Chapter 7

Biotechnology - Principles and Processes

Chapter Contents

Introduction

What is Biotechnology?

Biotechnology is technology based on biology, especially when used in agriculture, food science and medicine.

The term brings to mind many different things. Some think of developing new types of animals. Others dream of almost unlimited sources of human therapeutic drugs. Still others envision the possibility of growing crops that are more nutritious and naturally pest-resistant to feed a rapidly growing world population. This question elicits almost as many questions as there are people to whom the question can be posed.

This chapter deals with basic principles of biotechnology, the components central to the process of gene cloning such as DNA manipulative enzymes and vectors which transport the desired gene into host cell. Latter part of the chapter turns our focus to PCR process and applications along with obtaining the desired product on large scale using bioreactors.

In its purest form, the term biotechnology refers to the use of living organisms or their products to modify human health and the human environment. However, it is used in a restricted sense today, to refer to those processes which use genetically modified organisms to achieve the same on a larger scale. Further, many other processes/techniques are also included under biotechnology. For example, *in-vitro* fertilisation leading to a 'test-tube' baby, synthesising a gene and using it, developing a DNA vaccine or correcting a defective gene, are all part of biotechnology.

The **European Federation of Biotechnology (EFB)** has given a definition of biotechnology that encompasses both traditional view and modern molecular biotechnology. The definition given by EFB is as follows :

The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services.

PRINCIPLES OF BIOTECHNOLOGY

The two core techniques that enabled us to combine the genetic elements of two or more living cells or that enabled birth of modern biotechnology are :

(i) **Genetic Engineering :** Techniques to alter the chemistry of genetic material (DNA and RNA), to introduce these into host organisms and thus change the phenotype of the host organism. The recombinant DNA thus created is called rDNA or chimeric DNA which has properties of DNA from multiple sources.

(ii) **Bioprocess engineering :** Maintenance of sterile (microbial contamination-free) ambience in chemical - engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

In these techniques, functional lengths of DNA can be taken from one organism and placed into the cells of another organism. For example, we can cause bacterial cells to produce human molecules, cows can be made to produce more milk for the same amount of feed and we can synthesize therapeutic molecules that have never before existed.

Let us now understand the conceptual development of the principles of genetic engineering.

Traditional hybridisation procedures used in plant and animal breeding, very often lead to inclusion and multiplication of undesirable genes along with desired genes. The techniques of genetic engineering which include creation of recombinant DNA, use of gene cloning and gene transfer overcome this limitation and allow us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organism.

