

SYLLABUS for M. Sc. BIOTECHNOLOGY
Choice Based Credit System (As per NEP 2020)
Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur
Effective from 2024-2025

M. Sc. Biotechnology Semester III
Paper 8 (Code: MBT3T08)

Immunology

Course Outcomes:

CO1: Understand basic concepts of Immunology.

CO2: Appreciate and assess the various immunological techniques used for public health.

CO3: Apply their knowledge of immunological experiments to demonstrate innate, humoral or cytotoxic T lymphocyte responses

UNIT I: Immune system

Innate and adaptive immune mechanisms: **Innate Immune Mechanisms:** Components of innate immunity-Physical & Chemical Barriers-Cellular Defences and cells involved. Phagocytosis, inflammation and soluble mediators.

Adaptive Immune Mechanisms: Humoral and Cellular Immunity. Overview of antibody mediated immune response and Cell mediated immune response

Organs of the immune system: Primary and Secondary lymphoid organs; Lymphatic system; Mucosal and Cutaneous associated Lymphoid tissue (MALT&CALT);

Cells of immune system: Haematopoiesis, Properties and Functions of Lymphocytes: B cells and T cells, NK cells, Eosinophils, Basophils, Neutrophils, Mast cells, Macrophages, Dendritic cells. Membrane bound receptors of lymphoid cells, lymphocyte trafficking

UNIT II: Antigens and Immunoglobulins

Antigens: Immunogens, Concept of haptens, determinants, conditions of antigenicity, antigenicity and immunogenicity, super antigen.

Immunoglobulin: Deducing antibody Structure. Antigenic determinants on Immunoglobulin –Isotopes, Allotypes and Idiotype

Structure and properties of immunoglobulin classes. Theories of antibody formation, hybridoma technology for monoclonal antibodies and designer monoclonal antibodies.

Multiple myelomas and structural basis of antibody diversity. Freund's adjuvants and its significance.

Unit III Expression and Regulation of Immune Response:

Immune responses generated by B and T lymphocytes; T lymphocyte and B lymphocyte development and maturation, antigen processing and presentation, generation of humoral and cell mediated immune response, activation of B and T lymphocytes, cytokines and their role in immune regulation, T cell regulation, MHC restriction, immunological tolerance.

Cell mediated cytotoxicity: Mechanism of Cytotoxicity of T cell and NK mediated lysis, antibody dependent cell mediated cytotoxicity, and macrophage mediated cytotoxicity.

Complement system: Classical, alternate, lectin pathway of complement activation.

Regulation of complement activation.

Transplantation immunology: MHC, HLA typing, types of grafts, grafts rejection, GVH reactions. Mechanism of graft rejection, and prevention of graft rejection.

Unit IV Clinical immunology and Immunoassays:

Immuno-assays: RID, ELISA, ELISA-PCR, RIA, Western Blotting, immunofluorescence and

related application.

Immune deficiencies and autoimmunity, Hypersensitivity, tumour immunology.

Vaccines: Live, killed, attenuated, sub unit vaccines, recombinant DNA and protein-based vaccines, plant-based vaccines, reverse vaccinology; Peptide vaccines, conjugate vaccines.

References:

1. Kindt, T. J., Goldsby, R. A., Osborne, B. A., & Kuby, J. (2006). Kuby Immunology. New York: W.H. Freeman.
2. Brostoff, J., Seaddin, J. K., Male, D., & Roitt, I. M. (2002). Clinical Immunology. London: Gower Medical Pub.
3. Murphy, K., Travers, P., Walport, M., & Janeway, C. (2012). Janeway's Immunobiology. New York: Garland Science.
4. Paul, W. E. (2012). Fundamental Immunology. New York: Raven Press.
5. Goding, J. W. (1996). Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry, and Immunology. London: Academic Press.
6. Parham, P. (2005). The Immune System. New York: Garland Science

M. Sc. Biotechnology Semester III
Paper 9 (Code: MBT3T09)
Biophysical Techniques

Course Outcomes:

CO1: Remember and comprehend techniques and instrumentation involved in studying basic biological phenomenon focusing on Spectrophotometry, Chromatography, Electrophoresis, Centrifugation and radioactivity.

CO2: Evaluate the application of each technique in providing solution to biotechnological problems

UNIT I: Spectrophotometry

Principle, Instrumentation & applications: UV-Visible spectrophotometry, fluorescence spectrophotometry, absorption and emission spectrophotometry, Infrared (IR) Spectroscopy, Nuclear Magnetic Resonance (NMR) Spectroscopy, and their applications.

Basic introduction to Raman and Mass spectrophotometry.

UNIT II: Chromatography

Principles, techniques and applications: Partition chromatography, adsorption chromatography, Gel filtration chromatography (GPC/SEC), Affinity chromatography and Ion exchange chromatography, Gas chromatography. Applications of all types of chromatography. Concept of GLC, HPLC, and HPTLC. Integrating chromatography with Mass Spectrometry: GCMS, LCMS.

