

Shivaji University, Kolhapur

M. Phil Biotechnology

BT 101 / BC101 Research Methodology

(100 marks)

(Common with Biochemistry M. Phil)

1) Structure and functions biomolecules

a) Purification of enzymes, antibodies and glycoproteins –

Enzyme assay, enzyme activity and specific activity determination. Cell disintegration and extraction techniques, separation of proteins by fractionation (ammonium sulphate, organic solvents). Ion exchange chromatography, molecular sieve chromatography, affinity chromatography, HPLC, ultra filtration.

b) Ultracentrifugation

c) Electrophoretic methods -

Gel electrophoresis, isoelectric focusing and immunoelectrophoresis, capillary electrophoresis, pulse field electrophoresis.

d) Enzyme kinetics -

K_m, V_{max}, alternative plotting methods, advantages and disadvantages.

2) Immobilization

Various methods for immobilization of enzymes, antibodies and whole cells. Applications of immobilization techniques in industries.

3) Distillation solvent extraction.

4) Immunology techniques

Ag-Ab interactions, double diffusion, immunoelectrophoresis, Western blotting, radio immune assay. ELISA, FACS.

5) Isolation-

Identification and characterization of DNA, RNA, plasmids. Agarose gel electrophoresis, ethidium bromide staining.

6) Tracer techniques

Measurement of alpha, β radiations, labeling of nucleic acid and proteins. Autoradiography, liquid scintillation, half life of isotope, measurement of radioisotopes.

7) Determination of biomolecule structure (applications only)

Use of the technique for determination of molecular structure viz. x-ray diffraction, NMR, UV-visible Spectroscopy, CD/ORD, GCMS, IR-spectroscopy. Principles and applications of fluorescence staining and transmission electron microscopy.

8) Biostatistics-

Mean, Standard error, standard deviations, correlation and regression, one way analysis of variance, student's test, Tukey Kramer multiple comparisons test.

9) Bioinformatics

Introduction to internet and its applications, clustal W, oligo primer analysis, SPDBV, primer design.

Suggested readings:

1. Biostatistics : A foundation for Analysis in the Health Sciences 7/E Wayne W. Daniel, Wiley Series in Probability and Statistics.
2. Introductory Statistics. Fifth Edition. (2004) Prem S. Mann. John Wiley and Sons (ASIA) Pvt. Ltd.
3. Bioinformatics Methods and Applications Genomics, Proteomics, and Drug Discovery (S. C. Rastogi, N. Mendiratta, and P. Rastogi).
4. Introduction to Bioinformatics, (Atwood, T. K. and Parry-Smith, D. J).
5. Protein Purification by Robert Scopes, Springer Verlag Publication, 1982
6. Tools in Biochemistry David Cooper
7. Methods of Protein and Nucleic acid Research, Osterman Vol I – III
8. Centrifugation D. Rickwood
9. Practical Biochemistry, V th edition, Keith Wilson and Walker.

BT 102 Genetic Engineering.

(100 marks)

1. DNA and 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. Packing of DNA, supercoiled DNA, nucleosome, Inverted repeats, repetitive DNA sequence, satellite DNA.

Transcription and translation.

Labeling of DNA: Nick translation, random priming, radioactive and non-radioactive probes, use of Klenow enzyme, T4 DNA polymerase, bacterial alkaline phosphatase, polynucleotide kinase.

2. Tools in genetic engineering:

a. Cloning Vectors

Plasmids, bacteriophages, Cloning in M13 mp vectors, phagemids, Lambda vectors; insertion and replacement vectors, EMBL, λ DASH, λ gt10/11, λ ZAP etc. Cosmid vectors. Artificial chromosome vectors (YACs, BACs), Animal Virus derived vectors-SV-40, vaccinia/baculo & 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

b. Restriction analysis: DNA modifying enzymes and restriction enzymes for GE, 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.

