BACHELOR WITH BIOTECHNOLOGY AS MAJOR

4th SEMESTER

BTG 422J1: BIOTECHNIQUES

CREDITS: THEORY – 3, PRACTICAL – 1

MAXIMUMMARKS: 75, MINIMUM MARKS: 27

- Course Learning Objective: This course is designed to expose student to different techniques, handling instruments understand their working and applications for research and analysis.
- **Course outcome:** A student will be able to;
  - separate, purify and characterize different bio molecules using centrifugation and chromatographic techniques.
  - analyze, separate and identify nucleic acids and proteins by different electrophoretic and blotting techniques.
  - prepare specimens and use different types of microscopes for observation and use of UV-Vis spectroscopy for different applications.

Unit -1 15 hours

Centrifugation: General principle of centrifugation, sedimentation coefficient, preparative and analytical centrifugation, differential centrifugation & density-gradient centrifugation, ultracentrifugation and its applications.

Chromatography: Principle, working and applications of thin-layer chromatography, ion-exchange chromatography, gel filtration and affinity chromatography. HPLC.

Unit -2 15 hours

Electrophoresis: General principle and types; Principle, procedure and applications of native poly acrylamide gel electrophoresis, sodium dodecyl sulphate polyacrylamide gel electrophoresis, isoelectric focusing, two-dimensional gel electrophoresis and agarose gel electrophoresis, pulse field gel electrophoresis.

Blotting techniques: Southern, northern & western blotting; **PCR**: principle, methodology and applications.

Unit -3 15 hours

Microscopy: Principle, working and applications of light microscopy - bright-field, dark-field, phase-contrast, fluorescence & confocal microscopy, electron microscopy- TEM and SEM; Staining-principle and procedure of simple staining, negative staining & differential staining.

Spectroscopy: Beer-Lambert's law. Principle, working and applications of ultraviolet/visible light spectroscopy (UV/Vis spectroscopy).

PRACTICALS (1 CREDITS: 15 hours) Maximum Marks: 25, Minimum Marks: 9

1. Use of microscope – simple attaining and differential staining
2. Separating cells from broth/plasma separation from blood.
3. Paper chromatography/TLC
4. SDS-PAGE.
5. Amplification of a gene by PCR/demonstration.
6. Agarose gel electrophoresis.

BOOKS RECOMMENDED

1. Principles and Techniques of Biochemistry and Molecular Biology: Wilson, K. and Walker, J, Cambridge University Press.
2. Physical Biochemistry - Applications to Biochemistry and Molecular Biology: Freifelder, D., W. H. Freeman and Company.
3. Molecular Cloning - A Laboratory Manual: Sambrook, J. and Russell, D. W., Cold Spring Harbor Laboratory Press.

BACHELOR WITH BIOTECHNOLOGY AS MINOR

4th SEMESTER

BTG 422N: BIOTECHNIQUES

CREDITS: THEORY – 3, PRACTICAL – 1

MAXIMUMMARKS: 75, MINIMUM MARKS: 27

- Course Learning Objective: This course is designed to expose student to different techniques, handling instruments understand their working and applications for research and analysis.
- **Course outcome:** A student will be able to;
  - separate, purify and characterize different bio molecules using centrifugation and chromatographic techniques.
  - analyze, separate and identify nucleic acids and proteins by different electrophoretic and blotting techniques.
  - prepare specimens and use different types of microscopes for observation and use of UV-Vis spectroscopy for different applications.

Unit -1 15 hours

Centrifugation: General principle of centrifugation, sedimentation coefficient, preparative and analytical centrifugation, differential centrifugation & density-gradient centrifugation, ultracentrifugation and its applications.

Chromatography: Principle, working and applications of thin-layer chromatography, ion-exchange chromatography, gel filtration and affinity chromatography. HPLC.

Unit -2 15 hours

Electrophoresis: General principle and types; Principle, procedure and applications of native poly acrylamide gel electrophoresis, sodium dodecyl sulphate polyacrylamide gel electrophoresis, isoelectric focusing, two-dimensional gel electrophoresis and agarose gel electrophoresis, pulse field gel electrophoresis.

Blotting techniques: Southern, northern & western blotting; **PCR**: principle, methodology and applications.

