U. Satyanarayana.

Semester III [Core (CR)]

| Course No: BT23301CR | Credits: 4 |
|---|---|
| Course Title: Genetic engineering | |
| Maximum Marks: 100 [80 (SEE) + 20 (IA)] | |

Course Objective: The objective of genetic engineering course is to familiarise students with fundamentals of DNA recombinant technology and advanced aspects of genetic engineering.

Unit I

Recombinant DNA Technology Tools: Restriction endonucleases: Historical perspective. Nomenclature. Different types of restriction-modification systems and their characteristic features.Blunt end and cohesive end cutters with examples.Isochizomers, neoisoschizomers and isocaudemers.Restriction modification enzymes and their importance in DNA recombinant technology (Dam, DCM methylases). DNA ligases: E.coli and T4 DNA ligases. Chemistry of T4 DNA ligase reaction.DNA Phosphatases and their role in recombinant DNA technology.DNA Pol I and Klenow fragment and their role in recombinant DNA technology. Vectors: Plasmids: General features of plasmid vectors. Molecular regulation of high and low copy number plasmids. Characteristics features of pBR322, pUC series of plasmid vectors.General scheme of cloning in plasmid vectors. Selectable marker genes used in plasmid vectors and their mechanism of action. Molecular details of blue-white selection. Expression plasmid vectors: transcriptional and translation regulatory elements in expression plasmids. Characteristics feature of inducible plasmid expression vectors.yeast plasmid vectors: General features and mode of selection. Transformation of plasmid DNA in bacterial cells (Physical and chemical methods). Bacteriophages as cloning vectors: lambda-phage vectors: General characteristics features. Insertional lambda phage vectors and replacement lambda phage vectors .General scheme of cloning in lambda phage vectors. In-vitro packaging and its importance. M13 vectors: General features and scheme of cloning in M13 phage. Phagemid vectors: General features and their importance. Cosmid vectors: General characteristics and scheme of cloning in cosmid vectors. YACs: General characteristic features and scheme of cloning in YACs. BACs: General characteristic features and their importance.

## Unit II

Genetic engineering techniques:Polymerase chain reaction: Principle and methodology. Source of template DNA (genomic DNA, Plasmid DNA and RNA). Features of an ideal primer.Primer design with restriction sites at the ends. Degenerate primers and their importance. DNA polymerases for PCR: characteristic features of error prone (Taq) and high fidelity DNA polymerases. Different types of PCR (nested, asymmetric, multiplex). Applications of PCR. Reverse Transcription PCR (RT- PCR): Principle and methodology. Different methods of first strand and second strand cDNA synthesis and cDNA library sysnthesis. Characteristic features of different reverse transcriptases (RT) used in RT-PCR. Real-Time PCR: Principle and methodology. Ct value and its importance.Different methods of fluorescent detection and probes (SYBER green, Taqmann probe, Molecular beacon probes, Scorpion probes). Melting curves and their importance. Quantification and normalization of raw data.Applications of Real-Time PCR.

## Unit III

Site-Directed mutageneis: M13 vector based methods, plasmid vector based methods (single primer and double primer methods), PCR based methods. Protein engineering: Different methods and application of protein engineering. Hetrologous protein expression systems: Expression in bacterial systems: Promoters and translation elements used in expression vectors. Inducible promoter systems.Expression and purification of GST fusion proteins. Expression in yeast: Various promoters elements used in expression vectors. Inducible expression systems in yeast (Gal and CUP1 system).Pichiapastroris as yeast expression systems. Expression in Insect cell line (Sf9/21): Baculovirus expression vectors. structure and construction of recombinant Basmid vectors and expression of protein in baculoviral vectors.Expression in mammalian cells.Mammalian expression vectors. Viral and cellular promoter used in expression vectors. Importnace of kozak in expression vectors and codon optimization. Tet- Off/On Inducible systems.Expression of proteins with fusion tags (HA, His, Myc, Flag, GFP) and their significance. In- vitro transcription and translation and its application

