BIOTECHNOLOGY

- Biotechnology can be defined as the use of micro-organisms, plant or animal cells or their components or enzymes from organisms to produce products and processes (services) useful to human beings.
- The **European Federation of Biotechnology** (EFB) defines biotechnology as the integration of natural science and organisms, cells, parts thereof and molecular analogues for products and services.
- The term biotechnology was coined in 1917 by a Hungarian Engineer, **Karl Ereky** to describe a process for large scale production of pigs.

- Gene manipulation is a fast emerging science. It started with the development of recombinant DNA molecules. It is named variously as **DNA manipulation biotechnology** or **recombinant DNA technology or genetic engineering.**
- The technology mostly involves cutting and pasting of desired DNA fragments. It is based on two important discoveries in bacteria; (i) Presence of *plasmids in bacteria* which can undergo replication alongwith and independent of chromosomal DNA. (ii) *Restriction endonucleases* **(Arber, Nathan** and **Smith** 1970; Nobel Prize in 1978) which can break DNA at specific sites, They are appropriately called **molecular scissors.**
- **Berg** (1972) was able to introduce a gene of SV-40 into a bacterium with the help of lambda phage. Berg is often considered **"father of genetic engineering".** He was awarded Nobel Prize in 1980.
- The science of recombinant technology took birth when **Cohen** and **Boyer** (1973) were able to introduce a piece of antibiotic resistance gene containing foreign DNA into plasmid of *Salmonella typhimurium.* This modified plasmid was then inserted into E. *coli* to get clones of recombinant DNA.

OLD BIOTECHNOLOGY (TRADITIONAL BIOTECHNOLOGY)

- Microorganisms were first used to produce some organic compounds like citric acid. They were also used to produce antibodies. The levels of production of penicillin yield have been improved. But the types of products are not changed. They remain the same as those obtained from the natural strains/cell lines.
- In all these processes, only the natural capabilities of the organisms and cells are exploited. These activities are called **old biotechnology.**

MODERN BIOTECHNOLOGY

- Human insulin is also produced from a transgenic *Escherichia coli* stain that contains and expresses the insulin gene. Proteins produced by transgenes are called **recombinant proteins.** The production technologies based on genetic engineering are termed as **modern biotechnology.** It developed during 1970.
- A definition of biotechnology which covers both traditional views and modem molecular biotechnology has been given by European Federation of Biotechnology (EFB). According to EFB, "Biotechnology is the integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services."

RECOMBINANT DNA TECHNOLOGY

- **Recombinant DNA technology** includes formation of recombinant DNA and introduction of

recombinant DNA into an appropriate host. Recombinant DNA is the DNA formed by combining DNAs from different organisms.

- Formation of recombinant DNA requires careful handling of the genetic material; therefore, the term **genetic engineering** is also used for recombinant DNA technology.

PRINCIPLES OF MODERN BIOTECHNOLOGY

- The two main techniques that gave birth to modem biotechnology are as follows:
- **(i) Genetic Engineering.** The techniques which change the chemistry of genetic material (DNA and RNA) to introduce these into host organisms and thus alter the phenotype of the host organism are called genetic engineering (= Recombinant DNA technology).
- **(ii) Chemical Engineering.** It involves maintenance of microbial contamination free (sterile) ambient surrounding to have growth of only the desired micro-organism/eukaryote in large quantities for the manufacture of biotechnological products such as antibiotics, vaccines, enzymes, medicines, hormones, etc.

CONCEPTUAL DEVELOPMENT OF THE PRINCIPLES OF GENETIC ENGINEERING

- The sexual reproduction is more useful than asexual reproduction. It provides opportunities for variations and formation of new combinations of genetic makeup. Some variations are beneficial to the organisms as well as population.
- Asexual reproduction preserves the genetic characters. Traditional hybridisation methods used in plant and animal breeding very often include and multiply undesirable genes alongwith the desired genes.

