CLASS XII

There will be two papers in the subject:

***Paper I:** Theory……..* *3 hours ... 70 marks*

***Paper II**: Practical……..* *3 hours ... 15 marks*

Project Work…….. *...10 marks*

Practical File…….. *... 5 marks*

PAPER I: THEORY- 70 Marks

*There will be **one** paper of **three** hours duration divided into **two** parts.*

***Part 1 (20 marks)** will consist of compulsory short answer questions, testing knowledge, application and skills relating to elementary/fundamental aspects of the entire syllabus.*

***Part 2 (50 marks)** will consist of **eight** questions out of which the candidates will be required to answer **five** questions. Each question in this part shall carry 10 marks.*

1. Molecular Biology

(i) Nucleic acids and their estimation: an understanding of nucleic acids, their biochemical structure.

DNA as the genetic material (Hershey and Chase experiment).

DNA (B-DNA)– physical and chemical structure; definition, double helical model of DNA, (Watson and Crick's); Nucleotide and nucleoside; Chargaff's Law, method of replication of DNA, various replicative enzymes in both prokaryotic and eukaryotic organisms, example topoisomerases, helicase, SSBs polymerases, primases, ligases. Concept of semi conservative (with respect to Messelson and Stahl experiment and Taylor et.al experiment on Vicia faba using radiolabelled thymidine) and semi-discontinuous replication, (leading and lagging strands), okazaki fragments.

RNA – definition, various types of RNAs such as mRNA, tRNA (Clover leaf model with diagram; brief introduction to L-shaped model), rRNA their structure and functions.

Techniques of nucleic acid estimation – colorimetry and UV-visible spectrophotometry.

(ii) Protein Synthesis: synthesis of different RNAs, and the complete mechanism of polypeptide chain formation.

Concept of central dogma.

From genes to proteins:

(a) Concept of transcriptional unit, promoter, structural and terminator region; concept of split gene - intron and exon; monocistronic and polycistronic RNA, hnRNA;

(b) Transcription – explanation of the complete process including enzymes involved in the process; Post-transcriptional changes and their significance in eukaryotes – polyadenylation, capping and RNA splicing;

(c) Concept of reverse transcription;

(d) Genetic code – properties of genetic code, start and stop codons, anticodons.

*(e) The translation of RNA to protein – complete mechanism of chain initiation, elongation and termination, the role of tRNA, mRNA and rRNA in protein synthesis. **(Post translational changes not included).***

(iii) Gene regulation in prokaryotes

Operon concept – lac operon and trp operon.

2. Genetic Engineering

(i) Introduction to gene cloning and genetic engineering: concept of cloning and vectors.

Tools of recombinant DNA technology, types of restriction endonucleases and other enzymes used in gene cloning; techniques involved in extraction and purification of DNA from bacterial, plant and animal cells.

Selection of host cells: eukaryotic and prokaryotic.

Vectors: Characteristics and types such as plasmids -pBR322, pUC (in pBR322- presence of two antibiotic resistant genes and in pUCpresence of lac Z gene to be taught), cosmids, phages (M13 and λ), YACs, BACs (to be taught with reference to stability and their carrying capacity), animal and plant viruses (CaMV, retrovirus, SV40 – only names of viruses, no details).

Transfer of recombinants into host cells –

(a) Vectorless methods - basic concept of transformation, transfection, electroporation, liposome mediated gene transfer, microinjection, biolistic

(b) Vector-mediated method - Agrobacterium tumefaciens induced gene transfer.

Methods of identification of recombinants- Direct selection (green fluorescent selection) and Insertional inactivation (Blue-white selection, antibiotic resistance).

A basic understanding of DNA libraries – construction of genomic and cDNA libraries.

Construction of a recombinant DNA molecule.

(ii) Innovations in Biotechnology: produced by using modern biotechnological tools, (select examples of products already available)

(a) Plants: Production of Flavr Savr tomatoes, Bt-crops and Golden rice.

(b) Healthcare: Production of recombinant hepatitis-B vaccine, Humulin, interferon and edible vaccines.

(c) Animal: Dolly the cloned sheep, Sources and characteristics of stem cells and their applications.

(d) Environmental biotechnology: bioremediation using oil-eating bacteria as an example.

(e) Industrial biotechnology: applications of industrial enzymes – rennet, subtilisin, amylase, papain.

(iii) Gene analysis techniques: various techniques involved in recombinant DNA technology.

DNA probes – definition and use.

Low resolution mapping techniques: gel electrophoresis, southern blotting (details of the technique to be taught), western and northern blotting (a brief idea and their uses).

