

# Savitribai Phule Pune University

(Formerly University of Pune)

# Three Year B. Sc. Degree Program in INDUSTRIAL MICROBIOLOGY

**Syllabus** 

# (To be implemented from Academic Year 2021-22)

T.Y.B.Sc. (Industrial Microbiology)

Choice Based Credit System Syllabus To be implemented from Academic Year 2021-2022

# Savitribai Phule Pune University, Pune-411007

#### T.Y B.Sc. Industrial Microbiology (Vocational)

#### CBCS pattern (2019)

#### To be implemented from- 2021-2022

#### Introduction

The 3-year B.Sc. Vocational Course in Industrial Microbiology is conducted as a part of the Savitribai Phule Pune University approved course in B.Sc. Microbiology. Revised choice based credit system has been implemented for F.Y.B.Sc. (2019) and S.Y.B.Sc. (2020). Theory and practical courses of 8 credits from the subject of Industrial Microbiology are offered as one of the subjects among the four subjects at the F.Y.B.Sc level and among the three subjects at S.Y.B.Sc. level.

First two years of Industrial Microbiology learning makes a student equipped with basic knowledge and understanding of Microbiology as well as concepts in Industrial Microbiology. The final year courses have been designed in such a way that student will be able to learn the state- of the art techniques required by Industry/Academia/Research domain after he/she completes graduation. The Students after completion of their graduation in Industrial Microbiology will be aware of core subjects such as Bioreactor design and maintenance, parameter optimization and quality control and assurance which are essentials for a student aspiring for a job/higher education in pharmaceutical sector. They will also have an understanding of subjects matter associated with entrepreneurship and intellectual property rights making them desirable candidates for recruitment.

**Eligibility :**The student should have offered Industrial Microbiology (Vocational) at F.Y.B.Sc and S.Y.B.Sc and passed as per SPPU rules.

**Course Structure:** The student of Industrial microbiology should take four theory papers (Paper I to Paper IV), two practicals (Practical Paper I to II) and One skill enhancement elective from General microbiology syllabus **per semester**.

The syllabus of industrial microbiology specific courses is given below, which include two theory courses (Paper V and Paper VI), one practical course (Practical paper III) and one Skill enhancement course **per semester**.

Old Paper Codes	Paper No.	Paper titles 2013 pattern	New Paper Codes	Paper No.	Paper titles 2019 pattern
VOC-IND- MIC – 335	Ι	Pollution Control Technology	IMB 355	Ι	Applications of Microbial Systems
VOC-IND- MIC – 336	II	Animal and Plant Tissue Culture	IMB 356	II	Cell Culture Technology
Voc-IND-MB 337	III	Practical Based on Theory Paper	IMB 359	III	Practical course-III Practicals based on IMB 355 and IMB 356
VOC-IND- MIC – 346	II	Entrepreneurship Development	IMB 365	Ι	Bio-entrepreneurship and IPR
VOC-IND- MIC – 345	I	Mol. Bio. and Recombinant DNA Technology	IMB 366	II	Recombinant DNA technology
Voc-IND-MB 337	III	Practical Based on Theory Paper	IMB 369	III	<b>Practical course-III</b> Practicals based on IMB 365 and IMB 366

Semester	Theory/ Practical/ Elective	Paper codes	Paper Titles
		MB 351	Medical Microbiology I
	Discipline	MB 352	Immunology I
	Specific	MB 353	Enzymology
	Elective Course	MB 354	Genetics
	Theory	IMB 355	Applications of Microbial Systems
Semester V		IMB 356	Cell Culture Technology
	Discipline	MB 357	Practical course-I
	Specific Elective Course	MB 358	Practical course-II
	Practical	IMB 359	Practical course-III
	Skill	IMB 3510	Plant tissue culture
	Enhancement courses	MB 3511	Dairy Microbiology

# New Syllabus Paper Distribution: Semester V

# New Syllabus Paper Distribution :Semester VI

Semester	Theory/ Practical/ Elective	Paper codes	Paper Titles
	D: !!	MB 361	Medical Microbiology II
	Discipline Specific	MB 362	Immunology II
	Elective	MB 363	Metabolism
	Course Theory	MB 364	Molecular Biology
		IMB 365	Bioentreprenurship and IPR
Semester VI		IMB 366	Recombinant DNA technology
	Discipline	MB 367	Practical course I
	Specific Elective	MB 368	Practical course II
	Course Practical	IMB 369	Practical course III
	Skill	IMB 3610	Introduction to Bioinformatics
	Enhancement course	MB 3611	Nanobiotechnology

