

Biotechnology: Principles and Processes

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- **Biotechnology** involves the use of living organisms or enzymes derived from them to produce products and processes beneficial to humans.

Traditional Perspective

- In its broad sense, biotechnology includes various microbe-mediated processes, such as:
 - Curd making
 - Bread fermentation
 - Wine production
- These are age-old examples where microorganisms are used for human benefit.

Modern Perspective

- In contemporary usage, the term biotechnology is applied in a restricted sense, focusing mainly on processes that involve **genetically modified organisms (GMOs)** for large-scale production.
- It also encompasses several advanced techniques, such as:
 - In vitro fertilisation leading to the birth of a 'test-tube' baby
 - Gene synthesis and its application
 - DNA vaccine development
 - **Gene therapy** for correcting defective genes

Definition by the European Federation of Biotechnology (EFB)

- The **EFB** provides a definition that integrates both traditional and modern molecular biotechnology approaches:
 - "The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services."

Principles of Biotechnology

Core Techniques of Modern Biotechnology

Modern biotechnology is founded on two **core techniques**:

A. Genetic Engineering

- Involves techniques to alter the chemistry of genetic material (**DNA** and **RNA**) and introduce these into host organisms.
- This process enables modification of the host organism's phenotype by incorporating desirable traits.
- Overcomes limitations of traditional breeding:
 - Sexual reproduction creates genetic variations but may also transmit **undesirable genes**.
 - **Asexual reproduction** preserves genetic makeup without introducing variation.
 - Traditional hybridisation often transfers both desired and undesired genes.
 - Genetic engineering allows for the isolation and introduction of only the desired gene(s) into the target organism.

B. Bioprocess Engineering

- Deals with maintenance of sterile (microbial contamination-free) conditions in large-scale chemical engineering processes.
- Ensures the **growth of only the desired microorganisms or eukaryotic cells** for producing biotechnological products such as:
 - Antibiotics
 - Vaccines
 - Enzymes

Concept of Genetic Engineering

A. Introduction of Alien DNA

- When a foreign (alien) DNA is introduced into an organism, it generally **cannot replicate independently** unless it becomes integrated into the host's genome.

B. Integration into Host Chromosome

- Integration occurs when the alien DNA becomes part of a **chromosome** that contains an origin of replication (ori).
- The origin of replication is a specific DNA sequence that initiates the process of DNA replication.

C. Replication of Alien DNA

- By linking the alien DNA to an origin of replication, it gains the ability to **replicate and multiply** within the host organism.

D. Cloning

- The process of producing multiple identical copies of a DNA sequence in this manner is known as **cloning**.

First Artificial Recombinant DNA

The **first recombinant DNA** was successfully created in **1972** by **Stanley Cohen** and **Herbert Boyer**.

Process Steps

1. Isolation of the Gene

- Extracted an antibiotic resistance gene from a plasmid of *Salmonella typhimurium* using **restriction enzymes** (molecular scissors).

2. Gene Linking to Plasmid

- The isolated gene was attached to a native plasmid DNA - an autonomously replicating, circular, extra-chromosomal DNA molecule.

3. Joining DNA Fragments

- The enzyme **DNA ligase** joined the cut ends, forming a new circular DNA molecule (recombinant DNA).

4. Vector Function

- The recombinant plasmid served as a **vector**, similar to how a mosquito acts as a vector for malaria, to deliver the gene into *Escherichia coli* cells.

5. Replication in Host

- Inside *E. coli*, the recombinant DNA replicated using the host's **DNA polymerase** enzyme, producing **multiple copies** of the antibiotic resistance gene.

Three Basic Steps in Genetic Modification

1. **Identification** of DNA with desirable genes.
2. **Introduction** of the identified DNA into the host organism.
3. **Maintenance and transfer** of the introduced DNA in the host, ensuring its inheritance by the progeny.

Tools of Recombinant DNA Technology

- Modern **genetic engineering** or **recombinant DNA technology** requires several essential tools, including:
 - Restriction enzymes
 - Polymerase enzymes
 - Ligases
 - Vectors
 - Host organisms
- Below is a detailed explanation of the key tools:

Restriction Enzymes

Discovery

- In **1963**, two enzymes responsible for restricting bacteriophage growth in *Escherichia coli* were discovered:
 1. One enzyme added methyl groups to DNA.
 2. The other enzyme cut DNA, called **restriction endonuclease**.

First Isolated Restriction Enzyme

- **Hind II** was the first restriction endonuclease to be isolated (five years later).
- It cut DNA at a specific recognition sequence of six base pairs.
- Example: **Hind II** always recognises a specific sequence and cuts at the same position.

Recognition Sequences

- These specific base pair sequences are called **recognition sequences**.
- Over **900 restriction enzymes** have been discovered from more than **230 bacterial strains**, each recognising different sequences.

