Chapter 9: Biotechnology; Principles and Processes

(i) Principles of Biotechnology; (ii) Tools of recombinant DNA Technology; (iii) Processes of Recombinant DNA Technology.

Biotechnology is the technique of using live organisms or enzymes from organisms to produce products and processes useful to humans. Many processes like ***in vitro*** fertilization leading to '**test-tube' baby**, synthesizing gene and using it, developing a **DNA vaccine** or **correcting a defective gene** are also parts of Biotechnology.

Principles of Biotechnology:

Modern biotechnology is based on two main principles-

- **Genetic engineering:** It is the technique in which genetic material (DNA & RNA) is chemically altered and introduced into host organisms to change the phenotype.
- **Bioprocess engineering: Maintenance of sterile ambience** in chemical engineering processes for growing desired microbe/eukaryotic cell for the manufacture of antibiotics, vaccines, enzymes etc.

Cloning is the process of creating genetically identical copies of organisms, cells, or DNA sequences. Cloning has applications in **agriculture, medicine**, and **research**, but also raises ethical concerns regarding human cloning and animal welfare.

Recombinant DNA technology (rDNA) or also called "**Genetic Engineering**" deals about, the production of new combinations of genetic material (artificially) in the laboratory. These "recombinant DNA" (rDNA) molecules are then introduced into host cells, where they can be propagated and multiplied.

Basic steps in rDNA:

- 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.

Tools of recombinant DNA Technology

1. **Restriction Enzymes (Molecular Scissors):**

   Restriction enzymes, also known as **restriction endonucleases**, are enzymes that recognize specific DNA sequences and cleave the DNA at or near those sequences. When

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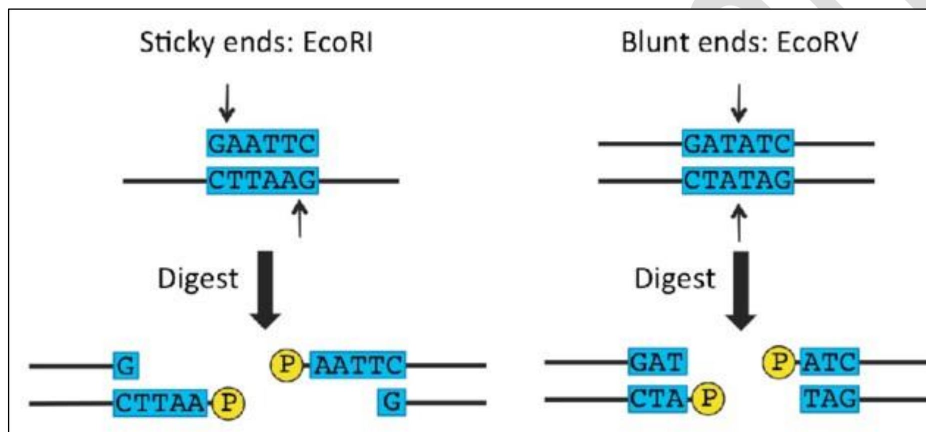
restriction enzymes cut DNA, they can produce two types of ends: **sticky ends and blunt ends**.

- **Sticky ends** refer to DNA ends with single-stranded overhangs, enabling them to base-pair specifically with complementary sequences, crucial for DNA fragment joining in techniques like DNA cloning.
- **Blunt ends** refer to DNA ends that are fully base-paired with no overhangs, making them unable to base-pair specifically but suitable for direct ligation, as in certain molecular biology techniques.



Restriction enzymes belong to a larger class of enzymes called **Nucleases**. These are of two kinds; **Exonucleases** and **Endonucleases**.

Exonucleases remove nucleotides from the ends of the DNA whereas, **Endonucleases** make cuts at specific position within the DNA.

Example, the first restriction endonuclease – **Hind II**, always cut DNA molecules at a particular point by recognizing a specific sequence of six base pairs. This specific base sequence is known as the **Recognition Sequence** for Hind II.

Each restriction endonuclease recognizes a specific **Palindromic Nucleotide Sequences** in the DNA.

What are Palindromes?

The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same.

An example of a palindrome sequence commonly recognized by restriction enzymes is "**GAATTC**," which is recognized by the enzyme **EcoRI**.

