Course title: Biotechnology laboratory – Part 1			
Course code: BBP 105	**No. of credits:** 7	**L-T-P:** 7-0-196	**Learning hours:** 203
Pre-requisite course code and title (if any): None			
Department: Department of Biotechnology			
Course coordinator: Prof. Shashi Bhushan Tripathi	**Course instructor:** Prof. Shashi Bhushan Tripathi / Dr. Anandita Singh /Dr.Chaithanya Madhurantakam		
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Course type: Core	**Course offered in:** Semester1		

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.

Course objectives:

1. To introduce the students to standard techniques of molecular biology and GLPs (good laboratory practices).
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	P
Suggested practical				
1	Introduction to laboratory safety and safe practices in biotechnology laboratory. Introduction to the Good Laboratory Practices (GLP) regulatory regime of the Organization for Economic Co-operation and Development (OECD).	7	0	0
2	**Analytical Techniques and Biochemistry-** 1. Preparing various stock solutions, working solutions, buffers solution. 2. To prepare an CH_3COOH-CH_3COONa buffer system and validate the Henderson-Hasselbach equation. 3. Quantitative analysis by UV-Vis spectrophotometer- determination of unknown concentration of KMnO4 or BSA Solution.by plotting a standard graph and validating Beer- Lambert's Law. 4. Glucose assay by dinitro salicylic- determination of concentration of given unknown glucose solution by DNS/glucose assay by dinitro salicylic acid. 5. To determine concentration of unknown protein by Bradford protein assay method. 6. To determine concentration of unknown protein by Lowry method/Lowry assay method. 7. Enzyme kinetic analysis of catechol oxidation by catechol oxidases from apple/potatoes/etc. 8. Effect of temperature and enzyme inhibitor on enzyme activity. 9. To perform catalase assay on given plant tissue 10. To Perform Lysozyme crystallization using vapor diffusion methods. 11. Overexpression of the target gene in a heterologous system 12. To purify a histidine tagged protein using Ni- NTA (Nitrilo -triacetic acid) affinity chromatography	0	0	84

	13. To perform Ion exchange chromatography for purification of target protein 14. To perform gel exclusion chromatography for purification of target protein till homogeneity 15. To perform SDS- PAGE for the protein sample			
3	**Essential techniques in microbiology and molecular biology-** 1. Estimation of bacterial titre using colony counts from serial dilutions 2. Growth of bacterial culture and preparation of growth curve 3. Isolation of pure bacterial cultures from mixed cultures. 4. Qualitative and quantitative analysis of DNA. 5. Isolation and restriction enzyme analysis of DNA from soil samples. 6. Methylation analysis DNA using restriction enzymes. 7. Gel purification of DNA by silica binding. 8. Preparation of electrocompetent bacteria and estimating their transformation frequency.	0	0	28
4	**Isolation of nucleic acids and manipulation-** 1. PCR and optimization of factors affecting PCR 2. PCR based genotyping for confirmation of transgene insertion in plants 3. Isolation, qualitative and quantitative analysis of total cellular RNA from eukaryotic cell systems 4. 1st strand c-DNA synthesis and RT-PCRs 5. Restriction digestion of plant gDNA with rare and frequent cutters (restriction enzymes) 6. Purification of plasmids from E. *coli* cells (Alkaline Lysis method and spin-column based methods) 7. Linearization of plasmid vectors 8. Screening of recombinant plasmid vectors by PCR based genotyping of inserts and restriction enzyme based release of inserts	0	0	35
5	**Plant and Animal Biotechnology-** 1. Preparation of stock solutions for plant tissue culture media, vitamins and hormones 2. Sterilisation of explants and initiation of cultures for micropropagation 3. Initiation of various explants for direct and indirect organogenesis 4. Embryo culture 5. Control of phenolics under tissue culture conditions 6. *In vitro* and *ex vitro* hardening 7. Isolation of genomic DNA from plants 8. ISSR/RAPD fingerprinting for clonal uniformity testing 9. Cell viability assay 10. Sub-culturing and maintenance of cell lines 11. Genomic DNA isolation from blood/ cell cultures	0	0	49

Evaluation criteria:

1. Attendance: 5%
2. Preparation of lab record(s) throughout the semester: 25%
3. End semester evaluation: 70% (Following components would be included)
   a) Spotting: 15 %
   b) Viva-voce: 15 %
   c) Experiment(s) assigned on the day of the exam: 40%

Learning outcomes:

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:

1. Able to work in biotechnology lab and perform experiments
2. Able to analyses experimental data and critical thinking.

Employability:

1. Academic and industrial research
2. Industries based on biotechnology, pharmacy, and agriculture.

Materials-

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

1. https://nptel.ac.in/

Journals

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. Dr. Manoj Shrivastava, Principal Scientist, Centre for Environment Science and Climate Resilient Agriculture (CESCRA), Nuclear Research Laboratory (NRL), Indian Agricultural Research Institute, New Delhi-110012
2. Dr. Rakesh Singh, M. Tech. (IIT, BHU) Ph.D. (NIPB, IARI) Principal Scientist (Plant Biotechnology) Division of Genomic Resources ICAR-NBPGR, New Delhi-110012