SYLLABUS FOR RESEARCH ENTRANCE TEST (RET) FOR PhD. IN BIOTECHNOLOGY

SECTION-I: RESEARCH METHODOLOGY

Microscopic techniques: Principles and application of light, phase contrast, fluorescence, confocal, scanning and transmission electron microscopy, cytophotometry and flow cytometry, fixation and staining, Fluorescence *in-situ* hybridization (FISH), GISH (Genomic *in-situ* hybridization).

Accessing microbial diversity using molecular methods such as Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), Amplified rRNA restriction analysis, terminal Restriction Fragment Length Polymorphism (T-RFLP), 16S rDNA sequencing, metagenomics

Introduction to Bioinformatics, use of Internet and search engines (WWW, HTML, URLs, Netscape, Explorer, Google, PUBMED), database management system, database browsing, data retrieval, sequence and genome database, databases such as GenBank, EMBL, DDBJ, Swissprot, PIR, TIGR, TAIR, Searching for sequence database like FASTA and BLAST algorithm, multiple sequence alignment, phylogenetic analysis and detection of open reading frames (ORFs).

Molecular evolution and phylogenetic tree, Gene predictions, Introduction to computational structural biology, *in-silico* methods for structural predictions, Homology threading and modeling, *ab-initio* modelling; Validation of *in-silico* determined 3D structures of proteins, Computer aided drug design-tools and applications.

Selection of sample or sampling, theory: qualitative, random and non-random sample. Collection of data, their classification, tabulation, graphic representation and diagrammatic representation, measures of central tendency and dispersion: mean, median, mode, range, standard deviations, variance, idea of two types of errors and level of significance, test of significance (F & I test); chi-square tests, sample linear regression and correlation.

Introduction to science of omics-genomics, proteomics, metabolomics, transcriptomics, comparative genomics, nutrigenomics, lipidomics, cytomics, toxicogenomics, pharmacogenomics;whole genome sequencing strategies, first, second, third and fourth generation sequencing technologies, genome mapping-physical and genetic mapping techniques.

Principles and applications of thin layer chromatography, gas chromatography, HPLC and FPLC, Principles and applications of X-ray diffraction, NMR, ESR, ORD/CD, fluorescence, UV, IR, visible and mass spectroscopy.

Principles and applications of tracer techniques in biology: Effect of radiation on biological systems, radioactive isotopes and their half life, autoradiography, radiation dosimetry, Cerenkov radiation, liquid scintillation spectrometry.

The WTO and other international agreements; Intellectual properties , copyrights, trademarks, trade secret, patents, geographical indications, etc.; Protection of plant variety and farmers right act; Indian patent act and amendments, patent filing; Convention on biological diversity; Implications of intellectual property rights on the commercialization of biotechnology products.

Biosafety and risk assessment issues, regulatory framework, National biosafety policies and law, The Cartagena protocol on biosafety, WTO and other international agreements related to biosafety; Cross border movement of germplasm; Risk management issues-containment.

General principles for the laboratory and environmental biosafety; health aspects; toxicology, allergenicity, antibiotic resistance etc. Impact on environment; gene flow in natural and artificial ecologies; Sources of gene escape, tolerance of target organisms, creation of superweeds/superviruses etc.

Ecological aspects of GMOs and impact on biodiversity; Monitoring strategies and methods for detecting transgenics; Radiation safety and non-isotopic procedures; Benefits of transgenics to human health, society and the environment.

SECTION-II: BIOTECHNOLOGY

MICROBIOLOGY

Microbial diversity and systematics, Modern approaches to bacterial taxonomy, polyphasic classification, General characteristics of primary domains and of taxonomic groups belonging to Bacteria, Archaea and Eukarya, Nomenclature and outline of bacterial classification as per Bergey's Manual.

Methods in Microbiology: Theory and practice of sterilization, Pure culture techniques, Principles of microbial nutrition, Construction of culture media, Enrichment culture techniques, Isolation and culture of aerobic and anaerobic bacteria, Culture collection, preservation and maintenance of microbial cultures.

Metabolic Diversity among Microorganism: Microbial Nutrition: nutritional types and modes of nutrition in bacteria, Extremophiles. Microbial growth: The definition of growth, mathematical expression of growth, growth curve, measurement of growth and growth yields; Synchronous growth and Continuous culture.

Chemotherapy/Antibiotics Antimicrobial agents; Antibiotics: Penicillins and Cephalosporins and Broad- spectrum antibiotics, sulfa drugs, Antifungal antibiotics, Mode of action, Molecular mechanism of drug resistance. Bacterial Genetic System: Transformation, Conjugation, Transduction, Recombination, bacterial genetic map with reference to *E coli.*

MOLECULAR BIOLOGY

Prokaryotic and eukaryotic genome organization, structural elements of chromosome and construction of artificial chromosome. DNA replication: Enzymes, accessory proteins and mechanisms of prokaryotic and eukaryotic DNA replication.