Sem	Paper Code	Paper	Paper title	credits	Lectures/ Week				Evaluation	
					Th . T	ut Pr.	CA	UE 7	Tot.	
	IMB 355 TC	Ι	Applications of Microbial Systems	2	3		15	35	50	
	IMB 356 TC	II	Cell culture Technology	2	3		15	35	50	
V	IMB-359 PC	III	Practical course III	2		5	15	35	50	
	IMB-3510 SEC	SEC	Plant tissue culture	2	2	2.5	15	35	50	
	IMB 365 TC	Ι	Bio-entrepreneurship and IPR	2	3		15	35	50	
VI	IMB 366 TC	II	Recombinant DNA technology	2	3		15	35	50	
	IMB 369 PC	III	Practical Course III	2		5	15	35	50	
	IMB-3610 SEC	SEC	Introduction to Bioinformatics	2	2	2.5	15	35	50	

New Syllabus structure on lectures and evaluation

#### T.Y.B.Sc. Industrial Microbiology Semester V IMB-356: Applications of Microbial Systems

[2 Credits; 36 Lectures]

[1 credit=15hrs x 60 mins = 900mins/50mins=18 lectures]

Course Learning Outcomes: The Student will,

- understand various methods of wastewater treatment
- able to describe the role of microbes in wastewater treatment
- understand ETP design and wastewater treatment
- able to describe the role of microbes in dairy and agriculture industry
- understand significance of dairy fermented product in human health.

Credits	Topics	Number of lectures
	Pollution control technology	or lectures
I	<ol> <li>Waste water characterisation and treatment         <ul> <li>a) Wastewater characteristics and wastewater analysis</li> <li>b) Physical treatment methods</li> <li>c) Chemical treatment methods</li> <li>d) Biological treatment methods</li> <li>e) Sludge characterisation and treatment</li> </ul> </li> </ol>	1 2 2 2 2
	<ul> <li>2) Advanced treatment methods and ETP design <ul> <li>a) Tertiary waste water treatment methods</li> <li>b) <i>In situ</i> Bio-remediation</li> <li>c) Removal of ROC</li> <li>d) ETP design and EIA</li> </ul> </li> <li>Student activity: Construct a flowchart for Effluent treatment plant</li> </ul>	2 2 2 3
	Dairy and Agricultural Microbiology 3) Dairy Microbiology a) Basics of Dairy Microbiology- microbiology of starter cultures and dairy products	1
II	b) Nutritional and therapeutic importance of fermented dairy products	1
Π	<ul> <li>c) Probiotics, prebiotics and functional dairy foods</li> <li>d) Detail study including definition, process and microbial culture/consortium of Functional Dairy Products: Fermented milk products, functional dairy products, and therapeutic applications.</li> <li>e) Health benefits of functional fermented dairy products: such as dahi, lassi, yoghurt, kefir, cheese, fermented whey drinks.</li> </ul>	1 4 2

4) Agricultural Microbiology	
a) Microbes in soil and Nutrient recycling by microbes, Agro-	3
wastes	2
b) Sustainable agriculture using microorganisms	3
c) Biofertilizers, Biopesticides and Biofuel	3
(Definition, Examples, Production process in short)	•
Student activity- Product based layout of Industry, Guest lectures by	
Industry experts	

References:

- 1. George T and Metcalf & Eddy (2003) Wastewater Engineering Treatment Disposal Reuse McGraw Hill Company
- Marth, Elmer H and James L Steele eds. Applied Dairy Microbiology, 2<sup>nd</sup> Edition(2018), T&F publisher India, ISBN-10: 9781138367609
- 3. Prescott, S.C. and Dunn, C.G., 1983, Industrial Microbiology, Reed G. (Ed.). AVI Tech books.
- Nduka Okafor(2007), Modern Industrial Microbiology and Biotechnology Science Publisher, Edenbridge Ltd, ISBN 978-1-57808-434-0

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# T.Y.B.Sc. Industrial Microbiology Semester V IMB-356: Cell culture technology

[2 Credits; 36 Lectures]

[1 credit=15hrs x 60 mins = 900mins/50mins=18 lectures]

- Development of basic knowledge on mammalian cell culture.
- Understanding media constituents and media formulation strategies for mamalian cell culture.
- Knowledge on cell lines and their application.
- Application of cell culture to make products required for human health
- Apply cell and molecular techniques to in vitro situations.

