Tool in Biotechnology (2nd Ed.). Springer.

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
		Lecture	Tutorial	Practical/ Practice		
Recombinant DNA Technology and Proteomics **ALS BOT GE 04**	**4**	**2**	**0**	**2**	NIL	**NIL**

Objectives:

- To illustrate the use of modern techniques for the manipulation and analysis of DNA sequences.
- To learn to clone, analyse and modify the genetic material
- To understand the applications of recombinant DNA technology for the generation of commercial biotechnological products of diverse usage.
- To gain knowledge about biosafety and ethical concerns associated with recombinant DNA technology.

- To acquaint the students with proteome and its analysis
- To train students in strategizing research topics employing genetic engineering techniques.

Learning Outcomes:

Students would learn about

- technical know-how on modern techniques involved in manipulation and analysis of nucleic acids, Gene cloning for the creation of genetically modified organisms (GMOs).
- details of restriction endonucleases, marker and reporter genes, the repertoire of various vectors, construction of genetic libraries, screening methods and gene identification.
- applications of PCR, hybridization techniques and sequencing in basic and applied experimental biology.
- biosafety and ethical issues associated with rDNA technology
- designing and conducting experiments involving genetic manipulation.

Theory:

Unit 1. Introduction to Recombinant DNA technology and Gene cloning:

Hours: 05

Introduction to rDNA and Genetic Engineering, Restriction endonucleases - Discovery, Nomenclature and applications of Type I - Type IV, Gene cloning - steps and applications, Bacterial transformation, strategies for selection and screening, Introduction to marker and reporter genes (GUS, GFP and Luciferase).

Unit 2. Vectors in gene cloning and transfer: Hours: 09

Plasmids (pBR322, pUC18/19, Blue-white screening and α-complementation); Lambda based vectors and derivatives (Insertion vectors, replacement vectors, cosmids, phagemids); Artificial Chromosomes (BACs and YACs), plant transformation vectors (Ti plasmid), Gene construct, Protein Expression Vectors for use in *E. coli*. Construction of genomic and cDNA libraries, screening methods for locating the desired gene (Replica plating, Complementation screening, Heterologous gene probe-based hybridizations). Biosafety concerns.

Unit 3. Polymerase chain reaction (PCR), nucleic acid hybridization and sequencing technologies: Hours: 07

PCR technique and its applications, RT-PCR, Hybridization based assays (Southern blotting and hybridization and detection of RFLPs), Northern and Western blotting and hybridization, Restriction maps: construction and importance in navigating genomes, Sanger's di-deoxy chain termination method of sequencing and autoradiography and fluorescence dye chemistry, slab gel-based electrophoresis (semi-automated) to capillary-based gel electrophoresis (automated sequencing).

Unit 4. Proteomics: **Hours:09**

Introduction and Scope of Proteomics, Post-translational modifications, Protein separation techniques - Electrophoresis (PAGE, SDS-PAGE, 2D-gel electrophoresis) and Column chromatography, Protein identification through Mass Spectroscopy - principle, ionization (MALDI, MALDI-TOF, ESI), Structural Proteomics - through NMR and X-ray crystallography, protein-protein interaction.

Practical:

- Isolation of genomic and plasmid DNA from bacteria.
- Quantification of extracted DNA by DPA (Diphenylamine) method.
- Estimation of proteins by Lowry's method.
- Restriction digestion and AGE (Agarose gel electrophoresis) of DNA.
- Restricting Mapping of linear and circular DNA.
- Study of techniques using digital resources/demonstration: PCR, RT-PCR, Real-time PCR, Southern, Northern and Western blotting and hybridization.
- Study of techniques using digital resources/demonstration: SDS-PAGE, 2D-PAGE, MALDI, NMR, X-ray crystallography.
- Study of applications of rDNA technology using digital resources/ in silico studies: recombinant insulin, interferon and human growth hormone.
- Demonstration of equipment used in rDNA technology: Thermocycler, Laminar air flow, Autoclave, Incubator shaker, Refrigerated centrifuge.

Essential/recommended readings

1. Green, M.R., & Sambrook, J. (2012). *Molecular Cloning: A Laboratory Manual* (4th Ed.). Cold Spring Harbor.
2. Wink, M. (2011). *An Introduction to Molecular Biotechnology: MolecularFundamentals, Methods and Applications in Modern Biotechnology* (2ndEd.). Wiley.

3. Glick B.R., & Patten C.L. (2022). *Molecular Biotechnology: Principles & Applications of Recombinant DNA* (6th Ed.). ASM Press.
4. Snustad, D. P., & Simmons. M.J. (2012). *Principles of genetics* (6th ed.). John Wiley & Sons.
5. Brown, T.A. (2010). *Gene cloning and DNA analysis: an introduction.* John Wiley &Sons.

Suggestive readings

1. Primrose, S. B., & Twyman, R. (2009). *Principles of gene manipulation and genomics.*Wiley.
2. Howe, C. J. (2007). *Gene cloning and manipulation*. Cambridge University Press.
3. Liebler D. C. (2002) *Introduction to Proteomics: Tools for the New Biology*. Humana Press Inc.
4. Scopes R. K. (1994) Protein Purification: Principles and Practice, Springer.
5. Albala J.S. & Smith I.H. (Eds.). (2003). *Protein Arrays, Biochips and Proteomics: The Next Phase of Genomic Discovery* (1st ed.). CRC Press.

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