FISH BIOTECHNOLOGY

Course Contents

FBT 501 FUNDAMENTALS OF MOLECULAR BIOLOGY 2+1

Objective

To provide knowledge of basic molecular processes involving nucleic acids and protein structure, synthesis and maintenance within a living cell.

Theory

UNIT I

Nucleic Acids: Genetic material, Structures of DNA and RNA; Stereochemistry of bases and secondary structures; Organisation of the nucleic acids - chromatin structure.

UNIT II

DNA replication: Models of DNA replication in prokaryotes and eukaryotes; Mechanics of DNA replication; Enzymes; Structure and function of DNA polymerases; Types of priming.

UNIT III

Transcription: Prokaryotes – Bacterial RNA polymerase, initiation, elongation and termination, types of RNA polymerases; Eukaryotes – enzymes and mechanics, post transcriptional modifications; Structure and synthesis of rRNA and tRNA.

UNIT IV

Translation: Genetic code, codon bias, types and structures of ribosomes, tRNA structure, Wobble hypotheisis, mechanisms of initiation, elongation, termination, and post-translational modifications in prokaryotes and eukaryotes and the factors involved in various steps, concept of polysomes and protein structure.

UNIT V

DNA recombination: Molecular models – homologous and site-specific recombination; crossing over; Holliday junction; transposition.

UNIT VI

Mutations: Types, mutagens – nitrous acid, UV, aflatoxin, bleomycin.

UNIT VII

DNA Repair: Types and mechanisms.

UNIT VIII

Gene transfer: Molecular mechanisms of conjugation, transduction,

transfection and transformation.

Practical

Nucleic acid isolation (genomic/plasmid DNA and RNA); Agarose gel electrophoresis; Nucleic acid quantification; Protein purification and separation in polyacrylamide gel electrophoresis (SDS-PAGE); Preparation of competent cells and transformation.

Suggested Readings

Boyer R. 1999. *Concepts in Biochemistry*. Cole Publ. Co.

Glick BR & Pasternak JJ. 2005. *Molecular Biotechnology: Principles and Applications of Recombinant DNA Technology*. ASM Press.

Lewin B. 2008. *GENES – IX*. Jones & Bartlet Publ.

Primrose SB. 1987. *Modern Biotechnology*. Blackwell.

Watson JD, Baker TA, Bell SP, Gann A, Levine M, Losick R & Inglis C. 2007. *Molecular Biology of the Gene*. 6th Ed. Benjamin Cummings Publ.

FBT 502 BASIC CONCEPTS OF CELL BIOLOGY 2+1

Objective

To outline the basic structure, growth and differentiation of prokaryotic and eukaryotic cell, sub cellular components and their function.

Theory

UNIT I

Prokaryotic and eukaryotic cell architecture: Cell theory; diversity of cell size and shape.

UNIT II

Organization and function of sub-cellular organelles: Cell membrane; cytoplasm; endoplasmic reticulum; Golgi apparatus; lysosomes; mitochondria; nucleolus; peroxisomes and sub-nuclear structures.

UNIT III

Principles of membrane transport: Active/passive membrane transport (Case study - Osmoregulation in freshwater and marine fishes) ion channels; carrier proteins; cell signaling.

UNIT IV

Cell division: Cell cycle and its regulation; Cell growth and differentiation.

UNIT V

Cell motility: Actin-myosin filaments; flagella; cilia.

UNIT VI

Protein sorting: Secretion and targeting; vesicular traffic; endocytosis; exocytosis; protein translocation and secretary pathways.

Practical

Microscopic techniques- bright field, phase contrast and fluorescent microscopy; Microtomy; Sub-cellular fractionation and their functional integrity; Chromosome preparation; Histochemical techniques.

Suggested Readings

Alberts B, Johnson A, Lewis J*,* Raff M, Roberts K & Walter P*. 2002*. *Molecular Biology of the Cell*. 4th Ed.Science Publ.

Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Zipursky L & Darnell J. 2004. *Molecular Cell Biology*. 5th Ed. WH Freeman.

Scott FG. 1998. *Developmental Biology*. 2nd Ed. Sunderland Sianuer Associates.

Wilson EB. 1900. *The Cell in Development and Inheritance*. 2nd Ed. The MacMillan Co.

FBT 503 GENE STRUCTURE AND REGULATION OF EXPRESSION 2+1

Objective

To understand the structure of genes in prokaryotes and eukaryotes and the significance of cis and trans acting genetic elements in the regulation of gene expression.

Theory

UNIT I

Gene structure: Promoters, UTRs, ORFs, exons, introns, termination signal, mono- and polycistronic genes, Gene clustering; Overlapping genes in (Phi X174 virus).

UNIT II

Bacteriophage genome: Organization and life cycle of Lambda and M13.

UNIT III

Regulation of gene expression in Prokaryotes: Operon concept (Lac/Trp); SOS response, bidirectional promoters.

UNIT IV

Regulation of gene expression in Eukaryotes: DNA protein interactions (zinc fingers, leucine zippers, helix turn helix, Z-DNA); transcription factors, promoters, enhancers, repressors, insulators, attenuators, IRES, alternative splicing.

UNIT V

RNA in gene regulation : Antisense RNA, microRNA, ribozymes.

UNIT VI

Case study: Molecular regulation of growth hormone expression in carp/or Molecular regulation of Na+K+ ATPase in gills and kidney cells of freshwater and marine fishes.

UNIT VII

Expression analysis – Techniques to test the up and down regulation of specific genes like Micro array and Real time PCR.

UNIT VIII

Epigenetics - DNA methylation, genetic imprinting, histone modifications, chromatin remodeling.

UNIT IX

Inhibitors of transcription and translation : Mode of function and resistance mechanism – Actinomycin D, α–amanitin, Rifampicin, Tetracyclin, Streptomycin, Chloramphenicol, Kanamycin, Cyclohaxamide, Diptheria toxin, Ricin.

UNIT X

Site-directed mutagenesis and its applications.

Practical

Expression studies of a gene controlled by lacz promoter – Induction, blue/white selection, cell extract separation by PAGE and western blotting; lambda plaque formation on E. coli lawn; Seperation of gill extract on PAGE and histochemical staining of Na+ K+ ATPase of fish kept at different salinities; retrieval of gene information from ensemble and NCBI, BLAST.

Suggested Readings

Boyer R. 1999. *Concepts in Biochemistry*. Cole Publ. Co.

Lewin B. 2008. *GENES IX*. Jones & Bartlet Publ.

Primrose SB & Twyman RM. 2006. *Principles of Gene Manipulation and*

Genomics. 7th Ed. Blackwell.

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FBT 504 GENETIC ENGINEERING AND ITS APPLICATION 2+1
IN FISHERIES

Objective

To detail the basic steps in recombinant DNA technology and its application in optimization of production, health and environment in fisheries.

Theory

UNIT I

Recombinant DNA technology: DNA modifying enzymes - types of restriction endonucleases (Type I, II and III), DNA/RNA modifying enzymes (alkaline phosphatases, kinases, exonucleases, ligases, terminal transferases); Vectors - plasmids (replication, copy number control and compatibility), phagemids, cosmids, high capacity vectors (eg. BAC), shuttle vectors; Adapters, linkers, ligation, transformation and selection.

UNIT II

Hosts: Prokaryotic (selected *E. coli* strains) and eukaryotic (selected yeast strains).

UNIT III

DNA amplification: PCR – principle, types and applications; T/A cloning of amplified products; Structure and function of DNA polymerase and reverse transcriptase.

UNIT IV

Genomic DNA library: Construction, screening (PFGE) and applications; chromosome walking.

UNIT V

cDNA library: Construction, screening (PFGE) and clone characterization.

