Subject Code : RCCSBI4

CORE COURSE VII- rDNA TECHNOLOGY

UNIT- I

Outline process of genetic engineering and recombinant DNA technology, Isolation of genes, exonuclease & endonuclease, Concept of restriction and modification - Restriction endonucleases, DNA modifying enzymes, Ligases. Different Kinds of Vectors - Plasmids, Phage vectors, Cosmids, Phagemids, Virus vectors, Shuttle vectors and expression vectors- YAC, BAC- *S. cerevisiae* system as a model.

UNIT -II

Host-vector system - Cloning vectors for *E. coli.*, Cloning vectors for Eukaryotes-methods of transformation - Cloning strategies, construction of genomic libraries and cDNA Libraries. Probe construction, recombinant selection and screening, Molecular cloning.

UNIT -III

Analysis of expression. Analysis of recombinant DNA (Selection methods – antibiotics, expression basis, GUS expression), sequencing (chemical degradation; chain termination and automated sequence). mutagenesis, altered expression and engineering genes. Site-directed mutagenesis.
DNA amplification using polymerase chain reaction (PCR): key concepts, Analysis of amplified products. Southern blot, Northern blot and Western blot Applications of PCR : Ligase chain reaction. RFLP, RAPD, DNA Finger printing.

UNIT -IV

Application of rDNA Technology in plants: Transgenic plants with reference to virus and pest resistances, herbicide tolerance and stress tolerance (cold, heat and salt); cytoplasmic male sterility; delay of fruit ripening; resistance to fungi and bacteria, Bio-pharmaceuticals and secondary metabolite production.

UNIT -V

Application of rDNA Technology in animals: Transgenic animals –pharmaceutical production; insulin production. farm animal protection; Gene therapy – haemopoietic cells, genetically engineered bone marrow cells, skin fibroblasts, hepatocytes, myoblast and genetically modified lymphocytes – Recombinant Technology in the production
of vaccines.

REFERENCES

1. Old, R.W and S.B. Primrose. 1996. Principles of Gene Manipulation: An Introduction to Genetic Engineering. Blackwell Scientific Publications, Oxford.
2. Glover, DM. and BD. Hames .1995. DNA Cloning: A Practical Approach.. IRL Press, Oxford.
3. Innis, M.A., D.H. Gelfand and J.J. Sninskey .1995. PCR Strategies.. Academic Press, San Diego.
4. Persing, D.H., K T.F Smith, F.C. Teower and T.J.While. 1993. Diagnostic Molecular Microbiology. ASM Press, Washington D.C.
5. Watson J.D.,Gilman M., Witkowski,J., and Zoller M. 1992. Recombinant DNA. Scientific American Books, New York.
6. Tvan R.S. 1997. Recombinant Gene Expression Protocols. Humana Press Inc., Tokowa.