

# Contents

Preface.....	xxix
Author .....	xxxix

<b>Chapter 1</b> Introduction to Biotechnology.....	1
Learning Objectives.....	1
1.1 What Is Biotechnology? .....	1
1.1.1 Definitions of Biotechnology .....	2
1.2 Animal Biotechnology .....	3
1.3 Agricultural Biotechnology.....	4
1.4 Medical Biotechnology.....	4
1.5 Industrial Biotechnology .....	5
1.6 Environmental Biotechnology.....	6
1.7 Other Emerging Fields of Biotechnology.....	6
1.7.1 Nanobiotechnology.....	6
1.7.2 Bioinformatics .....	7
1.7.3 Pharmacogenomics .....	8
1.7.4 Regenerative Medicine.....	8
1.7.5 Therapeutic Proteins .....	8
1.8 History of Biotechnology .....	9
1.8.1 Ancient Biotechnology .....	9
1.8.2 Modern Biotechnology.....	10
1.9 Human Genome Project .....	10
1.9.1 Need for Human Genome Project.....	12
1.10 Major Scientific Discoveries in Biotechnology .....	12
1.11 Biotechnology as the Science of Integration .....	13
1.12 Bio-Revolution.....	14
1.13 Ethical and Regulatory Issues in Biotechnology.....	15
1.14 Future of Biotechnology .....	15
Problems.....	17
Section A: Descriptive Type .....	17
Section B: Multiple Choice .....	17
Section C: Critical Thinking.....	18
Assignment.....	18
Online Resources.....	18
References and Further Reading .....	18

<b>Chapter 2</b> Genes and Genomics.....	19
Learning Objectives.....	19
2.1 Introduction .....	19
2.2 Cell as the Building Block of Life.....	19

2.3	Classification of Cells.....	20
2.3.1	Prokaryotic Cell.....	20
2.3.2	Eukaryotic Cell.....	20
2.4	Extracellular Organization.....	21
2.4.1	Cell Membrane.....	21
2.4.2	Cell Capsule.....	22
2.4.3	Flagella.....	22
2.5	Intracellular Organization.....	22
2.5.1	Cytoplasmic Constituents.....	22
2.5.1.1	Mitochondria and Chloroplasts.....	23
2.5.1.2	Ribosomes.....	23
2.5.1.3	Endoplasmic Reticulum.....	23
2.5.1.4	Golgi Apparatus.....	23
2.5.1.5	Lysosomes and Peroxisomes.....	23
2.5.1.6	Centrosome.....	23
2.5.1.7	Vacuoles.....	23
2.5.1.8	Cytoskeleton.....	24
2.5.2	Nuclear Constituents.....	24
2.5.2.1	Deoxyribonucleic Acid.....	24
2.5.2.2	Ribonucleic Acid.....	25
2.5.2.3	Messenger Ribonucleic Acid.....	26
2.5.2.4	Transfer RNA.....	26
2.5.2.5	Ribosomal RNA.....	26
2.5.2.6	Small Nuclear RNA.....	27
2.5.2.7	Nucleoli.....	28
2.5.2.8	Chromatin.....	28
2.6	Macromolecules.....	28
2.6.1	Proteins.....	28
2.6.2	Carbohydrates.....	29
2.6.2.1	Simple Sugars.....	29
2.6.2.2	Complex Carbohydrates.....	29
2.6.3	Lipids.....	30
2.6.3.1	Nucleic Acid.....	31
2.7	Genes and Genetics.....	31
2.7.1	Mendelian Genetics.....	31
2.7.2	Modern Genetics.....	33
2.8	Cell Division.....	33
2.8.1	Meiosis.....	34
2.8.2	Mitosis.....	34
2.9	DNA Replication.....	36
2.9.1	Role of DNA Polymerase in Replication.....	36
2.9.2	DNA Replication within the Cell.....	37
2.9.2.1	Replication Fork.....	38
2.9.2.2	Leading Strand.....	38
2.9.2.3	Lagging Strand.....	38
2.9.3	Regulation of DNA Replication.....	38
2.9.4	Termination of Replication.....	39
2.10	DNA Interactions with Proteins.....	39
2.11	DNA-Modifying Enzymes.....	40
2.11.1	Topoisomerases and Helicases.....	40
2.12	DNA Methylation.....	40

## Contents

2.13	DNA Mutation.....	41
2.14	Tools of Biotechnology.....	42
2.14.1	Polymerase Chain Reaction .....	42
2.14.1.1	Principle and Practice of PCR.....	43
2.14.1.2	PCR Optimization.....	45
2.14.2	Types of PCR Reactions.....	45
2.14.2.1	Allele-Specific PCR .....	45
2.14.2.2	Polymerase Cycling Assembly .....	45
2.14.2.3	Asymmetric PCR.....	45
2.14.2.4	Helicase-Dependent Amplification .....	46
2.14.2.5	Hot-Start PCR.....	46
2.14.2.6	AFLP-PCR.....	46
2.14.2.7	Alu PCR.....	46
2.14.2.8	Colony PCR.....	46
2.14.2.9	Inverse PCR.....	46
2.14.2.10	Ligation-Mediated PCR .....	47
2.14.2.11	Methylation-Specific PCR.....	47
2.14.2.12	Miniprimer PCR.....	47
2.14.2.13	Multiplex Ligation-Dependent Probe Amplification.....	47
2.14.2.14	Multiplex PCR.....	47
2.14.2.15	Nested PCR .....	47
2.14.2.16	Overlap-Extension PCR .....	48
2.14.2.17	Quantitative PCR .....	48
2.14.2.18	Reverse Transcription PCR.....	48
2.14.2.19	Solid Phase PCR.....	49
2.14.2.20	Thermal Asymmetric Interlaced PCR .....	49
2.14.2.21	Touchdown PCR (Step-Down PCR) .....	49
2.14.2.22	Universal Fast Walking .....	49
2.14.3	Applications of PCR.....	49
2.14.3.1	Diagnostic Assay.....	49
2.14.3.2	Genetic Engineering.....	50
2.14.3.3	Forensic DNA Profiling.....	50
Problems.....		50
Section A: Descriptive Type .....		50
Section B: Multiple Choices.....		50
Section C: Critical Thinking.....		52
Assignment.....		52
References and Further Reading .....		52

<b>Chapter 3</b>	<b>Proteins and Proteomics.....</b>	<b>55</b>
	Learning Objectives.....	55
3.1	Introduction .....	55
3.2	Significance of Proteins.....	56
3.2.1	Proteins for Body Functions.....	56
3.2.1.1	Nonessential Amino Acids .....	56
3.2.1.2	Essential Amino Acids .....	57
3.2.1.3	Other Amino Acids.....	58
3.2.2	Proteins as Enzymes .....	58
3.2.3	Proteins in Cell Signaling and Ligand Binding .....	58
3.2.4	Structural Proteins.....	59

