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and recombinant plasmids into the same medium.

Bacterial cells take up recombinant plasmid, especially, if they are treated with calcium chloride to make them more permeable. Thereafter, as the cell reproduces, a bacterial clone forms and each new cell contains at least one plasmid. Therefore, each of the bacteria contains the gene of interest, which will express itself and make a product.

26.2.3 Identification of transformed clone:

The transformed clone can be identified by adding a particular antibiotic (for which resistant gene is found in plasmid) into the medium. As the transformed clone has got resistance against the antibiotic, so it remains alive and continues to grow, because the gene of interest is inserted inside the other antibiotic resistant gene.whereas all the untransformed clones are killed by the antibiotic. From this transformed bacterial clone, the cloned gene can be isolated for further analysis or its protein product can be separated.

26.3 POLYMERASE CHAIN REACTION

The polymerase chain reaction (PCR) is a revolutionary technique in molecular biology to amplify (cloning) a single or a few copies of a piece of DNA, to generate thousands to millions of copies. It was originally invented by Kary Mullis in 1983; later on he was awarded the Nobel Prize in Chemistry in1993. PCR is a process that 'amplifies' or 'copies' a piece of DNA repeatedly until there is an amount which is great enough to observe visually. It is based upon *in vitro* replication process which is carried out by DNA polymerase enzyme. In this technique DNA polymerase is compelled to polymerize a given piece of DNA again and again, so that multiple copies are produced, thus, the technique is known as **polymerase chain reaction (PCR).**

26.3.1 Components of PCR technique:

The following are the components required for carrying out a PCR reaction:

1. Template DNA
2. Deoxyribo-nucleoside tri-phosphates (dNTPs)
3. Primers
4. *Taq* polymerase

Fig: 26.8 PCR Machine

1- Template DNA or Target DNA: It is the piece of DNA to be cloned or amplified. It may be a useful gene found in the genomic DNA or the piece of DNA of infecting organisms.

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2- Deoxyribo-nucleoside tri-phosphates (dNTPs): These are free nucleotides that act as raw material for the synthesis of new DNA fragments. There are four different types of dNTPs (dATP, dGTP, dCTP, dTTP) required in this process.

3- Primers: DNA polymerase is unable to initiate polymerization unless primers are attached. Two sets of primers—forward primer and the backward primer are used in this technique that select 3' ends of target DNA fragment by annealing with its complementary sequences.

4- *Taq* polymerase: The Taq DNA polymerase is a temperature-tolerant enzyme isolated from *Thermus aquaticus*, a bacterium found in hot springs. It catalyzes the synthesis of DNA. This enzyme is stable and active at near-boiling temperatures. Therefore, it is well suited for carrying out PCR reactions. These are basic components required for the assembly of a PCR reaction. They form a mixture called **PCR mixture** or **reaction mixture**. The PCR mixture is placed in an instrument called **thermal cycler** or **PCR machine**. Thermal cycler regulates the temperature during various steps of PCR reaction according to the need.

26.3.2 Mechanism of PCR reaction:

A PCR-amplification cycle consists of three basic steps. They are **denaturation**, **primer annealing**, and **extension or polymerization**. Its time duration, temperatures and sequence of the steps have to be programed in the thermal cycler.

Fig: 26. 9 A hot spring in Yellowstone National Park, the habitat for *Thermus aquaticus*.

Denaturation: In the denaturation step, the template is heated to 94°C for one minute or up to five minutes. At this high temperature the DNA undergoes complete denaturation and the double-stranded DNA (dsDNA) becomes single-stranded (ssDNA). Each single ssDNA can act as the template for the *in vitro* DNA synthesis.

Primer annealing: The next step is the primer annealing. In this step the two primers, the forward primers and the backward primers, anneal or hybridize to the single-stranded template DNA at its complementary regions. Annealing is usually carried out at a lower temperature depending on the length and sequence of the primers. In standard cases it is 54°C and approximate time required for this step is 2 minutes.

Extension or Polymerization: The final step in each cycle is the primer extension or polymerization 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

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extension reaction or polymerization of dNTPs is standardized at 72°C. This step takes just one minute to be completed.

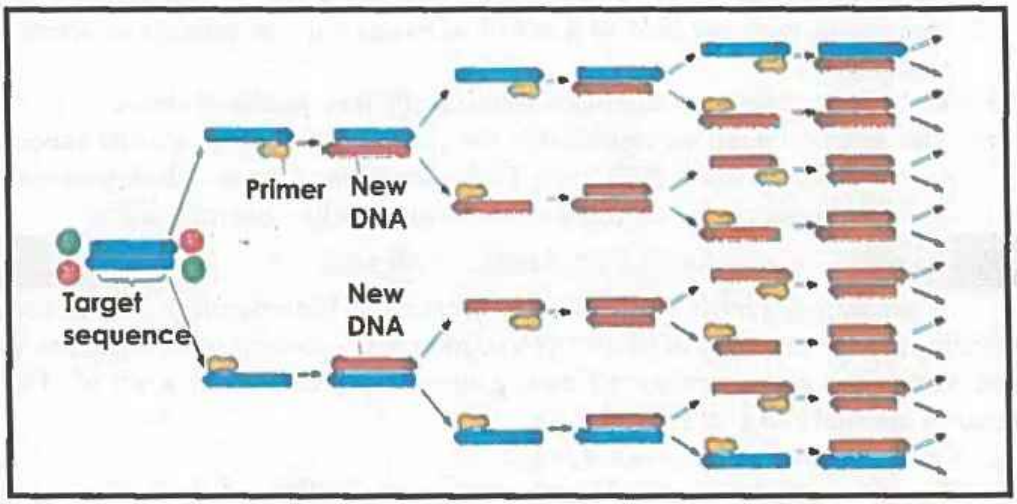

Fig: 26.10 Mechanism of PCR reaction

At the end of first cycle one target DNA molecule is converted in to two molecules. The second cycle immediately starts with the denaturation by heating at 94°C, so that 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 and thus the cycle of denaturation, 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.

26.3.3 Applications 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.

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- PCR has also shown its impact in criminology. The DNA of the suspects and the DNA sample recovered from the crime scene can be analyzed 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 in sex cells—sperm and egg.

26.4 GENOMEIC LIBRARY

A genomic library is a collection of bacterial or bacteriophage clones, each containing at least one copy of every DNA sequence in a genome of an organism. In single library the entire genome of an organism is represented as a set of DNA fragments inserted into a vector molecule.

26.4.1 Construction of Genomic Library:

To construct a genomic library, the genomic DNA of the organism is extracted and is cut into fragments of suitable sizes by any of the following three methods.

- The genomic DNA is digested completely by a restriction enzyme that converts it into fragments of suitable sizes. The restriction enzyme cuts at all relevant restriction sites and produces a large number of short fragments with sticky ends. The disadvantage of this is that genes containing restriction sites within the reading frame may be cut into two or more fragments and may be cloned separately.
- The genomic DNA can be fragmented un-enzymatically by means of mechanical shearing such as **sonication**, which produces longer DNA fragments. The disadvantage in this case is that the ends of the fragments produced are not uniform and need enzymatic modification for insertion into a cloning vector.
- The third method for fragmenting genomic DNA is by partial enzymatic digestion with a restriction enzyme.

26.4.2 Locating gene of interest from DNA libraries:

A DNA probe is a small, fluorescently or radioactively labeled DNA molecule that is used to locate similar or complementary sequences among a long stretch of DNA molecule or bacterial colonies such as genomic or cDNA libraries or in a genome.