## Unit IV

Studying protein-protein interaction: Yeast Hybrid systems: Two hybrids based on split transcriptional activation, Split ubiquitin system, SOS recruitment system. Reverse two hybrid. Yeast three hybrid systems for protein-protein, protein-RNA interactions and protein ligand interaction. Transfections: Transient and stable transfection in animal cell.Physical, chemical and biological transfection agents.Repoter assays: Repoter genes and applications (Chlorophenicolacetytransferase (CAT), Luciferase (Firefly and Renilla), living colours (Green fluorescent, yellow fluorescent and their application in co- localization studies). Dual luciferase assay and its application. Gene knock-downs: Antisence RNA technology with examples from animals. RNA interference: Methodology and applications. Transgenics: Gene knock-in: Various methods of making transgenics (animals). Gene knock outs: Methodology based on Cre-LoXp system. Conditional and specific knock-outs. Gene editing: CRISPR-Cas9 system: Biology and mechanism. Re-engineering of CRISPR-Cas9 tools for Gene expression, repression, epigenome editing, etc. CRISPR-Cas9 and Base editing: Adenosine Base editing (ABEs) and cytosine base editing (CBEs).

Learning Outcomes:

After successful completion of the course, the students are expected to gain knowledge in genetic engineering concepts. The academic knowledge provides them a strong platform for performing various research-based endeavours in genetic engineering

Books Recommended:


1) Principles of Gene Manipulation and Genomics by Sandy B. Primrose, Richard Twyman: Blackwell Publishing Professional.
2) Analysis of Genes and Genomes by Richard J. Reece: Wiley.
3) Molecular Biotechnology - Principles and Applications of Recombinant DNA by Glick, Bernard R.; Pasternak, Jack J.; Patten, Cheryl L: ASM Press..

Semester III [Core (CR)]

| Course No: BT23302CR | Credits: 4 |
|---|---|
| Course Title: Plant Biotechnology | |
| Maximum Marks: 100 [80 (SEE) + 20 (IA)] | |

Course Objectives:
The aim of this course is to provide skilled knowledge of biotechnology for the improvement of plants. The course deals with the concept of plant totipotency and its regulation .How to propagate plants in vitro by using tissue culture, Understanding the mechanism of genetic transformation of plants using agrobacterium system for the production of disease resistant, stress tolerant and to have altered nutrient content.

## Unit I

General structure, organization & Molecular basis of Shoot Apical Meristem(SAM) & Root Apical meristem (RAM).Totipotency of Plant cell, Plant cell cycle, Role of various hormones in regulating plant cell cycle, Micropropagation (Seed V/S Soma), Stages & methods of micropropagation.Production of virus free plants. Tissue culture media (Composition & preparation),Role of micro, macro nutrients & other components present in tissue culture media, Commonly used media (Murashige and Skoogetc) Initiation and Maintenance of callus and suspension culture, Single cell clones Organogenesis: Basis, applications & control of Somaclonal variation. Somatic embryogenesis- acquisition of embryogenic competency, factors & genes influencing the embryogenic competency of cell during somatic embryogenesis, Synthetic seeds. Embryo rescue.

## Unit II

Protoplast isolation (mechanical & enzymatic methods), maintenance, purification, viability, Culture and fusion (Spontaneous & induced fusion, sodium nitrate, calcium ion, PEG, electrofusion).Identification & Selection of hybrid cells and regeneration of hybrid plants; Symmetric & Asymmetric hybrids, Cybrids-formation and applications. Anther, pollen and ovary culture for the production of haploid homozygous lines, Molecular mapping, Introduction to genetic and physical maps, physical mapping

## Unit III

Plant Transformation Technology; Morphology of Agrobacterium tumefaciens, Features of Ti Plasmids, Opines and its Types, Basis of tumor formation, Factors influencing binding of

Agrobacterium to plant, Mechanism of T-DNA transfer & Role of virulent proteins in (Formation of T-DNA strand, movement of T-Complex & Integration of T-DNA into Plant genome), Features of Binary vectors & its Types (pBIN19, pGreen, pCAMBIA, etc), Promoters used in Ti vectors (CaMV 35S and other promoters), Use of reporter genes(Opine synthase, CAT, GUS, LUX, GFP) and selectable markers (antibiotic & herbicide resistant genes, Metabolic intermediates etc) Generation of marker free plants (using Cre- Lox & other Excision techniques), Vector less or direct DNA transfer (Particle bombardment, Electroporation, WHISKERS, Pollen tube entry, Floral dip, Liposome mediated,etc). Plant transformation for productivity and performance with special example of Herbicide resistance (Glyphosate &Phosphinothricin resistance), Insect resistance (Bt based plants), Disease resistance (Role of R-proteins & other molecules), long shelf fruit and flowers, Stress tolerance (water deficit stress, Role of osmoprotectants and other molecules).

