



Solapur University, Solapur

M.Sc. Part-II Semester III & IV Biotechnology

(Revised semester pattern syllabus)

(w.e.f. June, 2014)

Syllabus for M.Sc. Part II Biotechnology

SEMESTER- III (THEORY)

Code	Title of the paper	Marks
MBT - 301	Advanced analytical techniques	100
MBT - 302	Fermentation Technology	100
MBT - 303	Research Methodology & IPR	100
MBT - 304	Plant Biotechnology	100

SEMESTER- III (PRACTICAL)

Code	Title of the paper	Marks
MBT PR- 305	Advanced analytical techniques & Fermentation Technology	100
MBT PR- 306	Plant Biotechnology and Research Methodology	100
	Seminar	25
	Total Marks	625

SEMESTER- IV (THEORY)

Code	Title of the paper	Marks
MBT - 401	Animal Biotechnology	100
MBT - 402	Industrial & Environmental Biotechnology	100
MBT - 403	Stem Cell Technology & Regenerative medicine	100
MBT - 404	Genetic Engineering	100

SEMESTER- IV (PRACTICAL)

Code	Title of the paper	Marks
MBT PR – 405	Genetic Engineering, Stem Cells, Animal & Industrial Biotechnology	100
MBT PR – 406	Project	100
	Seminar	25
	Total Marks	625

As per the credit system, the assessment of Theory paper of 100 marks weightage will be as: 70 marks theory assessment by University examination and 30 marks internal assessment by the Department. For internal assessment of candidate, periodical tests/seminars/ viva/oral / quiz etc. may be suitably adopted.

M.Sc II Sem – III (Biotechnology)

MBT 301 Advanced analytical techniques

UNIT I - Microscopy & Centrifugation: (8)

Light microscope, Fluorescence microscope, Phase contrast microscope, Electron microscope, confocal microscopy. Centrifugation: Small bench top centrifuges, large capacity refrigerated centrifuges, High speed refrigerated centrifuges, preparative and analytical ultra centrifuge, Molecular weight determination.

UNIT II - Chromatographic techniques: (8)

Principles of chromatography, size exclusion, ion exchange and affinity chromatography. High performance liquid chromatography (HPLC), Gas liquid chromatography (GLC), Thin layer chromatography (TLC), Paper chromatography, GC-MS, LC-MS, Chromatofocussing.

UNIT III - Electrophoresis: (10)

General principles, factors affecting migrating rates, factors affecting migration of ions, supporting media, Techniques of electrophoresis: low and high voltage, capillary electrophoresis, electro osmotic flow techniques, instrumentation, zone electrophoresis and applications. Electrophoresis of proteins: SDS-PAGE, Native gels, Gradient gel, Iso-electric focusing, 2-D gel electrophoresis (2-D PAGE), cellulose acetate electrophoresis, continuous flow electrophoresis; Detection, estimation and recovery of proteins, Western blotting. Electrophoresis of nucleic acids: agarose gel electrophoresis of DNA, DNA sequencing gels, Pulse field gel electrophoresis, electrophoresis of RNA, Capillary electrophoresis.

UNIT IV - Electrochemical techniques: (10)

Principles of electrochemical techniques, redox reactions, pH electrode, ion-sensitive and gas-sensitive electrodes, The Clark oxygen electrode, Biosensors. Spectroscopic techniques: Properties of electromagnetic radiation, interaction with matter. Gamma ray spectroscopy, X-ray spectroscopy, UV and Visible spectroscopy, Infrared and Raman spectroscopy, Electron spin resonance spectroscopy, Nuclear magnetic resonance spectroscopy, Circular dichorism spectroscopy, Atomic spectroscopy, MALDI Tof, Lasers, Spectrofluorimetry, turbidometry and nephelometry, X-ray crystallography.

UNIT V - Radio isotope techniques: (9)

The nature of radioactivity, detection and measurement of radioactivity: detection based on gas ionization- Geiger Muller counter- principles and applications. Detection based on excitation-

Liquid Scintillation counter-principle and applications. Supply, storage and purity of radio labelled compounds, specific activity, inherent advantages and restrictions of radiotracer experiments, safety aspects, applications- of radio isotopes in biological sciences. Flow cytometry, ELISA, Immuno blotting.