Credit	Topic		No. of Lectures
	1)	<b>Basics of Cell Culture</b> Introduction, Definition of terms – Tissue culture, Organ culture, Primary, Secondary, Continuous and Established cell lines	3
	2)	Organ culture Scope and techniques e.g. plasma clot, agar gel. Grid method etc. Advantages ,Limitations , Applications	2

	3) Cell culture	
Ι	a) Monolayers-anchorage dependency, types of substrate	3
	b) Suspension culture- types	
	c) Nutrient requirements, growth media, sterilization, growth conditions.	2
	d) Culture techniques- explants disaggregation, subculture, primary and continuous cell lines, maintenance of cell lines e) Large scale culture of animal cells – types of reactors, immobilization, hollow fibre reactor	
	4) Applications of ATC	
	<ul><li>a) Somatic cell fusion and hybridoma technology</li><li>b) cell culture products -viral vaccines, recombination proteins,</li></ul>	2
	growth factors, cytokines, interferons, monoclonal and hybrid antibodies	2
	Student activity: To visit and study the ATCC website	
	<ul> <li>5) Advances in animal tissue culture <ul> <li>a) In vitro fertilization and embryo transfer – test tube babies</li> <li>b) Gene transfer to animal cells and Transgenic animals and their economic importance</li> </ul> </li> </ul>	3 3
II	<ul> <li>c) Stem cell culture and application- Introduction to stem cells - Definition, properties, proliferation, culture of stem cells</li> <li>d) Medical applications of stem cells</li> </ul>	3
	Ethical and legal issues in use of stem cells.	4
	6) Types of stem cells:	
	a) Stem Cell biology and therapy, types embryonic stem cell, Adult stem cell, Stem Cell Biology and Therapy, Embryonic Stem	3
	Cells, culture and the potential benefits of stem cell technology b) Therapeutic applications of stem cells	2
	L	1

References:

1. Adams, R.L.P., 1980, Cell culture for Biochemists, Laboratory techniques in Biochemistry and Molecular Biology, Elsevier, Amsterdam

2. Freshney, R. I. (ed), 1992, Animal cell culture : a Practical approach (2nd ed.) Oxford University Press, New York

3. Freshney, R.I. (2005) Culture of Animal Cells: A Manual of Basic Technique. Wiley-Liss: Hoboken, NJ, USA. ISBN 9780471453291

#### T.Y.B.Sc. Industrial Microbiology Semester V IMB -359 -Practical Course III

# [2 Credits; 78 Lectures]

# [1 credit=15hrs x 130 mins = 1950mins/50mins=39 lectures]

78 L distributed as 60 L for performing practicals and 18 L for internal evaluation

#### **Course Learning Outcome**

- Skills to perform wastewater analysis with respect to BOD and COD
- Understand design of Effluent treatment plant
- Microbiological Analysis of fermented product and Bio-fertilizer.
- Isolating and Culturing lymphocyte
- Observation and estimation of lymphocytes

Sr. No.	Practicals based on Applications of Microbial Systems	Number of practicals
1	Estimation of solid contents in wastewater	1
2	Biological oxygen demand	1
3	Chemical oxygen demand	1
4	Designing of Effluent treatment plant	1
5	Estimation of total viable cells in fermented product	1
6	Estimation of total viable cells in Bio-fertilizer	1
	Practicals based on Cell culture Technology	
7	Lymphocyte culture – medium preparation and sterility check	1
8	Isolation of Lymphocytes using HiSep	1
9	Observing growth of lymphocytes Observe for contamination, Counting lymphocytes	2
10	Sub-culturing of Lymphocytes	1
11	Demonstration – Fibroblast culture	1

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### T.Y.B.Sc. Industrial Microbiology Semester V Skill Enhancement Course

# IMB -3510 Plant Tissue Culture

2 credit course: 1.5 credit Theory+0.5 credit Practical

- Know the technique of preparation of plant tissue culture media.
- *Knowledge about various techniques for plant tissue culture.*
- Knowledge about laboratory setup for plant tissue culture.

