ISC

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YEAR 2028



BIOTECHNOLOGY

(878)

Developed by:
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CISCE

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- Minimum monitoring.
- Allowing schools to evolve their own niche.
- Catering to the needs of the children.
- Giving freedom to experiment with new ideas and practices.
- Diversity and plurality - the basic strength for evolution of ideas.
- Schools to motivate pupils towards the cultivation of:
  Excellence - The Indian and Global experience.
  Values - Spiritual and cultural - to be the bedrock of the educational experience.
- Schools to have an 'Indian Ethos', strong roots in the national psyche and be sensitive to national aspirations.

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

S. NO.	UNIT	WEIGHTAGE (Marks)
1.	**Molecular Biology**	15
2.	**Genetic Engineering**	23
3.	**Cell culture technology**	20
4.	**Bioinformatics**	12
	Total	70

1. Molecular Biology

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

Units of nucleic acids: Nucleotides. Difference between nucleosides and nucleotides.

DNA – its structure (as suggested by Watson and Crick), Chargaff's Equivalence Rule, DNA as the genetic material (Hershey and Chase experiment).

RNA and its types – structure and roles of mRNA-monocistronic and polycistronic types, tRNA, rRNA.

tRNA- an introduction to Clover leaf model and inverted L-shaped model.

Replication of DNA- Messelson-Stahl Experiment and Taylor et. al experiment, with reference to leading and lagging strands, enzymes and proteins involved in replication in prokaryotes viz. Topoisomerase, helicase, SSBPs, primase, DNA polymerase, DNA ligase.

DNA proofreading and repair by DNA Polymerase I and II.

Estimation methods for nucleic acids using UV-visible spectrophotometry and colorimetry.

(ii) Protein Synthesis: Central dogma, transcription and translation.

Concept of central dogma.

(a) Transcriptional Unit: promoter region, structural region and terminator region; Split genes – (exons and introns); hnRNA.

(b) Process of transcription - Initiation, Elongation and termination including enzymes involved.

(c) Post-transcriptional changes their significance in *eukaryotes – polyadenylation, capping and RNA splicing; role of snRNA in splicing.*

(d) *Genetic code – and their properties: with special reference to start and stop codons.*

(e) *Translation* process - *Initiation, elongation and termination,*

Post translational - modification in proteins (i.e. glycosylation, protein folding, proteolytic degradation, intein splicing).

Reverse transcription - process (an exception to Central Dogma.

(iii) Gene regulation in prokaryotes

Types of Operons: Inducible and Repressible.

Lac operon and trp operons – their structure and working.

2. Genetic Engineering

(i) Introduction to gene cloning and genetic engineering: DNA extraction from the source cell, host selection.

Construction of recombinant DNA.

Tools: enzymes such as restriction endonucleases – (Type I, II and III), DNA ligase, alkaline phosphatase, Taq Polymerase, RNase, S1 nuclease, polynucleotide kinase, alongside exonuclease, DNA polymerases I, II and III. DNA isolation from bacterial cells, plant cells and animal cells and its purification using CTAB method.

Vectors used in gene cloning ex. plasmids – (pBR322, pUC), Phages (M13 and λ), Phagemids, Cosmids, cloning vectors, expression vectors, YACs, and BACs and their specific carrying capacities.

(a) *Transformation of host cells:*

- *Vector-less: electroporation, liposome mediated, microinjection and biolistics.*
- *Vector mediated method: Agrobacterium mediated.*

(b) *Identification of recombinants-Direct selection by Green Fluorescent Protein (GFP), Insertional inactivation (antibiotic resistance gene, Blue-white selection).*

(c) *Genomic DNA and cDNA libraries.*

(ii) Innovations in Biotechnology:

(a) *Development of Flavr Savr tomatoes, Bt-crops, Golden rice.*

(b) *Gene editing, Gene therapy using CRISPR-Cas9 technology to insert knockout genes for gene therapy of sickle cell anaemia and cancer.*

(c) *Development of Humulin, recombinant interferons.*

(d) *Recombinant Hepatitis B vaccine, mRNA based vaccine against Covid-19, stem cell therapy.*

(e) *Transgenic animals ex. Dolly Sheep, Rosie cow, ATryn goat, development of artificial meat without slaughter.*

(f) *Bioremediation using oil-eating bacteria (Super Bug). Mycoremediation* (*Pleurotus ostreatus to digest diesel and motor oils), Phycoremediation (Chlamydomonas for heavy metals and crude oils).*

(g) *Industrial enzymes: Rennet, Subtilisin, Amylase, Papain – their sources and applications.*

(iii) Gene analysis:

(a) *DNA probes – definition and use.*

(b) *Process and applications of Gel electrophoresis, Southern blotting, Western and Northern blotting.*

(c) *DNA sequencing – Sanger's Method of Chain termination and automated DNA sequencing method.*

(d) *Site directed mutagenesis with an example of human insulin.*

(e) *Process and application of Polymerase chain reaction (PCR), RT-PCR, DNA finger printing.*

3. Cell culture technology

Different cell culture technologies and their applications such as microbial, plant tissue and animal cell cultures.

(i) Microbial culture:

Fermentation process and growth kinetics-batch, fed batch, continuous cultures -principles, products obtained and graphs.

Process of turbidostat and chemostat- forms of open continuous culture.

Bioprocess engineering like bioreactor designing – its components, downstream processing with respect to antibiotic production.