Separation and Isolation of DNA fragments (DNA of interest):

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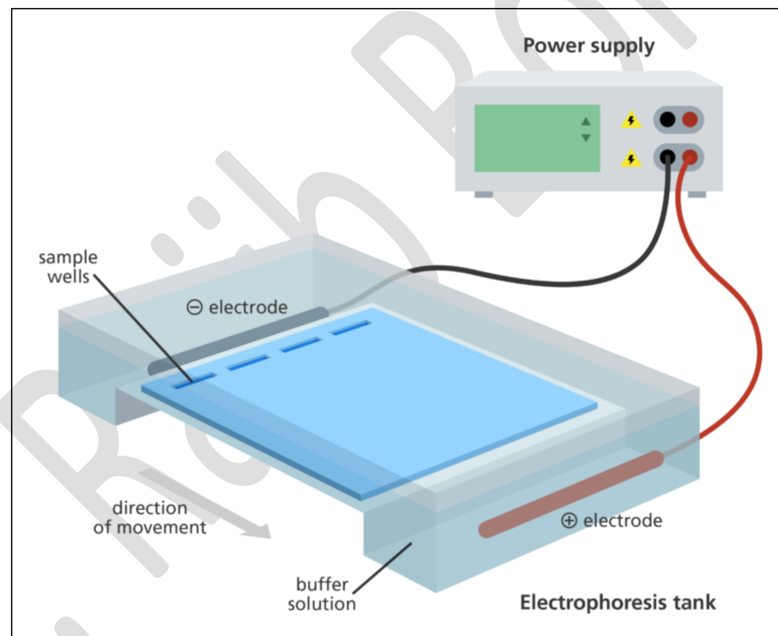
- The cutting of DNA by **restriction endonucleases** results in the fragments of DNA.
- These fragments can be separated by a technique known as **Gel Electrophoresis.**
- The DNA fragments are separated according to their size.
- The separated DNA fragments can be visualized only after staining the DNA with **Ethidium bromide** followed by exposure to **UV radiation**. Now DNA fragments appear bright orange coloured bands.
- The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as **Elution**.
- These DNA fragments are purified and used in constructing recombinant DNA with cloning vector.



2. Cloning Vectors (Vehicles for Cloning):

Vector serves as a vehicle to carry a foreign DNA sequence into a given host cell.

Salient features of a Vector:

- It should contain an **origin of replication** (ori) so that it is able to multiply within the host cell.
- It should incorporate a **selectable marker** (**antibiotic resistance gene**), which will allow to select those host cells that contain the vector from amongst those which do not.

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- The vector must also have atleast one unique restriction endonuclease recognition site to enable foreign DNA to be inserted into the vector during the generation of a recombinant DNA molecule.
- The vector should be relatively small in size. The most commonly used vectors are – **Plasmids** and **Bacteriophages**.

Insertional inactivation:

The most efficient method of screening for the presence of recombinant plasmids is based on the principle that the cloned DNA fragment disrupts the coding sequence of a gene. This is termed as **Insertional Inactiviation**.

For example, the powerful method of screening for the presence of recombinant plasmids is referred to as **Blue-White selection**. This method is based upon the insertional inactivation of the lac Z gene present on the vector. The lac Z gene encodes the enzyme **beta-galactosidase**, which can cleave a chromogenic substrate into a **blue coloured** product. If this lac Z gene is inactivated by insertion of a target DNA fragment into it, the development of the blue colour will be prevented and it gives white coloured colonies. By this way, we can differentiate recombinant (**white colour**) and non-recombinant (**blue colour**) colonies.

3. **Competent Host (Introduction of recombinant DNA into host cells):**

In rDNA technology, the most common method to introduce **rDNA** into living cells is transformation, during which cells take up DNA from the surrounding environment.

1. Simple chemical treatment with **divalent calcium ions** increases the efficiency of host cells (through cell wall pores) to take up the **rDNA** plasmids.
2. **rDNA** can also be transformed into host cell by incubating both on ice, followed by placing them briefly at **42°C (Heat Shock)**, and then putting them back **on ice**. This enables the bacteria to take up the recombinant DNA.
3. **In Microinjection method**, rDNA is directly injected into the nucleus of cells by using a glass micropipette.

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4. **Biolistics / Gene gun method**, it has been developed to introduce rDNA into mainly plant cells by using a **Gene / Particle gun.** In this method, microscopic particles of **gold / tungsten** are coated with the DNA of interest and bombarded onto cells.
5. The last method uses "**Disarmed Pathogen**" Vectors (***Agrobacterium tumefaciens***), which when allowed to infect the cell, transfer the recombinant DNA into the host.