3.3	Protein Biosynthesis .....	60
3.3.1	Transcription Stage.....	60
3.3.2	Transcription in Prokaryotes and Eukaryotes.....	61
3.3.3	Stages of Transcription.....	61
3.3.3.1	Pre-Initiation.....	61
3.3.3.2	Initiation.....	63
3.3.3.3	Promoter Clearance .....	63
3.3.3.4	Elongation.....	63
3.3.3.5	Termination Stage.....	63
3.3.3.6	Reverse Transcription .....	64
3.3.3.7	RNA Splicing.....	64
3.3.3.8	Spliceosomal Introns .....	64
3.3.3.9	Spliceosome Formation and Activity .....	64
3.3.3.10	Self-Splicing .....	65
3.3.3.11	tRNA Splicing .....	65
3.3.3.12	RNA Export.....	65
3.3.4	Translation.....	65
3.3.4.1	Post-Translational Modification.....	66
3.4	Protein Structure .....	67
3.4.1	Protein Prediction Methods.....	68
3.5	Protein Folding.....	69
3.5.1	Tools of Protein Folding.....	69
3.5.1.1	Circular Dichroism .....	70
3.5.1.2	Dual Polarization Interferometry .....	70
3.5.1.3	Vibration Circular Dichroism of Proteins .....	70
3.5.1.4	Protein Folding with High Time Resolution.....	70
3.5.1.5	Energy Landscape Theory.....	70
3.6	Protein Modification.....	71
3.6.1	Protein Modification by Phosphorylation .....	71
3.6.2	Protein Modification by Ubiquitination .....	71
3.6.3	Additional Modifications.....	71
3.7	Protein Transport.....	71
3.8	Protein Dysfunction and Degradation.....	71
3.9	Regulation of Protein Synthesis .....	74
3.9.1	Stages of Gene Expression .....	74
3.9.1.1	Operon Model for Gene Regulation .....	75
3.9.1.2	General Transcription Factors .....	75
3.9.1.3	Enhancers .....	75
3.9.1.4	Induction and Repression.....	75
3.10	Regulatory Protein.....	77
3.11	Methods for Protein Analysis.....	77
3.11.1	Protein Identification and Quantification.....	77
3.11.1.1	Native Gel .....	78
3.11.1.2	SDS-PAGE.....	78
3.11.1.3	QPNC-PAGE.....	79
3.11.1.4	Protomap.....	79
3.11.1.5	Western Blot .....	79
3.11.1.6	Cellular Techniques .....	79
3.11.1.7	Enzyme-Linked Immunosorbent Assay.....	81
3.12	Protein Purification.....	82

## Contents

3.13	Tools of Proteomics .....	83
3.13.1	2D Electrophoresis .....	83
3.13.2	Mass Spectrometry .....	83
3.13.3	Protein Microarray .....	84
3.13.4	Two-Hybrid Screening .....	85
3.13.5	Protein Structure Prediction .....	86
3.13.6	PEGylation .....	86
3.13.7	High-Performance Liquid Chromatography .....	86
3.13.8	Shotgun Proteomics .....	86
3.13.9	Top-Down Proteomics .....	86
	Problems .....	87
	Section A: Descriptive Type .....	87
	Section B: Multiple Choice .....	87
	Section C: Critical Thinking .....	88
	Assignment .....	88
	References and Further Reading .....	88

<b>Chapter 4</b>	<b>Recombinant DNA Technology .....</b>	<b>91</b>
	Learning Objectives .....	91
4.1	Introduction .....	91
4.2	Making of Recombinant DNA .....	91
4.2.1	Steps in Making a Recombinant DNA Product .....	91
4.2.2	Methods Involved in Making Recombinant DNA Product .....	92
4.2.2.1	Transformation .....	92
4.2.2.2	Nonbacterial Transformation .....	93
4.2.2.3	Phage Introduction .....	93
4.3	Significance of Recombinant DNA Technology .....	93
4.4	Role of Restriction Enzymes in rDNA Technology .....	93
4.4.1	Types of Restriction Enzymes .....	94
4.4.1.1	Type I Restriction Enzymes .....	94
4.4.1.2	Type II Restriction Enzymes .....	94
4.4.1.3	Type III Restriction Enzymes .....	95
4.4.2	Nomenclature of Restriction Enzymes .....	95
4.4.3	Recognition Sequences for Type II Restriction Enzymes .....	96
4.4.4	Cleavage Pattern of Type II Restriction Enzymes .....	96
4.4.5	Modification of Cut Ends .....	97
4.5	Steps in Gene Cloning .....	97
4.6	Synthesis of Complete Gene .....	98
4.7	Polymerase Chain Reaction and Gene Cloning .....	99
4.7.1	Comparison of PCR versus Gene Cloning .....	99
4.8	Significance of Vectors in rDNA Technology .....	99
4.8.1	Properties of Good Vectors .....	100
4.8.2	Cloning and Expression Vectors .....	100
4.8.3	Applications of Viral Vectors .....	101
4.9	Classification of Vectors .....	101
4.9.1	Bacterial Vectors .....	101
4.9.1.1	<i>E. coli</i> Vectors .....	101
4.9.1.2	Plasmid Vectors .....	102

4.9.2	Viral Vectors .....	102
4.9.2.1	Retroviruses .....	103
4.9.2.2	Lentiviruses .....	104
4.9.2.3	Adenoviruses .....	105
4.9.2.4	Adeno-Associated Viruses .....	105
4.9.3	ARS Vectors.....	105
4.9.4	Minichromosome Vectors .....	106
4.9.5	Yeast Artificial Chromosome Vectors.....	107
4.9.6	Vectors for Animals .....	107
4.9.7	SV40 Vectors .....	108
4.9.8	Bovine Papillomavirus Vectors.....	108
4.10	Integration of the DNA Insert into the Vector.....	108
4.10.1	Both Ends Cohesive and Compatible .....	109
4.10.2	Both Ends Cohesive and Separately Matched.....	109
4.10.3	Both Ends Cohesive and Unmatched .....	110
4.10.4	Both Ends Flush/Blunt .....	110
4.10.5	One End Cohesive and Compatible, the Other End Blunt .....	110
4.11	Introduction of the Recombinant DNA into the Suitable Host .....	110
4.12	Increased Competence of <i>E. coli</i> by CaCl <sub>2</sub> Treatment.....	110
4.13	Infection by Recombinant DNAs Packaged as Virions .....	111
4.14	Selection of Recombinant Clones.....	111
4.15	Identification of Clones Having Recombinant DNAs .....	111
4.16	Selection of Clone Containing a Specific DNA Insert.....	112
4.16.1	Colony Hybridization .....	112
4.16.2	Other Approaches for Developing Specific Probes.....	112
4.16.3	Complementation .....	113
4.16.4	Unique Gene Products.....	113
4.16.5	Antibodies Specific to the Protein Product .....	113
4.16.6	Colony Screening with Antibodies .....	113
4.16.7	Fluorescence Activated Cell Sorter.....	114
4.17	Applications of Recombinant DNA Technology.....	114
4.17.1	Genetically Modified Organisms .....	114
4.17.1.1	Transgenic Microbes.....	115
4.17.1.2	Transgenic Animals.....	115
4.17.1.3	Transgenic Plants.....	115
4.17.1.4	Cisgenic Plants.....	115
4.18	DNA Sequencing .....	116
4.18.1	Maxam and Gilbert Procedure.....	117
4.18.2	Enzymatic Procedure .....	117
4.18.3	Automated DNA Sequencing .....	118
4.18.4	Current Challenges in DNA Sequencing .....	118
4.18.5	Trends in DNA Sequencing.....	119
4.18.5.1	High-Throughput Sequencing.....	119
4.18.5.2	<i>In Vitro</i> Clonal Amplification.....	119
4.18.5.3	Parallelized Sequencing .....	119
4.18.5.4	Sequencing by Ligation .....	120
4.18.5.5	Microfluidic Sanger Sequencing.....	120
4.18.5.6	Other Sequencing Technologies .....	120
4.19	Microarrays .....	120
4.20	DNA Chips .....	120
4.21	Isolation of Desired DNA.....	121

