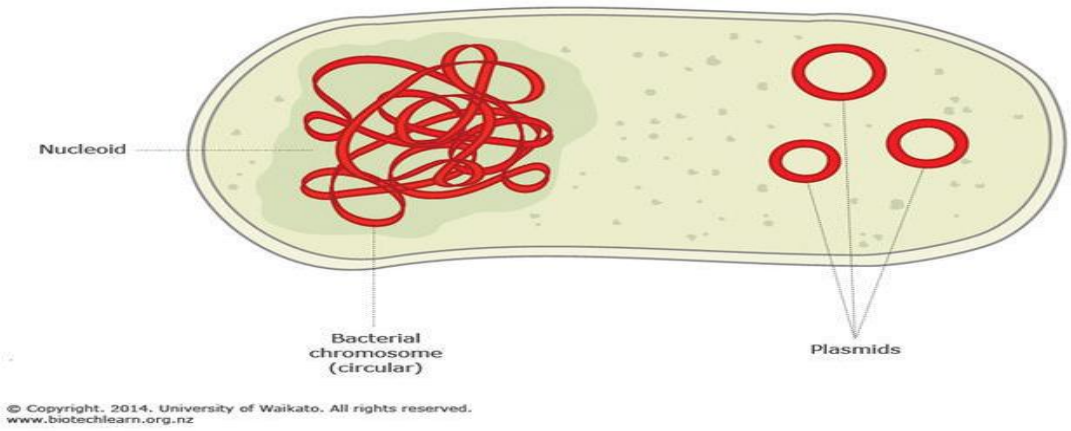
Plasmids

Plasmid Definition:

- They are extrachromosomal elements that control their own replication, which replicate independently of chromosomal DNA.
- They are different than chromosomal DNA and have separate genes.
- Plasmids are mainly found in bacteria and some eukaryotic microbes (as yeast).
- They are small circular double-stranded DNA with size range 1kb to over 100 kb.
- The number of copies of a plasmid in each cell is tightly controlled with a general rule that small plasmids tend to have a high copy number, sometimes over 100 copies per cell, whereas larger plasmids may be present in one or a few copies per cell.
- Plasmids usually carry at least one gene, and many of the genes that plasmids carry are beneficial and with specific functions to their host organisms.
- Multiple plasmids can coexist in the same cell, each with different functions.



Note: linear plasmids have been observed in *Borrelia burgdorferi*, the causative agent of Lyme disease.

Reproduction in plasmid:

Plasmids replicate independently of the host chromosome, but some plasmids called episome (def) are able to insert or integrate the host cell chromosome, then their replication is integrated by the chromosome.

Functions

Plasmids have many different functions:

1. They may contain genes that enhance the survival of an organism, either by killing other organisms or by defending the host cell by producing toxins.
2. Some plasmids facilitate the process of replication in bacteria.

