BIOTECH FINISHING SCHOOL

SYLLABUS FOR

POSTGRADUATE DIPLOMA IN BIOTECH FINISHING SCHOOL PROGRAM

STREAM
PROTEIN EXPRESSION AND SCALE-UP

Choice Based Credit System and Continuous Assessment and Grading Pattern Syllabus

POSTGRADUATE DIPLOMA IN BIOTECH FINISHING SCHOOL
STREAM – PROTEIN EXPRESSION AND SCALE-UP

Scheme of Study – 2012-13 onwards

I SEMESTER			
Course Code	**Course title**	**Credit pattern (L:T:P)**	**Credits**
BTFS101	**Lab to products: From DNA to Proteins**	2:1:2	5
BTFS102	**Recombinant proteins: Industrial perspective**	2:1:2	5
BTFS103	**Fermentation process and soft skills**	2:1:2	5
BTFS104	**Downstream processing**	2:1:2	5
Total credits			**20**
II SEMESTER			
Course Code	**Course title**	**Credit pattern (L:T:P)**	**Credits**
BTFS201	**Industry Internship – Project work**	0:0:10	20
Total credits			**20**
		TOTAL CREDITS FOR THE PROGRAM	**40**

Paper 1 Lab to products: From DNA to Proteins (40 hrs)

Unit-1

Major top ten Biotech industries in India and their products. State-of the art facilities available in these industries. Guidelines and basic principles of current good manufacturing practices.

DNA
DNA amplification methods, DNA polymerases, DNase and DNA ligase - Industrial orientation/commercialization. Sequencing techniques, Gene therapy

Practicals
Restriction digestion Ligation and PCR, sequencing techniques commercialization of the same, Isolation of DNA, cDNA conversion. Primer designing using software

RNA
Types of RNA its structure and regulation. RNA extraction, mRNA isolation and cDNA conversion. RNA polymerases, Reverse Transcriptase and RNase. RNA interference technology.

Practicals
GTC method of mRNA isolation, Agarose Gel electrophoresis, Designing of SiRNA, BLAST, FASTA, Multiple Sequence alignment.

Unit-2

Proteins and Recombinant protein therapeutics
Chemical synthesis of peptides - Khorana's solution phase and Merrifield's solid phase synthesis-Industrial Application. Importance of peptides in research and industrial use (eg. Antibody production, biophysical studies, cyclic peptides etc.). How determination of peptides/amino acids can help in identification of proteins (Mass Spec). Introduction to proteases.

Structure function and their applications: Insulin, Interferon alpha, Interferon gamma, Interleukin-2, Gm-CSF,G-CSF, Hepatitis B vaccine, Erythropoietin, Strptokinase, EGF, Chymotrypsin, Modification of proteins to increase their life.
Clotting, Haemophilia, Anticoagulants, Thrombolytic agents, tissue plasminogen activator, streptokinase.

Monoclonal antibodies as therapeutics: antibodies, hybridoma technology, FDA approved therapeutic antibodies, humanization. Methods for production of vaccines.

Practicals
Determination of molecular weight of proteins by SDS-PAGE, Gel filtration technique, Isolation of splenocytes from myeloma cells and fusion of splenocytes

Paper 2 Recombinant proteins: Industrial perspective (40 hrs)

Unit-1

Cloning

General introduction to cloning and transformation techniques; Cloning tools- vectors, hosts, codon optimization, enzymes; Site directed mutagenesis methods; Engineering protein expression; Expression of various membrane proteins, cytosolic, carrier etc.

Protein expression in bacteria

Applications of expression vectors, small scale isolation and regulation of protein expression, screening of recombinants, general considerations for purification of fusion proteins, detection / analysis of fusion proteins. Problems and troubleshooting of protein expression. Optimization of expression. Characteristics of small scale and large scale expression.

Protein expression in yeasts

General protein expression and regulation mechanisms in yeast *Saccharomyces cereviseae,* cloning and expression vectors in yeasts- Yip, Yep and Ycp vectors. Recombinant protein expression in yeasts- example and methodology used, advantages and disadvantages of *S. Cereviseae* as host; General protein expression and regulation mechanisms in *Pichia* species, cloning and expression in *Pichia pastoris*- example and methodology used, advantages and disadvantages of *P. pastoris* as host, other yeasts used for protein expression.

Practicals

Bacmid vectors, pET vectors in bacteria, pcDNA mammalian vector – characteristic, yeast vectors, antibody markers using kit method.

Unit-2

Construction of expression vectors, transfection methods, transient and transduction methods. Multiplication of infection cloning strategies- advantages and disadvantages, protein production and purification methods, characterization of target protein and functional studies.

