M Sc biotechnology course of study

Second Semester

Course Title: Genetic Engineering

Course No.: BT 521
Credits: 2

Objectives
At the end of the course the students should be able to:

- explain the various techniques of molecular biology and their uses in genetic engineering.
- know the uses of PCR in various field of molecular biology
- describe the various ways of genetic manipulation and transformation, and explain how the transformation can be proved by molecular techniques.
- describe artificial ways of inducing mutation and explain the role of mutation in molecular biology
- describe the types of DNA-library and explain the various methods of library screening
- explain the methods of DNA sequencing

Course Description

Introduction & Importance of Genetic Engineering **1hr**
Basic techniques of Gene cloning **4 hrs**
Introduction, Basic techniques: Restriction enzymes and restriction digestion, other enzymes used for DNA manipulation: synthesis, joining and modification, gel-electrophoresis: Principal, types, process and uses

PCR techniques **3 hrs**
DNA polymerase and RNA-polymerase used in PCR, Role of Mg^{++} and NTPs., PCR techniques and its multiple uses. The use of PCR in gene assembly: multiplex PCR, Overlap extension PCR. Real time quantitative PCR. PCR in molecular diagnostics, application of RT-PCR in gene expression

DNA cloning and esxpression **10 hrs**
Cloning vectors: plasmid, polylinker, lamda vector (phagemids), cosmid, Artificial chromosomes, marker and reporter gene, genetic transformation of E.Coli and selection, DNA recombination without ligase: topoisomerase, cre-lox recombination, Gate way method etc

DNA library: genomic library, cDNA library, expression library, substraction library
Cloning strategies, cloning in bacteria other than *E.coli*, cloning in yeast and other fungi, gene manipulation of animals and plants, Analysis of transcriptome. expression analysis of protein
Expression systems: Recombinant DNA technology, Synthesis of protein through expression vector, fusion protein, .Prokyaryotic: expression system in *E.coli,Bacillus* expression, eukaryotic : Pichiaexpression system; expression in insects system (Baculovirus expression system); Protein expression in mammalian cells

Identification, isolation and sequencing of cloned DNA **7 hrs**
Direct selection of gene, Oligonucleotide probes (radioactive and nonradioactive) Nucleic acid hybridization: southern blotting, northern blotting,

Library screening by membrane hybridization, Western Blotting and immunoscreening for expression library
Methods of DNA sequencing: Maxam and Gilbert, Sanger and Coulson, improvement in the methods, Pyrosequencing, next generation sequencing, Microarray and sequencing

Method of transformation **3 hrs**
Direct transformation: electroporation, microinjection, microprojectile bombardment, direct uptake of DNA fragment
Indirect method: through Ti-plasmid, conjugation, transduction

Functional genomics **5 hrs**
Mutation and induced mutation, The use of PCR in site directed mutagenesis and protein engineering, knockout mutation, role of transposons in mutation, isolation and analysis of mutants, genetic mapping of mutation, cloning of mutated gene, proteomics and genomics.

Microarray techniques and its uses **1 hr**