SACRED HEART COLLEGE (AUTONOMOUS)

Department of BOTANY

MASTER OF SCIENCE IN BOTANY

Course plan

Academic Year 2015 – 16

Semester IV

COURSE PLAN

TISSUE CULTURE AND MICROBIAL BIOTECHNOLOGY

.Basic Reference

1. Hamish A Collin, Sue Edwards (1998). *Plant tissue culture*. Bios scientific publishers.
2. R A Dixon, R A Gonzales (2004). *Plant cell culture, a practical approach* (II Edn). Oxford University Press.
3. S S Bhojwani, M K Razdan (1996). *Plant tissue culture: Theory and Practice*. Elsevier.
4. Susan R. Barnum (1998). *Biotechnology an introduction*. Thomson Brooks/cole.
5. Nicholas C Price, Lewis Stevens (1999). *Fundamentals of enzymology* (III Edn). Oxford university press.
6. Trever Palmer (2004). *Enzymes: Biochemistry, Biotechnology, Clinical chemistry*. T Palmer/Harwood Publishing Limited.
7. E M T El-Mansi, C F A Bryce, A L Demain, A R Allman (2007). *Fermentation Microbiology and Biotechnology* (II Edn). Taylor & Francis.
8. L Gamborg, G C Philips (Eds.) (2005). *Plant cell, tissue and organ culture: Fundamental methods*.Narosa Publishinh House.
9. *In vitro cultivation of plant cells*. Biotechnology by open learning. Elsevier.

John L Ingraham, Catherine A Ingraham (2000). *Introduction to microbiology* (II Edn). Brooks/Cole

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1.	Date	Topic	Method	Remarks
1	Session 1	(a) Brief history and important milestones in plant tissue culture. (b) Cellular totipotency. (c) Types of cultures: organized structures - meristem, shoot tip, node, embryo, root cultures; unorganized structures - callus, suspension and protoplast cultures.	Presentation/Chalk and Board	
2.	Session 2			
3	Session 3	Adventitious regeneration: Direct regeneration, indirect regeneration. Factors influencing adventitious regeneration; genotype, explant – orientation of explant, position on mother plant.	Presentation/Chalk and Board/Assignment	
4	Session 4			
5	Session 5			

6	Session 6	Somatic embryogenesis: General aspects, initiation of embryogenic cultures, maturation of somatic embryos, regeneration of plants, factors regulating somatic embryogenesis, differences between somatic and zygotic embryos. Encapsulation of somatic embryos, synthetic seed production; desiccated and hydrated types. Applications and limitations of synthetic seeds	Presentation/Chalk and Board/Assignment	
7	Session 7	Differentiation of cells in callus - tracheid formation, factors influencing vascular differentiation. Organogenic differentiation: factors influencing shoot bud differentiation, induction of organogenic differentiation	Presentation/Chalk and Board	
8	Session 8			
9	Session 9			
10	Session 10	(a) Isolation of somaclonal variants, molecular basis of somaclonal variation. (b) Origin of somaclonal variation – pre-existing variability, in vitro induced variability; Reasons– changes in ploidy level, changes in chromosome structure, gene mutations, gene amplifications, changes in extra nuclear genes, activation of transposable elements, DNA methylation. (c) Applications of somaclonal variation.	Presentation/Chalk and Board	
11	Session 11			
12	Session 12			
13	Session 13			
14	Session 14	(a) Haploids: Androgenesis - pretreatment of anther/pollen grains, media and growth regulators, Induction and stage of pollen development, regeneration, androgenic embryos, factors affecting androgenesis. Microspore culture - protocol, advantages over anther culture. (b) Gynogenesis: Developmental stage at inoculation, in vitro maturation of embryo sacs, origin of embryos, triggering factors – pretreatment, medium. Uses and limitations of haploid plants. (c) Triploids: importance of triploid plants, conventional production of triploid plants, endosperm culture-advantages and limitations	Presentation/Chalk and Board	
15	Session 15			
16	Session 16			
17	Session 17			
18	Session 18			
19	Session 19	(a) Isolation and purification of protoplasts, culture of protoplasts, cell division and callus formation, plant regeneration.		
20	Session 20			
21	Session 21			
22	Session 22			