Insertion of Foreign DNA into Host Cells: Transformation, Transfection: Chemical and physical methods, liposomes, microinjection, macroinjection, electroporation, biolistics, somatic cell fusion,

c. 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.

d. Expression strategies and methods for producing industrially important molecules:

Various expression vectors in bacteria and eukaryotes including shuttle vectors. Induced expression strategies and protocols. Chimeric constructs, Expression of industrially important products.

3. Analytical techniques:

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.

PCR – design and optimization, use to engineer DNA, amplification of specific sequences from a cDNA library, use in diagnosis of diseases; 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.

DNA sequencing- Maxam-Gilbert method, Sanger's Dideoxy chain termination method, Enzymatic DNA sequencing Automated DNA sequencing method. Sequencing strategies and analysis, and applications. RNA sequencing

Human genome sequencing: Genetic and physical mapping techniques, sequencing strategies, and gene annotation. Chemical Synthesis of oligonucleotides.

Gene silencing techniques: Introduction to siRNA and siRNA technology, micro RNA, construction of siRNA vectors, principle and application of gene silencing. Gene knockouts and Gene Therapy: Creation of knock out mice, disease model, somatic and

germ-line therapy in vivo and ex-vivo, suicide gene therapy, gene replacement, gene targeting.

Applications of genetic engineering:

Genetic diseases-Detection and Diagnosis, Gene therapy – ex vivo, in vivo, gene delivery systems, viral and non viral; DNA marker technology in plants, Transgenics, application in global gene expression analysis. Applications of recombinant DNA technology in medicine, agriculture, veterinary sciences. DNA fingerprinting, Genetically engineered biotherapeutics and vaccines and their manufacturing, Transgenic plants and transgenic animals.

Suggested readings:

1. Sambrook J, Fritsch E. F. and Maniatis (1989) Molecular cloning, vol. I, II, III, II nd edition, Cold spring harbor laboratory press, New York.
2. DNA Cloning : A practical approach D.M. Glover and D.B. Hames, RL Press, Oxford, 1995
3. Molecular and cellular methods in Biology and Medicine, P.B. Kaufman, W. Wu , D. Kim and L.J. Cseke, CRC Press Florida 1995
4. Methods in Enzymology Guide to Molecular Cloning Techniques, Vol. 152 S.L. Berger and A. R. Kimmel, Academic Press Inc, San Diego, 1996
5. Methods in Enzymology Gene Expression Technology, Vol. 185D. V. Goedel, Academic Press Inc, San Diego, 1990
6. DNA Science: A First Course in Recombinant Technology, D. A. Mickloss and G. A Freyer, Cold Spring Harbor Laboratory Press, New York, 1990
7. Molecular Biotechnology, 2nd Ed. S. B. Primrose, Blackwell Scientific publishers, Oxford, 1994
8. Milestones in Biotechnology, Classic Papers on Genetic Engineering, J. A. Davis and W. S. Reznikoff, Butterworth-Heinemann Boston 1992
9. Route Maps in Gene Technology, M. R. Walker, and R. Rapley, Blakwell Science, Oxford, 1997
10. Genetic Engineering : An Introduction to Gene Analysis and Exploitation in Eukaryotes, S. M. Kingsman, Blackwell Scientific Publications, Oxford, 1998

BT 103A Advanced Techniques in Cell Culture

(100 marks)

Basic techniques in tissue culture

Introduction to Cell & Tissue Culture. Design & lab setup of Tissue Culture laboratory, Tissue culture Media (Composition preparation), Types of culture.

Plant cell culture, plant transformation technology & its applications.

Role of Plant Hormones in growth & development of Plants.

Micro propagation (Organogenesis, Somatic Embryogenesis, Shoot tip culture, Rapid clonal propagation, Embryo Culture & Embryo Rescue, Acclimatization of Plants)

Invitro mutagenesis. Cryopreservation, Slow growth & DNA Banking for germ plasm conservation.

