Broad Syllabus for Ph. D Entrance Test in Molecular Biology Examination March 2021

The Syllabus mentioned here has been sourced from M.Sc Life Sciences and M. Sc Biotechnology syllabus.

Part I

Research Methodology

The syllabus for research methodology is the same as mentioned in PET life Sciences syllabus Part I - Research Methodology portion.

Kindly follow the link pasted below to access the syllabus on research methodology.

https://mu.ac.in/wp-content/uploads/2021/02/PET-Life-Sciences-syllabus.pdf

Part II

Subject Specific Syllabus

1) **Molecular Biology of Life processes**
2) **Protein Biology**
3) **Molecular Techniques**
4) **DNA Typing and Omics**
5) **Genetic Engineering**

1) Molecular Biology of Life processes

DNA Replication and Recombination:

DNA replication: Unit of replication and enzymes, replication origin and replication fork, fidelity and processivity of replication, extrachromosomal replicons (plasmid).

Recombination: Homologous (General and Site specific) and Non Homologous recombination in prokaryotes and eukaryotes. Mechanisms and proteins involved

Transcription: Classes of RNA molecules - structure and function, Transcription factors and machinery - Enzymatic Synthesis of RNA, Basic features of RNA synthesis, E. coli RNA polymerase, transcription activators and repressors, transcription in prokaryotes- initiation, elongation and termination, Transcription in Eukaryotes - formation of initiation complex, capping, elongation & Termination. Eukaryotic rRNA genes, formation of eukaryotic tRNA molecules, RNA Polymerases of eukaryotes, RNA polymerase II Promoters, Eukaryotic Promoters for RNA polymerase III, hypersensitive sites, Upstream activation sites and enhancers

RNA Processing: Capping and polyadenylation, Splicing mechanisms - major and minor, Alternative splicing pathways, Self splicing introns, Splicing of tRNA precursors, rRNA precursors, small RNAs, Micro RNAs, RNA editing, RNA transport, exon shuffling.

Translation: Outline of Translation, The Genetic Code, The Decoding System, Codon Anticodon interaction, the special properties of the prokaryotic Initiator tRNAfMet, Transfer RNA genes, Protein Synthesis in prokaryotes and eukaryotes - formation of initiation complex, initiation factors and their regulation, elongation and elongation factors, termination,

aminoacylation of tRNA, tRNA-identity, aminoacyltRNA synthetase, translational proofreading, suppressors, Inhibitors and Modifiers of protein synthesis.
Ribosomes: the special properties of the prokaryotic and eukaryotic ribosomes, ribosome biogenesis and its regulation.

Regulation of Gene expression in Prokaryotes: General aspects of Regulation, transcriptional regulation - inducible and repressible system, positive regulation and negative regulation; Operon concept – Lactose, Tryptophan, Arabinose and galactose operon, relative positions of Promoters and Operators, Regulons, Master switches, Regulation of Translation, Regulation of the synthesis of Ribosomes, Unregulated changes in gene expression, Feedback Inhibition. RNA interference, mRNA half-life, riboswitches, ribozymes

Gene expression in Eukaryotes: Regulatory strategies in Eukaryotes, Gene alteration (Gene loss, Gene amplification, Gene rearrangement: the joining of coding sequences in the immune system), Regulation mediated through Transcription factors, Regulation of enhancer activity, role of chromatin changes in regulating gene expression, role of nucleosome remodeling and posttranslational modifications in transcription initiation, methylation and epigenetics, RNA processing, RNA splicing, RNA degradation and RNA interference in regulation of gene expression, Regulation of gene expression in plant cells by light. Transcriptional Control by hormones and signaling factors, Translational control, Diseases associated with defects in regulation

2) Protein Biology

Protein Structure and Function: Primary, secondary, tertiary, quaternary and quinary structures. Bond that characterize the protein structure, domains, motifs, folds, signatures, repeats and consensus sequences, physical and chemical properties of proteins, Relationship between structure and function.

Protein Folding, modification and processing: polypeptide processing – cleavage of signal peptide, propeptide, protein folding- role of chaperones, heat shock proteins and other factors, protein splicing, post- translational modification of proteins – Addition of chemical groups, give examples and explain its role in regulation of gene expression and enzyme activity, protein targeting, mutational effects.

Protein-protein and protein-DNA interactions: Classifications and specificity: protein domains, protein networks and complexes, structural properties of Interacting proteins, forces of interaction, thermodynamics and energetic, regulation, significance, Methods of studying and

theoretical prediction of protein – protein interactions, biotechnological and medical applications Protein-DNA interactions – sequence specific DNA binding, DNA binding motifs, thermodynamics, methods of study, medical and biotechnological applications

3) Molecular Techniques

Essentials of Gene cloning
Clone: meaning, Overview of the procedure, Gene library, Hybridization, Importance of DNA Cloning, Principles of Cell-based DNA Cloning and cell independent DNA cloning

Isolation, identification and manipulation of genes
Purification and Separation of nucleic acids – Extraction and Purification of nucleic acids, Detection and Quantitation of Nucleic acids, Gel Electrophoresis.

Modification of nucleic acids- Cutting and Joining DNA – Restriction Endonucleases, Ligation, Alkaline Phosphate, Double Digest, Modification of Restriction Fragments ends, Other Ways of joining DNA Molecules, ligation independent gene cloning, methylases, DNA polymerases, Nucleases, T4 polynucleotide kinases.

