BIOTECHNOLOGY

Chapter 26

Major Concept

In this Unit you will learn:

- Cloning of Genes
- DNA Sequencing
- DNA Analysis
- Genomic Maps
- Tissue culture
- Transgenic Bacteria, Plants and Animals
- Biotechnology and Healthcare
- Scope and Importance of Biotechnology

Biotechnology is a multidisciplinary field of biology which deals with the application of biological phenomenon by using living organisms, cells and bio-molecules for the welfare of human beings. The basic aim of biotechnology is the improvement of human health, agriculture and environment. The history of biotechnology starts when farmers tried to improve their crop yields and develop better breeds of animal by cross-breeding techniques. The modern biotechnology develops **recombination DNA technology** to get the same result without any failure or chances of failure. The recombinant DNA is developed by using different techniques and this sub-branch of biotechnology is called **Genetic engineering** which deals with the manipulation in material of an organism to get desired results.

26.1 CLONING OF GENES

Gene cloning is a technique that involves the creation of multiple copies, of a specific DNA fragment, when a gene is identified and cloned it can be used for various purposes e.g., gene therapy, genetic engineering and production of different pharmaceutical products. There are two possible ways of gene cloning (a) Recombinant DNA technology (b) Polymerase chain reaction.

Recombinant DNA Technology: In this technique a series of procedures involve to join DNA segments taken different sources. It involves following steps:

i) Identification and isolation of gene of interest.
ii) Insertion of gene of interest into vector.
iii) Introduction of recombinant DNA into Host cell.
iv) Selection and isolation of Transformed cell.
v) Expressing of recombinant gene (protein synthesis).

26.1.1. Techniques of Gene Cloning by Recombinant DNA technology.

As we have discussed that recombinant DNA technology is a technique used to manipulate and modify DNA by combining DNA taken from different sources. This combination develops a new DNA molecule which was not found in nature before. This new form of

DNA is Called recombinant DNA (rDNA). When this DNA insert in an organism if it expresses, it transforms the living organism e.g., pUC19 is a recombinant gene if insert in E. coli bacteria it transforms ampicillin sustible bacteria into ampicillin resistant bacteria. This technology of gene cloning requires following techniques:

i) Identification and Isolation of gene of interest:

The gene of interest is the part of segment of DNA which is to be cloned. The gene of interest is identified from genome of an organism by using radioactive small fragment of DNA called **probe.** When it is identified it is directly cleaved from a Chromosomal DNA by using particular DNA scissors Called **restriction endonucleases** (Enzyme). This ensures, that the gene of interest in obtained and ready for furthers manipulation.

ii) Insertion of gene of interest into vector (Joining of gene of interest)

The gene of interest is ligated (joined) to another DNA entity called **cloning vector**. The cloning vector is usually a plasmid i.e., a small DNA molecule found as extra chromosomal DNA found in bacteria or sometimes yeast. The combination of the gene and the cloning vector form a new recombinant DNA molecule.

iii) Introduction of Recombinant DNA into Host cell

The recombinant DNA molecule is transferred into host cells. This process is called **transformation** and it involves incorporating the recombinant DNA into host cell's genome. It can be performed by putting host cell and recombinant DNA into same medium, the host are treated with **calcium chloride** which make their membrane more permeable for recombinant DNA. After this process, host cell reproduces into medium which produce clone of host cell and each new cell contain at least one recombinant Plasmid. In this way clone of recombinant genes are produced.

iv) Selection and Isolation of transformed cell:

The host cells which successfully take up the recombinant DNA (with gene of interest) are identified and selected. This selection

is achieved by the use of specific marker present in cloning vector. The markers develop resistance in host cell towards certain antibiotics or develop colours which make difference between transformed and non-transformed cell.

v) Expressing of recombinant gene (Protein Synthesis):

The recombinant gene, within the host cell is transcribed and translated in-vitro under suitable condition. As a result, production of specific protein occurs which is encoded by cloned recombinant DNA. This protein can be further purified and used for various applications in research medicine or industries.

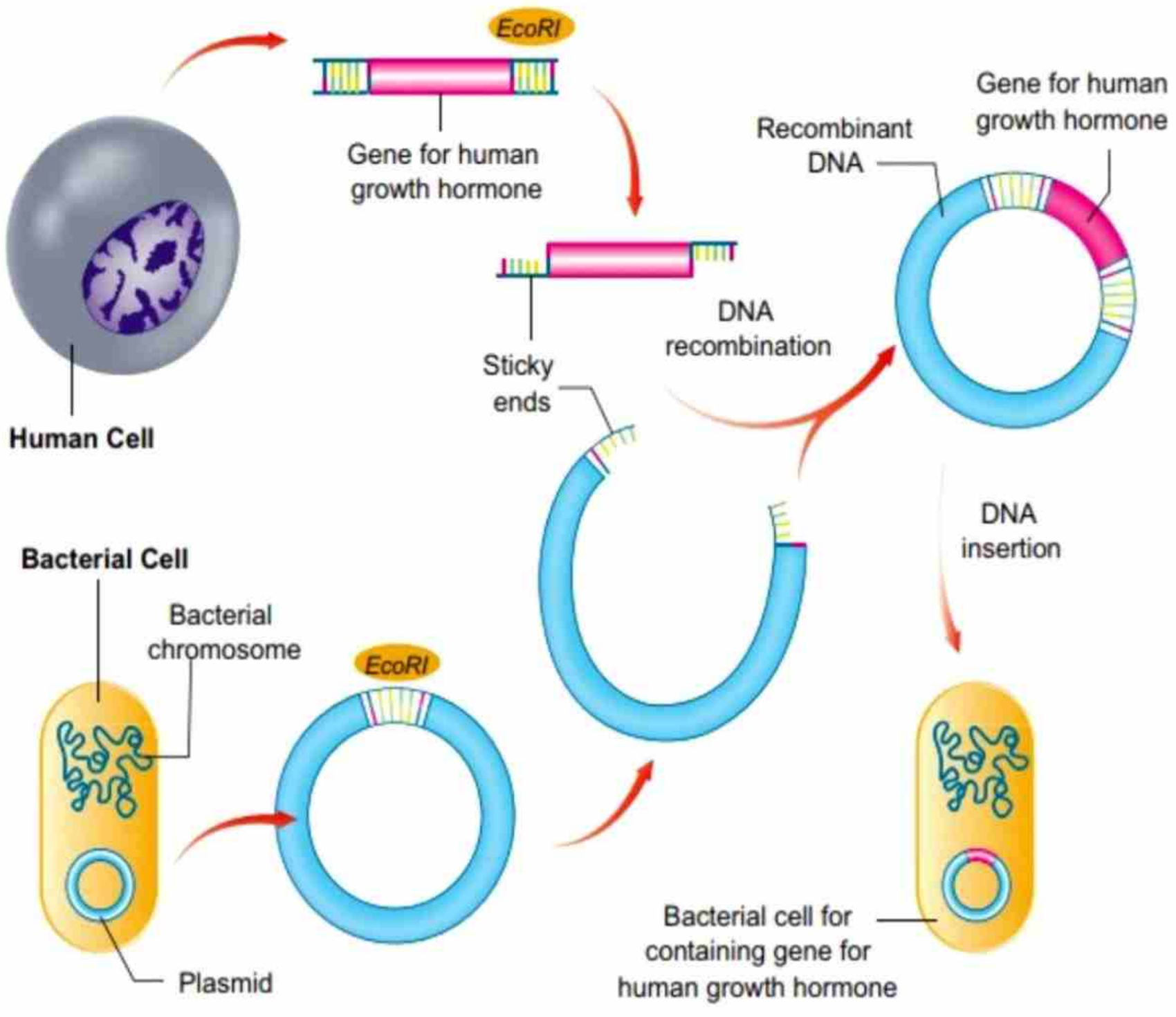

Fig.26.1 Recombinant DNA technology

26.1.2 Role of restriction endonucleases and ligases in gene cloning

Some enzymes play important role in gene cloning. They play crucial role of DNA fragmentation and pasting of fragments during development of recombinant DNA.

(a) Restriction Endonuclease and its role

They are also called restriction enzyme. They are commonly known as **(DNA) molecular scissors** because they break the phosphodiester bond at specific sequence of nitrogenous base to cut the DNA at site called **restriction site**.

Naturally these enzymes are synthesized by bacteria as defense protein to cut the DNA of invader. In this way, they restrict the activity of pathogenic DNA therefore called **restriction enzyme**. These enzymes cleave both strands of DNA molecule at or near the recognition site. These sites usually have **palindromic sequences**. A palindromic sequence is a four to eight base-pairs in DNA in which nucleotides are arranged symmetrically in reverse order. Restriction enzyme usually make **sticky ends** some of these enzymes are also make blunt end. The sticky end joins with plasmid by base pairing. Some examples of restriction enzymes are λ-Hindiii, EcoR1 etc.

