# Solapur University, Solapur

# B.Sc. II (Biotechnology, Semester III&IV)

Syllabus 2011-2012

#### SOLAPUR UNIVERSITY, SOLAPUR B.Sc. II Biotechnology Syllabus W.E.F. June- 2011 Semester III

Paper No	Paper Title	Paper periods	Marks
BT- 201	Bt-201 (I) Genetics-I	40	50
	(Mendelian and Population Genetics)		
	Bt-201 (II) Molecular Biology-I		
	(Molecular biology of Gene)	40	50
BT- 202	Bt-202 (I) Methods in Biotechnology –I	40	50
21 202	(Biophysical Techniques)		
	Bt-202 (II) Environmental Biotechnology-I		
	(Pollution and Toxicology)	40	50
BT- 203	Bt-203 (I) Bioenergetics and Metabolism-I	40	50
	(Bioenergetics and Thermodynamics)		
	Bt-203 (II) Immunology-I		
	(Cells and organs of Immunity)	40	50
	Semester III - Total Marks		300

#### Semester IV

Paper No	Paper Title	Paper periods	Marks
BT- 204	Bt-204 (I) Genetics-II	40	50
	(Microbial Genetics)		
	Bt-204 (II) Molecular Biology-II		
	(Gene Regulation and Expression)	40	50
BT- 205	Bt-205 (I) Methods in Biotechnology -II	40	50
	(Biochemical Techniques)		
	Bt-205 (II) Environmental Biotechnology-II		
	(Bioenergy and Bioremediation)	40	50
BT- 206	Bt-206 (I) Bioenergetics and Metabolism-II	40	50
	(Metabolism)		
	Bt-206 (II) Immunology-II		
	(Humoral and Cell Mediated Immunity)	40	50
	Semester IV - Total Marks		300

### Practicals

Bp 207	Techniques in Molecular Genetics	100	25 P
Bp 208	Methods in Advanced Biotechnology	100	25 P
Bp 209	Techniques in Bioenergetics and Immunology	100	25 P

\* Practical examination will be conduct at the end of semester IV.

#### Semester- III Bt-201(I) Genetics - I

#### (Mendelian and Population Genetics) 40L

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Unit I-

Mendelism: - Introduction, Law of segregation, and Law of independent Assortment, Modifications of Mendelian ratios: Co-dominance, Interaction of genes- complementary (9:7), supplementary gene (9:3:4), inhibitory gene (13:3), epistasis (12:3:1)

#### Unit II-

Linkage and crossing over- linkage groups, mapping of chromosome, three point testcross. Extra chromosomal inheritance- Genetic system in mitochondria, chloroplast, and plasmid. Multiple alleles- ABO blood groups in human, fur colour in rabbit, self-incompatibility in plants, and eye colour in drosophila.

#### Unit III-

Chromosome- morphology, Heterochromatin & euchromatin, Lampbrush chromosome, polytene chromosome, Role of chromosome in heredity. Chromosomal aberrations-Structural changes- Deletion, Duplication, Inversion, and Translocation. Numerical changes- Aneuploidy, Euploidy.

#### Unit IV-

Population genetics- Hardy-weinberg law, gene frequency, factors affecting gene frequency, significance of population genetics. Genetic basis of evolution, evolution of some crop plants- Wheat, Cotton, Brassica,

## Bt-201(II) Molecular Biology –I (Molecular biology of Gene) 40L

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Unit - I

DNA as the genetic material - Structure of DNA, Organization of Genomes - Viral, Bacterial, Organization of Eukaryotic Genomes, Chromosomal organization and Structure (Nucleosome, Solenoid Model) And Histones, Telomeres, Centromeres, Heterocromatin and Euchromatin.

Unit - II 10L Genome complexity- Molecular nature of Gene and Genetic code. Gene definition Introns and Exons, regularity sequences. Micro and mini satellites, LINES and SINES, Pseudogenes and Cot curve. RNA: Types structure and functions.

Unit - III 10L Replication- Semiconservative synthesis. The replicon model. Rolling circle model of replication. Mechanism and enzymology of DNA replication in Prokaryotes and Eukaryotes.