		(b) Protoplast fusion (somatic hybridization) – chemical, mechanical, electrofusion. Selection, isolation of heterokaryons, cybrids and their applications. Applications of protoplast culture.		
23	Session 23	(a) Culture conditions for producing secondary metabolites, selection of high yielding lines, elicitation, immobilization of cells. (b) Hairy root culture – advantages of using hairy root culture, establishment of hairy root culture and production of secondary metabolites	Presentation/Chalk and Board/Assignment	
24	Session 24			
25	Session 25			
26	Session 26			
27	Session 27			
28	Session 28	(a) Importance, methods of conservation: In situ and ex situ conservation. (b) In vitro conservation, short and medium term storage, cryopreservation technique– importance of cryopreservation, pretreatment, freezing methods, cryoprotectants, vitrification.	Presentation/Chalk and Board	
29	Session 29			
30	Session 30			
31	Session 31	(a) Cell immobilization: Methods, advantages and applications. (b) Enzyme immobilization: Preparation, applications, enzymes as biosensors. (c) Enzyme engineering	Presentation/Chalk and Board	
32	Session 32			
33	Session 33			
34	Session 34	(a) Regenerative medicine, methods and applications of tissue engineering. (b) Stem cells – embryonic stem cell and adult stem cells – potential applications	Presentation/Chalk and Board	
35	Session 35			
36	Session 36			
37	Session 37			
38	Session 38	(a) Screening of microbes for metabolite production. Selection of media, sterilization of media. (b) Bioreactors – airlift, stirred tank, bubble column, rotary drum. Fermentation process - batch, fed batch, continuous fermentation. Submerged and solid state fermentation Process control during fermentation- pH, aeration, agitation, temperature, foam control. (c) Downstream processing. (d) Large scale production of antibiotics - penicillin, streptomycin, industrial chemicals - ethanol, acetone, butanol, lysine. Microbial insecticides. Commercial production of enzymes and their uses - amylase, cellulase, polygalacturonase.	Presentation/Chalk and Board	
39	Session 39			
40	Session 40			
41	Session 41			
42	Session 42			
43	Session 43			
PRACTICALS				
44	Session 44	1. Preparation of the stock solutions of MS medium. 2. Preparation of selective medium for drought or salinity resistance. Preparation of MS soild medium from stock solutions containing auxin and cytokinin, NaCl or PEG, and inoculation.	Laboratory	
45	Session 45			
46	Session 46			
47	Session 47			
48	Session 48			
49	Session 49			
50	Session 50			

51	Session 51	3. Preparation of synthetic seeds. 4. Find out the uninucleate stage of anther and anther culture. 5. Dissect out an embryo from any seed and culture it on a suitable solid medium. 6. Isolation of microbes producing amylase.		
52	Session 52			
62	Session 62			
63	Session 63 - 72			

COURSE PLAN
BIOTECHNOLOGY & GENETIC ENGINEERING

Course Objectives:

1. Students will have a better understanding on the principles of Genetic engineering
2. The students will be able to describe and discuss the tools and techniques of biotechnology.
3. Students will be able to think critically about the gene cloning procedure and its merits and demerits.
4. Insight into the important aspects of advanced transgenic technology.
5. Student gains practical knowledge on various genetic engineering techniques.\
6. At the end of the course the students will be able to critically evaluate the ethical, legal and social impacts of biotechnology.