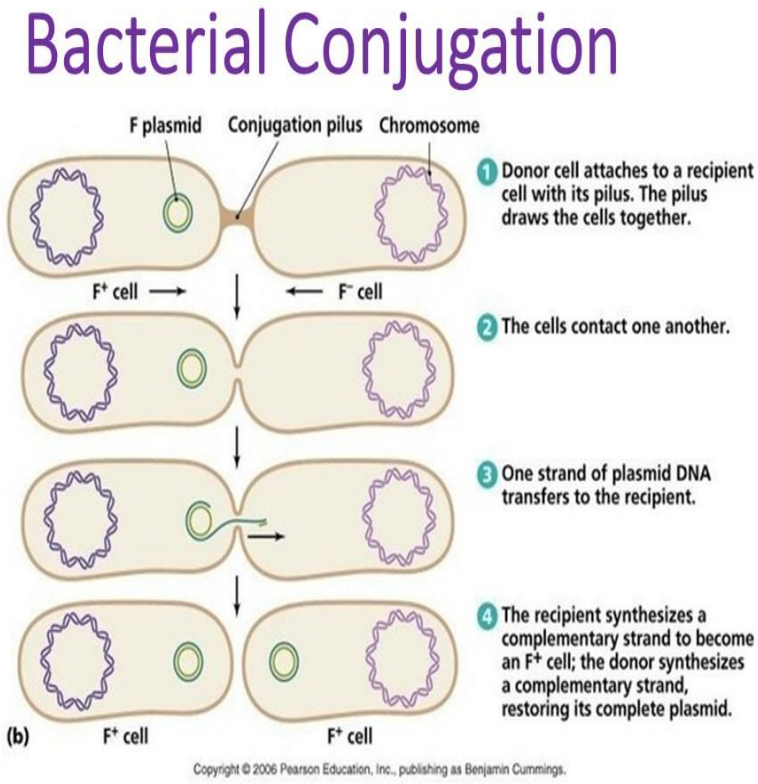
Types of plasmids

General types: Conjugative and non-conjugative & incompatibility.

Based on function: Fertility (F-plasmids), Resistance (R-plasmids), Virulence (V-plasmids), Degradative (D-plasmids) & Col plasmids.

Conjugative:

Bacteria reproduce by sexual conjugation, which is the transfer of genetic material from one bacterial cell to another, either through direct contact or a bridge between the two cells. Some plasmids contain genes called transfer genes that facilitate the beginning of conjugation.



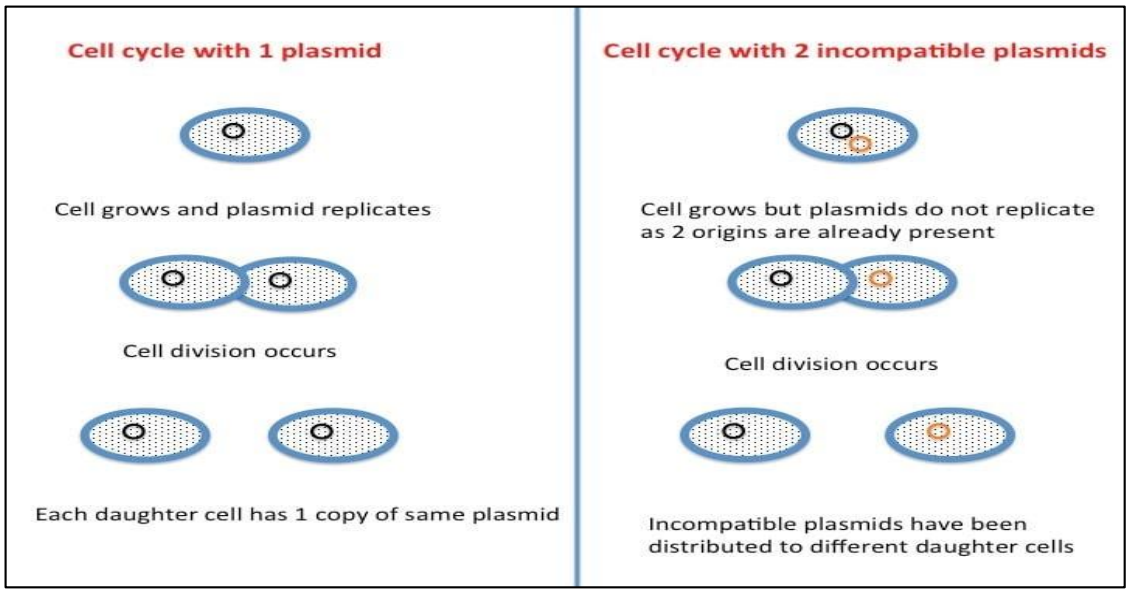
Non-conjugative:

Non-conjugative plasmids cannot start the conjugation process, and they can only be transferred through sexual conjugation with the help of conjugative plasmids.

Incompatibility:

Plasmid incompatibility is usually defined as the failure of two co-resident plasmids to be stably inherited together in the absence of external selection i.e. if the introduction of a second plasmid negatively affects the inheritance of the first, the two are considered to be incompatible. Plasmids can be seen as selfish entities in evolutionary terms. Having gained territory in a bacterial cell, they will try to prevent any other plasmid co-residing with them. The number of plasmids in a cell is governed by elements encoded within the origin of replication (*ori*). It is not possible to maintain two different plasmids that use the same mechanism for replication in a single cell. Therefore, plasmids fall into compatibility groups' base on their replication strategy and you cannot use two plasmids in the same cell system if the plasmids belong to the same compatibility group.