Unit – IV 10L DNA damage. DNA Repair- Mismatch, Excision, Recombination, SOS repair. mechanisms and disorders. General Recombination and Transposition

#### BT-202(I) Methods in Biotechnology – I (Biophysical Technique)

Unit I

Colorimetry: - principle, Beer – Lambert's Law, instrumentation, working, Applications. Nephelometry and Turbidimetry:- theory, factors affecting measurements, Instrumentation, working, applications. pH meter:- Definition – acids and bases; pH – definition, dissociation of acids and bases, measurements of pH – pH indicators, accurate measurement of pH, glass electrode, operation and calibration of pH electrode, errors in pH measurements.

Unit II Centrifugation:-

Centrifugation:- Introduction, Sedimentation and Relative Centrifugal g Force, Rotor Types Swinging-Bucket Rotors, Fixed-Angle Rotors, Vertical Rotors, Density Gradients, Types of Centrifugation Techniques, Differential Centrifugation, Rate-Zonal Centrifugation, Isopycnic Centrifugation, Analytical Ultracentrifugation. Microscopy:-Introduction, image formation in light and electron microscopy, optical principles of microscopy, design and practice of Transmission Electron Microscopy and Scanning Electron Microscopy.

Unit III

Unit IV

Nature and types of radiations, preparation of labeled biological samples. Detection and measurement of radioactivity, GM counter, Scintillation counter, Autoradiography, Flow cytometry. Safety measures in handling radioisotopes. RIA, non radiolabelling.

VIS–Spectrophotometry:- theory, deviation from Beer's law, instrumentation, applications. UV–Spectrophotometry:- Introduction, theory of UV spectrophotometer, Instrumentations, applications. IR Spectrophotometry:- range of IR spectrophotometer, theory, instrumentation, applications. Atomic absorption spectroscopy: principle, instrumentation, applications

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#### Bt-202(II) Environmental Biotechnology - I (Pollution and Toxicology)

Unit I

Soil pollution: Waste dumping, soil solute increase, alkalinity, effect of plastics, hospital wastes, industrial & domestic wastes, heavy metals, pesticide residues, fertilizers. Water pollution: Point and non-point pollution, eutrophication, mass mortality of fishes, thermal effluents, agricultural drainages, oil pollution, heavy metal pollution, industrial effluents.

#### Unit II

Air pollution – Sources –oxides of carbon, Nitrogen, Sulphur. Hydrocarbons- Transport and Diffusion – Effects of air pollutants on life and properties – Acid rain- Ozone depletion- Global warming and their consequences, Radioactive pollution – thermal pollution – noise pollution – dioxins, radio activity hydrocarbon, lead pollution.

#### Unit III

Eco-toxicology: Introduction to ecotoxicology, Principles of toxicology, Types of toxic substances - degradable and non-degradable; Influence of ecological factors on the effects of toxicity; Sigmoid relationships, Corollary of toxicology Toxicants in the Environment: Toxic substances in the environment, their sources and entry roots, Eco-system influence on the fate and transport of toxicants; Transport of toxicants by air and water; Transport through food chain - bio-transformation and bio-magnification

#### Unit IV

Man and Environmental Toxins: Routes of toxicants to human body – entry through inhalation, skill absorption, indigestion and injection; Response to toxin exposures – Dose response, Frequency response and cumulative response; Lethal and sub-lethal doses; Dose- Response relationships between chemical and biological reactions. Analysis of NOEL, LD 50, LC 50 and MLD; Detoxification in human body - detoxification mechanisms, organs of detoxification

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#### BT-203(I) Bioenergetics and Metabolism - I (Bioenergetics and Thermodynamics)

#### Unit I

Principles of thermodynamics and their application in Biochemistry- introduction, thermodynamic system, thermodynamic state functions, first and second laws of thermodynamics, concept of free energy, standard free energy, determination of  $\Delta G$  of a reaction, relation between equilibrium constant and standard free energy change, biological standard state and standard free energy change in coupled reactions.

