LS431A	Life Sciences Practicals II	12 Credits

Coordinator: Dr. Niti Puri

Participating Faculty Members (Module-wise):

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I. **Molecular Biology**: Prof. P.C. Rath*, Prof. K. Natarajan
II. **Plant Physiology**: Prof. A.K. Nandi*, Prof. S. Chakraborty, Dr. Amarjeet Singh
III. **Animal Physiology**: Prof. A.C. Mondal*, Dr. S.K. Jha, Prof. R.P. Singh
IV. **Developmental Biology**: Prof. S. Saran*, Prof. A.K. Sarkar
V. **Immunology**: Dr. N. Puri*
VI. **Computational Biology and Bioinformatics**: Dr. D. Kaur, Prof. S. Gourinath, Prof. N. Ramchiary*, Dr. K. Kar

	S.No.	Topic	Faculty Name/ Contact Hours
Molecular Biology (2 credits)	1.	DNA Structure: Preparation of a ball and stick model of B-DNA. Draw the chemical structures of A,T and G,C base pairs, show different groups/ bonds in the DNA and study the structural features.	PCR/10* (*overlapping with other experiments)
	2.	Genomic DNA isolation from yeast *Candida albicans*/ mouse cell line and DNA quantification	KN/2
	3.	Primer designing for PCR-based cloning of genes for expression in *E.coli*- use of various tools and genome sequence databases	KN/2
	4.	Titration of template concentrations and PCR amplification; Quantification of amplicons by gel electrophoresis and clean-up of DNA.	KN/1
	5.	Preparation of Insert DNA: Restriction digestion of PCR fragment with six-base cutter/s, gel analysis and clean-up and quantify DNA. DNA ready for ligation.	KN/1
	6.	Preparation of vector DNA: Restriction digestion of pET28b vector with compatible six-based cutter/s; verification of RE digestion by gel analysis; purification of vector DNA and quantification by gel analysis.	KN/1
	7.	Preparation of LB medium, LB agar plates with antibiotics and other reagents.	PCR/1
	8.	Streaking of *E. coli* DH5α strain on LB+Agar+ Nalidixic acid plate	PCR/1
	9.	Preparation of competent *E. coli* DH5α cells	PCR/2
	10.	Transformation of *E. coli* DH5α cells with pBluescript, pET28b plasmid DNA and recombinant DNA clones	PCR/2
	11.	Isolation of plasmid DNA from overnight culture of the transformed colony/ clone.	PCR/1
	12.	Agarose gel electrophoresis of plasmid DNA preparation	PCR/1

	13.	Restriction enzyme digestion of the plasmid DNA and clone verification Restriction enzyme digestion of bacteriophage λ DNA/ genome by Hind III and Hind III+EcoR I; preparation of restriction map for the double digest.	PCR/1
	14.	Blue/ white screening of the recombinant DNA clones in *E.coli.*	PCR/2
Plant Physiology (2 credits)	1.	Regeneration and transformation of tobacco using *Agrobacterium tumefaciens*.	SC/6
	2.	Study of photosynthetic efficiency in normal and stressed plants	AJS/1
	3.	Separation of Chlorophyll pigments and study of absorption spectra	AJS/2
	4.	Effect of plant hormones on growth and physiology	AJS/4
	5.	Stress-associated cell death and reactive oxygen species accumulation studies	AKN/ 2
Animal Physiology (2 credits) Animal Histology & Animal Physiology	1.	Histology of liver, kidney & brain; Histology of normal and carcinomas of different human organs	RPS/1
	2.	Tissue processing for histological preparations	ACM/1
	3.	Section cutting; Microtomy (Rotary), Vibrotomy, Cryo-sectioning	ACM/1
	4.	Staining: Haematoxyline/ Eosin staining for different organs and tissues	ACM/2
	5.	Preparation of permanent slides	ACM/1
	6.	Identifying characters for sections of different tissues or organs stained with H&E.	ACM/1
	7.	Histology of Blood: Preparation & staining of human blood film	ACM/1
	8.	(i) Determination of blood group (ii) Study of haemin crystal (iii) Measurement of arterial blood pressure	ACM/1
	9.	Immunohistochemistry of brain sections and cancer tissues: Staining, microscopic evaluation and quantification of biomarkers and determination of proliferation index	RPS/2
	10.	Demonstration and application of rodent's stereotaxic instruments.	SKJ/1
	11.	Demonstration of recorded brain waves. Identification of vigilant states in the recorded brain waves.	SKJ/1
	12.	Identification of alpha, beta, delta, theta and gamma brain waves in recorded the EEG.	SKJ/1

