Contents

Preface to the Fifth Edition xvii

1 **The Development of Molecular Biotechnology** 1

Emergence of Molecular Biotechnology 1

Recombinant DNA Technology 3

Commercialization of Molecular Biotechnology 4

Concerns and Consequences 7

SUMMARY 9

REFERENCES 9

REVIEW QUESTIONS 10

2 **Fundamental Technologies** 11

Molecular Cloning 11

Preparation of DNA for Cloning 11

Insertion of Target DNA into a Plasmid Vector 16

Transformation and Selection of Cloned DNA in a Bacterial Host 20

Cloning Eukaryotic Genes 24

Recombinational Cloning 28

Genomic Libraries 30

Genome Engineering using CRISPR Technology 32

Polymerase Chain Reaction 35

Amplification of DNA by PCR 36

Cloning PCR Products 39

Quantitative PCR 39

Chemical Synthesis of DNA 42

Synthesis of Oligonucleotides 42

Assembling Oligonucleotides into Genes 48

Gene Synthesis by PCR 50

DNA Sequencing Technologies 50

Dideoxynucleotide Procedure 53

Pyrosequencing 55

Sequencing using Reversible Chain Terminators 57

Sequencing by Single Molecule Synthesis 57

Sequencing Whole Genomes 59
Preparation of Genomic DNA Sequencing Libraries 60
High-Throughput Next-Generation Sequencing Strategies 61
Genome Sequence Assembly 63
Sequencing Metagenomes 64
Genomics 64
Transcriptomics 67
Proteomics 72
Metabolomics 85
SUMMARY 87
REFERENCES 89
REVIEW QUESTIONS 91

3 Production of Recombinant Proteins 93
Protein Production in Prokaryotic Hosts 93
Regulation of Transcription 94
Increasing Translation Efficiency 98
Increasing Protein Stability 102
Increasing Protein Secretion 106
Facilitating Protein Purification 110
DNA Integration into the Host Chromosome 115
Heterologous Protein Production in Eukaryotic Cells 120
Posttranslational Modification of Eukaryotic Proteins 120
General Features of Eukaryotic Expression Systems 122
Yeast Expression Systems 124
Baculovirus–Insect Cell Expression Systems 136
Mammalian Cell Expression Systems 143
Protein Engineering 153
Directed Mutagenesis 154
Random Mutagenesis 158
Examples of Protein Engineering 162
SUMMARY 171
REFERENCES 173
REVIEW QUESTIONS 175

4 Molecular Diagnostics 177
Immunological Approaches to Detect Protein Biomarkers 178
Antibodies 178
Agglutination 183
Enzyme-Linked Immunosorbent Assays 183
Protein Arrays to Detect Polygenic Diseases 189
Immunoassays for Protein Conformation-Specific Disorders 191
DNA-Based Diagnostic Approaches 193
Hybridization Probes 193
PCR-Based Detection Methods 200
DNA Microarrays 208
Whole Genome Sequencing to Assess Genetic Disease Risk 214

Detecting RNA Signatures of Disease 215
Detection of Disease-Associated Changes in Gene Expression Using Microarrays 215
Detection of RNA Signatures of Antibiotic Resistance in Bacteria 216
Detection of miRNA Signatures of Cancers 217

Biofluorescent and Bioluminescent Systems 219
Fluorescent Proteins 219
Luciferase 221
Microbial Biosensors 222

SUMMARY 224
REFERENCES 225
REVIEW QUESTIONS 227

5 Protein Therapeutics 229

Pharmaceuticals 230
Human Interferons 231
Human Growth Hormone 235
Tumor Necrosis Factor Alpha 237
Extending Protein Half-Life 238

Enzymes 240
DNase I 240
Alginate Lyase 242
Phenylalanine Ammonia Lyase 245
α_1-Antitrypsin 247
Glycosidases 248
Masking Nonhuman Epitopes 249
Engineering Bacteriophages 250
Targeting Mitochondria 253

Lactic Acid Bacteria 255
Interleukin-10 255
Leptin 258
An HIV Inhibitor 258
Insulin 260

