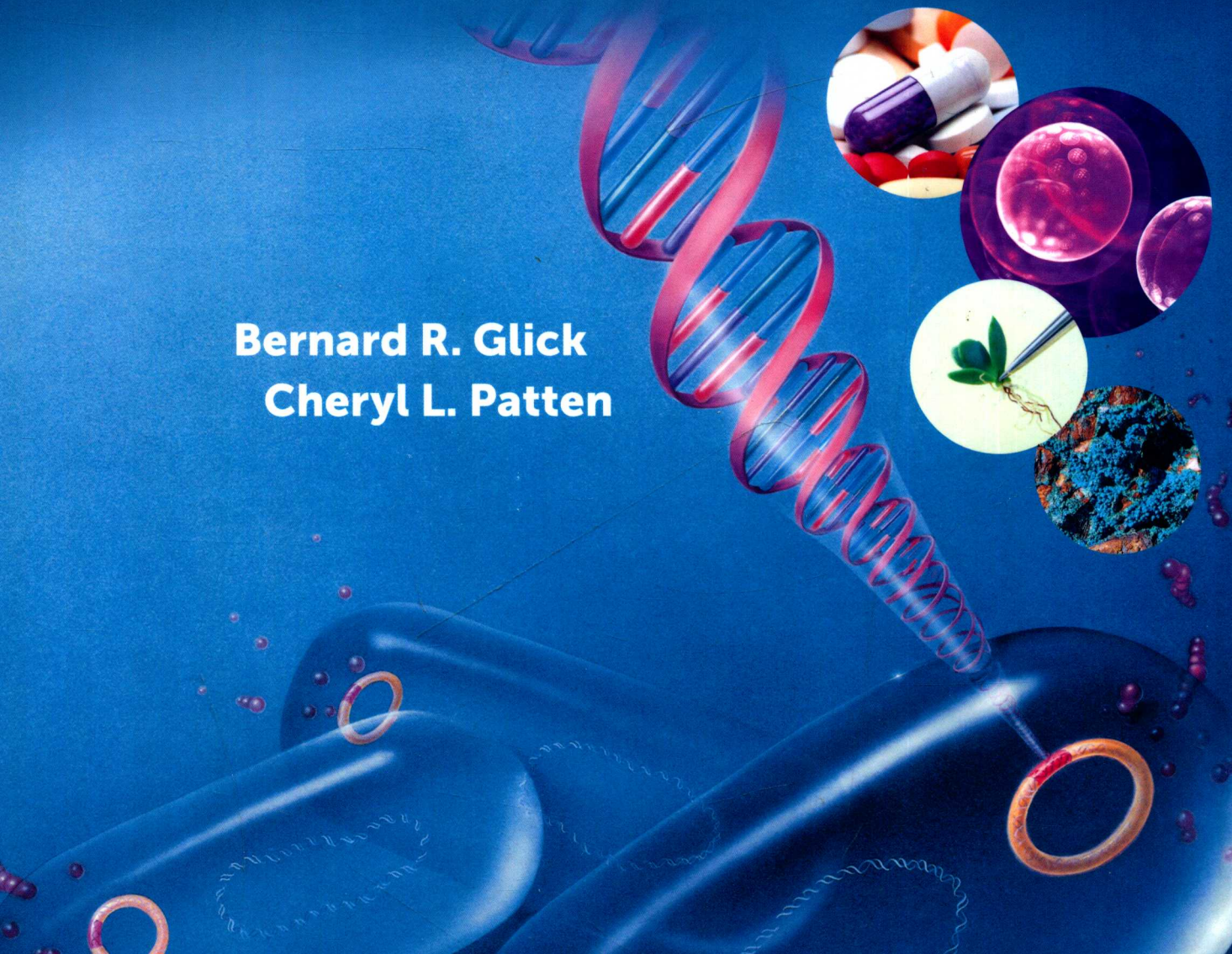


FIFTH EDITION

molecular biotechnology

Principles and Applications
of Recombinant DNA

Bernard R. Glick
Cheryl L. Patten



FIFTH EDITION

molecular biotechnology

Principles and Applications
of Recombinant DNA

Bernard R. Glick

Department of Biology, University of Waterloo
Waterloo, Ontario, Canada

Cheryl L. Patten

Biology Department, University of New Brunswick,
Fredericton, New Brunswick, Canada



WASHINGTON, DC

Copyright © 2017 American Society for Microbiology. All rights reserved.
No part of this publication may be reproduced or transmitted in whole or
in part or reused in any form or by any means, electronic or mechanical,
including photocopying and recording, or by any information storage and
retrieval system, without permission in writing from the publisher.

