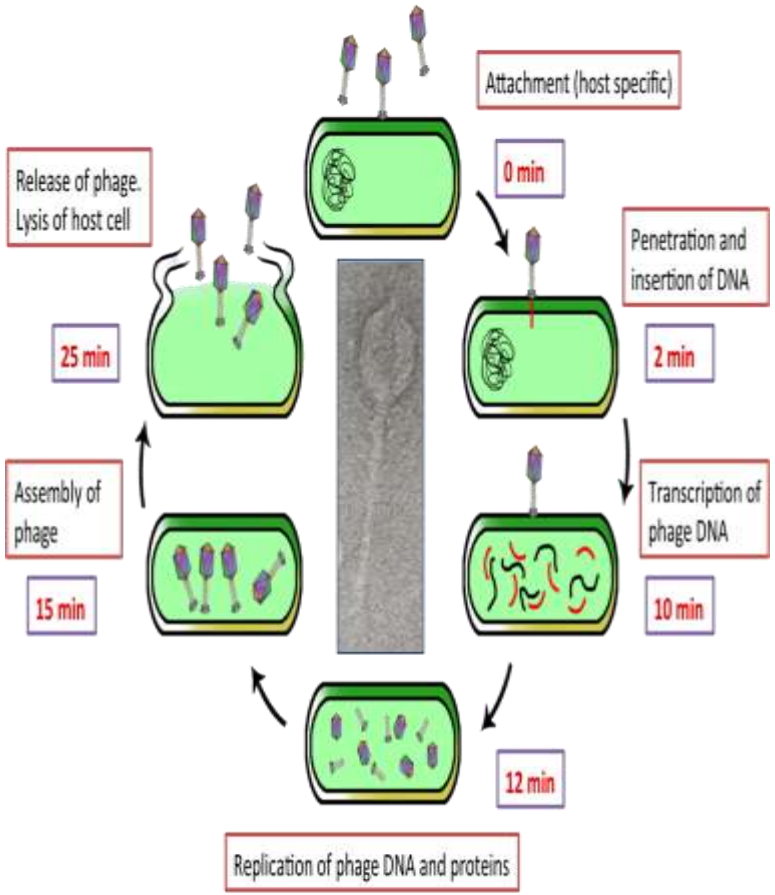


# Bacterial genetics

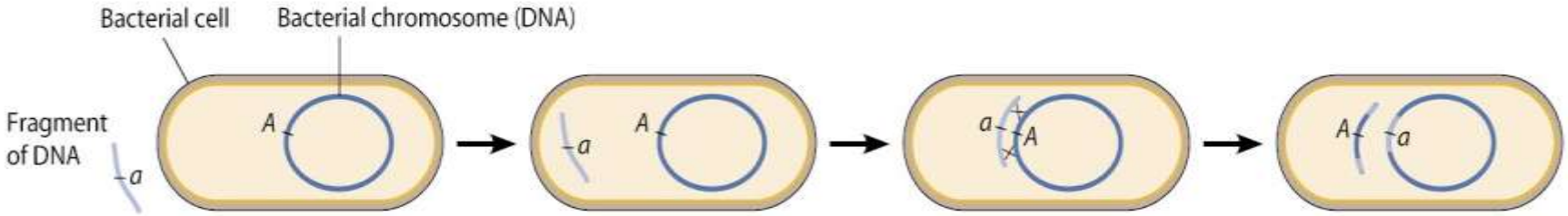
Similarly another plasmids e.g. drug resistant plasmid (**R factor**) can promote chromosomal transfer from different bacteria.

