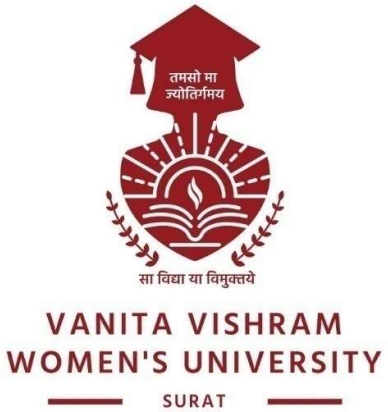
VANITA VISHRAM WOMEN'S UNIVERSITY
SCHOOL OF SCIENCES
DEPARTMENT OF MICROBIOLOGY



MASTER OF SCIENCE (M.Sc.) MICROBIOLOGY PROGRAMME
under Learning Outcomes-based Curriculum Framework (LOCF)
for Post Graduate (PG) Education

SEMESTERS 3
Core Courses (CC)

Syllabus applicable to the students seeking admission in the
M.Sc.- Microbiology
under LOCF
w.e.f. the Academic Year 2021-2022

SEMESTER 3
CORE COURSE PAPER 8

MB21120 MOLECULAR BIOLOGY

Course Objectives:

The purpose of this course is to introduce the student to the advanced concepts in molecular biology. Students will gain an understanding of molecular mechanisms of DNA replication, DNA repair, transcription, translation, and gene regulation in prokaryotic and eukaryotic organisms. The student will study the techniques and experiments used to understand these mechanisms.

Course learning outcomes : By the end of this course the students-

CO1: Is able to describe structure of DNA and RNA, organization of eukaryotic genome CO2: Is able to compare and contrast the mechanisms of bacterial and eukaryotic DNA replication, DNA repair, transcription

CO3: Is able to explain concepts in DNA repair mechanisms, and recombination as a molecular biology tool

CO4: Is able to explain various levels of gene regulation in both prokaryotic and eukaryotic organisms

CO5: Is able to describe post-transcriptional processes, RNA editing, RNAi and miRNA CO6: Is able to describe translation mechanism in prokaryotes and eukaryotes, regulation of translation, and post-translational processing

CO7: Is able to describe post-translational processes

THEORY COURSE
(4+1 Credits)

Unit-1	**The nature of Genetic material**: The structure of DNA and RNA; melting of DNA, superhelicity, organization of microbial genomes, organization of eukaryotic genomes, chromatin arrangement, nucleosome formation.	**15 Lectures**
Uni-2	**DNA replication**: Arrangement of replicons in a genome, various modes of replication, continuous, discontinuous synthesis, various replication enzymes, replication fork and priming, leading and lagging strand, elongation, termination, specific features of replication in prokaryotes and eukaryotes, action of topoisomerases, telomere maintenance and chromatin assembly, single stranded DNA replication, relationship between DNA replication and cell cycle, and DNA copy number maintenance.	**15 Lectures**
Unit-3	**Transcription & Post-transcriptional processes:** Transcription machinery of prokaryotes, various transcription enzymes & cofactors, initiation, elongation & termination, sigma factors, transcription machinery of eukaryotes, various forms of RNA polymerase & cofactors, initiation, elongation and termination, promoters, enhancers, silencers, activators, effect of chromatin structure, regulation of transcription, RNA processing, splicing, capping and polyadenylation, rRNA and tRNA	**15 Lectures**

	processing, RNA Editing; RNAi and miRNAs, Antisense RNA, Post transcriptional gene regulation.	
Unit-4	**Translation & Post-translational processes**: The genetic code and protein structure, Mechanisms of translation in prokaryotes, Mechanisms of translation in eukaryotes, initiation complex, ribosomes and tRNA, factors, elongation and termination, *in vitro* translation systems, polycistronic/ monocistronic synthesis, Regulation of translation, RNA instability, inhibitors of translation, stringent response in bacteria, Protein modification, folding, chaperones, transportation. The Signal Hypothesis. Protein degradation.	**15 Lectures**

Reference Book

1. Molecular Biology by D.P. Clarke, N. Pazdernik. 2nd edition. Academic Press. 2012.
2. Molecular Cloning: A laboratory manual by J. Sambrook, D. Russell. 4th edition. Cold Spring Harbor laboratory Press. 2012.
3. DNA Technology: The Awesome Skill by I. Edward Alcamo. Harcourt Academic Press. 2001.
4. Molecular Biology of the Gene by J. Watson, T. Baker, S. Bell, A. Gann, M. Levine, R. Losick. 7th edition. Pearson. 2014.
5. Gene Cloning and DNA Analysis: An Introduction by T.A. Brown. 7th edition. Wiley Blackwell Publishers. 2016.

