Bacteriology

2nd year / 2024-2025

Lec(8): Bacterial Genetics

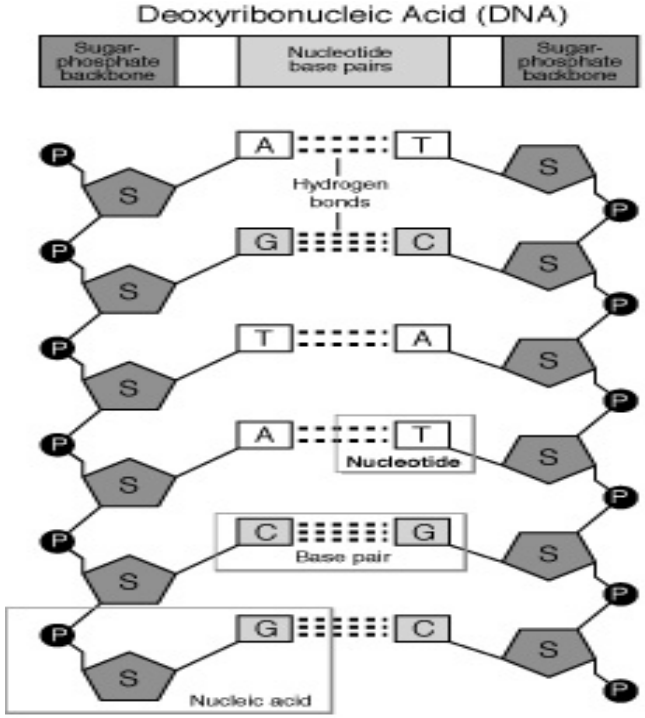
Bacterial Genetics

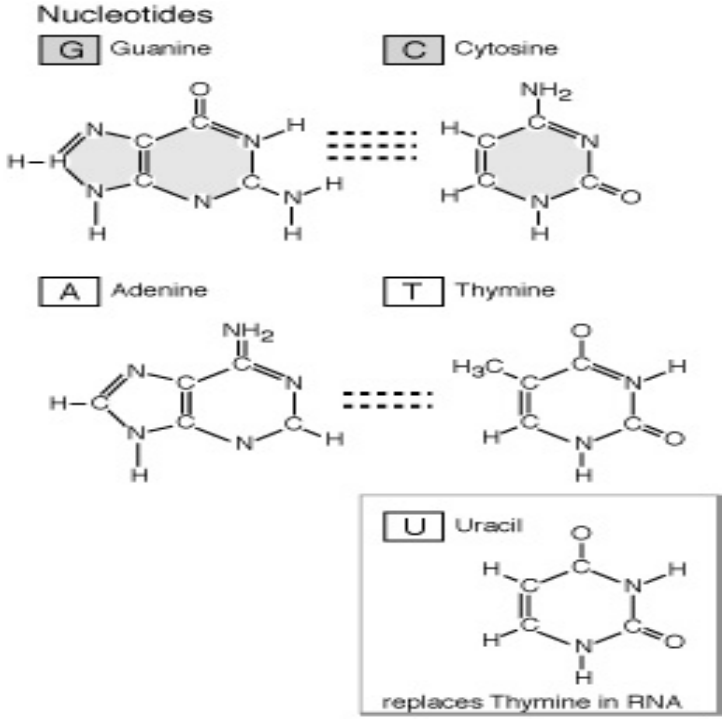
A bacterial genome is the total amount of DNA in an organism, the genome of each species contains unique arrangement of genes. The genome of prokaryotes such as bacteria consists of a few thousand genes and it is typically a single circular chromosome. The first bacterial genome to be completely sequenced was that of *Haemophilus influenzae* (1995). The first archaeal genome to be completely sequenced was that of Methanococcus sp (1997).

