BACHELOR WITH BIOTECHNOLOGY AS MAJOR

4th Semester

BTG 422J3: RECOMBINANT DNA TECHNOLOGY

CREDITS: THEORY – 4, PRACTICAL – 2

MAXIMUM MARKS: 100, MINIMUM MARKS: 36

Objective: *Through this course, students will learn about the different tools used in recombinant DNA technology and its applications.*

Expected Learning Outcomes: *At the end of the course students should be able to;*

1. *Use different enzymes for cloning, modification and amplification of DNA.*
2. *Select and use the suitable vector for cloning and screening of transformants.*
3. *Express recombinant proteins and purify them.*
4. *Make cDNA library, edit and target different genes.*

THEORY (4 CREDITS: 60 HOURS)

UNIT I 15 Hours

Introduction to Recombinant DNA technology, tools of recombinant DNA technology: Restriction endonucleases (types, nomenclature, cleavage pattern-blunt and cohesive end cutters), DNA polymerases (pol I, Klenow fragment, Taq), DNA ligases, kinases, phosphatases, nucleotidyl transferase, exonucleases, reverse transcriptase. Use of linker and adapters, homopolymer tailing.

Unit II 15 Hours

Plasmid vectors- general features. Features of pBR322 and pUC. Bacteriophage vectors: insertion and replacement, M13, cosmids, phagemids, YAC and BAC. Basic cloning methodology in plasmid vectors: Vector and insert preparation, ligation, competent cells, transformation (heat-shock and electroporation) screening of recombinants (antibiotic resistance and blue-white screening). Preparation of probe – radioactive and non radioactive labeling. Sequence based screening (colony, hybridization, PCR)

UNIT III 15 Hours

Recombinant protein expression in heterologous systems: expression in E.coli (inducible promoter system), yeast, insect and mammalian systems. Recombinant protein purification using tags (His, GST, Flag, HA). Reporter genes (luciferase, CAT, GFP, GUS) and their applications. In vitro transcription and translation and its applications.

Unit IV 15 Hours

Genomic and cDNA library construction, screening of libraries. Gene knock downs: antisense RNA technology and RNA interference. Gene knock out by Cre-LoxP system. Gene editing by CRISPR-CAS system. Gene targeting: Site directed mutagenesis (single primer extension, double primer extension, PCR based mutagenesis). Protein engineering for increased thermal stability, activity, shelf life.

PRACTICALS (2 CREDIT: 60 HOURS) Maximum Marks: 50, Minimum Marks: 18

1. DNA/plasmid isolation from bacterial/plant/any other cell.
2. Preparation of competent cells.
3. Restriction digestion of DNA.
4. Transformation of competent cells by heat shock.
5. Easy (TA) Cloning.
6. Blue-white screening
7. Agarose gel electrophoresis.
8. Educational tour to different labs/institutes.

Suggested Readings:

1. Molecular Biotechnology: Principles and Applications of Recombinant DNA by Bernard R. Glick, Cheryl L. Pattern, ASM Press.
2. Gene Cloning and DNA Analysis: An Introduction by Brown TA Wiley-Blackwell
3. Principles of Gene Manipulation and Genomics by Primrose SB, Twyman R and Old B Wiley- Blackwell.
4. Gene Cloning and Manipulation by Christopher Howe, Cambridge University Press
5. Analysis of Genes and Genomes by Reece J Richard, Wiley-Blackwell