Naming Convention

- **First letter** → Genus name
- **Next two letters** → Species name
- **Strain letter** → Denoted by a capital letter after species initials
- **Roman numerals** → Order of discovery in that strain
- Example: **EcoRI** from *Escherichia coli* RY13 (R = strain, I = first enzyme from that strain).

Types of Nucleases

- **Exonucleases** → Remove nucleotides from the ends of DNA.

- **Endonucleases** → Cut DNA at specific positions within the strand.

Palindromic Sequences

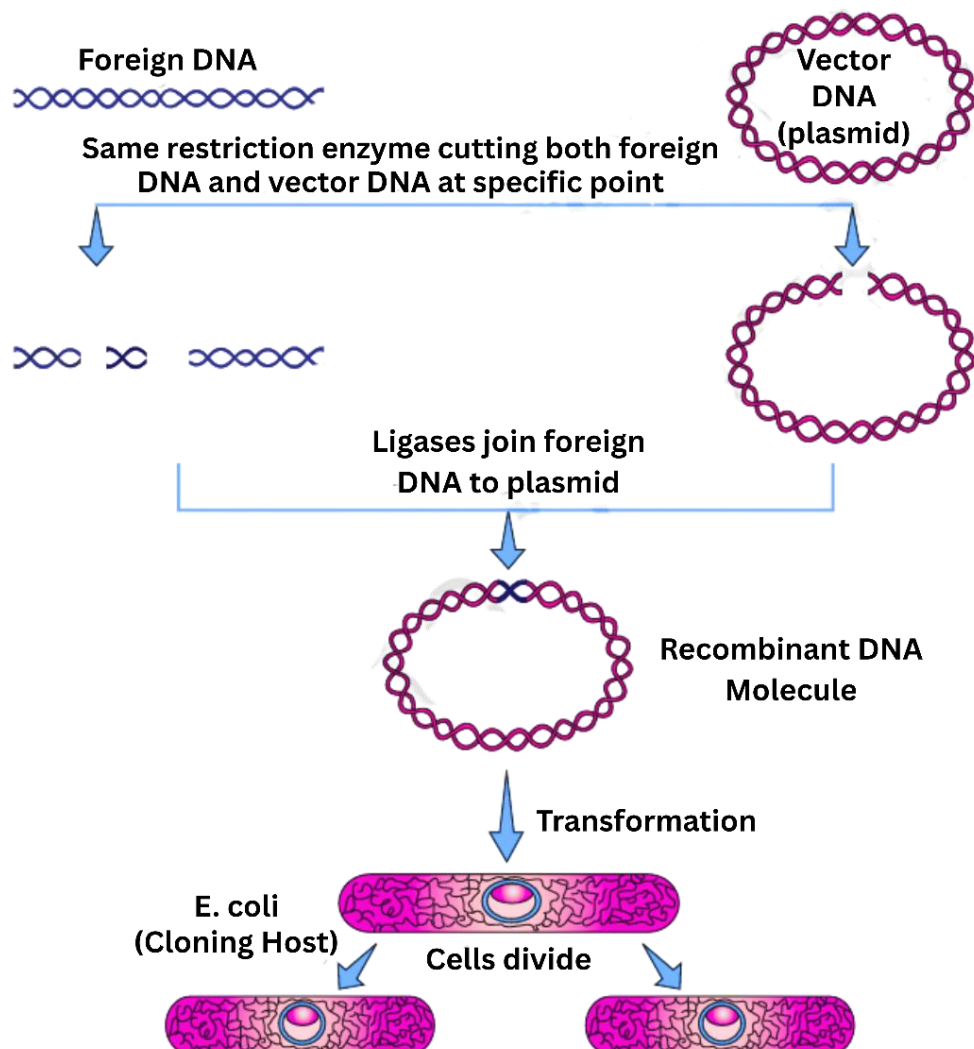
- A **palindrome** is a sequence that reads the same in both directions.
- In DNA, palindromic nucleotide sequences have identical reading in **5' → 3'** and **3' → 5'** directions.

5' — GAATTC — 3'

3' — CTTAAG — 5'

Sticky Ends

- Restriction enzymes cut **away from the centre** of the palindrome, creating overhanging single-stranded sticky ends.
- Sticky ends can form hydrogen bonds with complementary sequences, facilitating **ligation** by **DNA ligase**.



Use in Genetic Engineering

- DNA fragments cut by the same restriction enzyme have matching sticky ends and can be joined to create **recombinant DNA molecules**.
- Cutting vector DNA and source DNA with different enzymes will not allow recombinant formation.



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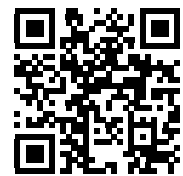


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