

# **CENTRE OF BIOTECHNOLOGY PROPOSED REVISED SYLLABUS**

**Recommended by Academic Program Committee, IIDS**

## **COURSE CURRICULA**

**Semester I** (20 credits)

**Papers** (12 credits)

Paper 1: Analytical Techniques (3 credits)

Paper 2: Biochemistry (4 credits)

Paper 3: Genetics and Microbiology (3 credits)

Paper 4: Biostatistics and Computer Applications in Biotechnology (2 credits)

**Laboratory Technique I** (8 credits)

**Semester II** (20 credits)

**Papers** (12 credits)

Paper 1: Biophysical Chemistry (4 credits)

Paper 2: Cell Biology (2 credits)

Paper 3: Molecular Biology (4 credits)

Paper 4: Fermentation Technology (2 credits)

**Laboratory Technique II** (8 credits)

**Semester III** (20 credits)

**Papers** (12 credits)

Paper 1: Animal Cell Biotechnology (2 credits)

Paper 2: Genetic Engineering (4 credits)

Paper 3: Immunology (4 credits)

Paper 4: Plant Biotechnology (2 credits)

**Laboratory Technique III** (8 credits)

**Semester IV** (20 credits)

**Papers**

**Elective Papers (any two of the following)** (2 credits each)

Elective 1: Proteomics

Elective 2: Environmental Biotechnology

Elective 3: Nanobiotechnology

Elective 4: Molecular Therapeutics

**Project work** (16 credits)

**Grand total of credits = 80**

**Semester-I; Paper: 1**

## **ANALYTICAL TECHNIQUES**

**(3 credits)**

**1. Basic techniques:** Buffer preparations; pH measurement; Cell disintegration; Dialysis and ultra filtration.

**2. Spectroscopy:** Principles and applications of UV-Visible, Fluorescence and Infra Red spectroscopy.

**3. Chromatography:** Principles and applications of Paper and Thin layer chromatography; Size exclusion, Ion exchange, Hydrophobic, Reverse phase and Affinity chromatography; HPLC and FPLC.

**4. Electrophoresis:**

Theory and application of Polyacrylamide and Agarose gel electrophoresis; Different variants of polyacrylamide gel electrophoresis (PAGE) like native, SDS-PAGE, 2D-PAGE, Blotting Techniques: Southern, Western and Northern blotting, Immunoblotting, Immunoelectrophoresis, Immunofluorescence, ELISA.

**5. Centrifugation:** Sedimentation, Analytical ultra centrifugation, Preparative ultra centrifugation: zonal and equilibrium density gradient ultracentrifugation

**6. Radioactivity:** Concept of radioactivity; Radioactivity counting methods with principles of different types of counters; Autoradiography; Applications of radioactive tracers in biology.

**7. Microscopy:** Principles and applications of Simple, Compound and Phase contrast microscope, Fluorescence microscope, confocal microscope, Time lapse imaging, Electron microscopy: SEM & TEM, Cryo-Electron microscopy

**Text books/References:**

1. Principles and Techniques of Practical Biochemistry by Keith Wilson and John Walker (5<sup>th</sup> edition, 2000).
2. Physical Biochemistry, application to Biochemistry and Molecular Biology by David Freifelder (2<sup>nd</sup> edition, 1982).
3. Analytical Biochemistry by D. Holme and H. Peck (3<sup>rd</sup> edition, 1998).
4. Protein Purification: Principles and Practice by Robert K. Scopes (3<sup>rd</sup> edition, 1993).

**Semester-I; Paper: 2**

**BIOCHEMISTRY**

**(4 credits)**

1. **Chemical foundations of Biology:** Composition of living matter, Water-properties, pH, pKa, acids, bases, buffers; weak bonds, covalent bonds.
2. **Protein:** physical and chemical properties of amino acids; Primary, secondary, tertiary and quaternary structure; Globular and fibrous proteins; Amino acid composition and primary structure analysis, Structure-function relationship in model proteins like ribonuclease A, myoglobin and hemoglobin.
3. **Carbohydrates:** mono, di and polysaccharides; Structural and functional role; Glycoprotein and Glycolipid.
4. **Lipids:** Structure and properties of storage and membrane lipids; Lipoproteins; Structural organization of biological membrane.
5. **Nucleic acids:** Structure and properties of purines, pyrimidines, nucleosides, nucleotides, helical structure of DNA. Different forms of DNA. Denaturation and renaturation of DNA.
6. **Enzyme catalysis:** General principles of catalysis; Quantitation of enzyme activity and efficiency; Enzyme characterization and Michaelis-Menten kinetics; Relevance of enzymes in metabolic regulation, activation, inhibition and covalent modification; single substrate enzymes.
7. **Metabolic pathways:** Energy concepts and energy rich compounds; Glycolysis, gluconeogenesis, pentose phosphate pathway, citric acid cycle and oxidative phosphorylation; Fatty acid biosynthesis and oxidation.
8. **Vitamins:** Structure and biological properties

**Text books/References:**

1. Biochemistry by Jeremy M. Berg, John L. Tymoczko and Lubert Stryer (6<sup>th</sup> edition, 2008).
2. Lehninger Principles of Biochemistry by David L. Nelson and Michael M. Cox (5<sup>th</sup> edition, 2009).
3. Biochemistry by V.Voet and J.G. Voet (4<sup>th</sup> edition Dec 2010).

