

Contents

Preface xix

Preface to the First Edition xxi

I Fundamentals of Molecular Biotechnology 1

- 1 The Molecular Biotechnology Revolution 3
 - Recombinant DNA Technology 3
 - Emergence of Molecular Biotechnology 5
 - Commercialization of Molecular Biotechnology 7
 - Concerns and Consequences 10
 - SUMMARY 12
 - REFERENCES 13
 - REVIEW QUESTIONS 13
- 2 Molecular Biotechnology Biological Systems 14
 - Prokaryotic and Eukaryotic Organisms 14
 - Escherichia coli* 15
 - Saccharomyces cerevisiae* 17
 - Secretion Pathways in Prokaryotic and Eukaryotic Organisms 18
 - Eukaryotic Cells in Culture 21
 - SUMMARY 22
 - REFERENCES 22
 - REVIEW QUESTIONS 22
- 3 DNA, RNA, and Protein Synthesis 23
 - Structure of DNA 23
 - DNA Replication 27

Decoding Genetic Information: RNA and Protein	28
Translation	34
Regulation of mRNA Transcription in Bacteria	38
Regulation of mRNA Transcription in Eukaryotes	42
SUMMARY	45
REFERENCES	46
REVIEW QUESTIONS	46

4 Recombinant DNA Technology 47

Restriction Endonucleases	49
Plasmid Cloning Vectors	57
Plasmid Cloning Vector pBR322	58
Transformation and Selection	60
Other Plasmid Cloning Vectors	61
Creating and Screening a Library	64
Making a Gene Library	64
Screening by DNA Hybridization	67
Screening by Immunological Assay	73
Screening by Protein Activity	73
Cloning DNA Sequences That Encode Eukaryotic Proteins	75
Vectors for Cloning Large Pieces of DNA	80
Bacteriophage λ Vectors	80
Cosmids	83
High-Capacity Bacterial Vector Systems	86
Genetic Transformation of Prokaryotes	86
Transferring DNA into <i>E. coli</i>	86
Electroporation	86
Conjugation	87
SUMMARY	89
REFERENCES	89
REVIEW QUESTIONS	90

5 Chemical Synthesis, Sequencing, and Amplification of DNA 91

Chemical Synthesis of DNA	91
The Phosphoramidite Method	92
Uses of Synthesized Oligonucleotides	96
DNA Sequencing Techniques	101
Dideoxynucleotide Procedure for Sequencing DNA	102
Automated DNA Sequencing	106
Using Bacteriophage M13 as a DNA Sequencing Vector	107
Primer Walking	110
PCR	110
Gene Synthesis by PCR	115
Cycle Sequencing	115

SUMMARY	118
REFERENCES	119
REVIEW QUESTIONS	120

6 Manipulation of Gene Expression in Prokaryotes 121

Gene Expression from Strong and Regulatable Promoters	122
Regulatable Promoters	122
Increasing Protein Production	126
Large-Scale Systems	126
Expression in Other Microorganisms	128
Fusion Proteins	130
Cleavage of Fusion Proteins	131
Uses of Fusion Proteins	131
Surface Display	135
Unidirectional Tandem Gene Arrays	136
Translation Expression Vectors	138
Increasing Protein Stability	142
Protein Folding	142
Overcoming Oxygen Limitation	145
Use of Protease-Deficient Host Strains	145
Bacterial Hemoglobin	145
DNA Integration into the Host Chromosome	146
Removing Selectable Marker Genes	149
Increasing Secretion	151
L-Form Bacteria	153
Metabolic Load	155
SUMMARY	159
REFERENCES	159
REVIEW QUESTIONS	161

7 Heterologous Protein Production in Eukaryotic Cells 163

<i>Saccharomyces cerevisiae</i> Expression Systems	165
<i>S. cerevisiae</i> Vectors	166
Intracellular Production of Heterologous Proteins in <i>S. cerevisiae</i>	169
Secretion of Heterologous Proteins by <i>S. cerevisiae</i>	171
<i>Pichia pastoris</i> and Other Yeast Expression Systems	171
Baculovirus-Insect Cell Expression System	174
Baculovirus Expression Vector System	175
Increasing the Yield of Recombinant Baculovirus	175
Construction of an <i>E. coli</i> -Insect Cell Baculovirus Shuttle Vector	177
Mammalian Glycosylation and Processing of Precursor Proteins in Insect Cells	180
Mammalian Cell Expression Systems	181
Selectable Marker Systems for Mammalian Expression Vectors	185

