SHIVAJI UNIVERSITY
KOLHAPUR
DEPARTMENT OF BIOTECHNOLOGY

M. Sc. PART -II
Revised Syllabus for Credit System
(WITH EFFECT FROM ACADEMIC YEAR 2013-2014)

SEMESTER-III

GE  341: Genetic Engineering
FT  341: Fermentation Technology-I
IM  341: Immunology
BT  341: Plant Biotechnology
LC BT 341: Laboratory Course V
LC BT 342: Laboratory Course VI

SEMESTER-IV

AB   441 : Animal Cells in Biotechnology
AGP 441 : Advances in Genomics and Proteomics
APB 441 : Advances in Plant Biotechnology
IOM 441 : Industrial Organization and Management
LC BT 441: Laboratory Course VII
LC BT 442: Laboratory Course VIII (Project Work)
Work load for M. Sc II

(Each Semester) | Theory | Practicals
----------------|--------|----------
Seminars        | 16 hrs | 16 hrs   |
                | 2 hrs  | (for 1 batch) |
Oral Exam       | 2 hrs  |           |
----------------|--------|-----------|

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20 hrs
SEMESTER-III
GE 341: Genetic Engineering
IM 341: Immunology
PB 341: Plant Biotechnology
LC BT 341: Laboratory Course V
LC BT 342: Laboratory Course VI

SEMESTER-IV
AGP 441: Advances in Genomics and Proteomics
APB 441: Advances in Plant Biotechnology
LC BT 441: Laboratory Course VII
LC BT 442: Laboratory Course VIII (Project Work)

Following courses offered by Dept. of Biotechnology are optional or choice based. Instead of these courses, students can opt for courses offered by other departments as part of Choice Based Credit System (CBCS pattern).

Sem I BSI 141: Biostatistics and Bioinformatics with Computer Orientation
Sem. III: FT 341: Fermentation Technology-I
    IOM 441: Industrial Organization and Management

Department of Biotechnology will offer following three courses as part of CBCS pattern

Sem III: PB 341: Plant Biotechnology (pre-requisites: MB 241, GE 341)
Sem. IV: AGP 441: Advances in Genomics and Proteomics (pre-requisites: MB 241, GE 341)
    APB 441: Advances in Plant Biotechnology (pre-requisites: PB 341)

These choice based courses are open to be opted by students studying M. Sc Biochemistry, Microbiology, Botany, Zoology, Environmental Biotechnology.

(LS141 represents: LS: Course name, 1: Semester, 4: credit allotted to the course, 1: Chronological order within that category)

Core Theory courses: 12 x 4 = 48 credits
Core Practical courses: 6 x 4 = 24 credits
SEMESTER III

GE 341: Genetic Engineering
(Prerequisite: MB 241)

UNIT-I

DNA & BASICS OF RECOMBINANT DNA TECHNOLOGY

Structure of DNA: A-, B-, Z-, and triplex DNA, measurement of properties, spectrophotometric, CD, AFM, and electron microscope analysis of DNA structure. Restriction analysis: Types of restriction enzyme, Type I, II and III, restriction modification systems, type II restriction endonucleases and properties, isoschizomers and neoschizomers, mcr/mrr genotypes, Cohesive and blunt end ligation, linkers, adaptors, homopolymeric tailing. Labeling of DNA: Nick translation, random priming, radioactive and non-radioactive probes, use of Klenow enzyme, T4 DNA polymerase, bacterial alkaline phosphatase, polynucleotide kinase. Hybridization techniques: Northern, Southern and Colony hybridization, Fluorescence in situ hybridization

Restriction maps and mapping techniques, DNA fingerprinting, chromosome walking & chromosome jumping

DNA-Protein Interactions: Electro mobility shift assay, DNase I footprinting, methyl interference assay