Basics of Tumor formation, Hairy root, features of Ti & Ri Plasmid ,Mechanism of DNA transfer role of Virulence gene, Use of Ti & Ri as vectors, Binary vectors, Use of 35s & other promoters genetic markers methods of nuclear transformation viral vectors & their applications, Multiple gene transfers vector less or direct DNA transfer ,Use of reporter gene, Particle bombardment ,electroporation, Microinjection, Transformation of monocots, Transgene stability & gene silencing in Plant transformation. Applications of Plant Transformation for Productivity & performance Herbicide resistance like atrazine, Insect resistance Bt gene ,non Bt like protease inhibitors, Virus resistance, disease resistance, antibiotic stress, post harvest losses long shelf life of fruits & flowers.

Chloroplast transformation, Advantage vectors & success with tobacco & potato
Metabolic engineering & Industrial products

Plant secondary metabolites, control mechanisms & manipulation of Phenyl

Propanol pathway, Shikimate pathway, Alcoloids, Industrial enzymes,

Biodegradable plastics, Therapeutic proteins, lysozomal enzymes, Antibodies, edible vaccines, Purification strategies, oleosin partitioning technology

Integration of Genetic Engineering of Plants in Agriculture

Diseases resistant, Biotic & Abiotic stress resistant, Enhancement of nutritional value of crop Plants & molecular farming

Animal cell culture and tissue engineering.

Cell lines, cell culture growth kinetics, Basic Techniques of mammalian cell culture (Open and closed cell-cultures, Primary Cell culture),

Cell surgery and Cell Fusion Methods (Preparation of anucleated cells and polykaryon cells, preparation of ghost RBCs, Preparation of mini cells, micro cells, Surgical manipulation of *in vitro* fertilization, Hybridoma cell preparations, 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, 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. An introduction to Plant Tissue Culture 2nd edn. Razdan, M. K, Science Publishers, USA.
2. Textbook of plant biotechnology, Chawla P.K.2002,Oxford&IBH,New Delhi.
3. Bhojwani, S. S. and M. K. Razdan 1996.Plant Tissue Culture: Theory and Practice, Elsevier Pub.
4. Chrispeels, M. J. 2002.Plant Tissue Culture:Genetical Aspects. Jones and Bortlett Publishers, International.
5. Chopra V. L. et al 1999. Applied Plant biotechnology. Science Publishers Inc.
6. Verpoorte, R. and A.W. Alfermann (Eds) 2000.Metabolic Engineering of plant secondary metabolism, lower Academic Publisher.
7. Kuchler, R.J., Biochemical Methods in cell culture and Virology, Dowden, Hutchinson and Ross,Inc. Strausberg, USA, 1977
8. Morgan, S. I. Animal cell culture, 1993,Bio Scientific Publishers Ltd, Oxford.
9. Freshney,R.I.Culture of Animal cells:A Manual of Basic Technique, 1994, John Wiley and Sons Inc. Publication, USA.
10. Butler, M.Mammalian, cell Biotechnology: A Practical Approach (1991),

IRL Press, Oxford.

11. Jenni P.Mather and David Barnes, eds; Animal cell culture Methods, Methods in cell Biology, vol.57, Academic Press.
12. Cell Culture: Methods in enzymology, Vol-58, Academic Press 1979 or recent.

BT103B Bioremediation and Waste Water Treatment Technologies.

(100 marks)

Bioremediation, biotransformation and biodegradation.

1. Bioremediation, In situ and Ex situ bioremediation, constraints and priorities of bioremediation, Evaluating Bioremediation, Bioremediation of VOCs.
2. Biodegradation. Factors affecting on process of biodegradation. Methods in determining biodegradability. Contaminant availability for biodegradation.
3. Xenobiotics, Persistence and biomagnification of xenobiotic molecules. Microbial interactions with xenobiotics. Phase I and Phase II reactions. Cyt P 450 mediated reactions. Use of microbes (bacteria and fungi) and plants in biodegradation and Biotransformation.
4. Sources of heavy metal pollution, Microbial interactions with inorganic pollutants Microbial metal resistance, Microbial transformation, accumulation and concentration of metals, Biosorption Biotechnology and heavy metal pollution.

