PARVATIBAI CHOWGULE COLLEGE OF ARTS AND SCIENCE, (AUTONOMOUS)

DEPT. OF BIOTECHNOLOGY

TYBSc BIOTECHNOLOGY

SYLLABUS (Sem V & VI)

for

ACADEMIC YEAR

2020 - 2021

COURSE STRUCTURE

SEMESTER	CORE		ELECTIVE			
I	**BIO-I.C-1** Biomolecules	**BIO-I.C-2** Cell Biology	--------	--------	--------	--------
II	**BIO-II.C-3** Fundamental Genetics	**BIO-II.C-4** Basic Microbiology	--------	--------	--------	--------
III	**BIO-III.C-5** Molecular Biology		**BIO-III.E-1** Basics of Plant and Animal Sciences	**BIO-III.E-2** Metabolism of Biomolecules	**BIO-III.E-3** Biostatistics	**BIO-III.E-4** Enzymology
IV	**BIO-IV.C-6** Immunology		**BIO-IV.E-5** Plant and Animal Physiology	**BIO-IV.E-6** Tools & Techniques in Biotechnology	**BIO-IV.E-7** Evolution and Anthropology	**BIO-IV.E-8** Molecular genetics
V	**BIO-V.C-7** Concepts in Genetic Engineering		**BIO-V.E-9** Molecular medicine	**BIO-V.E-10** Environmental Biotechnology	**BIO-V.E-11** Plant Biotechnology	**BIO-V.E-12** Bioinformatics
VI	**BIO-VI.C-8** Industrial Biotechnology		**BIO-VI.E-13** Bioethics and Biosafety	**BIO-VI.E-14** Advanced Cell Biology	**BIO-VI.E-15** Food Biotechnology	**BIO-VI.E-16** Animal Cell Culture

BIO-V.C-7: CONCEPTS IN GENETIC ENGINEERING

COURSE TITLE: CONCEPTS IN GENETIC ENGINEERING (THEORY)
COURSE CODE: BIO-V.C-7
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45
PRE-REQUISITES: Completion of BIO-III.C-5- Molecular Biology

Course Objective

The course aims to introduce the students to the principles and techniques involved in Genetic Engineering through the use of genetic material and vehicles for suitable manipulation of genes.

Course Outcomes

On the successful completion of the course, students will be able to:
CO1: understand the functions of several enzymes and vectors used in cloning.
CO2: acquaint to the versatile tools and techniques employed in recombinant DNA technology.
CO3: Explain the construction of DNA & c DNA library
CO4: Procure skills for selection of recombinants
CO5: Acquire skills on techniques of plasmid isolation

BIO-V.C-7: CONCEPTS IN GENETIC ENGINEERING (THEORY)

Module I **15 hrs**

Introduction to genetic engineering

2 hrs

Aims; principles; applications; ethical issues involving recombinant DNA technology and genetic engineering

DNA modifying enzymes **3 hrs**

Nucleases- endonucleases (restriction enzymes recognition sequences, cleavage pattern); exonucleases**;**DNA ligases**;**reverse transcriptases**;**polynucleotide kinases**;**alkaline phosphatases**;**nucleotidyltransferases

Vehicles for gene cloning **10 hrs**

Vectors - properties of ideal cloning vectors**;** plasmids – properties, classification**;** Vector for Prokaryotes - pBR322, pUC 18**;** bacteriophages as cloning vectors - lambda bacteriophages; features-insertional vectors and replacement vectors & M13 Bacteriophage; cosmids, phagemids and phasmids- definition, features with examples; vectors for cloning in *Saccharomyces cerevisiae* (examples and features)**;** shuttle vectors - any one example; vectors for plant – Ti plasmid

Module II **15 hrs**

DNA insertion into vector **3 hrs**

Ligation; linkers; adaptors, homopolymer tailing

Transformation methods **8 hrs**

Methods, advantages and disadvantages: competence (transformation in bacteria); microinjection; lipofection; electroporation; macroinjection; sonication; silicon carbide fibre vortex; DNA co-precipitation; ultrasonication; laser induced; *Agrobacterium* mediated transfers

Identification of recombinants **4 hrs**

Principle and importance of identification of recombinants; antibiotic resistance (amp, tet resistance); lacZ selection; colony hybridization; *cI*selection

Module III **15 hrs**

DNA isolation methods and analysis **5 hrs**

Isolation of genomic DNA & plasmid DNA; principle of plasmid isolation; spectrophotometric analysis of DNA; agarose gel electrophoresis; purification of DNA

Amplification of nucleotide sequences **3 hrs**

Polymerase chain reaction (principles, components & method of PCR)

DNA sequencing **5 hrs**

Significance and importance of DNA sequencing**;** Maxam Gilbert's method**,** Sanger's method**,** Automatic DNA sequencer

Genomic / cDNA libraries **2 hrs**

Preparation of genomic library; cDNA library**;** Screening of libraries

BIO-V.C-7: CONCEPTS IN GENETIC ENGINEERING (PRACTICAL)

COURSE TITLE: CONCEPTS IN GENETIC ENGINEERING (PRACTICAL)
COURSE CODE: BIO-V.C-7
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. Plasmid DNA isolation by alkaline lysis method
2. Plasmid DNA isolation by boiling method
3. Plasmid DNA separation on agarose gel
4. Molecular size determination of the plasmid
5. Preparation of competent cells in bacteria
6. Transformation in bacteria using suitable plasmid (pUC 18)
7. Selection of transformed colonies
8. Deciphering the DNA sequence from a sequencing gel photograph by Maxam and Gilbert's method and Sanger's method
9. Demonstration of Polymerase Chain Reaction (PCR)

REFERENCES

1. Brown, T.A. (2006) Manipulation of purified DNA. In:Gene cloning & DNA analysis An Introduction, 5th Ed. Blackwell publishing, Ltd, UK
2. Jogdand, S.N. (2008). Gene Biotechnology, 2nd edition, Himalaya Publishing House, Mumbai.
3. Primrose, S.B. & Twyman, R.M. (2009). Principles of Gene Manipulation and Genomics, Blackwell Publishing.
4. Purohit, S.S. (2009). Biotechnology: Fundamentals and Applications, Student Edition.
5. Singh, B.D. (2008). Biotechnology: Expanding Horizons, Kalyani publishers.
6. Verma P.S and Agarwal V.K. (2009). Genetic Engineering, S.Chand & Company LTD, New Delhi.
7. Watson, J.D., Tooze, J. & Kurtz, D.T. (1983). Recombinant DNA: A short Course, Scientific American Books (WH Freeman), New York.

