First Semester 2015-16 **02.08.2016**

COURSE HANDOUT (PART II)

In addition to part-I (General Handout for all courses) printed on page 1 of the Time Table booklet, this portion gives further specific details regarding the course.

Course No. : **BIO F418**
Course Title : **GENETIC ENGINEERING TECHNIQUES**
Instructor in-Charge : **SANDHYA MARATHE**
Lab Instructors : **Rini Dhawan**

1. Scope and Objective of the Course:

This course aims to give the student hands-on experience of the essential techniques used in the molecular biology laboratory with specific emphasis on DNA manipulation. Laboratory practicum will be complemented with lectures explaining the principles of the experiments being performed.

2. Textbook:

- Nicholl, D. S. T. An Introduction to Genetic Engineering. (3rd Edition) Cambridge University Press, 2008

3. Reference books:

- Brown, T.A. Gene Cloning and DNA Analysis: An Introduction. United Kingdom: Wiley-Blackwell, 2010.
- Sambrook J., MacCallum P. and David Russell. Molecular Cloning: A Laboratory Manual (3rd edition, three-book set). New York, USA: CSHL Press, 2001.
- Metzenberg, Stan. Working with DNA. Oxford: Taylor and Francis, 2007.

4. List of Experiments:

i. Isolating single colonies of *E. coli*
ii. Rapid isolation of plasmid DNA from bacteria (mini-prep) using: (a) alkaline lysis and (b) column-based method
iii. Agarose gel electrophoresis of DNA
iv. Learning to use DNA analysis software
v. Restriction enzyme digestion of DNA
vi. Gel extraction of DNA from agarose gel
vii. DNA ligation and creation of recombinant plasmid
viii. Making competent bacteria by chemical treatment
ix. Chemical transformation of bacteria
x. Selection of recombinant clones (e.g. blue-white screening)
xi. Primer designing

xii. Polymerase Chain Reaction (PCR)

xiii. Single-step gene knockout in bacteria

a. Primer design for knockout

b. Preparation of electrocompetent cells & electroporation

c. Screening for gene knockout

Note: These experiments may not be performed in the same order as they appear above. Protocols for each experiment will be made available in advance before the lab commences.

5. Lecture Plan:

Lect. #	Learning objective	Topics	Chapter # of textbook
1,2	Isolating and analyzing DNA	• Purification of plasmid and genomic DNA • Quantitation and electrophoresis of DNA	3
3,4	Learning about prokaryotic vectors for gene cloning	• Plasmids – types and characteristics • Other cloning vectors – an overview	5
5,6	Learn about restriction enzymes	• Restriction enzymes and DNA digestion • Restriction mapping	4
7,8	Cloning strategies	• Uses of DNA ligase, polymerase, phosphatase, kinase, topoisomerase • Ligation reaction – linkers and adaptors • Design of a gene cloning experiment	4, 6
9	Knowing about the bacterial host *E. coli*	• *E. coli* and its versatility as a host • Knowing genotypes of strains • Transformation procedures for *E. coli*	5, notes
10	Selection and screening procedures	• Different selection procedures	8
11,12	Polymerase Chain Reaction	• Basic working – reaction and primer design • Post-PCR analysis – results vs. artifacts • Cloning of PCR products	7

13	Recombineering	• Single step gene knockout in *E. coli*	Ref. material will be provided

6. Evaluation scheme:

Component	Duration	Weight	Date and Time	Remarks
Mid-semester Test	1 hour	10%	<TEST_1>	-
Mid-semester lab	-	10%	-	-
Laboratory Quizzes and Assignments	10 – 15 min. each	10%	-	Surprise tests
Lab Assessment & performance	-	35%	-	OB, See *note (A)* & *(B)* overleaf
Comprehensive Lab	-	15%	-	OB
Comprehensive Exam	2 hours	20%	<TEST_C>	OB/CB

Notes:

(A) Every student would be assessed on the following criteria during the regular lab sessions: endeavor to perform the assigned tasks, scientific integrity, punctuality, maintenance of lab decorum and ability to work in a group. Besides the regular assessment, periodic pre-announced laboratory tests/assignments shall also be given.

(B) Before arriving in the lab, the objective and brief theory of the experiment(s) planned for that day should be written in the record book. All calculations, observations and results for the experiment(s) must be recorded during the lab hours, and should be gotten checked by the instructor, preferably on the same day. *Copying contents of the record from other fellow students is strictly forbidden, and would be treated as indulgence in malpractice.*

7. Attendance Policy:

It is expected that the student attend 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 class group. If failure to complete the task due to absence is anticipated, it is the student's responsibility to inform the instructor prior to the scheduled laboratory.

8. Grading Policy:

Award of grades would be guided in general by the histogram of marks. Decision for borderline cases would be based on the student's attendance in classes and instructors' overall assessment of the individual's sincerity to endeavor.

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

10. Make-up Policy:

For a foreseen absence, make-up request should be made to the instructor-in-charge. Reasons for unanticipated absence that qualify one for make-up include medical or similar personal emergencies only. Normally, make-ups for regular laboratory sessions are not arranged.

11. Notices:

Wherever necessary, course announcements shall be displayed in the notice board of Department of Biological Sciences only.

Instructor-in-Charge
BIO F418