UNIT 26

BIOTECHNOLOGY

Major Concept

26.1 Cloning of Genes
26.2 DNA Sequencing
26.3 DNA Analysis
26.4 Genome Maps
26.5 Tissue Culture
26.6 Transgenic Bacteria, Plants and animals
26.7 Biotechnology and Healthcare
26.8 Scope and importance of Biotechnology

Learning Outcomes

Students will be able to:

- Define gene cloning and state the steps in gene cloning.
- Describe the techniques of gene cloning through recombinant DNA technology.
- Explain the role of restriction endonucleases and DNA ligases in gene cloning.
- Describe the selection and isolation of the gene of interest.
- Explain the properties and the role of vectors in recombinant DNA technology.
- State the steps for the integration of DNA insert into the vector.
- Briefly state the technique applied for the selection of the vectors that take up the DNA insert.
- Describe the steps involved in gene amplification through polymerase chain reaction.
- Describe the procedure for the construction of genomic library.
- Explain the Maxam *I* Gilbert procedure and the Sanger-Coulson method of DNA sequencing.
- Describe the principles of Gel Electrophoresis as being used in gene sequencing.
- Introduce the automated DNA sequencing as based on the Sanger-Coulson method.
- Describe the purposes and mechanism of DNA analysis.
- Describe the terms of genome analysis, genome map and genetic markers.
- State the history of the human genome project admiring James Watson as its first director.
- Describe the goals of the human genome project.
- Predict some of the possible benefits that can be derived after the completion of the human genome project.
- Define following terms related to plant tissue culture; explants, callus, micro-propagation, plantlets, somatic embryogenesis, soma clonal variation.
- Explain tissue culture and differentiate between the organ culture and cell culture.
- Differentiate between the callus culture and suspension culture techniques.

- Describe the anther culture, ovary culture, meristem culture and embryo culture techniques.
- Briefly describe the techniques used for, applications and limitations of animal tissue culture.
- State the objectives of the production of transgenic bacteria, transgenic plants and transgenic animals.
- Describe different methods applied for the introduction of DNA into plant and animals cells / embryos.
- Describe the role of biotechnology in the production of insect, virus and herbicide resistant plants.
- State the notable human gene transfers in different animal species and describe their potential applications and future prospects.
- State the role of transgenic bacteria in making biotechnology products.
- List some of the ecological concerns surrounding transgenic bacteria.
- Describe the ways in which genetic engineering improves farm animals.
- Describe how biotechnologists are able to combat health problems by producing vaccines.
- State the role played by biotechnology in disease diagnosis (DNA/RNA probes, monoclonal antibodies).
- Describe what products biotechnologists obtain for use in disease treatment.
- Explain the current methods employed for gene therapy (*ex-vivo* and *in-vivo* methods).
- Explain with example gene therapies in the detection and treatment of some genetic diseases.
- Explain the role of successful gene therapy for cystic fibrosis.
- Explain the scope and importance of biotechnology in promoting human welfare.
- List the hazards and social/ethical implications of using gene technology in human.

Introduction

Biotechnology is any technique that uses living organisms to make products. As far back in recorded history as biblical times, biotechnology was used; *e.g.* yeast was used to bake bread and for the fermentation of wine and the production of cheese. In 1862, Louis Pasteur discovered that fermentation is caused by micro-organisms and is not an inorganic chemical process. The people became aware that they were using living things to make everyday products. Since Mendel's work was rediscovered in 1900, geneticists made startling advances which have led to new era of DNA technology. Modern techniques and technologies enable us to remove genes from one organism and insert them into another in order to produce desired substances, such as insulin, growth hormone, drugs and vaccines. This knowledge is being used to clean up environmental pollutants, kill insect pests, increase fertility of soil, *etc.*

Nowadays the field of biotechnology as has become a vast field, but this unit deals with a brief introduction of biotechnology, called **Genetic Engineering** (deals with manipulation or alteration in genetic material of an organism).

26.1 Cloning of Genes

Cloning of genes produces many identical copies (clones) of a single gene. The clones of genes are used in many chemical processes. There are two possible ways of cloning of gene *i.e.* recombinant DNA technology and polymerase chain reaction (PCR). The recombinant DNA technology is used when a very large quantity of a gene is required while PCR is used to create an optimum number of copies within a laboratory in a test tube.

26.1.1 Gene Cloning through Recombinant DNA Technology

Recombinant DNA technology is popularly known as genetic engineering, which is a series of procedures that are used to join together (recombine) two or more segments of DNA from different sources. The recombinant DNA (rDNA) is also called **Chimeric DNA**.

Cloning

It is a technique for developing a large number of genes, identical cells, tissues or organisms.

In order to produce recombinant DNA, following components are required:

- To get gene of interest (desired gene), which is to be cloned.
- **Molecular scissors (restriction endonuclease)** to cut out the gene of interest.
- Vector or molecular carriers are needed to carry gene of interest.
- The enzyme (ligase), which is responsible for the formation of phosphodiester linkage between two adjacent nucleotides.
- Expression system, (such as bacteria) in which gene of interest along with vector is inserted, to get large number of desired genes or specific chemical products.

26.1.2 Role of Molecular Scissors and DNA Ligase

Molecular scissors are also called **restriction endonucleases**. These natural enzymes of bacteria were first isolated by **Hamilton D. Smith** (1970), at **John Hopkin University** in Baltimore, Maryland, USA. He observed that this enzyme has property to cut the DNA of virus to prevent replication of viral DNA in bacteria, different species of bacteria produce different restriction endonucleases, which cut the DNA at very specific sequence of **four to eight nucleotides** arranged symmetrically in reverse order. Such sequences are called **palindromic sequence** and their cutting points are called **restriction site**. So far over **3,000 restriction enzymes** have been studied in detail and more than 600 of these are available commercially (230 different DNA sequences). These enzymes are routinely used for DNA modification in laboratories, and they are a vital tool in molecular cloning, twenty enzymes are frequently used in recombinant DNA technology. (Fig.26.1)

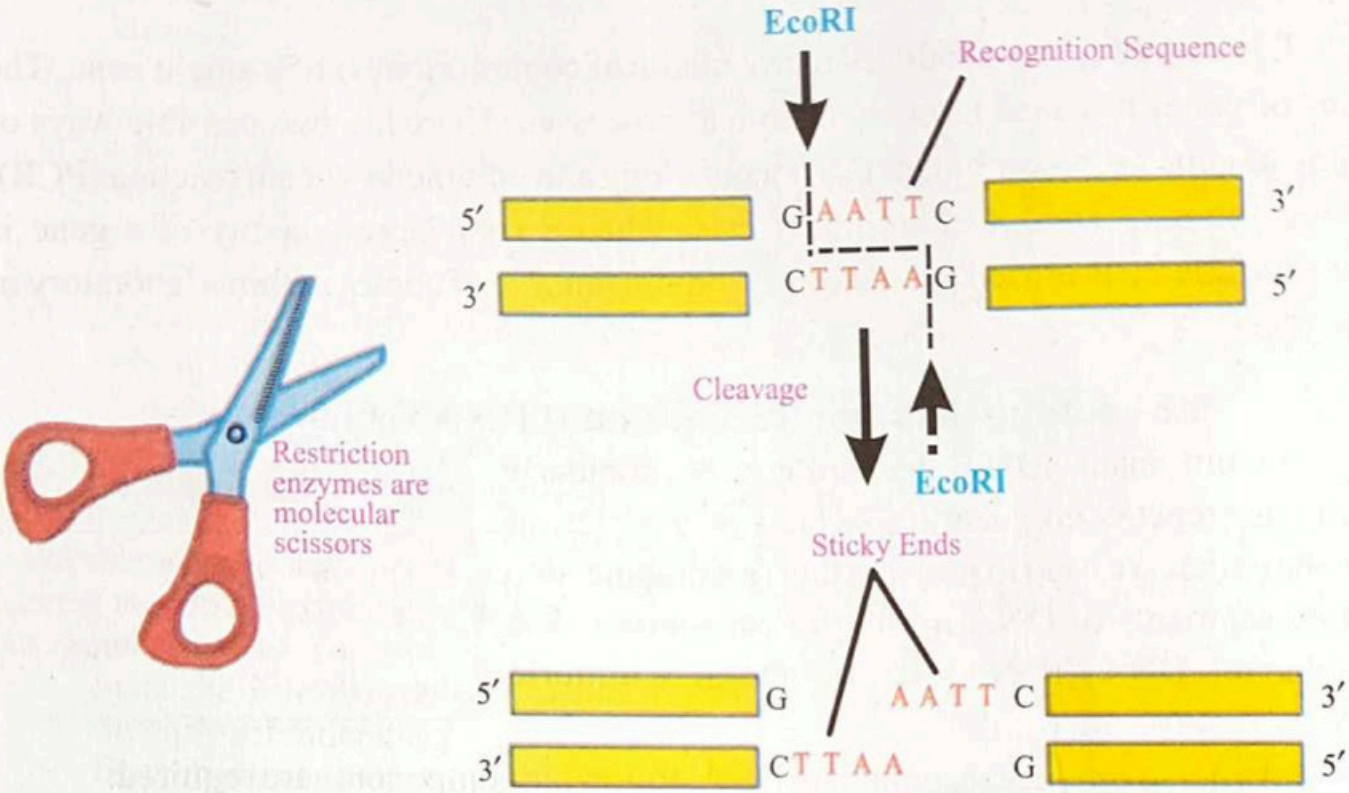


Fig. 26.1: Molecular Scissors

EcoRI (isolated from ***Escherichia coli***) is most commonly used restriction enzyme, which cut double stranded DNA into single stranded complementary ends, whose palindromic sequence has (AATT) bases at the cleavage site.

The complementary ends of the two DNA molecules are called **sticky ends** (a staggered cut) because these can bind by complementary base pairing. Thus foreign DNA with same palindromic sequence is easily inserted into vector DNA.

Extra Information

Restriction enzymes are named after their host of origin. *e.g.* xhal from Xanthomonas holcicola, hind II and III from Haemophilus influenzae.

Gene of Interest

The desired genes can be acquired by three possible ways.

- Genes can be isolated from the chromosomes by cutting the chromosomes on the flanking sites of the gene. For this purpose, restriction endonucleases are used. (These enzymes are obtained from bacteria, which they use for their own protection against viruses. The restriction enzyme cut down the viral DNA, but does not harm to the bacterial chromosome).
- The gene of interest can also be synthesized in the laboratory from mRNA, using reverse transcriptase, which are naturally found in retro viruses. This DNA molecule (gene) is called complementary DNA (cDNA).
- If the genes of interest are small, then they can be synthesized artificially by a

machine called DNA synthesizer. This machine can create specific DNA molecules of desired sequence of nucleotides.

Role of Molecular Carriers or Vectors

Vectors are another major component required to make a rDNA molecule for gene cloning. It acts as a vehicle for carrying foreign DNA into a host cell for multiplication. One common type of vector is a **plasmid** (small extra chromosomal circular DNA molecules in bacteria, contain genes for antibiotic resistance and fertility, *etc.*). The commonly used vectors are plasmids, **Lambda phage** DNA), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs).

DNA Ligase: It acts as **molecular glue** that assists to seal the foreign pieces of DNA into the vector to form recombinant or chimeric DNA. The complimentary ends of two DNA molecules are joined by hydrogen bonding and ligase is responsible for the formation of the phosphodiester linkage between adjacent nucleotides.

Expression of Recombinant DNA / Expression system

The most suitable organism which acts as vector to multiply and express the recombinant DNA, is bacteria, because they have short generation time and simplicity of its genetic system. The bacterial cells easily take up recombinant plasmid, especially if they are treated with calcium chloride to make them more permeable. Therefore, as the bacterium reproduces, each daughter bacterium contains the gene of interest, which will express itself and make a product *e.g. Agrobacterium, tumefaciens*.

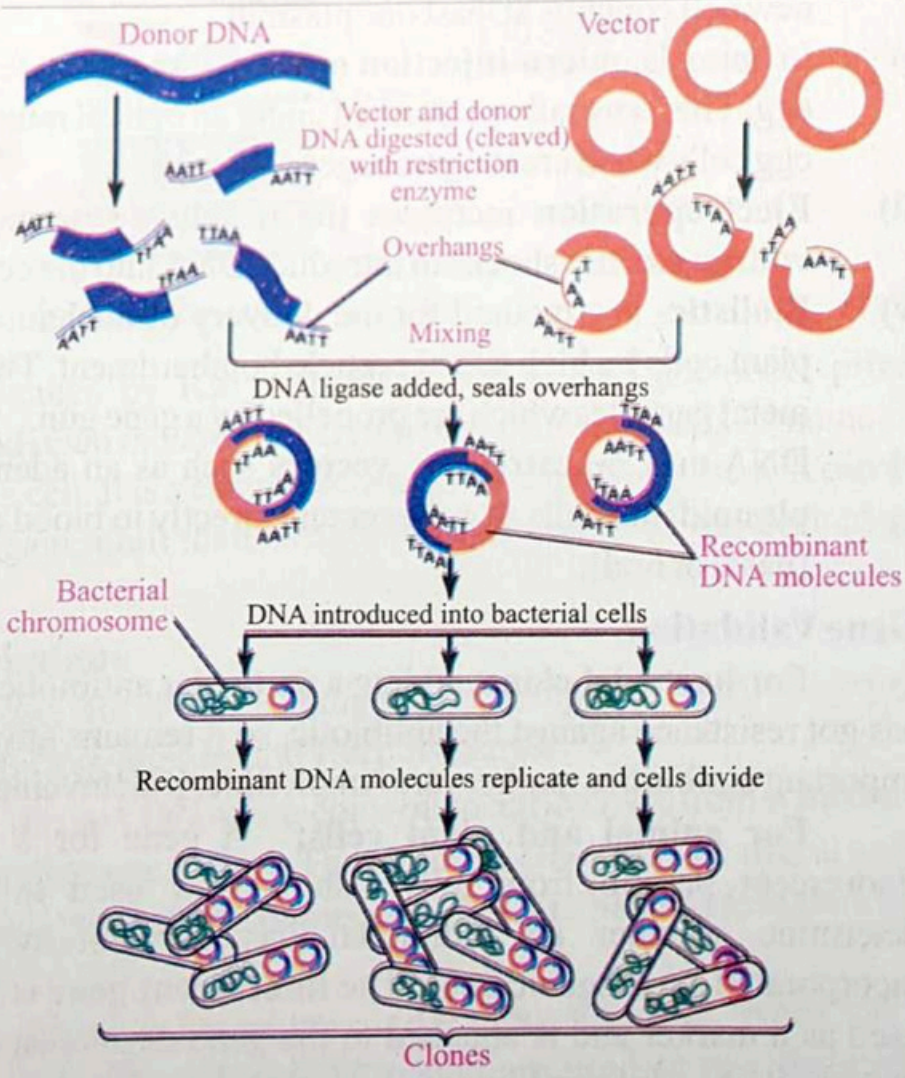


Fig.26.2: Formation of Recombinant DNA in Bacteria

Steps for creation of Recombinant DNA (Plasmid)

The simplified steps outlining how to create a recombinant DNA and

transgenic species is cut, copy and paste.

