UNIVERSITY OF MUMBAI
No.UG/ 426 of 2004

CIRCULAR:-

Attention of Principals of the affiliated colleges in the Faculty of Science is hereby invited to the Ordinances. Regulations and syllabus relating to the Diploma and Advanced Diploma Course in Molecular Microbiology and they are hereby informed that the recommendation made by the Board of Studies in Microbiology at is meeting held on 17th March. 2004 has been accepted by the Academic Council at its meeting held on 2nd April. 2004 *vide* item No.4.55 and subsequently approved by the Management Council at its meeting held on 17th April. 2004 *vide* item No.20 and that in accordance therewith the Diploma and Advance Diploma Courses in Molecular Microbiology has been introduced by the University with effect from the academic year 2004-2005.

They are further informed that in exercise of the powers conferred on the Management Council under Section 54(1) of the Maharashtra Universities Act.1994 the Management Council has made the Ordinance 5414 relating to the syllabus scheme of papers and standard of passing for the Diploma and Advanced Diploma Courses in Molecular Microbiology is as per *Appendix* and that the same has been brought into force with effect from the academic year 2004-2005 and 2005-2006 respectively.

They are also informed that in exercise of the powers conferred on the Management Council under Section 55(1) of the Maharashtra Universities Act. 1994 the Management Council has approved the Regulations 4739 and 4740 relating to the syllabus. scheme of papers and standard of passing for the Diploma and Advanced Diploma Courses in Molecular Microbiology is as per *Appendix* and that the same has been brought into force with effect from the academic year 2004-2005 and 2005-2006 respectively.

Mumbai 400 032.
12th October,2004.

for I/c REGISTRAR

AC/4.5/2.4.2004
MC/20/17.4.2004

To.
The Principals of the affiliated colleges in the Faculty of Science

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No.UG/ 426-A of 2004. MUMBAI-400 032 12th October,2004

Copy forwarded with compliments for information to :-

1. The Dean. Faculty of Science.
2. The Chairman. Board of Studies in Microbiology

A.C. Item No. 4.55 dt. 2/4/2004

UNIVERSITY OF MUMBAI

Syllabus for
Diploma / Advanced Diploma Courses
in
Molecular Microbiology

(with effect from the academic year 2004-2005 and 2004-2006 respectively.)

Syllabus for Diploma / Advanced Diploma Courses in Molecular Microbiology

R. 4739	No. of Lectures	60 / year	
	No. of Practicals	60 / year	
	Duration	3 years	
O. 5414	Eligibility	All students offering biological sciences at B.Sc.	
R. 4740	Fee Structure	Certificate	Rs. 3,000/-
		Diploma	Rs. 3,500/-
		Advanced Diploma	Rs. 4,000/-

Note :-

1) The Certificate / Diploma and Advanced Diploma Courses is designed as a three-year integrated course and the syllabus of the course expects continuity from first year to second year and to third year.
2) On the successful completion of first year, student will be awarded a certificate, on the successful completion of second year, a Diploma and after successful completion of third year, an Advance Diploma, as per the directive.
3) The emphasis is on core Molecular Biology. It is expected that basics of biology and Chemistry/biochemistry etc.are covered in regular B.Sc. course. This will avoid overlapping of syllabi.
4) Examination pattern will be similar to current B.Sc. course, FY and SY examination may be conducted by Colleges on behalf of university and final examination by University.