## Contents

4.22	cDNA Library.....	122
4.22.1	Problems in cDNA Preparation.....	123
4.22.2	Isolation of mRNA.....	123
4.23	Preparation of cDNA.....	123
4.24	Genomic Library.....	123
4.24.1	Construction of a Genomic Library.....	123
4.25	DNA Libraries.....	124
4.26	Chemical Synthesis Gene.....	125
4.26.1	Phosphodiester Approach.....	125
4.26.2	Phosphotriester Approach.....	125
4.26.3	Phosphite Triester Approach.....	125
4.27	Applications of Synthetic Oligonucleotides.....	126
	Problems.....	126
	Section A: Descriptive Type.....	126
	Section B: Multiple Choice.....	126
	Section C: Critical Thinking.....	127
	Assignments.....	128
	References and Further Reading.....	128
<b>Chapter 5</b>	<b>Microbial Biotechnology.....</b>	<b>131</b>
	Learning Objectives.....	131
5.1	Introduction.....	131
5.2	Structural Organization of Microbes.....	132
5.2.1	Structure.....	132
5.2.2	Intracellular Organization.....	133
5.2.3	Extracellular Organization.....	134
5.3	Microbial Metabolism.....	136
5.3.1	Heterotrophic Microbial Metabolism.....	136
5.3.2	Fermentation.....	137
5.3.3	Aerobic Respiration.....	138
5.3.4	Denitrification.....	139
5.3.5	Nitrogen Fixation.....	139
5.4	Microbial Growth.....	140
5.4.1	Phases of Microbial Growth.....	140
5.4.2	Factors That Influence Microbial Growth.....	141
5.5	Microbial Genetics.....	142
5.5.1	Mutations.....	142
5.5.1.1	Auxotrophic Mutant.....	142
5.5.1.2	Resistant Mutant.....	143
5.5.1.3	Metabolic Mutant.....	143
5.5.1.4	Regulatory Mutant.....	143
5.5.2	Spontaneous Mutations.....	143
5.5.3	Induction of Selective Mutations.....	144
5.5.4	Induced Mutations.....	145
5.6	Genetic Recombination in Bacteria.....	145
5.6.1	Bacterial Transformation.....	145
5.6.2	Bacterial Transduction.....	146
5.6.2.1	Types of Transduction.....	147
5.6.2.2	Stages of Transduction.....	149
5.6.3	Conjugation Mechanism in Gene Recombination.....	150

5.7	Transposable Genetic Elements.....	151
5.7.1	Types of Transposable Genetic Elements.....	152
5.7.1.1	Insertion Sequences.....	152
5.7.1.2	Transposons.....	152
5.8	Use of <i>E. coli</i> in Microbial Cloning.....	152
5.8.1	Genetic Simplicity.....	152
5.8.2	Growth Rate.....	152
5.8.3	Safety.....	153
5.8.4	Conjugation and the Genome Sequence.....	153
5.8.5	Ability to Host Foreign DNA.....	153
5.9	Pathogenic Bacteria.....	153
5.10	Application of Microbes.....	154
5.10.1	Microbes and Agriculture.....	154
5.10.2	Nitrogen Fixers.....	154
5.10.3	Biopesticides and Bioweedicides.....	154
5.10.4	Acetone Butanol Fermentations.....	155
5.10.5	Microbes in Recovery of Metals and Petroleum.....	155
5.10.6	Microbes in the Paper Industry.....	155
5.10.7	Microbes in Medicine.....	155
5.10.8	Microbes in Synthetic Energy Fuels.....	156
5.10.9	Microbes and Environment Cleaning.....	156
5.11	Food Microbiology.....	156
5.11.1	Microbes Associated with Food Spoilage.....	157
5.11.2	Meat and Fish.....	157
5.11.3	Poultry and Eggs.....	157
5.11.4	Breads and Bakery Products.....	157
5.11.5	Other Foods.....	157
5.11.6	Importance of Microbes in Foods.....	158
5.11.7	Food Fermentation.....	158
5.12	Microbial Biotechnology.....	159
5.12.1	Production of Enzymes by Microorganisms.....	159
	Problems.....	159
	Section A: Descriptive Type.....	159
	Section B: Multiple Choice.....	160
	Section C: Critical Thinking.....	160
	References and Further Reading.....	161
<b>Chapter 6</b>	<b>Agricultural Biotechnology.....</b>	<b>163</b>
	Learning Objectives.....	163
6.1	Introduction.....	163
6.2	Plant Breeding.....	163
6.2.1	Classical Breeding.....	164
6.2.2	Plant Breeding by Traditional Techniques.....	165
6.2.2.1	Selection.....	165
6.2.2.2	Hybridization.....	165
6.2.2.3	Polyploidy.....	166
6.2.3	Modern Plant Breeding.....	166
6.2.3.1	<i>In Vitro</i> Cultivation.....	166
6.2.3.2	<i>In Vitro</i> Selection and Somaclonal Variation.....	167
6.2.3.3	Somatic Hybrid Plants.....	167



6.2.3.4	Breeding by Restriction Fragment Length Polymorphism.....	169
6.2.3.5	Plant Breeding by Gene Transfer.....	169
6.2.3.6	Agrobacterium-Mediated Gene Transfer.....	169
6.2.3.7	Particle Bombardment .....	170
6.2.3.8	Electroporation and Direct DNA Entry into Protoplasts.....	170
6.2.3.9	Transgene Expression .....	170
6.2.3.10	Selection and Plant Regeneration .....	171
6.2.3.11	Reverse Breeding and Doubled Haploids .....	172
6.2.3.12	Genetic Modification .....	173
6.3	Plant Diseases.....	174
6.3.1	Diseases Caused by Fungi.....	174
6.3.2	Diseases Caused by Oomycetes .....	174
6.3.3	Diseases Caused by Bacteria.....	175
6.3.4	Diseases Caused by Plant Virus.....	175
6.3.5	Diseases Caused by Nematodes .....	176
6.3.6	Diseases Caused by Protozoa.....	176
6.3.7	Diseases Caused by Parasitic Plants .....	176
6.4	Applications of Molecular and Genetic Tools in Agriculture .....	176
6.4.1	Expression of Viral Coat Protein to Resist Infection in Agriculture .....	176
6.4.2	Expression of Bacterial Toxin in Agriculture Using Molecular Techniques.....	176
6.5	Herbicide-Tolerant Plants .....	177
6.6	Pigmentation in Transgenic Plants .....	177
6.7	Altering the Food Content of Plants.....	178
6.8	Gene Transfer Methods in Plants .....	178
6.9	Target Cells for Gene Transformation.....	179
6.10	Vectors for Gene Transfer.....	179
6.10.1	Structure and Functions of Ti and Ri Plasmids .....	179
6.11	Transformation Techniques Using Agrobacterium .....	181
6.11.1	Requirements of Transgenic Plants.....	181
6.11.2	Explants Used for Transformation .....	182
6.11.3	Marker Genes for Selection and Scoring of Cells.....	182
6.11.4	Neomycin Phosphotransferase Gene.....	182
6.12	$\beta$ -Glucuronidase GUS Gene .....	182
6.13	Agroinfection and Gene Transfer.....	183
6.14	DNA-Mediated Gene Transfer .....	183
6.14.1	Microinjection and Macroinjection .....	183
6.15	Electroporation for Gene Transfer.....	184
6.16	Liposome-Mediated Gene Transfer.....	184
6.17	Gene Transformation Using Pollen .....	184
6.18	Application of Transgenic Plants.....	185
6.18.1	Detoxification or Degradation of Herbicides .....	185
6.18.2	Crystal (Cry) Proteins.....	185
6.18.3	Toxic Action of Cry Proteins.....	185
6.18.4	Expression of Cry Genes in Plants.....	186
6.18.5	Insect Resistance to Cry Proteins.....	186
6.18.6	Virus Resistance.....	186
6.18.7	Drought Resistance .....	186

