Lahore University of Management Sciences

BIO300/500 – Methods in Molecular Biology Laboratory/Advanced Methods in Biology

Fall 2023-24

Instructor	Dr Muhammad Tariq, Dr Khurram Bashir, Dr Amir Faisal, Dr Shaper Mirza, Dr Zaigham Shahzad; Dr. Muhammad Shoaib
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Course Basics				
Credit Hours	4			
Lecture(s)	Nbr of Lec(s) Per Week	3	Duration	30 minutes
Labs (per week)	Nbr of Lec(s) Per Week	3	Duration	3:30 hours
Tutorial (per week)	Nbr of Lec(s) Per Week		Duration	

Course Distribution	
Core	Biology Core
Elective	
Open for Student Category	All biology major at 300 and 500 level
Close for Student Category	Non-biology major

COURSE DESCRIPTION

This lab course is designed to introduce fundamentals of molecular and biochemical tools used in a molecular biology lab to understand biological processes. It kicks off with a brief introduction to bioinformatics comprising of exercises and hands on experiments involving homology searches (both DNA and protein searches) and primer design for various applications. Major part of lab comprises of independent modules in molecular biology, biochemistry and genetics which introduce students to molecular cloning, agrobacterium mediated transformations, protein expression and purification through chromatography and enzymatic assays. In addition, classical and molecular genetics module involves experiments using Drosophila to understand various concepts of development, chromatin, and gene regulation. A thirty-minute pre-lab lecture is followed by hands on lab experiments which will expose students to cutting edge ideas and questions of molecular and cell biology.

COURSE PREREQUISITE(S)

- For BIO300: (BIO212 & BIO216) OR (BIO212 & BIO221) OR (BIO216 & BIO221)
- For BIO500: Graduate-standing

COURSE OBJECTIVES

- Introduction to fundamental techniques of molecular and cell biology and biochemistry
- Hands on training of these techniques in the laboratory
- Analysis and interpretation of the results

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Learning Outcomes	
• • •	After this course students should be able to: understand fundamentals of molecular tools perform experiments addressing a specific question analyze the data and interpret the results to answer the biological question

Grading Breakup and Policy
The final grade will comprise of a project (35%), attendance-plus-class participation including –ve participation with negative marks (5%), five quizzes (40%) and reports 20%

Examination Detail	
Midterm Exam	Yes/No: No Combine Separate: Duration: Preferred Date: Exam Specifications:
Final Exam	Yes/No: Yes Combine Separate: Combine Duration: 3 hours Exam Specifications:

COURSE OVERVIEW			
Week/ Lecture/ Module	Topics	Recommended Readings	Objectives/ Application
Week1/day1/ Module: Plant hormonal signaling (ZS, KB)	**Genetics of auxin signaling in plants.** • How does auxin pathway affect plant root development? • Seed sterilization of wild type and auxin signaling mutants • Plant growth medium (minimal) preparation and sterilization. • Preparation of soil containing pots for plant cultivation	**Handouts**	Lecture by ZS + Teaching lab.
Week1/day2/ Module: Plant hormonal signaling (ZS, KB)	**In vitro plant cultures and plant cultivation on soil** • Sowing wild type and mutant plants for auxin signaling under different conditions on agar plates.	**Handouts**	Teaching lab

	• Sowing of wild type and mutant plants on soil.		
Week1/day3/ Module: Plant hormonal signaling (ZS, KB)	**Database search to identify genes and mutants for auxin signaling pathways (T-DNA express, BLAST, TAIR)** • Identify mutant lines for auxin signaling related genes using T-DNA express. • Retrieve DNA sequences of Arabidopsis auxin responsive transcription factor (ARF) genes from TAIR and using BLAST (list of AGIs will be provided). • Generate phylogenetic trees.	**Handouts**	Lecture by ZS + Computer lab
Week2/day1/ Module: Plant hormonal signaling (ZS, KB)	**Genetics of ethylene signaling in plants.** • How does ethylene signaling pathway influence plant growth? • Sowing seeds of wild type and ethylene signaling mutants under light and dark environments.	**Handouts**	Lecture by ZS + Teaching lab.
Week2/day2/ Module: Plant hormonal signaling (ZS, KB)	**Plant imaging and computational analysis for high-throughput phenotyping.** • EZ-Root-VIS • Image J	**Handouts**	Lecture by ZS + Computer lab
Week2/day3/ Module: Plant hormonal signaling (ZS, KB)	**Computational analysis of root systems of wild type and auxin mutants.** • Scanning agar plates to obtain root images. • Image analysis using EZ-Root-VIS	**Handouts**	Teaching lab + Computer lab
Week3/day1/ Module: Plant hormonal signaling (ZS, KB)	**Genotyping assay for homozygous mutant identification in segregating population.** • Principle of Polymerase chain reaction (PCR). • Primer design • PCR amplification • Agarose gel preparation	**Handouts**	Lecture by ZS + Computer lab
Week3/day2/ Module: Plant hormonal signaling (ZS, KB)	• Agarose gel electrophoresis • Ethylene mutant scanning • Image analysis to measure hypocotyl growth using Image J.	**Handouts**	Teaching lab + Computer lab
Week3/day3/ Module: Plant hormonal signaling (ZS, KB)	**Data analysis and representation.** • Statistical test (T-test & ANOVA) to analyze quantitative data. • Preparing publication quality figures; images, bar charts, box	**Handouts**	Computer lab