**Semester-I; Paper: 3**

## **GENETICS AND MICROBIOLOGY**

**(3 credits)**

**1. Mendelian genetics:** Laws of dominance, segregation & independent assortment; Incomplete dominance, complementary genes, epistasis, lethal genes, duplicate genes, and multiple allelism. Extrachromosomal inheritance: mitochondrial & chloroplast inheritance. Linkage & crossing over; sex linkage & sex determination, Population & Evolutionary genetics; Gene Mapping

**2. Microbial gene transfer mechanisms:** conjugation, transduction, transformation: genetic systems of *Neurospora*, *Yeast*, *E. coli*, *Arabidopsis* and *Drosophila*

**3. Bacterial mutants & mutation:** Isolation, Useful phenotypes (auxotrophic, conditional, lethal, resistant); Mutation rate, Types of mutations (base pair changes; frameshift; insertion; deletion; tandem duplication), Reversion vs suppression; Mutagenic agents; Molecular mechanism of mutations, practical applications of mutations; Assay of mutagenic agents (Ames test); variations in chromosome number and structure, DNA repair mechanisms

**4. Study of microorganisms:** General characteristics and salient features related to structure, function, physiology and significance of cyanobacteria, actinomycetes, fungi, yeast, viruses, rickettsia & mycoplasma. Ultrastructure of a bacterial cell: spore, cell wall, flagella, cell membrane, capsule, pili. Microbial growth: Batch, fed-batch, continuous kinetics, synchronous growth, yield constants, methods of growth estimation, stringent response, death of a bacterial cell.

**5. Microbial systematics, Molecular Taxonomy, Basic microbiological techniques:** Microscopy, Pure culture, nutrition, enrichment, sterilization, disinfection, safety in the microbiological laboratory.

**6. Study of ecophysiological, biochemical and nutritional aspects of phylogenetically diverse representative groups of organisms:** extremophiles - thermophiles, psychrophiles, halophiles, methanogens, archaeobacteria, Nitrogen fixing organisms and nitrogen fixing genes, Mycorrhiza: types and its functions

**7. Microbial Ecology:** interactions among microbial populations, microbial interaction with animals, microbial interaction with plants

**8. Diseases of humans:** Bacterial meningitis, Botulism, Poliomyelitis, African trypanosomiasis, salmonellosis, giardiasis, hepatitis and AIDS.

**9. Antibiotics:** types & mode of action, resistance to antibiotics

### **Text books/References:**

- 1) Pleczar MJ Jr., Chan ECS and Kreig NR., Microbiology, 5<sup>th</sup> Edition, 1993
- 2) Lansing Prescott, John Harley and Donald Klein, Microbiology
- 3) R.Y.Stanier, General Microbiology
- 4) D. Peter Snustad, Michael J. Simmons, John B. Jenkins, PRINCIPLES OF GENETICS

**Semester-I; Paper: 4**

**BIostatistics AND COMPUTER APPLICATION IN BIOTECHNOLOGY**

**(2 credits)**

**UNIT: I BIostatistics**

Mean, Median and Mode, Standard deviation, use of Chi square, t and F tests, Analysis of one way and two way classification, regression lines and correlation, Method of least squares, Multiple Regression law for three variables of Permutation and combination, Concept of Probability theory, Conditional Probability, random variable, discrete and continuous distribution function (simple cases only), Binomial, Poisson and normal distribution.

**UNIT: II COMPUTER APPLICATION**

- 1) Organisation of Personal computers, block diagram level, distinction between hardware and software, computer memory, hard-disk and CD-ROM, computer peripherals, introduction to DOS and Windows operating system, user environment, files and folders, file operations, directory structure
- 2) Word processing, presentation graphics, spreadsheet, office automation packages.
- 3) Application of computers in biology: General concept of databases, search and queries, Internet access to biological databases, protein sequence and secondary structure analysis, DNA sequence analysis, RNA secondary structure analysis, Human Genome project

**Text books/References**

1. Introductory Probability and Statistical Application by Paul Meyer
2. Mathematical Statistics by Goel
3. Introductory Statistics, 6<sup>th</sup> Edition by Prem S. Mann, Wiley, 2006
4. Handbook of Computer Communication Standard vol.3, Stalling W.
5. Developing Bioinformatics Computer Skill by O'Reilly

