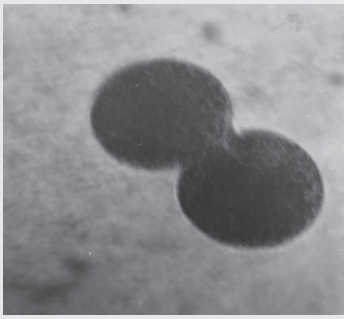
19BT312 GENETIC ENGINEERING



Hours Per Week :

L	T	P	C
3	-	2	4

Total Hours :

L	T	P
45	-	30

WA/RA	SSH/HSH	CS	SA	S	BS
5	50	-	8	3	5

COURSE DESCRIPTION AND OBJECTIVES:

The course is oriented at making the student understand about the process of gene expression and its regulation. Also to give awareness about different vectors used for gene transfer, enzymes, cloning methods, expression and detection of clones, molecular methods and markers and applications of r-DNA technology.

COURSE OUTCOMES:

Upon completion of the course, the student will be able to achieve the following outcomes:

COs	Course Outcomes	POs
1	Acquaint knowledge on gene expression and regulation mechanisms.	1,2
2	Apply gene manipulation techniques to produce GMO's.	1, 6, 7
3	Analyze structure and organization of different vectors used in gene transfer.	2, 6, 7, 8
4	Design primers for amplification of genes.	3

SKILLS:

- ✓ *Design and construct vectors.*
- ✓ *Identify restriction patterns for molecular scissors.*
- ✓ *Perform stable transformation.*
- ✓ *Realize gene silencing.*

UNIT - I **L-9**

GENE REGULATION IN PROKARYOTES AND EUKARYOTES: Prokaryotes - lactose, tryptophan and arabinose operons; Repressors and activators; Sigma switch in *Bacillus subtilis*; Eukaryotes - gene regulation, promoters and enhancer elements; Gene rearrangement; Gene amplification; Epigenetic regulations - methylation, glycation and acetylation.

UNIT - II **L-9**

PLASMIDS, TRANSPOSONS / VECTORS FOR GENE TRANSFER: Plasmids - definition, types, identification, classification, transfer of plasmids; Host restriction in transfer; Transposable elements - definition, types of bacterial transposons, mechanisms of transposition and excision, detection of transposition in bacteria, retroviruses, applications of transposons, retrotransposons; Enzymes involved in genetic engineering; Different types of cloning vectors - plasmid (pUC 19), lambda phage, cosmid, M13, BAC, YAC and YEP.

UNIT - III **L-9**

EXPRESSION AND DETECTION OF CLONES: Cloning strategies, construction of prototype vector (pBR 322), Genomic and cDNA library construction and application; Detection of clones and its expression.

UNIT - IV **L-9**

MOLECULAR TECHNIQUES: Purification of genomic DNA from living cells, Manipulation of purified DNA; Introduction of DNA into living cells - methods of Gene transfer, DNA hybridization, DNA sequencing, DNA fingerprinting; Blot analysis - Southern, Northern & Western blot; Dot and slot blot; PCR - principles, designing of primers, methodology, identification of PCR product, types of PCR, RT - PCR, multiplex PCR, application of PCR technology.

UNIT - V **L-9**

MOLECULAR MARKERS AND APPLICATIONS OF R-DNA TECHNOLOGY: Molecular markers - RFLP, RAPD, AFLP; 16s r-DNA typing, gene chip and microarray applications in disease profile and phylogeny; Gene cloning in medicine (Insulin, Blood clotting factor VIII); Introduction to Gene therapy (*Ex vivo* & *In vivo*), Case study of ADA as an example, Advantages and limitations of Gene therapy and novel technologies.

LABORATORY EXPERIMENTS

LIST OF EXPERIMENTS **TOTAL HOURS: 30**

1. Isolation of plasmid DNA by alkaline lysis method from *E.coli*.
2. Optimization of PCR reaction mixture to Amplify gene by polymerase chain reaction (PCR).
3. Restriction analysis of plasmid DNA and analysis by agarose gel electrophoresis.
4. Preparation of competent cells by calcium chloride treatment for plasmid transformation.
5. Setting up of ligation reaction using T4 DNA ligase. And dot blot Techniques for quantification.
6. Transformation of chemically competent *E. coli* with the ligation mixture, plating and analysis of transformants.
7. Setting up a dephosphorylation reaction using alkaline phosphatase enzyme.
8. Cloning of gene into a plasmid vector and transformation to *E. coli*.
9. Primer design to amplify genes.

TEXTBOOK:

1. T. A. Brown, “Gene Cloning and DNA Analysis, An Introduction”, 7th edition., Blackwell, 2016.
2. Bernard R Glick and Jack J Pasternak, “Molecular Biotechnology: Principles and applications of recombinant DNA”, 5th edition, ASM Press, 2017.

REFERENCE BOOKS:

1. R.W. Old and S.B. Primrose, “Principles of gene manipulation”, 6th edition, BlackWell Scientific Publications, 2001.
2. Jeff Hardin, Gregory Paul Bertoni, Lewis J. Kleinsmith and Wayne M Becker “Becker’s World of the Cell”, 8th edition, Pearson, 2016.
3. S.B. Primrose, “Principles of Gene manipulation and Genomics”, 5th edition, Blackwell Scientific Publications, 2006.
4. D. Freifelder, “Essentials of Molecular Biology”, 7th edition, Narosa Publishing House, 2006.