SEMESTER III

Name of the Programme: M.Sc. Biotechnology

Course Code: GBT-600

Title of the Course: RECOMBINANT DNA TECHNOLOGY

Number of Credits: 3

Effective from AY: 2022-23

Pre-requisites for the Course:	General concepts in genetics and molecular biology	
Course Objectives:	The students will understand the use of 1) various enzymes and techniques for manipulating DNA. 2) various DNA vectors and their use in creating recombinant DNA molecules 3) recombinant DNA modification techniques and heterologous gene expression used for creating applications for biological research and biotechnology industries.	
Content:	**MODULE I** ● Enzymes used in Molecular biology: restriction endonucleases and methylases; DNA ligase, Klenow enzyme, T4 DNA polymerase, polynucleotide kinase, alkaline phosphatase; nucleases, Topoisomerase, thermostable polymerase, Terminal deoxynucleotide polymerase and others. ● Cohesive and blunt end ligation; linkers; adaptors; ● Homopolymer tailing; labelling of DNA: nick translation, ● Random priming, radioactive and non-radioactive probes, ● Hybridization techniques: northern, southern, south-western and far-western and colony hybridization, fluorescence in situ hybridization. Plasmids; Bacteriophages; M13mp vectors; pUC19 and pBluescript vectors, phagemids; Lambda vectors; Insertion and Replacement vectors; Cosmids; Artificial chromosome vectors (YACs; BACs); Principles for maximizing gene expression vectors; pMal; GST; pET-based vectors; Protein purification; His-tag; GST-tag; MBP-tag etc.; Intein-based vectors;	**No. of hours** 15

	Inclusion bodies; methodologies to reduce formation of inclusion bodies; mammalian expression and replicating vectors; ● Baculovirus and Pichia vectors system, ● Plant based vectors, Ti and Ri as vectors, yeast vectors, shuttle vectors.	
	MODULE II ● Principles of PCR: primer design; fidelity of thermostable enzymes; DNA polymerases; types of PCR – multiplex, nested; real time PCR, touchdown PCR, hot start PCR, colony PCR, cloning of PCR products; T vectors; proofreading enzymes; ● PCR based site specific mutagenesis; PCR in molecular diagnostics; viral and bacterial detection; ● Sequencing methods; enzymatic DNA sequencing; chemical sequencing of DNA; automated DNA sequencing; RNA sequencing; chemical synthesis of oligonucleotides; mutation detection: SSCP, DGGE, RFLP. ● Insertion of foreign DNA into host cells; transformation, electroporation, transfection; ● construction of libraries; isolation of mRNA and total RNA; reverse transcriptase and cDNA synthesis; cDNA and genomic libraries; construction of microarrays – genomic arrays, cDNA arrays and oligo arrays; study of protein DNA interactions: electrophoretic mobility shift assay; ● DNase I footprinting; methyl interference assay, chromatin immunoprecipitation; protein-protein interactions using yeast two-hybrid system; phage display.	15
	MODULE III ● Gene silencing techniques; introduction to siRNA; siRNA technology; Micro RNA; construction of siRNA vectors; principle and application of gene silencing; gene knockouts and gene therapy; ● Development of transgenic plants; debate over GM crops; introduction to methods of genetic	**15**

	manipulation in different model systems e.g. fruit flies (Drosophila), worms (C. elegans),Frog (Xenopus sp), fish (zebra fish) and chick. ● Transgenics - gene replacement; gene targeting; creation of transgenic and knock-out mice; disease model; introduction to genome editing by CRISPR-CAS with specific emphasis on Chinese and American clinical trials; ● Cloning genomic targets into CRISPR/Cas9 plasmids; electroporation of Cas9 plasmids into cells; purification of DNA from Cas9 treated cells and evaluation of Cas9 gene editing; in vitro synthesis of single guide RNA (sgRNA); using Cas9/sgRNA complexes to test for activity on DNA substrates; evaluate Cas9 activity by T7E1 assays and DNA sequence analysis; Applications of CRISPR/Cas9 technology	
Pedagogy:	Lectures, tutorials, assignments	
References/ Readings:	1. T. A. Brown, Gene Cloning and DNA Analysis: An Introduction, Wiley-Blackwell Publishers, 2016. 2. T. A Brown, Genomes, New York: Garland Science Publisher, 2017. 3. J. W. Dale, M. von Schantz and N. Plant, From Genes to Genomes: Concepts and Applications of DNA Technology, Wiley-Blackwell publisher, 2011. 4. H. K. Das, Textbook of Biotechnology, Wiley Publisher, 2017. 5. M. R. Green and J. Sambrook, Molecular Cloning: A Laboratory Manual.CSH Press, 2012. 6. V. Hunter and F. Strickland, Applications of Recombinant DNA Technology. ED-TECH Press, 2018. 7. A. J. Nair, Introduction to Biotechnology and Genetic Engineering. Laxmi Publications Pvt. Ltd, 2008. 8. S. Primrose and R. B. Twyman, Principles of Gene Manipulation and Genomics, Blackwell Publishing Limited, 2006. 9. M. K. Sarwar, I. A. Khan and D. Barp, Applied Molecular Biotechnology: The Next Generation of Genetic Engineering CRC Press, 2016. 10. V. Singh and P Dhar, Genome Engineering via CRISPR-Cas9 System, Elsevier Publisher, 2020.	
Course	The students will be able to	

Outcomes:	1. create recombinant DNA molecules and evaluate their expression. 2. Exploit relevant tool/techniques as well as vector and host for cloning and expression. 3. Design experiments for generating applications for use in medical animal and plant biotechnology. 4. Devise strategies for creating transgenic and understand CRISPER technology

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