Genetic engineering

Genetic engineering: the process of using recombinant DNA technology to alter (changing or manipulating) their genetic makeup of an organisms it involves the direct manipulation of one or more genes most often a gene from another species is added to an organisms' genome to give it desired phenotype.

Recombinant DNA: fragments of DNA from two different species such as bacterium and a mammals spliced together in the laboratory into single molecule .

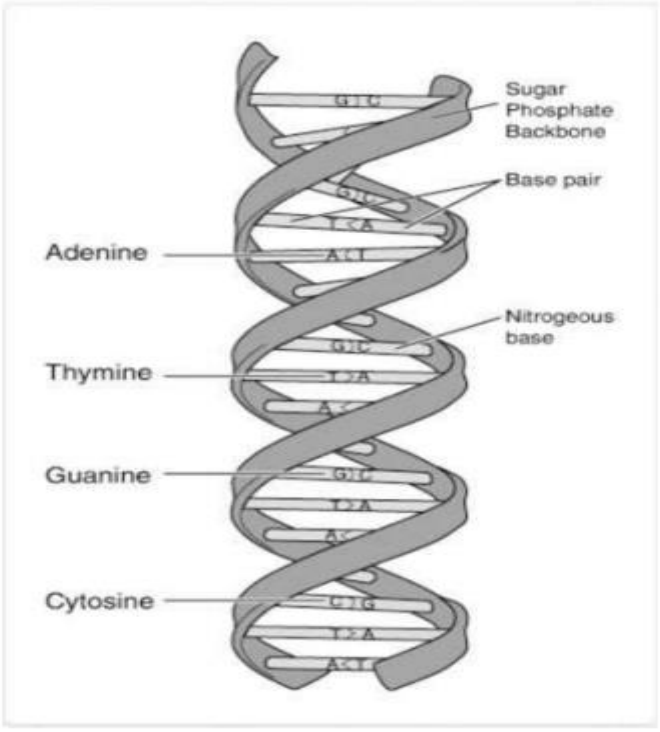
-What is a Gene?

Gene defined as the basic unit of heredity a sequence of DNA nucleotides on a chromosome that encodes A protein tRNA or rRNA molecule.

Base pair: bp two complementary nitrogenous molecules that are connected by hydrogen bonds.

A :Adenine
T: Thiamine
G: Guanine
C: Cytosine

Q/ When we say base pair and when we say nucleotide?
Answer: A nucleotide is composed of a phosphate group, 5-carbon sugar, and nitrogenous base. A nitrogenous base is formed by either a single ring pyrimidine or a double ring purine.