6.18.8	Modification of Seed Protein Quality .....	187
6.18.8.1	Introduction of an Appropriate Transgene .....	187
6.18.8.2	Modification of Endogenous Genes.....	187
6.18.8.3	Successful Examples .....	188
6.18.9	Co-Suppression of Genes.....	188
6.18.10	RNA-Mediated Interference .....	188
6.18.11	Biochemical Production in Plants.....	189
6.18.12	Plant-Derived Vaccines.....	189
6.18.12.1	Edible Vaccines .....	189
6.18.12.2	Recombinant and Subunit Vaccines.....	190
6.18.13	Hirudin A Polypeptide.....	190
6.18.14	Phytase as an Enzyme .....	190
6.18.15	Polyhydroxybutyrate Biodegradable Plastic Substrate .....	191
6.19	How Safe Are Transgenic Plants? .....	191
6.20	Bioengineered Plants .....	191
6.20.1	Golden Rice.....	192
6.20.2	Tomato “Flavr Savr” .....	193
6.21	Genetically Modified Maize.....	194
6.22	Terminator Technology .....	195
6.22.1	Types of Terminator Technology .....	195
6.22.1.1	V-GURT .....	195
6.22.1.2	T-GURT.....	196
6.22.2	Benefits of Terminal Technology .....	196
6.22.3	Concerns of Terminal Technology.....	196
Problems.....		197
Section A: Descriptive Type .....		197
Section B: Multiple Choice .....		197
Section C: Critical Thinking.....		198
Assignment.....		198
References and Further Reading .....		198

<b>Chapter 7</b>	<b>Animal Biotechnology .....</b>	<b>201</b>
	Learning Objectives.....	201
7.1	Introduction .....	201
7.2	History of the Use of Animals in Research.....	203
7.3	Drug Testing in Animals is Mandatory.....	203
7.4	Most Commonly Used Animals in Research.....	203
7.4.1	Invertebrates.....	204
7.4.2	Fish and Amphibians.....	205
7.4.3	Vertebrates.....	205
7.4.3.1	Rodents .....	205
7.4.3.2	Cats and Dogs.....	205
7.4.3.3	Primates and Nonprimates .....	205
7.5	Application of Animal Models.....	206
7.5.1	Use of Animals in Basic Research.....	206
7.5.2	Use of Animals in Applied Research.....	207
7.5.2.1	Genetic Diseases.....	207
7.5.2.2	Virology.....	207
7.5.2.3	Neurological Disorders .....	207
7.5.2.4	Organ Transplantation .....	208

## Contents

	7.5.2.5	Drug Efficacy Testing .....	208
	7.5.2.6	Toxicological Analysis.....	209
	7.5.2.7	Cosmetics Testing .....	210
7.6		Animal Models.....	210
	7.6.1	<i>Caenorhabditis elegans</i> .....	211
	7.6.2	<i>Drosophila melanogaster</i> .....	212
	7.6.3	Laboratory Mouse .....	213
	7.6.4	Rhesus Monkey .....	214
	7.6.5	<i>Xenopus laevis</i> .....	214
	7.6.6	Zebrafish.....	215
	7.6.7	Bioengineered Mosquito .....	216
7.7		Animal Biotechnology .....	216
	7.7.1	Use of Animals in Antibody Production.....	216
		7.7.1.1 Monoclonal Antibodies in Diagnostic Applications.....	217
		7.7.1.2 Monoclonal Antibodies in Therapeutic Applications.....	217
		7.7.1.3 Recombinant Antibodies .....	218
	7.7.2	<i>In Vitro</i> Fertilization and Embryo Transfer .....	218
		7.7.2.1 Embryo Transfer in Cattle .....	218
	7.7.3	Animal Cell Culture Products.....	220
	7.7.4	Animal Cloning.....	220
		7.7.4.1 Cloning of Extinct and Endangered Species .....	221
	7.7.5	Transgenic Animals .....	222
		7.7.5.1 Transgenic Cow .....	224
		7.7.5.2 Transgenic Pigs.....	225
		7.7.5.3 Transgenic Goat.....	225
		7.7.5.4 Transgenic Sheep.....	225
		7.7.5.5 Transgenic Chickens.....	226
		7.7.5.6 Transgenic Primates .....	226
7.8		Biotechnology and Fish Farming .....	226
	7.8.1	Mariculture.....	227
	7.8.2	Polyculture.....	227
	7.8.3	Aquatic Biotechnology .....	227
		7.8.3.1 Transgenic Fish.....	227
		7.8.3.2 Green Fluorescent Protein .....	228
		7.8.3.3 Antifreeze Proteins.....	228
		7.8.3.4 Transgenic Salmon .....	229
		7.8.3.5 Transgenic Mussel .....	229
		7.8.3.6 Fugu Fish .....	229
		7.8.3.7 <i>Squalus acanthias</i> .....	230
		7.8.3.8 <i>Limulus polyphemus</i> .....	230
7.9		Regulations of Animal Testing.....	231
	7.9.1	Alternatives to Animal Testing .....	231
		7.9.1.1 <i>In Vitro</i> Cell Culture Technique .....	231
		7.9.1.2 Synthetic Membranes .....	232
		7.9.1.3 Statistics Instead of Animal Testing .....	232
		7.9.1.4 Newer Scanning Techniques.....	232
		7.9.1.5 Computer Models .....	232
		Problems.....	233
		Section A: Descriptive Type .....	233
		Section B: Multiple Choices.....	233
		Section C: Critical Thinking.....	234

