

Semester I

PAPER - PGD MB 101 : BIOPHYSICAL TECHNIQUES-I

Quantification of Proteins:

Principles of Spectrophotometry: ultraviolet- visible absorption spectrophotometry, visible recording of spectra for proteins and nucleic acids and calculation of concentration of protein and nucleic acids from spectrum. Fluorescence spectroscopy, mass spectrometry (6 periods)

Separation of Proteins

Gel Filtration chromatography: Separation based on size, principle, types of gel filtration beads, preparation of slurry, packing of column, determination of void volume, separation of proteins by filtration, determination of molecular weight, storage of columns. (6 periods)

Ion Exchange chromatography: Separation based on charge, types of ion exchangers and general properties, selection of ion exchanger, selection of buffer, operating methods, batch operation and column operation packing and development of column, various gradients for elution, effect of flow rate, volume of gradient and fraction size on separation, high pressure liquid chromatography, fast protein liquid chromatography (8 periods)

Affinity Chromatography: Separation based on affinity, principle, activation of matrix, ligands, methods used for elution, metal chelate chromatography, hydrophobic and covalent chromatography (6 periods)

Thin Layer chromatography: Principles of thin layer chromatography, systems for separation of various molecules, activation of Silica plates, elution of material from silica gel. (2 periods)

Gas liquid chromatography: Principle, instrumentation, detectors. (2 periods)

Purification of proteins: using salts, organic solvents, organic polymers. Dialysis and membrane filtration. (2 periods)

Enzymes: Basic features of enzymes, catalysis, estimation of V_{max} and K_m using Lineweaver – Burke plot, enzyme inhibition, specific activity. (6 periods)

Tissue Culture: concept of totipotency, callus, plant tissue culture laboratory set up, tissue culture media, phytohormones, cybrids, cell, tissue and organ culture, somatic embryogenesis, organogenesis, applications (somatic hybridization, embryo rescue, virus-free plants, somaclonal variations etc). (6 periods)

Animal tissue culture: primary culture, cell lines, continuous cell lines (transformation, anchorage independence, contact inhibition etc) applications. (6 periods)

Suggested Reading:

1. Biochemistry and Molecular Biology, Keith Wilson & John Walker (6th Edition, 2008) Cambridge University Press
2. Biochemistry Laboratory: Modern Theory and Techniques Rodney Boyer (International Edition, 2009) Benjamin Cummings
3. Physical Biochemistry Freifelder (2nd edition, 1982) W.H.Freeman and Co
4. Principles of Biochemistry (Lehninger) Nelson and Cox, (5th edition, 2008) W.H.Freeman and Co.
5. Modern Industrial Microbiology and Biotechnology, Nduka Okafor (Science Publishers, 2007)
6. Bioinformatics Attwood
7. Bioinformatics David Mount
8. Plant Tissue Culture Theory and Practice, Bhojwani and Razdan ,2008,Elsevier
9. Culture of Animal Cells, Freshney (4th edition, 2000) Wiley-Liss Inc.

PAPER - PGD MB 102 : RECOMBINANT DNA TECHNOLOGY- I

Concept of gene manipulation :

Restriction enzymes: various types, their properties, nomenclature, creating new restriction sites by DNA manipulation.

DNA methylation systems in E.coli (dam, dcm, M EcoKI). (8 periods)

Various DNA modifying enzymes used in cloning (DNA polymerases :DNA Polymerase I, Klenow fragment, T4DNA Polymerase, T7 DNA Polymerase), RNA Polymerases(T3, T7, SP6), Reverse Transcriptase (AMV, MoMLV), Ligases (T4 DNA ligase, E.coli DNA ligase), Taq polymerase etc (5 periods)

Cloning vectors : Biology of plasmids (conjugative, nonconjugative, relaxed and stringent control of copy number , incompatibility) Plasmid based vectors(one step and two-step selection); Biology of Lambda phage (lytic versus lysogenic cycle), λ bacteriophage based vectors (insertional and replacement),in vitro packaging; Biology of M13 bacteriophage, M13 phage based vectors, phagemids