Fig: 26.11 DNA Probe is added to locate similar sequences of DNA.

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26.5 DNA SEQUENCING

To understand the complexities of gene structure, its expression, its regulation, protein interactions, and molecular mechanisms of genetic diseases—the detailed and exact sequences of the bases in DNA is very essential. The main principle of any DNA sequencing method is:

1. To generate piece of DNA of different sizes all starting from the same point and ending at different points.
2. Separation of these different sized pieces of DNA by gel electrophoresis.
3. Reading of sequence from the gel.

For generation of different sized DNA fragments, two different sequencing methods were developed during the late 1970s. They are: Sanger method and Maxam-Gilbert method.

26.5.1 Sanger's method:

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

Procedure:

Before the DNA can be sequenced, it has to be denatured into single strands using heat because only one strand that acts as template is required in this procedure. 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 labeled "G", "A", "T" and "C". 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

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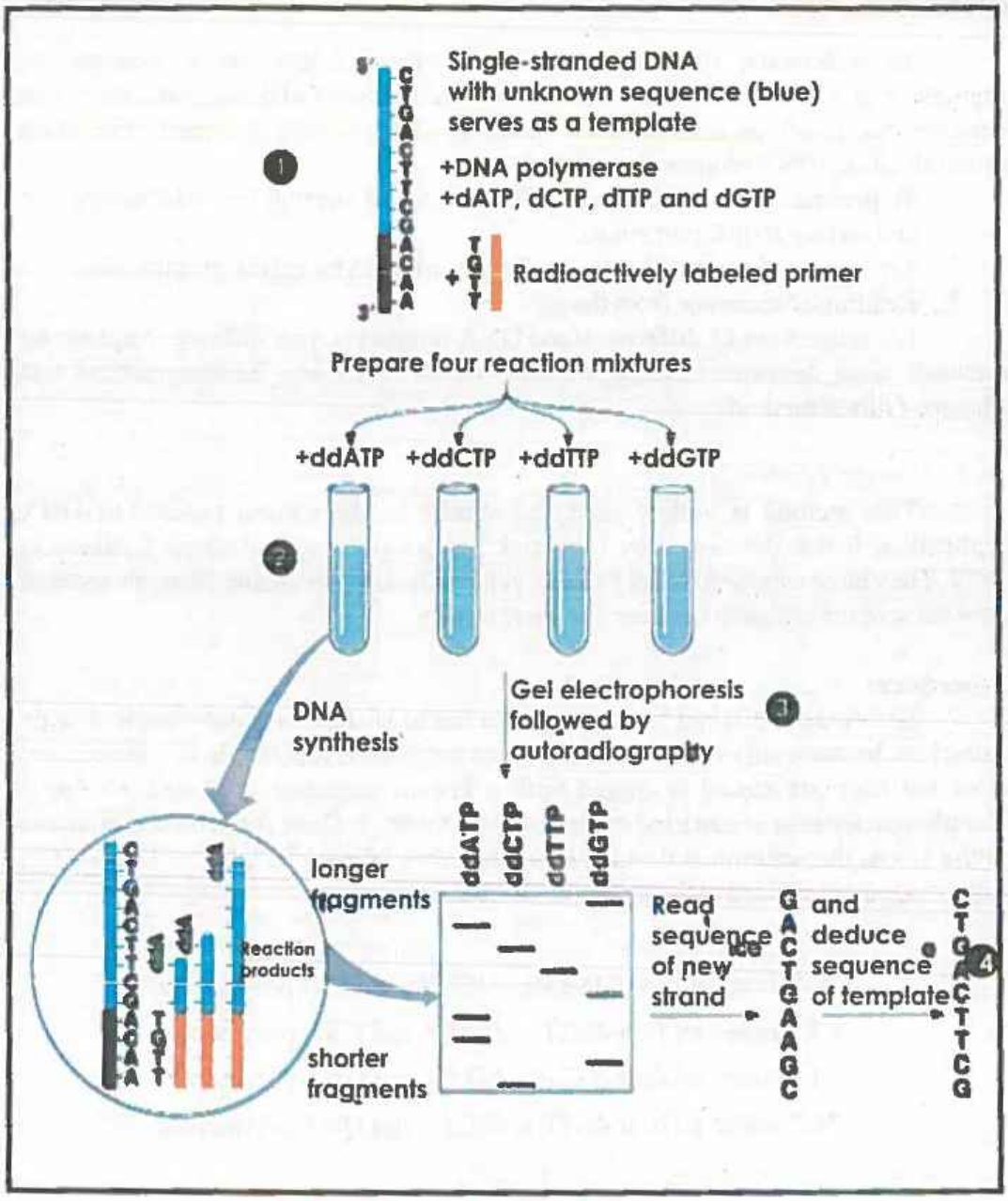

Fig: 26.12 Sanger's method of DNA sequencing

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As shown above, all of the tubes contain a different ddNTP present, and each at about one-hundredth the concentration of the normal precursors. Now all these test 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 occasion a dideoxynucleotide is incorporated into the chain in place of a normal nucleotide, which results in a chain-terminating event. For example if we looked at only the "G" tube, we might find a mixture of the following products:

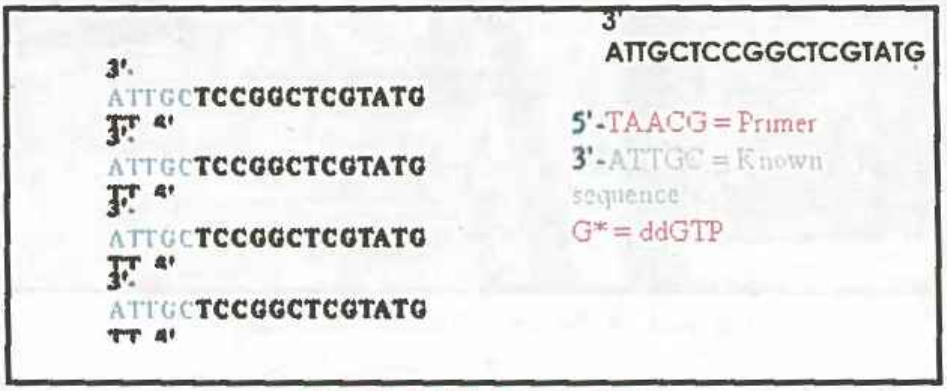

Fig: 26.13 DNA sequencing

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 polyacrylmide 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 labeling the DNA.

26.5.2 Gel electrophoresis:

Gel electrophoresis is a technique used in molecular biology to separate charge bearing polymers (proteins, RNA or DNA) under the influence of electric field. DNA electrophoresis is used to separate DNA fragments primarily by size. The types of gels most commonly used for DNA electrophoresis are agarose (for relatively large DNA molecules) and polyacrylamide (for high resolution of short DNA fragments). The freshly prepared gel is in liquid form but when it is poured in a gel caste to form a thin slab of gel and is allowed to be cooled, it turns into solid state.

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At one end of the gel slab some partial holes are made which are called "wells", they are partial in the sense that they are not opened from other side of the slab. Later on various samples of mixture of different sized DNA fragments are poured into these wells.