Environmental impacts on agriculture.

1. Biodegradation of agricultural chemicals. GM crops and their impact on environment.
2. Biological nitrogen fixation, Phosphate solubilization. Biofertilizers.
3. Biological control of insect pests, role of biopesticides/insecticides. Biocontrol of plant pathogens Integrated pest management-practical implementation, ecology and IPM.

Water pollution monitoring.

1. Methods of monitoring.
2. Biological methods- Detection methods for DO, BOD, Pathogen monitoring by heterotrophic plate count, multiple tube method, membrane filtration methods, Other emerging techniques such as enzyme detection, hybridization, PCR, gene probe technology etc. Strategies for controlling pathogen transfer.
3. Chemical methods- Detection methods for COD, pH, alkalinity, TSS, TDS, Total organic carbon, oil, grease etc.

Effluent treatment systems

1. Sewage and waste water treatments systems. Primary, secondary and tertiary treatments. Measurement of treatment efficiencies. Biological treatments- aerobic versus anaerobic treatments. Environmental pollution control- Bioremediation, Bioaugmentation and Biostimulation.
2. Biofilms in treatment of waste water. Biofilm development and biofilm Kinetics. Aerobic Biofilms.
3. Bioreactors for waste water treatments. Reactors types and design. Reactors in series. Development and optimization of membrane bioreactor process for use in sanitary and industrial sewage treatment.

Biotechnological application of hazardous waste management and management of resources.

1. Use of microbial systems.
2. Phytoremediation. Waste water treatment using aquatic plants, root zone treatment.
3. Development of new biocatalysts to be applied in waste water biotechnology.
4. Need for management of resources. Role of environmental biotechnology in management of resources. Reclamation of wasteland, biomass production, Biogas and biofuel production. Development of environmentally friendly processes such as integrated waste management.

Suggested readings:

1. Biotreatment Systems , Volume II ; D.L. Wise.
2. Advances in Biotechnological Process ; Mizrahi & Wezel.
3. Bruce Rittman, Perry L. McCarty. Environmental Biotechnology: Principles and Applications. McGraw-Hill 2nd edition (July 25, 2000) ISBN: 0072345535.
4. Raina M. Maier , Ian L. Pepper, Charles P. Gerba. Environmental Microbiology. Publisher: Academic Press; (February 23, 2000).
5. Martin Alexander. Biodegradation and Bioremediation. Academic Press; 2nd edition (April 15, 1999) ISBN: 0120498618.
6. Gabriel Bitton (Author). Wastewater Microbiology, 2nd Edition. Wiley-Liss;

2nd edition (February 16, 1999) ISBN: 0471320471.

7. Milton Wainwright. An Introduction to Environmental Biotechnology.
Kluwer Academic Publishers, Boston. Hardbound, ISBN 0-7923-8569-1.
July 1999, 192.
8. M.N.V. Prasad, Kazimierz Strzalka. Physiology and Biochemistry of Metal
Toxicity and Tolerance in Plants. Kluwer Academic Publishers, Dordrecht
Hardbound, ISBN 1-4020-0468-0. February 2002, 460 pp.
9. Biotechnology for Solving Agricultural Problems; Danforth & Bakst.
10. Introduction to Environmental Microbiology; R. Mitchell.

BT103C Bioinformatics

(100 marks)

Biological databases

Genomes, DNA sequences. Sequence databases: GeneBank, European Molecular Biology Laboratory (EMBL) Nucleotide sequence databank, DNA Data Bank of Japan (DDBJ), Protein databases; primary databases and secondary databases, database formats. Structural databases; Protein Data bank (PDB), Nucleic Acid Data Bank (NDB), Molecular modeling Data Bank (MMDB).