UNIT III: Electrophoresis

Principle, procedure, & applications: Gel electrophoresis (Agarose, SDS-PAGE, Native- PAGE), Disc gel electrophoresis, Temperature Gradient Gel Electrophoresis (TGGE) and Denaturing Gradient Gel Electrophoresis (DGGE), Pulsed field gel electrophoresis, Capillary electrophoresis.

UNIT IV: Centrifugation & Radioactivity

Centrifugation: Basic principles, Mathematics & theory (RCF, Sedimentation coefficient etc). Types of centrifuge: microcentrifuge, high speed & ultracentrifuges with specific applications. Differential & density gradient centrifugation, Isolation of cell components using centrifugation technique.

Radioactivity: Radioactivity, Radioactive & stable isotopes, Pattern and rate of radioactive decay, Units of radioactivity. Applications of isotopes in Biotechnology: Principles of tracer techniques, Its advantages and limitations, Distribution studies, Isotope dilution technique, Metabolic studies, Clinical applications. Radioimmunoassay.

References:

1. Lakowicz, J. R. (2006) Principles of Fluorescence Spectroscopy. 3rd edition. Springer.
2. Cantor, C. R. and Schimmel, P. R. (1980) Biophysical Chemistry. Part II. 1st edition. W. H. Freeman & Co.

3. Banwell, C N. and McCash, E. M. (1994) Fundamentals of Molecular Spectroscopy. 4th Edition, McGrawHill.
4. Hollas, J. M. (2004) Modern Spectroscopy. 4th Edition. Wiley.
5. Campbell, I. D. & Dwek R. A. (1984) Biological Spectroscopy. Addison-Wesley.
6. Tinoco. I. et al. (2014) Physical Chemistry: Principles and Applications in Biological Sciences. Pearson Education.
7. Methods in Molecular Biophysics, Igor N S, N Zaccai & J Zaccai, (2007) Cambridge
8. Principle of Biochemistry, D Voet, J Voet and CW Pratt, 3rd Ed,
9. Essential Biophysics, Narayanan, New Age Publ 6. Handbook of Molecular Biophysics (Methods & Application), 2009, HG Bohr, Wiley
10. Cantor, C. R. and Schimmel, P. R. (1980) Biophysical Chemistry. Part III. 1st edition. W. H. Freeman & Co.
11. Jackson, M. B. (2006) Molecular and Cellular Biophysics. Cambridge University Press.

M. Sc. Biotechnology Semester III
Paper 10: (Code: MBT3T10)
Fundamentals of Genetic Engineering

Course Outcomes:

CO1: Remember and understand fundamental concepts of Genetic Engineering

CO2: Illustrate and compare different techniques involved in Genetic Engineering

CO3: To develop an approach towards applications of molecular biology techniques in developing recombinant molecules.

CO4: To understand the concept of DNA sequencing

UNIT I: Introduction and tools for genetic engineering: Impact of genetic engineering on modern society; general requirements for performing a genetic engineering experiment; restriction endonucleases and methylases; DNA ligase, Klenow enzyme, T4 DNA polymerase, polynucleotide kinase, alkaline phosphatase; cohesive and blunt end ligation; linkers; adaptors; homopolymeric tailing.

Vectors: General characteristics of vectors, Plasmids, pBR 322, pBR325, pUC 18 and 19 vectors, Bacteriophages- lambda & M13 mp vectors; Bluescript vectors, Phagemids; Insertion and Replacement vectors; Cosmids; Artificial chromosome vectors (YACs; BACs)

Expression vectors: mammalian, Baculovirus and *Pichia* vectors system; Plant based vectors- Ti and Ri as vectors, yeast vectors, shuttle vectors, Animals vectors-SV40, Bovine papilloma virus vector & retroviral vectors.

UNIT II: Gene manipulation, Cloning, Gene Libraries & Microarrays

Gene manipulation & cloning: Insertion of foreign DNA into host cells; Cloning from mRNA-isolation & purification of mRNA and total RNA; reverse transcriptase and cDNA synthesis, Berg's terminal transferase method (dA:dT joints); Boyer-Cohen-Chang experiment (cohesive ends); Isolation of plasmids, cloning cDNA in plasmid vectors, cloning cDNA in bacteriophage vectors.

Gene Libraries: Construction of Genomic DNA library and its applications; Construction of cDNA Library- method, problems to be addressed, advantages and disadvantages compared to the genomic DNA library & its applications.

Construction of microarrays: genomic arrays, cDNA arrays and oligo arrays.

UNIT III: Transformation Techniques

Transformation Techniques: Purification of vector DNA, restriction digestion, end modification, cloning of foreign genes, (from mRNA, genomic DNA, synthetic DNA) transformation screening, selection, expression and preservation. Transformation and transfection techniques, preparation of competent cells of bacteria, chemical methods- calcium phosphate precipitation method, liposome mediated method, physical methods- Electroporation, gene gun method. Method of DNA transfer to yeast, mammalian and plant cells, transformation and transfection efficiency.