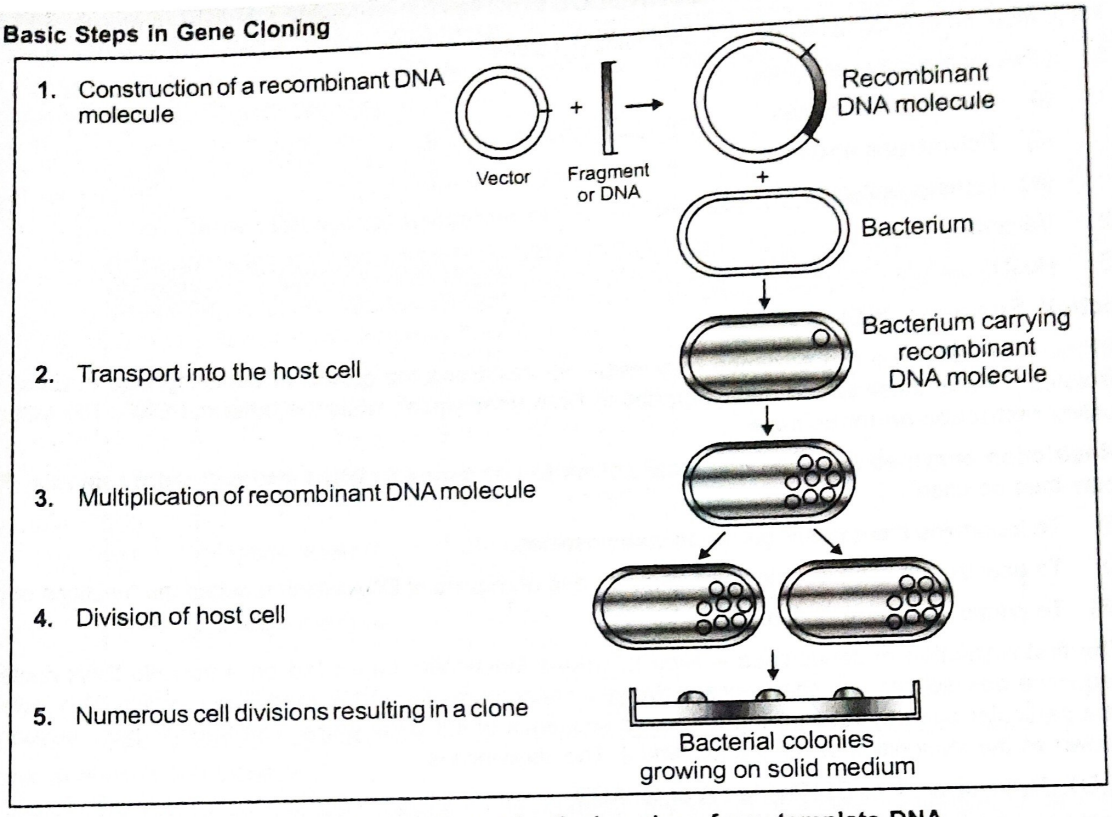
Note :

What is gene cloning?

The easiest way to answer this question is to follow the steps in a gene – cloning experiment :

1. A fragment of DNA, containing the gene to be cloned, is inserted into a circular DNA molecule called a vector, to produce a recombinant DNA molecule.
2. The vector transports the gene into a host cell, which is usually a bacterium although other types of living cell can be used.
3. Within the host cell the vector multiplies, producing numerous identical copies, not only of itself but also of the gene that it carries.
4. When the host cell divides, copies of the recombinant DNA molecule are passed to the progeny and further vector replication takes place.
5. After a large number of cell divisions, a colony, or clone, of identical host cells is produced. Each cell in the clone contains one or more copies of the recombinant DNA molecule; the gene carried by the recombinant molecule is now said to be cloned.

Basic Steps in Gene Cloning



- Therefore, **Cloning is making multiple identical copies of any template DNA.**

Let us now focus on the first instance of the construction of an artificial recombinant DNA molecule. The construction of the first recombinant DNA emerged from the possibility of linking a gene-encoding antibiotic resistance with a native **plasmid** (autonomously replicating circular extra-chromosomal DNA) of *Salmonella typhimurium*. **Stanley Cohen** and **Herbert Boyer** accomplished this in 1972 by isolating the antibiotic resistance gene by cutting out a piece of DNA from a plasmid which was responsible for conferring antibiotic resistance. The cutting of DNA at specific locations became possible with the discovery of the so-called '**molecular scissors**' - **restriction enzymes**. The cut piece of DNA was then linked with the plasmid DNA. These plasmid DNA act as **vectors** to transfer the piece of DNA attached to it into the host organism. The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme **DNA ligase**, which acts on cut DNA molecules and joins their ends. This makes a new combination of circular, autonomously replicating DNA created *in-vitro* and is known as **recombinant DNA**. When this DNA is transferred into *Escherichia coli*, a bacterium closely related to *Salmonella*, it could replicate using the new host's **DNA polymerase** enzyme and make multiple copies. The ability to multiply copies of antibiotic resistance gene in *E.coli* was called **cloning** of antibiotic-resistance gene in *E.coli*.

Thus, there are three basic steps in genetically modifying an organism that are listed below :

(i) Identification of DNA with desirable genes.

(ii) Introduction of the identified DNA into the host.