1. **Cut:** A gene of interest is removed from the cell of an organism, using molecular scissors.
2. **Copy:** Now multiple copies are made (known as gene cloning). This step is usually carried in bacteria (some time phage) *e.g. Escheria coli* is the regulating system.
3. **Paste:** The genes are inserted (Pasted) into a bacterium or an egg cell or a plant cell of another species. And (after fertilization in case of animal) become part of the newly formed transgenic organism's DNA.
4. The transgenic organism develops into a mature organism with the new gene "Switched on" to function. (Fig.26.2)

Transformation of Expressing system/ Gene delivery Techniques

i) **Agrobacterium mediated transformation** take up recombinant plasmid, especially, if they are treated with calcium chloride to make them more permeable. Thereafter, as the cell reproduces, a bacterium clone forms and each new cell contains at least one plasmid.

ii) In animals, **micro-injection of DNA** directly injects DNA into the nucleus of an egg. This is usually performed under an optical microscope to introduce DNA into egg cells when creating transgenic species.

iii) **Electroporation** increases the membrane permeability by applying a high-voltage electric shocks to introduce DNA into the cells.

iv) **Biolistics** is a method for the delivery of nucleic acid (recombinant genes) into plant cells by high speed particle bombardment. The technique uses DNA-coated metal particles which are propelled by a gene gun.

v) DNA may be carried by **vectors** such as an adenovirus, liposome or bacterial plasmid into cells. It either enters directly to blood or may be delivered by aerosol (nasal or oral).

Gene Validation

For bacterial clone, adding a particular antibiotic to transformed clone, if clone has got resistance against the antibiotic, so it remains alive and continues to grow, some important antibiotics markers are Ampiciline, Kalamycine, Streptomycine, *etc.*

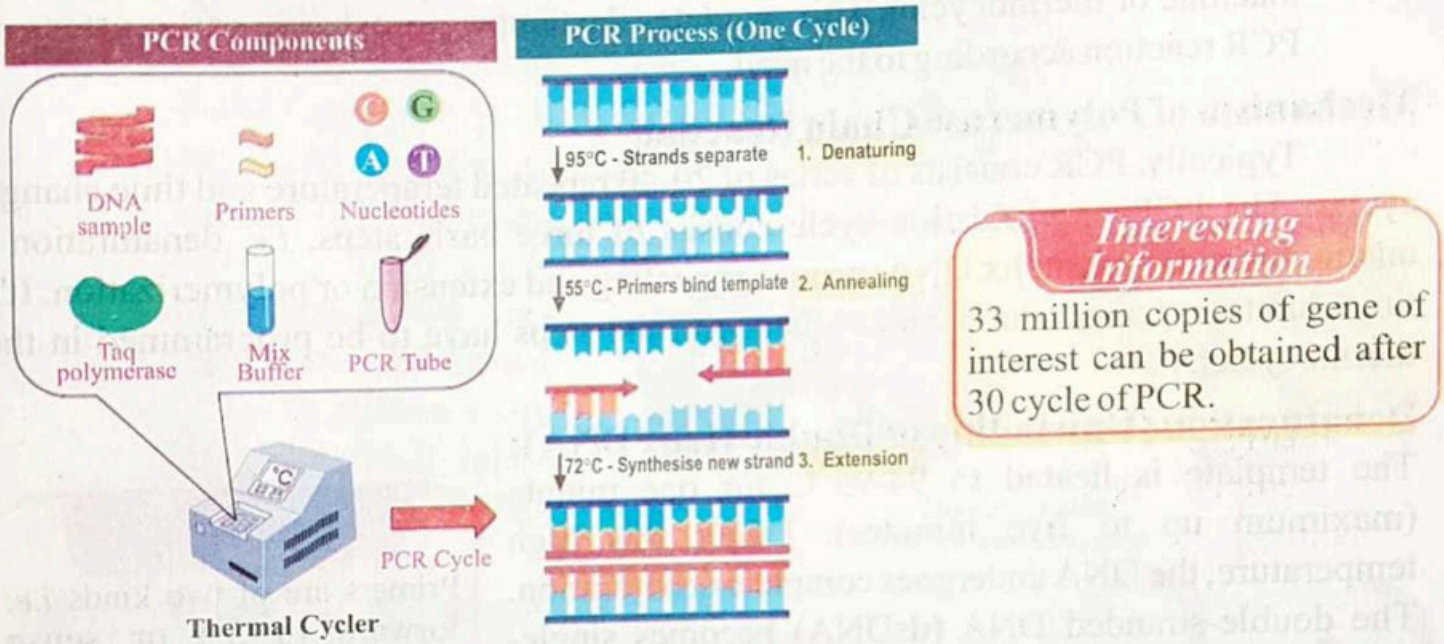
For animal and plant cells: A gene for a fluorescent protein from jelly fish is now used to determine whether an individual has successfully incorporated a transgenic gene. The **fluorescent gene** is used as a marker and is attached to the gene of interest that will be inserted into prospective transgenic

Liposomes are minute spherical sacs of molecule enclosing water droplet, carrying genes or drugs.

organism. Thus resulting offspring (cell) gives fluorescence under certain lightening conditions.

26.1.3 Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction "PCR" is a type of reaction which can create millions of copies of a single gene or any specific small piece of DNA quickly in a test tube. (Fig.26.3)



Interesting Information

33 million copies of gene of interest can be obtained after 30 cycle of PCR.

Fig. 26.3: Polymerase Chain Reaction

It was originally invented by **Kary Mullis** in 1983, who got noble prize in chemistry in 1993. It is based upon *in vitro* replication process. PCR takes its name from DNA replication enzyme in a cell. It is a chain reaction because polymerase will carry out replication over and over again, until there are millions of copies of the desired gene (DNA).

Components of PCR Technique

The components, required for PCR technique are template DNA, deoxyribo-nucleoside triphosphates (dNTPs), primers and Taq polymerase.

i) **Template DNA or Target DNA** is a copy of specific DNA from a mixture of DNA molecules which is to be copied. The template DNA may be useful gene or piece of DNA of infected organisms or pieces of DNA to be used for DNA finger printing.

ii) **Deoxyribo-Nucleoside triphosphates (dNTPs)** are four types of free nucleotides *i.e.* dATP, dGTP, dTTP, dCTP which are used as raw material for replication of new DNA pieces.

iii) **Primers:** Before carrying out PCR, primers sequence of about 15-20 bases are needed because DNA polymerase does not start the replication process. These primers are complementary to the base on either side of the target DNA.

iv) **Taq Polymerase:** The basic components required to copy template DNA is **thermocycler** and temperature tolerant (thermostable) enzyme. This enzyme is extracted from the bacterium ***Thermus aquaticus*** which lives in hot springs, it can withstand high temperature up to 115C. PCR is done in an automatic PCR machine or thermocycler. This regulates the temperature during various steps of PCR reaction according to the need.

Mechanism of Polymerase Chain Reaction

Typically, PCR consists of series of 20-40 repeated temperature and time change cycles. The PCR amplification cycle consist of three basic steps, *i.e.* denaturation / unwinding of double helix DNA, primer annealing and extension or polymerization. It's time duration, temperatures and sequence of the steps have to be programmed in the thermocycler.

Denaturation (Unwinding of Double Helix DNA):

The template is heated to 94-95°C for one minute (maximum up to five minutes). Under this high temperature, the DNA undergoes complete denaturation. The double-stranded DNA (dsDNA) becomes single-stranded (ssDNA). Thus each single ssDNA can act as the template for the *in vitro* DNA synthesis.

Interesting Information

Primers are of two kinds *i.e.* forward (3′-5′) or sense primers. Reverse primer (5′-3′) or Anti sense primer.

Primer annealing (Hybridize): In this step the two primers, the forward primers (3′-5′) and the backward primers (5′-3′), anneal or hybridize to the single-stranded template DNA at its complementary regions. This step is usually carried out at a lower temperature depending on the length and sequence of the primers. (In standard case 54-55°C) for approximately 1-2 minutes.

Interesting Information

Polymerase chain reaction is the reaction which is used for production of number of desired gene copies within test tube.

Extension of Polymerization

It is final step of PCR, in which the Taq DNA polymerase synthesizes new DNA strands to the 3′ ends of primers using dNTPs. The optimum temperature for carrying out the primer extension reaction or polymerization of dNTPs is standardized at 72°C and take one minute to be completed.

At the end of first cycle one target DNA molecule is converted into two daughter molecules. The second cycle immediately starts with the denaturation by heating at 94°C.

As a result all the newly synthesized. DNA are also denatured to single strands, which again act as templates. It will again be followed by the primer annealing and extension continues resulting in the amplification of the selected DNA sequence at an exponential rate *i.e.* the number of existing DNA molecules become doubled after each cycle.

The Application of Polymerase Chain Reaction

- To diagnose viral infections, genetic disorders and cancer.
- To identify criminals in forensic laboratories (DNA finger Printing).
- To determine the evolutionary history of human population. It has been possible to sequence DNA taken from a 76,000 year's old **mummified human brain** and from a 17 to 20 million years old plant fossil following PCR amplification.
- Detection of micro-organisms in food samples, water and in the environment with the help of species-specific primers.
- Reactions substances for DNA sequencing.

26.1.4 Genomic Library

A **genome** is a full set of genes or chromosomes of an individual. (Fig.26.4)

Genomic Library

A genomic library is a collection of bacterial or bacteriophage clones, each clone containing a particular

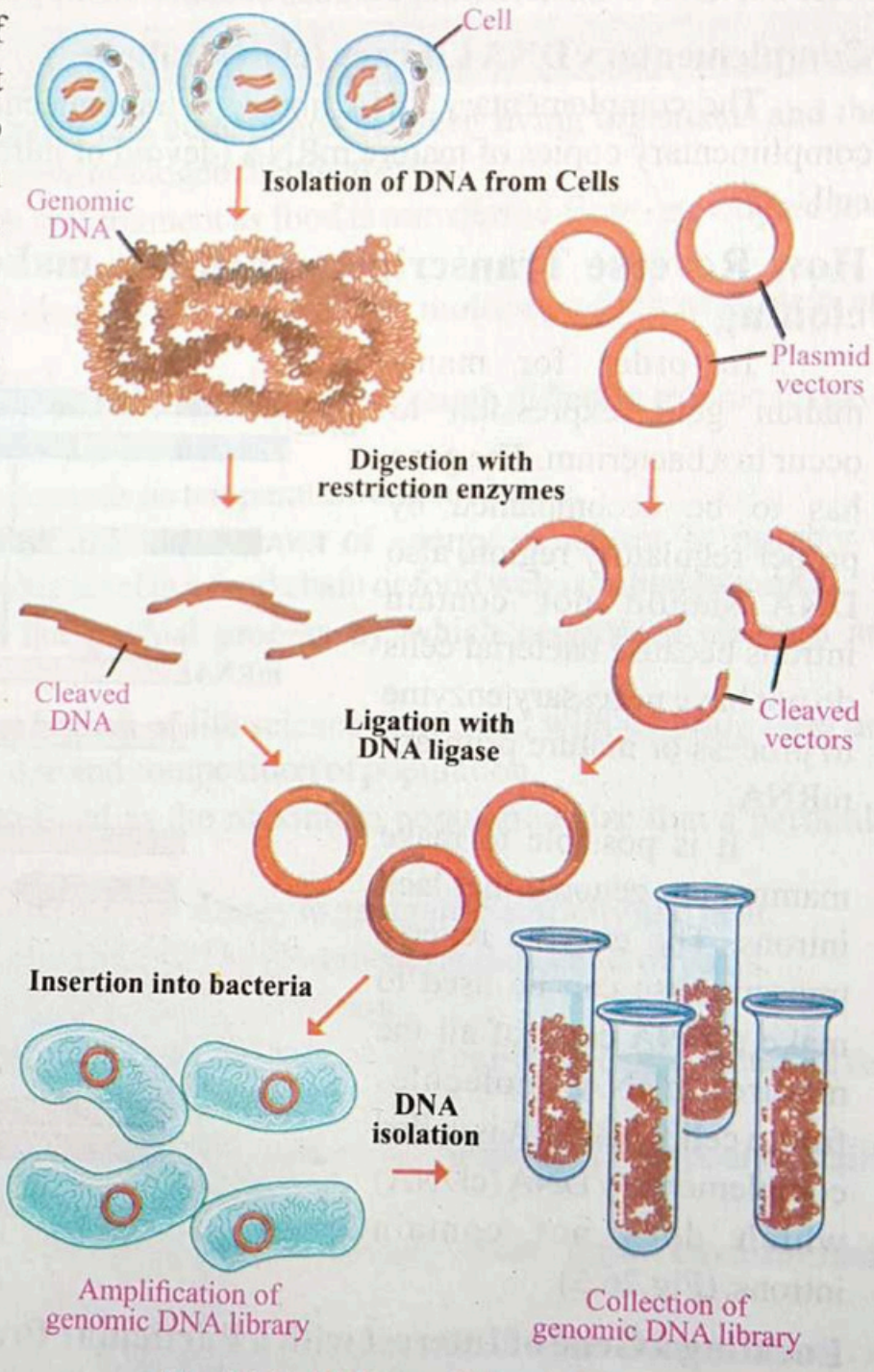


Fig. 26.4: Making a Genomic Library

segment of DNA from the source cell. The collection of genomic libraries of different organisms is called **gene bank.**

Making a Genomic Library

For making a genomic library, an organism's DNA is simply sliced into pieces by a molecular scissor and pieces are put into vectors (cut with the same molecular scissor *i.e.* plasmids or viruses) that are taken up by host bacteria. The ligase enzyme connects DNA pieces which is now called recombinant DNA. The entire collection of these bacterial or bacteriophage clones contains all the genes of that organism.

Complementary DNA Library (cDNA Library)

The complementary DNA library is the collection of clone of DNA, which is complimentary copies of mature mRNA (devoid of introns) separated from a particular cell.