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1. https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/v/dna-sequencing (DNA sequencing)
2. https://www.khanacademy.org/science/high-school-biology/hs-molecular-genetics/hs-biotechnology/v/the-polymerase-chain-reaction-pcr (PCR)
3. https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/bacterial-transformation-selection (Transformation in bacteria using pUC 18)

BIO-V.E-9 MOLECULAR MEDICINE

COURSE TITLE: MOLECULAR MEDICINE (THEORY)
COURSE CODE: BIO-V.E-9
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45
PRE-REQUISITES: Completion of BIO-IV.E-8 -Molecular Genetics

Course Objective

Molecular medicine is the application of molecular biology and molecular genetics to the understanding of human health and disease. It aims to understand the underlying origins and mechanisms of human diseases and to find novel ways of preventing, diagnosing and treating diseases

Course Outcomes

On the successful completion of the course, students will be able to:
CO1: Understand the historical aspects of molecular medicine
CO2: Understand the underlying genetic factors of common diseases
CO3: Describe molecular and cellular therapies for the same

CO4: Gain a basic knowledge on cancer genetics and pharmaco-genetics
CO5: Understand the importance of maintaining public health

BIO-V.E-9 MOLECULAR MEDICINE (THEORY)

Module I **15 hrs**

Historical aspects **2 hrs**

History of molecular medicine – foundations (1869 – 1980s); the modern era (1980s – 2000s); The Human Genome project (1990 – 2000)

Gene structure and expression **3 hrs**

Exons, introns, alternative splicing, epigenetic changes

Genetic factors in common diseases **6 hrs**

Hypertension; coronary heart disease; autism; alzheimer disease; haemochromatosis; age-related macular degeneration

Complex genetic traits **4 hrs**

Multifactorial disorders – diabetes, dementia, schizophrenia; novel mechanisms for DNA and disease – mitochondrial inheritance, genomic imprinting, mosaicism, chimerism

Module II **15 hrs**

Cancer genetics **5 hrs**

Differentiation between genetic and environmental factors in cancer; oncogenes – types and function; tumour-suppressor genes – "two hit hypothesis"; genetics of common cancers – breast, ovarian and prostate cancer

Introduction to Omics **3 hrs**

Genomics, Proteomics, Metabolomics, Phenomics, Metagenomics

DNA Tests **4 hrs**

Direct Detection; indirect detection - DNA scanning; linkage analysis; classes of DNA tests and function of each type; validity of DNA tests

Delivering genetics and genomics to consumers **3 hrs**

Definitions and marketplace, types of direct-to-consumer (DTC) DNA tests; Pros and Cons of DTC DNA Tests

Module III **15 hrs**

Molecular and cellular therapies **8 hrs**

Recombinant DNA products – Factor VIII (Haemophilia); vaccines; somatic cell gene therapy; examples of gene therapy trials – ADA, haemophilia, cancer, eye disease, HIV; RNA therapies – RNA interference (RNAi), ribozymes; regenerative medicine – cloning, stem cells.

Pharmacogenetics **3 hrs**

Drug metabolism; genetic variations revealed by effects of drugs; pharmacogenetics– maturity -onset diabetes of the young (MODY); neonatal diabetes; pharmacogenomics; adverse effects; efficacy

Public health **4 hrs**

Preventive medicine; population screening (cystic fibrosis, sickle cell anaemia, newborn screening); changing behaviour (familial hypercholesterolemia); DNA testing in the workplace – predisposition to disease; detecting exposure to toxins; litigation, identity

BIO-V.E-9: MOLECULAR MEDICINE (PRACTICAL)

COURSE TITLE: MOLECULAR MEDICINE (PRACTICAL)
COURSE CODE: BIO-V.E-9
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. Investigation of Genetic Factors in any four common diseases
2. Study of mitochondrial inheritance, genomic imprinting, mosaicism and chimerism with one example of each
3. A study on the types of DNA tests for diagnosis of diseases
4. Investigation of Molecular Mechanisms of any one type of Cancer
5. Understanding concepts relating to genomics and proteomics
6. A study on RNA therapies and regenerative medicine
7. Application of pharmacogenetics in drug metabolism
8. An investigation into the screening programmes adopted in various countries
9. Submission of a report on the molecular mechanisms and therapy for any one disease

REFERENCES

1. Trent, R.J. (2005). Molecular Medicine – an Introductory Text, Elsevier Academic Press.
2. Trent, R.J. (2012). Molecular Medicine – Genomics to Personalized Health Care, Fourth Edition, Elsevier Inc.
3. Turnpenny, P.D. & Ellard, S. (2007). Emery's Elements of Medical Genetics, 13th Edition, Churchill Livingstone Elsevier.

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2. https://onlinelibrary.wiley.com/doi/abs/10.1002/bies.201400138 (Eukaroytic gene expression)
3. https://onlinelibrary.wiley.com/doi/full/10.1002/wrna.1276 (Alternative splicing)
4. http://journals.tubitak.gov.tr/medical/issues/sag-15-45-5/sag-45-5-3-1406-146.pdf (genetic factors in Alzheimer's disease and age related macular degeneration)
5. https://www.spandidos-publications.com/br/7/2/105 (genetic factors in Alzheimer's disease)

6. https://www.sciencedirect.com/science/article/pii/S014067361501315X (genetic factors in hemochromatosis)
7. https://link.springer.com/article/10.1007/s10815-017-0895-5 (Genomic imprinting)
8. https://www.sciencedirect.com/science/article/abs/pii/S0168952515000669 (Mosaicism review)
9. https://www.sciencedirect.com/science/article/abs/pii/S0090825817300744 (Cancer genetics)
10. https://www.nature.com/articles/nrg.2018.4 (Omics)
11. https://www.nature.com/articles/nrg3908 (DNA testing Linkage analysis)
12. https://onlinelibrary.wiley.com/doi/abs/10.1002/ajmg.c.31390 (Pharmacogenetics)

BIO-V.E-10: ENVIRONMENTAL BIOTECHNOLOGY

COURSE TITLE: ENVIRONMENTAL BIOTECHNOLOGY (THEORY)
COURSE CODE: BIO-V.E-10
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45

Course Objective

The main aim of this course is to introduce the students to the hazards of our environment, the effects of pollution on living systems, solutions to protect the environment for sustainable development.

