# Biotechnology

# Question Paper 2016

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**Maximum Marks: 80**
**Time allowed: Three hours**

- Candidates are allowed additional 15 minutes for only reading the paper. They must NOT start writing during this time.
- Answer Question 1 (Compulsory) from Part I and five questions from Part II, choosing two questions from Section A, two questions from Section B and one question from either Section A or Section B.
- The intended marks for questions or parts of questions are given in brackets [ ].
- Transactions should be recorded in the answer book.
- All calculations should be shown clearly.
- All working, including rough work, should be done on the same page as, and adjacent to the rest of the answer.

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## Part-I
## (Answer all questions)

**Question 1.**
(a) Mention any one significant difference between each of the following : [5]
(i) Ligases and Helicases
(ii) Introns and Exons
(iii) Gel electrophoresis and Gel permeation
(iv) Sucrose and Starch
(v) Plasmids and Phages

(b) Answer the following questions : [5]
(i) Why is the nutrient medium autoclaved before using it for cell culture?
(ii) Name the enzyme that can synthesize DNA at a high temperature.
(iii) Why are restriction enzymes also called as molecular scissors ?
(iv) Name the nitrogenous bases present in RNA.
(v) Why is Agrobacterium tumifaciens called as the natural genetic engineer of plants ?

(c) Write the full form of each of the following :
(i) YAC
(ii) NCBI

(iii) RAM
(iv) SNP
(v) EMBL

(d) Explain briefly : [5]
(i) Somatic hybridization
(ii) Promoter gene
(iii) Site directed mutagenesis
(iv) DNA probes
(v) Primer

**Answer:**
(a) (i) Ligases are the enzymes which help in linking up of okazaki (DNA) segments produced on the parent strand with 5'-3' direction. Hclicases help in unwinding the DNA helix, using ATP hydrolysis as a source of energy.

(ii) Introns are the intervening sequences which do not appear in the mature or processed RNA.
Exons are the coding sequences or expressed sequences that form functional and processed RNA.

(iii) Gel Electrophoresis is a technique by which negatively charged DNA fragments are separated by forcing them to move towards the anode under an electric field through a medium/matrix.

Gel permeation or filtration involves that molecules of different sizes can be separated from each other on the basis of their ability to enter the pores within the beaded gel, followed by passing down a column containing the gel. The technique is used in protein purification.

(iv) Sucrose is a disaccharide formed of glucose and fructose molecules while starch is a polysaccharide molecule formed of large number of glucose molecules arranged in the form of chains.

(v) Plasmids are the extra-chromosomal, self replicating, circular, double stranded DNA molecules present in bacteria.
Phages are the viruses which infect bacteria/cell. lyse it, integrate its DNA into it and replicate with the host chromosome.

(b) (i) The nutrient medium is autoclaved to make it sterilise i.e., free from microbes.

(ii) DNA polymerase isolated from a bacterium Thermus aquations.

(iii) Restriction enzymes are called ‘molecular scissors’ because they make cuts at specific positions/recognition sites within both strands of DNA.

(iv) Adenine, guanine, uracil, cytosine.

(v) Agrobacterium tumefaciens is able to deliver a piece of DNA known as ‘T-DNA’ to transform normal plant cells into tumour cells. By manipulating its Ti plasmid, it has now been modified into a useful cloning vector for delivering gene of our interest into a variety of plants.

(c) (i) YAC = Yeast Artificial Chromosome.
(ii) NCBf = National Centre for Bioinformatics Information.
(iii) RAM = Random Access Memory.
(iv) SNP = Short Nucleotide Polymorphism.
(v) EMBL = European Molecular Biology Laboratory.

(d) (i) Somatic hybrids are the hybrid plants produced through the fusion of protoplasts from two different varieties of plants each haring a desirable character. The hybrid protoplasts can be further grown to form a new plant.

(ii) Promoter gene a gene haring a regulatory sequence of DNA that initiates the expression of a gene.

(iii) Site directed mutagenesis involves induction of specified or desired changes in the base sequence at specified sites of genes, most successfully achieved by overlap extension PCR.
Or
The process of nucleotide changes in the cloned genes by specific in mutagenesis.

(iv) DNA probes are short 15-30 bases long, labelled oligonucleotides (RNA – DNA) used to detect complementary nucleotide sequences after hybridization and the auto radiograms gives many bands of different sizes.

(v) Primer is a short oligonucleotide that hybridizes with the template strand and gives a 3'- OH end at which a DNA polymerase start synthesis of DNA chain.

**Part-II**
**(Answer any five questions)**

**Question 2.**
(a) With reference to amino acids, explain :
(i) Any one physical and any one chemical property of amino acids.
(ii) Essential and non-essential amino acids.
(b) Briefly outline the various steps involved in the gene cloning technique
(c) List any four characteristics of genetic code.

