GUJARAT TECHNOLOGICAL UNIVERSITY

Program Name: Master of Science (Industrial Biotechnology)

Level: PG

Course / Subject Code: IB01001031

Course / Subject Name : Genetic Engineering

1. Learning Outcomes

Learning Outcome Component	Learning Outcome (Learner will be able to)
Understand various approaches to conducting genetic engineering	• Gain strong theoretical knowledge of this technology.
Value applications of genetic engineering in biological research as well as in biotechnology industries	• 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.
Effective Communication	• Communicate concepts and ideas effectively.
Professional & Ethical Behaviour	• Transparency, honesty and ethical reasoning in handling mutants and biomolecules.

LO – PO Mapping: Correlation Levels:

1 = Slight (Low); 2 = Moderate (Medium); 3 = Substantial (High), "-"= no correlation

Sub Code: 1310103	PO1	PO2	PO3	PO4	PO5	PO6	PO7
LO1: Understand various approaches to conducting genetic engineering	2	2	3	3	2	2	3
LO2: Value applications of genetic engineering in biological research as well as in biotechnology industries	3	3	3	3	3	3	3
LO3: Effective Communication	3	3	3	2	3	2	1
LO4: Professional & Ethical Behaviour	2	2	2	3	2	2	3

2. Course Duration: The course duration is 45 sessions of 60 minutes each.

3. Course Contents:

Module No:	Module Content	No. of Sessions	70 Marks (External Evaluation)
1	**Introduction and tools for genetic engineering** Impact of genetic engineering in modern society; general requirements for performing a genetic engineering experiment; restriction endonucleases and	7	12

	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.		
2	**Different types of vectors** 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 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.	9	12
3	**Different types of PCR techniques** 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.	7	12

4	**cDNA analysis** 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.	9	12
5	**Gene silencing and genome editing technologies** 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 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.	13	22

6	**Practicals** 1. Concept of lac-operon: a) lactose induction of β-galactosidase. b) Glucose Repression. c) Diauxic growth curve of E. coli. 2. UV mutagenesis to isolate amino acid auxotroph. 3. Phage titre with λ phage/M13. 4. Genetic Transfer-Conjugation, gene mapping. 5. Plasmid DNA isolation and DNA quantitation. 6. Restriction Enzyme digestion of plasmid DNA. 7. Agarose gel electrophoresis. 8. Polymerase Chain reaction. 9. DNA Ligation. 10. Preparation of competent cells. 11. Transformation of E.coli with standard plasmids, Calculation of transformation efficiency. 12. Confirmation of the insert by Colony PCR and Restriction mapping 13. Expression of recombinant protein, concept of soluble proteins and inclusion body formation in E.coli, SDS-PAGE analysis 14. Purification of His-Tagged protein on Ni-NTA columns a) Random Primer labeling b) Southern hybridization	—	(30 marks)

4. Pedagogy:

- ICT enabled Classroom teaching
- Practical / live assignment
- Interactive classroom discussions

5. Evaluation:

Students shall be evaluated on the following components:

	Internal Evaluation	**(Internal Assessment – 20 Marks)**
A	• Continuous Evaluation Component	10 marks
	• Class Presence	5 marks
	• Record maintenance	5 marks
B	**Mid-Semester Examination**	**(Internal assessment-30 Marks)**
C	**End-Semester Examination**	**(External assessment-70 Marks)**

6. Reference Books:

No	Author	Name of the Book	Publisher	Year of Publication / Edition
1	Old, R. W., Primrose, S. B., & Twyman, R. M	Principles of Gene Manipulation and Genomics	Oxford: Blackwell Scientific Publications	Latest edition
2	Green, M. R., & Sambrook, J.	Molecular Cloning: a Laboratory Manual.	Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press	Latest Edition
3	Brown, T. A.	Genomes	New York: Garland Science Pub	Latest Edition

Note: Wherever the standard books are not available for the topic appropriate print and online resources, journals and books published by different authors may be prescribed.

7. List of Journals/Periodicals/Magazines/Newspapers / Web resources, etc

- https://www.nature.com/ng/
- https://www.neb.uk.com/support/download-literature

Course Outcomes:
On completion of this course, students should be able to:

- Strong theoretical knowledge of technology.
- Able to take up biological research as well as placement in the relevant biotech industry.