High capacity vectors: cosmids, P1 phage based vectors, PACs, yeast artificial chromosomes, bacterial artificial chromosomes. Advantages of each vector. (12 periods)

Covalent linkage of DNA fragments to vector molecules: linkers, adapters, conversion adaptors, homopolymer tailing (recovery of DNA insert after homopolymer tailing). (2 periods)

Generation of genomic and cDNA libraries: (mRNA source, integrity, enrichment techniques, different methods of first strand and second strand of cDNA synthesis)

Limitations of cDNA synthesis (5' end RACE, 3' end RACE) (6 periods)

Solid phase synthesis of DNA: (phosphoramidite based). (2 periods)

Selection and screening of recombinant clones: Radiolabelled probe preparation via nick translation, random priming, 3' end labeling, 5' end labeling, Guessmers and degenerate probes, Non radioactive probes preparation using Biotin, Digoxigenin. (6 periods)

Sequence dependent and independent screening: PCR based, colony and plaque hybridization, functional screening, immunological screening, gain of function screening. HRT, HART (4 periods)

Suggested Reading:

1. Principles of Gene Manipulation and Genomics
S.B. Primrose & R.M. Twyman (7th Edition,2006) Blackwell Publishing
2. Molecular Cloning (A Laboratory Manual)
Sambrook and Russell (3rd Edition,2001) CSHL Press

PAPER - PGD MB 103: IMMUNOLOGY -I

Overview of the immune system : historical background, innate immunity, toll like receptors (8 periods)

Organization of the immune system: primary & secondary lymphoid organs, myeloid cells, lymphoid cells, dendritic cells and natural killer cells (4 periods)

Antigens : immunogenicity and antigenicity, factors that influence immunogenicity, haptens, carrier, epitopes, cross reactivity (4 periods)

Antibodies : structure of immunoglobulins, immunoglobulin subtype, B cell receptor, isotype, allotype, diotype, Monoclonal antibodies : preparation of lymphocytes, myeloma cells, fusion protocol, selection, cloning and culturing of monoclonal antibody secreting hybridoma cell line, engineering of antibodies (6 periods)

Antigen antibody interactions : affinity, avidity, cross reactivity, precipitation reactions, agglutination reactions, immunofluorescence, fluorescence activated cell sorter, complement tests, ELISA, RIA (8 periods)

The major histocompatibility complex : structure and cellular distribution of MHC molecules, peptide binding by MHC, MHC and immune responsiveness (4 periods)

Antigen processing and presentation : Cytosolic and Endocytic pathway (2 periods)

The response of B cells to antigen : B cell maturation, activation and proliferation, signaling pathways leading to B cell activation, germinal centers and formation of plasma cells, memory cells, class switching (6 periods)

Generation of antibody diversity : multi gene organization of immunoglobulin genes,
mechanism of gene rearrangement (6 periods)

Suggested Reading

1. Immunology by Janis Kuby (Freeman and Company),6th edition,2007
2. Immunobiology by Janeway , Travers, Walport, Sclomchik 9 Garland publishing) 6th edition, 2005

PRACTICAL PAPER – PGD MBL 104 : BIOPHYSICAL TECHNIQUES-I

1. Spectrophotometric analysis of nucleic acids.
Protein estimation at λ_{280} .
Effect of solvent perturbation on absorption by a chromophore
2. Determination of void volume and partition coefficient by Gel filtration
3. Purification of proteins on ion exchange chromatography
4. Purification of proteins on affinity chromatography
5. Thin layer chromatography
6. Ammonium sulphate fractionation and dialysis
7. Assay of enzyme activity (standardization of assay conditions)
Determination of optimum pH, K_M and V_{max} .
8. Agarose gel electrophoresis:
Determination of molecular weight of unknown DNA sample

Suggested Reading:

1. The Tools of Biochemistry Terrance G. Cooper(Wiley Interscience, 2011 reprint)
2. Purifying Proteins for Proteomics Richard J. Simpson , 2004(CSHL Press)
3. Molecular Cloning (A Laboratory Manual)
Sambrook and Russell (3rd Edition,2001) CSHL Press