High resolution techniques: DNA sequencing- sequencing by chain termination, automated DNA sequencing. Site directed mutagenesis.

DNA amplification by Polymerase chain reaction (PCR)– applications of PCR, steps and application of DNA profiling or DNA finger printing.

3. Cell culture technology

A brief idea of tools and techniques involved in cell culture technology and their applications in microbial, plant tissue and animal cell cultures respectively.

(i) General tools and techniques used in cell culture technology

(a) Instruments - centrifuge, LAF hood and biosafety cabinets, pH meter, autoclave, vortex mixer, hot air oven, magnetic stirrer, weighing balance, micro filtration unit, incubator, CO_2 incubator, inverted microscope, bioreactor (diagram, its components and their function)-stirred tank and sparged type (brief idea only), use of T flasks to propagate animal cells.

Only uses of the above instruments to be studied.

(b) Sterilization techniques for culture room, apparatus, transfer area, media, vitamins, and living material;

(c) Cryopreservation (need and steps).

(d) Cell counting (direct counting by haemocytometer), cell viability by Evan's blue stain and cell sorting (FACS only)

(e) *Types of media (synthetic /defined, semi-synthetic/differential, complex/natural)*

Preparation of media: microbial media- LB agar and LB broth; Plant media-MS and White's media; Animal media- RPMI, DMEM and FBS - brief idea only. (includes inorganic and organic macronutrients and micronutrients, antibiotics, growth regulators for plants: auxins and cytokinins).

Importance of pH and solidifying agents.

(ii) Microbial culture and its application.

Fermentation process and growth kinetics- batch culture, fed batch culture, continuous culture (with the help of graphs only): Definition of turbidostat and chemostat: Products and application-SCP (definition and use), industrial enzyme-subtilisin (source and its use).

(iii) Plant tissue culture and its application.

Isolation of single cell by mechanical and enzymatic methods, synchronisation of cell culture by chemical methods like starvation, inhibition and mitotic arrest.
Cellular totipotency-definition of cellular differentiation, de-differentiation, re-differentiation. Application of plant cell culture technology ***(methodology not required, only brief idea needed):***

(a) Haploid production-androgenesis and gynogenesis and their significance.
(b) Triploid production-understanding and need for triploid production and its application (seedless crops).
(c) In-vitro pollination- concept and its application.
(d) Zygotic embryo culture- concept and its application, Embryo rescue (brief idea only).
(e) Somatic hybridisation-protoplast fusion (Pomato).
(f) Micropropagation and its significance.
(g) Developing virus free plants and synthetic seeds.
(h) Biodegradable plastics (concept of PHB).

(iv) Animal cell culture and its application.

Primary cell culture with mechanical and enzymatic disaggregation and its drawbacks; Types of cell-lines: finite, continuous, adherent and suspension; scale up-mono layer by Roller bottle, application of animal cell culture-tissue, hybridoma technology, tissue engineering (definition only).

4. Bioinformatics

(i) Introduction to bioinformatics; global bioinformatics databases and data retrieval tools; genomics, different types of sequences, types of sequence analysis.

Introduction to bioinformatics: definition and need.

An introduction to global bioinformatics databases (nucleotide and protein databases). Information sources such as EMBL, NCBI, DDBJ, SWISSPROT, GenBank, GENSCAN.

Data retrieval tools- ENTREZ, Taxonomy Browser.

(ii) Genomics: Definition, introduction, tools used in Genomics and its applications.

Definition of genomics. Types of genomics- structural and functional. Basic criteria in selecting the organism for its genome sequencing. Different types of sequences – cDNA, genomic DNA, ESTs (Expressed Sequence Tags) and STSs (Sequence Tagged Sites) and the different softwares (example gene scan).

Types of sequence analysis by using BLAST and FASTA –global, local, pair wise and multiple.

Human Genome Project - its objectives, the countries involved, its achievements and significance.

DNA microarray technology – definition and application only.

Concept of Single Nucleotide Polymorphisms (SNPs).

(iii) Proteomics: definition, introduction and databases.

Types of Proteomics – structural, functional and expression; Important protein databases available for the public on the internet like PDB (Protein Data Bank), PIR (Protein Identification Resources).

PAPER II

PRACTICAL WORK – 15 marks

Candidates are required to complete the following experiments.