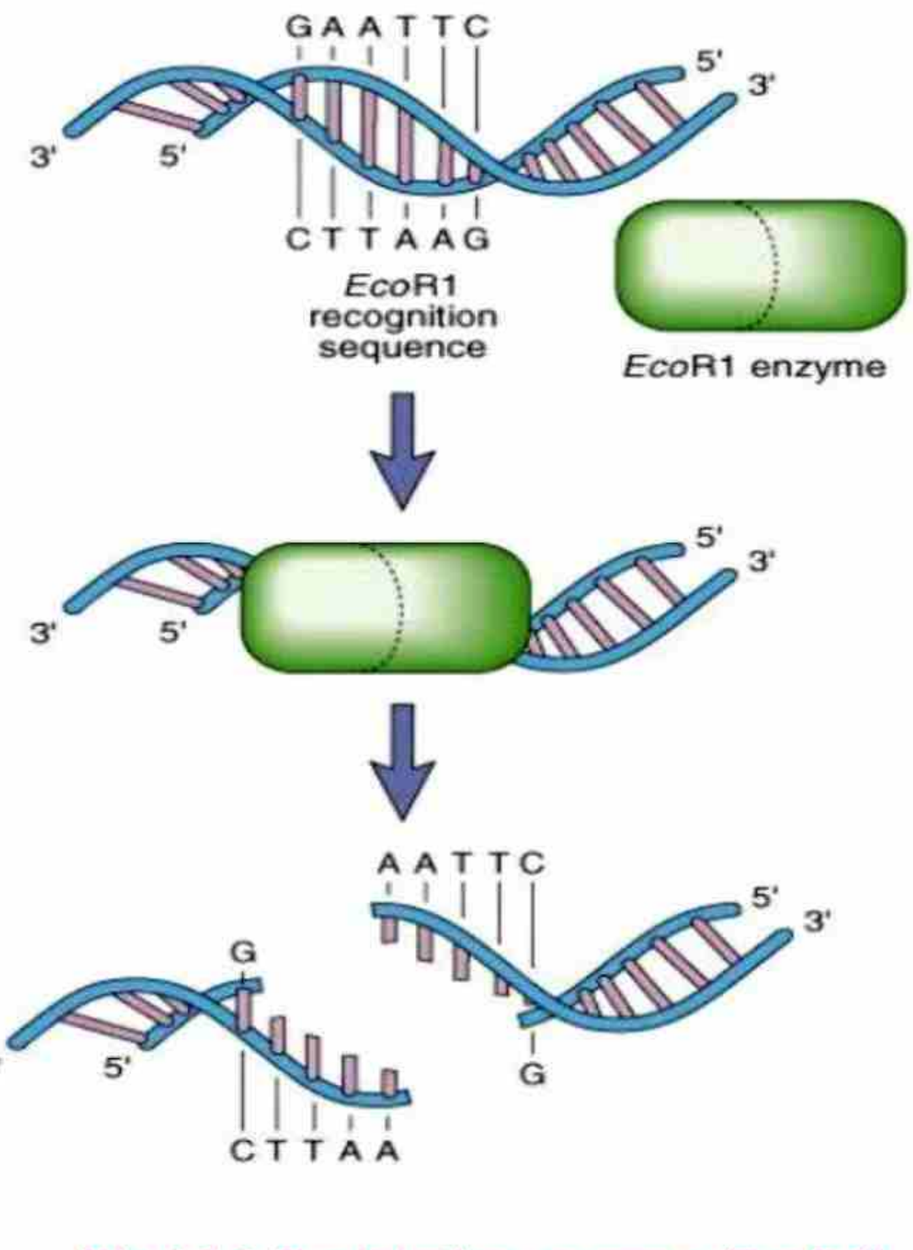

Fig.26.2 Restriction enzyme (EcoR1)

(b) DNA Ligase and its role (Molecular Glue):

Another enzyme use in developing recombinant DNA is DNA Ligase, which catalyze the formation of phosphodiester bond between adjacent nucleotides of DNA. These enzymes join the nucleotides of gene of interest with nucleotides of vector at sticky ends to form rDNA,

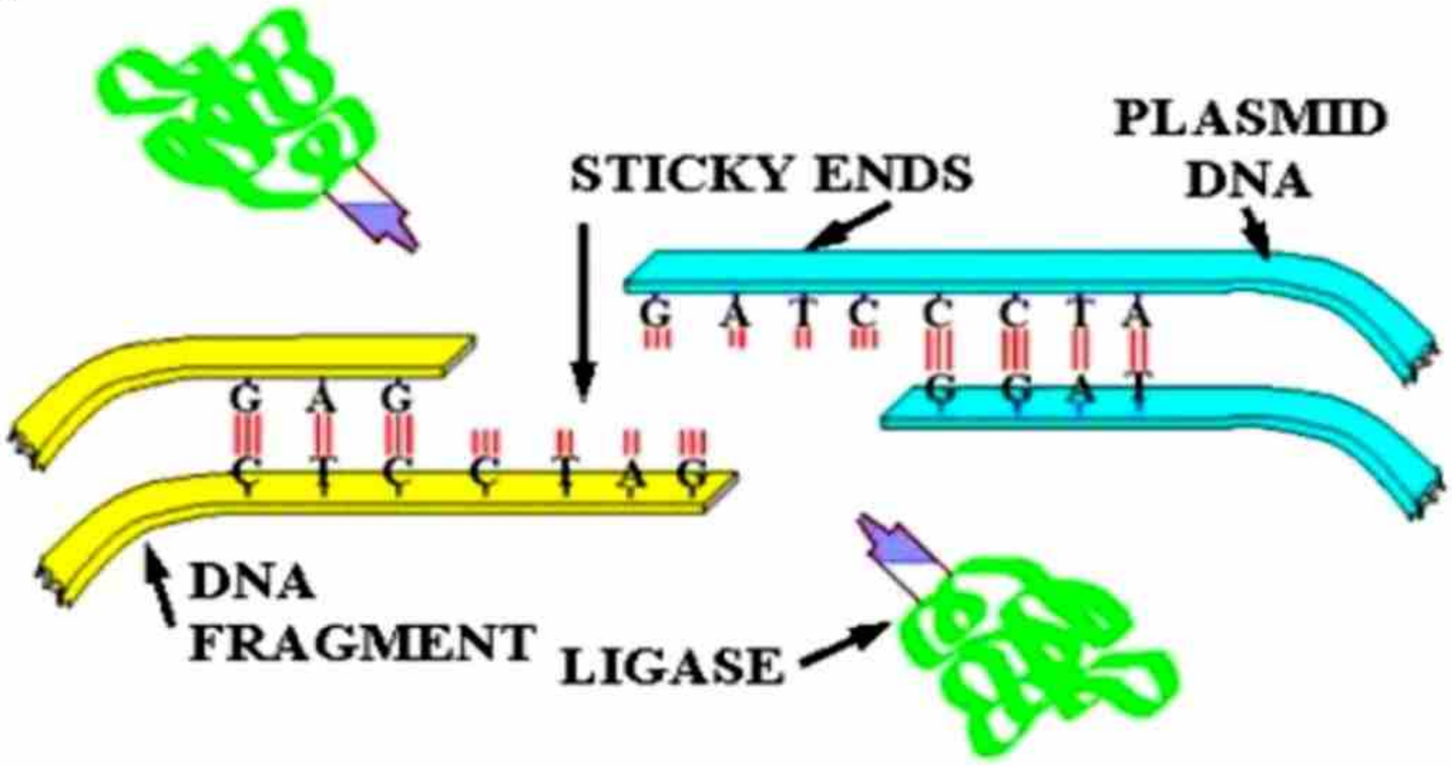

Fig.26.3 DNA ligase

26.1.3. Selection and Isolation of Gene of Interest

There are different methods used to select gene of interest. Usually, a gene library or DNA library is constructed where a comprehensive collection of cloned DNA fragments taken from cell, tissues or organism are present. The genes of interest are also identified by DNA probes. DNA probes are complementary fragments of know unique sequences comprising the gene of interest. The probes are radioactively labeled. Once the probes are added the Hybridize or attach only to the gene of interest and this help in identification of the desired fragment of DNA.

After identification the fragment of DNA is cut by restriction enzyme. These fragments are isolated by gel electrophoresis techniques or they are clone of by PCR method. The isolated gene can be cloned into vector and need for various applications.

26.1.4. Role of Vector in Recombinant DNA Technology

Vectors are DNA molecules used to transport the gene of interest into host cells. In host cell gene interest can be replicated

and expressed. Usually small extra chromosomal DNA of bacteria i.e. plasmid used as Vectors. Vector must possess following characters:

i) It should a DNA molecule has origin of replication site.
ii) It have antibiotic resistant gene.
iii) It has restriction sites of different enzymes

Some of examples of vectors are, Plasmid, Lambda (λ) phage DNA, yeast artificial Chromosome, Cosmid (It is a combination of Plasmid and Phage DNA) etc.

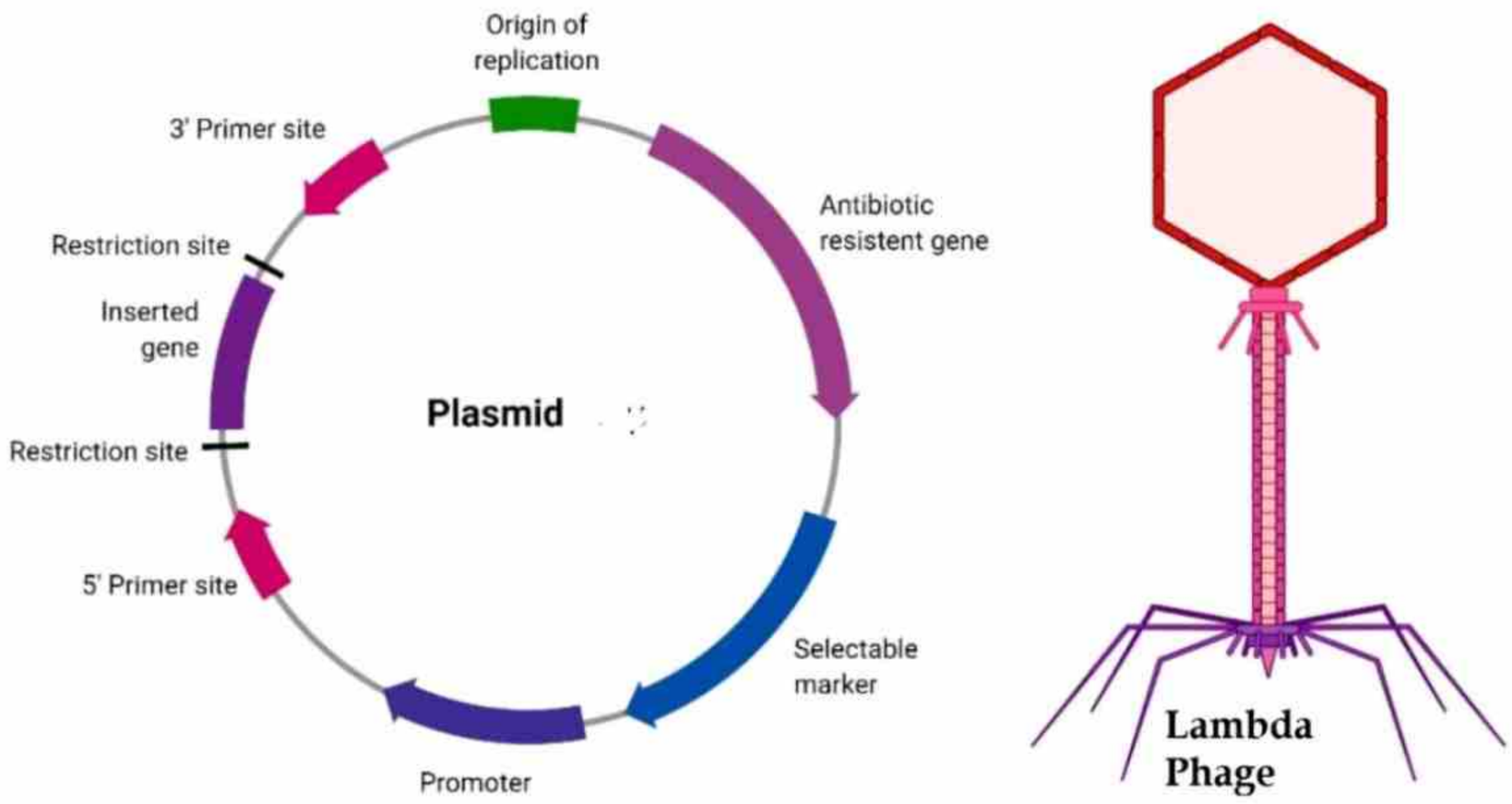

Fig.26.4 Plasmid and Lambda phage DNA

26.1.5 Steps for integration of DNA (gene of interest) insert into vector:

The integration of gene of interest into vector involves several following steps:

1st step:

The vector and gene of interest are digested by same restriction enzymes to create sticky ends.