UNIT VI

DNA and protein sequencing: Principle, types and applications.

UNIT VII

Application of rDNA technology : Transgenesis – fish as a model organism, target genes, methods of gene transfer, transgenic screening techniques; Production of diagnostics and vaccines; biofactories, biosensors, waste water treatment, probiotics, GMOs - Biosafety

regulations and ethical issues related to biotechnological products; patent laws and IPR issues.

UNIT VIII

Optimization of recombinant protein expression in prokaryotes and eukaryotes.

UNIT IX

Nucleic acid hybridization: Southern, Northern and Western blotting; DNA probes and their labeling.

Practical

Cloning strategies – insert and vector preparation, ligation, preparation of competent cells, transformation, clone confirmation techniques (horizontal slot lysis/colony PCR); Southern hybridization, probe Labeling methods; Primer designing; DNA sequencing and analysis.

Suggested Readings

Brown TA. 1998. *Recombinant DNA*. Academic Press.

Brown TA. 2002. *Genomes*. 2nd Ed. John Wiley & Sons.

Lewin B. 2008. *GENES IX*. Jones & Bartlet.

Primrose SB, Twyman RM. 2006. *Principles of Gene Manipulation and Genomics*. 7th Ed. Blackwell.

Sambrook J & Russel WD. 1989. *Molecular Cloning: A Laboratory Manual*. Vols. I-III.Cold Spring Harbour.

FBT 505 MOLECULAR AND IMMUNOGENETICS 1+1

Objective

To acquaint the students with techniques used to estimate genetic variation among individuals and populations for various purposes and DNA diversity generated by somatic recombination of immunoglobulin genes.

Theory

UNIT I

Biochemical markers: Allozyme polymorphism and its application in estimating population genetic parameters.

UNIT II

Molecular markers: RAPD, RFLP, AFLP, EST, SNP, Minisatellites and Microsatellites and application in population genetic analysis and gene mapping, FISH – principle and application.

UNIT III

Analysis: Interpretation of gels and data analysis using various softwares. DNA sequence polymorphism and related software for alignment and analysis.

UNIT IV

Immunogenetics: Molecular biology of Ig synthesis, genetic basis of antibody diversity, humoral B-cell immunoglobulins, T-cell receptors and MHC.

Practical

Biochemical markers: Allozyme polymorphism. Molecular Markers: RAPD, RFLP, AFLP, Minisatellites and Microsatellites.Interpretation of gels and data analysis.

Suggested Readings

Caetano-Anolles G & Gresshoff PM. 1998. DNA *Markers: Protocols, Applications and Overviews*. Wiley-VCH.

Pasteur N, Pasteur G, Bonhomme F, Catalan J & Britton-Davidian J. 1988. *Practical Isozyme Genetics*. John Wiley & Sons.

Sambrook J & Russel WD. 1989. *Molecular Cloning: A Laboratory Manual*. Vols. I-III.Cold Spring Harbour.

FBT 506 BIOINFORMATICS 1+1

Objective

To learn the application of information technology for the fish genetics studies.

Theory

UNIT I

Introduction to bioinformatics: history, definition, scope and applications; Fields related to bioinformatics.

UNIT II

Data base: mining tools, submission of DNA sequences; Sequence alignment and database searching, similarity search, FASTA, BLAST.

UNIT III

Information networks: internet; Gene bank sequence database, EBI-net; NCBI, Genome net.

UNIT IV

Genomics: genome diagnostics, genome projects, genome analysis.

UNIT V

Proteomics: protein information resources, primary and secondary protein data bases, analysis packages, predictive methods, ESTs.

UNIT VI

Phylogenetic analysis; Comparative genome analysis; Microarray bioinformatics.

Practical

Internet search: retrieving information from different data base like NCBI, protein information sources; Preparation of data base; Use of genome analysis packages: genetics data base; Searching by similarity; Phylogenetic analysis; Accessing and submission to gene banks; BLAST, sequence alignments, comparisons.

