

IV SEMESTER

BCH 551: GENETIC ENGINEERING: HARDCORE

Lecture Hours: 56 hours

Total Credits: 04

Course objectives

- To study the concept of gene cloning.
- To elucidate the sequence and identify the clones using various molecular techniques.
- Maintenance of animal cell and plant tissue culture laboratory.
- Applications of fermentor.

Unit I

14 hrs

Principle of Gene Cloning I: Isolation and purification of nucleic acids (DNA and RNA) from living cells. DNA manipulative enzymes - ligases, polymerases, endonucleases Type II, Sticky and blunt ends, isoschizomers. Ligation: blunt end and sticky end ligation, use of linkers and adapters, homo polymer tailing, Vectors: Plasmids (construction of pBR322, pUC8 and pUC18 plasmids), virus based vectors (lambda phage and M13), phagemid, cosmid, Yeast cloning vectors, bacterial artificial vectors, plant vectors, expression vectors, cDNA cloning.

Unit II

14 hrs.

Principle of Gene Cloning II – Gene library construction, Direct selection, insertional inactivation of marker gene, visual screening, immunological detection method, colony and plaque hybridization. Transformation: Microinjection, electroporation, lipofection, calcium phosphate method, protoplast fusion, biolistic method. Introduction to plant tissue culture and animal cell culture, Laboratory design, aseptic conditions, equipment and materials for cell culture. Different constituents of culture medium, types of media.

Unit III

14 hrs

Cell culture techniques: Preparation of primary culture; disaggregation of tissue and primary cultures, chick embryo, HUVEC, characterization of cultures, ploidy, cell doubling time. Cell lines: Characteristics and routine maintenance, cell separation techniques. Measurement of viability and cytotoxicity. Scaling-up of animal cell culture; bioreactors used in animal cell culture and their applications. Industrial applications: Fermenter - stirred fermenter, micro-carrier, encapsulation, hollow fiber chambers, packed glass bead reactors. Cell immobilization techniques. Plant cell culture: Micro propagation, callus culture, haploid production, somatic embryogenesis, somatic hybridization, cybridization and somaclonal variation. Production of disease-free plants.

Unit IV

14 hrs

Molecular Techniques: Polymerase chain reaction, reverse transcriptase-PCR, qPCR, technique and applications, applications of PCR, concepts of DNA sequencing, Sanger's method, automated fluorescent DNA sequencing shot gun sequencing, chromosome walking, Blotting Techniques - Dot blot, Southern, Northern, Western blot, DNA foot print assay, DNA finger print assay, gel retardation assay, nuclease