UNIT -II

CLONING VECTORS

Gene Cloning Vectors: Plasmids, bacteriophages, Cloning in M13 mp vectors, phagemids, Lambda vectors; insertion and replacement vectors, EMBL, λDASH, λgt10/11, λZAP etc. Cosmid vectors. Artificial chromosome vectors (YACs, BACs), Animal Virus derived vectors-SV-40, vaccinia/bacculo & retroviral vectors. Expression vectors; pMal, GST, pET-based vectors. Protein purification; His-tag, GST-tag, MBP-tag etc. Restriction proteases, intein-based vectors. Inclusion bodies, methodologies to reduce formation of inclusion bodies. Baculovirus and pichia vectors system
UNIT- III
CLONING METHODOLOGIES

Insertion of Foreign DNA into Host Cells: Transformation, Transfection: Chemical and physical methods, liposomes, microinjection, macroinjection, electroporation, biolistics, somatic cell fusion, gene transfer by pronuclear microinjection, Plant transformation technology: Basis of tumor formation, hairy root, features of Ti and Ri plasmids, mechanism of DNA transfer, role of virulence genes, use of Ti and Ri as vectors. Cloning and expression in yeasts (Saccharomyces, Pichia etc.), animal and plants cells, methods of selection and screening, cDNA and genomic cloning, expression cloning, jumping and hopping libraries, southwestern and far western cloning, yeast two hybrid system, phage display, Construction of cDNA libraries in plasmids and screening methodologies, Construction of cDNA and genomic DNA libraries in lambda vector. Principles in maximizing gene expression, Site-directed mutagenesis.

UNIT- IV
PCR AND ITS APPLICATIONS

Primer design, Fidelity of thermostable enzymes, DNA polymerases, multiplex, nested, reverse transcriptase, real time PCR, touchdown PCR, hot start PCR, colony PCR, cloning of PCR products, T-vectors, proof reading enzymes, PCR in gene recombination, deletion, addition, overlap extension, and SOEing, site specific mutagenesis, PCR in molecular diagnostics, viral and bacterial detection, PCR based mutagenesis.

Sequencing methods: Enzymatic DNA sequencing, Chemical sequencing of DNA, principle of automated DNA sequencing, RNA sequencing.


Other applications: Transgenics, Genome projects and their implications, application in global gene expression analysis. Applications of recombinant DNA technology in medicine, agriculture, veterinary sciences.

Suggested readings
FT 341: Fermentation Technology-I (Prerequisite: LS 141, TB 241, GE 341) (60)

UNIT I (15)

Upstream processing, microbial cell growth, kinetics and stoichiometry, various Methods for growth measurement, strain improvement by mutation, genetic engineering etc. Over production of metabolites, alternative carbon and nitrogen sources and their composition. Development of inocula for industrial fermentation, design of industrial production media. Alternate metabolic routes for utilization of carbon sources with their regulation and inter-linkage especially for glucose and hydrocarbons, preservation and maintenance of microbes.

UNIT II (15)

Fermentation, Design of fermenter, Construction materials, Various sterilization techniques for solid, liquid and gases, Aeration and agitation, foam, Auxillary equipments. Control of various parameters – online and offline monitoring, rheological properties of fermenter, role of computer in fermenter operation,

UNIT III (15)

Batch, fed-batch and continuous fermentation. Effluent treatment, scale up and scale down. Types of fermentors, solid state fermentation, process economics, fermentation economics.

UNIT IV (15)

Downstream Processing, Principle, methodology, instrumentation an applications of cell homogenization techniques liquid-liquid extraction centrifugation, filtration, distillation, ultrafiltration, precipitation, adsorption chromatography, ion exchange chromatography, gel filtration and affinity chromatography in clarification, concentration, isolation and purification of various metabolites from fermented media

Suggested Readings

IM 341: Immunology  
(Prerequisite: B Sc Life Science)  

UNIT I  


UNIT II  

Immunity to infection, antigen processing and presentation, MHC, complement system. Bacterial, viral, protozoal and parasitic infections with reference to (Diphtheria, influenza virus, malaria and helminthes) with specific representative examples of each group. Vaccines – Active and passive immunization, DNA vaccines, multivalent subunit vaccines, synthetic peptide vaccines.

UNIT III  


UNIT IV  

Immunotechnology, antigen antibody interactions: principles, types and applications of agglutination, precipitation, complement fixation, viral neutralization, immunodiffusion, immunoelectrophoresis, ELISA and RIA. Monoclonal antibodies – Hybridoma technology and various cellular technologies. Automation in immunological techniques – auto analyzers used in immunology, FACS etc.