Course Outcomes

On the successful completion of the course, students will be able to:
CO1: Explain the scope of Environmental Biotechnology.
CO2: Understand the basic ecological concepts, various pollution, its measurements & remediation.
CO3: Describe the various eco-friendly bio-products.
CO4: Assess the quality of the water sample through various parameters like MPN test, dissolved oxygen concentration, biological oxygen demand, chemical oxygen demand and nitrates of water sample.
CO5: Understand the working of sewage treatment plant.

BIO-V.E-10: ENVIRONMENTAL BIOTECHNOLOGY (THEORY)

Module I **15 hrs**

Basic ecological concepts and principles **3 hrs**

Structure (biotic and abiotic components); food chains and food webs; ecological pyramids; productivity and eco-energetic (10% law)

Anthropogenic activities, its effects and control **12 hrs**

Air pollution: Major air pollutants and their sources, Impacts of air pollution on human health, animals, plants and climate; removal of gaseous contaminants and odour: bioscrubbers, biotrickling filters and biofilters/biobeds

Water pollution: Principal forms of water pollutants and their sources;wastewater treatment: activated sludge process, rotating biological discs, oxidation ponds, trickling filters

Soil pollution: Soil pollution and their sources; treatment of solid wastes: hazardous; non hazardous; composting and vermi-technology

Module II **15 hrs**

Pollution monitoring **10 hrs**

Bio indicators: concept and examples (indicators of water quality; air pollution indicators); choice of criteria: visual rating; genotoxicity; metabolic rating; applications (two each); using plant test systems and animal test systems; tests for assessing Genetic damage: AMES test; cyto-genetic assay; membrane damage; concept and applications of molecular biology in environmental monitoring: reporter gene: concept and applications of biosensors in pollution detection

Pollution abatement: Bioremediation **5 hrs**

Bioremediation: definition, microbial bioremediation, phytoremediation; microbial desulphurization of coal (direct and indirect mechanisms)

Module III **15 hrs**

Pollution abatement: biodegradation **6 hrs**

Biodegradation: basis of biodegradation, concepts of use of mixed microbial populations; Biodegradation of two xenobiotics: aromatic hydrocarbons (benzene) and alkanes

Biosorption: principle; use of fungi and algae (2 examples each); genetically engineered microorganisms - superbug (*Pseudomonas* sps.)

Eco-friendly Bio-products **7 hrs**

Biogas (biomethanisation) production; bioethanol production; bio hydrogen production: anaerobic bacteria and photolysis photosynthetic algae; biodiesel production; bioplastics: biopol and biolac; biopesticide

Scope of environmental biotechnology **2 hrs**

Scope of environmental biotechnology

BIO-V.E-10: ENVIRONMENTAL BIOTECHNOLOGY (PRACTICAL)

COURSE TITLE: ENVIRONMENTAL BIOTECHNOLOGY (PRACTICAL)
COURSE CODE: BIO-V.E-10
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. Determination of dissolved oxygen concentration of water sample by Winkler's method
2. Determination of biological oxygen demand (BOD) of the given sample

3. Determination of chemical oxygen demand (COD) of the given sample ($KMnO_4$/ $K_2Cr_2O_7$ method)
4. Determination of TS (total solids) of the given water sample
5. Isolation of xenobiont degrading bacteria by selective enrichment
6. Determination of nitrates from water sample
7. Visit to an effluent /sewage treatment plant and preparation of report
8. Detection of coliforms for determination of the purity of potable water (MPN, Presumptive, confirmatory and confirmed tests)

REFERENCES

1. Agarwal S.K. (2009). Environmental Biotechnology, APH Publishing Corporation New Delhi.
2. Anjaneyulu Y. (2005). Introduction to environmental Science, BS publications, India.
3. Chatterji A.K. (2009). Introduction to Environmental Biotechnology, 2nded, Prentice Hall of India Pvt. Ltd. New Delhi.
4. Jogdand B.N. (2008). Environmental Biotechnology (Industrial Pollution Management), Himalaya Publishing House, Mumbai.
5. Santra S.C. (2001). Environmental Science, New central book agency (P) Ltd. Calcutta.
6. Singh B.D. (2008). Biotechnology, 3rd edition, Kalyani Publishers.
7. Thakur I.S. (2006). Environmental Biotechnology: Basic concepts and applications, I.K. International Pvt. Ltd. New Delhi.

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1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2781844/ (Anthropogenic activities and its effects)
2. https://www.khanacademy.org/science/high-school-biology/hs-ecology/hs-human-impact-on-ecosystems/a/hs-human-impact-on-ecosystems-review (Anthropogenic activities and its effects)
3. https://www.sciencedirect.com/book/9780128000212/microbial-biodegradation-and-bioremediation (Anthropogenic activities and its effects)
4. https://www.intechopen.com/books/biodegradation-life-of-science/biodegradation-involved-microorganisms-and-genetically-engineered-microorganisms (Bioremediation & biodegradation)
5. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4463667/ (Bioremediation & biodegradation)
6. https://www.intechopen.com/books/biofuels-state-of-development/prospective-biodegradable-plastics-from-biomass-conversion-processes (Eco-friendly Bio-products)
7. https://www.epa.gov/ingredients-used-pesticide-products/what-are-biopesticides (Eco-friendly Bio-products)
8. https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/biopesticide (Eco-friendly Bio-products)

BIO-V.E-11: PLANT BIOTECHNOLOGY

COURSE TITLE: PLANT BIOTECHNOLOGY (THEORY)
COURSE CODE: BIO-V.E-11
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45

Course Objective

This course aims at introducing the concept of in vitro culture of plants including set up of a plant tissue culture laboratory, instruments and sterilization techniques. This course will help the students to understand that various parts of the plant may be cultured, with each type of culture having specific applications. Plant tissue culture also lends itself for production of transgenic plants which have various applications.