- Hands on exposure to plant tissue culture.
- Employment skill in commercial plant tissue culture laboratory.

Credit	Theory Topics	No. of
		Lectures
	1.Plant Tissue Culture basics	
	a) Introduction to Plant tissue culture	2
	b) Potency of plant calls/differentiation and de-differentiation	1
	c) Growth factor requirements, Nutrient media, Propagation and preservation of plant tissues, Role of growth hormones	5
1.5	2.Types of cell cultures	
1.5	a) Callus culture	1
	b) Anther culture – Haploid plants	1
	c) Ovary culture	1
	d) Meristem culture	1
	e) Embryo culture	1
	3. Stages of Micropropagation	
	Selection of plant, Ex-plant preparation, Surface sterilization, Inoculation and incubation, Sub-culturing and Hardening	3
	4. Applications of PTC	5
	Protoplast fusion, Transgenic plants, Synthetic seeds, Virus free plants and Plantibodies	

# **Skill Enhancement Course**

# IMB -3510 Plant Tissue Culture

Sr No	Practicals Title	No of Practicals
1	<ul> <li>a) Preparation of Plant Tissue Culture Media, Plant selection, Explant preparation and preparation of explant for culturing</li> <li>b) Callus culture – inoculation of explant. Observation for growth of callus.</li> </ul>	2
2	Studying different characteristics of callus	1
	or Differentiation of Callus – Shooting/Rooting	

#### **References:**

- 1) Razdan, M. K. 2002. Introduction to plant tissue culture. 2nd ed. Science, India, USA
- 2) Trigiano, R. N. and D. J. Gray. 2000. Plant tissue culture concepts and laboratory exercises. 2nd ed. CRC press, USA

- 3) Bhojwani, S. S. and M. K. Razdan. 1996. Plant tissue culture: theory and practice, a revised edition. Elsevier Science, Netherlands
- 4) Chawla, H. S. 2002. Introduction to plant biotechnology, 2nd ed. Science, India, USA.
- 5) Thorpe, T. A. 1981. Plant tissue culture : methods and applications in agriculture. Academic Press, New York, USA.
- 6) Dodds, J. H. and L. W. Roberts. 1985. Experiments in plant tissue culture. 2nd ed. Cambridge University, New York, USA.

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#### T.Y.B.Sc. Industrial Microbiology Semester VI IMB-365 Bio-entrepreneurship and Intellectual Property Rights [2 Credits; 36 Lectures]

[1 credit=15hrs x 60 mins = 900mins/50mins=18 lectures]

- Provides students with the necessary knowledge on how to bridge science and business.
- Development of the interdisciplinary skills required to produce business plan.
- Knowledge on Business forms, model and funding
- Analyze different types of intellectual property rights
- Protection of products derived from industrial microbiology research

Credits	Topics	Number of lectures
	<ol> <li>Bio-entrepreneurship</li> <li>Concept of entrepreneurship, Historical background, need and scope of entrepreneurship in modern society, Entrepreneurial behaviour, attributes and skills.</li> </ol>	1
I	<ul> <li>2) Elements of entrepreneurship         <ul> <li>a) Key elements of entrepreneur, Entrepreneurial process, Entrepreneurial culture, Environment of Entrepreneurship, Socio economic origins of Entrepreneurship</li> </ul> </li> </ul>	1
	b) Barriers of Entrepreneurship and means to reduce those, types of Entrepreneurs, Characteristics of Entrepreneur.	1
	3)Introduction and Overview of the Biotechnology Industry Translational biotechnology industry overview (commercialization pathways for drug, medical device, diagnostic companies)	3