## 2. Trasduction:

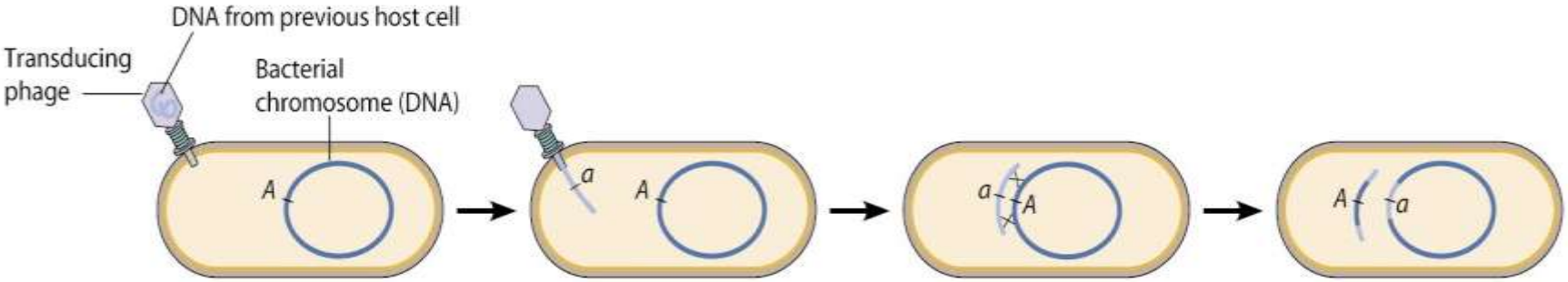
In transduction, bacterial donor DNA is carried in a phage coat & transferred to recipient by the mechanism used for phage infection. So, transduction is phage-mediated recombination in bacteria. Temperate phage is preferred vehicle for gene transfer, because infection of recipient bacteria minimize cell lysis & favor survival of recombinant strain. Furthermore, recipient bacteria carrying a prophage may form a repressor that renders the cell immune to lytic infection, such cell may continues take up bacterial DNA from transducing plasmids.



# Bacterial transduction




**(a) Transformation.** Transformation involves uptake by the bacterial cell of exogenous DNA, which occasionally becomes integrated into the bacterial genome by two crossover events (indicated by X's). The exogenous DNA will be detectable in progeny cells only if integrated into the bacterial chromosome, because the fragment of DNA initially taken up does not normally have the capacity to replicate itself autonomously in the cell. (The main exception is an intact plasmid.)



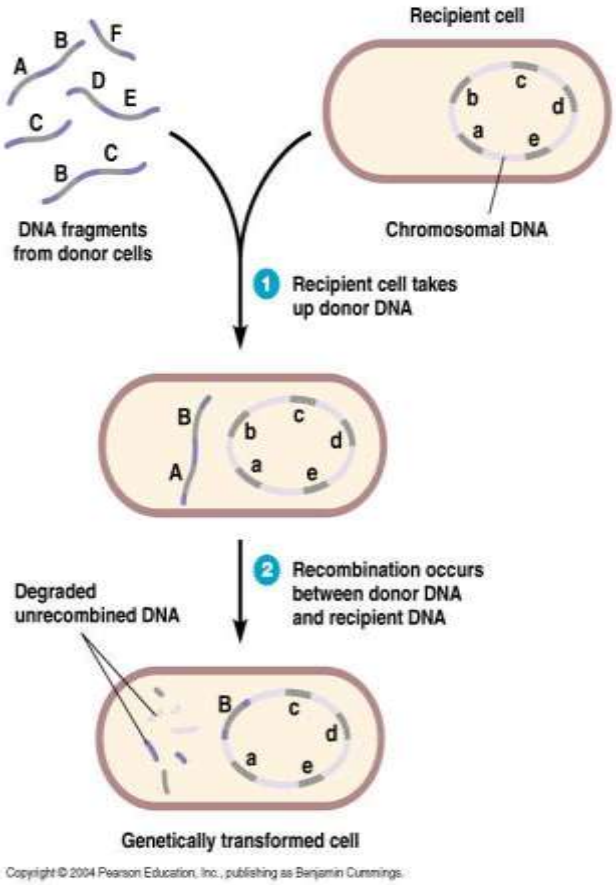

**(b) Transduction.** Transduction involves the introduction of exogenous DNA into a bacterial cell by a phage. Once injected into the host cell, the DNA can become integrated into the bacterial genome in the same manner as in transformation. In both cases, linear fragments of DNA that end up outside the bacterial chromosome are eventually degraded by nucleases.

