ADIKAVI NANNAYA UNIVERSITY: RAJMAHENDRAVARAM
Single Major B.Sc. Biotechnology (w.e.f:2023-24A.B)

SEMESTER-III
COURSE 5: PLANT AND ANIMAL BIOTECHNOLOGY

Theory Credits: 3 3 hrs/week

I. LEARNING OUTCOMES

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

1. Learn about plant tissue culture techniques and secondary metabolites production.
2. Learn about transgenesis and molecular markers.
3. Learn about animal tissue culture techniques
4. Learn about transgenic animals and gene therapy.
5. Learn about Bioethics, Biosafety and IPR.

II. Syllabus

Unit – I Plant tissue culture techniques & secondary metabolites production

1. totipotency, media preparation – nutrients and plant hormones; sterilization techniques; establishment of cultures – callus culture, cell suspension culture
2. applications of tissue culture-micro propagation; Somatic embryogenesis
3. synthetic seed production; protoplast culture and somatic hybridization - applications. Cryopreservation, Plant secondary metabolites- concept and their importance

Unit – II Transgenesis and Molecular markers

1. Plant transformation technology—Agrobacterium-mediated Gene transfer (Ti plasmid), hairy root features of Ri plasmid, Transgenic plants as bioreactors.
2. Herbicide resistance – glyphosate, Insect resistance- Bt cotton
3. Molecular markers - RAPD, RFLP and DNA fingerprinting-principles and applications.

Unit – III Animal tissue culture techniques

1. cell culture media and reagents; culture of mammalian cells, tissues and organs; primary culture, secondary culture, cell lines,stem cell cultures;
2. Tests: cell viability and cytotoxicity, Cryopreservation.
3. Transfection methods (calcium phosphate precipitation, electroporation, Microinjection) and applications.

Unit – IV Transgenic animals & Gene Therapy

1. Production of vaccines, diagnostics, hormones and other recombinant DNA products in medicine (insulin,somatostatin, vaccines),IVF,
2. Concept of Gene therapy,
3. Concept of transgenic animals – Merits and demerits -Ethical issues in animal biotechnology

Unit V Bioethics, Biosafety and IPR

1. Bioethics in cloning and stem cell research, Human and animal experimentation, animal rights/welfare.
2. Bio safety-introduction to biological safety cabinets; primary containment for biohazards; biosafety levels; GLP,GMP
3. Introduction to IP-Types of IP: patents, trademarks & copyright

III . Skills Outcome

On Successful Completion of this Course, Student shall be able to

1. Learn about different plant tissue media
2. Learn about the induction of callus from explants
3. Learn about plant propagation of through various tissue culture
4. Learn about cell lines

Learn about cell viability by various methods

SEMESTER-III
COURSE 5: PLANT AND ANIMAL BIOTECHNOLOGY

Practical Credits: 1 2 hrs/week

1. plant culture media and composition of MS media
2. Raising of aseptic seedlings
3. Induction of callus from different explants
4. Plant propagation through Tissue culture (shoot tip and Nodal culture)
5. Establishing a plant cell culture (both in solid and liquid media)
6. suspension cell culture
7. Cell count by hemocytometer.
8. Establishing primary cell culture of chicken embryo fibroblasts.
9. Animal tissue culture – maintenance of established cell lines.
10. Animal tissue culture – virus cultivation.
11. Estimation of cell viability by dye exclusion (Trypan blue).
12. ELISA – Demonstration

V. REFERENCES

1. .Introduction to Plant Tissue Culture..M.K. Razdan ,2003,Science Publishers

2. Plant Tissue Culture, kalyan Kumar De,199 M7,New Central Book Agency

3. Plant Tissue Culture : Theory and Practice By S.S. Bhojwani and A. Razdan,1998

4. Biotechnology – By U. Satyanarayana ;1997

5. Plant Cell, Tissue and Organ Culture, Applied and Fundamental Aspects By Y.P.S. Bajaj and A. Reinhard ,2001

6. Introduction to Plant Tissue Culture,M. K. Razdan, 2003,Science Publishers

7. A Textbook of Biotechnology,R C Dubey,S. 2014,Chand Publishing

8. Elements of Biotechnology,P. K. Gupta, 1994,Rastogi Publications

9. R. Ian Freshney, "Culture of animal cells – A manual of basic techniques" 4th edition, John Wiley & Sons, 2000 ,Inc, publication, New York