Course outcomes

On the successful completion of the course, students will be able to:
CO1: This paper aims at introducing the concept of in vitro culture of plants including set up of a plant tissue culture laboratory, instruments and sterilization techniques.
CO2: This paper will help the students to understand that various parts of the plant may be cultured, with each type of culture having specific applications.
CO3: Plant tissue culture also lends itself for production of transgenic plants which have various applications.
CO4: On completion of this module, the student will be able to understand all about plant biotechnology in terms of set up of a laboratory, culture of explants
CO5: In addition, the students will be able to understand genetic engineering methods for production of transgenic plants.

BIO-V.E-11: PLANT BIOTECHNOLOGY (THEORY)

Module I **15 hrs**

History of plant tissue culture **2 hrs**

International and Indian scientists

Laboratory organization **4 hrs**

Washing and drying facility; general laboratory and media preparation area; transfer area; culture room; growth chambers and green house (ideal conditions for incubation and maintenance of cultures/plants).

Sterilization techniques **2 hrs**

Sterilization techniques used in plant tissue culture – steam, dry, filter, ultra violet, alcohol, flame and chemical (explants)

Plant tissue culture media **4 hrs**

Major and minor inorganic nutrients; vitamins; carbon source; hormones; complex organic additives and their functions;composition of some commonly used plant tissue culture media – MS, White's, Nitsch, Gamborg B5

Totipotency **2 hrs**

Totipotency and its Importance; Various parts of the plant serving as Explants

Organogenesis **1 hr**

Root and shoot regeneration and applications

Module II **15 hrs**

Organ culture and its applications **5 hrs**

Root; shoot tip/meristem; anther and pollen; ovary and ovule embryo

Callus and cell suspension cultures **4 hrs**

Callus culture – principle; characteristics of callus tissue; applications; cell suspension culture – principle; isolation; growth patterns; concept of batch and continuous culture; viability testing

Somaclonal variation **2 hrs**

Concept; isolation of variants; mechanisms of somaclonal variation and applications

Somatic embryogenesis and artificial seeds **2 hrs**

Somatic embryogenesis – principle; procedure and applications; artificial seeds – methods of production and applications

Applications of Tissue Culture in Plant Sciences **2 hrs**

Micropropagation; gene conservation banks; forestry

Module III **15 hrs**

Protoplast culture and somatic hybridization **4 hrs**

Protoplast culture – principle; isolation of protoplasts (mechanical and enzymatic); methods of culture; checking viability; somatic hybridization - protoplast fusion (spontaneous and induced); selection of hybrid protoplasts; applications of somatic hybridization

Production of secondary metabolites **2 hrs**

Classification of secondary metabolites with examples; production using culture methods - callus culture; cell suspension culture; hairy root culture (A. rhizogenes); immobilized cell systems

Gene transfer in plants **4 hrs**

Introduction to Agrobacterium tumefaciens and Ti plasmid; Agrobacterium based vectors (cointegrate and binary vectors); co-culture method and in plant transformation; direct methods of gene transfer – electroporation, chemical methods, particle gun method and microinjection

Applications of transgenic plants **5 hrs**

Insect resistance (BT toxin); drought and salt tolerance; herbicide resistance; increasing shelf life of fruits; improvement of vitamin content (golden rice) and edible vaccines

BIO-V.E-11: PLANT BIOTECHNOLOGY (PRACTICAL)

COURSE TITLE: PLANT BIOTECHNOLOGY (PRACTICAL)
COURSE CODE: BIO-V.E-11
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. Washing, Packing and Sterilization of Glassware
2. Preparation of Stock solutions for Murashige and Skoog (MS) medium
3. Preparation, sterilization and pouring of MS medium
4. Aseptic germination of seedling
5. Callus induction from hypocotyl and carrot cambial explants and subculturing
6. Shoot tip culture
7. Regeneration of shoot/root from callus
8. Setting up of cell suspension culture and checking viability by Evan's blue method
9. Setting up an in vitro culture from seed embryo (embryo culture)
10. Encapsulation of somatic/true embryo (synthetic seeds) and Regeneration of Plants from Synthetic Seeds

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1. Chawla, H.S. (2002) Introduction to Plant Biotechnology, Science Publishers Inc. USA.
2. De, K.K. (2008) Plant Tissue Culture, New Central Book Agency Pvt. Ltd.
3. Jha, T.B. & Ghosh,B. (2005) Plant Tissue Culture, University Press (India) Pvt. Ltd.
4. Singh, B.D. (2005) Plant Biotechnology, Kalyani Publishers.

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1. https://www.sciencedirect.com/science/article/abs/pii/S0140196301908845 (Tissue culture technology)
2. https://www.researchgate.net/publication/272493719_Plant_Cell_Tissue_and_Organ_Culture_Biotechnology_and_Its_Application_in_Medicinal_and_Aromatic_Plants (organ culture)
3. https://link.springer.com/article/10.1007/BF02632054 (transgenic plants)
4. https://www.nature.com/articles/nbt0188-56 (protoplast culture)
5. https://link.springer.com/chapter/10.1007/978-981-10-2961-5_2 (applications)

BIO-V.E-12: BIOINFORMATICS

COURSE TITLE: BIOINFORMATICS (THEORY)
COURSE CODE: BIO-V.E-12
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45

Course Objective

This Course aims at introducing the importance of the basics of computers, concept of Human Genome Project, storage of biological information and tools and techniques of bioinformatics used and their importance in the field of biotechnology.

Course Outcomes

On the successful completion of the course, students will be able to:
CO1: Explain the scope of Bioinformatics
CO2: Understand the basic concept of biological databases, various types and applications of biological databases.
CO3: Describe the various applications of BLAST and FASTA in understanding differences in evolutionary patterns
CO4: Assess the mutations and genetic disorders and understand the importance of drug design in silico
CO5: Will be able to construct evolution tree, cladogram, retrieve and biological information accessed through various information resources.

