BIOTECHNOLOGY PRINCIPLES AND PROCESSES

1. INTRODUCTION :

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

Parameters	Genetic Engineering	Bioprocess Engineering
Organisms Involved	Microbes	Genetically Modified Organisms
Production	Small Scale	Large Scale
Examples/ Technique Include	Curd, Bread or Wine Making	In vitro fertilisation leading to a 'test -tube' baby

EFB (European Federation of Biotechnology)

- 'The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'.
- Correcting a defective gene.
- It encompasses both traditional view and modern molecular biotechnology.
- Developing a DNA vaccine.
- Synthesising a gene & using it.

2. PRINCIPLES OF BIOTECHNOLOGY/ CORE TECHNIQUES INVOLVED IN MODERN BIOTECHNOLOGY

Parameters	Traditional Biotechnology	Modern Biotechnology
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.

Note :
The ability to multiply copies of antibiotic resistanse gene in E.coli was called cloning of antibiotic resistance gene in E.coli.

3. ADVANTAGES OF BIOTECHNOLOGY OVER OTHER TECHNIQUES

I. Methods : Asexual reproduction

Advantage - Preserves Genetic Information

Disadvantage - No Variations

II. Methods : Sexual Reproduction

Advantage - Provides opportunities for variations and formulation of unique combinations of genetic setup.

Disadvantage - Some of which may be harmful to the organism as well as the population

III. Methods : Traditional hybridisation

Advantage - Used in plant and animal breeding.

Disadvantage - Very often lead to inclusion and multiplication of undesirable genes along with desirable genes.

IV. Methods : Genetic engineering

Advantage - Allows us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into target organism.

Disadvantage - ----

4. THREE BASIC STEPS IN GENETICALLY MODIFYING ORGANISMS

(1) Identification of DNA with desirable genes (2) Introduction of the identified DNA into the host (3) Maintenance of introduced DNA in the host and transfer of the DNA to its progeny

5. KEY TOOLS OF RECOMBINANT DNA TECHNOLOGY

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

Enzymes : Most commonly used enzymes in genetic engineering are
- Nucleases
- DNA Polymerase
- Ligases

Nucleases : Catalyse the cleavage of nucleic acids.

Exonucleases : Remove nucleotides from the ends of the DNA

Endonucleases : Make cuts at specific positions within the DNA i.e. at recognition /palindromic sequence

Note: Palindromic sequence reads same on the two strands (from 5' → 3' and 3' → 5' direction) when orientation of reading is kept same

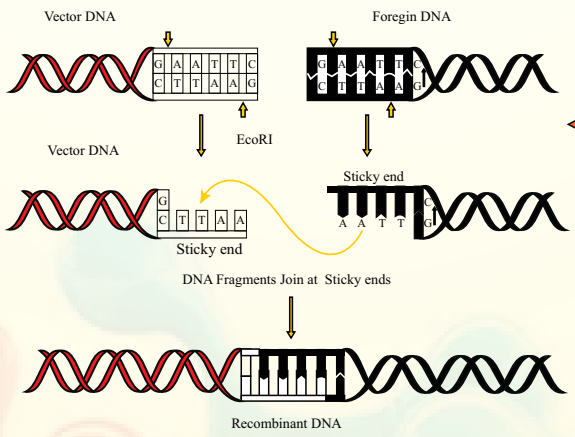
In the year 1963, the two enzymes responsible for restricting the growth of bacteriophage in Escherichia coli were isolated

Methylase : Add methyl groups to bacterial DNA

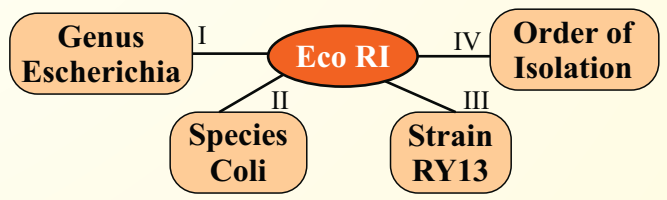
Restriction endonuclease / Molecular scissors : Cut the DNA of bacteriophage

6. ENZYMES

- **Restriction endonuclease**
  More than 900 restriction enzymes have been isolated from over 230 strains of bacteria (prokaryotic cell) each of which recognise different recognition sequences.
- **Functions :**
  1. 'Inspecting' the length of DNA s equence
  2. Binds to the "specific recognition sequence"
  3. Cuts the two strands of ds DNA at specific points in their sugar-phosphate back bone sand leaves single stranded portions at the ends.
  4. These overhanging stretches and called sticky ends.