Nucleic acids:

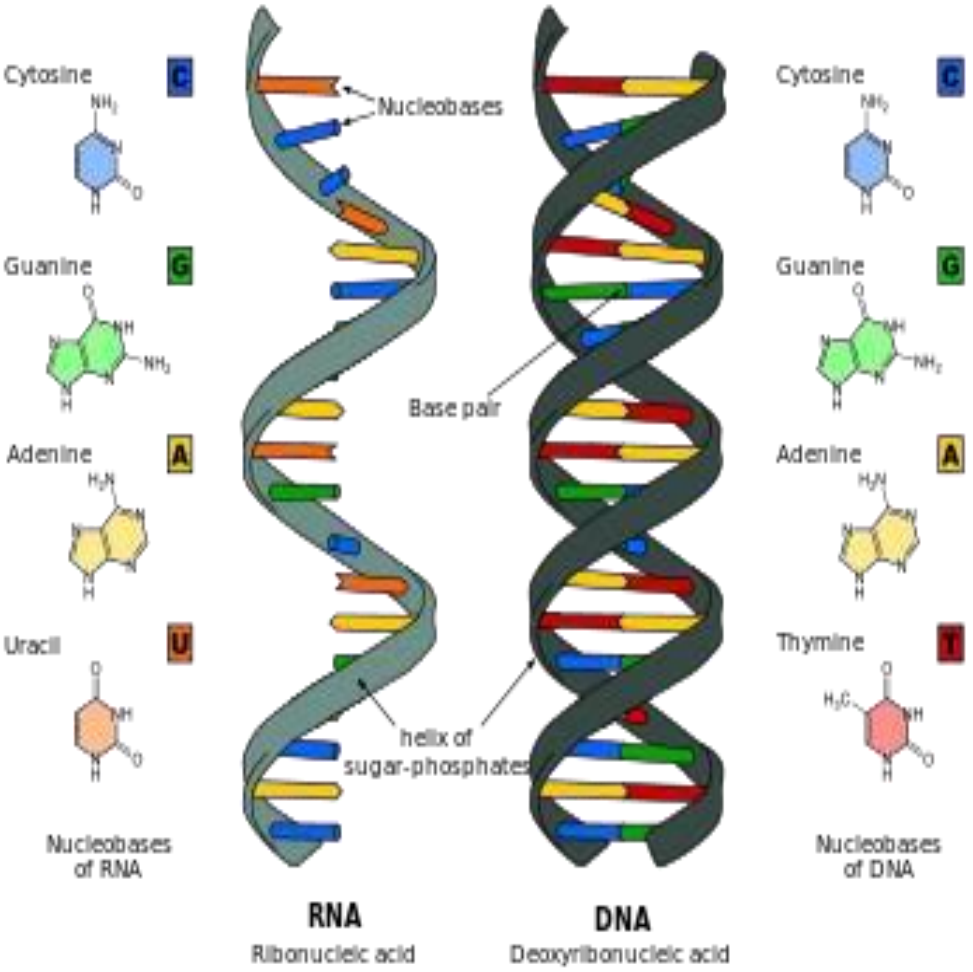
Nucleic acids are biopolymers, or large biomolecules, essential for all known forms of life. Nucleic acids, which include DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), are made from monomers known as nucleotides.
Each nucleotide has three components: a 5-carbon sugar, a phosphate group, and a nitrogenous base. If the sugar is deoxyribose, the polymer is DNA. If the sugar is ribose, the polymer is RNA. When all three components are combined, they form a nucleotide. Nucleotides are also known as phosphate nucleotides.
The nucleobases found in the two nucleic acid types are different: adenine, cytosine, and guanine are found in both RNA and DNA, while thymine occurs in DNA and uracil occurs in RNA.
Nucleic acids are among the most important biological macromolecules (others being amino acids/proteins, sugars/carbohydrates, and lipids/fats). They are found in abundance in all living things, where their function in encoding, transmitting and expressing genetic information, in other words, information is conveyed through the nucleic acid sequence,

or the order of nucleotides within a DNA or RNA molecule. Strings of nucleotides strung together in a specific sequence are the mechanism for storing and transmitting hereditary or genetic information via protein synthesis. Nucleic acids were discovered by Friedrich Miescher in 1869. Experimental studies of nucleic acids constitute a major part of modern biological and medical research, and form a foundation for genome and forensic science, as well as the biotechnology and pharmaceutical industries.





