17BT007- r-DNA Technology

Hours Per Week:

L	T	P	C
3	-		3

Total Hours:

L	T	P
45	-	2

BS	SA	CS	WA/RA	SSH	S
5	8	1-5	5	40	1-5

Course Description and Objectives:

The course is oriented towards understanding the processes of gene expression and regulations. The objective of the course is to provide awareness about different vectors used for gene transfer, enzymes, cloning methods, expression and detection of clones. It is also aimed to provide insights into molecular methods, markers and applications of r-DNA technology.

Course Outcomes:

The student will be able to:

- *Gain knowledge on gene expression and regulations.*
- *Analyze structure and organization of different vectors used in gene transfer.*
- *Understand and handle enzymes used in gene manipulation.*
- *Perform cloning methods, expression and detection of clones.*
- *Excel in molecular techniques, markers and applications of r-DNA technology.*

SKILLS TO BE ACQUIRED:

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

ACTIVITIES:

- *Culture bacterial cells.*
- *Isolaton of bacterial and plasmid DNA.*
- *Handling micropipettes to deal with molecular enzymes.*
- *Experimentation on gel electrophoresis.*
- *Preparation of competent cells.*

UNIT - I **L-9**

PLASMIDS, TRANSPOSONS / VECTORS FOR GENE TRANSFERS: Plasmids- definition, types, identification, classification, purifications and transfer of plasmids. Host restriction in transfer; Transposable elements- definition, detection of transposition in bacteria, types of bacterial transposons, mechanisms of transposition and excision; 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 - II **L-9**

EXPRESSION AND DETECTION OF CLONES: Cloning strategies; sequencing; DNA fingerprinting; Blot analysis- Southern, Northern, Western blot; Dot and slot blot; PCR- principles, designing of primers, methodology and applications of PCR.

UNIT - III **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.

UNIT - IV **L-9**

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

UNIT - V **L-9**

HISTONES, RNA AND EPIGENETIC MECHANISMS: Types of histones and their participation in compact and relaxed genomes; DNA methylation; Histone modifications; Acetylation; RNA silencing; Micro RNA; RNAi-mediated gene regulation; Methods of detecting epigenetic mechanisms – interplay of epigenetic mechanisms in development, differentiation, regeneration and aging.

LABORATORY EXPERIMENTS

LIST OF EXPERIMENTS Total hours: 30

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

TEXTBOOK:

1. T.A.Brown, "Gene Cloning and DNA analysis", 5th edition, Blackwell Scientific Publications, 2006.

REFERENCE BOOKS:

1. S.B. Primrose, "Principles of Gene manipulation and Genomics", 5th edition, Blackwell Scientific Publications, 2006.

2. D. Freifelder, "Essentials of Molecular Biology", 7th edition, Narosa Publishing House, 2006.