Modules for SDP Winter School 2019

Method of Teaching: A blend of Class Room Teaching and Hands-on-Training

Classroom Location: Skill Development Center

Course Duration: 6 weeks

Timings: 10 AM to 5 PM Monday to Saturday

What will you learn?
By completion of this training, successful applicants will be able to:

1. Learn good practices and basic lab concepts
2. Bioinformatics concepts and tools for gene, protein and big data analysis
3. Biotechnological tools and techniques applicable in research and pharma industry
4. Gene expression and analysis
5. Fundamentals of gene cloning and protein expression
6. Cell culture and cell imaging

Approach to Learning
Each module uses a variety of approaches including: class room lessons, discussions and hands on training of specific modules.

Workload Expectations
Each module is a 5-6h/day course running over a period of six weeks. Expect to spend about 5-6 hours a day on course tasks, in addition to classroom or hands on training.

Attendance

We follow ungraded attendance policy where your attendance is strongly encouraged but not graded. Take the advantage of the course by attending all the sessions/trainings scheduled. In case of scheduled absence, please let your instructor(s) know much in advance. Please note that instructor(s) will not review the information that you missed.

Copyright: Guidance for Attendees

Module 1- Good Lab Practices and Introductory Biochemistry

This is an introductory session for the candidates which comprises of short talks on course outlay, good lab practices and basics of biochemistry all serving as essential components for research purposes.

Instructors: Dr. T. Prasad and Dr. Pallabi Mitra

Date	Topic	Learning Activities and Assignments
14th Nov	Introductory remarks by Dean and Course description by Coordinator	Participants will be introduced to the core structure of the course followed by a small interaction session with Dean, coordinator and instructors
15th Nov	Good Laboratory Practices	Good laboratory practices
16th Nov	Preparation of General Laboratory reagents and Buffers	Concepts of buffers and Molarity and solution chemistry

Module 2- Applications of Bioinformatics in Genomic Era

Course Contents

1. Introduction to Bioinformatics and different biological databases
2. Methods for pair-wise and multiple sequence alignment
3. Introduction to Next-generation sequencing: Mapping and analysis

You are required to complete the following assignments.

Course Schedule

Date	Topic	Learning Activities & Assignments
18th Nov	Bioinformatics: Introduction, biological databases and tools	Participants will learn basic of bioinformatics databases and tools. Activities will include learning basic unix commands and sequences retrieval from NCBI and Uniprot databases.
19th Nov	Pair-wise sequence Alignment part I: Dotplots, Dynamic Programing, Substitution matrices	. Activity will include pair-wise sequence alignment using dotplot and EMBOSS suite.
20th Nov	Pair-wise alignment part II: BLAST and FASTA	Hands on training on using online and standalone blast to find homologs. Activities will include different types of BLAST searches (PSI-BLAST, PHI-BLAST etc.)
21st Nov	Multiple Sequence Alignment: Phylogenetics	Learning activities will include identification of patterns from multiple sequence alignment and construction of rooted and unrooted phylogenetic trees
22nd Nov	Next generation sequencing part I	Head on training on mapping reads obtained from next generation sequencing experiments
23rd Nov	Next generation sequencing part II	Activities will include downstream analysis in RNASeq analysis

Module 3- Analytical Tools and Techniques in Biotechnology

Instructor: Dr. Parul Mishra

Objective: The module is designed to offer a comprehensive hands-on training to participants in various analytical tools and techniques to develop the skill-set required to enhance job opportunities in biotechnology industry and academia.

This module will consist of the following sub-modules and experiments:

1. **Protein technology and Bioassay Development:** Protein concentration estimation, protein analysis using Native-PAGE and SDS-PAGE, Western blotting, Enzyme assay and calculation for kinetic parameters (Km, Vmax, Kcat).
2. **Antibiotic susceptibility tests:** Quantitative and qualitative determination of minimal inhibitory concentration (MIC) of various antibiotics against bacteria.
3. **Immunotechnology:** Analyzing antigen-antibody interaction using Enzyme-linked Immunosorbent Assay (ELISA) and Immunodiffusion.

