Biotechnology

Question Paper 2017

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.

Part -1 (20 Marks)
(Answer all questions)

Question 1.
(a) Mention any one significant difference between each of the following : [5]
(i) Reducing sugar and non-reducing sugar.
(ii) Triploids and haploids.
(iii) Lac operon and Trp operon
(iv) Blunt end and sticky end
(v) Spectroscopy and colorimetry

(b) Answer the following questions : [5]
(i) Who developed the microbe called super bug, which was designed to degrade spilled oil ?
(ii) Name any two growth regulators used in a culture medium.
(iii) What is an apoenzyme ?
(iv) How is the disease albinism caused ?
(v) State any one limitation of gynogenesis.

(c) Write the full form of each of the following : [5]
(i) AFLP

(ii) SSBs
(iii) BAC
(iv) CIMAP
(v) PAGE

(d) Explain briefly : [5]
(i) Polyadenylation
(ii) Lock and key model of enzyme action
(iii) Edible vaccine
(iv) Vascular differentiation
(v) Seedless crops

Answer:
(a) (i) Reducing sugar possess a free aldehyde (–CHO) and ketone (–C = 0) group while non-reducing have no free aldehyde and ketone group.

(ii) Plants produced from pollen grains or egg cells of ovules are called haploid plants. Plants produced from endosperm are called triploid plants.

(iii) Lac-operon is under negative gene control.
Trp-operon is under positive gene control.

(iv) Blunt ends : Some restriction enzymes cut both the strands of a DNA molecule at the same site so that the resulting termini or ends have blunt or flush ends in which the two strands end at the same point.

Sticky ends : Most of the restriction enzymes produce staggered cuts in which the two strands of DNA double helix are cleaved at different locations producing two protuding end (3' or 5') called cohesive or sticky ends. They readily pair with each other under annealing conditions. When fragments generated by a single restriction enzyme from different DNA are mixed they join together due to their sticky' ends.

(v) Spectroscopy is the study of the interaction between matter and electromagnetic radiation. Colorimetry involves the estimation of the concentration of a colored substance in a solution by comparing the intensity' or depth of its color with that of a solution having known concentration of the substance while passing visible light of the same intensity through both the solutions.

(b) (i) Ananda Mohan Chakrabarty engineered a new species of Pseudomonas bacteria in 1971. This is the oil spill disester bacteria (super bug).

(ii) Growth regulator hormones : These are Auxin and Cytokinins. Auxins (Indole acetic acid, 1-napthaleneacetic acid), Cytokinins (6-benzylaminopurine, zeatin).

(iii) Apoenzyme: Protein that forms an active enzyme system by combination with a coenzyme and determines the specificity' of this system for a substrate.

(iv) Albinism: It results from inheritance of recessive alleles due to non-conversion of tyrosine into melanin.

(v) Limitation of Gynogenesis:

- Frequency' of responding ovules is quite low i.e., 1-5 %
- Successful only is relatively small number of species.

(c) (i) AFLP: Amplified Fragment Length Polymorphism.

(ii) SSB's : Single-Strand Breaks or Single-Strand DNA Binding Proteins.

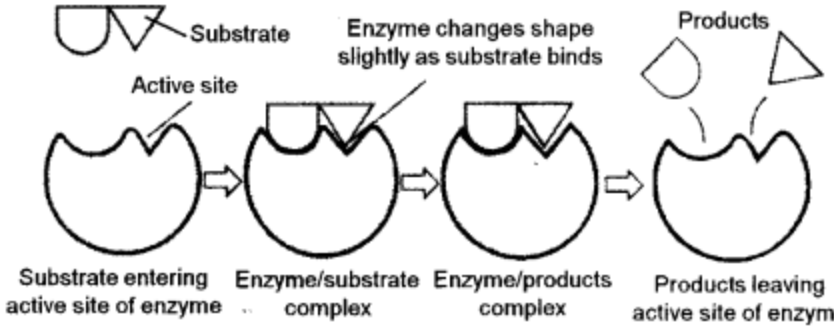
(iii) BAC : Bacterial Artificial Chromosome.

(iv) CIMAP: Central Institute of Medicinal and Aromatic Plants.

(v) PAGE : Poly Acrylamide Gel Electrophoresis

(d) (i) Polyadenylation: It is the addition of a poly (A) tail to a messenger RNA. In eukaryotes, polyadenylation is part of the process that produces mature messenger RNA (mRNA) for translation.

(ii) Lock and key mechanism : This model was proposed by Emil Fisher in 1898. It is also called the template model. According to this model the union of the substrate and the enzyme takes place at the active site, more or less in a manner in which a key fits in a lock and results in the formation of an enzyme substrate complex. As the two molecules are involved, this hypothesis is also known as the concept of inter-molecular fit. The ES complex is highly unstable and almost immediately this complex breaks to produce the end product of the reaction and regenerate the free enzyme. The ES complex results in the release of energy.



