MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

Ph. D Course Work Papers

Biotechnology

(with effect from the academic year 2018-19 onwards)

Course	Name of the course	Credit	Exam hrs/ week	Marks				
				Maximum			Passing Minimum	
				IA	EA	TOTAL	Ext.	Total
CORE I	Advanced Research Methodology	4	4	25	75	100	38	50
CORE II	Advanced Molecular Biology	4	4	25	75	100	38	50
CORE III	Advanced Bioinformatics	4	4	25	75	100	38	50
CORE IV	Natural Products	4	4	25	75	100	38	50
CORE-V	Advances in Microbial Biotechnology	4	4	25	75	100	38	50
CORE-VI	Molecular Toxicology	4	4	25	75	100	38	50
CORE-VII	Tissue Culture	4	4	25	75	100	38	50

Objectives of the Program

- To equip the scholars with a better understanding in specific area of research
- To enrich the researchers in proper usage of techniques in Biotechnology
- To enlighten the scholars to critically think and perform research and exercising them to write high quality manuscripts and thesis

Eligibility for Admission in Ph.D

M.Sc/M.Phil/M.Tech/LifeSciences/biotechnology/Microbiology/Biochemistry/Environmental Sciences/Environmental Biotechnology through Entrance examination conducted by the University.

Choice of Course Work

1. Candidates with PG qualification should obtain 16 credits as per UGC Regulations in the following options:
   4 Course works of 4 credits each for a total of 16 credits (or)
   3 Course works of 4 credits each & 1 mini project of 4 credits for a total of 8 credits
2. Candidates with M.Phil. Qualification should earn 8 credits as per UGC regulations in the following options
   2 Course works of 4 credits each for a total of 8 credits (or)
   1 Course work of 4 credits & 1 mini project of 4 credits for a total of 8 credits

Mini Project

As per University Norms

Advanced Research Methodology

L	T	P	C
4	0	0	4

Preamble:

To equip the students with the updated methodologies, techniques and instruments.

Outcome:

To obtain a thorough knowledge regarding the reagent preparations, experimental protocols and instruments.

Unit: I: Preparation of Solutions

Types of Solutions - Standard Solutions, Stock Solution, Satuarated Solution, Solution of Acids, Expression of Concentration - Molarity (M), Molality (m), Preparation of one Molar (1M) Solutions, Normality (N), Mass Percent % (w/w), Percentage by Volume or % (v/v), Volume/Weight (V/W), Parts per Million (ppm), Parts per Billion (ppb), pH; Buffers and their preparation. (14L)

Unit: II: Microscopy & Microtechnique

Microscopy - Principle, Working Mechanism and applications of Light, Phase Contrast, Fluorescent, Darkfield, SEM, TEM and STEM. Preparation of Whole mount and sections, staining, mounting and preparation of permanent slides; Cyto and Histochemical techniques. (11L)

Unit: III: Quantitative & Molecular Techniques

Quantification of carbohydrate, protein, lipid, fatty acids and aminoacids (Proximate composition), Estimation of hydrolytic and detoxication enzymes. Molecular Techniques - Principle, mechanism and application of SDS, PAGE, AGE, PCR, RT-PCR; Basic principle and applications of chromatography, UV spectrophotometer. (12L)

Unit: IV: Biostatistics

Parametric - Student T test, F Test, Z -Test, Correlation, Regression and Co-efficient, ANOVA (One-way, Two-way), MANova, Ancova, Non-parametric - Chi-square, Wilcoxon Signes Rank Test, Mann-Whitney Test, Kolmogorov-Snirnow Tests, SPSS, Sigma Plot and MiniTab or Biological data analysis. (13L)

Unit: V: Manuscript, Thesis and Project Writing

Research Processing, Writing of Report, Research paper and Review Articles, Project, Proof Correction - symbols, MS word review option and other tools; Palgiarism Checking, Impact Factor, h index, citation index, Funding agencies - DST, DBT, CSIR, ICMR, ICAR, MoEF, MoEs. (10L)

(Total: 60L)

References:

1. Rodney F. Boyer, 2012. Biochemistry laboratory: Modern Theory & Techniques, Second Edition, Prentice Hall.
2. Rajan Katoch, 2011. Analytical Techniques in Biochemistry & Molecular Biology, Springer, New York.

