Birla Institute of Technology & Science, Pilani
Hyderabad Campus

FIRST SEMESTER 2023-24

Course Handout (Part-II)

Date: 11.08.2023

In addition to part I (General Handout for all courses appended to the time table) this portion gives further specific details regarding the course.

Course No. : **BIO F311**
Course Title : **Recombinant DNA Technology**
Instructor In Charge : **VIDYA RAJESH**
Lecture and Tutorial Instructors : **VIDYA RAJESH and Devika P.**

1. **Course Description:** Recombinant DNA technology is an interdisciplinary field encompassing biochemistry, microbiology, molecular biology, genetic engineering, and cell biology. It will focus mainly on the tools and techniques available to create and manipulate chimeric DNA molecules to gain basic theoretical understanding of recombinant technology through lectures, interactive tutorials, and quizzes. Emphasis will be placed on procedures to create chimeric molecules using examples from actual experimental work.
2. **Scope and Objective:** The course aims to introduce the students to the versatile tools and techniques of genetic engineering and recombinant DNA technology that can be applied to virtually any research question that involves a molecular approach. Vector designing, PCR, qPCR, DNA sequencing, in-vitro mutagenesis, cloning in prokaryotic and eukaryotic systems and whole genome approaches will be covered. The specific learning objectives are listed in the table below.
3. **Text Book (T1):** Gene Cloning & Manipulation, by Christopher Howe, 2nd Edition (First South Asia Edition, 2016), Cambridge University Press
4. **Reference Book (R1):** S. B. Primrose and R.M. Twyman Principles of Gene Manipulation and Genomics. 7th Edition, 2006; Blackwell Publishing;
   Reference Book (R2): Sambrook and Russell. Molecular Cloning: A laboratory Manual. Vols 1-3, 2001; CSH Press.
   Reference Book (R3): Desmond S. T. Nicholl. An introduction to Genetic Engineering. Third Edition. 2008, Cambridge)

Course Plan: Divided into 3 modules

MODULE 1: BASIC TOOLS AND FACTS				
Lect.	**Learning Objective**	**Topics to be covered**	**Reference for basic content**	**Advanced content**
1	Introductory concepts	Overview of rDNA technology	R1, R3: Chapter 1	-
2-3	About DNA and RNA	Review of nucleic acid chemistry; Genes and Genome, size complexity, Phosphodiester chemistry-based synthesis	R3: Chapter 2 Lecture notes	-
4-6	Basic tools & techniques	Cutting DNA; restriction endonucleases; other DNA modifying enzymes; joining DNA; ligases; Plasmid transformation, purification and electrophoresis; blotting techniques. Restriction mapping	T1: Chapter 1	R1: Chapter 3 R3: Chapter 3 and 4
7-9	Polymerase Chain Reaction	Principle of PCR; primer design; melting temperature; applications of PCR; modifications of PCR: inverse PCR; RNA-PCR; real-time PCR.	T1: Chapter 2	R1: Chapter 2, R3 Chapter 7
10-11	DNA sequencing	Principle of sequencing; Sanger sequencing; cycle sequencing; pyrosequencing; analysing DNA sequence data	R3: Chapter 3 Lecture notes	R1: Chapter 7
MODULE 2: ENGINEERING RECOMBINANT MOLECULES BASIC METHODOLOGY AND TECHNIQUES				
12-15	Plasmid vectors	Plasmid biology; plasmid replicons & copy number; examples of common plasmids; selection markers; cloning and expression vectors; expression modules	T1: Chapter 3	R1: Chapter 4 R3: Chapter 5
16-18	Cloning: making recombinant	Basic cloning; sticky and blunt ends; ligation; linkers & adaptors; homopolymer tailing; TA cloning for PCR products	T1: Chapter 3	R1: Chapter 3 R3: Chapter 6,8

	DNA molecules			
19-21	Alternate vectors for *E. coli*	Bacteriophage and cosmid vectors; molecular aspects of lambda and M13 life cycle relevant to vector design; BACs and PACs	T1, Chapter 4	R1, Chapters 4, 5 R3: Chapter 6
MODULE 3: ADVANCED APPLICATIONS AND POSSIBILITIES FOR FUTURE INNOVATIONS				
22-27	Making libraries and Library screening	Library screening; screening by hybridization; PCR screening; expression screening; functional screening; Southwestern and Northwestern screening; screening for other functions	T1, Chapter 5 and 6	R1, Chapter 6 R3: Chapter 6
28-30	Modification & mutagenesis; protein engineering	Restriction enzyme-based and oligonucleotide-directed mutagenesis; The single primer method; PCR-based mutagenesis; gene inactivation techniques; creating chimeric proteins	T1, Chapter 7	R1, Chapter 8
31-34	Expression and uses of cloned DNA	Expression of RNA and protein using cloned DNA; Expression strategies; purification of recombinant proteins; reporter genes and tags	T1, Chapter 8	R1, Chapter 6
35-41	Cloning in bacteria other than *E. coli* and other organisms; Gene transfer into animal cells	Gram stain; bacterial transposons; IncP, IncQ and IncW plasmids for cloning in Gram negative (other than *E. coli*); cloning in Gram positive bacteria; multigene assembly using *B. subtilis* Different methods of DNA transfection (physical and chemical); transient and stable transfection; selection markers (endogenous; dominant, amplifiable markers); viral vectors and introduction to their design	T1: Chapter 9 R1, Chapter 10	R1: Chapter 10-14
42	Futuristic road ahead	Where we are heading and what impact and transformations can this knowledge bring about. Whole organism cloning, targeting gene therapy, Overview of gene editing CRISPR-CAS9 and other advanced methods, Implications, Ethics and Perceptions	R3: Chapter 13-15; Lecture	R1: Part III and IV, commentaries and research papers.

5. Evaluation Scheme:

Evaluation Component	Duration	Weightage (%)	Date & Time	Remarks
Mid Sem examination	90 mins	30 (60)	12/10 - 4.00 - 5.30PM	Closed Book
Quizzes	30 mins	20 (40)	One each before and after mid sem	Tutorial (Closed book)
Assignment	1	10 (20)	During semester	October end or November (Open Book)
Comprehensive examination	3 hours	40 (80)	16/12 FN	Closed book (20%) + Open Book (20%)

6. Chamber Consultation Hour: Will be announced in the Class.

7. Notices: All notices, concerning the course will be displayed on CMS

8. Grading policy: Award of grades will be guided in general by the histogram of marks. Decision on border line cases will be taken based on individual's sincerity, attendance in classes, and the section instructor's assessment of the student. Students missing one or more component of evaluation completely may be given NC.

9. Make-up policy: For midsem and Compre, make-up will be granted only if candidate is sick and hospitalized. No make-up will be granted for surprise quizzes and assignments under any circumstances.

10. Academic Honesty and Integrity Policy: Academic honesty and integrity are to be maintained by all the students throughout the semester and no type of academic dishonesty is acceptable.

INSTRUCTOR-IN-CHARGE
BIO F311