Title of the Course: GENETIC ENGINEERING [T]

Course Code: MIC-623
Number of Credits: 3, Theory
Contact hours: 45
Effective from Academic Year: 2022-23

Prerequisites	Knowledge of bacterial and animal genetics, basic molecular biology and microbiology.	
Objective:	• Introduces the fundamental and state-of the-art tools and techniques required for molecular cloning and protein expression. • Elaborates the applications of genetic engineering in agriculture, therapeutics, industry and bioremediation.	
Content:		
1.	**Introduction to genetic engineering and tools involved in genetic manipulation**	**(20)**
1.1	**Introduction to genetic engineering**	**1**
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 kinase, Alkaline phosphatase, S-1 Nuclease, Mung bean nuclease, RNases.	**3**
B.	Gene cloning systems/Hosts: Gene cloning in *E. coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and other microbial eukaryotes	**2**
C.	Cloning vectors: Plasmids (Col plasmid, pUC19, pBR322 and their derivatives), λ phage based vectors, cosmid vectors, phasmid vectors, shuttle vectors, high capacity cloning vectors (BAC and YACs).	**3**
D.	Sequencing vectors: pUC 19 and M-13 phage vector.	**2**
E.	Manipulation of gene expression in Prokaryotes; Strong and regulatable promoters (lac, trp, tac, SV 40, T7, T3) for induction of gene expression; Prokaryotic expression vectors – pET, pGEX-2T and others; Fusion proteins; Genetic manipulation to increase recombinant protein stability and secretion	**2**
F.	Construction of recombinant DNA molecule and its transfer to appropriate host (bacteria/yeast/plant cell/animal cell) using suitable techniques: transformation, electroporation, transfection, gene gun.	**2**
G.	Gene cloning strategies: Cohesive end and blunt end cloning, universal TA cloning, shotgun cloning and directed cloning; genomic DNA cloning, reverse-transcriptase mediated synthesis of cDNA and cDNA cloning, screening of gene libraries for recombinant clones.	**2**

H.	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, real time PCR, microarray, DNA sequencing using Sanger's dideoxy chain termination method, capillary sequencing and next-generation sequencing; chromosome walking, hybrid release and hybrid arrest translation to screen clones, site directed mutagenesis.	**3**
2.	**Genetic Engineering in Biology, forensics and medicine**	**(10)**
A.	Screening of genetic diseases using DNA probes (DNA diagnostics)	**2**
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.	**6**
C.	Application of recombinant DNA technology in solving parental disputes and criminal cases (DNA fingerprinting).	**2**
3.	**Genetic Engineering in Agriculture**	**(05)**
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.	
D.	CRISPR-Cas mediated gene editing for improvement of farm animals and crops	
4.	**Applications of Genetic Engineering in Industry**	**(05)**
A.	Genetic manipulation of microbes to over-produce industrially valuable enzymes.	
B.	Production of recombinant pharmaceuticals, nutraceuticals and other biomolecules.	
C.	Production of fermentation products using recombinant organisms.	
D.	Production of microbial SCPs.	
5.	**Genetic engineering of microbes for biomonitoring, bioremediation and biohydrometallurgy.**	**(05)**
	Genetic manipulation of microbes to develop biosensors for monitoring toxic organic and inorganic pollutants, bioremediation of xenobiotics, toxic heavy metals and organometals,	

	Biohydrometallurgy for recovery of precious metals	
Pedagogy:	Lectures/tutorials/assignments	
References/ Readings	• Brown, T.A., Gene cloning and DNA Analysis: An Introduction, Blackwell Science (2020).	
	• Davis, L. G., Dibner, M. D. & Battey, J. F., Basic Methods in Molecular Biology, Elsevier (1994).	
	• Gerhardt, P., Methods for General and Molecular Bacteriology, Elsevier (2007).	
	• Glick, B.R., Pasternak, J.J. & Patten, C.L., Molecular Biotechnology: Principles and Applications of Recombinant DNA, ASM Press (2022).	
	• Glover, D. M., Gene cloning: The Mechanics of DNA Manipulation, Springer-Science+Business Media, B. V (2013).	
	• Green, M.R. & Sambrook, J., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (2012).	
	• Grinsted, J. & Bennett, P.M., Methods in Microbiology, Vol. 21, Plasmid Technology, Academic Press (1990).	
	• Old, R.W. and Primrose, S.B., Principles of Gene Manipulation: An introduction to Genetic Engineering, University of California Press (2014).	
	• Williamson, R., Genetic Engineering, Volumes 4-7, Academic Press (1997).	
Course Outcomes	• Apply tools and techniques involved in molecular cloning. • Formulate strategies for effective protein expression in prokaryotic hosts. • Evaluate the applications of genetic engineering techniques in medical and forensic fields • Appraise the potential of GMOs in industry and bioremediation.	