FOURTH SEMESTER

Course Name: Plant and Animal Tissue Culture Techniques

Mode: Offline BMGN 4201

Credits: 4

<u>Aim of the Course:</u> To acquaint students with the basic knowledge of in vitro plant and animal tissue culture techniques.

<u>Course Objectives</u>: To impart basic knowledge about the introduction and history of tissue culture, concept on the cell culture and also the In vitro culture: approaches & methodologies. To acquaint students with the details about Tissue nutrition in culture condition and in dept knowledge of Tissue culture methodologies, Tissue culture methodologies and get a concept of the plant and animal Organ Culture

SI	Graduate attributes	Mapped modules
CO1	The student will get an introduction and history of tissue culture.	M1
CO2	The students will get a clear concept on the cell culture	M2
CO3	The student shall get a concept of the In vitro culture: approaches & methodologies	M3
CO4	To acquaint students with the details about Tissue nutrition in culture condition	M4
CO5	The student shall be master to know the Tissue culture methodologies	M5
CO6	The student will get an introduction to the Cloning & Selection of specific cell types	M6
CO7	The student shall get a concept of the plant and animal Organ Culture	M7

Learning Outcome/Skills: To impart basic knowledge about the following

- Introduction to Techniques
- Basic concepts in cell culture
- In vitro culture: approaches & methodologies
- Tissue nutrition
- Tissue culture methodologies
- Cloning & Selection of specific cell types
- Organ Culture

Module					
	Content	Total	% of	Bloom Level	Remarks, if
Number		Hours	questions	(applicable)	any

1 V1 /	Organ Culture	0	15	1,2,3,4	
M6 M7	Cloning & Selection of specific cell types Organ Culture	10 8	18	1,2,3,4	NA NA
M5	Tissue culture methodologies	12	20	1,2,3,4	NA
M4	Tissue nutrition	12	20	1,2,3,4	NA
M3	In vitro culture: approaches & methodologies	8	14	1,2,3,4	NA
M2	Basic concepts in cell culture	6	9	1,2,3	NA
M1	Introduction to Techniques	4	6	1,2,3	NA

Detailed Syllabus

Module 1:

Introduction to Techniques - Introductory history, Laboratory organization, Media, Aseptic manipulation. **(Total Hours: 3)**

Module 2:

Basic concepts in cell culture - cell culture, Cellular Totipotency (Total Hours: 4)

Module 3:

In vitro culture: approaches & methodologies - preparation steps for tissue culture, surface sterilization of plant tissue material, basic procedure for aseptic tissue transfer, incubation of culture (Total Hours: 6)

Module 4:

Tissue nutrition: Growth Hormones - Plant cells (Composition of culture media, Growth hormones, Vitamins, Unidentified supplements, selection of media); Animal cells (substrate on which cells grow, Feeder layer on substrate, gas phase for tissue culture, media and supplements).

(Total Hours: 9)

Module 5:

Tissue culture methodologies - Plant cells (Types of cultures -Callus Culture, Cell Suspension Culture, Organ Microculture, plant micro-propagation, Somatic Embryogenesis); Animal cells (Source of tissue, primary culture, differentiation of cells, growth kinetics, animal cell lines and their origin and characterization).

(Total Hours: 9)

Module 6:

Cloning & Selection of specific cell types – cloning, somatic cell fusion and HAT selection, Medium suspension fusion, selection of Hybrid clone, production of monoclonal antibodies

(Total Hours: 8)

Module 7:

Organ Culture - Culture of embryonic organs, whole embryo culture, culture of adult organs. **(Total Hours: 6)**

Suggested Readings:

- 1. Razdan, M. K. (2003). Introduction to Plant Tissue Culture. Enfield, NH: Science.
- 2. Culture of Animal Cells. A Manual of Basic Technique and Specialized Applications Book by R. Freshney

Course Name: Bio-analytical Tools

Mode: Offline BMGN 4202 Credits: 4

Aim of the Course: To acquaint students with different essential bio-analytical tools

<u>**Course Objectives:**</u> To impart basic knowledge about the bio-analytical tools and principles of centrifugation, microscopy, spectroscopy, chromatography, X-ray crystallography and other different tools.

SI	Graduate attributes	Mapped modules
CO1	The student will get an introduction to the Principle, types and applications of Different microscopy	M1
CO2	The students will get a clear concept on the Principle, types and applications of Absorption spectroscopy	M2
CO3	The student shall get a concept of the Principle, types and applications of centrifugation	M3
CO4	To acquaint students with the details about concepts the Principle, types and applications of chromatography	M4
CO5	The student shall be master to know the Principle, types and applications of spectrometry	M5
CO6	The student will get an introduction to the Principle, and applications of X-ray crystallography	M6
CO7	The student shall get a concept of the Principle and applications of NMR spectroscopy	M7

Learning Outcome/Skills: To impart basic knowledge about the following

- Principle, types and applications of Different microscopy
- Principle, types and applications of Absorption spectroscopy

- Principle, types and applications of Centrifugation
- Principle, types and applications of Spectrometry
- Principle, types and applications of Chromatography
- Principle, and applications of X-ray crystallography
- Principle and applications of NMR spectroscopy

