

**Paper No- 13**  
**SEMESTER - V**  
**RECOMBINANT DNA TECHNOLOGY**

**THEORY**

**MARKS: 100**

**Unit 1 Introduction to genetic engineering** **No. of lectures: 12**

- 1.1 Milestones in genetic engineering and biotechnology
- 1.2 **Restriction modification systems:** Types I, II and III. Mode of action, nomenclature, applications of Type II restriction enzymes in genetic engineering
- 1.3 **Analysis of restricted DNA:** Agarose gel electrophoresis and Southern blotting
- 1.4 **DNA modifying enzymes and their applications:** DNA polymerases. Terminal deoxynucleotidyltransferase, kinases and phosphatases, and DNA ligases
- 1.5 **Cloning:** Use of linkers and adaptors
- 1.6 **Transformation of DNA:** by chemical method

*(Chapter 3, Principles of Gene Manipulation by Primrose SB, Twyman RM and Old RW, 6<sup>th</sup> Ed., Blackwell Science Publishers, 2001, Pages: 26-42)*

**Unit 2 Vectors** **No. of lectures: 12**

- 2.1 **Cloning Vectors:** Definition and Properties
- 2.2 **Plasmid vectors:** pBR and pUC series
- 2.3 Bacteriophage lambda and M13 based vectors
- 2.4 Cosmids, BACs, YACs
- 2.5 **Expression vectors:** *E.coli* lac and T7 promoter-based vectors, yeast YIp, YEp and YCp vectors, Baculovirus based vectors, mammalian SV40-based expression vectors

*(Chapters 4,5,9, 10, Principles of Gene Manipulation by Primrose SB, Twyman RM and Old RW, 6<sup>th</sup> Ed., Blackwell Science Publishers, 2001, pages 43-71, 158-163, 179-193).*

**Unit 3 DNA Amplification and DNA sequencing** **No. of lectures: 10**

- 3.1 **PCR:** Basics of PCR, RT-PCR, Real-Time PCR
- 3.2 **Sanger's method of DNA Sequencing:** traditional and automated sequencing
- 3.3 Primer walking and shotgun sequencing

*(Chapter 9, Gene Cloning and DNA Analysis : An Introduction by Brown TA, 4<sup>th</sup> Ed., Blackwell Science Publishers, 2001, pages 179-185).*

*(Chapter 7, Principles of Gene Manipulation by Primrose SB, Twyman RM and Old RW, 6<sup>th</sup> Ed., Blackwell Science Publishers, 2001, pages 120-132).*

**Unit 4 Construction and Screening of Genomic and cDNA libraries** **No. of lectures: 6**

- 4.1 **Genomic and cDNA libraries:** Preparation and uses
- 4.2 **Screening of libraries:** Colony hybridization and colony PCR
- 4.3 Chromosome walking and chromosome jumping

*(Chapter 6, Principles of Gene Manipulation by Primrose SB, Twyman RM and Old RW, 6<sup>th</sup> Ed., Blackwell Science Publishers, 2001, Pages: 86-109).*

*(Chapter 8, Gene Cloning and DNA Analysis by Brown TA, 6<sup>th</sup> Ed., Blackwell Publishing, Oxford, 2006).*

**Unit 5 Applications of DNA Technology** **No. of lectures: 8**

- 5.1 **Gene delivery:** Microinjection, electroporation, biolistic method (gene gun), liposome and viral-mediated delivery, *Agrobacterium* - mediated delivery

## **5.2 Products of recombinant DNA technology: Products of human therapeutic interest - insulin, hGH**

*(Chapters 6 & 10, DNA Technology - The Awesome Skill by Alcamo E, 2nd Ed, Academic Press, 1996, pages 123-136 and 226-228)*

*(Chapter 10, Principles of Gene Manipulation by Primrose SB, Twyman RM and Old RW, 6<sup>th</sup> Ed., Blackwell Science Publishers, 2001, pages 174-176)*

### **PRACTICALS**

**MARKS: 50**

1. Isolation of Plasmid DNA from *E.coli*
2. Digestion of DNA using restriction enzymes and analysis by agarose gel electrophoresis
3. Ligation of DNA fragments
4. Interpretation of sequencing gel electropherograms
5. Amplification of DNA by PCR

### **SUGGESTED READING**

1. Brown TA. (2010). Gene Cloning and DNA Analysis. 6<sup>th</sup> edition. Blackwell Publishing, Oxford, U.K.
2. Clark DP and Pazdernik NJ. (2009). Biotechnology: Applying the Genetic Revolution. Elsevier Academic Press, USA
3. Primrose SB and Twyman RM. (2006). Principles of Gene Manipulation and Genomics, 7<sup>th</sup> edition. Blackwell Publishing, Oxford, U.K.
4. Sambrook J and Russell D. (2001). Molecular Cloning-A Laboratory Manual. 3rd edition. Cold Spring Harbor Laboratory Press
5. Wiley JM, Sherwood LM and Woolverton CJ. (2008). Prescott, Harley and Klein's Microbiology. McGraw Hill Higher Education
6. Brown TA. (2007). Genomes-3. Garland Science Publishers
7. Primrose SB and Twyman RM. (2008). Genomics: Applications in human biology. Blackwell Publishing, Oxford, U.K.

### **ONLINE READING MATERIAL**

1. <http://www.amazon.com/Genomes-3-T-Brown/dp/0815341385>