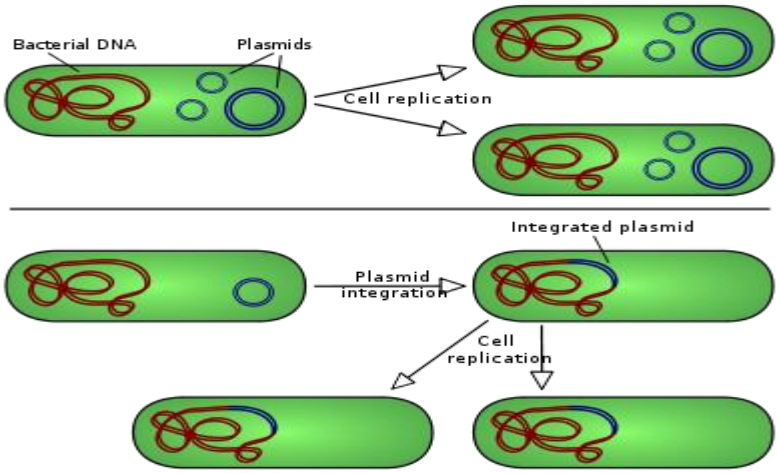
Note: Incompatibility only becomes an issue if your work requires that two plasmids be maintained together, so **before** you start out big cloning experiments where you require two plasmids make sure they don't belong to the same group.



Fertility (F-plasmids):

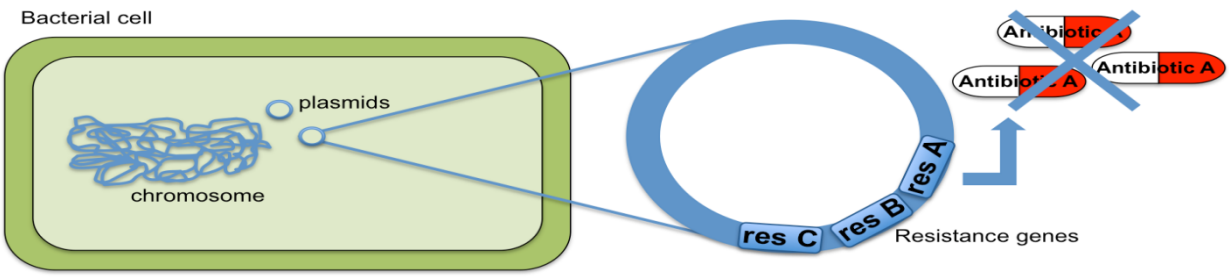
Fertility plasmids contain transfer (*tra*) genes that allow genes to be transferred from one bacterium to another through conjugation by expressing sex pilli. These make up the broad category of conjugative plasmids. F-plasmids are episomes (can be inserted into chromosomal DNA). Bacteria that have the F-plasmid are known

as F positive (F+), and bacteria without it are F negative (F-). When an F+ bacterium conjugates with an F- bacterium, two F+ bacterium result.



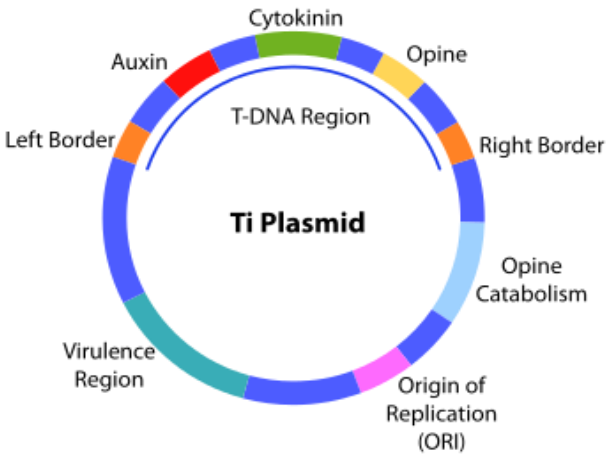
Resistance (R-plasmids):

Resistance plasmids contain genes that help a bacterial cell defend against environmental factors such as antibacterial agents, antibiotics….. Some resistance plasmids can transfer themselves through conjugation. When this happens, a strain of bacteria can become resistant to those agents. R-plasmids are very important in clinical microbiology and can cause threat to treatment of bacterial infections.



