Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore

M.Sc. (Biotechnology)

Semester II

SUBJECT CODE	Category	SUBJECT NAME	TEACHING &EVALUATION SCHEME								
			THEORY			PRACTICAL		Th	T	P	CREDITS
			END SEM University Exam	Two Term Exam	Teachers Assessment*	END SEM University Exam	Teachers Assessment*				
MSBT201	**DC**	**Immunology**	60	20	20	0	0	3	0	0	3

Legends: **L**-Lecture; **T**-Tutorial/Teacher Guided Student Activity; **P-**Practical; **C** - Credit;
***Teacher Assessment** shall be based following components: Quiz / Assignment / Project / Participation in Class, given that no component shall exceed more than 10 marks.

Course Objectives:

1. Study of structural features of components of immune system and their function.
2. Study of mechanism of Immune response
3. Study of clinical immunology and vaccinology

Course Outcomes:

1. Understanding the components of immune system and their mechanism
2. Applications of immunology and development of vaccines

UNIT-I: Fundamental Concepts and Overview of the Immune System:

Types and components of innate and acquired immunity; phagocytosis; complement and inflammatory responses; pathogen recognition receptors (PRR) and pathogen associated molecular pattern (PAMP); innate immune response; mucosal immunity. Antigens: Immunogenecity versus Antigenicity, Factors influencing immunogenecity, immunogens, haptens; Epitopes - Properties of B-cell epitopes and T-cell epitopes. Cells of the Immune System: B and T Lymphocytes; T-cell sub-sets; Antigen Presenting Cells, Major Histocompatibility Complex: MHC genes, HLA typing, MHC and immune responsiveness and disease susceptibility, Organs of immune system, Primary lymphoid organs (Bone marrow and Thymus); Secondary lymphoid organs (lymph nodes, spleen and mucosal-associated lymphoid tissue).

UNIT-II: Immune Responses Generated by B and T Lymphocytes

Immunoglobulins - basic structure, classes & subclasses of immunoglobulins, antigenic determinants; multi-gene organization of immunoglobulin genes; B-cell receptor; Immunoglobulin superfamily; basis of self & non-self-discrimination; Clonal selection, kinetics of immune response, memory; B cell maturation, activation and differentiation; generation of antibody diversity; Monoclonal Antibodies - Formation and selection of hybrid cells; Production of Monoclonal Antibodies and their clinical uses.

T-cell maturation, activation and differentiation and T-cell receptors; functional T Cell subsets; cell-mediated immune responses, ADCC; cytokines. Antigen processing and presentation- endogenous antigens, exogenous antigens, non-peptide bacterial antigens and super-antigens.

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MSBT201 Immunology

UNIT-III: Antigen-antibody Interactions

Epitopes and epitope mapping, Affinity, Avidity, cross reactivity, specificity, non-peptide bacterial antigens and super-antigens; Hapten-carrier system.

Precipitation, agglutination and complement mediated immune reactions; Advanced immunological techniques - RIA, ELISA, Western blotting, ELISPOT assay, immune fluorescence, flow cytometry and immunoelectron microscopy; Surface Plasmon resonance, Biosensor assays for assessing ligand –receptor interaction, CMI techniques- lymph proliferation assay, Mixed lymphocyte reaction, Cell Cytotoxicity assays, Apoptosis, Microarrays, Transgenic mice, Gene knockouts.

UNIT-IV: Clinical Immunology

Immunity to infection: bacteria, viral, and parasitic infections; hypersensitivity: Type I-IV; autoimmunity; types of autoimmune diseases; treatment of autoimmune diseases; transplantation: immunological basis of graft rejection; HLA typing, clinical transplantation and immunosuppressive therapy; Hypersensitivity reactions and their types.

Tumor immunology: tumor antigens; immune response to tumors and tumor evasion of the immune system, cancer immunotherapy; immunodeficiency: primary immunodeficiencies, acquired or secondary immunodeficiencies, autoimmune disorder, anaphylactic shock, immunosenescence, immune exhaustion in chronic viral infection, immune tolerance, NK cells in chronic viral infection and malignancy.

