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 adopters, 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

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protection assay. RFLP, RAPD. Applications in agriculture medicine, industry, GM foods, negative impact of genetic engineering, gene knock out.

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.

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
3. Culture of Animal Cells, Ian Freshney
4. Plant Tissue culture, S. S. Purohith
5. Principles and Techniques of Biochemistry and Molecular Biology, ed., Keith Wilson & John Walker, March 2010, Cambridge Univ. Press.

BCH 552: METABOLISM OF NITROGEN CONTAINING COMPOUNDS: HARDCORE

Lecture hours: 56 **Total Credits: 04**

Course objectives:

- To have a clear picture of nitrogen cycle.
- To learn amino acid metabolism and also urea cycle.
- To have a knowledge of degradation and biosynthesis of individual amino acids.
- To understand metabolisms of heme and nucleotides.

Unit I **14 hrs.**

Nitrogen Cycle: Introduction, biological and non-biological nitrogen fixation, *nif*genes, regulation and utilization of nitrate and nitrite, regulation of nitrate reductase. Assimilation of ammonia, formation of amino acid amides by glutamine synthetase and its regulation. **Amino acid Metabolism:** General metabolic reaction of amino acids–transamination, pseudo-transamination, glucose – alanine cycle, oxidative deamination (glutamate dehydrogenase), minor pathways of amino acid degradation – trans-deamination, amino acid oxidase, and non – oxidative deamination (α–deaminase, dehydratase, asparaginase and glutaminase). Urea cycle– regulation and metabolic disorders. Biosynthesis of creatine and creatine phosphate, polyamines– putrescine, spermidine and spermine, glutathione (γ-glutamyl cycle), physiologically active amines (γ-amino butyric acid, serotonin, histamine and catecholamines – dopamine, epinephrine and epinephrine).

Unit II **14 hrs.**

Degradation of the individual amino acids: Pathways in animal, plant and microbial systems; Amino acids forming from pyruvate (alanine, glycine, threonine, serine, cystine and cysteine), oxaloacetate (aspartic acid and asparagine), α- ketoglutarate (glutamic acid, glutamine, arginine, histidine and proline),

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