Date	Topic	Learning Activities and Assignments
25th Nov	Protein Technology and Bioassay Development	Introductory Lecture Quantitative protein estimation using Lowry's, Biuret and BCA methods Native-PAGE SDS-PAGE Data analysis
26th Nov	Protein Technology and Bioassay Development	Introductory Lecture Western blotting Data analysis
27th Nov	Protein Technology and Bioassay Development	Introductory Lecture Enzyme bioassay (determining catalase activity) Data analysis and calculation of kinetic parameters (Km, Vmax, Kcat).
28th Nov	Antibiotic susceptibility tests	Introductory Lecture Preparation of culture media. Disc diffusion test. Data analysis and compilation
29th Nov	Antibiotic susceptibility tests	Introductory Lecture Broth dilution test Data analysis
30th Nov	Immuno-technology	Introductory Lecture ELISA Immunodiffusion (Radial Immunodiffusion and Ouchterlony double diffusion technique) Data analysis Module Feedback

Module 4- Gene Expression Analysis

Instructor: Dr. Vengal Rao

Date	Topic	Learning Activities and Assignments
Pre-Course Activities		
2nd Dec	**Isolation, Quantification and Analysis of RNA:** i) RNA isolation (Trizol method) ii)Quantitation of RNA (Spectrophotometrically) iii) mini gel running for analysis	Different types of RNA, Precautions for RNA isolation, DNA and Protein contamination of RNA preparation
3rd Dec	**cDNA synthesis and analysis:** i) cDNA synthesis ii) semiquantitative analysis of prepared cDNA by PCR using standard primers(GAPDH/ actin etc)	Reverse Transcriptase, Oligo dT, Random hexamers, RT PCR
4th Dec	iv) Real time PCR	Real time PCR, CT values, analyzing results
5th Dec	Isolation and characterization of exosomes	Introduced to differential centrifugation based fractionation
6th Dec	Isolation of miRNAs from Exosomes	Concepts of miRNA and their role in gene expression
7th Dec	Small scale miRNA profiling of exosomes	miRNA expression analysis

Module 5- Recombinant DNA Technology and Downstream Processing

Instructor: Dr. Pallabi Mitra

Date	Topic/ Hands on activity	Learning Activities and Assignments
9th Dec	**Isolation, quantification and analysis of DNA:** i) Plasmid DNA isolation (manual method and plasmid isolation kit) ii) Qualitative and quantitative analysis of isolated DNA (spectrophotometric estimation and agarose mini gel running)	A) Cloning in plasmid vectors: plasmid vectors, selecting a plasmid vector for your work, Plasmid vector maps,, Designing cloning strategies B) Different methods of DNA isolation followed by quantitation and estimation of DNA by analyzing band intensity on agarose gel C) Troubleshooting session
10th Dec	**Cloning a gene of interest in plasmid Vector:** i)Primer Designing ii)PCR amplification of gene of interest ii)Restriction digestion iii)Ligation	A) Key enzymes for cloning: i) DNA Polymerases ii)Restriction enzymes iii)Ligases B) Polymerase Chain Reaction: primer designing, Tm calculation, standardizing cycling conditions. C) Troubleshooting session D)Preparation for next day's practical
11th Dec	**Transformation of the plasmid construct carrying the gene of interest:** i) Preparation of competent *E. coli* ii) Transformation of *E. coli* using plasmid DNA containing your gene of interest by heat shock	A) Competent *E. coli* cells: different methods of preparation of competent cells, determining the efficiency of your competent cells, checking the cells for contamination. B) Different methods of transforming *E. coli.* Selection and screening for positive colonies. C) Troubleshooting session D)Preparation for next day's practical