(iii) Edible vaccine are the antigenic proteins that induce B-cells to secrete antibodies. Transgenic crop plants can be constructed which produce vaccine to be eaten i.e., edible vaccine on a large scale at low cost. In 1990, first report of production of edible vaccine in tobacco was establish. There are many advantages associated with edible vaccine such as no problem of storage, easy delivery', low cost of production. These provide similar effects as the recombination vaccines.

(iv) Vascular differentiation : In vascular differentiation, first identify and clone transcripts that are produced in differentiating vascular tissues, then approach gene function by assessing phenotype in plants with loss of function, created by insertion mutagenesis of RNA interference.

(v) A seedless fruit is a fruit developed to possess no mature seeds. As consumption of seedless crops e.g., fruit is generally easier and more convenient, they are considered commercially valuable. Most commercially produced seedless fruits have been developed from plants whose fruits normally contain numerous relatively large hard seeds distributed throughout the flesh of the fruit.

Seedless fruits can develop in one of two ways : either the fruit develops with fertilization (parthenocarpy), or pollination triggers fruit development, but the ovules or embryos abort without producing mature seeds (stenospermocarpy). Seedless banana and watermelon fruits are produced on triploid plants, whose three sets of chromosomes make it very unlikely for meiosis to produce fertile gametes.

One disadvantage of most seedless crops is a significant reduction in the amount of genetic diversity in the species. As genetically identical clones, a pest or disease that affects one individual is likely capable of affecting each of its clones.

Part – II (50 Marks)
(Answer any five questions)

Question 2.
(a) Briefly explain the structure of tRNA. Write its function in protein synthesis.

(b) With reference to lipids, explain its :
(i) Building blocks.
(ii) Any two chemical properties.
(c) What is DNA probe ?

Answer:
(a) Transfer RNA (tRNA) : It is also called soluble or sRNA. There are over 100 types of tRNA's. Transfer RNA constitutes about 15% of the total RNA. tRNA is the smallest RNA with 70-85 nucleotide's and sedimentation coefficient of 4S. The nitrogen bases of several of its nucleotide's get modified e.g., pseudouridine (φ), dihydrouridine (DHU), inosine (I). This causes coiling of the otherwise single- stranded tRNA into L-shaped form (three dimensional, Klug, 1974) or clover-like form (two dimensional, Holley, 1965). About half of the nucleotides are based paired to produce paired stems. Five regions are unpaired of single-stranded —AA-binding site, T φ C loop, DHU loop, extra arm and anticodon loop,

(i) Anticodon. It is made-up of three nitrogen bases for recognizing and attaching to the codon of tRNA.
(ii) AA-Binding site. It lies at the 3' end opposite to the anticodon and has CCA—OH group (5' ends bears G). Amino acid or AA-binding site and anticodon are the two recognition sites of tRNA.
(iii) T φ C loop. It contains pseudouridine. The loop is the site for attaching to ribosomes.
(iv) DHU loop. The loop contains dihydrouridine. It is binding site for aminoacyl synthetase enzyme,
(v) Extra arm. It is a variable site arm or loop which lies between T φ C loop and anticodon. The exact role of extra arm is not known.

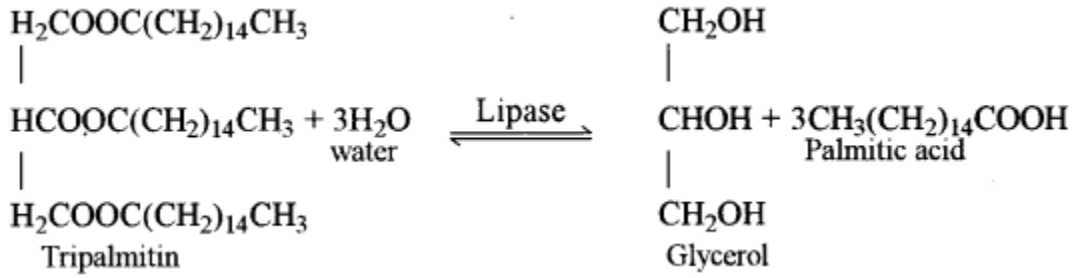
Functions :
(i) tRNA is adapter molecule which is meant for transferring amino acids to ribosomes for synthesis of polypeptides. There are different tRNAs for different amino acids. Some amino acids can be picked up by 2-6 tRNA's. tRNA's carry specific amino acids at particular points during polypeptide synthesis as per codons of wRNA. Codons are recognised by anticodons of tRNA's. Specific amino acids are recognized by particular activating or aminoacyl synthetase enzymes.