2nd step:

The digested fragments are purified to isolate the desired size fragments.

3rd step:

The purified vector and DNA insert are ligated together through ligase.

4th step:

The ligated DNA is introduced into host cells through transformation using methods like electrophoresis, chemical transformation or heat shock method. The selected clones can be scaled up and used for various applications, including protein expression therapy or genetic modification.

The techniques applied for the selection of vector

The techniques which applied for selection of vector are based on the presence of suitable **markers**. The markers a may be genetic elements like **antibiotic resistance gene** or gene which produce different colours in host or **fluorescent protein genes** identify and select only those host cells that have successfully up the vector with gene of interest.

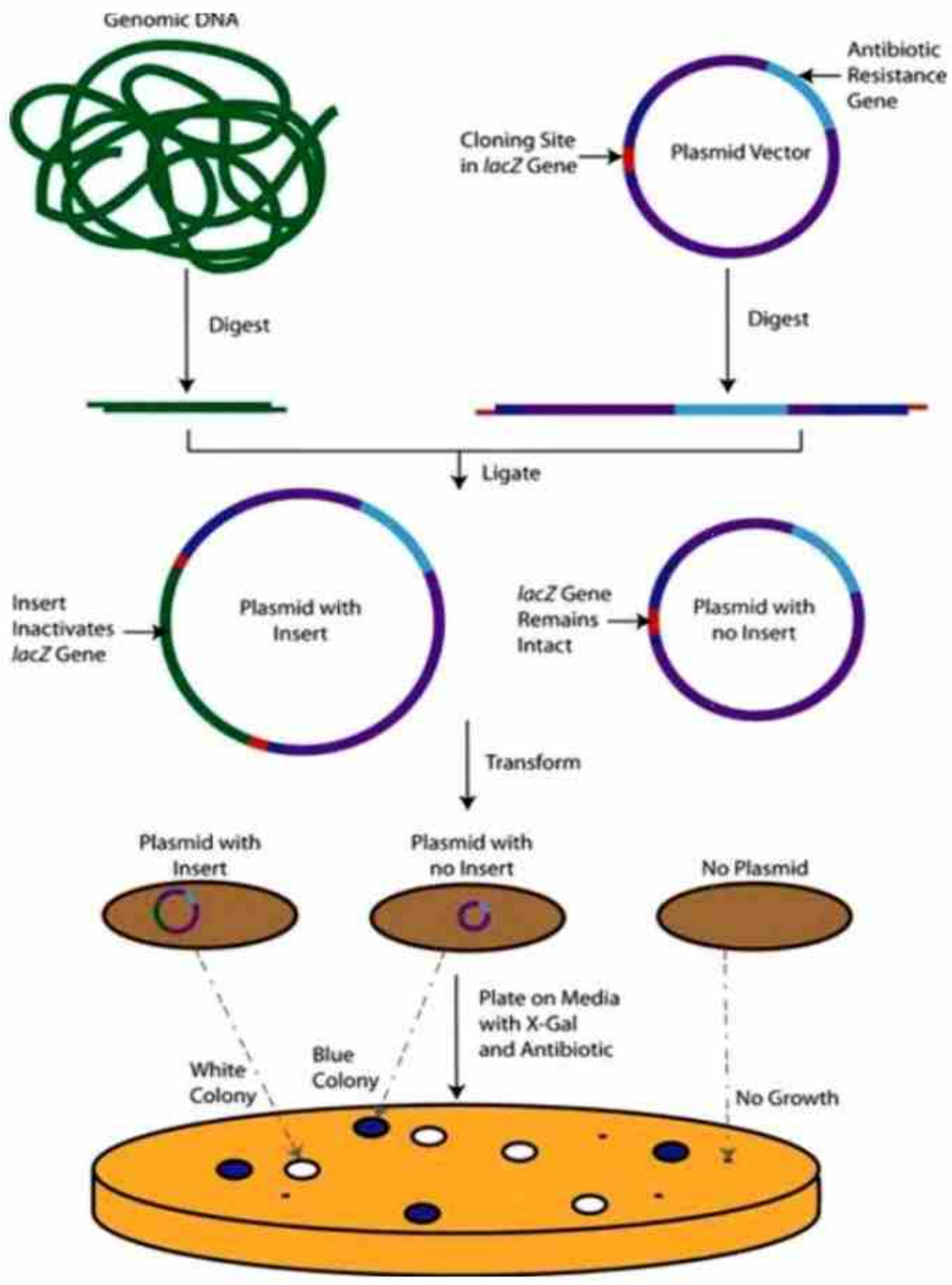

Fig.26.5 Selection of clones

(a) Antibiotic resistance: Amp^R gene is the gene if present in plasmid the host can grow on a medium containing Ampicillin.

(b) Colour producing gene: Lac^Z gene contain vector if present in plasmid the host bacteria can hydrolyze X-Gal (a modified sugar) and produce blue colour in bacteria which shows the presence of vector. When gene is inserted, the bacteria will not produce blue colour.

(c) Fluorescent protein: Another approach is use of Gene florescent protein (GFP). The vector contains FPG with gene of interest, when

vector with gene of interest is taken up by host cell the FPG also express and produce fluorescence which can be observed under fluorescence microscope.

By incorporating selectable markers into vectors biotechnologists can identify and propagate the host successfully.

26.1.7 Gene Amplification through Polymerase Chain Reaction (PCR):

As we have discussed earlier that there are two methods of gene cloning. One this method is **Polymerase Chain Reaction** (PCR). It is a technique used for amplifying (cloning) a specific DNA fragment or gene into millions of copies. It takes place in vitro in specific conditions and in the presence of enzymes. Following are the steps in gene replication through PCR:

Fig.26.5 PCR Machine

Denaturation: The first step in PCR is denaturing where the, double stranded DNA template is heated to high temperature. i.e. 94-98^{0}C to separate the two strands.

Primer Annealing: The temperature is then lowered abruptly up to 50-65 ^{0}C. The primer, a short DNA which is complementary to the ends of the target sequence are added. The primer anneals to the template DNA. It provides a starting point for DNA synthesis.

Extension or polymerization: A heat stable DNA polymerase enzyme e.g., Taq polymerase, is added, along with a supply of nucleotides. The temperature is raised up to 72^{0}C be which is the optimal temperature for DNA polymerase to extend the primer and synthesize a new complementary strand of DNA.

Repeat Cycle: The above steps are repeated for multiple time i.e., 25-40 cycles each time the amount of DNA become double. The exact

number of cycles" depends in the length of the target sequence, and the amount of starting template DNA.

PCR has revolutionized field of biology and medicine, allowing the amplification and analysis of DNA sequences from variety of sources e.g., Clinical samples, fossils and environmental samples.

Table. 26.1 Applications of polymerase chain reaction (PCR)

Application	Description
Medical Diagnosis	PCR is used for the diagnosis of genetic diseases, including cancer, HIV, and genetic disorders. It amplifies specific sequences of DNA, allowing the detection of disease-causing mutations even at very low concentrations.
Forensic Analysis	It is used in forensic analysis to amplify small amounts of DNA from crime scenes or evidence samples. Amplified DNA can then be analyzed to identify suspects, match DNA profiles, and establish the presence or absence of specific genetic markers.
Environmental Monitoring	It is used to detect and quantify microorganisms in environmental samples. It is used to monitor water quality, identify pathogens in food, and detect the presence of harmful bacteria and viruses.
Genetic Research	In genetic research, PCR amplifies DNA sequences for further analysis. It generates DNA fragments that can be cloned, sequenced, and used for a wide range of molecular biology techniques.
Paternity Testing	It compares DNA samples from a child and a potential father to establish paternity with high accuracy. Its high sensitivity, specificity, and versatility make it a powerful tool for DNA analysis and diagnostics

26.1.8 Genome Library

A genome library is a collection of DNA fragment representing an organism's entire genome. These DNA fragments of an organism are cloned in different bacteria or bacteriophage, so we can also say that the genome library is a collection of bacteria or bacteriophage

clone, which contain DNA of a genome of an organism. The Fragments of genome of an organism are inserted into vectors.

These all bacteria are collected and labelled and stored in a freezer below -30^{0}C. The whole sequences of these DNA fragments and their products are also known. Following are the steps for creating genome library.

i) Extract and purify chromosomal DNA of an organism.

ii) Digest the genome with restriction enzyme to create DNA fragments.

iii) Insert DNA fragment into specific vectors, which creates a large port of recombinant molecules.

iv) These rDNAs are taken by host bacteria which transform them, it creates a DNA library.

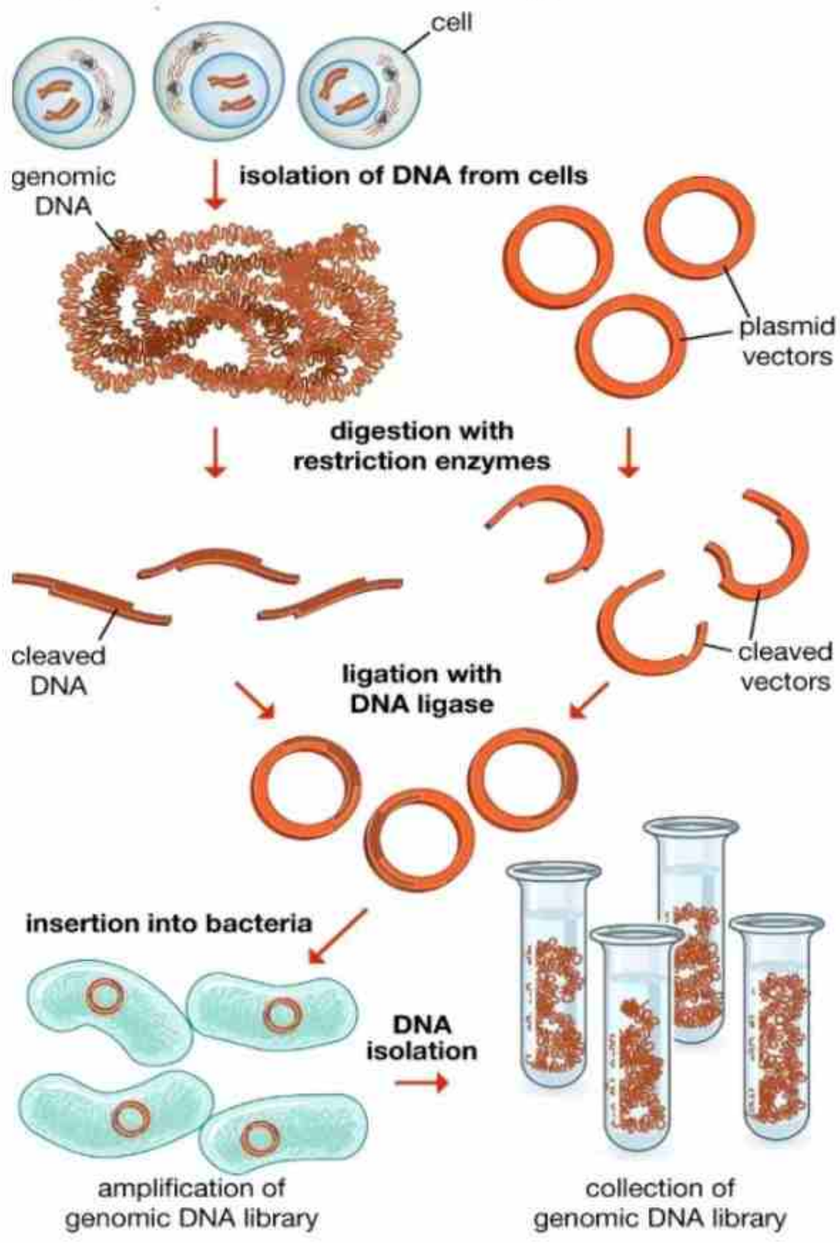

Fig.26.6 Genomic Library

26.2 DNA SEQUENCING

As we know that DNA is made up of 4 types of deoxyribonucleotides. Each fragment by DNA (gene) has specific sequences of their four nucleotides. To determine the exact sequences of these nucleotides called DNA sequencing.