1. Paper Chromatography – separation of photosynthetic pigments

   Take any leaf. Extract chlorophyll in 80% acetone. Take a strip of paper or prepare a thin layer of silica gel on a slide. Load chlorophyll extract at one end of the paper/gel. Keep paper or gel in the rising medium in test tube or jar for about 30 minutes. The rising medium should have methanol/ acetic acid, n-butanol or benzene. The rising fluid should always be at the bottom below the point of loading of chlorophylls. After 30 minutes, three spots: yellow, bluish green and light green will be observed corresponding to carotenes, chlorophyll A & chlorophyll B.

2. Preparation of buffers – phosphate, acetate and borate buffers

   This experiment should be done to make the basics clear to the students. Basic calculation for buffer preparation should be known. The approach should be to utilize easily available chemicals at reasonable costs. Phosphate, borate and acetate buffers can give the range of pH 4 - pH 9.2

3. Preparation of culture media

   (i) Bacterial culture Media - Luria Bertani (L.B.) media - Peptone/ Tryptone, yeast extract and NaCl. (Nutrient broth / Nutrient Agar).

   (ii) Plant Tissue culture medium (Sugars + Coconut milk + Agar Agar).

4. Sterilization of culture medium and other materials.

   (i) Dry Physical method – heat or radiation.

   (ii) Wet Physical methods – steam sterilization.

   (iii) Chemical Sterilization/ Surface sterilization Disinfection with 70% alcohol and Sodium hypochlorite solution carbolic acid

5. Preparation of various forms of culture media – Petri plate, slant and suspension.

   Luria Bertani (L.B) media to be prepared, autoclaved and cooled to 60 degrees C. To prepare nutrient plates the media is poured into presterilized petri-dishes under a LAF. To prepare slants the media is poured into several test tubes, plugged and kept in a tilted position (at an angle of 45°) until it sets.

6. Inoculation and incubation of *Lactobacillus* on the culture medium in the Petri plate.

   Use of inoculation loop or inoculation needle for the purpose.

7. Identification of bacteria by Gram +ve and Gram –ve (from curd /saliva and/or soil solution)

   (i) Prepare a bacterial smear on a slide (ii) Stain with crystal violet stain. (iii) Rinse with water. (iv) Add a few drops of iodine solution. (v) Add few drops of 90 % ethanol (vi) Counterstain with safranin solution (vii) Observe the red and blue colonies under the microscope

8. Action of enzymes on starch under: (a) variable temperature (b) variable substrate concentration – plotting of K_m value by graph

   (i) Soluble starch solution (0.5% - 1%) to be prepared. Test with iodine. Collect saliva, dilute 1: 5, add 1 ml of saliva to 10 ml of starch solution. Incubate for 15 minutes. Again test for presence of starch with iodine. Also test for the presence of reducing sugars in solution. Repeat the same process at the variable volumes of starch

   (ii) To study the effect of variable temperature on the activity of the enzyme salivary amylase.

9. Isolation of DNA from plants

   Take half a ripe and peeled banana into a beaker and add 50 ml of extraction fluid (1.5gm table salt +10 ml liquid detergent +90 ml distilled water). Place the beaker in a water bath set at 60 degrees C for 15 minutes. Stir gently with a glass rod. Filter 5ml of cooled content into a clean test tube and add 5ml of cold 90% ethanol. DNA molecules separate out and appear as white fibres. [DNA can also be extracted from pea seeds and soaked wheat grains]

10. DNA estimation by colorimeter by DPA method.

11. Protein estimation by colour reaction – Bradford test.

    Bradford's Assay is a Dye binding assay based on the differential change of colour of a dye in response to various concentrations of proteins. Bradford's assay can be performed for qualitative as well as quantitative assessment of proteins in a sample.

    Dilute 1 volume of Bradford's dye with 4 volumes of distilled water. Filter the dye through Whatman filter paper and store at room

temperature in a brown glass bottle. Take different aliquots of standard Bovine Serum Albumin (BSA solution), for example (0.2, 0.4, 0.6, 0.8 and 1.0 ml) in different test tubes Make up the volume to 1ml with distilled water. To each tube add 2ml of Bradford's dye. Extent of colour development can be made by rough estimate using + signs to show the concentration of protein in the sample. Alternatively, OD can be read using colorimeter or spectrophotometer. Take the unknown sample to be estimated and perform the experiment. Similarly read the OD and note the corresponding concentration of protein in it using the graph.

12. Cell viability test by Evan's blue dye.
13. Isolation of milk protein – wet weight and dry weight.

    Milk proteins are isolated by adding 0.4 N HCl into the milk sample. Casein start coagulating at its isoelectric point (i.e. at pH 4.6). The precipitate is filtered and weighed to quantify the protein present.
14. Chromatography to find adulteration in spices by using mixer of turmeric and metanil yellow.
15. Demonstration of cell counting by haemocytometer by using diluted blood.
16. Experiment to show the process of saponification.