(ii) They hold peptidyl chains over the mRNA's. The initiator tRNA has the dual function of initiation of protein synthesis as well as bringing in of the first amino acids. There is, however, no tRNA for stop signals.

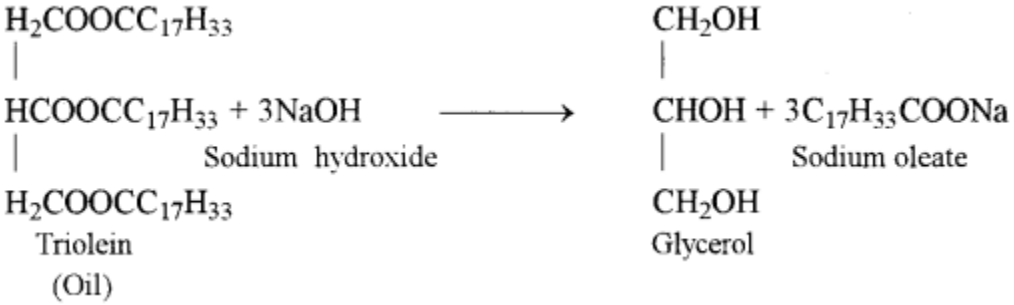
(b) (i) The building blocks of lipids are the long chain hydrocarbons, glycerol and cholesterol.
They are special group of lipids. Building blocks of lipids show various types of

reactivities of ester linkage and the degree of unsaturation of hydrocarbon chain. Simple fatty acids, Sphingosine, Glycerol and Cholesterol these are the building blocks of lipids.

(ii) Chemical properties of Lipids :
Hydrolysis: On hydrolysis with alkali or lipolytic enzyme lipases, fats are broken down into their components fatty acids and glycerols.



Saponification : The hydrolysis of fats by alkali to produce glycerol and soaps (salts of fatty acids) is known as saponification.



(c) DNA Probe : It is a solution of radioactive, single-stranded DNA or oligodeoxy nucleotides (a DNA segment of few to several nucleotide's). The name probe signifies the fact that this DNA molecule is used to detect and identify the DNA fragment in the gel membrane that has a sequence complementary to the probe. The probe hybridizes with the complementary DNA on the membrane to the greater extent with a low non-specific binding on the membrane. This step is known as hybridization reaction.

Question 3.
(a) Explain the process involved in the transcription of DNA to mRNA. [4]
(b) What are stem cells ? Explain the various types of stem cells. [4]
(c) Name any two chemicals used to determine the amino acid sequence in protein. [2]
Answer:
(a) Transcription is the process of creating a messenger RNA strand from DNA, performed by the enzyme RNA polymerase. Transcription always occurs in a 5'→ 3' direction, with polymerase moving 3'→ 5' along the DNA strand.

Transcription Initiation : There are three steps in transcription :
(1) Initiation: RNA synthesis begins after the RNA polymerase attaches to the DNA and unwinds it. RNA synthesis will always occur on the template strand.

(2) Elongation: RNA polymerase unwinds the DNA double helix and moves downstream and elongates the RNA transcript by adding ribonucleotides in a '5 →3' direction. Each ribonucleotide is added to the growing mRNA strand using the base pairing rules (A binds with T, G binds with C). For each C encountered on the DNA strand a G is inserted in the RNA, for each Q a C and for each T, an A is inserted. Since there is no T in RNA, U is inserted whenever an A is encountered. After RNA polymerase has passed, the DNA restores its double stranded structure.

(3) Termination: When the mRNA is complete, the mRNA is released and the RNA polymerase releases from the DNA.
Two post transcriptional changes that occur in the mRNA formed are :
RNA transcripts Eukaryotas are modified or processed, before leaving the nucleus to produce functional mRNA. It is processed in two ways :
(1) 5' capping: Capping of the pre-mRNA involves the addition of 7-methylguanosine to the 5' end.,

(2) 3' poly adenylation : The pre-mRNA processing at the 3' end of the RNA molecule involves cleavage of its 3' end and then the addition of about 200 adenine residues to form a poly (A) tail. The cleavage and adenylation reaction occur if a polyadenylation signal sequence (5' – AAU AAA-3') is located near the 3' end of the pre-mRNA molecule, followed by another sequence, which is usually (5'-CCA-3')

(b) Stem cells are the cell which are capable to divided and renew and also produce progeny. These can differentiate into a variety of different cells types, e.g., tissues continuously renew themselves throughout the life. The stem cells have two properties which increases their importance.
(i) They have potential to form more differentiated cells and
(ii) these are self-renewing because each division of a stem cells creates at least one stem cell.