## Semester-I

### PRACTICALS

(8 credits)

- 1) To prepare acetate buffer and validate Henderson-Hasselbach equation.
- 2) Estimation of protein by Folin's method.
- 3) Estimation of protein by Biuret method
- 4) Estimation of Carbohydrates by Anthrone method
- 5) Detection of amino acids by ninhydrin method
- 6) Detection of aminoacids by paper chromatography
- 7) Detection of aminoacids by thin layer chromatography
- 8) Estimation of DNA by DPA method
- 9) Estimation of RNA by Orcinol method
- 10) Separation of proteins by SDS-PAGE
- 11) To determine protein sensitivity limit using Coomassie staining & Silver nitrate staining of protein
- 12) Separation of proteins by centrifugation
- 13) Enzyme purification and assay
- 14) Effect of pH and temperature on enzyme activity
- 15) Sterilization, disinfection, safety in microbiological laboratory
- 16) Preparation of media for growth of micro organisms
- 17) Isolation and maintenance of microorganism by plating, streaking and serial dilution.
- 18) Staining and enumeration of microorganisms.
- 19) Growth curve, measure of bacterial population.

Semester-II; Paper: 1

## BIOPHYSICAL CHEMISTRY

(4 credits)

**1. Interaction in biological systems:** Intra and inter molecular forces, electrostatic interactions, hydrogen bonding, van der Waal interactions, hydrophobic interactions, disulfide bond.

**2. Protein Structure:** Conformational properties of polypeptide, Ramachandran plot. Primary and secondary structure of proteins; alpha helix, beta sheet and random coil Tertiary structure; concept of domain and fold, Quaternary structure; Oligomeric proteins and cooperativity, Metalloproteins, Structural features of membrane proteins, Intrinsically disordered proteins.

**3. Multiple equilibrium:** Titration of proteins to evaluate total and net charge; Scatchard and hill plots; Protein stability, denaturation, unfolding equilibrium; Kinetics and thermodynamics of protein folding; Protein refolding and aggregation; Effect of solvent and temperatures on the protein stability and folding. Differential scanning calorimetry.

**4. Methods for the structure analysis:** Far-UV and near UV-Circular Dichroism (CD); Fluorescence, single molecule fluorescence spectroscopy, fluorescent probes ; Hydrogen-Deuterium (H-D) exchange; Fourier-transform Infra Red (FT-IR) spectroscopy; Mass spectrometry (ESI and MALDI-TOF); Nuclear magnetic resonance (NMR) spectroscopy; X-ray crystallography.

### Text books/Reference:

1. Introduction to Protein Structure by Carl Branden and John Tooze (2<sup>nd</sup> edition, 1999)
2. Proteins structure and molecular properties by Thomas E. Creighton ( 2<sup>nd</sup> edition , 1992)
3. Principles of Physical Biochemistry by Kensal E. van Holde, Curtis Johnson and Pui Shing Ho (2<sup>nd</sup> edition 2005).
4. Protein structure: A practical approach by Thomas E. Creighton (2<sup>nd</sup> edition, 1997)
5. Latest reviews from journals



## CELL BIOLOGY

(2 credits)

### 1. Cell Structure and Methods in Cell Biology

Cell: structural and functional organization, Cell motility, Other sub cellular organelle like Nucleus, Endoplasmic reticulum, Golgi, Mitochondria, Lysosomes; Fractionation of sub cellular organelles, Principles and applications of the microscopy, Cell counting.

### 2. Bio-membrane structure and Function

Plasma Membrane: organization and properties, Dynamics transport across membrane, Cell signaling: Types of receptors (Intracellular and cell surface), signal transduction by membrane bound, cytosolic and nuclear receptors via various pathways

### 3. Endo-membrane System and Cellular Motility

General organization of protein transport within and outside the cell, Mechanisms of endocytosis and exocytosis, Protein sorting and secretion, Vesicular transport, Mechanism of intracellular digestion

### 4. Cell Dynamics

Cell dynamics, cytoskeleton and cell surface, Microfilaments: Structural organization, cell motility and cell shape; Microtubule: Structural and functional organization, cilia, flagella, centriole; Intermediate filaments, Cell-cell interactions and cell matrix interaction

### 5. Cell Cycle & Cell Death

Mitosis, Meiosis, Eukaryotic Cell cycle and its regulation, Apoptosis, Cancer biology - Mechanism of carcinogenesis, tumor suppressor genes and oncogene.