UNIT-V: Vaccinology

Active and passive immunization; live, killed, attenuated, subunit vaccines; vaccine technology: role and properties of adjuvants, recombinant DNA and protein based vaccines, plant-based vaccines, reverse vaccinology; peptide vaccines, conjugate vaccines; antibody genes and antibody engineering: chimeric, generation of monoclonal antibodies, hybrid monoclonal antibodies; catalytic antibodies and generation of immunoglobulin gene libraries, idiotypic vaccines and marker vaccines, viral-like particles (VLPs), dendritic cell based vaccines, vaccine against cancer, T cell based vaccine, edible vaccine and therapeutic vaccine.

BOOKS:

1. Brostoff, J., Seaddin, J. K., Male, D., &Roitt, I. M. (2002). Clinical Immunology. London. Gower. Medical Pub.
2. Goding J. W. (1996). ; Monoclonal Antibodies in Cell Biology, Biochemistry, and Immunology. London: Academic Press
3. Kindt, T. J., Goldsby, R. A., Osborne, B. A., & Kuby, J. (2019). Kuby Immunology. New York: W.H. Freeman. 8th Edition
4. Murphy, K., Travers, P., Walport, M., &Janeway, C. (2012). Janeway's Immunobiology. New York: Garland Science.
5. Parham, P. (2005). The Immune System. New York: Garland Science.
6. Paul, W. E. (2012). Fundamental Immunology. New York: Raven Press.

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			THEORY			PRACTICAL		Th	T	P	CREDITS
			END SEM University Exam	Two Term Exam	Teachers Assessment*	END SEM University Exam	Teachers Assessment*				
MSBT202	**DC**	**Bioprocess Technology and Down Stream Processing**	60	20	20	0	0	3	0	0	3

Legends: **L**-Lecture; **T**-Tutorial/Teacher Guided Student Activity; **P**-Practical; **C** - Credit;
***Teacher Assessment** shall be based following components: Quiz / Assignment / Project / Participation in Class, given that no component shall exceed more than 10 marks.

Course Objectives:

1. Theoretical and practical understanding of bioreactors, their types and different aspects of fermentation processes.
2. Knowledge of exploitation of microorganisms and their processes in the manufacture of microbial products.
3. Study of quality control and regulatory framework of bioprocess industries.

Course Outcomes:

1. Understanding the design and operating systems for manufacture of biological products
2. Methods for discovering new microorganisms for industrial application
3. Quality control and marketing of biological products

UNIT–I: Fundamentals of Bioprocess Engineering

Bioprocess engineering and its components; Fermentation Processes – conventional fermentation v/s Biotransformation; Solid State, Dual/Multiple, Aerobic, Anaerobic, Batch, fed-batch and Continuous Fermentation. Bioreactors – types and designs; Operational Kinetics, Kinetic modelling and model structure; Growth linked and Non-growth linked products; Material Balances and Energy Balances; Isolation, Screening and Maintenance of Industrially important microbes; Strain improvement by mutation, protoplast fusion, Para-sexual fusion and genetic engineering; Culture Preservation and Inoculum Development

UNIT–II: Upstream Processing

Medium formulation for optimal growth of microbes and product formation in fermentation; Ingredients for mammalian cell culture and plant cell culture; Design of sterilization process, sterilization of bioreactor, sterilization of media, Maintenance of aseptic conditions; Theory and Designing of depth filters; Thermal death kinetics of microbes; Monitoring of process variables; Types of Sensors, measurement and control of mass transfer, aeration and agitation; PID control; Computer control of variables; Scale-up and scale-down.

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MSBT202 Bioprocess Technology and Down Stream Processing

UNIT–III: Down Stream Processing

Bio separation: filtration, centrifugation, sedimentation, flocculation; Cell disruption (Physical, Chemical and enzymatic methods); Extraction (Liquid-liquid, Aqueous two phase, Supercritical fluid); Distillation, Purification by chromatographic techniques; Reverse osmosis and ultrafiltration; Drying; Crystallization, Whole Broth Processing.