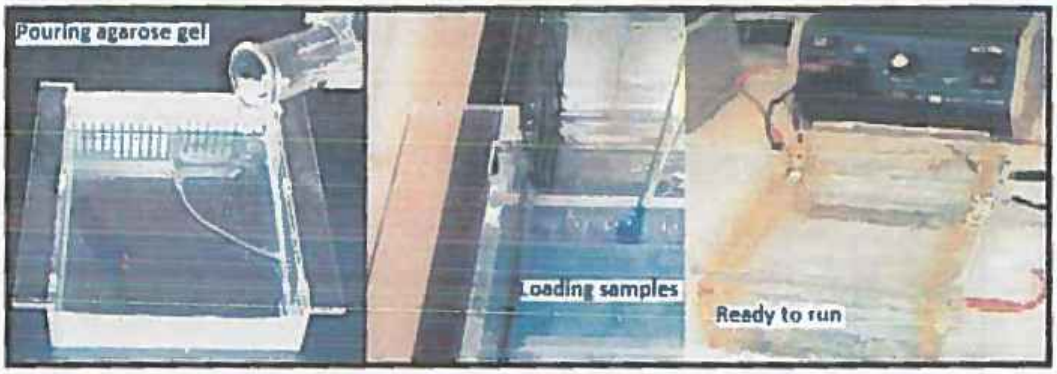

Fig: 26.14 Steps in Gel Electrophoresis technique.

The gel slab is suspended in a buffer solution which is used to establish electric field. The buffers used for the separation are Tris-Borate-EDTA or Tris-Acetate-EDTA. To establish an electric field, a positive electrode at one end and a negative electrode at another end of buffer solution are inserted. As the electric current run between the electrodes, the DNA fragment of different length present in the wells begin to move from negative pole to positive pole because DNA molecules have negative charges due to the phosphate groups.

The DNA fragments migrate relative to its size because the distance a DNA fragment travels is inversely proportional to its length so the smaller fragments move faster through the gel matrix than larger fragments.

Although, the movements of the fragments also depend upon charges, number of strands (single or double), shape of the molecules (linear or circular) and the concentration of the gel (pore size).

When the movement of fragments is stopped, the DNA molecules are appeared as band at different positions of gel but still bands cannot be viewed until they are labeled with florescent dyes or radioactive probes.

Following the separation, the gels can be stained by DNA binding florescent dyes that bind to the DNA molecules and are typically viewed under UV illumination. DNA bands can also be transferred from gel to the nitrocellulose membrane for autoradiography (X ray imaging).

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In the image of gel pattern, some bands are appeared thick and some are thin, thick bands represent the high concentration of same sized fragments while thin bands show low concentration. If a particular sized fragment is to be used for further analysis, the piece of gel containing that band can be cut and its DNA can be purified again.

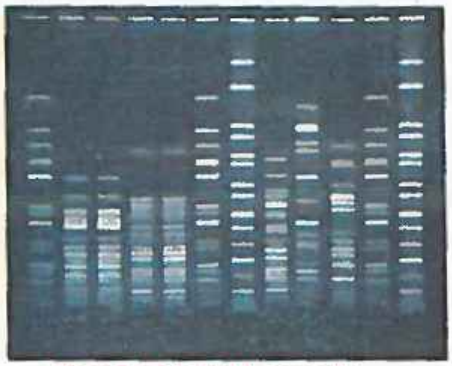

Fig: 26.15 DNA bands

26.5.3 Automated DNA Sequencing

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

There is no need for radiolabeling and autoradiography. The use of fluorescently labeled ddNTPs (dideoxynucleotide triphosphates) 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 labeled 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.

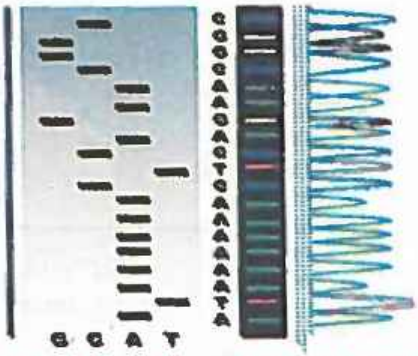

Fig: 26.16 After electrophoresis the colored bands can be monitored using UV-laser beams.

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26.5.4 Maxam-Gilbert Method:

In 1976-1977, **Allan Maxam** and **Walter Gilbert** developed a DNA sequencing method which is also called 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 first be end labeled, at one end only. This is accomplished by kinase treatment with 32P ATP, which transfers radioactive phosphorus from ATP to 5' end of each strand. Dimethylsulphoxide (DMSO) is then added and the labeled DNA samples heated to 90°C. This results in the breakdown of base pairing and dissociation of DNA molecules into its two component strands.

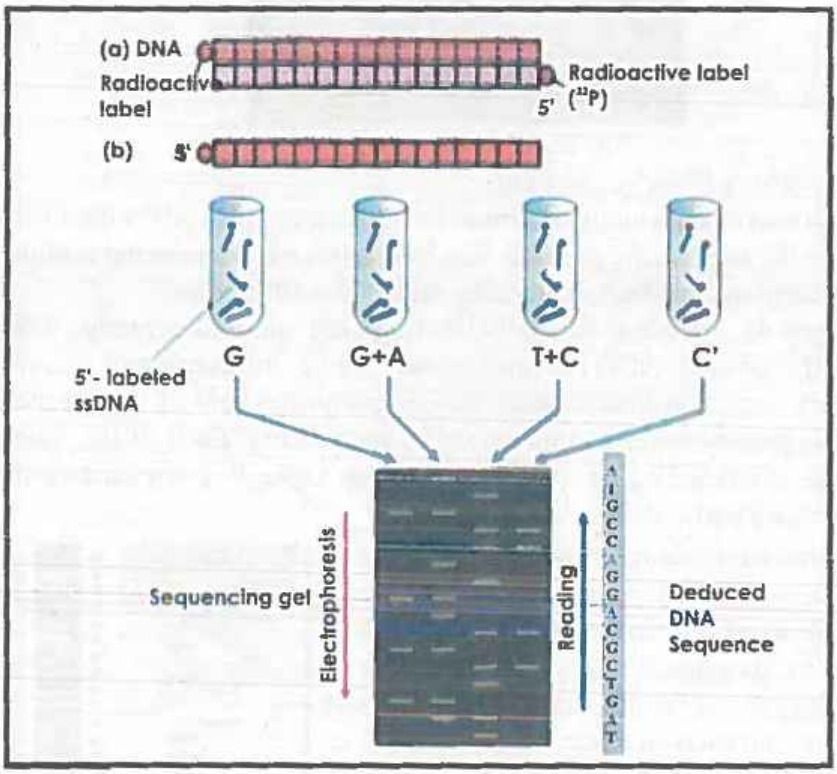

Fig: 26.17 Maxam and Walter Gilbert developed a DNA sequencing method.

The two strands are separated from one another by gel electrophoresis, one strand is purified from the gel and divided into four samples (G, A+G, T+C, and C), each of which is treated with one of the cleavage reagents and modifying chemical reagents.

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The former are used to cut DNA strand at specific point while the later cause a chemical modification in the nucleotides they are specific for, making them to susceptible to cleavage.

26.6 DNA ANALYSIS

DNA analysis or DNA fingerprinting is an examination method that emerged in the 1980s and is credited to **Alec Jeffrey**, an English geneticist who made first DNA fingerprint in 1985. Every species has unique genetic sequences. DNA analysis allows any type of organism to be identified by analyzing its genetic sequences. This method can also clarify questions of identification within a species. Identification within a species can present more of a challenge than determining between two different species. For example, it is much easier to determine whether a victim was attacked by a bear or human than it is to determine which human perpetrated an attack.

Human DNA not only has a record of each person's individuality but also a shared history of the evolution of our species, and the code that can provide insight into a person's future health. So Today, DNA analysis has become a standard practice for defining paternity or maternity, predisposition to disease, embryonic health, criminal guilt or innocence, and so on. DNA can be analyzed in various ways for these purposes.