Data base: mining tools, submission of DNA sequences; Sequence alignment and database searching, similarity search, FASTA, BLAST.

Suggested Readings

Attwood TK & Smith DJP. 1999. *Introduction to Bioinformatics*. Addison Wesley Longman.

Baxevanis AD & Ouellettee BF. 2002**.** *Bioinformatics, A Practical Guide to the Analysis of Genes and Proteins*. John Wiley & Sons.

Brown SM. 2000. *Bioinformatics: A Biologist's Guide to Biocomputing and the Internet*. Eaton Publ.

Campbell MA & Heyer LJ. 2003. *Discovering Genomics, Proteomics, and Bioinformatics***.** Benjamin Cummings.

Lesk AM. 2008**.** *Introduction to Bioinformatics.* Oxford University Press.

Mount DW. 2001. *Bioinformatics: Sequence and Genome Analysis*. Cold Spring Harbor Press.

Rashidi HH & Buehler LK. 2005. *Bioinformatics Basics: Applications in Biological Sciences and Medicine*. CRC Press.

FBT 507 CELL AND TISSUE CULTURE 1+1

Objective

To impart knowledge on cell and tissue culture techniques and their application in health management, gene banking and genetic characterization.

Theory

UNIT I

Introduction: Structure and Organization of animal cell; Equipments and materials for animal cell culture technology.

UNIT II

Cell lines and media: Primary and established cell line cultures; media supplements – their metabolic functions; serum and protein free defined media and their application.

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UNIT III

Cell culture: Basic techniques of cell culture in vitro; development of primary cultures, cell separation, maintenance of cell lines; biology of cultured cells, transformation and differentiation of cell cultures.

UNIT IV

Characterization of cell lines: Measurement of viability and cytotoxicity assays; measuring parameters of growth; karyotyping, isozyme assays, cryopreservation, assessment of contaminants.

UNIT V

Cell cloning: Micromanipulation, cell transformation, application of fish cell culture, scaling-up of cell culture.

UNIT VI

Stem cells: Stem cell cultures, embryonic stem cells and their applications; cell culture based vaccines, organ and histotypic cultures; measurement of cell death; apoptosis; three dimensional culture and tissue engineering.

UNIT VII

Cell hybridization: Somatic cell fusion, hybridoma technology, Production and Application of monoclonal antibodies.

Practical

Principles of sterile techniques and cell propagation; Preparation of different cell culture media; Primary cell culture techniques; Establishing cell lines: isolation, characterization identification of cell lines; Pure culture techniques; Maintenance and preservation of cell lines; Propagation of cells in suspension cultures; Hybridoma technology: strategy and techniques; Production of monoclonal antibodies.

Suggested Readings

Barnes D & Mathur PJ. 1998. *Methods in Cell Biology*. Vol. 57. *Animal*

Cell Culture Methods. Academic Press.
Basega R. (Ed.). 1989. *Cell Growth and Division: A Practical Approach*. IRL Press.
Butler M & Dawson M. (Ed.). 1992. *Cell Culture*. Bios Scientific Publ.
Clynes M. 1998. *Animal Cell Culture Techniques*. Springer.
Freshney I. 1994. *Culture of Animal Cells: A Manual of Basic Techniques*. 4th Ed. Wiley-Liss.
Harrison AM, Rae FI & Harris A. 1997. *General Techniques of Cell Culture*. Cambridge University Press.
Lan FR. 1994. *Culture of Animal Cells*. 3rd Ed. Wiley-Liss.
Masters RW. 2000. *Animal Cell Culture-Practical Approach*. Oxford University Press.

FBT 508 MARINE BIOTECHNOLOGY 1+1

Objective

To outline an overview on the potential marine resources for bioactive compounds, pharmaceuticals and the application of biotechnological tools to combat marine pollution.

Theory

UNIT I

Introduction: Historical background, overview of the present status of marine biotechnology, commercially important and potential species, micro-algae, macro-algae, aquaculture.