**Answer:**
(a) They are the building blocks of molecular proteins.
(i) Amino acids are the organic acids (with carboxylic group-COOH) having amino group ($-NH_2$) generally attached to a-carbon or carbon next to the carboxylic group. Amino acids condenses to produce peptide (-NHCO-) bond.

(ii) Essential amino acids are essential for our body but they are not synthesised inside our body e.g., valine, isoleucine, lysine etc. They are supplemented through diet. Non-essential amino acids are those which are synthesised through transformation and transamination inside our body e.g., serine, alanine etc.

(b) The various steps involved in gene cloning technique are :

- Identification and isolation of desired DNA. .
- Amplification of gene of interest using PCR.
- Fragmentation/cutting of desired DNA and vector DNA by restriction enzymes.
- Ligation/joining of desired DNA fragment into vector using ligase enzyme.
- Transferring the recombinant DNA into host cell/organism by transformation, transfection, electroporation, microinjection (vectorless transfer), particle bombardment gun (Biolistics) (Vectorless transfer) or by Agrobacterium/retrovirus mediated gene transfer..
- Culturing the host cell for obtaining the foreign gene product/recombinant protein.
- Extraction of desired products or downstream processing.

(c) The characteristics of genetic codes are :

- Codes are universal.
- Codes are unambiguous and specific.
- Code is degenerate i.e., some amino acids are coded by more than one codon.
- Codons are read in a continuous fashion i.e., there are no commas or punctuation's.

**Question 3.**
(a) Describe the three dimensional structure of DNA as proposed by Watson, Crick and Wilkins. Name a biochemical technique that was used by them to confirm the structure of DNA. [4]

(b) Explain the role of the following enzymes during the process of protein synthesis : [4]
(i) RNA polymerases and amino acyl tRNA synthetase.
(ii) Start codons and end codons.
(c) Why are auxins and cytokinins used in plant tissue culture ? [2]

**Answer:**
(a) Watson, Crick and Wilkins described the structure of DNA but also indicated how it could be replicated and transfer from one organism to its off spring.
Salient features of double helix structure of DNA include :

- Double helix made of two polynucleotide chains.
- Sugar and phosphate form backbone and N-base project inside.
- The two chains are antiparallel with one chain has the polarity of 5' → 3', the other has 3'→ 5.
- Presence of double hydrogen bonds between A=T, and triple bond to between G = C The . purine always comes opposite to pyrimidines to create uniform distance.
- Two chains are coiled in a right handed fashion.
- Pitch (one turn) of helix is 3.4 nm (= 34 A) contains 10 bp with 0.34 nm (3.4 A) gap between adjacent bps. (1 nm = 10-9 m)
- Two chains of DNA are 20 A far apart, due to the pairing of purines with pyrimidines. This distance remains constant.
- The plane of one base pair stacks over the other in double helix to confer stability

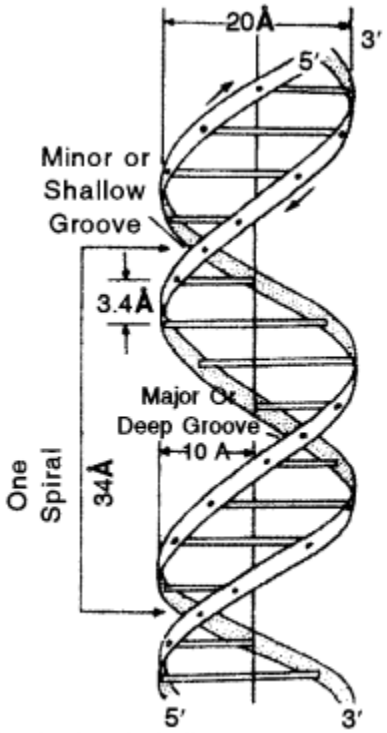

**Fig.** *Coiling in double helix or duplex of DNA.*

Rosalind Franklin confirmed this structure of DNA through X-Ray crystallography.

(b) (i) RNA polymerizes get associated transiently with initiation sigma factor (σ). They do not require a primer and can initiate the synthesis of a new chain on the template strand. It binds to the promoter site to start synthesis. The first nucleotide is labelled as + 1 and is called the transcription start site. It polymerizes ribonucleotides in 5' → 3' direction over 3' → 5' template DNA.

In prokaryotes, a single RNA polymerase transcribes all m-RNA, r-RNA and t-RNA. However, in case of Eukaryotas there are three different polymerizes i.e., RP+1 for r-RNA, RP-II for m-RNA and RP-III for t-RNA.
Amino acyl t-RNA syntheses couple each amino acid to its appropriate set of t-RNA molecules. There are 20 synthesizes for each of the 20 natural amino acids.