Suggested Readings:

1. Keith Wilson and John Walker. Practical Biochemistry- principles and techniques; Cambridge University press, London, UK.
2. David T Plummer, Tata McGraw- Hill publishing company limited; McGraw office, New Delhi.
3. C.R. Kothari, 2nd Edition, 2004. Research methodology- methods and techniques. New Age International (P) limited publishers, New Delhi.
4. Instrumental methods of chemical analysis - P.K. Sharma
5. Biophysical chemistry - Upadhyay. Upadhyay and Nath
6. A Biologist's guide to principle and techniques of practical biochemistry - Brigian L. Williams.
7. Handbook of Biomedical Instrumentation - R.S. Khandpur, Tata McGraw Hill.

MBT 302 Fermentation Technology

UNIT - I Bio Processing – Introduction

(10L)

Bioprocess technology for the production of cell biomass and primary/secondary metabolites, ts etc.; Microbial production, purification and bioprocess enzymes; Production and purification of recombinant proteins on a large scale; Chromatographic and membrane based bioseparation methods; Isolation, identification and initial selection of microbial strains – culture preservation, stock culture maintenance, storage of culture and culture collection resources ,modification of genetic structure to increase product formation, optimal nutritional and physical requirements for growth – microbial nutrition, growth measurements, growth curve and optimization of nutritional and physicochemical factors, Process strategy –primary and secondary metabolites kinetic and process rate, reactor design, product recovery, waste treatment Microbial cell cultivation systems – Introduction, Batch cultivation system, continuous growth cultivation system, Fed-batch cultivation system, recycling cultivation system, inoculums cascading system, solidstate and solid-substrate cultivation system- principles, general features, microbial basis of processes, importance of inoculums, bioreactor design Immobilized cells/enzyme systems – alginate, ion exchange resin, polyurethane foam, cell aggregation/flocculation, covalent coupling, immobilized bioreactor design and biosensors

UNIT – II Upstream Processing

(12L)

Fermentation Technology – Introduction, Microbial Growth, Applications of Fermentation - Microbial Biomass, Microbial Metabolites, Microbial Enzymes, Transformation Processes, Recombinant Products, types – submerged and solid state, The Fermentation Process - The Mode of operation of Fermentation Processes, Bioreactors- description and modeling, lab scale fermentation, large scale fermentation, cell culture bioreactors, Media for microbial fermentations, media for cell culture, media and air sterilization, oxygen transfer and mixing – scale up implications, in cell culture reactors, Strategies for fermentation with recombinant microorganisms, Anaerobic fermentations, fermentation monitoring and control, data analysis for design and control, design of aseptic aerated fermentors, biotransformations and enzyme reactors Bioprocess Engineering: Kinetics of microbial growth, substrate utilization and product formation; Simple structured models; Sterilization of air and

media; Batch, fed-batch and continuous processes; Aeration and agitation; Mass transfer in bioreactors; Rheology of fermentation fluids; Scale-up concepts; Design of fermentation media; Various types of microbial and enzyme reactors;

UNIT - III Downstream processing (10L)

Protein Extraction and Purification – Introduction, Cell Disruption: Enzymic Methods of Cell Disruption, Chemical Methods of Cell Lysis – Alkali and Detergents, Physical Methods of Cell Lysis -Osmotic Shock, Grinding with Abrasives, Solid Shear and Liquid Shear, Initial Purification - Debris Removal, Batch Centrifuges, Continuous-flow Centrifugation, Basket Centrifuges and Membrane Filtration, Aqueous Two-phase Separation, in vitro protein refolding, Precipitation, Ultrafiltration, electrokinetic separation of proteins, lyophilization and spray drying, Design of Proteins for Purification.

UNIT – IV Measuring, Modeling and control (10L)

Measurements and control: Common instruments in process analysis and control, Methods and Instruments in fermentation gas analysis, Biosensors, Characterization of Bioreactors, Determination of Cell Concentration, optimization of sampling, Cell Models, Stirred Tank Models, Tower Reactor Models, Process Models, Control of Bioreactor Systems, Automation, Control of Downstream Processing.