Advanced Molecular Biology

L	T	P	C
4	0	0	4

Objectives of the paper: The course is to teach the students the following areas: Isolation and characterization of DNA, RNA and protein molecules.

Outcome of the paper: The students will be trained in the area of the characterization of DNA, RNA and protein molecules. The knowledge will be helpful to understand and also solve the molecular level problem in our local community.

Unit 1 (Biomolecules): Isolation of DNA, RNA molecules using Tri- reagent, preparation of protein lysate using different buffer constitutions and the functions of the reagents in the buffers. Purity check of DNA and RNA molecules. Quantification & storage of DNA, RNA and protein molecules.

Unit 2 (PCR): Working concentration & storage of dNTPs & primers. Length of primers. Designing primers for the given DNA fragment for analytical PCR. Designing primer for amplification of DNA fragments for protein over expression purpose. Agarose gel electrophoresis, Reason for the followings: 1. Smear in the PCR amplified product 2. More than one band; 3. Primer dimer, 4. DNA in the well, 5. No band and band in the unexpected size, 6. Primer degradation etc. storage of PCR products. Restriction analysis, cloning, and sequencing of PCR products. Removal of template DNA by Dpn-1. Differences between Taq DNA polymerase, Pfu DNA polymerases and the differences in their PCR products. Gradient PCR and q-PCR

Unit 3 (Proteomics): SDS-PAGE, MALDI-TOF, MS-MS for identification of protein, Immunoblot, primary antibodies, secondary antibodies, fluorescent dyes in different wave length, Horse radish peroxidase and alkaline phosphatase conjugated secondary antibodies. Protein markers and prestained markers.

Unit 4 (Sequencing): Types of DNA sequencing. Next Generation DNA sequencing. Whole exon sequencing, Transcriptome analysis, comparison of cytochrome-c oxidase & 16S RNA molecule and identification of organisms. Finding promoter, intron, exon, ORF, SNPs, mutations and insertion and deletion in a given sequence.

Unit 5 (Manipulations of gene expression): Anti-sense technique, siRNA, micro RNA, pseudo genes, TALEN nuclease and CRISPR Cas9. Difference between the above techniques.

References:

1. Molecular cell biology 7th edition by Harvey Lodish.

2. Principles of gene manipulation and genomics 7th edition by S.B. Primrose.

3. Molecular biology of the cell 5th edition by Bruce Alberts.

4. From Genes to Genomes: Concepts and Applications of DNA Technology 3rd edition by Jeremy W Dale and Malcolm von Schantz.

Advanced Bioinformatics

L	T	P	C
4	0	0	4

Objectives of the paper: The course is to teach the students the genetic relationship with organisms and the structural aspects of biomolecules.

Outcome of the paper: The students will be trained in the area of the molecular evolution and molecular depth of drugs and their applications.

Unit 1 (Comparison of Biomolecules): Basic shell programming & python programming. Different format of DNA, RNA, and protein molecules, Pairwise and multiple aligment tools, BLAST tool and their applications. Identification of new species, SNPs and mutations.

Unit 2 (Genome): Human, mouse, *Drosophila* and *Arabidopsis* genome projects in NCBI, 1000 human genome project, Flybase, and TAIR.

Unit 3 (NGS analysis and Annotation): Next Generation sequencing, NGS data analysis, Assembly: reference based denovo and related tools. Functional annotation and comparison. Differential gene expression & transcriptome analysis

Unit 4 (RNA molecules): structure of RNA, prediction of coding and noncoding RNA & related tools, types of noncoding RNA molecules. RNA editing, guide RNA, designing of siRNA,

Unit 5 (Molecular docking): Analysis of protein sequences, 3D structure of proteins, and structure of ligand in pdb format. Binding efficiency, structure based function predication, uses of different docking tools, Ramachandran plot. Computer aided drug designing (CADD).

