# 11. BIOTECHNOLOGY: PRINCIPLES & PROCESSES

## TOOLS OF RECOMBINANT DNA TECHNOLOGY

### 1. Restriction Enzymes (molecular scissors)

- The enzymes that cut DNA at specific sites into fragments.
- They belong to a class of enzymes called ***nucleases***.
- In 1963, two enzymes responsible for restricting growth of bacteriophage in *E. coli* were isolated. One enzyme added methyl groups to DNA. The other ***(restriction endonuclease)*** cut DNA.

#### Naming of the restriction enzymes:

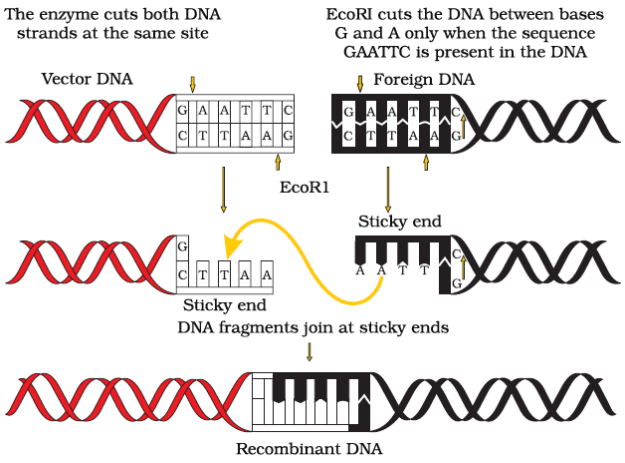
- First letter indicates genus. The second two letters indicate species of prokaryotic cell from which they were isolated. E.g. ***EcoRI*** comes from *E. coli* RY 13 (R = the strain. Roman numbers = the order in which the enzymes were isolated from that strain of bacteria).

#### Types of Restriction enzymes:

- **Exonucleases:** They remove nucleotides from the ends of the DNA.
- **Endonucleases:**
- They cut at specific positions within the DNA. E.g. EcoRI.
- They bind to specific recognition sequence of the DNA and cut the two strands at specific points.
- The first restriction endonuclease is **Hind II.** It cuts DNA molecules by recognizing a specific sequence of 6 base pairs. This is called the **recognition sequence** for Hind II.
- Restriction endonuclease recognizes a specific **palindromic nucleotide sequences** in the DNA. It is a sequence of base pairs that read the same on the two strands in 5′ → 3′ direction and in 3′ → 5′ direction. E.g. Palindromic nucleotide sequence for EcoRI is

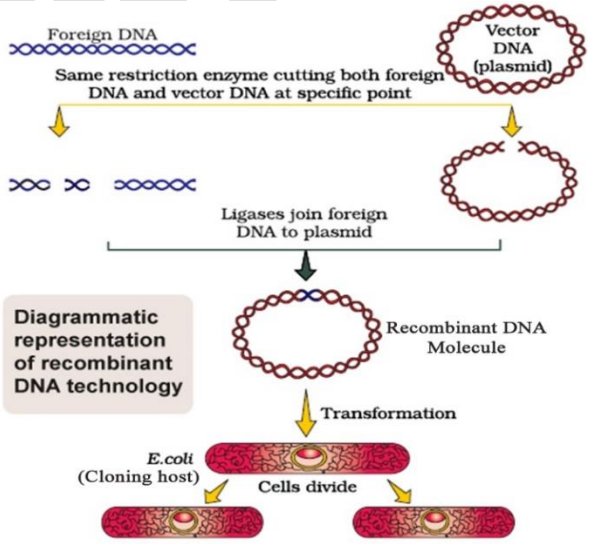
  5′—— GAATTC —— 3′
  3′—— CTTAAG —— 5′

- Restriction enzymes cut the strand a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. This leaves single stranded overhanging stretches at the ends. They are called **sticky ends**. They form H-bonds with their complementary cut counterparts. This stickiness facilitates action of the enzyme ***DNA ligase.***




*Steps in formation of recombinant DNA by EcoRI*

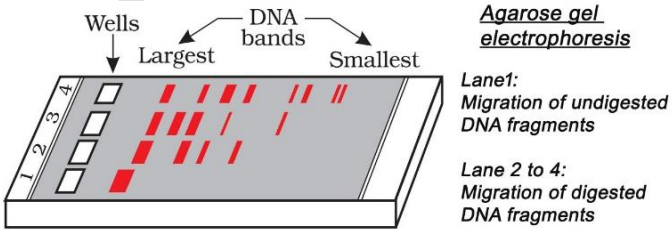
- When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of sticky-ends and these are joined together by *DNA ligases*.




