

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).

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. 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, two-dimensional electrophoresis. Manipulating gene expression: Transcriptional fusions, translational fusions, *In-vitro* mutagenesis: Oligonucleotide directed mutagenesis, deletions, Insertional mutagenesis, direct single base mutagenesis.

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 T.A., Stanley Thorne Publ. 1999
- 6) An introduction to genetic engineering. Nicoll D.S.T., Cambridge Univ Press., 2012
- 7) Principles of Genome Analysis and Genomics. Primrose S.B., Twyman R.M., Blackwell Publ. 2002