References:

1. Bioinformatics: Sequence and Genome Analysis 2nd edition by David W Mount.

2. A Primer of Genome Science 3rd edition by Greg Gibson and Spencer V. Muse.

3. Essential bioinformatics by Jin Xiong.

4. Proteins: Structures and Molecular Properties 2nd edition by Thomas E. Creighton.

5. Molecular Biology of the Gene 7th edition by James D Watson.

Natural Products

L	T	P	C
4	0	0	4

Objectives of the paper: The course is to teach the students the knowledge about natural resources and methods of extraction of valuable products

Outcome of the paper: The students will be trained in the area of understanding & utilization of natural products.

UNIT 1 Bioresources: Biomedical potential of marine and terrestrial natural products – Isolation techniques, structural elucidation techniques and mode of action. Application in various field of biology of Secondary Metabolites isolated from both marine and terrestrial natural products

UNIT 2 Marine resources: Important products isolated from marine organisms and their uses – Agarose, Agar, Alginates, Carrageenans, chitin, chitosons and glucosanins, marine flavourants, Lectins, heparin and carotene. Single cell Protein. Packing and storage.

UNIT 3 Biofuel: Sources of biomass- Ethanol from biomass, Methane from biomass, Hydrogen from biomass.

UNIT 4 Phytochemicals: carbohydrates and derived products - drugs containing glycosides, tannins, lipids (fixed oils, fats and waxes), volatile oils and terpenoids, enzymes and proteins, alkaloids. Biological testing of herbal drugs - Preliminary phytochemical screening for plant products - Qualitative chemical tests - Chromatography (TLC and HPLC).

UNIT 5: Pharmaceutically important products from marine and terrestrial organisms pharmaceutical surfactants, antimicrobial compounds, hormone like materials, vitamins, immunomodulators, anticancer and cytotoxic compounds. NMR, FTIR, Single crystal preparation, X-ray diffraction, 3D structure of compounds.

REFERENCES:

1. Marine natural products: chemical and biological perspectives Paul J. Scheuer Academic Press, 392 pages

2. Bioactive Marine Natural Products Bhakuni, Dewan S., Rawat, D.S. 2005, XV, 400 p.

3. Marine natural products Hiromasa Kiyota, K. Fujiwara, T. Nagata, 2010 - 301 pages

4. Drugs from the Sea, Nobuhiro Fusetani, 2000 - 158 pages

5. Herbal plants and Drugs, Agnes Arber, 1999. Mangal Deep Publications.

6. Contribution to Indian Ethnobotany by Editor S.K.Jain, 1991 Scientific Publishers.

7. New Natural products and Plants drugs with Pharmacological, Biological (or)

8. Therapeutical activity, H.Wagner and P.Wolff, 1979. Springer, New Delhi.

9. Ayurvedic drugs and their plant source, V.V.Sivarajan and Balachandran Indra, 1994. Oxford IBH publishing Co.

Advances in Microbial Biotechnology

L	T	P	C
4	0	0	4

Preamble:

To have an in depth insight into fermentation concepts, understanding the usage of microbes as biocontrol agents, its usage in environment and industries.

Outcome:

To enrich the minds of students with microbes utility in different fields.

Unit 1

Brief history of Fermentation; Fermentation- General Concepts, Applications of Fermentation; Range of fermentation process- Microbial biomass, enzymes, metabolites, recombinant products, transformation process; Component parts of a fermentation process. Fundamentals of Microbial Biotechnology Microbial life: Microbial Cell Cultivation Systems, Cycles of Matter/Microbial Ecology (C, N, S, Fe, Cu, etc.) Methods in Microbial Biotechnology; Recombinant Gene Expression in Prokaryotes and Eukaryotes Protein Engineering .

Unit 2

Types of fermentations- Aerobic and anaerobic fermentation, Submerged and solid state fermentation; Factors affecting submerged and solid state fermentation; Aeration and agitation- Effect of aeration and agitation on fermentation, Oxygen requirement and oxygen supply, Oxygen transfer kinetics; Determination of KLa value; Effect of agitation and microbial biomass on KLa value; Newtonian and non-Newtonian fluids; Foam and antifoams, their effect on oxygen transfer; Fermentation economics.

Unit 3

Microbes as Biocontrol Agents (Baculoviruses, entomopathogenic fungi, *Bacillus thurinigiensis*, *Bacillus sphaericus*, *Bacillus popilae*, Microbe derived inhibitors Biology of nitrogen fixation, preparation of different Types of inoculants (nitrogen fixers phosphate solubilizers, plant growth promoting Rhizobacteria, PGPR), composting.

