BTH 452 GENETIC ENGINEERING

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 DNAmolecules 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 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