DNA	RNA
DNA is a double stranded molecule	**RNA is a single stranded molecule**
sugar is deoxyribose	**sugar is ribose**
DNA is responsible to storing and transferring genetic informations	**RNA directly codes for amino acids and as acts as a messenger between DNA and ribosomes to make proteins like mRNA (messenger ribonucleic acid)**
DNA is stable under alkaline conditions	**Is not stable**
Purine basses : Adenine، Guanine **Pyrimidine bases: Thymine, Cytosine**	**Purine basses : Adenine، Guanine** **Pyrimidine bases: Uracil, Cytosine**



DNA: **DNA is a molecule that carries most of the genetic instructions used in the development, functioning and reproduction of all known living organisms and many viruses. DNA stores biological information. The DNA backbone is resistant to cleavage, and both strands of the double-stranded structure store the same biological information. Biological information is replicated as the two strands are separated. A significant portion of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences.**

The two strands of DNA run in opposite directions to each other and are therefore anti-parallel. Attached to each sugar is one of four types of nucleobases (informally, bases). It is the sequence of these four nucleobases along the backbone that encodes biological information. Under the genetic code, RNA strands are translated to specify the sequence of amino acids within proteins. These RNA strands are initially created using DNA strands as a template in a process called transcription.

Prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm. Within the chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.

Structure of chromosome

In contrast to the linear chromosomes found in eukaryotic cells, most bacteria have single, covalently closed, circular chromosomes. Not all bacteria have a single circular chromosome: some bacteria have multiple circular chromosomes, and many bacteria have linear chromosomes and linear plasmids. Multiple chromosomes have also been found in many other bacteria, including *Brucella*, *Leptospira interrogans*, *Burkholderia* and *Vibrio cholerae*.

***Borrelia* and *Streptomyces* have linear chromosomes and most strains contain both linear and circular plasmids. The chromosome of *E coli* has a length of approximately 1.35 mm, several hundred times longer than the bacterial cell, but the circular DNA is then looped and supercoiled to allow the chromosome to fit into the small space inside the cell.**

Codon

A set of three base pairs constitutes a codon, which codes for a single amino acid. The "triplet code" is said to be degenerate or redundant because more than codon may exist for the same amino acid. For example, the codons AGA, AGG, CGU, CGC, CGA and CGG all code for arginine. There are 64 codons, of which 3 (UAA, UAG and UGA) are nonsense codons. They don't code for any amino acid, but act as stop codons. There are specific codons which code for start and stop sequences. The start codon (AUG) indicates the beginning of the sequence to be translated, and the stop codons (UAA, UGA, UAG) terminate the protein synthesis. With the exception of methionine, all amino acids are coded for by more than one codon. The DNA in a gene that are expressed into the protein product are called exons and the non-coding DNA segments are called introns. There are no introns in bacterial chromosome. A segment of DNA carrying codons specifying a particular polypeptide is called a cistron or a gene.

Flow of genetic information

The central dogma of molecular biology is that DNA carries all genetic information. The flow of genetic information includes the replication of DNA to make more DNA, the transcription of the DNA into mRNA and the translation of mRNA into proteins.

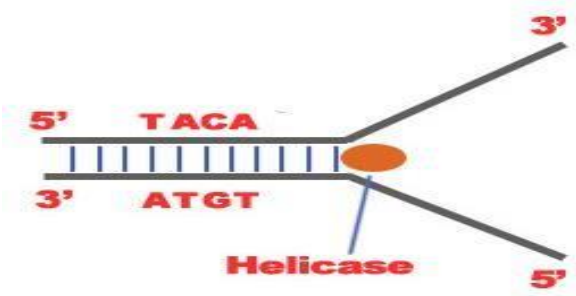
Replication of DNA first involves the separation of the two strands of DNA followed by synthesis of new identical DNA strand by enzymes called DNA polymerases.. The RNA strand is synthesized by enzymes called RNA polymerases. The RNA sequence will be complementary to the DNA sequence. The mRNA strands are then guided to the ribosomes for protein translation. Amino acid residues are brought to the mRNA strand on the ribosomes by transfer RNA (tRNA).