(ii) In prokaryotes, AUG acts as a start/initiation codon as well as codes for methionine. GUG acts as start codon for valine in Eukaryotes. UAA, UAQ UGA function as stop codons and do not code for any amino acid.

(c) Role of auxin :

- It helps in the formation of callus and in the development of xylem along with the promotion of cambial activity.
- It is used for the elongation and enlargement of plant cell and it inhibits the promotion of growths of apical and lateral buds.

Role of cytokinin:

- It stimulates the cell division process.
- It helps in the morphogenesis of plant cell, along with auxins.

**Question 4.**
(a) Explain how DNA technology has been used to create the following : [4]
(i) Dolly
(ii) Hepatitis B vaccine
(b) Write short notes on : [4]
(i) Batch culture and continuous culture.
(ii) Salinity resistance in crops.
(c) Name any two chemicals used to prepare the gel for gel electrophoresis. [2]

**Answer:**
(a) (i) Dolly – The first cloned animal
Using nuclear transfer technique, the world's first mammalian clone – Dolly, was born in February 1996. In 1995, Ian Wilmut and his research group (Scotland) took out udder from a six year old sheep A called clone mother, and put in a special solution. Nucleus

of udder cell was taken out and put in a solution. At the same time an unfertilized egg was taken out from another sheep B called egg mother. Nucleus of the egg was removed and nucleated egg was put in a culture medium. The nucleus of udder cell and nucleated egg cell were put together followed by mild electric shock. Consequently nucleus was taken up by the nucleated cell. This cell was incubated onto growth medium then transferred into a surrogate mother. A little lamb Dolly was born in February, 1996.

(ii) It is true that proteins (vaccines) stimulate immune system and cause to secrete specific antibodies. Such specific amino acid sequences in the protein that stimulate immune response are called epitopes. Based on selected epitopes recombinant vaccines may be produced on commercial level which can prove more effective and safer than the conventional vaccines.

Working on these lines, a recombinant Hepatitis B vaccine was produced by cloning the synthetic gene (for the surface antigen of the virus) in yeast cells. This gene expressed well in yeast cells and produced 22 nm particles of hepatitis B virus (HBV) surface antigen (as produced in patients) infected with hepatitis B virus. The recombinant vaccine has high immunogenecity. This product has been marketed as a vaccine for protection against HBV infection.

(b) Tissues and cells cultured in a liquid medium produce a suspension of single cells and cells clumps of few to many cells, are called suspension culture. Suspension cultures are of two types:

(i) Batch culture: In a batch culture, the same medium and all the cells produced are retained in the culture vessel e.g., culture flasks (100-250 ml), fermenters (variable size) etc. The cell number of biomass, of a batch culture exhibits a typical sigmoid curve represented by lag phase (cell number or biomass remain unchanged), log phase (rapid increase in cell number) and finally stationary phase (cell member does not change). The lag-phase lasts about 3-4 cell generation. The stationary phase is forced on the culture.

- due to depletion of nutrients
- and due to accumulation of wastes.

They are maintained by subculturing. They are unsuitable for studies on cell growth and metabolism because of constant change in cell density and nutritional status.

Continuous culture: Here the cell population is maintained in a steady state by regularly replacing a portion of used or spend medium. Such culture systems are either

- closed or
- open system.

In closed system, cells are separated from the used medium, taken out for replacement and added back to the culture so that biomass keeps on increasing. In contrast, both cells and used medium are taken out from open continuous system and replaced by equal volumes of fresh medium.

(ii) A plant which bears a foreign gene for desired function of other organism is called transgenic crop. Using biotechnological approaches stress/salinity tolerant plants can be produced. They secrete stress-related osmolytes such as sugars (fructans and trehalose) sugar alcohols (mannitol) amino acids (betaine, glycine and proline) and other proteins. For example, betaine is highly effective osmolyte that accumulates in some plants during, water stress or high salinity: Betaine is synthesised both in bacteria and plants. A transgenic tobacco was prepared by transferring E.coli bet A. gene through Ti-plasmid. The transgenic tobacco was 80% salt tolerant (i.e. 300 mM) than the normal tobacco.

(c) (i) Agarose gels
(ii) Polyacrylamide gels.

**Question 5.**
(a) Give the step wise procedure of the Southern Blotting technique. Mention any two differences
between Southern Blotting technique and Northern Blotting technique. [4]
(b) Explain the principle and any two applications of each of the following biochemical techniques:
(i) Ion Exchange Chromatography
(ii) Colorimetry [4]
(c) What is the cause of inborn metabolic disorders? Give any two examples of these disorders. [2]

**Answer:**
(a) Southern Blotting (Hybridization) Technique : In 1975, Edward M. Southern developed the technique of DNA separation and its hybridization. Therefore, in his honor this technique is known as ‘Southern blotting or Southern hybridization technique’. A specific DNA fragment can be separated and identified in a heterologous population of DNA molecules on the basis of binding of DNA probe with its complementary DNA strand.

