Ph. D Entrance Test Syllabus For Biotechnology Engineering, UIET, M.D. University , Rohtak

1.Organization of structure and functions of prokaryotic and eukaryotic cells:

a) Cell wall and Cell Membrane: physical structure of model membranes in prokaryotes and eukaryotes, lipid bilayer, membrane proteins, other constituents; diffusion, osmosis, active transport, regulation of intracellular transport and electrical properties.

b) Structural organization and functions of cell organelles: nucleus, mitochondria, Golgi bodies, endoplasmic reticulum, lysosomes, Chloroplast, peroxisomes, vacuoles. Cytoskeletons structure and motility function.

c) Organization of genomes: genes and chromosomes, Operon, unique and repetitive DNA, interrupted genes, gene families, structure of chromatin and chromosomes, heterochromatin, euchromatin, transposons.

d) Cell division and cell cycle: Mitosis and meiosis, their regulation, Cell cycle and its regulation, Apoptosis, Necrosis and Autophagy.

e) Cell transformation and cancer, oncogenes and proto-oncogenes, tumor suppressor genes, metastasis. Therapeutic interventions of uncontrolled cell growth.

2. Biomolecular structure and function:

a) Covalent structure of Amino acids, proteins, nucleic acids, carbohydrates and lipids.

b) Forces that stabilize biomolecules: electrostatic and van der Waal‟s interaction, hydrogen bonding. Interactions with solvents, Hydrophobic effect.

c) Protein Structure: Structural characteristics of α-helix, β-sheet and β-turn. Ramachandran plot. Protein domains and domain architecture. Quaternary structure of proteins.

d) Conformation of Nucleic acids: Structural characteristics of A, B and Z-DNA. 3D structure of t-RNA, ribozymes and riboswitches

e) Basic Thermodynamics: Laws of thermodynamics. Concepts of ΔG, ΔH and ΔS.

f) Physical properties of water and their role in biology. Concepts of pH, ionic strength and buffers.

g) Chemical kinetics: Concepts of order and molecularity of a chemical reaction. Derivation of first and second order rate equation, measurement of rate constants.

h) Concept of activation energy.

i) Enzymology: Introduction to enzymes. Types of enzymatic reaction mechanisms, Michaelis-Menten kinetics. Competitive, Non-competitive and Un-competitive inhibition. Bi-substrate reaction kinetics. Allostery.

3. Cellular processes:

a)DNA replication, repair and recombination (Unit of replication, enzymes involved,

replication origin and replication fork, fidelity of replication, extrachromosomal replicons, DNA damage and repair mechanisms, homologous and site-specific recombination).

a) Transcription of various types of RNAs and their processing and modifications. Transcription factors and machinery including RNA polymerases, formation of initiation complex, elongation and termination of transcription. Regulation of transcription: activators (enhancers) and repressors, Locus control regions. Structure and function of different types of RNA and mRNPs. RNA transport, localization and function.

b) Protein synthesis, processing and transport of proteins: Ribosome, mRNA structure, genetic code, aminoacylation of tRNA, aminoacyl tRNA synthetase. Mechanism of translation: Initiation, elongation and termination factors and translational proof-reading. Regulation of Translation- global vs mRNA-specific. Translation inhibitors, Post- translational modifications of proteins. Protein trafficking and transport.

c) Control of gene expression at transcription and translation level: Regulation of gene expression in viruses, prokaryotes and eukaryotes, role of chromatin, chromatin remodelling and gene silencing, Epigenetic regulation.

4. <u>Genetics, Phylogeny & Evolution:</u>

a) Chromosomal inheritance: Principles of Mendelian inheritance, codominance, incomplete dominance, gene interactions, pleiotropy, genomic imprinting, linkage and cross-over, sex-linked inheritance, Population Genetics and Hardy-Weinberg equilibrium.

b) Extrachromosomal inheritance: Maternal inheritance (mitochondria and chloroplast)

c) Gene concept: Allele, multiple alleles, pseudoalleles.

d) Genetic analysis: Linkage maps, mapping with molecular markers, tetrad analysis, gene transfer in bacteria: transformation, conjugation, transduction, sex-duction, fine structure analysis of gene.

e) Mutation: Spontaneous, induced, lethal, conditional, reversion, mutagenic suppression, germinal and somatic mutation, insertion, deletion, duplication, translocation, transposition, ploidy.

f) DNA finger printing and its applications, DNA bar coding, marker assisted selection and QTL mapping.

g) Species concept in archaea, bacteria and eukarya.

h) Phylogenetic analysis and evolutionary relationship among taxa, MLST.