How Reverse Transcriptase helps to make Mammalian Gene for cloning

In order for mammalian gene expression to occur in a bacterium. The gene has to be accompanied by proper regulatory region, also DNA should not contain introns because bacterial cells do not have necessary enzyme to process or mature prim-ary mRNA.

It is possible to make mammalian genome that lack introns. The enzyme reverse transcript-ase can be used to make a DNA copy of all the mature mRNA molecules from a cell. This DNA is called complementary DNA (cDNA) which does not contain introns. (Fig.26.5)

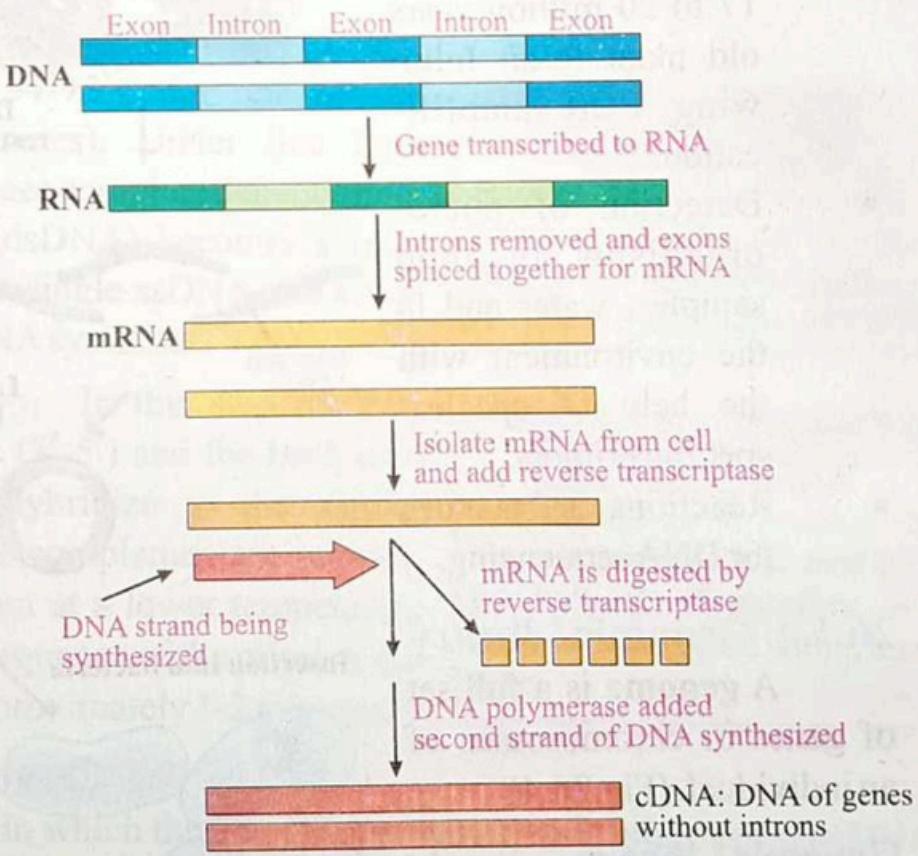


Fig. 26.5: Formation of cDNA

Locating a Gene of Interest with a Particular Probe

A **particular probe** is a single stranded nucleotide sequence that will hybridize

(pair) into a certain piece of DNA, it is used to search a genetic library for a certain gene.

Location of the probe is possible because the probe is either radioactive isotopes of phosphorous or fluorescent dye. To search gene from genomic library, following steps are taken.

- Bacterial cells, each carrying a particular DNA fragment, can be plated onto agar in a petri dish.
- Now probe is placed on these petridishes, probe is made of nine or more nucleotides.
- The probe hybridizes (form pairing) into the gene of interest.
- The genes can be isolated from the fragment.
- Now this particular fragment can be cloned further or even analyzed for its particular DNA sequence.

26.2 DNA Sequencing

DNA sequencing is the process of determining the order of the four chemical building blocks known as "bases" that make up the DNA molecule. The sequence tells scientist the kind of genetic information that is carried in a particular segment. One of the important tools used in various DNA sequencing techniques is gel electrophoresis.

26.2.1 Gel Electrophoresis

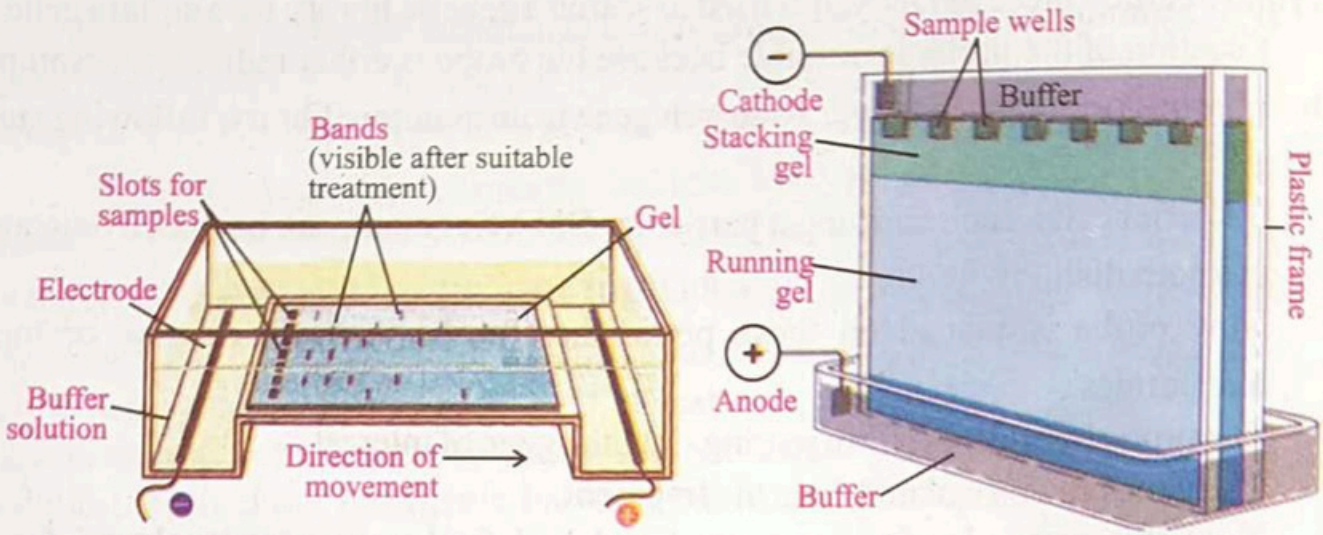
This laboratory technique is used to separate fragments of DNA, RNA and proteins in an effort to identify its origin.

Enzymes are used to chop up the long filaments of DNA into varying size of fragments. The DNA fragments are placed into small wells (holes) in the gel made of agarose or polyacrylamide, which are aligned along one end.

Interesting Information

Agarose is used for relatively large DNA molecules (more than 50 nucleotides) and **poly acrylamides** for high resolution of short DNA fragments (less than 50 nucleotides).

The gel is exposed to an electric current, positive on one side and negative on the other. DNA fragment travel is inversely proportional to its length. The effect is that the biggest, heaviest, and light charged particles do not move easily through the gel. They get stuck very close to the wells they were in at the beginning and most charged particles pass through the gel to the other side with less difficulty. Intermediate particles are distributed in between. In the end, the fragments of the different size leave a banded pattern of DNA on the gel. (Fig.26.6)



Horizontal Gel Electrophoresis *Vertical Gel Electrophoresis*

Fig.26.6:

Visualization of Fragments

To visualize DNA fragments, the gel can be stained by DNA binding florescent dyes that bind to the DNA molecules and are typically viewed under ultraviolet trans-illuminator. DNA bands can also be transferred from gel to nitrocellulose membrane for auto radiography (x-ray imaging).

Now we can observe that some bands are appeared thick and some are thin. The thick represent the high concentration of same size fragments where as thin bands exhibit low concentration. If a particular sized fragment is to be used for further analysis then the piece of gel containing that band can be cut and its DNA can be purified again.

26.2.2 DNA sequencing Techniques

In the late 1970s, methods were developing that allowed the nucleotide sequence any purified DNA fragment to be determined simply and quickly. These methods are based upon three steps.

i) To generate piece of DNA of different sizes. All starting from the same point and ending at different point.

ii) Separation of these different pieces of DNA on agarose gel.

iii) Reading of sequence from the gel.

26.2.3 Methods of DNA sequencing

Three methods are generally used to generate DNA fragments (which are of different sized).

i) Sanger-Coulson Method

ii) Maxam Gilbert Method

iii) Automated DNA Sequencing / Next generation sequencing

i) Sanger-Coulson Method / Dideoxy method

In this method **dideoxyribonucleoside triphosphates (dd NTP's)** are used to terminate DNA synthesis at different sites. Therefore, this method is also called as dideoxy method. (Fig.26.7)

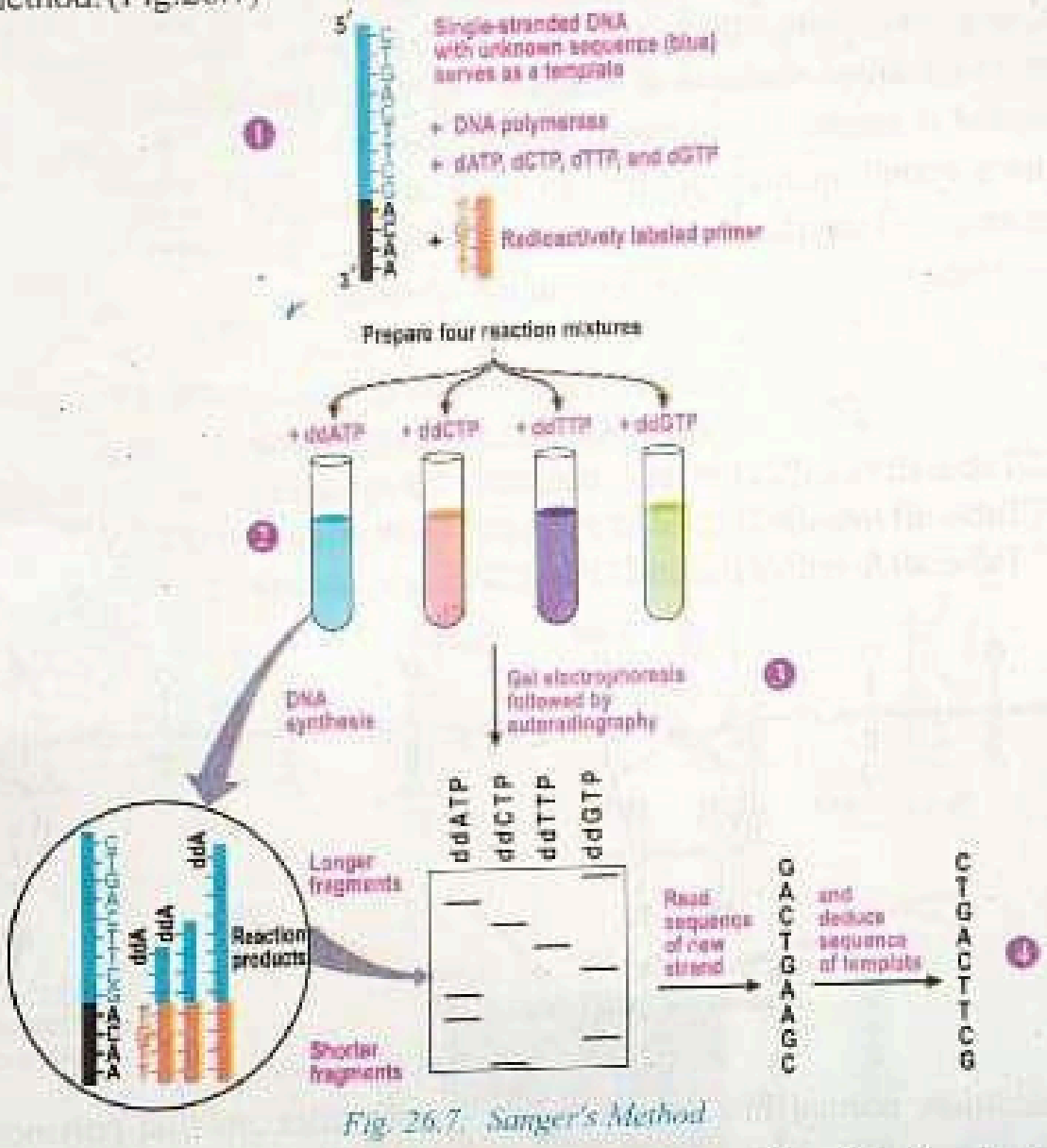


Fig. 26.7 Sanger's Method

It was developed by Frederick Sanger along with Andrew Coulson in 1977. They were awarded Nobel Prize in 1980 on this achievement. Sanger's method now has become the standard because of its practicality thus called Sanger's method. Sanger method is widely used and similar to the natural process of DNA synthesis.

Procedure:- The modified dideoxyribonucleotides are used which are same as deoxyribonucleotides except, they contain a hydrogen group on the 3′ carbon instead of a hydroxyl group (OH^-). When dideoxyribonucleotide integrated to sequence, it prevents

the addition of further nucleotides. This occurs because a phosphodiester bond cannot form between dideoxynucleotides and the next incoming nucleotides. Therefore, the DNA chain is terminated and replication ceased. For the sequencing of DNA, their double strand has to be denatured into single strands, using heat because only one strand that acts as template. Now the template strand is tagged with a known sequence at 3′ end, so that a complimentary primer can bind on the known sequence. Once the primer is attached to the DNA, the solution is divided into four tubes labelled "G", "A", "T" and "C".

Dideoxyribonucleotide triphosphate

Is a chain terminating precursor of DNA synthesis that block further polymerization when added to the end of DNA strand by DNA polymerase. These nucleotides lack 3′-OH hydroxyl group necessary for continued 5′-3′ DNA synthesis.