The DNA sequencing process involves break down of a large DNA molecule into small fragments and then reading of its nitrogenous base arrangement by different method, which gives sequences of letters A, G, C, T. These sequences can be analyzed and compared to other DNA sequences, to understand different genetic makeup of an organism, disorders and develop treatments.

There are several methods of DNA sequencing, each have its own advantages, disadvantage and limitations. Some common

methods include Sanger sequencing method, next generation sequencing automated sequencing method, Maxam - Gilbert procedure.

26.2.1. (a) Maxam-Gilbert method

It is a DNA Sequencing method develops in early 1970s by Allan Maxam and Walter Gilbert. It is also known as the chemical cleavage method. In this method Chemicals are used to cleave the DNA at specific nucleotide. It involves following four major steps:

i) DNA fragmentation: The Genome DNA is fragmented into specific size using chemicals and enzymes.

ii) End Labeling: These fragments are now labelled at one end with a radioactive or fluorescent marker to aid in detection.

iii) Chemical cleavage: The labelled fragments are then subjected to specific chemical treatments that cleave the DNA at specific nucleotide residue, producing fragments of varying length.

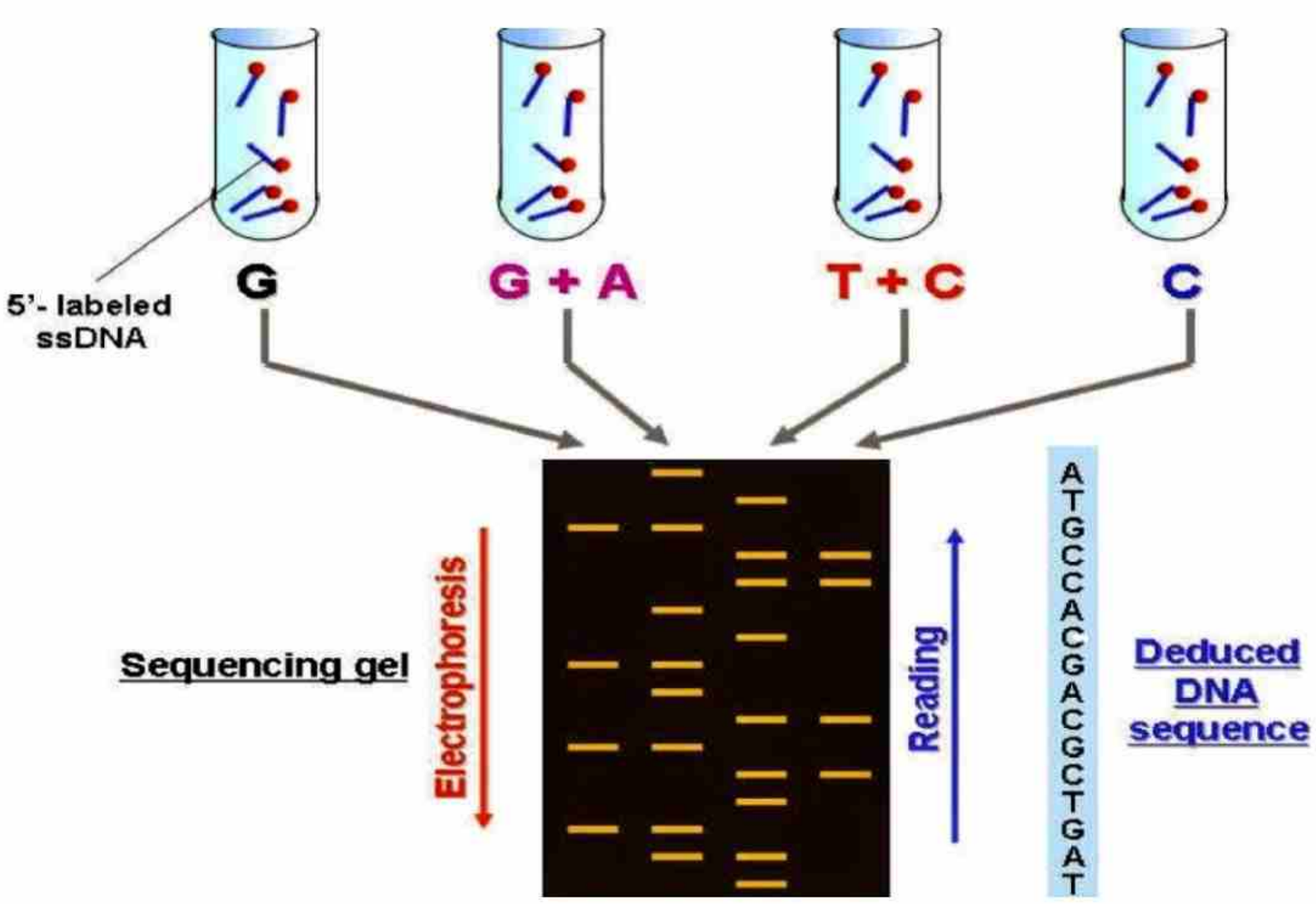

Fig.26.7 Maxam-Gilbert Method

iv) Electrophoresis: Finally, the fragments separated by size, using gel electrophoresis and the sequence can be read, based on the position of cleavage site.

This method has the advantage of able to sequence DNA fragments up to about **500 bases in length**. The disadvantage of this method in that it requires toxic chemicals, time to consume and labour intensive.

26.2.1(b) Singer-Coulson Method

Another early developed method of gene sequencing is called Singer Coulson method or dideoxy Chain-termination method. It is more reliable, efficient and widely used method of gene sequencing in late 1970s and early 1980s. In this method a modified nucleotide, are called dideoxy nucleotides (dd NTPs). These modified nucleotides do not contain 3'-hydoxyl group, which is required for DNA elongation. Then dd NTP's are incorporated into growing DNA chains by DNA polymerase, which result chain termination. In this way, a series of DNA fragments of different length are generated. These fragments are separated by Gel-electrophoresis and the sequence can be determined by reading the band pattern from the gel. By this method longer fragments up to several thousand bases in length can be sequenced.

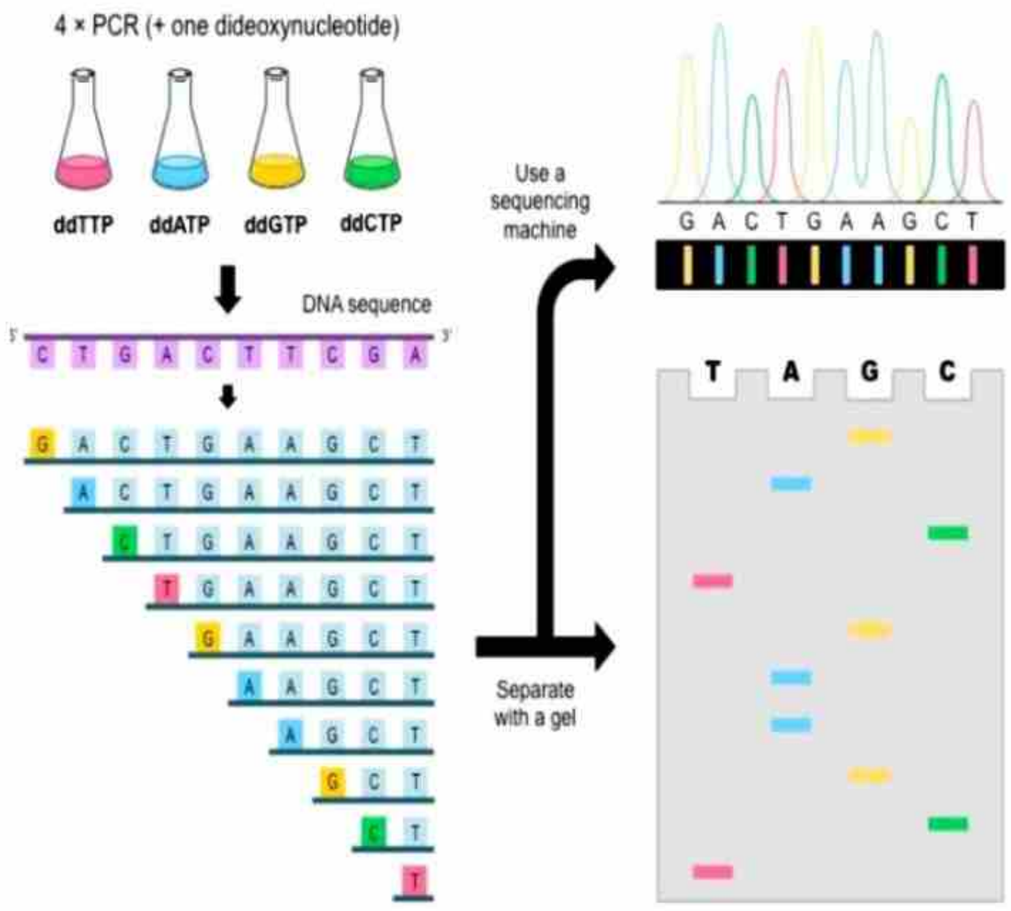

Fig.26.8 Singer-Coulson Method

26.2.2 Gel Electrophoresis and its Principle:

As we have discussed in XI-Biology that the **electrophoresis** is a technique to separate different sized charged fragments of polymers (Protein, DNA and RNA) under the influence of electric current in an **electric cell**. In gel Electrophoresis these molecules move from the tiny pores of semi-solid gel medium of agarose and polyacrylamide. The polymers of different sized are loaded into the well of developed in gel material this gel is placed in an electric cell which is filled with an electrolytic solution. When electric current applied, the different sized DNA fragment begins to move to opposite electrode. (In the case of DNA, they move toward +ve pole) through, pores of gel. After sometimes the different sized molecules are separated in the shape of bands in the gel.