10. Daniel R. Marshak, Richard L. Gardner, David Gottllieb "Stem cell Biology" edited by Daniel 2001,Cold Spring Harbour Laboratory press, New York

11. M.M. Ranga, Animal Biotechnology; Agrobios (India) ,2006.

VI. CO-Curricular Activities

a) **Suggested C0-Curricular Activities**

1. Assignments
2. Seminars, Group Discussions on related topics
3. Charts on different medias
4. Visit to plant tissue culture lab

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SEMESTER-III

COURSE 6: MOLECULAR BIOLOGY

Theory Credits: 3 3 hrs/week

I. LEARNING OUTCOMES

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

1. Learn about genome structure and organization.

2. Learn about mechanism and enzymes of DNA replication.

3. Learn about enzymatic synthesis and features of transcription.

4. Learn about regulation of gene expression.

5. Learn about genetic code and protein synthesis.

II. Syllabus

Unit I Genome Structure

1. Watson and Crick model of DNA; Genome organization with specific reference to prokaryotic and eukaryotic genomes; Genome size.
2. Concepts of Genetic Material, Gene, Chromosome and Genome.
3. Experiments to prove DNA as genetic material (Griffith experiment, Hershey- Chase experiment)

Unit II DNA Replication

1. Enzymology of replication (DNA polymerase I, pol II and III, helicases, topoisomerases, single strand binding proteins, DNA melting proteins, primase.
2. Proof of semiconservative replication, Replication origins,
3. Rolling circle replication of DNA

Unit III Transcription:

1. Enzymatic synthesis of RNA: Basic features of transcription, the structure of prokaryotic RNA polymerase (core enzyme and hollo enzyme, sigma factor),
2. concept of promoter (Pribnow box, -10 and -35 sequences),
3. Four steps of transcription (promoter binding and activation, RNA chain initiation, chain elongation, termination and release). Reverse transcription.

Unit IV Gene Expression and regulation

1. Regulation of gene expression; Clustered genes
2. the operon concepts - Negative and positive control of the Lac Operon, trp operon,
3. Control of gene expression. Poly and Mono cistronic m-RNA,

Unit V Genetic Code and Protein Synthesis

1. Genetic code: Features of genetic code, Structure of m RNA, brief structure of tRNA,
2. The adaptor hypothesis, attachment of amino acids to tRNA.
3. Codon-anticodon interaction - the wobble hypothesis. Initiation, elongation, termination protein.

III . Skills Outcome

On Successful Completion of this Course, Student shall be able to

1. Learn about Quantitative estimation of Nucleic Acids
2. Learn about isolation of DNA from different sources
3. Learn about purity analysis of DNA

SEMESTER-III
COURSE 6: MOLECULAR BIOLOGY

Practical | Credits: 1 | 2 hrs/week

1. Effect of UV radiations on the growth of microorganisms.
2. Determination of absorption maxima of DNA and RNA and their quantification
3. Quantitative estimation of RNA
4. Quantitative estimation of DNA
5. Isolation of plasmid DNA from bacteria
6. Isolation of genomic DNA from *E.coli*
7. Isolation of DNA from sheep liver
8. Isolation of DNA from plant leaves (Rice or Tobacco or any other plant)
9. Separation of DNA by Agarose gel Electrophoresis
10. Purity analysis of the Nucleic acids

V. REFERENCES

1. Cell and Molecular Biology, 8th edition. De Robertis, E.D.P. and De Robertis, E.M.F. 2006; Lippincott Williams and Wilkins, Philadelphia.
2. Cell Biology, (2017), De Robertis & De Roberis, Blaze Publishers & Distributors Pvt. Ltd.
3. The Cell: A Molecular Approach. 5th edition. Cooper, G.M. and Hausman, R.E. 2009. ASMPress & Sunderland, Washington, D.C.; Sinauer Associates, MA.
4. The World of the Cell, 7thedition, Becker, W.M., Kleinsmith, L.J., Hardin. J. and Bertoni, G. P. 2009 Pearson Benjamin Cummings Publishing, San Francisco.
5. David A. Thompson. 2011. Cell and Molecular Biology Lab. Manual.
6. P.Gunasekaran. 2007. Laboratory Manual in Microbiology. New Age International.
7. D O Hall, S E Hawkins. 1974. Laboratory Manual of Cell Biology. British Society for Cell Biology, Published by Crane, Russia.
8. Mary L. Ledbetter. 1993. Cell Biology: Laboratory Manual. Edition: 2. Published by Ron Jon Publishing. Incorporated.
9. Gunasekaran, P. 2009. Laboratory Manual in Microbiology. 1st Edition. New Age International Publishers.
10. Dr. T. Sundararaj. Microbiology Laboratory Manual. 2005. Dr.A.L. MPGIBMS, University of Madras, Taramani, Chennai – 600 113.
11. James G. Cappuccino and Natalie Sherman. 2013. Microbiology: A Laboratory Manual. 10th Edition. Benjamin Cummings.
12. Dr. David A Thompson. 2011. Cell and Molecular Biology Lab Manual.
13. George M. Malacinski. 2013. Freifeder's Essentials of Molecular Biology. Narosa Publishing House.

