REVISED CURRICULUM

M. Tech

PHARMACEUTICAL BIOTECHNOLOGY

Department of Biotechnology
Ministry of Science & Technology,
Government of India

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CONTENTS

LIST OF ELECTIVES

Contents for electives are given separately.

SEMESTER - I

Enzyme & Microbial Technology - 3 Credits

Unit I

Isolation, development, preservation and improvement of industrially important micro-organisms; Isolation of auxotrophic mutants; Isolation of revertant mutants and use of recombinant systems for the improvement of industrial microorganisms; Metabolic pathways: Regulatory mechanisms of metabolic pathways in industrial strains; Bioenergetics-basic principles; Equilibria and concept of free energy; Coupled processes; Glycolysis and Glycolytic enzymes regulation; TCA cycle and enzyme regulation; Oxidative phosphorylation and enzyme regulation; Fatty acid metabolism; Principles of metabolic regulation: Regulatory steps; Signals and second messengers; ATP yield and calculations; Solutions of numerical problems in Biosynthetic pathways.

Unit II

Enzymology: Source of enzymes; Production, isolation and purification of enzymes; Characterization in terms of pH, temperature, ionic strength, substrate and product tolerance, effects of metal ions etc.; Enzyme kinetics: Enzymes as biological catalysts; Enzyme action: Active site, Functional group, Enzyme substrate complex, Cofactors; Michaelis-Menten equation; Enzyme inhibition; Order of reaction; Methods of plotting enzyme kinetics data; Enzyme turnover; Solutions of numerical problems; Energy yielding and energy-requiring reactions; Calculation of equilibrium constants; Activation energy etc.; Multisubstrate enzymes and kinetic mechanisms; Enzyme induction, repression, covalent modification, isoenzymes, allosteric effect.

Unit III

Immobilized enzyme technology: Different techniques of immobilization of enzymes and whole cells; Advantages and disadvantages of immobilization; Kinetics of immobilized enzymes; Design and operation of immobilized enzyme reactors; Calculations of diffusional resistances and Thiele's modulus; Multi step immobilized enzyme systems; Solutions of numerical problems; Application and future of immobilized enzyme technology.

Unit IV

Enzymes in organic solvents and ionic liquids: Various organic solvents and ionic liquids used in biocatalysis; Potential of biocatalysis in organic solvents and ionic solvents; Enzyme engineering: Random and rational approach of protein engineering; Directed evolution and its applications in the field of biocatalysis; Various approaches of creating variant enzyme molecules; Future of biocatalysis; Ideal Biocatalyst.

Unit V

Biocatalysis; Advantages and disadvantages of biocatalysis over chemical catalysis; Different types of biocatalysis: Microbial, enzymatic and immobilized system of biocatalysis; Current industrial biocatalysis; Biocatalysis with different enzymes: Lipase, amidase/aminopeptidase, Acylase, Hydantoinase, lyases, Oxido-reductase, Nitrilase, Epoxide hydrolase, Hydroxylase, Aldolases, Decarboxylase; Micro-organisms in

degradation of xenobiotics and removal of heavy metals; Stereoselective production of drug intermediates; Biocatalysis for the synthesis of some chiral pharmaceutical intermediates such as synthesis of ACE inhibitors; Synthesis of anti-cholesterol drugs by biocatalytic routes; Calcium channel blocking drugs; Potassium channel openers; Anti-arrythmic compounds; Anti-psychotic compounds; Anti-infective drugs; Anti-inflammatory drugs; Antiviral agents; Prostaglandin synthesis.

Texts/References

1. Nelson and Cox, Principles of Biochemistry, 4th Edition, W. H. Freeman, 2004.
2. Donald Voet and Judith Voet, Biochemistry, 3rd Edition, John Wiley, 2007.
3. J. Rehm and G. Reed, Enzyme Technology, Vol. 7a, VCH-Verlag.
4. Biotol Series (This series has many volumes pertaining to different subjects including white, red, blue and green biotechnology)

Biochemical Engineering Fundamentals - 3 Credits

Unit I

Kinetics of microbial growth; Substrate utilization and product formation; Structured and unstructured model of growth; Equations for substrate utilization and product formation; Yield coefficients; Numericals related to growth and product kinetics.

Unit II

Agitation and aeration: Need of agitation and aeration in aerobic fermentation; Effect of agitation on aeration; Different types of agitational methods; Rheological behaviour of fluids; Newton's law of viscosity etc.

Unit III

Sterilization of air and medium: Different methods of sterilization; Kinetics of sterilization; Batch and continuous sterilization; Advantages and disadvantages thereof; Calculation of del factor and solving of numerical; Detailed studies on the batch, continuous and fed-batch bioreactors; Air sterilization; Solving of different types of numericals associated with sterilization.

Unit IV

Mass transfer: Mass and energy balance in microbial processes; Resistances encountered in fermentation medium by the oxygen molecule; Role of dissolved oxygen concentration in the mass transfer; Determination of mass transfer co-efficient (K_La); Factors effecting K_La and their relationship; Solving of different types of numericals associated with mass transfer.

