

## 22BT302 GENETIC ENGINEERING

Hours Per Week :

L	T	P	C
3	0	2	4

**PREREQUISITE KNOWLEDGE:** Cell and Molecular biology.

### COURSE DESCRIPTION AND OBJECTIVES:

The aim of this core-course is to acquaint the students to versatile tools and techniques employed in genetic engineering and recombinant DNA technology. This course provides theoretical bases for the application of versatile DNA modifying enzymes, cloning strategies, vector types, host genotype specificities for selection and screening of recombinants and/or recombinant transformants. Students will also be introduced to prominent nucleic acid labelling techniques, construction of genomic and cDNA library and whole genome sequencing.

### MODULE-1

#### UNIT-1

9L+0T+6P=15 Hours

#### PROKARYOTES

Lactose, tryptophan and arabinose operons, Repressors and activators, Eukaryotes – gene regulation, promoters and enhancer elements, Plasmids- definition, types, identification, classification, transfer of plasmids, Enzymes involved in genetic engineering, Different types of cloning vectors - plasmid (pUC19), lambda phage, cosmid, M13, BAC, YAC and YEP.

#### UNIT-2

15L+0T+10P=25 Hours

#### APPLICATIONS OF GENETIC ENGINEERING

Sigma switch in *Bacillus subtilis*, Gene rearrangement, Gene amplification, Epigenetic regulations – methylation, glycation and acetylation, Host restriction in transfer, Transposable elements definition, types of bacterial transposons, mechanisms of transposition and excision, detection of transposition in bacteria, retroviruses, applications of transposons, retrotransposons, Cloning strategies, construction of prototype vector (pBR322), Genomic and cDNA library construction and application, Detection of clone and its expression.

#### PRACTICES:

- Isolation of plasmid DNA by alkaline lysis method from *E. coli*.
- Restriction analysis of plasmid DNA and analysis by agarose gel electrophoresis.
- Cloning experiments using the chosen gene and a bacterial plasmid in a prokaryotic host.
- Setting up a dephosphorylation reaction using alkaline phosphatase enzyme.

### MODULE-2

#### UNIT-1

9L+0T+6P=15 Hours

#### MOLECULAR TECHNIQUES

Purification of genomic DNA from living cells, manipulation of purified DNA; Introduction of DNA into living cells - methods of gene transfer, DNA hybridization, blot analysis - southern, northern & western blot, Dot and slot blot, PCR-Principles, designing of primers, methodology, identification of PCR product.



source: <https://leaps.org/our-genetically-engineered-future-is-closer-than-you-think/>

**UNIT-2****5L+0T+10P=25 Hours****APPLICATIONS OF RDNA TECHNOLOGY**

DNA sequencing, DNA fingerprinting; Types of PCR, RT - PCR, multiplex PCR, application of PCR technology; Molecular markers: RFLP, RAPD, AFLP; 16S r-DNA typing, gene chip and microarray applications in disease profile and phylogeny; Gene cloning in medicine (Insulin, Blood clotting factor VIII); Gene therapy (Ex vivo & In vivo), case study of ADA as an example, advantages and limitations of gene therapy and novel technologies.

**PRACTICES:**

- Primer design to amplify genes.
- Optimization of PCR reaction mixture to Amplify gene by polymerase chain reaction (PCR).
- Preparation of competent cells by calcium chloride treatment for plasmid transformation.
- Setting up of ligation reaction using T4 DNA ligase and Dot Blot Techniques for quantification.
- Transformation of chemically competent *E.coli* with the ligation mixture, plating and analysis of transformants.

**COURSE OUTCOMES:**

Upon successful completion of this course, students will have the ability to:

CO No.	Course Outcomes	Blooms Level	Module No.	Mapping with POs
1	Analyze gene expression and regulation mechanisms.	Analyze	1	2, 4, 5,9,10
2	Apply gene manipulation techniques to produce GMO's.	Apply	1	2, 5, 6, 8,9,10
3	Evaluate structure and organization of different vectors used in gene transfer.	Evaluate	1	1,3,4,5,9,10
4	Design primers for amplification of genes.	Design	2	3,5,6,7,9,10

**TEXT BOOKS:**

1. T.A. Brown, "Gene Cloning and DNA Analysis: An Introduction", 8th edition, Wiley, 2020.
2. Bernard R Glick and Cheryl L. Patten, "Molecular Biotechnology: Principles and applications of recombinant DNA", 5th edition, ASM Press, 2017.

**REFERENCE BOOKS:**

1. R. M. Twyman and S.B. Primrose, "Principles of gene manipulation and genomics", 7th edition, Black Well, 2006.
2. G M Malacinski, "Freifelder's Essentials of Molecular Biology", 4th edition, Jones & Bartlett, 2015.
3. Jeff Hardin, Gregory Paul Bertoni and Lewis J. Kleinsmith, "Becker's World of the Cell", 8th edition, Pearson Education, 2013.

**SKILLS:**

- ✓ Analysis of digested DNA samples.
- ✓ Competent cell preparation.
- ✓ Selection of recombinant clones.
- ✓ Design of primers.