Assignment.....	234
References and Further Reading.....	234
<b>Chapter 8 Environmental Biotechnology.....</b>	<b>237</b>
Learning Objectives.....	237
8.1 Introduction .....	237
8.1.1 Ecosystem.....	237
8.1.2 Biomes.....	237
8.1.3 Wilderness.....	238
8.1.4 Geological Activity .....	238
8.1.5 Oceanic Activity.....	239
8.2 Factors Affecting the Environment .....	239
8.2.1 Global Warming .....	239
8.2.1.1 Carbon Footprint .....	239
8.2.1.2 Destruction of Forests.....	240
8.2.1.3 Air Pollution .....	241
8.2.1.4 Major Carbon Dioxide Emission Countries .....	241
8.2.1.5 Greenhouse Effect .....	241
8.2.1.6 Acid Rain.....	243
8.2.1.7 Ocean Acidification .....	243
8.2.1.8 Health Hazards due to Pollution.....	244
8.3 Environment Protection by Biotechnology .....	244
8.3.1 Bioremediation .....	245
8.3.1.1 Mycoremediation.....	246
8.3.2 Waste Water Treatment .....	247
8.3.2.1 Pretreatment Phase .....	247
8.3.2.2 Screening Phase.....	248
8.3.2.3 Sedimentation Phase.....	248
8.3.2.4 Secondary Treatment Phase .....	248
8.3.2.5 Activated Sludge.....	248
8.3.2.6 Filter Beds.....	249
8.3.2.7 Biological Aerated Filters .....	249
8.3.2.8 Nutrient Removal.....	249
8.3.2.9 Nitrogen Removal.....	250
8.3.2.10 Phosphorus Removal .....	250
8.3.2.11 Disinfection of Wastewater.....	250
8.3.2.12 Sludge Disposal .....	251
8.3.3 Biofuels.....	251
8.3.3.1 Biodiesel .....	252
8.3.3.2 Biogas .....	253
8.3.3.3 Bioalcohols .....	253
8.3.3.4 Bioethers .....	254
8.3.3.5 Syngas.....	254
8.3.3.6 Solid Biofuels.....	254
8.3.3.7 Second-Generation Biofuels .....	254
8.3.3.8 Third-Generation Biofuels.....	254
8.3.3.9 International Biofuel Efforts.....	255
8.3.3.10 Future of Biofuels .....	255
8.3.4 Biodegradable Plastic .....	255
8.3.5 Biodegradation by Bacteria .....	256

## Contents

8.3.6	Oil-Eating Bacteria.....	257
8.3.7	Bioaugmentation and Bioaugmentation.....	258
8.3.8	Bioleaching.....	258
8.3.9	Single-Cell Protein and Biomass from Waste.....	260
8.3.10	Vermitechnology.....	260
8.3.11	Biosorption.....	261
	8.3.11.1 Bacteria.....	261
	8.3.11.2 Fungi.....	262
	8.3.11.3 Algae.....	262
8.3.12	Genetically Engineered Organisms.....	262
Problems.....		262
	Section A: Descriptive Type.....	262
	Section B: Multiple Choice.....	263
	Section C: Critical Thinking.....	263
	Debate.....	264
	Field Visit.....	264
References and Further Reading.....		264
<b>Chapter 9</b>	<b>Medical Biotechnology.....</b>	<b>267</b>
Learning Objectives.....		267
9.1	Introduction.....	267
9.1.1	Vaccine Development.....	267
	9.1.1.1 Killed Vaccines.....	268
	9.1.1.2 Attenuated Vaccines.....	268
	9.1.1.3 Toxoid Vaccines.....	269
	9.1.1.4 Subunit Vaccines.....	269
	9.1.1.5 Conjugate Vaccines.....	269
	9.1.1.6 Experimental Vaccines.....	269
	9.1.1.7 Valence Vaccines.....	269
	9.1.1.8 Vaccine Production.....	269
	9.1.1.9 Making of Influenza Vaccines.....	270
	9.1.1.10 Large-Scale Production of Vaccines.....	272
	9.1.1.11 Economics Involved in Vaccine Production.....	273
	9.1.1.12 Trends in Vaccine Research.....	274
	9.1.1.13 Issues Related to Vaccines.....	275
	9.1.1.14 Synthetic Peptides as Vaccines.....	275
9.2	Antibody Production.....	275
9.2.1	Applications of Monoclonal Antibodies.....	277
	9.2.1.1 Diagnostic Test.....	277
	9.2.1.2 Cancer Treatment.....	277
9.2.2	Hybridoma Technology.....	277
	9.2.2.1 Purification of Monoclonal Antibodies.....	278
9.2.3	Recombinant Monoclonal Antibodies.....	279
9.2.4	Constraints in Making Monoclonal Antibodies.....	279
9.3	Therapeutic Proteins.....	279
9.3.1	Growth Factors.....	280
9.4	Stem Cell Transplantation.....	282
9.4.1	Adult Stem Cells.....	282
	9.4.1.1 Dental Pulp-Derived Stem Cells.....	282
	9.4.1.2 Hematopoietic Stem Cells.....	282

9.4.1.3	Mammary Stem Cells.....	283
9.4.1.4	Mesenchymal Stem Cells .....	283
9.4.1.5	Neural Stem Cells.....	284
9.4.1.6	Olfactory Adult Stem Cells.....	284
9.4.1.7	Clinical Applications of Stem Cells .....	284
9.4.1.8	Cancer Treatment.....	285
9.4.1.9	Spinal Cord Injury .....	286
9.4.1.10	Corneal Repair.....	286
9.4.2	Embryonic Stem Cells.....	286
9.4.2.1	How Were Embryonic Stem Cells Discovered? .....	287
9.4.2.2	Cell Line Contamination .....	288
9.4.2.3	Immunorejection.....	288
9.4.2.4	Alternative Approach to Creating Embryonic Stem Cells.....	288
9.4.2.5	Embryonic Stem Cells as Models for Human Genetic Disorders.....	289
9.4.2.6	First Clinical Trial .....	289
9.5	Bioengineered Skin .....	289
9.6	Bioengineered Organ Transplantation.....	290
9.7	Gene Therapy .....	290
9.7.1	<i>Ex Vivo</i> Gene Therapy.....	291
9.7.1.1	Gene Therapy Using Viral Vectors .....	291
9.7.1.2	Nonviral Methods of DNA Transfer.....	295
9.7.2	<i>In Vivo</i> Gene Therapy .....	296
9.7.3	Problems with Gene Therapy.....	297
9.7.3.1	Short-Lived Nature of Gene Therapy .....	298
9.7.3.2	Immune Response .....	298
9.7.3.3	Viral-Induced Toxicity.....	298
9.7.3.4	Not for Multi-Gene Disorders.....	298
9.7.3.5	Induced Mutagenesis .....	298
9.7.4	Genetic Counseling .....	298
9.8	Molecular Diagnostics.....	299
9.8.1	DNA Fingerprinting.....	299
9.8.2	Techniques of DNA Profiling.....	300
9.8.2.1	Restriction Fragment Length Polymorphism Analysis.....	300
9.8.2.2	PCR Analysis.....	301
9.8.2.3	Short Tandem Repeats Analysis .....	301
9.8.2.4	Amplified Fragment Length Polymorphism Analysis .....	302
9.8.2.5	Y-Chromosome Analysis.....	302
9.8.2.6	Mitochondrial DNA Analysis.....	302
9.8.2.7	Confirmation of Genetic Relationship.....	302
9.8.2.8	Fake DNA Evidence .....	303
9.8.2.9	Criminal DNA Data.....	303
9.9	Artificial Blood.....	303
9.10	Organ Transplant.....	304
9.10.1	History of Organ Transplant .....	304
9.10.2	Types of Transplants.....	306
9.10.2.1	Autograft.....	306
9.10.2.2	Allograft .....	306