Suggested readings

2. Immunology by Roitt I. M., Brostoff J. and Male D. Gower medical publishing London.
BT-341: Plant Biotechnology
(Prerequisite: B Sc Life Science)

UNIT-I

Plant physiology and its significance in agriculture; physical properties and chemical constitution of protoplasm, concept of diffusion and osmosis, plant cell water relation – imbibition, absorption and translocation of water and nutrients, transpiration, guttation, Photosynthesis-modern concept and the factors affecting photosynthesis, nitrogen fixation

UNIT-II


UNIT III

Initiation, maintenance and importance of callus culture, suspension culture and single cell culture, types and applications of single cell culture, organ culture, protoplast culture and its applications, isolation and types of protoplasts fusion, seed dormancy and germination, somatic embryogenesis its types and applications, encapsulation and production of synthetics seeds, development of somatic hybrids to overcome the incompatibility barriers.

UNIT IV

Anther culture and production of haploid plants and homozygous lines, embryo culture and embryo rescue, pollen, ovary culture and their applications, germ plasm preservation. Micropropagation: principle, vegetative regeneration by-shoot tip, meristem, axillary and adventitious shoot initiation, organogenesis and production of pathogen free plants, hardening of tissue cultured plants, industrial applications of micropropagation technique.

Suggested Readings


**LC BT 341: Laboratory Course V**

*(Prerequisite: LC BC 141, LC BC 142)*

1. Estimation of IAA
2. Determination of activity of enzyme IAA oxidase
3. Estimations amino acids (arginine and histidine)
4. Induction of beta galactosidase in *E. coli*.
5. Study of mutations by Ames test.
6. Assay of antibiotics and demonstration of antibiotic resistance.
7. Isolation of organic acid and amine producers and biochemical characterization of isolated microbes.
8. One step growth curve of coliphage.
9. Isolation of Streptomycin resistant mutants.
10. Transduction.
11. Conjugation.
12. Isolation of genomic DNA

**LC BT 342: Laboratory Course VI**

*(Prerequisite: LC BC 141, LC BC 142)*

1. Plant tissue culture
2. Preparation of Media
3. Surface Sterilization
4. Organ Culture
5. Callus Culture, organogenesis
6. In vitro rooting and acclimatization
7. Protoplast isolation and culture
8. Anther Culture/ Production of haploids
9. Cytological examination of regenerated plants
10. Agrobacterium culture, selection of transformants, GUS assay.
11. Synseed preparation
12. Immunological techniques
14. ELISA
15. Antibody capture
16. Antigen capture
17. Dot ELISA
18. Precipitin Reaction - Ring test- Immunodiffusion.
19. Immunofluorescence
20. Latex Agglutination
22. Rocket Immunodiffusion.

Suggested Readings
1. Practical Biochemistry: An Introductory Course by Fiona Frais.
5. Hawk’s Physiological Chemistry ed. by Bernard L. Oser.
SEMESTER IV

AB- 441: Animal Cells in Biotechnology  
(Prerequisite: B Sc Life Science)  

UNIT I  

Laboratory and introduction of cells: Equipments and materials for animal cell culture technology. Design of Tissue Culture Laboratory. Equipments : Laminar Flow Hoods, CO₂ incubator, Open and closed cultures, Microscopes, centrifuge, Refrigerators and Freezers, pipetting aids, Miscellaneous small items of equipments, Materials, filters, Miscellaneous Items. Basic Aseptic Techniques. Storage shipping and safety. Characters of cells and behavior: Cells and tissue types. Behavior of cells in culture: Primary cell lines permanent/Established cell lines/Transformed cell lines. Tumor/cancer originated cells.