BIO-V.E-12: BIOINFORMATICS (THEORY)

Module I **15 hrs**

Introduction to Computers in Biology **3 hrs**

Introduction to use of computers, internet and software in biology; Role of computers in medicine and research

DNA, RNA and Proteins and HGP **5 hrs**

Background of DNA, RNA and Proteins, ORF**;** Review of transcription and translation**;** Introduction to HGP; objectives; achievements of HGP; Ethical and Social issues

Introduction to bioinformatics **3 hrs**

Definition; scope of bioinformatics; bioinformatics vs computational biology; components of bioinformatics and applications

Information resources **4 hrs**

Introduction and objectives of NCBI, NLM, NIH, EBI and SRS

Module II **15 hrs**

Biological databases **7 hrs**

Types of data and biological databases; Primary databases: GenBank, EMBL, DDBJ; Secondary databases: Swiss-PROT, PDB & PIR; Composite databases: OWL & PROSITE

Structural databases **5 hrs**

X-ray crystallography, PDB, MMDB, CATH and SCOP; Visualization of proteins – Cn3D and Rasmol

Literature databases **3 hrs**

Pubmed; MedLINE and OMIM

Module III **15 hrs**

BLAST and FASTA **4 hrs**

Introduction to BLAST and FASTA and their types

Sequence alignment tools **6 hrs**

Sequence alignment - Pairwise and Multiple; Clustal-W Omega; T-coffee

Phylogeny **5 hrs**

Introduction to phylogeny and cladistics; Cladogram and Phylogenetic tree construction; structure and types of phylogenetic trees; differences between cladogram and phylogenetic tree; Applications of phylogeny.

BIO-V.E-12: BIOINFORMATICS (PRACTICAL)

COURSE TITLE: BIOINFORMATICS (PRACTICAL)
COURSE CODE: BIO-V.E-12
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. Introduction to Bioinformatics & its Applications
2. Study of Human Genome Project
3. Usage of NCBI resources
4. Biological data search using NCBI – Protein or amino acid sequences
5. Biological data search using NCBI – DNA or gene sequences
6. Biological data search using NCBI – Literature & Structure databases
7. Database search & Pairwise sequence alignment using NCBI BLAST :BLASTp&BLASTn
8. Multiple sequence alignment using Clustal-W
9. Construction of phylogenetic tree using Clustal-W
10. DNA sequence analysis to find restriction enzymes sites using NEBcutter
11. Visualization of protein structures using Cn3D/ Rasmol

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1. Harisha, S. (2007). Fundamentals of Bioinformatics, I.K. International Publishing House, Mumbai.
2. Ignacimuthu, S. (2005). Basic Bioinformatics, Narosa Publishing House, New Delhi.
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4. Murthy,C.S.V. (2003). Bioinformatics, Himalaya Publishing House, Mumbai.
5. Rastogi, S.C., Mendiratta, N. &Rastogi, P. (2004). Bioinformatics: Concepts, Skills and Applications, CBS Publishers.
6. Xiong, J. (2006). Essential Bioinformatics, Cambridge University Press.

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2. www.pellegrini.mcdb.ucla.edu › wp-content › uploads › sites › 2017/07 (Phylogenetic tree construction)
3. https://vlab.amrita.edu/?sub=3&brch=273&sim=1432&cnt=1 (Phylogenetic tree construction)
4. https://www.ck12.org/biology/phylogeny-and-cladistics/lesson/Cladistics-Advanced-BIO-ADV/ (Phylogeny and Cladistics)
5. https://science.jrank.org/pages/5210/Phylogeny/ (Phylogeny and Cladistics)
6. https://pediaa.com/difference-between-cladogram-and-phylogenetic-tree/ (Phylogeny and Cladistics)
7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1186895/ (X-ray crystallography and protein structure determination)

BIO-VI.C-8: INDUSTRIAL BIOTECHNOLOGY

COURSE TITLE: INDUSTRIAL BIOTECHNOLOGY (THEORY)
COURSE CODE: BIO-VI.C-8
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45
PRE-REQUISITES: Completion of BIO-II.C-4-Basic Microbiology

Course Objective

This course is designed to introduce the students to the basic concepts in Industrial Biotechnology. The paper covers concepts in Industrial Biotechnology, mainly introducing the basics of upstream processes in fermentation technology on an industrial scale.

Course Outcomes

On the successful completion of the course, students will be able to:

CO1: Understand and explain various features of a fermentor.

CO2: Comprehend various concepts of Upstream and Downstream processes.

CO3: Describe the production processes of fermentation products like wine or vinegar at the industrial level.

CO4: Design small scale experiments to produce common enzymes like amylase.

CO5: Prepare basic fermentation products like wine, vinegar, etc.

BIO-VI.C-8: INDUSTRIAL BIOTECHNOLOGY (THEORY)

Module I **15 hrs**

Fermentation equipment and its use **10 hrs**

Definition of fermentor/bioreactors; structure of ideal fermentor**;** definition and uses of impellers and their types; spargers and their types;baffles; headspace**;** controls and sensors (temperature, pH, antifoam, dissolved oxygen and carbon dioxide sensor)**;** types of reactors (definition, description, diagram and uses)-stirred tank reactors; bubble columns; airlift bioreactors (internal and external loop); fluidised bed; packed bed column, photobioreactors; tray bioreactors

Screening and selection of microorganisms **3 hrs**

Primary screening-definition; techniques; crowded Plate; auxanography; enrichment; indicator dye; secondary screening- definition and features; giant colony technique

Stock cultures **2 hrs**

Cryogenic preservation**;**aims of preservation of cultures; definition of working and primary stock cultures**;** techniques of preservation- serial subculture , sterile soil, water , silica gel; sterile mineral oil; lyophilisation

Module II **15 hrs**

Types of fermentation processes **3 hrs**

Continuous; submerged; surface/solid state; batch; fed-batch

Fermentation media **5 hrs**

Characteristics of an ideal; production media; media composition – crude, synthetic; media; sterilization -Heat, radiation, chemical methods and filtration; batch and continuous sterilization, inoculum preparation

