Principles of DNA Manipulation

Genetic engineers have developed genetic recombination techniques to manipulate gene sequences in plants, animals and other organisms to express specific traits. Applications for genetic engineering are increasing as engineers and scientists work together to identify the locations and functions of specific genes in the DNA sequence of various organisms. Once each gene is classified, engineers develop ways to alter them to create organisms that provide benefits such as cows that produce larger volumes of meat, fuel- and plastics- generating bacteria, and pest-resistant crops.

Genetic engineering is the process of transferring specific genes from the chromosome of one organism and transplanting them into the chromosome of another organism in such a way that they become a reproductive part of the new organism. The process that produces the resulting recombinant DNA involves four steps:

1. The desired DNA is cleaved from the donating chromosome by the action of restriction enzymes, which recognize and cut specific nucleotide segments, leaving a "sticky end "on both ends. The restriction enzymes also splice the receiving chromosome in a complementary location, again leaving "sticky ends" to receive the desired DNA.
2. The desired DNA fragment is inserted into a vector, usually a plasmid, for transfer to the receiving chromosome. Plasmids are an ideal vector because they replicate easily inside host bacteria and readily accept and transfer new genes. Plasmids are circular DNA molecules found in the cytoplasm of

bacteria that bond with the desired DNA fragment with the help of the joining enzyme, DNA ligase, to create the resulting recombinant DNA.

3. When the host cell reproduces, the plasmids inside also reproduce, making multiple clones of their DNA. Because the plasmid DNA contains the desired as well as unwanted DNA clones, the entire product is referred to as a gene library. The desired gene is similar to one book in that library.
4. To recover the desired DNA, the current technology is to screen unwanted cells from the mixture and then use gel electrophoresis to separate the remaining genes by movement on an electric grid. Gel electrophoresis uses a positively charged grid to attract the negatively charged DNA fragments, thereby separating them by size, because the smaller ones will migrate the most. Radioactive or fluorescent probes are added, which attract and bind with the desired DNA to produce visible bands. Once isolated, the DNA is available for commercial use.

The term genetic engineering initially referred to various techniques used for the modification or manipulation of organisms through the processes of heredity and reproduction. As such, the term embraced both artificial selection and all the interventions of biomedical techniques, among them artificial insemination, in vitro fertilization (e.g., "test-tube" babies), cloning, and gene manipulation. In the latter part of the 20th century, however, the term came to refer more specifically to methods of recombinant DNA technology (or gene cloning), in which DNA molecules from two or more sources are combined either within cells or in vitro and are then inserted into host organisms in which they are able to propagate.