Disclaimer: To the best of the publisher's knowledge, this publication
provides information concerning the subject matter covered that is accurate
as of the date of publication. The publisher is not providing legal, medical, or other
professional services. Any reference herein to any specific commercial products,
procedures, or services by trade name, trademark, manufacturer, or otherwise
does not constitute or imply endorsement, recommendation,
or favored status by the American Society for Microbiology (ASM).
The views and opinions of the author(s) expressed in this publication
do not necessarily state or reflect those of ASM, and they shall not be
used to advertise or endorse any product.

Library of Congress Cataloging-in-Publication Data

Names: Glick, Bernard R., author. | Patten, Cheryl L., author.
Title: Molecular biotechnology : principles and applications of
recombinant DNA / Bernard R. Glick, Cheryl L. Patten.
Description: 5th edition. | Washington, DC : ASM Press, [2017]
Identifiers: LCCN 2017011321 | ISBN 9781555819361 (hardcover)
Subjects: LCSH: Biotechnology. | Genetic engineering. | Molecular biology.
Classification: LCC TP248.2 .G58 2017 | DDC 660.6—dc23
LC record available at <https://lcn.loc.gov/2017011321>

All Rights Reserved
Printed in Canada

10 9 8 7 6 5 4 3 2 1

Address editorial correspondence to
ASM Press, 1752 N Street NW
Washington, DC 20036-2904, USA

Send orders to ASM Press, P.O. Box 605, Herndon, VA 20172, USA
Phone: 800-546-2416; 703-661-1593
Fax: 703-661-1501
E-mail: books@asmusa.org
Online: <http://www.asmscience.org>

Cover illustration: Terese Winslow, CMI
Cover and interior design: Susan Brown Schmidler
Illustrations: Patrick Lane, ScEYence Studios
Cover Photo Credits
Plant - © KYTan/Shutterstock.com
Cells - © Jurk Peter/Shutterstock.com
Pills - © RidvanArda/Shutterstock.com
Copper Bubbles - © tucsonazrealestateblog.com

FIFTH EDITION

molecular biotechnology

Principles and Applications
of Recombinant DNA

*The two of us thank our life partners, Marcia Glick and Patrick Patten,
for the enormous support and encouragement that they have
provided throughout this endeavor*

B. R. Glick
C. L. Patten

Preface to the Fifth Edition

BASED ON THE DEVELOPMENT OF RECOMBINANT DNA technology, molecular biotechnology emerged as a new research discipline in the late 1970's. Since those early days, there has been a veritable explosion of knowledge in the biological sciences. With the advent of PCR, chemical DNA synthesis, DNA sequencing, monoclonal antibodies, directed mutagenesis, genomics, proteomics, metabolomics, and more recently, specific genome modification techniques, our understanding of and ability to manipulate the biological world has grown exponentially. When the first edition of *Molecular Biotechnology: Principles and Applications of Recombinant DNA* was published in 1994, nearly all of the transgenic organisms that were produced included only a single introduced gene. Now, 23 years later it is common for researchers to engineer organisms by both modifying the activity and the regulation of existing genes and also by introducing entire new pathways. In 1994, only a handful of products produced by this new technology had been commercialized. Today, as a consequence of molecular biotechnology hundreds of new therapeutic agents are available in the marketplace with many more in the pipeline as well as dozens of transgenic plants. DNA technologies have become a cornerstone of modern forensics, paternity testing and ancestry determination. A number of new recombinant vaccines have been developed, with many more on the horizon. The list goes on and on. Molecular biotechnology has clearly lived up to its promise and all of the original hype that has existed since the late 1970s. Worldwide there are several thousand biotechnology companies, in virtually every corner of the globe, employing hundreds of thousands of scientists. When the exciting science being done at universities, government labs and research institutes around the world is factored in, the rate of change and of discovery in the biological sciences is absolutely astounding. This fifth edition of *Molecular Biotechnology*, building upon the fundamentals that were established in the previous four editions, endeavors to provide readers with a window on some of the major developments in this growing field. Given the enormity of the field of molecular biotechnology, we have had to be highly selective in the material we included in this edition. Moreover, the window that we are looking through is moving. This notwithstanding, we both expect and look forward to the commercialization of many of the discoveries that are discussed here, and in the future to the development of many new approaches, insights, and discoveries.

