Recombinant DNA Technology

This is a four credit course, which includes class room teaching, practical laboratory experiments and industry visits. It can be selected individually by the students and does not comprise a consolidated course program.

Prerequisites: Basic biological science background.

Evaluation: Continuous evaluation which includes seminars, practical experiments, field visits, reports, discussions, debates and at least two written tests

Learning objectives: The student will have an understanding genetic engineering and what it involves - the enzymes, the vectors required and the various cloning strategies. In addition the advantage and disadvantages of genetic engineering, the ethical and moral issues and the application of genetic engineering in various fields.

Unit –I
Restriction – modification systems, Deoxyribo nuclease- exo-nuclease and endo nucleases- Restriction enzymes- type-I, II, and III. S1 Nuclease, DNA Ligases, Alkaline phosphatase, DNA polymerase,

Unit –II
Cloning strategies:- shot gun cloning, cDNA cloning, -advantages and disadvantages; Construction of genomic DNA and cDNA libraries. Cloning Vectors -plasmids, lambda phage, SV40. Phagemids- Construction of artificial chromosome vectors-BAC & YAC, Expression systems and their applications. Human Genome

Unit –III
Recombinant DNA-tailing, cohesive ends, Use of linkers, blunt end methods. In vitro packaging, Host vector systems. Probe construction, recombinant selection and screening, Southern hybridization, Colony hybridization, Plaque hybridization

Unit –IV
Applications - RT-PCR/ Inverse PCR/ Nested PCR/LAMP; Molecular Markers RAPD, RFLP, DNA fingerprinting-microsatellites and mini satellites, SNPs, ESTs, Barcoding/ Sequencing of DNA/ Microarray techniques/ SAGE/DNA footprinting

Unit –V

Site directed mutagenesis, direct gene transfer and molecular chimeras. Antisense technology/ Gene transfer in animals and plants; Microinjection, electroporation, biolistics, direct gene transfer using PEG, calcium chloride, calcium phosphate, Vector mediated gene transfer-Agrobacterium mediated transfer

Unit –V

Heterologous protein expression in prokaryote and Eukaryotes- Expression in E. coli, yeasts and mammalian cells. Advantages and disadvantages of the various expression systems, cloning of genes into vectors, production and subsequent characterization of the recombinant protein.

References:

1. From genes to Clones-Ernst L. Winnaker. 1st Indian reprint 2003,VCH Panima Educational Book Agency.