Unit V

Dimensional analysis: Heat transfer in bioreactors; Counter current and co-current system of heat transfer; Scale-up: Principles and criteria; Different methods of scale-up and the detailed analysis with case studies; Scale down; Instrumentation and control of bioprocesses, Scale up problems; Solving of different types of numericals associated with scale up.

Texts/References

1. Michael L. Shuler and Fikret Kargi, Bioprocess Engineering: Basic Concepts, 2nd Edition, Prentice Hall, 2001.
2. Pauline M. Doran, Bioprocess Engineering Principles, 1st Edition, Academic Press, 1995.
3. James E. Bailey and David F. Ollis, Biochemical Engineering Fundamentals, 2nd Revised Edition, McGraw-Hill, 1986.
4. Biotol Series (This series has many volumes pertaining to different subjects including white, red, blue and green biotechnology)

Molecular Biology - 3 Credits

Unit I

Genome organization

Organization of bacterial genome; Structure of eukaryotic chromosomes; Role of nuclear matrix in chromosome organization and function; Matrix binding proteins; Heterochromatin and Euchromatin; DNA reassociation kinetics(Cot curve analysis); Repetitive and unique sequences; Satellite DNA; DNA melting and buoyant density; Nucleosome phasing; DNase I hypersensitive regions; DNA methylation & Imprinting

Unit II

DNA Structure; Replication; Repair & Recombination

Structure of DNA - A-,B-, Z- and triplex DNA; Measurement of properties-Spectrophotometric, CD, AFM and Electron microscope analysis of DNA structure; Replication initiation, elongation and termination in prokaryotes and eukaryotes; Enzymes and accessory proteins; Fidelity; Replication of single stranded circular DNA; Gene stability and DNA repair- enzymes; Photoreactivation; Nucleotide excision repair; Mismatch correction; SOS repair; Recombination: Homologous and non-homologous; Site specific recombination; Chi sequences in prokaryotes; Gene targeting; Gene disruption; FLP/FRT and Cre/Lox recombination.

Unit III

Prokaryotic & Eukaryotic Transcription

Prokaryotic Transcription; Transcription unit; Promoters- Constitutive and Inducible; Operators; Regulatory elements; Initiation; Attenuation; Termination-Rho-dependent and independent; Anti-termination; Transcriptional regulation-Positive and negative; Operon concept-lac, trp, ara, his, and gal operons; Transcriptional control in lambda phage; Transcript processing; Processing of tRNA and rRNA

Eukaryotic transcription and regulation; RNA polymerase structure and assembly; RNA polymerase I, II, III; Eukaryotic promoters and enhancers; General Transcription factors; TATA binding proteins (TBP) and TBP associated factors (TAF); Activators and repressors; Transcriptional and post-transcriptional gene silencing

Unit IV

Post Transcriptional Modifications

Processing of hnRNA, tRNA, rRNA; 5'-Cap formation; 3'-end processing and polyadenylation; Splicing; RNA editing; Nuclear export of mRNA; mRNA stability; Catalytic RNA.

Translation & Transport

Translation machinery; Ribosomes; Composition and assembly; Universal genetic code; Degeneracy of codons; Termination codons; Isoaccepting tRNA; Wobble hypothesis; Mechanism of initiation, elongation and termination; Co- and post-translational modifications; Genetic code in mitochondria; Transport of proteins and molecular chaperones; Protein stability; Protein turnover and degradation

Unit V

Mutations; Oncogenes and Tumor suppressor genes

Nonsense, missense and point mutations; Intragenic and Intergenic suppression; Frameshift mutations; Physical, chemical and biological mutagens; Transposition - Transposable genetic elements in prokaryotes and eukaryotes; Mechanisms of transposition; Role of transposons in mutation; Viral and cellular oncogenes; Tumor suppressor genes from humans; Structure, function and mechanism of action of pRB and p53 tumor suppressor proteins; Activation of oncogenes and dominant negative effect; Suppression of tumor suppressor genes; Oncogenes as transcriptional activators.

Text/References

1. Benjamin Lewin, Gene IX, 9th Edition, Jones and Barlett Publishers, 2007.
2. J.D. Watson, N.H. Hopkins, J.W Roberts, J. A. Seitz & A.M. Weiner; Molecular Biology of the Gene, 6th Edition, Benjamin Cummings Publishing Company Inc, 2007.
3. Alberts et al; Molecular Biology of the Cell, 4th edition, Garland, 2002.

Spectral Analysis - 2 Credits

Unit I

Ultra violet and visible spectroscopy: Energy levels and selection rules; Woodward-Fieser; Fieser-Kuhn and Nelson rules; Influence of substituent; Ring size and strain on spectral characteristics; Solvent effect; Stereochemical effect; Non-conjugated interactions; Spectral correlation with structure.

Unit II

Infrared spectroscopy (IR): Characteristic regions of the spectrum; Influence of substituents, ring size, hydrogen bonding, vibrational coupling and field effect on frequency; Determination of stereochemistry and spectral interpretation with examples.