Unit -3 15 hours

Microscopy: Principle, working and applications of light microscopy - bright-field, dark-field, phase-contrast, fluorescence & confocal microscopy, electron microscopy- TEM and SEM; Staining-principle and procedure of simple staining, negative staining & differential staining.

Spectroscopy: Beer-Lambert's law. Principle, working and applications of ultraviolet/visible light spectroscopy (UV/Vis spectroscopy).

PRACTICALS (1 CREDITS: 15 hours) Maximum Marks: 25, Minimum Marks: 9

1. Use of microscope – simple attaining and differential staining
2. Separating cells from broth/plasma separation from blood.
3. Paper chromatography/TLC
4. SDS-PAGE.
5. Amplification of a gene by PCR/demonstration.
6. Agarose gel electrophoresis.

BOOKS RECOMMENDED

1. Principles and Techniques of Biochemistry and Molecular Biology: Wilson, K. and Walker, J, Cambridge University Press.
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BACHELOR WITH BIOTECHNOLOGY AS MAJOR

4th SEMESTER

BTG 422J2: Molecular Biology

CREDITS: THEORY – 4, PRACTICAL – 2

MAXIMUM MARKS: 100, MINIMUM MARKS: 34

- Course Learning Objective: aim of this course is to understand information flow at molecular level, appreciate the functions of DNA, RNA and protein and how these regulate different biological processes.
- **Course outcome:** A student will be able to;
  - analyze the different properties of nucleic acids and genomes.
  - interpret and predict the role of different enzymes and proteins involved in replication of DNA, mutation and repair.
  - illustrate the process of gene expression, factors involved, processing and regulation of expression.
  - describe how the language of the nucleic acids is translated into proteins and its regulation.

UNIT – I 15 hours

DNA as genetic material (Griffith, Avery-MacLeod-McCarty and Hershey and Chase experiments), RNA as genetic material (Fraenkel Conrat experiment). DNA structure - features of double helix, forms of DNA (A, B, Z, H). Forces stabilizing DNA. DNA topology, Genome and C-value paradox, Genome complexity (Cot curve, repetitive, non-repetitive sequences). Organization of prokaryotic and eukaryotic genomes.

UNIT – II 15 hours

DNA Relication in prokaryotes, modes of replication (semi-conservative, conservative, dispersive, continuous, discontinuous and bi-directional replication), origin of replication (prokaryotic and eukaryotic), Enzymes and proteins involved: DNA polymerases, helicases, topoisomerase and ligase, proof reading, leading and lagging strand synthesis. Rolling circle replication (λ and M13). End replication of linear DNA (telomerase). DNA Mutations: Base substitution, missense, non sense, deletion, insertion, frame shift, silent). DNA damage: radiation, alkylation and oxidative. DNA repair: photo-reactivation, base excision, nucleotide excision, mismatch and recombination repair.

UNIT – III 15 hours

Transcription in prokaryotes: RNA polymerase, role of σ factor, promoter, initiation, elongation and termination. Operon concept, positive and negative regulation with reference

to *lac* and *trp* operons. Eukaryotic transcription: RNA polymerases, promoters, promoter clearance, enhancers, silencers, transcription factors/domains (zinc finger domains, leucine zippers, basic domains). Post-transcriptional processing - 5' cap formation, splicing, polyadenylation. Brief outline of rRNA and tRNA processing. Inhibitors of transcription.

UNIT – IV 15 hours

Protein translation machinery: ribosomes, mRNA, tRNA, charging of tRNA, aminoacyl tRNA synthetases, translation initiation (prokaryotes and eukaryotes). Genetic code – its salient features, Wobble hypothesis, reading frames, mechanism of elongation and termination in prokaryotes and eukaryotes. Inhibitors of translation. Post-translational modifications of proteins. Translation regulation.

PRACTICALS (2 CREDITS: 30 hours) Maximum Marks: 50, Minimum Marks: 18

1. Isolation of genomic DNA from bacterial cells.
2. Qualitative analysis of DNA by agarose gel electrophoresis.
3. Isolation of RNA from cells.
4. Quantitative estimation of RNA and DNA by spectrophotometry.
5. DNA denaturation and renaturation curves and calculation of Tm.
6. Lab visits/ Field trip/ subject tour etc.