Processes of Recombinant DNA Technology.

rDNA technology involves several steps in specific sequence such as,

1. Isolation of DNA
2. Fragmentation of DNA by restriction endonucleases
3. Isolation of desired DNA fragment
4. Ligation of the DNA fragment into a vector
5. Transferring the recombinant DNA into the host
6. Culturing the host cells in a medium at large scale and extraction of the desired product.

1. **Isolation of DNA:**
    - DNA should be isolated in pure form, without macromolecules. Hence cell wall can be broken down by treating the bacterial cells / plant or animal tissue with enzymes such as **Lysozyme (bacteria), cellulose (plant cells), chitinase (fungus).**
    - DNA should be removed from its histones proteins and RNAs. This can be achieved by using enzymes **ribonuclease** for RNA and **Proteases** for histone proteins.
    - Finally purified DNA precipitates out after the addition of **chilled Ethanol**.
2. **Fragmentation of DNA:**
    - Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme.
    - DNA is a negatively charged molecule, hence it moves towards the positive electrode (anode).

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- After having cut the source DNA as well as the vector DNA with a specific restriction enzyme, the cut out gene of interest from the source DNA and the cut vector with space are mixed and ligase is added.
- This results in the preparation of recombinant DNA.

3. **Amplification of Gene of Interest using PCR:**
    - PCR stands for **Polymerase Chain Reaction**. In this reaction, multiple copies of the gene of interest is synthesized ***in vitro*** using two sets of primers and the enzyme DNA polymerase.
    - The process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times. Such repeated amplification is achieved by the use of a themostable DNA polymerase (**Taq DNA Polymerase** – isolated from a bacterium, ***Thermus aquaticus***). The amplified fragment if desired can now be used to ligate with a vector for further cloning.
4. **Insertion of Recombinant DNA into the Host Cell / Organism**:
    - There are several methods of introducing the ligated DNA into recipient cells. If a recombinant DNA bearing gene for resistance to an antibiotic (ampicillin) is transferred into ***E.coli*** cells, the host cells become transformed into ampicillin-resistant cells.
    - If we spread the transformed cells on agar plates containing ampicillin, only transformants will grow, untransformed recipient cells will die. The ampicillin resistance gene in this case is called a **selectable marker**.
5. **Obtaining the Foreign Gene Product:**
    - The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory. The cultures may be used for extracting the desired protein and then purifying it by using different separation techniques.

Bioreactors

To produce in large quantities, the development of bioreactors, where large volume of culture can be processed, was required. Thus, bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human cells.

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- **A bioreactor** provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).

Stirred-tank reactor:

- It is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. Alternatively air can be bubbled through the reactor.
- The bioreactor has an **agitator system**, **an oxygen delivery system** and **a foam control system, a temperature control system, pH control system** and **sampling ports** so that small volumes of the culture can be withdrawn periodically.

Downstream Processing:

The processes include **separation and purification**, which are collectively referred to as downstream processing. Strict quality control testing for each product is also required.

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NCERT EXERCISES

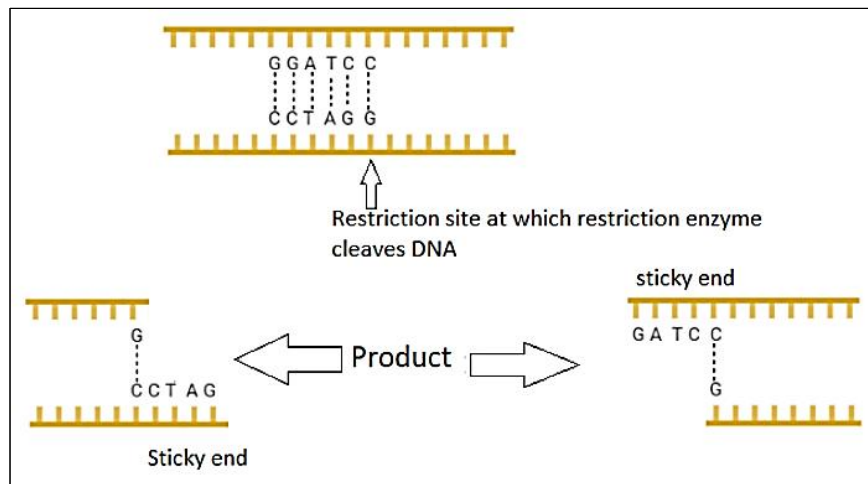
1. Can you list 10 recombinant proteins which are used in medical practice? Find out where they are used as therapeutics (use the internet).