Unit III

Nuclear magnetic resonance spectrometry (NMR) (Part I): Magnetic nuclei; Chemical shift and shielding; Relaxation processes; Chemical and magnetic non-equivalence; Local diamagnetic shielding and magnetic anisotropy; Spin-spin splitting, Pascal's triangle.

Unit IV

Nuclear magnetic resonance spectrometry (NMR) (Part II): Coupling constant; Mechanism of coupling; Quadrupole broadening and decoupling; Effect of conformations and stereochemistry on the spectrum; Diastereomeric protons; Virtual coupling; Long range coupling-epi, peri, bay effects; Shift reagents-mechanism of action, spin decoupling and double resonance.

Unit V

Mass spectrometry (MS): Molecular ion and metastable peak; Fragmentation patterns; Nitrogen and ring rules; McLafferty rearrangement; Electron and chemical ionization modes; Applications.

Text/References

1. Petre Stoica and Randolph L. Moses, Introduction to Spectral analysis, Prentice Hall, 1997.
2. Gwilym M. Jenkins and Donald Watts, Spectral Analysis and its application, Emerson-Adams Press, 1998.
3. Donald L Pavia, Gary M Lampman, George S Kriz, Introduction to spectroscopy: A guide for students of organic chemistry, 3rd Edition, Harcourt College Publishers, 2001.

Separation Techniques - 2 Credits

Unit I

Chromatography: General principles; Classification of chromatographic techniques; Normal and reversed phase; Bonded phase; Separation mechanisms.

Unit II

Column chromatography: Merits and demerits; Short-column chromatography and flash chromatography; Vacuum liquid chromatography (VLC); Medium pressure liquid chromatography; High pressure liquid chromatography (HPLC).

Unit III

TLC; HPTLC; Over pressure layer chromatography (OPLC); Centrifugal chromatography.

Unit IV

Counter-current chromatography; Droplet counter-current chromatography; Ion-exchange; Affinity; Size exclusion and ion-pair chromatography.

Unit V

Gas chromatography; Introduction to GC-MS and LC-MS techniques.

Text/References

1. P D Sethi, HPLC Quantitative Analysis of Pharmaceutical Formulations, CBS Publishers & distributors, 2008
2. Yuri V Kazakevich, Rosario Lo Brutto, HPLC for Pharmaceutical Scientists, 1st Edition, Wiley Intersciences, 2007.
3. Ronald W Rousseau, Handbook of separation process technology, Wiley Intersciences Publication, New York, 1987.

Biostatistics - 2 Credits

Unit I

Statistics: Introduction, its role and uses; Collection; Organization; Graphics and pictorial representation of data; Measures of central tendencies and dispersion; Coefficient of variation.

Unit II

Probability: Basic concepts; Common probability distributions and probability distributions related to normal distribution; Sampling: Simple random and other sampling procedures; Distribution of sample mean and proportion.

Unit III

Estimation and Hypothesis testing: Point and interval estimation including fiducial limits; Concepts of hypothesis testing and types of errors; Student-t and Chi square tests; Sample size and power; Experimental design and analysis of variance: Completely randomized, randomized blocks; Latin square and factorial designs; Post- hoc procedures.

Unit IV

Correlation and regression: Graphical presentation of two continuous variables; Pearson's product moment correlation coefficient; its statistical significance; Multiple and partial correlations; Linear regression; Regression line; Coefficient of determination; Interval estimation and hypothesis testing for population slope; Introduction to multiple linear regression models; Probit and logit transformations.

Unit V

Non-parametric tests: Sign; Mann-Whitney U; Wilcoxon matched pair; Kruskal wallis and Friedman two way anova tests. Spearman rank correlation; Statistical techniques in pharmaceutics: Experimental design in clinical trials; Parallel and crossover designs; Statistical test for bioequivalence; Dose response studies; Statistical quality control.

Texts/References

1. P.S.S. Sundar Rao, P.H.Richard, J.Richard, An introduction to Bio-statistics, Prentice Hall of India(P) Ltd., New Delhi, 2003.
2. Gupta S.P, Statistical Methods, Sultan Chand & Sons, New Delhi, 2005.
3. Jerrold H. Zar, Bio Statistical Analysis, Tan Prints(I) Pvt. Ltd., New Delhi, 2003.
4. Goulden, Methods of Statistical Analysis, Asia Publishing Co., New Delhi, 1962.

Lab on Biochemistry & Analytical Techniques - 4 Credits

1. To prepare an Acetic-Na Acetate Buffer system and validate the Henderson-Hasselbach equation.
2. To determine an unknown protein concentration by plotting a standard graph of BSA using UV-Vis Spectrophotometer and validating the Beer- Lambert's Law.
3. Titration of Amino Acids and separation of aliphatic, aromatic and polar amino acids by TLC.
4. AN ENZYME PURIFICATION THEME (such as *E.coli* Alkaline phosphatase or any enzyme of the institutions choice).

(a) Preparation of cell-free lysates
(b) Ammonium Sulfate precipitation
(c) Ion-exchange Chromatography
(d) Gel Filtration
(e) Affinity Chromatography
(f) Generating a Purification Table
(g) Assessing purity by SDS-PAGE Gel Electrophoresis
(h) Assessing purity by 2-D gel Electrophoresis
(i) Enzyme Kinetic Parameters: Km, Vmax and Kcat.