Sequence alignments

Introduction, Protein sequences, physicochemical properties based on sequence, sequence comparison. Pair-wise sequence alignment, gaps, gap-penalties, scoring matrices, Smith-Waterman and Needleman-Wunsch algorithms for sequence alignments, multiple sequence alignment, comparison, composition and properties, useful programs, ClustalW, BioEDIT, BLASTp, Phylogenetic analysis tools- Phylip, ClustalW, Online phylogenetic analysis.

Gene expression and DNA microarray

Introduction, Basic steps for gene expression, genome information and special features, coding sequences (CDS), untranslated regions (UTR's), cDNA library, expressed sequence tags (EST). Approach to gene identification; codon-bias detection, detecting functional sites in the DNA. Internet resources for gene identification, detection of functional sites. Types of microarrays; Tools for microarray analysis; soft-finder, xCluster, MADAM, SAGE, Applications of microarray technology.

Structural biology

Nucleic acid structures, RNA folding, RNA loops, conformational study, various ribose ring conformations, ribose-ring puckering, protein-protein interactions, protein-ligand interactions, DNA-binding proteins, RNA-binding proteins, Ramachandran plot, , 3-dimensional structures of membrane proteins, importance of 3^{10} helix and loops, biophysical aspects of proteins and nucleic acids.

Protein structure prediction

Protein Structure Prediction; Homology modeling, prediction of protein structure from sequences, functional sites, Protein folding problem, protein folding classes, protein identification and characterization; structure determination by X-ray and NMR.

Molecular modeling

Introduction, force field, quantum chemistry, Schrödinger equation, potential energy functions, energy minimization, local and global minima, saddle point, grid search, , various approximations; LCAO, HF, semi-empirical calculations; single point calculations, full-geometry optimization methods, ZDO, MNDO, CNDO, NDDO, AM1, PM3, RM1, conformational search, Z-matrix, docking, molecular modeling packages.

Molecular mechanics

Definition, balls and springs, force fields, bond-stretching, bond-bending, dihedral motions, out of plane angle potential, non-bonded interaction, coulomb interactions, conformational search, united atoms and cut-offs, Derivative methods; First-order methods; Steepest descent, conjugate gradient, Second order methods; Newton-Raphson method.

Molecular dynamics

Introduction, Newton's equation of motion, equilibrium point, radial distribution function, pair correlation functions, MD methodology, periodic box, algorithm for time dependence; leapfrog algorithm, Verlet algorithm, Boltzman velocity, time steps, duration of the MD run.

Fundamentals of Unix system

Introduction about UNIX, differentiation between UNIX and other Operating systems, various programs in the UNIX System, structure of the UNIX system, applications of the UNIX systems, basic UNIX commands, file access permissions, the file system hierarchy, terminating process, running jobs at background, controlling running programs, overview of system administration, managing deisk space, shutting down UNIX system.

Bioinformatics applications

Agriculture, Molecular biology, Environment, Biotechnology, Neurobiology, Drug Designing, Biomedical genome medicines.

Reference books:

1. Bioinformatics Methods and Applications Genomics, Proteomics, and Drug Discovery (S. C. Rastogi, N. Mendiratta, and P. Rastogi).

2. Introduction to Bioinformatics, (Atwood, T. K. and Parry-Smith, D. J).
3. An introduction to Computational Biochemistry. (C. Stain Tsai, A John Wiley and Sons, Inc., publications).
4. Developing Bioinformatics Computer Skills. (Cynthia Gibas and Per Jambeck).
5. Molecular Modelling for Beginners (Alan Hinchliffe).
6. A user guide to the UNIX system (Rebecca Thomas and Jean Yates)
7. <http://www.ncbi.nlm.nih.gov>