**LS431A** **Life Sciences Practicals II** **12 Credits**

**Coordinator**: Dr. Niti Puri

*Participating Faculty Members (Module-wise):*

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

| | S.No. | Topic | Faculty Name/ Contact Hours |
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| **Molecular Biology (2 credits)** | 1. | Visualization of different forms of DNA using Pymol/Molmol DNA Structure: Preparation of a ball and stick model of B-DNA.<br>Draw the chemical structures of A,T and G,C base pairs, show different groups/ bonds in the DNA and study the structural features. | PKV,SD, PKG/10* (*overlapping with other experiments) |
| | 2. | Genomic DNA isolation from plant/human cell line and DNA quantification | PKV,SD, PKG/3 |
| | 3. | Primer designing for PCR-based cloning of genes for expression in *E.coli*- use of various tools and genome sequence databases | PKV,SD, PKG/2 |
| | 4. | Titration of template concentrations and PCR amplification; Quantification of amplicons by gel electrophoresis and clean-up of DNA. | PKV,SD,PKG /1 |
| | 5. | Preparation of Insert DNA: Restriction digestion of PCR fragment with six-base cutter/s, gel analysis and clean-up and quantify DNA to be ready for ligation. | PKV,SD,PKG/1 |
| | 6. | Preparation of vector DNA: Restriction digestion of suitable vector with compatible six-based cutter/s; verification of RE digestion by gel analysis; purification of vector DNA and quantification by gel analysis and NanoDrop. | PKV,SD,PKG/1 |
| | 7. | Ligation of Insert and vectors digested with compatible restriction enzymes with T4 DNA ligase | PKV,SD,PKG/1 |
| | 8. | Preparation of LB medium, LB agar plates with antibiotics and other reagents. | PKV,SD,PKG/1 |
| | 9. | Streaking of *E. coli* DH5α/BL21DE3 strain on LB+Agar+ antibiotic/s | PKV,SD,PKG/1 |
| | 10. | Preparation of competent *E. coli* DH5α /BL21DE3 cells | PKV,SD,PKG/2 |
| | 11. | Transformation of *E. coli* DH5α/BL21DE3 cells with suitable vector plasmid DNA and recombinant DNA clones | PKV,SD,PKG/2 |

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| | 12. | Isolation of plasmid DNA from overnight culture of the transformed colony/ clone and<br>Confirmation of positive clones by colony PCR or blue/white screening of the recombinant DNA clones in *E. coli* | PKV,SD,PKG/2 |
| | 13. | Agarose gel electrophoresis of plasmid DNA preparation | PKV,SD,PKG/1 |
| | 14. | Restriction enzyme digestion of the plasmid DNA and clone verification; preparation of restriction map of clones. | PKV,SD,PKG/2 |
| **Plant P hysiology (2 credits)** | 1. | Regeneration and transformation of tobacco using *Agrobacterium tumefaciens*. | SC/6 |
| | 2. | Study of photosynthetic efficiency in healthy and stressed plants | SC/1 |
| | 3. | Separation of Chlorophyll pigments and study of absorption spectra | SC2 |
| | 4. | Effect of plant hormones on growth and physiology | AKN/4 |
| | 5. | Stress-associated cell death and reactive oxygen species accumulation studies | AKN/ 2 |
| **Animal Physiology (2 credits)**<br>**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<br>(ii) Study of haemin crystal<br>(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

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| **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 culture media, buffer, and reagents for experiments. | BCM/1 |
| 8. | Day 2: Studying the development of *Drosophila* | BCM/1 |
| 9. | Day 3: Continue with the development of *Drosophila* | BCM/1 |
| 10. | Day 4: Study of meroistic oogenesis by dissecting and observing the ovaries of *Drosophila* | BCM/1 |
| 11. | Day 5: Study of expression of developmental genes in larval imaginal discs and pharate adults | BCM/1 |
| 12. | Day 6: Observation and mounting of homeotic gene mutants in *Drosophila* | BCM/1 |
| 13. | Day 7: Cuticular preparation of *Drosophila* embryos and studying segmentation defects in mutants | BCM/1 |
| 14. | Day 8: Observing different model systems used in Developmental Biology | BCM/1 |

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| **Immunology** | | |
| 1. | Counting of immune cells: Differential Leukocyte count; Total Leukocyte count | NP/SD/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/SD/2 |

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| | 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/SD/2 |
| | 4. | Activation of complement by classical pathway: Lysis of antibody coated cells | NP/SD/1 |
| **Immunology (2 credits)** | 5. | Adaptive humoral immune responses: Checking antibody titer in immunized mouse serum by indirect ELISA (comparison of immunized versus unimmunized) | NP/SD/2 |
| | 6. | Use of Antigen-antibody interaction in immunological techniques for antigen detection and quantitation (Immunodiffusion/ haemagglutination; immunoprecipitation/western blotting) | NP/SD/1 |
| | 7. | Tissue typing (MHC-typing) by Mixed lymphocyte reaction (MTT/CFSE-FACS) | NP/SD/1 |
| | 8. | T-cell subset determination (CD4 and CD8) by Immunophenotyping experiment/labeling/ running sample on Flowcytometry followed by analysis | NP/SD/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/SD/2 |
| | 10. | NK cell-mediated killing of tumor cell targets by MTT assay (non-radioactive method) | NP/SD/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