3. Biotechnology Process and Application

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)**

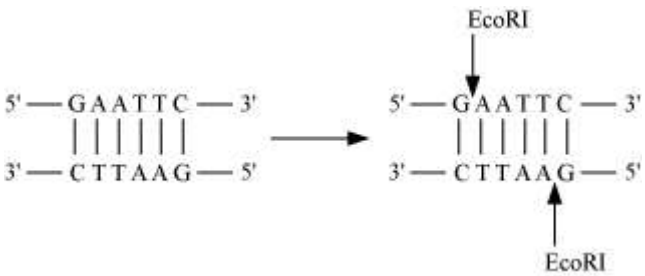
- First letter represents the genus of bacteria
    - The next two letters represent the species from which the enzyme is isolated.
    - The second capital letter represents the name of the strain.
    - The roman number indicates the order in which the enzyme is isolated.

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 electrophrosis 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 tumifaciens* acts as a vector for cloning genes in plants.
- *Retroviruses* can be disarmed and used as cloning vectors in animals.

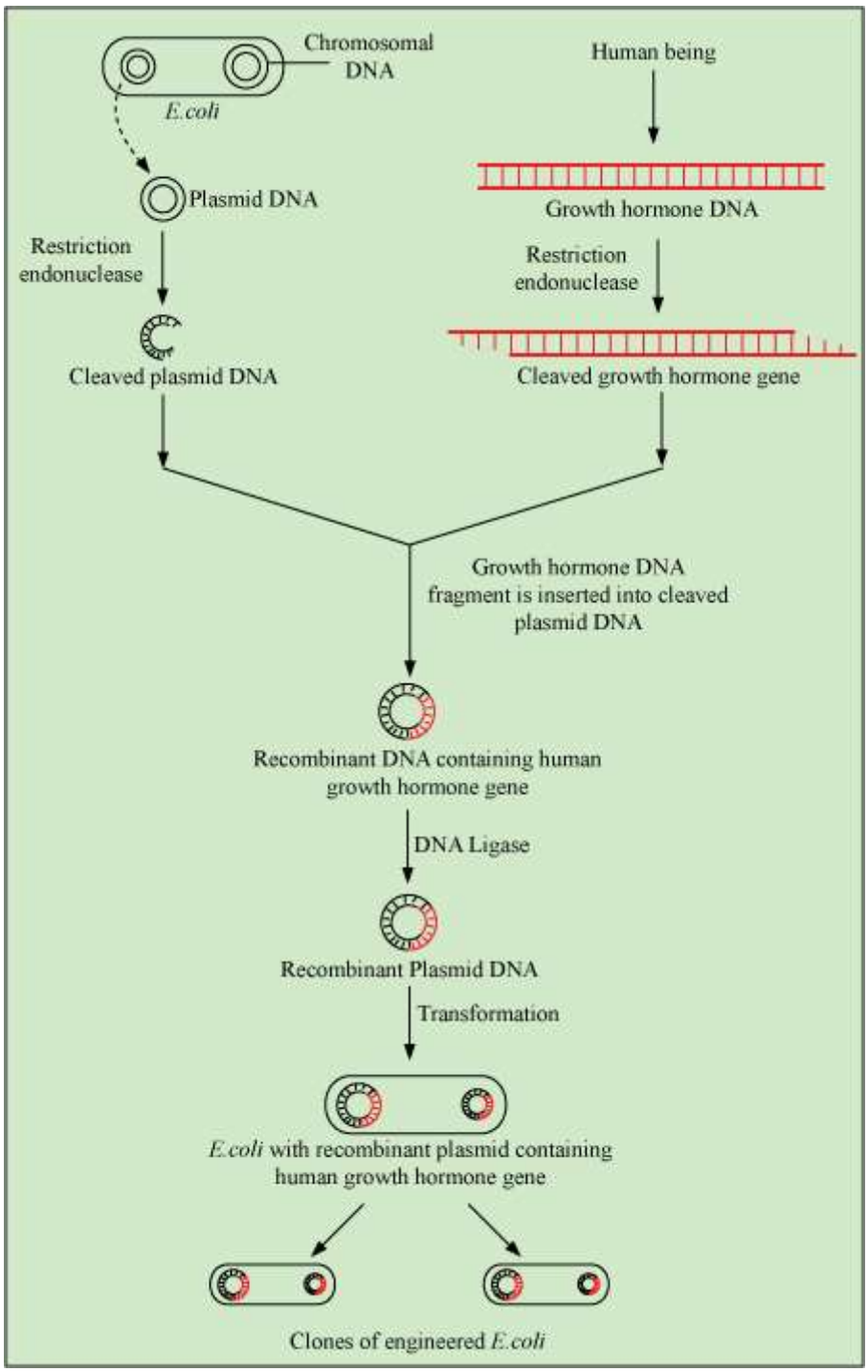
(iii) Transformation: It is the method of uptaking foreign DNA particle into a bacterial cell. The following methods are used for transformation.

- **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**
    - 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**



- **Biotechnology** deals with genetically modifying living organisms (microbes, plants and animals) to produce several useful products.
- **Applications of biotechnology –**
  - **Therapeutics**
  - **Diagnostics**
  - **Genetically modified crops**
  - **Food processing**
  - **Bioremediation**
  - **Waste treatment**

- **Energy production**

- Genetically modified organisms (GMO) are produced by the manipulation of the genetic material of organisms.
- **Genetically modified crops** have several advantages. Genetic modification increases a crop's tolerance to abiotic factors; it increases the efficiency of mineral uptake by the roots of a crop, etc. It also decreases the post-harvest losses in crops.
- The bacterium, *Bacillus thuriengiensis* is used for producing Bt-toxin.
- It acts as a bio-pesticide in plants.

Isolation of toxin-producing gene (Bt) from bacteria

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Insertion of Bt gene into plants

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Gene provides resistance against insects

- The Bt-toxin gene is insect-group specific and is coded by the 'cry' gene.
  - Proteins coded by *cryIAc* and *cryIIAb* make the plant resistant for cotton bollworms.
  - Proteins coded by *cryIAb* make the plant resistant to corn borer.
- **RNA-interference (RNAi):** It is a gene-silencing mechanism that prevents translation of mRNA. It is a method of cellular defence. It involves –

Introduction of DNA

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Formation of sense and antisense strand in host cell

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Formation of dsRNA

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Initiation of RNA interference

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Slicing of mRNA

- **Transgenic animals:** They carry foreign genes that are purposely introduced into their genome; for example, mice, sheep, cows, fish, rabbit.
- **Transgenic animals are used for –**
  - Studying the regulation of genes
  - Understanding the development of diseases
  - Producing useful biological products
  - Testing the safety of vaccines
  - Testing the toxicity of drugs
- The manipulation of microbes/plants/animals has raised certain ethical issues.
- GEAC (Genetic Engineering Approval Committee) in India takes decisions regarding the validity of GM researches and the safety regarding genetically modified organisms.

- **Biopiracy:** It is the theft or robbery of biological resources without the knowledge of the concerned authority.
- **Patent:** It is an exclusive right which is granted for an invention, which could be a product or a process that provides, in general, a new way of doing something, or offers a new technical solution to a problem.
- Patents are awarded on the basis of novelty, non-obviousness, and utility.