UNIT IV: Screening of recombinants & DNA sequencing

Screening of recombinants: Screening by complementation, Hybridization techniques-southern, northern & western hybridization; colony lift, immunoprecipitation, south-western screening. Purification strategies of expressed His- tagged proteins.

DNA sequencing: Sanger Nicolson dideoxynucleotide method, Maxam-Gilbert chemical cleavage method, multiplex DNA sequencing, automated DNA sequencing. RNA sequencing. Basic idea of oligonucleotide synthesis. Next Generation Sequencing (NGS) methods.

References:

1. Nicholl D.S.T. *Introduction to Genetic Engineering*. Cambridge (3rd Ed.) University press.UK. 2008.
2. Old R.W., Primrose S.B. *Principles of gene manipulation - An introduction to genetic engineering (5th Ed.)*, Blackwell Scientific Publications, UK. 1996.
3. Ernst-L Winnacker, *From Genes to Clones: Introduction to Gene Technology*. WILEY-VCH Verlag GmbH, Weinheim, Germany Reprinted by Panima Publishing Corporation, New Delhi. 2003.
4. Benjamin Lewis, *Genes VIII (3rd Ed.)* Oxford University & Cell Press, NY. 2004.
5. Robert Williamson. *Genetic Engineering (1st Ed.)* Academic Press. 1981. USA.
6. Rodriguez. R.L (Author), Denhardt D.T. *Vectors: A Survey of Molecular Cloning Vectors and Their Uses (1st Ed.)* Butterworth-Heinemann publisher. UK. 1987.
7. Ansubel F.M., Brent R., Kingston R.E., Moore D.D. et al. *Short protocols in molecular biology (4th Ed.)*, Wiley publishers. India. 1999.
8. Sambrook J et al. *Molecular cloning Volumes I, II and III*. Cold Spring Harbor laboratory Press, New York, USA. (1989, 2000).
9. Terence A Brown. *Genomes, (2nd Ed.)* BioScientific Publishers. UK. 2002.
10. S. B. Primrose, Richard M. Twyman. *Principles of gene manipulation and genomics (7th Ed.)* John Wiley & Sons publishers. 2006.
11. Brown, T. A. (2006). *Genomes (3rd ed.)*. New York: Garland Science Pub.

Paper 11: Elective (Code: MBT3T11)
A) Plant Biotechnology (Code: MBT3T11 A)
OR
B) Animal Biotechnology (Code: MBT3T11 B)

Course Outcomes:

CO1: Remember and understand the concept of Plant Biotechnology

CO2: Differentiate between different plant tissue culture techniques

CO3: Comprehend different techniques to produce better crop via applying the principles of biotechnology

CO4: Understand concepts of Plant Metabolic Engineering

UNIT I: Concepts, methods of plant regeneration & techniques in plant tissue culture

History and types of plant tissue culture techniques, Concept of totipotency, types of culture media for plant tissue culture. Solid and liquid media. Role of auxin, cytokinin, and other growth regulators. Explants used for plant tissue culture, Callus and suspension cultures: initiation and maintenance of callus and suspension cultures; Direct and indirect organogenesis, embryogenesis; Soma clonal variation, Ovule, ovary and Endosperm culture, Embryo culture.

UNIT II: Plant tissue culture techniques for crop improvement

Micropropagation- Meristem tip culture and virus indexing, Anther and microspore culture- Production of haploid plants: anther, pollen and ovary cultures for production of haploid plants and homozygous lines *In vitro* fertilization- Embryo rescue and wide hybridization- Protoplast culture and fusion, selection of hybrid cells and regeneration of hybrid plants, symmetric and asymmetric hybrid, cybrid. application variation- *In vitro* mutagenesis- *In vitro* germplasm conservation; Introduction to *in vitro* conservation, cryopreservation, slow growth cultures and DNA banking for germplasm conservation. Genetic engineering of protoplast and haploid cells

UNIT III: Gene Transfer Methods & Transgenic Plants

Gene transfer methods in plant tissue culture: Agrobacterium mediated Gene transfer Method, Marker free transformation; Crop engineering using CRISPR Advanced technologies – cis genesis and intragenesis. Molecular techniques for analysis of transgenics (copy number, transgene stability, silencing; segregation), co-integrate and binary vector system – Direct gene transfer methods – biolistic gun, microinjection, and electroporation. Chloroplast transformation. Transgene design-Promoters & Marker genes. Factors influencing transgene expression.

Transgenic plants and its application: Herbicide resistance, phosphinothricine glyphosate, sulfonyl urea, atrazin, insect resistance, virus resistance, coat protein mediated nucleocapsid gene, disease resistance, chitinase, 1-3 beta glucanase, RIP, Antifungal proteins, thionins, PR proteins, nematode resistance, abiotic stress, post harvest losses, long shelf life of fruits and flowers, use of ACC synthase, polygalacturanase, ACC oxidase, male sterile lines, bar and barnase systems, carbohydrate composition and storage, ADP glucose pyrophosphatase.