5. Techniques in Biotechnology

a) Concepts of precision and accuracy in experimental measurements. Concept of signal to noise ratio.

b) Biostatistics: Measures of Central Tendency. Fundamental ideas of probability and probability distributions: Binomial, Poisson and Gaussian distributions. Concept of the Central Limit Theorem. Hypothesis testing: Use of Student‟s t and χ^2 tests. Correlation and regression. Basic concepts of design of Experiments.

c) Biochemical Methods: Chromatography: Ion exchange, Gel Filtration and Affinity chromatography. Electrophoresis: Native and SDS-PAGE. Isoelectric focusing. 2D-PAGE and its applications.

d) UV/Vis spectrophotometry. Beer-Lambert‟s law and its use in determination of protein/ nucleic acid concentration.

e) Fluorescence Spectroscopy: Basic concepts of excitation and emission. Quenching, Stern-Volmer Plots. Theory and applications of FRET and fluorescence lifetime measurements.

f) Fundamentals of CD, IR and Raman spectroscopy and their use in the study of biomolecular conformation.

g) Centrifugation: Basic concepts of centrifugation. Calculation of g value from RPM. Density gradient centrifugation. Sedimentation velocity and Sedimentation equilibrium. Separation of sub-cellular components and macromolecules using high speed and ultracentrifugation.

h) Microscopy: Bright field, phase contrast, fluorescence, confocal, and electron microscopy.

i) Fundamentals of X-ray, NMR and cryo-electron microscopy for determination of biomolecular structure.

6. Recombinant DNA Technology:

a) Enzymes used in Recombinant DNA technology.

b) Isolation and purification of DNA (genomic and plasmid) and RNA. Various methods of separation, characterization of nucleic acids including Southern and Northern hybridizations.

c) Molecular cloning of DNA or RNA fragments in bacterial and eukaryotic systems. Expression of recombinant proteins using bacterial, animal and plant vectors and their purification. Western blotting.

d)Ge neration of genomic and cDNA libraries. Plasmid, phage, cosmid, BAC and YAC vectors. In vitro mutagenesis and deletion techniques, gene knock out in bacterial and eukaryotic organisms.

e)Isolation and amplification of specific nucleic acid sequences, PCR, RT PCR and qRT PCR

f)DNA sequencing methods, strategies for genome sequencing.

g)Methods for analysis of gene expression at RNA and protein level, large scale expression, such as micro array based techniques.

h)Analysis of DNA polymorphism: RFLP, RAPD and AFLP techniques.

7. **Bioinformatics & Computational Biology:**

1. Major Bioinformatics Resources: Sequence databases, Gene Expression database: GEO, SAGE, 3D Structure Database: PDB, NDB, Knowledge driven Databases & utility, Pattern Sequence: InterPro, Prosite, Pfam, ProDom, Gene Ontology
2. Database Searches: Keyword-based searches using tools like ENTREZ and SRS Sequence-based searches: BLAST and FASTA
3. Sequence Analysis, Basic concepts: Sequence similarity, identity and similarity, definitions of homologues, orthologues, paralogues, Tandem and Interspersed repeats, repeat finding.
4. Scoring Matrix, Pairwise sequence alignments, Multiple sequence alignments (MSA), Application in Taxonomy and phylogeny, Comparative genomics.
5. Structural Biology: 3-D structure visualization and simulation, Basic concepts in molecular modeling: different types of computer representations of molecules. External coordinates and Internal Coordinates, Molecular Mechanics, Force fields etc.
6. Proteins: Secondary structure elucidation using Peptide bond, phi, psi and chi torsion angles, Ramachandran map, anatomy of proteins – Hierarchical organization of protein structure –like CATH,SCOP, FSSP .
7. DNA & RNA secondary and tertiary structures, t-RNA tertiary structure
8. Classification and comparison of protein 3D structures: Secondary structure prediction: Algorithms viz. Chou Fasman, GOR methods, Tertiary Structure prediction: Fundamentals of the methods for 3D structure prediction (sequence similarity/identity of target proteins of known structure, fundamental principles of protein folding etc.) Homology/comparative Modeling, fold recognition, threading approaches, and *ab initio* structure prediction methods. CASP. Computational design of Promoters, Proteins & Enzymes.
9. Application in drug design: Chemical databases like NCI /PUBCHEM. Fundamentals of Receptor-ligand interactions. Structure-based drug design: Identification and Analysis of Binding sites and virtual screening. Ligand based drug design: Structure Activity Relationship – QSARs & Pharmacophore etc. *In silico* predictions of drug activity and ADMET.