# Bacterial genetics

**Transformation:**

This process involves the transfer of free (nacked) DNA containing genes on a segment of chromosomal or plasmid DNA from a lysed donor bacterium to a competent recipient.

**Natural transformation** is unusual among bacteria. Many bacteria unable to undergo natural transformation, can be forced to incorporate plasmids by treatment with calcium chloride & temperature shock. This process is the cornerstone of modern molecular biology, because it enable DNA from diverse biological sources to be incorporated as part of well-characterized bacterial replicon.



# Bacterial genetics

## Genetic engineering:

Specified DNA fragments can be isolated & amplified, & their genes can be expressed at high levels. The nucleotide specificity required for cleavage by restriction enzymes allows fragments containing genes or parts of genes to be covalently bound to plasmids " **vector**" that can be inserted into bacterial host. Bacterial colonies or **clones** carrying specified genes can be identified by **hybridization** of DNA or RNA with chemical or radiochemical **probes**.

The protein products encoded by these genes can be recognized either by enzyme activity or by immunological techniques.

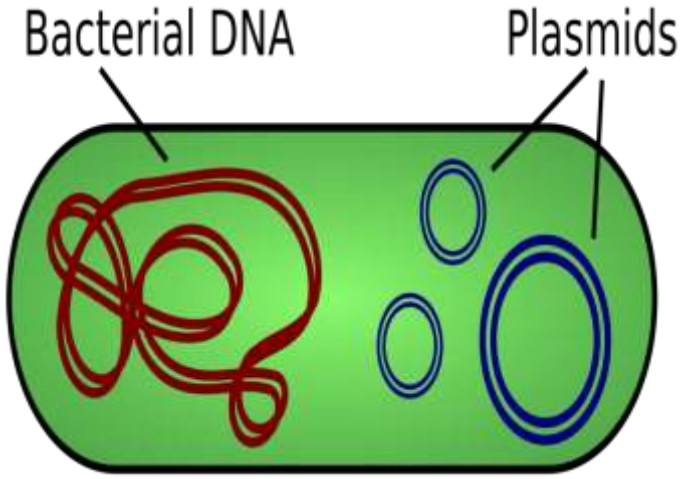
The protein products of isolated genes offers great promise as **vaccine** because it can be prepared without genes that encode the replication of viral nucleic acid. Moreover, protein ,e.g. insulin can be prepared in large quantities from bacteria that express cloned genes.

# Bacterial genetics

## Mobile genetic elements:

## Plasmids:

Although most bacteria carry all the genes necessary for survival on their chromosome, many bacteria contain small additional genetic elements, termed plasmids, which also located in the cytoplasm. **Plasmids** are small genetic elements capable of independent replication in bacteria & yeast. plasmids carry genes associated with specialized functions & genes that mediate their transfer from one organism to another. plasmids are frequently involved in the genetic engineering, because of their small size, that permits their genetic manipulation & after alteration can be easily introduced to the cell. In pathogenic bacteria, plasmids encode virulence factors & antibiotic resistance.