Detection and assay of fermentation products **5 hrs**

Physical or chemical assay- titration and gravimetric assay; turbidity analysis and cell determination; spectrophotometric assay; chromatographic partition assay; biological assay-concept benefits and drawbacks; diffusion assay;turbidimetric and growth assay; end point assay; metabolic response assay; enzymatic assay

Scale up of fermentations and increasing product yields **2 hrs**

Significance of scale up; pilot fermenters**;** increasing product yields by mutagens-physical and chemical mutagens/strain improvement

Module III **15 hrs**

Downstream processing **10 hrs**

Biomass: separation of cells – flocculation; floatation; filter aids and filtration (surface, depth); centrifugation- batch centrifuge eg. tubular bowl centrifuge; continuous centrifuge eg. basket centrifuge; disintegration in brief: mechanical eg: ultrasonication; homogenisers and use of ballotini; non mechanical eg. thermallysis; chemical detergent solubilisation, organic solvents; enzymatic methods eg. lysozyme

Broth: Enrichment: evaporation, membrane filtration, liquid-liquid extraction, precipitation, adsorption

Purification: chromatography

Formulation - crystallization and drying (convection drying eg. spray dryers, freeze drying)

Industrial production **5 hrs**

Organisms; fermentation media and conditions;downstream processing and uses -alcohol /Wine; penicillin,vinegar

BIO-VI.C-8: INDUSTRIAL BIOTECHNOLOGY (PRACTICAL)

COURSE TITLE: INDUSTRIAL BIOTECHNOLOGY (PRACTICAL)
COURSE CODE: BIO-VI.C-8
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. A study on the phases of growth of microorganisms during batch fermentation (equipment: Erlenmeyer flask, medium: nutrient broth, inoculum: *E.coli*).
2. Parts of a fermentor
3. Preparation and sterilization of medium for batch fermentation process
4. Batch fermentation using fermentor
5. Preparation and sterilization of medium for fed-batch fermentation process
6. Fed-batch fermentation
7. Decontamination and sterilization of the fermentor
8. Primary screening of antibiotic producing bacteria by crowded plate technique
9. Secondary screening for antibiotic producers by Giant Colony Technique
10. Production of wine (from pineapple or any other fruit/vegetable) using yeast
11. Production of vinegar from toddy
12. Estimation of total reducing sugars and acidity (total and volatile) in wine and vinegar (before and after fermentation)

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3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4027325/ (Isolation and Screening)
4. https://www.youtube.com/watch?v=3pL2X-8-eVk (Fractional Distillation)
5. https://www.sciencedirect.com/science/article/pii/S2095809917304241 (Photobioreactors)

BIO-VI.E-13: BIOETHICS AND BIOSAFETY

COURSE TITLE: BIOETHICS AND BIOSAFETY (THEORY)
COURSE CODE: BIO-VI.E-13
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45

Course Objective

This course aims at introducing the importance of the basic concepts of bioethics and biosafety and their relationship with several fields such as ecology, agriculture, medicine, chemistry and advances brought about in the field of biology and medicine. The course deals with answers to ethical questions that arise in the relationships among life sciences and their importance in the field of biotechnology.

Course Outcomes

On the successful completion of the course, students will be able to:

CO1: Understand the importance of general safety measures in laboratories and biosafety guidelines

CO2: Justify the design of confinement facilities at different Biosafety levels,

CO3: Demonstrate good laboratory practices

CO4: Discuss the relevance of intellectual property rights to biotechnological innovations,
CO5: Describe the standard operating procedures for disposal of various types of wastes from the Biotechnology laboratory

BIO-VI.E-13: BIOETHICS AND BIOSAFETY (THEORY)

Module I **15 hrs**

Introduction to Bio-safety **6 hrs**

Introduction to Biological Safety Cabinets; Primary Containment for Biohazards; Biosafety Levels: Physical containment, Biological containment, Biosafety Levels of Specific Microorganisms; Recommended Biosafety levels for infectious agents and infected animals

Safety in Laboratories **4 hrs**

General safety measures, Hazards: Physical, Biological and Chemical, Spillage and waste disposal

International and Indian biosafety guidelines **5 hrs**

Biosafety guidelines in India; International biosafety guidelines: OECD, FAO, WHO, CAC and other organisations

Module II **15 hrs**

Introduction to bioethics **5 hrs**

Introduction to bioethics; social and ethical issues in biotechnology: issues related to test tube babies; bioethics in plant genetic engineering; bioethics in animal genetic engineering

Introduction to IPR **10 hrs**

Introduction to intellectual property; protection of intellectual property; property rights: trade secret, patent, copyright, plant variety protection; plant breeders' right: history, PPVFR, UPOV, requirements for PBR, need and benefit for PBR, breeders exemption, farmer's privilege, farmer's right; world intellectual property organization (WIPO), GATT & TRIPs ; patent status – international Scenario; patenting of biological materials; significance of patents in India

Module III **15 hrs**

Case studies **3 hrs**

Patenting Basmati rice; Revocation of patents-turmeric and neem

Protection of biotechnological inventions **6 hrs**

Patenting of genes and DNA sequences; gene patents and genetic resources; farmers rights; plant breeder's rights; patenting of life forms;broad patents in biotechnology

Regulatory affairs **3 hrs**

Good laboratory practices; good manufacturing practices

Biosafety of GMOs and GEMs **3 hrs**

Planned introduction and field trials of: GMOs and GEMs

BIO-VI.E-13: BIOETHICS AND BIOSAFETY (PRACTICAL)

COURSE TITLE: BIOETHICS AND BIOSAFETY (PRACTICAL)
COURSE CODE: BIO-VI.E-13
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. General safety measures and study of safety notices
2. Study of preventive measures and first aid during laboratory hazards
3. Case study on handling and disposal of radioactive waste
4. Case study on handling and disposal of medical/microbial waste
5. Study of Good Laboratory Practices
6. Study of Good Manufacturing Practices
7. Study of components and design of a Biosafety laboratory
8. A case study on clinical trials in India with emphasis to ethical issues
9. Planning of establishment of a hypothetical biotechnology industry in India
10. Study of steps of a patenting process

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3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3217699/ (Introduction to IPR)
4. https://www.wipo.int/export/sites/www/about-ip/en/iprm/pdf/ch1.pdf (Introduction to IPR)
5. http://www.fao.org/fileadmin/user_upload/gmfp/docs/Biosafety%20Brochure.pdf (Biosafety of GMOs)
6. https://www.hindawi.com/journals/isrn/2011/369573/ (Biosafety of GMOs)

BIO-VI.E-14 ADVANCED CELL BIOLOGY

COURSE TITLE: ADVANCED CELL BIOLOGY (THEORY)
COURSE CODE: BIO-VI.E-14
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45
PRE-REQUISITES: Completion of BIO-I.C-2- Cell Biology

Course Objective

The course will give a detailed description of how eukaryotic cells receive, transmit and respond to environmental signals, cellular regulation of cell cycle progression and cell death. The principal and working of the essential tools used in cell biology will also be covered.