UNIT IV: Plant Metabolic engineering & Molecular marker aided breeding

Molecular pharming – plant production of vaccines, antibodies and therapeutic proteins.

Plant metabolic engineering and industrial products: Plant secondary metabolites. Control mechanisms and manipulation of phenylpropanoid pathway, shikimate pathway, alkaloids, industrial enzymes, biodegradable plastics, polyhydroxybutyrate, lysosomal enzymes, antibodies, edible vaccines, purification strategies, oleosin partitioning technology. Analysis of transgenic plants.

Molecular marker aided breeding: RFLP maps, linkage analysis, RAPD markers, STS, microsatellite, SCAR (sequence characterized amplified regions), SSCP (single strand conformational polymorphism), QTL, map based cloning, molecular marker assisted selection. Biosafety of transgenic plants – regulation of transgenic plants in India. Green House Technology

References:

1. Chawla, H. S. (2000). Introduction to Plant Biotechnology. Enfield, NH: Science.
2. Razdan, M. K. (2003). Introduction to Plant Tissue Culture. Enfield, NH: Science.
3. Slater, A., Scott, N. W., & Fowler, M. R. (2008). Plant Biotechnology: an Introduction to Genetic Engineering. Oxford: Oxford University Press.
4. Buchanan, B. B., Gruissem, W., & Jones, R. L. (2015). Biochemistry & Molecular Biology of Plants. Chichester, West Sussex: John Wiley & Sons.
5. Umesha, S. (2013). Plant Biotechnology. The Energy And Resources.
6. Slater, A., Scott, N. W., & Fowler, M. R. (2003). Plant Biotechnology: The Genetic Manipulation of Plants. Oxford: Oxford University Press.
7. Bhojwani, S.S and Dantu P. 2013. *Plant Tissue Culture – An Introductory Text*. Springer Publications.
8. Gamborg, O.L and G.C. Philips (eds.). 2013. *Plant Cell, Tissue and Organ culture-Lab Manual*. Springer Science & Business media.

A) Animal Biotechnology (Code: MBT3T11 B)

Course Outcomes:

CO1: Remember and Understand the Fundamental Concepts of Animal Cell Culture techniques

CO2: Understand different approaches and techniques involved in creating transgenic animals

CO3: Understand and envision the future Commercial aspects of Animal Cell culture

UNIT I:

Introduction and history of cell culture technology. Importance of animal cell culture.

Equipment and materials for animal cell culture technology. Tissue culture techniques:

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

Primary and secondary culture, continuous cell lines, suspension cultures, organ cultures, 3D tissue culture, etc.

Types and composition of cell culture media: Natural, synthetic, growth supplements. balanced salt solutions and simple growth medium. chemical, physical and metabolic functions of different constituents of culture medium, role of carbon di oxide, serum and supplements. Growth requirements of different cell types

UNIT II:

Behaviour of cells in culture: growth, differentiation and metabolism. Cell division, growth pattern. Estimation of cell number

Characteristics of cells: contact inhibition, anchorage dependence, cell-cell interaction, etc.

Apoptosis (death domain, role of cytochrome C), Senescence, viability and cytotoxicity

Cell cloning, cell synchronization and cell manipulation.

Various methods of separation of cell types, advantages and limitations; flow cytometry

UNIT III:

Development of cell lines. Transformation of cells, tissue engineering

Characterization and maintenance of cell lines. Scaling up.

Stem cells, iPSCs and their applications. Embryonic stem cells.

Hybridoma Technology

Cryopreservation and revival of cells. Cell culture contaminants.

UNIT IV:

An introduction to *In-vitro* fertilization, embryo transfer technology, microinjection and transgenesis.

Commercial applications of cell culture: Tissue culture as a screening system; cytotoxicity and diagnostic tests. Mass production of biologically important compounds (e.g. Vaccines). Harvesting of products, purification, Different assays for confirmation of purified products.

References:

1. Gordon, I. (2005). Reproductive Techniques in Farm Animals. Oxford: CAB International.

2. Levine, M. M. (2004). New Generation Vaccines. New York: M. Dekker.
3. Pörtner, R. (2007). Animal Cell Biotechnology: Methods and Protocols. Totowa, NJ: Humana Press.
4. Animal Tissue Culture by Ian Freshney.