UNIT – V Process Validation and regulatory issues (3L)

Analytical protein chemistry, biotechnology facility design and process validation,

References

- 1) Bailey J.E and Ollis D.F. (1986) Biochemical Engineering Fundamentals 2nd ed. McGraw Hill Book Company, N. Delhi.
- 2) Aiba S, Humphrey A. E. and N. F. Millis (1973) Biochemical Engineering, 2nd Edition University of Tokyo Press, Tokyo, Japan.
- 3) Stanbury P.F., Whitaker A, and Hall S.J. (1997) Principles of Fermentation Technology 2 nd ed. Aditya Books Pvt. Ltd, N.Delhi.
- 4) Mukhopadhyaya S.N. (2001) Process Biotechnology Fundamentals. Viva Books Pvt. Ltd.
- 5) Rehm H.J and Reed G. (1985) Biotechnology vol. I & II. VCH, Basel.
- 6) Fermentation Microbiology and Biotechnology, Second Edition
edited by E. M. T. El-Mansi, C. F. A. Bryce, Arnold L. Demain, A.R. Allman

MBT 303- RESEARCH METHODOLOGY & IPR

UNIT - I Research: (8)

Definition, Importance and Meaning of Research, Objectives of research, Characteristics of Research, Types of Research. Steps in Research; Identification, Selection and Formulation of Research Problem, Research Design, Formulation of Hypothesis, Review of Literature.

UNIT - II Sampling Techniques & Parametric Tests: (10)

Sampling theory, Types of Sampling, Steps in Sampling, Sample Size, Advantages and limitations. Collection of Data: Primary Data, Data Collection Methods, Secondary Data, Relevances, Limitations and Cautions, Testing of significance Mean, Proportion, Variance and Correlation, Testing for Significance of Difference between Means, Proportions, Variances and Correlation Co efficient. Chi-square tests, ANOVA

UNIT - III Thesis and Manuscript writing: (12)

Abstract, Introduction, Materials and Methods, Results and Discussion, Summary and Conclusion, References (IMRAD). Preparation of Manuscript; Author instructions, modes of paper communication, criteria for publication. Computer and internet application in Research. Presentation of a scientific Paper, Preparation of Oral Presentation and Poster Presentation for conferences. Use of Audio-Visual aids in Presentation.

UNIT - IV Introduction to IPR and Patents: (10)

Intellectual property, Protection of Intellectual property, World organizations, forms of protection- patent, copyright, trademark, geographical indications, trade secrets. Criteria and procedure of patenting, patenting biological material. Patent procedure in India. Types of patenting, Patenting of biological materials with examples and case studies.

UNIT - V Plant breeder's right: (5)

UPOV, Breeders exemption, Plant variety protection in India. Farmer's right, advantages and disadvantages of PBR. Technology transfer- Introduction, types of technology transfer and Indian scenario.

Reference Books:

1. Statistical Methods by S.P. Gupta.
2. Research Methodology, Method and Techniques by C.R. Kothari or by Santosh Gupta.
3. Research Methodology by Gurumani.
4. Text book of Biotechnology, P K Gupta
5. Text book of Biotechnology, B D Singh.

MBT – 304 Plant Biotechnology

UNIT I: Plant Physiology and Basic Techniques in Plant Tissue Culture **09**

Plant Nutrition: Microelements and micronutrients in plant metabolism, Functions & Deficiency diseases. Plant Hormones: Types & Mechanism of Action. Role of Hormones in growth of Plants. Cell & Tissue Culture. Lab setup of Plant Tissue Culture laboratory Tissue culture Media, Initiation and Maintenance of callus & Suspension culture, single cell clones.

UNIT II: Micro propagation **08**

Organogenesis, Somatic Embryogenesis, Synthetic seeds. Shoot tip culture/ Auxiliary bud culture, Rapid clonal propagation. Embryo Culture & Embryo Rescue. Acclimatization of Plants. Somaclonal Variations /Invitro mutagenesis Selected successful examples of Plants of Diverse Origin using Tissue Culture technology, Rescue of endangered plants.