References:

1. James D Watson, Amy A Caudy, Richard M Myers, Jan A Witkowski (2007). Recombinant DNA (III Edn). W H Freeman.
2. Primrose, R M Twyman (2006). Principles of gene manipulation and genomics (VII Edn). Blackwell publishing.
3. T A Brown (2002). Genomes (II Edn). Bios.
4. Smita Rastogi, Neelam Pathak (2010). Genetic engineering. Oxford.
5. Bernard R Glick, Jack J Pasternak, Cheryl L Pattein (2010). Molecular biotechnology: Principles and applications of recombinant DNA. ASM press.

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Sl. No.	Session	Topic/Module	Method of teaching	Remarks
1	1	Isolation and purification of genomic DNA from bacteria	PPT / Lecture	
2	2	Isolation and purification of plasmid DNA from bacteria	PPT / Lecture	
3	3	Isolation and purification of plant genomic DNA	PPT / Lecture	
4	4	Isolation and purification of RNA	PPT / Lecture	
5	5	Vectors - necessary properties of a vector	PPT / Lecture	
6	6	Plasmids - pBR322, pUC	PPT / Lecture	
7	7	Virus - Lambda phage, M13	PPT / Lecture	
8	8	Artificial chromosomes – YAC, BAC, PAC, HAC	PPT / Lecture	
9	9	Shuttle vectors, expression vectors	PPT / Lecture	

10	10	Direct Gene Transfer Methods	PPT / Lecture	
11	11	Restriction endonucleases – naming, types and reaction	PPT / Lecture	
12	12	Ligases – reaction, methods of blunt end joining -	PPT / Lecture	
13	13	linkers and adaptors	PPT / Lecture	
14	14	Topocloning and Gateway cloning	PPT / Lecture	
15	15	Creation of recombinant DNA, Introduction of recombinant DNA into host cell	PPT / Lecture	
16	16	Preparation of competent host cells, transformation	PPT / Lecture	
17	17	Selection of transformed cells	PPT / Lecture	
18	18	Identification of recombinant cells – insertional inactivation	PPT / Lecture	
19	19	Methods of screening and selection of recombinant cells – Lac Z system	PPT / Lecture	
20	20	GFP	PPT / Lecture	
21	21	Agrobacterium tumefaciens mediated gene transfer in plants	PPT / Lecture	
22	22	binary vector and cointegrate vector	PPT / Lecture	
23	23	Creation of Bt plants	PPT / Lecture	
24	24	Golden rice	PPT / Lecture	
25	25	Flavr Savr Tomato	PPT / Lecture	
26	26	Phosphodiester and phosphotriester	PPT / Lecture	
27	27	phosphite-triester method	PPT / Lecture	
28	28	Phosphoramidite method	PPT / Lecture	
29	29	Automated DNA synthesis	PPT / Lecture	
30	30	Artificial genome synthesis	PPT / Lecture	
31	31	Applications of protein engineering	PPT / Lecture	
32	32	Protein modification by site-directed mutagenesis, combinatorial methods	PPT / Lecture	
33	33	Design and operation, types. Applications - medical, food and agriculture, industrial, pollution monitoring	PPT / Lecture	
34	34	GMOs as biosensors	PPT / Lecture	
35	35	Inducible expression systems	PPT / Lecture	
36	36	site-specific recombination for in vivo gene manipulation	PPT / Lecture	
37	37	gene targeting using antisense RNA and RNAi	PPT / Lecture	
38	38	In vitro mutagenesis - site-directed mutagenesis	PPT / Lecture	
39	39	Genomic and cDNA library	PPT / Lecture	
40	40	Procedure for the construction of a genomic library using phage λ system	PPT / Lecture	

