



GUJARAT TECHNOLOGICAL UNIVERSITY

Integrated Master of Science (Biotechnology)

Semester: 7

Subject Name: Genetic Engineering

Subject Code: 1370403

Prerequisite: Students should have prior understanding of basic molecular biology, including DNA/RNA structure and function, gene expression, and recombinant DNA technology. Familiarity with basic microbiology and biochemistry will aid in comprehending experimental systems and enzymatic tools used in genetic manipulation.

Rationale: This course provides foundational and advanced knowledge of molecular tools and techniques used in genetic engineering. It equips students with the ability to manipulate genetic material for research, industrial, agricultural, and therapeutic purposes. Emphasis is placed on practical applications such as recombinant DNA technology, gene expression, genome editing (e.g., CRISPR-Cas9), and gene therapy, making the course highly relevant to biotechnology, molecular biology, and biomedical sciences.

Course Scheme:

Teaching Scheme			Total Credits	Assessment Pattern and Marks				Total Marks
L	T	PR		Theory		Practical		
			ESE (E)	PA(M)	ESE (V)	PA (I)		
4	0	0	4	70	30	0	0	100

Course Content:

Sr. No.	Course Content	No. of Hours	% of Weightage
1	<u>Introduction and tools for genetic engineering</u> Impact of genetic engineering in modern society; general requirements for performing a genetic engineering experiment; restriction endonucleases and methylases; DNA ligase, Klenow enzyme, T4 DNA polymerase, polynucleotide kinase, alkaline phosphatase; 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.	7	15
2	<u>Different types of vectors</u> 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; Inclusion bodies; methodologies to reduce	9	15



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	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.		
3	<p><u>Different types of PCR techniques</u></p> <p>Principles of PCR: primer design; fidelity of thermostable enzymes; DNA polymerases; types of PCR – multiplex, nested; reverse-transcription PCR, real time PCR, touchdown PCR, hot start PCR, colony PCR, asymmetric PCR, cloning of PCR products; TA cloning vectors; proof reading 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.</p>	7	15
4	<p><u>cDNA analysis</u></p> <p>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 footprinting; methyl interference assay, chromatin immunoprecipitation; protein-protein interactions using yeast two-hybrid system; phage display.</p>	9	20
5	<p><u>Gene silencing and genome editing technologies</u></p> <p>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; creation of transgenic plants; debate over GM crops; introduction to methods of genetic manipulation in different model systems e.g. fruit flies (Drosophila), worms (C. elegans), frogs (Xenopus), 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</p>	13	35



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	by T7E1 assays and DNA sequence analysis; Applications of CRISPR/cas9 technology. Applications of gene therapy/gene editing - antiviral strategies, cancer immunotherapy, hematologic disorders, liver-targeted gene editing, neuromuscular disorders, ocular disorders etc., examples of Chinese and American clinical trials.		
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Reference Books:

- Old, R. W., Primrose, S. B., & Twyman, R. M Principles of Gene Manipulation and Genomics Oxford: Blackwell Scientific Publications Latest edition
- Green, M. R., & Sambrook, J. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press Latest Edition
- Brown, T. A. Genomes New York: Garland Science Pub Latest Edition

Course Outcome:

After Completion of the Course, Student will able to:

No	Course Outcomes	RBT Level
1	Gain strong theoretical knowledge of this technology.	UN
2	In conjunction with the practicals in molecular biology & genetic engineering, the students should be able to take up biological research as well as placement in the relevant biotech industry.	AN
3	Communicate concepts and ideas effectively.	EL
4	Transparency, honesty and ethical reasoning in handling mutants and biomolecules.	CR

*RM: Remember, UN: Understand, AP: Apply, AN: Analyze, EL: Evaluate, CR: Create