The genomic DNA is isolated from the clone and digested with restriction enzymes. The DNA fragments are separated by agarose gel electrophoresis. Different DNA bands are formed on agarose gel which represents DNA fragments of varying sizes. These

fragments are transferred from gel to nylon or nitrocellulose membrane. The process of DNA transfer is called ‘blotting’.

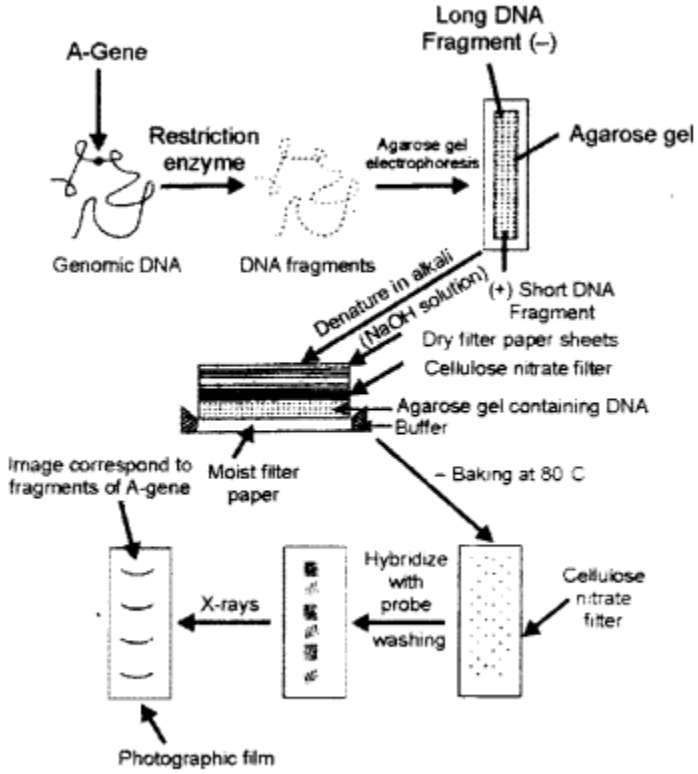


**Fig.** *Southern blotting technique*

A nitrocellulose membrane is put over the gel. Many layers of filter paper are placed over nitrocellulose membrane. This assembly is put in a container having NaOH solution. NaOH denatures DNA and results in formation of single stranded DNA. DNA fragments are transferred from gel to membrane by capillary action.

The DNA fragments are fixed to membrane by using UV radiation or baking at 80°C. The pattern of DNA bands on membrane corresponds to the position of DNA on gel. The membrane is put in solution containing radio labelled DNA probe and incubated for some time. DNA probe hybridizes complementary DNA fragments fixed on membrane. It is gently washed at 12°C and dried. The membrane is exposed through a photographic film. DNA bands formed on photographic film corresponds to the original position of DNA fragments present on agarose gel.

Southern Blotting technique involves separation and identification of a specific DNA fragment. In Northern Blotting technique the RNA is analysed rather than DNA. During southern blotting NaOH denatures DNA to form single stranded DNA which are transferred from gel to nitro cellulose membrane. In northern blotting total RNA molecules are extracted and then mRNA molecules are isolated by using oligo (dT) cellulose Chromatography. RNA samples separated are transferred to a nylon membrane.

(b) (i) Ion Exchange Chromatography Principle: It is based on reversible exchange of ions in solution with ions electrostatically bound to some sort of insoluble support medium. Separation is obtained since different molecules have different degree of interaction with ion exchanger due to difference in their charges, charge densities and distribution of charge on their surface.

Applications:

- A technique for separating two proteins differing by only one charged amino acid.
- It is capable of separating species with very minor differences in their properties.

The ion-exchange may be

- Anion exchanger or
- Cation exchanger.

(ii) Colorimetry:
Principle : It is based on the use of interaction of light energy with coloured solutions of certain molecules as when light passes through a coloured solution, some wavelengths are absorbed more than others. The amount of light absorbed is proportional to the intensity of colour and hence to the concentration of the compound.

Applications:

- Quantitative estimation i.e. concentration in solution.
- Detect and identification of biomolecules

(c) Inborn metabolic disorders are due to change in the gene, for the particular character. Some autosomal genes become recessive and are transmitted to the progeny.
Examples:

1. Albinism
2. Cystic fibrosis
3. Phenylketonuria
4. Sickle-cell anaemia
5. Alkaptonuria

Alkaptonuria: This was one of the first metabolic diseases described by Garrod in 1908. It is an inherited metabolic disorder produced due to deficiency of an oxidase enzyme requiredfor breakdown ofhomogentisic acid (also called alcapton, hence, alkaptonuria is also written as alcaptonuria). Lack of the enzyme is due to the absence of the normal

form of gene that controls the synthesis of the enzyme. Hence, homogentisic acid then accumulates in the tissues and is also excreted in the urine. The most commonly affected tissues are cartilages, capsules of joints, ligaments and tendons. The urine of these patients if allowed to stand for some hours in air, turns black due to oxidation of homogentisic acid.