We have throughout endeavored to make the text reader friendly by minimizing the use of technical jargon and unnecessary abbreviations. Moreover, when an important term appears for the first time in the text, it is followed in parentheses with a synonym or brief explanation. The overall size of this edition has been pared down significantly compared to the fourth edition, done, in large measure, by removing some older material that has come to be common knowledge within the past 10–20 years. In addition, to facilitate the book's flow and ease of understanding, in a number of instances, two or more figures have been combined into a single figure. Endeavoring to be as up-to-date as possible, this edition expands the discussion of interfering RNA and explains CRISPR technology in detail, providing examples of their use in both gene therapy and transgenic plants.

Each chapter opens with an outline of topics and concludes with a summary and list of review questions to sharpen students' critical thinking skills. All of the key ideas in the book are illustrated by the more than 500 full-color figures and elaborated in more than 80 tables. After introducing molecular biotechnology as a scientific and economic venture in Chapter 1, the next two chapters explain the detailed methodologies of molecular biotechnology. These chapters provide a solid scientific base for the remainder of the book. Chapters 4 to 8 present examples of microbial molecular biotechnology covering such topics as the production of metabolites, new vaccines, both protein and nucleic acid therapeutic agents, diagnostics, bioremediation, and biomass utilization. Chapter 9 describes some of the key components of large-scale fermentation processes using recombinant microorganisms. Chapters 10 to 12 describe the molecular biotechnology of plants and animals. The book concludes in Chapter 13 with a discussion of the interaction of molecular biotechnology with society including controversies that have occurred as a consequence of this technology, coverage of the regulation of molecular biotechnology and patents.

Throughout the text we have relied extensively upon the recent published work of many researchers. In all cases, although not cited directly in the body of a chapter, the original published articles are cited in the references section of the appropriate chapter. In some cases, we have taken "pedagogic license" and either extracted or reformulated data from the original publications. Clearly, we are responsible for any distortions or misrepresentations from these simplifications, although we hope that none has occurred. The references sections also contain other sources that we used in a general way, which might, if consulted, bring the readers closer to a particular subject.

BERNARD R. GLICK
CHERYL L. PATTEN

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
	<i>Staphylococcus aureus</i> 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**

The Development of Molecular Biotechnology



**Emergence of Molecular
Biotechnology**
Recombinant DNA Technology
**Commercialization of Molecular
Biotechnology**
Concerns and Consequences
SUMMARY
REFERENCES
REVIEW QUESTIONS

Emergence of Molecular Biotechnology

LONG BEFORE WE KNEW that microorganisms existed or that genes were the units of inheritance, humans looked to the natural world to develop methods to increase food production, preserve food, and heal the sick. Our ancestors discovered that grains could be preserved through fermentation into beer, that storing horse saddles in a warm, damp corner of the stable resulted in the growth of a saddle mold that could heal infected saddle sores, that intentional exposure to a “contagion” could somehow provide protection from an infectious disease on subsequent exposures, and that plants and animals with enhanced production traits could be developed through cross breeding. Following the discovery of the microscopic world in the 17th century, microorganisms have been employed in the development of numerous useful processes and products. Many of these are found in our households and backyards. Lactic acid bacteria are used to prepare yogurts and probiotics, insecticide-producing bacteria are sprayed on many of the plants from which the vegetables in our refrigerator are harvested, nitrogen-fixing bacteria are added in the soil used for cultivation of legumes, the enzymatic stain removers in laundry detergent come from a microorganism, and antibiotics that are derived from common soil microbes are used to treat infectious diseases. These are just a few examples of traditional biotechnologies that have improved our lives. Up to the early 1970s, however, traditional biotechnology was not a well-recognized scientific discipline, and research in this area was centered in departments of chemical engineering and occasionally in specialized microbiology programs.

In a broad sense, biotechnology is concerned with the manipulation of organisms to develop and manufacture useful products. The term “biotechnology” was first used in 1917 by a Hungarian engineer, Karl Ereky, to describe an integrated process for the large-scale production of pigs by