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	plots, and indicating significance indices.		
Week4 Module: Cell Biology (AF)	• Mammalian tissue culture. • Cell splitting, counting, and freezing • Drug testing in non-adherent cells	**Handouts**	This session will consist of a short lecture followed by demo by the LI
Week5 Module: Cell Biology (AF)	• Transfection methods. Transfection of GFP tagged protein in mammalian cells • Microscopy: Main concepts, light pathways etc • Cell staining for immunofluorescence microscopy	**Handouts**	
Week6 Module: Cell Biology (AF)	• IC50 evaluation of drugs in adherent cells (SRB proliferation assay) • Cell cycle analysis through FACS • Quiz	**Handouts**	
Week7/day1/ Module: Biochemistry (MS)	**Molecular Cloning, expression and purification of HCV NS5a gene** • Gene amplification via PCR • Preparation of agarose gel		
Week7/day2/ Module: Biochemistry (MS)	• Agarose gel electrophoresis for confirming PCR amplification. • Restriction digestion • Restricted fragment gel elution • Ligation	**Handouts**	This session will consist of a short lecture followed by demo by the LIs.
Week7/day3/ Module: Biochemistry (MS)	• Transformation into Top cells • Purification of plasmid containing gene of interest	**Handouts**	This session will consist of a short lecture followed by demo by the LIs
Week8/day1/ Module: Biochemistry (MS)	**Transformation to a bacterial strain for protein expression** • Prepare agar plates • Transform into BL21 cells • Plate the transformed cells to grow	**Handouts**	This session will consist of a short lecture followed by demo by the LIs
Week8/day2/ Module: Biochemistry (MS)	• Protein purification from inclusion bodies • *In vitro* refolding • Protein fractionation by salting out method • Purification of protein through affinity chromatography • Running SDS gel	**Handouts**	This session will consist of a short lecture followed by demo by the LIs
Week8/day3/ Module: Biochemistry (MS)	• Size exclusion column chromatography • Protein concentration and dialysis	**Handouts**	This session will consist of a short lecture followed by demo by the LIs
Week9/day1/ Module: Biochemistry (MS)	• Enzyme catalyzed reactions • Preparation of reagents and (product) standard solutions	**Handouts**	This session will consist of a short lecture followed by demo by the LIs