Virulence (V-plasmids):

Virulence plasmids contain *vir* genes. When a virulence plasmid is inside a bacterium, it turns that bacterium into a pathogen, which is an agent of disease. Bacteria that cause disease can be easily spread and replicated among affected individuals. Eg *Agrobacterium tumefeciens* present in soil and cause crown gall diseases to plant. *Escherichia coli* (*E. coli*) found naturally in the human gut and in other animals, but certain strains of *E. coli* can cause severe diarrhea and vomiting.



Agrobacterium sp

Degradative (D-plasmids):

Degradative plasmids help the host bacterium to digest unusual compounds that are not commonly found in nature, such as xylene, toluene, and salicylic acid. These plasmids contain genes for special enzymes that break down specific compounds. Degradative plasmids are conjugative. Eg plasmids of *Pseudomonas putida*.

Col plasmids:

Col plasmids contain genes that make bacteriocins (also known as colicins), which are proteins that kill other bacteria and thus defend the host bacterium. Eg ColE1 of *E. coli*.

Plasmid Constructions:

There are a number of features of plasmids that are useful in gene cloning. The plasmids used in routine gene cloning tend to be **less than 10 kb** to make them easy to purify and to manipulate.

– Plasmids have an "**origin of replication**" (ori) which directs the replication of the plasmid and ensures that the cell contains many copies of the plasmid which are distributed between the daughter cells when the cell divides. As long as the gene that you have cloned is part of a DNA molecule with an origin of replication, that is, cloned into a plasmid, it will also be copied when the plasmid is copied.

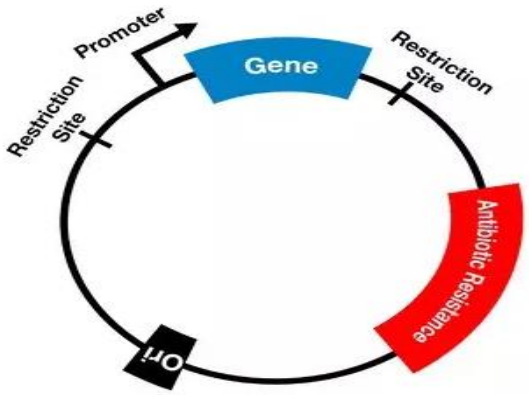
– Plasmids commonly used in cloning contain a selectable marker, usually an **antibiotic resistance gene**. This means that you can tell which bacteria contain the

plasmid simply by spreading them onto an agar plate containing the antibiotic. Those that contain the plasmid will grow and eventually form a visible colony, and all the cells within that colony will carry copies of the plasmid. Any bacteria that do not contain the plasmid will be killed by the antibiotic, and so cannot give rise to a non-cloned colony.

– **Gene**: A DNA sequence encoding a particular protein that a researcher has inserted into the plasmid to study.

– **Promoter**: A DNA sequence that allows the cell to produce the protein encoded by the gene.

– **Restriction sites**: DNA sequences that allow a researcher to cut and paste components of plasmids together.



Vector: It is a DNA sequence that can transport foreign genetic material from one cell to another cell, where the genes can be further expressed and replicated. It also has the ability to self-replicate.

The six major types of vectors are:

- **Plasmid:** It is first vectors used in gene cloning.
  Circular extrachromosomal DNA that autonomously replicates inside the bacterial cell. pBR322, pUC18, F-plasmid are some of the examples of plasmid vectors.
- **Phage:** More efficient than plasmids for cloning large DNA inserts. 53 kb DNA can be packaged in the bacteriophage. Phage λ and M13 phage are commonly used bacteriophages in gene cloning.
- **Cosmids:** Another circular extrachromosomal DNA molecule that combines features of plasmids and phage.