5. Biophysical methods (Circular dichroism spectroscopy, fluorescence spectroscopy).
6. Determination of mass of small molecules and fragmentation patterns by Mass Spectrometry

Lab on Molecular Biology - 4 Credits

1. Plasmid DNA isolation and DNA quantitation: Plasmid minipreps
2. Restriction digestion
3. Preparation of competent cells.
4. Agarose gel electrophoresis
3. Restriction Enzyme digestion of DNA
4. Purification of DNA from an agarose gel
5. DNA Ligation
6. Transformation of E.coli with standard plasmids, Calculation of transformation efficiency
7. Cloning of genomic DNA in standard plasmid vectors
8. Confirmation of the insert, Miniprep of recombinant plasmid DNA Restriction mapping
9. Polymerase Chain reaction, using standard 16srRNA eubacterial primers
10. RFLP analysis of the PCR product
11. Transformation of yeast *Saccharomyces cerevisiae*

Communication Skills

Process of communication

Concept of effective communication- Setting clear goals for communication; Determining outcomes and results; Initiating communication; Avoiding breakdowns while communicating; Creating value in conversation; Barriers to effective communication; Non verbal communication- Interpreting non verbal cues; Importance of body language, Power of effective listening; recognizing cultural differences

Presentation skills

Formal presentation skills; Preparing and presenting using Over Head Projector, Power Point; Defending Interrogation; Scientific poster preparation & presentation; Participating in group discussions

Technical Writing Skills

Types of reports; Layout of a formal report; Scientific writing skills: Importance of communicating Science; Problems while writing a scientific document; Plagiarism; Scientific Publication Writing: Elements of a Scientific paper including Abstract, Introduction, Materials & Methods, Results, Discussion, References; Drafting titles and framing abstracts

Computing Skills for Scientific Research

Web browsing for information search; search engines and their mechanism of searching; Hidden Web and its importance in Scientific research; Internet as a medium of interaction between scientists; Effective email strategy using the right tone and conciseness

Texts/References

1. Mohan Krishna and N.P. Singh, Speaking English effectively, Macmillan, 2003.

Immunology & Immunotechnology - 3 Credits

Unit I

Immunity: Innate and adaptive; Immune response memory, specificity and recognition of self and non-self; Immunogenicity; Antigenicity; Physiology of immune response; Epitope analysis; Synthetic peptides and immune response; Immunity to virus, bacteria, fungi.

Cells and organs of the immune system: Lymphoid cells, T cells, B cells, monocytes, phagocytes, mast cells and basophils; Primary and secondary lymphoid organs; Interplay between cells.

Unit II

Humoral immunity: Antigen-antibody interactions; Affinity; Avidity; Immunoglobulins; Molecular mechanism of generation of antibody diversity; Molecular biology of IgG.

Cell mediated immunity: T cell subset and surface marker; T cell-dependent and -independent markers; Structure and function of MHC; Association of MHC with disease susceptibility; Structure of T cell antigen receptor; T cell cloning.

Unit III

Natural immunity: Inflammation; Stimuli; Chemotaxis; Arachidonic acid metabolite and cytokines; vascular modifications, healing and fibrosis.

Natural killer cells: Function; Mechanism of lysis; Recognition structures; Phosphorylation.

Unit IV

Immune memory: B-cell memory; Significance; Mutations and switches in memory cells; T-cell memory; Lack of mutations and switches in T-cell memory, activation, super activation, loss of memory.

Immune tolerance: B-cell tolerance; Reversible and irreversible tolerance; Antigen induced tolerance; Induction; T-cell tolerance; Partial engagement of signal transducer; Self-antigens; Molecular consequence of tolerance

Hypersensitive reaction; Immunosuppression; Autoimmune disorders: its molecular mechanism; Immunodeficiency disorders (AIDS); Tumor immunology.

Unit V

Immunobiotechnology: Hybridoma; Vaccines; Viral, bacterial peptides; Genetically engineered production of lymphokines; Second generation antibodies: a brief outline;

Immunological Techniques: Immunodiffusion; Immunoblot; Immunofluorescence; Immunoaffinity; ELISA; Agglutination; Immunoprecipitation; Immunoelectrophoresis; Biotinylation; Avidin-streptavidin cross-linking; immunogens; Immunomodulations.

Texts/References

1. Kuby, RA Goldsby, Thomas J. Kindt, Barbara, A. Osborne Immunology, 6th Edition, Freeman, 2002.
2. Brostoff J, Seaddin JK, Male D, Roitt IM., Clinical Immunology, 6th Edition, Gower Medical Publishing, 2002.
3. Janeway et al., Immunobiology, 4th Edition, Current Biology publications., 1999.
4. Paul, Fundamental of Immunology, 4th edition, Lippencott Raven, 1999.
5. Goding, Monoclonal antibodies, Academic Press. 1985.