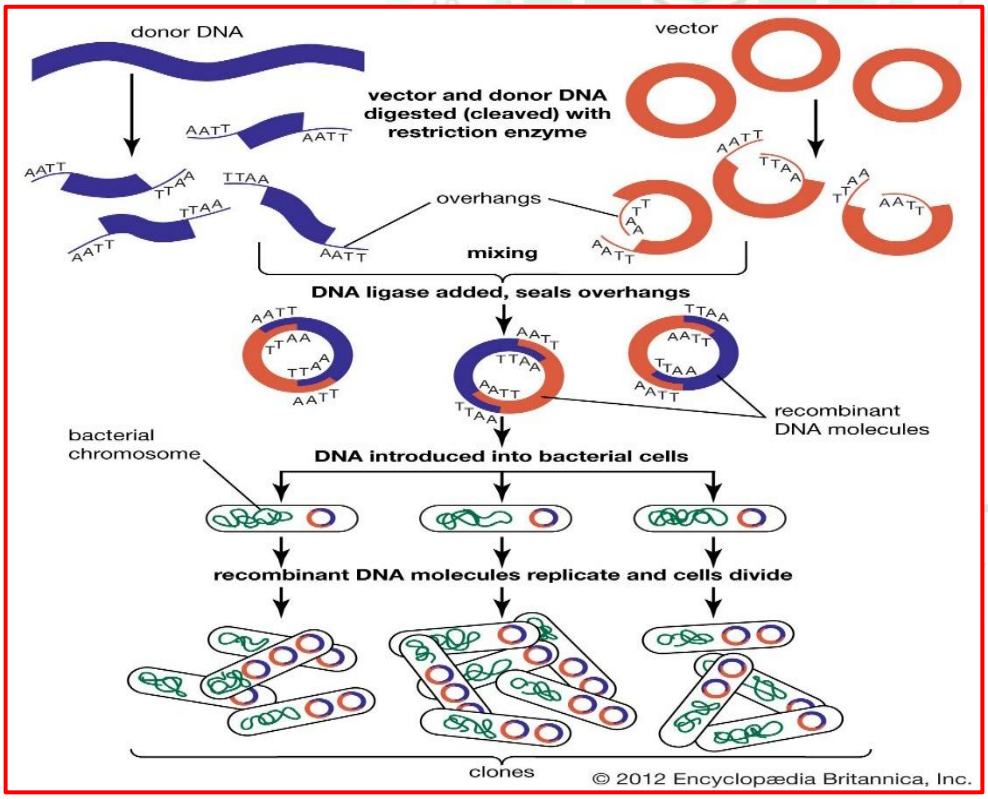
Recombinant DNA Technology

Recombinant DNA technology, joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic

combinations that are of value to science, medicine, agriculture, and industry. Since the focus of all genetics is the gene, the fundamental goal of laboratory geneticists is to isolate, characterize, and manipulate gene.

Although it is relatively easy to isolate a sample of DNA from a collection of cells, finding a specific gene within this DNA sample can be compared to finding a needle in a haystack. Consider the fact that each human cell contains approximately 2 meters (6 feet) of DNA. Therefore, a small tissue sample will contain many kilometers of DNA.

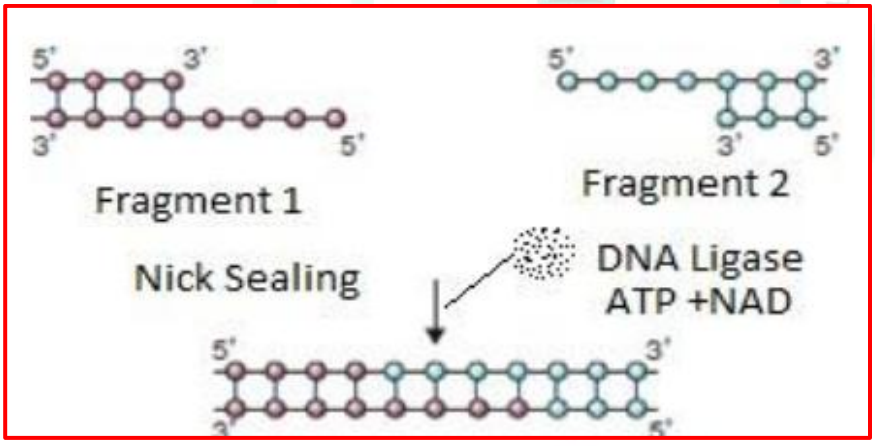
However, recombinant DNA technology has made it possible to isolate one gene or any other segment of DNA, enabling researchers to determine its nucleotide sequence, study its transcripts, mutate it in highly specific ways, and reinsert the modified sequence into a living organism.

