POOL OF DISCIPLINE SPECIFIC ELECTIVES

DISCIPLINE SPECIFIC ELECTIVE COURSE (DSE -07): Recombinant DNA Technology and Proteomics

CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course (if any)
		Lecture	Tutorial	Practical/ Practice		
Recombinant DNA Technology and Proteomics BOT-DSE-07	**4**	**2**	**0**	**2**	Class XII pass with Biology/ Biotechnology	**Nil**

Learning Objectives: This course structure is designed to:

- familiarize the students with the essential knowledge and technical skills/ methodology involved in creating recombinant DNA molecules.
- provide knowledge on generating modified organisms, synthesize a product or modify a biological process by tailoring and/or incorporating DNA from one organism into another.

Learning outcomes:

After completion of the course students will:

7. be able to identify, locate, isolate and functionally characterize DNA sequences/genes.
8. Get familiarized with technologies used to create recombinant DNA.
9. be able to design strategies adopted to generate genetically modified organisms for various applications.
10. be aware of the application of recombinant DNA in pharmaceuticals, agriculture, environment management, etc.

Unit 1: **Enzymes inrecombinant DNA technology** **04 hours**

Nucleases: DNAses, RNAses, Restriction endonucleases (discovery, classification, isoschizomers and cleavage action), exonucleases, polymerases (DNA, RNA, Reverse transcriptase, *Taq* polymerase), ligases, kinases, alkaline phosphatase.

Unit 2: Cloning vectors **04 hours**

Plasmids (basic features and types - pBR322, pUC18, pUC19, TA vectors), lambda vectors (insertion and replacement vectors), M13, cosmids and phagemids, pBluescript II; Artificial chromosomes as vectors (BACs, YACs). Expression vectors and shuttle vectors, YeP; strategies for over-expression of proteins

Unit 3: Isolation and cloning of target DNA **03 hours**

PCR, Strategies: isolation/generation of target sequence (restriction-based and PCR-based), generation of compatible cohesive ends, linkers and adaptors.

Unit 4: **Creating and screening DNA libraries** **03 hours**

Construction of genomic and cDNA libraries, screening and identification of target sequence by DNA hybridization and immunological methods.

Unit 5: **Introduction of DNA into host cell** **06 hours**

Preparation and transformation of competent bacterial cells (heat shock and electroporation). DNA delivery into plant cells and protoplasts: *Agrobacterium* mediated (disarmed Ti plasmid), electroporation, microinjection, liposomes and biolistic methods). Selection and identification of transformants (alpha-complementation, antibiotic resistance and reporter genes (GUS and GFP).

Unit 6: Protein purification and Identification **03 hours**

Chromatography-based methods (ion exchange chromatography and affinity chromatography), antibody-based methods (ELISA and Western blotting).

Unit 7: **Proteomics** **04 hours**

Introduction to proteomics: gel-based methods (Native and SDS PAGE, 2D gel electrophoresis, differential gel electrophoresis), mass spectrometry.

Unit 8: Applications **03 hours**

Application of recombinant DNA technology and Proteomics in medicines (insulin, vaccines), agriculture (insecticide delta endotoxin, golden rice, antisense strategy in tomatoes).

Practicals **60 hours**

15. Plasmid DNA isolation using Bacterial cultures.
16. Agarose Gel electrophoresis of plasmid DNA
17. Quantification of DNA by spectrophotometry
18. Extraction of protein and its Quantification by Lowry's method

19. Constructing Restriction map of linear and circular DNA using the data provided
20. Study of recombinant DNA techniques through photographs (Biolistic technique, electroporation, microinjection, PCR, western blotting, artificial chromosomes YAC, BAC, Cosmid, Phagemid, Ti plasmid)
21. Demonstration of SDS-PAGE and affinity Chromatography

Suggested reading:

- Brown, T. A. (2016) Gene Cloning an Introduction: Chapman & Hall.
- Zlatanova, J. and Van Holde, K.E. (2016) Molecular Biology Structure and Dynamics of Genomes and Proteomes: Taylor and Francis; .
- Glick, Bernard R, Jack J. Pasternak, Patten Cheryl L. 2018. Molecular Biotechnology; principles and applications of recombinant DNA, ASM Press, Washington.
- Lovric, J., 2011. Introducing Proteomics. Wiley-Blackwell
- S.B. Primrose, R. M. Twyman, R.W.Old. 2001. Principles of Gene manipulation: Blackwell Science; 2001

Additional reading:

- Banks, K (2022) Introduction to Proteomics. Larsen & Keller Education
- Kreuzer, H. Massey, A (1996) Recombinant DNA and Biotechnology; A guide for teachers; ASM Press.

Note: Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.