### 6. Cell Differentiation

Cell differentiation, hormones and growth factors; Stem cell differentiation, Blood cell formation, Fibroblast and their differentiation, Mating cell type in yeast, Surface antigen changes in Trypanosomes

### Text books/References

- 1) Alberts et al (2007). Molecular Biology of the Cell. Garland
- 2) Lodish et al (2004). Molecular Cell Biology. Freeman
- 3) Karp (2005). Cell and Molecular Biology. John Wiley
- 4) Lewin (2007). Genes IX. Jones and Barlett
- 5) Cooper (2007). The Cell: A molecular Approach. ASM Press
- 6) Latest reviews and research articles

**1.) Genes and Chromosomes**

Organization of bacterial genome; DNA structure, Structure of eukaryotic chromosomes; Complexity of genome and its reassociation kinetics (Cot curve analysis); Clusters and repeats; Chromatin: Heterochromatin and Euchromatin; Nucleosome structure and its phasing: DNase sensitivity, DNA methylation and imprinting

**2.) Replication in prokaryotes & eukaryotes**

Replication initiation and its regulation, elongation and termination in prokaryotes and eukaryotes; Enzymes and accessory proteins; Fidelity; Replication of single stranded circular DNA

**3.) Repair & Recombination**

Gene stability and DNA repair enzymes: Photoreactivation, Nucleotide excision repair, Mismatch correction, SOS repair; Recombination: Homologous and non-homologous, Site specific recombination, RecBCD system in prokaryotes, Lambda recombination, Cre/Lox and FLP/FRT recombination

**4.) Prokaryotic & Eukaryotic Transcription**

Prokaryotic Transcription; Transcription unit; Promoters-Constitutive and Inducible; Operators; Regulatory elements: Attenuation, Positive and negative regulation, Transcriptional control in lambda phage; Operon concept- lac, trp, ara, his and gal operons; Initiation, Elongation and Termination steps of prokaryotic transcription; Transcript processing recognition, Promoters and enhancers, Transcription factors: TATA binding proteins (TBP) and TBP associated factors (TAF), Activators and repressors; Transcriptional and post-transcriptional gene silencing; Processing of hnRNA, tRNA, rRNA; 5'-Cap formation; 3'-end processing and polyadenylation; Splicing; RNA editing; Nuclear export of mRNA; mRNA stability; Catalytic RNA.

**5.) Translation & Transport**

Translation machinery; Ribosomes; Steps of translation and its mechanism in prokaryotes and eukaryotes: Initiation, elongation and termination; Genetic codon and its properties; Co- and post translational modifications; Genetic code in mitochondria; Transport of proteins and molecular chaperons; Protein stability; Protein turnover and degradation

**6.) Mutation & Transposition**

Nonsense, missense and point mutations; Intragenic and Intergenic suppression; Frameshift mutations; Physical, chemical and biological mutagens; Transposition – Transposable genetic elements in prokaryotes and eukarotes; Mechanisms of transposition; Role of transposons in mutation.

**Text books/References:**

1. Lewin's GeneX-10<sup>th</sup> Edition Jones and Bartlett Publishers 2010.
2. J.D. Watson, N.H. Hopkins, J.W. Roberts, J.A. Seitz & A.M. Weiner; Molecular Biology of the Gene, 6<sup>th</sup> Edition, Benjamin Cummings Publishing Company Inc, 2007.
3. Alberts et al; Molecular Biology of the Cell, 4<sup>th</sup> edition, Garland, 2002

**Semester-II; Paper: 4**

**FERMENTATION BIOTECHNOLOGY**

**(2 credits)**

1. Fermentation technology: fermentation media, Design, operation & applications of fermentors.
2. Isolation and screening of commercially important microbes, media formulation, Strain improvement; microbial growth kinetics, effect of environmental conditions on microbial growth.
3. Industrial production of: ethanol, citric acid, acetic, fumaric and gluconic acid, solvents (glycerol, acetone and butanol), antibiotics (penicillin, streptomycin, tetracycline), amino acids (lysine & glutamic acid), pectolytic enzymes (Pectinases, Inverstase, proteases and lipases) and Vitamins (Vit B<sub>12</sub>, Riboflavin)
4. Immobilization of enzymes (or whole cells): immobilization techniques, carriers used, and characteristics of free vs. immobilization enzymes. Design & operation of immobilized enzyme reactors, applications of immobilized enzymes.
5. Downstream processing: removal of microbial cells & solid matters, foam separation, precipitation, membrane filtration, centrifugation, cell disruptions, liquid-liquid extraction, chromatography, membrane process & crystallization.
6. Bioreactor design, its functions, type of reactors.