DNA replication: is the process of producing of two identical replicas from one original DNA molecule. This biological process occurs in all living organisms and is the basis for biological inheritance. DNA is made up of two strands and each strand of the original DNA molecule serves as a template for the production of the complementary strand, a process referred to as semiconservative replication. Cellular proofreading and error-checking mechanisms ensure near perfect fidelity for DNA replication

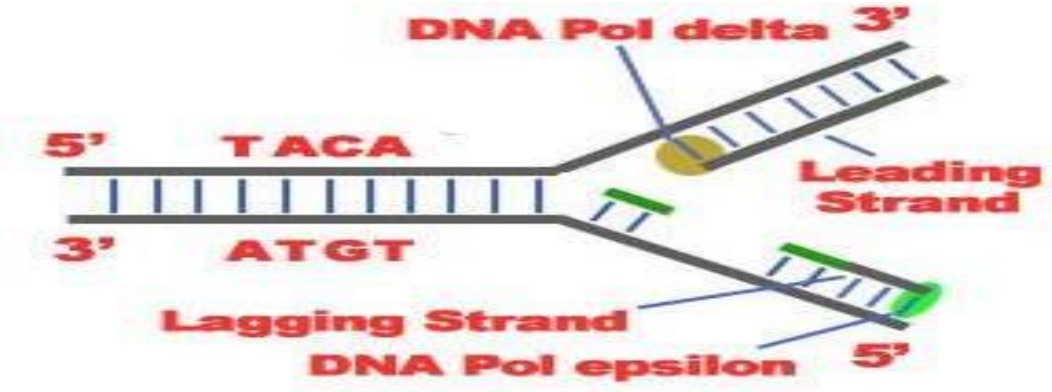
Steps of DNA Replication

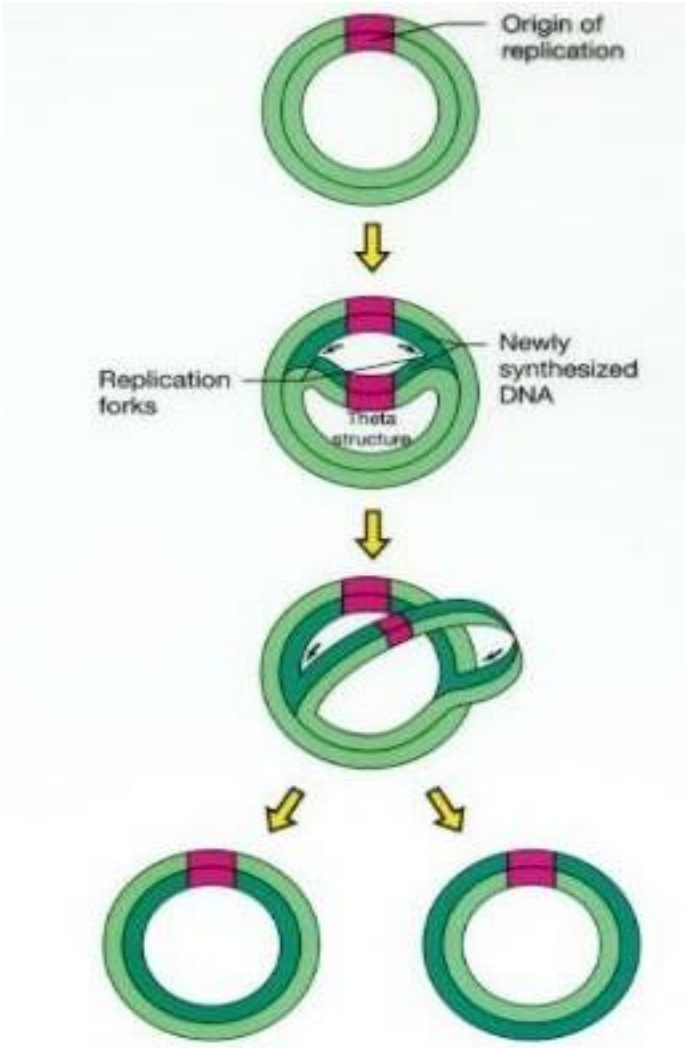
1-Initiation: The first major step for the DNA Replication to take place is the breaking of hydrogen bonds between bases of the two antiparallel strands. The unwinding of the two strands is the starting point. The splitting happens in places of the chains which are rich in A-T. That is because there are only two bonds between Adenine and Thymine (there are three hydrogen bonds between Cytosine and Guanine)