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UNIT II

Marine Resources: Biodiversity, marine natural products, valuable chemicals, biomedical and bioactive compounds from marine organisms, commercial bio-products from marine organisms; green fluorescent protein from jelly fish and its application, marine organisms as a source of polysaccharides, antiviral, anticancer and anti-inflammatory compounds; and commercially important enzymes - Xylanase, agarase, proteases, chitinases, amylase, lipases, cellulase, phytase.

UNIT III

Environmental Biotechnology: Marine biotechnology for economic development and environmental problem solving, bio-film and bioremediation, bio-sensor and transgenic marine organisms; unculturable

bacteria- occurrence, characteristics, characterization and exploitation; metagenomic library of unculturable bacteria, marine pollution and its control; genetically engineered microbes for waste water treatment; Red sea tide and its control, biofouling and prevention.

UNIT IV

Gene mining : Identification of genes responsible for novel proteins, rDNA technology for the large scale production of novel proteins, pharmaceutical, cosmetic and neutraceuticals and their use in drug designing - for various finfish and shellfish bacterial and fungal toxins.

UNIT V

Fermentation technology: Types – batch, continuous; Down stream processing of commercially important compounds.

Practical

Extraction of bioactive compounds from seaweeds, microalgae, sponges and test their efficiency microbiology, biochemistry and molecular assays, isolation of marine algae, plankton and its culture method, methods for isolation of viable and unculturable bacteria from sea, recombinant DNA technology to produce commercially important enzymes.

Suggested Readings

Colwell RD. 1984. *Biotechnology in the Marine Sciences*. Proceedings of the First Annual MIT Sea Grant Lecture and Seminar.

Fingerman M, Nagabushana M & Thompson R. 1998. *Recent Advances in Marine Biotechnology*. Vol.II. Science Publ.

Fusetani N. 2000. *Drugs From Sea*. Karger Publ.

Kamely D, Chakraborty A & Omenn GS. 1990. *Biotechnology and Biodegradation*. Portfolio Publ. Co.

Karl DM. 1995. *Microbiology of Deep Sea Hydrothermal Vents*. CRC Press.

Omura S. 1992. *The Search for Bioactive Compounds from Microorganisms*. Springer.

FBT 509 AQUACULTURE BIOTECHNOLOGY 1+1

Objective

To provide an overview of the application of biotechnological tools in fish breeding, feed, health, processing and other facets in fisheries.

Theory

UNIT I

Fish Breeding: Synthetic hormones for induced breeding- GnRH analogue structure and function.

UNIT II

Transgenesis : Methods of gene transfer in fishes, single gene traits, screening for transgenics, site of integration, applications, regulation of GMOs, IPR, Evaluation of GFP transgenics.

UNIT III

Gene Bank and conservation: Cryopreservation of gametes and embryos.

UNIT IV

Feed Technology: Micro encapsulated feeds, micro coated feeds, microparticulate feeds and bio-encapsulated feeds, mycotoxins and their effects on feeds.

UNIT V

Health Management: DNA and RNA vaccines, molecular diagnosis of viral diseases, PCR, Dot-blot, ribotyping of pathogenic microbes, RNAi, Biofilms and its impact on health management, genetically modified microorganisms as probiotics, immunostimulants, bioremediation of soil and water.

UNIT VI

Algal Biotechnology: Microalgae - indoor and mass culture methods, biotechnological approaches for production of important microalgae, single cell protein from Spirulina, raceway system of micro algae culture, vitamins, minerals and omega3 fatty acids from micro algae, enrichment of micro algae with micronutrients.

UNIT VII

Post harvest biotechnology: Delaying of spoilage, detection of toxic substances and pathogenic microbes, biosensors for toxins.

UNIT VIII

Application of nanotechnology in aquaculture.