- **Phagemids:** These are artificial vectors used in combination with M13 phage.They possess multiple cloning sites and an inducible lac gene promoter.
- **Bacterial Artificial Chromosomes:** Based on bacterial mini-F plasmids. These are used to study genetic disorders.
- **Yeast Artificial Chromosomes.** This is an artificial chromosome that contains telomeres with origins of replication, a yeast centromere, and a selectable marker for identification in yeast cells.
- **Retroviral Vectors:** is a type of hybrid plasmid that contains a Lambda phage *cos* sequence. Cosmids (*cos* sites + plasmid = cosmids) DNA sequences are originally from the lambda phage. The vector also contains viral and cellular gene promoters, such as the CMV promoter, to enhance expression of the gene of interest in the target cells. Cosmids can contain 37 to 52 (normally 45) kb of DNA, limits based on the normal bacteriophage packaging size. They can replicate as plasmids if they have a suitable origin of replication (ori). They are often used as a cloning vector in genetic engineering. Cosmids can be used to build genomic libraries.
- **Human Artificial Chromosome.** This type of vector is potentially useful for gene delivery into human cells, and a tool for expression studies and determining human chromosome function. It can carry a very large DNA fragment.

All engineered vectors have an origin of replication (a replicator), a cloning site (located where the insertion of foreign DNA neither disrupts replication or inactivation of essential markers), and a selectable marker (typically a gene that provides resistance to an antibiotic.)

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Gene Cloning

Cloning is the method of producing identical genes through different procedures. Method of gene cloning is useful in studying the structure and function of genes in detail.

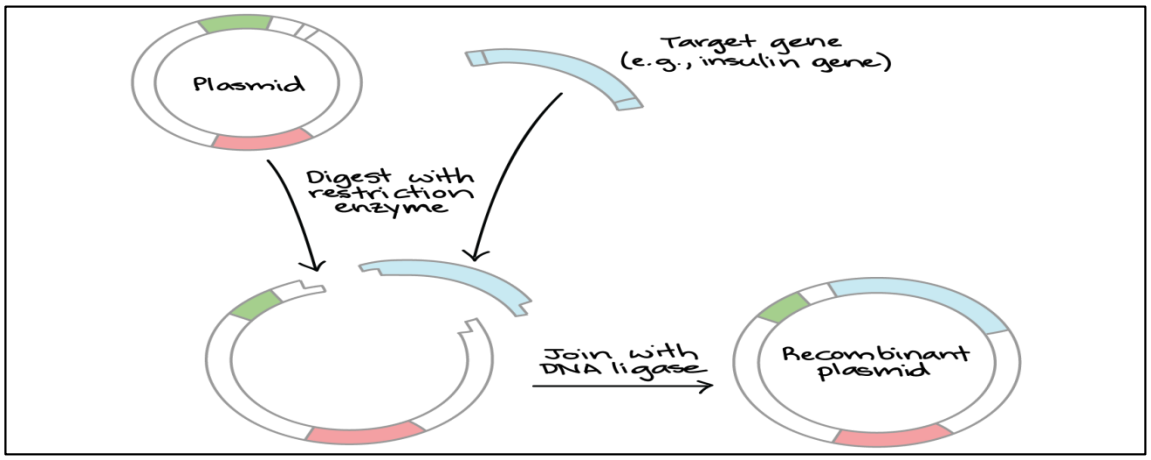
Definition:

Gene cloning involves taking a piece of DNA from the organism where it naturally occurs and putting it into a cloning host such as the bacterium *Escherichia coli*. It is then possible to study the cloned DNA or produce the protein encoded by the gene.

For many applications you may want subsequently to transfer the cloned DNA into another organism, but the initial cloning steps are almost always performed in *E. coli*.