Various types of stem cells : On the basis of development potential, these are divided into following levels:

- Totipotent Cells : which give rise to entire organism.
- Pluripotent Cells : w hich form totipotent cells and also give rise to most but not all of cell types that are necessary for foetal development.
- Multipotent Cells : cells which are formed after further differentiation of pluripotent cells. These can give rise to a limited number of cells types.
- Unipotent Cells : are also formed from further differentiation of multipotent cells. These give rise to a single cell type.

(c) There are many chemicals which are used to determine the amino acid sequence in protein. Sanger's reagent.(1-fluoro-2, 4-dinitrobenzene) and dansyl derivatives such as dansyl chloride are used for amino acid sequencing.

Question 4.
(a) Explain the following methods of selection of recombinant cells: [4]
(i) Insertional inactivation.
(ii) Blue white colony.
(b) Enumerate the steps involved in regenerating a plant from a single cell [4]
(c) What is wobble effect ? [2]

Answer:
(a) (i) Insertional inactivation: Harder problem to solve is to determine which of the transformed colonies comprise cells that contain recombinant DNA molecules, and which contain self- ligated vector molecules. Insertional inactivation is the inactivation of a gene by inserting a fragment of DNA into the middle of its coding sequence. Any future products from the inactivated gene will not work because of the extra codes added to it. Recombinants can, therefore, be identified because the characteristic coded by the inactivated gene is no longer visible.

pBR322 contains genes which code for ampicillin resistance and tetracycline resistance. BamHI cuts in the middle of the gene which codes for tetracycline resistance. If a gene is inserted here, the plasmid loses it ability to code for tetracycline resistance. Thus, the plasmid containing the recombinant gene is resistant to ampicillin but sensitive to tetracycline. To screen, we use replica plates.

(ii) The blue-white screen is a molecular technique that allows for the detection of successful ligation's in vector-based gene cloning. DNA of interest is ligated into a vector. The vector is then transformed into competent cell (bacteria). In this method, a reporter gene lac Z is inserted in the vector. The lac Z encodes for the enzyme β-galactosidase which breaks a synthetic substrate X-gal (5-bromo-4-chloro-indolyl, (β-D-galacto-pyranoside) into insoluble blue colored product. The competent cells are grown in the presence of X-gal. If the ligation was successful, the bacterial colony will be white because p- galactosidase is not synthesized due to the inactivation of lac Z; if not, the colony will be blue. This technique allows for the quick and easy detection of successful ligation, without the need to individually test each colony. An example of such a vector is the artificially reconstructed plasmid pUC 19.

(b) Basic technique of Plant Tissue Culture : The basic technique of plant tissue culture involves the following steps :
Preparation and Sterilization of Suitable Nutrient Medium : Suitable nutrient medium as per objective of culture is prepared and transferred into suitable containers. Culture

medium is rich in sucrose, minerals, vitamins, and hormones. Yeast extract, coconut milk, are also added. The culture is completely sterilized in an autoclave.

Selection of explants : Selection of explants such as shoot tip should be done.

Sterilisation of explants : Surface sterilisation of the explants by disinfectants (e.g sodium hypochlorite or mercuric chloride) and then washing the explants with sterile distilled water is essential.

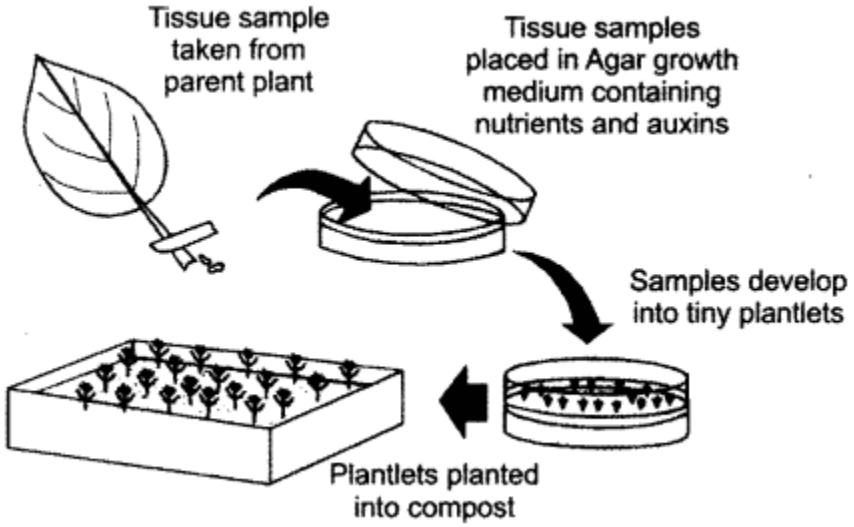
Inoculation : Inoculation (transfer) of the explants into the suitable nutrient medium (which is sterilised by autoclaving to avoid microbial contamination) in culture vessels under sterile conditions is done.