#### Unit II

Biological oxidation- reduction reactions- introduction, redox potential, relation between standard reduction potential and free energy change (derivation and numericals included). High energy phosphate compounds- introduction, phosphate group transfer- free energy of hydrolysis of ATP and sugar phosphates along with reasons for high  $\Delta G$ 

#### Unit III

Enzyme catalysis: nature of non enzymatic and enzymatic catalysis, measurement and expression of enzyme activity-enzyme assays, Definition of IU, Katal, role of non protein organic molecules and inorganic ions, coenzyme, prosthetic groups, role of vitamins as coenzymes. Role of cofactors in enzyme catalysis: NAD/ NADP+, FMN/ FAD, biocytin, cobamide, lipoamide, TPP, tetrahydofolate and metal ions with special emphasis on coenzyme functions, acid base catalysis, covalent catalysis, proximity and orientation effects, strain and distortion theory. Mechanism of action of chymotrypsin, carboxypeptidase and lysozyme.

#### Unit IV

Enzyme Kinetics: Factors affecting enzyme activity : enzyme concentration, substrate concentration, p and temperature. Derivation of Michaelis Menten equation for uni substrate reactions. Km and its significance. Lineweaver Burk's plot and its limitations. Importance of Kcat/ Km. Bi- substrate reactions. Kinetics of zero and first order reactions. Reversible and irreversible inhibition, competitive, non competitive and uncompetitive inhibitions, determination of Km and Vmax in presence and absence of inhibitors.

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#### Bt-203(II) Immunology-I (Cells and organs of Immunity)

Unit I 10L Hematopoisis: Introduction, regulation at genetic level, factors involved in hematopoisis, programmed cell death. Cell of immune system: B lymphocytes, T lymphocytes, natural killer cells, mononuclear phagocytes, dendritic cells, follicular dendritic cells. Organs of immune system: Structure and functions of primary lymphoid organs ( thymus, bone marrow, lymphatic system), secondary lymphoid organs (lymph nodes, spleen), mucosa associated lymphoid tissue, cutaneous associated lymphoid tissue.

#### Unit II

Major histocompatibility complex: Introduction, three classes, allelic forms, (structure and function) Cytokines: Introduction, properties,function,cytokines receptors,cytokines related diseases,cytokine based therapies. Complement system: Introduction, functions, components, general account on complement activation (classical, alternative and lectin pathways), complement deficiencies

#### Unit III

Antigen : Introduction, immunogenicity, antigenicity, types of antigens, Haptens, properties of immunogen, role of biological system inimmunogenecity (genotype of animal, immunogen dosage, route of Administration), adjuvants, epitopes.

Antibody: Introduction, basic structure, antibody binding site, antibody mediated effector functions, structure and biological function of antibody classes, antigenic determinants, introduction and uses of monoclonal antibodies and abzymes.

#### Unit IV

Antigen – antibody interactions: Principles and applications of interaction, strength of interactions, cross – reactivity, features of interactions, measurement of antigen – antibody, precipitation ( ring test, slide test, tube test), immunodiffussion (Oudin procedure, Oakley Fulthroe procedure, Ouchterlony procedure), mmune-electrophoresis, Electroimmunodiffussion (CIE, Rocket electrophoresis), agglutination (slide test, tube test, passive agglutination test, agglutination inhibition test), complement fixation test, immunofluorescence test, Radioimmunoassay, ELISA.

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#### Bt-204(I) Molecular Genetics Genetics-II (Microbial Genetics)

Unit I-Plasmids- Properties, Types, Classification, Plasmid stability, Isolation, detection, purification of plasmid DNA, transfer of plasmid DNA, plasmid replication, plasmid as vector.

#### Unit II-

Mutagenesis- Spontaneous, induced, lethal mutations, Mutagens-chemical and physical, methods of isolation of auxotrophic mutants, drug resistance mutants, analysis of mutations in biochemical pathway

#### Unit III-

Transposable elements- Terminology, insertion sequences, detection of transposition in bacteria, types of bacterial transposons. Transposition- structure of transposons and target sites, replicative and non-replicative transposition, excision of transposons, genetic phenomenon mediated by transposons in bacteria.