Microbiology & Industrial Applications - 3 Credits

Unit I

Introduction, aims and scope: Organization and function of prokaryotic and eukaryotic cells; Structure and function of cell organelles-surface structure, special organelles, cellular reserve materials; Distinguishing features of various groups of micro organisms: Actinomycetes, bacteria, moulds, yeasts and algae and their broad classification; Characteristics of selected groups of microbes: Archaebacteria and microorganisms of extreme environment; Control of micro organisms by physical and chemical agents; pure culture concept and cultural characteristics.

Unit II

Microbial nutrition and growth principles: Growth measurement techniques; Assimilation of carbon, nitrogen and sulphur; Isolation and preservation: Isolation of organisms from various sources and long term preservation and improvement of cultures

Host–Pathogen interactions: Microbes infecting humans, veterinary animals and plants; Pathogenicity islands and their role in bacterial virulence.

Unit III

Biochemical pathways: Energy transduction in microbial systems, phosphoketolase, Entner doudoroff and glyoxylate pathways; Anaerobic respiration; Microbial pathogenicity; Recycling of energy sources: Bioassays; Recycling of carbon, nitrogen and sulphur; Role of microbes in agriculture, public health, medicine and industry.

Unit IV

Industrially important microbial metabolites: Process technology for the production of primary metabolites e.g. baker's yeast, ethanol, acetone-butanol, citric acid, lactic acid, amino acids, polysaccharides, nucleosides and bioplastics; Production of secondary metabolites- penicillin, cephalosporins, streptomycin, vitamins etc.

Unit V

Industrially important bioprocesses: Applications of enzymes in pharmaceutical industry, therapeutics and clinical analysis; Production and use of glucose isomerase, amidase/aminopeptidase; amylase, cellulase, penicillin acylase, lipase, oxido-reductase; protease, hydantoinase, epoxide hydrolase; nitrilase, hydroxylase, aldolases; decarboxylase. etc. for the production of different types of drugs and drugs intermediates, future directions; Biomass production from agro-residues; Biofertilizers and biopesticides.

Texts/References

1. Pelczar MJ Jr., Chan ECS and Kreig NR., Microbiology, 5th Edition, Tata McGraw Hill, 1993.
2. Crueger and A Crueger, Biotechnology: A Textbook of Industrial Microbiology, Sinaeur Associates, 1990.
3. G Reed, Prescott and Dunn's, Industrial Microbiology, 4th Edition, CBS Publishers, 1987.
4. M.T. Madigan and J.M. Martinko, Biology of Microorganisms, 11th Edition, Pearson Prentice Hall, USA, 2006
5. Whitaker et al, Principles of Fermentation Technology, Indian Edition, Hall Books, 2007.
6. S. J. Pirt, Principles of microbe and cell cultivation, 3rd Edition, Wiley, 1975.

Genetic Engineering - 3 Credits

Unit I

Basics Concepts

DNA Structure and properties; Restriction Enzymes; DNA ligase, Klenow enzyme, T4 DNA polymerase, Polynucleotide kinase, Alkaline phosphatase; Cohesive and blunt end ligation; Linkers; Adaptors; Homopolymeric tailing; Labeling of DNA: Nick translation, Random priming, Radioactive and non-radioactive 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

Unit II

Cloning Vectors

Plasmids; Bacteriophages; M13 mp vectors; PUC19 and Bluescript vectors, Phagemids; Lambda vectors; Insertion and Replacement vectors; EMBL; Cosmids; Artificial chromosome vectors (YACs; BACs); Animal Virus derived vectors-SV-40; vaccinia/bacculo & retroviral vectors; Expression vectors; pMal; GST; pET-based vectors; Protein purification; His-tag; GST-tag; MBP-tag etc.; Intein-based vectors; Inclusion bodies; Methodologies to reduce formation of inclusion bodies; Baculovirus and pichia vectors system, Plant based vectors, Ti and Ri as vectors, Yeast vectors, Shuttle vectors

Unit III

Cloning Methodologies

Insertion of Foreign DNA into Host Cells; Transformation; Construction of libraries; Isolation of mRNA and total RNA; cDNA and genomic libraries; cDNA and genomic cloning; Expression cloning; Jumping and hopping libraries; Southwestern and Far-western cloning; Protein-protein interactive cloning and Yeast two hybrid system; Phage display; Principles in maximizing gene expression

Unit IV

PCR and Its Applications

Primer design; Fidelity of thermostable enzymes; DNA polymerases; Types of PCR – multiplex, nested, reverse transcriptase, real time PCR, touchdown PCR, hot start PCR, colony PCR, cloning of PCR products; T-vectors; Proof reading enzymes; PCR in gene recombination; Deletion; addition; Overlap extension; and SOEing; Site specific mutagenesis; PCR in molecular diagnostics; Viral and bacterial detection; PCR based mutagenesis, Mutation detection: SSCP, DGGE, RFLP, Oligo Ligation Assay (OLA), MCC (Mismatch Chemical Cleavage, ASA (Allele-Specific Amplification), PTT (Protein Truncation Test)

Unit V

Sequencing methods; Enzymatic DNA sequencing; Chemical sequencing of DNA; Automated DNA sequencing; RNA sequencing;

Chemical Synthesis of oligonucleotides; Introduction of DNA into mammalian cells; Transfection techniques; Gene silencing techniques; Introduction to siRNA; siRNA technology; Micro RNA; Construction of siRNA vectors; Principle and application of gene silencing; Gene knockouts and Gene Therapy; Creation of knock out mice; Disease model; Somatic and germ-line therapy- in vivo and ex-vivo; Suicide gene therapy; Gene replacement; Gene targeting; Transgenics; cDNA and intragenic arrays; Differential gene expression and protein array.