SUMMARY	187
REFERENCES	187
REVIEW QUESTIONS	189

8 Directed Mutagenesis and Protein Engineering 190

Directed Mutagenesis Procedures 191

Oligonucleotide-Directed Mutagenesis with M13 DNA	192
Oligonucleotide-Directed Mutagenesis with Plasmid DNA	194
PCR-Amplified Oligonucleotide-Directed Mutagenesis	195
Random Mutagenesis with Degenerate Oligonucleotide Primers	198
Random Mutagenesis with Nucleotide Analogues	201

Error-Prone PCR 201

DNA Shuffling 203

Mutant Proteins with Unusual Amino Acids 204

Protein Engineering 205

Adding Disulfide Bonds 205

Changing Asparagine to Other Amino Acids 209

Reducing the Number of Free Sulfhydryl Residues 209

Increasing Enzymatic Activity 211

Modifying Metal Cofactor Requirements 212

Decreasing Protease Sensitivity 214

Modifying Protein Specificity 215

Increasing Enzyme Stability and Specificity 218

Altering Multiple Properties 219

SUMMARY 221

REFERENCES 222

REVIEW QUESTIONS 223

II Molecular Biotechnology of Microbial Systems 225

9 Molecular Diagnostics 227

Immunological Diagnostic Procedures 228

Enzyme-Linked Immunosorbent Assay 229

Monoclonal Antibodies 230

Formation and Selection of Hybrid Cells 231

Identification of Specific Antibody-Producing Hybrid Cell Lines 232

DNA Diagnostic Systems 234

Hybridization Probes 235

Diagnosis of Malaria 236

Detection of *Trypanosoma cruzi* 236

Nonradioactive Hybridization Procedures 237

Molecular Beacons 240

DNA Fingerprinting 241

Random Amplified Polymorphic DNA	242
Bacterial Biosensors	244
Molecular Diagnosis of Genetic Disease	245
Screening for Cystic Fibrosis	246
Sickle-Cell Anemia	246
The PCR/OLA Procedure	248
Padlock Probes	250
Genotyping with Fluorescence-Labeled PCR Primers	250
SUMMARY	253
REFERENCES	253
REVIEW QUESTIONS	254

10 Therapeutic Agents 256

Pharmaceuticals	257
Isolation of Interferon cDNAs	257
Human Interferons	258
Human Growth Hormone	259
Tumor Necrosis Factor Alpha	260
Optimizing Gene Expression	261
Therapeutics Produced and Delivered by Intestinal Bacteria	262
Enzymes	263
DNase I	263
Alginate Lyase	265
Phenylalanine Ammonia Lyase	266
α_1 -Antitrypsin	267
Monoclonal Antibodies as Therapeutic Agents	267
Structure and Function of Antibodies	269
Preventing Rejection of Transplanted Organs	270
Treating Brain Tumors	270
Chemically Linked Monoclonal Antibodies	271
Human Monoclonal Antibodies	272
Hybrid Human-Mouse Monoclonal Antibodies	274
Production of Antibodies in <i>E. coli</i>	277
Phage Combinatorial Libraries	277
Shuffling CDR Sequences	280
Single-Chain Antibodies	282
Nucleic Acids as Therapeutic Agents	283
Antisense RNA	284
Antisense Oligonucleotides	284
Ribozymes	288
Chimeric RNA-DNA Molecules	291
Interfering RNAs	292
Antibody Genes	294
Treating Genetic Disorders	295
Human Gene Therapy	298
Prodrug Activation Therapy	303
SUMMARY	304

REFERENCES	305
REVIEW QUESTIONS	307

11 Vaccines 309

Subunit Vaccines	312
<i>Herpes Simplex Virus</i>	312
Foot-and-Mouth Disease	313
Peptide Vaccines	315
Genetic Immunization: DNA Vaccines	317
Attenuated Vaccines	322
<i>Cholera</i>	322
<i>Salmonella</i> Species	324
<i>Leishmania</i> Species	325
<i>Herpes Simplex Virus</i>	326
Vector Vaccines	327
Vaccines Directed against Viruses	327
Vaccines Directed against Bacteria	333
Bacteria as Antigen Delivery Systems	335
SUMMARY	336
REFERENCES	337
REVIEW QUESTIONS	338