Incubations : Growing the culture in the growth chamber or plant tissue culture room, having the appropriate physical condition (i.e., artificial light; 16 hours of photoperiod), temperature (- 26°C) and relative humidity (50-60%) is required.

Regeneration: An unorganized mass of cells developing from explants is called callus. The callus gives rise to embryoids which can develop into w hole plant if the medium is provided with proper concentration of hormones. This property of developing every somatic cell into a full fledged plant is called totipotency. Regeneration of plants from cultured plant tissues is carried out.

Hardening: Hardening is gradual exposure of plantlets to an environmental conditions.

Plantlet transfer: After hardening, plantlets are transferred to the greenhouse or field conditions following acclimatization (hardening) of regenerated plants.



(c) Wobble effect:

The wobble effect is an effect caused by the redundancy found in the genetic code. Each amino acid is coded by a 3 nucleotide sequence on the wRNA. The triplets are referred to as codons. Though there are only 21 amino acids, 61 of the 64 possible codons found on the mRNA code for amino acids (the other 4 codons signal translation stop and start points). Thus, each amino acid can be coded by more than one codon. For any amino acid, the first 2 nucleotide's in the codon are always identical. It is the 3rd nucleotide that can change. This is where the wobble comes in.

Question 5.
(a) Discuss the working of PCR technique in detail.
(b) Explain the principle and any two applications of each of the following biochemical techniques:
(i) Iso-electric focusing.
(ii) Centrifugation.
(c) Where do we find the following carbohydrates:
(i) Glycogen
(ii) Chitin

Answer:
(a) Working mechanism of PCR: The action of PCR involves several cycles. However, there are three steps in one amplification cycles, e.g., denaturation (melting), annealing and polymerisation.

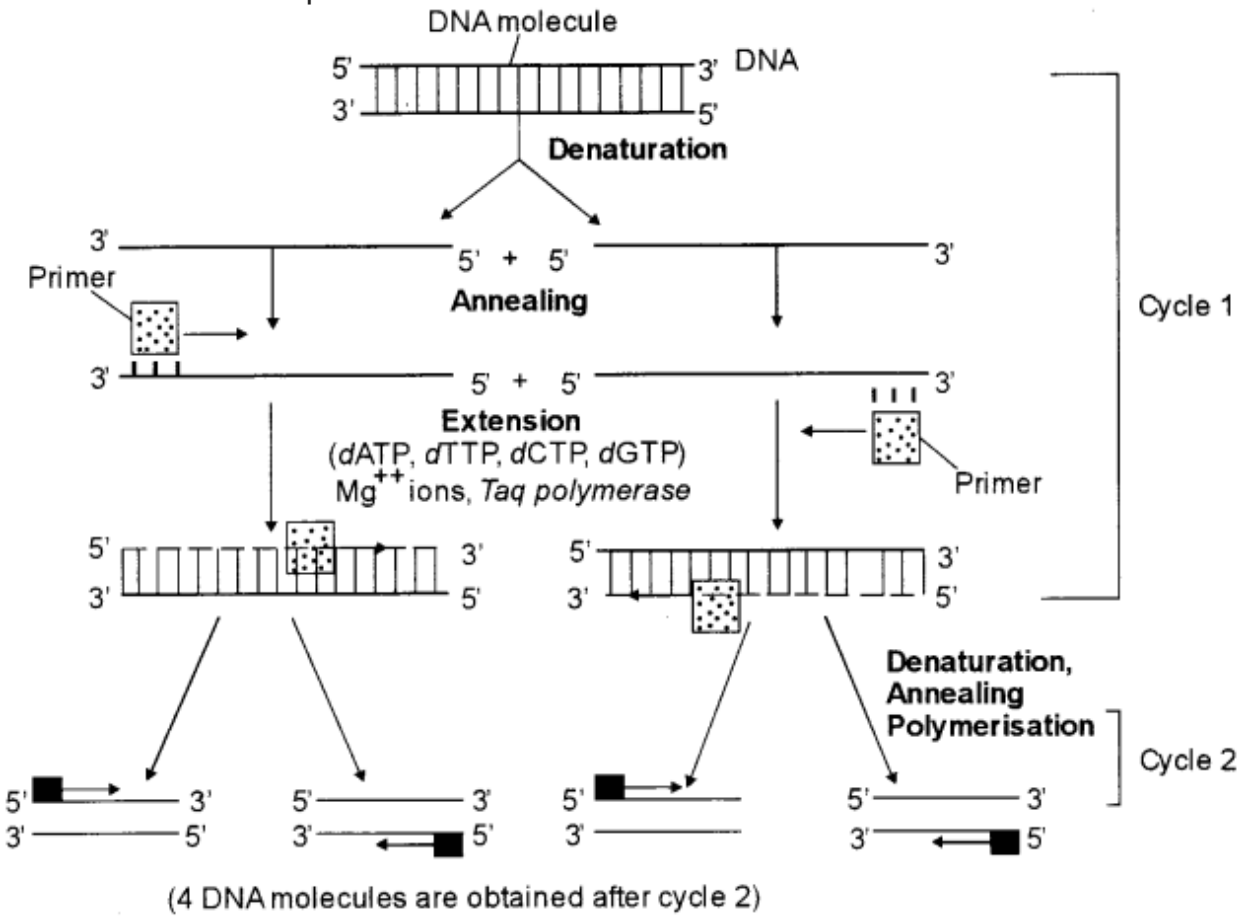
Denaturation : The two strands of DNA are separated by applying a high temperature (95°C). After separation each strand acts as template for DNA synthesis.