Fine structure of gene, molecular basis of spontaneous and induced mutations and their role in evolution; DNA damage and repair, DNA amplification and rearrangement. Anti-sense and Ribozyme Technology: Inhibition of splicing, polyadenylation and translation, disruption of RNA structure and capping, Biochemistry of ribozyme, hammerhead, hairpin and other ribozymes, applications of anti-sense and ribozyme technologies.

Transcription: Organization of transcriptional units, mechanisms of transcription and its regulation in prokaryotes and eukaryotes, Operon concept, attenuation and antitermination controls, RNA processing (capping, polyadenylation, splicing), DNA methylation, heterochromatization, General and specific transcription factors, regulatory elements and mechanism of transcription regulation, transcriptional and post-transcriptional gene silencing, environmental regulation of gene expression.

Translation: Genetic code, Prokaryotic and Eukaryotic translation, mechanisms for initiation, elongation and termination, regulation of translation, co-and post- translational modifications of proteins. Homologous Recombination and Site-specific recombination.

BIOCHEMISTRY

Classification, structure and properties of amino acids; primary, secondary, tertiary, quaternary and domain structure of proteins, forces stabilizing protein structure, Ramachandran plot, DNA-protein and protein-protein interactions, protein folding, protein misfolding and related diseases; protein sequencing.

Primary and secondary structure of nucleic acids, Watson-Crick model of DNA, structural polymorphism of DNA and RNA, three dimensional structure of RNA, biosynthesis of purines and pyrimidines.

Classification and structure of carbohydrates, polysaccharides, glycoproteins and peptidoglycans, glycolysis, TCA cycle, oxidative phosphorylation, glycogen synthesis and breakdown, gluconeogenesis, interconversion of pentoses and hexoses. Classification, structure and functions, biosynthesis of fatty acids, oxidation of lipids, triglycerides, phospholipids, sterols.

CELL BIOLOGY

Structure of prokaryotic and eukaryotic cells, Cellular organelles: Plasma membrane, cell wall, cytoskeleton- their structural organization; Mitochondria; Chloroplast; Nucleus and other organelles and their organization and function, genetic constitution of mitochondria and chloroplast, artificial membrane Liposomes.

Transport of nutrients, ions and macromolecules across membranes, Cell cycle: Mitosis, meiosis, role of cyclins and cyclin dependent kinases, regulation of Cdk-cyclin activity, Cdk inhibitors, induction of cancer with respect to cell cycle, molecular events and regulation in model systems, cell surface receptors, second messenger system, MAP kinase pathways, mechanism of signal transduction pathway.

Molecular biology and biochemistry of cancer, oncogenes, tumor suppressor genes, chemical carcinogenesis, Cellular basis of differentiation and development- cell division, gametogenesis and fertilization, differential gene activity and cell differentiation, Morphogenetic determinants in egg cytoplasm, genetic regulation of early embryonic development in Drosophila, homeotic genes.

RECOMBINANT DNA TECHNOLOGY

Molecular tools and their applications: Restriction endonucleases, polymerases, nucleases, kinases, topoisomerases, gyrases, methylases and ligases. Cloning vectors: Plasmids, Bacteriophages, Cosmids, Phagemids, Artifical chromosomes (BAC, PAC, MAC).

Construction and screening of genomic and cDNA libraries, EMSA (Electrophoretic mobility shift assay), DNA footprinting, Primer extension, SI mapping, RNase protection assay, Reporter assays, Principles and techniques of nucleic acid hybridization, Southern, Northern and Western hybridization/blotting, DNA microarray-fabrications, variations and applications, Serial Analysis of Gene Expression (SAGE).

Polymerase chain reaction: principle, different ingredients of PCR, primer-designing, variations-standard PCR, Touch down PCR, Hot- start PCR, Asymmetric PCR, Inverse PCR, Long PCR, High Fidelity PCR, Multiplex PCR, Nested PCR, Reverse transcriptase PCR, Real Time quantitative PCR, Applications of PCR in different fields.

Expression strategies for heterologous genes: vector engineering, codon optimization, host engineering, expression in bacteria, yeast, insects, mammalian cells and plants, *in-vitro* transcription and translation, T-DNA and transposon tagging.

ENZYME TECHNOLOGY

Nomenclature and classification of enzymes, general properties of enzymes, active sites, cofactors and specificity.

Isolation, purification and large scale production of enzymes with principles and applications of the involved techniques, viz gel filtration, ion exchange and affinity chromatography, centrifugation and electrophoretic techniques.