Second Year: Diploma course in Molecular Microbiology (60 Lectures)

Theory Syllabus

Molecular Biology of gene and genomes

I. Molecular analysis and manipulation of genes [20]

a) Detection and amplification of nucleic acid sequences and genes
   1. PCR – Principle, procedure, reaction constituents, interference, applications
   2. Error prone PCR and High-fidelity DNA polymerases.
   3. cDNA technology – Advantages, enzymes used for cDNA library construction, model scheme of cDNA synthesis and maintenance.
   4. Blotting Techniques – Types of, definitions, applications to molecular biology.
   5. Advances in blotting / hybridization techniques – Slot / Dot blot, North Western blotting, Far Western analysis,

b) Gene microarrays
   1. Concept of transcriptome and use of macro / microarrays for its detection and monitoring.
   2. concept and construction of array
   3. techniques of analysis of transcriptome,
   4. advantages and limitation of analysis

II. Sequence analysis of genes and genomes [10]

a) Methods of DNA sequencing – Sangers, Maxim & Gilbert, Automated sequencing Detailed review of all methods with reference to Principle, applications, length of DNA sequenced, advantages and limitations

III. Maintenance of DNA sequences / gene copies – genomic libraries [20]

a) Construction methods - Shotgun, Partial Digestion, advantages and disadvantages of both methods.
b) Introduction to high capacity vectors – YAC, BAC, PAC and human artificial chromosome, their uses in library construction
c) Expression libraries and vectors used for DNA expression libraries, Phage display strategy
d) Methods to identify desired clones – colony hybridization and colony PCR
e) Advantages and disadvantages of genomic DNA library construction
f) Transformation and related methods
g) Recombinant selection and screening
h) Methods of studying gene function - Gene transfer to plants and animal

IV. Engineering genes and genomes – recombination, site directed mutagenesis [10]

(Brief overview of physiology of the processes, enzymes involved, differences between site-specific and homologous recombination and their applications to gene engineering)

Second Year: Diploma course in Molecular Microbiology (60 Lectures)

Practical syllabus

1. Enrichment and culturing of lambda phage
2. Isolation of DNA from Lambda Phage – restriction to obtain DNA ladder
3. Specific ligation into Multiple Cloning Site (MCS) of plasmid
4. Isolation of genomic DNA from *E.coli* or *S.marcescens* for Construction of genomic DNA library from *E.coli* or *S.marcescens* and screening for clones with desired genes.
5. Mutation mapping using genomic DNA library
6. Identification of recombinants using reporter genes
   a. Blue – white screening
   b. Green Fluorescence Protein (GFP)
7. PCR demonstration - DNA amplification using thermal cycler or water baths
8. Southern Blotting (Demonstration)
9. Demonstration of macroarray technique
10. Analysis of macroarray data available on internet using various software / techniques.

Third Year: Advanced Diploma course in Molecular Microbiology (60 Lectures)

Theory Syllabus
Molecular biology of Proteins and proteomes

I. Regulation of genetic expression – [05]
   a) Differences in plants, animals and bacteria, common themes of regulation, Relevance of transcription factors, methods of post transcriptional silencing in eukaryotes, limitation to array analysis.

II. Analysis of regulation of genetic expression [12]
   a) Experimental approaches – uses of knockouts and hyper expression models, foot printing analysis
   b) Bioinformatics approaches – identification of transcription factor binding sites, establishment of networks in cellular regulation, tools for whole genome analysis – BLAST, FASTA

III. Protein purification [12]
   a) Identification of appropriate source, Obtaining relevant sub cellular fractions, concentration of desired protein (Gel filtration)
   b) Definition of enzyme units, specific activity and their significance
   c) Precipitation methods – Ammonium sulfate and Organic Solvent, Principle and differences in bcth methods, comparison between toxicity of both methods and utilization
   d) Dialysis
   e) Methods to assay for protein activity, fold purification calculation
   f) Chromatographic techniques to obtain pure protein samples – ion exchange, affinity chromatography.

IV. Protein sequence and structural analysis [12]
   a) Basics of protein structure analysis – primary, secondary, tertiary and quaternary structure, structural elements – family, superfamily and folds, their use in analysis of protein structure.
   b) Methods for determination of protein sequence (primary structure) – N and C terminal sequencing, Mass spectrometric analysis, chemical reactions of the process.
   c) Secondary protein structure determination – NMR, CD
   d) Methods to analyze tertiary structure of proteins – Crystallographic techniques, X ray diffraction
   e) Bioinformatics approaches – methods to determine protein structure based on homology analysis, advantages and disadvantages of computational approach.

