Chapter - 15
Genetic Engineering

During the last century, knowledge about the cell and its functions has increased. In this development of knowledge. Genetics provided basic and important infomations about structure of DNA, its replication, formation and gene expression which are known today. Specifically in previous three decades we got success in knowing about gene or DNA sequencing, gene cloning and in developing the gene transfer techniques. This was possible only because of identical genetic code in both prokaryotic and eukaryotic organisms. In 1970, **Hargovind Khurana** synthesized gene in test tube and provided a strong basis for recombinant DNA techniques Biotechnology is considered as the most important of all the recent branches of biology. The purpose of which is to improve the quality of inheritance of various living animals and plants. Many techniques have been adopted to achieve this objective which are collectively known by various names such as DNA cloning or genetic engineering etc.

Recombinant DNA is a modified DNA which is produced by addition of a foreign DNA into the basic DNA of any organism.

Desired effective techniques for manipulation of DNA of any organism are called **recombinant DNA** technology. Genetic engineering means useful changes in the inheritance of any organism by the manipulation of genes.

Recombinant DNA technology was discovered by **Stanley Cohen**, **Herbert Boyer** et al. In 1973 they demonstrated the recombinant in ***Salmonella*** and ***E. coli***. In this chapter we will also understand the basic concept of recombinant DNA technology under the broad topic genetic engineering.

General Process of Genetic Engineering

The main technique of genetic engineering is **recombinant DNA technology**. Its main steps are as follows :

(1) Identification and isolation of desired gene

(2) Selection of cloning vectors

(3) Insertion of desired gene in cloning vector.

(4) Multiplication of recombinant DNA in host cell.

(5) Identification of cloned gene and its transfer into other organism.

(6) Expression of desired gene

To get recombinant DNA by genetic engineering the following tools (components) are required.

(1) Restriction endonuclease enzyme

(2) Vector

(3) Host cell or Receptor cell

(4) Gene bank

1. Identification and isolation of desired gene –

Restriction Endonuclease enzyme is used for identification and isolation of desired gene. **Werner Arber and Hamilton O. Smith** discovered the restriction endonuclease enzyme in 1970.

Restriction Endonuclease –

(A) These enzymes are like molecular scissors, which cut the DNA molecule at a specific site.

(B) These enzymes are naturally found in ***E. coli, Bacillus, Streptococcus, Thermus aquaticus*** etc.

(C) Restriction endonuclease enzymes are of three types –

Type I Endonuclease (RI), Type II Endonuclease (RII), Type III Endonuclease (R III). Type II Endonuclease (R II) is mainly used in gene cloning and restriction mapping.

Example :- *Eco* RI, *Hind* II etc.

Nomenclature of restriction enzyme –

(I) The first letter of enzyme represents the genus from which it has been isolated. This is written in capital letter always.

(ii) The two letters after this, represent the species of that genus. These are written in small letter. These three letters are written in italics.

Example :- *Eco* = *E.coli (From Escherichia coli)*

Hin* = *H. influenzae
(From Haemophilus influenzae)

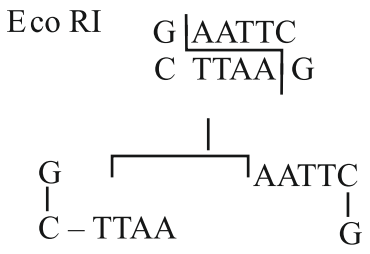
(iii) As a fourth letter those strain of a genus is written from which it has been isolated.

Example :- *Eco* R = from R strain of *E. coli*

(iv) If more than one restriction enzymes are obtained from one organism then these are represented by Roman numbers.

Example :- *Eco* RI, *Eco* RII etc.

Eco RI enzyme identify the following sequence of DNA and cut it between base G and base A.



Now if the DNA fragments obtained from different sources are mixed together in the presence of DNA ligases enzyme, then both DNA fragments form a double stranded structure after forming phospho di ester bond.

Other enzymes used in genetic engineering are:

1. RNA dependent DNA polymerase enzyme- This enzyme polymerises the nucleotides of DNA strand on RNA template.

2. DNA dependent DNA polymerase enzyme- This enzyme polymerised the nucleotides of complementary DNA strand on template DNA.