UNIT III: Protoplast Culture, Anther Culture and Cryopreservation **08**

Protoplast Isolation, Culture, Fusion, Selection of Hybrid Cells and Regeneration of Hybrid Plants, Symmetric and Asymmetric hybrids. Anther, Pollen and Ovary culture for production of Haploid Plants and Homozygous lines. Cryopreservation, Slow growth & DNA Banking for germ plasm Conservation.

UNIT IV: Plant Transformation Technology. **10**

Basics of Tumor formation, Hairy root, features of Ti & Ri Plasmid, Mechanism of DNA transfer role of Virulence gene, Use of Ti & Ri as vectors, Binary vectors, Use of 35s & other promoters genetic markers methods of nuclear transformation viral vectors & their applications, Multiple gene transfers vector less or direct DNA transfer ,Use of reporter gene, Particle bombardment ,electroporation, Microinjection, transformation of monocots, Transgene stability & gene silencing in Plant transformation.

UNIT V: Applications of Plant Biotechnology **10**

Commercial micro propagation. Metabolic engineering & Industrial products Plant secondary metabolites, control mechanisms & manipulation of Phenyl Propanol pathway, Shikimate pathway, Alcoloids, Industrial enzymes, Biodegradable plastics, Therapeutic proteins, lysozomal enzymes, Antibodies, edible vaccines, Purification strategies, oleosin partitioning technology. Integration of Genetic Engineering of Plants in Agriculture Diseasesresistant, Biotic & Abiotic stress resistant, Enhancement of nutritional value of crop Plants & molecular farming, Applications in Biodiversity conservation.

References

1. An introduction to Plant Tissue Culture 2nd edn. Razdan, M. K, Science Publishers, USA.
2. Textbook of plant biotechnology, Chawala P.K.2002, Oxford & IBH, New Delhi.
3. Bhojwani, S. S. and M. K. Razdan 1996.Plant Tissue Culture:Theory and Practice, Elsevier Pub.
4. Chrispeels, M. J. 2002. Plant Tissue Culture: Genetical Aspects. Jones and Bortlett Publishers, International.
5. Chopra V. L. et al 1999. Applied Plant biotechnology. Science Publishers Inc.
6. Verpoorte, R. and A.W. Alfermann (Eds) 2000.Metabolic Engineering of plant secondary metabolism, lower Academic Publisher.
7. Chawla HC (2004) – Introduction to plant biotechnology (Science Publ)
8. Davies K (Ed) (2004) – Plant pigments and their manipulation – Annual plant reviews, vol 14 Blackwell Publ)
9. Altman A, Hasegawa PM (Ed) (2012) – Plant Biotechnology and agriculture. Prospects for the 21th century (Academic press).
10. Bhojwani SS. & Razdan MK (1996). - Plant Tissue Culture: Theory & Practice (Elsevier)
11. Hou CT, Shaw JF (2009) – Biocatalysis and agricultural biotechnology (CRC Press)
12. Slater A, Scott NW, Fowler MR (2008) – Plant Biotechnology: the genetic manipulation of plants (Oxford Press)
13. Vasil IK, Thorpe TA (1994) – Plant cell and tissue culture (Springer)
14. H K Das Textbook of Biotechnology 4th edition

PRACTICALS

MBT PR 305 - Advanced analytical techniques & Fermentation Technology

1. Electrophoresis of proteins under native and denaturing conditions (PAGE)
2. Separation of proteins / pigments using column chromatography
3. Demonstration of techniques: gas chromatography high performance liquid chromatography (HPLC) and atomic absorption spectroscopy AAS.
4. Separation and identification of amino acid mixture by Paper chromatography technique.
5. Thin layer chromatographic separation of sugars and membrane lipids.
6. Separation of proteins (hemoglobin & cytochrome c) using molecular sieve chromatography.
7. Determination of capacity of ion exchange resin [Dowex- 50]
8. Purification of protein by ion exchange chromatography.[DEAE cellulose chromatography]
9. Absorption spectra of dsDNA and ssDNA melting
10. To find out isoelectric point of amino acids
11. Fermentation of wheat bran/ cellulose
12. Analysis of spent broth
13. Purification and precipitation secreted proteins from spent broth
14. 2-D gel electrophoresis of precipitated protein
15. Estimation of cellulase activity of cellulose degraders
16. Estimation of cellulose/xylanase activity in broth and precipitated protein fraction
17. Production of algal biomass
18. Microbial load of canned foods
19. Analysis of preserved food stuff for presence of pathogens
20. Detection of viable, non-viable cells in fermented products.

