MOHANLAL SUKHADIA UNIVERSITY, UDAIPUR

B. Sc. BIOTECHNOLOGY III YEAR TDC (2016-17)

Paper II : *Principles of Recombinant DNA Technology*

Unit-I

Genetic Engineering – Introduction, definition, scope and importance, molecular tools for genetic engineering; restriction endonucleases- types, nomenclature, recognition sequences, cleavage pattern; DNA ligases-properties and functions, ligation techniques. Vectors – general characteristics of vectors, desirable characters such as size, ori site, selection/ markers gene, restriction sites and MCS, cloning and expression vectors. Plasmids- pBR-322, pUC vectors, Ti-plasmid, M13 derived pUC vectors.

15 Credit hours

Unit-II

Vectors for cloning large DNA fragments- bacteriophage, λ vectors, cosmids, YAC, BAC, creation of recombinant DNA- cloning and selection of individual gene, DNA amplification by PCR: RAPD, RFLP, AFLP. Transformation techniques: preparation of competent cells of bacteria: exogenously supplied chemical methods Calcium chloride heat shock method, methods of DNA transfer:, liposome mediated method and electroporation, *Agrobacterium* T-DNA mediated method, gene gun method; determination of transformation/ transfection efficiency.

15 Credit hours

Unit-III

Gene libraries – genomic library and cDNA library, reverse transcriptase, Colony hybridization, screening by DNA hybridization, immunological assay and protein activity, labelling of DNA, RNA and proteins: use of radioactive isotopes, non-radioactive labelling, relative advantages, *in vivo* labelling, nick translation, random primer labelling, autoradiography, blotting techniques southern and northern.

15 Credit hours

Unit-IV

In vitro translation, protein profiling and its significance, fusion proteins, polyacrylamide gel and 2D gel electrophoresis, Western blotting, gel retardation assay, T-DNA and transposon mediated gene tagging, chloroplast transformation and its utility, DNA microarrays to study gene expression etc., Basics of protein engineering and design.

15 Credit hours

Unit-V

Antisense and ribozyme technology: Molecular mechanism of antisense molecules, inhibition of splicing, polyadenylation and translation, disruption of RNA structure and capping, biochemistry of ribozyme, hammerhead, hair pin and other ribozymes, strategies for designing ribozymes, application of antisense and ribozyme technologies.

15 Credit hours

Suggested Readings

1. Christopher, H. Gene cloning and Manipulation. Cambridge University, Press.
2. Nicholl, D.S.T. An introduction to genetic engineering. Cambridge University Press.
3. Sambrook, Russell and Maniatis. Molecular Cloning : A Laboratory Manual (Vol. I, II and III). Cold Spring Harber Laboratory.
4. Glover, D.M. and Hames, B.D. DNA Cloning : A practical approach. IRL Press. Oxford.
5. Brown, T.A. Gene cloning. Blackwell Publisher.
6. Kreuzar, H. and Massey, A. Recombinant DNA technology. A.S.M. Press, Washington.
7. Llibelli, Lanza and Campbell. Principles of Cloning. Academic Press.