Biotechnology

Biotechnology deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.

For example, in vitro fertilization leading to a 'test-tube' baby, synthesizing a gene and using it, developing a DNA vaccine or correcting a defective gene, are all part of biotechnology.

PRINCIPLES OF BIOTECHNOLOGY

(i) **Genetic engineering:** Techniques to alter the chemistry of genetic material (DNA and RNA), to introduce these into host organisms and thus change the phenotype of the host organism.

(ii) **Bioprocess engineering:** Maintenance of sterile (microbial contamination-free) ambience in chemical engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

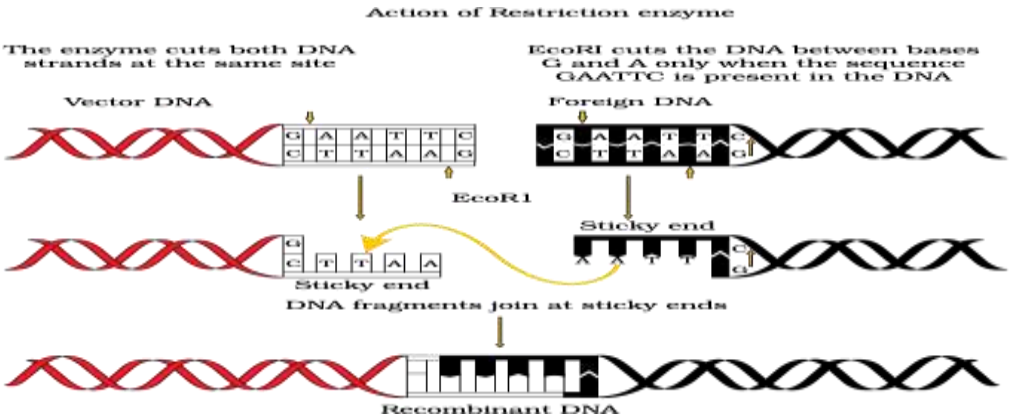
TOOLS OF RECOMBINANT DNA TECHNOLOGY

Restriction Enzymes

First restriction endonuclease–Hind II, today we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria each of which recognize different recognition sequences.

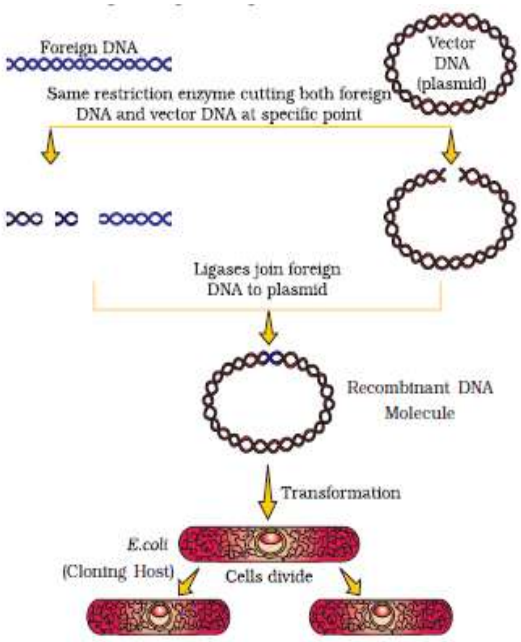
Restriction enzymes belong to a larger class of enzymes called nucleases. These are of two kinds; exonucleases and endonucleases. Exonucleases remove nucleotides from the ends of the DNA whereas; endonucleases make cuts at specific positions within the DNA.

Each restriction endonuclease functions by 'inspecting' the length of a DNA sequence. Once it finds its specific recognition sequence, it will bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar -phosphate backbones. Each restriction endonuclease recognizes a specific palindromic nucleotide sequences in the DNA.



Steps in formation of recombinant DNA by action of restriction endonuclease enzyme – EcoRI

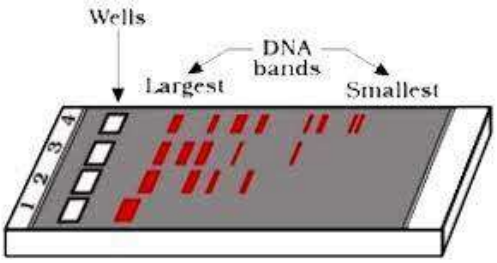
Restriction endonucleases are used in genetic engineering to form 'recombinant' molecules of DNA, which are composed of DNA from different sources/genomes.



Diagrammatic representation of recombinant DNA technology

Separation and isolation of DNA fragments: fragments of DNA can be separated by a technique known as gel electrophoresis.

The separated DNA fragments can be visualized only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation.



A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

PROCESSES OF RECOMBINANT DNA TECHNOLOGY

(1) Isolation of the Genetic Material (DNA)
(2) Cutting of DNA at Specific Locations
(3) **Amplification of Gene of Interest using PCR –** PCR stands for Polymerase Chain Reaction. In this reaction, multiple copies of the gene (or DNA) of interest is synthesized in vitro using two sets of primers (small chemically synthesized

oligonucleotides that are complementary to the regions of DNA) and the enzyme DNA polymerase.

(4) **Insertion of Recombinant DNA into the Host Cell/Organism –** Recombinant DNA bearing gene for resistance to an antibiotic (e.g., ampicillin) is transferred into E. coli cells, the host cells become transformed into ampicillin-resistant cells. The ampicillin resistance gene in this case is called a selectable marker.

(5) **Obtaining the Foreign Gene Product –** foreign gene gets expressed under appropriate conditions. Small volume cultures cannot yield appreciable quantities of products. To produce in large quantities, the development of bioreactors. in which raw materials are biologically converted into specific products.

(6) **Downstream Processing –** separation and purification.