DISCIPLINE SPECIFIC ELECTIVE COURSE-18 (BIOMED-DSE-18)

ADVANCED MOLECULAR BIOLOGY AND GENETIC ENGINEERING

CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course
		Lecture	Tutorial	Practical/ Practice		
Advanced Molecular Biology and Genetic Engineering	4	2	-	2	XII Passed	Basic knowledge of Biological Science

Learning Objective:

- The course aims to teach an in-depth understanding of how those basic principles are applied in developing advanced techniques, enabling students to analyze the genomes and proteomes of any organism.
- The students would gain perspective on the transition of applications of molecular techniques performed in prokaryotes to the complex eukaryotes.

- As the course progresses, students will gain proficiency in gene editing tools such as CRISPR-Cas9 in creating transgenic organisms and in genetic engineering; they will learn the importance of molecular interactions, including protein-protein protein-DNA interactions.
- Finally, students will be primed to the significance of responsible molecular biology research practices and understand the ethical, regulatory and social aspects of RDT and genetic engineering.

Learning Outcomes:

- Based on this learning, they will appreciate how the recombinant DNA technology, which provides the ability to isolate, manipulate and express genes derived from any cell type, is helpful in creating therapeutic genes and recombinant proteins in human medicines.
- In-depth understanding of transcription, translation, and post-transcriptional modification process.
- Understanding the applications of these molecular processes and comprehending the next-generation techniques used in genome sequencing and analysis.
- Understand and apply molecular techniques in producing transgenic organisms and recombinant proteins as therapeutics.
- Students would appreciate the recent advances in Molecular Biology, Genetic Engineering and advanced high throughput sequencing methods that are leading to whole genome

sequencing of diverse organisms, and creating recombinant proteins in human medicine.

SYLLABUS **30 hours**

Unit-I: Basic Understanding of Molecular Biology (3 Hours)

Structure of DNA and its forms (duplex, triplex, quadruplex); Structure and versatility of RNA; Structure of Proteins; Formation of nucleosome, chromatin and genome structure; Replication, Mutation and Repair of DNA. Mechanisms of Transcription, RNA-splicing and Translation. The Genetic Code, Transcriptional Regulation in Prokaryotes and Eukaryotes, Regulatory RNAs.

Unit II: Genome Dynamics- Recombination and Transposable Elements (9 Hours)

Homologous Recombination in DNA –Strand invasion, Holiday Junction model and Double strand break and repair and various enzymes involved; Homologous recombination in eukaryotes; genetic consequences of recombination.

Site-Specific Recombination in DNA- Role of enzymes in Site Specific recombination - Tyrosine recombinases, gamma integrase, Hin recombinase

Transposition- Prokaryotic transposable elements- IS elements, Composite transposons, Tn-3 elements; Eukaryotic transposable elements- Ac-Ds system in maize and P elements in Drosophila; Uses of transposons; Eukaryotic Viruses.

Unit-III: Enzymes and Vectors used in recombinant DNA Technology (8 Hours)

Restriction Modification system; Introduction to rDNA technology: Molecular gene cloning by Gibson Assembly; Restriction and modification enzymes used in rDNA technology. Reverse transcriptase for cDNA synthesis

Prokaryotic and Eukaryotic Vectors for cloning & expression with one example each: Expression and purification of recombinant proteins using a therapeutic gene of interest as an example; Importance of fusion proteins. Importance of genome organization of λ bacteriophage for understanding cloning vectors. Yeast vectors and expression system.

Unit-IV: Applications of rDNA Technology in Genetic Engineering (10 Hours)

a. Construction of cDNA library: Preparation and cloning of cDNA, Applications of cDNA library. A comparative analysis of Northern hybridization & Microarray methods for studying transcriptomes; Phage Display and Immunoprecipitation for protein-protein interactions; South-Western hybridization and DNase-foot printing for DNA-Protein interaction
b. Basic concepts of transgenic organisms:Role of reporter genes, CRISPR-CAS; Functional analysis of cloned genesin transgenics; Production of transgenic plants, animals and microbes with one example of each; Applications of transgenic animals.
c. Production of recombinant proteins for their application in human medicine: Three generations of recombinant hormones as therapeutics with one example of each; Recombinant enzymes - Streptokinase synthesis and application in myocardial infarction.
d. Vaccines: DNA vaccines and Reverse Vaccinology

e. Ethical and legal concerns in transgenics and recombinant therapeutics: Ethical issues in genetic engineering, patenting genes, cloning, genetic testing & screening; The legal & socio-economic impact of Biotechnology; Biosafety regulatory framework for the production of genetically modified organisms (GMOs) & their release in the environment. Cartagena Protocol on biosafety.