MBT PR 306 - Plant Biotechnology and Research Methodology

1. Preparation of Media.
2. Surface Sterilization
3. Organ Culture
4. Callus Culture, organogenesis.
5. In vitro rooting and acclimatization.
6. Protoplast isolation and culture.
7. Anther Culture/ Production of haploids.
8. Cytological examination of regenerated plants.
9. Agrobacterium culture, selection of transformants, GUS assay.
10. Synseed preparation
11. Preparation of Poster and oral presentation for conferences.
12. Preparation of Manuscript for publication.
13. Statistical analysis of biological data by Chi square test, ANOVA.
14. Presentation of Scientific paper.
15. A report on IPR.
16. Case studies of biotechnological and biological patents.
17. Karyotyping

M.Sc II Sem – IV (Biotechnology)

MBT 401 Animal Biotechnology

UNIT - I Tissue Culture Laboratory (8L)

Introduction of animal cells & Tissue Culture Laboratory, Equipments and Materials for animal Cell Culture Technology, Design of Tissue Culture Laboratory, Equipments : Laminar Flow Hoods, CO₂ incubator, Open and closed cultures, Microscopes, centrifuge, Refrigerators and Freezers, pipetting aids, Miscellaneous small items of Equipments, Materials, filters, Miscellaneous Items. Basic Aseptic Techniques, Storage, shipping and safety.

UNIT - II Introduction to Tissue Culture Techniques: (7L)

Definition, principle and significance of tissue culture. Maintenance of sterility and use of antibiotics, Mycoplasma and viral contaminants. Various systems of tissue culture - their distinguishing features advantages and limitations. Culture medium: Logic of formulation (natural media, synthetic media, and sera). Methodology: i. Primary culture: Behaviour of cells, properties, utility. ii Explant culture. iii. Suspension culture

UNIT - III Animal Cell & Organ Culture: (15L)

Cell lines: Definition, development, maintenance and management and Cell adaptation. Established cell lines: Their characteristic features and utility, Cross contamination hazards.Characteristics of cells in culture. Contact inhibition, anchorage dependence & independence, cell-cell communication etc, Cell senescence. Cell and tissue response to tropic factors, Culturing of different cells. Designing of an experiment in tissue culture and response assessment. Significance of various controls. Growth studies: Cell proliferation, cell cycle, mitosis in growing cells. Organ culture: Methods, behaviour of organ explant, and utility of organ culture. Organ transplants. Freeze storing of cells and transport of cultures. Mass production of biologically important compounds. Harvesting of products, purification and assays. Propagation of viruses (viral sensitivity of cell lines). Cell cloning and cell synchronization. Separation of cell types: Various methods: advantages and limitations; Flow cytometry. Nuclear transplantation, Cell hybridization, Transfection studies.

UNIT - IV Applications of Tissue Culture (10L)

Commercial applications of animal tissue culture: Tissue culture as a screening system. Cytotoxicity and diagnostic tests. Development and preparation of vaccines against infecting organisms, mammalian cloning.Establishment of cell lines from tissues of genetic diseases.

Workings of a commercial laboratory (Design, aseptic techniques and control of contamination, quarantine, pathological indexing, packaging, cost analysis, marketing).

UNIT - V Transgenic Animals & Applications (5L)

Introduction to transgenic animals, Method of production, Examples of transgenic animals and their commercial applications, ethical issues of transgenic animals.

Suggested Readings:

1. Kuchler, R.J., Biochemical Methods in cell culture and Virology, Dowden, Hutchinson and Ross, Inc. Strausberg, USA, 1977.
2. Morgan, S.I. Animal cell culture, 1993, Bio Scientific Publishers Ltd, Oxford.
3. Freshney, R.I. Culture of Animal cells: A Manual of Basic Technique, 1994, John Wiley and Sons Inc. Publication, USA.
4. Butler, M. Mammalian, cell Biotechnology: A Practical Approach (1991), IRL Press, Oxford.
5. Jenni P. Mather and David Barnes, eds; Animal cell culture Methods, Methods in cell Biology, vol.57, Academic Press.
6. Cell Culture: Methods in enzymology, Vol-58, Academic Press 1979 or recent.

