Birla Institute of Technology & Science, Pilani
Hyderabad Campus

ISEMESTER 2022-2023

Course Handout (Part II)

Date: 05.08.2022

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

Course No.:	**BIO G561**
Course Title:	**Advance recombinant DNA tech**
Instructor-in-Charge:	**Nishith Gupta**
Co-instructors:	**Ratnesh Kumar Srivastav**
	Mohammad Mustafa
	Keith Joseph Henderson
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Course Description : Recent advances in high--throughput genomics, proteomics and large--scale mutagenesis; genomics techniques like transcriptome arrays and arrays for whole genome analysis; proteomics analysis techniques like 2D PAGE and MS; understanding genome and protein structures and protein interactions through yeast/bacterial two--hybrid systems; large scale mutagenesis and interference.

1. Scope and Objectives of the Course:

This theory and lab-based course module is designed to teach students the basics and advanced techniques of genetic manipulation. The lecture series and hands-on experiencewill help themfathom the principles of transgenic procedures widely utilized in biological research.The course will focus on three prokaryotic and eukaryotic model organisms namely bacteria (*E. coli*), yeast (*S. cerevisiae* and/or *P. pastoris*) andmammalian cells (HEK and/or HeLa). Not least, the course will also teach methods of genomeengineering in intracellular protozoan parasites(*Toxoplasma gondii*, *Plasmodium falciparum*).

Intended Learning Outcomes: After successful completion of this course, students will be able to:

- Comprehend various aspects of recombinant DNA technology
- Understand methodology of genome manipulation in several prokaryotic and eukaryotic models
- Learn recombinant protein expression in bacteria and yeast
- Understand fundamentals of functional complementation in prokaryotes and eukaryotes

- CRISPR-Cas-based system for basic and advanced genomeengineering
- Ethics associated with genomemanipulation technologies

2. Textbook and Reference Books:

Textbook (T)

(1) Primrose and Tywman, Principles of Gene Manipulation and Genomics, WILEY
(2) Burton E. Tropp, Molecular Biology: Genes to Proteins, Third Edition
(3) Genome Engineering via CRISPR-Cas9 Systemby Vijai Singh, Publisher: Academic Press

Reference Book (R)and **reviews**

(1) Sambrook J., MacCallum P. and Russell D. Molecular Cloning: A Laboratory Manual (3rd edition, three-book set). New York, USA: CSHL Press, 2001.
(2) Eldon T Enger, Frederick C. Ross and David B. Bailey, Thirteenth Edition, Concepts in Biology
(3) Yeast Protocols Handbook; (https://www.med.upenn.edu/robertsonlab/assets/user-content/documents/Yeast%20Protocols%20Handbook.pdf)
(4) Karbalaei, M., Rezaee, S. A., &Farsiani, H. (2020). Pichia pastoris: A highly successful expression system for optimal synthesis of heterologous proteins. Journal of cellular physiology, 235(9), 5867–5881. https://doi.org/10.1002/jcp.29583
(5) Komor, A. C., Badran, A. H., & Liu, D. R. (2017). CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes. Cell, 169(3), 559. https://doi.org/10.1016/j.cell.2017.04.005
(6) Boris Striepen andDominique Soldati-Favre , Parasite Genomics: Genetic manipulation of *Toxoplasma gondii*,

3. List of Experiments:

(Note: Experiments may not necessarily be undertaken in the order listed below)

1. Preparation of *E.coli* competent cells (DH5α, BL21-DE3, XL1-blue and M15) and determination of transformation efficiency
2. Isolation of plasmid DNA and assessing the quantity and quality using spectrophotometer and agarose gel electrophoresis
3. PCR amplification of different genes using plasmid, genomic DNA and cDNA as the templates
4. Restriction digestion and ligation of the vector and PCR products to clone into the suitable expression vectors (Directional and non-directional cloning). Gibson assembly mediated cloning.
5. Transformation, and selection of positive clones (colony PCR, diagnostic cut and sequencing)
6. Functional complementation assay in *E. coli*

7. Methods of yeast culture (liquid and solid) at permissive and non-permissive temperature
8. Functional complementation assays in yeast
9. Protein expression in bacteria and yeast using denatured polyacrylamide gel electrophoresis
10. Immunoblot to confirm the recombinant protein expression
11. Mammalian cell culture and transfection(*time permitting*)
12. Bioinformatics tools required for genome engineering (Construct design, data base mining *etc*.)