Enzyme kinetics: Enzymatic reaction mechanisms, Michaelis-Menten equation, Effect of substrate, pH, temperature and inhibitors on enzyme activity.

Mechanism of enzyme action and regulation: Active and regulatory sites, chemical modification, feedback inhibition, positive and negative cooperativity, allosteric enzymes.

Isozymes, multienzyme complexes, artificial enzymes, catalytic antibodies, Enzyme engineering-strategies, directed evolution, degradation of unnatural substrates.

Industrial enzymes: In detergent, food, leather, dairy, medicines and chemical industries.
Enzyme immobilization: Introduction, methods, applications and limitations.

IMMUNOLOGY

Introduction: Phylogeny of Immune System, Innate and acquired immunity, Clonal nature of immune response, Primary and secondary immune response, Organization and structure of lymphoid organs, Cells of the immune system: Haematopoesis and differentiation, lymphocyte trafficking, B lymphocytes, T- lymphocytes, Macrophages, dendritic cells, natural killer and

lymphokine activated killer cells, Eosinophils, Neutrophils and mast cells. Antigens and superantigens. Structure and function of immunoglobulins.

Major histocompatibility complex; Antigen processing and presentation, BCR and TCR, generation of immunological diversity, Complement system.Cell- mediated cytotoxicity : Mechanism of T cell and NK cell mediated lysis, antibody dependent cell mediated cytotoxicity, macrophage mediated cytotoxicity, effector mechanism.

Regulation of immune response:Generation of humoral and cell medicated immune responses, Activation of B-and T-lymphoeytes, cytokines and their role in immune regulation, Immunological tolerance, Genetic control of immune responses. Immunoprophylatic intervention: Basic concepts of vaccination and different types of vaccines.

Hypersensitivity, Autoimmunity, Tumor immunology, AIDS and other immune-deficiencies.Antigen and antibody interactions, Immunodiffusion, Immunoelectrophoresis, RIA, ELISA, Hybridoma technology and monoclonal antibodies.

PLANT BIOTECHNOLOGY

History of plant cell and tissue culture; Culture media; various types of culture; callus, suspension, nurse, root, meristem, etc.; *In vitro* differentiation; organogenesis and somatic embryogenesis.Micropropagation; Anther and microspore culture; Somaclonal variation; *In vitro* fertilization; *In vitro* germplasm conservation; Production of secondary metabolites; Synthetic seeds.

Embryo culture and embryo rescue; Protoplast isolation, culture and fusion; selection of hybrid cells and regeneration of hybrid plants; symmetric and asymmetric hybrids, cybrids. Conventional versus non-conventional methods for crop improvement; Present status and recent developments on available molecular markers, transformation and genomic tools for crop improvements. Molecular marker-aided breeding, QTL, molecular marker assisted selection.

Plant transformation technology: *Agrobacterium* mediated, Particle bombardment, Electroporation; transgene stability and gene silencing. Chloroplast Transformation, Genetic engineering for resistance against abiotic (drought, salinity, flooding, temperature, etc.) and biotic (insect pest, fungal, viral and bacterial diseases, weeds, etc.) stresses; Genetic engineering for increasing crop productivity by manipulation of photosynthesis, nitrogen fixation and nutrient uptake efficiency; Genetic engineering for quality improvement (protein, essential amino acids, vitamins, minerals nutrients, etc.) etc.

Metabolic Engineering and Industrial Products: Plant secondary metabolites, control mechanisms and mainipulation of phenylpropanoid pathway, shikimate pathway; alkaloids, biodegradable plastics, therapeutic proteins, edible vaccines, purification strategies.

ANIMAL BIOTECHNOLOGY

Introduction to animal cell and tissue culture, its advantages and limitations, Applications of animal cell and tissue culture.
Basic techniques in animal cell culture: Disaggregation of tissue and setting up of primary culture, established cell line cultures, maintenance of cell culture, culture media and role of serum in cell culture, organ culture.

Biology and characterization of the cultured cells, measurement of growth, measurement of viability and cytotoxicity.
Scale up of animal cell culture, cell cloning, cell synchronization and transformation.

Stem cell cultures: Embryonic and adult stem cells, their isolation, culture and applications, animal cloning.
Transgenic animals: Construction of transgenic animals, gene knockouts, ethical and biosafety considerations.

Gene therapy: Genetic disorders, vector engineering, types of gene therapy, strategies of gene delivery, targeted gene replacement/augmentation, gene editing, gene correction, gene silencing.
Molecular markers linked to disease resistance genes, Application of RFLP in forensic, disease prognosis, genetic counseling and pedigree analysis.

BIOPROCESS TECHNOLOGY

Introduction to bioprocess technology, bioreactors, Isolation, preservation and maintenance of industrial microorganisms, kinetics of microbial growth and death, media for industrial fermentation, air and media sterilization.