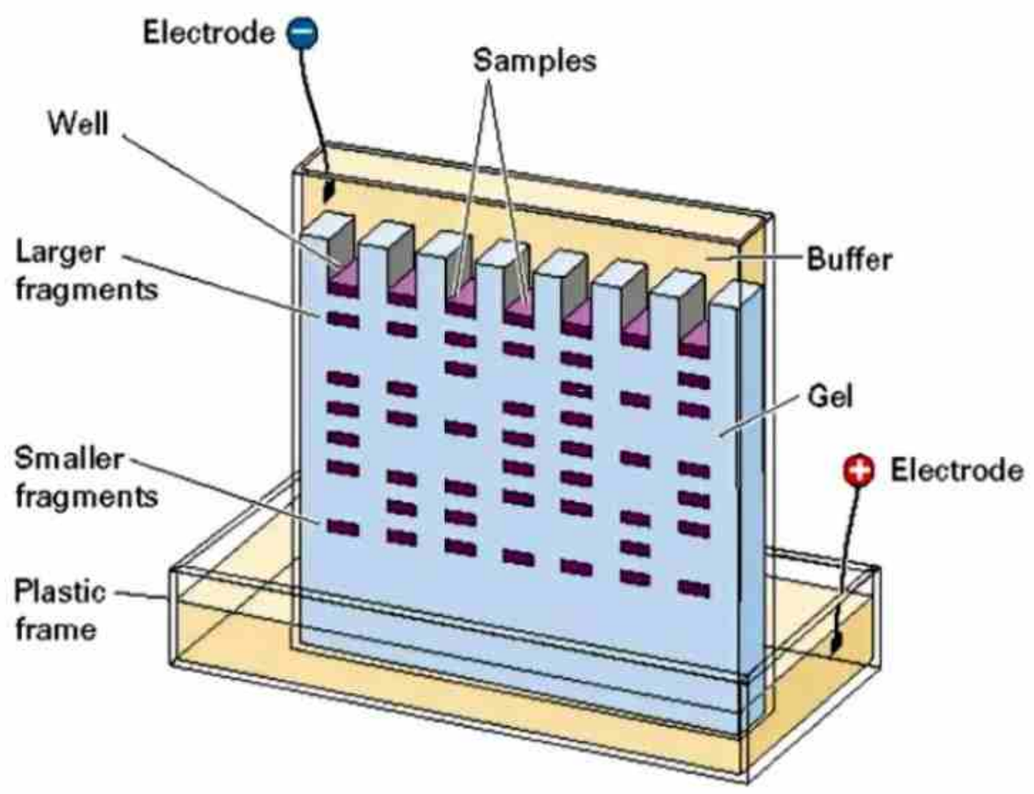

Fig.26.9 Electrophoresis

Principle of Gel electrophoresis

The separation of DNA fragment by gel electrophoresis in based on the size, charges and distance traveled by fragment of DNA molecules. It is proportional to its length, so the smaller fragment moves faster through gel matrix than larger fragments. The movement also depends upon charges, number of strands, shapes of molecules and concentration of gel per size.

Visualization of fragments: After completion of electrophoresis, the DNA fragments are visualized by staining the gel with fluorescent dye, such as **ethidium bromide** or **SYBR Green**. The DNA fragments appear as bands or Smears under UV light, with the smallest fragments closest to the bottom of the gel.

26.2.3 The automated DNA sequencing based on the Sanger-Coulson

Automated DNA sequencing highly efficient method of DNA sequencing based on the Sanger-Coulson method. The key features of automated DNA sequencing are:

i) Fluorescently labelled dideoxy nucleotide (ddNTP):

The fluorescent labelled ddTNPs are used instead of radioactive ddTNPs. Each of the four ddTNP's are labelled with a distinct colour fluorophore, allowing for the simultaneous sequencing of all four nucleotides.

ii) Capillary electrophoresis:

In automated method capillary electrophoresis is used instead of Gel electrophoresis. The DNA fragments are separated in at thin capillary filled with a polymer matrix. The capillary is placed in an instrument that applies an electric current for DNA fragment separation and migration. As the fragment pass through a laser beam, the florescent labels are excited, producing a signal which is detected by a detector.

The data generated is analyzed by computer software by converting signals into a sequence of nucleotides. **Bioinformatics** tools are software applications as computational method used to store analyze and interpret biological automated DNA sequencing has several advantages over traditional methods. It is faster, performing sequencing of thousands of bases per day. It also requires accurate than manual method. It also requires less starting material and suitable for application.

6.3 DNA ANALYSIS

A set of techniques used to study DNA structure, function and properties. It is essential in genetics, forensics, medicine and biotechnology. Techniques, like DNA sequencing, PCR and gel electrophoresis enable the detection of genetic mutation, identification of disorders analysis of gene expression and exploration of evolutionary history. It is also used to diagnosis of diseases, developing drugs, enhancing agriculture yield and solving crimes.

6.3.1 Purpose and mechanism of DNA analysis

On DNA Analysis It is found that each individual has a specific percentage of DNA which does not code for protein but repeated frequently on the genome of that individual. These repetitive units vary in length from organism to organism of the same species each of such repetitive units are 20-40 base pair lung. These variable and unique lengths of non-functional DNA are passed on to the offering along with the compliment of gene in a Mendelian fashion. The differences in DNA electrophoresis pattern among individuals are called, **Restriction fragment length Polymorphisms** (RFLPs pronounced as Riff Lips). It is also called **DNA finger print**.

It is used to settle down the disputes over parentage relationship identification of individual during accidents. It is also used to identify criminals from blood, semen, saliva and hair follicle etc. left at the scene of crime. It has also spectacular potential for medicine, for instance in the parental diagnosis of individual disorders.

Mechanism of DNA analysis:

Numbers of techniques are used for analysis. Restriction Fragment Length polymorphism (RFLP) is one of the first method used in DNA analysis. Following are the steps to make a DNA finger prints by using this method:

i) Collection of DNA sample: A small fraction of DNA sample is collection from blood saliva, semen or hair follicle. It can amplify to get clones by PCR.

ii) Digestion and separation of RFLP: The collected DNA sample is digested by specific restriction enzymes it produces different sized DNA fragments. This mixture of DNA is separated by electrophoresis.

iii) Denaturation of fragments: The electrophoresed gel is placed into an alkaline solution (NaOH) to denature the dsDNA. The denaturation improves binding of the negatively charged DNA to positively charged membrane and separating it into ssDNA for later hybridization to the probe.

iv) Blotting: In this step transfer of DNA at nitrocellulose membrane perform. A sheet of nitrocellulose membrane placed on the top of alkaline gel. The ssDNA bind with membrane by ion exchange interaction because the DNA is negatively charge which the membrane is +vely changed. The membrane is then baked in a vacuum or regular oven at 80^0C for 2 hours for permanent attachment of ssDNA with membrane.

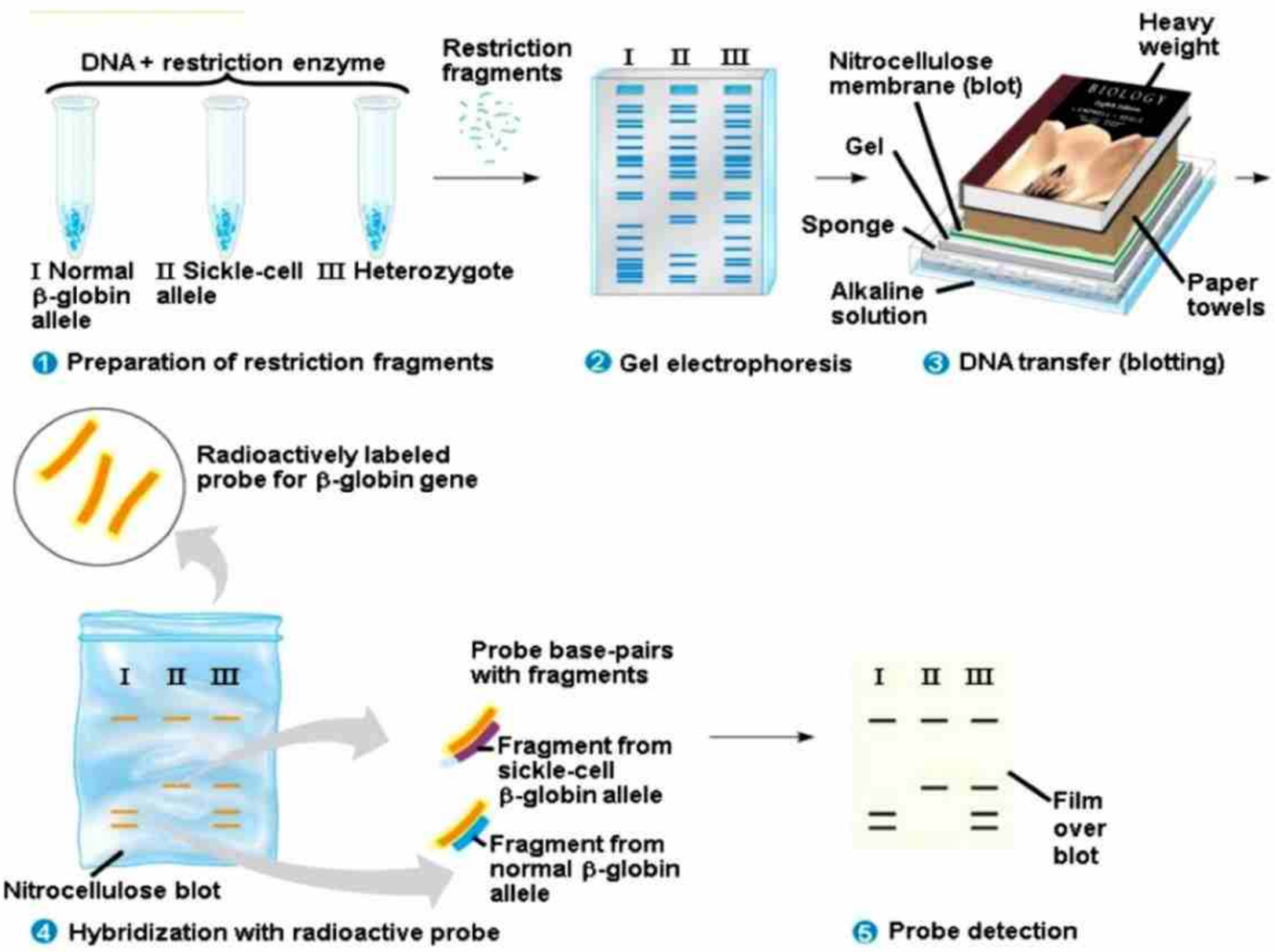

Fig. 26.10 DNA analysis

Labelling of RFLPs: The membrane is then expose to radioactive probe to hybridize the denatured ssDNA fragments in all bands. The radioactive DNA can be observed by autoradiography.