**Text books/ References:**

1. P F Stanbury, S. Hall, A. Whitaker. Principles of Fermentation Technology, Second Edition. Publisher Butterworth-Heinemann
2. Crueger, W. and Crueger, A. Biotechnology: A Textbook of Industrial Microbiology. Panima Publshers
3. AH Patel. A text book of Industrial Microbiology by, Macmillan Publishers India

## Semester-II

### PRACTICALS

(8 credits)

- 1) Protein concentration determination by using molar extinction coefficient.
- 2) Protein conformational studies by Fluorescence and CD.
- 3) Thermal unfolding of protein and calculation of thermodynamic parameters from temperature scanning
- 4) Effect of solvent conditions on thermal stability of protein.
- 5) Microscopy: a) simple, b) compound c) phase contrast microscopes.
- 6) Study of Permanent Slides
- 7) Haemocytometer: calibration and measurement of biological samples.
- 8) Electron microscopy: Demonstration and good photographs for interpretation.
- 9) Isolation of organelles
- 10) Blood smear identification of leucocytes by Giemsa stain
- 11) To study dye binding property using different proteins
- 12) Isolation of genomic DNA from plants/bacteria/fungus.
- 13) Quantification and purity determination of isolated genomic DNA by UV-spectrophotometry and agarose gel electrophoresis.
- 14) Preparation of competent cells
- 15) Transformation of plasmid DNA (pBR 322/pUC 18/pUC 19) into DH 5 $\alpha$  strain of *E.coli*, calculation of transformation efficiency.
- 16) Isolation of plasmid DNA by alkaline lysis and phenol method.
- 17) Restriction digestion
- 18) Ligation of foreign DNA into cloning/expression vector
- 19) Polymerase chain reaction
- 20) RFLP analysis of the PCR product

## ANIMAL CELL BIOTECHNOLOGY

(2 credits)

### 1. Introduction to Tissue Culture Techniques

Introduction to tissue culture: Definition, principle and significance of tissue culture. Animal tissue culture, Maintenance of sterility and use of antibiotics, Mycoplasma and viral contaminants.

Various systems of tissue culture - their distinguishing features, advantages and limitations.

Culture medium: Logic of formulation (natural media, synthetic media, and sera).

Methodology: i. Primary culture: Behaviour of cells, properties, utility, ii Explant culture. iii. Suspension culture.

### 2. Animal Cell Organ Culture

Cell lines: Definition, development, maintenance and management and Cell adaptation.

Established cell lines: Their characteristic features and utility, Cross contamination hazards.

Contact inhibition, anchorage (in) dependence, cell-cell communication etc., Cell senescence.

Cell and tissue response to tropic factors, Culturing of different cells.

Designing of an experiment in tissue culture and response assessment. Significance of various controls.

Growth studies: Cell proliferation, cell cycle, mitosis in growing cells.

Organ culture: Methods, behaviour of organ explant, and utility of organ culture.

Organ transplants. Freeze storing of cells and transport of cultures.

Mass production of biologically important compounds.

Harvesting of products, purification and assays.

Propagation of viruses (viral sensitivity of cell lines).

Cell cloning and cell synchronization (environmental and chemical induction).

Separation of cell types: Various methods: advantages and limitations; Flow cytometry.

Nuclear transplantation, Cell hybridization, Transfection studies.

### 3. Applications of Tissue Culture

Commercial applications of animal tissue culture: Tissue culture as a screening system.

Cytotoxicity and diagnostic tests.

Development and preparation of vaccines against infecting organisms, mammalian cloning.

Establishment of cell lines from tissues of genetic diseases.

Gene therapy: somatic and germ line gene therapy

Applications of Genetic manipulations.

### 4. Stem cell concept and technologies

Committed cells and late development

Stem cells, Embryonic stem cells, differentiation.

ES cell technologies, Transgenics and knock outs.

Concept of Cell replacement therapy and regenerative medicine

Human cloning and Bioethics.

**Text books/ References:**

- 1) Animal Cell Culture Technique, Ed. Martin, Clynes. Springer, 1998
- 2) Animal Cell Culture-Practical Approach, 3<sup>rd</sup> Edition, Ed. John R.W. Masters, Oxford University Press, 2000
- 3) Stem Cells, C.S.Potten, Elsevier, 2006
- 4) Stem Cell Biology and Gene Therapy, Peter J. Quesenberry, 1<sup>st</sup> Edition, Willy – Less, 1998

### 1.) Basic Concepts

DNA structure and properties; Restriction Enzymes; DNA ligase, Klenow enzyme, DNA polymerases, Polynucleotide kinase, Alkaline phosphatase; Linkers; Adaptors; Homopolymer tailing; Labeling of DNA: Nick translation, Random priming, Radioactive and non radioactive probes, Fluorescent probes; Hybridization techniques: Northern, Southern and Colony hybridization, Fluorescence in situ hybridization; Chromatin immunoprecipitation; DNA-Protein Interactions- Electromobility shift assay, DNaseI footprinting; Methyl interference assay.