Unit 4

Introduction to the use of microbes in environmental Applications, Bioremediation, bioaugemntation, Bioemulsifiers, biosurfactants, MEOR, Leaching of ores, Microbial Fuels (Methane, Hydrogen), Functional Metagenomics , syntrophic biodegradation of hydrocarbon contaminants.

Unit 5

Microbial production of organic acids, solvents and beverages (Citric acid, acetic acid, ethanol, acetone-butanol, beer, wine) therapeutic agents,(Streptomycin, cephalosporin, Anticancer agents , Vaccines aSiderophores, Ergot alkaloids),enzymes, vitamins and amino acids(proteases, amylases and lipases , B2 and B12, lysine, glutamic acid and tryptophan) and other microbial products(Microbial polysaccharides: Xanthan and Dextran, Biosurfactants, Steroid transformation, Polyhydroxyalkanoates: PHA and PHB).

References:

1. Stanbury, P. F., Whitaker and Hall, A. S. J., Principles of Fermentation Technology. Butterworth-Heinemann.
2. Shuler, M.L. and Karg, I F., Bioprocess Engineering Basic Concepts, Prentice Hall.
3. Microbial Biotechnology by A. N. Glazer and H. Nikaido.
4. SubbaRao, N. S. (1999) Soil Microbiology Science Pub Inc.

Molecular Toxicology

L	T	P	C
4	0	0	4

Preamble:

To introduce students about the chemicals and metabolic toxicity with the aim to provide sufficient knowledge about the technologies involved in toxicity assessment.

Outcome:

To nurture the minds with the lethal dose informations which is mandatory for any toxicological experiments.

UNIT I

Introduction to Toxicology: Various types of toxicity (Acute, subacute, subchronic and chronic). Chemical interactions (Additive effect, potentiation, synergism and antagonism), Dose response relationship (ED50, LD50 EC50, LC50.)

UNIT II

Routes of exposure, absorption, distribution, elimination. IN VITRO and IN VIVO models in toxicological studies. Toxicity - Factors affecting toxicity. General concepts in toxicology; Passage of a chemical through the body absorption, distribution, metabolism, Excretion.

UNIT III

Role of Phase I metabolism in toxicity: Introduction, Cytochrome P450-mediated Phase I metabolism; Flavin monooxygenase-mediated Phase I metabolism. Role of Phase II metabolism in toxicity: Introduction, Glucuronide conjugation; sulphate conjugation; Glutathione conjugation.

UNIT IV

Co-ordinated responses to toxicity: Introduction, Immediate responses to toxic insult, coordination of the response to reactive chemicals, repair of cellular damage, regulation of apoptosis and necrosis. Role of genetics in toxic response: introduction, mechanisms of genetic control, tools for studying genetic responses to toxic insult.

UNIT V

Technologies for toxicity testing: Genomics-analysis of variation within the genome, Reporter gene assays, Transgenics; Transcriptomics-Microarray analysis, real time quantitative RT-PCR; Proteomics- 2D-gel electrophoresis, MALDI-TOF mass spectroscopy, protein chip analysis; Metabonomics; Bioinformatics.

References:

1. Subramanian, M.A., 2004. Toxicology Principles and Methods, MJP Publishers, Chennai.
2. Plant, N. (2003). Molecular Toxicology. Bios Scientific Publishers, New York.
3. Hodgson, E. and Smart, R.C. (2001). Introduction to Biochemical Toxicology. John Wiley & Sons,Inc. New York.
3. Keohavong, P. and Grant, S.G. (2005). Molecular toxicology Protocols. Humana Press, New York.

4. Josephy, P.D. and Mannervik, B. (2006). Molecular Toxicology. Oxford University Press.

Tissue Culture

L	T	P	C
4	0	0	4

Objectives

The goal of Cell Culture Techniques is for Scholars to identify and understanding the novel research findings through the necessary practical skills for the isolation of both animals and plant cells for in vitro studies, maintenance and manipulation of animal and plant cells in vitro and in vivo, and application of molecular techniques to in vitro situations.