(iii) Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

TOOLS OF RECOMBINANT DNA TECHNOLOGY

Tools required to accomplish genetic engineering include :

1. DNA manipulative enzymes
   (i) Restriction enzymes
   (ii) Polymerase enzymes
   (iii) Ligases enzymes
2. Vectors
3. Host organism

Restriction Enzymes (RE)

In the year 1963, the two enzymes responsible for restricting the growth of bacteriophage in *E.coli* were isolated. One of these added methyl groups to DNA (methylase), while the other cut DNA. The latter was called **restriction endonuclease.**

Restriction enzymes serve as chemical knives to cut genes (= DNA) into defined fragments. These may then be used

(i) To determine the order of genes on chromosomes.

(ii) To analyze the chemical structure of genes and of regions of DNA which regulate the functions of gene.

(iii) To create new combinations of genes.

The first restriction endonuclease – *Hind* II, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterised five years later. It was found that *Hind* II always cuts DNA molecules at a particular point by recognising a specific sequence of **six base pairs**. This specific base sequence is known as the recognition sequence for *Hind* II. The sequence is

5′ GT (Pyrimidine : T or C) (Purine : A or G) AC3′

3′ CA (Purine : A or G) (Pyrimidine : T or C) TG5′

Besides *Hind* II, we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences.

Note : Restriction enzymes are obtained only from prokaryotes. It is their natural defense mechanism against bacteriophage infection.

The convention for naming these enzymes is the first letter comes from the name of the genus and the second two letters come from the species of the prokaryotic cell from which they were isolated. For example, *EcoR*I comes from *Escherichia coli* RY13. In *EcoRI*, the letter 'R' is derived from the name of strain "Rough". Roman number following the names indicate the order in which the enzymes were isolated from that strain of bacteria.

Restriction enzymes belong to a larger class of enzymes called **Nucleases**.

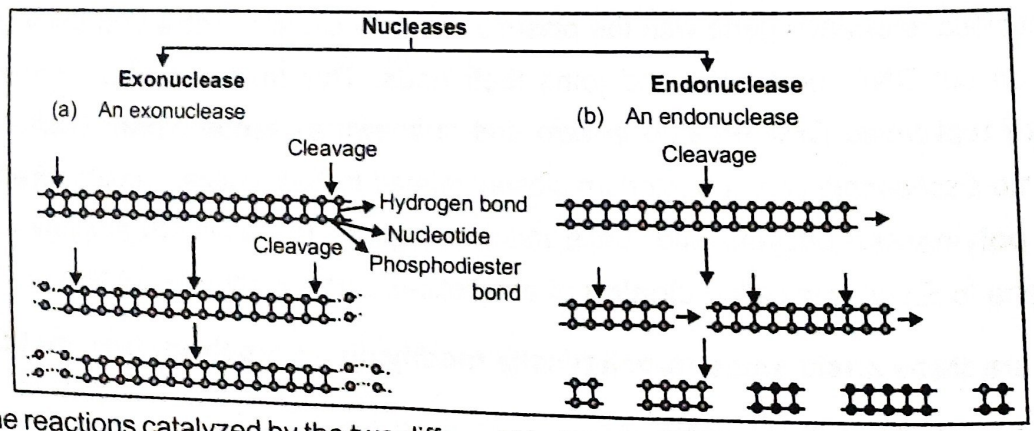


Fig. : The reactions catalyzed by the two different kinds of nuclease. (a) An exonuclease, which removes nucleotides from the end of a DNA molecule. (b) An endonuclease, which breaks internal phosphodiester bonds.

Each restriction enzyme recognises a specific palindromic nucleotide sequence in DNA. A **palindrome** is a word, phrase, number or other sequence of units that can be read the same way in either direction

Palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept same. These sequences may range from 4–8 nucleotides in length usually.

For example, the following sequences reads the same on the two strands in 5′ → 3′ direction. This is also true if read in the 3′ → 5′ direction.