Albinism: It is caused by the absence of the enzyme tyrosinase which is essential for the synthesis of the pigment from dihydroxyphenylalanine. The gene for albinism (a) does not produce the enzyme tyrosinase but its normal allele (A) does. Thus, only homozygous individual (aa) is affected by this disease. Albinos (individuals with albinism) lack dark pigment melanin in the skin, hair and iris. Although albinos have poor vision yet they lead normal life.

**Question 6.**
(a) Discuss the significance of each of the following techniques used in cell culture technology: [4]
(i) Androgenesis and gynogenesis
(ii) In-vitro pollination
(b) Enumerate any two post transcriptional changes in the mRNA to produce a completely mature mRNA. [4]
(c) State any four achievements of the Human Genome Project. [2]

**Answer:**
(a) (i) Androgenesis involves production of haploid plants by development of an egg cell containing male nucleus. The female nucleus is eliminated before fertilization. Gynogenesis includes production of haploid plants by the development of an unfertilized egg cells because of delayed pollination (through use of abortive pollen pre-exposed to ionizing radiations or using an alien pollen). It is found in inter-specific crosses of potato.

(ii) In vitro pollination : A laboratory procedure, in which pollination and fertilisation occur under in vitro condition, offer an opportunity for producing hybrid embryoids among plants that cannot be crossed by conventional method of plant breeding. To overcome interspecific and intergeneric incompatibility cases, the style or part of it can be excised and pollen grains are either placed on the cut surface of ovary or transferred through a hole in the wall of ovary. This technique is called intra-ovarian pollination. It is applied successfully in Papaver somniferum, Argemone mexicana etc. It also involves direct pollination of cultured ovules or excised ovules together with placenta.

(b) Post-transcriptional changes : Modification at 5' end of primary RNA (Heterogenous RNA or hnRNA) transcript is called capping. During capping, an extra guanosine residue in first added to the terminal nucleoside triphosphate of the primary RNA transcript. Guanosine residue is further modified by addition of methyl groups. Capping prevents

the 5' end of TORNA from being digested by exonucleases. It aids in the transport of m-RNA out of the nucleus and plays an important role in the m-RNA translation.

Addition of a string of adenosine residues, forming a poly (A) tail at 3' end of the TO-RNA.

The process of removal of those parts of primary transcript, corresponding to introns and joining together of exons, is known as splicing.

(c) Achievement of Human Genome Project:

- Human genome is largely consisting of – 3 × 109 base pairs a lot of repeated sequences.
- 3 billion base pairs were translated into biologically meaningful information by using computers and it led to a new field of bioinformatics.
- Human genome sequencing will enable a new approach to biological research.
- Understanding of function of biological systems increases in public and private sectors due to knowledge from DNA sequences.
- More than 1200 genes are associated with common human disorders such as Cardio- . Vascular diseases, diabetes, (endocrine disease), Alzheimer disease (neurological disorder).
- Efforts for health care are being made to design drugs, genetically modified diets, nutraceuticals etc.

**Question 7.**
(a) Explain the method used for the construction of a genomic DNA library. Also state how a genomic DNA library differs from a cDNA library. [4]
(b) Discuss any four vector-less methods of transfer of foreign DNA into the host cells. [4]
(c) Define glycosidic bond and peptide bond. [2]

**Answer:**
(a) DNA Library: DNA library is a collection of DNA fragments of one organism, each carries by a plasmid or virus and cloned in an appropriate host. A DNA probe is used to locate specific DNA sequence in the library. A collection representing the entire genome is called genomic (DNA) library. An assortment of DNA copies of messenger RNA produced by a cell is known as a complimentary DNA (cDNA) library.

Construction of Genomic Library: The process of subdividing genomic DNA into clonable elements and inserting them into host cells is called creating a library.

A complete library, by definition, contains the entire genomic DNA of the source organism and is called as genomic library. A genomic library' is a set of cloned fragments of genomic DNA.

The process of creating a genomic library includes four steps:

1. In the first step the high molecular weight genomic DNA is separated and subjected to restriction enzy me digestion by using two compatible restriction enzy mes.
2. In the second step, the fragments are then fractionated or separated by using agarose gel electrophoresis to obtain fragments of required size.
3. These fragments are then subjected to alkaline phosphatase treatment to remove the phosphate. In the third step, the dephosphorylated insert is ligated into vector which could be a plasmid, phage or cosmid, depending upon the interest of the researcher.
4. In the last step, the recombinant vector is introduced into the host by electroporation and amplified in host.