- **The technique of genetic engineering includes**
    - (i) formation of 'recombinant DNA (rDNA)',
    - (ii) use of gene cloning and
    - (iii) gene transfer.
- It checks this limitation and permits to isolate only one or a set of desirable genes without introducing undesirable genes into the target organism.
- A piece of DNA which is introduced into the alien (foreign) organism would not be able to multiply itself in the organism but when it gets incorporated into the genetic material of the recipient, it may multiply and be inherited alongwith the host DNA, because the alien piece of DNA has become part of a chromosome which possesses the ability to replicate.
- There is a specific DNA sequence called the *origin of replication* in a chromosome that is responsible for initiating replication. Thus, an alien DNA linked with the origin of replication can replicate and multiply itself in the host organism. It is also called the cloning, *i.e.,* forming multiple identical copies of any template DNA. Thus genetic engineering is alternately called recombinant DNA technology or gene cloning.
- First case of construction of An Artificial Recombinant DNA. The first recombinant DNA was constructed by **Stanley Cohen and Herbert Boyer in 1972**. They cut the piece of DNA from a plasmid carrying antibiotic-resistance gene in the bacterium *Salmonella typhimurium* and linked it to the plasmid of Escherichia coli.
- The vector transfers the piece of DNA attached to it. As we know that the female Anopheles mosquito acts as an insect vector to transfer the malarial parasite into human body. In the similar manner a plasmid can be used as vector to carry an alien piece of DNA into the host organism.
- The linking of antibiotic resistance gene with the plasmid vector is in the presence of the enzyme DNA ligase. DNA ligase acts on cut DNA molecules and joins their ends. Now a combination of circular automatically replicating DNA is created in vitro, it is called recombinant DNA.

- When this recombinant DNA is transferred into Escherichia coli, a bacterium closely related to *Salmonella,* it could replicate in the presence of the new host's DNA polymerase enzyme and make multiple copies. The ability to multiply copies of antibiotic resistance gene in E. coli was termed as cloning of antibiotic resistance gene in *E. coli.*

- **Three Basic steps in creating genetically modified organism (GMO) or transgenic organism. These are as follows.**
    - (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

- Three types of 'biological tools" are used in the formation of recombinant DNA. These are as follows:
    - (A) Enzymes
    - (B) Cloning Vectors (Vehicle DNA).
    - (C) Competent host (for transformation with recombinant DNA).

Different kinds of specific enzymes are used in genetic engineering (recombinant DNA technology). These are briefly described below :

- **(1) Lysing Enzymes.**
- These enzymes are used to open up the cells to get DNA for genetic experiments. Lysozyme is usually used to dissolve the bacterial cell wall. In plant cell, cell wall is made up of cellulose, while in fungi, it is made up of chitinase.