SEMESTER 3
CORE COURSE PAPER 9
MB21130 RECOMBIANAT DNA TECHNOLOGY

Course Objectives:

The objective of this course is to make the student familiar with the currently used techniques to manipulate/ analyze DNA, RNA and proteins. The student will be made familiar with the methods used to clone genes, make and screen libraries, and the various applications of the polymerase chain reaction. The student will be taught about the methods currently used to carry out genome wide analyses and global analyses of transcription and protein expression. The student will be made familiar with how recombinant DNA technology has been exploited in the study of biology as well as in the production of pharmaceutical products.

Course learning outcomes :

Upon successful completion of the course, the student:

CO1: Will be familiar with the use of various cloning vectors, and methods of DNA, RNA and protein analysis.

CO2: Will be able to describe the various applications of PCR, and know how to make and screen genomic and cDNA libraries.

CO3: Will be able to understand the methods by which DNA is sequenced and will gain insights into how entire genomes of organisms are sequenced.

CO4: Will have learnt about promoter analyses, the many uses of the reporter genes, and methods

to study the transcriptome.

CO5: Will be aware of the different bacterial and eukaryotic systems available for overexpression of proteins.

CO6: Will have learnt about different methods to analyze protein-DNA and protein-protein interactions, protein engineering, and methods for proteome analyses.

CO7: Will know about the creation of plant and animal transgenics, and about animal cloning methods.

THEORY COURSE
(4+1 Credits)

Unit-1	**Basics of DNA cloning, PCR and construction of cDNA and genomic DNA libraries:** Simple cloning and cloning using linkers and adaptors. Cloning into various kinds of vectors – plasmids, phages lambda and M13, phagemids, cosmids, P1 phage, PACs, BACs and YACs. Selection and screening of clones. Concept of PCR and various thermophilic enzymes used in PCR. Gradient PCR versus Touchdown PCR. Designing primers. Cloning PCR products. Long PCR, Inverse PCR, Vectorette PCR, RT-PCR, 5' and 3' RACE, Real Time PCR, Multiplex PCR. Steps in the construction of cDNA versus genomic DNA libraries. Screening libraries by colony hybridization and colony PCR.	**15 Lectures**
Uni-2	**Genome sequencing:** DNA sequencing by Sanger's method – traditional and cycle sequencing. Physical mapping by restriction fragment fingerprinting of BAC clones and STS mapping. E-PCR. Whole genome shotgun sequencing. Clone-by-clone shotgun sequencing of genome – preparation of BAC/YAC library, selection of BACs, subclone library construction, random shotgun phase and finishing phase followed by sequence authentication. Genome annotation at the nucleotide level, protein level and process level. Next Generation sequencing methods**.**	**15 Lectures**
Unit-3	**Transcriptional analysis of gene expression and transcriptomics:** Gene expression analysis by Northern Blotting, RT-PCR, EST analysis and the use of reporter genes. Enzymatic and bioluminescent reporters. Reporters used in protein localization and trafficking studies. Promoter analysis – deletion analysis and linker scanning analysis coupled to reporter assays, mapping transcriptional start sites by S1 nuclease mapping, primer extension studies or 5' RACE. Transcriptome analysis by DD-PCR and EST analysis, DNA microarrays (cDNA arrays and oligo arrays), Serial Analysis of Gene Expression (SAGE), RNA-seq.	**15 Lectures**
Unit-4	**Analysis of protein-DNA and protein-protein interactions, protein engineering and proteome analysis:** Gel retardation assay, DNA foot-printing by DNase I and chemical methods, yeast one-hybrid assay, ChIP- chip, ChIP-seq. Yeast two hybrid, three-hybrid, split hybrids and reverse hybrid. Co-immunoprecipitation, pull-down, far-western. Use of GFP and its variants in FRET analysis, use of BiFC. Phage display. Insertional and deletion mutagenesis. Site directed mutagenesis by conventional and PCR-based methods. Proteome analysis by 2D gel	**15 Lectures**

	electrophoresis coupled to mass spectrometric analysis. Principles and use of MALDI-TOF and LC-MS platforms. PMF verses MS/MS. Protein arrays and their applications.	