PRACTICAL PAPER - PGD MBL 105: RECOMBINANT DNA TECHNOLOGY- I

1. Preparation and sterilization of LB medium.
2. Obtaining isolated colonies of E.coli by streak plate and spread plate method.
3. To study the growth curve of *E.coli* DH5 α
4. Isolation of chromosomal DNA of *E.coli*
5. Isolation of plasmid DNA by the alkaline lysis method (maxi-preparation and mini-preparation) and the boiling lysis method.
6. Digestion of plasmid DNA with restriction enzymes
7. Recovery of DNA from low-melting temperature agarose gel: organic extraction etc.

Suggested Reading:

1. Molecular Cloning (A Laboratory Manual)
Sambrook and Russell (3rd Edition, 2001) CSHL Press
2. Gene Cloning and DNA Analysis T.A.Brown, (6th Edition, 2010) Blackwell Publishing
3. Prescott, Harley and Klein's Microbiology Wiley, Sherwood, Woolverton
(7th edition, 2008) McGraw Hill

PRACTICAL PAPER - PGD MBL 106 : IMMUNOLOGY -I

1. Quantitative precipitation test
2. Immuno diffusion : Single radial immunodiffusion, double immunodiffusion
3. Immuno electrophoresis
- 4. Electroimmunoprecipitation: Counter immunoelectrophoresis, Rocket immunoelectrophoresis, Crossed immunoelectrophoresis**
5. Staining of precipitin bands in gel
6. Identification of human blood groups and Rh factor
7. Passive agglutination using inert particles like SRBC, latex particles

8. Inhibition of agglutination using latex particles
9. Preparation of lymphocytes from spleen and blood
10. Immunization of rabbit to raise polyclonal antiserum

Suggested Reading

1. Practical Immunology by Hudson & Hay (Blackwell Publishing) 4th edition 2002
2. Handbook of Immunoprecipitation by Nils H. Axelsen (Blackwell Publishing) 1984

IInd Semester

THEORY PAPER - PGD MB 201 : BIOPHYSICAL TECHNIQUES -II

Separation of macromolecules by electrophoresis:

Theory of polyacrylamide gel electrophoresis: native and SDS PAGE, reducing and non reducing gels, detection of protein bands in gels- Coomassie blue staining, silver staining, fluorescence staining, molecular weight determination by SDS PAGE recovery of proteins from the gel, affinity staining, isoelectric focusing of proteins, Two dimensional gel electrophoresis, gradient gel electrophoresis, Differential gel electrophoresis(DIGE).

Theory of agarose gel electrophoresis, Pulsed Field Gel Electrophoresis. (8 periods)

Blotting Techniques: Southern blot and factors affecting DNA transfer, Northern blot, Western blot; colony and plaque lift, dot blot. (5 periods)

Centrifugation : Principle, instrumentation and applications (5 periods)

Radio active materials: Types, precautions for handling, methods of measurements and applications. Autoradiography. (6 periods)

Fundamentals of fermentation technology: Batch, fed batch and continuous cultures, stirred tank reactors and airlift fermentors, downstream processing. (6 periods)

Additional methods to identify associated proteins: Analysis of protein–protein interactions: Yeast two-hybrid systems, analyzing protein interaction s by fluorescence resonance energy transfer (FRET), protein fragment complementation(PCA), Mass Spectroscopy (MS), library based methods (surface display) Protein microarrays. (5 periods)

Bioinformatics and computational biology: An overview

Biological databases and Archives: sequence databases, structure databases, microbial databases, and eukaryotic databases. (4 periods)

Genomics: Genome and genes, gene organization, prokaryotic and eukaryotic protein structure,

control switches, ORF, promoters, ESTs, genome analyses, gene prediction, statistical models, mathematical models, sequence alignment, comparative genomics, genomics in preservation of endangered species, SNPs. (4 periods)