### 2.) Cloning Vectors

Plasmids; PUC19 and Bluescript vectors; Phagemids; Lambda vectors: Insertion and Replacement vectors; m13 mp vectors; Cosmids; Artificial chromosome vectors (YACs and BACs); Animal Virus derived vectors: SV-40; vaccinia/baculo & retroviral vectors; Expression vectors: pMal, GST, pET-based vectors, Fusion protein purification strategies: His-tag; GST-tag; MBP-tag etc.; Intein-based vectors; Inclusion bodies, Methodologies to reduce formation of inclusion bodies; Plant based vectors, Ti and Ri as vectors, Yeast vectors, Shuttle vectors

### 3.) Cloning Methodologies and Gene Expression

Insertion of foreign DNA into Host Cells; Transformation; Transfection; Transduction; Construction of libraries: cDNA and genomic libraries; cDNA and genomic cloning; Expression cloning; Southwestern and Far-western cloning; Protein-protein interactive cloning and Yeast two hybrid system; Phage display; Principles in maximizing gene expression

### 4.) PCR and its Applications

Primer design; DNA polymerases: Fidelity and Proof reading; Types of PCR-multiplex, nested, reverse transcriptase, real time PCR, touchdown PCR, hot start PCR, colony PCR; PCR in gene recombination; Deletion; Addition; Overlap extension and SOEing; Site specific mutagenesis; PCR in molecular diagnostics; Mutation detection: SSCP, DGGE, RFLP, Molecular polymorphism, RFLP, RAPD, STS, AFLP, SNP markers; Construction of genetic map; Oigo Ligation Assay (OLA), MCC (Mismatch Chemical Cleavage), ASA (Allele-Specific Amplification), PTT (Protein Truncation Test)

**5.) Sequencing methods:** Enzymatic DNA sequencing, Chemical sequencing of DNA, Automated DNA sequencing, RNA sequencing; Introduction of DNA into mammalian cells; Transfection techniques; Gene silencing techniques: siRNA technology; Micro-RNA; Construction of siRNA vectors; cDNA and intragenic arrays; Differential gene expression and microarray technology.

**Text books/References:**

1. S.B. Primrose, R.M. Twyman and R.W.Old; Principles of Gene Manipulation. 6<sup>th</sup> Edition, S.B. University Press, 2001.
2. J. Sambrook and D.W. Russel; Molecular Cloning: A Laboratory Manual, Vols 1-3, CSHL, 2001.
3. Brown TA, Genomes, 3<sup>rd</sup> ed. Garland Science 2006.
4. Technical Literature from Stratagene, Promega, Novagene, New England Biolab etc.



## IMMUNOLOGY

(4 credits)

### 1. Introduction to immune system

Immune system overview, innate and acquired immune system, Components of immune system, Antigens, Structure and function of antibody, Monoclonal antibodies, Immunological Methods, Techniques in humoral and cellular immunology, Antigen-antibody interactions, B and T cell receptors and coreceptors

### 2. Immunoglobulin and T-cell receptor genes

Organization of Ig gene loci, Molecular mechanisms of generation of antibody diversity, Expression of Ig genes, Regulation of Ig gene transcription, Antibody engineering, Organization of TCR gene loci, Generation of TCR diversity

### 3. The HLA complex

Organization of HLA complex, Structure of class I and II HLA molecules, Expression of HLA genes, HLA polymorphism

### 4. Generation and regulation of immune responses

Antigen processing and presentation, MHC-restriction, Cytokines, T Cell Maturation, activation and differentiation, B Cell Generation, Activation and differentiation, Clonal selection and immunological memory, Complement system, Leukocyte, Activation and Migration, Cell mediated cytotoxic responses, Regulation of immune responses, Immunological tolerance

### 5. Immunological Disorders

Primary and secondary immunodeficiencies, Autoimmune disorders, Hypersensitive reactions, Cytokine-related diseases

### 6. Immune system in human health

Immune response to infectious diseases and malignancy, Concept of immunotherapy, Vaccines, Transplantation immunology

#### Text books/References:

- 1) Kuby (2006). Immunology. Freeman
- 2) Abbas et al (2007). Cellular and Molecular Immunology. Saunders
- 3) Benjamin et al (2003). Immunology – A Short Course. Wiley-Liss
- 4) Roitt (2003). Essential Immunology. Blackwell

## PLANT BIOTECHNOLOGY

(2 credits)

### 1. Plant Tissue Culture

Historical perspective; Totipotency; Organogenesis; Somatic embryogenesis; Artificial seed production; Micropropagation; Somaclonal variation; Androgenesis and its applications in genetics and plant breeding; Germplasm conservation.

### 2. Protoplast culture and Somatic Hybridization

Protoplast isolation; Culture and its applications; somatic hybridizaods and applications;

### 3. Agrobiology

*Agrobacterium*-plant interaction; Virulence; Ti and Ri plasmids; Opines and their significance; T-DNA transfer; Disarming the Ti plasmid.