3. Ligases – The function of this enzyme is attachment of the end of DNA fragment on template.

4. Lysozymes - These enzymes dissolve the cell wall of bacteria so that DNA of bacteria can be isolated.

5. Alkaline phosphatases – It cuts the phosphate at 5' end of circular DNA and help in keeping the DNA linear, so that the foreign DNA can be inserted in it. This enzyme prevent the circular nature of DNA to form again.

2. Selection of cloning vectors –

After the isolation of the gene a vector is required which enters the host cell with desired gene and replicate its DNA.

The properties of vectors are follows –

(1) It should have the ability to replicate autonomously in the host cell.

(2) It should be easily introduced into the host cell and should be isolated again.

(3) A vector should contain specific restriction sites which can be broken easily by restriction endonuclease enzyme. The foreign DNA can be inserted easily at restriction site.

(4) The vector should have marker site that allow easy detection of transformed cells.

(5) Transformation should be easy and perfect.

(6) For the expression of desired foreign DNA, the vector should have some regulatory elements like promoter, operator etc.

Natural and man made both type of vectors can be used in ***E. coli***. The main vectors used in this are–

1. Plasmid
2. Bacteriophage
3. Cosmid

1. Plasmid – In 1952 **Lederberg** saw it first in bacterial cell as an extra chromosome. Its main properties are -

(1) They are found in bacterial cell apart from the main chromosome.

(2) They are circular, double stranded and spiral molecules.

(3) They are capable of independent replication.

(4) They are not essential for the growth and survival of bacteria.

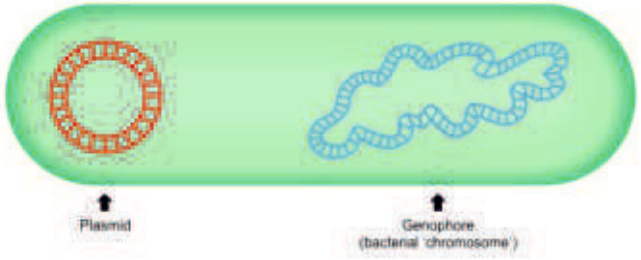


Fig. 15.1 Plasmid in a bactrial cell

(5) They contain specific restriction site where the desired gene can be integrated.

(6) They have maker sites

(7) Plasmid may contain three gene to thousand genes. The most usable plasmid is **pBR322**. It has two marker sites *Tet* R (Tetracyclin resistant) and *Amp* R (Ampicillin resistant).It contain recognition site for 12 different restriction enzymes.

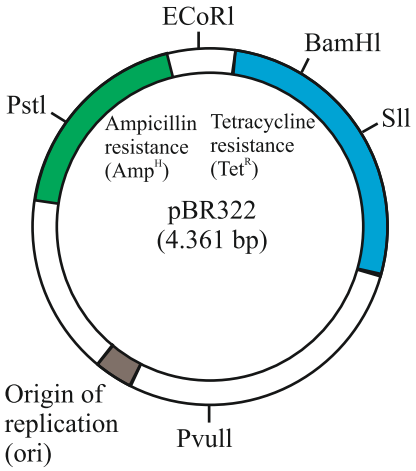


Fig. 15.2 Structure of pBR 322 plasmid

Foreign DNA is integrated between *Tet* R and *Amp* R gene with the help of restriction enzyme.

First of all, the restriction enzyme cut the plasmid to make it linear for integration of desired gene in it. After that desired DNA of approximately 5-10 kb (Kilobase) length is inserted between both ends (plasmid DNA and desired DNA). At the state of duplication the extra chromosomal structure is called **episome/Plasmid.**