Practical 1 (Code: MBT3P06)
Practical on Plant Biotechnology or Animal Biotechnology

A) Practical Plant Biotechnology (Code: MBT3P06 A)

1. Preparation and sterilization of standard tissue culture media.
2. Sterilization of explants
3. Establishment of callus/cell suspension cultures
4. Induction of Embryo/Ovary culture;
5. Induction of Anther/ pollen culture
6. Micropropagation – Explant establishment, shoot multiplication, root induction
7. Protoplast isolation and culture.
8. *In vitro* grown plantlets hardening and transfer to soil
9. Extraction and quantification of secondary metabolites from callus of medicinal plants
10. Agrobacterium mediated genetic transformation.
11. Artificial seed preparation.
12. Isolation of plant genomic DNA

B) Practical Animal Biotechnology (Code: MBT3P06 B)

1. Preparation of animal cell culture media.
2. Filter sterilization and sterility test.
3. Media storage, serum inactivation.
4. Chick Embryo Fibroblast culture to learn primary cell culture
5. Lymphocyte culture to learn primary culture of non-adherent cells
6. Maintenance of established cell lines
7. Cell counting using Neubauer chamber.
8. Cryopreservation of Established and primary cells
9. Revival of cryopreserved established cell lines.
10. Cell fusion.
11. Cell transformation by viruses.
12. Lyophilization of local germplasm
13. Viability Staining of animal cells
14. MTT based Cytotoxicity assay

M.Sc. Biotechnology Semester III
Research Project (RP) Minor (Code: MBT3P07)

Course Outcomes:

CO1: Attain in-depth knowledge of the chosen area of research.

CO2: Develop competence in research design and planning.

CO3: Be able to create, analyse and critically evaluate different technical solutions.

CO4: Develop ability to conduct research independently.

CO5: Be able to perform analytical techniques/experimental methods.

CO6: Develop report writing & communication skills.

M. Sc. Biotechnology Semester IV
Paper 12 (Code: MBT 4T12)
Applied Molecular Biology

Course Outcomes:

CO1: Gain knowledge of Recombination and Genome Mapping and its application in Biotechnology

CO2: Comprehend the concept of Antisense, Ribozymes and Epigenetics and their application

CO3: Understand the importance of Polymerase chain reaction and its application in biotechnology

CO4: Understand basic concept of Cancer Biology and stem cells.

UNIT I: Recombination and Genome Mapping

Homologous recombination: Mechanism of recombination, Holliday, White house and Radding models, FLP/FRT and Cre/Lox recombination, RecA and other recombinases.

Molecular mapping of genome: Genetic and physical maps, choice of mapping population, southern and fluorescence in situ hybridization for genome analysis, RFLP, RAPD, and AFLP analysis, molecular markers linked to disease resistance genes, application of molecular markers in forensic investigation, disease prognosis, genetic counselling, pedigree.

UNIT II: Polymerase Chain Reaction and its different types

Principles of PCR: primer design; calculation of annealing temperature, preparation of reaction mixture and their function, construct of thermal cycler, gradient cyclers, choice and types of thermostable DNA polymerases.

Types of PCR – multiplex, nested; reverse-transcription PCR, real time PCR, touchdown PCR, hot start PCR, colony PCR, asymmetric PCR,

Cloning of PCR products; T-vectors; proof reading enzymes; PCR based site specific mutagenesis; PCR in molecular diagnostics; viral and bacterial detection.

UNIT III: Antisense, Ribozymes and Epigenetics

Antisense and ribozyme technology: Antisense technology and the molecular mechanism, ribozyme technology, biochemistry of ribozyme, hammerhead ribozymes, applications of antisense and ribozyme technologies.

Epigenetics: Epigenetic markers, chromatin remodeling, chromatin marking systems, chemical modification of histones - methylation, acetylation, phosphorylation, ubiquitination, and sumoylation, chemical modification of DNA – DNA methylation and DNA methyltransferases (DNMTs)

UNIT IV: Cancer

Cancer biology – Morphological properties and growth characteristics of cancer cells; types of cancers, differences between benign and malignant tumors; Tumor markers on cells; genetics of cancer - oncogenes (ras, myc), suppressor genes (p53, Rb), Basics of Cancer Genomics.

DNA Viruses and cancer: Polyoma virus, SV40, adenovirus

Basic idea of Cancer stem cells

Angiogenesis: Brief idea of healthy vasculature, definition of angiogenesis, basic process of tumor induced angiogenesis, Hypoxia induced factor (HIF), basics of pro- and anti- angiogenic factors

Metastasis: Epithelial-mesenchymal transition, stages of metastatic progression, prerequisites for metastasis (properties a cell must acquire for metastasis), secondary tumor formation

References:

1. Old, R. W., Primrose, S. B., & Twyman, R. M. (2001). *Principles of Gene Manipulation: an Introduction to Genetic Engineering*. Oxford: Blackwell Scientific Publications.
2. Green, M. R., & Sambrook, J. (2012). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
3. Brown, T. A. (2006). *Genomes* (3rd Ed.). New York: Garland Science Pub.
4. Judit Pongracz and Mary Keen, *Medical Biotechnology 1st Edition*, Elsevier publications, 2008.
5. Jogdand S N, *Medical Biotechnology 2nd Edition*. Himalaya publishers 2008.
6. Kleinsmith J Lewis, *Principles of Cancer Biology 1st Ed.*, Pearson 2016.
7. Weinberg Robert, *The Biology of Cancer 2nd Ed.*, W.W. Norton & Company 2013.
8. McKinnel Robert, Damjanov Ivan, *The Biological Basis of Cancer*. Cambridge University Press 2006.

