Syllabus on Research Methodology

1.Separation techniques:

Chromatography − Paper, TLC, adsorption, partition, ion-exchange, reverse phase, gel, filtration, affinity, GLC, HPLC and FPLC.

Electrophoresis −Theory of electrophoresis and electrical parameters in electrophoresis, paper electrophoresis, gel electrophoresis, SDS-PAGE, Disc gel, gradient gel, isoelectric focusing, gel electrophoresis of nucleic acids and its applications, pulse field electrophoresis, continuous electron electrophoresis.

2.Spectroscopy

Absorption Spectroscopy: UV -VIS, NIR, and their application in biology and medicine, **Fluorescence Spectroscopy: protein fluorescence,** Use in biology, energy transfer, **Single Molecule spectroscopy:** PCS and FCS, **Resonance spectroscopy**: NMR, ESR, CD spectroscopy- CD of peptide, protein and DNA, **NIR spectroscopy** - Application in Biology Medicine and Agriculture.

3.Biophysical methods:

Crystallography: Symmetry in Crystal, Point and Space Group, Miller Indices, Unit Cell, Atomic Scattering Factor, Structure Factor. Laue and Bragg's Law of Diffraction, Ewald's Construction, Concept of Reciprocal Lattice and Fourier Transformation, Relation between Structure Factor and Electron Density, Phase problem.

Microscopy: Light Microscopy (Bright, Dark Field and Phase contrast Microcopy, Resolving power and Magnification). **Electron Microscopy** (Working principle, Image, formation process and Contrast, Image defects, Optimum resolution, Sample preparation and contrast enhancement techniques). Comparison between SEM, STEM, STM, AFM

Other Techniques: Viscosity of protein/DNA solution, dialysis, ultrafiltration, centrifugation - moving boundary and zonal centrifugation, RCF and RPM, density gradient centrifugation, isoelectric precipitation, solvent fractionantion.

Radio isotope Techniques: Types of radiation used in biology, properties of α, β and γ- rays, radioisotope tracer techniques, measurement of radioactivity (GM, scintillation and gamma counters), autoradiography, Radiation protection−safety measures, radiation Dose measurements− ionizing and nonionizing radiations, basic concepts of radiation biology.

Immunotechniques: Hybridoma technique for monoclonal antibody production; Precipitation reactions, Radial immunodiffusion, double immunodiffusion, Immunoelectrophoresis, agglutination reaction, ELISA, RIA, immunofluoresence, western blot, immunohistochemistry, flow cytometry.

Recombinant DNA Methods:

Tools : Plasmids (F, R & Col plasmids, copy number & its Control, replication of ColE1 plasmid, plasmid incompatibility, plasmid amplification), Restriction enzymes (nomenclature, types, characteristics of type II R.E, modification, restriction map), Cloning vectors (pBR322, pUC, λ-vectors, cosmid, M13 vectors, phagemid, shuttle vectors), brief overview of vectors based on plant & animal viruses, Artificial chromosomes (YAC, BAC, HAC etc.).
General Methods: Manipulation of DNA (by nucleases, ligases, polymerases modifying enzymes), Construction of chimeric DNA (linker, adaptor, homo- polymer tailing), Introduction of DNA into cells (chemical method, electroporation microinjection, gene gun etc.), Gel electrophoresis (polyacrylamide, agarose, pulse-field), Nucleic acid blotting (Southern, Northern, Western, South-Western), Construction of libraries (genomic, cDNA, subtraction), Selection of a clone from library (screening by nucleic acid hybridization, immunoscreening, two-hybrid screening), DNA sequencing (manual & automated), RFLP, Genetic fingerprinting, Gel retardation & DNA footprinting, PCR (reaction conditions, thermostable DNA polymerases, characteristics of primers, cloning of PCR products, RT-PCR, real-time PCR, clinical diagnosis, RAPD), In vitro mutagenesis, protein engineering, Production of proteins from cloned genes (expression vectors, problems in *E.coli*, GST-MBP-His tagging for protein purification), Genetic mapping (SNPs, VNTRs, microsatellites), Microarray technique to study global gene expression, Gene Knock-out technique, Antisense & RNA interference, brief overview of Protein array techniques.

Fermentation Technology: Batch – fed batch – continuous fermentation, Bioreactors, Large-scale fermentation system, Harvesting and disrupting microbial cells, Downstream processing.

Industrial Microbiology: Industrially important microbial strains, Biochemical principle for industrial production of primary metabolites (lysine, glutamic acid, vitamins, alcohol, butanol, acetone, glycerol and citric acid) and secondary metabolites(streptomycin and penicillin), Production of enzymes of industrial use (amylase, protease) Improvement of Microbial strains.

Recombinant DNA in Medicine & Industry: Production of recombinant pharmaceuticals: Recombinant insulin, Human growth hormone, Complex human proteins, Antibiotics, Gene Therapy: Ex Vivo & In Vivo, Viral & non-viral gene delivery systems, Prodrug activation therapy, Nucleic acid therapeutic agents.

Engineering Animals: Transgenic mice methodology (retroviral vector, DNA microinjection, Embryonic stem cell) & its application, transgenic cattle (sheep, goats, pigs), Transgenic birds & fish.

Engineering Animals: Transgenic mice methodology (retroviral vector, DNA microinjection, Embryonic stem cell) & its application, transgenic cattle (sheep, goats, pigs), Transgenic birds & fish.

4. Bio-computational Method:
Introduction of Biocomputing: Fundamental aspects; Usefulness of the subject Overview of biological databases: Basics of biological databases; Architecture of the biological databases. Fundamental aspects of NCBI, Uniprot, PDB Fundamentals of sequence alignments: Introduction; Statistical aspects of sequence alignments; Overview of the methodologies of sequence alignment

5. Mathematical Methods
Functions and their graphical representation with application in biology: Linear Power-Periodic-Logarithmic-Exponential functions.

Properties of Function: Maxima – Minima – Pt. of inflection of the functions and applications in biology viz., pk value, Tm ; rate of change of function.

Differentiation and Integration: Simple differentiation, integration as a measure of area and simple integrals, statement of different biological and biophysical problems with their boundary conditions and setting up of differential equations, solution of 1st order and 2nd order differential equations, partial differentiation and Euler's criteria of exact differential.

Matrix Algebra: Determinants with examples from biology, matrix as operation of reflection-rotation-inversion-magnification-translation-symmetry, applications in biology.

6. Biostatistics:
Statistical evaluation of results: Estimation of standard error, confidence limits, significance tests.

Tests: Simple tests based on normal distribution, normal approximation to binomial andPoisson distribution, one and two –tailed tests, use of t-test for small samples, χ^2- test of goodness of fit , ANOVA**,** Correlation and linear regression, methodof least squares.

Regression: Use of regression to analyse wetland data (extinction coefficient, K_m, V_{max} etc.)

Use of R: Using R to read biological database and perform statistical test.