Then reagents are added to these samples as follows:

"G" Tube: all four dNTP's, ddGTP and DNA polymerase

"A" Tube: all four dNTP's, ddATP and DNA polymerase

"T" Tube: all four dNTP's, ddTTP and DNA polymerase

"C" Tube: all four dNTP's, ddCTP and DNA polymerase

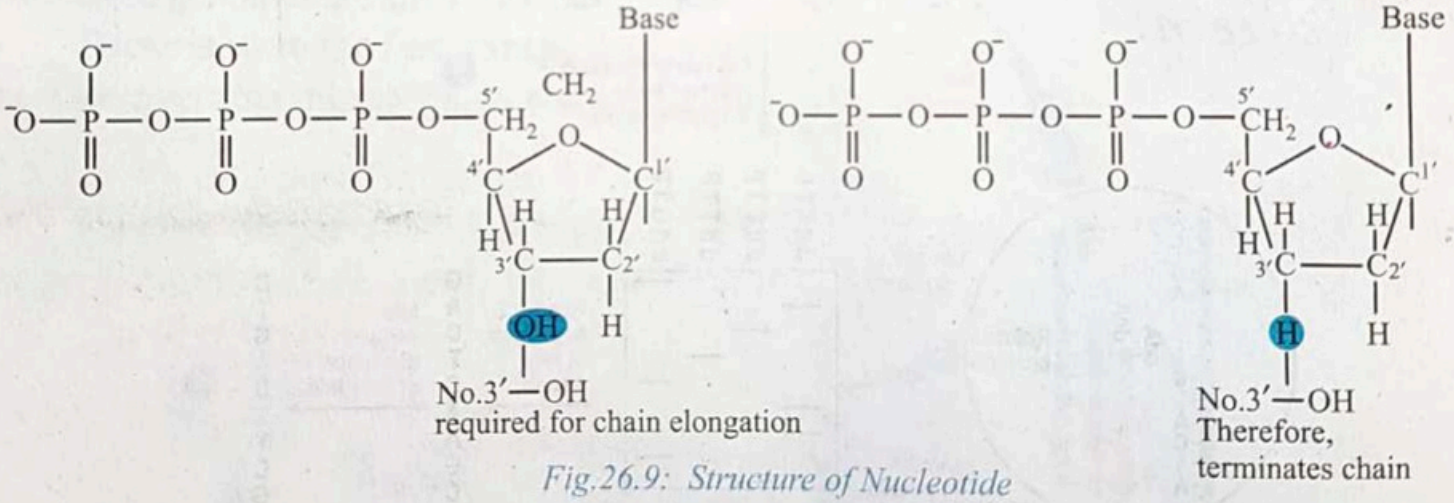


Fig.26.9: Structure of Nucleotide

In addition, normal free A, T, G, and C nucleotides and Taq polymerases are dissolved in these tubes. Now all tubes are placed in PCR machine so that sequencing reaction can start. As the DNA is synthesized, nucleotides are added on to the growing chain by the DNA polymerase. However, on this occasion a dideoxynucleotide is incorporated into the chain in place of a normal nucleotide, which results in a chain terminating event. For example, if the tube is containing ddTTP then only those fragments will be produced that will terminate on "T", same mechanism takes place in other tubes.

Importance: - The key to this method is that all the reactions start from the same

nucleotide and end with a specific base. Thus in a solution where the same chain of DNA is being synthesized over and over again, the new chain will terminate at all positions where the nucleotide has the potential to be added because of the integration of the dideoxynucleotides. In this way, bands of all different lengths are produced. Once these reactions are completed, the DNA is once again denatured in preparation for electrophoresis. The contents of each of the four tubes are run in separate lanes on a polyacrylamide gel in order to separate the different sized bands from one another. After the contents have been run across the gel, the gel is then exposed to either UV light or X-rays, depending on the method used for labelling the DNA.

ii) Maxam-Gilbert Method

This DNA sequencing method was developed by Allan Maxam and Walter Gilbert (1976-77). It is also known as chemical cleavage method because it is based on chemical modification of DNA and subsequent cleavage at specific bases.

Procedure: - The DNA to be sequenced must denature so that the two strands can be separated from each other, one strand is purified and divided into four samples (G, A + G, T + C and C). Each sample is treated with one of the modified chemical reagents and cleavage reagents. The former cause a chemical modification in the nucleotides they are

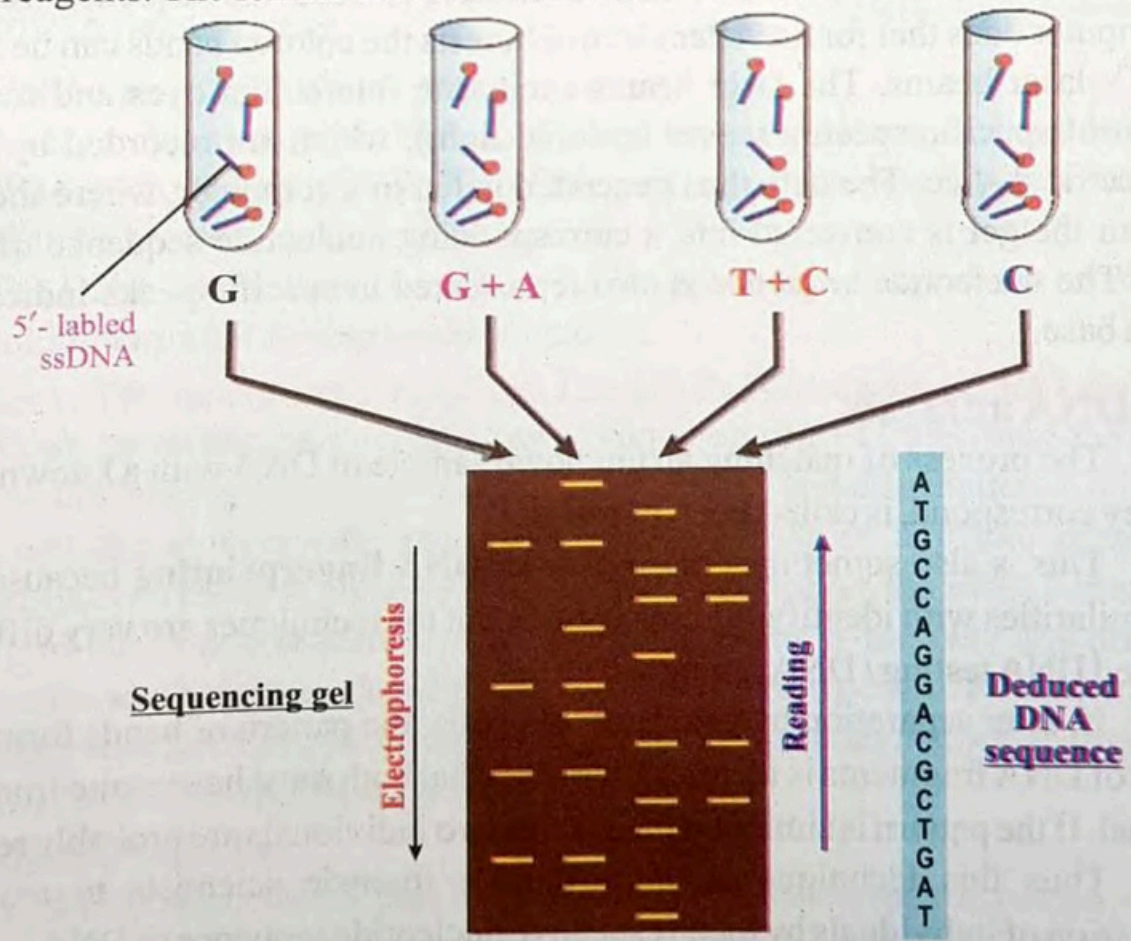


Fig.26.8: Maxam Gilbert DNA Sequencing Method

specific for, making them susceptible to cleavage while the later are used to cut DNA strand at specific point. For example, in the sample "G" the unknown DNA fragment will be cleaved from all those points where G is present. Similarly, in sample "A+G" the fragment will be cleaved from A as well as from G and so on. In other samples when all chemical reactions are completed then the products are run through gel electrophoresis and finally the sequence is read from autoradiograph of gel pattern. (Fig.26.8)

iii) Automated DNA Sequencing

Automatic sequencing machines have greatly improved the quality as well as the speed of the sequencing process. The basic principal of sequencing is quite same in manual and automated DNA sequencing except few differences.

There is no need for radiolabelling and autoradiography. The use of fluorescently labelled ddNTPs (dideoxynucleotide triphosphate) has made the reading very easy, convenient, and automatic with the help of UV laser detectors. Thus, it has greatly improved the speed of sequencing. Each of the four types of ddNTPs can be labelled with a specific dye, so that a specific color can be attributed to the presence of a particular nucleotide or base.

After electrophoresis, we don't even have to 'read' the sequence from the gel. The computer does that for us. After electrophoresis the colored bands can be monitored using UV-laser beams. The laser beams excite the fluorescent dyes and result in the emission of specific spectral waves (colored light), which are recorded by a specific photoelectric device. The data thus generated is fed to a computer, where the emission data from the gel is converted into a corresponding nucleotide sequence of the DNA sample. The nucleotide sequence is also represented in specific peaks indicating each nitrogen base.

26.3 DNA analysis

The process of matching an unknown sample of DNA with a known sample to see if they correspond, is called **DNA profiling**.

This is also sometimes referred to as **DNA fingerprinting** because there are some similarities with identifying fingerprints, but the techniques are very different (it is also called DNA testing / DNA typing).

If, after separation by gel electrophoresis, the pattern of bands formed by two samples of DNA fragments is identical, it means that both must have come from the same individual. If the pattern is similar, it means that two individuals are probably related.

Thus this technique is employed by forensic scientists to assist in the identification of individuals by their respective nucleotide sequence of DNA.

First DNA fingerprint was made by **Alec Jeffrey**, an English geneticist 1985.

26.3.1 Use/ Applications/ Purposes of DNA Analysis

- DNA fingerprinting can be used in paternity suits when the identity of someone's biological father needs to be known for legal reasons.
- At crime scene, forensics specialists can collect samples such as blood or semen, which contain DNA.
- To identify crime and catastrophe victims.
- To match organ donors with recipients in transplant programs.
- To detect bacteria and other microorganisms that pollute water, air, food, soil.
- Establishment of paternity and other family relationships.

26.3.2 Procedure/ Mechanism of DNA Analysis

There are many procedures that can be used for DNA analysis. One of the most important methods is restriction fragment length polymorphism (RFLPs). The RFLPs (pronounced as Riflips) refers to different sized fragments of DNA produced by a particular restriction enzyme. Following are the key steps of DNA analysis/DNA fingerprinting.

Extra Information

Human share about 98% of their genes with chimpanzees, 92% with mice, 76% with Zebrafish, 51% with fruit fly and 18% with E. coli bacteria.

1. Collection of DNA Samples

A very small fraction of DNA is sufficient for analysis because it can be copied for several times with the help of PCR. Thus DNA can be collected even from a drop of blood or cell of single hair root, or from single sperm.

The DNA samples can be collected from mummified organisms or from fossils when evolutionary relationship has to be studied.

Placement of Randomised Fragment Length Polymorphism (RFLPs)

As we know that every person has a unique set of RFLPs because the restriction site of a particular enzyme is always different in number and distribution in all human on earth except the monozygotic twins. Therefore, RFLPs of any two persons, when compared, one can easily analyze their individuality. However, the entire human has 99% similarity in nucleotide sequence of their genomes, this is the only 1% difference in genome sequence that establishes the individuality of every person. Placement of RFLPs is the digestion of DNA samples by a particular restriction enzyme, which produces a set of different sized DNA fragments (RFLPs).

Separations of RFLPs

The DNA fragments are then **electrophoresed** on two types of gel. An **agarose gel** to separate them by size. The mixture of RFLPs is loaded in **polyacrylamide gel** and run for electrophoresis. Fragments of various lengths begin to move at different rates from

negative to positive pole within the gel. When the movement is stopped, the gel is proceeded for further treatments in order to observe banding pattern.

Southern Blotting

This method was invented by British biologist Edwin southern. A southern blot is a method routinely used in molecular biology for detection of a specific DNA sequence in DNA samples. Southern blotting is the combination of two types *i.e.* transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization. (Fig.26.10)

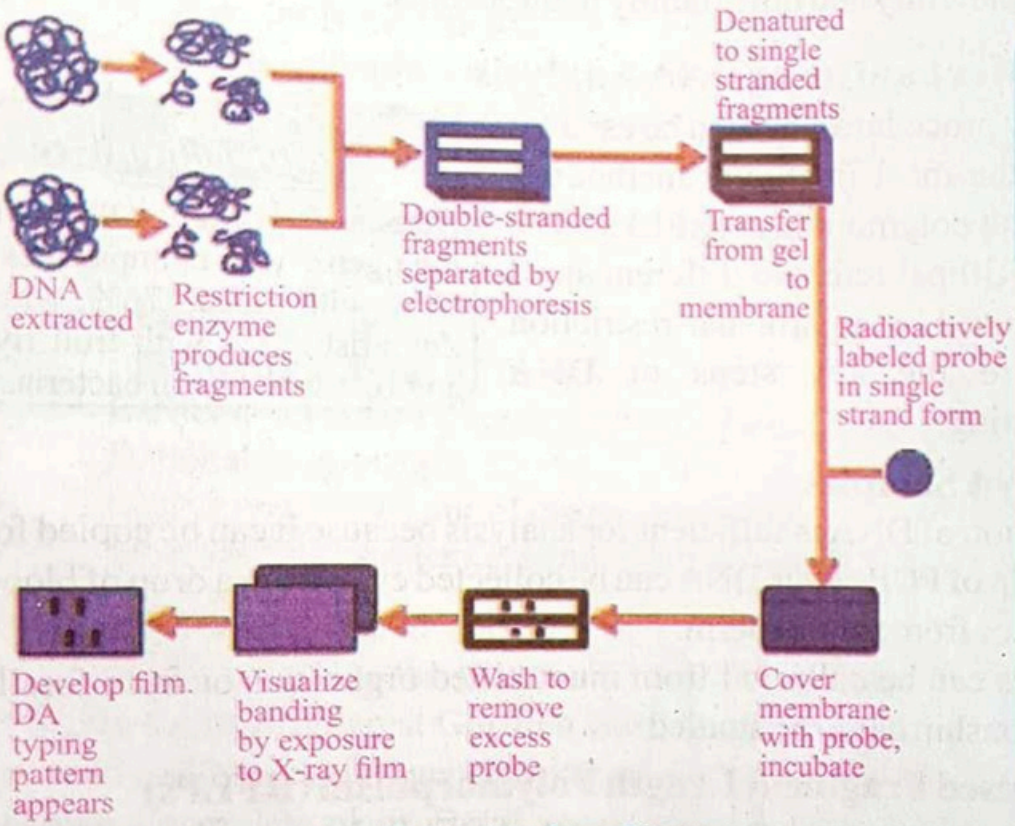


Fig. 26.10: Southern Blotting

Extra Information

The human genome is like a book" which contains 23 units (*i.e.* each unit is chromosomes), each unit possesses several thousand stories (*i.e.* genes). Each story is made up of paragraphs called exons, which are interrupted by advertisements called introns. Each word written in letters are called bases, which are thymine, guanine, cytosine and adenine (TGCA).