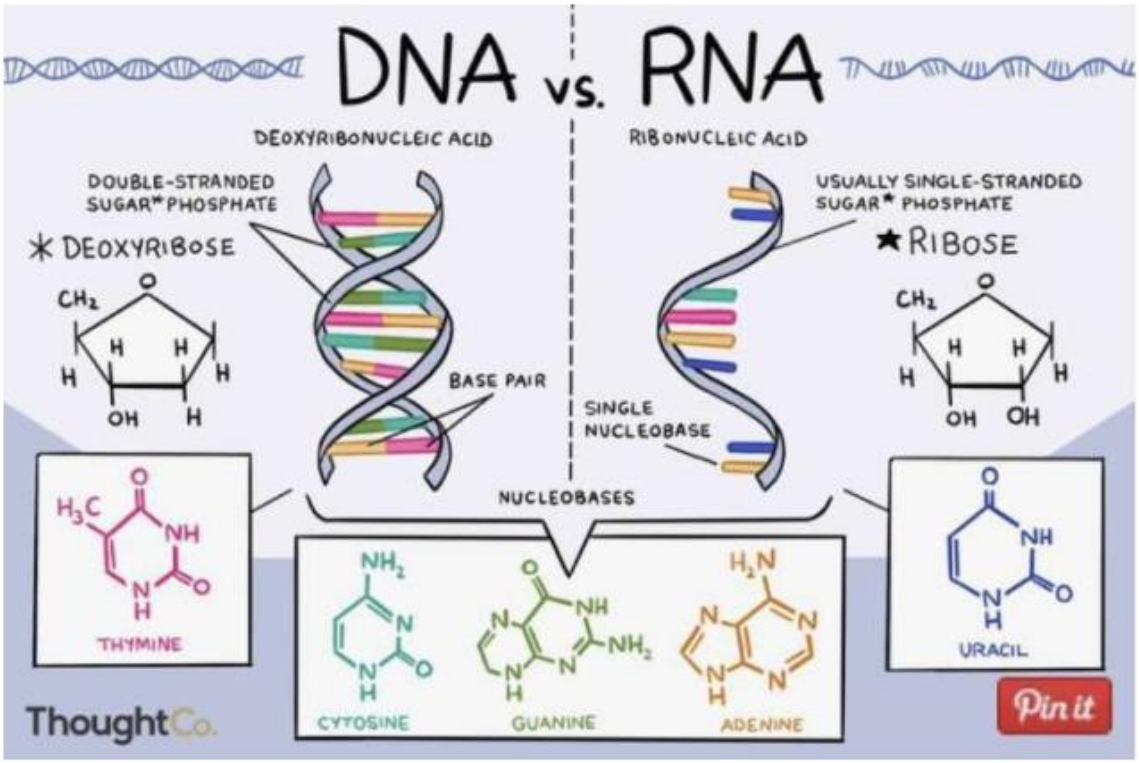
DNA structure

Types of bonds in DNA molecules:

1. **Hydrogen bond** between strands of dsDNA

2. **Glycosidic bond** between sugar (deoxyribose in DNA or ribose in RNA) and nitrogen bases.

3. **Phosphodiester bond** between two nucleotides in same strand.

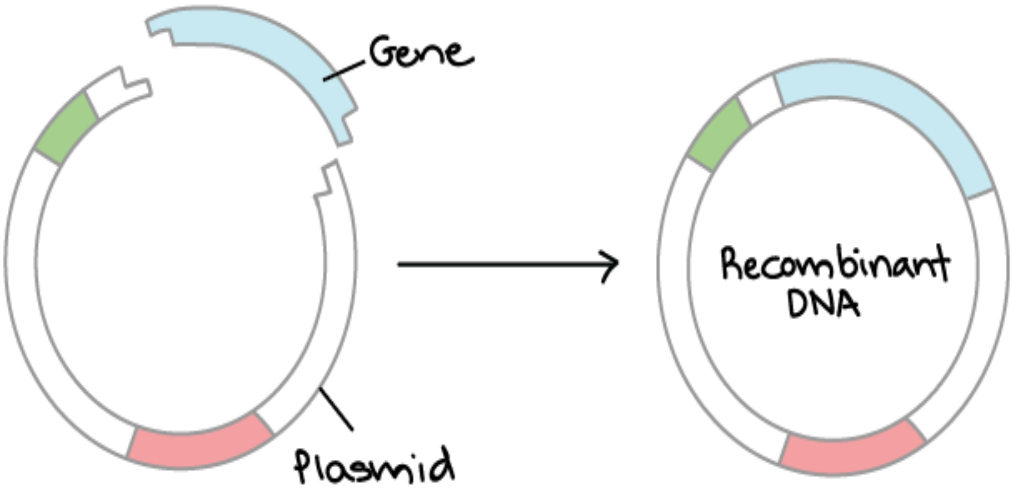


Start codon: ATG

Stop codons:TAA, TGA, TAG

What is Molecular cloning?

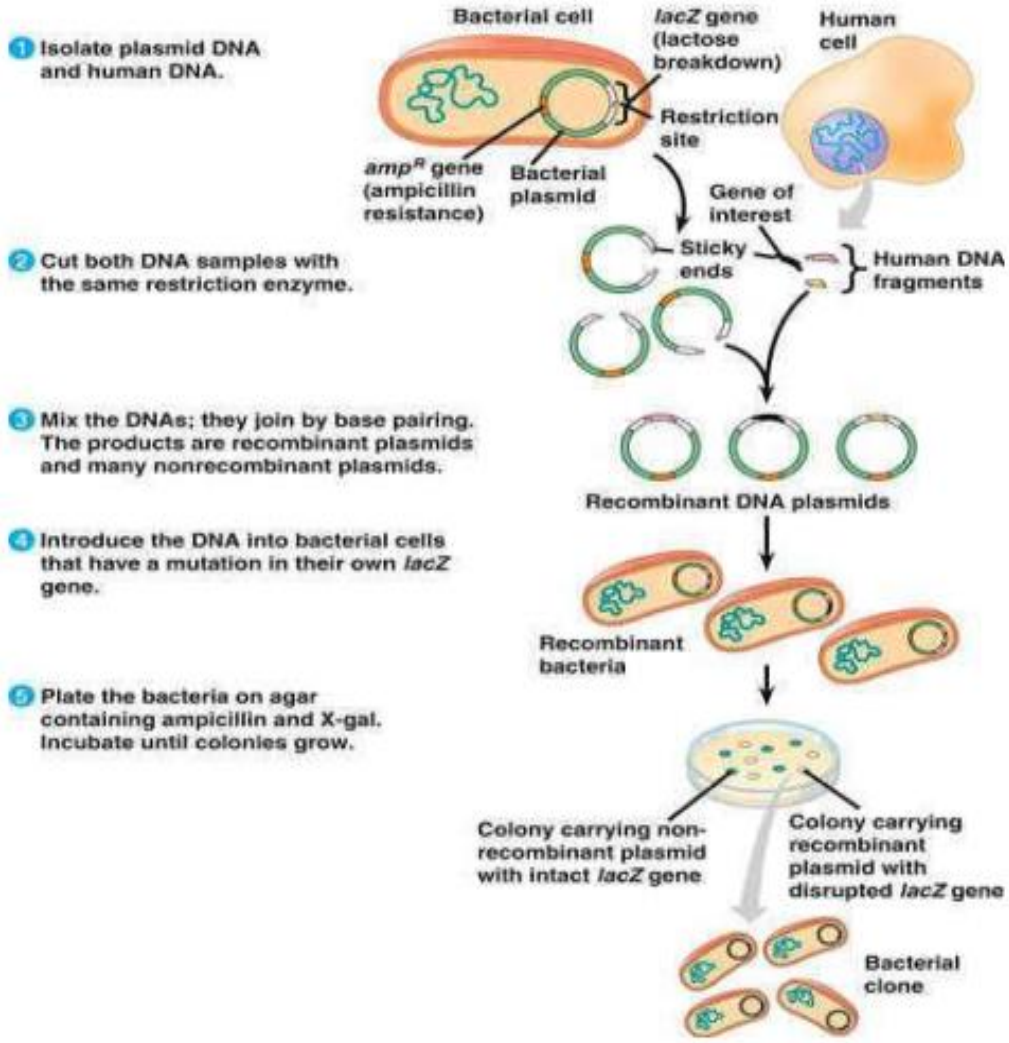
Molecular cloning is an essential technique in molecular biology and biotechnology laboratories. It is a useful method to study a gene, modify the gene, reintroduce the modified gene into the natural host or another host, or to produce protein. This can be achieved by combining a piece of DNA into a plasmid (a recombinant DNA) to make more identical recombinant DNA in a living host. The insertion is done using enzymes that "cut and paste" DNA, and it produces a molecule of **recombinant DNA**, or DNA assembled out of fragments from multiple sources.