26.6.1 Procedure:

There are several techniques that can be used for DNA analysis. **Restriction fragment length polymorphism (RFLP)** was one of the first methods used in DNA analysis. Following are the key steps to make a DNA fingerprint by using this method.

Fig: 26.18

Collection of DNA samples:

For DNA analysis, very small fraction of DNA is sufficient because it can be amplified several times with the help of PCR. Therefore it can be collected even from a small trace of blood or from the cells of single hair root.

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

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Placement of RFLP:

RFLP refers to the different sized fragments of DNA produced by a particular restriction enzyme. 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 (identical) twins. Therefore RFLPs of any two persons, when compared, one can easily analyze their individuality. However, the entire human have 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 RFLP is the digestion of DNA samples by a particular restriction enzyme, which produces a set of different sized DNA fragments (RFLPs)

Separation of RFLPs:

The DNA fragments are then electrophoresed on 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 rate 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:

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 combinations of two steps i.e. transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization. The method is named after its inventor, the British biologist Edwin Southern.

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.

Fig: 26.19 bands visible through autoradiography.

26.6.2 Applications of DNA analysis:

Today DNA analysis has wide range of application in different fields of life. It can be used to:

- Identify potential suspects whose DNA may match evidence left at crime scenes

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- Exonerate persons wrongly accused of crimes
- Identify crime and catastrophe victims
- Establish paternity and other family relationships
- Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers)
- Detect bacteria and other organisms that may pollute air, water, soil, and food
- Match organ donors with recipients in transplant programs
- Determine pedigree for seed or livestock breeds

26.7 GENOME MAPS

The genome is a collection of all the genes found in one complete set of chromosome. So a diploid organism has two copies of genome while egg or sperm has one.

26.7.1 Genome maps:

Just like the road maps and street maps of a city, which guide us to reach a specific location, the genome maps are used by the scientists searching for a specific gene somewhere within the vast human genome.

They have available to them two broad categories of maps: genetic maps and physical maps, which are being used for genome analyses. A genetic map, like an interstate highway map, provides an indirect estimate of the distance between two locations (loci). On the other hand, physical maps mark an estimate of the true distance, in measurements called base pairs, between locations (loci) interest. To continue our analogy, physical maps would then be similar to street maps, where the distance between two sites of interest may be defined more precisely.

For Your Information

In his marvelous book, Genome, Matt Ridley wrote: "Imagine that the human genome is a book. There are 23 chapters, called chromosomes. Each chapter contains several thousand stories, called genes. Each story is made up of paragraphs called exons, which are interrupted by advertisements called introns. Each paragraph is made up of words called codons. Each word written in letters are called bases, which are Cytosine, Guanine, Adenine, Thiamine or shortly A,G,T,C."

26.7.2 Genetic markers:

Just like interstate maps have cities and towns that serve as landmarks, genetic maps have landmarks known as genetic markers, or "markers" for short. The term marker is used very broadly to describe any observable variation that results from an alteration, or mutation, at a single genetic locus.

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A marker may be used as one landmark on a map if, in most cases, that stretch of DNA is inherited from parent to child according to the standard rules of inheritance. Markers can be within genes that code for a noticeable physical characteristic such as eye color, or a not so noticeable trait such as a disease. DNA-based reagents can also serve as markers. These types of markers are found within the non-coding regions of genes and are used to detect unique regions on a chromosome. DNA markers are especially useful for generating genetic maps when there are occasional, predictable mutations that occur during meiosis—the formation of gametes such as egg and sperm that, over many generations, lead to a high degree of variability in the DNA content of the marker from individual to individual.

26.7.3 Commonly Used DNA Markers

- **RFLPs, or restriction fragment length polymorphisms**
- **VNTRs, or variable number of tandem repeat polymorphisms**
- **Microsatellite polymorphisms**
- **SNPs, or single nucleotide polymorphisms**

26.7.4 Genome Analysis:

Due to rapid development of genome studies, a new branch of biotechnology has emerged called genomics which deals with exploration and analysis of complete DNA sequence of an organism's genome. This field was not really possible until the publication of the genome of Haemophilus influenzae in 1995. Before this Fred Sanger also had sequenced the first bacteriophage genome for which he later won the Nobel Prize. Beside it human genome project was also launched in 1990.

26.7.5 Human Genome Project (HGP)

The Human Genome Project (HGP) is an international scientific research project which is based on the exploration and analysis of human genome. It was originally founded by the U.S. Department of Energy and the National Institutes of Health in 1990. They have established National Human Genome research Institute (NHGRI), who has completed this task in 2003. James D. Watson was appointed as first director of this institute but at the time of completion of the project, the institute was being led by Dr. Francis Collin. Although this project was funded initially by the US government but later on Welcome Trust (U.K.) became a major partner; additional contributions came from Japan, France, Germany, China, and others.

26.7.6 Major Goals of HGP:

Major goals and objective of this project were to

- identify all the approximately 20,000-25,000 genes in human DNA,
- determine the sequences of the 3 billion chemical base pairs that make up human DNA,
- store this information in databases,

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- 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.

26.7.7 Benefits of HGP

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

Molecular Medicine:

- Improved diagnosis of disease
- Earlier detection of genetic predispositions to disease
- Rational drug design
- Gene therapy and control systems for drugs
- Pharmacogenomics "custom drugs"

Bioarchaeology, Anthropology, Evolution, and Human Migration:

- Study evolution through germ line mutations in lineages
- Study migration of different population groups based on female genetic inheritance
- Study mutations on the Y chromosome to trace lineage and migration of males
- Compare breakpoints in the evolution of mutations with ages of populations and historical events.

26.8 TISSUE CULTURE

The propagation of a plant by using a plant part or single cell or group of cells in a test tube under very controlled and hygienic conditions is called "Tissue Culture". Tissue culture is often a generic term that refers to both organ culture and cell culture. The initial plant part which is used to develop tissue culture is called **explant**. It may be complete organ (seed, leaf, and twig) or single cell (protoplast) or a piece of tissue. On the basis of explant tissue culture is variously called as **cell culture** or **organ culture**.

26.8.1 Procedure of Tissue Cultures:

A typical tissue culture method consists of following steps:

Sterilization:

Tissue culture is performed under aseptic conditions. Sterilization refers to the decontamination because living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with

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microorganisms, so surface sterilization of starting materials (explants) in chemical solutions (usually Sodium or calcium hypochlorite or mercuric chloride) is required. The glassware which is to be used in the procedure should also be sterilized.

Media preparation:

Soil is a natural medium of plant growth which is supposed to be very complex for a tiny explant, so in tissue culture technique, explants are grown on artificial media in which composition is kept under control. Solid or liquid media are used depending upon the need, which are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones.

Inoculation:

Inoculation refers to the placement of explant onto the surface of a solid culture medium, but is sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired. This is done in laminar flow, a sterile chamber. After the placement of explant, it is ensured that petri plates or test tube should be air tight. Next, these glass wares are shifted to the growth room or incubator.

Development of callus:

First the explant is allowed to grow into and unorganized mass of cells, callus, which is then shifted to the new media for the development of shoots and roots. A balance of both auxin and cytokinin will often produce an unorganized mass of cells, the callus.