MBT – 402 Industrial & Environmental Biotechnology

UNIT - I Application of enzymes in industrial processing (10L)

Textile Processing, Leather Processing, Paper & Pulp Processing, laundry, Role of Cellulases, hemicellulases Pectinases – pectin methylesterases, pectin depolymerises, glucose oxidase, catalase, lysozyme, sulfhydryl oxidase, glucose isomerise, β -1, 4-galacturonidase in Baking, Fruit Processing, Cereal Extraction, Brewing, Grain Processing, Protein Processing & Flavours, Dairy Processing, enzymes used in meat industry, dairy industry – enzyme from rennet and rennet substitutes, production of aroma and texture, enzymatic synthesis of aspartame, enzymes in starch processing and baking industry.

UNIT - II Biomass processing: (08L)

Production and processing of microbial biomass for nutrition, microorganisms–bacteria, yeast, fungi, algae, production of microbial biomass as nutritional protein source. baker's yeast production, mushroom production, algal biomass production. Enrichment of animal feed–protein enriched starch, protein enriched whey, conversion of lignocelluloses into feed using cellulases and hemicellulases of white-rot fungi, role of phytases, Silage fermentation, from crop residues and by products.

UNIT - III Biotransformations (10L)

Perspectives in Biotransformation, Biotransformations with Lipases, Esterases, Cleavage and Formation of Amide Bonds, Nitriles, Redox Enzymes, alkaloids, Yeast, Dehydrogenases Characteristics, Design of Reaction Conditions, and Applications, Hydroxylation and Dihydroxylation. Solid Waste Treatment, Carbon-Carbon Bond Formation, Addition, Elimination and Substitution Reactions – Lyases, Halocompounds, Phosphorylation.

UNIT - IV Products of Primary and Secondary Metabolism (10L)

Classical Methods, Potential Source of Energy and Chemical Products, Microbial Production of Glycerol and Other Polyols, Microbial Production of Ethanol/Acetone/Butanol/Isopropanol, Microbial Production of 2, 3-Butanediol. Lactic Acid, Citric Acid, Gluconic Acid, Vinegar, PHB. Technical Production and Use of Amino Acids, Nucleotides and Related Compounds, Extracellular Polysaccharides – dextran, xanthan gum, alginate, Biosurfactants.

Products of Secondary Metabolism

General Aspects of Secondary Metabolism, Regulation of Bacterial Antibiotic Production, Screening of Novel Receptor-Active Compounds of Microbial Origin, Antibiotic Biosynthesis,

Cyclosporins: recent developments in biosynthesis, Secondary Products from Plant Cell Cultures & filamentous fungi.

UNIT - V Environmental Biotechnology

(07L)

Composting – physical and chemical factors, microbiology, health risk from pathogens, odour sources, Processing of simple polymer biomass (starch, sugar, oil, protein) from various agricultural crops – grain, cassava, potato and sugar crop –sugarcane, fatty acid and oil containing crops – oil palm. Bioenergy production – Biofuels, pretreatment of biomass, biofuel in the form of gas – hydrogen and methane (biogas), biofuel in form of liquid – ethanol and diesel.

References:

- 1) Moo-Young M. ed. (1985) Comprehensive Biotechnology vol: I & II, Pergamon Press N.Y.
- 2) Ratledge C and Kristiansen B. eds. (2001) Basic Biotechnology 2nd ed. Cambridge Univ Press Cambridge.
- 3) Old R.W and Primose S.D (1995) Principles of Gene Manipulation 5th ed. Blackwell Scientific Pub. Oxford.
- 4) Bailey J.E and Ollis D.F. (1986) Biochemical Engineering Fundamentals 2nd ed. McGraw Hill Book Company, N. Delhi.
- 5) Aiba S, Humphrey A. E. and N. F. Millis (1973) Biochemical Engineering, 2nd Edition University of Tokyo Press, Tokyo, Japan.
- 6) Stanbury P.F., Whitaker A, and Hall S.J. (1997) Principles of Fermentation Technology 2nd ed. Aditya Books Pvt. Ltd, N.Delhi.
- 7) Mukhopadhaya S.N. (2001) Process Biotechnology Fundamentals. Viva Books Pvt. Ltd. N.Delhi.
- 8) Rehm H.J and Reed G. (1985) Biotechnology vol. I & II. VCH, Basel.
- 9) Stainer R. Y. Ingrahm J. L., Wheelis M. L. and Painter P. R. (1987) General Microbiology 5th Edition, Macmillan Press Ltd. London.