d) Host-pathogen interaction: Recognition and entry processes of different pathogens like bacteria, viruses and protozoans into animal and plant host cells, alteration of host cell behavior by pathogens, virus-induced cell transformation, pathogen-induced diseases in animals and plants, cell-cell fusion in both normal and abnormal cells.

e) Cell signaling: Hormones and their receptors, cell surface receptor, signaling through G-protein coupled receptors, signal transduction pathways, second messengers, regulation of signaling pathways, bacterial and plant two-component systems, light signaling in plants, bacterial chemotaxis and quorum sensing.

f) Cellular communication: General principles of cell communication, cell adhesion and roles of different adhesion molecules, tight junctions, communicating junctions, extracellular matrix, integrins, neurotransmission and its regulation. Regulation of hematopoiesis, differentiation and development

8. <u>Agricultural Biotechnology</u>:

1. <u>Tissue Culture, Transgenic Technologies and Biotechnology</u>

a) Totipotency; Tissue culture media; Plant hormones and morphogenesis; embryogenesis; Cell suspension culture; Micropropagation – shoot tip culture, somatic embryos, artificial seeds; Applications of tissue culture; shoot tip culture; Wide hybridization, Anther culture and dihaploids.
b) Production of alkaloids and other secondary metabolites; Protoplast isolation and purification; Protoplast culture; Protoplast fusion; Somatic hybrids; Cybrids.
c) Direct transformation of protoplasts using PEG; electroporation; Transformation by particle bombardment; Chloroplast transformation. Ti plasmid-based transformation; Ti and Ri plasmids, T-DNA genes, borders, Ti plasmid virulence genes and their functions, Monocot transformation, binary vector; Floral dip transformation; Targeted gene delivery and methods of detection
d) Promoters, Plant selectable markers; Reporter genes; Selectable marker elimination; Transgene silencing and strategies to avoid transgene silencing.
e) Genetic engineering of crops; Codon optimization in the expression of genes in plants, Commercial status of transgenic plants; Herbicide resistance, glyphosate, sulfonyl urea, phosphinothricin, atrazine; Pest resistance, Bt toxin; Protease inhibitor; GNA and other lectins; a-amylase inhibitor; nematode resistance; Genetic engineering for male sterility-Barnase-Barstar; Delay of fruit ripening; polygalacturanase, ACC synthase, ACC oxidase; Improved seed storage proteins; Improving and altering the composition of starch and plant oils; Golden rice for ß-carotene accumulation; Production of antibodies and pharmaceuticals in plants.
f) Biofuels, *Bacillus thuringiensis*: molecular basis of insecticidal activity. Agriculturally important microorganisms and their application. Environmental pollution, Bioremediation, Biodiversity and conservation

2. <u>Molecular Breeding and Genomics</u>

a) Principles of plant breeding, breeding for self and cross pollinated crops. Heterosis breeding limitations of conventional breeding.
b) Molecular markers: Restriction based and PCR based; DNA profiling using different assays- RFLP, RAPD, AFLP, ISSR, SNP etc. Development of SCAR and SSR markers.
c) Gene flow in plants: Development of mapping population - Marker Assisted Selection (MAS), screening and validation; Trait related markers and characterization of genes involved; Mapping genes on specific chromosomes; QTL mapping; Gene pyramiding.
d) Development of ESTs. Molecular markers for plant genotyping and germplasm analysis;

Fidelity analysis; settling IPR issues.

e) Marker Assisted Breeding for various traits, Foreground and background selection, gene introgression and pyramiding, Non-gel based techniques for plant genotyping.

f) Genome organization, Structural and Functional genomics, Proteomics, Metabolomics, Nutrigenomics, interactomics, Metagenomics.

3. Abiotic and Biotic Stress Biology

a) Abiotic stress: Acclimation and crop adaptation to water, salinity, photo oxidative, heavy metal, heat and cold, nutrient stress. Metabolite engineering for abiotic stress tolerance, Functional genomics of stress tolerance. Induced systemic tolerance.

b) Biotic stress: Plant response to pathogens and herbivores, biochemical and molecular basis of host plant resistance , toxins of fungi and bacteria, systemic and induced resistance, pathogen derived resistance, signaling - gene for gene hypothesis, genetic engineering for biotic stress resistance, gene pyramiding. Induced systemic resistance, systemic acquired resistance.

9. Animal Biotechnology:

1. Immunology*:* Cells of immune system, clinical and transplantation immunology, tumor immunology and immunodeficiency. Nude and SCID mice biology and immunosuppression.

