

301: GENETIC ENGINEERING

UNIT I

1. The recombinant DNA Technology : General concept and Principle of cloning
2. Enzymes: Nucleases and restriction endonucleases - properties and types; phosphomonoesterases; polynucleotide kinase; DNA ligase; DNA polymerase I; RNA Dependent DNA Polymerase; terminal deoxynucleotidyl transferase; poly A polymerase
3. Prokaryotic host-vector system: Characteristics of *E. coli* as host; vectors for cloning in *E. coli* (plasmid, bacteriophage and plasmid-phage)
4. Other Prokaryotic host vector systems: Characteristics of Gram positive and Gram negative organisms as host and suitable vectors for cloning; Shuttle Vectors

UNIT II

1. Design and characteristics of expression vectors for cloning in prokaryotes
2. Factors affecting expression of cloned genes in prokaryotes
3. Cloning in Yeast: Properties of yeast as host for cloning and different types of vectors designed for cloning in yeast
4. Cloning in animals system: Animal system as a model host, Methods of introduction of foreign DNA in animal system; Vectors for cloning in animal system - SV 40, bovine papilloma virus, adenovirus, vaccinia virus, baculovirus and retrovirus vectors

UNIT III

1. Methods for Constructing rDNA and cloning: Inserts; vector insert ligation; infection, transfection and cloning
2. Methods for screening and selection of recombinant clones
3. DNA Libraries: Types, advantages and disadvantages of different types of libraries; Different methods for constructing genomic and full length cDNA libraries
4. Gross anatomy of cloned insert - size, restriction mapping and location

UNIT IV

1. Fine anatomy of DNA segment - General principle of chemical and enzymatic methods of nucleotide sequence analysis and advantages of automatic gene sequencers.
2. Localization of cloned segments in genomes - molecular and chromosomal location
3. Methods for determination of copy number of a cloned gene in genome
4. Mutant construction: Introduction, deletion, insertion and point mutation

UNIT V

1. Principles and applications of Blotting techniques - Southern, Northern, Western and South-Western; Polymerase Chain reaction, Oligonucleotide synthesis, site directed mutagenesis, in-situ hybridization,
2. Principle and applications of Gel Mobility Shift Assay, DNA Fingerprinting and DNA Footprinting, Restriction fragment length polymorphism, Chromosome mapping and chromosome painting
3. Applications of Recombinant DNA Technology in Medicine
4. Biosafety measures and Regulations for genetically engineered products

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Practical Exercises

1. Bacterial Culture and antibiotic selection media. Preparation of competent cells
2. Isolation of plasmid DNA
3. Isolation of phage DNA
4. Quantitation of nucleic acids
5. Restriction mapping of plasmid DNA
6. Cloning in plasmid/phagemid vectors
7. Preparation of helper phage and its titration
8. Preparation of single stranded DNA template
9. Gene expression in *E. coli* and analysis of gene product
10. Polymerase Chain Reaction

Reference Books

1. Recombinant DNA - By Watson et al
2. Principles of Gene Manipulation, by Old and Primrose
3. Gene Cloning: An introduction, by Brown
4. Biotechnology: Theory and Techniques (Vol I & II), by Chirikjian
5. Molecular Genetics of Bacteria, Dale
6. Molecular Cloning (Vol I, II & III), by Sambrook & Russell
7. Applied Molecular Genetics, by Miesfeld
8. Genes and Genome, by Singer & Berg
9. Molecular Biotechnology, by Glick & Pasternak
10. Plant Molecular Biology (Vol I & II), by Gilmartin & Bowler

Note: All text books are of latest editions:

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302: ENZYME TECHNOLOGY

UNIT I

1. Enzyme: Historical aspects, classification and nomenclature, EC number.
2. Mechanism of enzyme catalysis and action
3. Sub cellular localization and organization of enzymes
4. Methods of enzyme assay: continuous and sampling techniques, coupled enzyme assay and methods and significance of enzyme turnover number; specific activity

UNIT II

1. Enzyme purification techniques: objectives and strategy; methods of homogenization; method of isolation; purification and crystallization
2. Criteria of purity and tabulation of purification data; stable storage of enzymes
3. Characterization of purified enzyme
4. Coenzymes, Cofactors and Isoenzymes

UNIT III

1. Enzyme Kinetics: Equilibrium and steady state theory, rate equation and determination of K_m and V_{max}
2. Factors affecting rate of enzyme reaction: pH, temperature and pressure
3. Enzyme inhibition: reversible and irreversible inhibition, their type, inhibitor constant and its significance
4. Rapid reaction techniques

UNIT IV

1. Protein- ligand binding: types, cooperativity, Hill and Scatchard plot, Allosteric enzymes : Models of allostery, types and kinetics
2. Regulation of enzymes
3. Mechanism of action of Chymotrypsin; Ribonuclease; Lysozyme; Metallo-enzymes
4. Degradation of enzymes

UNIT V

1. Enzyme immobilization; techniques; experimental procedures and effect of immobilization on kinetic parameters
2. Principle and Industrial application of immobilized systems
3. Enzymes in Medical diagnosis and enzyme therapy
4. Enzymes during aging

Practical Exercises :

1. Protein estimation methods: Lowry, Bradford and Spectrophotometric.
2. Urease estimation in plant tissues
3. Assay of Acid phosphatase in plant seeds
4. Assay of Alkaline phosphatase in Kidney and Liver
5. Determination of optimum pH, temperature & time
6. Determination of K_m value of alkaline phosphatase
7. Acetylcholinesterase estimation in Rat /Goat Brain
8. Enzyme purification: Ammonium sulphate precipitation, Ion exchange chromatography, molecular sieve chromatography.

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9. Checking of purity of enzyme by PAGE
10. Molecular weight determination of enzyme by Gel Filtration
11. Immobilization of HRP (Horse reddish peroxidase).
12. Kinetic properties of Immobilised HRP
13. Sub-cellular fractionation of rat liver and marker enzyme assays.

Reference Books

1. The Nature of Enzymology by R.L. Foster
2. Enzymes by Dixon and Webb
3. Fundamentals of Enzymology by Price and Stevens
4. Enzyme Catalysis and Regulation by Hammes
5. Enzyme Reaction Mechanisms by Walsch
6. The Enzymes vol I and II by Boyer
7. Enzyme Structure and Mechanism by Alan Fersht
8. Enzyme Assays: A Practical Approach by Eisenthal and Danson
9. Enzyme Biotechnology by G. Tripathi
10. Practical Biochemistry by Plummer.
11. Practical Biochemistry by Sawhney and R. Singh

Note: All text books are of latest editions.

Ajay Kumar