Subtilisin, Production of SCP - large scale production of mycoprotein (using Yeast) by fermentation.

(ii) Plant tissue culture:

Cell isolation, synchronisation of isolated cells using chemical methods.
Totipotency – definition and concept of cellular differentiation, de-differentiation, re-differentiation with respect to callus formation.

(a) Process of Haploid production (androgenesis and gynogenesis) and Triploid production (endosperm culture).

(b) Process of In-vitro pollination and Embryo rescue.

(c) Embryo culture, Somatic hybridisation - Pomato.

(d) Process of Micropropagation, Virus resistant plants by RNAi.

(e) Somatic embryogenesis and production of synthetic seeds, PHB (bioplastics).

(iii) Animal cell culture:

Primary culture, secondary culture or Sub-culture - suspension (with suspension cell line) and monolayer (adherent cell line), cell-lines and its types: finite and continuous. Scale up using Roller bottle culture technique.

Process of hybridoma technology, tissue engineering and CAR-T therapy by genetically engineered T-cells to cure cancer.

4. Bioinformatics

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

Definition and need.

Bioinformatics databases: EMBL, NCBI, DDBJ, SWISSPROT, GenBank.

Data retrieval tools- ENTREZ, Taxonomy Browser.

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

Definition of genomics. Types: Structural, Functional, and Metagenomics. Sequences – cDNA, ESTs STSs.

Tools: BLAST and FASTA; global, local, pair wise and multiple sequence alignments.

Rice Genome Project and Human Genome Project - objectives, achievements, countries involved, people contributing in it.

DNA microarray: Definition and application.

Concept of SNPs.

(iii) Proteomics:

Types - Structural, functional and expression.

Databases: PDB, PIR, SWISSPROT.

Protein Engineering - definition, principle and application.

AI powered Drug Designing and Drug Targeting (AlphaFold2 as AI for vaccine against Covid-19 and cancer therapy).

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 (LB, MS) as slants and suspensions.

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

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

   Medium to be prepared, autoclaved and cooled to 60 degrees C. To prepare nutrient plates the media is poured into pre-sterilized 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^{o}) until it sets.

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

   Use of inoculation loop or inoculation needle for the purpose.

6. 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

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

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

(ii) 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.

8. 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].

9. DNA estimation by colorimeter by DPA method.
10. 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.

11. Cell viability test by Trypan blue dye.
12. 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.

13. Demonstration of cell counting by haemocytometer by using diluted blood.
14. Experiment to show the process of saponification.
15. Separation of amino acids by paper chromatography.

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 CISCE.

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.

LIST OF SUGGESTED PROJECTS:

1. Effluent analysis.
2. Role of biotechnology in brewing industries – viz. production of beer, wine etc.
3. Drug designing and drug targeting through AI and proteomics.
4. Vaccine/drug development.
5. Diagnosis of diseases by modern techniques like ELISA, RIA and Antibody targeting.
6. DNA Fingerprinting.
7. Methods of identifying microbes (various staining techniques and biochemical reactions).
8. Tissue Culture and its applications.
9. Stem Cell Technology.
10. Nanotechnology.
11. Bioinformatics.
12. Cloning – gene and animal.
13. Forensic Biotechnology.
14. Ethical, Legal and Social Issues (ELSI) related to Biotechnology/ GMOs.
15. 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 ABBREVIATIONS TO BE STUDIED

1. AI: Artificial Intelligence
2. BAC: Bacterial Artificial Chromosomes
3. BLAST: Basic Local Alignment Search Tool
4. CAR: Chimeric Antigen Receptor
5. COVID-19: Corona Virus Disease 2019
6. CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
7. Cas9: CRISPR-associated protein 9.
8. CTAB: Cetyl Trimethyl Ammonium Bromide
9. DDBJ: DNA Database/ Data Bank of Japan
10. ddNTP: Dideoxy Nucleoside triphosphate
11. DMEM: Dulbecco Modified Eagle Medium
12. EMBL: European Molecular Biology Laboratory
13. EST: Expressed Sequence Tag
14. FACS: Fluorescence Activated Cell Sorting
15. FASTA: Fast All
16. FBS: Foetal Bovine Serum
17. HEPA: High Energy Particulate Air
18. HGP: Human Genome Project
19. hnRNA: heterogeneous nuclear RNA
20. LAF: Laminar Air Flow
21. IFN: Interferon
22. LB medium: Luria and Bertani Medium
23. MS medium: Murashige and Skoog medium
24. NCBI: National Centre for Biotechnology Information
25. PAGE: Polyacrylamide Gel Electrophoresis
26. PCR: Polymerase Chain Reaction
27. PDB: Protein Database/ Data Bank
28. PHB: Poly 3–Hydroxyl Butyrate
29. PIR: Protein Information Resource
30. RFLP: Restriction Fragment Length Polymorphism
31. RNA: Ribonucleic acid
32. RNAi: RNA Interference
33. RPMI medium: Roswell Park Memorial Institute medium
34. RT PCR: Reverse Transcription Polymerase Chain Reaction
35. SCP: Single Cell Protein
36. SDS – PAGE: Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis
37. SNP: Single Nucleotide Polymorphism
38. snRNA: Small Nuclear RNA
39. SSBs: Single Strand Binding Proteins
40. STS: Sequence Tagged Site
41. Taq: *Thermus aquaticus*
42. VNTR: Variable Number of Tandem Repeats
43. YAC: Yeast Artificial Chromosome

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