## Contents

9.10.2.3	Isograft.....	307
9.10.2.4	Xenograft.....	307
9.11	Cloning.....	307
9.11.1	Molecular Cloning.....	308
9.11.2	Clonal Cell Technology.....	308
9.11.3	Clonal Embryo.....	308
9.11.4	Reproductive Cloning.....	309
9.11.5	Human Cloning.....	310
Questions	.....	310
Section A:	Descriptive Type.....	310
Section B:	Multiple Choice.....	310
Section C:	Critical Thinking.....	311
Assignment	.....	312
References and Further Reading	.....	312
<b>Chapter 10</b>	<b>Nanobiotechnology.....</b>	<b>315</b>
Learning Objectives	.....	315
10.1	Introduction.....	315
10.2	Nanotechnology.....	315
10.3	Nanobiotechnology.....	316
10.4	Applications of Nanobiotechnology.....	317
10.4.1	Nanomedicine.....	317
10.4.1.1	Drug Delivery.....	318
10.4.1.2	Cancer Diagnostics.....	321
10.4.1.3	<i>In Vivo</i> Drug Imaging.....	322
10.4.1.4	Nanonephrology.....	323
10.4.1.5	Gene Therapy Using Nanotechnology.....	324
10.4.1.6	Antimicrobial Techniques.....	325
10.4.2	Neuro-Electronic Interfaces.....	325
10.4.3	Molecular Nanotechnology.....	325
10.4.4	Nanorobots.....	325
10.4.5	Cell Repair Machines.....	326
10.4.6	Nanosensors.....	326
10.4.7	Nanoparticles.....	327
10.4.7.1	Classification of Nanoparticles.....	328
10.4.7.2	Characterization of Nanoparticles.....	328
10.4.7.3	Nanoparticles and Safety Issues.....	328
10.5	Nanotechnology in the Food Industry.....	329
10.6	Water Pollution and Nanotechnology.....	329
10.7	Research Trends.....	330
Problems	.....	330
Section A:	Descriptive Type.....	330
Section B:	Multiple Choice.....	331
Section C:	Critical Thinking.....	331
References and Further Reading	.....	331
<b>Chapter 11</b>	<b>Product Development in Biotechnology.....</b>	<b>333</b>
Learning Objectives	.....	333
11.1	Introduction.....	333

11.2	Methods of Scientific Enquiry.....	334
11.2.1	Characterizations of Scientific Investigation.....	335
11.2.2	Scientific Inventions .....	335
11.3	Commercialization of Scientific Discovery.....	336
11.4	Business Plan.....	336
11.4.1	Project Feasibility.....	336
11.4.1.1	Market Research.....	336
11.4.1.2	Significance of a Project.....	337
11.4.1.3	Technical Outline .....	337
11.4.1.4	Time Plan.....	337
11.4.1.5	Project Cost.....	337
11.4.1.6	Legal and Regulatory Issues.....	337
11.4.1.7	Quality of Product .....	337
11.5	Biotechnology Product Development .....	337
11.5.1	Infrastructure Requirements .....	339
11.5.1.1	Research and Development Facility .....	339
11.5.1.2	Animal Testing Facility or Preclinical Lab .....	339
11.5.1.3	Bioequivalence Lab .....	340
11.5.1.4	Clinical Trial Center .....	341
11.5.1.5	Manufacturing Plant .....	341
11.5.1.6	Formulation Lab .....	341
11.5.1.7	Quality Assurance and Quality Control Lab.....	343
11.6	Phases of Biotechnology Product Development.....	343
11.6.1	Preclinical Studies.....	344
11.6.2	Phase 0.....	344
11.6.3	Phase I.....	344
11.6.3.1	Single Ascending Dose.....	344
11.6.3.2	Multiple Ascending Doses.....	344
11.6.3.3	Food Effect .....	345
11.6.4	Phase II.....	345
11.6.5	Phase III .....	345
11.6.6	Phase IV .....	346
11.7	Biotechnology Entrepreneurship.....	346
11.7.1	Starting a Biotechnology Company .....	346
11.7.1.1	Grants.....	346
11.7.1.2	Private Investors .....	347
11.7.1.3	Angel Investors .....	347
11.7.1.4	Venture Capitalists.....	347
11.7.1.5	Bank Loans.....	347
11.8	Biotechnology Industry: Facts and Figures .....	347
11.8.1	Capital Investment in Biotechnology .....	348
11.8.2	Mergers and Acquisitions of Biotechnology Companies .....	349
11.9	Formation of a New Biotechnology Company .....	349
11.10	Successful Bioentrepreneurship .....	350
11.11	Biotechnology Products and Intellectual Property Rights.....	350
11.11.1	Patenting, Licensing, and Partnership in the Biotechnology Industry .....	350
11.11.2	Intellectual Property Protection .....	351
11.11.3	Intellectual Property Rights for Plants.....	352
11.11.4	Patents and Biotechnology Products .....	352



## Contents

11.11.5	International Treaties .....	353
11.11.5.1	Patent Cooperation Treaty .....	354
11.11.5.2	World Intellectual Property Organization.....	355
11.11.5.3	Agreement on Trade-Related Aspects of Intellectual Property Rights .....	355
11.11.5.4	Issues with Biotechnology Patents .....	355
11.11.5.5	Patent Infringements .....	356
11.12	Biotechnology Stock Investment: Pros and Cons.....	357
11.12.1	Products in the Pipeline .....	357
11.12.2	Collaboration and Merger .....	357
11.12.3	Experienced Management.....	358
11.12.4	Cash Flow.....	358
11.13	Marketing Trends in Biotechnology .....	358
11.14	Role of Regulators in Biotechnology Product Development.....	358
11.14.1	World Health Organization .....	358
11.14.2	International Conference on Harmonization .....	359
11.14.3	United States Food and Drug Administration .....	360
11.14.4	Good Laboratory Practice.....	360
11.14.4.1	GLP Requirements.....	361
11.14.4.2	National Legislation .....	361
11.14.4.3	Facilities .....	362
11.14.4.4	GLP Inspection and Enforcement .....	362
11.14.5	Good Manufacturing Practice.....	362
11.14.5.1	Cleanroom Facility.....	363
11.14.5.2	Cleanroom Classifications .....	363
11.14.5.3	GMP Enforcement .....	363
11.15	Certification and Accreditation .....	364
11.15.1	International Operating Procedures.....	364
11.15.1.1	ISO 14644-1 .....	364
11.15.1.2	ISO 14698-1 .....	364
11.15.1.3	ISO 14698-2 .....	364
11.15.2	British Standard 5295.....	365
Problems	.....	365
Section A: Descriptive Type	.....	365
Section B: Multiple Choice	.....	365
Section C: Critical Thinking	.....	366
Field Trip	.....	366
References and Further Reading	.....	366
<b>Chapter 12</b>	<b>Industrial Biotechnology .....</b>	<b>369</b>
Learning Objectives	.....	369
12.1	Introduction .....	369
12.2	Fermenter or Bioreactor.....	370
12.3	Principle of Fermentation.....	370
12.3.1	Aerobic Fermentation.....	371
12.3.1.1	Submerged Culture Method.....	371
12.3.1.2	Semisolid/Solid-State Methods .....	372
12.3.2	Anaerobic Fermentation.....	372
12.3.2.1	Batch Fermentation Process .....	372
12.3.2.2	Continuous Fermentation Process .....	373