Steps:

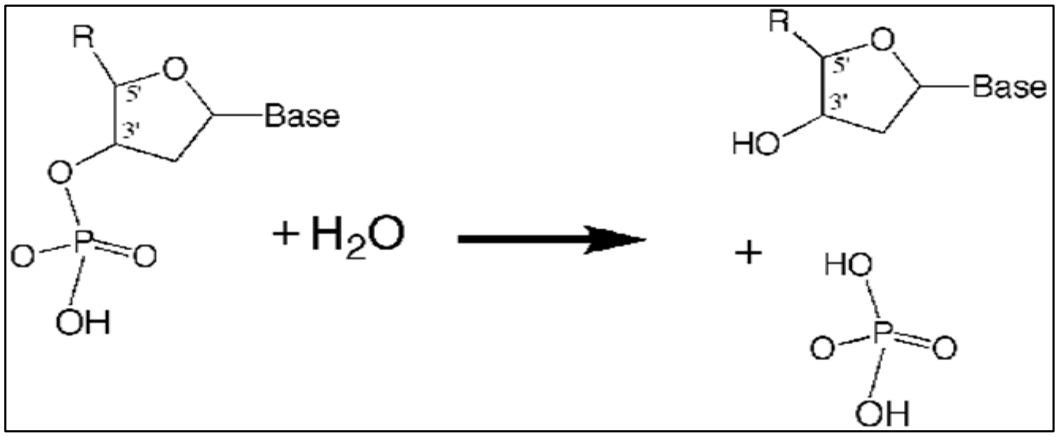
DNA is cut into fragments and introduced into a new host, usually *E. coli*, where it is copied. However, you cannot simply introduce fragments of DNA into a cell or organism as they will probably be degraded, and even if it is not it will not be replicated and passed on when the cell divides. To make sure that the piece of cloned DNA is copied and passed on it is necessary to put it into a vector which will ensure that it is copied every time that the cell copies its own DNA and that a copy is passed on to each daughter cell at cell division. This involves cutting the vector and joining in the piece of DNA that you want to clone. This cutting (in a very precise and reproducible manner) and joining of DNA fragments is done using enzymes. The new molecule that you have thus created is introduced into your host cell by a process called transformation. Once in the host it will be copied and passed on every time the cell divides making many copies or clones of the original fragment.



To perform gene cloning we need groups of enzymes to cut DNA and rejoin it again.

Nucleases

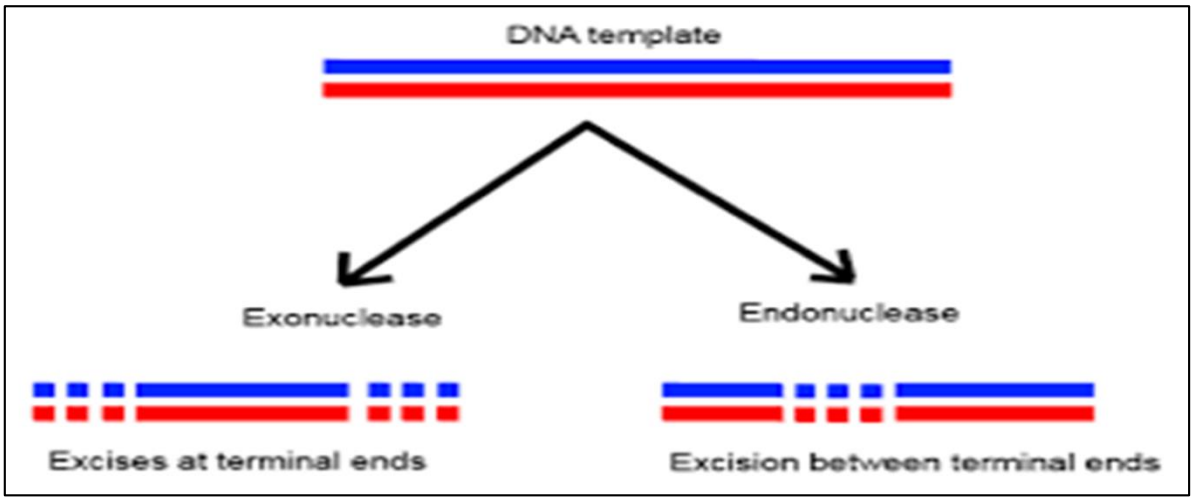
A nuclease is an enzyme that degrades nucleic acids by hydrolyzing the phosphodiester bond that joins the sugar residues. Nucleases are critical components to biological processes involving nucleic acids.