Unit IV

Molecular farming: Methodology involved in the production of Golden rice, Metabolic engineering of carbohydrates (Starch and fructan production), lipids (production of shorter & longer chain fatty acids, Modification of the degree of saturation). Production of Biodegradable plastic, Production Therapeutic protein in plants (Hirudin, Glucocerebrosidase, etc), Purification strategies for proteins-Oleosin partitioning Technology, Plantibodies (full length, scFv, Minibody, Diabody, Bispecific) Edible Vaccines, Manipulation of Shikimate pathway for the production of Vitamin E, Chloroplast Transformation (Mechanism & Advantages), Principle & applications of Gene termination technology, Concerns about Genetically modified plants.

Learning Outcomes:
Concepts of molecular mechanism involved in dedifferentiation & re- differentiation of plant cell using plant hormones to alter cell cycle. Core methodology involved in tissue culture for micropropagation of plants. Applied knowledge how plant can be used as an expression system for production of bioactive compounds at industrial scale. How to address the social and scientific concerns of genetically modified plants

Books Recommended:


1) Plant Biotechnology: The Genetic Manipulation of Plants Adrian Slater Nigel W. Scott Fowler: Oxford University Press.
2) Introduction to Plant Biotechnology: H S Chawla: Science Publishers, Inc.
3) Plant propagation by Tissue Culture : Edwin F. George,Michael A Hall: Springer-verlag.
4) Agrobacterium: From Biology to Biotechnology: Tzfira,Tzvi,Citovsky,Vitaly: Springer verla

Semester III [Core (CR)]

| Course No: BT23303CR | Credits: 4 |
|---|---|
| Course Title: Bioprocess engineering and Fermentation Technology | |
| Maximum Marks: 100 [80 (SEE) + 20 (IA)] | |

Course Objectives: The objective of the course is to provide students with the knowledge of fermentation, bioreactor technology, and thus applications of the chemical engineering principles in biological systems.

## Unit I

Sterilization: Types of sterilization. Thermal death kinetics of microorganism. Heat sterilization of liquid medium, Batch mode, Continuous mode, Problems & Examples. Air sterilization. Fermentation overview: Inoculum development. Various types of Fermentation: submerged fermentation, aerobic and anaerobic fermentation. Bioreactor operations: Different types of bioreactors, Configuration of Bioreactors and their main components. Modes of bioreactor operation.Important bioreactor accessories.

## Unit II

Basic concepts, Kinetics of Cell Growth: Kinetics of batch culture, Growth kinetics for continuous culture, Material balance for CSTR. Fundamentals of material and energy balance for processes with/without chemical reaction: Biomass Balances (Cells) in a Bioreactor, Material Balance in Terms of Substrate in a Chemostat, Modified Chemostat. Problems & Examples. Metabolic stoichiometry: Biomass and Product Yields, YX/S and YP/S .Overview of biosynthetic mechanisms.

## Unit III

Whole cell immobilization and their applications.Single cell protein. Cell disruption: mechanical, enzymatic, and chemical methods. Pre-treatment strategies. Solid-liquid separation: filtration, centrifugation, Adsorption, Problems/Examples. Liquid-liquid extraction, Solvent selection, Operating Conditions, Mode of Operation, Extractor Type Design Criteria. Membrane separation: ultrafiltration (Theory, Experimental set-up) reverse osmosis, dialysis, lyophilization. Precipitation of proteins by salting out, isoionic& semisynthetic polyelectrolyte methods.