Recombinant Antibodies 261
Hybrid Human–Mouse Monoclonal Antibodies 264
Human Monoclonal Antibodies 268
Antibody Fragments 270
Combinatorial Libraries of Antibody Fragments 274
A Combinatorial Library of Full-Length Antibodies 277
Shuffling CDR Sequences 278
Dual-Variable-Domain Antibodies 280
Anticancer Antibodies 281
Antibodies Against Various Diseases 284
Antiobesity Antibodies 287
Enhanced Antibody Half-Life 290

SUMMARY 292
REFERENCES 292
REVIEW QUESTIONS 295

6 Nucleic Acids as Therapeutic Agents 297

Targeting Specific mRNA and DNA Sequences 299
Antisense RNA 299
Aptamers 302
Ribozymes and DNAzymes 307
Interfering RNA 311
Zinc Finger Nucleases 315
CRISPR-Cas System 317
Nanozymes 318
Nanoparticles 319

Viral Delivery Systems 319

Nonviral Delivery Systems 325
Direct Injection 325
Lipids 327
Bacteria 328
Dendrimers 331
Antibodies 332
Aptamers 332
Transposons 334

Gene Therapy 335
Prodrug Activation Therapy 335
Promoterless Gene Targeting 337

SUMMARY 338

REFERENCES 339

REVIEW QUESTIONS 341

7 Vaccines 343

Vaccination 343

Current and Future Vaccines 345

Subunit Vaccines 347
Herpes Simplex Virus 348
Cholera 350
SARS 350
Staphylococcus aureus 351
Human Papillomavirus 353
Foot-and-Mouth Virus 354
Streptococcus 356
Delivery 357

Peptide Vaccines 359
Malaria 359

Genetic Immunization: DNA Vaccines 363
Delivery 364
Cancer 370
Dental Caries 370

Attenuated Vaccines 372
Herpes Simplex Virus 372
Cholera 374

Salmonella Species 375
Leishmania Species 378

Vector Vaccines 378
Vaccines Directed against Viruses 378
Vaccines Directed against Bacteria 388
Bacteria as Antigen Delivery Systems 392

Monoclonal Antibody Passive Immunity 396
Influenza Virus 396

SUMMARY 397

REFERENCES 398

REVIEW QUESTIONS 400

8 Industrial and Environmental Uses of Recombinant Microorganisms 403

Restriction Endonucleases 403

Small Biological Molecules 405
L-Ascorbic Acid 407
Indigo 410
Amino Acids 412
Lycopene 417
Antibiotics 418
Biopolymers 429

Microbial Degradation of Xenobiotics 434
Genetic Engineering of Biodegradative Pathways 436

Utilization of Starch and Sugars 445
Commercial Production of Fructose and Alcohol 446
Increasing Alcohol Production 448
Improving Fructose Production 453

Utilization of Cellulose and Hemicellulose 454
Lignocellulosics 455
Cellulase Genes 457
Direct Conversion of Biomass to Ethanol 462
Zymomonas mobilis 464

Lipids from Cyanobacteria 467

Hydrogen Production 468

SUMMARY 470

REFERENCES 471

REVIEW QUESTIONS 474

9 Large-Scale Production of Proteins from Recombinant Microorganisms 475

Principles of Microbial Growth 476
Batch Fermentation 477
Fed-Batch Fermentation 479
Continuous Fermentation 480

Maximizing The Efficiency of The Fermentation Process 481
High-Density Cell Cultures 483
Increasing Plasmid Stability 484
Quiescent *E. Coli* Cells 485
Protein Secretion 486
Reducing Acetate 489

Bioreactors 491

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 498

Harvesting Microbial Cells 501

Disrupting Microbial Cells 502

Downstream Processing 504
Protein Solubilization 506
Utilizing an Immobilized Enzyme 507
Magnetic Separation of Proteins 507

Large-Scale Production of Plasmid DNA 508

SUMMARY 511

REFERENCES 512

REVIEW QUESTIONS 514

10 Genetic Engineering of Plants: Methodology 515

Plant Transformation with the Ti Plasmid of *A. Tumefaciens* 516

Ti Plasmid-Derived Vector Systems 522

Microprojectile Bombardment 526

Chloroplast Engineering 527
Very High Level Protein Expression 529

Use of Reporter Genes in Transformed Plant Cells 532

Manipulation of Gene Expression in Plants 533
Transient Gene Expression 533
Plant Promoters 536
Targeted Gene Editing 538
Facilitating Protein Purification 539
Protein Glycosylation 541