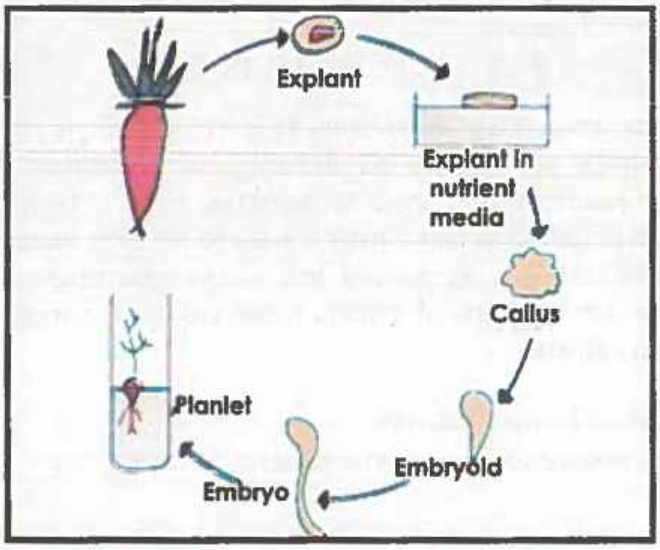

Fig: 26.20 Major steps in tissue culture technique.

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Development of plantlets:

As the callus grows, pieces are typically sliced off and transferred to new media (sub cultured) to allow for growth or to alter the morphology of the callus. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard.

As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants.

26.8.2 Types of tissue culture:

There are several types of tissue cultures which are primarily based upon type of explant used.

i. Callus culture:

When explants are cultured on the appropriate medium, usually with both an auxin and a cytokinin, can give rise to an unorganized, growing, and dividing mass of cells. This is called callus culture. Any plant tissue can be used as an explant.

ii. Cell-suspension cultures:

Cell-suspension culture is developed from the callus, when it is placed into a liquid medium and then agitated, single cells and/or small clumps of cells are released into the medium. Under these cells continue to grow and divide, eventually producing a cell-suspension culture. It can be scaled up by repeated sub culturing into fresh medium. Large cell clumps can be removed during subculture of the cell suspension.

Fig: 26.21 Callus of *Nicotiana tabacum*

Cell suspension cultures are very useful to obtain some drug compound which are generally obtained from specific parts of adult plant because the cell suspension cultures 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.

iii. Protoplasts culture:

Protoplasts are plant cells with the cell wall removed. Protoplasts are most commonly isolated from either leaf mesophyll cells or cell suspensions.

Protoplast cultures can be used to develop whole plants by organogenesis or **somatic embryogenesis** (synthetic embryos that are developed from somatic cells). Genetic variations can also be induced in these somatic embryos, if they are exposed

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to chemical of physical mutagens. Such variations are called as **somaclonal variations**. Protoplasts also act as ideal targets for transformation by a variety of means.

Fig: 26.12 Callus of *Nicotiana tabacum*

iv. Meristem Culture:

Meristems are the rapidly dividing and growing tissues, especially found at the apices of roots or shoots and in some other plant parts. A tissue culture in which meristems are used as explants is called meristem culture. Generally root apical or shoot apical meristems are used for meristem culture.

Meristem culture is mostly used for micropropagation and to obtain virus or parasite free plants because the whole plant may be infected by virus, bacteria or fungi but meristems are devoid of viruses due to the absence of vascular system in them.

v. Anther culture:

Anther culture is also called as microspore culture or pollen culture. It is a technique in which mature anthers or microspores are cultured in a suitable medium, the haploid cells (tube cells) present in the microspore begin to divide and produce haploid callus.

A haploid callus can be grown into a haploid plant; a diploid plant can also be obtained if chromosomal doubling is induced by the colchicine treatment.

26.8.3 Animal Cell Culture:

An important aspect of any biotechnological processes is the culture of animal cells in artificial media. These animal cells in culture are used in recombinant DNA technology, genetic manipulations and in a variety of industrial processes. Now-a -days it has become possible to use the cell and tissue culture in the areas of research which have a potential for economic value and commercialization. The animal cell cultures are being extensively used in production of vaccines, monoclonal antibodies, pharmaceutical drugs, cancer research, genetic manipulations etc.

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Techniques of animal cell culture:

Animal cells can grow in simple glass or plastic containers in nutritive media but they grow only to limited generations. A cell culture which is initiated by the cells removed from an animal's organ is called as **primary cell culture**; where as the primary culture is subcultured in fresh media to establish **secondary cultures**.

The culture of native tissue that retains most of the in vivo histological features is regarded as **organ culture** while the culturing of the cells for their re-aggregation to form a tissue-like structure represents **histotypic culture**. Another culture technique involves the recombination of different cell types to form a more defined tissue or an organ is known as **organotypic culture**.

Among the essential requirements for animal cell culture are special incubators to maintain the levels of oxygen, carbon dioxide, temperature, humidity as present in the animal's body, and, the synthetic media with vitamins, amino acids and fetal calf serum.

Synthetic media are prepared artificially by adding several organic and inorganic nutrients, vitamins, salts, serum proteins, carbohydrates, cofactors etc.

Different types of synthetic media can be prepared for a variety of cells and tissues to be cultured. Synthetic media are of two types- Serum containing media (media containing serum) and serum- free media (media without serum).

Fig: 26.23 Cell Culture Incubator

Applications of Animal Cell Culture:

The animal cell cultures are used for a diverse range of research and development. These areas are:

a) Production of antiviral vaccines.

d) Genetic manipulation, which is easy to carry out in cells or organ cultures.
e) Production of monoclonal antibodies requires cell lines in culture.
f) Production of pharmaceutical drugs using cell lines.
g) Chromosome analysis of cells derived from womb.
h) Study of the effects of toxins and pollutants using cell lines.
i) Use of artificial skin.
j) Study the function of the nerve cells.

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26.9 TRANSGENIC ORGANISMS

Combining genes from different organisms is known as recombinant DNA technology, and the resulting organism is said to be **"genetically modified (GM)," "genetically engineered (GE)," or "transgenic."** In other words the free living organisms in the environment that have had a foreign gene inserted into them are called transgenic organisms. Bacteria were the first transgenic organisms, first transgenic bacterium was produced in 1973, since then many transgenic organisms such as animals, plants, and bacteria have been produced. Genetic Modification consists of a special set of techniques that alter the genetic makeup of the organisms.

26.9.1 Transgenic Bacteria:

Bacteria were the first organisms to be modified in the laboratory, due to their simple genetics. The first example of this occurred in 1978 when **Herbert Boyer** working at a University of California laboratory took a version of the human insulin gene and inserted into the bacterium *Escherichia coli* to produce synthetic "human" insulin.

Role of transgenic bacteria in making biotechnology products:

Transgenic bacteria are now being used in a variety of ways, and are particularly important in producing large amounts of pure human proteins for use in medicine. Genetically modified bacteria are used to produce the protein insulin to treat diabetes. Similar bacteria have been used to produce clotting factors to treat haemophilia, and human growth hormone to treat various forms of dwarfism. Some transgenic bacteria have been produced that synthesize tissue plasminogen activator (tPA), a protein used by the heart patients to treat thrombotic disorders as it dissolves clotted blood masses; and interferons which are used for treating viral infections. These recombinant proteins are much safer than the products they replaced, since the older products were purified from cadavers and could transmit diseases.

For Your Information

Both strains of *P. syringae* occur naturally, but recombinant DNA technology has allowed for the synthetic removal or alteration of specific genes, enabling the creation of the ice-minus strain. Modifying *P. syringae* may have unexpected consequences for climate. A study has shown that its ice nucleating proteins may play an important part in causing ice crystals to form in clouds. If humans increase the frequency of bacteria lacking these proteins then it could potentially affect rainfall.