4. Course Plan:

Lect. #	**Learning objective(s)**	**Topics to be covered**	**Chapter #**
1	Getting Oriented	Introduction to the course; Mode of evaluation	1-2& 26 (T1) 1& 5 (T2) 1 (R1)
		Overview of the rDNA technology and its utility	
2-4	DNA, RNA& Protein: The molecular basis of life and central dogma	DNA and replication in prokaryotes & eukaryotes	1,3 (T2) 8 (R2)
		Types of RNA and transcription	
		Protein translation	
5-6	Bacterial host (*E. coli*)	*E. coli* and its versatility; knowing genotypes of strains	2, 12 (T1), 1, 2 (R1)
		Transformation procedures used for *E. coli*	
7-8	Vectors for gene cloning	Characteristics of an ideal cloning vector	4,5,6 (T1) 2,3 (R1)
		Plasmid types;difference between prokaryotic and eukaryotic expression vectors	
9	Nucleic acid isolation and analysis	Plasmid and genomic DNA	4,5,6 (T1) 1,6 (R1)
		RNA and cDNA	
10	Restriction enzymes	Restriction enzymes and DNA digestion	3(T1), 17 (T2)
		Restriction mapping	
11	Other enzymes for DNA manipulation	DNA and RNA-dependent polymerases	3 (T1), 5, 13 (T2), 6,9,8,12 (R), Research article
		DNA ligase,phosphatase, kinase, and topoisomerase. Introduction of Gibson assembly	
12	Polymerase Chain Reaction (PCR)	Different types of PCR reactions, Primer design;	2 (T1), 7 (T2) 1, 8 (R), 5, 6 (T1), 1, 8 (R)
		site-directed mutagenesis; post-PCR analysis – results *vs.* artifacts, PCR for selecting positive colonies	
13	Techniques for DNA, RNA &gene analysis	Southern, Northern, RT-PCR, SAGE	2,7, 9, 20(T1)
		Sequencing	

			5 (T2)
14- 15	Protein expression	Characteristics of expression hosts Techniques to analyze recombinant protein expression(SDS-PAGE and Western blotting) Functional complementation in *E. coli*	~~3,6,8, A9~~ 5, 18, 20 (T2) A9 (R)
16- 17	Yeast as a host	Yeastas amodel for protein expression Functional complementation and two-hybrid system for protein-protein interaction analysis	11, 23 (T1), 16 (T2) Research articles
18- 19	Geneticengineering inhigher eukaryotes	Genome engineering in *C. elegans, Drosophila*, Zebra fish, mouse and human cells	12 (T1) Research articles
20- 21	Genome manipulation in parasitic protists	Gene knockout, knockdown in *Toxoplasma gondii* and *Plasmodium falciparum*	Research articles
22-23	Applications and Ethics	Sequencing and analyzing genomes, medical and forensic applications, Hybridoma, Bioethics	26 (T1), 3 (T2)
24-31	Omics	Genomics (Deep sequencing, large-scale mutagenesis) Transcriptomics (microarray and RNA seq) Proteomics (MS, 2D PAGE). Application of proteomics to understand PTMs Metabolomics, Glycomics ,Lipidomics	19- 25 (T1), Research articles
32-37	CRISPR-Cas9 system	Use of various methods of gene cloning in research and biotechnology	1, 2,3 (T3)
38-39	Miscellaneous and Feedback session	Interactive session with students	--

5. Evaluation scheme:

Component	Duration	Marks	%	Date and Time	Venue	Remarks
Mid-semester	90 min	40	20	02/11 1.30 - 3.00PM		Closed book (20%)
Laboratory Evaluation	-	100				TOTAL – Open Book (50%)
Continuous evaluation in lab and theory course		40	20			Quiz, presentation, interactions
Lab-based evaluation		60	30			Assignments, notebook maintenance
Comprehensive exam	180 min	60	30	23/12 FN		Closed book (20%)

Notes:
For the Observation component: Every student would be assessed on the following criteria during the lab sessions: how successfully and efficiently the student is engaged with assigned experimental tasks, scientific integrity, punctuality to the lab, maintenance of lab decorum, and ability to work in a group. Besides regular evaluation, pre-announced laboratory assignments could also be given. During classes, students will be assigned to present a relevant research article to evaluate their understanding.

6. Attendance Policy:

It is expected that the student attends every laboratory session and theory class. Individual students may be assigned specific tasks, forming part of the planned experiment, to be done before or during the lab hours, the completion of which may be required for the entire group. If failure to complete the task due to absence is anticipated, the studentmust inform the instructor before the scheduled laboratory.

7. Grading Policy:

Award of grades would be guided in general by the histogram of marks. Decision for borderline cases would be based on the individual's sincerity, attendance in classes and the instructor's assessment.

8. Chamber Consultation Hour: To be announced in the class.

9. Make-up Policy: Clause 4.07 of BITS *Academic Regulations* booklet should be consulted. Make-up can be requested only for the two class tests.

10. Notices:

All course announcements shall be displayed in CMS and/or in the Biological Sciences departmental notice board only.

11. **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 G561