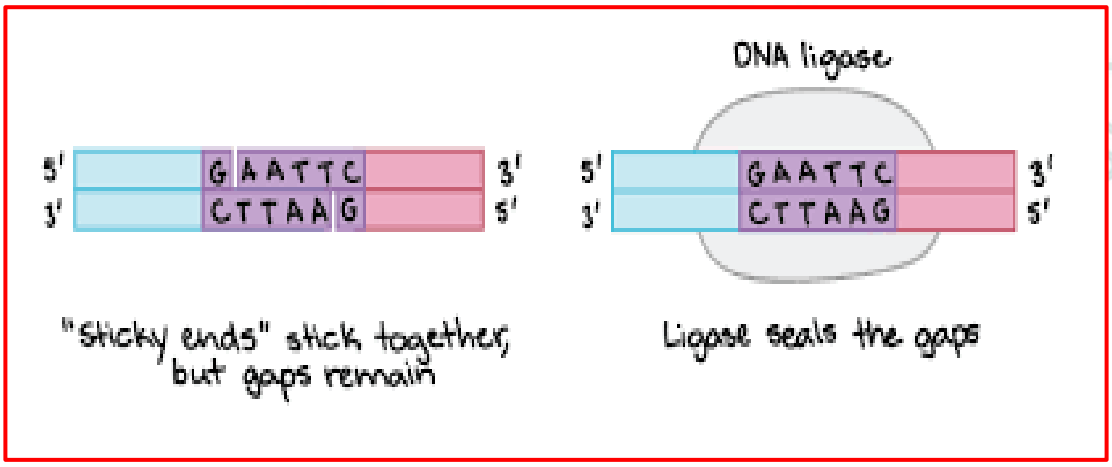


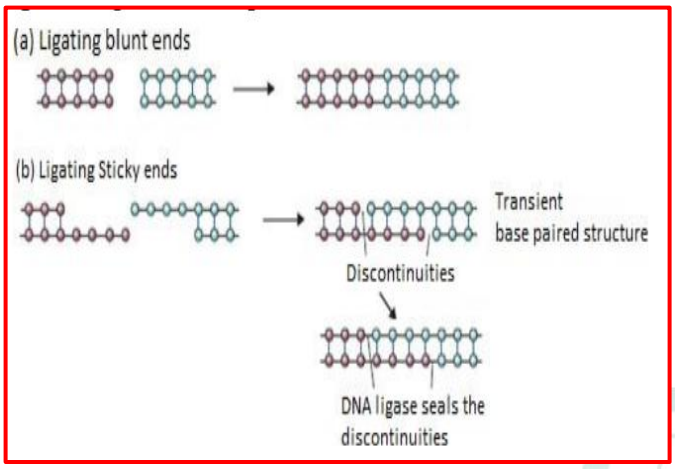
Enzymes use in Genetics Engineering:

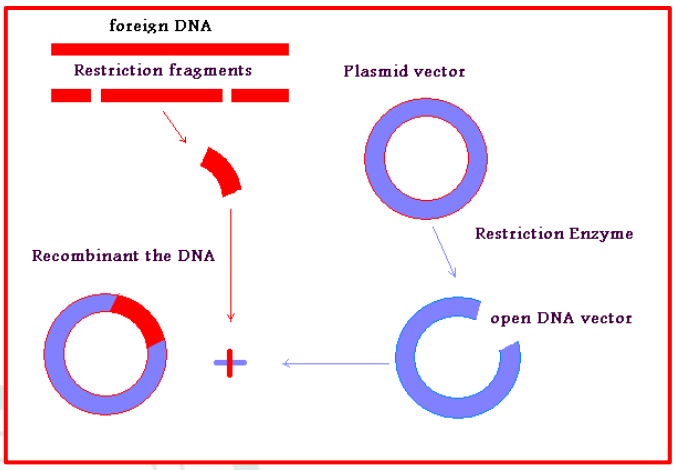
a. Ligases:

In molecular biology and genetics engineering, ligase enzymes are called molecular glue or molecular paste. The action of ligase is opposite to that of RE restriction endonuclease. In the cell the function of DNA ligase is to repair single-stranded breaks (“discontinuities”) that arise in double-stranded DNA molecules during DNA replication. DNA ligases from most organisms can also join together two individual fragments of double-stranded DNA. Ligase catalyzes the ligation or joining of nucleic acid fragments by forming a phosphodiester bond between the directly adjacent 3’-OH and 5’-P termini.









Nucleases

Nucleases are enzymes that hydrolyze nucleic by breaking the phosphodiester bond that holds the nucleotides together. Nucleases are most important classes of enzymes involved in molecular biology and genetic engineering. Nucleases are usually further divided into endonucleases and exonucleases. Restriction enzymes are good examples of endonucleases, which cut within a DNA strand. A second group of nucleases, whichdegrade DNA from the termini of the molecule, are known as exonucleases.

DNA polymerases

DNA polymerases are enzymes that synthesize DNA molecules from deoxyribonucleotides, the building blocks of DNA. These enzymes are essential to DNA replication and usually work in pairs to create two identical DNA strands from a single original DNA molecule. During this process, DNA polymerase “reads” the existing DNA strands to create two new strands that match the existing ones.

Reverse transcriptase

Reverse transcriptase is a common name for an enzyme that functions as a RNA- dependent DNA polymerase. They are encoded by retroviruses, where

they copy the viral RNA genome into DNA prior to its integration into host cells.

Vectors:

Gene cloning vector deals with the transfer of the desirable gene into a host cell. The cell which received desired gene is call host cell.

Characteristics of a cloning vectors

1. it must be small in size
2. It must be self-replicating inside host cell
3. It must possess restriction site for Restriction Endonuclease enzymes
4. Introduction of donor DNA fragment must not interfere with replication property of the vector
5. It must possess some marker gene such that it can be used for later identification of recombinant cell
6. it must possess multiple cloning site

Types of cloning vectors used in gene cloning:

1. Plasmid -psBR322
2. Bacteriophage-Lambda phage,
3. Bacterial artificial chromosome (BAC):
4. Yeast artificial chromosome (YAC):
5. Virus-SV40,
6. Cosmid vectors