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Transgenic bacteria are also being used for bioremediation (removal of environmental pollutants by organisms). Such bacteria are also used to cleanup and recovery from an oil spill.

Ecological concerns surrounding transgenic bacteria:

Where lots of benefits are being obtained from genetically engineered bacteria, there are some ecological concerns also associated with these bacteria. One of the main issues regarding this is the possibility that hazardous new pathogens might be created.

26.9.2 Transgenic Plants:

The first field trials of genetically engineered plants occurred in France and the USA in 1986, when tobacco plants were engineered to be resistant to herbicides. In most cases the aim of developing transgenic plant is to introduce a new trait to the plant which does not occur naturally in this species. Examples include resistance to certain pests, diseases or environmental conditions, or the production of a certain nutrient or pharmaceutical agent.

Methods of gene transformation in plants:

There are many techniques of gene transformation have been developed; in all techniques gene of interest is introduced into single or few plant cells which are then allowed to regenerate in a suitable culture medium, upon a successful tissue culture procedure a transgenic plant is obtained.

Agriculturally improved transgenic crops:

During the last two decades, a tremendous progress has been made in the development of transgenic plants using the various techniques of genetic engineering. As per estimates recorded in 2002, transgenic crops are cultivated world-wide on about 148 million acres 587 million hectares) land by about 5.5 million farmers.

For Your Information

The first genes available for genetic engineering of crop plants for pest resistance were Cry genes (popularly known as Bt genes) from a bacterium ***Bacillus thuringiensis***. These are specific to particular group of insect pests, and are not harmful to other useful insects like butter flies and silk worms. Transgenic crops with Bt genes (e.g. cotton, rice, maize, potato, tomato, brinjal, cauliflower, cabbage, etc.) have been developed. This has proved to be an effective way of controlling the insect pests and has reduced the pesticide use.

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Transgenic plants have many beneficial traits like:

- Tolerance against biotic stresses (viral, bacterial infections, pests and weeds) and abiotic stresses (physical actors such as temperature, humidity, salinity, drought, waterlogging etc.), weedicide or Herbicide tolerance e.g. glyphosate resistance and phosphinothricin resistance.
- Insect resistance like transgenic crops with Bt genes (e.g. cotton, rice, maize, potato, tomato, brinjal, cauliflower, cabbage, etc.)
- Delayed fruit ripening,
- Improvements in nutritional contents

Some of the commercially grown transgenic plants in developed countries are: "Roundup Ready" soybean, "Freedom II squash", "High- lauric" rapeseed (canola), "Flavr Savr" and Endless Summer" tomatoes. During 1995, full registration was granted to genetically engineered Bt gene containing insect resistant 'New Leaf' (potato), 'Maximizer' (corn), 'Boll Gard' (cotton) in USA.

DO YOU KNOW?

In July 2000, researchers from the team that produced Dolly reported success in producing transgenic lambs in which the transgene had been inserted at a specific site in the genome and functioned well.

26.9.3 Transgenic Animals:

A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. Transgenic animals have the potential to improve human welfare in:

- agriculture, such as larger sheep that grow more wool
- medicine, such as cows that produce insulin in their milk
- industry, such as goats that produce spider silk for materials production

Methods of creation of transgenic animals:

In comparison to that for larger vertebrates, mice have become the model animal used in the field of transgenics because of their small size and low cost of housing, short generation time, and, fairly well defined genetics.

The insertion of gene is, however, a random process, and there is a high probability that the introduced gene will not insert itself into a site on the host DNA that will permit its expression.

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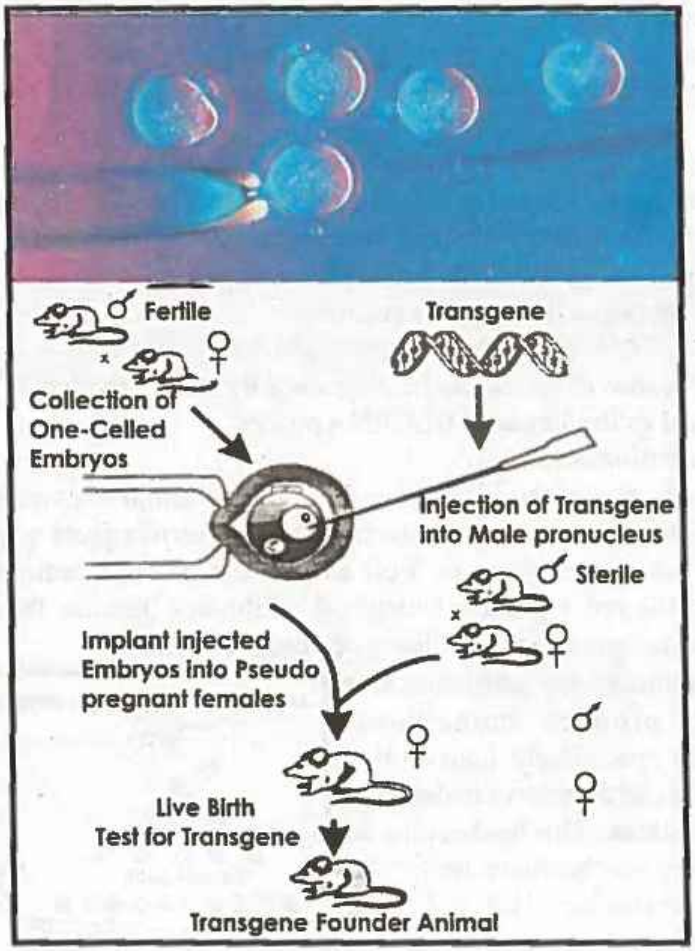

Fig: 26.24 Procedure for creation of transgenic animal.

The three principal methods used for the creation of transgenic animals are DNA microinjection, embryonic stem cell-mediated gene transfer and retrovirus-mediated gene transfer.

26.10 BIOTECHNOLOGY AND HEALTHCARE

The tools and techniques of biotechnology have opened up new doors when it comes to researching and learning more about the human body and what goes wrong with it when problems arise. Due to being able to understand the molecular base of health and disease this has lead scientists to improve methods of treating and preventing those diseases.

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Biotechnology has made a huge difference in human health care and has now enabled scientists to develop products which can give quicker and more accurate tests, therapies that have a lot less side effects and vaccines which are safer than ever before.

6.10.1 Development of vaccine in biotechnology:

Biotechnology is used in three different ways in the development of vaccine:

a) Separation of a pure antigen using a specific monoclonal antibody.

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

c) Synthesis of peptides to be used as vaccines.

26.10.2 Role of Biotechnology in Diagnosis of diseases:

Many human diseases can be diagnosed by using products of biotechnology like monoclonal antibodies and DNA/RNA probes.

Monoclonal Antibodies:

The response of the immune system to any antigen, even the simplest, is polyclonal. That is, the system manufactures antibodies of a great range of structures both in their binding regions as well as in their effector regions. Monoclonal antibodies (mAb) are a group of identical antibodies because they are made by identical immune cells that are all clones of a unique parent cell.

