

## **MB2PG07 - MOLECULAR BIOLOGY & GENETIC ENGINEERING**

**Number of Hours / Week: 4**

**Credits: 4**

### **UNIT I**

**DNA Replication** – Process of DNA replication, semi-conservative, discontinuous uni and bidirectional, Okazaki fragments, DNA polymerases in eukaryotes and prokaryotes, Klenow fragment, modes of replication, theta, rolling circle, d-loop replication, Primosome, SSB, Helicase, Ligase, methylation and control, repetitive DNA sequences, minisatellite, microsatellite, DNA protein interaction DNA Linking number and topoisomerase, Inhibition of replication.

### **UNIT II**

**Transcription.** -Process of transcription, stages in transcription, RNA polymerases in prokaryotes and eukaryotes, sigma factor in prokaryotes, Rho dependant and Rho independent termination. Enhancers, Transcription factors in Eukaryotes, Differences in transcription between prokaryotes and Eukaryotes, post transcriptional modifications-Polyadenylation, capping, r-RNA processing, Splicing-Spliceosome, lariat structure, Group 1, II and III Introns Ribozyme, Importance of ribozyme, properties, application, RNase P, RNase III, RNase H. monocistronic and polycistronic m-RNA, Joint transcript of r-RNA and t-RNA in prokaryotes and their processing, Transplicing, alternate splicing, inhibitors of Transcription.

Molecular mechanism of gene regulation in prokaryotes-Transcriptional regulation in prokaryotes; Inducible & repressible system, positive and negative regulation; Operon concept, structure of operon, Lac, Trp, Ara operon, Catabolic repression, Attenuation. Role of Hormones in gene regulation.

**RNA World, RNA based technology-** Molecular mechanism of Ribozyme, Antisense RNA, siRNA, MicroRNA, Riboswitches & their applications; Telomerase structure and function Nucleic acid as therapeutic agent

### **UNIT III**

#### **Translation**

Process of translation. Stages in translation, genetic code, properties, wobble hypothesis, eukaryotes and prokaryotes ribosomes, m-RNAs, t-RNAs, aminoacyl t-RNA synthetases, protein factors initiation complex, peptidyl transferase, releasing factors, differences between prokaryotic and eukaryotic systems, inhibition of translation. Post translation modification by cleavage, self assembly assisted self assembly chaperones, acylation, phosphorylation, acetylation and glycosylation, Histone acetylation and deacetylases, chromosome remodeling

complex. Intein splicing. Protein targeting, co-translational import, post translational import, SRP- structure and function, Blobel's concept, Lysosome targeting, M6P address Glycosylation core glycosylation terminal glycosylation, Dolichol phosphate.

#### **UNIT IV**

##### **Tools and techniques for Genetic Engineering- 1**

History of rDNA Technology, Cohen And Boyer Patents. Enzymes used in genetic engineering with special reference to restriction enzymes, ligases, and other DNA modifying enzymes. modification of restriction fragments, vaccinia topoisomerases, Cloning strategies - Use of linkers, adaptors, TA cloning, and homopolymer tails . Nucleic acid hybridization - Colony hybridization, plaque hybridization; Blotting techniques – Southern, Northern.

#### **UNIT V**

##### **Tools and techniques for Genetic Engineering- 2**

Vectors- Plasmid- pSC101, pBR322, pUC their development, features and selection procedures; Bacteriophages-  $\lambda$  and M13, Cosmids- features, advantages and cosmid cloning schemes; Phagemids- pEMBL, pBluescript, pGEM3Z , pSP64. Shuttle vectors- YAC. Ti-plasmids. Expression vectors. Construction of genomic libraries and cDNA libraries, procedures for recombinant selection and library screening, Chemical synthesis of DNA , DNA sequencing - plus and minus sequencing, Sanger's dideoxy sequencing, Maxim and Gilbert's method. Advanced sequencing procedures – pyrosequencing, Illumina, ABI / SOLiD and their applications. Principles, techniques and applications- PCR, RFLP, RAPD, AFLP, Foot and Finger printing etc.

#### **UNIT VI**

##### **Applications of Genetic Engineering**

DNA chips and microarray, gene screen technology; site directed mutagenesis, gene knockout techniques, Genetic markers, Gene transfer in plants and animals. Applications of transgenic technology. Animal cloning- stem cell technology, somatic cell nuclear transfer, Plant cell culture based techniques. Applications of Molecular Biology in forensic sciences, medical science etc.

#### **References**

1. M. Fogiel, J. A. Stone, Research and Education Association., (1995) *The genetics problem solver : a complete solution guide to any textbook*. REA's problem solvers (Research and Education Association, Piscataway, N.J.
2. Anthony. J. F. Griffiths (2002), *Modern genetic analysis*. 2<sup>nd</sup> ed. (W. H. Freeman ; Basingstoke : Palgrave, New York.

3. EJ Wood, Chris Smith (1996) *Cell biology*. 2<sup>nd</sup> ed. Chapman and Hall, London.
4. Gerald Karp.(2009) *Cell and Molecular Biology: Concepts and experiments*. 6<sup>th</sup> ed. John Wiley & Sons.
5. Geoffrey M Cooper, Robert E Hausman (2007) *The cell: a molecular approach*. 4<sup>th</sup> Ed. ASM Press.
6. EDP DeRobertis , EMF DeRobertis. (1995).*Cell and Molecular Biology*. 6<sup>th</sup> ed. B.I Waverly.
7. Bernard R Glick, Jack J Pasternak (2010) *Molecular Biotechnology. Principles and Applications of recombinant DNA*. 4<sup>th</sup> ed.
8. Sandy B Primrose , Richard Twyman (2009) *Principles of Gene Manipulation and Genomics*. 7<sup>th</sup> ed. John Wiley & Sons.
9. Terry A Brown. (2010) *Gene Cloning and DNA Analysis: An Introduction*. 6<sup>th</sup> ed. John Wiley & Sons.
10. Benjamin Lewin, Jocelyn E. Krebs, Elliott S. Goldstein, Stephen T. Kilpatrick (2009) *Lewin's Genes X*. 10<sup>th</sup> ed. Jones and Bartlett Publishers International, London.
11. James D Watson. (2008) *Molecular biology of the Gene*. 6<sup>th</sup> ed. Pearson/ Benjamin Cummings.
12. Harvey F Lodish. (2004) *Molecular Cell Biology*. 5<sup>th</sup> ed. W.H Freeman.