	<ul><li>4) Forms of Businesses</li><li>a) Sole proprietorship, partnership, Joint Stock Company, cooperative organization etc. Meaning and definition ,</li></ul>	3
	Relative merits and demerits of each form b) Types of Small Scale Industry, LLP etc.	1
	5) Starting Business Startup- Biotechnology business models, corporate structure (LLC, LLP, C-Corp, S-Corp, etc.) Meaning, scope and importance of marketing, Marketing strategy, Market segmentation, marketing channels. Marketing mix and its effect. Digital marketing	2
	<ul> <li>6) Business Funding         <ul> <li>a)Funding process - organizations promoting</li> <li>Entrepreneurship, Banks- Government and Commercial, Co-operative societies, Govt/Public sources of finance Sources of finance</li> <li>b)Role of Funding agencies, government and commercial</li> </ul> </li> </ul>	4
	Role of various funding corporations and funding institutes Student activity- Pitch an idea in front of panel of venture capitalis	
	7) Intellectual Property Rights	
	a) Basic concept of intellectual properties (Patents, Trademarks and Copyrights)	4
п	<ul> <li>b) Theories of Intellectual Property Rights, Need for protecting Intellectual Property- Policy Consideration- National Perspectives and International demands</li> </ul>	6
	<ul> <li>8) Types of Intellectual Property</li> <li>a) Origin and Development, Role of International Institutions</li> <li>b) World Intellectual Property Organisation (WIPO)</li> <li>c) Function of WIPO</li> <li>d) Membership of WIPO</li> <li>e) Agreement between the WIPO and the WTO</li> </ul>	4
	9) Dispute Settlement New Treaties, Commercialisation of Intellectual Property Rights by Licensing, Determining Financial Value of Intellectual Property Rights.	4
	Student activity- Read and discuss a patent application	
Reference	I S-	

References-

- Vasant Desai Dynamics of Entrepreneurial development & management (2011), Himalaya Publishing House; Sixth edition ISBN-13 :978-9350244548
   Commission on Life Sciences, Putting Biotechnology to Work: Bioprocess Engineering (1992), The
- National Academy Press. DOI:10.17226/2052

- 3. David H. Holt Entrepreneurship New Venture Creation (1992): Prentice Hall India Learning Private Limited ISBN-13 : 978-8120312814
- Venkataraman M. An Introduction to Intellectual Property Rights 1<sup>st</sup> Edition, (2014), ISBN-13 :978-8191045727

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# T.Y.B.Sc. Industrial Microbiology Semester VI IMB-366 Recombinant DNA technology

[2 Credits; 36 Lectures]

[1 credit=15hrs x 60 mins = 900mins/50mins=18 lectures]

- Understanding the basic steps of gene cloning and the role of enzymes and vectors responsible for gene manipulation, transformation and genetic engineering.
- At the end of the course, the students will have sufficient scientific understanding of the techniques in *Recombinant DNA technology*.
- Summarize various applications of rDNA technology in human health care
- Describes the impact of Recombinant DNA technology on Medicine
- Understand, advances in Recombinant DNA technology

Credit	Торіс	No of
		Lectures
Ι	1) Techniques in Recombinant DNA Technology:	
	a) Basic concepts in molecular cloning	2
	b) Types of vectors- Plasmids ,Cosmids, Bacteriophages,	3
	Artificial chromosomes- PAC, BAC, YAC, Megaplasmids	2
	c) Screening and selection of transformants, Blue and white screening, Replica plate method	3
	d) PCR– primers, cloning, PCR products, RT-PCR and other modifications, Types of heat resistant enzymes used,	3
	e)Types of DNA sequencing, DNA fingerprinting- process and application, Microarray-technique and its applications, Site-	5
	directed mutagenesis, protein engineering	

	10) Advances in Recombinant DNA Technology:	
	a)Advances in Genomics and Proteomics	2
II	b) Introduction to metagenomics (Omics) Metagenomics in Microbiology-, overview of metagenomic library construction advantages and applications	5
	<ul> <li>c)Human Genome Project</li> <li>d)Impact of RDT on Medicine- New diagnostics, Detection of pathogens, genetic disorders, fetal DNA analysis</li> <li>e)Novel routes of vaccines</li> <li>f) Industrial applications – generation of novel proteins, production of cells, proteins, small molecules</li> </ul>	2 4 1 4
	Student activity: Designing an entire experiment for cloning the gene of interest in desired host	

References:

- 1. Old, S. B. Primrose (1994) Principles of gene Manipulations., Blackwell Scientific Publications.
- 2. Brown T. A. Gene Cloning and DNA Analysis: An Introduction, 7th Edition (2013), Wiley-Blackwell.
- 3. Sambrook and Green , Molecular Cloning: A Laboratory Manual (*Fourth Edition*), (2012), Cold Spring Harbour Laboratory, ISBN :0879693096
- 4. Russel P.J., iGenetics: A molecular Approach 3rd edition (2010) Pearson
- 5. Primrose, S. B. and Twyman, R. M. (2006). Principles of Gene Manipulation and Genomics, 7th Ed. Blackwell Publishing: U.S.A.
- 6. Lewin's GENES X (2011). Jocelyn Krebs, Stephen T. Kilpatrick, Elliott S. Goldstein Editors. Jones & Bartlett Learning, USA.