12.4	Production of Biomolecules Using Fermenter Technology .....	373
12.4.1	Gluconic Acid.....	373
12.4.2	Citric Acid .....	374
12.4.3	Acetone Butanol.....	374
12.4.4	Itaconic Acid .....	374
12.4.5	Gibberellic Acid .....	374
12.4.6	Lactic Acid .....	375
12.4.7	Amino Acids .....	375
12.4.8	Enzymes .....	376
12.4.9	Proteases.....	376
12.4.10	Amylases .....	376
12.5	Development Process of Microbial Products .....	376
12.5.1	Isolation and Screening of Microorganisms .....	376
12.5.1.1	Isolation of Microorganisms.....	377
12.5.1.2	Screening of Microorganisms .....	378
12.5.2	Inoculum Development .....	378
12.5.3	Culture Media .....	379
12.5.4	Contamination.....	379
12.5.5	Sterilization .....	379
12.5.5.1	Heating.....	379
12.5.5.2	Radiation.....	380
12.5.5.3	Chemicals .....	380
12.5.5.4	Filtration .....	380
12.5.6	Strain Improvement.....	380
12.5.6.1	Mutant Selection.....	380
12.5.6.2	Selective Isolation of Mutants.....	381
12.6	Upstream Bioprocess .....	382
12.6.1	Industrial Microbial Culture .....	382
12.6.2	Mammalian Cell Culture .....	383
12.6.2.1	Manipulation of Cultured Cells.....	383
12.6.2.2	Generation of Hybridomas.....	383
12.6.3	Nonmammalian Culture Methods .....	384
12.6.3.1	Industrial Plant Cell Culture.....	384
12.6.3.2	Bacterial/Yeast Culture Methods .....	384
12.6.3.3	Viral Culture Methods.....	384
12.7	Downstream Bioprocess.....	384
12.7.1	Stages in DSP .....	385
12.7.1.1	Removal of Insolubles.....	385
12.7.1.2	Product Isolation.....	385
12.7.1.3	Product Purification.....	385
12.7.1.4	Product Polishing.....	385
12.8	Bioprocess Automation .....	385
12.8.1	Modeling Individual Unit Operations .....	386
12.8.2	Simple Mass Balance .....	387
12.8.3	Mass and Heat Balance .....	387
12.8.4	Rate-Based Model .....	387
12.8.5	Batch Processing .....	387
12.8.6	Continuous Processes.....	388
12.8.7	Dynamic Simulation.....	388
12.8.8	Water Consumption.....	388
12.8.9	Waste Recycling .....	388

## Contents

12.9	Industrial Application of Microbes .....	388
12.9.1	<i>Corynebacterium</i> .....	388
12.9.2	<i>Bacillus</i> .....	389
12.9.3	<i>Saccharomyces cerevisiae</i> .....	389
12.9.4	<i>Pseudomonas</i> .....	389
12.9.5	<i>Clostridium</i> .....	390
12.9.6	Thermophiles .....	390
12.10	Industrial Production of Healthcare Products .....	391
12.10.1	Antibiotic Manufacturing.....	391
12.10.1.1	Penicillin Production.....	391
12.10.1.2	Cephalosporins Production .....	391
12.10.1.3	Streptomycin Production.....	391
12.10.2	Steroids Production .....	393
12.10.3	Textile Production .....	393
12.10.4	Microbial Synthesis of Vitamin B12.....	394
Problems.....		394
Section A: Descriptive Type .....		394
Section B: Multiple Choice .....		395
Section C: Critical Thinking .....		395
Laboratory Assignment .....		395
References and Further Reading .....		396
<b>Chapter 13</b>	<b>Ethics in Biotechnology .....</b>	<b>397</b>
Learning Objectives.....		397
13.1	Introduction .....	397
13.2	Genetically Modified Foods and Plants .....	398
13.3	Use of Animals as Experimental Models.....	400
13.3.1	Disadvantages of Animal Testing .....	401
13.3.2	Attacks on Researchers .....	401
13.3.3	Regulations for Animal Testing in the United States.....	402
13.3.4	Role of Animal Welfare Groups .....	402
13.3.5	Future of Animal Testing.....	402
13.3.6	Decide for Yourself .....	403
13.4	Use of Humans as Experimental Models .....	403
13.4.1	Bioethical Implications .....	404
13.5	Xenotransplantation.....	404
13.6	Genetic Screening .....	406
13.7	Biometrics.....	406
13.8	DNA Fingerprinting .....	407
13.9	Organ Donation and Transplantation .....	408
13.10	Euthanasia .....	408
13.11	Neuroethics.....	408
13.12	Assisted Reproductive Technology .....	409
13.13	Embryonic Stem Cell Research.....	409
13.13.1	In Favor of Embryonic Stem Cell Research.....	410
13.13.2	Against Embryonic Stem Cell Research .....	411
13.13.3	Current Status.....	411
13.14	Human Cloning .....	411
Questions.....		412
Section A: Descriptive Type .....		412

Section B: Multiple Choice .....	413
Section C: Critical Thinking.....	413
Assignments .....	413
References and Further Reading .....	414
<b>Chapter 14</b> Careers in Biotechnology .....	417
Learning Objectives.....	417
14.1 Introduction .....	417
14.2 Education and Investment in Biotechnology.....	417
14.3 Research and Development in Biotechnology.....	419
14.4 Biotechnology Industry and Products .....	421
14.5 Biotechnology Status in the United States .....	421
14.6 Career Opportunities in Biotechnology .....	422
14.7 Entry-Level Job Positions in Biotechnology .....	423
14.7.1 Research and Development Division.....	423
14.7.1.1 Glass Washer .....	423
14.7.1.2 Laboratory Assistant .....	423
14.7.1.3 Research Associate.....	424
14.7.1.4 Research Assistant.....	424
14.7.1.5 Postdoctoral Fellow .....	424
14.7.1.6 Media Preparation Technician .....	424
14.7.1.7 Greenhouse Assistant.....	424
14.7.1.8 Plant Breeder .....	424
14.7.2 Quality Control.....	425
14.7.2.1 Quality Control Analyst .....	425
14.7.2.2 Quality Control Engineer .....	425
14.7.2.3 Environmental Health and Safety Specialist .....	425
14.7.2.4 Quality Assurance Auditor.....	425
14.7.2.5 Validation Engineer.....	425
14.7.2.6 Validation Technician.....	425
14.7.3 Clinical Research .....	426
14.7.3.1 Clinical Research Administrator.....	426
14.7.3.2 Clinical Coordinator.....	426
14.7.3.3 Clinical Programmer.....	426
14.7.3.4 Biostatistician .....	426
14.7.3.5 Clinical Data Specialist.....	426
14.7.3.6 Drug Experience Coordinator .....	427
14.7.3.7 Clinical Research Associate.....	427
14.7.3.8 Animal Handler.....	427
14.7.3.9 Animal Technician .....	427
14.7.3.10 Technical Writer.....	427
14.7.4 Product and Development .....	427
14.7.4.1 Product Development Engineer.....	427
14.7.4.2 Production Planner/Scheduler .....	428
14.7.4.3 Manufacturing Technician .....	428
14.7.4.4 Packaging Operator .....	428
14.7.4.5 Manufacturing Research Associate.....	428
14.7.4.6 Instrument/Calibration Technician .....	428
14.7.4.7 Biochemical Development Engineer.....	429
14.7.4.8 Process Development Associate.....	429