## PROCESSES OF RECOMBINANT DNA TECHNOLOGY

### Cutting of DNA at Specific Locations

- Purified DNA is incubated with the **restriction enzyme.** As a result, **DNA digests.** These DNA fragments are separated by a technique called **gel electrophoresis**.




- **Agarose gel electrophoresis** is employed to check the progression of a restriction enzyme digestion. DNA is negatively charged. So it moves towards the anode. DNA fragments are separated according to their size through sieving effect of the agarose gel (a polymer extracted from sea weeds). The smaller sized fragment moves farther.
- The process is repeated with the vector DNA also.
- DNA fragments can be seen as bright orange coloured bands when they are stained with **ethidium bromide** and exposed to UV radiation.
- DNA bands are cut out from agarose gel. It is called **elution.** The cut-out **gene of interest** and cut **vector** are mixed and ***ligase*** is added. It creates **recombinant DNA.**

### Amplification of Gene of Interest using PCR

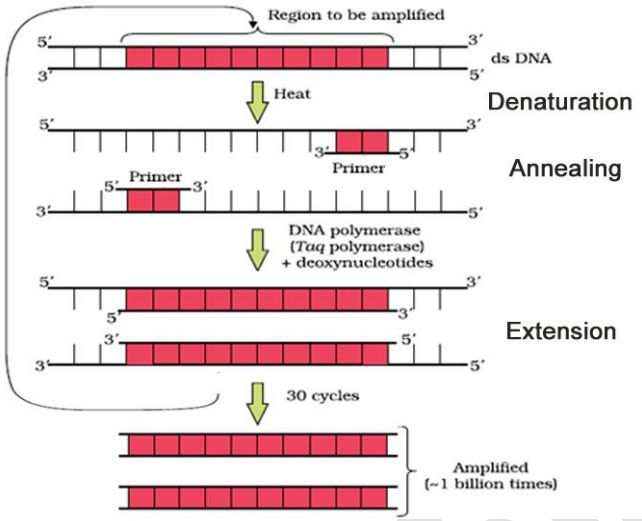
- **Polymerase Chain Reaction (PCR)** is the synthesis of multiple copies of the gene of interest *in vitro* using 2 sets of **primers** & the enzyme ***DNA polymerase.***
- Primers are small chemically synthesized oligonucleotides that are complementary to the regions of DNA.

## Steps of PCR:

- **Denaturation:** It is the heating of target DNA (gene of interest) at high temperature ($94^0$ C) to separate the strands. Each strands act as template for DNA synthesis.
- **Annealing:** It is the joining of the two primers (at $52^0$ C) at the 3' end of the DNA templates.
- **Extension:** It is the addition of nucleotides to the primer using a thermostable *DNA polymerase* called ***Taq polymerase.*** It is isolated from a bacterium, ***Thermus aquaticus*****.** It remains active in high temperature during the denaturation of double stranded DNA.

  Through continuous replication, the DNA segment is amplified up to 1 billion copies.

  The amplified fragment can be used to ligate with a vector for further cloning.




## Obtaining the Foreign Gene Product

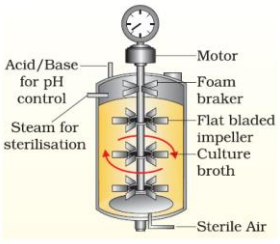
- The aim of recombinant DNA technology is to produce a desirable protein.
- If a protein encoding foreign gene is expressed in a heterologous host, it is called a **recombinant protein**.



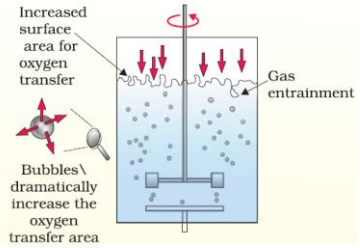
- The cells with foreign genes can be grown in laboratory. The cultures are used to extract the desired protein and purify it by using separation techniques.
- The cells can also be multiplied in a **continuous culture system.** Here, the used medium is drained out from one side while fresh medium is added from the other. It maintains the cells more physiologically active and so produces a larger biomass. It yields more desired protein.

## Bioreactors

- These are the vessels in which raw materials are biologically converted to specific products, enzymes etc., using microbial, plant, animal or human cells.
- A bioreactor provides the optimal growth conditions (pH, temperature, substrate, salts, vitamins, oxygen) to get desired product.
- The most commonly used bioreactors are of stirring type **(stirred-tank bioreactor).**




Simple stirred-tank bioreactor




Sparged stirred-tank bioreactor through which sterile air bubbles are sparged

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. Alternatively, air can be bubbled through the reactor.

The bioreactor has

- An agitator system
- An oxygen delivery system
- A foam control system
- A temperature control system
- pH control system
- Sampling ports (for periodic withdrawal of the culture).

## ☞ EXAM CAPSULE VIDEOS