5′ —— GAATTC —— 3′
3′ —— CTTAAG —— 5′

Table : Recognition Sequences of Several Restriction Endonucleases

Enzyme	Microbial origin	Recognition site	Type of ends after restriction enzyme digestion
Bam HI	*Bacillus amyloliquefaciens*	5′–G↓G–A–T–C–C–3′ 3′–C–C–T–A–G–G↑–5′	Sticky ends
Eco RI	*Escherichia coli*	5′–G↓A–A–T–T–C–3′ 3′–C–T–T–A–A–G↑–5′	Sticky ends
Hind III	*Haemophilus influenzae*	5′–A↓A–G–C–T–T–3′ 3′–T–T–C–G–A↑–A–5′	Sticky ends
Pst I	*Providencia stuartii*	5′–C–T–G–C–A↓G–3′ 3′–G–A↑–C–G–T–C–5′	Sticky ends
Sal I	*Streptomyces albus*	5′–G↓T–C–G–A–C–3′ 3′–C–A–G–C–T↑–G–5′	Sticky ends
Sma I	*Serratia marcescens*	5′–C–C–C↓G–G–G–3′ 3′–G–G–G↑–C–C–C–5′	Blunt ends

Working of Restriction Enzymes

Each restriction endonuclease functions by '**inspecting**' the length of a DNA sequence. Once it **finds** its specific recognition sequence, it will **bind** to the DNA and **cut** each of the two strands of the double helix at specific points in their sugar-phosphate backbones.

Some restriction enzymes (RE) such as *EcoRI* cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. This leaves single-stranded portions at the ends. There are overhanging stretches called **sticky ends or cohesive ends or staggerred ends** on each strand (shown in figure below). These are named so because they form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase.

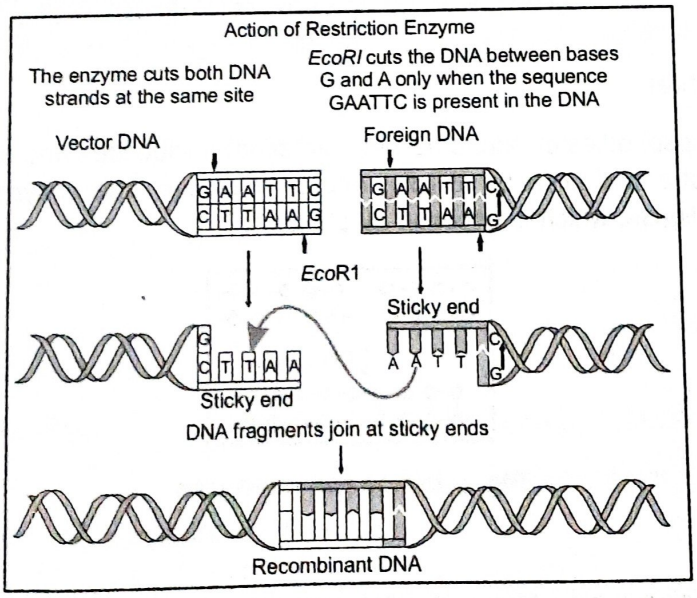


Fig. : Steps in formation of recombinant DNA by action of restriction endonuclease enzyme - *EcoRI*

Some restriction enzymes cut the strand of DNA in the centre of palindrome. Such ends are called **blunt ends** or **flush ends**.

e.g., Sma I

5′— C C C ↓ G G G —3′
3′— G G G ↑ C C C —5′

5′—CCC GGG—3′
3′—GGG CCC—5′

Restriction endonucleases are used in genetic engineering to form '**recombinant**' molecules of DNA(rDNA), which are composed of DNA from different sources/genomes.

When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of 'sticky-ends' and, these can be joined together (end-to-end) using **DNA ligases** (shown in figure below).

