Birla Institute of Technology & Science, Pilani
Hyderabad Campus

FIRST

SEMESTER 2021-2022

Course Handout (Part II)

Date: 20.08.2021

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 F418**
Course Title:	**GENETIC ENGINEERING TECHNIQUES**
Instructor-in-Charge:	**Nishith Gupta**
Co-instructors:	**Naresh Patnaik**
	Rolly Kumari
	Murali Krishna Ramgopal
	Peddiraju NVSJ Swaroop

1. Course Description:

Experiments involving common molecular biology techniques used for gene manipulation of bacteria and parasites; gene cloning methods in bacteria – from isolation of plasmids to screening of recombinant clones; polymerase chain reaction (PCR) and its applications; gene and protein expression analysis; DNA sequencing; designing of constructs for making transgenic parasites; usage of cloning software

2. Scope and Objectives of the Course:

This course aims to give students hands-on experience of the essential techniques used in the molecular biology laboratory, with specific emphasis on DNA manipulation. Students will also learn the theoretical basis underlying the experiments.

3. Textbook and Reference Books:

Textbook (T)

(1) Metzenberg, Stan. Working with DNA. Oxford: Taylor and Francis, 2007. Molecular Parasitology by Hugo H. Mejia-Madrid

(2) Primrose and Tywman, Principles of Gene Manipulation and Genomics, WILEY

Reference Book (R)

(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) Parasite Genomics: Genetic manipulation of *Toxoplasma gondii*, Boris Striepen and Dominique Soldati-Favre

3. List of Experiments:

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

1. Understanding of genetic manipulation using *E. coli*
2. Solutions and buffers
3. Preparation of *E.coli* competent cells
4. Determination of transformation efficiency using competent cells
5. Isolation of plasmid DNA
6. Agarose gel electrophoresis and quantification of plasmid DNA
7. Restriction digestion of the vector, dephosphorylation and verification of the vector quality
8. Restriction enzyme digestion of the PCR products and gel-purification for directional cloning into appropriate cloning vectors
9. Setting up ligation reactions and transformation of competent bacterial cells
10. Screening colonies for identification of recombinant plasmids
11. Sequence verification of the recombinant clones
12. Scaling up the recombinant plasmid clones and their purification

4. Lecture Plan:

Lect. #	Learning objective(s)	Topics to be covered	Chapter in the Text Book
1	Getting oriented	Introduction to the course; mode of operation in the online course; how to maintain the lab record notebook. Overview of the gene cloning procedure	1-3 (T) 1 (R)
2	Knowing the bacterial host (*E. coli*)	*E. coli* and its versatility; knowing genotypes of strains; transformation procedures for *E. coli*	4 (T), 5 (R)
3	Vectors for gene cloning	(i) Plasmids – types, characteristics of an ideal cloning vector (ii) Difference between prokaryotic and eukaryotic expression vectors (iii) Vectors for CRISPR-Cas9 system	4 (T) 5 (R) 15 (R)
4	Isolating and analyzing DNA	(i) Purification of plasmid DNA (ii)Purification of genomic DNA from bacteria (iii) Quantitation and electrophoresis of DNA	4 (T) 3 (T) 2 (T)
5	Restriction enzymes	Restriction enzymes and DNA digestion; restriction mapping	5 (T)
6	Other enzymes for DNA manipulation	DNA ligase, polymerase, phosphatase, kinase, topoisomerase.	6 (T), 4 (R)
7	Selecting the correct clone	Direct selection; selection from gene libraries	4 (T), 8 (R)
8	Polymerase Chain Reaction	How to perform a PCR reaction in the lab; Primer design; post-PCR analysis – results *vs.* artifacts	7 (T, R) 6 (R)
9-10	Techniques for DNA and gene analysis	Southern, Northern, RT-PCR, SAGE, mutagenesis, etc.	8 (T) 3,6,8 (R)

11	Protein expression	Expression hosts, vectors and techniques (SDS-PAGE and Western blotting)	4 (T) 6,10 (R)
12	Some applications of genetic engineering	Sequencing and analyzing genomes, medical and forensic applications, transgenic parasites	3,9,11 (R)
13	Putting it all together; Feedback session	Use of various methods of gene cloning in research and biotechnology	8 (T)

5. Evaluation scheme:

Component	Duration	Weightage (%)	Date , Time and venue	Nature of Component
Mid-semester	90 min	**25**	21/10/2021 3.30 - 5.00PM	Open Book
a) Lab components		**25**	Lab Quiz	Quiz based (Closed Book)
b) Experiment-based evaluation		**25**		Quiz based (Closed Book)
Comprehensive exam	120 min	**25**	21/12 FN	Open Book

Notes:
(i) **For Observation component**: Every student would be assessed (during the regular or online session) on the following criteria: his/her attendance in the class, capability of asking scientific questions and interaction with the instructor. Besides the common assessment, laboratory assignments would be given to observe their sincerity and level of participation.

6. Attendance Policy: It is expected that the student attends every laboratory session and theory class. It is the student's responsibility to inform the instructor about their absence beforehand.

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, participation in the lecture/tutorial classes and the instructor's evaluation of the student's academic capability.

8. Office Consultation ID: gupta.nishith@hyderabad.bits-pilani.ac.in

9. Make-up Policy: Make-up decisions will be taken based upon evidence and/or prior approval by the course I/C. Under no circumstances make up will be considered for the lab component and assignments.

10. Notices: All course announcements shall be displayed in the CMS and/or ion the Biological Sciences department 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 F418