VI. CO-Curricular Activities

a) **Suggested C0-Curricular Activities**

1. Assignments
2. Seminars, Group Discussions on related topics
3. Charts on Replication, Transcription, and Translation

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SEMESTER-III
COURSE 7: GENETIC ENGINEERING

Theory Credits: 3 3 hrs/week

I. LEARNING OUTCOMES

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

1. Learn about the history and tools of genetic engineering
2. Learn about vectors used in genetic engineering
3. Learn about Hybridization techniques
4. Learn about vectors and their screening techniques
5. Learn about gene editing tools

II. Syllabus

UNIT-I

1. Basics, history, scope, and recent developments in Genetic Engineering; guidelines; strategies in plant and animal genetic engineering.
2. Molecular tools in genetic engineering- Restriction enzymes: Endo & Exonucleases. Modifying enzymes
3. Ligation (cohesive & blunt end ligation) – linkers & adaptor.

UNIT-II

1. Cloning vectors: plasmid - definition, properties and types. pUC19 & pBR322- phage vectors (λ & M13),
2. Cosmid vectors, Shuttle and expression vectors; YAC (S.cerevisiae as a model)& BAC (E.coli);
3. Screening and selection of recombinants; Gene transfer methods

UNIT-III

1. Hybridization techniques: Probes (radioactive & non-radioactive), detection.
2. Polymerase Chain Reaction (PCR) – Principle , Applications and types of PCR
3. Labeling of DNA- Nick translation, Random priming method & labeling by primer extension.

UNIT-IV

1. Construction of genomic & c DNA libraries.
2. Vector engineering & codon optimization, strategies of gene delivery, invitro translation
3. Expression in bacteria, yeast, insects, plant & mammalian cells

UNIT-V

1. Chromosome engineering, targeted gene replacement,
2. gene editing, gene regulation & silencing. Site-directed mutagenesis.
3. DNA sequencing – Maxam Gilbert (chemical) & Sanger's, Nicolson sequencing, Pyrosequencing. Gene therapy, Human Genome Project.

III . Skills Outcome

On Successful Completion of this Course, Student shall be able to

1. Learn about problems in genetic engineering
2. Learn about restriction digestion
3. Learn about isolation of Plasmid
4. Learn about activity of enzymes

SEMESTER-III
COURSE 7: GENETIC ENGINEERING

Practical | Credits: 1 | 2 hrs/week

1. Problem in Genetic engineering.
2. Transformation in Bacteria using plasmid
3. Restriction digestion of DNA and its electrophoretic separation.
4. Ligation of DNA molecules and their testing using electrophoresis.
5. Activity of DNAase and RNAse on DNA and RNA.
6. Isolation of Plasmid DNA
7. Demonstration of PCR

V. REFERENCES

1. Textbook of Biotechnology - 2007, By H.K. Das (Wiley Publications)
2. Principles of Gene Manipulation - 7th edition, 2006, By R.W. Old & S.B. Primrose, Publ: Blackwell
3. Molecular Biology & Biotechnology- 1996, By H.D. Kumar, Publ: Vikas
4. Molecular Biotechnology - 4th edition, 2010, G.R. Click and J.J. Pasternak, Publ: Panima
5. Genes and Genomes – 1991, By Maxine Singer and Paul Berg
6. Genes VII- 2000, By B. Lewin - Oxford Univ. Press
7. Molecular Biology - 4th Edition, 2008, By D. Freifelder, Publ: Narosa Publishing house New York, Delhi
8. Brown TA. (2006). Gene Cloning and DNA Analysis. 5th edition. Blackwell Publishing, Oxford, U.K.
9. Clark DP and Pazdernik NJ. (2009). Biotechnology-Applying the Genetic Revolution. Elsevier Academic Press, USA.
10. Glick, B.R., Pasternak, J.J. (2003). Molecular Biotechnology- Principles and Applications of recombinant DNA. ASM Press, Washington
11. Primrose SB and Twyman RM. (2006). Principles of Gene Manipulation and Genomics, 7thedition. Blackwell Publishing, Oxford, U.K.
12. Sambrook J, Fritsch EF and Maniatis T. (2001). Molecular Cloning-A Laboratory Manual. 3rdedition. Cold Spring Harbor Laboratory Press.

