



GUJARAT TECHNOLOGICAL UNIVERSITY

Bachelor of Engineering
Subject Code: 3150401
ADVANCED MOLECULAR BIOLOGY

5TH SEMESTER

Type of course: Professional Core

Prerequisite: Basic Knowledge of Cell Biology and Molecular Biology

Rationale: The objectives of this subject is to teach regulation of central dogma, various approaches to conducting genetic engineering and their applications in biological research as well as in biotechnology industries.

Teaching and Examination Scheme:

Teaching Scheme			Credits C	Examination Marks				Total Marks
L	T	P		Theory Marks		Practical Marks		
				ESE (E)	PA (M)	ESE (V)	PA (I)	
4	0	2	5	70	30	30	20	150

Contents:

Sr. No.	Topics	Teaching Hrs.	Module Weightage
1	Regulation of Transcription: Prokaryotes : Gene regulation: Repressors, activators, positive and negative regulation, Constitutive and Inducible, small molecule regulators, operon concept: lac, trp, his operons, attenuation, anti-termination, stringent control, translational control, DNA re-arrangement, two component system; regulatory RNA – riboswitch, tmRNA, antisense RNA; transcriptional control in lambda phage. Eukaryotes: Regulation of RNA processing: capping, splicing, polyadenylation; mRNA stability and degradation: degradation and surveillance pathways; RNA editing; Nuclear export of mRNA; Catalytic RNA: Group I and Group II introns splicing, Peptidyl transferase; Regulatory RNA and RNA interference mechanisms, miRNA, non-coding RNA; Silencers and insulators, enhancers,	10	17%



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	<p>mechanism of silencing and activation, histone modifications and chromatin remodeling; Methods for studying DNA-protein interaction: EMSA, DNase I footprinting, methylation interference assay, chromatin immunoprecipitation.</p>		
2	<p>Genetic Engineering:</p> <p>General requirements for performing a genetic engineering experiment; biology of restriction, types and properties of restriction endonucleases and methylases; DNA ligase, Klenow enzyme, T4 DNA polymerase, polynucleotide kinase, alkaline phosphatase; cohesive and blunt end ligation; linkers; adaptors; homopolymeric tailing; labelling of DNA: nick translation, random priming.</p> <p>Biology of plasmids and bacteriophages; Plasmids & M13 mp vectors; pUC19 series and Bluescript vectors, phagemids; Lambda vectors; Insertion and Replacement vectors; Cosmids; Artificial chromosome vectors (YACs; BACs); Principles for maximizing gene expression (RNA and protein), protein 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; protein refolding; mammalian expression and replicating vectors; Baculovirus and Pichia vector system, wheat germ line expression system, plant based vectors, Ti and Ri as vectors, yeast vectors, shuttle vectors, reporters and reporter assays; Yeast two-hybrid system.</p> <p>Introduction of foreign DNA into host cells; transformation, electroporation, transfection; construction of genomic and cDNA libraries, phage display; strategies for library screening; radioactive and non-radioactive probes</p> <p>Techniques: Northern, Southern, South-western and Far-western and colony hybridization, fluorescence, in situ hybridization, PCR and its</p>	28	47%



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	variants Principles of PCR and alternative methods to PCR (LAMP, NASBA, SDA, RCA, LCR); Primer design; properties of thermostable DNA polymerase enzymes and proofreading; types of PCR – multiplex, nested, inverse, reverse-transcription PCR, real time PCR, touchdown PCR, hot start PCR, colony PCR, asymmetric PCR, RNA isolation, reverse transcriptase, cDNA synthesis, RNA quantitation, cloning of PCR products; T-vectors; PCR-based site directed mutagenesis and protein engineering		
3	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; introduction to methods of genetic manipulation in different model systems e.g. fruit flies (<i>Drosophila</i>), worms (<i>C. elegans</i>), and mouse model 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	9	15%
4	Molecular mapping and marker assisted selection Molecular markers - hybridization and PCR based markers RFLP, RAPD, STS, SSR, AFLP, SNP markers; DNA fingerprinting-principles and applications; introduction to mapping of genes/QTLs; marker-assisted selection - strategies for Introducing genes of biotic and abiotic stress resistance in plants: genetic basis for disease resistance in animals; molecular diagnostics of pathogens in plants and animals; detection of meat adulteration using DNA based methods.	7	12%
5	Methods for Gene Variation: Conjugation, Transduction, Recombination, Transposons	6	9%

Suggested Specification table with Marks (Theory): (For BE only)



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Distribution of Theory Marks

R Level	U Level	A Level	N Level	E Level	C Level
05	10	30	20	05	0

Legends: R: Remembrance; U: Understanding; A: Application, N: Analyze and E: Evaluate C: Create and above Levels (Revised Bloom's Taxonomy)

Reference Books:

1. Krebs JE, Goldstein ES and Kilpatrick ST (2014) Lewin's Gene XI, Jones and Barlett Publishers.
2. RF Weaver Molecular Biology, 5th edition (2012) McGraw Hill Higher Education
3. Watson JD, Baker TA, Bell SP, Gann A, Levine M & Losick R (2014) Molecular Biology of the Gene, 7th Edition, Cold Spring Harbor Laboratory Press, New York.
4. Old, R.W.; Primrose, S.B.; & Twyman, R.M.; (2001). Principles of Gene Manipulation: an introduction to Genetic Engineering. Oxford: Blackwell Scientific Publications.

Course Outcome:

Sr. No.	CO Statement	Marks % Weightage
CO-1	To explain and summarize fundamental knowledge of regulation of transcription in prokaryotes and eukaryotes.	25%
CO-2	To use of various tools and vectors for gene manipulation and cloning methods, individually and collaboratively that are typical of molecular biology laboratory investigations	25%
CO-3	To gain knowledge of gene silencing and editing tools and methods and appreciate their relevance for investigating specific contemporary biological questions.	20%
CO-4	To gain knowledge of various molecular markers and apply the same in the field of biotechnology	20%
CO-5	To understand various techniques of gene variation conjugation, transduction, recombination and trasposition	10%

LIST OF PRACTICALS:

1. Gene transfer conjugation – gene mapping
2. Restriction Digestion of plasmid DNA
3. DNA ligation
4. Preparation of competent cells.
5. Transformation of *E.coli* with standard plasmids and calculation of standard efficiency.



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6. Phage titer assay
7. Single stranded confirmation polymorphism
8. Transposon assay

List of Open Source Software/learning website:

- 1) NPTEL
- 2) MIT Open course lecture