Reference Book

1. Molecular Biology by D.P. Clarke, N. Pazdernik. 2nd edition. Academic Press. 2012.
2. Molecular Cloning: A laboratory manual by J. Sambrook, D. Russell. 4th edition. Cold Spring Harbor laboratory Press. 2012.
3. DNA Technology: The Awesome Skill by I. Edward Alcamo. Harcourt Academic Press. 2001.
4. Molecular Biology of the Gene by J. Watson, T. Baker, S. Bell, A. Gann, M. Levine, R. Losick. 7th edition. Pearson. 2014.
5. Gene Cloning and DNA Analysis: An Introduction by T.A. Brown. 7th edition. Wiley Blackwell Publishers. 2016.

SEMESTER 3
CORE COURSE PAPER 10
MB21140 MICROBIAL GENETICS

Course Objectives:

The objective of this course is to understand how microorganisms can be used as tools to understand various biological phenomena. The student will become familiar with methods of transfer of genetic material in bacteria, and will understand the biology of lytic and lysogenic phages. The student will be acquainted with the different modes of gene regulation in bacteria and the importance of bacterial transposition and its applications.

Course learning outcomes : By the end of this course the students-

CO1: Can discuss the importance of mutation analysis, can analyze mutations by complementation and recombination tests, and can design a strategy to create gene replacement in bacteria

CO2: Is able to explain how plasmid copy number is regulated, can differentiate between Hfrstrains and strains carrying F plasmid, and can construct a genetic map of bacterial genome using conjugation-based method

CO3: Is able to compare and contrast generalized versus specialized transduction, knows how to construct genetic linkage maps using two-factor and three factor cross, is able to discuss the basis of natural competence in bacteria.

CO4: Is able to list the events in the lytic and lysogenic phases of lambda phage life cycle and the regulatory factors and events involved.

CO5: Can list the outcomes of transposition events, can design strategies to mutagenize bacteria using transposons, can explain the construction of conditional knockouts

CO6: Can differentiate between positive and negative regulation of gene expression, inducible and repressible systems. Can describe the regulation of the lac, trp, gal,ara and tol operons.

CO7: Will have learnt about the model organisms used in biological studies.

THEORY COURSE
(4 Credits)

Unit-1	**Genetic analysis of bacteria:** Importance and uses of mutation analysis. Inheritance in bacteria, types of mutations, spontaneous and induced mutagenesis, isolating mutants, selecting mutants, mutant enrichment. Reversions versus suppression. Complementation tests, recombination tests & gene replacements. Cloning genes by complementation. Cloning genes by marker rescue.	**15 Lectures**
Uni-2	**Gene transfer and mapping by conjugation:** Basis of fertility in bacteria. Self-transmissible and mobilizable plasmids. Molecular mechanism of gene transfer by conjugation – genes and proteins involved. Regulation of gene transfer by conjugation. Hfr strains. Mapping bacterial genomes using Hfr strains. Chromosomal DNA transfer by plasmids – by integrated plasmids, by chromosome mobilization and by creation of prime factors. Transfer systems in gram positive bacteria. Ti plasmid transfer system and its application in creating transgenics.	**15 Lectures**
Unit-3	**Lytic bacteriophages and gene transfer by transformation and transduction:** Lytic development cycle, replication and regulation of expression of genes in phage T4 and phage T7. Natural transformation and competence. Molecular basis of natural transformation – DNA uptake competence systems in gram positive and gram negative bacteria. Regulation of competence in *B.subtilis*. Importance of natural transformation. Artificially induced competence. Generalized versus specialized transduction: T4, lambda phage. Mapping bacterial genes by transduction.	**15 Lectures**
Unit-4	**Transposons, Gene regulation and Model organisms used in genetic studies:** Transposons-Discovery, Classes, regulation of transposition activity, Molecular mechanisms of transposition, Control of gene expression. Positive gene regulation, negative gene regulation and attenuation, using the *lac*, *gal*, *trp*, *ara*, *tol*operons. Yeast (*Saccharomyces cerevisiae*), fruitfly (*Drosophila melanogaster*), nematode worm (*Caenorhabditis elegans*), mouse (*Mus musculus*), Arabidopsis (*Arabidopsis thaliana*).	**15 Lectures**

Reference Book

1. Molecular Genetics of Bacteria by L. Snyder, J. Peters, T. Henkin, W. Champness. 4th edition. ASM Press. 2013.
2. Fundamental Bacterial Genetics by N. Trun, J. Trempy. 1st edition. Wiley-Blackwell Publishing. 2004.
3. Modern Microbial Genetics edited by U.N. Streips, R.E. Yasbin. 2nd edition. Wiley-Liss Publishers. 2002.