In principle, all the DNA from the source organism is inserted into the host, but this is not fully possible as some DNA sequences escape the cloning procedure. Genomic library is a source of genes and DNA sequences. A genomic library is a set of cloned fragments of genomic DNA. Prior information about the genome is not required for library construction for most organisms. In principle, the genomic DNA, after the isolation, is subjected to RE enzyme for digestion to generate inserts.

cDNA libraries V/s Genomic libraries:

- Genomic library is a mixture of fragments of genomic DNA while cDNA obtained from ZM-RNA may cloned to give rise to a cDNA library. Genomic library contains DNA fragments that represent genes as well as those that are not genes. In contrast cDNA library contains only those genes that are expressed in the concerned tissue/organism. In both cases, a mixture of fragments is used for cloning to establish the library.
- Use of cDNA is absolutely essential when the expression of an eukaryotic gene is required in a prokaryote.
- Eukaryotic cDNAs are free from intron sequences.
- As a result of the above, they are smaller in size than the corresponding genes, i.e.. the genes that encoded them.
- A comparison of the cDNA sequence with the corresponding genome sequence permits the delineation of intron/exon boundaries.
- The contents of cDNA libraries from a single organism will vary widely depending on the developmental stage and the cell type used for preparation of the library. In

contrast the genomic libraries w ill remain essentially the same irrespective of the developmental stage and the cell type used.
- A cDNA library will be enriched for abundant mRNAs. but may contain only a few or no clones representing rare mRNAs.

(b) Transfection: Transfection is the transfer of foreign DNA into cultured host cells mediated through chemicals. The charged chemical substances such as cationic liposomes, calcium phosphate of DEAE dextran are taken and mixed with DNA molecules. The recipient host cells are overtaxed by this mixture. Consequently the foreign DNA is taken up by the host cells.

Electroporation (Electric Field-mediated Membrane Permeation): In electroporation an electric current at high voltage (about 350 V) is applied in a solution containing foreign DNA and fragile host cells. This creates transient microscopic pores in cell membrane of naked protoplasts. Consequently foreign DNA enters into the protoplast through these pores. The transformed protoplasts are cultured in vitro which regenerate respective cell walls.

Microinjection : In this technique foreign DNA is directly and forcibly injected into the nucleus of animal and plant cells through a glass micropipette containing very fine tip of about 0.5 mm diameter. It resembles with injection needle. In 1982, for the first time Rubin and Spradling introduced Drosophila gene into P-element and microinjected into embryo.

Particle Bombardment Gun (Biolistics): This technique was developed by Stanford in 1987. In this method macroscopic gold or tungsten particles are coated with desired DNA. A plastic micro-carrier containing DNA coated gold/tungsten particles is placed near rupture disc. The particles are bombarded onto target cells by the bombardment apparatus. Consequently foreign DNA is forcibly delivered into the host cells

(c) Glycosidic bond (- O -) is the bond formed between the OH group attached to an anomeric carbon atom of a monosaccharide can easily dehydrate with an -OH group attached to another monosaccharide leading to the formation of a disaccharide.

Peptide bond (-NHCO-) is the bond formed between the carboxylic group (COOH) of one amino acid w ith the amino group (NH2) of another amino acid, with elimination of w ater to form a dipeptide.

**Question 8.**
(a) Explain the secondary and the quaternary structure of proteins. Mention any two important functions of proteins. [4]
(b) Discuss the method used for DN A sequencing by A utomated DNA sequencing

technique. [4]
(c) Give any one example each of in-situ and ex-situ conservation. [2]

**Answer:**
(a) Secondary Structure (2° structure) of Proteins: It is development of new steric relationships amongst the amino acids for protecting their peptide bonds through formation of intrapolypeptide and interpolypeptide hydrogen bonds. Secondary' structure is of three types – α-helix β-pleated and collagen helix. The prefixes α and β signify the first and second types of secondary structure discovered by Pauling and Corey (1951).

(i) α-Helix: The polypeptide chain is spirally coiled, generally in a clockwise or right-handed