Primer annealing : Since nucleotide sequence of each oligonucleotide primer is complementary to 3' end of single stranded template, the primers anneals (hy bridizes) the each template. Annealing is done at low temperature based on length and sequence of the primers. The annealing temperature varies, but the too low temperature favours mispairing. The annealing temperature (°C) can be calculated using the formula T = 2 (AT) + 4(G + C)

Extension (Polymerization): It is the final step of amplification cy cle. In the presence of Mg^{++} and dNTP's (deoxynucleotide triphosphates, e.g., dATP, dGTP, dCTP, dTTP), Taq DNA, polymerase (at 70°C for 60-90 seconds) synthesis through onward extension of primer in 5' -» 3' direction on single DNA template. The concentration of Mg^{++} is maintained between 1 and 4 mM. Thus, in the first step, the target DNA is copied from the primer sites for various distances until the start of second cycle.

The second cycle starts and doubles the DNA molecules synthesized in the first cycle. The second cycle is started with heating of double stranded DNA to result in single

DNA. Each single stranded DNA again acts as template;. e., DNA molecules polymerized in first cycle act as template in second cycle. Following the above events all the single stranded DNA molecules of second cycle are converted into the double stranded DNA. The third and onward cycles are repeated in the same ways to get more DNA products. Always after n number of cycles, 2n molecules of DNA are generated using single stranded DNA as template.



(4 DNA molecules are obtained after cycle 2)

(b) (i) Isoelectric focusing (IEF), also known as electrofocusing. is a technique for separating different molecules by their electric charge differences. It is a type of zone electrophoresis usually performed on proteins in a gel.

(ii) Centrifugation: On the basis of physical properties, cells are separated. For example, the larger and smaller cells are separated by centrifugation. Different components of homogenate are separated on the basis of their shape, size and density. For the separation of cell components high speed centrifuges are used .e.g., zonal centrifuge, density gradient centrifuge.

(c) (i) Glycogen is also known as animal starch and it is the reserve of carbohydrates in animals. It occurs in algae, fungi and yeasts. Glycogen is dextro-rotatory and its hydrolysis yield D- glucose.

(ii) Chitin : It is hard,tough substance that occurs widely in nature particulary in the shells of arthropods such as crab, insects and spider. The walls of hyphae are composed of slightly different chitin. Chemically, chitin is a polysaccharide drived from glucose.

Question 6.
(a) Describe the procedure of sequencing of DNA by Sanger's method. [4]
(b) Explain any two physical and any two chemical methods used to synchronize suspension cultures. [4]
(c) Name any two industrial enzymes and give their uses. [2]

Answer:
(a) DNA sequencing: It is the determination of the precise sequence of nucleotide's in a sample of DNA.

Sanger dideoxy method: The most popular method for DNA sequencing is called the dideoxy method or Sanger method (named after its inventor, Frederick Sanger, who was awarded the (1980) Nobel prize in chemistry).

Procedure : The DNA to be sequenced is prepared as a single strand.
This template DNA is supplied with

a mixture of all four normal (deoxy) nucleotides in ample quantities

- dATP
- r/GTP
- c/CTP
- dT TP

a mixture of all four dideoxynucleotides, each present in limiting quantities and each labelled with a ” tag that fluoresces a different color:

- ddATP
- ddGTP
- ddc TP
- ddlTP

DNA polymerase I

Because all four normal nucleotides are present, chain elongation proceeds normally until, by chance, DNA polymerase inserts a dideoxy nucleotide (shown as colored letters) instead of the normal deoxynucleotide (shown as vertical lines).

If the ratio of normal nucleotide to the dideoxy versions is high enough, some DNA strands will succeed in adding several hundred nucleotides before insertion of the dideoxy version halts the process.

At the end of the incubation period, the fragments are separated by length from longest to shortest. The resolution is so good that a difference of one nucleotide is enough to separate that strand from the next shorter and next longer strand. Each of the four dideoxynucleotides fluoresces a different color when illuminated by a laser beam and an automatic scanner provides a printout of the sequence.