Protein expression in insect cells using baculovirus- advantages and disadvantages, methods, purification modules and protein expression analysis. Interpretation and scale-up

Protein expression in mammalian cells- advantages and disadvantages. Interpretation and scale-up. Compare prokaryotic and eukaryotic expression system, control of expression, promoters, translation difference, codon bias selection, secondary modifications, downstream processing.

Biosimilars

Introduction to biologics, defining biosimilars, differences between biosimilars and generics, technical challenges associated with production of biosimilar molecules, regulatory aspects of biosimilar molecules. Current status of biosimilars in different countries.

Practicals

Media preparation Growing of SF 31 cells Expression of Fusion proteins (GST, HIS, FC – tag) in DH5α cells. Growing of mammalian cells – NIH 3T3, HEK 293. Western blot.

Paper 3 Fermentation process (40 hrs)

Unit -1

Different types of fermentation (Solid liquid, surface etc). Detailed study of the design and construction of fermenters; Different process variables (measures to control the same). Industrial fermentations of importance (With Specific example to two products); scale-up processes-need for scale-up, factors affecting scale-up.

Microbes of industrial importance (Specific examples), strain improvement, Inoculum build up (Stages involved) and its importance.

Practicals

Kinetics of cell growth, Sterilization of air and media, scale-up of microbial process, growth curve of microorganisms antibiotic sensitivity of microbes, use of antibiotic discs

Unit -2

Fermentative process for the production of Proteases (Types of fermentation eg solid state, submerged, liquid surface etc) Fungal proteases: Extracellular products, Bacterial proteases: Intracellular products.

Proteases Enzyme definition with EC no importance of EC no ,enzyme classification ,what are proteases, world production, demand and supply, production in India ,major producer of protease (global as well as national scenario), Microorganisms producing Proteases Eg (Fungal, yeasts bacteria etc).

Culture maintenance, Preservation of cultures, strain improvement techniques)

Practicals

Production of protease in lab fermenter.

Paper 4 Downstream processing (40 hrs)

Unit -1

Chromatographic techniques
Principles and applications of TLC, adsorption, ion exchange, gel filtration, affinity, GLC, chromatofocusing, Liquid chromatography, HPLC and Gas chromatography.

Spectroscopic techniques
Colorimetry, Turbidometry, spectrophotometer, fluorimetry. Flame photometry Principles-Beer-Lambert's law, limitation, extinction coefficient. Mass spec and its applications.

Electrophoretic techniques
Polyacrylamide gel electrophoresis, SDS-PAGE, 2D-Electrophoresis, Isoelectric focusing, Agarose gel electrophoresis, separation of proteins, nucleic acids, visualizing separated components - staining, fluorescence, PAS staining, zymogram and reverse zymogram, pulsed field electrophoresis, high voltage electrophoresis, capillary electrophoresis.

Unit – 2

Ultra centrifugation
Construction of preparative and analytical ultra centrifuge, Schlieren optics for molecular weight determination, Svedberg's constant, sedimentation velocity and Sedimentation equilibrium. Step and gradient centrifugation.

Practicals
Reactions of proteins-colour reactions and precipitation techniques; estimation of proteins-Spectrophotometric methods.

GLP and GMP Safety awareness, documentation requirements.

C**ommunication skills**

Data Analysis

Seminar and paper presentation

Intellectual Property Rights

Technology transfer Biosafety

Note:

The scheme of study is prepared according to CBCS-CAGP.

The coverage of syllabus is through L: T: P pattern (Lecture: Tutorial: Practical).

Syllabus to be covered using lectures, tutorials and group discussions.

Practical exercises may be covered through case studies.

At least six lectures to be covered by industry/institute professionals.

REFERENCE BOOKS:

MOLECULAR BIOLOGY:

1. Molecular Cell Biology, 4th edition
-Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell.
2. Molecular Biology of the Cell
-Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter
3. Molecular Biotechnology
Principles & application of r-RNA
- Bernard R. Glick & Jack. J. Pasternak.
4. Principles of Gene Manipulation
- Sandy Primrose, Richard Twyman & Bob Old.
5. Cells
- Benjamin Lewin, Lynne Cassimeris, Vishwanath R. Lingappa , George Plopper
6. Production of Recombinant Proteins - Novel Microbial and Eukaryotic Expression Systems
http://www.cplbookshop.com/contents/C1627.htm
-Edited by Gellissen, Gerd

FERMENTATION TECHNOLOGY:

7. Principles of Fermentation Technology
-by Peter F. Stanbury, P. F. Stanbury, Allan Whitaker, Stephen J. Hall
8. A Text Book of Industrial Microbiology
-Cruger and Cruger
9. Fermentation Biotechnology-Principles, Process and Products -Ward,O.P