M. Sc. Biotechnology Semester IV
Paper 13 (Code: MBT 4T13)
Genetic Engineering and Its Application

Course Outcomes:

CO1: Understand the concepts of Prokaryotic and Eukaryotic Transformation

CO2: Describe the expression of heterologous genes and the vectors involved in it.

CO3: Appreciate technology involved in industrial products of Protein engineering.

CO4: Explain and illustrate techniques like Phage display, gene therapy and transgenics

UNIT I: Transformation & Transfection methods

Transformation: Mechanisms of DNA uptake by bacterial cells and its application in genetic engineering

Transfection: Chemical and physical methods, Viral vectors. Polyethylene glycol, DEAE-dextran, calcium phosphate coprecipitation, dimethyl sulfoxide, liposomes, microinjection, macroinjection, electroporation, biolistics, somatic cell fusion, gene transfer by pronuclear microinjection. Application of Transfection Methods in gene therapy, protein production, and functional genomics.

UNIT II: Plant transformation technology

Plant transformation technology: *Agrobacterium*-plant interaction; Basis of tumor formation, hairy root, features & use of Ti and Ri plasmids, mechanism of DNA transfer, role of virulence genes, Genetic transformation - *Agrobacterium*-mediated gene delivery; cointegrate and binary vectors and their utility, use of 35S and other promoters, genetic markers, use of reporter genes, use of scaffold attachment regions, methods of nuclear transformation, viral vectors and their application, Biological and physical transformation methods. Chloroplast transformation. Application of genetic transformation such as herbicide resistance, insect resistance, and nutritional enhancement in transgenic crops.

UNIT III: Expression of heterologous genes

Expression of heterologous genes: expression of eukaryotic genes in bacteria, expression of heterologous genes in yeast, insect and mammalian cells.

Salient features of expression vectors.

Processing of recombinant proteins: Refolding and stabilization.

Industrial Products of Protein engineering

Production of Insulin & Thaumatin

UNIT IV: Phage Display, Gene Therapy & Transgenics

Phage Display: Production of monoclonal bodies by phage display technique using filamentous phage vectors.

Gene Therapy: somatic and germline, gene replacement, in vivo and ex vivo gene delivery, retrovirus gene transfer system, advantages and disadvantages of adenovirus, adeno-associated virus, herpes virus vectors, gene correction, replacement/augmentation, editing, regulation and silencing. Gene therapy of human diseases.

Transgenics - gene replacement; gene targeting; creation of transgenic and knock-out mice; disease model.

References:

1. Chawla, H. S. (2000). *Introduction to Plant Biotechnology*. Enfield, NH: Science.
2. Slater, A., Scott, N. W., & Fowler, M. R. (2008). *Plant Biotechnology: an Introduction to Genetic Engineering*. Oxford: Oxford University Press.
3. Glick, B. R., & Pasternak, J. J. (2010). *Molecular Biotechnology: Principles and Applications of Recombinant DNA*. Washington, D.C.: ASM Press.
4. Brown, T. A. (2006). *Gene Cloning and DNA Analysis: an Introduction*. Oxford: Blackwell Pub.
5. Primrose, S. B., & Twyman, R. M. (2006). *Principles of Gene Manipulation and Genomics*. Malden, MA: Blackwell Pub.
6. Slater, A., Scott, N. W., & Fowler, M. R. (2003). *Plant Biotechnology: The Genetic Manipulation of Plants*. Oxford: Oxford University Press.
7. Lemoine, N.R., Vile R.G. (2000). *Understanding of Gene Therapy (1st Ed)*. Garland Science

M. Sc. Biotechnology Semester IV
Paper 14 (Code: MBT 4T14)
Bioinformatics

Course Outcomes:

CO1: Locate and use the main databases at the NCBI and EBI resources.

CO2: Know the difference between databases, tools, repositories and be able to use each one to extract specific information.

CO3: Use selected tools to run simple analyses on genomic sequences.

CO4: Understand the importance of protein modelling and protein structure prediction

Unit I Basics of Bioinformatics

Introduction to Bioinformatics: Definition and scope, Historical development and milestones, Importance and applications in biology and medicine; **Biological Databases:** Types of biological databases, Primary vs. secondary databases, **Key Databases:** NCBI, EMBL-EBI, DDBJ, UniProt, PDB, KEGG, Ensembl, Navigating NCBI and UniProt; **Gene and Protein Annotation:** Techniques for gene prediction, Functional annotation of genes, Basics of protein structure; **Introduction to genomics:** Genome sequencing technologies, Applications of genomics.

Introduction to proteomics: Techniques in proteomics, Applications of proteomics in research; **Bioinformatics Applications in Medicine:** Personalized medicine, Pharmacogenomics; **Future Trends in Bioinformatics:** Big data and bioinformatics AI and machine learning in bioinformatics.