Ans:

1. **Insulin:** Diabetes treatment.
2. **Erythropoietin (EPO):** Anemia therapy.
3. **Human Growth Hormone (HGH):** Growth disorders treatment.
4. **Tissue Plasminogen Activator (tPA):** Blood clot dissolution.
5. **Granulocyte Colony-Stimulating Factor (G-CSF):** Neutropenia prevention.
6. **Interferons:** Viral infections and cancer treatment.
7. **Factor VIII:** Hemophilia A treatment.
8. **Factor IX:** Hemophilia B treatment.
9. **Recombinant Factor VIIa:** Bleeding disorder management.
10. **Monoclonal Antibodies:** Targeted therapy for cancer, autoimmune diseases, and other conditions.

2. Make a chart (with diagrammatic representation) showing a restriction enzyme, the substrate DNA on which it acts, the site at which it cuts DNA and the product it produces.

Ans:



3. From what you have learnt, can you tell whether enzymes are bigger or DNA is bigger in molecular size? How did you know?

Ans: DNA molecules are larger than enzymes. DNA, being the genetic material, consists of long strands of nucleotides that encode genetic information. Enzymes, on the other hand, are typically smaller in size compared to DNA molecules. They are proteins that catalyze biochemical reactions and are composed of chains of amino acids.

4. What would be the molar concentration of human DNA in a human cell? Consult your teacher.

Ans: The molar concentration of human DNA in a human diploid cell is as follows:

⇒ Total number of chromosomes × 6.023×10^{23}

⇒ $46 \times 6.023 \times 10^{23}$

⇒ 277.05×10^{23} moles

⇒ 2.77×10^{25} moles

Hence, the molar concentration of DNA in each diploid cell in humans is 277.05×10^{25} moles.

5. Do eukaryotic cells have restriction endonucleases? Justify your answer.

Ans: No, eukaryotic cells do not have restriction endonucleases. Eukaryotic cells generally lack restriction endonucleases, unlike prokaryotic cells. While some unicellular eukaryotes may possess these enzymes, they are not as abundant or diverse. Eukaryotic cells have alternative mechanisms to deal with foreign DNA, such as RNA interference and immune responses, and the presence of a nuclear membrane further reduces the need for restriction endonucleases.

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6. Besides better aeration and mixing properties, what other advantages do stirred tank bioreactors have over shake flasks?

Ans: The shake flask method is used for a small-scale production of biotechnological products in a laboratory, whereas stirred tank bioreactors are used for a large-scale production of biotechnology products.

Stirred tank bioreactors have several advantages over shake flasks:

(i) Small volumes of culture can be taken out from the reactor for testing.

(ii) It has a foam breaker for regulating the foam.

(iii) It has a control system that regulates the temperature and pH.

7. Collect 5 examples of palindromic DNA sequences by consulting your teacher. Better try to create a palindromic sequence by following base-pair rules.

Ans: The palindromic sequence is a certain sequence of the DNA that reads the same whether read from 5' → 3' direction or from 3' → 5' direction. They are the site for the action of restriction enzymes. Most restriction enzymes are palindromic sequences.

Five examples of palindromic sequences are-

(i) 5'-AGCT-3'
3'-TCGA-5'

(ii) 5'-GAATTC-3'
3'-CTTAAG-5'

(iii) 5'-AAGCTT-3'
3'-TTCGAA-5'

(iv) 5'-GTCGAC-3'
3'-CAGCTG-5'

(v) 5'-CTGCAG-3'
3'-GACGTC-5'

8. Can you recall meiosis and indicate at what stage a recombinant DNA is made?

Ans: Meiosis is a process that includes the reduction in the amount of genetic material. It is of two types, namely meiosis I and meiosis II. During the Pachytene stage of prophase I, crossing over of chromosomes takes place where the exchange of segments between non-sister chromatids of homologous chromosomes takes place. This results in the formation of recombinant DNA.

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9. Can you think and answer how a reporter enzyme can be used to monitor transformation of host cells by foreign DNA in addition to a selectable marker?

Ans: A reporter gene can be used to monitor the transformation of host cells by foreign DNA. They act as a selectable marker to determine whether the host cell has taken up the foreign DNA or the foreign gene gets expressed in the cell. The researchers place the reporter gene and the foreign gene in the same DNA construct. Then, this combined DNA construct is inserted in the cell. Then, the reporter gene is used as a selectable marker to find out the successful uptake of genes of interest.