VI. CO-Curricular Activities

a) **Suggested C0-Curricular Activities**

1. Assignments
2. Seminars, Group Discussions on related topic
3. Visit to instrumentation labs

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SEMESTER-III
COURSE 8: METABOLISM

Theory Credits: 3 3 hrs/week

I. LEARNING OUTCOMES

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

1. Learn about Carbohydrate metabolism
2. Learn about Lipid metabolism
3. Learn about Amino Acid metabolism
4. Learn about nomenclature and specificity of enzymes
5. Learn about enzyme kinetics of enzyme

II. Syllabus

Unit I : Carbohydrate metabolism

1. Anabolism & catabolism , Photosynthesis – light and dark reactions. C3 cycle, C4 pathway,
2. Glycolysis – formation of lactate and pyruvate, TCA cycle and its regulation
3. gluconeogenesis, HMP stunt pathway , Disorders of Carbohydrate metabolism- Diabetes mellitus

Unit II : lipids metabolism

1. Denovo synthesis of Fatty Acids , Biosynthesis & degradation of TAG (Triacyl Glycerol),
2. Disorders of Lipid metabolism
3. Biosynthesis of cholesterol , Ketogenesis

Unit III :Amino acid Metabolism

1. General reactions of amino acids, deamination, decarboxylation & transamination.
2. Urea cycle. Biosynthesis of creatine
3. Inborn errors of aromatic and branched-chain amino acid metabolism.

UNIT IV Enzymes:

1. Difference between chemical and biological catalyst, definitions of Holoenzyme apoenzyme coenzyme
2. Classification and nomenclature of enzymes.
3. Enzyme specificity , interaction between enzyme and substrate -lock and key and induced fit models.

UNIT – V Enzyme kinetics:

1. Michaelis-Menten equation, Factors affecting enzyme activity- substrate concentration, enzyme concentration,pH a n d temperature.
2. Enzyme inhibition kinetics - competitive, uncompetitive, and non-competitive
3. Immobilized enzymes and their applications

III . Skills Outcome

On Successful Completion of this Course, the Student shall be able to

1. Learn about assay of enzymes from various sources
2. Learn about estimations of glucose by various methods
3. Learn about titrations of glucose and carbohydrates

SEMESTER-III
COURSE 8: METABOLISM

Practical Credits: 1 2 hrs/week

1. Immobilization of enzymes / cells by entrapment in alginate gel 19. Effect of temperature / pH on enzyme activity
2. Assay of protease activity.
3. Assay of alkaline phosphatase
4. Preparation of starch from Potato and its hydrolysis by salivary amylase
5. Isolation of urease and demonstration of its activity
6. Estimation of amino acids by ninhydrin method
7. Estimation of protein by Biuret method
8. Estimation of glucose by DNS method
9. Estimation of glucose by Benedicts titrimetric method
10 Estimation of total carbohydrates by anthrone method

V. REFERENCES

1. Understanding enzymes: Palmer T., Ellis Harwood ltd., 2001.
2. Enzyme structure and mechanism. Alan Fersht, Freeman & Co. 1997
3. Principles of enzymology for food sciences: Whitaker Marc Dekker 1972.
4. Principles of Biochemistry, White. A, Handler, P and Smith.
5. Biochemistry, Lehninger A.L.
6. Biochemistry, Lubert Stryer.
7. Review of physiological chemistry, Harold A. Harper.
8. Text of Biochemistry, West and Todd.
9. Metabolic pathways – Greenberg.

VI. CO-Curricular Activities

a) **Suggested C0-Curricular Activities**

1. Assignments
2. Seminars, Group Discussions on related topics
3. Charts on cycles – carbohydrate , lipid, amino acid metabolism