Date	Topic/ Hands on activity	Learning Activities and Assignments
12th Dec	**Screening and selection of positive colonies:** i)Screening Bacterial Colonies using colony PCR ii) Confirmation of cloned construct iii) Preparation for bacterial expression of the gene of interest	A) Colony PCR based screening for positive colonies. B) Confirmation and verification of gene of interest in the recombinant plasmid construct using restriction digestion and other methods. C) Proceed with verified construct for downstream processing. D) Troubleshooting session E)Preparation for next day's practical
13th Dec	**Expressing cloned gene for protein production, purification and analysis:** i) Expression of cloned genes in *E. coli* using IPTG-inducible promoters: primary and secondary inoculation and IPTG induction.	A) Choosing an appropriate expression system for the gene of interest. B) Fusion Proteins-various tags, optimization of expression of foreign proteins in bacterial system. C) Troubleshooting session D)Preparation for next day's practical
14th Dec	ii)Preparation of cell extract for purification of soluble proteins expressed in *E. coli* (enzymatic lysis by lysozyme) iii)Purification of poly-histidine-tagged proteins by immobilized metal affinity chromatography	A) Chromatography based purification methods B)Discussion on standardization of protein purification protocols C)Troubleshooting session

Module 6- Molecular Imaging

This module introduces basic principles molecular imaging and good practices of cell culture techniques while providing rigorous hands-on training on tissue culture and imaging.

Instructor (s): Dr. Hari Prasad Gorantla and Dr. Prasad Tammineni
Email: haribioster@gmail.com; prasadtammineni@uohyd.ac.in;

Module Description

Molecular Imaging is an emerging research area that enables the visualization of biological processes occurring at cellular and sub-cellular level in microcellular environment. The course covers the basic scientific principles behind various fluorophores and their biological applications. In addition, this course also includes good tissue culture practices whilst providing rigorous hands on training in cell culture and basic confocal imaging.

Course Contents

4. Basic Principles and good Practices of Tissue culture
5. Methods of gene delivery into Cellular Systems
6. Introduction to Molecular Imaging and its applications in Biology
7. Fluorophores and Molecular probes: Basic Principles and Applications

Course Assignments

You are required to complete the following assignments.

Assignment Name	Due Dates
Assignment 1: Thaw Cells and Keep them ready for module	3 Days prior to the course
Assignment 2: Seed Cells for Transfections	Day II
Assignment 3: Transfection	Day III
Assignment 4: Immunocytochemistry	Day V and Day VI

Course Schedule

Please review this schedule of tasks. See above for specific assignment due dates.

Date	Topic	Learning Activities & Assignments
Pre-Course Activities	Talk to the instructor(s) to thaw cells and make them ready for the module	At least 3 days prior to the start of the module, talk to instructors to have the cells ready for training. (Assignment I) Make note of course dates/time and match them to your schedule
16th Dec	Introduction of the module and class room teaching on Basics of Cell Culture Techniques	Attendees will learn about the core structure of the module and cell culture techniques
17th Dec	Hands on training of Cell Culture and Class room teaching on Transfection methods	Learning the sterile techniques of Cell line maintenance and passage. (Assignment II) Get familiar with various cell transfection techniques used for gene manipulation
18th Dec	Hands on training on Cell Transfections and Class room teaching on Introduction to "Molecular Imaging Techniques"	Hands on training on using lipid based methods to introduce gene of interest into cellular systems (Assignment III), while learning the basic principles of molecular imaging.
19th Dec	Class room teaching and Discussion on "Fluorophores and Molecular Reporters in Imaging"	Learn about various fluorophores and reporter plasmids and their applications in biology.
20th Dec	Hands on session of Immunocytochemistry Part I and Demonstration of Zeiss Microscopy and STED Microscopy	Learn about anticipated problems of immunocytochemistry and possible solutions. Hands on training of immunocytochemistry Part I (Assignment IV)
21st Dec	Hands on Session of Immunocytochemistry Part II and Image capturing using Zeiss microscopy	Attendees will be given a demonstration of our imaing systems and their applications. Hands on training of immunocytochemistry Part II (Assignment IV).
Final Course Task(s)	Submit the Course Evaluation Survey	Take 15 minutes to fill out the anonymous course evaluation survey to make the course even better.

Module 7- Bioprocess Engineering

Instructor: Dr. T. Prasad and Dr. Vengal Rao

Date	Topic	Learning Activities and Assignments
23rd	Demonstration of Bioreactor: Components and its applications in down-stream processing	Basic principles and outline of Bioreactor system
24th	Culture Harvesting and Preparing for batch purification	Concepts of batch culturing using Bioreactor for large scale purification
25th	Batch Purification using Acts purification system	Demonstration of ACTA purification system