Nomenclature / Naming of enzyme



- **Ligase :** When source DNA and vector DNA are cut by the same restriction enzyme there 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 and this stickiness facilitates the action of the enzyme DNA ligase.

7. CLONING VECTORS

Vectors are vehicles for delivering foreign DNA into recipient cells.

Vectors used at present are engineered in such a way that they help easy linking of foreign DNA & selection of recombinants from non recombinants

FEATURES OF CLONING VECTORS

Origin of Replication (ori)
- Sequence from where replication starts.
- Responsible for **Controlling Copy Number** of the linked **DNA**.
- Those vectors are preferred which support **High Copy Number.**

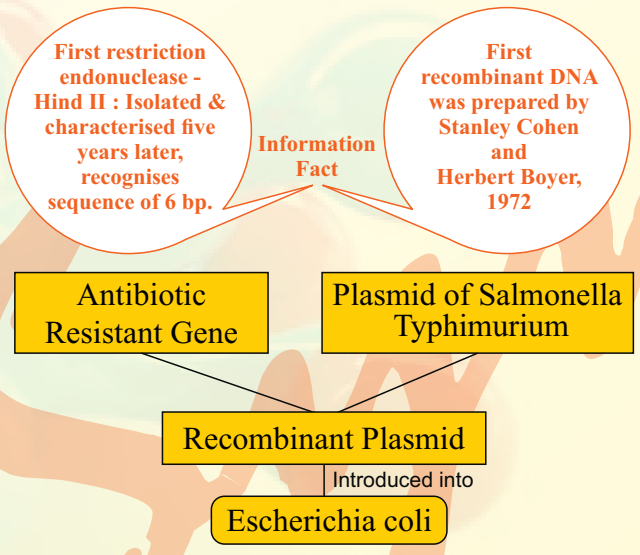
Selectable Marker
- Helps in selection of transformants.
- Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for E.coli.
- The normal E.coli cells do not carry resistance against any of these antibiotics.

Cloning Sites / Restriction Sites
- Single recognition site for a restriction enzyme within the vector is a preferable feature.
- Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning.
- The ligation of alien DNA/ **gene of interest (GOI)** is carried out at a restriction site present in one of the antibiotic resistant genes.

Information Fact

First restriction endonuclease - Hind II : Isolated & characterised five years later, recognises sequence of 6 bp.

First recombinant DNA was prepared by Stanley Cohen and Herbert Boyer, 1972

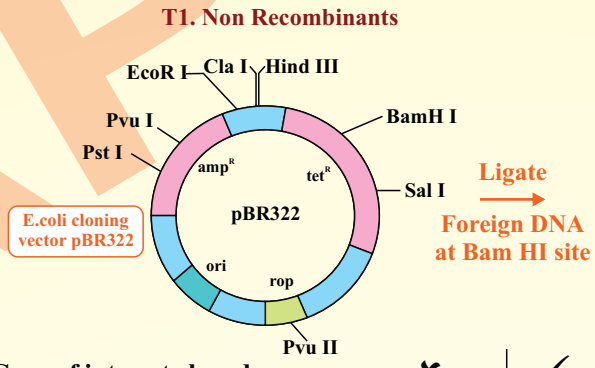


Motivational Syn-टेन्स

Your dedication & Teacher's guidance make a perfect example of recombination technology.

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

- **Insertional inactivation:** Insertion of GOI within antibiotic resistance gene/selectable marker results in inactivation/formation of the coded product.
- **Hypothesis:** Insertion of GOI at Bam HI site in tet^R .
- If transformation fails – Non transformants are obtained in antibiotic lacking agar medium but they don't grow on antibiotic rich medium.
- If transformation successful **Transformants** obtained are of **two types (T1 & T2)*.**



		T1	T2
Gene of interest cloned	:	✗	✓
Resistance to ampicillin	:	✓	✓
Resistance to tetracycline	:	✓	✗ {due to inactivation}

- ✶ **All transformants are not recombinants but all recombinants are transformants.**
- ✶ **One antibiotic resistant gene helps in selecting the transformants whereas the other antibiotic resistant gene helps in selection of recombinants.**
- ✶ **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.**

8. OTHER CLONING VECTORS

Selection of recombinants due to inactivation of antibiotic resistant gene as in pBR322 is a cumbersome procedure because it requires simultaneous plating of two plates having different antibiotics.