Practical

Induced breeding of carps, *Spirulina* culture, identification of selected algae, cryopreservation of gametes, diagnosis of WSSV, microencapsulation, ribotyping, HAACP methods, preparation of agar, PCR

amplification and cloning of growth hormone gene, transgenesis, chromosomal manipulation- androgenesis, gynogenesis, triploidy, tetraploidy.

Suggested Readings

Lakra WS, Abidi SAH, Mukherjee SC & Ayyappan S. 2004. *Fisheries Biotechnology*. Narendra Publ. House.

Nagabhushanam R, Diwan AD, Zahurnec BJ & Sarojini R. 2004. *Biotechnology of Aquatic Animals*. Science Publ.

Nair PR. 2008. *Biotechnology and Genetics in Fisheries and Aquaculture*. Dominant Publ.

Pandian TJ, Strüssmann CA & Marian MP. 2005. *Fish Genetics and Aquaculture Biotechnology*. Science Publ.

Reddy PVGK, Ayyappan S, Thampy DM & Gopalakrishna. 2005. *Text Book of Fish Genetics and Biotechnology*. ICAR.

FBT 601 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

Protein localization and trafficking: Co-translational and post-translational translocation, post-translational modifications; Protein transport through ER - Golgi system; Anterograde and retrograde transport; Exo- and endocytosis; Clathrin coated vesicles; membrane fusion and protein localization; Ubiquitin pathway for protein degradation.

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 (Cycline dependent kinases); Cell differentiation; Apoptosis: programmed cell death – genetic pathways for PCD, anti and proapoptotic proteins.

UNIT V

Epigenetics : DNA imprinting, histone modifications, histone code.

UNIT VI

Oncogenes and tumour suppressor genes: Viral and cellular oncogenes, tumour suppressor genes; Structure, function and mechanism of action of pRB and p53 tumour suppressor proteins.

UNIT VII

RNA interference: History, molecular mechanisms and applications of antisense RNA, microRNA, siRNA and Ribozymes.

Practical

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 softwares available online; multiple alignment, tandem repeat identification, promoter analysis, antisense/siRNA design.

Suggested Readings

Alberts B, Johnson A, Lewis J, Raff M, Roberts K & Walter P. *2002. Molecular Biology of the Cell*. 4th Ed. Science Publ.

Boyer R. 1999. *Concepts in Biochemistry*. Cole Publ. Co.

Lewin B. 2008. *GENES IX*. Jones & Bartlet.

Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Zipursky L & Darnell J. 2004. *Molecular Cell Biology*. 5th Ed. WH Freeman.

Scott GF. 1998. *Developmental Biology*. 2nd Ed. Sunderland Sianuer Associates.

Watson JD, Baker TA, Bell SP, Gann A, Levine M, Losick R & Inglis C. 2007. *Molecular Biology of the Gene*. 6th Ed. Benjamin Cummings Publ.

Wilson EB. 1900. *The Cell in Development and Inheritance.* 2nd Ed. The MacMillan Co.

FBT 602 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.

UNIT V

Strategies for optimizing recombinant gene expression in eukaryotic systems; Downstream processing of recombinant proteins.

UNIT VI

Transgenesis : Fish as a model organism, methods of gene transfer, strategies for gene targeting (homologous sites/ cre-lox recombination system); specialized vectors for high efficiency transgenesis – eukaryotic transposon vectors, retroviral vectors, etc., Transgene: integration and detection techniques, an overview of transgenics developed in fisheries sector – food/or ornamental; Fish as biosensors and biofactories.

UNIT VII

Gene function analysis: Gene knock-outs, gene silencing by RNAi, morpholinos, etc; site directed and transposon mediated mutagenesis.

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.

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Suggested Readings

Boyer R. 1999. *Concepts in Biochemistry*. Cole Publ. Co.

Brown TA. 2002*. Genomes*. 2nd Ed. John Wiley & Sons.

Lewin B. 2008. *GENES IX.* Jones & Bartlet.