Module					
Number	Content	Total Hours	% of questions	Bloom Level (applicable)	Remarks, if any
THEORY					
M1	Microscopy Techniques	8	13	1,2, 3, 4	NA
M2	Absorption spectroscopy	9	16	1,2,3,4	NA
M3	Centrifugation	8	13	1,2,3,4	NA
M4	Chromatography	9	16	1,2,3,4	NA
M5	Spectrometry	8	13	1,2,3,4	NA
M6	X-ray crystallography	9	15	1,2,3,4	NA
M7	NMR spectroscopy	9	14	1,2,3,4	NA
Total Theory		60	100		
	TOTAL	60			

Detailed Syllabus

Module 1:

Microscopy Techniques

Simple microscopy, phase contrast microscopy, fluorescence and electron microscopy (TEM and SEM), pH meter **(Total Hours: 8)**

Module 2:

Absorption spectroscopy

Absorption Spectroscopy – Simple theory of the absorption of light by molecules, Beer-Lambert law, Instrumentation for measuring the absorbance of visible light, Factors affecting the absorption properties of a Chromophore. Principle of absorption fluorimetry,

(Total Hours: 9)

Module 3:

Centrifugation

Centrifugation – Basic Principle of Centrifugation, Instrumentation of Ultracentrifuge (Preparative, Analytical), Factors affecting Sedimentation, Standard Sedimentation Coefficient, Rate-Zonal centrifugation, sedimentation equilibrium Centrifugation. Cell fractionation techniques, isolation of sub-cellular organelles and particles.

(Total Hours: 8)

Module 4:

Chromatography

Introduction to the principle of chromatography. Paper chromatography, thin layer chromatography, column chromatography: silica and gel filtration, affinity and ion exchange chromatography, gas chromatography, HPLC. Introduction to electrophoresis. Starch-gel, polyacrylamide gel (native and SDS-PAGE), agarose-gel electrophoresis, pulse field gel electrophoresis, immuno-electrophoresis, isoelectric focusing, Western blotting.

(Total Hours: 9)

Module 5:

Spectrometry

Mass spectrometry (MALDI, ESI) and Introduction to Biosensors and Nanotechnology and their applications. Radioactive labeling & counting, Autoradiography.

(Total Hours: 8)

Module 6:

X-ray crystallography

X-Ray Crystallography – X-ray diffraction, Bragg equation, Reciprocal lattice, Miller indices & Unit cell, Concept of different crystal structure, determination of crystal structure [concept of rotating crystal method, powder method]. **(Total Hours: 9)**

Module 7:

NMR spectroscopy

NMR Spectroscopy – Basic principle of NMR spectroscopy, Experimental technique & instrumentation, Chemical shift, hyperfine splitting, Relaxation process.

(Total Hours: 9)

Suggested Readings:

1. Karp, G. 2010. Cell and Molecular Biology: Concepts and Experiments. 6th Edition. John Wiley& Sons. Inc.

2. De Robertis, E. D. P. and De Robertis, E.M.F. 2006. Cell and Molecular Biology. 8th edition. Lippincott Williams and Wilkins, Philadelphia.

3. Cooper, G.M. and Hausman, R.E. 2009. The Cell: A Molecular Approach. 5th edition. ASM, Press & Sunderland, Washington, D. C.; Sinauer Associates, MA.

4. Becker, W.M., Kleinsmith, L.J., Hardin. J. and Bertoni, G. P. 2009

5. The World of the Cell. 7th edition. Pearson Benjamin Cummings Publishing, San Francisco.

Course Name: Genetics and Karyotyping

Mode: Offline BMGN 4203 & BMGN 4293 **Credits: 5(3+2)**

Aim of the Course: To acquaint students with the basics of Genetics and Karyotyping

Course Objectives: To impart basic knowledge about the To impart basic knowledge about Prokaryotic Genomes, Genome organization and Fine structure of the Gene, Eukaryotic Chromosome, Unique genetic features of plants, Chromosomal variation in Number & Structure, Human Cyto-Genetics and Plant and Animal Chromosome Preparation and Karyotyping.

SI	Graduate attributes	Mapped modules
CO1	To acquaint students about the Prokaryotic Genomes	M1
CO2	To acquaint students with the basic concept of Genome organization and Fine structure of the Gene	M2
CO3	To acquaint students with an overview of Eukaryotic Chromosome	M3
CO4	To acquaint students with Unique genetic features of plants	M4
CO5	To acquaint students with the basic knowledge of Chromosomal variation in Number & Structure	M5
CO 6	To acquaint students with the Human Cyto-Genetics	M6
CO7	To acquaint students with the Plant and Animal Chromosome Preparation and Karyotyping	M7

Learning Outcome/Skills: To impart basic knowledge about the following

- Prokaryotic Genomes
- Basic concept of Genome organization and Fine structure of the Gene
- Overview of Eukaryotic Chromosome
- Unique genetic features of plants
- Basic knowledge of Chromosomal variation in Number & Structure
- Human Cyto-Genetics
- Plant and Animal Chromosome Preparation and Karyotyping