2. Primary culture, secondary culture, sub-culturing, Cell lines, cloning & selection. Media, serum free media (advantage & disadvantages). Large scale culturing, Preservation and maintenance of anial cell lines. Cryopreservation, Cell culture products, Hybridoma technology.

3. Gene transfer (transfection) methods, Embryonic stem cell transfer, In vitro fertilization and embryo transfer.

4. Gene therapy, Animal cloning & ethical issues. Genetic diagnostic methods and microarray technology. Tissue and organ transplant, vaccines &peptide vaccines, Proteins as therapeutic agents, Applications, delivery and targeting of therapeutic proteins. Engineering human interferons and human growth hormones. Enzymes as therapeutic agents: Use of genetically engineered DNase I and alginate Lyase for treatment of Cystic Fibrosis

10. Biochemical Engineering and Industrial Biotechnology:

1. Introductory Mathematics: Calculus review, Ordinary differential equations, Second and higher order differential equations, Linear algebra, Numerical methods

2. Engineering Principles: Material and energy balance, Steady state energy and material balance, Properties of substances, Introduction to transport phenomena, momentum transfer, heat and mass transfer, Introduction to mass transfer equipments

3. Thermodynamics in Biological Systems: Biological systems as open non-equilibrium systems, Failure of classical thermodynamics in describing biological processes, Concepts of thermodynamics flux and force, Concept of entropy production, Constitutive equations, Thermodynamics of coupled biochemical reactions, Thermodynamic analysis of oxidative phosphorylation, Glycolytic oscillations; biological clocks

4. Biochemical engineering: Microbial growth kinetics-substrate utilization-product formation-aerobic fermentation methods,types, fluid properties, Sterilization methods, kineticsof batch,fed- batch and continuous bioreactors; Mass and energy balance in microbial process, effects of dissolved oxygen,heat transfer-counter current and co-current

system of heat transfer, scale-up and scale down methods, problems and trouble shooting.

4. Bioprocess Engineering and Technology: Principles of microbial growth and factors affecting growth, Growth kinetics and substrate utilization in batch, fed-batch and continuous systems, Introduction to bioreactors: batch and fed-batch, plug flow, continuous, enzyme reactors, Mass transfer of oxygen, aeration and agitation, Fermentation technology (Description of industrial processes: antibiotics, organic acids, alcohol, bioplastics, vitamins, enzymes; biotransformation of steroids)

6.Downstream Processing in Biotechnology: Separation of cell mass, filtration, methods of cell disruption-solid shear and liquid shear methods; Concentration methods-evaporation, distillation, crystallization, Extraction (solvent, aqueous two-phase, super critical), Drying ,phase separation;whole broth extraction, protein precipitation, adsorption; Membrane based purification: Cross flow filtration, Microfiltration, preparative isoelectric focusing; Effluent treatment-aerobic and anaerobic process for waste water treatment, BOD, COD, stabilization etc

7. Bioprocess Plant Design: General design information, Material and energy balance, Scale up and scale down issues, Scale up and downstream processes. Selection and specifications of bioprocess equipments, Facility design aspects. Utilities.

11.Environmental Biotechnology:

1. Basic Ecological Concepts and Principles: Ecosystem: types, development and evolution; Homeostasis, energy transfer in ecosystem, Energy budget, trophic structure, food chain, food web, ecological efficiency, biogeochemical cycles
2. Chemistry of organic and inorganic chemicals polluting Environment (air, water and soil)
3. Environmental Pollution; Types, Detection and Measurement of Pollutants; Environmental monitoring techniques
4. Water Pollution: sources, measurement and management;
5. Waste Water Treatment systems: primary, secondary and tertiary treatments; Biological Treatment Processes, Biochemistry and Microbiology of Aerobic and Anaerobic Treatment, Bioreactors for waste water treatment,,Disinfection and Disposal
6. Treatment of Typical Industrial Effluents: Dairy, Distillery, Sugar, and Antibiotic Industries.
7. Management of municipal, biomedical and agricultural solid waste.
8. Environmental Pollution control: concepts of bioaugmentation, biostimulation, biodegradation, biosorption, biofilms in the bioremediation of xenobiotics, petroleum hydrocarbons, pesticides and heavy metals, evolution of biodegradative pathways.
9. Environment friendly technologies: Biosurfactants, biofertilizers,biopesticides, microbially enhanced oil recovery, resource management, integrated waste management; production of biomass, biogas and biofuel from waste.
10. Pollution monitoring: chemical, biological and molecular methods; Environmental impact assessment, Biodiversity and its conservation, GMOs and Biosafety.
11. Global environmental problems: Ozone depletion, UV-B and green house gases

