Ph.D (Fish Biotechnology)

Course Structure – At a Glance

A. MAJOR COURSES **15 Credits**

A1. CORE COURSES 9 Credits

1	FBT 601	Advances in Molecular and Cell Biology	2+1
2	FBT 602	Genetic Engineering of Eukaryotes	2+1
3	FBT 603	Genetic Engineering of Bacteria and Viruses	2+1

A2. OPTIONAL COURSES 6 Credits

1	FBT 604	GMOs and Biosafety Issues	2+0
2	FBT 605	Functional Genomics and Proteomics	2+1
3	FBT 606	Bio-Process Technology	1+0
4	FBT 607	Aquaculture Genomics	2+1
5	FBT 608	NGS platforms and data analysis	0+1

B. MINOR COURSES (Courses from other relevant disciplines) **8 Credits**

C. SUPPORTING COURSES (Compulsory) **5 Credits**

(Course work total **28 Credits)**

D. SEMINAR **2 Credits**

E. RESEARCH THESIS **45 Credits**

Total **75 Credits**

Detailed syllabus- PhD

FBT601	**ADVANCES IN MOLECULAR AND CELL BIOLOGY**	**2+1**
Objective	To provide a deeper understanding of the molecular and cellular processes involved in the functioning, maintenance and death of living cells.	
Theory		
Unit I	Content of the genome: genome size and complexity, C-value paradox, repetitive and non- repetitive DNA, Cot curve, evolution of interrupted genes, cluster and repeats, gene families, pseudogenes, evolutionary clock.	
Unit II	Flow cytometry: FACS and MACS	
Unit III	Signal transduction: active and passive transport, carrier proteins (uniporter/ symporter/antiporter), ion channels (ligand and voltage gated channels), G-proteins, signaling pathways (Ras/MAPK, JAK-STAT).	
Unit IV	Cell cycle and growth regulation: cell cycle check points, cyclins, CDKs (Cyclin dependent kinases); Cell differentiation; Apoptosis: programmed cell death – genetic pathways for PCD, anti and proapoptotic proteins, Ubiquitin pathway for protein degradation, Proteasome.	
Unit V	Epigenetics: DNA imprinting, histone modifications, histone code.	
Unit VI	Oncogenes and tumour suppressor genes: Viral and cellular oncogenes, tumour suppressor genes.	
Practical	Safe laboratory practices, DNA sequence analysis for identification of cis acting elements – kozak sequence, intron-exon boundaries, poly A signal, terminators, promoters, transcription factor binding sites, zinc finger motif, cellular localization signals using bioinformatics software available online; multiple alignment, tandem repeat identification, promoter analysis, antisense/siRNA design.	

FBT602	**GENETIC ENGINEERING OF EUKARYOTES**	**2+1**
Objective	To provide in-depth knowledge on the techniques available for genetic engineering of eukaryotes and strategies to optimize recombinant protein production in eukaryotic expression systems.	
Theory		
Unit I	Eukaryotic expression systems: Yeast expression system - host strains, special features, types of vectors (yeast episomal vectors, integrating vectors and YACs), yeast two hybrid system.	
Unit II	Insect cell expression system - special features, types, baculoviral expression vectors, polyhedron promoters.	
Unit III	Mammalian cell expression system - special features, selectable markers; Transfection: principle, types, selection; transduction by viral vectors,	

	construct design (strong and constitutive promoters, inclusion of introns).
Unit IV	Fish cell expression systems: Tissue specific promoters, constitutive promoters and applications. Strategies for optimizing recombinant gene expression in eukaryotic systems; Downstream processing of recombinant proteins.
Unit V	Transgenesis: methods of gene transfer, integration and detection techniques, *In situ* hybridization; strategies for gene targeting (homologous sites/cre-lox recombination system); specialized vectors for high efficiency transgenesis – eukaryotic transposon vectors, retroviral vectors, etc., an overview of transgenics developed in fisheries sector
Unit VI	Fish as a model organism: Gene function analysis - gene knockouts, gene silencing by RNAi, morpholinos, etc; site directed and transposon mediated mutagenesis. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) knockdown technology.
Practical	Gene transfer experiments (electroporation, microinjection); Northern blotting, Western, Southern blotting for confirming integration and expression of transgene; Gene library: construction of cDNA and genomic DNA libraries; Screening: DNA hybridization, immunological assay and protein activity.

FBT603	**GENETIC ENGINEERING OF BACTERIA AND VIRUSES**	**2+1**
Objective	To provide knowledge on various techniques available to produce genetically engineered microbes and their application, design of viral vectors for efficient gene delivery.	
Theory		
Unit I	Recombinant protein expression in Bacteria: Optimization of expression; fusion proteins, purification of recombinant proteins - inclusion bodies, extracellular targeting, engineering of signal sequences, electroporation.	
Unit II	Genetic engineering of Virus: Use of animal viruses like vaccinia, herpes, retrovirus, baculovirus and adenovirus as cloning vectors, design of viral vectors - special features, cis acting regulatory elements; strategies to optimize recombinant protein production, pro's and con's of using viral vectors as gene delivery vehicles; vectors based on bacteriophage lambda, P1 and M13, special features and their application in optimizing recombinant protein production.	
Unit III	Scope and application of genetic engineering in Virus: Efficient gene delivery strategies, host-pathogen interaction, antigenic proteins, vaccination approaches, DNA vaccines, diagnostics; non-antibiotic selectable markers for live vaccines and gene therapy, methods for detection of viral infection, estimation of viral load by Real Time PCR, etc.	