Module Number	Content	Total Hours	% of questions	Bloom Level (applicable)	Remarks, if any
THEORY					
M1	Prokaryotic Genomes	3	10	1,2, 3, 4	NA
M2	Genome organization and Fine structure of the Gene	7	15	1,2,3,4	NA

	Eukaryotic Chromosome	7			
M3			15	1,2,3,4	NA
	Unique genetic features of plants	7			
M4			15	1,2,3,4	NA
	Chromosomal variation in Number &	7			
M5	Structure		15	1,2,3,4	NA
M6	Human Cyto-Genetics	7	15	1,2,3,4	NA
M7	Plant and Animal Chromosome Preparation and Karyotyping	7	15	1,2,3,4	NA
Total Theory		45	100		
<u>Practical</u>	·	30			
	TOTAL				
		75			

Detailed Syllabus

Module 1:

Prokaryotic Genomes - Physical organization of bacterial genomes (Structure of the bacterial nucleoid, Replication and partitioning of the bacterial genome and Genome of Archaea).

(Total Hours: 3)

Module 2:

Genome Organization and Fine Structure of the Gene : Genes and Gene numbers, C value paradox, Denaturation and Renaturation of DNA- Tm values and Cot curves, Repetitive and non-repetitive DNA, Inverted and Tandem repeats, Satellite DNA, Gene clusters-Histone, rRNA

(Total Hours: 7)

Module 3:

Eukaryotic Chromosome- Macro-molecular organization. Primary and Secondary constriction, Sat-bodies, telomeres. Heterochromatin and Euchromatin and its significance. Ultra structure of chromosome- Nucleosome model and Nucleosome Structure.

(Total Hours: 7)

Module 4:

Unique Genetic Features of Plants - Ability to photosynthesize, Totipotency of plant cells, Hermaphroditism and ability to reproduce both sexually and asexually, Double fertilization, Alternation of generations, Mitosis in haploid state, Chloroplast Genome Organization, Mitochondrial Genome Organization.

(Total Hours: 7)

Module 5:

Chromosomal variation in Number & Structure– Euploidy, Non-disjunction & Aneuploidy, Aneuploid segregation in plants and animal, Polyploidy in Plants & Animals, Induced Polyploidy, applications of Polyploidy, Chromosomal Mosaics, Polytene chromosome in Diptera, structural chromosomal variation, Chromosomal aberrations & evolution. **(Total Hours: 7)**

Module 6:

Human Cyto-Genetics–Banding techniques, classification, use of Human Cyto-genetics in Medical science, , viable monosomies & trisomies, chromosomal deletions & duplications, genetics of chromosomal inversions & translocations, human traits.

(Total Hours: 7)

Module 7:

Plant and Animal Chromosome Preparation and Karyotyping- Basic procedures of chromosome Preparation from Different Plant Parts. Peripheral Blood and Bone Marrow Culture- Specimen procurement, culture procedure, harvesting, slide making (plant and animal). Chromosome Staining (Plant and Animal)- Aceto orcein and feulgen staining for plants – principle and methods, conventional giemsa staining, differential staining techniques. Photomicrograph and Image Processing –basic concepts. Chromosome Analysis and Karyotyping methods. Plant chromosomes grouping, Chromosome Identification- individual band position and characteristics, Interpretation.

(Total Hours: 7)

PRACTICAL

BMGN 4293

Paper Name-Lab on Genetics and Karyotyping

(Where ever wet lab experiments are not possible the principles and concepts can be demonstrated through any other material or medium including videos/virtual labs etc.)

Credit:2

Total Hours:30

1. Preparation of Mitotic Chromosome from human Leucocytes.

- 2. Preparation of Mitotic Chromosome from plant tissue (Onion).
- 3. Problems based on Re-association Kinetics

4. Karyotyping with the help of photographs

Suggested Readings:

1. Gardner, E.J., Simmons, M.J., Snustad, D.P. (2006). Principles of Genetics. VIII Edition John Wiley & Sons.

2. Snustad, D.P., Simmons, M.J. (2009). Principles of Genetics. V Edition. John Wiley and Sons Inc.

3. Klug, W.S., Cummings, M.R., Spencer, C.A. (2009). Concepts of Genetics. IX Edition. Benjamin Cummings.

4. Russell, P. J. (2009). Genetics- A Molecular Approach. III Edition. Benjamin Cummings.

5. Griffiths, A.J.F., Wessler, S.R., Lewontin, R.C. and Carroll, S.B. IX Edition. Introduction to Genetic Analysis, W. H. Freeman & Co.4. Theory and problems in Genetics by Stansfield

6. Barch MJ et al. The AGT cytogenetics Laboratory Manual; 3rd ed,1007, LippincottRaven; New York

7. Arun Kumar Sharma and Archana Sharma :2014 Chromosome Techniques Theory and Practice, Butterworth-Heinemann, Oxford

8. Purandare Hema & Chakravarty Amit: Human cytogenetics Techniques& clinical applications,2000, Bhalani Publishing House, Mumbai

9. Culture of Animal cells-a manual of basic Techniques:R IAN Freshney (Wiley Publication)