VI Semester

Paper No- 15: Medical Biotechnology Marks: 150

Preamble: The unique preposition of this paper is that the students learn the basic techniques and methods used in the diagnosis and therapy of various human diseases and in the production of biopharmaceuticals. The concepts of cloning and expression of the desired gene is explored. This paper aims to train students to understand how biological systems are applied in the advancement of medical biotechnology.

THEORY **Total No of Lectures = 48**

Unit I: **Introduction to Biotechnology** **(1 Lecture)**
(Chapter 1: T.A. Brown; Chapter 1: Primrose)

Brief history and Importance

Unit II: **Basic techniques** **(4 Lectures)**
(Chapter 2: Primrose)

Agarose gel electrophoresis, Southern, Northern and Western blotting and hybridization, use of enzymatic and chemiluminiscent methods for detection of proteins, detection of nucleic acids by radioactive and fluorescent probes.

Unit III: **Manipulation of DNA** **(5 Lectures)**
(Chapter 3, 4 and 5: T. A. Brown; Chapter 3 and 4: Primrose)

Isolation and purification of genomic and plasmid DNA, Restriction and modification systems, type I-IV restriction endonucleases, nomenclature and sequence recognition, restriction mapping. Joining of DNA molecules: role of DNA ligase, adaptors, linkers, homopolymer tailing

Unit IV: **Cloning Vectors** **(8 Lectures)**
(Chapter2, 6 and 7: T.A.Brown; Chapter 5: Primrose)

Basic biology of plasmids, brief life cycle of phages (lambda and M13), Plasmid vectors (pBR322 and pUC vectors, T-vectors) and phage vectors (Bacteriophage vectors- replacement and insertion vectors), cosmids, phasmids, *in vitro* packaging, expression vectors, example of prokaryotic and eukaryotic expression vectors, inducible and constitutive expression vectors with one example each.

Unit V: **Cloning and expression of cloned genes in prokaryotic and eukaryotic Cells(Chapter 6, 7 and 11: T.A.Brown)** **(6 Lectures)**

Challenges in expression of foreign proteins in heterologous host, factors affecting the expression host cell physiology, promoters, codon choice, plasmid copy no. etc., expression in eukaryotic cells (yeast and mammalian expression system, Baculovirus system), Shuttle vectors,

Bacterial transformation and selection and screening of transformants (blue/white and antibiotic selection methods).

Unit VI: **Polymerase chain reaction (PCR) (4 Lectures)**
(Chapter 9: T.A. Brown)

Principle and applications, primer-design, detailed understanding of PCR and RT- (Reverse transcription) PCR.

Unit VII. **Construction of genomic and cDNA libraries, screening and selection of recombinants (6 Lectures)**
(Chapter 5 and 6: Primrose; Chapter 8: T.A.Brown)

Immunochemical methods of screening, nuclei acid hybridization (Colony and Plaque hybridization), different methods of preparation of gene probe. Hybrid Release Translation and Hybrid Arrest Translation.

Unit VIII. **Random and site-directed mutagenesis (4 Lectures)**
(Chapter 8: Primrose)

Methods in Random mutagenesis: any two, methods in Site-directed mutagenesis: oilgonucleotide-directed mutagenesis, PCR-based method, screening and identification of mutants. Protein engineering concept and examples of Subtilisin, and alpha-Antitrypsin (AAT)

Unit IX:Application of Medical Biotechnology (8 Lectures)
(Chapter 26: T.A.Brown)

(a) Production of recombinant biomolecules: Insulin, somatostatin, Factor VIII and interferons.
(b) DNA Profiling: Introduction, DNA profiling based on STRs, minisatellites, RFLP, AFLP, VNTRs, SNPs and their applications.
(c) Gene Therapy: Strategies and limitations, somatic and germline gene therapy, different vectors (viral and non viral) and their comparison, treatment for genetic and infectious diseases.

Unit X: Biosafety and ethical issues in biotechnology (2 Lectures)
(Chapter 13 and 14:T. A. Brown)

PRACTICALS

1. To understand the method of digesting DNA with different restriction enzymes.
2. To maintain and store the *E.coli* DH5 alpha cells.
3. Preparation of Competent Cell (Calcium Chloride Treatment).
4. To prepare insert and vector for ligation.
5. To perform ligation reaction using T4 DNA ligase.
6. Transform competent bacterial cells with foreign DNA.

7. To identify recombinants by blue-white screening and PCR.

ESSENTIAL BOOKS

1. Gene cloning and DNA analysis, 6th edition (2010), T.A. Brown. Wiley-Blackwell.
2. Principles of Gene Manipulation and Genomics, 7th edition (2006), S.B. Primrose and R.M. Twyman. Blackwell Scientific.

SUGGESTED READINGS

1. Molecular Biotechnology: Principles and Applications of Recombinant DNA, 4th edition (2009),Bernard R. Glick, Jack J. Paternack, Cheryl I. Patten. ASM press.
2. DNA Replication, 2nd edition (1992), Arthur Kornberg; University Science Books.
3. Genomics: The Science and Technology behind the Human Genome Project, 1st edition (1999), Cantor and Smith; John Wiley and Sons.
4. Molecular Cloning: A Laboratory Manual, 4th edition (2012), Three-volume set by Michael R. Green, Joseph Sambrook; Cold Spring Harbor Laboratory Press.