41	41	Identification of desirable clones from library – hybridization probing, colony and plaque hybridization probing, immunological screening	PPT / Lecture	
42	42	Locating and isolating a gene - in situ hybridization, positional cloning, chromosome walking and jumping.	PPT / Lecture	
43	43	PCR - Procedure and applications, variants of PCR - Real time PCR and its applications	PPT / Lecture	
44	44	In vitro mutagenesis- Oligonucleotide directed, Error- prone PCR, Cassette Mutagenesis. Applications of In vitro mutagenesis.	PPT / Lecture	
45	45	Blotting techniques - procedure and applications of southern, northern, western, and dot blotting.	PPT / Lecture	
46	46	Microarray (gene chip) technology.	PPT / Lecture	
47	47	Procedure and applications of DNA profiling, Footprinting.	PPT / Lecture	
48	48	Procedure and applications of ELISA	PPT / Lecture	
49	49	RIA, Immunoprecipitation	PPT / Lecture	
50	50	flow cytometry, FISH, GISH	PPT / Lecture	
51	51	Approaches to gene therapy- somatic cell and germline therapy, vectors used in gene therapy	PPT / Lecture	
52	52	In vivo and ex vivo therapy	PPT / Lecture	
53	53	Gene therapy of SCID, Cystic fibrosis, gene augmentation therapy	PPT / Lecture	
54	54	Problems and fears associated with gene therapy.	PPT / Lecture	
55	55	Uses of GM microbes: Bacteria and yeast - producing useful proteins, basic genetic research.	PPT / Lecture	
56	56	Applications of GM animals: In basic research, producing novel proteins; disease studies, prevention and cure diseases.	PPT / Lecture	
57	57	Uses of transgenic plants: Herbicide, insect and disease resistance, stress resistance. Genetic engineering for increasing nutritional and other novel qualities in plants.	PPT / Lecture	
58	58	Need for regulation, regulatory agency in India – GEAE.	PPT / Lecture	
59	59	Patents – issues relating to patenting living organisms, their genes and other bioresources	PPT / Lecture	

60	60	Potential impact of GMOs on the ecosystem.	PPT / Lecture	
61	61	GM food – effect on health and environment.	PPT / Lecture	
62	62	Ethical problems of rDNA technology	PPT / Lecture	
63	63	Economic problems of rDNA technology	PPT / Lecture	
64	64	Potential misuse of modern molecular biology tools and techniques, bioweapons, bioterrorism	PPT / Lecture	
65	65	Isolation of plant genomic DNA and its quantification	Practical lab	
66	66	Isolation of plasmids and its purification	Practical lab	
67	67	Isolation of bacterial genomic DNA and its quantification by using UV spectrophotometer	Practical lab	
68	68	Separation of DNA by agarose gel electrophoresis	Practical lab	
69	69	Separation of proteins by PAGE	Practical lab	
70	70 - 72	PCR	Practical lab	

COURSE PLAN

GENOMICS, PROTEOMICS & BIOINFORMATICS

COURSE OBJECTIVES:

- ✳ Aware about the unprecedented booming of bio-economy in our country
- ✳ Understanding the advent of omic technology revolution in genomics proteomics
- ✳ An exposure to omic technologies and its applications, the course has been incorporated as a complete courses for major omic approaches and its allied softwares.
- ✳ Openness in the availability genomic data, many *in silico* researches can also be envisage after the course.
- ✳ Highly futuristic and have a perfect insight into the modern technologies in genomics and proteomic.

Basic Reference

1. 1. S B Primrose, R M Twyman (2006). *Principles of gene manipulation and genomics* (VII Edn). Blackwell publishing.
2. Robert J Brooker (2009). *Genetics: analysis & principles* (III Edn). McGraw Hill.
3. James D Watson, Amy A Caudy, Richard M Myers, Jan A Witkowski (2007). *Recombinant DNA* (III Edn). W H Freeman.
4. T A Brown (2002). *Genomes* (II Edn). Bios.
5. Leland H Hartwell, Leroy Hood, Michael L Goldberg, Ann E Reynolds, Lee M Silver, Ruth C Veres (2004). *Genetics:From genes to genomes* (II Edn). McGraw Hill.
6. *Science*, 16 february 2001, Vol. 291.
7. Jeremy M Berg, John L Tymoczko, Lubert Stryer, Gregory J Gatto Jr. (2007).