Autoradiography

After hybridization, excess probes are washed from the membrane, and the pattern of hybridization is visualized on X-ray film by exposing the membrane to an X-ray source. This technique is known as autoradiography. The banding pattern, which was originally obtained in the gel due to the separation of RFLPs, is now developed on an X-ray film.

26.4 Genome Maps

As you know **gene** is the basic unit of biological information. Hereditary characteristics pass from parents to offspring through genes in their gametes. The collection of all the genes found in one complete set of chromosome is called **genome.**

The **haploid organism or egg and sperm** have one copies of genome while **diploid organism (cell)** contains two copies of genome.

Genome Analyses

For the study of genome of various organisms, a new sub branch of biotechnology has been created called **genomics** which deals with exploration and analysis of complete DNA sequence of an organism's genome. The genome of ***Haemophilus influenzae*** was published in 1995.

Frederick Sanger also sequenced the first bacteriophage genome. The project was also launched in 1990. Thus so far the genomes of many organisms have been described by the new branch of biotechnology.

Genome Maps

The genome analysis helps us to develop two broad categories of maps *i.e.* genetics maps and physical maps. The **genetic maps** exhibit the sequence of gene loci along the length of DNA. The **physical mapping** is used to find the order and physical distance between DNA base pairs by DNA markers. This technique can determine the sequence of DNA base pair with high accuracy.

Genetic markers

The genetic maps have landmarks called genetic markers, contain variable number of **tandem repeats** (short tandem repeats and single nucleotide polymorphism). These genetic markers are useful for generating genetics maps when there are occasional, predictable mutations that occur during meiosis. Over many generations, lead to a high degree of variability in the DNA content of the marker from individual to individual.

26.4.1 Human Genome Project (HGP)

In 1990, international cooperative venture called the human genome project (HGP) set out to sequence the complete human genome because the genome of an organism is a catalogue of the bases. HGP hoped to determine the order of all the bases A, T, C and G in human DNA. This project was originally founded by the **U.S. Department of Energy and National institutes of health**. They have established **National Human Genome Research institute (NHGRI)**, who completed this task in 2003, the first director of NHGRI was James D. Watson later this institute was being led by Dr. Francis Collin. This institute was first founded by US government later on **"Welcome Trust"** of UK became a major partner, additional contributions for china, France, Japan, Germany and others. In

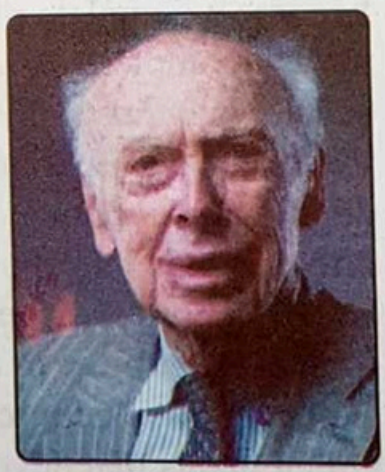


Fig.26.11: James Whatson

2003, the project announced that it had succeeded in achieving its goal. Finally, it came to know that human genome comprises 3.22 billion nucleotides and approximately 20,000 to 25,000 genes.

26.4.2 Major Goals of Human Genome Project

The goals and objectives of HGP were as under:

- To identify all the approximately 20,000 to 25,000 genes in human genome.
- To determine the sequences of the chemical base pairs those, make up human DNA which are about 3.22 billion.
- To store this information in database.
- Improve tools for data analysis.

Transfer related technologies to the private sector, and address the **ethical, legal, and social issues (ELSI)** that may arise from the project.

Benefits of HGP

Some potential benefits from human genome project are expected in the following fields:

Molecular Medicine

- Improved diagnosis of diseases.
- Earlier detection of genetic predisposition to certain diseases.
- Rational drug design.
- Gene therapy and control systems for drugs.
- **Pharmacogenomics** *i.e.* study how genes affect a person's response to drugs.

26.5 Tissue Culture

Tissue culture is the growth of a tissue in an artificial liquid culture medium. In 1902, the German botanist **Gottlieb Haberlandt** revealed that plant cells are **totipotent,** *i.e.* each cell has the full genetic potential of the organism. Its mean a single cell could become a complete plant. But it wasn't until 1958 that **British botanist F.C. Steward** grew a complete carrot plant from a tiny piece of phloem. He provided the cells with sugars, minerals and vitamins, but he also added coconut milk. (Later it was discovered that coconut milk contains the plant hormone **cytokinin**). When the cultured cells began dividing, they produced a **callus** (an undifferentiated group of cells), which later differentiated into shoot, root and developed into complete plants. The initial plant part which is used to develop tissue culture is known as **explants**. It may be a single cell (protoplast) a piece of tissue or complete organ (seed, leaf and twig). Thus on the basis of explants tissue culture is variously called **cell culture** or **organ culture. Plantlets** are young plants that developed during tissue culture. **Micro-propagation** is one of tissue culture techniques. It is the production of a large number of plants from a small plant material.

Somatic embryogenesis is an artificial process in which a plant or embryo is derived from a single somatic cell. Somatic embryos are formed from plant cells that are not normally involved in the development of embryos, *i.e.* ordinary plant tissue. No endosperm or seed coat is formed around a somatic embryo.

The plants produced from tissue culture are genetically identical to the original plant from which they are grown, so they are called **soma clones.** But a number of observations have indicated that this is not the case, sometime genetic variation observed among progeny of plants regenerated from somatic cells cultured *in vitro*. This is called **soma clonal variation.**

Differences between Cell culture and Organ Culture

Cell culture is a technique where cells are artificially grown under laboratory conditions (*in vitro*). While organ culture deals with the culture of the isolated organs such as root, stem, anther, *etc.*

Under laboratory condition (*in vitro*). There are different names for organ culture depending upon the organ utilized for the culture.

26.5.1 Methods of Tissue Culture

Many methods of tissue culture have been developed which are primarily based upon type of explants used such as meristem, anther, ovary, embryo culture, callus culture and suspension culture. *etc.*

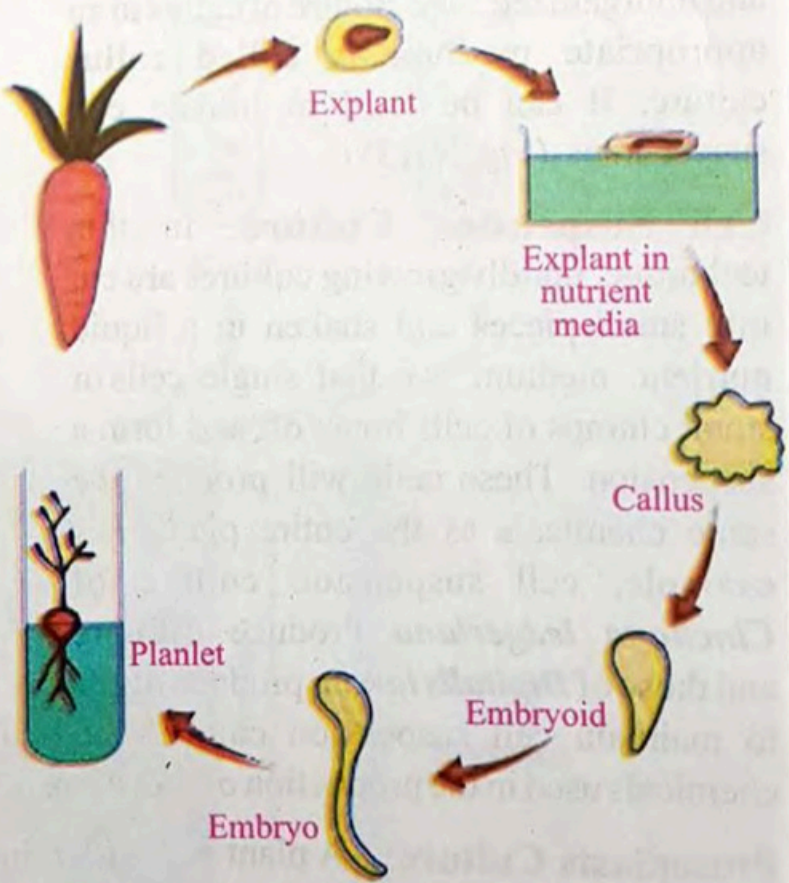


Fig.26.12: Tissue Culture

Meristem Culture

Meristems are tissues in plants that consist of undifferentiated cells capable of rapid cell division. A tissue culture in which meristems are used is called **meristem culture**. Tissue culture technique have by now led to micro-propagation, or commercial method of producing thou-sands, even millions of identical seedlings in limited amount of space. The correct proportions of auxins and cytokinin are added to a liquid medium with a single root tip, many new shoots

develop from a single shoot tip. When these are removed more shoots form. The advantage of meristem culture is that it is germ free (devoid of viruses) due to absence of vascular system in them, as the vascular system has prime importance in the travelling of viruses through the body. (Fig.26.12)

Anther Culture Technique

In this technique mature anthers are cultured in a medium containing vitamins and growth regulators the haploid tube cells within the pollen grains divide, producing pro-embryos consisting of as many as 20-40 cells. When the pollen grains rupture releasing haploid embryos. The experimenter can now generate a haploid plant, or chemical agent can be added that encourage chromosomal doubling. After chromosomal doubling the resulting plants are diploid but homozygous for all their alleles. Anther culture is a direct way to produce plants that express recessive alleles. If the recessive alleles govern desirable traits, the plants have these traits.

Differences between Callus Culture and Cell Suspension Culture

Callus Culture: Callus is formed by the proliferation of the parent tissue. The cells of a callus are parenchymatous, amorphous and unorganized. The culture of callus in an appropriate medium is called callus culture. It can be used to initiate cell suspensions. (Fig.26.13)

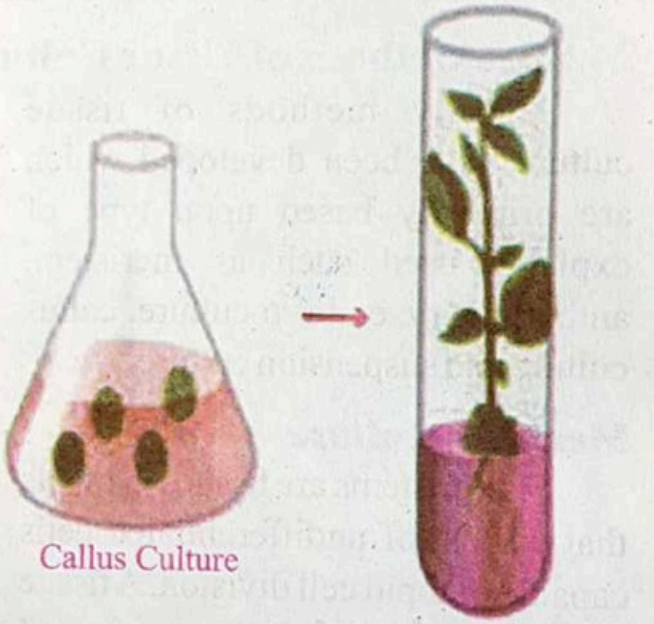


Fig.26.13: Callus Culture

Cell Suspension Culture: In this technique, rapidly growing cultures are cut into small pieces and shaken in a liquid nutrient medium so that single cells or small clumps of cells break off and form a suspension. These cells will produce the same chemicals as the entire plant. For example, cell suspension culture of ***Cinchona ledgeriana*** Produce **quinine** and those of ***Digitalis lanata*** produce **digitoxin**. Scientists imagine that it will be possible to maintain cell suspension cultures in bioreactors for the purpose of producing chemicals used in the production of drugs, cosmetics and agricultural chemicals.

Protoplasts Culture: - A plant cell with removed cell wall is called **protoplast**. It is commonly isolated from either leaf mesophyll cells or cell suspensions. Protoplast culture can be used to develop whole plant by **somatic embryogenesis** (synthetic

embryos that developed from somatic cells). Genetic variation can also be induced in these somatic embryos, if they are exposed to chemical of physical mutagens. These variations are known as **soma clonal** variations. Protoplasts also act as ideal targets for transformation by a variety of means.

Embryo Culture: - It is the culture of mature embryos; zygote or seed embryos that are often used as explants in plant tissue cultures. This embryo develops properly when nourishing tissues, endosperms were present in seed during the development.

Ovary Culture: - This culture is the ***in vitro* technique** which is carried outside on a suitable nutrient medium in an unpollinated flower to grow new haploid plants. The process of ovary culture is referring as **gynogenesis.** The gynogenesis or ovary culture is based on the regeneration principles, where an ovary can regenerate into a fully differentiated plant.

26.5.2 Methods of Animal Cell Culture

The biotechnological processes are helpful to culture the animal cells in artificial media but unlike plant and bacterial cells, these cells can grow only to a limited generation. The growth (division) of cells also depends on the source of the tissue isolated, such as some cell cannot divide and grow *e.g.* nerve cells, lens cells while some other can repeatedly divides and grow to limited generation *e.g.* fibroblast. The cultured cells are being used in variety of process *e.g.* in recombinant DNA technology, a variety of industrial processes (vaccine, monoclonal antibiotics and drugs production), genetic manipulation, cancer research, *etc.*

Techniques of Animal Cell Culture

We can use plastic containers to culture animal cells. A cell culture which is initiated by the cells isolated from a tissue or animal's organ is known as **primary cell culture.** If primary culture is sub cultured in fresh media to establish **secondary cultures**, it is called secondary culture or cell line.

The culture of native tissue that retains most of the *in vivo* histological features is regarded as organ culture while the culture of the cells for their reaggregation to form a tissue like structure represents **histotypic culture.** The culture of different cell types to form tissue or organ is called **organotypic culture**.

A special incubator is needed for animal cell culture that has capacity to maintain level of carbon dioxide, oxygen, temperature and all other substances which are necessary for this purpose.

There are two different patterns of animal cell growth *i.e.* anchorage dependent and anchorage independent.

Anchorage dependence can be defined as an increase in proliferation which is seen when cells are allowed to attach to a solid surface.

Anchorage independence is a condition in which a cell maintains its capacity to spread, divide and function despite of absence of a stable or inert surface to anchor with.

