MANGALORE UNIVERSITY

DEPARTMENT OF BIOCHEMISTRY

MSc Biochemistry

HARDCORE BCH 551:BIOTECHNOLOGY

Total Number of Lecture Hours:56hours **Total Number of Credits:04**

Course objectives

- To study the concept of genecloning.
- To elucidate the sequence and identify the clones using various moleculartechniques.
- Maintenance of animal cell and plant tissue culturelaboratory.
- Applications offermenter.

Course outcome:

- The student would understand the methods involved in gene cloning in using vectors in various hostcells.
- Selection and identification of clone by different methods of transformation in plants andanimals
- DNA isolation, amplification of DNA by PCR, blotting techniques and applications ofbioengineering.
- Positive and negative impacts of genetic engineering.

UnitI **14 hrs.**

Basic Principle of Gene Cloning: 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. Vectors: Plasmids, Lambda phage, Cosmid, phagemid, Yeast cloning vectors, bacterial artificial vectors, plant vectors, SV 40, expression vectors, Ligation: blunt end and sticky end ligation, use of linkers and adopters, homo polymer tailing, cDNA cloning.

Unit II **14 hrs.**

Cloneidentification–

Directselection,insertionalinactivationofmarkergene,visualscreening,immunologicaldetection method, colony and plaque hybridization. Transformation: Microinjection, electroporation, lipofection, calcium phosphate method, protoplast fusion, biolistic method. Cell culture techniques: Introduction to plant and animal tissue/cell culture, Laboratory design, aseptic conditions, equipment and materials for cell culture. Different constituents of culture medium, types ofmedia.

Unit III **14 hrs.**

Animalcellculture: Preparationofprimaryculture;disaggregationoftissueandprimarycultures,chickembryo,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-freeplants.

Unit IV

14 hrs.

Techniques:DNAsequencing,shotgunsequencing,chromosomewalking,PCR,applicationsofPCR,RT-PCRtechnique andapplications,RealtimePCRforquantification.BlottingTechniques-Dotblot,Southern,Northern,Westernblot,DNA footprint assay, DNA fingerprint assay, gel retardation assay, nuclease protection assay. RFLP, RAPD. Applications in agriculture medicine, industry, GM foods, negative impact of genetic engineering, gene knockout.

REFERENCES:

Gene cloning and DNA Analysis: An Introduction, Sixth edition, T A Brown

Molecular Biotechnology: Principles and Application of

Recombinant DNA, Glick and Pasternak Culture of

Animal Cells, Ian Freshney

Plant Tissue culture, S. S. Purohith