Programme: M.Sc. (Microbiology)

Course Code: MIO 108

Title of the Course: GENETIC ENGINEERING (T)

Number of Credits: 3

Effective from Academic Year: 2018-19

Prerequisites	Knowledge of bacterial and animal genetics, basic molecular and microbiology is a prerequisite.	
Objective:	This course aims to introduce the fundamental tools and techniques required for molecular cloning, with emphasis on DNA editing to protein expression in wide variety of hosts. Applications of genetic engineering in agriculture, therapeutics and industry will be covered.	
Content:		
1.	**Introduction to genetic engineering and tools involved in genetic manipulation**	**(16)**
1.1	**Introduction to genetic engineering**	
1.2	**Tools and techniques involved in genetic manipulation**	
A.	DNA modifying enzymes: restriction endonucleases, exonucleases, DNA ligases (T4 DNA Ligase and *E.coli* DNA ligase), Terminal DNA transferase, DNA Polymerases (Taq, Amplitaq, vent, Exo-vent, Pfu, T4 etc), Reverse transcriptase, T4 polynucleotide kinases, Alkaline phosphatase, S-1 Nuclease, Mung bean nuclease, RNases.	
B.	Gene cloning systems/Hosts: Gene cloning in *E. coli* and other organisms such as *Bacillus subtilis*, *Saccharomyces cerevisiae* and other microbial eukaryotes.	
C.	Cloning vectors: plasmid (pUC19, pBR 322), λ phage based vectors, cosmid vectors, Phasmid vectors, shuttle vectors, High capacity Cloning vectors (BAC and YACs).	
D.	Sequencing Vectors: pUC 19 and M-13 Phage vector.	
E.	Expression vectors: Prokaryotic (pET, pGEX-2T and others). Characteristics of expression vectors: strong bacterial and viral promoters (lac, trp, tac, SV 40, T7, T3) for induction of gene expression.	
F.	Construction of rDNA molecule and it's transfer to appropriate host (bacteria/yeast/plant cell/animal cell) using a suitable technique: transformation, electroporation, transfection, gene gun.	
G.	Other Recombinant DNA techniques: Use of radioactive and non-radioactive nucleotides for DNA probe preparation and detection of hybrids, Gel retardation assay, Restriction mapping, RFLP, PCR, RT-PCR, Real time PCR, Microarray, DNA sequencing using Sanger's Dideoxy chain termination method and automated sequencer; chromosome walking, Hybrid release and hybrid arrest translation to screen clones, site directed mutagenesis.	

2.	**Application of Genetic Engineering in Biology, forensics and medicine**	**(10)**
2.1	**Application of genetic engineering in DNA diagnostics and production of recombinant drugs, vaccines and hormones**	
A.	Screening of Genetic diseases using DNA probes (DNA diagnostics).	
B.	Production of recombinant proteins and drugs (insulin, tissue plasminogen activator, erythropoietin, human growth hormones, Antibodies (including bispecific antibodies), vaccines, interferons, DNA vaccines: merits and demerits, Edible vaccines- merits and demerits.	
C.	Application of recombinant DNA technology in solving parental dispute and criminal cases (DNA finger printing).	
2.2		
A.	Manipulation of gene expression in Prokaryotes; , gene expression from strong and regulatable promoters, Developing fusion proteins and separation of cloned protein by protease induced cleavage.	
B.	Genetic manipulation to increase recombinant protein stability and secretion using signal sequences.	
3.	**Application of Genetic Engineering in Agriculture**	**(05)**
3.1		
A.	Development of transgenic crops resistant to insect pests, bacterial, fungal and viral pathogens.	
B.	Strategies to develop transgenic crops and horticulture plants using various tools of recombinant DNA technology: Development of Bt Brinjal, Golden Rice and flavr savr tomato.	
C.	Importance of *Agrobacterium tumefaciens* in genetic manipulation of plants (Role of Ti plasmids), Role of *Bacillus thuringiensis* (*Bt* genes) to develop insect pest resistant crops.	
4.	**Application of Genetic Engineering in Industry**	**(02)**
4.1	**Genetic engineering of microbes for production of enzymes, biomolecules and fermentation products.**	
A.	Genetic manipulation of microbes to over-produce industrially valuable enzymes.	
B.	Production of microbial SCPs.	
5.	**Application of Genetic engineering in Bioremediation, Biorecovery and Biomonitoring of xenobiotics, metals and organometals.**	**(03)**
5.1	**Genetic engineering of microbes for bioremediation and biomonitoring of toxic environmental pollutants, Biohydrometallurgy**	
A.	Microbial bioremediation of xenobiotics by recombinant microbes.	
B.	Bioremediation of toxic heavy metals and organometals by recombinant microbes.	
C.	Biohydrometallurgy using recombinant microbes for recovery of precious metals.	

Pedagogy:	Lectures/tutorials/assignments/self-study	
References/ Readings	Old, R. W. and Primrose, S. B., Principles of Gene Manipulation: An introduction to Genetic Engineering, University of California Press.	
	Glick, B. R., Pasternak, J. J. and Patten, C. L., Molecular Biotechnology: Principles and Applications of Recombinant DNA, ASM Press.	
	Williamson, R., Genetic Engineering, Volumes 4-7, Academic Press.	
	Glover, D. M., Gene cloning: The Mechanics of DNA Manipulation, Springer-Science+Business Media, B. V.	
	Green, M. R. and Sambrook, J., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York.	
	Davis, L. G., Dibner, M. D. and Battey, J. F., Basic Methods in Molecular Biology, Elsevier.	
	Gerhardt, P., Methods for General and Molecular Bacteriology, Elsevier.	
	Grinsted, J. and Bennett, P. M., Methods in Microbiology, Vol. 21, Plasmid Technology, Academic Press.	
Learning Outcomes	1. Understanding of tools and techniques involved in molecular cloning. 2. Overall understanding about the importance of GMOs, GMPs and other engineered products in science and industry.	