MBT- 403 Stem Cell Technology & Regenerative medicine

UNIT - I Introduction: 07

Historical Background, Types of Tissue Culture, Biology of Cultured Cells and the Culture Environment., Cell Adhesion, Cell Proliferation and Differentiation, Energy Metabolism, Incubation and Culture, Laboratory and Equipments - Design, Layout, and Specifications, Laboratory Maintenance

UNIT - II Cell Culture: 08

Sterility and Standard Procedures to be followed, Culture Vessels and Substrates, Media and Supplements- Preparation, Storage, Physicochemical Properties and Validation, Primary Culture-Initiation, Isolation of the Tissue, Types, Sub- Culture, Cell Lines.

UNIT - III Stem Cells: 05

Basics, Properties and Classification. Types of Stem cells – Hematopoietic Stem Cells, Mesenchymal Stem Cells, Embryonic Stem Cells, Fetal Stem Cells, Stem cells from adult organs.

UNIT - IV Stem Cell Technology: 13

Characteristics, Isolation, Culture and Characterization protocols, Extra Cellular Matrices, Morphogenesis and Tissue Engineering, Principles of Tissue Culture, Bioreactor Design, Mechanochemical Regulation of Cell Behaviour, In Vitro and In Vivo Synthesis of Tissues and Organs Micro-Scale Patterning of Cells and their Environment ,Three-Dimensional Scaffolds

UNIT - V Regenerative Medicine: 12

Tissue Engineering and Transplantation Technique, Immunoisolation Techniques , Modes of Cell and Tissue Delivery, Regeneration of Bone and Cartilage, Islet Cell transplantation and Bioartificial Pancreas Bioprinting of Organs and Tissues, Stem Cells in Gastrointestinal , Liver, Pancreas, Kidney, Heart, Spinal Cord and Lung Regeneration, Stem Cells in Eye Diseases and Disorders.

Recommended Text Books:

1. R. Ian Freshney, Culture of Animal Cell; A manual of Basic Technique 5th Ed. 2005,
2. John Wiley.
3. J. M. Davis. Basic Cell Culture, 2nd Ed., 2005, Oxford University Press (OUP).
4. N. Jenkins: Animal Cell Biotechnology; Methods & Protocols, 2005, Humana Press
5. Richard Twyman. Gene Transfer to Animal Cells (Advanced Methods). Taylor & Francis; 1 edition (February 11, 2005)
6. Maureen A. Harrison, Ian F.Rae. General Techniques of Cell Culture. Cambridge

University Press 1997.

7. R. Lanza, J. Gearhart et al (Eds), Essential of StemCell Biology. (2009), Elsevier Academic press.
8. R. Lanza and I. Klimanskaya, Essential Stem Cells Methods. (2009)
9. J. J. Mao, G. Vunjak-Novakovic et al (Ed): Translational Approaches in Tissue Engineering & Regenerative Medicine 2008, Artech House, INC Publications.
10. Robert Lanza et al. Principles of Tissue Engineering, 3rd Edition. Academic Press; 3 edition (August 21, 2007)
11. Stein et al. Human Stem Cell Technology and Biology: A Research Guide and Laboratory Manual. Wiley-Blackwell; 1 edition (January 4, 2011)
12. Lanza et al. Handbook of Stem Cells, Two-Volume Set: Volume 1-Embryonic Stem
13. Cells; Volume 2-Adult & Fetal Stem Cells(v. 1).Academic Press (September 28, 2004)

MBT – 404 Genetic Engineering

UNIT I: Tools for Genetic Engineering- 08

DNA manipulation enzymes- Exonucleases, Endonucleases, Restriction enzymes, Ligase. Gene cloning Vectors-Properties and structure of natural and artificial plasmids, Bacteriophages (λ and M13), Cosmids, animal and plant viruses, Phagmids.