UNIT–IV: Industrial Production and Recovery process:

Microbial enzymes, Biofuels and industrial chemicals, Health care products, Food and beverage fermentations, Food additives and supplements, Microbial biomass production, biotransformation, Recombinant Vaccines, Large scale animal and plant culture cultivation; Cell immobilization, production of biomass and applications

UNIT–V: Quality Control (QC), Quality assurance (QA) and Fermentation Economics

Roles and responsibilities of QC and QA departments; Common Quality control tests; Standard Operating Procedures (SOP) & Good Manufacturing Practices (GMP); Regulations on use and distribution of Biotechnology products. Market analysis, equipment and operational costs.

BOOKS:

1. Arvind H Patel (2016). Industrial Microbiology (2nd Ed) Laxmi Publications.
2. Casida L E (2019) Industrial Microbiology (2nd Ed) New Age International Publisher.
3. Demain A. L. & Davies J. E. (2nd Ed.)Manual of Industrial Microbiology and Biotechnology (1999).
4. El-Mansi, M., & Bryce, C. F. (2007). Fermentation Microbiology and Biotechnology. Boca Raton: CRC/Taylor & Francis.
5. Stanbury, P. F., & Whitaker, A. (2010) Principles of Fermentation Technology. Oxford: Pergamon Press.
6. Shuler, M. L., & Kargi, F. (2002) Bioprocess Engineering: Basic Concepts. Upper Saddle River, NJ: Prentice Hall.

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			THEORY			PRACTICAL		Th	T	P	CREDITS
			END SEM University Exam	Two Term Exam	Teachers Assessment*	END SEM University Exam	Teachers Assessment*				
MSBT203	**DC**	**Genetic Engineering and its Applications**	60	20	20	0	0	3	0	0	3

Legends: **L**-Lecture; **T**-Tutorial/Teacher Guided Student Activity; **P**-Practical; **C** - Credit;
***Teacher Assessment** shall be based following components: Quiz / Assignment / Project / Participation in Class, given that no component shall exceed more than 10 marks.

Course Objectives:

1. Study of tools used in genetic engineering
2. Construction of genomic libraries
3. Applications of genetic engineering

Course Outcomes:

1. Understanding the techniques and the tools used in genetic engineering
2. Understanding genetic transformations and their applications

UNIT–I: Introduction and Tools for Genetic Engineering

Restriction endonucleases (types and classification) and methylases; DNA ligase, Klenow enzyme, T4 DNA polymerase, polynucleotide kinase, alkaline phosphatase; Cohesive and blunt end ligation; linkers; adaptors; homopolymeric tailing; labeling of DNA: nick translation, random priming, radioactive and non-radioactive probes, hybridization techniques: northern, southern, western, south-western, Zoo blots, far-western and colony hybridization, fluorescence *in situ* hybridization (FISH).

UNIT–II: Vectors, Transformation and Related Techniques

Plasmids; Bacteriophages; M13mp vectors; PUC19 and pBluescript vectors, phagemids; Lambda vectors; Insertion and Replacement vectors; Cosmids; Artificial chromosome vectors (YACs; BACs); Expression vectors: pMAL, GST, pET-based vectors; Mammalian expression and replicating vectors; Baculovirus and Pichia vectors system, yeast vectors, shuttle vectors, Intein-based vectors; Competent cell preparation methods; Transformation methods for bacteria, plant and animal cells; Screening of transformants- selection markers (antibiotic resistance and genes of essential metabolism), hybrid arrest and hybrid release translation, insertional inactivation and alpha complementation for recombinant selection, reporter genes (GUS assay, luciferase).

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MSBT203 Genetic Engineering and its Applications

UNIT–III: PCR and Related Techniques

Polymerase chain reaction: Thermal profile and reaction components; Optimization (touch down/hotstart and gradient PCR); Primer design; Fidelity of thermostable enzymes; DNA polymerases; Types of PCR and their applications: Conventional PCR, AP-PCR, Anchored-PCR, Inverse-PCR, Multiplex-PCR, Nested PCR, Reverse Transcription-PCR, DDRT-PCR and Real Time-PCR. PCR based cloning: T/A cloning, TOPO cloning and gateway cloning.

UNIT–IV: Construction and Screening of Genomic and cDNA Libraries

Cloning- conventional & recombination based; Construction of genomic libraries; Chromosome walking and chromosome jumping for positional cloning of genes; Construction of EST and cDNA libraries; Construction of subtractive and normalized cDNA libraries; Methods of library screening: Types of probes and their construction methods, hybridization based (using radiolabeled and non-radiolabeled probes) and Immuno-screening methods.

