

Course title: Biotechnology laboratory – Part 2			
Course code: BBP 106	No. of credits: 7	L-T-P: 0-0-210	Learning hours: 210
Pre-requisite course code and title (if any): None			
Department: Department of Biotechnology			
Course coordinator: Dr. Vivek Kumar Singh		Course instructor: Prof Anandita Singh /Prof. Ramakrishnan Sitaraman / Dr. Shashi Bhushan Tripathi / Dr. Chaithanya Madhurantakam / Dr. Vivek Kumar Singh	
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Course type: Core		Course offered in: Semester 2	
Course description: The objective of this laboratory course is to introduce students to experiments related to biotechnology. The course is designed to teach students the utility of set of experimental methods in biotechnology in a problem-oriented manner. The list of experiments given in each module is representative and includes experiments. Part A will be common for both the streams. Part B1 is only for Microbial Biotechnology stream whereas Part B2 is only for Plant Biotechnology stream. The instructor may choose experiments for student's laboratory training as per requirements.			
Course objectives: 1. To introduce the students to standard techniques of molecular biology. 2. To impart intensive hands-on-training using molecular tools in a research project mode. 3. To train the students in designing experiments with appropriate controls.			
Course contents			
Module	Topic	L	T
Suggested practical			
	PART A: Common to both streams		154
	<p>I- Genotyping methods and analysis of data-</p> <ol style="list-style-type: none"> 1. Genotyping of natural populations with ISSR markers 2. Genotyping of natural populations with SSR markers 3. Analysis of molecular data using MS Excel- marker attributes 4. Analysis of molecular data using GeneAlex- Cluster analysis 5. Analysis of molecular data using Corehunter or PowerCore- Core collections 6. Genotyping of mapping populations with SSR markers 7. Genotyping of mapping populations with ISSR markers 8. Construction of linkage maps with marker data 9. Identification of QTLs using mapping populations 10. GWAS using SNP data 11. Marker-trait associations in natural populations 12. ISSR fingerprinting for clonal uniformity testing 13. Processing of fastq files by FastQC/FastP (quality, trimming etc.) <p>II- Molecular biology techniques-</p> <ol style="list-style-type: none"> 1. Isolation of total cellular RNA from diverse plant tissue samples, qualitative and quantitative assessment 2. Synthesis of first strand cDNA using M-MuLV reverse transcriptase 3. RT-PCR for analysing spatio-temporal expression pattern of candidate genes 4. Designing artificial miRNAs 5. Analysis of relative expression levels using qRT PCR 6. qRT PCR for protein coding/ miRNA genes 7. Quantitation of relative expression levels (delta delta CT method) 		

	<p>by Livak and Schmittgen and RQ method by Knight et al. (2009)</p> <p>8. Overlapping PCR for joining promoter elements to CDS for construction of artificial gene</p> <p>III- Analytical Techniques</p> <ol style="list-style-type: none"> 1. Macromolecular analysis by Dynamic Light Scattering (DLS) <ol style="list-style-type: none"> a. To detect aggregate formations of a protein using DLS b. To detect the size of a protein molecule and to analyse the protein-ligand complex through DLS analysis. 2. ELISA Assays: <ol style="list-style-type: none"> a. To determine the Ag conc. by sandwich ELISA b. To determine the Ab capture by Ab capture ELISA method. c. To determine the Ag conc. by Ag capture ELISA method. d. To perform Dot-ELISA to detect an antigen. 			
PART B1: Microbial Biotechnology				56
	<p>I- Immuno-Techniques and Assays-</p> <ol style="list-style-type: none"> 1. Immunodiffusion and Immuno-precipitation assays: <ol style="list-style-type: none"> a. To study immunodiffusion techniques by single radial Immunodiffusion. b. To perform Ouchterlony double diffusion. 2. To determine antibody concentration by using quantitative precipitin assay. 3. Antibody Titrations: <ol style="list-style-type: none"> a. To detect titre value of antibodies, present in serum due to the infection of Salmonella genus causing enteric or Typhoid Fever by quantitative tube agglutination test. b. To detect the titre value of antibodies, present in test serum by using quantitative tube agglutination test <p>II- Techniques in microbiology-</p> <ol style="list-style-type: none"> 1. Isolation and identification of a probiotic strain from a fermented drink 2. 16S rRNA amplification and sequencing of a mixed culture. 3. Isolation and assay of phages from the environment. 4. Examination of bacterial motility using soft agar medium 5. Sporulation of bacteria 6. Evaluating environmental bacterial isolates for antibiotic production 			
PART B2: Plant Biotechnology				
	<ol style="list-style-type: none"> 1. Selfing and emasculation, setting up of controlled crosses 2. Making rooted cuttings in Sweet Basil (effect of different rooting mixtures) 3. Effect of salt stress/ABA on stomatal conductance/proline concentration 4. Seed viability testing and grow out test 5. Pollen viability testing 6. Histochemical staining for transgene expression 7. Plant genetic transformation 8. Generation of Arabidopsis transgenics by floral dip method 9. Micrografting 10. Root system architecture analysis 			56

Evaluation criteria:				
<ol style="list-style-type: none"> 1. Attendance: 5% 2. Preparation of lab record(s) throughout the semester: 25% 3. End semester evaluation: 70% (Following components would be included) <ol style="list-style-type: none"> a) Spotting: 15 % b) Viva-voce: 15 % c) Experiment(s) assigned on the day of the exam: 40% 				
Learning outcomes:				
<ol style="list-style-type: none"> 1. Ability to conduct experiments with adequate safety precautions. 2. Capacity to compare and evaluate various approaches in solving a given experimental problem. 3. Ability to design and interpret molecular biology experiments. 4. Proficiency in defining a research problem, drawing logical inferences from results and documenting outcomes in systematic manner. 				
Pedagogical Approach:				
Laboratory experiments, demonstration, writing and experiments result analysis.				
Skill Set:				
<ol style="list-style-type: none"> 1. Able to work in biotechnology lab and perform experiments. 2. Able to analyses experimental data and critical thinking. 				
Employability:				
<ol style="list-style-type: none"> 1. Academic and industrial research. 2. Industries based on biotechnology, pharmacy, and agriculture. 				
Materials-				
<ol style="list-style-type: none"> 1. Study material and laboratory protocol will be provided by course instructor. 2. "Biochemistry Laboratory: Modern Theory and Techniques" Rodney Boyer, second Edition, Pearson Education, 2012. 3. "Analytical Techniques in Biochemistry and Molecular Biology" Rajan Katoch, Springer, 2011. 4. "Molecular cloning: A laboratory manual" Sambrook, Joseph. & Russell, David W. & Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y : Cold Spring Harbor Laboratory, 2001. 5. "DNA and protein sequence analysis. A Practical approach" Bishop M.J., Rawlings C.J. (Eds.)1997. 				
Website				
<ol style="list-style-type: none"> 1. https://nptel.ac.in/ 				
Journals				
<ol style="list-style-type: none"> 1. Peer reviewed relevant scientific journals. 				
Advanced Reading Material:				
Will be provided by instructor, if require.				
Additional information (if any)				
List of experiments given in each module are representative, instructor may choose any of them for student's laboratory training as per requirements.				

Student responsibilities:

1. Class attendance.
2. Study of course materials as specified by the instructor.
3. Regular submission of given class assignments.

Course Reviewers

1. Prof. Bijoy Neog, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam
2. Dr. Rupesh Chaturvedi, Ramalingaswami Fellow, National Agri-food Biotechnology Institute, Mohali, Punjab