Proteomics: atomic view of proteins, the hierarchical nature of protein architecture, protein folding, protein structure prediction, homology models, threading/fold recognition, Ab-initio models, protein-protein interactions, proteins as drug targets, phylogenetic analyses (4 periods)

Suggested Reading:

1. Biochemistry and Molecular Biology, Keith Wilson & John Walker (6th Edition, 2008) Cambridge University Press
2. Biochemistry Laboratory: Modern Theory and Techniques Rodney Boyer (International Edition, 2009) Benjamin Cummings
3. Physical Biochemistry Freifelder (2nd edition, 1982) W.H. Freeman and Co
4. Principles of Biochemistry (Lehninger) Nelson and Cox, (5th edition, 2008) W.H. Freeman and Co.
5. Modern Industrial Microbiology and Biotechnology, Nduka Okafor (Science Publishers, 2007)
6. Introduction to Bioinformatics, Attwood, Parry- Smith, Phukan, 2007, Pearson Education
7. Bioinformatics, CSHL Press 2001, David Mount
8. Plant Tissue Culture Theory and Practice, Bhojwani and Razdan ,2008, Elsevier
9. Culture of Animal Cells, Freshney (4th edition, 2000) Wiley-Liss Inc.

THEORY PAPER - PGD MB 202: RECOMBINANT DNA TECHNOLOGY II

Heterologous protein expression of cloned DNA in E.coli: Expression vectors (lac promoter, tryptophan promoter, Lambda cI promoter, arabinose promoter based) optimization of protein expression (using upstream and downstream signals) Fusion proteins, cell-free translation systems. RNAi vectors. (4 periods)

DNA transformation in yeast: methods of gene transfer to yeast (YIp, YEp, YCp, YRp, shuttle vectors), optimization of protein expression. (4 periods)

Gene transfer to plants: Biolistics, protoplast mediated, electroporation, Agrobacterium mediated transfer (Ti plasmid, disarmed vectors, cointegrate vectors, binary vectors), virus-mediated transfer (CaMV), in planta transformation, signals for optimization of protein synthesis. (4 periods)

Gene transfer to animal cells: chemical transfection, lipofection, electroporation, gene-gun, microinjection, transient and stable transformation, optimization of protein synthesis, use of reporter genes. (4 periods)

Characterization of cloned DNA : Restriction mapping, DNA sequencing (dideoxy chain termination, chemical degradation, pyrosequencing, shotgun sequencing and contig assembly). (5 periods)

Polymerase Chain Reaction and its applications: components of the PCR, importance of primer designing, various thermostable enzymes vs Taq polymerase. RAPD etc (5 periods)

DNA markers: VNTRs and DNA fingerprinting, SNPs, RFLPs. (4 periods)

Modification of cloned DNA : Site directed mutagenesis(cassette mutagenesis, primer extension method, overlap extension method, megaprimer method), selection against parental phenotype. Protein engineering (4 periods)

Applications of recombinant DNA technology : Transgenic animals, (4 periods)
Transgenic plants, Gene therapy, Pharmaceutical products.

Genomics : organization of genomes, organization of nuclear DNA, mapping and sequencing genomes. (5 periods)

Analysis of the transcriptome: RNA expression level profiling with microarrays, MPSS, SAGE, ESTs, loss of function - Knock out ,knock down, antisense RNA and RNAi, (5 periods)

Safety of recombinant DNA technology and ethical issues (Patenting): Restriction and regulation for the release of Bt crops etc. (4 periods)

Suggested Reading:

1. Principles of Gene Manipulation and Genomics
S.B. Primrose & R.M. Twyman (7th Edition,2006) Blackwell Publishing
2. Molecular Cloning (A Laboratory Manual) Sambrook and Russell (3rd Edition, 2001)
CSHL Press

THEORY PAPER - PGD MB 203 : IMMUNOLOGY- II

The response of T cells to antigens : T cell receptor, T cell accessory membrane molecules, thymic selection of T cell repertoire, organization and rearrangement of TCR genes, cell mediated immune response : generation of cytotoxic cells, CTL mediated cytotoxicity, response of NK cells (6 periods)