Unit IV- 10L Methods of gene transfer in bacteria- Transformation, Conjugation-Mechanism of conjugation, F plasmid, conjugation in other bacteria. Transduction-Specialized and generalized. Recombination- general (homologous), site specific and non-homologous.

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#### Bt-204.II

# Molecular Biology –II (Regulation and gene expression) 40L

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Unit I 10 L Transcription- Organization of proteins and RNA coding genes, types of RNA transcripts and their role. Promoters and RNA polymerases in prokaryotes and eukaryotes, events in mRNA transcription. And processing. The mRNA processing, hnRNPs and snRNPs

Unit II 10 L Regulation of transcription- Regulation of transcription in prokaryotes, Regulation of transcription

Unit III 10L Translation – Ribosome's and t- RNA, Events of protein synthesis in prokaryotes and eukaryotes. Regulation of translation and drugs. Post – translational modifications.

Regulation of gene action- Operon concept. Repression and induction of genes. Regulation of operon in Lac operon and Trp operon

Unit IV

#### BT-205(I)

### Methods in Biotechnology - II (Biochemical Technique) 40L

Electrophoresis: Basic principles of electrophoresis, support media, theory and application of paper, starch gel, agarose, native and denaturing PAGE, isoelectric focusing, Southern Northern and Western blotting.

Introduction and types of chromatography, paper, thin layer, gas, Gel permeation, ionexchange, HPLC, FPLC and affinity chromatography and instrumental details of each. Applications of Chromatographic techniques in Biology.

#### Unit III

Unit I

Unit II

Protein Determination: BCA Assay, Bradford Assay, Lowry Assay, Gels: SDS Gels Electrophoresis, SDS Electrophoresis Without Stacker, Native Gels, Staining Gels: Fixing, Staining, Drying, Precipitation and Concentration: Denaturing Precipitation, Native Precipitation, Concentration, Blotting: Protein Staining on Blots, Blocking, Immunostaining, Ca2+ Binding, Ligand Staining, and Autoradiography of Gels and Blots.

#### Unit IV

Proteomics: Introduction, Sample Taking, 2D Gel Electrophoresis, Mass Spectroscopy of Peptides and Proteins, Mass Spectrometers, Sample Preparation for MALDI, The Possibilities of MALDI and ESI. Microsequencing: Preparing the Protein, the Edman Degradation, Carboxyterminal Sequencing.

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#### Bt-205(II) Environmental Biotechnology - II (Bioenergy and Bioremediation)

Unit I

Human energy requirement, Energy use pattern in different parts of the world and its impact on the environment; Energy use pattern in India; Sources of energy and their classification; Energy forms and transformation Sun as source of energy: Source of sun's energy, Solar spectrum, solar radiation – absorption, reflection, scattering and diffusion in the atmosphere, Albedo, Global energy

balance

Unit II

Bio-energy: Biomass composition and types; Conversion processes – pyrolysis, charcoal production, compression, gasification and liquefaction; Energy plantation; Biogas – production and uses, anaerobic digestion; Environmental constrains; Energy from solid Wastes - Sources, types, energy production

Unit III

Bioremediation for Water Environment: Biochemical, and ecological Foundations of Bioremediation, Ex-situ Decontamination of Groundwater -Characterizing the Site and Contaminant Complexity -Selecting the Bioremediation Option, Process Optimization, In-situ Bioremediation of Groundwater -Factors Affecting Bioaugmentation -Delivery Systems for Oxygen, Nutrients, and Innoculation, Landfill Leachate Biotreatment Technologies, Industrial Wastewater Biotreatment Technologies.

Unit IV

Bioremediation for Soil Environment: Environment of Soil Microorganisms, Soil Organic Matter and Characteristics, Pesticides and Microorganisms, Petroleum Hydrocarbons and Microorganisms, Industrial solvents and Microorganisms, Biotechnologies for Ex-Situ Remediation of Soil, Biotechnologies for in-Situ Remediation of Soil, Phytoremediation Technology for Soil Decontamination

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#### BT-206(I)

#### Bioenergetics and Metabolism - II

### (Metabolism)

Carbohydrate Metabolism: Reactions and energetics of glycolysis. Alcoholic and lactic acid fermentation. Entry of fructose, galactose, mannose etc. Reactions and energetics of TCA cycle, gluconeogenesis, glycogenesis and glycogenolysis; Reactions and physiological significance of pentose phosphate pathway. Regulation of glycolysis and TCA cycle. Photosynthesis: a brief review.