Given almost any substance, it is possible to produce monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine. Not only can antibodies be used therapeutically, to protect against disease; they can also help to diagnose a wide variety of illnesses, and can detect the presence of drugs, viral and 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**

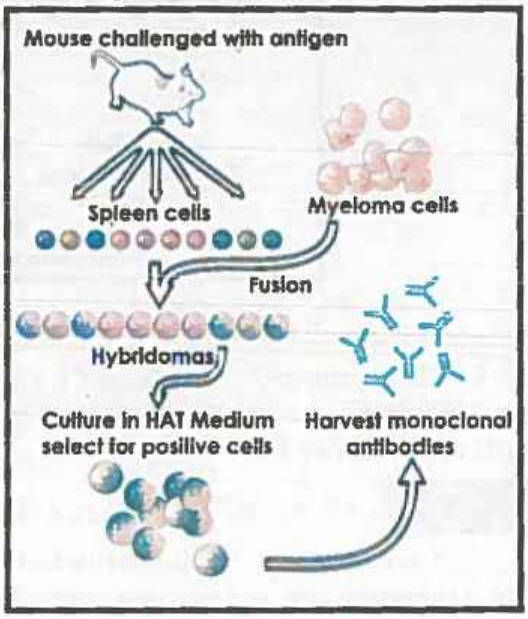

Fig: 26.25 Production of monoclonal antibodies.

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DNA/RNA Probes:

In biotechnology, a probe is a florescent or radioactive labeled fragment of DNA or RNA of variable length (usually 100-1000 bases long), which is used in DNA or RNA samples to detect the presence of nucleotide sequences that are complementary to the sequence in the probe. Such probes are widely used in diagnosis of many viral and bacterial diseases.

Diagnosis of diseases caused by protozoa and helminthes

The monoclonal antibodies and DNA probes are being used as very sensitive tools in biotechnology to diagnose the diseases caused by protozoa and helminthes. 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. Readymade 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 helminthes.

26.10.3 Gene Therapy:

Gene therapy is a technique for correcting defective genes responsible for disease development. Researchers may use one of several approaches for correcting faulty genes:

- A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common.
- An abnormal gene could be swapped for a normal gene through homologous recombination.

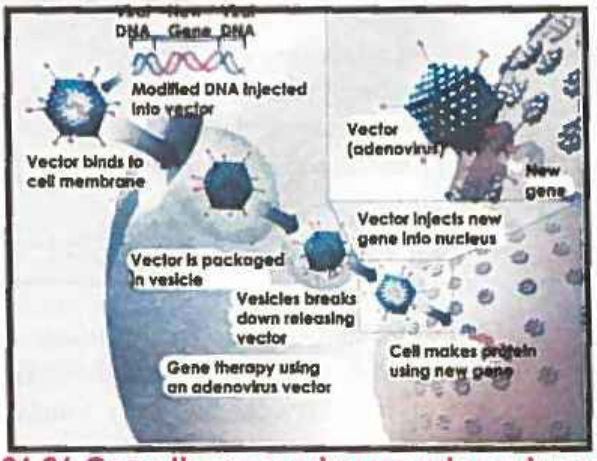

Fig: 26.26 Gene therapy using an adenovirus vector.

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- The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.
- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

Mechanism of gene therapy:

In gene therapy treatment normal gene is either delivered directly into the body ***(in vivo)*** or into the cells outside the body then these transgenic cells are again implanted into the body ***(ex vivo)***. In both cases, a "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene. A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that 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.

Target cells such as the patient's liver or lung cells are infected with the viral vector. The vector then unloads its genetic material containing the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state.

Besides virus-mediated gene-delivery systems, there are several non-viral options for gene delivery. The simplest method is the direct introduction of therapeutic DNA into target cells. This approach is limited in its application because it can be used only with certain tissues and requires large amounts of DNA.

Another non-viral approach involves the 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's membrane.

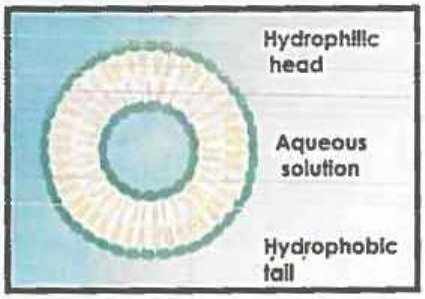

Fig: 26.27 Liposome

26.10.4 Cystic fibrosis:

An inherited disease, cystic fibrosis affects the mucus and sweat glands. People with severe symptoms can have serious lung and digestive problems, while people with a mild form of the disease may not have any symptoms until they are adolescents or young adults.

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Normally, mucus is watery. It 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 your cells. In people with cystic fibrosis, the gene does not work effectively. As a result, cells that line the passageways of the lungs, pancreas, and other organs produce abnormally thick, sticky mucus. This mucus obstructs the airways and glands, which causes the characteristic signs and symptoms of cystic fibrosis.

Gene Therapy of Cystic fibrosis: In 1989, experts discovered the gene that causes cystic fibrosis and identified it as the cystic fibrosis transmembrane conductance regulator or CFTR. The discovery of this defective gene posed new possibilities of a cure. Experts proposed gene therapy as a plausible method for curing the disease. Gene Therapy is the process of creating a healthy version of the flawed CFTR gene and infusing it into the affected cells in the body, particularly into the lungs of cystic fibrosis-inflicted patients.

26.11 SCOPE AND IMPORTANCE OF BIOTECHNOLOGY

During 1970s, biotechnology emerged as new discipline, as a result of marriage of biological science with technology. It has been possible due to revolutionary discoveries made in these two areas. Biotechnology is not a pure science, but an integrated effort of these two, the root of which lies in biological science. 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 man and other forms of life". Biotechnology is "the science of applied biological process". Importance of biotechnology is highlighted in the following fields.

26.11.2 Biochips & biological computers:

Biochip is the result of marriage of microchips business with biotechnology. In future, there is the possibility of developing of biological computers.

The development 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. Advances in these areas are giving scientists new methods for unraveling

Fig: 26.28 Biochip

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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.

26.11.3 Mycorrhiza: Mycorrhiza is a symbiotic association between certain fungi and roots of higher plants. This association is very beneficial for the growth of plants. In most of the cases plant seedling fails to grow if the soil does not contain inoculum of mycorrhizal fungi.

In recent years, use of biotechnologically produced inoculum of mycorrhizal fungi has increased its significance due to its multifarious role in plant growth and yield, and resistance against climatic and edaphic stresses, pathogens and pests.

Fig: 26.29 Mycorrhizal roots

26.11.4 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.

26.11.5 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 nanometers.Nanotechnologists are imparting their expertise in the development of such nano particle that can be used for efficient drug delivery at the target cells and in the diagnosis of diseases.

26.11.6 Scope of biotechnology: Biotechnology is one of 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 human mankind and the universe as a whole.

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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 processes.

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

- **Communications/media** - reporting, writing, editing
- **Computer Science** - data base development, bioinformatics, web site development, etc.
- **Pharmaceutical companies,** i.e Drug development
- **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 counseling, gene therapy and gene testing uses biotechnological tools.
- **Bio power plants.**
- **Bio-processing industry e.g.** enzyme technology, paper technology, metabolic engineering, protein engineering, food processing etc
- **Agriculture and animal husbandry**
- **Legal field** involving issues related to intellectual property rights, patency, 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.
- **Millitary** - With the fear of Biological warfare looming large on the human civilization, a biotechnologist is needed in pathogen identification, in the development of protection against the chemical and biological warfare, and in doing 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

Fig: 26.30 DNA finger printing is used in crime detection.

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26.11.7 Hazards and social/ethical implication 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 lead to some concerns and controversies raised by a number of groups, 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.

26.11.8 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 these 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 Organisms into the Environment".** The main areas of consideration for safety aspects in biotechnology are the following:

- How to dispose-off spent microbial biomass and purify the effluents from biotechnological processes?
- The toxicity of the allergy associated with microbial production.
- How to deal with the increase in the number of antibiotic resistant pathogenic microorganisms?
- How to evaluate the pathogenicity of the genetically engineered microorganisms to infect humans, plants and animals?
- How 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.