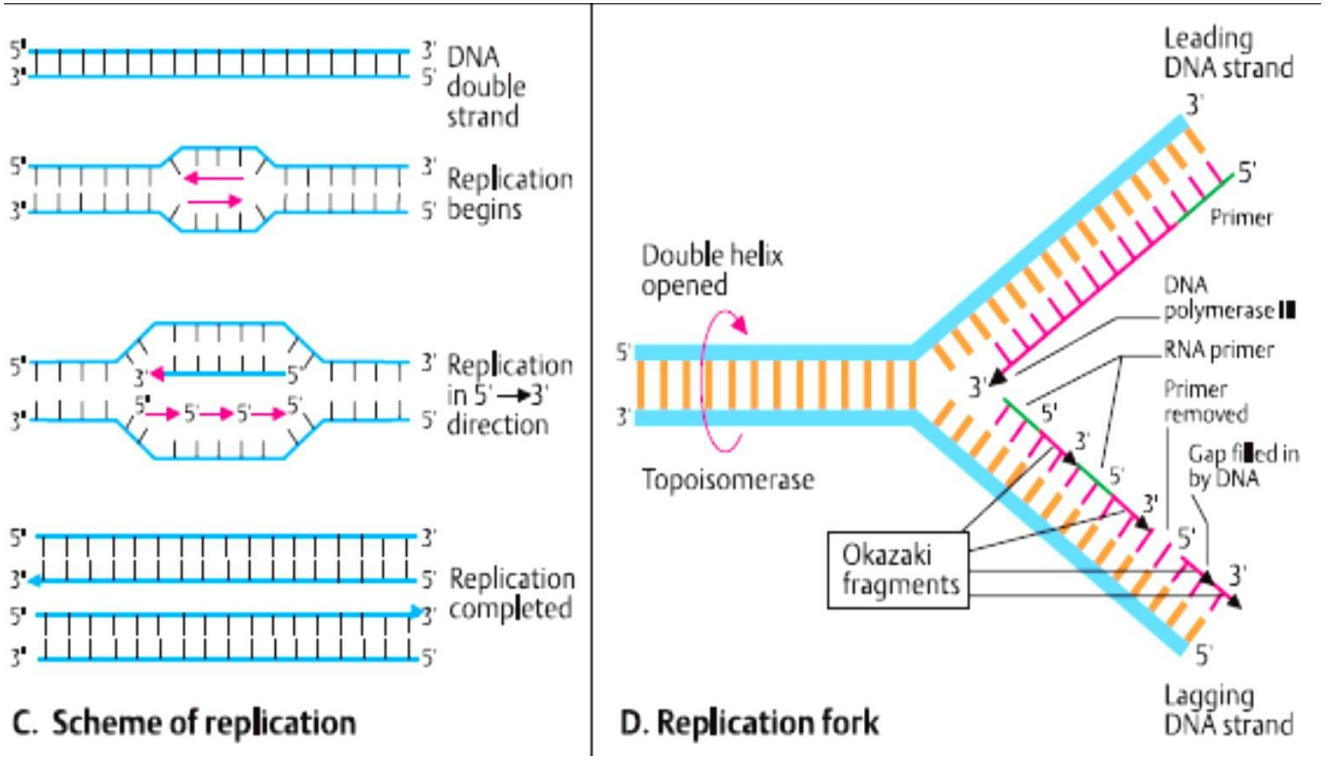
Helicase is the enzyme that splits the two strands. The initiation point where the splitting starts is called "origin of replication". The structure that is created is known as "Replication Fork".



