

## **Approval: 9<sup>th</sup> Senate Meeting**

**Course Name:** Genetic Engineering

**Course Number:** BY507

**Credit:** 3-0-2-4

**Prerequisites:** - IC 136 - Understanding basic Biotechnology & its Applications or Consent of Faculty member

**Students intended for:** UG/PG

**Elective or Compulsory:** Elective

**Semester:** Odd/Even

**Course Preamble:** This course will advance the knowledge of genetic manipulations of the organisms for the B.Tech. students. Genetic engineering techniques have been applied in several areas of Life Sciences and Biotechnology industries and are developing day by day. By the end of this course, the students are expected to know how to apply genetic principles in order to understand their applications in the development of biotechnology products such as enzymes, therapeutic proteins etc.

### **Course Outline:**

#### **Module 1 [4 Lectures]**

**Course Introduction:** What is Genetic Engineering? What will you learn? – A general outline of the course. Scope of the genetic manipulation methods in basic and applied Sciences

Outlook: what is the use of what you will learn here?

Gene and importance of gene cloning and analysis of the cloned DNA. History of genetic manipulations and its milestone discoveries

#### **Module 2 [4 Lectures]**

##### **Principles of gene cloning and DNA analysis:**

The early development of genetics. Invention of the DNA modifying tools and techniques for the gene cloning. Polymerase chain Reaction. Screening positive clones and confirmation of the cloned DNA with sequencing .

#### **Module 3 [6 Lectures]**

##### **DNA Manipulative Enzymes:**

Nucleases, ligases, polymerases, other DNA modifying enzymes. Enzymes for cutting DNA: restriction endonucleases. The discovery and function of the restriction endonucleases. Type II restriction endonucleases cut DNA at specific nucleotide sequences. Production Blunt and sticky ends of the DNA.

Restriction digestion of DNA. Analysis of the result of the restriction endonuclease reaction. Separation of the DNA and other molecules by gel electrophoresis. Visualizing DNA molecules in agarose gel. Estimation of the sizes of the DNA and restriction site mapping.

Ligation: Joining DNA molecules together. The mode of action of DNA ligase sticky ends, blunt ends, linkers and adaptors.

**Practical Classes will follow on this module.**

**Module 4 [16 Lectures]**

**Vectors for the gene cloning:**

Bacteriophages: The phage infection cycle, Lysogenic phages. Gene organization in the  $\lambda$  DNA molecule, the linear and circular forms of  $\lambda$  DNA. M13—a filamentous phage. Viruses as cloning vectors for other organisms.

Introduction of phage DNA into the bacterial cells: In vitro packaging of  $\lambda$  cloning vectors. Introduction of DNA into non-bacterial cells: Transformation of individual cells.

Cloning Vectors for E. coli: Cloning vectors based on E. coli plasmids.

More sophisticated/commercialized E. coli plasmid cloning vectors: pUC8—a lac selection plasmid, pGEM3Z—in vitro transcription of cloned DNA, cloning vectors based on M13 bacteriophage, how to construct a phage cloning vector, hybrid plasmid–M13 vectors. Insertion and replacement vectors

Cloning of long DNA fragments using a cosmid and other high-capacity vectors.

Cloning vectors for animals and insects. Viruses as cloning vectors for mammals, marker rescue extends the scope of direct selection. The scope and limitations of marker rescue.

Identification methods based on detection of the translation product of the cloned gene. A tutorial will follow this module on Software and online/freeware tools for analyzing restriction sites in DNA sequence. Vector NTI software for vector mapping.

**Module 5 [4 Lectures]**

Functional Genomics: Introduction to Gene knock-down and knock-out methods for bacteria, plant, Drosophila and Mouse organism.

**Module 6 [12 Lectures]**

**Applications of Genetic Engineering in Biotechnology:** The applications of Gene cloning and DNA analysis in Biotechnology. Production of protein from the the transgenic organism. Special vectors for expression of foreign genes in the E. coli. The promoter and its importance for an expression vector. Examples of promoters used in E.coli expression vectors. Expression cassettes and gene fusions. General problems with the production of recombinant protein in E. coli. Problems resulting from the sequence of the foreign gene. Problems caused by the host (E. coli). Production of recombinant protein by eukaryotic cells. Recombinant protein from yeast and filamentous fungi. Saccharomyces cerevisiae as the host for production of recombinant protein and advantages of this expression system.

. Protein production in mammalian and insect cells. Molecular Pharming—recombinant protein from live animals and Plants.

Gene cloning and DNA analysis in Agriculture: The gene addition/transfer approaches for plant genetic engineering. The  $\delta$ -endotoxins of Bacillus thuringiensis as an example (bt otton).

Gene cloning and DNA Analysis in Medicine. Production of the recombinant pharmaceuticals. Recombinant insulin: Synthesis and expression of artificial insulin genes. Synthesis of other recombinant human proteins and vaccines.

### **Text & Reference Books:**

A nascent textbook mentioned below will be used as appropriate and several recent papers from peer reviewed journals like Nature, Science, Molecular Therapy, PNAS, Biochemistry, JBC etc.

### **Reference Books:**

1. T. A. Brown. Gene Cloning and DNA Analysis: An Introduction, **6/e, Wiley, 2010.**
2. Sandy Primrose And Richard Twyman. Principles of Gene Manipulation and Genomics, **7/e Wiley-Blackwell, 2006.**
3. Desmond S. T. Nicholl. An Introduction to Genetic Engineering, **3/e Cambridge University Press, 2008.**