Autoradiography: After hybridization membrane is washed to remove excess amount of probe, the pattern of hybridization in observed by exposing the membrane on x-ray film. It is called **autoradiography**.

26.4. Genome Map:

Total DNA present on the chromosomes of Eukaryotic call called **Genome**. Each Chromosome carry specific genes at specific position of chromosome the position of gene at chromosome of gene at chromosome called **gene loci**. In diploid cell these genes are present in two sets which in haploid cells these are found in one set. It requires to know that a specific chromosome have genes of what of type of characters and what are their position at that Chromosome to find out the nature and position of genes at particular chromosome called **Genome Mapping**.

26.4.1 Genome Analysis

To make the Genome map the biologist perform analysis of the DNA of each chromosome by these techniques discussed earlier. The branch of biotechnology which deals with genome analysis is called **genomics**. This branch covers the analysis of complete DNA sequence of organism's genome.

Genome Map

Genome map is just like the road map and street map of a city which guide us to reach at specific location. It is used to search a specific gene at specific chromosome. There are two types of maps. (a) Genetic Maps (b) Physical maps. Genetic map shows the sequence of gene chromosomes while physical map shows the sequence of nucleotides in the DNA.

Genetic Markers: DNA Sequences or Variations used to identify or distinguish individual population or traits called Genetic markers like RFLPs. These markers are used in genome analysis to map genes, identify disease causing mutations biodiversity and evolution.

26.4.2 The history of the human genome project.

The Human Genome Project (HGP) was an international scientific research project that aimed to sequence and maps the entire human genome. The project was initiated in 1990 by the US National Institutes of Health (NIH) and the Department of Energy (DOE), with the participation of many international collaborators. James Watson, a prominent molecular biologist and one of the co-

discoverers of the structure of DNA, was appointed as the first director of the HGP in 1990. Watson was known for his pioneering work in molecular biology, and his leadership and vision were instrumental in the project's success. The project was completed in 2003, ahead of schedule and under budget, and its results were published in several landmark papers in the journals Nature and Science.

26.4.3 Goals of the human genome project.

The Human Genome Project (HGP) was a scientific research initiative aimed at mapping and sequencing the entire human genome, which is the complete set of genetic instructions that determines the traits and functions of an individual.

The primary goal of the HGP was to determine the complete sequence of the human genome, including all the genes, non-coding regions, and repetitive sequences. The HGP aimed to identify and annotate the function and location of all the genes and other functional elements in the human genome, including regulatory regions, splice sites, and other features. It aimed to identify new genes and genetic variations associated with human diseases, traits, and drug responses, using approaches like linkage analysis, association studies, and functional genomics.

Ethical, legal, and social implications: The HGP recognized the need to address the ethical, legal, and social implications of the genomic information and technologies generated by the project, and established programs to study and address these issues. However, the completion of the HGP laid the foundation for new discoveries and applications in personalized medicine, gene therapy, and other areas of health and biotechnology.

26.4.4 Benefits of the human genome project:

It revealed that humans have 23 pairs of chromosomes and showed us where genes and other important parts of the genome are located. The HGP also found differences in the structure of the genome, like deletions and duplications that can affect our health. By identifying all the estimated 20,000-25,000 genes in the human genome, researchers can study how these genes work and their role

in diseases. They have also discovered genetic variations linked to diseases like cancer, diabetes, and heart disease, which helps develop better ways to diagnose and treat these conditions.

26.5 TISSUE CULTURE

Tissue culture involves the isolation of cells or tissues from a living organism, providing them with the necessary nutrients, growth factors, and conditions to support their growth and proliferation.

26.5.1 Basic Terminology

Term	Definition
Explants	Small pieces of plant tissue (leaves, stems, roots and flowers) taken from a parent plant used to initiate a culture of cells or tissues in vitro.
Callus	Mass of unorganized and undifferentiated cells that arise from the explant culture in vitro. It can be used as a source of cells for further tissue culture experiments or for inducing somatic embryogenesis.
Micropropagation	Tissue culture technique involving rapid multiplication of plants by producing multiple shoots or plantlets from a single explant. Widely used for high-quality planting materials and multiplication of rare and endangered plant species.
Plantlets	Small, fully developed plants produced in vitro through micropropagation or somatic embryogenesis. They can be transferred to soil and grown into mature plants.
Somatic embryogenesis	Tissue culture technique involving the induction of embryo formation from somatic cells in vitro. It can produce many genetically identical plant embryos and regenerate whole plants.
Soma clonal variation	Genetic variation that arises from the culture of plant cells or tissues in vitro. It can result from mutations, chromosomal aberrations, epigenetic changes, or other factors affecting the genetic stability of cultured cells. It can have positive or negative effects on plant phenotype and can be utilized in developing new plant varieties.

26.5.2 Tissue culture, organ culture and cell culture:

Tissue culture is a technique used to grow and maintain cells, tissues, or organs in vitro, outside the organism. It involves the aseptic culture of plant or animal cells, tissues, or organs in nutrient media under controlled conditions of temperature, light, and humidity.

Table. 26.2 Comparison between Organ Culture and Cell Culture

Feature	Organ Culture	Cell Culture
Definition	In vitro culture of whole organs or tissues	In vitro culture of isolated cells
Types	Slice, explant, suspension culture	Monolayer, suspension, spheroid culture
Complexity	More complex, includes multiple cell types	Less complex, single cell type
Maintenance	Requires specialized maintenance	Easy to maintain
Nutrient supply	May require more complex nutrient supply	Nutrient supply can be controlled precisely
Experimental use	Mimics physiological conditions	Allows for more precise experimentation
Applications	Tissue engineering, drug development, toxicology testing	Vaccine development, cancer research, gene expression studies

26.5.3 The callus culture and suspension culture techniques:

Both techniques have different applications in research and commercial production of plant-based products. Callus culture and suspension culture are two common techniques used in plant tissue culture. The main differences between the two techniques are as follows.

Table. 26.3 Comparison between Callus culture and Suspension Culture

	Callus culture	Suspension culture
Cell organization	Callus culture involves the growth of undifferentiated mass of cells, which are disorganized and heterogeneous	Suspension culture involves the growth of individual cells suspended in liquid media.
Culture conditions	It requires a solid medium, such as agar, for the cells to grow and proliferate	It requires a liquid medium that allows for the free movement of the cells.
Cell growth:	produces a compact mass of cells that can be induced to differentiate into specialized tissues or organs	Produces single cells that can be used for recombinant proteins, monoclonal antibodies, or other biologically active molecules.
Maintenance:	easy to maintain, as the cells can be sub-cultured by simply transferring a small piece of the callus to a new culture medium.	It requires more attention, as the cells need to be regularly sub-cultured to prevent clumping and to maintain optimal growth conditions.

26.5.4 The anther culture, ovary culture, meristem culture and embryo culture techniques:

Anther culture, ovary culture, meristem culture, and embryo culture are all techniques used in plant tissue culture to produce new plants with desirable traits.

Anther culture: Anther culture is a plant tissue culture technique that involves the culture of anthers (male reproductive organs) in a nutrient medium to induce their development into haploid plants. The technique is widely used in plant breeding programs to produce haploid plants, which can be used to develop homozygous lines through chromosome doubling. The process involves the removal of

anthers from the flowers of a donor plant, followed by their placement on a nutrient medium containing plant growth regulators such as auxins and cytokinins. The anthers are then allowed to grow and develop into haploid plants, which can be further propagated and used for breeding purposes. The anther culture technique is particularly useful for the rapid production of homozygous lines, which can be used to develop new crop varieties with desired traits such as disease resistance, improved yield, and quality

Ovary culture: Ovary culture involves the culture of immature ovules on a nutrient medium such as cytokinins and auxins. The cells in the ovary can be induced to divide and differentiate into embryos, which can be used to produce new plants. Ovary culture is used to produce haploid and diploid plants, depending on the method used.

Meristem culture: Meristem culture is a plant tissue culture technique that involves the isolation and culture of shoot apical meristems, which are regions of actively dividing cells located at the tips of plant shoots. The technique is used to produce disease-free plants from infected or diseased plant material, as the shoot apical meristem is often free of viruses, bacteria, and other pathogens.

Embryo culture: Embryo culture involves the culture of embryos, which can be obtained from seeds or by inducing the formation of embryos in tissue culture. Embryo culture is used to produce genetically identical plants, as embryos can be divided to produce multiple plantlets with the same genetic makeup.

Each technique has its own advantages and limitations and can be used for different applications, such as the production of haploid plants, disease-free plants, and genetically identical plants.

26.5.5 Techniques, Applications, and limitations of animal tissue culture:

Animal tissue culture involves the growth of animal cells in vitro under controlled conditions. This technique has a wide range of applications in basic and applied research, drug discovery,

biotechnology, and regenerative medicine. Here are some of the commonly used techniques:

Primary cell culture:

Primary cell culture involves the isolation of cells directly from animal tissues and their growth in vitro. This technique is used to study the behavior of cells in their native environment and to test the toxicity and efficacy of drugs. The limitations of primary cell culture include the limited lifespan of cells and the potential for contamination with microorganisms.

Cell line culture:

Cell line culture involves the establishment of immortalized cell lines from primary cells. These cell lines can be used to study cell behavior and function under different conditions, to screen for new drugs, and to produce recombinant proteins. The limitations of cell line culture include the potential for genetic drift, the lack of physiological relevance, and the potential for contamination with other cell lines or microorganisms.

Transfection and gene expression analysis:

Transfection involves the introduction of foreign DNA into animal cells, which can be used to study gene function and regulation. This technique can be used to express recombinant proteins, to knock down or overexpress specific genes, and to study the effect of genetic mutations on cellular processes. The limitations of transfection include the potential for toxicity, the low efficiency of gene transfer, and the potential for off-target effects.

3D culture:

3D culture involves the growth of animal cells in three-dimensional structures, which can mimic the architecture and function of native tissues. This technique is used to study tissue development, regeneration, and disease, and to test the toxicity and efficacy of drugs. The limitations of 3D culture include the complex and variable nature of the structures, the potential for contamination, and the high cost and technical expertise required.

Applications of Animal tissue culture:

Animal tissue culture is a widely used technique in modern biological research with many applications in various fields. Some of the most common applications of animal tissue culture include:

Cell-based assays: Animal tissue culture is used to create a standardized platform for cell-based assays that allow researchers to test the effects of various drugs, chemicals, or other substances on cells in a controlled environment.