UNIT II: Expression strategies- 06

Various expression vectors in bacteria and eukaryotes- Yeast, Baculoviruse, Mammalian and Shuttle vectors. Induced expression strategies and protocols. Expression of industrially important products.

UNIT III: In Vitro construction, screening and Isolation of rDNA Molecules- 11

Isolation of Vector and donar DNA and its purification, assembly of gene of interest and vector DNA, Amplification of Recombinant DNA Molecules. Definition and introduction to genomic library, Construction of Genomic library. C-DNA library construction, Preparation of primers and probes, Direct Screening, Indirect Screening, Colony hybridization, Immuno-Screening.

UNIT IV: Analytical techniques- 12

RFLP, RAPD, Microarray, DNA chips. Blotting techniques- Southern, Northern, Western. DNA sequencing- Maxam-Gilbert method, Sanger's Dideoxy chain termination method, Automated DNA sequencing method. Genetic and Physical mapping techniques. Principle and applications of transformation methods- Cacl2 method, Electroporation, Microinjection, Gene-gun.

UNIT V: Applications of rDNA technology: 08

Genetic diseases- Detection and Diagnosis, Gene therapy – *ex vivo*, *in vivo*, DNA marker technology in plants, DNA fingerprinting, Genetically engineered biotherapeutics and vaccines and their manufacturing, Transgenic animals and Bio-pharming.

References:-

1. Sambrook J, Fritsch E. F. and Maniatis (1989) Molecular cloning, vol. I, II, III, II nd edition, Cold spring harbor laboratory press, New York.
2. DNA Cloning : A practical approach D.M. Glover and D.B. Hames, RL Press, Oxford, 1995
3. Molecular and cellular methods in Biology and Medicine, P.B. Kaufman, W. Wu , D. Kim and L.J. Cseke, CRC Press Florida 1995

4. Methods in Enzymology Guide to Molecular Cloning Techniques, Vol. 152 S.L. Berger and A. R. Kimmel, Academic Press Inc, San Diego, 1996
5. Methods in Enzymology Gene Expression Technology, Vol. 185D. V. Goedel, Academic Press Inc, San Diego, 1990
6. DNA Science: A First Course in Recombinant Technology, D. A. Mickloss and G. A Freyer, Cold Spring Harbor Laboratory Press, New York, 1990
7. Molecular Biotechnology, 2nd Ed. S. B. Primrose, Blackwell Scientific publishers, Oxford, 1994
8. Milestones in Biotechnology, Classic Papers on Genetic Engineering, J. A. Davis and W. S. Reznikoff, Butterworth-Heinemann Boston 1992
9. Route Maps in Gene Technology, M. R. Walker, and R. Rapley, Blakwell Science, Oxford, 1997
10. Genetic Engineering : An Introduction to Gene Analysis and Exploitation in Eukaryotes, S. M. Kingsman, Blackwell Scientific Publications, Oxford, 1998

PRACTICALS

MBT PR 405 - Genetic Engineering, Stem Cells, Animal & Industrial Biotechnology

1. Isolation of plasmid DNA.
2. *In vitro* DNA ligation
3. Transformation of *E.coli*
4. Southern blotting and hybridization
5. RFLP
6. DNA amplification by PCR
7. Preparation of Cell Culture Media
8. MTT Assay
9. Cryopreservation and Retrieval of Cells
10. Isolation of Primary cells
11. Isolation of cells by enzyme digestion
12. Staining of cell cultures and observations under microscope
13. Virus propagation in cells, cytopathogenic response of cells to viruses.
14. In vitro assay of drugs, predictive test for anticancer drugs.
15. Immuno histochemical staining (oncogene expression).
16. Working of lab bench fermentor
17. Optimization of parameters for growth and product formation
18. Monitoring and control of fermentation parameters
19. Solid state fermentation – biogas production
20. Batch and continuous fermentation
21. Study of Characteristics of bacterial growth
22. Methods of measurement of growth

MBT PR 406 - PROJECT