Biochemistry. W H Freeman and company.
8. David P Clark (2010). *Molecular biology*. Elsevier.
9. D Peter Snustad, Michael J Simmons (2010). *Principles of genetics* (V Edn). John Wiley and Sons.
10. David A Micklos, Greg A Freyer with David A Crotty (2003). *DNA Science: A first course* (II Edn). L K Inter.
11. Benjamin A Pierce (2008). *Genetics: A conceptual approach* (IV Edn). W H Freeman and Company.
12. Anthony J F Griffiths, Susan R Wesler, Sean B Carroll, John Doebley (2008). *Introduction to genetic analysis* (X Edn). W H Freeman and Company.
13. Benjamin A Pierce (2008). *Genetics: A conceptual approach* (IV Edn). W H Freeman and Company
14. C W Sensen (2002). *Genomics and Bioinformatics*. Wiley – VCH.
15. T A Brown (2002). *Genomes* (II Edn). Bios.
16. William J Thieman, Michael A Palladino (2009). *Introduction to biotechnology* (II Edn). Pearson
17. George Acquaah (2005). *Understanding biotechnology*. Pearson.
18. Robert H Tamarin (2002). *Principles of genetics*. McGraw Hill.
19. Robert K Murray, David A Bender, Kathleen M Botham, Peter J Kennelly, Victor W Rodwell, P Anthony Weil (2009). *Harper's Illustrated Biochemistry* (XXVIII Edn). Mc Graw Hill.
20. *Nature*, 409 (6822): 860-921, 2001.
21. S R Pennington, M J Dunn (Edts) (2002). *Proteomics: From protein sequence to function*. Viva Books Private Limited.
22. Bernard R Glick, Jack J Pasternak, Cheryl L Pattein (2010). *Molecular biotechnology, principles and applications of recombinant DNA*. ASM press.
23. Burton E Tropp (2012). *Molecular biology: Genes to Proteins* (IV Edn). Jones and Bartlett Learning.
24. Jocelyn E Krebs, Elliott S Goldstein, Stephen T Kilpatrick (2011). *Lewin's Genes X* . Jones and Bartlett Publishers.
25. Teresa K Attwood, David J Parry-Smith, Simiron Phukan (2007). *Introduction to Bioinformatics*. Pearson Education.
26. Zhumur Ghosh, Bibekanand Mallik (2008). *Bioinformatics: principles and applications*. Oxford University press.
27. Orpita Bosu, Simminder Kaur Thukral (2007). *Bioinformatics: Databases tools and algorithms*. Oxford University press.
28. David W Mount (2001). *Bioinformatics: Sequence and genome analysis*. CBS publishers & distributors.
29. Jin Xiong (2006). *Essential Bioinformatics*. Cambridge University Press

1.	Date	Topic	Method	Remarks/Reference
1	Session 1	Genomics: Genome and Proteomics- basis and key concepts.	PPT, Videos, Discussion & Lecture	
2.	Session 2			

3	Session 3	Important findings of the completed genome projects: Human genome project, Rice genome project, Arabidopsis genome project, *E. coli* genome project, Wheat genome project, Tomato genome project.	PPT, Videos & Lecture + Seminar	
4	Session 4			
5	Session 5	Basic steps in genome sequencing. Shot gun sequencing of small genomes. Map based sequencing: Hierarchical shot gun sequencing (clone-by-clone approach) - steps involved; Whole genome shot gun approach - steps involved.	PPT, Videos & Lecture	
6	Session 6			
7	Session 7			
8	Session 8	Next generation sequencing strategies – Pyrosequencing	PPT, Videos & Lecture	
9	Session 9			
10	Session 10			
11	Session 11	Sequence assembly – methods used.	PPT, Videos & Lecture	
12	Session 12			
13	Session 13	Genome mapping: Genetic mapping and physical mapping. Cytogenetic and linkage map. Molecular markers – RFLP, RAPD, AFLP, SSLP, SNP.	Presentation/Chalk and Board	
14	Session 14			
15	Session 15			
16	Session 16		Presentation/Chalk and Board	
17	Session 17	Construction of linkage maps using molecular markers – E.g., RFLP maps. Physical mapping – restriction mapping, STS, SNP, EST.	Presentation/Chalk and Board	
18	Session 18			
19	Session 19			
20	Session 20		Presentation/Chalk and Board	
21	Session 21	Transcriptome, expression profiling (mRNA profiling).	PPTs and Lectures	
22	Session 22			

