CHAPTER

6 Biotechnology: Principles and Processes

INTRODUCTION

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

Principles of Biotechnology/Core Techniques Involved in Modern Biotechnology

Parameters	Genetic engineering	Bioprocess engineering
Definition	Techniques to alter the chemistry of genetic material to introduce these into host organisms and thus change the phenotype of host organism	Maintenance of sterile ambience in **chemical engineering processes** to enable growth of only the desired microbe/ eukaryotic cell in large quantities
Include	Creation of rDNA Gene cloning Gene transfer	Manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.
The ability to multiply copies of antibiotic resistance gene in *E. coli* was called **cloning** of antibiotic resistance gene in *E. coli*.		

Three Basic Steps in Genetically Modifying Organisms (GMO)

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

KEY TOOLS OF RECOMBINANT DNA TECHNOLOGY

- **(1) Enzymes**
- **(2) Vectors**
- **(3) Competent host cells**

ENZYMES

Restriction Endonuclease

- More than 900 restriction enzymes have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences.

First restriction endonuclease-***Hin*dII:** Isolated and characterised **in 1968** later, recognises sequence of **6** bp.

The first recombinant DNA was constructed by **Stanley Cohen** and **Herbert Boyer**, **1972**.

Functions:

- Cuts the two strands of dsDNA at specific points in their sugar-phosphate backbones and leaves single stranded portions at the ends.

Ligase

- When source DNA and vector DNA are cut by the same restriction enzyme, the resultant DNA fragments have the same kind of sticky-ends .
- Sticky ends are named so because they form hydrogen bonds with their complementary cut counterparts.
- Stickiness facilitates the action of the enzyme DNA ligase.

CLONING VECTORS

Vectors are vehicles for delivering foreign DNA into recipient cells.

Features of cloning vectors:

- **Origin of Replication (*ori*)**
- **Selectable Marker**
- **Cloning Sites/Restriction Sites**

Transformation: Procedure through which piece of foreign DNA is introduced in a host bacterium.

- **Insertional inactivation:** Insertion of gene of interest within antibiotic resistance gene/selectable marker results in inactivation.

All transformants are not recombinants but all recombinants are transformants.

- **Non-Transformants:** Hosts that do not take up the vector DNA (Non-recombinant).
- **Transformants:** Hosts that take up the vector DNA (Recombinant or Non-recombinant).
- **Recombinants:** Transformant hosts that take up the recombinant DNA (Vector DNA with desired DNA).
- **Non-Recombinants:** Transformant hosts that take up the nonrecombinant DNA (Vector DNA without desired DNA)
- rop → Codes for the proteins involved in the **replication** of the plasmid.

Plasmids as vectors:

- Extra-chromosomal, circular, double-stranded DNA.
- Replicate independent of the control of chromosomal DNA (autonomously).
- They may have 1 or 2 copies per cell or even 15-100 copies per cell.

OTHER CLONING VECTORS

Ti-plasmid of *Agrobacterium tumefaciens*

- *Agrobacterium tumefaciens*, a pathogen of several **dicot plants** is able to deliver a piece of DNA known as **'T-DNA'** to transform normal plant cells into a tumor and direct the tumor cells to produce the chemicals required by the pathogen.
- **Disarmed tumour inducing (Ti) plasmid** is used which is no more pathogenic to the plants but is still able to use the mechanism to deliver the genes of our interest into varieties of plants.

Bacteriophages

- High copy number than plasmid.

Retroviruses

- Retroviruses in animals have the ability to transform normal cells into cancerous cells.
- Disarmed retroviruses are used to deliver desirable genes into animal cells.

Methods of Transformation

1. **Micro-injection**
   - Recombinant DNA is directly injected into the nucleus of an animal cell.
2. **Biolistic/Gene gun**
   - Plant cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA.
3. **Heat-shock method**
4. **Disarmed pathogen vectors**

Competent Host for Transformation with recombinant DNA

- DNA is hydrophilic, so it can not pass through cell membranes.
- In order to force cell to take up alien DNA/rDNA, it must first be made 'competent' by treating with **ice cold calcium chloride** ($CaCl_2$).
- Entry of rDNA in host cell is due to transient pores created by heat shock (42°C) and not due to Ca^{2+} ions.
- Divalent cations increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

Process of Recombinant DNA Technology

1. **Isolation of the Genetic Material (DNA)**
2. **Fragmentation by restriction endonucleases**
3. **Separation and isolation of DNA fragments**
   - **Gel electrophoresis:**
     - Separation of negatively charged DNA molecules under an electric field through a medium/matrix.
     - Most commonly used matrix for DNA separation is agarose.
4. **PCR-Polymerase Chain Reaction**
   - *In vitro* **amplification of DNA (gene of interest)**
     - The amplified fragment if desired can now be used to ligate with a vector for further cloning.
5. **Ligation of the DNA fragment into a vector by DNA ligase**
6. **Insertion of recombinant DNA into the host cell**
   - Transformed host cells are selected with the help of selectable marker genes.
7. **Culturing of recombinant host cells (Biosynthetic stage)**
   - The cells harbouring cloned genes of interest may be grown in laboratory/ bioreactors.
   - **Bioreactors:** Vessels in which raw materials are biologically converted into specific products using microbial plant, animal or human cells and provide optimal growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).
8. **Downstream processing**
   - Separation and purification of the desired product/ recombinant protein from heterologous host (non native host).
   - Product has to be formulated with suitable preservatives.
   - Strict quality control testing is done for each product.
   - The downstream processing and quality control testing vary from product to product.
9. **Product is subjected for marketing as a finished product**

In Open Culture System/Continuous Culture System

- Used medium is drained out from one side.
- Fresh medium is added from the other to maintain the cells in their physiologically most active log/exponential phase.
- Larger biomass → Higher yields of desired protein.