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#### T.Y.B.Sc. Industrial Microbiology Semester VI Practical Course III: IMB -369

[2 Credits; 78 Lectures]

[1 credit=15hrs x 130 mins = 1950mins/50mins=39 lectures]

78 L distributed as 60 L for performing practicals and 18 L for internal evaluation Course Learning Outcomes: Upon successfully completing this course the students could be able to

- Develop Bio-based business idea and business plan.
  - Critically review/analyze patent
  - To get expertise in isolation and separation of DNA.
  - To perform gene amplification experiments and primer designing.

Sr. No.	Practicals based on Applications of Bioentreprenurship and IPR	Number of practicals
1	Presentation of two entrepreneurial ideas	2
2	Design business plan	1
3	Write claims for a patent to be filed for a novel product/process	1
4	Critical review of a patent application	1
5	Visit a start-up/ venture centre	1
	Practicals based on Recombinant DNA Technology	
6	Isolation of Genomic DNA from yeast	1
7	Performing Agarose/ Poly-acrylamide Gel electrophoresis for DNA sample	1
8	Demonstration – Polymerase Chain Reaction	1
9	Using a primer designing software	1
10	Using UV as a mutagenic agent to transform bacteria	1
11	Demonstration of DNA fingerprinting	1

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#### T.Y.B.Sc. Industrial Microbiology Semester VI Skill Enhancement Course

# **IMB -3610 Introduction to Bioinformatics**

2 credit course: 1.5 credit theory+0.5 credit Practical

Course Learning Outcomes: Student will get,

- To get introduced to the basic concepts of Bioinformatics and its significance in Biological data analysis
- Classify different types of Biological Databases
- Introduction to the basics of sequence alignment and analysis.
- To gain knowledge on various techniques, algorithms and tools employed in DNA sequencing, assembly and its applications
- To get exposed to various tools and methodologies used in bioinformatics.

Credit	Торіс	No. of
		Lectures
	1) Basics of bioinformatics	
	a) Introduction and biological databases Nucleic acid, proteins, genomes	2
	b) Structure data bases, search engine	2
	c) Sequence data forms and submission tools	1
1.5	<ul> <li>d) Scoring matrices for sequence alignments, algorithms pairwise sequence alignments</li> </ul>	3
	e) Database similarity searches-BLAST, FASTA, Gene bank sequence database	2
	<ul> <li>f) Submitting DNA sequences to databases and database searching</li> </ul>	2
	<ul> <li>g) Sequence alignment; pairwise alignment techniques, Multiple sequence alignment</li> </ul>	4
	h) Phylogenetic analysis and tree building methods	5
	Student activity: Building a phylogenetic tree based on the practical course	

# **Skill Enhancement Course**

# IMB -3613 Introduction to Bioinformatics

Sr No	Practical title	No. of Practicals
1	Study of resources available online – NCBI, PubMed, GenBank	1
2	Searching similarity within nucleotide sequences using BLAST tool	1
3	Alignment of Nucleotide sequences using online software's OR Building a phylogenetic tree using MEGA	1

References

1. Claverie, J.M. and Notredame C. 2003 Bioinformatics for Dummies. Wiley Editor.

2. Letovsky, S.I. 1999 Bioinformatics. Kluwer Academic Publishers.

3. Baldi, P. and Brunak, S. 2001 Bioinformatics: The machine learning approach, The MIT Press.

4. Setubal, J. and Meidanis, J. 1996 Introduction to Computational Molecular Biology. PWS Publishing Co., Boston.

5. Lesk, A.M. 2005, 2nd edition, Introduction to Bioinformatics. Oxford University Press.

6. Fogel, G.B. and Corne, D.W., Evolutionary Computation in Bioinformatics.

7. Mount, D.W., Bioinformatics: 2001, Sequence and Genome Analysis. CSHL Press.

8. Durbin R., Eddy S., Krogh A. and Mithchison G. 2007 Biological Sequence Analysis, Cambridge University Press.