## Unit IV

Microbial fermentation and production of small and macromolecules: Antibiotics and Pharmaceuticals (Penicillin, Streptomycin), Microbial Production of Organic Acid (Citric Acid, Lactic Acid, Vinegar ), Microbial Production of Vitamins. Production of Amino Acids (L-Glutamate, Lysine), Industrial Production of Ethanol by fermentation. Bakers yeast fermentation. Spirulina production. Alpha-amylase production, High Fructose corn syrup production. Cheese Production. Biodiesel production. Butanol production. Biopesticides. Biopolymer. Hepatitis B vaccine. Insulin. Biofertilizer. Biomethanation process.

Learning outcomes:

After completing this course, the students will be able to analyze the kinetics of cell and product formation under different types of culture conditions. The students will be able to develop control strategies for bioprocess operations, and will be able to select the appropriate methods for product purification.

Books Recommended:


1. M.L.Shuler and F.Kargi, "Bioprocess Engineering--basic Concepts", 2nd Edn. Prentice-hall of India Pvt Ltd
2. P.M.Doran, "Bioprocess Engineering Calculations", Elsevier India Pvt Ltd (2008).
3. C. Ratledge& B. Kristiansen, "Basic Biotechnology" 3rd Edn. Cambridge University Press
4. Peter F. Stanbury, Stephen J. Hall & A. Whitaker, "Principles of Fermentation Technology", Elsevier India Pvt Ltd.(2007).

Semester III [Core (CR)]

| Course No: BT23304CR | Credits: 2 |
|---|---|
| Course Title: Human Genetics | |
| Maximum Marks: 50 [40 (SEE) + 10 (IA)] | |

Course Objective:
Provide deep understanding of complex genetic principles and their human genetics applications. Equip students to analyze inheritance patterns, interpret genomic data, and grasp advanced techniques in genetics research

Unit I

Inheritance Patterns and Genetic Variation:Mendelian Inheritance and Single-Gene Patterns: Overview of Mendel's laws. Autosomal recessive and dominant disorders: inheritance patterns and examples.Non-Mendelian Inheritance: Sex-linked recessive and dominant disorders. Mitochondrial inheritance. Genomic imprinting and its impact on gene expression.Population Genetics: Sources of genetic variation, Hardy-Weinberg equilibrium, population structure, genetic drift, migration, and natural selection.

Unit II

Advanced in Human Genetics:Complex Trait Genetics and Genomics: Polygenic inheritance, heritability, genome-wide association studies (GWAS), and their applications.Disease-Causing Variants and Personalized Medicine: Genetic basis of rare and common diseases, cancer genomics, and personalized medicine.Cutting-Edge Techniques in Human Genetics: Emerging genomics technologies (long-read sequencing, single-cell genomics), gene editing techniques and their applications.Genetic Models and Traits: Use of animal models in human genetics research, induced pluripotent stem cells and their role in genetic studies.

Learning Outcome:
Grasp Mendel's laws, inheritance patterns, and exceptions.Understand sex-linked, mitochondrial inheritance, and genomic imprinting.Analyze genetic variation, assess Hardy-Weinberg equilibrium, comprehend evolutionary forces.Interpret polygenic inheritance, heritability, conduct GWAS.Evaluate disease genetics, explore personalized medicine, assess cancer genomics.Comprehend cutting-edge genomics like long-read sequencing, gene editing.Recognize animal models, understand induced pluripotent stem cells in genetics.

Books Recommended:

1) Thompsan and Thompsan: Genetics in Medicine, Elsevier publications.
2) Emery’s Elements of Medical Genetics. Elsevier

Semester III [Discipline Centric (DCE)]

| Course No: BT23301DCE | Credits: 2 |
|---|---|
| Course Title: Bioethics, Biosafety and Intellectual Property Rights | |
| Maximum Marks: 50 [40 (SEE)+10 (IA)] | |

Course Objective:
The main aim is to introduce students to Bioethics, its meaning, its philosophical foundations and bioethics principles.Imparting knowledge and skills that will enable students to develop ethical answers to these various issues especially related to research discoveries made in the field of biology.Identify the basic concepts of modern biology and explain how recent advancements in these areas have influenced current bioethical issues.