PROJECT WORK AND PRACTICAL FILE – 15 Marks

Project Work – 10 Marks

The Project Work is to be assessed by a Visiting Examiner appointed locally and approved by the Council.

Candidates are to creatively execute **one** project / assignment on an aspect of Biotechnology.

Teachers may assign or students may choose any one project of their choice. The report should be kept simple, but neat and elegant.

A list of suggested projects is as follows:

1. Effluent analysis.
2. A study of the technological details of malt preparation.
3. A study of the technological details of the brewing industry.
4. A study of the organisation of a fermenter.
5. Technological analysis of the process of drug development, drug designing and drug targeting.
6. A study of the technological details of vaccine development.
7. Diagnosis of diseases by modern techniques like ELISA, RIA and Antibody targeting.
8. DNA finger-printing.
9. DNA foot-printing.
10. Microbiological contaminants in food and food products.
11. Isolation of microbes from air, water and soil.
12. Methods of identifying microbes (various staining techniques and biochemical reactions).
13. Tissue Culture and its applications.
14. Stem Cell Technology
15. Nanotechnology
16. Bioinformatics
17. Genetic Engineering
18. Cloning
19. Instrumentation in biotechnology
20. Forensic Biotechnology
21. Ethical, Legal and Social Issues (ELSI) related to Biotechnology/ GMOs
22. Biopiracy- Case Studies

Practical File – 5 Marks

The Visiting Examiner is required to assess students on the basis of the practical file maintained by them during the academic year.

Suggested Evaluation Criteria for Project Work:

Format of the Project:

- Content
- Introduction
- Presentation (graphs, tables, charts, newspaper cuttings, diagrams, photographs, statistical analysis if relevant)
- Conclusion/ Summary
- Bibliography

LIST OF EQUIPMENT FOR BIOTECHNOLOGY PRACTICALS FOR CLASSES XI & XII

1. Table-top Centrifuge
2. Vortex - Mixer
3. Thermostatic water-bath
4. Spectrophotometer (UV visible range)/ Colorimeter
5. Refrigerator
6. Lactometer
7. pH meter
8. Hot air oven
9. Autoclave
10. Desiccators
11. Micro-filtration unit
12. Incubator
13. Magnetic stirrer with hot plate
14. Laminar flow cabinet
15. Weighing Balance (Electrical)
16. Hot plate
17. Binocular Microscope
18. Haemocytometer
19. Colony counter
20. Antiserum
21. Electrophoresis chamber
22. Micropipettes

LIST OF ABBREVIATIONS TO BE STUDIED

1. BAC: Bacterial Artificial Chromosomes
2. BLAST: Basic Local Alignment Search Tool
3. CTAB: Cetyl Trimethyl Ammonium Bromide
4. DBM: Diazo–benzyl oxy–methyl paper
5. DDBJ: DNA Database/ Data Bank of Japan
6. ddNTP: Dideoxy Nucleoside triphosphate
7. DMEM: Dulbecco Modified Eagle Medium
8. EBI: European Bioinformatics Institute
9. EMBL: European Molecular Biology Laboratory
10. EST: Expressed Sequence Tag
11. FACS: Fluorescence Activated Cell Sorting
12. FASTA: Fast All
13. FBS: Foetal Bovine Serum
14. HEPA: High Energy Particulate Air
15. HGP: Human Genome Project
16. IBPGR: International Board of Plant Genetic Resources
17. ICGEB: International Centre for Genetic Engineering and Biotechnology
18. IFN: Interferon
19. LB medium: Luria and Bertani Medium
20. MS medium: Murashige and Skoog medium
21. NCBI: National Centre for Biotechnology Information
22. NHGRI: National Human Genome Research Institute
23. PAGE: Polyacrylamide Gel Electrophoresis
24. PCR: Polymerization Chain Reaction
25. PDB: Protein Database/ Data Bank
26. PHB: Poly 3–Hydroxyl Butyrate
27. PIR: Protein Information Resource
28. RFLP: Restriction Fragment Length Polymorphism
29. RNA: Ribonucleic acid
30. RPMI medium: Roswell Park Memorial Institute medium
31. SCP: Single Cell Protein
32. SDS – PAGE: Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis
33. SNP: Single Nucleotide Polymorphism
34. SSBs: Single Stranded Binding Proteins
35. STS: Sequence Tagged Site
36. VNTR: Variable Number of Tandem Repeats
37. YAC: Yeast Artificial Chromosome