Unit II DNA sequence analysis and Multiple sequence analysis

DNA sequence analysis: gene bank sequence database; submitting DNA sequences to databases and database searching; sequence alignment; pairwise alignment techniques; motif discovery and gene prediction; local structural variants of DNA, their relevance in molecular level processes, and their identification; assembly of data from genome sequencing. Multiple sequence analysis; multiple sequence alignment; flexible sequence similarity searching with the FASTA3 program package; use of CLUSTALW and CLUSTALX, MUSCLE and BLAST for multiple sequence alignment; submitting DNA protein sequence to databases: where and how to submit, SEQUIN, genome centres; submitting aligned sets of sequences, updating submitted sequences, methods of phylogenetic analysis. File formats of sequences: FASTA, FASTQ, GenBank, GFF/GTF, SAM/BAM, VCF, PDB, EMBL.

Unit III Protein modelling

Protein modelling: introduction; force field methods; energy, 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.

Tools: Swiss model

Unit IV Protein structure prediction and virtual library

Protein structure prediction: protein folding and model generation; secondary structure prediction; analyzing secondary structures; protein loop searching; loop generating methods; homology modelling: 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; protein function prediction; elements of in silico drug design; Virtual library: Searching PubMed, current content, science citation index and current awareness services, electronic journals, grants and funding information.

Tools: Homology Modelling

Recommended Textbooks and References:

1. Lesk, A. M. (2002). Introduction to Bioinformatics. Oxford: Oxford University Press.
 2. Mount, D. W. (2001). Bioinformatics: Sequence and Genome Analysis. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
 3. Baxevanis, A. D., & Ouellette, B. F. (2001). Bioinformatics: a Practical Guide to the Analysis of Genes and Proteins. New York: Wiley-Interscience.
 4. Pevsner, J. (2015). Bioinformatics and Functional Genomics. Hoboken, NJ.: Wiley-Blackwell.
 5. Bourne, P. E., & Gu, J. (2009). Structural Bioinformatics. Hoboken, NJ: Wiley-Liss.
 6. Lesk, A. M. (2004). Introduction to Protein Science: Architecture, Function, and Genomics. Oxford: Oxford University Press.
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M. Sc. Biotechnology Semester IV
Paper 15: Elective (Code: MBT 4T15)
Paper 15A: Agriculture Biotechnology OR
Paper 15B: Medical Biotechnology

Paper 15A: Agriculture Biotechnology (MBT 4T15A)

Course Outcomes:

CO1: Learn and gain knowledge about applications of biotechnology in the field of Agriculture

CO2: Understand the importance of Genetic Engineering and Recombinant DNA Technology in crop improvement and crop protection

CO3: Understand the importance of advanced techniques in the field of agriculture

Unit I: Introduction to Agricultural Biotechnology

Overview of agricultural biotechnology Historical Development and milestones in agricultural biotechnology. Importance and applications of agricultural biotechnology. Tools and Techniques in agricultural biotechnology. Composting, Vermicomposting, Biofertilizers: Bacterial, Algal, Aquatic ferns, & Fungi as biofertilizers. Crop production technology. Ethical and social considerations in agricultural biotechnology. Plant Breeders and Farmer's Rights.

Unit II: Genetic Engineering in Crop Improvement

Principles of genetic engineering Gene transfer methods in planta. Transgenic crops development, benefits, and challenges. Novel Vaccines from plants. Molecular markers and their applications in crop improvement. Marker- assisted selection (MAS) and its role in breeding programs.

Unit III: Biotechnology for Crop Protection

Plant disease management using biotechnology approaches. Genetic engineering for insect resistance, herbicide resistance, stress resistance in crops. Biotechnological approaches for weed control. Molecular diagnostics for disease detection in crops. RNA interference (RNAi) technology in crop protection. Biotechnology in agriculture for development of biofuels.

Unit IV: Advanced Topics in Agricultural Biotechnology

Application of Genome editing techniques in agriculture (e.g., CRISPR-Cas9). Omics technologies (genomics, proteomics, metabolomics) in agriculture. Bioremediation and phytoremediation. Nanotechnology applications in agriculture. Concept of Controlled Environment Agriculture (CEA). Biosafety regulations and risk assessment in agricultural biotechnology.

Recommended Textbooks and References:

1. "Principles of Agricultural Biotechnology" by T.P. Reddy and G. Satyanarayana
2. "Agricultural Biotechnology: Challenges and Prospects" by Praveen C. Verma and Rajesh K. Singh
3. "Applied Agricultural Biotechnology" by Bhavbhuti M. Mehta and P. V. K. Sasidhar
4. "Agricultural Biotechnology: Opportunities, Benefits, and

Challenges" edited by P.K. Gupta and R.K. Salar

5. "Plant Biotechnology and Agriculture: Prospects for the 21st Century" edited by Arie Altman and Paul Michael Hasegawa.