#### Unit II

Unit I

Electron Transport Chain and Oxidative Phosphorylation: Structure of mitochondria, sequence of electron carriers, sites of ATP production, inhibitors of electron transport chain. Hypothesis of mitochondrial oxidative phosphorylation (basic concepts). Inhibitors and uncouplers of oxidative phosphorylation. Transport of reducing potential into mitochondria.

#### Unit III

Lipid Metabolism: Introduction, hydrolysis of triacylglycerols, transport of fatty acid into mitochondria,  $\beta$  oxidation of saturated fatty acids, ATP yield from fatty acid oxidation, biosynthesis of saturated and unsaturated fatty acids. Metabolism of ketone bodies, oxidation of unsaturated and odd chain fatty acids. Biosynthesis of triglyceridse and important phospholipids, glycolipids, sphingolipids and cholesterol. Regulation of cholesterol metabolism.

#### Unit IV

Amino Acid Metabolism: General reactions of amino acid metabolism Transamination, oxidative deamination and decarboxylation. Urea cycle, degradation and biosynthesis of amino acids, glycogenic and ketogenic amino acids. Nucleotide Metabolism: Sources of the atoms in the purine and pyrimidine molecules. Biosynthesis and degradation of purine ad pyrimidines. Regulation of purine and pyrimidine metabolism.

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#### BT-206(II)

#### Immunology - II

#### (Humeral and Cell Mediated Immunity) 40L

Unit I

Innate immunity: introduction, general account. Humoral immunity: Primary and secondary immune response, antibody production against (T cell independent antigen, T cell dependent antigen and antigen presented by antigen presenting cells), role of B cells, T cells, antigen presenting cells, MHC molecules and cytokines in antibody production, B cell receptors, B cell (maturation, activation, differentiation.) Cell mediated immunity: T cell receptors, types of cells, their role in immunity, T cell (maturation, activation, differentiation), role of cytokines and MHC molecules in CMI

Unit II

Hypersensitivity: Introduction, Gell and Coombs classification, types general mechanism and component staking part in hypersensitivity) Autoimmunity: Introduction, general mechanism, classification of autoimmune diseases (Hemolytic, organ specific, non-organ specific).

Unit III

Immunity to Bacteria, Protozoa, Helminthes and Viruses: Regarding nonspecific immunity, specific immunity and evasion of the immune response. Transplantation immunity: Allograft rejection, Graft-Versus-Host Disease, Grafts that are not rejected suppression of the allograft response.

Unit IV

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Immunohematology: ABO blood group system, Rh blood group system, applications of blood group, Hemolytic diseases of new born, detection of Rh antibodies, ABO hemolytic diseases. Vaccines: Introduction active and passive immunization, Live – attenuated vaccines, killed vaccines, subunit vaccines, conjugate vaccines, DNA vaccines, recombinant vector vaccines.

### **Bp**-207 Techniques in Molecular genetics

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1) Study of Mendelian traits in garden pea.

2) Problem sets in Mendelian inheritance, single point, two point crosses and gene interactions

- 3) Determination of 'chromosome count' in onion root tips.
- 4) Determination of 'chromosome count' in PMCs in onion.
- 5) Identification of mutant phenotypes- Body shape /nature of wings /eye colour in Drosophila.
- 6) Estimation of nucleic acids by colorimetric method
- 7) Demonstration of agarose gel electrophoresis for separation of nucleic acids
- 8) Electrophoretic analysis of protein.
- 9) Field work based on Mendelian genetics.
- 10) Isolation of bacterial DNA
- 11) Isolation of Plasmid DNA
- 12) Isolation of DNA from Animal Cell/ plant cell.
- 13) Isolation of RNA from yeast
- 14) Isolation of RNA from plant cells / tissue
- 15) Isolation of coliphages