12.Genomics and Proteomics:

a) Introduction to Genomics: Structure and organization of prokaryotic and eukaryotic genomes - nuclear, mitochondrial and chloroplast genomes; Computational analysis of sequences- finding genes and regulatory regions; Gene annotation; Similarity searches; Pairwise and multiple alignments; Alignment statistics; Prediction of gene function using homology, context, structures, networks; Genetic variation- polymorphism, deleterious mutation; Phylogenetics; Tools for genome analysis– PCR, RFLP, DNA fingerprinting, RAPD, Automated DNA sequencing; Linkage and pedigree analysis; Construction of genetic maps; Physical maps, FISH to identify chromosome landmarks.

b) Genome sequencing: Human genome project-landmarks on chromosomes generated by various mapping methods; BAC libraries and shotgun libraries preparation; Physical map-cytogenetic map, contig map, restriction map, DNA sequence; DNA sequencing and sequence assembly; Model organisms and other genome projects; Comparative genomics of relevant organisms such as pathogens and non-pathogens; Evolution of a pathogen. Taxonomic classification of organisms using molecular markers -16S rRNA typing/sequencing.

c) DNA Microarray technology: Basic principles and design: cDNA and oligonucleotide arrays; Applications: Global gene expression analysis, Comparative transcriptomics, Differential gene expression; Genotyping/SNP detection; Detection technology; Computational analysis of microarray data.

d) Proteomics: Outline of a typical proteomics experiment; Identification and analysis of proteins by 2D analysis; Spot visualization and picking; Tryptic digestion of protein and peptide fingerprinting; Mass spectrometry; ion source (MALDI, spray sources); analyzer (ToF, quadrupole, quadrupole ion trap) and detector; clinical proteomics and disease biomarkers; Prions; proteins in disease; Protein-protein interactions: Solid phase ELISA, pull-down assays (using GST-tagged protein), far western analysis, by surface plasmon resonance technique, Yeast two hybrid system, Phage display; Protein interaction maps; Protein arrays-definition, applications- diagnostics, expression profiling.

13. IPR, Biosafety & Bioethics:

a) Types of IP: Patents, Trademarks, Copyright & Related Rights, Industrial Design, Traditional Knowledge, Geographical Indications, Protection of New GMOs; International framework for the protection of IP. IP as a factor in R&D; IPs of relevance to Biotechnology and few Case Studies; Introduction to History of GATT, WTO, WIPO and TRIPS

b) Invention in context of "prior art"; Patent databases; Searching International Databases; Country-wise patent searches (USPTO, EPO, India etc.); Analysis and report formation

c) Types of patents; Indian Patent Act 1970; Recent Amendments; Filing of a patent application; Precautions before patenting-disclosure/non-disclosure; WIPO Treaties; Budapest Treaty; PCT and Implications; Role of a Country Patent Office; Procedure for filing a PCT

application

d) Patent application- forms and guidelines, fee structure, time frames; Types of patent applications: provisional and complete specifications; PCT and convention patent applications; International patenting-requirement, procedures and costs; Financial assistance for patenting-introduction to existing schemes; Publication of patents-gazette of India, status in Europe and US Patenting by research students, lecturers and scientists-University/organizational rules in India and abroad, credit sharing by workers, financial incentives, Patent infringement-meaning, scope, litigation, case studies and examples.

e) Biosafety: Introduction; Historical Background; Introduction to Biological Safety Cabinets; Primary Containment for Biohazards; Biosafety Levels; Biosafety Levels of Specific Microorganisms; Recommended Biosafety Levels for Infectious Agents and Infected Animals; Biosafety guidelines - Government of India; Definition of GMOs & LMOs; Roles of Institutional Biosafety Committee, RCGM, GEAC etc. for GMO applications in food and agriculture; Environmental release of GMOs; Risk Analysis;

Risk Assessment; Risk management and communication; Overview of National Regulations and relevant International Agreements including Cartagena Protocol.

f) Bioethics: Concepts; Philosophical considerations; Epistemology of Science; Ethical Terms; Principles & Theories; Relevance to Biotechnology; Ethics and the Law Issues: Genetic Engineering, Stem Cells, Cloning, Medical techniques, Trans-humanism, Bioweapons; Research concerns - Animal Rights, Ethics of Human Cloning, Reproduction and Stem Cell Research; Emerging issues: Biotechnology‟s Impact on Society; DNA on the Witness Stand - Use of genetic evidence in civil and criminal court cases; Challenges to Public Policy – To Regulate or Not to Regulate; Improving public understanding of biotechnology products to correct misconceptions.