Sambrook J & Russel WD. 1989. *Molecular Cloning: A Laboratory Manual.*Vols. I-III. Cold Spring Harbour.

FBT 603 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

Scope and application of genetic engineering in bacteria: Engineered microorganisms for bioremediation, biofouling, biosensing, biofermentation, probiotics and single cell protein.

UNIT III

Molecular biology of fish DNA/RNA viruses: Major groups of DNA/RNA viruses; their cis acting genetic elements and regulation of protein expression.

UNIT IV

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 lamda,

P1 and M13, special features and their application in optimizing recombinant protein production.

UNIT V

Scope and application of genetic engineering in Virus : Efficient gene delivery strategies, host-pathogen interaction, antigenic proteins, vaccination approaches, DNA vaccines, diagnostics : 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; RT-PCR.

Suggested Readings

Boyer R. 1999. *Concepts in Biochemistry*. Cole Publ. Co.

Brown TA. 2002*. Genomes*. 2nd Ed. John Wiley & Sons.

Lewin B. 2008. *GENES IX.* Jones & Bartlet.

Sambrook J & Russel WD. 1989. *Molecular Cloning: A Laboratory Manual.*Vols. I-III. Cold Spring Harbour.

FBT 604 BIOSAFETY AND PATENT LAWS 2+0

Objective

To provide an overview on the current status of genetically modified organisms and patent laws, biosafety guidelines 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 related to biotechnology products.

UNIT II

Biosafety regulations: Guidelines for environmental release of GMOs,

guidelines for import and shipment of GMOs, mechanism of implementation of biosafety guidelines at Institutional, national and international level, Role of national agencies in regulating GMOs; Acts and treaties related to bisafety of GMOs, Public awareness, perception and acceptance of products of biotechnology.

UNIT III

Patent laws: Global scenario of genetically modified organisms, Intellectual Property Rights (IPR), patent laws at institutional, national and international level.

Suggested Readings

DBT. 1998. *Background Document for Workshop on Biosafety issues Emanating from Use of Genetically Modified Organisms (GMOs)*. Bangalore.

Subbaram NR. 1998. *Handbook of Indian Patent Law and Practice*. Viswanathan Printers & Publ.

Tzotzos GT. 1995. *Genetically Modified Organisms - A Guide to Biosafety*. CABI.

FBT 605 FUNCTIONAL GENOMICS AND PROTEOMICS 1+1

Objective

To give an introduction to application of modern techniques for functional genome analysis.

Theory

UNIT I

Whole genome analysis: Preparation of ordered cosmid libraries, BAC libraries, Shotgun libraries and sequencing, conventional and automated sequencing.

UNIT II

DNA Microarray: Printing of oligonucleotides and PCR products on glass slides, nitrocellulose paper, genome analysis for global patterns of gene expression using fluorescent labeled cDNA or end-labeled RNA probes, analysis of SNP using DNA chips.

UNIT III

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 microarrrays,

UNIT IV

Subtractive hybridization and differential display for identification of genes expressed in specific conditions.

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.

Suggested Readings

Brenner SE & Levitt M. 2000. *Functional Genomics: A Practical Approach*. Oxford University Press.

Peruski LF & Peruski LH. 1997. *The Internet and New Biology:Tools for Genomic and Molecular Research*. ASM Press.

Schena M. (Ed.).1999. *DNA Microarrays: A Practical Approach*. Oxford University Press.

FBT 606 PROTEIN CHEMISTRY AND ENGINEERING 1+1

Objective

To provide an insight into the structure and function of proteins with a focus on state-of-the-art protein engineering to design novel proteins and their application.

Theory

UNIT I

Chemical and physical characteristics of proteins: Properties of amino acids, peptides, and proteins, chemical modification of proteins, Posttranslational modification of proteins, forces that determine protein structures, Secondary tertiary and quaternary structures of proteins, protein folding patterns, protein modules, protein structure based drug design.

