

BIOTECHNOLOGY: PRINCIPLES AND PROCESSES

The European Federation of Biotechnology (EFB) definition of biotechnology is as follows: 'the integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'.

- **Genetic Engineering** - it is the technique of altering the chemistry of genetic material (DNA and RNA) to introduce these into host organisms and thus changes the phenotype of the host organism.
- In chromosome There is a specific DNA sequence called the origin of replication, which is responsible for initiating replication. In genetic engineering the foreign DNA is linked with the origin of replication, so the foreign DNA can replicate and multiply itself in the host organism, which is also known as cloning or making multiple identical copies of any template DNA.
- **Stanley Cohen and Herbert Boyer in 1972 isolated the antibiotic resistance gene**, by cutting out a piece of DNA from a plasmid which was making multiple identical copies of any template DNA. The cutting of DNA at specific locations became possible with the discovery of the so-called molecular scissors - restriction enzymes.

Steps of Genetically Modifying an organism-

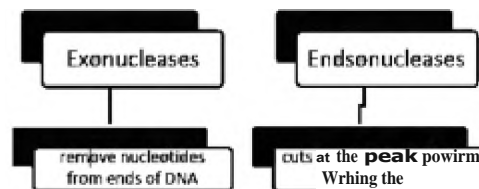
- I. Identification of DNA with desirable genes.
- II. Introduction of the identified DNA into the host.
- III. Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

Tools of Recombinant DNA Technology Includes

- Restriction Enzymes
- Polymerase enzymes
- Ligases
- Vectors host organisms

- **Restriction enzymes are responsible for restricting the growth of bacteriophage in E. coli** was called as restriction endonuclease. The first restriction endonuclease - Hind II always cut DNA molecule at a particular point by recognizing a specific sequence of six base pairs, called recognition sequence. Restriction enzymes belong to a group of enzymes called nucleases.

Nu LI EeNr:....



Each restriction endonuclease recognises a specific palindromic nucleotide sequence in the DNA. Palindromes are group of letters that form the same words when read both forward and backward for example "MALYALANr_

- Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome site between the same two bases on the opposite strands having sticky strand_ The stickiness of the strands facilitates the action of the enzyme DNA ligase.

Separation and isolation of DNA fragments

The fragment of DNA obtained by cutting DNA using restriction enzyme is separated by technique called gel electrophoresis. Negatively charged DNA fragments can be separated by forcing them to move towards the anode under an electric field through medium_ DNA fragments separate according to their size through sieving effect provided by agarose gel.

The separated DNA fragment can be visualized after staining the DNA with ethidium bromide followed by exposure to UV light. Separated bands of DNA are separated from agarose gel and extracted from gel, called elution. The DNA fragment purified this way is used for various applications.

Cloning Vector

Plasmids and Bacteriophages are commonly used vectors for cloning. They have ability to replicate within bacterial cells independent of the control of chromosomal DNA.

Following features are required to facilitate cloning into a vector-

{a} Origin of replication (ors)

(b) Selectable marker

{c} Cloning sites

{d} Vector for cloning genes in plants and animals



Agrobacterium tumefaciens (pathogen of dicot plant) is able to deliver a piece of DNA known as "T-DNA" to transform normal plant cells into a tumor and direct these tumor cells to produce the chemicals required by the pathogen.

Competent host

Now a day, DNA is directly introduced into host cell by microinjection in which DNA is directly injected into the nucleus of an animal cell. Bacterial or gene gun is also used to inject DNA into target host.

Production of Recombinant DNA Technology

Recombinant DNA technology involves several steps in specific sequence-

1. Isolation of DNA
2. Fragmentation of DNA by restriction endonucleases
3. Isolation of a desired DNA fragment
4. Ligation of the DNA fragment into vector
5. Transforming the recombinant DNA into the host
6. Culturing the host cells in a medium at large scale
7. Extraction of the desired product

Downstream Processing involves processes that make the product obtain ready for marketing. This process includes separation and purification called as downstream processing. Suitable preservatives are added to it and send for clinical trial in case of drug before releasing to market for public use.