(b) Cells in. suspension cultures vary greatly in size, shape of DNA and nuclear content. Moreover, the cell cycle time varies considerably within individual cells. Therefore, cell cultures are mostly asynchronous. This variation complicates studies of biochemical, genetic physiological and other aspects of cell metabolism. A synchronous culture is one in which the majority of cells proceed through each cell cycle phase (G, S,G_2 and M) simultaneously.

(A) Physical Methods:
Selection by Volume: Synchronization may be achieved on the basis of selecting the size of cell aggregates present even in the finest possible suspension cultures. Cell fractionation is employed for selection.

Temperature Shock: Low temperature shocks combined with nutrient starvation are reported to induce — synchronization of suspension culture.

(B) Chemical Methods:
Starvation : The principle of starvation is based on depriving suspension cultures of an essential growth compound leading to a stationary growth phase. Resupplying the missing compounds is expected to induce resumption of cell growth synchronously. Growth hormone starvation is also reported to induce synchronization of cell cultures.

Inhibition : Synchronization is achieved by temporarily blocking the progression of events in the cell cycle and accumulating cells in a specific stage using a biochemical inhibitor. On release the block cells with synchronously enter the next stage. Inhibitors of DNA synthesis (5-aminourail, 5-fluorodexypunne. hydroxyurea or excess thymidine) in cell cultures accumulate cells at the G_1/S boundary.

(c) Tw o industrial enzymes with uses :
Amylases (from fungi and plants) : Production of sugars from starch, such as in making high fructose corns syrup.
Rennin (derived from the stomach of young ruminant animals): Manufacture cheese used to hydrolyze protein.

Question 7.
(a) Briefly explain the essential features of a vector. [4]
(b) What is the principle of cryopreservation? Mention the steps of cryopreservation. [4]
(c) What is the importance of pH and solidifying agents in cell cultures ? [2]

Answer:
(a) Vectors are the DNA molecules that serve as vehicle to carry a foreign DNA sequence into a host.
Features:
A vector should have the following features :

- origin for replication (ori)
- have a selectable marker such as antibiotic resistance
- should be small in size.
- It must contain at least a unique recognition site for restriction enzyme.

Examples are : Plasmids, Viral DNA, Cosmids, YAC vector, BAC vector, etc.

(b) Cryopreservation involves cooling and storing cells in a frozen state at an extremely low temperature that allows them a higher chances of surviving when thawed. Orthodox seeds have a natural dormancy feature, which allows for their long term storage with little damage to DNA, provided they are kept in a cool, dry environment.

Steps of cryopreservation : Following are the steps of cryopreservation :
Freezing: Freezing generally requires a low molarity of cryoprotectants which are reletively less toxic for a wide range of plant cells. This can be used on a large number of samples which are processed simultaneously. In this technique we can use computerized freezer.

Vitrification : This step does not require an expensive controlled-freezing system. This is more suitable for plant cells which are sensitive to chilling.

(c) Importance of solidifying agents and pH in cell cultures : For preparing a cell culture or media we should always follow the criteria. Every nutrient and condition are important. Depending on the type of the plant cell or tissue used for culture, the composition of nutrient media vary. A cell culture generally require these constituents : Inorganic nutrients, carbon and energy sources, organic supplements, growth regulators, solidifying agents and pH.

Solidifying agents play important role to solidify the culture media properly. Mostly we use Agar for solidify the culture. Agar is a polysaccharide obtained from seaweed. Agar

gel do not react with constituents of media and it does not digested by the plant enzymes.

pH affects the uptake of ions. Always optimum pH of the medium should be maintained before sterilization of the medium. pH between 5.0 to 6.0 is an optimum pH which is required for growth and development of cultured tissues.

Question 8.
(a) Explain how DNA technology has been used to create the following: [4]
(i) Tomatoes with delayed ripening
(ii) Bt crops
(iii) Virus free crops
(iv) Biodegradable plastic.
(b) List the functions of the following bioinformatics tools: [4]
(i) GENSCAN
(ii) ENTREZ
(iii) FASTA
(iv) PIR
(c) Name any two media used in plant tissue culture. [2]

Answer:
(a) (i) Flaw Save tomato is a plant having delayed fruit ripening. This was genetically modified by introducing the genes from a fish to reduce the freezer damage of the tomatoes. This gives a long life to tomatoes.

(ii) Bt crops : Bt cotton and Bt brinjal, these are insect-resistant plants which are produced by introducing a cry gene of Bacillus thuringiensis that encode Cry protein.

(iii) Virus free crops : Transgenic papaya, peeper, tobacco and tomatoes; these are virus free plants. By using tissue culture method we can produce disease-resistant plants.