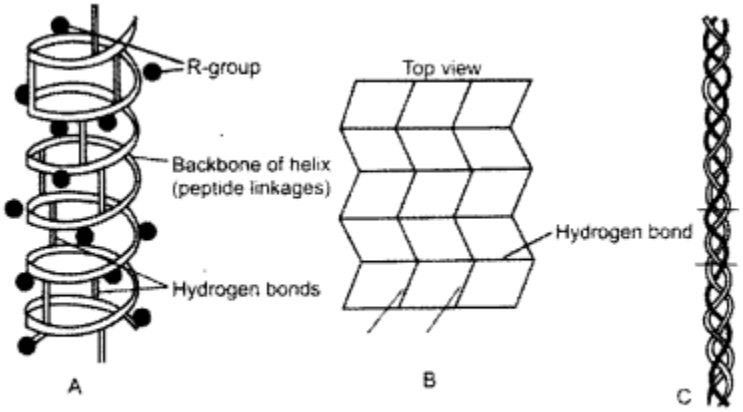


**Fig.** A. α-helix, B. β-Pleated sheet Tropocollegen (Collagen Helix)

fashion (Fig.). There are 3.6 amino acid residues per turn of the spiral. The spiral is stabilized by straight hydrogen bonds between imide group (-NH-) of one amino acid and carbonyl group (—CO—) of fourth amino acid residue. In this way all the imide and carbonyl groups become hydrogen bonded. R-groups occur towards the outer side of a-helix. a-helix is the final structure in certain fibrous proteins, e.g., keratin (hair, nail, horn), epidermis (skin).

β-Pleated Sheets : Two or more polypeptide chains come together and form a sheet. Condensation is little. How ever, tw isting does occur. The same polypeptide may fold over itself to form two strands for p-pleating. Adjacent polypeptide chains may occur in parallel (e.g., p-keratin) or anti-parallel (e.g., silk fibrin). Straight hydrogen bonds occur between imide (-NH-) group of one polypeptide and carbonyl (-CO-) group of adjacent polypeptide. Cross-linkages help in stabilization of β-pleated sheets.

Collagen Helix (Fig.) : Collagen has a large amount of glycine (25%) and proline (and hydroxyproline, 25%). It cannot form a-helix due to them. Three of its polypeptide each having about 1000 amino acid residues, come together with each forming an extended left-handed helix. They run parallel, form a right-handed super-helix that is stabilised by

hydrogen bonds amongst the three. The triple helix of collagen is often called tropo-collagen. Its one end is stabilised by -S-S- linkages amongst the three chains. Collagen occurs in those tissues where extensibility is limited, e.g., connective tissue, tendons, bones.

Quaternary Structure (4° Structure) of proteins : It is the last or fourth level of protein organisation found in only oligomeric proteins or multimers. The multimeric proteins are formed of two to several polypeptides. The monomers or polypeptide subunits are also called protomers. Protomers may be similar, e.g., two similar polypeptides in enzyme phosphorylase a. It is known as homogeneous quaternary structure. An oligomeric protein having dissimilar subunits shows heterogeneous quaternary structure, e.g., tetrameric haemoglobin with two a (141 amino acids each) and two β (146 amino acids each) polypeptide chains.

Functions of proteins:

- Major group of proteins are enzymes-biocatalyst.
- Myoglobin, a protein found in muscle store oxygen.
- Haemoglobin in RBC's transports gases in and out of lungs.
- Proteins as structural element; as hairkeratins and bio-membranes.

(b) Automatic DNA Sequencing : In this new method a different fluorescent dye is tagged to the ddNTPs. Using this technique a DNA sequence containing thousands of nucleotides can be determined in a few hours. Each dideoxynucleotide is linked with a fluorescent dye that imparts different colours to all the fragments terminating in that nucleotide. All four labelled ddNTPs are added to a single capillary tube. It is a refinement of gel electrophoresis which separates fastly. DNA fragments of different colours are separated by their respective size in a single electrophoretic gel. A current is applied to the gel. The negatively charged DNA strands migrate through the pores of gel towards the positive end.

The small sized DNA fragments migrate faster and vica versa. All fragments of a given length migrate in a single peak. The DNA fragments are illuminated with a laser beam. Then the fluorescent dyes are excited and emit light of specific wavelengths which is recorded by a special 'recorder'. The DNA sequences are read by determining the sequence of the colours emitted from specific peaks as they pass the detector. This information is fed directly to a computer which determines the sequence. A tracing electrogram of emitted light of the four dyes is generated by the computer (Fig.). Colour of each dye represents the different nucleotides. Computer converts the data of emitted light in the nucleotide sequences.