UNIT II  

Growth media, Physical requirements and Nutritional Requirements of Cells and growth media and cell culture growth kinetics, Natural media, Basal salt solution (BSS)-Various types Minimum Essential Medium( MEM). Antibiotics in media, Serum dependent defined media, Serum independent defined media – Cell specific media. pH, bulk ions, trace ions, CO₂, O₂ tension , Ascorbic acid, sugars, Vitamins coenzymes. Basic Techniques of mammalian cell culture, Open and closed cell-cultures, Primary Cell culture – Isolation and separation of cells, viable cell count, maintenance of cell culture, maintenance of stock culture, Antibiotic free stock cultures, Types of cell cultures – Monolayer, Suspension, Clonal culture, Mass culture-micro carrier culture (monolayer), Stem cell cultures (ESC)

UNIT III  

Biology and Characterization of cultured cells, Karyotyping, Contamination Testing of Culture Viability measurement and cytotoxicity, Measurement of growth parameters, Cell cycle analysis and Synchronization of cultures, Cell surgery Methods, Preparation of anucleated cells and polykaryon cells, Preparation of ghost RBCs. Preparation of mini cells, micro cells, Surgical manipulation of in vitro fertilization

UNIT-IV  

Cell Fusion Methods, Cell fusion techniques, Hybridoma cell preparations and their properties Use of Hybridoma technology: e.g. M AB and other related techniques, Mini cells, micro cells and anucleated cells in fusion and their application. Tissue Engineering: Capillary culture Units, Techniques for culturing differentiated cells: Reconstituted basement, membrane rafts, feeder layers. Use of Animal Cells in Culture, Mutant cell preparation, Evaluation of Chemical carcinogenicity, Cell malignancy Testing, Toxicity Testing, Karyotyping and cytogenetic characterization, Production of metabolic products, ,ESC applications, Pluripotent stem cell applications
Suggested Readings

1. Kuchler, R.J., Biochemical Methods in cell culture and Virology, Dowden,
2. Huchinson and Ross, Inc. Strausberg, USA, 1977
5. John Wiley and Sons Inc. Publication, USA.
7. Jenni P. Mather and David Barnes, eds; Animal cell culture Methods,
AGP 441: Advances in Genomics and Proteomics  
(Prerequisite: BC 142, MB 241 and GE 341)  

UNIT I  
ADVANCE NUCLEIC ACIDS TECHNIQUES  

Agarose gel electrophoresis, PFGE, Principles of PGFE, instrumentation, types and applications of PFGE. DGGE, Principles of DGGE, types and applications of DGGE, RT PCR, Real time PCR, principle of Real time PCR, types and application of Real time PCR, (quantitative and qualitative), Designing Primers and probes for RT PCR, types of probes and their chemistries. RNA interference and gene silencing (si-RNA, mi-RNA) technology, Mi RNA. Various blotting techniques.  

UNIT II  
DNA SEQUENCING TECHNOLOGIES  

DNA sequencing technologies: Different chemistries in DNA sequencing, Next Generation sequencing (Sanger’s sequencing, SOLiD, Pyrosequencing), application of next generation sequencing (NGS), genomic library, EST library, cDNA library, Whole genome sequencing, Introduction to the concept of Transcriptomics and Metagenomics, Applications of Transcriptomics and Metagenomics.  

UNIT III  
PHYLOGENETIC ANALYSIS  

Evolution, elements of phylogeny, methods of phylogenetic analysis, Phylogenetic tree of life, comparison of genetic sequence of organisms. Identification of microbial isolates by 16S rDNA amplification and sequencing, methods of study of unculturable microbial flora from environmental sample, 16S rDNA library, 16S rRNA library, Ribosomal Database Project (RDP), Introduction to phylogenetic analysis tools, Concept of operational taxonomic unit (OTU)/ phylotypes, rarefaction curve, DOTUR, MOTHUR, MEGA, Phylip, PAUP, ClustalW. DNA based molecular markers technologies: RAPD, RFLP, AFLP, SCAR, SSR, ISSR, t-RFLP, The principles and applications of DNA based molecular markers.  

UNIT IV  
MICROARRAYS AND PROTEOMICS  

Concept of microarrays; spotted arrays, oligonucleotide arrays, designing the experiment, microarray design, microarray experimentation, Applications of microarray technology. Chromatography: gel permeation, adsorption (ion exchange, affinity), partition, HPLC, protein purification. 2-D analysis and Maldi-Tof in Proteomics, Mass spectroscopy for protein analysis,
MALDI-TOF, Electrospray ionization, Tandem mass spectroscopy (MS/MS) analysis; tryptic digestion and peptide, fingerprinting (PMF), Protein Micro array in protein expression, profiling and Diagnostics.