16) Transfer of genetic material - Transduction / Transformation / Conjugation

17) Isolation of antibiotic resistant mutant by replica plate technique

#### **Bp** - 208 Methods in Advanced Biotechnology

- 1. Cell disruption by SDS/ EDTA/ Lysozyme/ Tolune
- 2. Purification of Amylase by dialysis.
- 3. Ammonium sulphate / Acetone precipitation of enzyme.
- 4. Immobilization of enzymes.
- 5. Maltose calibration curve by using colorimeter.
- 6. Growth curve by turbidimetry /Nephelometry.
- 7. Colourimetric estimation of inorganic phosphate.
- 8. UV spectra of protein and nucleic acid.
- 9. Elecrophoresis of RNA.
- 10. Electrophoresis of serum proteins.
- 11. SDS-PAGE for protein mol. Wt. Determination
- 12. Ion-exchange chromatography for protein/peptide purification
- 13. Gel permission chromatography for Enzyme purification
- 14. Tube Gel electrophoresis for Protein purification
- 15. Determination of Chlorides of water
- 16. Study of variation of Conductivity of water with temperature
- 17. Determination of transparency and turbidity of water
- 18. Determination of leaf area index by Planimeter Method
- 19. Estimation of sulphates by turbidometry.
- 20. Estimation of heavy metals in various samples by AAS.
- 21. Sampling techniques: wastewater analysis for physico-chemical haracteristics such as pH, conductivity, TDS, DO, BOD, COD, CO2,

alkalinity, nutrients, chlorides, hardness, settlability of solids.

- 22. Vermicomposting: analysis of composted material for NPK, moisture holding and microbial load.
- 23. Microbial degradation of textile dyes/pesticides/hydrocarbons and oils
- 24. Effect of heavy metals on microbial growth
- 25. Effect of pesticides on soil microorganisms
- 26. Field visit to river/lake and waste water treatment plants.

#### **Bp - 209 Techniques in Bioenergetics and Immunology**

1. Study of enzyme activity: effect of substrate concentration, temperature, pH and Hg metal on Amylase.

- 2. Separation of proteins by Gel Filtration chromatography.
- 3. Study of dialysis Precipitation of Ammonium sulphate and salt removal.
- 4. Isolation of cytochrome c, ATP.
- 5. Estimation of blood glucose level.
- 6. study of respiration by Manometer (Warburg).
- 7. Determination of the acid value of fat.
- 8. Study of Yeast Isocitrate dehydrogenase : an allosteric enzyme.
- 9. Electrophoresis: separation of isoenzymes of lactate dehydrogenase on polyacrylamide gel.

10. Photosynthesis: isolation of chloroplasts and separation of chlorophyll from Spinach leaves.

- 11. Spectrophotometer: assay of Hill Reaction and estimation of chlorophyll.
- 12. Study of respiration: effect of inhibitors on respiratory chain.
- 13. Induction of B-galactosidase in the strains of E. coli.
- 14. Study of leghaemoglobin: isolation and extimation from root nodules.
- 15. Study of nitrate reductase.
- 16. Study of bacterial photosynthesis.
- 17. Collection, separation and preservation of serum and plasma from animals.
- 18. Study of blood typing ABO and Rh.
- 19. Determination of blood clotting time.
- 20. Estimation of haemoglobin.
- 21. Enumeration of RBC, WBC from blood.
- 22. Study of differential count of WBC.
- 23. Quantitative precipitation test.
- 24. Latex agglutination test.
- 25. Coomb's test.
- 26. Oudin procedure.
- 27. Oakley Fulthorpe procedure.
- 28. Ouchterlony procedure.
- 29. Counter current immunoelectrophoresis.
- 30. Rocket immunoelectrophoresis.
- 31. Preparation of Flagellin antigen from Salmonella group microorganism.
- 32. Purification of immunoglobulin by ion exchange chromatography.
- 33. Demonstration of Immunofluorescence.
- 34. Visit to a pathology laboratory to observe ELISA, RIA.