(iv) Biodegradable plastic : The biodegradable plastic is made from lactic acid which is produced at the time of bacterial fermentation of plant materials like discarded stalks of corn. In the process, molecules of lactic acid are chemically grouped to form the biodegradable plastic. In fact, the biodegradable plastic is a material which has most of the properties of plastic except the property of being non-biodegradable. The biodegradable plastic polyhydroxyalkanates eg., polyhydroxylbutyrate (PHB) are obtained commercially by fermentation with bacterium Alcaligenes eutrophus. The genetically engineered Arabidopsis plants produced polyhydroxylbutyrate (PHB) globules in their chloroplasts without effecting plant growth and development. The large scale of polyhydroxylbutyrate (PHB) can be extracted from leaves as well as from transgenic plants.

(b) (i) GENSCAN: Genscan is a notable example of eukaryotic ab initio gene finders. It is one of the best gene finding algorithms for sequence alignment and gene prediction.

(ii) ENTREZ : This is an integrated information database retrieval system of NCBI. It is mainly used for accessing literature, sequences (both proteins and nucleotides) and structure (3D).

(iii) FASTA: It is a widely used programme for database similarity search. It performs optimised search for local alignment using a substituion matrix. FASTA format is used in a variety of molecular biology software suits.

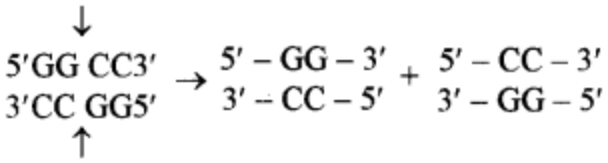
(iv) PIR : PIR is a non-redundant annotated protein sequence database and analytical tools.

(c) There are different types of culture media which are used in plant tissue culture. We use tham as root culture medium, organogenesis medium, cell and callus medium initially.
Following are the medias :
1. Ms medium
2. White medium
3. B5 medium
Ms medium is mostly used in the tissue culture.

Question 9.
(a) What are restriction enzymes? How do they work? What are the different types of restriction enzymes? [4]
(b) Define the term proteomics. Explain the various types of proteomics. [4]
(c) Differentiate between the following: [2]
(i) Local alignment and Global alignment.
(ii) FST and STS.

Answer:
(a) The restriction enzymes are called ‘molecular scissors’. Restriction enzymes are DNA-cutting enzymes present in bacteria. They are obtained from them for use in genetic engineering-rDN A technology. As sequence are cut within the DNA molecule, they are often called restriction endonucleases.

A restriction enzyme recognizes and cuts DNA only at a particular sequence of nucleotides. For example, the bacterium Hemophilus aegypticus produces an enzyme named Hae III that cuts DNA whenever it identifies the recognition sequence.



A cut is made between the adjacent G and C. Hae III cleaves both the strands of DNA at the . same base pairs producing “blunt” ends.
Examples : EcoRI, Hind III and Bam HI.

(b) Proteomics : It is the identification, analysis and large scale production of total protein components of an organism.
Types of Proteomics : Generally, proteomics have so many types but we will only discuss its following three types :

1. Expression proteomics
2. Structural proteomics
3. Functional proteomics

Expression proteomics is the quantitative study of protein expression between the samples which differ by some variables.

Structural proteomics generally deals with the study of the structure and nature of protein complexes which are present in a particular cell organelles.

Functional proteomics generally embraces all proteomics approaches related to devising its functions. In this we use proteomics methods for analysis of properties of molecular networks that are formed in a living cell. This is a complex process.

(b) Global vs local alignments: Illustration of global and local alignments demonstrating the ‘gappy’ quality of global alignments that can occur if sequences are insufficiently similar.

```
Global  FTFTALILLAVAV
        F--TAL-LLA-AV

Local   FTFTALILL-AVAV
        --FTAL-LLAAV--
```

Global alignments, which attempt to align every residue in every sequence, are most useful when the sequences in the query set are similar and of roughly equal size (This does not mean global alignments cannot end in gaps). A general global alignment technique is the Needleman-Wunsch algorithm, which is based on dynamic programming.

Local alignments are more useful for dissimilar sequences that are suspected to contain regions of similarity or similar sequence motifs within their larger sequence context. The Smith-Waterman algorithm is a general local alignment method also based on dynamic programming. With sufficiently similar sequences, there is no difference between local and global alignments.

EST is known as Expressed Sequence Tag. It is a unique stretch of DNA within a coding region of a gene that is useful for identifying full length genes and serves as a landmark for mapping. EST is a sequence tagged site derived form cDNA. STS is a short segment of DNA which occurs but once in the genome and whose location and base sequence are known. STS’s are detectable by PCR.