**Suggested readings**
1. Bernard R. Glick and Jack J. Pasternak, Molecular Biotechnology: Principles and Applications of Recombinant DNA.
5. Bioinformatics Methods and Applications Genomics, Proteomics and Drug Discovery. (Rastogi S. C. Mendiratta, and Rastogi P.)
7. Molecular Cloning by Sambruk and Russel (Maniatis)
APB 441: Advances in Plant Biotechnology

(Prerequisite: BT 341)

UNIT I

Plant protection: Diseases of field, vegetable, orchard and plantation crops of India and their control; causes and classification of plant diseases; principles of plant disease control biological control of diseases; seed health testing, Integrated pest management-concepts and components; host plant resistance-biological control of insect pests; genetic manipulation of insects for their control; pesticides, their formulation, classification and safe use; behavioural methods; use of computer modeling in pest and disease outbreak; insect growth regulators; biotechnological approaches in IPM;

UNIT II

Agricultural microbiology: Contributions of Beijerinck and Winogradsky-Rrole of microbes in carbon and nitrogen cycles-Influence of Rhizosphere on soil microorganism-various types of nitrogen fixing microorganism-Production of bacterial biofertilizers: Rhizobium, Azospirillum, Phosphobacteria etc.- Fungal biofertilizers; Ecto- and Endomycorrhizae- Azolla and BGA- Method of application for different biofertilizers, biopesticides.

UNIT III


UNIT IV

Transgenic techniques in plant biotechnology: introduction of foreign gene into plants, basics of tumor formation, hairy root culture and its uses, features of Ti & Ri plasmid, mechanism of DNA transfer, role of virulence gene, use of reporter gene, multiple gene transfers, vector less or direct DNA transfer, particle bombardment, electroporation, microinjection, chloroplast transformation. Applications of plant transformation for enhancing resistance to pests, productivity & performance, nutritional value, modification of ornamental plants, bioengineered food, edible vaccines, plantibodies, biopharming.
Suggested Readings

1. Slater, Plant Biotechnology, OUP
4. Concepts in Biotechnology D. Balasubramaniam, Bryce, Dharmalingam, Green, Jayaraman
5. Univ. Press, 1996
IOM- 441: Industrial Organization and Management
(Prerequisite: B. Sc. Life Science) (60)

UNIT I (15)


UNIT II (15)


UNIT III (15)


UNIT-IV (15)


Suggested Readings

5. N. D. Kapoor Mercantile Law.

The syllabus for this paper to be taught keeping in mind the aspects of commercialization, marketing and management of Biotechnological products. Seminars, Case studies, are included as a supportive work to clear concepts of Management.
LC BT 441: Laboratory Course VII  
(Prerequisite: B. Sc. Life Science)

1. Isolation of genomic DNA from plants.
2. Restriction digestion and analysis of DNA fragments by agarose gel electrophoresis.
3. Establishment of Molecular markers (RAPD/ISSR/RFLP).
4. Isolation of bacterial/fungal isolates and their preservation
5. Identification of bacterial/fungal isolates by 16S rDNA/18S rDNA amplification and sequencing.
6. Isolation of genomic DNA from environmental samples
7. Construction of 16S rDNA library, sequencing of clones and sequence analysis.
8. Preparation of competent cells (chemical or electro)
9. Isolation of plasmid DNA by miniprep/midiprep
10. Isolation of total RNA
11. Separation of RNA by denaturing gel electrophoresis
12. SDS page
13. Native Page
14. Identification and characterization of proteins resolved on 2D PAGE
15. Isolation of chloroplast
16. Tissue culture techniques: organ/anther/callus culture
17. Induction of somatic embryos
18. Protoplast isolation and cultures.
19. Genetic transformation for hairy root induction by *A. rhizogens*. 
Suggested readings