UNIT II

Structure Function Relationship of Proteins : DNA binding proteins, prokaryotic and eukaryotic transcription factors, DNA polymerases, membrane proteins and receptors, bacteriohodosin, photosynthetic centres, epidermal growth factor, insulin and ODGF receptors and their interaction with effectors, protein phosphorylation, immunoglobulins, nucleotide

binding proteins, enzyme serine proteases, ribonuclesase, lysozyme.
UNIT III
Protein-Protein and Protein-DNA Interactions: Biochemical, biophysical and computational methods to Study Protein-Protein Interactions and Protein-DNA Interactions.
UNIT IV
Protein and DNA Sequence Analysis: Web-based Literature Search, Sequence Retrieval and Sequence Analysis, Activities and Regulation of Protein Enzymes: Functions and Regulation of Enzymes, Regulation of the Activities of Enzymes and Other Proteins, Phosphorylation and Dephosphorylation.
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UNIT V
Protein Engineering and Protein Design : Protein data base analysis, methods to alter primary structure of proteins, examples of engineered proteins, protein design, principles and examples.
UNIT VI
Proteolysis in Cellular Regulation: Mechanism of Protein Degradation and Proteolysis Pathways.

Practical

Proteomics and sequence analysis tools - Identification and characterization (Aldente, FindMod, Popitam, Phenyx, pI/Mw, ProtParam), DNA -> Protein, similarity searches (BLAST), pattern and profile searches (ScanProsite), post-translational modification and topology prediction, primary structure analysis, secondary and tertiary structure tools (Swiss-PdbViewer), alignment and phylogenetic analysis, DNA mobility shift assay.

Suggested Readings

Creighton TE. 1992. *Protein: Structure and Molecular Properties*. 2nd Ed. WH Freeman.
Liebler DC. 2007. *Introduction to Proteomics: Tools for the New Biology*. Humana Press.
Twyman RM. 2004. *Advanced Text: Principles of Proteomics*. Garland Science/BIOS Scientific Publ.

FBT 607 RNA*i* TECHNOLOGY 1+1

Objective

To comprehend the basic process of RNAi and issues involved in their applications.

Theory

UNIT I

Introduction : Regulation of gene expression in prokaryotes and eukaryotes, types of RNA – rRNA, mRNA, tRNA, miRNA, siRNA, shRNA, tncRNA, gene knock down, gene knock out, 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.

UNIT II

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 III

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.

Practical

Softwares to design siRNA and target validation – ERNAi, optiRNAi, iRNAi; different methods of delivery – vector based, naked siRNA, chemically modified siRNA, gene expression analysis techniques after RNAi delivery – Real time PCR, hybridization techniques.

Suggested Readings

Hannon GJ. 2003. *RNAi, A Guide to Gene Silencing*. CHSL Press.

Schepers U. 2005**.** RNA *Interference in Practice. Principles, Basics, and Methods for Gene Silencing in C. elegans, Drosophils, and Mammals.* WILEY-VCH Verlag, GmbH.

Twyman RM. 2004. *Advanced Text: Principles of Proteomics*. Garland Science, BIOS Scientific Publ.

FBT 608 BIOPROCESS TECHNOLOGY 1+1

Objective

To learn the techniques for 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 auto mation and application of computers in bioprocessing, recombinant products with representative examples, biosafety and environmental monitoring of GEMs, Introduction to patents, Intellectual Property Rights in Biotechnology.

Practical

Downstream processing, separation and purification of compounds, Preparation of vaccines, Purification of protein and enzymes by precipitation, adsorbtion, chromatography and bioaffinity based methods.

Suggested Readings

Ratledge C & Kristiansen B. (Eds.). 2006. *Basic Biotechnology*. Cambridge University Press.

Renneberg R. 2007. *Biotechnology for Beginners*. Academic Press.

Waites MJ, Morgan NL, Rockey JS & Higton G. 2001. *Industrial Microbiology: An Introduction*. Wiley-Blackwell.