I Year & I Sem.; M.Tech. Biotechnology & Bioprocess Engg.	L	T	P	TO	C
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(BT533) r - DNA TECHNOLOGY

Objectives of the Course:

This course will focus on the concepts about the structure, synthesis and processing of nucleic acids and protein synthesis in prokaryotes and eukaryotes. Also to make the students aware about the classification and types of mutations and how they effect the gene and its expression and how DNA will repair the damage.Also to provide the awareness about the process of gene expression and its regulation, different vectors used for gene transfer, cloning methods and their expression & detection, r-DNA technology and its applications.

UNIT I : Replication, Transcription & Translation mechanisms in prokaryotes & eukaryotes: Mechanism of DNA replication in *E.coli*, Eukaryotic telomeres and its replication, Homologous and Heterologous recombination. Mechanism of transcription in prokaryotes and eukaryotes, Post transcriptional processing of RNA 's t-RNA, r-RNA, m- RNA splicing. The genetic code and Wobble Hypothesis, Protein synthesis in Prokaryotes and Eukaryotes & their differences. Post translation modifications.

UNIT II: Mutagenesis & Gene regulation in prokaryotes & Eukaryotes: Classifications and types of mutations, their actions and applications. Mutagenesis – different mechanisms of mutagenesis, site directed mutagenesis, DNA damage and repair mechanisms. Mutagenicity testing using microbial systems- Ames test; gene regulation in prokaryotes - *lac, trp*, arabinose & *gal* operons, catabolic repression, attenuation; gene regulation in eukaryotes - gene amplication, gene rearrangements, enhancers, silencers, regulatory transcription factors, structural motifs, DNA response elements.

UNIT III: Vectors, expression & detection of clones Different types of cloning vectors -plasmid – pUC 8, ? pGEM3Z Phagemids, 2 mm plasmid, YAC, BAC, P element, Transposons, types of mechanisms and applications of transposons, retrotransposons; Cloning strategies, construction of prototype vector (pBR 322), Genomic and cDNA library construction and application; Detection & Expression of cloned genes in yeast & *E. coli*.

UNIT IV : Molecular Techniques & Molecular Markers*:*

DNA sequencing, foot printing with DNase I, HRT & HART, Protein- Protein interactions - phage display & yeast 2 hybrid systems, chromosome walking, pulsed-field & field inversion gel electro phoresis, immunochemical detection & immunochemical screening, colony hybridization, plaque-lift procedure, differential screening & replicaplating. Nested PCR, RACE, Microsatellites, EST's, SNP's, antisense technology, DNA profiling.

UNIT V: Applications of r-DNA Technology:

Gene cloning in medicine (Insulin, Blood clotting factor VIII), High-level expression of proteins in different host systems (*E. coli*, yeast, insect, mammalian cells), Inclusion bodies, refolding techniques, Case study for important therapeutic proteins, monoclonal antibodies and quality control of recombinant proteins. Introduction to Gene therapy (*Ex vivo & In vivo*) & Advantages and limitations of Gene therapy and novel technologies.

TEXT BOOKS :

1. B.D. Singh, "Fundamentals of Genetics", 4th ed., Kalyani Publishers, 2007.
2. Channarayappa, "Molecular Biotechnology Principles and Practices", University Press, 2006.

REFERENCE BOOKS :

1. G.m. Malacinski, "Molecular Biology", 4th ed.,Friefelder's Essentials Narosa Publisishng House, 2006.
2. Becker, Kleinsmith, Hardin, "The World of the Cell:Frief elder's EssentialsFriefelder's Essential" 6th ed., Pearson Publishers. 2006.