Text/References

1. S.B. Primrose, R.M. Twyman and R.W.Old; Principles of Gene Manipulation. 6th 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, 3rd ed. Garland Science 2006
4. Selected papers from scientific journals.
5. Technical Literature from Stratagene, Promega, Novagen, New England Biolab etc.

Drug Metabolism - 1.5 Credits

Unit I

Biotransformation of drugs; Enzymes responsible for biotransformations; Microsomal and nonmicrosomal mechanisms.

Unit II

Factors influencing enzyme induction and inhibition; Extraction of drugs; Biliary and fecal excretion; Factors effecting drug metabolism; Drug metabolism in fetus and new born

Unit III

Models to study drug metabolism; Dose effect relationships; Adverse drug reactions and drug interactions; Toxic reactions; Allergic reactions; Idiosyncrasy; Acute poisoning and its treatment.

Text/References

1. Goodman & Gilman, Laurence L Brunton, The Pharmacological Basis of Therapeutics, 11th Edition, McGraw Hill, New York, 2005.
2. Thomas F. Woolf, Handbook of Drug Metabolism, Marcel Dekker, New York, 1999.
3. Bertram G. Katzung, Basic and Clinical Pharmacology, 8th Edition, Lange Meical, New York, 2000.

Pharmacological Screening & Assays - 1.5 Credits

Unit I

General principles of screening; Correlations between various animal models and human situations; Animal ethics.

Unit II

Pharmacological screening models for therapeutic areas such as hypertension, cerebral ischaemia, pain, epilepsy, depression, Parkinson's disease, Alzheimer's disease, diabetic, leishmania etc.

Unit III

Correlation between in-vitro and in-vivo screens; Special emphasis on cell-based assay, biochemical assay, radioligand binding assay; High through put screening; High through put pharmacokinetic analysis; Specific use of reference drugs and interpretation of results.

Bioinformatics - 2 Credits

Unit I

Bioinformatics basics: Computers in biology and medicine; Importance of Unix and Linux systems and its basic commands; Database concepts; Protein and nucleic acid databases; Structural databases; Biological XML DTD's; Pattern matching algorithm basics; Computational tools for DNA sequence analysis: GCG: The Wisconsin package of sequence analysis programs; Web-based interfaces for the GCG sequence analysis programs.

Unit II

Databases and search tools: Biological back ground for sequence analysis; Identification of protein sequence from DNA sequence; Searching of databases similar sequence; The NCBI; Publicly available tools; Resources at EBI; Resources on the web; Database mining tools.

Unit III

DNA sequence analysis: The gene bank sequence database; Submitting DNA sequence to the databases and database searching; Sequence alignment; Pair wise alignment techniques; Multiple sequence analysis; Multiple sequence alignment; Flexible sequence similarity searching with the FASTA3 program package; Use of CLUSTAL W and CLUSTAL X for the multiple sequence alignment; Submitting DNA protein sequence to databases: Where and how to submit, SEQUIN, genome centres; Submitting aligned set of sequences, updates and internet resources.

Unit IV

Protein Modeling: Introduction; Force field methods; Energ, Buried and exposed residues; Side chains and neighbours; Fixed regions; Hydrogen bonds; Mapping properties onto surfaces; Fitting monomers; rms fit of conformers; Assigning secondary structures; Sequence alignment- methods, evaluation, scoring; Protein completion: backbone construction and side chain addition; Small peptide methodology; Software accessibility; Building peptides; Protein displays; Substructure manipulations, Annealing.

Peptidomimetics: Introduction, classification; Conformationally restricted peptides, design, pseudopeptides, peptidomimetics and transition state analogs; Biologically active template; Amino acid replacements; Peptidomimetics and rational drug design; CADD techniques in peptidomimetics; Development of non peptide peptidomimetics.

Unit V

Protein Structure Prediction: Protein folding and model generation; Secondary structure prediction; Analyzing secondary structures; Protein loop searching; Loop generating methods; Loop analysis; Homology modeling: potential applications, description, methodology, homologous sequence identification; Align structures, align model sequence; Construction of variable and conserved regions; Threading techniques; Topology fingerprint approach for prediction; Evaluation of alternate models; Structure prediction on a mystery sequence; Structure aided sequence techniques of structure prediction; Structural profiles, alignment algorithms, mutation tables, prediction, validation, sequence based methods of structure prediction, prediction using inverse folding, fold prediction; Significance analysis, scoring techniques, sequence-sequence scoring. *The virtual library:* Searching MEDLINE, Pubmed, current content, science citation index and current awareness services, electronic journals, grants, and funding information.