2- Elongation: One of the most important steps of DNA Replication is the binding of RNA Primase in the initiation point of the 3'-5' parent chain. RNA Primase can attract RNA nucleotides which bind to the DNA nucleotides of the 3'-5' strand due to the hydrogen bonds between the bases. RNA nucleotides are the primers (starters) for the binding of DNA nucleotides.




Origin of replication
Replication forks
Newly synthesized DNA
Theta structure



Transcription (Translation): **is the process in which cellular ribosomes create proteins. In translation, messenger RNA (mRNA)—produced by transcription from DNA—is decoded by a ribosome to produce a specific amino acid chain, or polypeptide. The polypeptide later folds into an active protein and performs its functions in the cell. The ribosome facilitates decoding by inducing the binding of complementary tRNA anticodon sequences to mRNA codons. The tRNAs carry specific amino acids that are chained together into a polypeptide as the mRNA**

passes through and is "read" by the ribosome. The entire process is a part of gene expression. Breifly, translation proceeds in four phases:

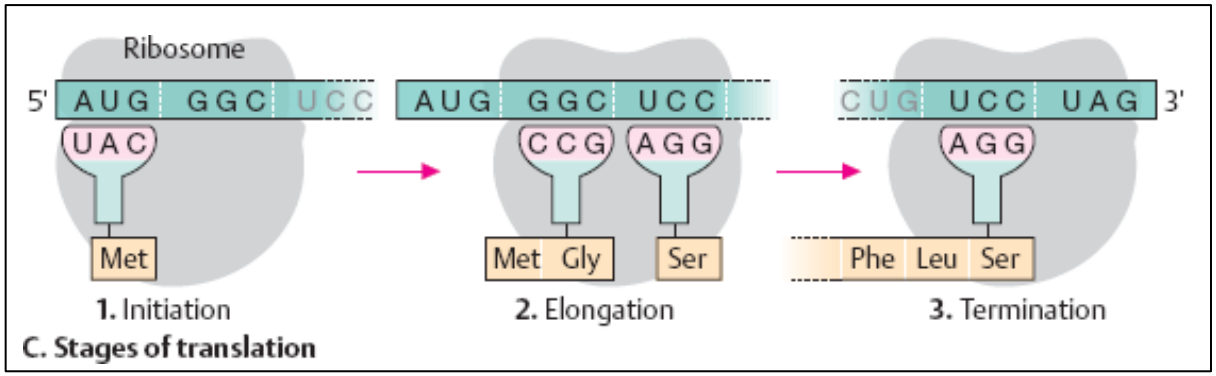
1-Initiation: The ribosome assembles around the target mRNA. The first tRNA is attached at the start codon.

2-Elongation: The tRNA transfers an amino acid to the tRNA corresponding to the next codon.

3-Translocation: The ribosome then moves (translocates) to the next mRNA codon to continue the process, creating an amino acid chain.

4-Termination: When a stop codon is reached, the ribosome releases the polypeptide. In bacteria, translation occurs in the cell's cytoplasm, where the large and small subunits of the ribosome bind to the mRNA.

In eukaryotes, translation occurs in the cytosol or across the membrane of the endoplasmic reticulum in a process called vectorial synthesis. In many instances, the entire ribosome/mRNA complex binds to the outer membrane of the rough endoplasmic reticulum (ER); the newly created polypeptide is stored inside the ER for later vesicle transport and secretion outside of the cell.