23	Session 23	Gene expression analysis using dot blotting and microarrays. Fabrication of microarrays – spotted arrays, *in situ* synthesis.	PPT, Videos & Lecture	
24	Session 24			
25	Session 25	Chromatin immunoprecipitation (ChIP) and its applications.	PPT, Videos & Lecture	
26	Session 26	(a) Orthologs and Paralogs Comparative genomics as a tool in evolutionary studies.	PPT, Videos & Lecture	
27	Session 27			
28	Session 28	(a) Gene identification by comparative genomics (b) Metagenomics.	PPT, Videos & Lecture	
29	Session 29	Proteome, proteomics.	PPT, Videos & Lecture	
30	Session 30	Separation and identification of cellular proteins by 2D gel electrophoresis and mass spectrometry.	PPT, Videos & Lecture	
31	Session 31			
32	Session 32			
33	Session 33	Introduction, aim and importance of bioinformatics.	PPT & Lecture	
34	Session 34	(a) Databases: primary and secondary databases (b) DNA sequence databases - Genbank, DNA databank, Nucleotide sequence databank (EMBL Bank). Specialised databases. (c) Protein databases - SWISS-PROT, PDB.	PPT, Videos & Lecture + Online experiment	
35	Session 35			
36	Session 36	Submission and retrieval of databases – BankIt, ENTREZ.	PPT, Videos & Lecture + Online experiment	
37	Session 37	Sequence analysis – significance. Methods of sequence alignment – paired sequence alignment, multiple sequence alignment, scoring matrices.	PPT, Videos & Lecture + Online experiment	
38	Session 38			
39	Session 39			
40	Session 40			

41	Session 41	Sequence comparison – dot matrix method, dynamic programming for sequence alignment; Global – Needleman-Wunch algorithm; Local - Smith Waterman algorithms.	PPT, Videos & Lecture	
42	Session 42			
43	Session 43	Database similarity search – query sequence search; BLAST - different versions; FASTA - different versions.	PPT, Videos & Lecture + Online experiment	
44	Session 44	Tools for multiple sequence alignment – CLUSTAL X/W.	PPT, Videos & Lecture + Online experiment	
45	Session 45			
46	Session 46	Gene prediction strategies, ORF search.	PPT, Videos & Lecture + Online experiment	
47	Session 47	RNA secondary structure prediction;	PPT, Videos & Lecture + Online experiment	
48	Session 48	Protein structure and function prediction - tools used.Bioinformatics for enzyme and protein design. Protein visualization tool – Rasmol	PPT, Videos & Lecture + Online experiment	
49	Session 49			
50	Session 50	Applications of bioinformatics in evolutionary studies – molecular phylogenetics, molecular clock.	PPT & Lecture	
51	Session 51	Construction of phylogenetic trees – MEGA, Phylip, Mr.Bayes, RaXML	PPT, Videos & Lecture + Online experiment	
52	Session 52			
53	Session 53			
54	Session 54			
55	Session 55	Computer assisted drug design - concept, methods and practical approaches.	PPT, Videos & Lecture	

56	Session 56	Various computational methods applied to design drugs.	PPT, Videos & Lecture + Online experiment	
	57 – 65	Seminar		
	66 – 72	Revision		