Texts/References

1. David W. Mount, Bioinformatics: Sequence and Genome Analysis 2nd Edition, CSHL Press, 2004.
2. A. Baxevanis and F. B. F. Ouellette, Bioinformatics: a practical guide to the analysis of genes and proteins, 2nd Edition, John Wiley, 2001.
3. Jonathan Pevsner, Bioinformatics and Functional Genomics, 1st Edition, Wiley-Liss, 2003.
4. P. E. Bourne and H. Weissig, Structural Bioinformatics, 2nd Edition, Wiley, 2008.
5. C. Branden and J. Tooze, Introduction to Protein Structure, 2nd Revised Edition Garland Publishing, 1998.

Downstream Processing of Biological Products - 1 Credit

Unit I

Characteristics of biological materials: pretreatment methods; Separation of cell mass: centrifugation, clarification and filtration; Different equipment use related to various unit operations; Solving of different types of numericals associated with the problems.

Unit II

Different methods of cell disruption; Advantages; Disadvantages; Solid shear method and liquid shear method; solving of different types of numericals associated with the problems.

Unit III

Different concentration methods: evaporation, distillation, crystallization, pervaporation, SCFE, solvent extraction, phase separation, drying etc., whole broth extraction, protein precipitaiton; extraction; adsorption; solving of different types of numericals associated with the problems.

Unit IV

Modern techniques: Electrophoresis; Chromatographic methods; Ultrafiltration; Reverse osmosis; Cross flow filtration; Microfiltration; Isoelectric focusing; Affinity based separations; Case studies; solving of different types of numericals associated with the problems.

Unit V

Treatment of Effluent and its disposal: anaerobic and aerobic process for waste water treatment; Definition of BOD, COD, stabilization etc.; Determination of BOD and COD in effluent; Characterization of the waste water; Kinetic analysis of waste water treatment; Solving of different types of numericals associated with the problems.

Texts/References

1. S N Mukhopadhay, Process biotechnology fundamentals, Viva Books, 2001.
2. Doran, Bioprocess Engineering, Academic Press, 2005.
3. Biotol series, Product Recovery in Bioprocess Technology, 1st Edition, Butterworth Heinemann Ltd., 1992.

Lab on Immunology - 3 Credits

1. Selection of animals, Preparation of antigens, Immunization and methods of bleeding, Serum separation, Storage.
2. Antibody titre by ELISA method.
3. Double diffusion, Immuno-electrophoresis and Radial Immuno diffusion.
4. Complement fixation test.
5. Isolation and purification of IgG from serum or IgY from chicken egg.
6. SDS-PAGE, Immunoblotting, Dot blot assays
7. Blood smear identification of leucocytes by Giemsa stain
8. Separation of leucocytes by dextran method
9. Demonstration of Phagocytosis of latex beads
10. Separation of mononuclear cells by Ficoll-Hypaque
11. Flowcytometry, identification of T cells and their subsets
12. Lymphoproliferation by mitogen / antigen induced
13. Lymphnode Immunohistochemistry (direct and indirect peroxidase assay)
14. Hybridoma technology and monoclonal antibody production.
15. Immunodiagnostics using commercial kits

Lab on Microbiology - 3 Credits

1. Sterilization, disinfection, safety in microbiological laboratory.
2. Preparation of media for growth of various microorganisms.
3. Identification and culturing of various microorganisms.
4. Staining and enumeration of microorganisms.
5. Growth curve, measure of bacterial population by turbidometry and studying the effect of temperature, pH, carbon and nitrogen.
6. Assay of antibiotics production and demonstration of antibiotic resistance.
7. Isolation and screening of industrially important microorganisms.
8. Determination of thermal death point and thermal death time of microorganisms.

Lab on Genetic Engineering - 2 Credits

1. Isolation of genomic DNA from *Bacillus subtilis** genome.
2. PCR amplification of *scoC* gene and analysis by agarose gel electrophoresis
3. Preparation of plasmid, pET-28a from *E.coli* DH5α and gel analysis.
4. Restriction digestion of vector (gel analysis) and insert with NcoI and XhoI
5. a. Vector and Insert ligation

   b. Transformation in *E.coli* DH5α.
6. Plasmid isolation and confirming recombinant by PCR and RE digestion.
7. Transformation of recombinant plasmid in *E.coli* BL21 (DE3) strain
8. Induction of ScoC protein with IPTG and analysis on SDS-PAGE
9. Purification of protein on Ni-NTA column and analysis of purification by SDS-PAGE
10. a. Random Primer labeling of scoC with Dig-11-dUTP

    b. Southern hybridization of *B. subtilis* genome with probe and non-radioactive detection.

*Any other bacterial strain can be used.

Animal and Plant Cell Technology - 2 Credits

Unit I

Animal cell metabolism: Regulation and nutritional requirement; Animal cell growth characteristics and kinetics.

Unit II

Transport of nutrients: Substrate and product transport through mammalian cell; Growth and mass transfer: Micro-carrier attached growth; Cell culture in continuous, perfusion and hollow-fiber reactor; Mass transfer in mammalian cell culture.

