

Biotechnology : Principles and Processes

Biotechnology is the field of molecular biology dealing with the techniques of using live microorganisms to produce products that are useful to humankind.

Genetic engineering

- It is a technique used by scientists for manipulating genetic material of living organisms.
- It involves artificial synthesis, isolation, modification and transfer of genetic material into a host organism to alter its phenotype.
- DNA cloning/Gene cloning: It is the method of making multiple identical copies of a single gene.
- Plasmid: It is a small, circular, extra-chromosomal genetic material that is capable of self replication.
- Characteristics of plasmid –
 - Has an origin of replication
 - Has a selectable marker
 - One or few cloning sites

Recombinant DNA technology

- It is a set of techniques for altering DNA. It includes the recognition and cloning of genes, the study of the expression of cloned genes and the production of a large number of gene products.

Construction of a Recombinant DNA

- Plasmid (autonomously replicating, circular, extra-chromosomal DNA) is isolated.
- Plasmid DNA is cut with a specific restriction enzyme at specific locations.
- The DNA of interest (to be inserted) is also cut with the same restriction enzyme.
- The DNA of interest is hybridised with the plasmid with the help of DNA ligase to form a **Recombinant DNA**.
- Recombinant DNA is then transferred into host for cloning.

i. Tools of recombinant DNA technology –

i. They are also known as molecular scissors.

- There are two types of restriction enzymes –

- **Endonuclease:** It removes the nucleotide from DNA fragments at specific positions within the DNA.
- **Exonuclease:** It makes a cut at the 5' or the 3' end of DNA.
- **Naming of restriction endonuclease enzyme (for example, in EcoRI)**
 - First letter represents the genus of bacteria
 - The next two letters represent the species from which the enzyme is isolated.
 - The second capital letter represents the name of the strain.
 - The roman number indicates the order in which the enzyme is isolated.

Restriction endonuclease recognises a particular sequence known as **palindromic sequence**.

- **Palindrome** is a sequence of base pairs that reads the same on the forward strand and the reverse strand.
- **Action of the restriction endonuclease enzyme**
 - The recognition sequence for the restriction enzyme EcoRI is
 - EcoRI recognises this particular palindromic sequence and makes a cut between the adjacent guanine and adenine nucleotides, thereby producing overhanging, single-stranded pieces called sticky ends.
 - The generated sticky ends can be joined by DNA ligase to form recombinant DNA molecule.
 - **Gel electrophoresis:** It is a technique used for separating and isolating the DNA fragments generated by the action of the endonuclease enzyme.
 - Electric field is applied to the electrophoresis matrix (commonly agarose gel) and negatively charged DNA moves towards anode.
 - Fragments get separated according to their size.

Cloning vectors: Examples include plasmids and bacteriophage. Cloning vectors consist of origin of replication (ori), selectable marker and one or few cloning sites.

- **Insertional inactivation:** It is a technique to select recombinant DNA on the basis of their ability to produce colour in the presence of chromogenic substrate.
- *Agrobacterium tumefaciens* acts as a vector for cloning genes in plants.
- *Retroviruses* can be disarmed and used as cloning vectors in animals.

(iii) Transformation: It is the method of uptaking foreign DNA particle into a bacterial cell. The following methods are used for transformation.

- **Microinjection:** It involves the injecting of recombinant DNA directly into the host cell using micropipettes.
- **Biolistics or gene gun:** It involves the bombardment of gold or tungsten particles coated with DNA into a plant cell.

Steps involved in recombinant DNA technology –

- **Isolation of genetic material:** This is the method of obtaining purified DNA. It is achieved by lysozyme in bacteria, by cellulase in plant cell and by chitinase in fungus.
- **Cutting of DNA at specific location:** It is performed by using restriction endonuclease and then separating the DNA fragments using agarose gel electrophoresis.
- **Amplification of gene:** The gene is amplified using polymerase chain reaction.
 - **Polymerase chain reaction** is a method of amplifying specific regions of DNA strand. It involves three steps – denaturation, annealing and extension.
 - This technique requires a set of primers and the thermostable DNA polymerase enzyme.
 - The thermostable DNA polymerase enzyme is isolated from the bacterium called *Thermus aquaticus*. The enzyme is resistant to denaturation by heat treatment.
- **Ligation of DNA fragments into the vector for cloning**
- **Insertion of recombinant DNA into recipient cells**
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 - It is done by making the cell competent to take up DNA from its surroundings. This method is known as transformation.
- **Obtaining the gene product**
 - It involves culturing host cells in a medium and then extracting the desired product.
 - **Bioreactors:** Used for large scale production of desired protein; two types of bioreactors used –
 - Simple stirred-tank bioreactor
 - Sparged stirred-tank bioreactor
- **Downstream processing:** It is the process of separation and purification of recombinant protein so that product is ready for marketing.
- **Diagrammatic representation of recombinant DNA technology**