Amplifying DNA : Primer design, PCR: basic features and application, types – standard, hot start PCR, touch-down PCR, Nested PCR, RT-PCR, Real time PCR, overlap PCR, RACE, Inverse PCR and others

Cloning Vectors and their properties – Essential components of vectors and their significance-ori, reporter genes, detection markers, linkers, polylinkers, adapters, promoters, RBS, signal sequences, termination sequence, fusion sequence, Plasmid vectors, Vectors based on the lambda Bacteriophage, Cosmids, M13 vectors, "TA" vectors, linear cloning vectors, Expression vectors, Vectors for cloning and expression in Eukaryotic cells, Super vectors: YACs and BACs, viral expression systems for mammals.

Specialized cloning systems - Cloning PCR products, cloning difficult DNA - promoters, terminators and repetitive DNA, cloning multiple genes in tandem, Cloning System for amplifying different sized fragments, Cloning System for producing single-stranded and mutagenized DNA, library construction.

Nucleic Acid Hybridization to detect genes - Principle and application - Preparation of nucleic probes, Principle of Nucleic acid hybridization, Nucleic acid hybridization assays – clone detection, southern, Northern hybridization and gene diagnosis, and microarrays

Other molecular techniques – Gene libraries, in situ hybridization, positional cloning, In Silico Gene discovery

Gene transfer: Bacterial Conjugation, Transformation, Transduction, Microinjection, Electroporation, Transfection, Microprojectile, Shot Gun method, Ultrasonication, Liposome fusion, Microlaser and biolistics

Tools for analyzing Gene Expression

Expression of recombinant proteins: Expression systems - *E. coli*, Yeast, insect cells, mammalian cell lines, cell free extracts, cell free systems, reticulocyte lysates, use recombinant baculoviruses. Expression vectors – bacterial, plant viruses as episomal expression vectors and mammalian expression vectors. Problems and optimization of expression – host compatibility, codon bias, media optimization, protein folding and solubilization, use of tags and fusion proteins

Reporter Genes – Commonly used reporter genes, Analysis of gene regulation, Purification and detection tags.

Protein purification and analysis

Protein precipitation and differential solubilization, proteins purification - immuno-precipitation, Centrifugation (ultra centrifugation), Sub cellular localization and fractionation of proteins, Chromatographic techniques (size exclusion, ion exchange, affinity, gel filtration, etc.), protein tags, Protein Analysis - Biochemical assays, Native and SDS polyacrylamide gel electrophoresis, 2-D gel electrophoresis, isoelectric focusing, Western blotting, determining enzyme kinetics and other physical and chemical properties of proteins, protein sequencing, multiprotein complexes, In situ analysis, ELISA, Light scattering, Multi detection GPC/SEC, Spectroscopy techniques - Fluorescence Spectroscopy, Circular dichroism, Mass spectroscopy, Isothermal titration calorimetry (ITC).

Analysis at the level of gene transcription – Northern blot, In situ hybridization, RNase protection assay, RT-PCR

Analysis of DNA protein interactions: Electrophoretic mobility shift assay (EMSA), DNase I footprinting, Chromatin immuno-precipitation assay.

Analysis of protein-protein interactions - Pull-down assay, Yeast two hybrid assay, Co-immunoprecipitation assay, Fluorescence resonance energy transfer (FRET).

4) DNA Typing and Omics

DNA Typing: DNA polymorphisms: the basis of DNA typing, Minisatellite analysis, Polymerase chain reaction based analysis, Short tandem repeat analysis, Mitochondrial DNA analysis, Y chromosome analysis, Randomly Amplified Polymorphic DNA (RAPD) analysis.

Genomics: DNA sequencing-chemical and enzymatic, genome sequencing – Random shotgun sequencing, WGS, Next-Generation Sequencing - Solexa, ion proton, Nanopore etc, BAC end sequencing, genetic mapping, Radiation hybrid mapping, HAPPY mapping, STS, ESTs, SNPs, Sequencing length polymorphism (AFLPs), FISH, Fibre fish, optical mapping and CGH, metagenomics.

Functional Genomics: identification and analysis of individual genes, positional cloning, Gene trap, High-throughput analysis of gene function-Tissue Arrays, SAGE, Substractive hybridization, Array methods, Macroarrays, Protein arrays, Mass spectrometry, Transcriptome and Bisulphite sequencing, RNA fingerprinting, cell based methods and assays, GFP Techniques and alternatives to GFP, FRET and Fluorescence Recovery After Photobleaching (FRAT)

Transcriptomics and Proteomics: Analysis of the transcriptome, Methods – RNA Seq, Microarrays, SAGE and Affymetrix Gene Chip arrays. Proteomics: Gel based - 2D PAGE, Isoelectric focusing, DIGE and Gel free proteomics - MALDI MS (Labeled and unlabelled analysis), Surface plasma resonance and Protein arrays

Other Omics Technologies: Metabolomics and global biochemical networks. Single Nucleotide Polymorphisms **-** Mapping disease-associated SNPs eg. Alzheimer's disease

5) Genetic engineering:

Changing genes: site-directed mutagenesis and Protein engineering: Primer extension is a simple method for site directed mutation, PCR based site directed mutagenesis, Random mutagenesis, Use of display techniques to facilitate the selection of mutant peptides – phage, cell suface, ribosome, RNA, DNA and *in vitro* compartmentalization, DNA shuffling, production of chimeric proteins.

Genetic engineering in animals: Production of transgenic mice, ES cells can be used for gene targeting in mice

Genetic engineering in plants: Use of *Agrobacterium* tumefaciens and *A. rhizogenes*, Ti plasmids, Strategies for gene transfer to plant cells, Direct DNA transfer to plants, Gene targeting in plants, Use of plant viruses as episomal expression vectors and tissue culture.

Antisense technology - Antisense oligonucleotides, RNA interference (RNAi), RNAi therapies

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