UNIT–V: Applications of Genetic Engineering

Gene down regulation- using antisense RNA, dsRNA and co-suppression, Knock-in and knock- out technology; Genome editing by CRISPR-Cas 9, TALENs & zinc finger nucleases; Site directed mutagenesis (PCR based methods); Detection and diagnosis of genetic diseases; Gene therapy – *ex vivo*, *in vivo*; Gene delivery systems – viral and non-viral; Transgenic animals and plants; Genetically engineered biotherapeutics (insulin, somatostatin, vaccines); Biosafety regulation: physical and biological contaminants.

BOOKS:

1. Brown, T. A. (2018). Genomes4 (4th Ed.). New York: Garland Science Pub.
2. Brown, T. A. (2020). Gene Cloning and DNA Analysis: An Introduction. (8th Ed.). Wiley-Blackwell.
3. Green, M. R., & Sambrook, J. (2012). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor. (4th Ed.). NY: Cold Spring Harbor Laboratory Press.
4. Glick, B. R., & Patten, C. L. (2017). Molecular Biotechnology: Principles and Applications of Recombinant DNA. (5th Ed.). American Society for Microbiology press.
5. NichollD. S.T. (2015). An Introduction to Genetic Engineering. (3rd Ed.). Cambridge University Press.
6. Primrose, S. B., & Twyman, R. M. (2006). Principles of Gene Manipulation and Genomics. (7th Ed.). Oxford: Blackwell Scientific Publications.

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			THEORY			PRACTICAL		Th	T	P	CREDITS
			END SEM University Exam	Two Term Exam	Teachers Assessment*	END SEM University Exam	Teachers Assessment*				
MSBT204	**DC**	**Biostatistics, Bioinformatics and Computational Biology**	60	20	20	0	0	3	1	0	4

Legends: **L**-Lecture; **T**-Tutorial/Teacher Guided Student Activity; **P**-Practical; **C** - Credit;
***Teacher Assessment** shall be based following components: Quiz / Assignment / Project / Participation in Class, given that no component shall exceed more than 10 marks.

Course Objectives:

1. Concepts in statistics and their application in biological studies
2. Basics of Bioinformatics and data bases
3. Applications of Bioinformatics

Course Outcomes:

1. Understanding the methods in statistics and their applications to biological problems
2. Understanding the tools of bioinformatics
3. Construction of molecular designs using the tools of bioinformstics

UNIT–I: Fundamentals of Biostatistics

Sampling methods; Types of sampling- random sampling, probability and non-probability sampling, stratified sampling; Statistical data distribution- normal and skewed distribution, coefficient of skewness, moments and kurtosis; Data presentation models- covariance models, spatial statistical model, multivariate spatial model, gaussian and non-gaussian random process models; Principles of hypothesis testing, significance level, null hypothesis; Comparison of means, t-test, Chi-square test; Covariance and correlation, Pearson's, Kendal's and Spearman's correlations, use of correlation and regression in biological analyses; Analysis of variance (ANOVA), Post hoc Tests- Tukey's test for pairwise comparison of treatments, Dunnet's test for comparison of treatment means with control, Duncan's multiple range test, Mann–Whitney U test.

UNIT–II: Bioinformatics Resources, Biological Databases and Sequence Analysis

Information Resources: NCBI, EBI, ExPASy Entrez & SRS System; Primary Sequence & Structure Databases: Genbank, ENA, DDBJ, SwissProt/Uniprot, EMBL, PIR, PDB, KEGG; Secondary Databases of Sequences and structure: Prosite, Pfam, SCOP, CATH, DSSP, FSSP, RNAbase; Genome Databases (at NCBI, EBI), High-throughput genomics sequence (EST, STS, GSS), ENSEMBL. Sequence File formats: fasta, genbank, embl, Swiss-prot, pdb, nbrf, pir and multiple sequences formats (Aln, Mega, Pileup, phylip etc.); Sequence Similarity Basics: Similarity, Identity, Homology, Scoring, Selectivity/Sensitivity, Gap cost, Linear and Affine Gap Penalty, Basic of scoring system and matrices (PAM, BLOSUM, GONNET ClustalW and ClustalX).