12 Synthesis of Commercial Products by Recombinant Microorganisms 340

Restriction Endonucleases	340
Small Biological Molecules	344
Synthesis of L-Ascorbic Acid	345
Microbial Synthesis of Indigo	349
Synthesis of Amino Acids	350
Removal of Lipids	354
Antibiotics	355
Cloning Antibiotic Biosynthesis Genes	356
Synthesis of Novel Antibiotics	360
Engineering Polyketide Antibiotics	361
Improving Antibiotic Production	365
Peptide Antibiotics	367
Biopolymers	367
Engineering <i>Xanthomonas campestris</i> for Xanthan Gum Production	367
Isolation of Melanin Biosynthesis Genes	369
Synthesis of an Animal Adhesive Biopolymer in Microbial Cells	370
Microbial Synthesis of Rubber	372
Microbial Production of Polyhydroxyalkanoates	372
SUMMARY	375
REFERENCES	375
REVIEW QUESTIONS	377

13 Bioremediation and Biomass Utilization 378

Microbial Degradation of Xenobiotics 378
Genetic Engineering of Biodegradative Pathways 382
Manipulation by Transfer of Plasmids 384
Manipulation by Gene Alteration 385
Utilization of Starch and Sugars 394
Commercial Production of Fructose and Alcohol 395
Improving Alcohol Production 396
Improving Fructose Production 398
Zymomonas mobilis 400
Silage Fermentation 404
Utilization of Cellulose 405
Components of Lignocellulose 406
Isolation of Prokaryotic Cellulase Genes 408
Isolation of Eukaryotic Cellulase Genes 410
Manipulation of Cellulase Genes 412
SUMMARY 413
REFERENCES 414
REVIEW QUESTIONS 415

14 Plant Growth-Promoting Bacteria 416

Nitrogen Fixation 417
Nitrogenase 419
Components of Nitrogenase 419
Genetic Engineering of the Nitrogenase Gene Cluster 421
Glycogen Synthase Mutants 425
Engineering Oxygen Levels 427
Hydrogenase 427
Hydrogen Metabolism 428
Genetic Engineering of Hydrogenase Genes 428
Nodulation 430
Competition among Nodulating Organisms 430
Genetic Engineering of Nodulation Genes 431
Growth Promotion by Free-Living Bacteria 435
Decreasing Plant Stress 438
Phytoremediation 440
Biocontrol of Pathogens 442
Siderophores 443
Antibiotics 445
Enzymes 447
Ice Nucleation and Antifreeze Proteins 447
Ethylene 449
Root Colonization 451
SUMMARY 451
REFERENCES 452
REVIEW QUESTIONS 453

15 Microbial Insecticides 455**Insecticidal Toxin of *B. thuringiensis* 456**

Mode of Action and Use 456

Toxin Gene Isolation 460

Engineering of *B. thuringiensis* Toxin Genes 462

Preventing the Development of Resistance 470

Genetic Engineering for Improved Biocontrol 472

Baculoviruses as Biocontrol Agents 473

Mode of Action 473

Genetic Engineering for Improved Biocontrol 475

SUMMARY 477

REFERENCES 478

REVIEW QUESTIONS 480

16 Large-Scale Production of Proteins from Recombinant Microorganisms 481**Principles of Microbial Growth 483**

Batch Fermentation 483

Fed-Batch Fermentation 485

Continuous Fermentation 486

Maximizing the Efficiency of the Fermentation Process 488

High-Density Cell Cultures 489

Bioreactors 490**Typical Large-Scale Fermentation Systems 494**

Two-Stage Fermentation in Tandem Airlift Reactors 495

Two-Stage Fermentation in a Single Stirred-Tank Reactor 496

Batch versus Fed-Batch Fermentation 497

Harvesting Microbial Cells 499**Disrupting Microbial Cells 502****Downstream Processing 504**

Protein Solubilization 506

Large-Scale Production of Plasmid DNA 506

SUMMARY 507

REFERENCES 507

REVIEW QUESTIONS 509

III Eukaryotic Systems 511**17 Genetic Engineering of Plants: Methodology 513****Plant Transformation with the Ti Plasmid of *Agrobacterium tumefaciens* 514**