	• Enzyme kinetic assay (over time and the effect of pH/Substrate concentration		
Week9/day2/ Module: Biochemistry (MS)	• Reaction setup • Incubation • Absorbance measurement on 96-well plate spectrophotometer • Interpretation of data (Michaelis-Menten equation	**Handouts**	This session will consist of a short lecture followed by demo by the LIs
Week9/day3/ Module: Biochemistry (MS)	• Enzyme inhibition assay (competitive/uncompetitive/non-competitive) i. Reaction setup ii. Incubation iii. Absorbance measurements • Interpretation of data • Final discussion	**Handouts**	This session will consist of a short lecture followed by demo by the LIs
Week10/day1/ Microbiology (SM)	**Study of Microbial Structures** *Gram staining*: Identification of gram positive and gram-negative bacteria *Capsular staining* Differentiation of bacteria on the bases of presence and absence of capsule	**Handouts**	The session will consist of a short lecture followed by a demo for staining techniques.
Week10/day2/ Microbiology (SM)	*Endospore staining:* Bacteria will be stained for the presence or absence of endospore. *DNA staining:* Bacterial DNA can be stained to differentiate between live and dead bacteria	**Handouts**	
Week10/day3/ Microbiology (SM)	**Microbial Nutrition and growth**: **Carbohydrate fermentation test: Goal:** *to determine sugars utilized by gram-positive and negative bacteria.* Inoculate bacteria in different sources of sugar and determine which sugars bacteria ferment	**Handouts**	Theory of carbohydrate utilization and principal and procedure of the test
Week11/day1/ Microbiology (SM)	**Isolation of pure culture** **Enrichment and Isolation of bacteria:** Identification of different bacteria in the same sample and enrichment of bacteria of bacteria of interest.	**Handouts**	Most specimen are contaminated with more than one species of bacteria. In this section students will learn about differentiating microbes in mixed culture and later their identification
Week11/day2/ Microbiology (SM)	**Antimicrobial testing: Goal;** *To determine the minimum inhibitory concentration of antibiotic for a non-pathogenic and pathogenic bacterium.* Bacteria will be incubated with different concentrations of drugs and growth will be measure after incubation for 16 hours **ELISA for detection of antibodies to bacterial antigen.**	**Handouts**	Common mechanism of antimicrobial resistance Principal and procedure of experiment
Week11/day3/ Microbiology (SM)	ELISA contd Quiz	**Handouts**	

Week12/day1/ Module: (Epi)Genetics (MT)	• Drosophila as a Model for Genetics and Development • Inheritance pattern of Eye Color in Drosophila • Set up two reciprocal crosses with Canton S and w1118 • Chromosome Mapping of Unknown gene. • Set up an individual cross of chromosome 2 and 3 balancers with Roc1a transgenic flies	**Handouts**	This session will consist of a short lecture followed by demo by the TAs.
Week12/day2/ Module: (Epi)Genetics (MT)	• Developmental Tissue Specific Gene Expression • • Dissection of larval discs from cross of ey-GAL4, Ptc-GAL4 (males) with UAS-LacZ (Virgins) • B-gal. staining of Imaginal Discs for tissue specific expression of developmental genes	**Handouts**	This session will consist of a short lecture followed by demo by the TAs. Students will perform independent experiments in groups.
Week12/day3/ Module: (Epi)Genetics (MT)	• Tissue Specific Gene Expression Continued • Analysis of B.gal stained Imaginal discs	**Handouts**	This session will consist of a short lecture followed by demo by the TAs. Students will perform independent experiments in groups.
Week13/day1/ Module: (Epi)Genetics (MT)	• Polytene Chromosomes: Identification of heat shocked Puffs and Banding Patterns • Identification of chromosomes on the basis of banding pattern and labelling of Puffs	**Handouts**	This session will consist of a short lecture followed by demo by the TAs. Students will perform independent experiments in groups.
Week13/day2/ Module: (Epi)Genetics (MT)	• Identification of Heterochromatin and Euchromatin regions on Polytene Chromosomes • Immunostaining of Polytenes with Anti H3K9me3 and H3K4me3 antibodies • Reverse Genetics: RNAi: Bathing of Dmel2 cell line with dsRNA for RNAi Experiment	Handouts	This session will consist of a short lecture followed by demo by the TAs. Students will perform independent experiments in groups.
Week13/day3/ Module: (Epi)Genetics (MT)	• Continuation of Immunostaining of Polytenes and visualization under fluorescent microscope.	**Handouts**	This session will consist of a short lecture followed by demo by the TAs. Students will perform independent experiments in groups.
Week14/day1/ Module: (Epi)Genetics (MT)	RNA Isolation from cell line and DNase treatment, Measurement of concentration of RNA cDNA synthesis and Actin PCR	**Handouts**	This session will consist of a short lecture followed by demo by the TAs. Students will perform independent experiments in groups.
Week14/day2/ Module: (Epi)Genetics (MT)	• Set up real time PCR on cDNA from RNAi experiment by determining expression of homeotic genes	**Handouts**	This session will consist of a short lecture followed by demo by the LIs Students will perform independent experiments in groups.
Week14/day3/ Module: (Epi)Genetics (MT)	• Analysis of real time PCR data using ΔΔct method	**Handouts**	

Textbook(s)/Supplementary Readings

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- Handouts
- Molecular Biology of Cell, Bruce Albert
- Molecular Cloning Protocols by Sambrook et al.
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