SARDAR PATEL UNIVERSITY
Programme: MSC (Genetics)
Semester: II
Syllabus with effect from: June 2010

Paper Code: PS02CGEN03	**Total Credits: 4**
Title Of Paper: Recombinant DNA Technology	

Unit	Description in detail	Weightage (%)
1	General strategies and steps involved in rDNA technology, Isolation of DNA from bacteria, plant and animals. **Restriction enzymes:** characteristics, Types; DNA ligase and other enzymes involved in gene manipulation. **Cloning vectors** - Plasmids, λ-bacteriophages, M-13 based vectors, Phagemids, Cosmids, YAC, BAC, PAC, HAC/MAC, plant and animal based viral vectors, expression vectors. **Introduction of DNA into different host systems**: chemical methods, Polyplexes, Liposome mediated transformation, Electroporation, Biolistic, Sonication, Viruses, Protoplast fusion.	**25 %**
2	**Polymerase Chain Reaction:** Principle, methodology and basic types of PCR-, Reverse Transcription, Real Time PCR, RACE, Inverse, Multiplex, Nested, Hot start PCR. Factors affecting PCR. Application and precautions. **DNA sequencing:** Maxam-Gilbert method, Sanger's method, Shot gun sequencing method, Mass spectroscopy based methods, Pyrosequencing, Automated DNA sequencing. Nucleic Acid Microarray. Generation of Genomic and cDNA libraries: Methods and strategies for preparation and screening of genomic and cDNA libraries, advantage and disadvantage of various methods, application of genomic and cDNA libraries.	**25 %**
3	**Characterization of cloned DNA:** Methodology and application of restriction mapping, S1 mapping, RFLP, RAPD, AFLP, SSR, SCAR, SNPs. **Selection and Screening of recombinant clones:** Direct and indirect methods. Probe preparation (radiolabelling and non- radiolabelling) Methods based on nucleic acid homology (Southern, northern, western blotting, subtractive, colony and plaque hybridization, in situ chromosomal hybridization, chromosomal walking); Protein Activity Assay – I, 2 and 3 yeast hybrid systems, Phage display, T-DNA and transposon tagging	**25 %**
4	**Expression Strategies for heterologous genes:** Vector engineering and codon optimization, host engineering, expression in bacteria, yeast, insect cells, mammalian cells and plants. **Processing of recombinant proteins**: Purification and refolding; characterization of recombinant proteins, stabilization of protein. **Application of rDNA technology:** Application in improvement of plants, animals and microbes, pharmaceutical products, molecular diagnostics, molecular pharming, gene therapy, evolutionary studies.	**25 %**

Basic Text & Reference Books:

- Molecular cloning: A laboratory manual, J. Sambrook, E. F. Fritsch and T. Maniatis 3rd Edition, Cold Spring Harbor, Laboratory Press, New York 2000. ISBN: 0-87969-577-3 (pbk)
- Current protocols essential laboratory techniques by Gallagher, Sean R. John Wiley & Sons, ISBN: 978-0-470-08993-4

- Recombinant DNA by Watson, James D. & Gilman, Michael et al 2nd ed., W.H. Freeman & Co. New York ISBN: 0-7167-2282-8.
- Principle of gene manipulation by Primrose, S. B. & Twyman R. M. & Old, R. W.Blackwell Science, USA. ISBN: 0-632-05954-0
- Principles of gene manipulation and genomics, S. B. Primrose and R. M. Twyman, 7th Edition, Blackwell Publishing, ISBN 978-1-4051-3544-3.
- Gene cloning and DNA analysis- An introduction, T.A. Brown, Blackwell Publishing, 5th edition, ISBN 13:978-14051-1121-8.
- Genetic engineering by Sandya Mitra, Macmillan India Ltd, Delhi, ISBN: 978-0333-92547-8
- Molecular Biotechnology – Principles and application of recombinant DNA, Bernard R. Glick and Jack J. Pasternak, 3rd Edition, ASM Press, Washington DC, ISBN 1-55581-269-4 DC, ISBN 1-55581-269-4.