V. Protein engineering [10]
   a) Establishment of structure function relationship
   b) Directed mutagenesis for generating desired changes in protein structure and function
   c) Correlation of active site residues to mechanism of protein function
   d) Theoretical treatment of designing a protein of desired function (e.g.: silk fiber)
   e) Successful examples of protein engineering

VI. Proteome analysis and interpretation [9]

a) Definition of proteome, dynamic nature of proteome and its relation to cellular functions and response. Its relation to genome, transcriptome and metaboleome.
b) Methods for comprehensive proteome analysis – 2D gels
c) Identification of proteins from gels – MALDI -TOF
d) Software and other computational approaches to study variation in expression profiles
e) Functional annotation of proteins and their correlation to observed physiological state

Third Year: Advanced Diploma course in Molecular Microbiology (60 Lectures)

Practical Syllabus

1. Development of a protein expression system
2. Induction of expression and electrophoretic techniques to monitor expression profiles
3. Protein purification of the expressed protein –
   a. Cell lysis to obtain protein (Sonication / Detergent treatment)
   b. Precipitation of the protein (Ammonium Sulfate)
   c. Gel filtration to separate cellular protein fractions and desalting
   d. Dialysis
   e. Chromatographic techniques as may be pertinent to the process
   f. Electrophoresis of fractions after different steps to monitor purity
   g. Methods of protein quantification relevant to molecular biology – Bradford's assay, Folin – Lowry Method
   h. Assay of the protein (Enzyme) and calculation of enzyme units, specific activity and fold purification
4. Affinity purification – His tag or equivalent system, active dyes affinity system
5. Use of various software / computational techniques in protein structure analysis
6. Demonstration of use of CD./ Dye affinity / Spectrophotometric techniques in structural analysis of proteins
7. Demonstration of 2D gel and MS interfacing for protein fingerprinting / analysis.
8. Survey of protein annotation efforts undertaken worldwide (Group project)
9. Western Blotting (Demonstration) – Antibodies may be raised by the students against any proteins and the primary antibody may be labeled and used for blotting.

List of text books and reference books

1. Freifielder David,; Microbial Genetics; 22nd Edition, (1990), Narosa publishing house,. New Delhi.
2. Lodish, Berk, Zipsursky, Matsudira, Baltimore, Darnell; Molecular Cell Biology, 4th Edition, (2000), W.H. Freeman and Co., New York.
3. Weaver R.F., Molecular Biology, 2nd Edition, (2002), McGraw Hill, Boston
4. Gardner, E.J., Simmons, M. J. and Snustdad, D. P.; Principles of Genetics,8th Edition, (2001). John Wiley and Sons, Singapore.
5. Maniatis, T., Fritsch, E.F., Sambrook, J:.Molecular Cloning-A Laboratory Manual, (1982), Cold Spring Harbor Laboratory.
6. Lewin, Benjamin; Genes VII; (2000), Oxford University Press, Oxford.
7. Russell, Peter J.; Genetics, 5th Edition, (1998), Addison Wesley Longman, Inc. California.
8. Old R. W and Primrose S.B.; Principles of Gene Manipulation, 5th Edition, (1994), Blackwell Science Ltd, London.
9. Old R. W and Primrose S.B.; Principles of Gene Manipulation, 6th Edition, (2002), Blackwell Science Ltd, London.
10. Baxevanis A. D. and Francis Ouellette B.F.; Bioinformatics, 2nd Edition, (2001), John Wiley and Sons, Singapore.
11. Attwood T. K. and Parry-Smith D. J.; Introduction to Bioinformatics, (2001), Pearson Education Ltd. New Delhi, India.
12. Krane D.E. and Raymer M. L.; Fundamental concepts of Bioinformatics, (2003), Pearson Education Ltd. New Delhi, India.