Programme: M.Sc. Marine Biotechnology
Course code: MBO 280
Title of the course: GENETIC ENGINEERING
Number of credits: 3
Effective from: 2019-2020

Course Objectives	To explain the various tools that are used in genetic engineering to create recombinants and its applications in biological research as well as in biotechnology industries	
Learning Outcomes	Given the impact of genetic engineering in modern society, students should be endowed with strong theoretical knowledge of this technology. In conjunction with the practicals in molecular biology & genetic engineering, the students should be able to take up biological research as well as placement in the relevant biotech industry.	
Content:	**MODULE I** • Enzymes used in Molecular biology: restriction endonucleases and methylases; DNA ligase, Klenow enzyme, T4 DNA polymerase, polynucleotide kinase, alkaline phosphatase; nucleases, Topoisomerase, thermostable polymerase, Terminal deoxynucleotide polymerase and others. • cohesive and blunt end ligation; linkers; adaptors; • homopolymer tailing; labelling of DNA: nick translation, • Random priming, radioactive and non-radioactive probes, • Hybridization techniques: northern, southern, south-western and far-western and colony hybridization, fluorescence *in situ* hybridization. • Plasmids; Bacteriophages; M13mp vectors; pUC19 and pBluescript vectors, phagemids; Lambda vectors; Insertion and Replacement vectors; Cosmids; Artificial chromosome vectors (YACs; BACs); Principles for maximizing gene expression vectors; pMal; GST; pET-based vectors; Protein purification; His-tag; GST-tag; MBP-tag *etc.*; Intein-based vectors; Inclusion bodies; methodologies to reduce formation of inclusion bodies; mammalian expression and replicating vectors; • Baculovirus and *Pichia* vectors system, • Plant based vectors, Ti and Ri as vectors, yeast vectors, shuttle vectors. **MODULE II** • Principles of PCR: primer design; fidelity of thermostable enzymes; DNA polymerases; types of PCR – multiplex, nested; real time PCR, touchdown PCR, hot start PCR, colony PCR, cloning of PCR products; T - vectors; proof reading enzymes; • PCR based site specific mutagenesis; PCR in molecular	12 hours 12 hours

	diagnostics; viral and bacterial detection; • Sequencing methods; enzymatic DNA sequencing; chemical sequencing of DNA; automated DNA sequencing; RNA sequencing; chemical synthesis of oligonucleotides; mutation detection: SSCP, DGGE, RFLP. • Insertion of foreign DNA into host cells; transformation, electroporation, transfection; • construction of libraries; isolation of mRNA and total RNA; reverse transcriptase and cDNA synthesis; cDNA and genomic libraries; construction of microarrays – genomic arrays, cDNA arrays and oligo arrays; study of protein - DNA interactions: electrophoretic mobility shift assay; • DNase I footprinting; methyl interference assay, chromatin immunoprecipitation; protein-protein interactions using yeast two-hybrid system; phage display. **MODULE III** • Gene silencing techniques; introduction to siRNA; siRNA technology; Micro RNA; construction of siRNA vectors; principle and application of gene silencing; gene knockouts and gene therapy; • creation of transgenic plants; debate over GM crops; introduction to methods of genetic manipulation in different model systems *e.g.* fruit flies (*Drosophila*), worms (*C. elegans*),Frog (xenopus), fish (zebra fish) and chick; • Transgenics - gene replacement; gene targeting; creation of transgenic and knock-out mice; disease model; introduction to genome editing by CRISPR-CAS with specific emphasis on Chinese and American clinical trials; • Cloning genomic targets into CRISPR/Cas9 plasmids; electroporation of Cas9 plasmids into cells; purification of DNA from Cas9 treated cells and evaluation of Cas9 gene editing; *in vitro* synthesis of single guide RNA (sgRNA); using Cas9/sgRNA complexes to test for activity on DNA substrates; evaluate Cas9 activity by T7E1 assays and DNA sequence analysis; Applications of CRISPR/cas9 technology	**12 hours**
References/ Reading	1. Brown, T. A. (2006). *Genomes* (3rd ed.). New York: Garland Science Pub 2. S. Primrose, R. Twyman, B. Old, and G. Bertola (2006). *Principles of Gene* 3. *Manipulation and Genomics*, Blackwell Publishing Limited; 7th Edition 4. Green, M. R., & Sambrook, J. (2012). *Molecular Cloning:*	

	A Laboratory Manual. 5. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. 6. Selected papers from Scientific Journals, particularly Nature & Science. *7.* Technical Literature from Stratagene, Promega, Novagen, New England Biolab. 8. Introduction to Biotechnology and Genetic Engineering (2008)A.J. Nair Laxmi Publications Pvt. Ltd 9. From Genes to Genomes: Concepts and Applications of DNA Technology 2011by Jeremy W. Dale, Malcolm von Schantz , Nicholas Plant Wiley-Blackwell publisher 10. Textbook of Biotechnology Paperback – 2017 by H.K. Das Wiley Publisher 11. Gene Cloning and DNA Analysis: An Introduction 2016 T. A. Brown Wiley-Blackwell; 7th edition 12. Applied Molecular Biotechnology: The Next Generation of Genetic Engineering (2016)Muhammad Sarwar Khan, Iqrar Ahmad Khan, Debmalya Barh. CRC press 1st Edition	