Practical **60 hours**

(Wherever wet lab experiments are not possible, the principles and concepts can be demonstrated through any other material or medium including videos/virtual labs etc.)

1. Gene-specific Primer Designing for PCR-based cloning.
2. Based on the provided restriction map of a plasmid vector, selection of REs for restriction digestion experiment.
3. Double Restriction Digestion of a recombinant plasmid and analysis using agarose gel electrophoresis.
4. RNA isolation and gel electrophoresis.
5. Checking the methylation status of provided genomic DNA using isochizomers such as DpnI and DpnII.
6. Bacterial transformation with a yeast vector that serves as a shuttle vector.
7. To perform a zymogram to assay the activity of an enzyme using Native Gel Electrophoresis.
8. Application of PCR technique in Forensics
9. Mandatory Project on **anyone** of the following topics (group of 5 to 10 students):
   9a. PCR-based cloning of a prokaryotic geneand over-expression of the recombinant protein on SDS PAGE.
   9b. Construct the design of an experiment/flow chart of techniques and methods learnt in this paper (and previous papers, too) to find a solution in Gene Therapy/production of a recombinant protein/in forensic, etc.

Essential Readings:

- Karp, G. (2010). Cell and Molecular Biology: Concepts and Experiments. (VI Edition): John Wiley & Sons. Inc.
- Becker, W.M., Kleinsmith, L.J., Hardin. J. and Bertoni, G. P. (2009). The World of the Cell.(VII Edition). San Francisco: Pearson Benjamin Cummings Publishing.
- Peter J. Russell. (2009). Genetics- A Molecular Approach. (III Edition). San Francisco,United States of America: Benjamin Cummings.
- Watson, J. D. Baker T. A. Bell, S. P. Gann, A. Levine, M. and Losick, R. (2013). 7thEdition. *Molecular Biology of the Gene.* New York, USA: Cold Spring Harbor LaboratoryPress,ISBN-13: 978-0-321-76243-6.
- De Robertis, E.D.P. and De Robertis, E.M.F. (2006). Cell and Molecular Biology. (VIII Edition). Philadelphia: Lippincott Williams and Wilkins.
- Malacinski, George M.; Freifelder, David (1998). Essentials of Molecular Biology. (III Edition) Jones & Bartlett Pub.

- Brown, T. A. (2016). 7th Edition. Gene cloning and DNA analysis: An introduction. New York, USA: John Wiley and Sons, ISBN- 978-1-119-07256-0.
- Primrose, S. B. and Twyman, R. B. (2006). 7th Edition. Principles of gene manipulation and genomics. Oxford, UK: Blackwell Scientific Publishers. ISBN: 978-1405135443.
- Bernard, R. G. Jack, J. P. and Cheryl, I. P. (2022). 6th Edition. Molecular biotechnology: Principles and applications of recombinant DNA. USA: ASM press, ISBN- 978168367368.

Suggested Readings:

- Kornberg,A.(2005).2nd Edition.*DNAreplication.*California,USA:UniversityScienceBooks,ISBN-13: 978-1891389443.
- Cox, M. M. Doudna J. A. and Donnell, M. O. (2012). 1st Edition. *Molecular biology:Principles and practice.* London, UK: W H Freeman & Co Publishers, ISBN-13: 978-0-716-7998-8.
- Green,M.R.andSambrook,J.(2012).4thEdition.*Molecularcloning:Alaboratorymanual,* New York, USA: Cold Spring Harbor Laboratory Press, ISBN-13:978-1936113422.
- Winnaeker E.L. (1987). From Genes to Clones: Introduction to Gene Technology. Publisher VCH. ISBN - 0895734206,9780895734204.
- D.M. Glower and B.D. Hames (1995). DNA cloning: A practical approach byIRLPRESS, Oxford. ISBN: 9780199634767.