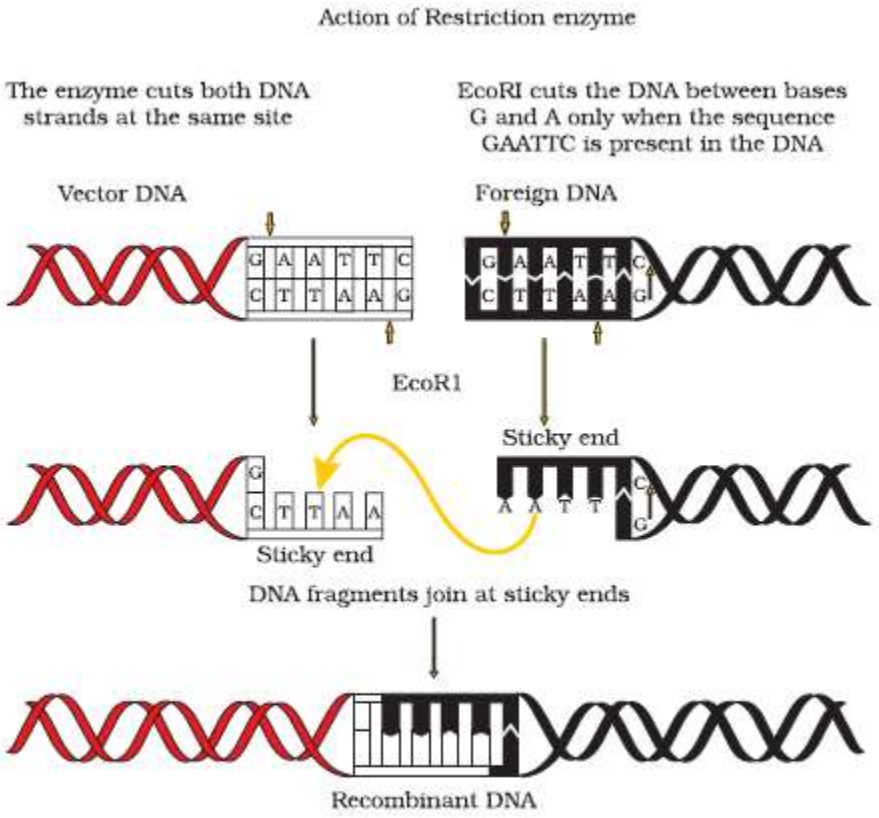
- **(2) Restriction Enzymes, (Cleaving Enzymes) - "The molecular Scissors".**
- These enzymes are used to break DNA molecules. They belong to a larger class of enzymes called nucleases. Restriction enzymes are of three types- exonucleases, endonucleases and restriction endonucleases.
- **(a) Exonucleases.** They remove nucleotides from the terminal ends (either 5' or 3') of DNA in one strand of duplex.
- **(b) Endonucleases.** They make cuts at specific position within the DNA. These enzymes do not cleave the ends and involve only one strand of the DNA duplex.
- **(c) Restriction endonucleases.** Restriction endonuclease was found by Arber in 1962 in bacteria. They act as "molecular scissors" or chemical scalpels. They recognize the base sequence at palindrome sites in DNA duplex and cut its strands, For example, restriction endonuclease EcoRI found in the colon bacteria *E. coli,* recognizes the base sequence GAATTC in DNA duplex and cuts its strands between G and A as shown below :

TYPES OF RESTRICTION ENDONUCLEASES

- Three main types of restriction endonucleases are **type I, type II and type III.**
- **Type I Restriction Endonucleases.** These enzymes consist of 3 different subunits. They require ATP, Mg^2+ and S-adenosyl methionine for restriction. Type I restriction endonucleases recognize specific sites within the DNA but do not cut these sites. Thus they are not used in recombinant DNA technology.
- **Type II Restriction Endonucleases.** These enzymes are simple and require Mg^2+ ions for restriction. Out of the three types, only type II restriction enzymes are used in recombinant DNA technology

because they can be used *in vitro* to recognize and cut within specific DNA sequence typically consisting of 4 to 8 nucleotides.

- **Type III Restriction Endonucleases.** These enzymes consist of two different subunits.They require ATP, Mg2+ and S-adenosyl methionine for restriction. They recognize specific sites within DNA but do not cut these sites; therefore, these restriction endonucleases are not used in recombinant DNA technology. They have intermediate properties between type I and type II.