Applications of Animal / Tissue Culture

The animal cell cultures are used to:

- Produce antiviral vaccines / pharmaceutical drugs using for cell lines.
- Amniocentesis (*i.e.* chromosomal analysis of fetus for various diseases).
- Produce monoclonal antibodies required for cell lines in culture.
- Helpful to study the function of the cell neurons.
- Use of artificial skin.
- Culture tumor cells, to know whether benign or cancerous tumor.
- To know the effects of toxins and pollutants using cell lines.

26.6 Transgenic Organisms (Bacteria Plants and Animals)

Modern genetic technology can be used to modify the genomes of living organisms. This process is also called **genetic engineering**. Gene of one species can be modified, or gene can be transplanted from one species to another. The organisms that have altered genomes are known as transgenic.

Bacteria were the first transgenic organisms produced in 1973, since then many other transgenic organisms (plants and animals) are produced by this method.

26.6.1 Transgenic Bacteria

Herbert Boyer (1978), University of California was able to produce first transgenic bacterium *(E. coli)*. He inserted human insulin gene in it. The bacteria

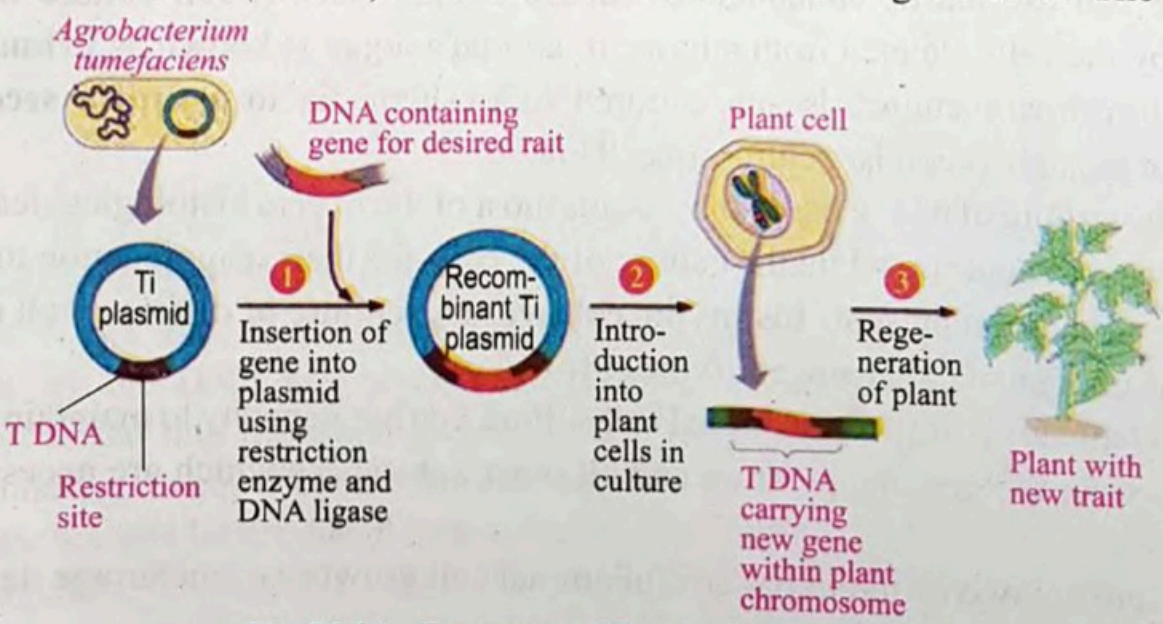


Fig.26.14: Formation of Recombinant Bacteria

synthesized human insulin "in Vats". Later many other human genes and genes for medicine were inserted in their plasmid or directly to their nuclear chromosome to get desirable products. (Fig.26.14)

- **Formation of Ice Crystal:** A strain of bacteria, named ***Pseudomonas syringae,*** commonly called **ice plus bacteria,** they have certain surface protein **(ice-nucleation –active protein (INA protein)** on their outer cell wall, which promotes ice crystal formation. Now a new strain of bacteria called ice minus in which gene encoding INA protein is removed, thus their surface lack INA "protein", therefore, provides a less favorable environment for ice formation. Now plants can be protected from cold stress if these bacteria are thrown on their surface.
- **Bioreactors:** Recombinant DNA technology is used to produce bacteria that reproduce in large **vats** called **Bioreactors**. If the foreign gene is replicated and actively expressed a large amount of protein products can be obtained.
- **Biotechnology products:** Many biotechnology products are made by transgenic bacteria, such as insulin, human growth hormone, clotting protein for haemophilia (haemophilia factor VII), hepatitis B vaccine, **interferon** (to treat viral infections), **tissue plasminogen activator (tPA)** to dissolve blood clot (used by heart patients).
- **Anti-insect toxin:** Bacteria that normally colonize the roots of corn plants have now been endowed with gene (from another bacterium) that code for an insect toxin. The toxin protects the roots from insects.
- **Cleaning up Beaches after Oil Spills:** Bacteria can be selected for their ability to grade a particular substance and this ability can be enhanced by genetic engineering *e.g.* naturally occurring bacteria may be changed to engineer bacteria which can clean up beaches after oil spills.
- **Importance of transgenic bacteria in industry:** Bacteria can be used by biofilters to prevent air born chemical pollutants from being vented into the air. Transgenic bacteria can also remove sulphur from coal before it is burned and help to clean up toxic waste dumps. One such bacteria was given genes that allowed it to clean up levels of toxin that would have killed other bacteria. Further these bacteria were given "suicide genes" that caused them to self-destruction when job had been completed.
- **Synthesis of organic chemicals:** Organic chemicals are often synthesized by having catalyst act on precursor molecules or by using bacteria to carry out the synthesis. Today, it is possible to manipulate genes that code for these enzymes. Biochemists discovered a strain of bacteria that is especially good for producing phenylalanine which is needed to make aspartame. **Aspartame** is **dipeptide sweetener** called as **"Nutra Sweet".** They isolated, altered and formed a vector for appropriate genes so that various bacteria could be genetically engineered to produce phenylalanine.

- **Extraction of Metals:** Many major mining companies already use bacteria to obtain various metals. Genetic engineering may enhance the ability of bacteria to extract copper, gold and uranium from low grade ore sources.
- **Bioleaching:** Some mining companies are testing genetically engineered organisms that have improved bioleaching capabilities.

Ecological Concerns Surrounding Transgenic Bacteria

There are some ecological concerns also associated with transgenic bacteria. Their uses may create serious hazard, such as if cancer cell genes were transferred into micro-organisms, antibiotic resistance may develop in many bacteria and some bacteria create pollution. Thus these transgenic bacteria can disturb the natural environment if their number exceed limits.

26.6.2 Transgenic Plants

The first transgenic plants (Tobacco plants) were made and cultivated in France and USA in 1986. In these tobacco plants herbicide resistant genes were inserted. In most cases to produce transgenic plants, the aim is to produce a new trait to the plant which does not occur naturally in the species. These plants can overcome environmental problems thus grow more successfully than natural species.

Methods of gene transformation in plants

Many techniques have been developed to introduce foreign genes or gene of choice into immature plant embryos or into plant cells. In all these techniques the cell wall of plant cells are removed, called protoplast cells.

Electroporation method

The protoplasts of plant cells are treated with an electric current while they are suspended in a liquid containing foreign DNA. The electric current makes tiny self-sealing holes in the plasma membrane through which foreign gene (DNA) can enter into cytoplasm and then to the nucleus of plant cell. This transgenic protoplast will develop into a complete plant.

Agrobacterium mediated transformation

In this technique, foreign DNA is inserted into the **tumour inducing plasmid (Ti plasmid)** of the bacterium, ***Agrobacterium tumefaciens.*** The plasmid of this pathogenic plant bacterium is widely used to produce recombinant DNA, therefore, known as **natural genetic engineering** of plants.

These bacteria have natural ability to insert **T- DNA (Transfer DNA)** of their plasmid into plant genome when they cause infection to plant at wound site. The **T-DNA** contains genes for auxin production, therefore, the infected plant cells are compelled to divide and redivide to produce a tumour often called **gall** (causes crown gall disease).

The gene of choice is cloned in the T. DNA region of tumour inducing plasmid in place of unwanted sequences. When these transgenic bacteria infect the plants, the genes of choice are entered into the nucleus of host cell. The transformed cells can be regenerated into whole plants.

Particle Gun

In 1987, **John C Sanford and Theodore M. Klein of Cornell University** developed another method of introducing DNA into a plant tissue culture callus. They constructed a device, called the **particle gun** which bombards a callus with DNA coated microscopic metal (gold or tungsten), then genetically altered somatic embryos develop into genetically altered adult plants. Many plants including corn and wheat varieties have been genetically engineered by this method. (Fig.26.15)

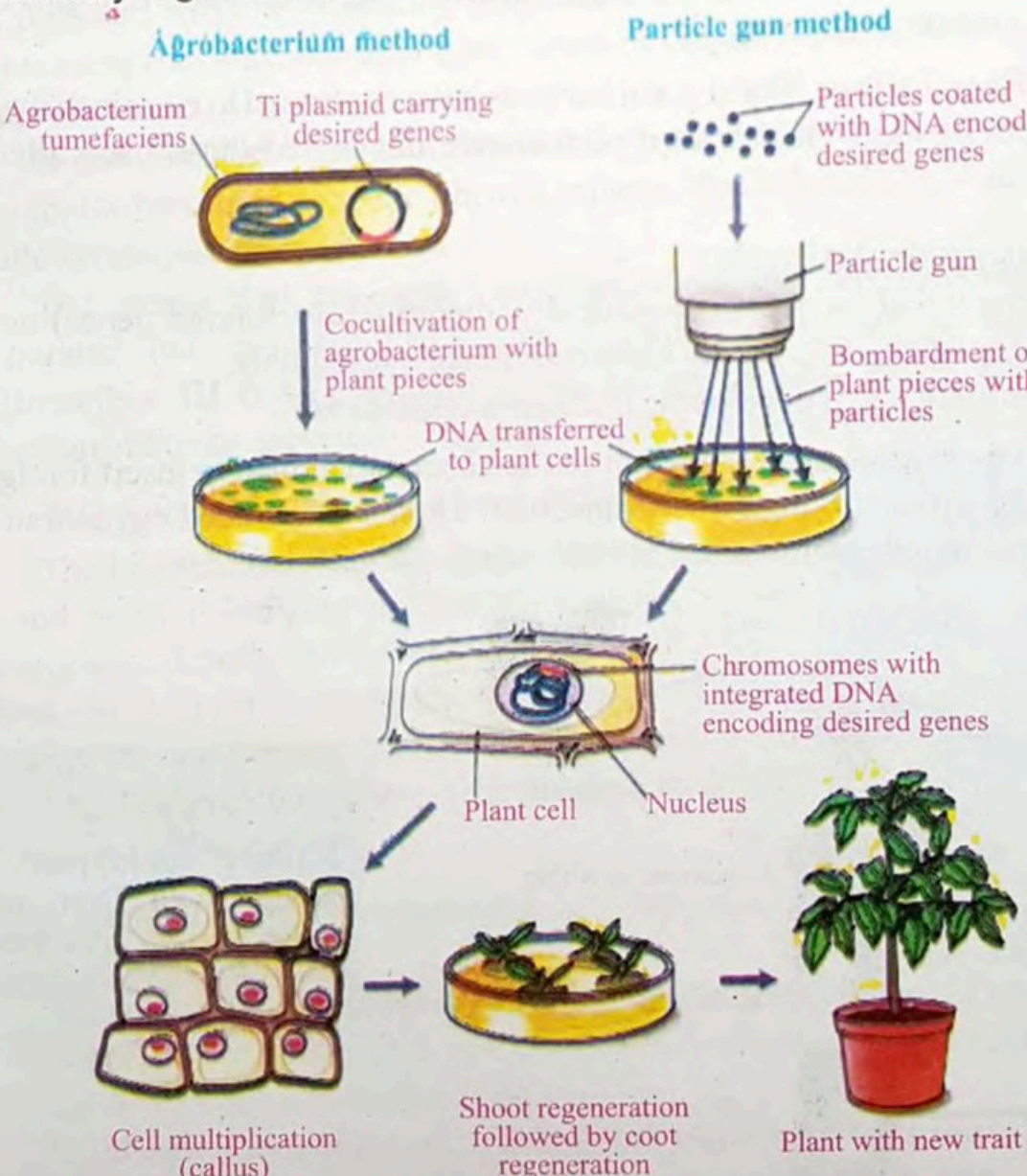


Fig. 26.15: Particle Gun Transformation

Advantages of Transgenic Plants

- **Resistance to pest:** Foreign genes transferred to cotton, corn and potato strains have made these plants resistant to pests because their cells now produce toxin against insects, *e.g.* Cotton, maiz.
- **Resistance to Herbicide:** Transgenic soybeans are resistant to herbicides. Some corn and cotton plants are also herbicide resistant, *e.g.* Grasses in lawn.
- **Increase in protein, oil and starch contents:** Improvements still to come for increase protein or starch content and modified oil or amino acid composition, *e.g.* Soya bean, potato, canola, maiz, golden rice.
- **Transgenic Wheat and Rice Versions:** To meet the increasing demand of rice, wheat and corn agribusiness, companies are trying to develop transgenic wheat and rice in addition to corn.
- **Green Revolution:** World grain harvests have continued to rise since the 1960's, when special high-yield hybrid plants were developed during so called green revolution.

26.6.3 Transgenic Animals

The animals that have been genetically altered (carry foreign genes) using the techniques of genetic engineering are known as transgenic animals.