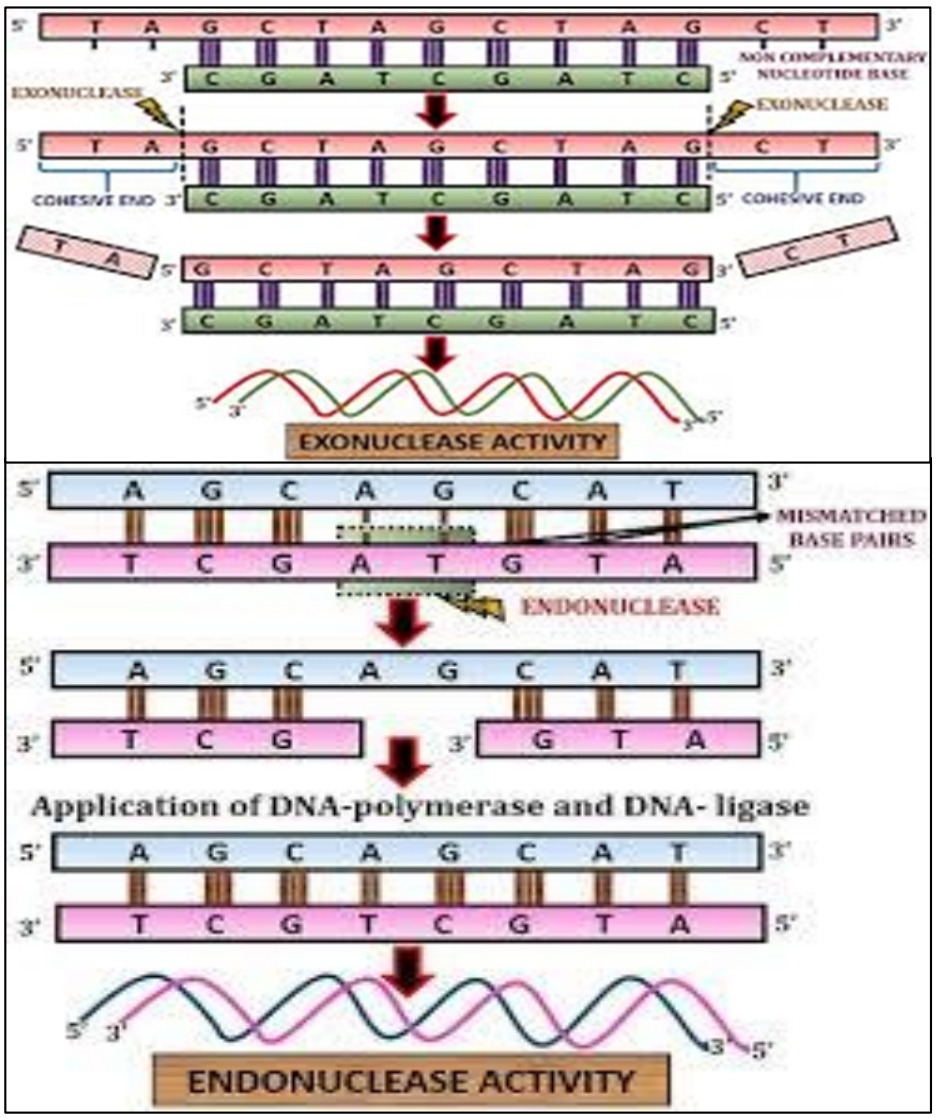


Some nucleases are DNA specific (DNase), some are RNA specific (RNase), and some degrade both DNA and RNA. Nucleases can also have a strong preference for either double-stranded or single-stranded nucleic acids. The nucleases are also characterized according to whether they degrade from an end of a nucleic acid molecule (exonuclease) or from within the nucleic acid molecule (endonucleases, restriction enzymes).

Furthermore, exonucleases are specific for either the 3′ end or the 5′ end of a molecule. Exonucleases degrade DNA by removing a single base per hydrolysis event and typically release mononucleotides. Endonucleases cleave nucleic acids internally

and leave either a (3′ hydroxyl and 5′ phosphate) or a (5′ hydroxyl and 3′ phosphate) at the site of cleavage.





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