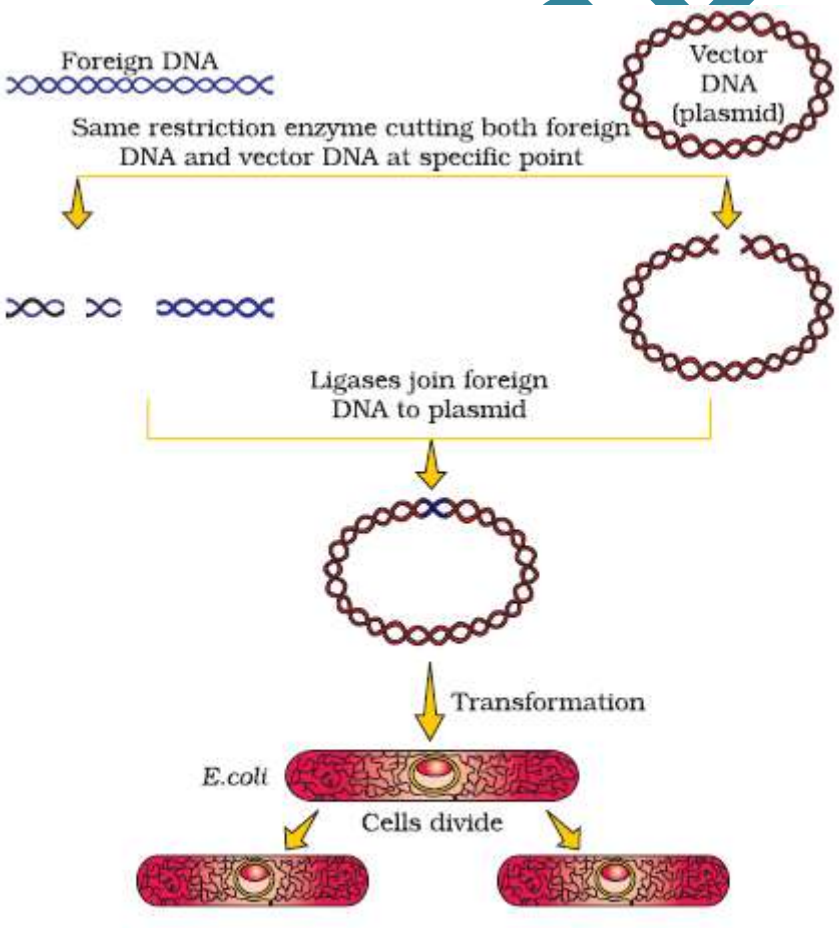


Steps in formation of recombinant DNA by action of restriction endonuclease enzyme - EcoRI

- The foundations of recombinant DNA (rDNA) were laid by the discovery of restriction enzymes. These enzymes are present in many bacteria where they function as a part of their defence mechanism called the Restriction Modification System. Molecular basis of this system was explained first by Werner Arber in 965.
- The Restriction-Modification system consists of two components; (i) A restriction enzyme which identifies the introduced foreign DNA and cuts into pieces called restriction endonucleases. The term 'restriction' refers to the function of these enzymes in restricting the propagation of foreign DNA of bacteriophages in the host bacterium.
- (ii) The second component is a modification enzyme that adds a methyl group to one or two bases usually 'within' the sequence reorganized by the restriction enzyme. Once a base in a DNA sequence is modified by addition of a methyl group, the restriction enzymes fail to recognize and could not cut that DNA. This is how a bacterium modifies and therefore, protects its own chromosomal DNA from cleavage by these restriction enzymes.
- *The first restriction endonuclease was Hin*d II (hin-dee-two). Its functioning depended on a specific DNA nucleotide sequence. It was isolated from *Haemophilus influenzae* Rd. It was found that *Hin* d 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 *Hin*d II. It produces blunt ends. Besides *Hin*d II, today we know more than 900 restriction enzymes that have been isolated from

over 230 strains of bacteria eac of which recognises different recognition sequences.

- The restriction endonuclease inspects the length of a DNA sequence. Once it recognise specific sequence, it binds to the DNA and cuts each of the two strands of the double her at specific points in their sugar phosphate back bones. Special sequence in the DNA recognise by restriction endonuclease is called palindromic nucleotide sequence.
- Restriction endonuclease recognizes palindromic sequences in DNA and cuts them. The palindromes are groups of letters that form the same words when read in bi-directions forward and backward.
- The palindromes in DNA are base pair sequences that are the same when read forward (left to right) or backward (right to left) from a central axis of symmetry. For example, following sequences read the same on the two strands in 5' - 3'when we read in the 3' - 5' direction.
- Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites but between the same two bases of the opposite strands. This leaves single stranded unpaired bases at cut ends. These ends with unpaired bases are called **sticky ends** or **cohesive ends**. The latter are named so because they form hydrogen bonds with their complementary cut counter parts. The sticky ends facilitate the action of the enzyme DNA ligase.
- EcoRI cuts the DNA between bases G and A only when the sequence GAATTC is present in the DNA.



Diagrammatic representation of recombinant DNA technology