Vaccine development: Tissue culture is used to grow viruses or bacteria for use in vaccine production, which helps to reduce the risk of contamination and increase the purity of the vaccine.

Cancer research: Tissue culture is used to grow cancer cells in vitro, which can help researchers to study the mechanisms of cancer development, test new treatments, and screen potential drugs.

Toxicity testing: Tissue culture is used in toxicology studies to determine the potential harmful effects of chemicals, drugs, or other substances on cells or tissues.

Regenerative medicine: Tissue culture is used to grow cells or tissues for transplantation, tissue engineering, and other regenerative medicine applications

26.6 TRANSGENIC BACTERIA, PLANTS AND ANIMALS:

Transgenic living thing are those having foreign gene is inserted in it.

26.6.1 Main Objectives behind Transgenic Bacteria, Plants and Animals

The objectives of producing transgenic bacteria, plants, and animals include:

Transgenic bacteria:

Transgenic bacteria are genetically modified by the introduction of foreign genes or DNA. Making transgenic bacteria

involves inserting a desired gene into the bacterial DNA through recombinant DNA technology. The gene of interest is first isolated and then inserted into a **plasmid**.

The plasmid is then introduced into a bacterial strain such as **Escherichia coli** (E. coli) through transformation. The use of transgenic bacteria for insulin production has allowed for large-scale production of insulin at a lower cost, making it more accessible to patients with diabetes. Once the transgenic bacteria are produced, they can be used for various applications such as the production of recombinant proteins, bioremediation, and gene therapy.

Transgenic plants: The production of transgenic plants aims to introduce new genes or modify existing ones to improve plant traits such as yield, disease resistance, and nutrient uptake, and to produce useful products such as pharmaceuticals, vaccines, and **biofuels**.

Biofuels are a type of fuel derived from renewable biological sources, such as plants. **Cellulose** can be broken down into simple sugars that can be fermented and distilled to produce biofuels such as ethanol. One example of a transgenic plant used in biofuel production is **switchgrass**, which has been engineered to produce higher levels of cellulose and to be more resistant to pests and diseases. Other transgenic plants used in biofuel production include corn, soybeans, and canola.

Transgenic animals: transgenic animals are genetically modified organisms that have had one or more genes from a different species introduced into their genome. The goal of producing animals that have desired traits, such as improved disease resistance, enhanced growth rate, or the ability to produce pharmaceuticals.

One modern application of transgenic animals is in the field of medicine. For example, transgenic animals can produce human proteins used to treat various diseases. One such example is **antithrombin**, a protein used to treat blood clotting disorders, in transgenic goat milk. This can be especially useful in areas where food is scarce, as it can increase the amount of food that is available.

26.6.2 Introduction of DNA into plant and animal cells/embryos

Method	Description
Electroporation	High voltage electrical pulse creates temporary pores in the cell membrane, allowing DNA to enter.
Microinjection	DNA is directly injected into the nucleus of the cell/embryo using a fine needle.
Biolistics or gene gun	DNA-coated tiny particles are propelled at high speed into cells/embryos using a gene gun.
Agrobacterium-mediated transformation	Agrobacterium tumefaciens bacterium is used to introduce DNA into plant cells. The bacterium transfers a portion of its own DNA (T-DNA) into the plant cell, which can be replaced with desired DNA.
Liposome-mediated transfection	Liposomes (tiny spheres made of lipids) encapsulate DNA, which is then introduced into cells/embryos.
Viral vectors	Modified viruses deliver DNA sequences to specific cells, commonly used for gene therapy in animals.

26.6.3 Role of biotechnology in the production of insect, virus, and herbicide resistant plants:

Biotechnology has played a significant role in insect, virus, and herbicide-resistant plants. By introducing specific genes into the plants, scientists have been able to enhance the plants' resistance to certain pests and environmental stressors, thus improving their productivity and yield.

Insect-resistant plants: Scientists have developed genetically modified (GM) plants that produce a toxin called **Bacillus thuringiensis** (Bt), which is toxic to certain pests, such as the European corn borer and cotton bollworm. The Bt toxin is produced by a gene transferred into the plant's genome, usually via Agrobacterium-mediated transformation. The toxin is highly specific

to the target pest, reducing the need for chemical pesticides, which can be harmful to the environment and non-target species.

Virus-resistant plants: Virus-resistant plants have been developed by introducing genes encoding viral coat proteins, which prevent viral replication and infection in the plant. The coat protein gene is either delivered through Agrobacterium-mediated transformation or viral vectors. Several virus-resistant crops have been developed through biotechnology to combat the threat of viral diseases to crops. These include genetically modified papaya that is resistant to the ringspot virus, tomato that is resistant to the Tomato yellow leaf curl virus, potato that is resistant to the Potato virus Y, and maize varieties, such as the Maize dwarf mosaic virus-resistant maize.

Herbicide-resistant plants: Herbicide-resistant plants are developed by introducing a gene encoding an enzyme that can detoxify the herbicide, making the plant resistant to the herbicide's effect. This allows farmers to use specific herbicides to control weeds without harming the crop. The most used herbicide-resistant crops are glyphosate-resistant, developed by Monsanto and known commercially as roundup Ready.

26.6.4 Human gene transfers in different animal species

There have been several notable human gene transfers into different animal species, with potential applications and prospects.

Mice: Mice are the most used animal models in biomedical research, and human gene transfers have been used extensively to create mouse models of human diseases. For example, scientists have introduced human genes into mice to study diseases such as cystic fibrosis, Alzheimer's disease, and cancer.

Sheep: Sheep have been genetically modified to produce human proteins in their milk, which can then be used to produce biotech products such as insulin, blood clotting factors, and growth hormones.

Pigs: Pigs have been genetically modified to produce organs that are compatible with human recipients. This technique is called xenotransplantation, and it could help to address the shortage of human organs for transplantation.

Fish: Fish have been genetically modified to produce human proteins, such as insulin and growth hormone, which can be used to treat human diseases. This technique is called aquaculture biotechnology, and it can produce biotech products at a lower cost than traditional manufacturing methods.

Primates: Non-human primates, such as monkeys, have been genetically modified to create animal models of human diseases, such as Parkinson's disease and HIV.

26.6.5 Role of transgenic bacteria in making biotechnology products:

Transgenic bacteria are important tools in biotechnology to produce various products. These bacteria are genetically engineered to express specific genes that enable them to produce a wide range of useful substances, such as enzymes, hormones, vaccines, and antibodies. One of the most common uses of transgenic bacteria is producing recombinant proteins, such as insulin and growth hormones. These proteins can be produced in massive quantities by inserting the gene for the protein of interest into the bacterial genome. The bacteria can then be grown in large-scale cultures and the protein harvested and purified. Transgenic bacteria are also used to produce vaccines. For example, the hepatitis B vaccine is produced using transgenic yeast or bacteria that express the hepatitis B virus surface antigen. The antigen can then be purified and used to produce the vaccine. It can be engineered to produce enzymes that break down cellulose to produce biofuels.

26.6.6 Some ecological concerns surrounding transgenic bacteria:

Transgenic bacteria can escape into the environment and transfer their modified genes to other bacteria, potentially creating new and unknown organisms that could have negative ecological

impacts. It is called Genetic Pollution. Transgenic bacteria can affect natural ecosystems by competing with native bacteria for resources, potentially leading to the displacement of native species. Bacteria may have unintended effects on non-target organisms, including beneficial insects and microorganisms. The use of antibiotic resistance genes in transgenic bacteria can potentially contribute to the spread of antibiotic resistance in the environment. The introduction of transgenic bacteria can potentially alter microbial ecology, including the composition and function of microbial communities in soil, water, and other environments.

26.6.7 Genetic engineering and farm animals:

Genetic engineering can benefit farm animals and agriculture in many ways. For example, it can introduce disease-resistant genes into farm animals, leading to better health and protection from common illnesses and infections. Additionally, genetic engineering can improve the growth rates of farm animals, leading to larger and more productive animals. It can also enhance nutrient utilization in animals, leading to better growth and overall health. Technology can even improve the quality of animal products, such as meat, milk, and eggs, by altering their composition. Moreover, genetic engineering can reduce the environmental impact of farming by improving feed utilization efficiency and decreasing waste produced by animals. However, proper regulation and monitoring of genetic engineering technologies are crucial to ensure their responsible and ethical use.

26.7 BIOTECHNOLOGY AND HEALTH CARE:

26.7.1 Biotechnologists produce vaccines to combat health problems Vaccine:

A vaccine is a biological preparation that provides immunity to a particular disease. It contains antigens that stimulate the body's immune system to produce antibodies to fight off specific pathogens, such as viruses or bacteria that cause the disease. Vaccines are usually administered through injection or oral route and can prevent or reduce the severity of the disease.

Table. 26.4 Some important vaccines developed by biotechnologists

Vaccine	Description
Hepatitis B vaccine	Made using recombinant DNA technology. The gene for hepatitis B surface antigen is inserted into yeast cells, which produce large quantities of the antigen. The antigen is purified to make the vaccine.
Human papillomavirus (HPV)	Made using genetic engineering. The gene for HPV surface protein is inserted into insect cells, which produce large quantities of the protein. The protein is purified to make the vaccine.
Influenza vaccine	Made using a combination of biotechnological techniques. Each year, the WHO identifies prevalent influenza strains, and a vaccine is produced containing appropriate influenza antigens.
COVID-19 vaccine	Developed by Pfizer-BioNTech, Moderna, and Johnson & Johnson, these vaccines use mRNA technology to instruct cells to produce a spike protein found on the surface of the coronavirus for immune response.
Malaria vaccine	Still in development, potential candidates use biotechnology. Sanaria's vaccine, for example, uses a weakened form of the malaria parasite to stimulate an immune response

26.7.2 Biotechnology and disease diagnosis (DNA/RNA probes, monoclonal antibodies):

Biotechnology plays a significant role in disease diagnosis through the development and use of various techniques, including DNA/RNA probes and monoclonal antibodies.

DNA/RNA probes: These probes are small molecules designed to bind to specific DNA or RNA sequences. In disease diagnosis, these

probes are used to detect the presence of specific pathogens or genetic mutations associated with certain diseases. This technique is particularly useful for detecting viral infections such as HIV, hepatitis B and C, and human papillomavirus (HPV). These probes are labeled with a fluorescent or radioactive marker, making it possible to detect their binding to the target DNA or RNA sequences. This allows for quick and accurate detection of pathogens or genetic mutation in clinical samples, such as blood, urine, or tissue.