### 4. Genetic Transformation

*Agrobacterium* mediated gene delivery; Cointegrate and binary vectors; Direct gene transfer-PEG-mediated, electroporation, particle bombardment and alternative methods; Screening markers; Chloroplast transformation.

### 5. Molecular Mapping & Marker Assisted Selection (MAS)

Quantitative and qualitative traits; MAS for genes of agronomic importance, e.g. insect resistance, grain quality and grain yield; Gene mapping and cloning; QTL mapping and cloning.

### 6. Strategies for Introducing Biotic and Abiotic Stress Resistance/Tolerance

Bacterial resistance; Viral resistance; Fungal resistance; Insects and pathogen resistance; Herbicide resistance; Drought, salinity, thermal stress, flooding and submergence tolerance.

### 7. Plants as Biofactories

Concept of biofactories; Fermentation and production of industrial enzymes; vitamins and antibiotics and other biomolecules; Secondary metabolite production; Production of pharmaceutically important compounds; Bioenergy generation.

#### Text books/References:

1. H. S. Chawla. Plant Biotechnology A Practical Approach Science Publishers, USA
2. Plant Biotechnology: Methods in Tissue Culture and Gene Transfer Book Description, Orient Longman Publishers
3. S.S. Bhojwani, M.K. Razdan - Plant Tissue Culture: Theory and Practice. Elsevier Science

## Semester-III

### PRACTICALS

(8 credits)

- 1) Electron microscopic observations of ultrastructure of animal viruses
- 2) To determine antibody titre or viral hemagglutination titre using microtitre plate reader
- 3) Immunoblotting and Dot blot assays
- 4) Protein profiling of total blood serum & ammonium sulphate purified fraction using SDS PAGE
- 5) Staining of cell cultures and observations under microscope
- 6) Growth studies. Cell count, protein estimation
- 7) Development and maintenance of a cell line
- 8) Virus propagation in cells, cytopathogenic response of cells to viruses
- 9) Preparation of tissue culture media
- 10) Micropropagation Techniques – Stem or nodal culture
- 11) Callus induction and growth measurement
- 12) Suspension culture techniques
- 13) Restriction digestion of  $\lambda$ -DNA with Hind III endonuclease.
- 14) Determination of molecular wt. of fragments obtained after Hind III digestion of  $\lambda$ -DNA.
- 15) Restriction digestion of vector (gel analysis) and insert with suitable restriction endonuclease.
- 16) Ligation of foreign DNA into linearized plasmid (pBR 322/pUC 18/pUC 19/any expression vector) and its transformation into DH 5 $\alpha$  strain of *E.coli*.
- 17) Recombinant screening by blue-white selection and confirmation of recombinant construct by agarose gel electrophoresis.
- 18) Plasmid isolation and confirming recombinant by PCR and RE digestion
- 19) Purification of protein on Ni-NTA column and analysis of purification by SDS-PAGE.
- 20) Strain differentiation of fungal isolates/plant varieties/bacterial isolates by RAPD analysis and cluster analysis.
- 21) Isolation of mRNA from bacterial/plant source.
- 22) Synthesis of cDNA, amplification of desired gene using end specific primers by PCR.

**Semester-IV; Elective: 1**

**PROTEOMICS**

**(2 credits)**

1. **Protein analysis:** concentration determination, amino acid composition, N-terminal sequencing, limited proteolysis, Posttranslational modifications like acetylation, glycosylation, hydroxylation, oxidation, sulfatation.
2. **Electrophoresis:** Basic principles; 1D and 2-D electrophoresis of proteins; Isoelectric focusing; Capillary electrophoresis.
3. **Mass spectrometry:** Principles, Electrospray ionization, Matrix assisted laser desorption ionization, Tandem mass spectrometry (MS-MS), H-D exchange coupled mass spectrometry, Peptide mass fingerprinting, Peptide sequencing by tandem mass spectrometry, Protein identification with MS/MS data.
4. **Protein Microarrays:** Proteomics Microchips; Nanospray LC/MS; Microarray MS chip (Ion trap); SELDI.
5. **Comparative Proteomics:** Differential display proteomics; Differential Gel electrophoresis.
6. **Quantitative proteomics:** SILAC, iTRAQ and label free analysis.
7. **Bioinformatics tools to proteomics.**
8. **Structural Proteomics.**

**Text books/References:**

1. Biochemistry by V.Voet and J.G. Voet (4<sup>th</sup> edition Dec 2010).
2. Introduction to Proteomics: Tools for the New Biology by Daniel C. Liebler, Humana Press, Totowa, NJ, 1st edition (December 2001)
3. An Introduction to Biological Mass Spectrometry”, C. Dass, Wiley, USA, 2002.
4. The Expanding Role of Mass Spectrometry in Biotechnology”, G. Siuzdak, MCC Press, San Diego, 2004.
5. Recent reviews and research articles from the journals

**Semester-IV; Elective: 2**

## **ENVIRONMENTAL BIOTECHNOLOGY**

**(2 credits)**

### **1.) Pollution**

Environment; basic concepts; Environmental pollution, its types and assessment techniques.