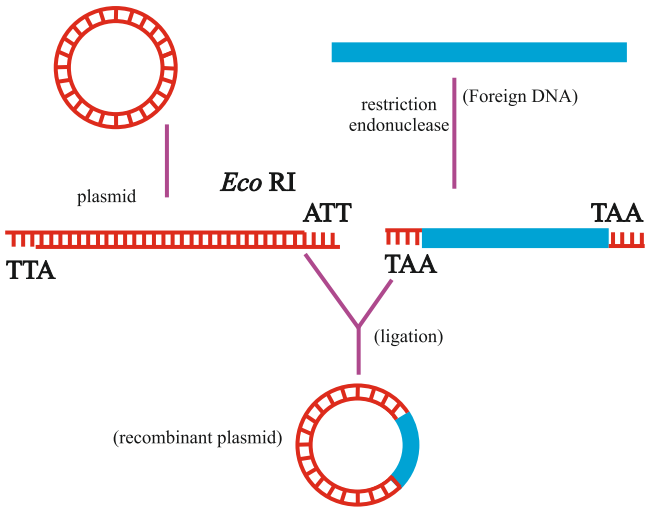


Fig. 15.3 Insertion of desired DNA in plasmid

The structure which replicate independently is called plasmid while the structure that replicate along with bacterial chromosome is called episome.

2. Bacteriophage– Viruses that infect the bacteria are called bacteriophage.

Example :- Lambda and M13-Bacteriophages are better vector than plasmids due to the following properties –

1. Large DNA fragments (24 kb) can be cloned in it.
2. Each bacteriophage produce a plaque area that makes testing easier.

Lambda bacteriophage (λ bacteriophage) is more important as vector than M13 because

1. They are bacteriophage of ***E. coli***
2. They have linear and double stranded helical DNA.

3. The unnecessary DNA part of λ phage can be removed so that vector molecule become small in size and large sized foreign DNA can be inserted in it.

3. Cosmid – It is a hybrid of plasmid and λ phage. It was developed by **Barbara Horn and John Collins**. Such plasmid in which DNA sequences of *cos* – site of λ bacteriophage are inserted are called cosmid.So cosmid have porperties of both plasmid as well as λ bacteriophage.

Cosmids replicate in bacterial cell like plasmid and their packing is like lambda particles due to presence of *cos*-site.

Example :- cosmid P^{LFR-5} this cosmid contain two cos-sites, 6 restriction enzyme sites, origin of replication site and tetra cyclin, resistant gene.

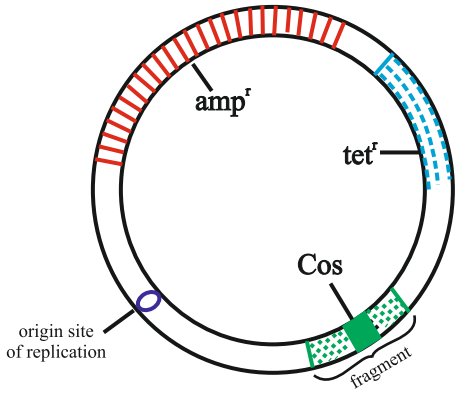


Fig. 15.4 Cosmid vector

Except plasmid, bacteriophage and cosmid some other vectors are also used in genetic engineering some of them are -

1. Phagemid
2. Shuttle vector
3. Transposons

Transposons are also known as **jumping gene**.

3. Insertion of desired gene in cloning vector–

For the insertion of desired gene in vector gene, similar restriction sites are made in both genes first and after that both are mixed. It is called ligation. The following enzymes are required in this process.

(1) Restriction endonuclease

(2) Methylases

(3) DNA ligases

(4) Alkaline phosphatases

(5) Reverse Transcriptase

(6) Terminal transferases

4. Multiplication of recombinant DNA in host cell–

Recombinant DNA is introduced into host cell by two ways -

1. Transformation
2. Transduction

In this process, the ***E. coli*** bacterial cell is most commonly used as host.

These processes have been studies earlier. In ***E. coli*** cell recombinant DNA multiplies.

5. Identification of cloned gene and its transfer in other organism –

When desired gene is integrated with vector then many unwanted products are also obtained. A special type of gene is used for removing unwanted products and for selection of recombinant DNA in host cell. This gene is called **marker gene** which produce special features in transformed cells. This gene is added into vector DNA

Example :- kanamycin resistant gene. Besides marker genes, there are some genes which produce specific phenotype. These are known as reporter gene which look different from other cell due to its phenotype.

Example :- *LUC* (Leuciferase) gene which is found in firefly and produces bioluminiscence.

6. Expression of desired gene –

The expression of cloned gene which is obtained from the desired gene, means the cloned gene derived from ***E.coli,*** is inserted into any microbes, plants or animals to get desired product.