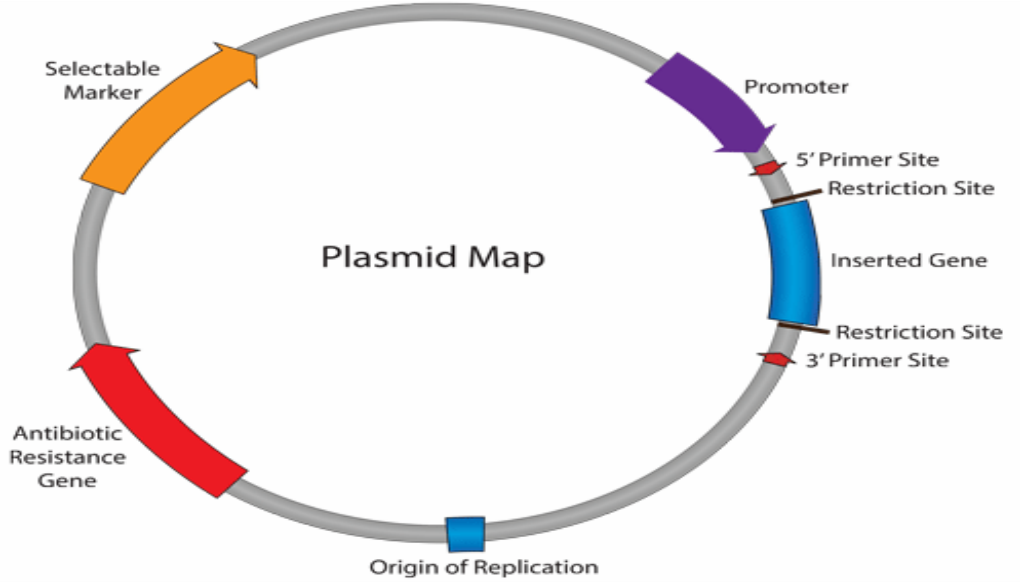


PLASMIDS:

Plasmids are extrachromosomal elements found inside a bacterium. These are not essential for the survival of the bacterium but they confer certain extra advantages to the cell. Number and size: A bacterium can have no plasmids at all or have many plasmids (20-30).

Plasmid: Usually they are closed circular molecules; however they occur as linear molecule in *Borrelia burgdorferi*. Their size can vary from 1 Kb to 400 Kb. Multiplication: Plasmids multiply independently of the chromosome and are inherited regularly by the daughter cells.

Key features of a typical plasmid vector are an origin of replication (to ensure the vector is copied within bacteria), a gene for antibiotic resistance (to ensure the vector is not lost by bacteria) and a set of recognition sites for restriction enzymes (to make it straight to insert foreign DNA into the vector).



Types of plasmids:

F factor: This is also known as fertility factor or sex factor. Most plasmids are unable to mediate their own transfer to other cells. Vertical (inheritance) or horizontal (transfer) transmissions maintain plasmids. F factor is a plasmid that codes for sex pili and its transfer to other cells. Those bacteria that possess transfer factor are called F+, such bacteria have sex pili on their surface. Those cells lacking this factor are designated F-. F-. The F factor plasmid is transferred to other cells through conjugation. An F- cell will become F+ when it receives the fertility factor from another F+ cell.

R factor: **Those plasmids that code for the transmissible drug resistance are called R factor. These plasmids contain genes that code for resistance to many antibiotics. R factors may be transferred by conjugation and its transfer to other bacteria is independent of the F factor. Bacteria possessing such plasmids are resistant to many antibiotics and this drug resistance are transferred to closely related species. R factors may simultaneously confer resistance to five antibiotics. They are usually transferred to related species along with RTF (resistance transfer factor).**

Heavy–metal resistance plasmid: **There are several bacterial strains that contain genetic determinants of resistance to heavy metals, such as Hg^{++}, Ag^{+}, $Cd^{++,}$ CrO_4, Cu^{++}, Ni^{++}, Pb^{+++}, Zn^{++}, and so forth. These determinants for resistance are often found on plasmids and transposons. Bacteria that have been found resistant to heavy metals are *E. coli, Pseudomonas aeruginosa*.**

Virulence plasmid: **Formation of invasin due to its virulence plasmid makes *Shigella flexneri* (a human intestinal pathogen) able to penetrate intestinal mucosa.**

Degradative plasmids: **consist of genes that equip the bacteria (e.g., Pseudomonas sps.) with special enzymes or enzyme system to enable them to digest unusual substances (Xenobiotics) like chlorinated aromatic or hydrocarbon compounds. For example, the camphor (CAM) plasmid of *P. putida* encodes enzymes for degradation of camphor, octane (OCT) plasmid helps it degrade octane, XYL–plasmid helps degrade xylene and toluene, NAH–plasmid helps degrade naphthalene, and SAL–plasmid helps it degrade salicilate. These plasmids are conjugative.**