Steps of cloning process

Cloning of any gene could be summarized by the following seven steps:

1- Isolation and purification of foreign DNA or target DNA (passenger DNA).
2- Suitable cloning vector isolation and purification, most of used vectors are plasmid or viruses.
3- Cutting DNA molecules by suitable restriction enzymes (R.E.).
4- Joining DNA molecules by DNA ligase.
5- Monitoring the cutting and joining of DNA molecules.
6- Transformation or transfection (introducing the recombinant molecules into the host).
7- Isolation and characterization of recombinants.



Materials for cloning:

To start with, it is important to prepare some important materials for cloning:

1- DNA Fragment

The source for a DNA fragment or "DNA insert" can be genomic DNA, complementary DNA, plasmid DNA, PCR product, or synthetic DNA. The DNA insert must contain particular sequences at the end of the fragments compatible with the prepared vector. You can add these particular sequences onto your DNA insert by PCR.

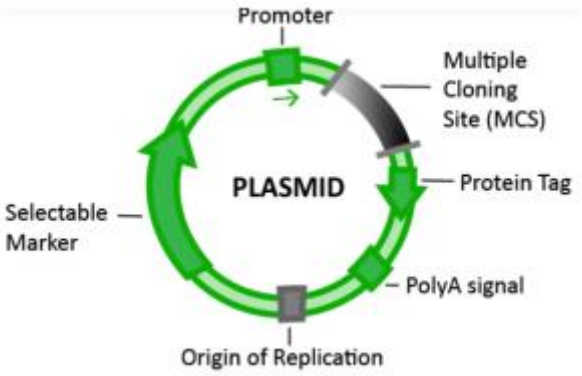
2- Vector

A vector is a DNA molecule which can carry a DNA insert to generate a recombinant DNA and replicate in a particular host. Examples of vectors are:

1- Cosmid: a large DNA vector containing λ phage DNA sequence. It can carry large DNA fragment up to 45 kilobases into the host.

2- **Artificial Chromosomes**: a large DNA vector which can perform the functions of a chromosome.

3- Plasmid: a small extrachromosomal circular DNA which can replicate in a cell, commonly used in cloning.



3- Competent Cells

After a DNA fragment is incorporated into the plasmid vector, the next cloning step is to perform a transformation step. In this transformation step, the recombinant DNA is introduced into the competent cell by chemical reaction or electroporation. Competent cells are cells which are temporarily permeable to extracellular DNA. The host organisms which are commonly used in the laboratories are Escherichia coli and Saccharomyces cerevisiae.

4- Selective Medium

It is a growth medium containing a selective agent to grow the transformed host. When you choose antibiotic selection for cloning, your growth medium must contain antibiotics. The most common antibiotics used for selection are Ampicillin, Kanamycin, and Chloramphenicol.