Example :- Insulin production in ***E. coli***

Those plants and animals in which foreign DNA is present are called transgenic plants or transgenic animals.

c-DNA (Complementary DNA) and Genomic Library

Complementary DNA – The copy of DNA produced on the template of RNA by **reverse transcriptase** is called complementary DNA.

c-DNA library – Inserting all or several complementary DNA of any organism in the host cell by gene cloning and maintaining it is called c-DNA library.

Formation of c-DNA Library –

(1) First of all, m RNA is obtained from the cell of tissue that produce functional protein.

Example :- Root of plants, leaf cell, from ovary of mammals

(2) Now, c-DNA is formed by each molecule of m RNA with the help of reverse transcriptase

(3) This c-DNA is cloned into vector and introduced in the bacterial cell.

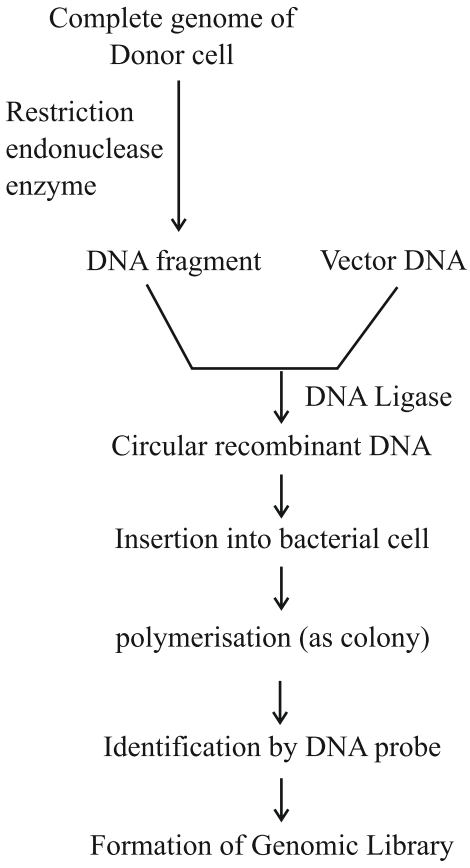
(4) After this, the identification and specification of c-DNA is done by hybridisation of probe.

c-DNA is used to express characteristics of eukaryotic genes in prokaryotic cell because they lack introns of eukaryotic gene.

Genomic Library –

A **genomic library** is a collection of the cloned fragments of the complete genome of any organism. The complete haploid DNA set of any organism is called its genome.

Genomic library is formed by isolating complete DNA of a cell. It is formed by following steps –



Molecular probes – The fragments of DNA or RNA with the help of which complementary DNA or RNA fragments present in some organism, can be identified are called **molecular probes**.

Molecular probes are of two types –

1. DNA probes 2. RNA probes

Significance of probes

(1) These are used in indentification of specific DNA fragments in genetic engineering research.

(2) Pollutant can be detected in food with the help of probes

(3) These are used in forensic science, in resolving disputed paternity issue and establishing family relationship.

(4) Probe are used in crop producing seeds and advanced species of plants.

Techniques of analysis of cloned genes

Specific techniques has been discovered during last decades for analysis of cloned genes. In this chapter some important techniques are being described.

1. Gel Electrophoresis – Electrophoresis is an analytical method which is normally used to purify and isolate the DNA fragments. In this method, negatively charged DNA fragments are separated based on their size, under electrified field. Two type of gel are used in this apparatus.

1. Agarose gel 2. Polyacrylamide gel

There are pores between gel molecules and it work as molecular sieves. The DNA fragments are stained and when they flow through these gel pores in electric field then some of the DNA fragment move faster and some others move slower, based on their size and form the bands of similar size at different locations. After this the gel is stained and when they are visualized in UV light then fragments are seen of bright orange colour.

2. Blotting techniques – In this method, DNA fragments which are obtained on agarose gel (By gel electrophoresis) are transferred and established on nitrocellulose filter. Then these are identified by hybridization with DNA probes. This process is called blotting technique.

