B.Sc. IV Year **Semester – VII**

BIM –E701
DSE-7 RECOMBINANT DNA TECHNOLOGY

MM : 100
Time : 3 hrs
L Credit
44
Total Hours: 60

Sessional : 30
ESE : 70
Pass Marks : 40

Learning objectives:

- To make students understand about the structure and function of biologically important molecules.
- To know the historical background of DNA structure and its role as genetic material.
- Become familiar with different tools and techniques used in genetic engineering and recombinant DNA technology.
- To understand the applications of DNA modifying enzymes, cloning strategies, vector types, and screening of recombinants
- Students will know how gene expresses and regulates in prokaryotic cells.

Learning outcomes:

At the end of course students will be able to

- Explain why DNA is the genetic material of bacteria.
- Explain the application of genetic engineering techniques in basic and applied experimental biology.
- Amplify the DNA using PCR for the diagnosis and DNA fingerprinting.
- Describe how protein synthesis occur in procaryotic cell and enzyme involved in it.

UNIT- I

Introduction to Genetic Engineering: Milestones in genetic engineering and biotechnology;Molecular Cloning- Tools and Strategies-Cloning Tools; Restriction modification systems: Types I, II and III. Mode of action, nomenclature, applications of Type II restriction enzymes in genetic engineering DNA modifying enzymes and their applications: DNA polymerases. Terminal deoxynucleotidyltransferase, kinases and phosphatases, and DNA ligases Cloning Vectors: Definition and Properties Plasmid vectors: pBR, Cosmids, Expression vectors.

(16 Lectures)

UNIT- II

Methods in Molecular Cloning: Transformation of DNA: chemical method, electroporation, Gene delivery: Microinjection, electroporation, biolistic method (gene gun), liposome and viralmediated delivery, *Agrobacterium* - mediated delivery DNA, RNA and Protein analysis: Agarose gel electrophoresis, Southern - and Northern – blotting techniques, DNA Western blotting. **(14 Lectures)**

UNIT- III

DNA Amplification and DNA sequencing PCR: Basics of PCR, Real-Time PCR, Sanger's method of DNA Sequencing: traditional and automated sequencing.

(09 Lectures)

UNIT- IV

Construction and Screening of Genomic and cDNA libraries: Genomic and cDNA libraries: Preparation and uses, Screening of libraries: Colony hybridization and colony PCR.

(09 Lectures)

UNIT - V

Applications of Recombinant DNA Technology: Products of recombinant DNA technology: Products of human therapeutic interest - insulin, hGH, antisense molecules. Bt transgenic - cotton, brinjal, Gene therapy, recombinant vaccines, protein engineering and site directed mutagenesis.

(12 Lectures)

Suggested Reading

1. Bruce Alberts. Molecular Biology of the Cells, W.W. Norton and Company, ISBN: 9780815344643
2. Dubey, R.C. *Advanced Biotechnology*. S. Chand & Co. P Ltd, New Delhi, p. 1161; ISBN: 81:219-4290-X.
3. Harvey, Lodish. Molecular Cell Biology, W.H.Freeman
4. Dubey, R.C. and Maheshwari, D.K. *Practical Microbiology*. 2nd ed., S. Chand & Co. P Ltd, New Delhi, p. 413. ISBN: 81:219-2559-2

DSE 7 SEMESTER VII / BIM-E751 (LAB COURSE CC-07)

The practicals based on BIM E701 will be performed.

A. To perform Bacterial DNA isolation and Southern analysis.

1. Bacterial DNA isolation and restriction digestion.
2. Agarose gel electrophoresis, staining and southern transfer.
3. Probe preparation and southern hybridization.
4. Washing and Blot development.

B. To perform plasmid isolation and restriction mapping.

5. Plasmid isolation and restriction digestion.
6. Agarose gel electrophoresis.

C. To perform acquiring antibiotic resistance through bacterial transformation.

7. Preparation of competent cells.
8. Transformation of competent *E. coli* with pBR322.