1. Practical Biochemistry : An Introductory Course by Fiona Frais.
5. Hawk’s Physiological Chemistry ed. by Bernard L Oser.
8. Developing Bioinformatics computer skills – Cynthia Gibas and Per Jambeck

LC BT 442: Laboratory Course VIII (Project Work) (Prerequisite: B. Sc. Life Science) (60)
Department of Biotechnology
Shivaji University, Kolhapur

A course under Choice Based Credit System (CBCS)

(Following points (minimum) should be covered while designing the CBCS course)

- Course code: **PB 341**
- Title of the course: **Plant Biotechnology**

Department at which course will be conducted: **Department of Biotechnology**

- Duration: **16 weeks**
- Contact Sessions: Theory – **60 hours** and Practical (if applicable): **.....hours**
- Credits: **4**
  
  (1 credit for 15 Theory hrs and 1 credit for 12 Practical hrs per week)

- Course Coordinator/Instructor: Dr. J.P. Jadhav, Dr. K.D.Pawar, Dr. P.K.Pawar

- Eligibility: Students should have studied following courses: MB 241, GE 341

- Intake: **60** Min: **(20)** Max.: **(60)**

- Course offered during: Odd semester

- Course Fee: **Rs. 2, 500**

- Course Contents: Unit 1/Unit 2/Unit 3/Unit 4

- Examination *(Method & Details)*

- Text Books / Reference Books:

- Any other information, if any.

Department of Biotechnology
Shivaji University, Kolhapur
A course under Choice Based Credit System (CBCS)

(Following points (minimum) should be covered while designing the CBCS course)

- Course code: APB 441
- Title of the course: Advances in Plant Biotechnology

Department at which course will be conducted: Department of Biotechnology

- Duration: 16 weeks
- Contact Sessions: Theory – 60 hours and Practical (if applicable): ..... hours
- Credits: 4
(1 credit for 15 Theory hrs and 1 credit for 12 Practical hrs per week)

- **Course Coordinator/Instructor:** Dr. J.P. Jadhav, Dr. K.D. Pawar, Dr. P.K. Pawar.

- **Eligibility:** Students should have studied following courses: **PB 341**

- **Intake:** **60** Min: **(20)** Max.: **(60)**

- **Course offered during:** Even semester

- **Course Fee:** **Rs. 2, 500**

- **Course Contents:** Unit 1/Unit 2/Unit 3/Unit 4

- **Examination:** (Method & Details)

- **Text Books / Reference Books:**
  1. Slater, Plant Biotechnology, OUP
  4. Concepts in Biotechnology D. Balasubramaniam, Bryce, Dharmalingam, Green, Jayaraman
  5. Univ. Press, 1996

- **Any other information, if any.**
Department of Biotechnology
Shivaji University, Kolhapur

A course under Choice Based Credit System (CBCS)

(Following points (minimum) should be covered while designing the CBCS course)

- **Course code:** AGP 441
- **Title of the course:** Advances in Genomics and Proteomics

Department at which course will be conducted: **Department of Biotechnology**

- **Duration:** 16 weeks
- **Contact Sessions:** Theory – 60 hours and Practical (if applicable) :.....hours
- **Credits:** 4
  
  (1 credit for 15 Theory hrs and 1 credit for 12 Practical hrs per week)
- **Course Coordinator/Instructor:** Dr. J.P. Jadhav, Dr. K.D. Pawar, Dr. P.K. Pawar.
- **Eligibility:** Students should have studied following courses: MB 241, GE 341
- **Intake:** 60 Min: (20) Max.: (60)
- **Course offered during:** Even semester
- **Course Fee:** **Rs. 2, 500**
- **Course Contents:** Unit 1/Unit 2/Unit 3/Unit 4
- **Examination:** (Method & Details)
- **Text Books / Reference Books:**
1. Bernard R. Glick and Jack J. Pasternak, Molecular Biotechnology: Principles and Applications of Recombinant DNA.
5. Bioinformatics Methods and Applications Genomics, Proteomics and Drug Discovery. (Rastogi S. C. Mendiratta, and Rastogi P.)
7. Molecular Cloning by Sambruk and Russel (Maniatis)

• Any other information, if any.