

perturbation, knockdown (interference RNA, small interference RNA), knockout technology, micro RNA.

References

- 1) Molecular Biology of the Cell. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J.D. Garland Publishing, Inc., 2002
- 2) The Cell - A Molecular Approach. Cooper, G.M. Sunderland: Sinauer Associates, Inc, 2000
- 3) Cell and Molecular Biology. De Robertis, E.D.P. and De Robertis, E.M.F. B.I. Waverly Pvt. Ltd., 1971
- 4) Developmental Biology. Gilbert, S.F. Sinauer Associates, Inc., 2000
- 5) Molecular cell Biology. Lodish, H., Berk, A., Zipursky, S.L. et al. WH. Freeman and Co, 2000
- 6) Cell & Molecular Biology – Concepts & experiments. Karp, G., Harris, D., Wiley & sons, 1999
- 7) Principles of Cell and Molecular Biology. Kleinsmith, L.J. & Kish, V.M. McLaughlin, S., Trost, K., Mac Elree, E., Harper Collins Publishers, 1995
- 8) Genes VII. Lewin, B. Oxford University Press, 2000
- 9) Molecular biology: genes to proteins. Tropp BE., Jones & Bartlett, 2010
- 10) Essential of Molecular biology. Freifelder D., Jones & Bartlett, 1985
- 11) Molecular Biology of Gene. Watson JD., Baker TA., Bell SP., et al., Pearson Edu. Inc. 2013
- 12) Molecular Biotechnology: Principles and applications of recombinant DNA. Glick BR. and Pasternak JJ. ASM Press, Washington, 2009

rDNA TECHNOLOGY

Hours: 52

Course outcome

After successful completion of the course, students will be able to:

- CO 1. Use the various tools and strategies utilized in the construction and production of recombinant DNA molecules *in vitro* and *in vivo*.
- CO 2. Learn the various techniques utilized for the introduction of recombinant DNA molecules in bacteria, yeast and mammalian cells.
- CO 3. Elucidate the steps involved in the genetic engineering from amplification of DNA molecules to cloning of molecules, and screening strategies for clone identification.
- CO 4. Understand the importance of high capacity vectors, plasmids and the various steps involved in genomic library preparation to understand complex genomes.
- CO 5. Differentiate between different gene mapping methods, analysis of gene expression by various methods, and techniques used for introduction of mutations

UNIT I (13 hrs)

Restriction – modification systems, Restriction enzymes – type I, II and III, specificity, sticky ends and blunt ends, isoschizomers. Double digests. DNA ligases, optimum ligation conditions. Enzymes to modify the terminals of DNA- Alkaline phosphatase, polynucleotide kinase, DNase I, S1 nuclease, DNA polymerase and Klenow fragment, Terminal nucleotidyl transferase, RNase H and DNA topoisomerase. Use of linkers, adapters and homopolymer tailing. Other methods of joining DNA molecules: TA cloning of PCR products, Construction of genomic libraries, construction of cDNA libraries, methods of cDNA synthesis; PCR: Design, optimization, types and applications.

UNIT II (13 hrs)

Essential features of vectors for transforming bacteria and yeast, animals and plants. Special vectors: Shuttle vectors, expression vectors, Construction of Artificial chromosomes vectors BACs, YACs and MACs. Cosmids, phagemids and phasmids. Fusion vectors. Viral vectors.

Techniques of introducing genes in Prokaryotes and eukaryotes: transformation, calcium phosphate method, DEAE- Dextran method, protoplast fusion/somatic cell hybridization. Liposome mediated transfer, microinjection, electroporation and gene gun.

UNIT III (13 hrs)

Identifying the right clones: Direct screening: Insertional inactivation of marker gene, visual screening, plaque phenotype. Indirect screening: Immunological techniques, Hybrid arrest translation, Hybrid select translation. Screening using probes: Construction of gene probes, hybridization and labeling. Nucleic acid hybridization – Southern blotting, colony hybridization, dot blot; Chromosome walking and chromosome jumping. DNA sequencing: Maxim and Gilbert's method, Sanger and Coulson's method, Messing's shot gun method, Automated sequencers; Analysis of genetic variation: Single nucleotide polymorphism, conserved and variable domains, RFLP, AFLP, EST, STS, SCAR, SSCP. DNA finger printing. Genome sequencing: overview, strategies (e.g. Human genome project), Next Gen Sequencing.

UNIT IV (13 hrs)

Mapping of DNA: Restriction mapping, DNA footprinting, mapping by somatic cell hybridization. Use of transposons in gene mapping. Analysis of gene expression: Analysis of transcription by Northern blot, RNase protection assay, Primer extension assay, *in-situ* hybridization, Fluorescent In-situ Hybridization (FISH) Technique, Comparative genomic hybridization. Comparing transcriptomes: Differential screening, subtractive hybridization, array based methods; Implication of Genetic engineering. Methods of studying promoter, reporter genes, locating the promoter, regulatory elements and DNA-binding proteins. Translational analysis: Screening expression libraries with antibodies – Western Blot, 2D-electrophoresis. Manipulating gene expression: Transcriptional fusions, translational fusions, *In-vitro* mutagenesis: Oligonucleotide directed mutagenesis, deletions, Insertional mutagenesis, direct single base mutagenesis. General guidelines for recombinant DNA research activity. Containment facilities and biosafety practices; Rules for import and export of biological materials.

References

- 1) From Genes to Clones, Winnacker E.L., Panima Educational Book agency, 1987,
- 2) Genes VII, Lewin, Oxford University Press, 2000
- 3) Principles of Gene Manipulation. Primrose S.B., & Twyman R.M. Blackwell scientific Pub. 2006.
- 4) Recombinant DNA Technology. Watson J.D. et al., Scientific American Book Series, 2006
- 5) Genetics: a molecular approach. Brown TA., Stanley Thornes Publ. 1999
- 6) An introduction to genetic engineering. Nicoll DST., Cambridge Univ Press., 2012
- 7) Principles of Genome Analysis and Genomics. Primrose SB., Twyman RM., Blackwell Publ. 2002

IMMUNOTECHNOLOGY (HARD CORE COURSE) Hours: 52

Course outcome

After successful completion of the course, students will be able to:

- CO 1. Understand the structure, components and functioning of the immune system, including toxins and toxin resistance
- CO 2. Understand the molecules related to immune system such as immunoglobulins, antigens and the genes associated with diversity and specificity, tissue histocompatibility