Example of reporter genes - lac Z gene, which encodes a green fluorescent protein in a jelly fish.

10. Describe briefly the following:

(a) Origin of replication

(b) Bioreactors

(c) Downstream processing

Ans:

(a) **Origin of replication** -Origin of replication is defined as the DNA sequence in a genome from where replication initiates. The initiation of replication can be either uni-directional or bi-directional. A protein complex recognizes the 'on' site, unwinds the two strands, and initiates the copying of the DNA.

(b) **Bioreactors** - Bioreactors are large vessels used for the large-scale production of biotechnology products from raw materials. They provide optimal conditions to obtain the desired product by providing the optimum temperature, pH, vitamin, oxygen, etc. Bioreactors have an oxygen delivery system, a foam control system, a PH, a temperature control system, and a sampling port to obtain a small volume of culture for sampling.

(c) **Downstream processing** - Downstream processing is a method of separation and purification of foreign gene products after the completion of the biosynthetic stage. The product is subjected to various processes in order to separate and purify the product. After downstream processing, the product is formulated and is passed through various clinical trials for quality control and other tests.

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11. Explain briefly

(a) PCR

(b) Restriction enzymes and DNA

(c) Chitinase

Ans: **a) PCR (Polymerase Chain Reaction):** PCR is a method used to amplify a specific segment of DNA. It involves three steps: denaturation, annealing, and extension. This technique is widely utilized in various fields such as molecular biology, genetics, forensics, and diagnostics to produce millions of copies of DNA from a small sample.

b) Restriction enzymes and DNA: Restriction enzymes are proteins that cut DNA at specific sequences, known as restriction sites. These enzymes are essential tools in genetic engineering for cutting and pasting DNA fragments. They allow scientists to manipulate DNA, creating recombinant DNA molecules used in research, biotechnology, and medicine. Restriction enzyme are categorized into two types:

(i) Exonuclease: It is a type of restriction enzyme that removes the nucleotide from either 5' or 3' ends of the DNA molecule.

(ii) Endonuclease: It is a type of restriction enzyme that makes a cut within the DNA at a specific site. This enzyme acts as an important tool in genetic engineering. It is commonly used to make a cut in the sequence to obtain DNA fragments with sticky ends, which are later joined by enzyme DNA ligase.

c) Chitinase: Chitinase is an enzyme that breaks down chitin, a polysaccharide found in the cell walls of fungi and exoskeletons of insects. It has applications in agriculture for controlling fungal infections in crops. Additionally, chitinase is being explored for its potential in biocontrol methods and various industrial processes. For example, the recognition site for enzyme ECORI is as follows

12. Discuss with your teacher and find out how to distinguish between

(a) Plasmid DNA and Chromosomal DNA

(b) RNA and DNA

(c) Exonuclease and Endonuclease

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Ans: a) Plasmid DNA and Chromosomal DNA

Plasmid DNA	**Chromosomal DNA**
Circular, extrachromosomal DNA molecule	Linear, double-stranded DNA molecule
Often found in bacteria and some eukaryotic cells	Present in the nucleus of eukaryotic cells and in the nucleoid region of prokaryotic cells
Multiple copies per cell	Single copy per cell
Typically carry non-essential genes, such as antibiotic resistance genes	Essential genes for cellular function, including those involved in growth, development, and metabolism

b) RNA and DNA

RNA	**DNA**
Usually single-stranded	Double-stranded helix
Contains Ribose sugar	Contains Deoxyribose sugar
Bases are Adenine, Uracil, Guanine, Cytosine	Bases are Adenine, Thymine, Guanine, Cytosine
Roles include protein synthesis, gene regulation, and as genetic material in some viruses	Main role is to carry genetic instructions from parents to offspring.

(c) Exonuclease and Endonuclease

Exonuclease	**Endonuclease**
Acts at the ends of nucleic acid molecules	Acts within the interior of nucleic acid molecules
Removes nucleotides from the ends of DNA or RNA molecules	Cleaves DNA or RNA at specific internal sites
Involved in proofreading and repairing DNA, as well as in processing RNA transcripts	Involved in DNA repair, recombination, and restriction enzyme activity
e.g. DNA polymerase I, Exonuclease III	e.g. EcoRI, HindIII

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