



FIRST SEMESTER 2022-2023

Course Handout (Part-II)

Date: 14.08.2022

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 : **SHUVADEEP MAITY**
Co-Instructor and Tutorial Instructor : **SHUVADEEP MAITY, Murali Krishna Ramgopal**

- 1. Course Description:** Recombinant DNA technology is an interdisciplinary field encompassing biochemistry, microbiology, immunology, molecular biology, genetic engineering, cell biology and chemical engineering. This course will deal with theoretical aspects underlying the practice of recombinant DNA technology. It will focus mainly on the tools and techniques available to create and manipulate chimeric DNA molecules.
- 2. Scope and Objective:** This course intends to provide interested students an opportunity to gain basic theoretical understanding of recombinant technology through lectures, interactive tutorials, and quizzes. It 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. 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. Second Edition. 2002 (Cambridge)

5. Course Plan: Divided into 3 modules:

MODULE 1: Basic Tools and facts

Lect.	Learning Objective	Topics to be covered	Reference
1	Introductory concepts	Overview of rDNA technology	R1, R3 Chapter 1
2-3	About DNA and RNA	Review of nucleic acid chemistry; Types of DNA and RNA, Genes and genome : Size complexity	R3 Chapter 2, Lecture notes
4-10	Basic tools & techniques	Purification and visualization of DNA, RNA, Tools to modify DNA: restriction endonucleases; other DNA modifying enzymes; joining DNA: ligases; Restriction mapping, Gel electrophoresis, Nucleic Acid hybridization.	T1, Chapter 1 R3 Chapter 3, Lecture notes
11-12	Polymerase Chain Reaction	Principle of PCR; primer design; melting temperature; applications of PCR; modifications of PCR: inverse PCR; nested PCR, RAPD and other acronyms; real-time PCR.	T1, Chapter 2 R1, Chapter 2
13-14	DNA sequencing	Principle of sequencing; Sanger sequencing; cycle sequencing; pyrosequencing; analysing DNA sequence data	Lecture notes R1, Chapter 7, R3 chapter 3, 9

MODULE 2: Engineering Recombinant Molecules – Basic methodology and techniques

Lect.	Learning Objective	Topics to be covered	Reference
15-21	Cloning: making recombinant DNA molecules – Vectors, transformation and	Basic cloning; sticky and blunt ends; ligation; Plasmid biology; plasmid replicons & copy number; examples of common plasmids; selection markers; cloning and expression vectors; expression modules	T1, Chapter 3 R1, Chapter 4 and 6, R3 Chapter 6

	Hosts	Modifications : linkers & adaptors; homopolymer tailing; TA cloning for PCR products	T1, Chapter 3 R1, Chapter 3
22-23	Alternate vectors for <i>E. coli</i>	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 5
MODULE 3: Advanced applications and Possibilities for Future Innovations			
24-28	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 6 R1, Chapter 6
29-32	Modification & mutagenesis; protein engineering	Restriction enzyme-based and oligonucleotide-directed mutagenesis; The single primer method; PCR-based mutagenesis; gene inactivation techniques; creating chimeric proteins; Gene editing techniques	T1, Chapter 7 R1, Chapter 8
33-36	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 R3 Chapter 10
37-42	Cloning in bacteria other than <i>E. coli</i> and other organisms; Gene transfer into animal cells	IncP, IncQ and IncW plasmids for cloning in Gram negative (other than <i>E. coli</i>); cloning in Gram positive bacteria; multigene assembly using <i>B. subtilis</i> ; 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 -14, Lecture Notes
43	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 , Reading material/Lecture notes/commentaries

6. Evaluation Scheme: Total course weightage: 100% (Maximum Marks: 200)

Evaluation Component	Duration	Weightage (%)	Date & Time	Remarks
Quiz 1	20 mins	10% (20 Marks)	Oct 1 – Oct 6 (during scheduled lecture or tutorial Hour)	Closed Book
Mid semester exam	90 mins	30% (60 marks)	TBD	Open Book (50%) + Closed book (50%)
Quiz 2	20 mins	10% (20) Marks	November 10-November 20 (during scheduled class hour)	Closed Book
Assignment 1	Presentation /GD (by students) followed by viva (by instructor)	10% (GD) + 5% (viva) = 15% (30 Marks)	During the semester (tutorial hour) TBD	Open Book
Comprehensive examination	2 hours	35% (70 Marks)	20th December FN	Open Book (50%) + Closed book (50%)

6. Virtual drop-in Hour (Chamber Consultation hour): Will be announced in the Class after mutual discussion.

7. Notices: All notices and concerning the course will be displayed on the course pages of CMS or through emails.

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 Instructor's assessment. Students missing one or more component of evaluation completely may be given NC.

9. Make-up policy: Only for genuine cases like medical emergency- with proper evidence and with prior permission (if applicable). I/C's decision will be final.

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 Rec. DNA Tech.