26.11.9 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.

Fig: 26.3 Signing ceremony of Biological Weapons Convention of 1972.

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26.11.10 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.

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 ensures that the plant breeder who developed a particular variety gets the exclusive rights for marketing the variety.

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KEY POINTS

- Biotechnology refers to the use of living organisms or their processes and products for the welfare of mankind.
- Gene cloning is the act of making copies, or clones, of a single gene. There are two possible ways of cloning of gene: recombinant DNA technology and polymerase chain reaction (PCR).
- Recombinant DNA Technology is an *in vivo* method which is used when gene cloning is required at industrial scale. For this purpose the following components or tools are required: gene of interest, molecular scissors, molecular carrier or vector, molecular glue and expression system
- Plasmid could be used as vectors which are derived mostly from bacteria and are the most widely used, versatile, and easily manipulated ones.
- DNA Ligase is enzyme responsible for the formation of the phosphodiester linkage between two adjacent nucleotides and thus joins two double-stranded DNA fragments, therefore it is called molecular glue.
- The polymerase chain reaction (PCR) is a technique in molecular biology to amplify (cloning) a single or a few copies of a piece of DNA, to generate thousands to millions of copies.
- A genomic library is a collection of bacterial or bacteriophage clones, each containing at least one copy of every DNA sequence in a genome of an organism. In single library the entire genome of an organism is represented as a set of DNA fragments inserted into a vector molecule.
- The main principle of any DNA sequencing method is to generate piece of DNA of different sizes all starting from the same point and ending at different points, separation of these different sized pieces of DNA by gel electrophoresis and reading of sequence from the gel.
- Gel electrophoresis is a technique used in molecular biology to separate charge bearing polymers (proteins, RNA or DNA) under the influence of electric field.
- DNA electrophoresis is used to separate DNA fragments primarily by size.
- The genome is a collection of all the genes found in one complete set of chromosome.

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KEY POINTS

- The Human Genome Project (HGP) is an international scientific research project which is based on the exploration and analysis of human genome. Major goals and objective of this project were to identify all the approximately 20,000-25,000 genes in human DNA, determine the sequences of the 3 billion chemical base pairs that make up human DNA, store this information in databases, 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.
- The animal cell cultures are being extensively used in production of vaccines, monoclonal antibodies, pharmaceutical drugs, cancer research, genetic manipulations etc.
- Combining genes from different organisms is known as recombinant DNA technology, and the resulting organism is said to be **"genetically modified (GM)," "genetically engineered (GE),"** or **"transgenic."**
- A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome.
- Gene therapy is a technique for correcting defective genes responsible for disease development.

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EXERCISE ?

1. Multiple choice questions

i. ***The type of gel most commonly used for short fragment DNA electrophoresis is:***

(a) agarose (b) DNA polymerase
(c) polyacrylamide (d) DNA ligase

ii. ***Cell suspension culture of Cinchona ledgeriana produce:***

(a) Quinine (b) Digitoxin
(c) Polludrin (d) Anti toxin

iii. ***Dideoxy ribonucleoside triphosphates are used to terminate DNA synthesis at different site. Which method involves this procedure?***

(a) Maxam-Gilbert's method (b) Sangar's method
(c) K.B. Mullis's Method (d) Gottlieb's method

iv. ***The gene of choice can also be synthesized in the laboratory from mRNA, using reverse transcriptase. This DNA molecule is called:***

(a) Complementary DNA (b) Replicative DNA
(c) Synthetic DNA (d) ssDNA

v. ***The gene of interest is joined with the sticky ends produced after cutting the plasmid with the help of another special enzyme known as:***

(a) DNA ligase (b) DNA polymerase
(c) Restriction endonuclease (d) Reverse transcriptase

vi. ***The enzyme DNA polymerase can work only in***

(a) 3' ⊥5' direction (b) 5' ⊥3' direction
(c) Both the direction (d) 5' ⊥5' direction

vii. ***In recombinant DNA technology a plasmid vector is cleaved by***

(a) Modified DNA ligase
(b) A heated alkaline solution
(c) The same enzyme that cleave the donor DNA
(d) The different enzyme other than that cleave the donor DNA

viii. ***Thermus aquaticus is the source of***

(a) Taq polumerase (b) vent polymerase
(c) both a and b (d) primase enzyme

ix. ***The complete set of chromosomal and extrachromosomal genes of an organism is called:***

(a) Genome (b) Gene pool
(c) Gene bank (d) Gene library

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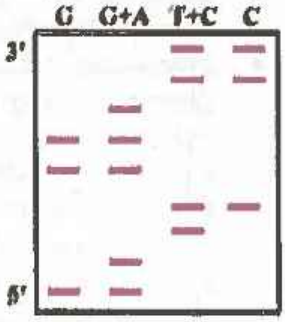
EXERCISE ?

x. *A totipotent cell means:*
   (a) An undifferentiated cell capable of developing into a system or entire plant
   (b) An undifferentiated cell capable of developing into an organ
   (c) An undifferentiated cell capable of developing into complete embryo
   (d) Cell which lacks the capability differentiate into an organ or system

2. Short Questions

i. Why don't the restriction enzymes destroy the DNA of the organism in which they are produced?
ii. What are the essential features of a vector?
iii. What are monoclonal antibodies?
iv. Name two conditions necessary for maintaining animal cells in culture which are different from plant cell culture
v. Give any two human proteins and their function which are produced biotechnologically.
vi. What are probes?
vii. What were the aims and objectives of human genome project?
viii. Differentiate between Maxam-Gilbert method & Sanger's method of gene sequencing.
ix. What is cDNA library?
x. What are the types of vector or carrier?
xi. What are the applications of PCR?
xii. Read carefully the diagram of gel pattern obtained through Maxam-Gilbert method of gene sequencing, and predicts the sequence of target DNA.



3. Long Questions

i. What are molecular scissors? Describe their sources and mode of action.
ii. Evaluate the process of gene sequencing with the help of Sanger's Method.
iii. Explain the process of gene cloning with the help of recombinant DNA technology.
iv. Explain the process of gene cloning with the help of Polymerase Chain Reaction.
v. Describe the procedure of DNA analysis.

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EXERCISE ?

v. Describe the procedure of DNA analysis.

4. **Analyzing and interpreting**

- Analyze and interpret the DNA of a child by comparing it with that of two individuals in case of disputed parenthood.

5. **Science, Technology & Society Connections**

- Describe the applications of polymerase chain reaction.
- State the importance and limitations of DNA analysis in foreign sick medicine and paleontology.
- Justify why the human genome project is regarded as the most ambitious project ever undertaken by man.
- Describe the major findings that have arisen from human genome project.
- Predict the applications of genetic engineering in crop improvement.
- Describe the role of genetic screening.
- Justify the need for genetic counseling.
- Describe briefly the accomplishments of the renowned genetic engineers working in privet or public sector institutions in his/her province.
- Suggest measures he/she would take to solve related problems by using knowledge gained in this chapter.
- Describe and analyze examples of technology that have extended or modified the scientific understanding of the genetic engineering.
- Investigate careers that require an understanding of biotechnology and genetic engineering.

6. **Online learning**

- www.ncbi.com
- www.biotechnology4u.com
- www.ornl.gov/hgmis
- www.vivo.colostate.edu/hbooks/genetics/biotech
- www.genetics-and-society.org