



MANGALORE UNIVERSITY

DEPARTMENT OF BIOCHEMISTRY

MSc BIOCHEMISTRY - III SEMESTER

BCH 551: **BIOTECHNOLOGY**

Total number of lecture hours: 42

Total number of credits: 03

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 fermenter.

Course outcome

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

Unit I

4hrs.

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

14hrs.

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. 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 of media.

Unit III

14 hrs.

Animal cell culture: 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 III

14 hrs.

Techniques: DNA sequencing, shotgun sequencing, chromosome walking, PCR, applications of PCR, RT-PCR technique and applications, Realtime PCR for quantification. Blotting Techniques- 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:

1. Gene cloning and DNA Analysis: An Introduction, Sixth edition, T A Brown
2. Molecular Biotechnology: Principles and Application of Recombinant DNA, Glick and Pasternak Culture of Animal Cells, Ian Freshney
3. Plant Tissue culture, S. S. Purohith