Course Outcomes

On the successful completion of the course, students will be able to:
CO1: Understand the theory behind the working of various techniques in cell biology.
CO2: Explain the processes of membrane transport and signal transduction.
CO3: Describe the regulation of the cell cycle events.
CO4: Isolate and visualize the subcellular organelles.
CO5: Prepare slides and identify various stages of Mitosis and Meiosis.

BIO-VI.E-14 ADVANCED CELL BIOLOGY (THEORY)

Module I **15 hrs**

Techniques in cell biology **10 hrs**

Review of 2D microscopy; confocal microscopy;transmission electron microscopy; scanning electron and atomic force microscopy; the use of radioisotopes; differential centrifugation; purification of proteins – precipitation;ion-exchange chromatography;gel filtration chromatography;affinity chromatography; polyacrylamide gel electrophoresis; two-dimensional gel electrophoresis; purification of nucleic acids-agarose, gel electrophoresis; ultracentrifugation, blotting techniques

Membrane potentials and nerve impulses **5 hrs**

The resting potential; the action potential; propagation of action potentials; neurotransmission

Module II **15 hrs**

Cell cycle and programmed cell death **10 hrs**

Overview of the cell cycle; regulation of cell cycle;events of mitotic phase; cytokinesis; events of meiosis; regulation of cell division; apoptosis (extrinsic and intrinsic pathway)

Membrane transport **5 hrs**

Review of structure and composition of cell membrane; transport across the nuclear envelope - simple diffusion and facilitated diffusion; passive transport - glucose transporter, anion

transporter; primary active transporters - P type ATPases, V type ATPases, F type ATPases; secondary active transporters –Na+-glucose symporter; ion channels - voltage-gated ion channels (Na+/K+ voltage-gated channel)

Module III **15 hrs**

Signal transduction **11 hrs**

The basic elements of cell signalling systems-autocrine, paracrine and endocrine types ; an overview of the major signalling pathways; mechanism and signal transduction of G protein-coupled receptors (GPCRs); Receptor protein-tyrosine kinases (RTKs); Ligand-gated channels; steroid hormone receptors; second messengers- cyclic AMP, phosphatidylinositol derived second messengers;role of calcium and NO as intracellular messengers

Cancer biology **4 hrs**

Development and causes of cancer; genetic basis of cancer; oncogenes; tumor viruses

BIO-VI.E-14 ADVANCED CELL BIOLOGY (PRACTICAL)

COURSE TITLE: ADVANCED CELL BIOLOGY (PRACTICAL)
COURSE CODE: BIO-VI.E-14
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. Identification of different stages of mitosis (in garlic root tip) `
2. Identification of different stages of meiosis (flower buds/ grasshopper testes)
3. Study of cell viability by trypan blue
4. Identification and study of cancerous cells using permanent slides/ photomicrographs
5. Study of plant, animal and human tumour viruses using photomicrographs
6. Differential centrifugation for separation of cellular components
7. Preparation of sucrose density gradient and separation of subcellular organelles
8. Visualization of nuclear fraction by acetocarmine stain and mitochondria by Janus green stain
9. Study of electron micrographs of subcellular organelles
10. Separation of photosynthetic pigments by TLC

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BIO-VI.E-15: FOOD BIOTECHNOLOGY

COURSE TITLE: FOOD BIOTECHNOLOGY (THEORY)
COURSE CODE: BIO-VI.E-15
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45

Course Objective

This course adds information about the role of microorganisms in many food industries both in production and spoilage processes and to understand the importance of the role of microorganisms in food industries in both beneficial and harmful ways.

Course Outcomes

On the successful completion of the course, students will be able to:

CO1: Understand the important spoilage microorganisms in foods and the factors influencing their growth

CO2: Demonstrate the principles of various food preservation techniques and the importance of food quality assurance

CO3: Appreciate the importance of microorganisms as food and fermented food

CO4: Assess the quality of the milk through various tests

CO5: Debate on the Pros and cons of GM foods

BIO-VI.E-15: FOOD BIOTECHNOLOGY (THEORY)

Module I **15 hrs**

History and development of food microbiology **2 hrs**

History of microorganisms in food**;** role and significance of microorganisms in foods

Factors influencing microbial growth in food **4 hrs**

Intrinsic and extrinsic factors responsible for food spoilage

Microorganisms involved in food spoilage **2 hrs**

Microorganisms involved in food spoilage: fruits vegetables, meat, eggs, bread

Food borne diseases **4 hrs**

Food poisoning: (bacterial toxin botulism and Staphylococcal toxin); fungal toxins: aflatoxin; food borne infections: gastroenteritis and Salmonellosis

Microorganisms as source of food **3 hrs**

Nutritive value and use of:Mushroom,SCP eg. Spirullina

Module II **15 hrs**

Milk Microbiology **6 hrs**

Sources of contamination; different microorganisms implicated in spoilage; milk borne diseases: listeriosis and scarlet fever; grading of milk by dye reduction test – MBRT and resazurin

Detection of food spoilage **6 hrs**

Methods of detection of food spoilage in any 1 type of food (example milk); traditional approaches in detection of spoilage (SCP, breeds smear, identification of specific; organisms by using selective and differential media); new approaches (examples gene probes, bioluminescence)