Unit I

Introduction to Bioethics.Ethics and Morality.Introduction to subject areas of Bioethics (Poverty, Birth control, ethics and religion, euthanasia, Environmental ethics). Bioethical Principles. Bioethics in Research. Conflicts of interest, Publication misconduct: definition, concept, problems that lead to unethical behavior and vice versa, types, Violation of publication ethics, authorship and contributor ship.Plagiarism and similarity index concepts.Laboratory Biosafety: Importance, Biosafety levels, Biosafety Guidelines.

Unit II

Bioethical and Biosafety issues concerning Stem cells and Cloning. Animal cloning. Controversies regarding Designer babies. Gene therapy. Ethical controversies on Organ Transplantation. Surrogacy. Ethical regulations on Surrogacy. Genetically modified crops. Ethical and Bio safety issues involved in GMO,s. Advantages and Disadvantages. Ethical Limits of Animal use. Animal experiments in light of Bioethics.

Intellectual property rights: Introduction, Categories, Examples and Importance. Entrepreneurship: Meaning, Types and its Importance.

Learning Outcome:
Students will be able to understand basics of bioethics, importance of this course, its relevance in research, publishing field and healthcare.Students will be able to understand the goal behind transgenic plants and animals, Ethical concerns and analysis. .

Books & Referencesrecommended:


1) Title: Bioethics, an introduction for the biosciences Author: Ben Mepham. Publisher: Oxford University, UKYear: 2013Edition: 2nd.
2) Title: Bioethics: An Anthology (Blackwell Philosophy Anthologies) Paperback..Authors: Helga Kuhse, UdoSchüklenk and Peter Singer. Publisher: John Wiley & Sons; Year: 2015. Edition: 3rd Revised edition.
3) Title: The International Law of Biotechnology: Human Rights, Trade, Patents, Health and the Environment (Principles of International Law series) .Author: Mathias

Semester III [Discipline Centric (DCE)]

| Course No: BT23302DCE | Credits: 3 |
|---|---|
| Course Title: Systems and computational Biology | |
| Maximum Marks: 75 [60 (SEE)+15 (IA)] | |

Course Objectives:

To introduce the concepts of systems biology to student. To expose the students to high through put methods like proteomics and next generation sequencing based methods

Unit I

Introduction to systems biology, Networks-definition, Representation of networks, Graph theory.Properties of networks, Degree, Degree distribution, Clustering coefficient, shortest-pathlength.Structure of biological networks.Types of networks- Random, Scale-free and Hierarchical networks.Emergent properties of networks.Cellular networks; genetic and molecular interaction networks. Significance of cellular networks (combinatorial-out puts, multitasking), Synthetic networks. Systems biology and future medicine

Unit II

Noise-noise and robustness of cellular processes, Sources of biological noise; Intrinsic and Extrinsic noise, Noise in gene expression; stochastic gene expression, cell-to-cell variation in gene expression (cell-to-cell variation in number of RNA and protein molecules). Single cell measurements -Methods to study cell-to-cell variability of RNA and proteins.Noise and cellular decision-making (microbes to mammals).Non-genetic cellular heterogeneity and response.

Unit III

Protein-Protein Interaction Networks (PPINs); Mass spectrometry LC-MS/MS, identification, generation, and computational analysis of PPINs.Genome sequencing; library preparations, barcoding and Next Generation Sequencing (NGS), reference genome alignment and de novo assembly.Transcriptomics; microarray, RNA-seq (including computational pipelines for data analysis (determination of RPKM values) and applications. Chromosome conformation capture; (3C, 4C, 5C and HiC) and computational pipelines for data analysis and visualization (HiC-Pro, HiGlass, Juice-box). Chromatin-immuno precipitation coupled to NGS (ChIP-seq) and computational pipelines for data analysis. Introduction to Galaxy platform for analysis of different omics and large data sets. Applications of Machine Learning (ML) and Artificial Intelligence (AI) in Biology; AlphaFold, prediction and subtyping of cancers

Learning Outcome: This advanced course will enable the students to think in terms of systemic/holistic perspective and understand the biological processes in a more realistic context. The students will also get familiar with proteomics and genomics based methods.

Books Recommended:

Introduction to Systems Biology, Edited by Sangdun Choi, HUMANA Press

Semester III [Discipline Centric (DCE)]

| Course No: BT23303DCE | Credits: 3 |
|---|---|
| Course Title: Laboratory Course-III | |
| Maximum Marks: 75 [60 (SEE)+15 (IA)] | |

Course Objectives:
To acquaint students with the principles and applications of genetic engineering techniques.