The three methods of developing transgenic animals are:

i) By micro- injection: This technique has been developed to insert foreign gene into the eggs of animals through micro-injection. Then the fertilized egg is transferred into the oviduct of female to implant, *e.g.* Dolly sheep. (Fig.26.16)

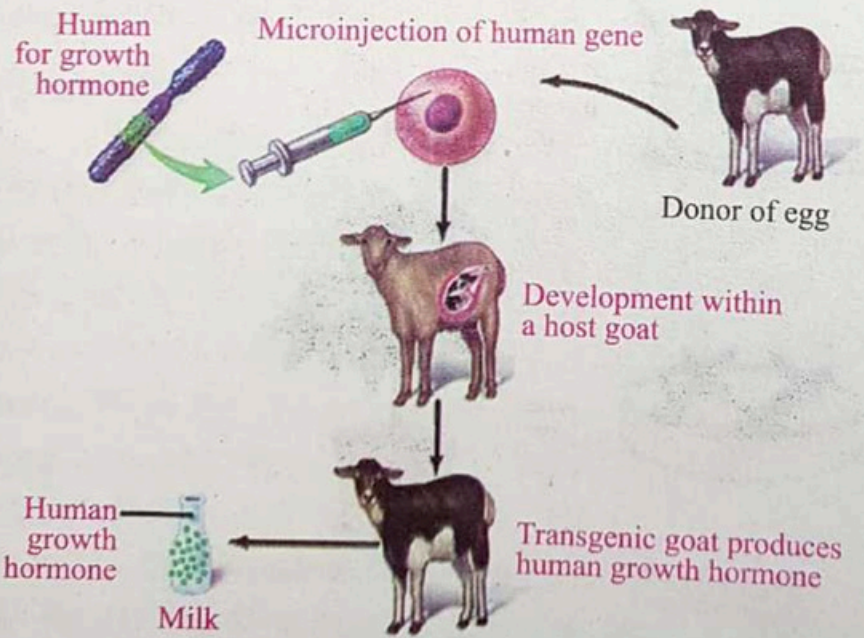


Fig. 26.16: Formation of Transgenic Goat

Extra Information

Alpha-1- Antitrypsin (AAT), produced in liver, protects lungs from infections. Its deficiency causes respiratory diseases.

ii) **Vortex Mixing:** In this method the egg is placed in an agitator with DNA and **silicon-carbide needle;** and the needles make tiny holes through which the DNA can enter. When these eggs are fertilized, the resulting offspring are transgenic animals.

iii) **Retrovirus-Mediated Gene Transfer:** In this method retroviruses are commonly used as vectors to transfer genetic material into the cells of blastocyst *in vitro* conditions. The blastocyst is then implanted into the womb of the female animal. Thus the resulting offspring is transgenic animal.

26.6.4 Importance of Genetic Engineered Farm Animals

Genetic engineering has also been used to produce **bovine growth hormone**, which are inserted into the animal's eggs to produce large size animals *e.g.* larger fishes, cows, pigs and sheep. Genetically engineered fishes are now being kept in ponds that offer no escape to wild because there is much concern that they will upset or destroy natural ecosystem.

In addition, the transgenic animals are also used to produce pharmaceuticals (drugs for the treatment of cystic fibrosis, cancer, blood disease) such transgenic animals are called **transpharmer animals**.

The genes that are code for therapeutically and diagnostic proteins are incorporated into animal's DNA and the proteins appear in animal's milk e.g. **Antithrombin III (hAT)** for blood clot during surgery, which prevent blood clot, production of human growth hormone in mice, which excrete it in their urine.

26.7 Biotechnology and Healthcare

The biotechnological techniques and tools create new and modern learning methods about human and other organisms body but sometime goes wrong when problems arise from it. Biotechnology has made great difference in our health care. It also has enable the researchers to develop products which fulfil our needs, safer vaccine and medicines.

Extra Information

Human Lactoferin (HLF) is a protein that helps to protect the body from infections and strengthens the immune system. This is found in human tears and lung secretion, protect them, from infection. Now these are produced by transpharmer animals.

26.7.1 Development of vaccine in biotechnology

There are three different ways are used in the development of vaccine:

a) Separation of a pure antigen using a specific monoclonal antibody, which is used for developing vaccine against pathogen.

b) Synthesis of an antigen with the help of a cloned gene or from cDNA.

c) Synthesis of peptides to be used as vaccines (protein region of pathogen for immune genic response).

26.7.2 Role of Biotechnology in diagnosis of diseases

Several human diseases can be diagnosed by using biotechnology products, such as monoclonal antibodies and DNA/RNA probes.

Monoclonal Antibodies

The response of the immune system to any antigen, even the simplest, is polyclonal; monoclonal antibodies (mAB) are a group of identical antibodies because they are made by identical immune cells which are all clones of a unique parent cell.

It is possible to produce monoclonal antibodies that specifically bind to the substance; they can then serve to detect or purify that substance. They can also help to diagnose a wide variety of illnesses; can detect the presence of drugs, viral, bacterial products, and other unusual or abnormal substances in the blood. Monoclonal antibodies are typically made by fusing **myeloma cells** (cancerous B-lymphocytes) with the spleen cells from a mouse that has been immunized with the desired antigen. The technique is called **somatic cell hybridization**

DNA/RNA Probes

A **probe** (in biotechnology) is a florescent or radioactive labelled piece of DNA or RNA of variable length (usually 100-1000 bases long), that is used in complementary to the sequence in the target DNA. These probes are widely used in diagnosis of many microbial diseases. Some specific DNA/RNA probes are useful to detect prenatal diseases, *e.g.* Short tandom Repeats (STR).

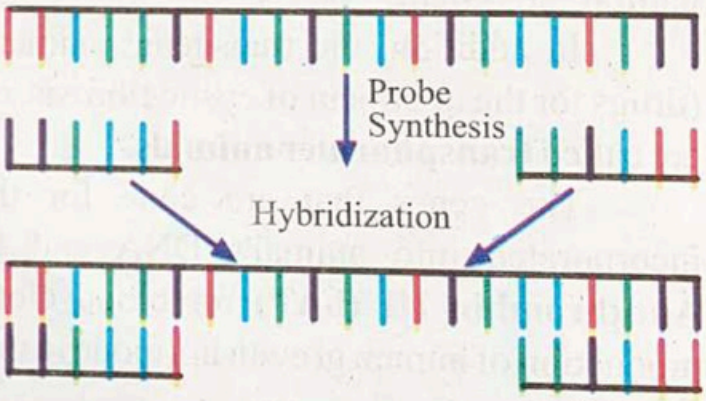


Fig.26.17

Diagnosis of diseases caused by protozoa and helminths

The monoclonal antibodies and DNA probes are being used as very sensitive tools in biotechnology to diagnose the diseases caused by protozoa and helminths. Monoclonal antibodies can be used through serological tests which takes only minutes as compared to conventional methods which require some weeks, as the bacteria and viruses have to be cultured *e.g.* in Herpes virus.

The DNA probes are more sensitive than monoclonal antibodies and the process takes hours instead of weeks. Ready made DNA probes for herpes virus and other human, animal and plant viruses are being prepared. Probes are now available for a number of human parasites from the group protozoa and helminths *e.g.* polio, hepatitis, *etc.*

26.7.3 Gene Therapy

It is a technique to replace defective genes (by normal genes) responsible for disease development. There are many approaches for correcting defective genes such as:

1. A normal gene may be inserted into a non-specific location within the genome to replace a non-functional gene. This approach is most common.
2. An abnormal gene could be swapped for a normal gene through homo-logous re-combination.
3. The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.
4. The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered. (Fig.26.17)

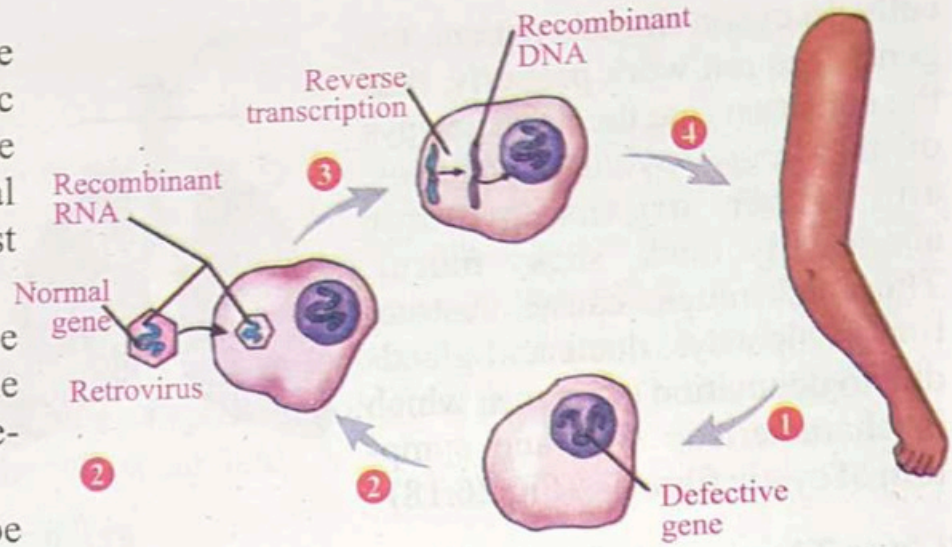


Fig. 26.17: Ex-vivo gene Therapy in Humans

Mechanism of gene therapy

This technique is done either "*in vivo*" or "*ex vivo*". During in vivo, the gene is delivered directly into the body, while *in vitro* into the cells outside the body. These transgenic cells are again implanted into the body in both cases. A "**normal gene**" is inserted into the genome to replace an "abnormal gene, diseases-causing gene". A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. The virus is most common vector which has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease causing genes and insert therapeutic genes. Besides, virus mediated gene delivery system, some non-viral approaches are also used *i.e.* creation of an artificial lipid sphere with an aqueous core. This **liposome**, which carries the therapeutic DNA, is capable of passing the DNA through the target cell membrane.

26.7.4 Cystic Fibrosis

It is an inherited disease, affects the mucus and sweat glands. The affected person with severe symptoms can have serious lung and digestive problems, while person with a mild form of the disease shows symptoms of disease after adolescence. The mucus of normal person is watery, which keeps the linings of certain organs moist and prevents them from drying out or getting infected. However, the cause of cystic fibrosis (CF) is a defect in the **Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)** gene that encodes a protein by which the movement of salt and water is controlled in and out of

cells. In cystic fibrosis patient, the gene does not work properly, thus the cells that line the passageways of the lungs, oviduct, pancreas, and other organs produce abnormally thick, sticky mucus. This thick mucus causes obstruction to air ways, ducts and glands due to deposition of mucus, which is characteristic sign and symptom of cystic fibrosis. (Fig.26.18)

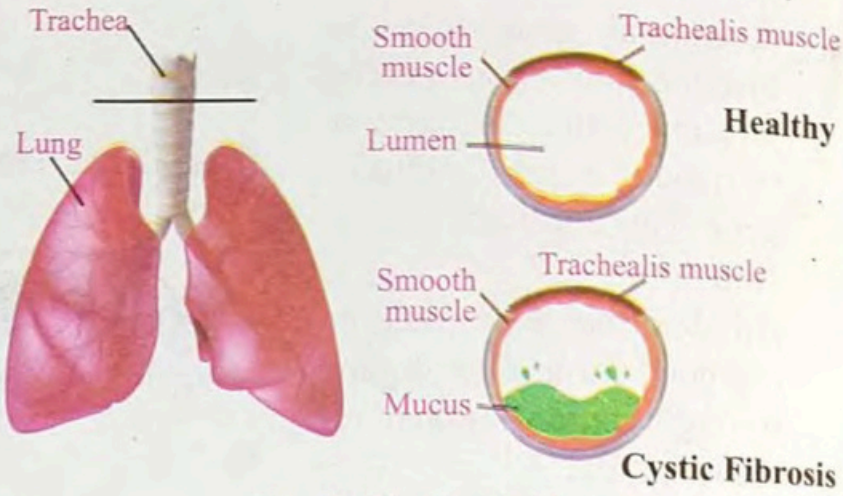


Fig. 26.18: Cystic Fibrosis

Gene Therapy of Cystic Fibrosis

The cystic fibrosis genes were identified in 1989, and were named as the **CFTR.** The discovery of this defective gene help scientists that it can be treated by gene therapy. Gene therapy is the process of creating a healthy version of the flawed CFTR gene and

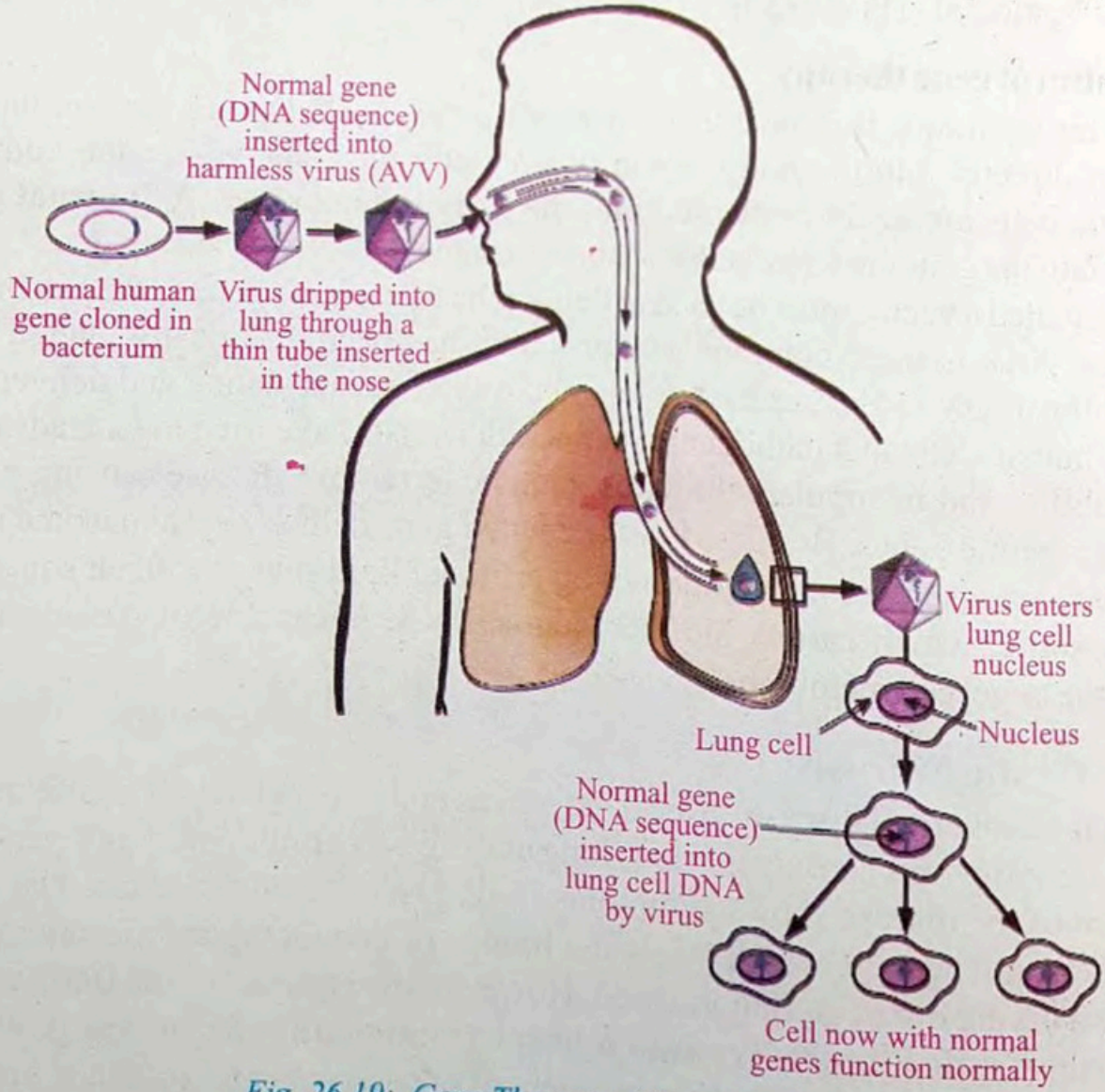


Fig. 26.19: Gene Therapy of Cystic Fibrosis

infusing it into the affected patients. (Fig.26.19)

Importance of Biotechnology

Biotechnology emerged as new discipline during 1970s. Although biotechnology is not a pure science, but an integration of biological science with technology created revolution in human life. Biotechnology is defined by different organizations in different ways. It has been broadly defined as, "the development and utilization of biological processes, forms and systems for obtaining maximum benefits to human and other forms of life". Biotechnology is "the science of applied biological processes". Importance of biotechnology is highlighted in the following fields.