To overcome the disadvantage of pBR322, alternative selectable markers (lac Z) acting as reporter enzyme have been developed which differentiate recombinants from nonrecombinants on the basis of their ability to produce colour in the presence of chromogenic substrate.

- lac Z gene coding for β-galactosidase acts as selectable marker in the plasmid.
- Experiment: Insert foreign DNA at lac Z gene + transformation in E.coli

Chromogenic substrate
- Fails → Blue coloured colonies → **Non-recombinants**
- Succeeds → White coloured colonies → **Recombinants**

- ◆ **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.

9. METHODS OF TRANSFORMATION

- **Micro - Injection:** Recombinant DNA is directly injected into the nucleus of an animal cell.
- **Heat Shock Method**
- **Disarmed Pathogen Vector**
- **Biolistic / Gene Gun:** Plant cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA.

10. 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 bemade 'competent' by treating with ice cold calcium chloride.
- ✶ Entry of rDNA in host cell is due to transient pores created by+2 heat shock (42°C) and not due to Ca ions.
- ✶ Divalent cations increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

11. PROCESS OF RECOMBINANT DNA TECHNOLOGY

A. Isolation of the Genetic Material (DNA)

- ❖ In majority of organisms, DNA is the genetic material
- ❖ Since DNA is enclosed within the membranes, we have to break the cell open to release DNA along with
- ❖ In order to get DNA in pure form (free from other macromolecules), it is treated with different enzymes like RNase, protease etc.

Bacteria - Lysozyme | Fungi - Chitinase | Plantcell - Cellulase

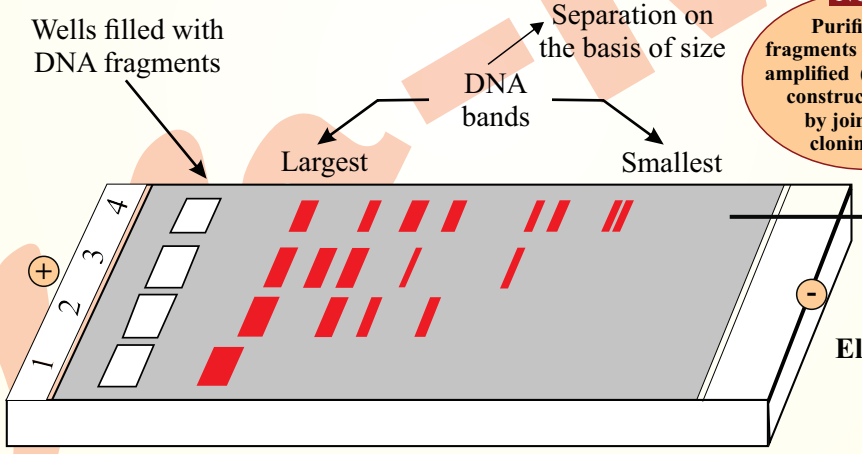
→ **B. Fragmentation by restriction endo-nucleases**

→ **C. Separation & 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**

NOTE: Purified DNA fragments are generally amplified (PCR) before constructing rDNA by joining with cloning vector.

