



**Second Semester 2015-16**

**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 : **Prabhat N Jha**  
Co-instructor : **Manoj Kannan**

**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:**

- Metzenberg, Stan. Working with DNA. Oxford: Taylor and Francis, 2007.

**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 (3<sup>rd</sup> edition, three-book set). New York, USA: CSHL Press, 2001.
- Nicholl, Desmond S.T. An Introduction to Genetic Engineering (3<sup>rd</sup> edition). New Delhi: Cambridge University Press India Pvt. Ltd., 2008.

**4. List of Experiments:**

- i. Isolating single colonies of *E. coli*
- ii. Making competent bacteria by chemical treatment
- iii. Chemical transformation of bacteria with a general-purpose cloning vector
- iv. Rapid isolation of plasmid DNA from bacteria (mini-prep) using: (a) boiling lysis method, and (b) alkaline lysis method
- v. Large-scale plasmid preparation from bacteria (maxi-prep)
- vi. Learning to use DNA analysis software
- vii. Agarose gel electrophoresis of DNA
- viii. Restriction enzyme digestion of DNA
- ix. Gel extraction of DNA from agarose gel



- x. DNA ligation and creation of recombinant plasmid
- xi. Selection of recombinant clones by various methods (e.g. blue-white screening)
- xii. Isolation of genomic DNA from (i) plant tissues, and/or (ii) bacteria
- xiii. Polymerase Chain Reaction (PCR)

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	Getting oriented to the course	<ul style="list-style-type: none"><li>• Introduction to the course; mode of operation in the lab; how to maintain the lab record notebook</li><li>• The gene cloning procedure – an overview</li></ul>	1 – 3
2	Knowing about the bacterial host <i>E. coli</i>	<ul style="list-style-type: none"><li>• <i>E. coli</i> and its versatility as a host</li><li>• Knowing genotypes of strains</li><li>• Transformation procedures for <i>E. coli</i></li></ul>	4
3	Learning about prokaryotic vectors for gene cloning	<ul style="list-style-type: none"><li>• Plasmids – types and characteristics</li><li>• Other cloning vectors – an overview</li></ul>	4
4, 5	Isolating and analyzing DNA	<ul style="list-style-type: none"><li>• Purification of plasmid DNA</li><li>• Quantitation and electrophoresis of DNA</li></ul>	2 – 4
6	Learn about restriction enzymes	<ul style="list-style-type: none"><li>• Restriction enzymes and DNA digestion</li><li>• Restriction mapping</li></ul>	5
7	Other enzymes for DNA manipulation	<ul style="list-style-type: none"><li>• Uses of DNA ligase, polymerase, phosphatase, kinase, topoisomerase</li></ul>	6
8	Cloning strategies and applications	<ul style="list-style-type: none"><li>• Ligation reaction – linkers and adaptors</li><li>• Design of a gene cloning experiment</li></ul>	4, 6



Lect. #	Learning objective	Topics	Chapter # of textbook
9	Selection and screening procedures	<ul style="list-style-type: none"> <li>• Direct selection</li> <li>• Selection from gene libraries</li> </ul>	4
10	Polymerase Chain Reaction	<ul style="list-style-type: none"> <li>• Basic working – reaction and primer design</li> <li>• Post-PCR analysis – results vs. artifacts</li> <li>• Cloning of PCR products</li> </ul>	7
11	Other selected techniques used for DNA analysis	<ul style="list-style-type: none"> <li>• Southern, Northern, RT-PCR, SAGE, dot blot analysis, mutagenesis, etc.</li> </ul>	8
12	Protein expression	<ul style="list-style-type: none"> <li>• Expression hosts and vectors</li> <li>• SDS-PAGE and Western blotting</li> <li>• Purification of recombinant proteins</li> </ul>	4
13	Putting it all together	<ul style="list-style-type: none"> <li>• Use of various methods of gene cloning in research and biotechnology</li> </ul>	8

#### 6. Evaluation scheme:

Component	Duration	Marks	Date and Time	Remarks
Mid-semester Test	1 hour	10%	15/3 2:00 -3:30 PM	-
Laboratory Quiz	10 – 15 min. each	10%	-	Multiple quizzes
Laboratory Tests, Assessments and Assignments	-	50%	Scheduled periodically	See <i>note (A)</i> overleaf
Laboratory Record	-	10%	-	See <i>note (B)</i> overleaf
Comprehensive Exam	2 hours	20%	6/5 FN	One section is open-book type



*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**