MSBT204 Biostatistics, Bioinformatics and Computational Biology

UNIT–III: Similarity Searching Tools and Phylogenetics

Pairwise sequence alignment: Brute Force method, Dot matrix method, Global (Needleman-Wunsch) and Local Alignment (Smith-Waterman) using Dynamic programming; BLAST and FASTA, Theory and Algorithms, variants of BLAST and FASTA, PSI-BLAST; Statistical Significance; Sequence Pattern and Profiles: Concepts of motif, pattern and profile; Multiple sequence alignment (MSA) algorithms, Methods of MSA (Progressive, Iterative, Block-Based Alignment); Protein profiles and Hidden Markov Model (HMM); Phylogenetics prediction methods: Basics, molecular clock, Substitution Models of evolution; Tree reconstruction methods: Distance based, character based method, statistical; Bootstrapping; Software and programs for sequence comparison and analysis; Phylogenetic analysis software.

UNIT–IV: Structural Bioinformatics

Major structural resources (PDB and PMDB); PDB File Format; Basic Structure Visualization- Visualization of major secondary structure and their role in protein structure, Visualization of various interactions: Polar (Hydrogen Bonds), Apolar (Hydrophobic, van der Waals, Pi stacking), Other (Salt Bridges, Coordination with ions) in protein structures and their role; Protein Structure Classification (SCOP and CATH); Protein Structure Prediction-Need and Concept of protein structure prediction, protein folding and model generation; Protein secondary structure prediction methods (Alignment-based and Single sequence-based secondary structure predictions); Tertiary structure prediction (Homology modeling and Fold Recognition, *ab initio* methods); Ramachandran Plot.

UNIT – V: Molecular Modeling

Introduction to modeling; Protein-ligand interactions; Pose prediction strategies in molecular docking: Rigid body docking, Flexible ligand docking (Conformational search method, Fragmentation method, Database method); Scoring Functions: Force field-based, Empirical, Knowledge-based; Application in Structure Based Drug Designing.

BOOKS:

1. Andrew, L. (2001). Molecular Modelling: Principles and Applications. (2nd Ed.). Publisher: Prentice Hall.
2. Baxevanis, A. D., Bader, G. D., & Wishart, D. S. (2020). Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins. (4th Ed.). Publisher: New York, John Wiley & Sons, Inc.
3. Burkowski, F. (2009). Structural bioinformatics: An algorithmic approach. (1st Ed.). Publisher: CRC Press.
4. Gu, J., & Bourne, P. E. (2003). Structural Bioinformatics (Methods of Biochemical Analysis). (2nd Ed.). Publisher: Wiley-Liss.
5. Mount, D. W. (2004). Bioinformatics: Sequence and Genome Analysis. (2nd Ed.). Publisher: Cold Spring Harbor Laboratory Press.
6. Rosner, B. (2015). Fundamentals of Biostatistics. (8th Ed.). Boston, MA: Duxbury Press.
7. Sunderrao, P.S.S. & Richards, J. (2014). Introduction to Biostatistics and Research Methods. (5th Ed.). Prentice Hall Pvt. Ltd. India.

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			THEORY			PRACTICAL		Th	T	P	CREDITS
			END SEM University Exam	Two Term Exam	Teachers Assessment*	END SEM University Exam	Teachers Assessment*				
MSBTL205	**DC**	**Immunology and Bioprocess Technology Laboratory**	0	0	0	30	20	0	0	8	4

Legends: **L**-Lecture; **T**-Tutorial/Teacher Guided Student Activity; **P-**Practical; **C** - Credit;
***Teacher Assessment** shall be based following components: Quiz / Assignment / Project / Participation in Class, given that no component shall exceed more than 10 marks.

PRACTICAL [Immunology]

1. Partial purification of IgG by ammonium sulphate fractionation and Dialysis.
2. Purification of IgG by column chromatography.
3. Serum separation and serological reactions (a) agglutination (b) precipitation.
4. Enzyme linked immune sorbent assay.
5. Isolation of lymphocytes from peripheral blood.
6. Ouchterlony double diffusion.
7. Single radial immune diffusion.
8. Rocket immune electrophoresis
9. Double diffusion, Immune-electrophoresis and Radial Immuno diffusion.
10. Blood smear identification of leukocytes by Giemsastain TICAL.