Production of Marker-Free Transgenic Plants 542
Removing Marker Genes from Nuclear DNA 543
Removing Marker Genes from Chloroplast DNA 545

SUMMARY 546

REFERENCES 547

REVIEW QUESTIONS 549

11 **Transgenic Plants 551**

Insect Resistance 551
B. thuringiensis Insecticidal Toxin 551
Increasing Expression of the *B. thuringiensis* Protoxin 555
Other Strategies for Protecting Plants against Insects 558
Preventing the Development of *B. thuringiensis*-Resistant Insects 564
Targeting Aphids 569

Virus Resistance 570
Viral Coat Protein-Mediated Protection 570
Protection by Expression of Other Genes 574

Herbicide Resistance 578

Fungus and Bacterium Resistance 583

Salt and Drought Stress 588

Fruit Ripening and Flower Wilting 592

Modification of Plant Nutritional Content 594
Amino Acids 594
Lipids 595
Vitamins 599
Iron 601
Gluten 602

Modification of Food Plant Taste and Appearance 603
Preventing Discoloration 603
Starch 605

Plants as Bioreactors 608
Antibodies 608
Poly(3-hydroxybutyric Acid) 610

Eddible Vaccines 611

Plant Yield 615
Altering Lignin Content 615
Increasing Oxygen Content 618

SUMMARY 619

REFERENCES 620

REVIEW QUESTIONS 624

12 **Transgenic Animals 625**

Transgenic Mice: Methodology 626
DNA Microinjection Method 627
Retroviral Vector Method 629
Engineered Embryonic Stem Cell Method 631
Conditional Gene Inactivation with the Cre–loxP Recombination System 637
Genome Editing with the CRISPR-Cas System 641
Gene Knockdown by RNA Interference 643

Transgenic Mice: Applications 644
Transgenic Disease Models: Alzheimer Disease 644
Transgenic Mice as Test Systems 647
Control of Transgene Expression 651
Conditional Control of Cell Death 654

Transgenic Livestock 656
Cloning Livestock by Somatic Cell Nuclear Transfer 656
Production of Pharmaceuticals 658
Production of Donor Organs 660
Disease Resistant Livestock 661
Improving Milk Quality 664
Improving Animal Production Traits 665

Transgenic Poultry 669

Transgenic Fish 673

SUMMARY 676

REFERENCES 676

REVIEW QUESTIONS 678

13 Molecular Biotechnology and Society 679

Development of Guidelines for Recombinant DNA Research 680

Deliberate Release of Genetically Modified Microorganisms 682
Environmental Concerns 682
Regulations 683

Regulation of Genetically Modified Foods 684
Food Ingredients Produced by Genetically Engineered Microorganisms 684
Genetically Modified Crops 687
Genetically Engineered Livestock 691

Societal Concerns About Genetically Modified Foods 692
Alteration of Nutritional Content of Food 692
Potential for Introducing Toxins or Allergens into Food 696
Potential for Transferring Transgenes from Food to Humans or Intestinal Microorganisms 698
Controversy About the Labeling of Genetically Modified Foods 700
Impact of Genetically Engineered Crops on Biodiversity 700
Who Benefits from Production of Genetically Modified Foods? 703
Environmental Benefits of Genetically Modified Crops 704
How do Views about Genetically Engineered Organisms Impact Trade? 705

Regulation and Safety of Medical Products of Biotechnology 706
New Biological Drugs 706
Genetic and Genomic Testing 709
Economic Issues 711

Patenting Biotechnology 714
Patenting 714
Patenting in Different Countries 716
Patenting Nucleic Acid Sequences 717
Patenting Living Organisms 719
Patenting and Fundamental Research 720
SUMMARY 721
REFERENCES 722
REVIEW QUESTIONS 724

Amino Acids of Proteins and Their Designations 725

Index 727