First of all **E. M. Southern** transferred the DNA fragments on nitrocellulose filter in 1975 and this technique was name as **Southern blotting technique**. DNA fragments are analyzed by this technique.

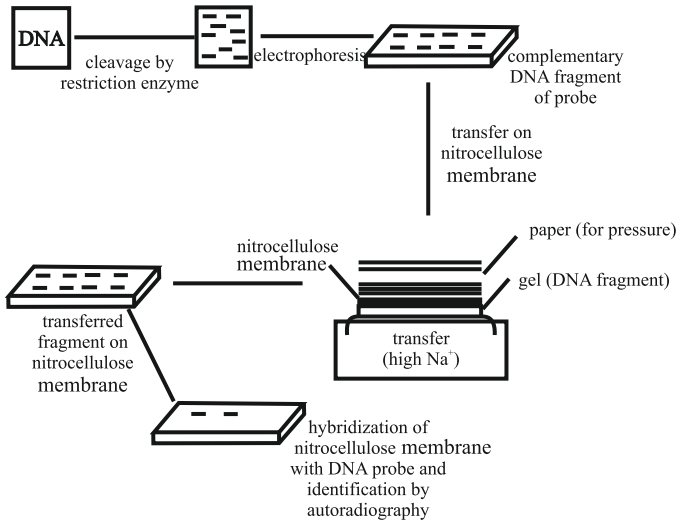


Fig. 15.5 Southern blotting technique

Alwin et.al. in 1979, transferred RNA fragments after gel electrophoresis, on Amino Benzyl Oxy Methyl Membrane (OBM) in place of nitro cellulose. This technique was named as **Northern blotting technique**. This technique is used for the analysis of RNA fragments. In 1979,**Tobin** et. al. first broke down the proteins into polypeptides with the help of sodium-do-decyl and transfer it onto nitro cellulose filter or nylon membrane with the help of gel electrophoresis and identified the protein on X-ray plate. This techniques is used for analysis of proteins this is called **western blotting**.

3. Polymerase Chain Reaction – (PCR) – Up to 1985, vectors were considered as necessary tool for replication of genes of prokaryotic and eukaryotic cells. But in 1989,**Mullis** discovered a strong technique for replication of DNA by which lacs of copies of one DNA copy can be obtained in very short time. This is known as **polymerase chain reaction.**

This technique includes three main steps which result in multiple DNA by repeating cycle again and again and by repeating 20-30 times, lacs of copies of DNA are formed. This reaction is completed in 'thermal cycler'. Each cycle is completed in 225 seconds.

Uses of PCR-

1. For molecular mapping and research objectives
2. In genetic engineering and gene therapy experiments
3. In study of polymorphism of DNA
4. Used in forensic science for identification of criminals.
5. Used in detection of hereditary diseases like hemophilia, sickle cell anemia etc.

4. DNA finger printing – This method was discovered by **Alec Jeffreys** and coworker in 1985. A person can be identified seperately due to presence of specific base sequence in DNA. DNA print or image is always the same whether it is taken

from the cell of any body organ of the person. In this method, DNA is cut into small fragments with the help of restriction endonuclease and are separated in the form of bands by electrophoresis. Then it is identified on X-ray plate by southern blotting technique with the help of UV light. This is called DNA finger printing.

Uses of DNA finger printing –

1. To find out parents of any child.
2. To find out about genetic disorders like haemophilia etc. before birth of infants.
3. To identify the rapists and murderers

5. Mapping Restriction Fragment Length Polymorphism (RELP) – When any restriction endonuclease cuts the fragments of genomic DNA of various strain or related species then it is called RELP.

In this method, the DNA of the related species is isolated and digested by enzyme and then separated by gel electrophoresis. These bands are transferred on nitro cellulose filter. This filter membrane is placed in the radio active probe with deviation. At the end, these are identified by auto radiography.

Uses of RFLP –

(1) Determine the relationship between different species or strain.

(2) To know the gene structure and function of quantitative symptoms.

(3) This is also used for DNA finger printing

(4) RFLP maps of corn, rice, wheat etc. have been prepared by this method.

Achievements of Recombinant DNA Technology –

Human got many achievement in medical science, agriculture science and industrial field with the help of biotechnology. By using this technique human can improve the varieties of the domestic animals and agricultural crops.