Ti Plasmid-Derived Vector Systems 517

Physical Methods of Transferring Genes to Plants 520

Microprojectile Bombardment	522
Use of Reporter Genes in Transformed Plant Cells	523
Manipulation of Gene Expression in Plants	525
Isolation and Use of Different Promoters	526
Targeted Alterations in Plant DNA	527
Targeted Alterations in Plant RNA	528
Targeting Foreign DNA to the Chloroplast Genome	529
Secretion of Foreign Proteins	532
Production of Marker-Free Transgenic Plants	532
Removing Marker Genes from Nuclear DNA	533
Removing Marker Genes from Chloroplast DNA	534
SUMMARY	535
REFERENCES	535
REVIEW QUESTIONS	537

18 Genetic Engineering of Plants: Applications 538

Development of Insect-, Pathogen-, and Herbicide-Resistant Plants 538

Insect-Resistant Plants	538
Virus-Resistant Plants	547
Herbicide-Resistant Plants	554
Fungus- and Bacterium-Resistant Plants	556

Development of Stress- and Senescence-Tolerant Plants 560

Oxidative Stress	561
Salt Stress	562
Fruit Ripening and Flower Wilting	564

Genetic Manipulation of Flower Pigmentation 566

Modification of Plant Nutritional Content 569

Amino Acids	569
Lipids	571
Vitamins	573
Iron	575

Modification of Food Plant Taste and Appearance 577

Preventing Discoloration	577
Sweetness	578
Starch	579

Plants as Bioreactors 582

Antibodies	583
Polymers	583
Foreign Proteins in Plants	584

Edible Vaccines 585

Plant Yield 586

Increasing Iron Content	587
Altering Lignin Content	587
Increasing Oxygen Content	588

SUMMARY	589
---------	-----

	REFERENCES	590
	REVIEW QUESTIONS	593
19	Transgenic Animals	594
	Transgenic Mice: Methodology	596
	Retroviral Vector Method	596
	DNA Microinjection Method	596
	Engineered Embryonic Stem Cell Method	599
	Genetic Modification with the <i>Cre-loxP</i> Recombination System	603
	Transgenesis with High-Capacity Vectors	607
	Transgenic Mice: Applications	609
	Transgenic Models for Alzheimer Disease	610
	Using Transgenic Mice as Test Systems	611
	Conditional Regulation of Gene Expression	613
	Conditional Control of Cell Death	615
	Cloning Livestock by Nuclear Transfer	616
	Transgenic Cattle, Sheep, Goats, and Pigs	618
	Transgenic Birds	623
	Transgenic Fish	626
	SUMMARY	629
	REFERENCES	630
	REVIEW QUESTIONS	632
20	Human Molecular Genetics	633
	Modes of Human Inheritance	634
	Genetic Linkage and Gene Mapping	636
	Detection and Estimation of Genetic Linkage in Humans	639
	Genetic Mapping of Human Chromosomes	643
	Genetic Polymorphism	643
	Restriction Fragment Length Polymorphism	644
	Short Tandem Repeat Polymorphism	647
	Comprehensive Human Linkage Maps	650
	Mapping of a Genetic Disease Locus to a Chromosome Location	650
	Whole-Genome BAC Map	652
	Radiation Hybrid Mapping	653
	Human Genome Sequence	655
	Detection of Mutations in Human Genes	657
	Single-Strand Conformation Analysis	657
	Denaturing Gradient Gel Electrophoresis	659
	Heteroduplex Analysis	660
	Chemical Mismatch Cleavage	661
	Direct DNA Sequencing	663
	Determining Gene Function	663
	SUMMARY	668
	REFERENCES	669
	REVIEW QUESTIONS	670

IV Regulating and Patenting Molecular Biotechnology 671

21 Regulating the Use of Biotechnology 673

Regulating Recombinant DNA Technology	674
Regulating Food and Food Ingredients	676
Chymosin	676
Tryptophan	677
Bovine Somatotropin	678
Deliberate Release of GMOs	680
Ice-Minus <i>P. syringae</i>	680
Open-Field Tests of Other GMOs	682
Controversy about GMOs	685
Human Gene Therapy	688
Development of a Policy for Somatic Cell Gene Therapy	688
Accumulation of Defective Genes in Future Generations	691
Human Germ Line Gene Therapy	692
Cloning Humans?	692
SUMMARY	693
REFERENCES	694
REVIEW QUESTIONS	695

22 Patenting Biotechnology Inventions 697

Patenting	698
Patenting in Different Countries	700
Patenting DNA Sequences	701
Patenting Multicellular Organisms	702
Patenting and Fundamental Research	703
SUMMARY	704
REFERENCES	705
REVIEW QUESTIONS	705

Glossary 707

Index 733