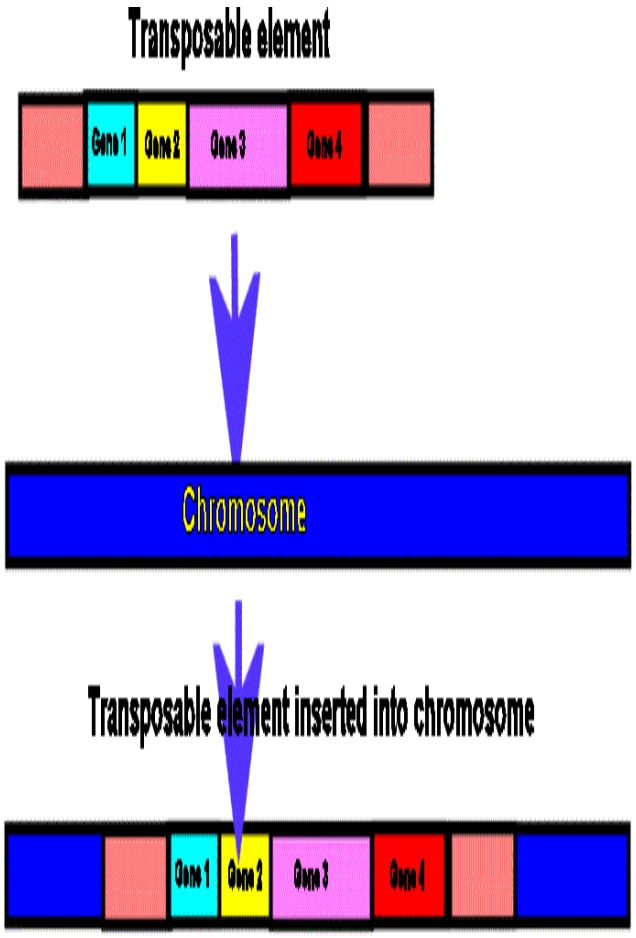
# Bacterial genetics

## Mobile genetic elements:

### Transposons:

Are genetic elements containing several kbp of DNA, including information necessary for their migration from one genetic locus to another. Almost all bacteria carry transposons, which differ from one species to another. Plasmids also carry transposones, which are important in the formation of high-frequency recombinant strains. Carrying on the plasmids, the transposons maintain its dissemination throughout a bacterial population.

Complex transposons carry genes for specialized functions, e.g. antibiotic resistance. Unlike plasmids, transposons do not contain genetic information necessary for their own replication. Therefore, they depend on their physical integration with a bacterial replicon.

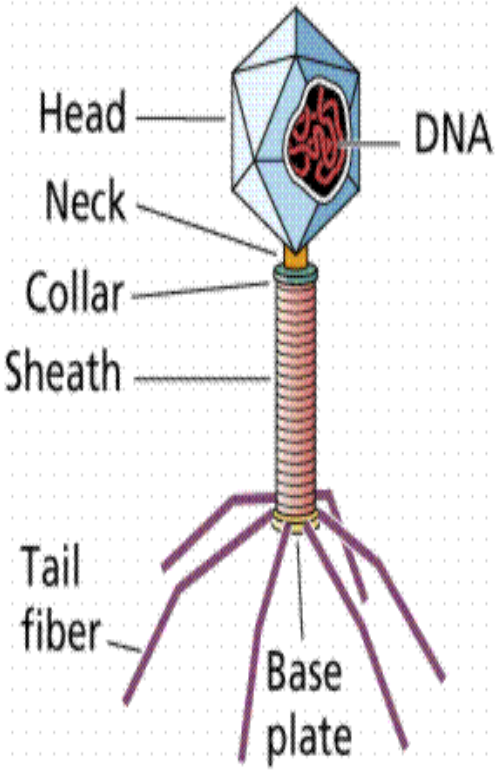


# Bacterial genetics

**Mobile genetic elements:**

**Bacteriophages ( phages):**

Viruses that infect bacteria. Phages have narrow specific host range. The phage genome may be ss- or ds- DNA or RNA. The NA of phage is surrounded by a protein coat. Many phages contain syringe-like structure that bind to receptors on the cell surface & inject the phage nucleic acid into the host cell. Replication of phage is similar to that of animal viruses.




**Types of phages:**

1. **Lytic phage:** produces many copies of themselves & kill their host cells. e.g. T2, T4 phage of *E. coli*
2. **Temperate phage:** are able to enter a non-lytic prophage state in which replication of its NA is linked to the replication of host cell DNA. Bacteria carry prophage are called lysogenic (e.g. *E. coli* phage lambda).
3. **Filamentous phage:** (e.g. *E. coli* phage M13) their filaments contain ss-RNA with protein that extruded from their host cell, which are debilitated but not killed by the phage infection.