Some of the important achievements of recombinant DNA technology are.

(i) Cloning of nitrogen fixation (*Nif*) gene.

(ii) Cloning of gene of haemophilia.

(iii) Cloning of gene of hepatitis B Virus.

(iv) Cloning of human growth hormore and insulin gene.

(v) Human Genome Project.

(vi) Cloning of penicillin G acylase gene for production of penicillin.

Important Points

1. Required effective programs for manipulation of DNA of any organism are called recombinant DNA technology.
2. Restriction endonuclease enzyme is also called molecular scissors because it cuts the phosphodiester bond at specific site of DNA.
3. Restriction endonuclease enzymes are of three types RI, RII, RIII
4. Transfer of desired DNA fragments into host cell is done by vector.
5. Main vectors are – plasmid, bacteriophage and cosmid
6. Most commonly used plasmid is pBR322
7. Bacteriophage which are used as vectors are Lambda and M13
8. *E. coli* is most commonly used as host.
9. Copy of DNA formed on RNA template by the reverse transcriptase enzyme is called complementary DNA or c-DNA.
10. Gel electrophoresis technique is used to purify the DNA and isolate it as a band.
11. Southern blotting technique is used for analysis of DNA, northern blotting for RNA and western blotting is used for protein analysis.
12. Lacs of DNA copies can be prepared by PCR technique in a very short time.
13. DNA finger printing is used for finding out the genetic disorders.
14. Human can improve the variety of crops and animals by using genetic engineering.

Practice Questions

Multiple Choice Questions

1. Which enzyme cut the DNA at specific site.

(A) Ligase
(B) Polymerase
(C) Restriction endonuclease
(D) All of the above

2. Restriction endonuclease enzyme is naturally found in -
(A) Bacteria (B) Virus
(C) Plants (D) Animals

3. Vector DNA is –
(A) Plasmid (B) c-DNA
(C) Synthesized DNA (D) All of the above

4. M13 is an example of -
(A) Plasmid (B) Bacteriophage
(C) Cosmid (D) All of the above

5. Which blotting technique is used to analyze DNA fragment
(A) Genomic DNA (B) Western
(C) Southern (D) Northern

6. Which of the following join the ends of DNA.
(A) Restriction endonuclease
(B) Ligases
© Lysozyme
(D) All of the above

7. Jumping genes are called –
(A) Phasmid (B) Plasmid
(C) Cosmid (D) Transposons

8. In 1989,Mullis discovered–
(A) Plasmid
(B) Polymerase chain reaction
(C) Southern blotting technique
(D) Western blotting technique

9. Which of the following is used in production of c-DNA
(A) t RNA (B) m RNA
(C) r RNA (D) DNA

10. The source of *Eco* R enzyme is
(A) Bacteria (B) Algae
(C) Plants (D) All of the above

Very Short Answer Questions

1. Who discovered the recombinant DNA technology?
2. Define recombinant DNA technology?
3. What are cloning vectors?
4. What are molecular probes?
5. What are marker genes? Give example
6. What are reporter gene? Give example
7. What is gene library?
8. What are cosmids?
9. Define the restriction endonuclease enzyme.
10. Write the name of gel which is used in Gel electrophoresis.
11. Write the full form of RFLP.

Short Answer Questions

1. What are cloning vectors? Describe briefly the various vectors which are used in recombinant DNA technology.
2. Write comment on pBR322 Plasmid.
3. Write comments on -
(i) Southern blotting technique.
(ii) DNA finger printing.
(iii) Polymerase Chain Reaction.
(iv) Nomenclature of restriction enzyme.
(v) Characters of cloning vectors.
4. Explain the process of formation of genomic library.
5. Describe briefly the use of bacteriophage as vector.

Essay Type Questions

1. Describe the various steps of recombinant DNA technique of genetic engineering.
2. Write the detailed description of blotting techniques.
3. Write detail note on plasmid.
4. What do you understand about molecular probes? Explain their uses.
5. Write the significance of genetic engineering?

Answer Key -

1. (C)	2. (A)	3. (A)	4. (B)
5. (C)	6. (B)	7. (D)	8. (B)
9. (B)	10. (A)		