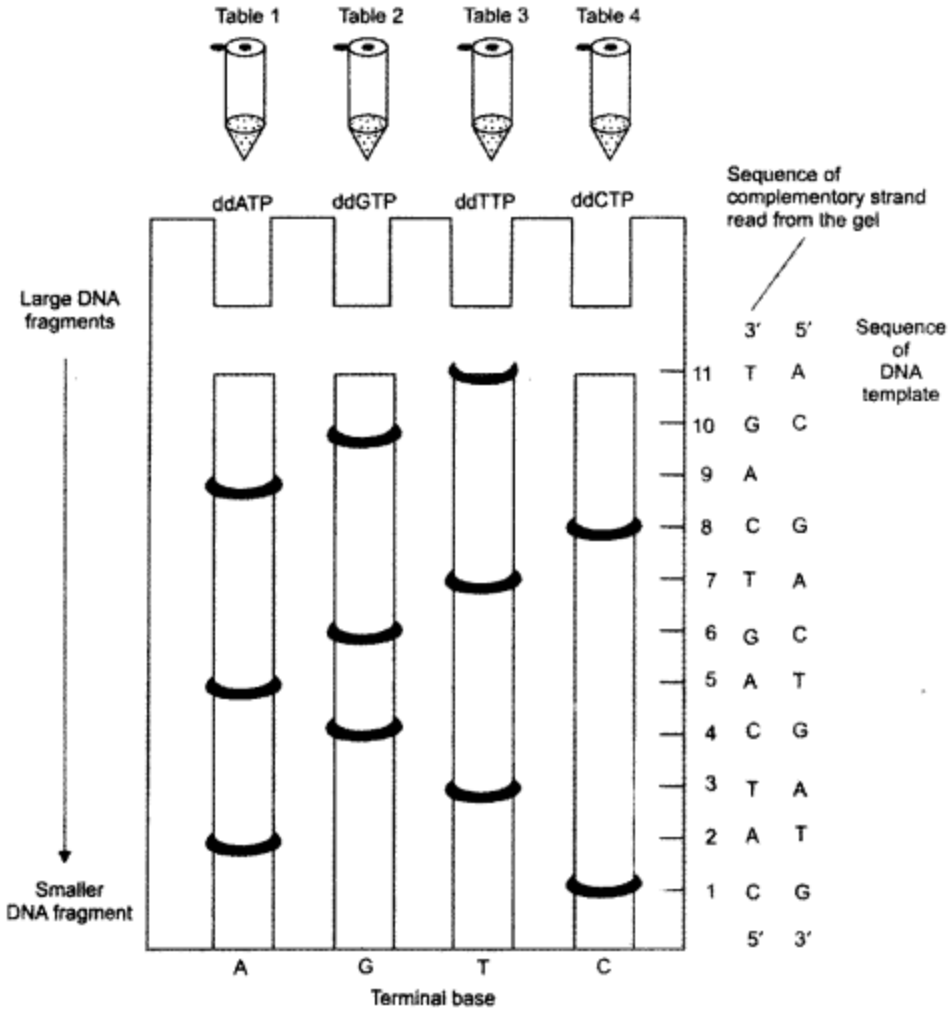


**Fig.** *Autoradiogram of electrophoresed DNA sequencing gel.*

(c) In situ conservation refers to 'on-site' conservation of plants and animals as such. It is a process of protecting plants or animals in its natural habitat by protecting or cleaning up the habitat, or by defending the species from the predators, e.g., National Park, Sanctuaries and Biosphere Reserve.

Ex situ conservation is 'off-site conservation' by protecting an endangered species of plant or animal by removing part of population from a threatened habitat and placing it in a new location such as wild area, zoos or botanical gardens, in vitro gene bank, germplasm-banks, in vivo gene bank.

**Question 9.**

(a) What is meant by the term genomics ? Mention the difference between structural genomics and functional genomics. [4]

(b) How do the following databases contribute towards managing biological data : [4]

(i) GDB and MGD

(ii) PDB and PIR
(c) Name any two organisms whose genomes have been completely sequenced. [2]

**Answer:**
(a) Genomics is a scientific discipline of mapping, sequencing and analysing the genome-the complete set of chromosomal and extra-chromosomal genes of an organism. Structural genomics deals with DNA sequencing, sequence assembly, construction of genetic, physical or sequence maps of high resolution of the organism.

Functional Genomics deals with reconstructing genome sequences and to find out the functions they do. It provides novel information about the genome. It helps in understanding of genes and functions of proteins and protein interactions.

(b) (i) GDB (Genome Database): It is the official central repository for genome mapping data created by Human Genome Project. Its central node is located at the hospital for sick children. GDB holds a vast quantity of data submitted by hundreds of investigators. The GDB has many useful genome resource web-links on its resource page.

MGD (Mouse Genome Database) is the primary public mouse genomic catalogue resource. The MGD includes information on mouse genetic markers and nomenclature, molecular segments, phenotypes, comparative mapping data, graphical display of linkage, cytogenetic and physical maps.

(ii) PDB (Protein Data Bank) : This database has the sequence of those proteins, nucleic acid, whose 3-D structures are known by crystallography or NMR spectroscopy. Source : NCBI-USA; EBI, UK.

PIR (Protein Information Resource): It is an integrated public bioinformatics resource to support genomic, proteomic and system biology, research and scientific studies.

(c) Organisms with completely sequenced genome : Phage λ , HIV, E.coli, Heliobacterpylori, Saccharomyces cerevisiae (yeast), Drosophila melanogaster (fruitfly). (any one organism)