Natural polymer, obtained from sea weeds | **Separate DNA fragments through seiving effect**

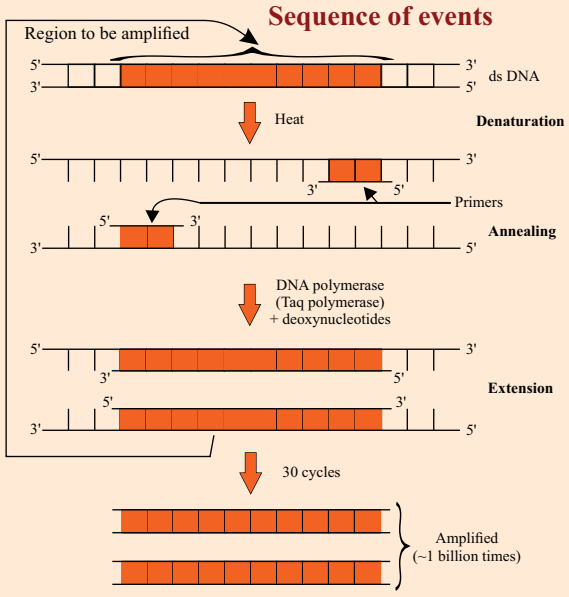


Gel → Stained with **Ethidium Bromide** → Exposed to **U.V Rays** → Appears **Bright Orange** Bands → Removal of DNA fragment from gel → Process → **Elution**

D. PCR - Polymerase Chain Reaction
Invitro amplification of DNA (gene of interest)

Reaction Mixture	Work / Function
Nucleotides	Formation of DNA chain
Primers	2 sets of chemically synthesised oligonucleotides, complementary to the regions of DNA
Taq-polymerase	Thermostable DNA polymerase, isolated from bacterium, Thermus aquaticus, remains active during high temperature induced denaturation of dsDNA. It extends the primers i.e. meant for chain elongation.
Genome DNA	Template DNA for gene of interest

Sequence of events



E. Ligation of the DNA fragment into a vector by DNA ligase

F. Insertion of Recombinant DNA into the host cell
Transformed host cells are selected with the help of selectable marker genes.

G. 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 human cells and provide optimal growth conditions (temperature, pH, substrate, salts, vitamins, oxygen)

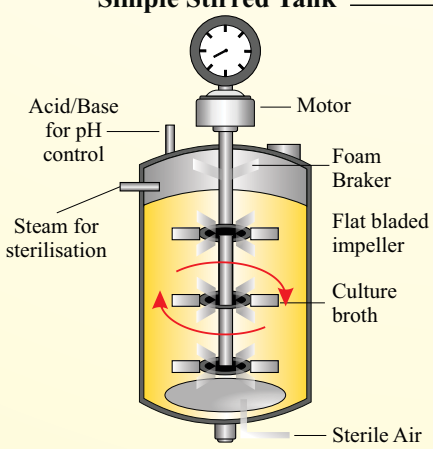
Parameters	Laboratory	Bioreactors
Culture	Small volume	Large volumes (100 - 1000 lts)
Maintaining optimal conditions	Not possible	✓
Growth rate of cell	Never optimal	Optimum
Production	Small scale	Large scale

Commonly used Bioreactors are stirred type having

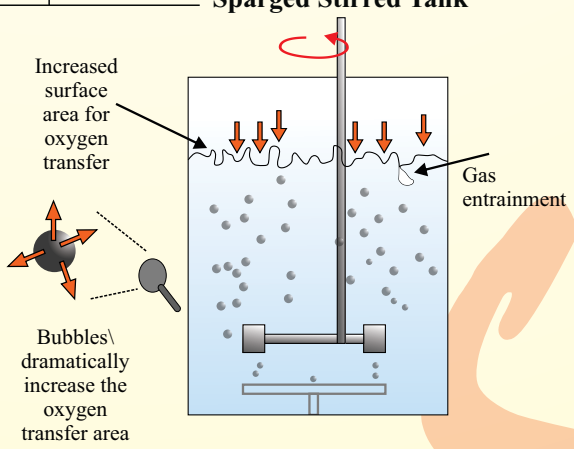
- Cylindrical or with curved base — Facilitate mixing of reactor contents
- Stirrer — Facilitate even mixing and oxygen availability throughout the bioreactor
- Agitator System
- Oxygen Delivery System
- pH Control System
- Foam Control System
- Sampling Ports — To withdraw small volumes of culture periodically

Types of stirred tanks

Simple Stirred Tank



Sparged Stirred Tank



H. Downstream processing

- Separation and purification of the desired product/recombinant protein from heterologous 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.

I. Product is subjected for marketing as a finished product

✶ **Note : In Open Culture System / Continuous Culture System** : Used medium is drained out from one side while 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.