PRACTICAL [Bioprocess Technology]

1. Screening and identification (Genus Level) of a production strain (enzyme /antibiotic) from soil samples. Maintenance of the isolated production organism (Agar slants/ glycerol stocks /soil culture/ lyophilization)
2. To Estimate the Monod Parameters for microbial growth kinetics.
3. Quantitative estimation of ethanol produced during Yeast fermentation.
4. To determine the residence time distribution (RTD) in Biochemical reactor.
5. To Determine the Oxygen transfer coefficient (KLa) in CSTR.
6. Isolation, screening and optimization of conditions for production:
   - Solid state fermentation: enzymes, alcohol
   - Submerged fermentation: enzymes, exopolysaccharide, organic acids and antibiotics
7. Rheological study of culture broth by Brookfield viscometer
8. Estimation, recovery and purification of fermentation products-enzymes, antibiotics, organic acids, exopolysaccharide
9. Immobilization of yeast biomass in sodium alginate gel.
10. Bio-separations

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MSBTL206	**DC**	**Genetic Engineering and Bioinformatics Laboratory**	0	0	0	30	20	0	0	8	4

Legends: **L**-Lecture; **T**-Tutorial/Teacher Guided Student Activity; **P**-Practical; **C** - Credit;
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PRACTICAL [Genetic Engineering]

1. PCR based amplification of DNA.
2. Restriction digestion of genomic or lambda DNA and size determination of the fragments on agarose gel.
3. Double digestion of DNA and restriction mapping, problems on restriction mapping.
4. Purification of DNA from agarose gel.
5. Vector and insert DNA ligation.
6. Preparation of competent cells.
7. Transformation of *E. coli,* and calculation of transformation efficiency.
8. Replica plate techniques.
9. Screening of recombinant transformants by alpha complementation / insertional inactivation.
10. Confirmation of clone by colony PCR.
11. Miniprep of recombinant plasmid DNA, restriction mapping.
12. Expression of foreign protein in heterologous host.
13. Concept of soluble proteins and inclusion body formation in *E.coli*, SDS-PAGE analysis.
14. *In vitro* site directed mutagenesis using PCR method.

PRACTICAL (Biostatistics]

1. Determination of Karl-Pearson's coefficient of correlation/ Spearman's rank correlation coefficient from the given grouped and ungrouped data.
2. Examples based on t – test, Chi-square test for goodness of fit and independent attributes.
3. Analysis of variance on the given data (ANOVA).
4. Measures of skewness and measures of Kurtosis (grouped and ungrouped data).

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MSBTL206 Genetic Engineering and Bioinformatics Laboratory

5. Using online resources like NCBI, PubMed (GenBank, UniProtKB, PDB).
6. Designing primers for PCR.
7. Bioedit as sequence handling tool.
8. Key word and accession number based database search and downloading bioinformatics data:
    a. Downloading DNA sequence data (Genbank/DDBJ/ENA)
    b. Downloading protein sequence data (Uniprot)
    c. Downloading protein structure data (PDB/MMDB) and visualization
    d. Downloading bioinformatics data from FTP servers (NCBI)
9. Pairwise (global and local) alignment of DNA and protein sequences.
10. Multiple sequence alignment of DNA and protein sequences and finding conserved sequences.
11. Searching similar sequences in databases using BLASTp, BLASTt and BLASTn.
12. Understanding ORF and gene prediction.
13. Making patterns (prosite syntax) and consensus sequence from multiple sequence alignments.
14. Phylogenetic analysis using PHYLIP or MEGA.
15. Basic Structure visualization using Deep View (Performing basic tasks like Selecting and Displaying structures, Colouring, Measuring distances and labeling).
16. Prediction of secondary structures of proteins online.
17. Prediction of protein tertiary structure using any method (CPH, MODELLER, SWISS Model, EasyModeler).
18. Molecular Docking using Auto Dock and Molecular visualization of docked complexes (using PyMOL or Chimera).