Practicals

- Restriction enzymes, cohesive and blunt end digestion of plasmid DNA,
- DH5-Alpha Competent cell preparation and competance calculation,
- Bacterial Transformation using plasmid DNA.
- Plasmid Isolation and purification,
- Polymerase chain Reaction (PCR): Primer design and PCR amplication of known DNA using plasmid template.
- Cloning DNA fragments in pUC vectors and selection of recombinant by blue-white selection.
- GST-fusion protein expression in E.coli and their affinity purification using GST tag.
- Basic idea of animal Cell Culture.

Learning Outcomes:
Students will acquire the knowledge on different techniques related to gene cloning, PCR amplification, Protein Purification and basic culture handling. Development of an ability to design and conduct genetic engineering experiments, as well as to analyze and interpret data.

Books Recommended:
Sambrook, J. and Russell, D.W. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Semester III [Generic Elective (GE)]

| Course No: BT23003GE | Credits: 2 |
|---|---|
| Course Title: Molecular Mechanism of Plant life | |
| Maximum Marks: 50 [40 (SEE)+10 (IA)] | |

Course Objectives:
The aim of this course is to study the organization root apical meristem and shoot apical meristem, floral development, mode of action of new plant hormones.

Unit I

Organization of Shoot & Root apical Meristem. Molecular mechanism of shoot, Root & Leaf development. Phyllotaxy. Transition of flowering: Induction of flowering, Regulatory Pathways of Flowering. Floral meristem & floral development (Arabidopsis &Antirrhium)

Unit II

Plant hormones (Auxin, Gibberellin, Cytokinin, Ethylene, Brassinosteroids, Abscisic acid, Strigolactones, Jasmonates, polyamines, Salicyclic acid, Nitric oxide) biosynthesis storage, breakdown and transport: physiological effects and mechanism of action. Changing the genome of plants-transgenic plants (methods, advantages & concerns).

Learning Outcomes:
Demonstrate the molecular mechanism of regulating the stem cells in plant meristems.Elucidates the role new hormones in plants

Books recommended:

1) Handbook of Plant Science by Keith Roberts (Volume I &II), Wiley-Interscience.
2) Molecular life of plants by Russel Jones, Helen Ougham, Howard Thomas, Susan Waaland, Wiley- Blackwell

Semester III [Generic Elective (GE)]

| Course No: BT23004GE | Credits: 2 |
|---|---|
| Course Title: Cancer Immunology | |
| Maximum Marks: 50 [40 (SEE)+10 (IA)] | |

Course Objectives:
The objective of this course is to introduce current concepts and advances in the area of cancer biology. The Students will understand the role of oncogenes and suppressor genes and get knowledge on cancer related mutagens and pathways and cancer therapy

Unit I

Oncogenes: Historical aspects, provirus, protovirus and oncogene hypothesis. Functional class of oncogenes (proto-oncogenes) Mechanism of carcinogenic transformation by oncogenes, viral oncogenes.Tumor suppressor genes- properties, mechanism of tumor suppressor genes in cancer induction with special reference to P53 gene. Inherited cancers.

Unit II

Tumor immunology and cancer diagnostics & therapy: Tumor immunology –Introduction, Mechanism of immune response to cancer, natural killer cells and cell mediated cytotoxicity. Biochemical, histological and radiological methods for cancer diagnosis Chemotherapy and radiotherapy strategies for cancer treatment.Cancer chemotherapeutic drugs.Types of radiation therapy.Immunotherapy of cancer – Rationale of immunotherapy, Tumor necrosis factor, interleukins, cytokines, interferons, vaccines, monoclonal antibodies.

Learning Outcomes:
Comprehend pathogenesis, molecular mechanisms and identification of cancerExplain cancer metastasis microenvironment and cancer therapy

Books Recommended:

1) Basic Immunology: Abul K. Abbas, Andrew H. Lichtman.
2) Janeway'sImmunobiology, Garland Sciernce
3) Essential Immunology by Delvis, Martin , Burton and Roitt