## Contents

14.7.4.9	Assay Analyst.....	429
14.7.4.10	Manufacturing Engineer .....	429
14.7.5	Regulatory Affairs .....	429
14.7.5.1	Regulatory Affairs Specialists .....	429
14.7.5.2	Documentation Coordinator.....	430
14.7.5.3	Documentation Specialist .....	430
14.7.6	Information Systems .....	430
14.7.6.1	Library Assistant.....	430
14.7.6.2	Scientific Programmer/Analyst .....	430
14.7.7	Marketing and Sales.....	430
14.7.7.1	Market Research Analyst .....	430
14.7.7.2	Systems Analyst .....	431
14.7.7.3	Sales Representative.....	431
14.7.7.4	Customer Service Representative.....	431
14.7.7.5	Technical Service Representative.....	431
14.8	Administration.....	431
14.8.1	Technical Recruiter .....	431
14.8.2	Human Resource Representative .....	432
14.8.3	Patent Administrator .....	432
14.8.4	Patent Agent .....	432
14.9	Which Job Is Good for Me?.....	432
14.10	Why Are R&D Jobs the Most Challenging?.....	433
14.10.1	Medical and Diagnostics Sectors .....	433
14.10.1.1	Detecting and Treating Hereditary Diseases .....	433
14.10.1.2	Heart Disease .....	434
14.10.1.3	Cancer .....	434
14.10.1.4	AIDS .....	434
14.10.1.5	Veterinary Medicine.....	434
14.10.1.6	Vaccines .....	434
14.10.1.7	Monoclonal Antibodies.....	434
14.10.1.8	Growth Hormones.....	434
14.10.2	Agricultural Sector.....	434
14.10.2.1	Crop Yield .....	435
14.10.2.2	Protein and Oil Content of Seeds.....	435
14.10.2.3	Environmental Conditions .....	435
14.10.2.4	Disease and Pest Resistance.....	435
14.10.3	Law Enforcement Sector.....	435
14.10.4	Product Manufacturing Sector .....	435
14.10.5	Microbial Engineering Sector.....	436
14.11	Salary and Incentives in Biotechnology.....	436
	Problems.....	437
	Assignments .....	437
	Field Visit .....	437
	References and Further Reading .....	437
<b>Chapter 15</b>	<b>Laboratory Tutorials.....</b>	<b>439</b>
15.1	Laboratory Experiments.....	439
15.1.1	Controlled Experiment.....	439
15.1.2	Observational Experiment.....	440
15.2	Laboratory Safety.....	440

15.3	Good Laboratory Practices for Biotechnology Labs.....	442
15.4	General Laboratory Techniques .....	443
15.4.1	Pipetting Technique.....	443
15.4.1.1	Plastic Pipette .....	443
15.4.1.2	Plastic Pipette Pump.....	443
15.4.1.3	Pipette with a Bulb .....	444
15.4.1.4	Micropipette.....	444
15.4.2	Centrifugation Technique.....	444
15.4.3	Spectrophotometer Technique.....	445
15.4.4	Aseptic Techniques .....	446
15.5	General Principles of Animal Handling.....	447
15.6	Animal Anesthesia .....	447
15.6.1	Anesthesia by Gas .....	447
15.6.2	Injectable Anesthesia.....	448
15.6.2.1	Intravenous Method .....	448
15.6.2.2	Intramuscular Method .....	448
15.6.2.3	Intraperitoneal Method .....	448
15.6.2.4	Subcutaneous Method.....	449
15.6.3	Animal Euthanasia.....	449
15.6.3.1	Criteria for Euthanasia.....	449
15.6.3.2	Surgical Operation.....	449
15.6.3.3	Use of CO <sub>2</sub> Chamber .....	450
15.7	Animal Histology .....	450
15.8	Blood Collection in Animals.....	451
15.9	Histology and Microscopy .....	452
15.9.1	Immunocytochemistry Technique.....	452
15.9.1.1	Direct Immunofluorescence.....	453
15.9.1.2	Indirect Immunofluorescence .....	454
15.10	Separation Techniques.....	455
15.10.1	Agarose Gel Electrophoresis (Basic Method).....	455
15.10.2	How Much Percentage Gel Will Be Made?.....	455
15.10.3	Which Gel Tank to Use? .....	455
15.10.4	How Much DNA Should Be Loaded? .....	455
15.10.5	Which Comb?.....	456
15.11	Microbiology Techniques .....	458
15.11.1	Isolation of Pure Culture .....	458
15.11.2	Streaking Bacteria for Single Colonies .....	459
15.11.3	Gram-Staining Procedure .....	459
15.12	Biochemistry Techniques .....	459
15.12.1	Estimation of Fat in Milk Samples.....	459
15.12.2	Protein Quantification by Bradford Assay .....	460
15.12.3	Indirect ELISA.....	461
15.12.4	Sandwich ELISA .....	462
15.12.5	Sonication of Bacteria .....	463
15.13	Molecular Techniques .....	463
15.13.1	Isolation of Genomic DNA from Blood .....	463
15.13.2	Isolation of DNA from Fresh or Frozen Tissue.....	464
15.13.3	Preparation of Genomic DNA from Bacteria.....	466
15.13.4	DNA Isolation Procedure .....	466
15.13.5	Polymerase Chain Reaction .....	467

## Contents

15.13.6 Semiquantitative RT-PCR .....	469
15.13.6.1 Role of Housekeeping Gene Transcript.....	469
15.13.6.2 Isolation of Total RNA .....	469
15.14 Genetic Techniques .....	471
15.14.1 Preparation of Human Metaphase Chromosomes.....	471
15.14.2 Structural Analysis of Human Chromosomes by Karyotype .....	472
15.14.3 DNA Amplification Fingerprinting Protocol .....	474
15.14.4 Single-Strand Conformation Polymorphism Technique .....	475
15.15 Agricultural Biotechnology .....	476
15.15.1 Plant DNA Isolation .....	476
15.15.2 Plant Regeneration by Protoplast Fusion.....	477
15.15.3 Simplified Arabidopsis Transformation .....	478
15.15.4 Agrobacterium-Mediated Gene Transfer via Hypocotyls.....	478
15.15.5 Isolation of DNA from Onion .....	479
15.15.6 Isolation of DNA from Wheat Germ.....	479
15.16 Microbial Biotechnology .....	480
15.16.1 Gram Positive/Negative Staining .....	480
References .....	480
<b>Glossary</b> .....	<b>481</b>
<b>Index</b> .....	<b>565</b>