Food quality assurance **3 hrs**

Food safety: HACCP system to food protection

Module III **15 hrs**

Food preservation **8 hrs**

Preservation by drying: solar drying, mechanical drying, salting, smoking)**;** preservation at high temperature: concept of TDP and TDT**;** pasteurization (LTHT, HTST, UHT processes); efficiency of pasteurization – phosphatase test, canning, hurdle technology**;** preservation at low temperature: freezing preservation by use of additives: acids, salts, sugars, antibiotics, ethylene oxide, antioxidants; preservation by radiation: UV, ionizing radiations,gamma and cathode rays, microwave processing; other methods: hydrostatic pressure cooking,modified atmosphere

Fermentation technology **3 hrs**

Fermented Food: process, microbiology involved and changes during fermentation of fermented food: sauerkraut; milk products: yogurt

GM foods **4 hrs**

Pros and cons of GM foods Eg: Golden rice, FlavrSavr tomato and BtBrinjal

BIO-VI.E-15: FOOD BIOTECHNOLOGY (PRACTICAL)

COURSE TITLE: FOOD BIOTECHNOLOGY (PRACTICAL)
COURSE CODE: BIO-VI.E-15
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. Plating of spoiled food on selective media
2. MIC of common food preservatives – (sugar/ salt)
3. MIC of chemical food preservatives – (sodium benzoate/ potassium metabisulphite)
   Milk Microbiology
4. Standard plate count
5. Grading of quality of milk using dye reduction test (MBDRT / Resazurin)
6. Pasteurisation of milk
7. Determination of efficiency of pasteurisation by phosphatase test
8. Determination of TDP and TDT

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BIO-VI.E-16: ANIMAL CELL CULTURE

COURSE TITLE: ANIMAL CELL CULTURE (THEORY)
COURSE CODE: BIO-VI.E-16
MARKS: 75
CREDITS: 3
TOTAL HOURS: 45

Course Objective

This course is designed to introduce the students to the basic concepts of Animal Cell Culture. The paper covers topics that explain animal cell culturing and methods involved in basic culturing of animal cells with a few applications to life sciences.

Course Outcomes

On the successful completion of the course, students will be able to:
CO1: Understand the basic concepts of animal cell culture.
CO2: Comprehend the various requirements and techniques for animal cell culture and importance of the same.
CO3: Understand the importance of primary and established cell lines for biotechnological applications.
CO4: Appreciate the various methods of characterization and growth assessment techniques in culturing animal cells.
CO5: Understand the applications of animal cells in the development of disease diagnostics and therapeutics.

BIO-VI.E-16: ANIMAL CELL CULTURE (THEORY)

Module I **15 hrs**

Introduction to animal cell culture **2 hrs**

Animal Tissue and Cell Culture (Definition and Concepts in brief)
History and Scope of Animal Tissue Culture

Requirements for animal cell culture **4 hrs**

Basic layout of an animal cell culture laboratory (washing room, media preparation & sterilization room, inoculation and aseptic culture room); equipment; culture vessels for tissue culture

Basics of an animal Cell **3 hrs**

Structure and organization of animal cell; an overview of developmental biology (importance in understanding differentiation of cells in culture)

Media in animal cell culturing **6 hrs**

Physico-chemical properties of culture media (pH, CO_2, O_2& Temperature); growth media – (types, advantages and disadvantages of each type); natural and artificial media; natural media – clots, biological fluid, tissue extracts, complex natural media; artificial media – serum

containing, serum- free media, chemically defined and protein- free media; basal salt solutions (BSS) – constituents (vitamins, amino acids, trace elements, inorganic ions); importance; uses and examples; serum as a complex supplement; growth factors in promoting proliferation of cells – uses and examples (EGF, FGF, PDGF)

Module II **15 hrs**

Basic techniques in animal cell culture **6 hrs**

Techniques in mammalian cell culture – source of cells; dissection/isolation of cells; mechanical and enzymatic disaggregation; types of cell cultures (organ culture, whole embryo culture, histotypic cultures, explants cultures)

Cell line cultures **6 hrs**

Primary and established cell line cultures; establishment of continuous cell lines – spontaneous transformation; chemical transformation; viral transformation; non- chemical methods; characteristics & maintenance of established / continuous cell lines; characteristics of normal and transformed cells (properties of transformed cells)

Normal cell growth, phases of growth in culture and synchronization of cells **3 hrs**

Eukaryotic cell cycle and basics of cell synchronization; apoptosis in cultured cells – Reasons for cell suicide; phases of cell growth (lag, log, stationary, decline); population doubling level; morphology

Module III **15 hrs**

Characterization and growth measurement of cultured cells **6 hrs**

Characterization – genetic and enzymatic methods (cytogenetics, karyotyping, Isoenzymes and immunological tests); growth measurement – direct method (particle counter, dye exclusion test, cytotoxicity assay); growth measurement – indirect method (MTT assay)

Cell separation methods **3 hrs**

Physical method of cell separation – separation based on cell size; cell density; cell surface charge; cell affinity; separation by flow cytometry

Applications of animal cell culture **6 hrs**

Stem cell culture (applications in Animal Cell Culture); artificial skin; artificial cartilage; special secondary metabolites / products (insulin, growth hormone, interferon, t-plasminogen); other valuable products obtained using animal cell cultures (emphasis on monoclonal and polyclonal antibodies)

BIO-VI.E-16: ANIMAL CELL CULTURE (PRACTICAL)

COURSE TITLE: ANIMAL CELL CULTURE (PRACTICAL)
COURSE CODE: BIO-VI.E-16
MARKS: 25
CREDITS: 1
TOTAL HOURS: 30

1. Washing of glassware and culture wares, preparation of animal cell culture media, sterilization
2. Introduction to use of instruments and sterile techniques in animal cell culture
3. Preparation of Basal Salt Solutions (DPBS) and filter sterilization
4. Preparation of culture media for animal cell culture (DMEM / RPMI 1640) using BSS.
5. Preparation of serum from goat blood & filter sterilization for animal cell culture
6. Culturing lymphocytes from blood cells using RPMI 1640
7. Dissection of chick embryo for culturing fibroblast cells
8. Estimation of cell viability using MTT & calculations of seeding density for animal cell cultures
9. Establishing a monolayer culture using warm trypsinization method
10. Establishing a monolayer culture using cold trypsinization method
11. Subculture of monolayer culture

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