Cytokines : properties, function of IL -1 to IL-5, IL-10, IL-12, IFNs, TNFs, cytokine receptors and signal transduction mediated by them, cytokine related diseases (4 periods)

The complement system : classical & alternate pathway, Lectin pathway, regulation of the pathway, biological consequences of complement activation (6 periods)

Hypersensitivity reactions : type I, II,III and IV (6 periods)

Vaccines : active and passive immunization, attenuated & inactivated vaccines, new approaches to vaccine development (4 periods)

Autoimmunity : organ specific and systemic autoimmune diseases (4 periods)

Transplantation immunology : types of grafts, tissue typing, immunological basis of graft rejection, immunosuppressive therapy (4 periods)

Immune response to infectious diseases: immune response to bacterial, viral, protozoan and helminth infections, genomics and the challenge of infectious diseases (10 periods)

Cancer and the immune system : oncogenes, tumor antigens and induction of immune response, immunotherapy for tumors (3 periods)

Regulation of the immune response : antigen & antibody mediated regulation, Jerne's theory (4 periods)

Suggested Reading

1. Immunology by Janis Kuby (Freeman and Company) 7th edition,2006
2. Immunobiology by Janeway , Travers, Walport, Sclomchik (Garland publishing) 6th edition, 2005

PRACTICAL PAPER – PGD MBL 204 : BIOPHYSICAL TECHNIQUES-II

1. Polyacrylamide gel electrophoresis
2. SDS gel electrophoresis of proteins (reducing and nonreducing) and determination of molecular weight of protein samples.
3. Isoelectric focussing of proteins and two dimensional gel electrophoresis
4. Southern blotting
5. Western blotting
6. Immunoblotting
7. Bioinformatics Exercises:
Databases: Protein data bank, Nucleic acid database, Genbank,
Sequence alignment using BLASTn, BLASTp, CLUSTALW.
Gene finding tools- GenScan, GLIMMER
Introduction to proteomics Protparam, GOR, nnPredict, SWISSMODEL
Visualization Softwares - Rasmol, JMOL

Suggested Reading:

1. The tools of Biochemistry by Terrance G. Cooper(Wiley Interscience)
2. Purifying Proteins for Proteomics by Richard J. Simpson (CSHL Press)
3. Introduction to Bioinformatics, Attwood, Parry- Smith, Phukan, 2007, Pearson Education
4. Bioinformatics, CSHL Press 2001, David Mount

PRACTICAL PAPER – PGD MBL 205 : RECOMBINANT DNA TECHNOLOGY-II

1. Preparation of competent cells of *E.coli*
2. Transformation of competent E.coli cells with plasmid DNA.
3. To study the effect of alkaline phosphatase on plasmid recircularization
4. To amplify a gene using PCR.
5. Calculation of the phage titre with a phage titration kit.

Suggested Reading:

1. Molecular Cloning (A Laboratory Manual)
Sambrook and Russell (3rd Edition,2001) CSHL Press
2. Gene Cloning and DNA Analysis T.A.Brown, (6th Edition,2010) Blackwell Publishing

PRACTICAL PAPER - PGD MBL 206: IMMUNOLOGY -II

1. Quantitative estimation of haemolytic complement activity in serum
2. Complement fixation test
3. Purification of antibodies from serum using salt fractionation and gel filtration
4. Purification of IgG by ion exchange chromatography

5. Preparation of IgG fraction using Protein A Sepharose column
6. Digestion of antibodies with pepsin and preparation of F(ab)₂ fragment using Sephadex G-100 chromatography
7. Linking of enzyme to antibodies using one step glutaraldehyde method
8. Dot ELISA
9. Determination of antibody titre by indirect ELISA
10. Measurement of antigens by Direct and Competitive ELISA

Suggested Reading:

1. Practical Immunology by Hudson & Hay (Blackwell Publishing) 4th edition 2002
2. Handbook of Immunoprecipitation by Nils H. Axelsen (Blackwell Publishing) 1984