Types of fermentation processes: Analysis of batch, fed-batch, and continuous bioreactors, stability of microbial reactors, analysis of mixed microbial populations, specialized bioreactors (pulsed, photobioreactors etc.), measurement and control of bioprocess parameters.

Downstream processing: Introduction, removal of microbial cells and solid matter, foam separation, precipitation, filtration, centrifugation, cell disruptions, liquid-liquid extraction, chromatography, Membrance process, Drying and crystallization, Whole cell immobilization and its industrial application.

Industrial production of chemicals: Alcohol (ethanol), acids (citric, acetic and gluconic), Solvents (glycerol, acetone, butanol), Antibiotics (penicillin, streptomycin, tetracycline). Amino acids (lysine, glutamic acid), Single cell protein, Use of microbes in mineral beneficiation and oil recovery, Introduction to food technology:Elementary idea of canning and packing, Sterilization and pasteurization of food products.,Technology of typical food/ food products (bread, cheese, idli), Food preservation.

ENVIRONMENTAL BIOTECHNOLOGY

Introduction to Environmental Science: Environmental Pollution: Classification of pollutants, Ecosystem structure and functions, abiotic and biotic component, Energy flow, food chain, food web, Ecological Pyramids-types, biogeochemical cycles. Air, Water, Soil, Noise and Thermal pollution: Their source, Effect and biotechnology based control measures. Solid waste pollution and its management.

Waste water Treatment: Biological treatment system (Oxidative ponds, aerobic and anaerobic ponds,facultative ponds, aerated ponds), Biological waste treatment, activated sludge treatment, microbialpollution in activated sludge, percolating filters, waste water treatment by biofilms. Treatment scheme of Dairy, Distillery, Tannery, Sugar, Fertilizers, Refinery, Chemical and Antibiotic waste.

Bioremediation & Phytoremediation: Biofeasibility, applications of bioremediation, Bioreduction, Phytoremediation. Microbial Leaching and biomining, Recovery of metals from solutions, Microbes in petroleum extraction, Microbial desulfurization of coal, microbial transportation of toxic metals, Biodegradation of chlorinated hydrocarbons and xenobiotic compounds, pesticides, oil spills, and toxic dyes industrial effluents.

Biofertilizers, biopestisides and Integrated pest management (IPM). Energy & Biofuels: Non-conventional or renewable sources of energy, Energy from Biomass, Biosensors and biochips. Ozone depletion, UV-B, Green-house effect and acid rain, their impact andbiotechnological approaches for management.

GENOMICS FOR CROP IMPROVEMENT

Introduction to science of omics for crop improvement, Introduction to the plant genome-nuclear, chloroplast and mitochondrial genomes, genome size and complexity, mapping of genome: genetic and physical maps, map-based cloning, molecular markers in plant genome analysis; RFLP, RAPD, STS, Microsatellite, SCAR (Sequence characterized amplified regions), SSCP (single strand conformational Polymorphism), and AFLP analysis, FISH and GISH for genome analysis.

Plant gene expression and regulation, functional genomics-expression analysis using microarrays, transposon tagging and Insertional mutagenesis - methods and significance, TILLING and EcoTILLING, Diversity Array Technology, transcriptomics.

Whole genome analysis: Genome size, strategies for sequencing genome, ordered genomic libraries (Cosmid, YAC, BAC libraries), Genome sequencing in plants–Principles and Techniques; Next generation sequencing technologies, Applications of sequence information in plant genome analyses; Comparative genomics, Detection of Single Nucleotide Polymorphism; Role of transcriptomics, proteomics and metabolomics in linking genome and phenome.

Marker assisted selection (MAS), Genomic assisted breeding approaches, Genomics andgenoinformatics for crop improvement; Integrating functional genomics information on agronomically/economically important traits in plant breeding, tagging of agronomically important traits, RNA interference in crop improvement.

PROTEOMICS AND NANOBIOTECHNOLOGY

Proteomics technology: Gel electrophoresis of protein- SDS-PAGE, Native gel electrophoresis, zymography, identification and analysis of proteins by 2D analysis, mass spectrometry, MALDI-TOF, NMR and X-ray crystallography.

Differential display proteomics, protein-protein interactions, yeast two hybrid system and phage display, GFP and RFP, western blot, metabolic engineering.

Chemical, physical and biological properties of biomaterials and bioresponse, biomineralization, biosynthesis and properties of natural materials (protein, DNA &polysaccharides).

Preparation and characterization of nanoparticles : nanoparticular carrier system, micro and nano fluidics, drug and gene delivery system, microfabrication, chip technologies, biosensors, nano-imaging.