Learning Outcome

- Develop basic aseptic skills for vertebrate and invertebrate cell culture.
- Understand media constituents and media formulation strategies for cell culture.
- Develop proficiency in vertebrate primary cell culture and the maintenance of cell lines.
- Apply cell and molecular techniques to in vitro situations.
- To know the different methods and equipments employed in the scale-up of animal and plant cell culture.

Unit I

Course Introduction - Animal models vertebrates and invertebrates Rabbit, Mouse, Drosophilla, Zebrafish, Earthworm, Bacteria, Fungi. Media Formulations for Cell Culture, Importance of Serum and Serum Free media, Preparation of primary cells from invertebrate, Insect cell culture, overview.

Unit II

Techniques and methods in animal cell culture- Animal Cell Culture: Historical Background, Importance and progress in Animal Cell Culture Technology, Biology of Animal Cells and cultured cells, Types of cells, Cellular Interactions, Organo-typic culture and specialized cell culture techniques, Maintaining the culture, Producing cell lines of a particular cell type, Quantization of cells in cell culture, harvesting of cells, Cell viability determination, characterization, differentiation and transformation cells, Culturing and Sub-Culturing of Animal

Cells, Growth Parameters, Primary Cell Culture Principles and Procedures - Primary cell culture: Keratinocytes; Adipocytes, Hepatocytes; Lymphocytes. Long term and short term storage of cells and reviving cells from cryo-preservation. cell culture application in pharmaceutical research.

Unit III

Basic techniques in plant tissue culture - Introduction to plant Cell & Tissue Culture. Design & lab setup of Tissue Culture laboratory, Tissue culture Media (Composition preparation), Types of culture. Role of Plant Hormones in growth & development of Plants. Micro propagation (Organogenesis, Somatic Embryogenesis, Shoot tip culture, Rapid clonal propagation, Embryo Culture & Embryo Rescue, Acclimatization of Plants) *In vitro* mutagenesis. Cryopreservation, Slow growth & DNA Banking for germ plasm conservation.

Unit IV

Plant cell culture, plant transformation technology & its applications. 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. Applications of Plant Transformation for Productivity & performance Herbicide resistance like atrazine, Insect resistance Bt gene, non Bt like protease inhibiters, Virus resistance, disease resistance, antibiotic stress, post harvest losses long shelf life of fruits & flowers. Chloroplast transformation, Advantage vectors & success with tobacco & potato Metabolic engineering & Industrial products.

Unit V

Cell Immortalization – Steps and process of species specific cell immortalization, Methods of immortalization: integration of SV40, hTERT, HPV E6/E7, EBV, and MycT58A, RasV12, and $p53^{-/-}$ Cell Immortalization Systems, impacts of immortalization in animal cells, Applications of immortalized cells in clinical research. Ongo genes and tumor suppressor genes.

References

1. Morgan, S. I. Animal cell culture, 1993,Bio Scientific Publishers Ltd, Oxford.
2. Freshney,R.I.Culture of Animal cells:A Manual of Basic Technique, 1994, John Wiley and Sons Inc. Publication, USA.

3. Butler, M.Mammalian, cell Biotechnology: A Practical Approach (1991), IRL Press, Oxford.
4. Jenni P.Mather and David Barnes, eds; Animal cell culture Methods, Methods in cell Biology, vol.57, Academic Press.
5. Cell Culture: Methods in enzymology,Vol-58,Academic Press 1979 or recent.
6. An introduction to Plant Tissue Culture 2nd edn. Razdan, M. K, Science Publishers, USA.
7. Textbook of plant biotechnology, Chawla P.K.2002,Oxford&IBH,New Delhi.
8. Bhojwani, S. S. and M. K. Razdan 1996.Plant Tissue Culture: Theory and Practice, Elsevier Pub.
9. Chrispeels, M. J. 2002.Plant Tissue Culture:Genetical Aspects. Jones and Bortlett Publishers, International.
10. Chopra V. L. et al 1999. Applied Plant biotechnology. Science Publishers Inc.
11. Verpoorte, R. and A.W. Alfermann (Eds) 2000.Metabolic Engineering of plant secondary metabolism, lower Academic Publisher.