Paper 15 B: Medical Biotechnology (MBT 4T15B)

Course Outcomes:

CO1: develop an ability to identify, organize and answer problems in Medical Biotechnology

CO2: understand the cellular and molecular pathogenesis of the various disease

CO3: understand molecular and cytological causes for various genetic disorders

CO4: understand various current diagnostics methods and therapies

CO5: develop novel diagnostic methods and therapeutics

CO6: Appreciate the importance of tissue engineering and stem cell technology

Unit I: Introduction to Medical Biotechnology and biology of disease

Overview of medical biotechnology. Importance and applications of medical biotechnology in healthcare. Ethical considerations and regulations in medical biotechnology.

The Genetic Basis of Disease: Chromosomal Abnormalities, Genome-Wide Association Studies.

Single-Gene Disorders (Thalassemia, Sickle-Cell Anaemia, Cystic Fibrosis, Huntington Disease), **Polygenic Disorders**, GWAS Strategies to Map Genes for Polygenic Disease (Breast Cancer, Type 1 Diabetes).

Microbial Pathogenesis: (Bacterial- MTB and Staphylococcal disease; Viral- Influenza and HIV) Evolution of MTB, Detection of MTB and Treatment of MTB. Evolution of Influenza, Detection of HIV and Influenza, Targets and Treatment of Influenza and HIV.

Unit II: Principles of molecular diagnostics

Detect Protein Biomarkers of Disease: Enzyme-Linked Immunosorbent Assays (Measuring Disease-Associated Proteins, Diagnosing Autoimmune Diseases),

Detection of Infectious Disease: Protein Arrays, Immunoassays for Protein Conformation, DNA-Based Approaches to Disease Diagnosis (Hybridization Probes, Allele-Specific Hybridization, Oligonucleotide Ligation Assay, Padlock Probes, Allele-Specific PCR, TaqMan PCR, Real-Time PCR To Detect Infectious Disease,

Overview of Genetic detection in pathology: Detection of Mutations, Epigenetic Markers, SNPs, RNA Signatures of Disease, Disease-Associated Changes in Gene, RNA Signatures of Antibiotic Resistance in Human Pathogens, miRNA Signatures of Cancers

Application of NGS analysis in disease diagnostics.

Unit III: Therapeutic Applications of Biotechnology

Principles of Drug Therapy: Concept of Pharmacokinetics, Drug-Receptor Interactions and Pharmacodynamics. Genomic medicine. Pharmacogenomics and personalized medicine.

Nucleic Acid based Therapeutic Agents: Treating Genetic and Nongenetic Disorders, Targeting Specific mRNAs and DNAs, Antisense RNA, Aptamers, Ribozymes, DNazymes, Interfering RNA, Zinc Finger Nucleases, Viral Delivery Systems, Gamma retrovirus, Adeno- Associated Virus, Adenovirus, Herpes

Simplex Virus, Nonviral Nucleic Acid Delivery Systems, Prodrug Activation Therapy.

Protein based Therapeutics Agents: Interferon, Human Growth Hormone, Tumor Necrosis Factor, Engineered Bacteriophages, Recombinant Antibodies, Human Monoclonal Antibodies, Anticancer Antibodies, Anti-anthrax Antibodies, Enhanced Protein and Antibody Half-Life. Overview of Therapeutic Enzymes.

Vaccines: Types, Development, production, and delivery systems.

Unit IV: Advanced Topics in Medical Biotechnology

Concept of Nanotechnology in medicine and targeted drug delivery.

Cell and tissue engineering, Organ culture, Classifications of cell transplantation, Bioengineered cells, Significance of cell and tissue engineering, Trends and Challenges of tissue engineering.

Concept of Synthetic biology and its application in medical biotechnology. Health risk and ethical issues associated with synthetic biology.

Stem cell technology in regenerative medicine. Stem cell technology, Classification of stem cells, Induced pluripotent stem cells, Applications of stem cells, Stem cell transplantation. Stem cell banks, Hurdles in ESC research.

Application of gene editing technology for disease diagnosis and therapeutic protein production.

Reference Books

1. BIOTECHNOLOGY IN MEDICAL SCIENCES Firdos Alam Khan Manipal University Dubai, UAE CRC Press
2. Medical Biotechnology by Bernard R. Glick, Terry L. Delovitch, and Cheryl L. Patten ASM Press
3. Biotechnology and Biopharmaceuticals by Rodney J. Y. Ho, Ph.D., FAAAS, FAAPS and Milo Gibaldi, Ph.D Wiley Blackwell publication
4. Essentials of Stem Cell Biology by Robert Lanza et al Academic Press
5. Lippincotts Illustrated Reviews: Pharmacology sixth edition by Karen Whalen

M. Sc. Biotechnology Semester IV
(Code: MBT 4P08)
Research Project (RP) Major

Course Outcomes:

CO1: Develop the aptitude to work on a scientific problem and look for alternative solution

CO2: Understand and apply the scientific method.

CO3: Develop the critical thinking ability and communication skills.

CO4: Write their finding in the form of a thesis and defend it by presenting it in front of their teachers and examiners.

CO5: Experience and embrace the habit of ethical practice in performing experiments and communicating them