Developmental Biology (2 credits) Plant and Animal Developmental Biology	**Plant Developmental Biology**		
	1.	Embryogenesis: sample preparation and microscopic visualization of developmental stages of *Arabidopsis* embryos.	AS/1
	2.	Shoot and root meristems: sample preparation, morphological and microscopic visualization of embryonic and post-embryonic shoot/root meristems	AS/1
	3.	Root growth and branching: morphological and microscopic observation of root growth and number or lateral roots (LRs); microscopical observation of stages of LR development in Arabidopsis	AS/1
	4.	Auxin localization in shoot and root using DR5:GUS reporter. GUS-staining of *Arabidopsis* seedlings, analysis and imaging of staining pattern in shoot and root tissue	AS/1
	5.	Analysis of root developmental/auxin pathway mutant for defect in root, comparison with wild type	AS/1
	6.	Leaf polarity- analysis of dorsoventral features. Observation of number and density/pattern of stomata and trichomes on leaf surfaces. Tissue fixation, processing, embedding and Histological analysis leaf tissues	AS/1
	Animal Developmental Biology		
	7.	Day 1: Preparation of media, buffer and reagents for experiments	SS/1
	8.	Day 2: Studying growth and development of *Dictyostelium discoideum*	SS/1
	9.	Day 3: Continue with development of *D.discoideum*	SS/1
	10.	Day 4: To study pattern formation in Dictyostelium using neutral red dye	SS/1
	11.	Day 5: LacZ staining of few mutants of *Dictyostelium*	SS/1
	12.	Day 6: Observing developmental mutants of *Drosophila*	SS/1
	13.	Day 7: Observing different model systems used in Developmental Biology	SS/1
Immunology (2 credits)	**Immunology**		
	1.	Counting of immune cells: Differential Leukocyte count; Total Leukocyte count	NP/1
	2.	Innate Immune Response 1: Phagocytosis of Fl-labeled microbes or damaged cells by phagocytes followed by assay of ROS production (Fluorescence microscopy/Flowcytometry)	NP/2
	3.	Innate Immune Response 2: Exocytosis/Release of inflammatory mediators by Mast cells in response to allergen challenge (Mediator release assessment by colorimetric assay)	NP/2
	4.	Activation of complement by classical pathway: Lysis of antibody coated cells	NP/1

	5.	Adaptive humoral immune responses: Checking antibody titer in immunized mouse serum by indirect ELISA (comparison of immunized versus unimmunized)	NP/2
	6.	Use of Antigen-antibody interaction in immunological techniques for antigen detection and quantitation (Immunodiffusion/ haemagglutination; immunoprecipitation/western blotting)	NP/1
	7.	Tissue typing (MHC-typing) by Mixed lymphocyte reaction (MTT/CFSE-FACS)	NP/1
	8.	T-cell subset determination (CD4 and CD8) by Immunophenotyping experiment/labeling/ running sample on Flowcytometry followed by analysis	NP/2
	9.	T-cell activation by mitogen (Con A). Study proliferation (MTT/CFSE-FACS) and detection of cytokines/chemokines secretion by activated immune cells using ELISA (sandwich ELISA).	NP/2
	10.	NK cell-mediated killing of tumor cell targets by MTT assay (non-radioactive method)	NP/1

Computational Biology & Bioinformatics

Computational Biology & Bioinformatics (2 credits)	1.	Database searching using Boolean operators (Advanced database searching) and data retrieval	DK/1
	2.	Sequence analysis of DNA for base compositions and Codon usage analysis, Use of Pattern finding algorithm to find patterns/motifs and statistical relevance using Python or in-silico tools.	DK/3
	3.	Pair-wise alignment and MSA with DNA/Protein sequence dataset, Phylogenetic tree overview	NR/KK/2
	4.	Query search against different databases, understanding search set options, program selection and different algorithm parameters, Overview of BLAST suite of programs – Computational approach – Statistical significance	DK/2
	5.	Predicting RNA secondary structure and Protein structures using in-silico tools (Homology Modelling, Ab-initio and Threading concepts).	DK/4
	6.	Next generation sequencing (NGS) data analysis: Hands on training of software, pipeline, tools and visualization of data	DK/NR/5
	7.	Hands on use of Molecular Docking using computational tools	SGN/KK/2

Further Reading:

1. **Molecular Biology**
   Molecular Cloning- A LABORATORY MANUAL 4th Edition Green, M.R. and Sambrook, J.

2. **Plant Physiology**
   Concerned literature/handout will be given by individual faculty members.

3. **Animal Physiology**
   1. diFiore's Atlas of Histology with Functional Correlations by Victor P Eroschenko
   2. Theory and Practice of Histological Techniques Eds. John D Bancroft, Marilyn Gamble
   3. Atlas of Histology with Functional Correlations Victor P. Eroschenko
   4. Histology for Pathologists. Stacey E. Mills (Ed.)

4. **Developmental Biology**
   1. Plant Physiology and Development, 6th Ed. by Eduardo Zeiger and Lincoln Taiz et al.
   2. Arabidopsis: A laboratory manual (CSHL press). Detlef Weigel and Jane Glazebrook
   3. Handouts for experiments will be given in the class.

5. **Immunology**
   Concerned literature/handout will be given by the faculty member

6. **Computational Biology & Bioinformatics**

   Textbook:
   1. Jonathan Pevsner. Bioinformatics and functional genomics. Wiley Blackwell, Third Edition, 2015
   2. Des Higgins and William Taylor. Bioinformatics: Sequence, Structure, and databanks. Oxford University Press, 2001

   Reference Book:
   1. Richard Durbin, Sean R. Eddy, Anders Krogh, Graeme Mitchison. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. Cambridge University Press, 1998