Biochips and Biological Computers

Biochips are the result of combination of microchips business with biotechnology. In future, there is the possibility of developing of biological computers. The formation and use of biochips is a major thrust of the rapidly growing biotechnology industry, which encompasses a very diverse range of research efforts including genomics, proteomics, computational biology, and pharmaceuticals, among other activities.

It reveals, the complex biochemical processes occurring inside cells, with the larger goal of understanding and treating human diseases. At the same time, the semiconductor industry has been steadily perfecting the science of **microminiaturization**. The merging of these two fields in recent years has enabled biotechnologists to begin packing their traditionally bulky sensing tools into smaller and smaller spaces, onto so-called biochips. These chips are essentially miniaturized laboratories that can perform hundreds or thousands of simultaneous biochemical reactions. Biochips enable researchers to quickly screen large numbers of biological analytes for a variety of purposes, from disease diagnosis to detection of bioterrorism agents.

Biofertilizers:- In recent years, use of microbial inoculants as a source of biofertilizers (nutrient inputs of biological origin for plant growth) has become a hope for most of countries, as far as economic and environmental viewpoints are concerned. Biologically fixed nitrogen is such a source which can supply an adequate amount of nitrogen to plants and other nutrients to some extent. It is a non-hazardous way of fertilization of field. Moreover, biologically fixed nitrogen consumes about 25 percent to 30 percent less energy than normally done by chemical process.

Nanotechnology:- A new and exciting sub-branch requiring biotechnologists is the field of nanotechnology. **Nanotechnology** gives us the capability to engineer the tiniest of objects, things at the molecular level. Nanotechnology includes the study and

manipulation of materials between 1 and 100 nanometres. Nanotechnologists are imparting their expertise in the development of such Nano particles that can be used for efficient drug delivery at the target cells and in the diagnoses of disease.

Scope of Biotechnology: - The biotechnology is the fastest growing field in the area of research and development. It is also called a technology of the future or technology of tomorrow because of its unprecedented impacts on the mankind and the universe as a whole.

Due to its interrelation with other fields such as industry, agriculture, computers, *etc.* it is going to create amazing opportunities for manipulating the biological systems and thereby understanding the mysteries of fundamental life process.

Students of biotechnology after completing their studies can have scope in the following fields:

- **Communications/media**-reporting, writing editing, *etc.*
- **Computer science**-data base development, bioinformatics, web site development, *etc.*
- **Pharmaceutical companies** *i.e.* drug development.
- **Genetic engineering** working in bioprocess chambers, instrumentation development, fermentation technology.
- **Research e.g.** cancer, genetically linked diseases, AIDS.
- **Diagnostic laboratories** funded by public and private sectors.
- **Waste management,** bio-monitoring bodies and pollution control boards.
- **Medicine** - The medical genetics, genetic counselling, gene therapy and gene testing, uses biotechnological tools.
- **Bio power plants,** *e.g.* There are many types of bio power plants including corn, sugar cane and bamboo, *etc.*
- **Bio-processing industry e.g.** enzyme technology, paper technology, metabolic engineering, protein engineering, food processing, *etc.*
- **Agriculture and animal husbandry,** Animal husbandry is the branch of agriculture concerned with animals that are raised for meet, fiber, milk, eggs, *etc.*
- **Legal field** involving issues related to intellectual property rights, patency, and copyrights related to the field of biotechnology. The issues related to Genetic and Paternity testing also requires the combined expertise of biotechnologist and a law expert.
- **Military** - With the fear of Biological warfare looming largely on the human civilization, a biotechnologist is needed in pathogen identification, in the development of protection against the chemical and biological warfare, and in conducting the risk assessment studies.

- **Crime and law** - With the use of DNA finger printing in forensic science it has become easy to create a data bank of the criminals and thereby catch the culprits faster.

Social/Ethical Implications of using Biotechnology

The field of biotechnology has had a lot of beneficial contribution in the area of healthcare, agriculture, food production, manufacture of industrial enzymes, and appropriate environmental management. However, the advancement in this field has also led to some concerns and controversies raised by a number of groups, None Governmental Organizations (NGOs), *etc.* ELSI is the short form to represent the **ethical, legal, and social implications** of biotechnology. ELSI broadly covers the relationship between biotechnology and society with particular reference to ethical and legal aspects.

Concerns about the genetically Modified Organisms (GMOs)

There are concerns regarding the biosafety, ethics and issues related to the release of GMOs in the environment. Many countries and NGOs have opposed the release of the GMOs due to several reasons. In order to address these issues, the United Nations has built up an informal working Group on biosafety. In 1991, this group prepared the "voluntary code of conduct for the release of organism into the environment". The main areas of consideration for safety aspects in biotechnology are the following:

- To dispose of spend microbial biomass and purify the effluents from biotechnological processes.
- The toxicity of the allergy associated with microbial production.
- To deal with the increase in the number of antibiotic resistant pathogenic microorganisms.
- To evaluate the pathogenicity of the genetically engineered microorganisms to infect humans, plants and animals.
- To prevent contamination, infection or mutation of the processed strains.
- The evaluation of the interaction of the genetically engineered microbes with the elements of natural environment.

Biological Warfare

Most of the countries of the world are signatories to the **Biological Weapons Conventions** of 1972. As a signatory, it is a voluntary pledge by a nation "Never to produce microbial or other biological agents or toxins, whatever may be their method of production, for use in wars. However, many people have expressed their concerns about the possible use of genetic manipulations for military purposes in the near future.

Intellectual Property

With the fast pace development in the field of biotechnology, the issues related to

legal characterization and the treatment of trade related biotechnological processes and products are of immense importance. These are popularly known as **Intellectual Property**. Intellectual property includes patents, trade secrets, copyrights, and trademarks. **Intellectual property Rights (IPR)** is a collective term applied to a number of different types of legal rights granted by each country. The rights to protect this property prohibit others from making, copying, using or selling the proprietary subject matter.

Interesting Information

Blue Biotechnology: The study of biotechnology of water and bodies of water.
Red Biotechnology: It is medical biotechnology.
Green Biotechnology: It is the plant biotechnology.

In biotechnology, the intellectual property covers the processes and products which result from the development of genetic engineering techniques through the use of restriction enzymes to create recombinant **DNA.** Another example of intellectual property is the development of crop varieties which are protected through "Plant Breeder's Rights or **PBRs.** The **PBRs** ensure that the plant breeder who developed a particular variety gets the exclusive rights for marketing the variety.

Science, Technology and Society (STS)

Describe the applications of polymerase chain reaction (PCR).

Application of PCR: PCR has application in almost all areas of molecular biology, genetics and in clinical areas.

- PCR is an efficient diagnostic technique used for the detection of specific infectious agents *e.g.* HBV, HCV, HIV.
- It is also used for the detection of microorganisms in food samples, water and in the environment with the help of species-specific primers.
- PCR can also be used for genome analysis and for generating markers for the construction of genetic and physical maps of organisms.
- There is the PCR-based cDNA synthesis known as RT-PCR (reverse transcriptase-PCR), which can be directly carried out with purified mRNA.
- Reactions for DNA sequencing are also simplified by introducing the PCR method.
- PCR has also shown its impact in criminology. The DNA of the suspects and the DNA sample recovered from the crime scene can be analysed by PCR techniques with the help of a set of identical random primers or specific primers.
- DNA fingerprinting is also made simple by PCR as described above.
- The genetic mutations responsible for certain genetic diseases and cancers can be detected using PCR tools. Early detection of genetic disease is even possible in embryonic conditions or even sex cells-sperm and egg.

SUMMARY

- The molecular genetics enables us to manipulate genetic material for the welfare of mankind.
- Nanotechnology is the branch of biotechnology that deals with dimensions and tolerances of less than 100 nanometres especially the manipulation of atoms and molecules.
- Genetic engineering deals with manipulation or alteration in genetic material of a living thing.
- Staggered restriction endonuclease enzymes cut the duplet DNA fragments in such a way that each piece shows single stranded projected end called sticky ends. The sticky ends also help the insertion of foreign DNA into vector DNA.
- The organisms that have had a foreign gene inserted into them are called genetically engineered (GE), or genetically modified (GM) or transgenic organism.
- PCR was developed by Kary B. Mullis in 1983.
- Tissue culture is often a genetic term that refers to both organ culture and cell culture.
- A DNA probe means a small single stranded DNA nucleotide sequence that will hybridize (pair) into certain piece of DNA, may be used to search a genetic library.
- The Taq DNA polymerase is a temperature tolerant enzyme isolated from *Thermus aquaticus*, a bacterium found in hot spring. It is used for carrying out PCR reactions.
- Biochips are the result of combination of microchips business with biotechnology.
- Gene therapy is a technique for correcting defective genes responsible for disease development.
- The three methods on which mechanism of any DNA analysis sequencing is based are (i) Sanger-Coulson Methods, Maxim Gilbert Method and automated DNA sequencing.
- Gene cloning is a technique for developing a large number of genes or genetically identical cells or organisms.
- The sign and symptom of Cystic Fibrosis are obstruction of the airways, ducts and glands due to deposition of mucus.
- A cell culture which is initiated by the cells removed from an animal's organ is called as primary cell culture.
- Gel Electrophoresis is a technique used in molecular biology to separate charge bearing fragments (proteins, nucleic acids) under the influence of electric field in a solid medium called gel made of agarose and polyacrylamide.

EXERCISE

SECTION-I: OBJECTIVE QUESTIONS

Multiple Choice Questions (MCQs)

A. Select the correct answer.

1. The enzyme which joints two pieces of DNA is:
   (a) DNA polymerase III (b) Restriction endonuclease
   (c) DNA ligase (d) DNA polymerase I
2. Antibody made by soybeans can be used as treatment for:
   (a) AIDS (b) Herpes Simplex
   (c) Genital Herpes (d) Hepatitis
3. Antithrombin III is biotechnical product produced in:
   (a) Sheep (b) Goat
   (c) Mice (d) Cow
4. Cell suspension cultures of *Digitalis lanata* produce.
   (a) Anti-toxin (b) Digitoxin
   (c) Polludrin (d) Quinine
5. EcoRl is commonly used as
   (a) Bacteriophage (b) Gene
   (c) Restriction enzymes (d) DNAase
6. Recombinant DNA is introduced into the host cell by means of
   (a) Phage (b) Vector
   (c) Bacterium (d) Fungus
7. The PCR was developed by K. Mullis in
   (a) 1970 (b) 1983
   (c) 1975 (d) 1978
8. Taq polymerase is an enzyme present in
   (a) Bacteria (b) Protozoans
   (c) Algae (d) Helminths
9. A complete set of genes of an individual is called as
   (a) Gene pool (b) Genome
   (c) Gene library (d) Recombinant gene
10. Those organisms which have had a foreign gene into them are called as:
    (a) Transgenic (b) Transmuted
    (c) Hermaphrodites (d) Polygenesis

11. The use of transgenic animal to produce pharmaceutical is termed as
(a) Gene pharming (b) Antibiotic
(c) Gene therapy (d) Antiviral

12. Transgenic soybeans are made to resist against
(a) Herbicides (b) Fungicides
(c) Insecticides (d) Pesticide

13. Which of the following is the genetic marker that is used in DNA finger printing.
(a) Primer (b) Probe
(c) RFLP (d) Intron

14. RFLP is a (an)
(a) Intron (b) Exon
(c) Anticodon (d) Codon

B. Fill in the Blanks.

1. Recombinant DNA technology is a series of procedures that is used to join ..... Segments.
2. First restriction enzyme was discovered in 1970, by .....
3. The collection of bacterial clone containing recombinant DNA is called ..... library.
4. Human genome comprises ..... billion nucleotides.
5. The initial plant part which is used to develop tissue culture is called .....
6. Interferons are used to treat ..... infections.
7. A plant, containing foreign gene is called .....
8. The transgenic animals which produce drugs are also called ..... animals.
9. DNA molecules with complementary sticky ends associated by ..... bond.
10. PCR is the abbreviation of .....

SECTION-II: SHORT QUESTIONS

C. Give the short answers of the following questions.

1. Write the role of restriction endonucleases in gene cloning.
2. Write note on Taq polymerase.
3. Briefly describe monoclonal antibodies.

4. Write short note on callus culture.
5. Define anther, explain briefly anther culture.
6. List some applications of animal cell culture.
7. Write methods of gene transformation in plants.
8. Define DNA/RNA probes.
9. Illustrate bio-fertilizers.
10. Write brief note on nanotechnology.
11. Explain Hazards and ethical implications of using biotechnology.
12. List some biotechnological products.
13. Write the process of the automated DNA sequencing.
14. Write short note about biochips.

SECTION-III: EXTENSIVE QUESTIONS

D. Give detailed answers of the following questions.

1. Explain method for production of recombinant DNA.
2. Describe uses of transgenic bacteria.
3. Write note on components of PCR technique.
4. Define genomic library and also explain it.
5. Write a detail note on gel electrophoresis.
6. Explain Sanger's methods of DNA sequencing techniques.
7. Describe automated DNA sequencing technique.
8. Write note on Human genome project. What are its applications?
9. Define cystic fibrosis, and also describe role of gene therapy in cystic fibrosis.
10. Explain the scope and importance of biotechnology in promoting human welfare.