4. Microbial Genetics by S.R. Maloy, J.E. Cronan, Jr., D. Freifelder. 2nd edition. Jones and Bartlett Publishers. 1994.

MB21150: PRACTICAL V

Marks: 100 Duration: 60 hours (4 credits)

Course Objectives:

The objective of this course is to train the student in basic molecular biology and microbial genetics techniques. The student will learn how to isolate, analyze, and manipulate DNA, amplify DNA, fingerprint microbes, overexpress and purify recombinant proteins. The student will become familiar with transferring genetic material into bacteria by transformation and conjugation methods.

Course Learning Outcomes:

The student:

CO1. Is able to perform restriction digestion and carry out its analysis by agarose gel electrophoresis.

CO2. Is able to perform restriction digestion and carry out its analysis by agarose gel electrophoresis.

CO3. Learns how to prepare competent cells and determine transformation efficiency

CO4. Learns how to do basic cloning

CO5. Is able to fingerprint microorganisms by RAPD analysis

CO6 Is able to overexpress recombinant proteins and analysis by SDS-PAGE

Contents:

1. To determine molecular weight of a fragment from band profile.
2. To analyze plasmid DNA by restriction digestion followed by agarose gel electrophoresis.
3. To analyze plasmid DNA by restriction digestion followed by polyacrylamide gel electrophoresis.
4. To prepare competent cells by chemical method and determine their transformation efficiency
5. To isolate genomic DNA.
6. To amplify a gene of genomic DNA using PCR.
7. Blue white selection for identification of recombinant vector.
8. To perform RAPD analysis for microbial identification.
9. Cloning and Expression of a protein X
10. Separation of protein using SDS-PAGE analysis.
11. Determine molecular weight of a protein from gel pattern.

Suggested Readings:

1. Molecular Cloning: A laboratory manual by Joseph Sambrook, David Russell, 4th edition. Cold Spring Harbor laboratory Press. 2012.
2. Sequence - Evolution - Function: Computational Approaches in Comparative Genomics by E.V. Koonin , M.Y. Galperin. Kluwer Academic, USA. 2003.
3. Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins edited by A. D. Baxevanis, B.F. Francis Ouellette . 3rd edition. Wiley and Sons. 2004.

MB21160: Practical VI

Course learning outcome:

The student:

CO1. Can find ORFs in given nucleotide sequence using ORF Finder.

CO2. Can create phylogenetic tree from the given nucleotide and protein sequence.

CO3. Can perform protein modelling using SWISS-MODEL.

CO4. Can analyse amino acid sequence to predict the structure and functions

Contents:

1. Isolation of a hyper producing mutant by treatment of Physical/chemical mutagen.
2. To find ORFs in given nucleotide sequence using ORF Finder.
3. To create phylogenetic tree from the given nucleotide and protein sequence.
4. To Study Protein Parameters using Protparam.
5. To study domain architecture using ExPASy PROSITE.
6. To perform protein modeling using SWISS-MODEL.
7. To create multiple sequence alignments
8. To visualize and understand structures from PDB using PyMol/DeepView.
9. To predict secondary structure of a given amino acid sequence.
10. To analyzed protein properties from sequence- PROTPARAM

Suggested Readings:

1. Molecular Cloning: A laboratory manual by Joseph Sambrook, David Russell, 4th edition. Cold Spring Harbor laboratory Press. 2012.
2. Sequence - Evolution - Function: Computational Approaches in Comparative Genomics by E.V. Koonin, M.Y. Galperin. Kluwer Academic, USA. 2003.
3. Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins edited by A. D. Baxevanis, B.F. Francis Ouellette. 3rd edition. Wiley and Sons. 2004.