Unit III

Scale up: Scale up of cell culture processes; Case studies; Special features and organization of plant cells; Totipotency; Regeneration of plants; Examples of regeneration from leaves, roots, stem etc.

Unit IV

Plant products: Plant products of industrial importance; Biochemistry of major metabolic pathways and products; Cell suspension culture development.

Unit V

Kinetics and scale up: Characterization; Kinetics of growth; Product formation and examples; Large scale production of secondary metabolites from suspension cultures-nutrient optimization; Cell growth regulators; Somaclonal variation; Plant cell reactors; Types of reactors; Comparison of reactor performance; Immobilized plant cell reactors; Novel design concepts; Genetic engineering: Genetic engineering of plant cells.

Texts/References

1. Biotol series, In vitro Cultivation of Plant cell, Butterworth Heinemann Ltd., 1994
2. Biotol series, In vitro Cultivation of Animal cell, Butterworth Heinemann Ltd. 1994.
3. M. M. Ranga, Animal Biotechnology, 3rd Revised Edition, Agrobios, 2007.
4. By Bhojwani & Rajdhan, Animal and Plant Biotechnology.

IPR & Biosafety - 3 Credits

Unit I

Introduction to Intellectual Property

Types of IP: Patents, Trademarks, Copyright & Related Rights, Industrial Design, Traditional Knowledge, Geographical Indications, Protection of New GMOs; International framework for the protection of IP

IP as a factor in R&D; IPs of relevance to Biotechnology and few Case Studies; Introduction to History of GATT, WTO, WIPO and TRIPS

Unit II

Concept of 'prior art'

Invention in context of "prior art"; Patent databases; Searching International Databases; Country-wise patent searches (USPTO, EPO, India etc.); Analysis and report formation

Unit III

Basics of Patents

Types of patents; Indian Patent Act 1970; Recent Amendments; Filing of a patent application; Precautions before patenting-disclosure/non-disclosure; WIPO Treaties; Budapest Treaty; PCT and Implications; Role of a Country Patent Office; Procedure for filing a PCT application

Unit IV

Patent filing and Infringement

Patent application- forms and guidelines, fee structure, time frames; Types of patent applications: provisional and complete specifications; PCT and convention patent applications; International patenting-requirement, procedures and costs; Financial assistance for patenting-introduction to existing schemes; Publication of patents-gazette of India, status in Europe and US

Patenting by research students, lecturers and scientists-University/organizational rules in India and abroad, credit sharing by workers, financial incentives

Patent infringement- meaning, scope, litigation, case studies and examples

Unit V

Biosafety

Introduction; Historical Backround; Introduction to Biological Safety Cabinets; Primary Containment for Biohazards; Biosafety Levels; Biosafety Levels of Specific Microorganisms; Recommended Biosafety Levels for Infectious Agents and Infected Animals; Biosafety guidelines - Government of India; Definition of GMOs & LMOs; Roles of Institutional Biosafety Committee, RCGM, GEAC etc. for GMO applications in food and agriculture; Environmental release of GMOs; Risk Analysis; Risk Assessment; Risk management and communication; Overview of National Regulations and relevant International Agreements including Cartagena Protocol.

Important Links

http://www.w3.org/IPR/

http://www.wipo.int/portal/index.html.en

http://www.ipr.co.uk/IP_conventions/patent_cooperation_treaty.html

www.patentoffice.nic.in

www.iprlawindia.org/ - 31k - Cached - Similar page

http://www.cbd.int/biosafety/background.shtml

http://www.cdc.gov/OD/ohs/symp5/jyrtext.htm

http://web.princeton.edu/sites/ehs/biosafety/biosafetypage/section3.html

Seminar

It will be based on the research project work to be undertaken by the student.

Lab on Pharmacology - 3 Credits

1. General animal handling: mice, guinea pig, etc.
2. Various routes of administration of drugs: intraveinous, intramuscular, intraperitoneal, intradermal, etc.
3. Dose response relationship of drugs
4. Acute toxicity testing of drugs
5. Determination of analgesic activity of a compound
6. Estimation of protein and haematological parameters.

Lab on Bioinformatics - 3 Credits

Background information on computers in general, all forms of remote computing, Text editing, Basics of the UNIX operating system, and the X environment, as well as requesting your new FSU HPC account; Molecular databases and how they are organized and accessed , Internet sequence and structural databases as well a brief introduction to the Wisconsin Package (aka Genetics Computer Group or GCG) and its graphical user interface (GUI) SeqLab and the on-site GCG sequence databases; Unknown DNA — rational probe design and analysis; DNA fragment contig assembly (GCG's SeqMerge) and restriction enzyme mapping; Database similarity searching and the dynamic programming algorithm; Gene finding strategies. How are coding sequences recognized in genomic DNA (pdf); Multiple sequence alignment, expectation maximization, profiles, and Markov models; Molecular evolutionary phylogenetic inference; Estimating protein secondary structure and physical attributes; Molecular modelling and visualization.