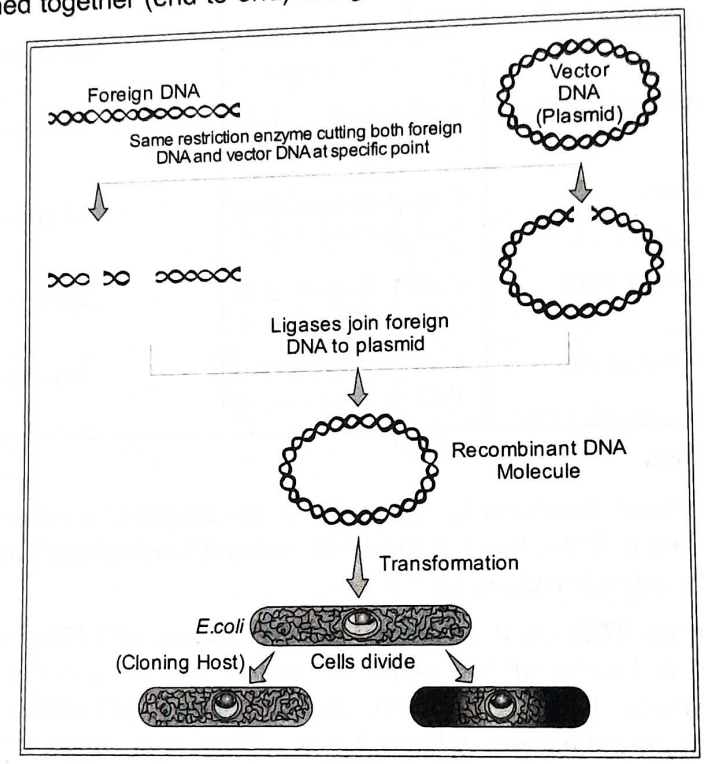


Fig. : Diagrammatic representation of recombinant DNA technology

Note : Unless vector and the source DNA are cut with same restriction enzyme the recombinant vector molecule cannot be created.

DNA Ligases (Molecular glue)

This enzyme forms phosphodiester bonds between adjacent nucleotides and covalently links two individual fragments of double-stranded DNA by utilising energy from cell. The enzyme used most often in rDNA technology is T_4 DNA ligase, which is encoded by phage T_4.

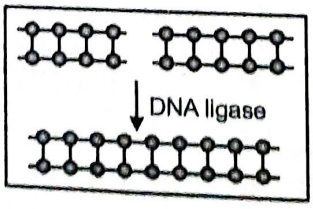


Fig. : Joining two molecules

DNA Polymerases

These enzymes synthesize a new strand of DNA complementary to an existing DNA template in 5′ to 3′ direction.

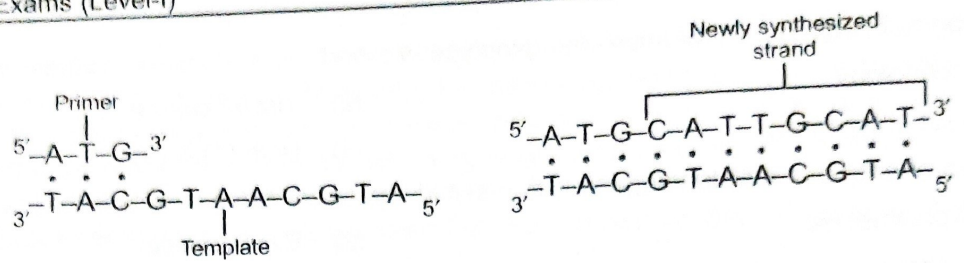


Fig. : Extension reaction

Usually DNA polymerase I is employed in genetic engineering.

Example 1 : *Discovery of which molecule made genetic engineering possible?*

Solution : Restriction endonucleases.

Example 2 : *How long is the recognition sequence of the first restriction enzyme isolated?*

Solution : Six base pairs.

Example 3 : *The antibiotic resistance gene isolated by Cohen and Boyer was inserted in plasmid native to which organism?*

Solution : *Salmonella typhimurium.*

Example 4 : *Give one word for following :*

Sequence of bases on DNA strands which read the same forward as well as backward in 5′ to 3′ direction.

Solution : Palindromic DNA.

Try Yourself

1. What do you understand by staggered ends?
2. What is the end product obtained after exonuclease activity?
3. What does 'co' represent in term *Eco*RI?
4. Name the enzyme used to combine two fragments of DNA.
5. What is a clone?