### **2.) Control, remediation and management**

Waste water treatment through chemical, microbial and biotech techniques; types of reactors; Bioremediation of organic pollutants like Hydrocarbons, substituted hydrocarbons, oil pollutants, surfactants, herbicides. Phytoremediation; Soil, Waste & Water management; Biofilters; Biosensors; Biofilms.

### **3.) Alternate source of energy**

Biomass as source of energy; Bioreactors; Biocomposting; Biofertilizer; Vermiculture; Organic farming; Bio-mineralization; Biofuels; Bioethanol and biohydrogen; Solid waste management.

### **4.) Environment and health**

Human biomonitoring; Environment pollution and children; Effect of carbon and other nanoparticles upon health; Gene and environment, Bioindicators and biosensors for detection of pollution, Biotechnology for Hazardous Waste Management, Persistent organic pollutants, Xenobiotics, Biological Detoxification of PAH, Biotechniques for Air Pollution Control.

### **Text books/References:**

1. Hans-Joachim Jördening, Josef Winter John. Environmental Biotechnology: Concepts and Applications Wiley & Sons, Ltd
2. T Srinivas Environmental Biotechnology (Paperback)
3. Hemant Rawat. Environmental Biotechnology. Eastern Book Corporation
4. Amann, R.I. Stromley, J. Stahl : Applied & Environmental Microbiology
5. Chattergy : Environmental Biotechnology
6. Peavy & Rowe : Environmental Pollution
7. Asthana & Asthana : Environment Problems & Solutions
8. Manahan : Environmental Chemistry
9. Saigo, Canninham : Environmental Science

**Semester-IV; Elective: 3**

**NANOBIOTECHNOLOGY**

**(2 credits)**

- 1.) Introduction to Nano-Biotechnology; Nanotechnology definition and concepts; Cellular Nanostructures; Nanopores; Biomolecular motors; Criteria for suitability of nanostructures for biological applications
  
- 2.) Basic characterization techniques; Electron microscopy; Atomic force microscopy; Photon correlation spectroscopy
  
- 3.) Thin films; Colloidal nanostructures; Nanovesicles; Nanospheres; Nanocapsules
  
- 4.) Nanostructures for drug delivery, concepts, targeting, routes of delivery and advantages
  
- 5.) Nanostructures for diagnostics and biosensors; Nanoparticles for diagnostics and imaging; Nanodevices for sensor development

**Texts books/References**

1. Multilayer Thin Films, Editor(s): Gero Decher, Joseph B. Schlenoff Publisher: Wiley-VCH Verlag GmbH & Co. KGaA ISBN: 3527304401
  
2. Bionanotechnology: Lessons from Nature Author: David S.Goodsell Publisher: Wiley-Liss ISBN: 047141719X
  
3. Biomedical Nanotechnology Editor: Neelina H. Malsch Publisher: CRC Press ISBN: 0-8247-2579-4

**Semester-IV; Elective: 4**

## **MOLECULAR THERAPEUTICS**

**(2 credits)**

- 1) Gene therapy, Drug targeting and drug delivery system: Intracellular barriers to gene delivery, Overview of inherited and acquired diseases for gene therapy, virus mediated gene transfer, Liposome and nanoparticles mediated gene delivery, Cellular therapy, Concept of tissue engineering, Role of scaffolds
- 2) HIV diagnostics and treatment.
- 3) Recombinant therapy, Clinical application of recombinant technology, Erythropoietin, Insulin analogs and its role in diabetes, Recombinant human growth hormone, Streptokinase and urokinase in thrombosis.
- 4) General concept of infectious disease, metabolic disorders and their diagnosis
- 5) Phage and their application, Immunotherapy, Monoclonal antibodies and their role in cancer, Role of recombinant interferons, Immunosuppressors in organ transplants, Role of cytokine therapy in cancer.
- 6) Gene silencing technology, Antisense therapy, siRNA, microRNA, Transgenics and their uses.

### **Text books/References:**

1. Bernhard Palsson and Sangeeta N Bhatia, Tissue Engineering, 2<sup>nd</sup> Edition, Prentice Hall, 2004
2. Pamela Greenwell, Michelle McCulley, Molecular Therapeutics: 21<sup>st</sup> century medicine, 1<sup>st</sup> Edition, Springer, 2008
3. Latest Reviews and Research articles

**Semester-IV**

**PROJECT WORK**

**(16 credits)**

Final year student will do research work on defined topic within Centre of Biotechnology, University of Allahabad for a duration of one semester under the guidance of Centre faculty.