DISCIPLINE SPECIFIC CORE COURSE –14 (BIOMED-DSC-14) MEDICAL BIOTECHNOLOGY

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	Department offering the course
		Lecture	Tutorial	Practical / Practice			
Medical Biotechnology **BIOMED-DSC-14**	**4**	**3**	**-**	**1**	**XII Passed**	**Basic knowledge of biology**	**Biomedical Science**

Learning objectives

- The objective of this course is to enable the students to comprehend the concepts of recombinant DNA technology and apply the gained knowledge towards cloning and expression of genes and purification of the recombinant proteins.
- In the process, students would get a grasp on the cutting-edge technologies used in the analysis of nucleic acids and expressed proteins. The course aims to give students training in modern molecular techniques and help them make a connection between biological concepts and the technologies developed for various applications in biotechnology.
- The course finally aims to augment students' understanding of the role biotechnology plays/can play in various aspects of human medicine and provide them the platform to appreciate the drivers of emerging innovations in medical biotechnology along with biosafety and ethical concerns.

Learning outcomes

- Students will learn the contemporary techniques being applied in the field of medical biotechnology which include PCR, Gene Cloning, Gel electrophoresis etc.
- Students will gain a comprehensive understanding of DNA manipulation techniques and how to create recombinant DNA molecules by making a suitable choice of vectors and expression hosts.

- An in-depth understanding of gene cloning, expression in prokaryotic and eukaryotic systems and on the production of recombinant proteins shall prepare students to apply the gained knowledge on different organisms.
- Having grasped the fundamentals of recombinant DNA technology, its robust potential and the limitations & challenges, students shall discern the applications of biotechnology in human medicine. Their gained knowledge shall be imbued with a deeper understanding of the safety and limitations of molecular tools used in the diagnostics of infectious diseases, production of biopharmaceuticals and gene therapy.

SYLLABUS OF BIOMED-DSC-14

Unit I: Introduction to Recombinant DNA Technology and its applications in Medical Biotechnology

(13 hrs)

Brief history and scope of molecular biotechnology, concept of manipulation of DNA, cloning vectors and gene cloning. Restriction and modification system: Type I-IV restriction endonucleases, nomenclature and sequence recognition, isochizomers, blunt end and sticky ends, restriction mapping. Joining of DNA molecules: role of DNA ligase enzymes, adaptors, linkers, homopolymer tailing.

Cloning vectors: bacterial plasmids (T-vector, pUC vector), Lambda phage-derived vectors (replacement and insertion vectors), Cosmids, *in vitro* packaging. Gene cloning: Blunt end and directional.

Unit II: Expression of cloned genes in prokaryotes (13hrs)

Prokaryotic expression vector (pET vector). Bacterial transformation (*E.coli*): Preparation of competent cells ($CaCl_2$ method), selection of the transformants (antibiotic-resistance) and screening (blue/white & by colony PCR). Challenges in the expression of foreign proteins in a heterologous host, Factors affecting the expression: Promoters, Codon usage, Plasmid copy number. Fusion proteins and tagged protein cleavage system. Gene Probe preparation, Use of enzymatic and chemiluminescent methods for the detection of proteins.

Unit III: Cloning and expression in a eukaryotic system (09hrs)

Concept of auxotrophic mutants of yeast (eg. *Saccharomyces cerevisiae*) as cloning host. Cloning vectors (yeast Integrative (yIP), Replicative (yRP) and Episomal (yEP) plasmid, YAC), Shuttle vectors. Expression in eukaryotic cells, screening and selection of recombinants. cDNA cloning.

Unit IV: Applications of Medical Biotechnology (10hrs)

(a) Production of recombinant biopharmaceuticals: Insulin and Factor VIII.

(b) Gene Therapy: Strategies and limitations, Somatic and germline gene therapy, Vectors used in gene therapy (viral and non-viral) and their comparison.

(c) Polymerase chain reaction (PCR): Principle and applications. Importance of RT PCR in diagnosis of infectious diseases.

(d) Biosafety and ethical concerns in medical biotechnology.

Practical (30 hrs)

The below listed practicals are based on a guided project: 'PCR-based gene cloning' where students need to work in a group (4-6 students) to perform *in vivo* gene cloning. For this, any prokaryotic gene of interest may be chosen.

1. Plasmid DNA isolation
2. Designing of gene-specific primers
3. PCR amplification of the desired gene
4. Agarose gel analysis of plasmid DNA and PCR product(s).
5. Restriction digestion of plasmid DNA (vector) and PCR product (insert)
6. Ligation of the insert and vector using T4 DNA ligase
7. Preparation of competent cells (*E.coli*) using the calcium chloride method
8. Transformation of competent bacterial cells with ligation mixture along with suitable controls.
9. Screening of transformants by blue/white selection OR by colony PCR.

Essential Readings

- 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-978-1-683-6736-8
- Brown, T. A. (2016). 7th Edition. *Gene cloning and DNA analysis: An introduction.* NewYork, 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.

Suggestive Readings

- Karp, G. (2020). 9th Edition. *Cell and molecular biology: Concepts and experiments.* NewJersey, USA: Wiley Publishers, ISBN-13: 978-1119598244
- Green, M.R. and Sambrook, J.(2012). 4th Edition, (three-volume set). Molecular cloning: A laboratory manual. New York, USA: Cold Spring Harbor Laboratory Press ISBN-13:978-1936113422.