BOOKS RECOMMENDED

1. Cell and Molecular Biology: Concepts and Experiments, Karp, G. John Wiley & Sons. Inc.
2. The Cell: A Molecular Approach. Cooper, G.M. and Hausman, R.E ASM Press
3. The World of the Cell, Becker, W.M., Kleinsmith, L.J., Hardin. J. and Bertoni, G. P. Benjamin-Cummings Pub
4. Molecular Biology of the Gene, J Watson, T Baker, S Bell, A Gann, M Levine, R Losick, Pearson
5. Lewins Genes XI J E. Krebs, S T. Kilpatrick, E S. Goldstein Jones & Bartlett Learning
6. Molecular Cell Biology Harvey Lodish, Arnold Berk, Chris A. Kaiser Monty Krieger, Anthony Bretscher, W H Freeman & Co
7. Molecular Biology, David Freifelder, Narosa Publishers.

BACHELOR WITH BIOTECHNOLOGY AS MAJOR

4th Semester

BTG 422J3: RECOMBINANT DNA TECHNOLOGY

CREDITS: THEORY – 4, PRACTICAL – 2

MAXIMUM MARKS: 100, MINIMUM MARKS: 36

- **Objective:** Through this course, students will learn about the different tools used in recombinant DNA technology and its applications.
- **Expected Learning Outcomes:** At the end of the course students should be able to;

- use different enzymes for cloning, modification and amplification of DNA.
- select and use the suitable vector for cloning and screening of transformants.
- express recombinant proteins and purify them.
- make cDNA library, edit and target different genes.

THEORY (4 CREDITS: 60 HOURS)

UNIT I 15 Hours

Introduction to Recombinant DNA technology, tools of recombinant DNA technology: Restriction endonucleases (types, nomenclature, cleavage pattern-blunt and cohesive end cutters), DNA polymerases (pol I, Klenow fragment, Taq), DNA ligases, kinases, phosphatases, nucleotidyl transferase, exonucleases, reverse transcriptase. Use of linker and adapters, homopolymer tailing.

Unit II 15 Hours

Plasmid vectors- general features. Features of pBR322 and pUC. Bacteriophage vectors: insertion and replacement, M13, cosmids, phagemids, YAC and BAC. Basic cloning methodology in plasmid vectors: Vector and insert preparation, ligation, competent cells, transformation (heat-shock and electroporation) screening of recombinants (antibiotic resistance and blue-white screening). Preparation of probe – radioactive and non radioactive labeling. Sequence based screening (colony, hybridization, PCR)

UNIT III 15 Hours

Recombinant protein expression in heterologous systems: expression in E.coli (inducible promoter system), yeast, insect and mammalian systems. Recombinant protein purification using tags (His, GST, Flag, HA). Reporter genes (luciferase, CAT, GFP, GUS) and their applications. In vitro transcription and translation and its applications.

Unit IV 15 Hours

Genomic and cDNA library construction, screening of libraries. Gene knock downs: antisense RNA technology and RNA interference. Gene knock out by Cre-LoxP system. Gene editing by CRISPR-CAS system. Gene targeting: Site directed mutagenesis (single primer extension, double primer extension, PCR based mutagenesis). Protein engineering for increased thermal stability, activity, shelf life.

PRACTICALS (2 CREDIT: 60 HOURS) Maximum Marks: 50, Minimum Marks: 18

1. DNA/plasmid isolation from bacterial/plant/any other cell.
2. Preparation of competent cells.
3. Restriction digestion of DNA.
4. Transformation of competent cells by heat shock.
5. Easy (TA) Cloning.
6. Blue-white screening
7. Agarose gel electrophoresis.
8. Educational tour to different labs/institutes.

BOOKS RECOMMENDED

1. Molecular Biotechnology: Principles and Applications of Recombinant DNA by Bernard R. Glick, Cheryl L. Pattern, ASM Press.
2. Gene Cloning and DNA Analysis: An Introduction by Brown TA Wiley-Blackwell
3. Principles of Gene Manipulation and Genomics by Primrose SB, Twyman R and Old B Wiley- Blackwell.
4. Gene Cloning and Manipulation by Christopher Howe, Cambridge University Press
5. Analysis of Genes and Genomes by Reece J Richard, Wiley-Blackwell