Monoclonal antibodies:

Monoclonal antibodies are laboratory-made antibodies designed to bind to specific antigens, such as proteins on the surface of pathogens or cancer cells. In disease diagnosis, monoclonal antibodies are used to detect these antigens in clinical samples. This technique is useful for diagnosing infectious diseases such as tuberculosis, syphilis, and Lyme disease. It is also used in the diagnosis of cancer, where monoclonal antibodies are used to detect specific tumor markers in blood or tissue samples. They are also used in imaging techniques, such as positron emission tomography (PET), to detect cancer cells in the body.

26.7.3 Products biotechnologists obtain for use in disease treatment:

Application	Description
Recombinant Proteins	Production of proteins using recombinant DNA technology for treating diseases like diabetes, growth hormone deficiency, and anemia.
Monoclonal Antibodies	Laboratory-made antibodies designed to target specific antigens, used for treating diseases such as cancer, rheumatoid arthritis, and multiple sclerosis.
Vaccines	Production of vaccines using recombinant DNA technology to stimulate the immune system and provide protection against diseases like influenza, hepatitis B, and human papillomavirus (HPV).

Application	Description
Gene Therapy Products	Development of gene therapy products to treat genetic disorders by replacing or repairing defective genes, targeting diseases like cystic fibrosis, muscular dystrophy, and sickle cell anemia.
Stem Cell Therapies	Utilization of stem cells to develop therapies for diseases such as Parkinson's disease, spinal cord injuries, and diabetes, enabling targeted and effective treatments for a wide range of conditions, improving patient outcomes and quality of life.

26.7.4 Current methods employed for gene therapy (in-vitro and in-vivo methods):

Gene therapy is a technique that involves the insertion, removal, or modification of genetic material within a patient's cells to treat or prevent disease. There are two main methods of gene therapy: in-vitro and in-vivo.

Therapy Name	Description
In-vitro Gene Therapy	Manipulation of cells outside the patient's body, followed by transplantation back into the patient
	a) Isolate patient's cells: Bone marrow or blood cells are isolated.
	b) Genetically modify cells: Cells are genetically modified using a viral vector or other delivery system.
	c) Expand modified cells: Modified cells are expanded to produce many cells with therapeutic genes.
	d) Transplant modified cells: Modified cells are transplanted back into the patient's body for treatment.

Therapy Name	Description
In-vivo Gene Therapy	Direct delivery of the therapeutic gene to the patient's cells or tissues within their body
	a) Deliver therapeutic gene: Gene is delivered directly using a viral vector or other system.
	b) Express therapeutic gene: Gene is expressed within patient's cells for protein production.
	c) Treat disease: Protein production leads to the treatment of the disease

26.7.5 Gene therapies in the detection and treatment of some genetic diseases:

Gene therapy has shown great promise in the detection and treatment of various genetic diseases. Here are some examples:

Hemophilia: Hemophilia is a genetic disorder that affects the blood's ability to clot properly. In-vivo gene therapy for hemophilia involves the delivery of a functional copy of the clotting factor gene to the liver cells using a viral vector. The goal of this therapy is to replace the defective gene with a functional one, leading to the production of normal clotting factors and the prevention of bleeding episodes.

Adrenoleukodystrophy (ALD): ALD is a genetic disorder that affects the nervous system and leads to the progressive destruction of the myelin sheath that covers nerve cells. In-vivo gene therapy for ALD involves the delivery of a functional copy of the ABCD1 gene to the brain cells using a viral vector. The goal of this therapy is to replace the defective gene with a functional one, leading to the production of normal ABCD1 protein and the prevention of nerve cell destruction.

26.7.6 Gene therapy for cystic fibrosis:

Cystic Fibrosis (CF): It is a genetic disease caused by a mutation in the CFTR gene that leads to the production of thick mucus in the lungs, pancreas, and other organs. In-vivo gene therapy for CF

involves the delivery of a functional copy of the CFTR gene to the lungs using a viral vector. The goal of this therapy is to replace the defective gene with a functional one, leading to the production of normal CFTR protein and improvement of lung function.

Gene therapy for CF involves the delivery of a functional copy of the CFTR gene to the lung cells of patients with the disease. The *CFTR* gene provides instructions for making a protein called the CF **transmembrane conductance regulator** (CFTR). This protein functions as a channel across the membrane of cells that produce mucus, sweat, saliva, tears, and digestive enzymes. The channel transports negatively charged particles called chloride ions into and out of cells. The transport of chloride ions helps control the movement of water in tissues, which is necessary to produce thin, freely flowing mucus. Mucus is a slippery substance that lubricates and protects the lining of the airways, digestive system, reproductive system, and other organs and tissues. The CFTR protein also regulates the function of other channels, such as those that transport positively charged particles called sodium ions across cell membranes. These channels are necessary for the normal function of organs such as the lungs and pancreas.

Genetic counseling:

Genetic counseling is a crucial process that helps individuals and families understand and manage genetic risks. It involves assessing family history and performing genetic testing to identify potential genetic disorders or mutations. Genetic counseling provides education, support, and helps individuals make informed decisions about family planning and medical management options. It also addresses ethical concerns related to genetic testing. Ultimately, genetic counseling empowers individuals and families to make informed decisions about their genetic health and care.

Genetic screening:

It is a vital medical procedure that involves analyzing an individual's DNA to detect genetic disorders and mutations. It serves various purposes such as early detection of diseases, carrier screening, prenatal screening, cancer screening, and pharmacogenomics. Early detection enables timely intervention and

better disease management, carrier screening helps with family planning, prenatal screening aids in informed decision-making, cancer screening identifies high-risk individuals for early detection, and pharmacogenomics enables personalized medicine for better treatment outcomes. In brief, genetic screening plays a crucial role in identifying risks, aiding family planning, and facilitating personalized medical care for improved disease management.

26.8 SCOPE AND IMPORTANCE OF BIOTECHNOLOGY

Biotechnology is of great importance due to its potential in addressing global challenges such as disease, hunger, and environmental degradation. It enables the development of new treatments, improved crop yields, and sustainable energy sources. Additionally, biotechnology has the power to transform industries, create economic opportunities, and drive technological advancements. With its positive impact on human health, food security, and sustainable development, biotechnology will continue to shape the future of science, technology, and society.

26.8.1 Social/ ethical implications of using gene technology in human:

The use of gene technology in humans carries significant social and ethical implications. There are health risks associated with gene therapy, including immune reactions, off-target effects on genes, and the potential for cancer or genetic diseases. The unequal access to genetic therapies raises concerns about social inequality, as lower-income individuals and those in developing countries may struggle to afford these treatments, leading to healthcare disparities. Ethical concerns arise regarding genetic enhancement and the creation of "perfect" humans, which could result in discrimination against those without desired traits. Genetic discrimination is another worry, with potential misuse of genetic information by insurers, employers, and others leading to privacy violations and increased social inequality. Additionally, unintended consequences such as genetic changes in future generations or the emergence of new genetic diseases must be carefully considered and addressed before widespread implementation of gene technology in humans.

SUMMARY

- Process of creating identical copies of DNA fragments or whole organisms.
- Allows for large amounts of specific DNA sequences.
- Used in genetic engineering, gene therapy, and agriculture.
- Technique used to determine the exact order of nucleotides in a DNA molecule.
- Crucial tool for studying genetic mutations and diseases.
- Has revolutionized fields such as genomics, personalized medicine, and forensics.
- Involves the identification, isolation, and manipulation of DNA molecules.
- Widely used in forensic science and paternity testing.
- Techniques include polymerase chain reaction (PCR) and gel electrophoresis.
- Detailed maps of the arrangement and location of genes on a chromosome.
- Used to identify genes associated with diseases and genetic disorders.
- Can aid in the development of new treatments and therapies.
- The growth of cells or tissues in a controlled environment outside of an organism.
- Used to study cellular processes, develop new medical treatments, and produce vaccines.
- It can be used to produce clones of plants and animals.
- Organisms that have had foreign genes introduced into their DNA.
- Used to improve crop yields, develop new treatments for diseases, and produce biopharmaceuticals.
- Can raise ethical concerns related to genetic engineering and genetically modified organisms (GMOs).

EXERCISE

1. Encircle the correct choice.

i) Cloning of genes creates an identical copy of a DNA sequence. The process of cloning is significant for what reason?
(a) To create a transgenic organism
(b) To diagnose genetic disorders
(c) To study gene function
(d) All the above

ii) DNA sequencing is a technique used to determine the sequence of nucleotides in a DNA molecule. What is the significance of DNA sequencing?
(a) To identify genetic variations
(b) To create a transgenic organism
(c) To study gene expression
(d) All the above

iii) DNA analysis is used to identify genetic disorders. What is the purpose of DNA analysis?
(a) Sequence of nucleotides in a DNA
(b) To study gene expression
(c) To identify genetic variations
(d) All the above

iv) Biotechnology has many applications in healthcare, including gene therapy, drug production, and diagnosis of diseases. What is the significance of biotechnology in healthcare?
(a) To study gene function
(b) To improve animal welfare
(c) To develop new materials
(d) All the above

v) Polymerase chain reaction (PCR) is a technique used to amplify DNA. What is the significance of PCR?
(a) To sequence DNA
(b) To identify genetic disorders
(c) To study gene expression
(d) All the above

vi) Gel electrophoresis is a technique used to separate DNA fragments based on size. What is the significance of gel electrophoresis?
(a) To study gene expression
(b) To sequence DNA
(c) To identify genetic variations
(d) All the above

vii) Sanger sequencing is a technique used to read the sequence of DNA. What is the significance of Sanger sequencing?
(a) To identify genetic disorders
(b) To sequence DNA
(c) To study gene expression
(d) All the above

viii) Southern blotting is a technique used to transfer DNA fragments from a gel to a membrane. What is the significance of Southern blotting?
(a) To identify genetic disorders
(b) To sequence DNA
(c) To study gene expression
(d) All the above

ix) Genomic maps are useful for:
(a) Identifying genes associated with specific diseases
(b) Creating genetically modified organisms
(c) Analyzing DNA methylation patterns
(d) Detecting protein-protein interactions

x) What is the significance of monoclonal antibodies in biotechnology?
(a) To study gene expression
(b) Used to diagnose diseases
(c) Genetically modified organisms
(d) They can be used to create new drugs

2. Write short answer of the following:

i) Why Amp^R and Lac^Z are used in construction of rDNA?
ii) What do you mean by RFLPs?
iii) How is biotechnology used in healthcare, and what are some of the potential benefits and challenges associated with its use?
iv) Why restriction enzymes are called molecular scissors?

v) What do you mean by palindrome?
vi) Enlist enzymes use in rDNA technology?

3. Give detailed answers to following questions:

i) What is DNA sequencing, and how has it been used to study genetic mutations and diseases?
ii) What is the role of DNA analysis in forensic science and paternity testing, and what are some of the methods used in this field?
iii) Describe the process of creating genomic maps and explain how this technology has been used to study genes and diseases.