Practical	Transformation of bacteria by electroporation, Southern and dot- blot transfer techniques; Restriction mapping of DNA; labeling of DNA probes; PAGE analysis for recombinant proteins. Preparation of primary and secondary monolayer cell culture, use of cell culture in virus cultivation and assay; Viral DNA isolation and restriction analysis; Culture and maintenance of bacteriophages; qRT-PCR.

FBT604	**GMOs AND BIOSAFETY ISSUES**	**2+0**
Objective	To provide an overview on the current status of genetically modified organisms and patent laws, biosafety guideline and regulatory mechanisms involved.	
Theory		
Unit I	Safety and ethical issues: guidelines for research on genetically modified organisms (GMOs), quality control of biologicals produced by rDNA technology, safety in the contained use and release of transgenic animals, ecological risk of engineered organisms/plants and remedial measures, ethical issues, public awareness and acceptance of products of biotechnology; animal ethics, regulation and compliance	
Unit II	Biosafety regulations: Guidelines for environmental release of GMOs, guidelines for exchange and shipment of GMOs, material transfer agreement (MTA), mechanism of implementation of biosafety guidelines at Institutional, national and international level, acts and treaties related to biosafety of GMOs.	
Unit III	Patent laws: Global scenario of genetically modified organisms, Intellectual Property Rights (IPR), patent laws at institutional, national and international level, patent search.	

FBT605	**FUNCTIONAL GENOMICS AND PROTEOMICS**	**2+1**
Objective	To give an introduction to application of modern techniques for functional genome analysis.	
Theory		
Unit I	Transcriptome analysis: DNA Microarray, Subtractive hybridization and differential display for identification of genes expressed in specific conditions. Trait specific and global libraries.	
Unit II	Types of RNAi—miRNA, siRNA, shRNA, tncRNA, gene knockdown, gene knockout, co-suppression, post transcriptional gene silencing, quelling, RNAi in *C. elegans*– landmark events in the discovery of RNAi components – dsRNA, Dicer, RISC complex, argonaute protein; mechanism of RNAi, miRNA	

	pathway, RNAi and origin of heterochromatin, CRISPR knockdown.
Unit III	Ribonuclease II super family: forms and functions in RNA, maturation, decay and gene silencing, RNA dependent RNA polymerase in gene silencing, RNAi in invertebrates – antiviral immunity by dsRNA in shrimps.
Unit IV	Delivery of RNAi : Bio-distribution, delivery and application, delivery reagents, target validation, detection methods, delivery systems – viral and nonviral delivery, RNAi as a tool against animal and human diseases – HIV, cancer; gene therapy.
Unit V	Proteome analysis: Two dimensional separation of total cellular proteins, isolation and sequence analysis of individual protein spots by mass spectroscopy, protein microarrays, advantage and disadvantage of DNA and protein microarrays
Practical	Analysis of SNP using DNA chips, printing of oligonucleotides and PCR products on glass slides, nitrocellulose paper, conventional and automated sequencing of DNA, protein sequencing by mass spectroscopy, protein microarrays.

FBT606	**BIOPROCESS TECHNOLOGY**	**1+0**
Objective	To learn the concepts of bulk processing, production and purification of biologicals.	
Theory		
Unit I	Raw materials for bioprocessing, comparison of chemical and biochemical processing based on energetics and environmental issues. Development of inocula, kinetics of enzymatic and microbial processes, optimisation studies, sterilization of media, air and equipment, modes of cell cultivation, general principles of bioreactor design and their operation -Downstream processing, separation and purification techniques, quality assurance testing, representative examples of microbial products, vaccines and vaccine development.	
Unit II	Immobilization of cells and enzymes: principles, methodology and applications, disintegration of cells, separation of solid and liquid phases, isolation and purification techniques for proteins and other products based on different physico-chemical properties, eg., precipitation, adsorption, chromatographic separations, bio-affinity based methods - Principles of bioprocess control, bioprocess automation and application of computers in bioprocessing, recombinant products with representative examples,	

FBT607	Aquaculture Genomics	2+1
Objective	To learn development and application of genomic tools in aquaculture species.	
Theory		
Unit I	Molecular markers in aquaculture and their application. Transcriptome associated markers - Type I markers	
Unit II	DNA marker maps: Radiation hybrid mapping and integrated maps	
Unit III	Genome analysis: Preparation of ordered cosmid libraries, BAC libraries, Shotgun libraries and sequencing, conventional and automated sequencing, Case studies (zebrafish, etc.). Metagenomics, NGS platforms – principles and applications.	
Unit IV	Genotype based sequencing, Case studies of linkage maps (Rohu, Tilapia, European seabass, Channel catfish, *Litopenaeusvannamei*).	
Unit V	QTL and Maker assisted selection, genomic selection, DNA chips case study	
Practical	Microsatellite enriched genomic library construction, screening by colony hybridization, probe labeling techniques, software related to linkage mapping, SNP discovery and analysis.	

FBT 607	NGS platforms and data analysis	0+1
Objective	To understand the basic principles of next generation sequencing technology, data processing and analysis.	
Practical	NGS platforms and NGS data files and formats, transcriptome sequencing, NGS experimental design and planning, introduction to Unix environment; NGS bioinformatics workflow steps following sequence generation, data analysis/quality filtering using FAST-Q screen, variant calling & NGS data assembly tools (reference guided and *de novo*), sequence alignment using BWA, genome annotation, SNP detection, RNAseq analysis using Trinity software, metagenomics analysis.	