DISCIPLINE SPECIFIC CORE COURSE –20:
PRINCIPLES OF RECOMBINANT DNA TECHNOLOGY

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		
MICROB-DSC801: **PRINCIPLES OF RECOMBINANT DNA TECHNOLOGY**	**4**	**3**	**0**	**1**	**Class XII pass with Biology/ Biotechnology/ Biochemistry**	

Learning Objectives

The Learning Objectives of this course are as follows:

- The main objective of this course is to enable students to develop a clear comprehension of the concepts of recombinant DNA technology and identify its potential.
- Students will get acquainted with the major tools used to manipulate DNA, and will become familiar with various methods and applications of cloning. They will be brought abreast with recent high throughput technologies and gain knowledge of recombinant products of agricultural and human importance.
- Students will be able to design a suitable strategy towards developing a genetically modified organism.

Learning outcomes

The Learning Outcomes of this course are as follows:

- Student will be able to describe and perform simple DNA cloning and use DNA restriction and DNA modifying enzymes.
- Student will be able to discuss the use of cloning and expression vectors.
- Student will be able to explain various gene delivery methods and basic as well as high throughput methods of DNA, RNA and protein analysis
- Student will be able to elaborate on DNA amplification and DNA sequencing methods.

- Student will be able to evaluate the applications of recombinant DNA techniques in the areas of agriculture and pharmaceutical.

SYLLABUS OF DSC-20

UNIT – I (6 hours)

Concept of gene cloning and enzymes used in recombinant DNA technology: Introduction to genetic engineering. Restriction endonucleases (RE), its types and nomenclature. Role of Type II enzymes in gene cloning: generation of cohesive and blunt ends, frequency of recognition sequences in a DNA molecule, star activity, isoschizomers and neoschizomers, partial and double digestion. DNA modifying enzymes: DNA polymerase I, Klenow fragment, alkaline phosphatase, T4 polynucleotide kinase, terminal deoxynucleotidyl transferase, DNA ligase.

UNIT – II (9 hours)

Cloning vectors and expression systems: Cloning vectors: nomenclature and properties. Plasmid vectors: pBR, pUC and pGEM series. Phage vectors: lambda (insertion and replacement) vectors, M13-based vectors. Phagemids, cosmids, artificial chromosomes. Conversion of blunt-ended DNA into DNA with cohesive ends via linkers, adaptors, and homopolymer tailing. Screening and selection of recombinants: insertional inactivation (including alpha complementation and inactivation of drug resistance cassette), use of suicide genes for counter-selection of non-recombinants. Expression vectors and its components: strong promoters (prokaryotic and eukaryotic), reporter genes, and gene fusions. Expression systems in *S. cerevisiae* (YIp, YEp, YRp and YCp vectors), *Pichia pastoris*, baculovirus-based expression vectors, mammalian SV40 based expression vectors

UNIT – III (10 hours)

Introduction of DNA into living cells and analysis of DNA, RNA and proteins: Physical methods of introduction of DNA into cells: microinjection, electroporation, biolistic particle delivery. Chemical methods: Calcium chloride-based method, liposome-mediated delivery. Biological Methods: viral-mediated delivery, Agrobacterium - mediated gene transfer. DNA and RNA analysis by agarose gel electrophoresis, Southern Blotting and Northern Blotting. Protein analysis by SDS-PAGE and western blotting. Probes labelling by random priming and nick translation. Techniques to identify interaction of DNA with proteins: Gel Retardation Assay and DNA Footprinting. Transcriptome analysis by Microarrays. Phage display.

UNIT – IV (14 hours)

Amplification and Sequencing of DNA and Construction of DNA libraries: PCR: Basic Reaction, primer designing, RT-PCR, Real-Time PCR. Applications of PCR. DNA Sequencing: by Sanger's Method. Automated DNA sequencing. Primer walking. Hierarchical versus whole genome shotgun sequencing. Human Genome Project. Introduction to Next Generation Sequencing (NGS) method: Illumina platform. Genomic and cDNA libraries: Construction and uses of genomic and cDNA libraries, their screening

by colony hybridization, colony PCR, immunoscreening and bioactivity assays.

UNIT – V (6 hours)

Applications of recombinant DNA technology: Recombinant Products of human therapeutic value: Insulin, recombinant vaccines. Gene therapy: Somatic and germline, strategies, applications, and current status. Gene cloning in agriculture: Bt cotton, antisense RNA technology (FlavrSavr tomato). Safety concerns with GM crops. Applications in forensics: DNA fingerprinting by RFLP.

Practical component

UNIT 1: (15 hours)

Analysis of DNA fragments by agarose gel electrophoresis: Determination of molecular weight of given DNA against a standard DNA molecular weight ladder by resolution on agarose gel electrophoresis followed by graphical analysis of the migration patterns. Restriction digestion analysis of given plasmid DNA: comparison of RFLP patterns between vector and gene clone (vector plus insert) by analysis on agarose gel electrophoresis. Ligation of Lambda HindIII fragments: comparative analysis of DNA before and after ligation by analysis on agarose gel electrophoresis. Cloning of GFP gene in bacteria OR cloning of gene into suitable vector followed by selection using alpha-complementation.

Unit 2: (15 hours)

DNA sequencing and DNA amplification: Introduction to DNA sequencing by Sanger's method using virtual lab and videos: traditional as well as automated methods. Interpretation of sequencing results: reading a sequence off a traditional autoradiogram as well as current sequencing electropherogram. Introduction to PCR: designing primers for amplification of a fragment of genomic DNA. Group experiment: amplification of bacterial rDNA using 16S rDNA primers- performance of PCR and analysis of results by agarose gel electrophoresis.

Essential/recommended readings

Theory:

1. Molecular Biotechnology: Principles and Applications of Recombinant DNA by B.R. Glickand C.L. Patten. 6th edition. ASM Press, USA. 2022.
2. Gene Cloning and DNA Analysis: An introduction by T. A. Brown. 8th edition. Wiley-Blackwell Publishing, UK. 2020.
3. Prescott's Microbiology by J. M. Willey, K. Sandman and D. Wood. 11th edition. McGrawHill Higher Education, USA. 2019.
4. Principles of Gene Manipulation and Genomics by S.B. Primrose and R.M. Twyman. 8th

Edition. Blackwell Publishing, UK. 2016.

5. Biotechnology by D.P. Clark, N.J. Pazdernik. 2nd edition. Academic Press, USA. 2015.

Practicals:

1. Gene Cloning and DNA Analysis: An introduction by T. A. Brown. 8th edition. Wiley-Blackwell Publishing, UK. 2020.

2. Molecular Cloning: A Laboratory Manual by M. Green and J. Sambrook Volumes 1-3. 4th edition. Cold Spring Harbor Laboratory Press, USA. 2012.

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