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
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
Contents

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

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

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

## 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
- α1-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
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
    Nanobodies 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

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
Contents

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

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

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

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
Contents

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

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
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
## Index

### A

**Acetobacter**, 407

**Acetobacter woodii**, formate synthesis, 469–470

**Acetoacterium woodii**, for mating, 469–470

**Acetobacter**, 407

**Acremonium chrysogenum**, 484

**Actinorhodin**, 424, 428

**Acute myeloid leukemia**, nucleic acid-based test, 710

**Acyclovir**
- chemical structure of, 286
- herpes simplex viruses, 284–285, 286

**Adalimumab (Humira)**, economics of, 711

**Adeno-associated viruses (AAV)**
- gene therapy with promoterless gene, 337
- life cycle of, 322
- viral delivery, 320, 321, 323
- Adenoviruses, viral delivery, 320, 323–324
- Adotrastuzumab emtansine (Kadcyla), 707

**Adenoviruses**, viral delivery, 320, 323–324

**Adotrastuzumab emtansine (Kadcyla)**, 707

**Agaricus bisporus**, 604–605

**Agglutination**, 183, 184

**Agrobacterium radiobacter**, 684

**Agrobacterium tumefaciens**
- bio synthesis of auxin and cytokinin, 521
- crown gall formation, 518, 520
- gene expression, 534–535
- genetic engineering, 516, 518–522, 546–547
- infection of plant, 558, 560
- plant transformation with Ti plasmid of, 516, 518–522
- Agropine, chemical structure of, 522
- **AIDS** (acquired immunodeficiency syndrome), 297
- antivirals for treatment of, 712
- phosphorothioate antisense oligonucleotide for, 301
- see also **Human immunodeficiency (HIV)/AIDS**

**Airlift reactors**, 491, 493–494
- configuration, 492
- two-stage, 497
- two-stage fermentation in, 495–496
- see also **Bioreactors**

**Aldotratuzumab emtansine (Kadcyla)**, 707

**Aequorea victoria**, 219, 540

**A β**, 540

**Agropine**, chemical structure of, 522

**AIDS** (acquired immunodeficiency syndrome), 297

**Aldotratuzumab emtansine (Kadcyla)**, 707

**Amino acids**
- L-citrulline, 415–416
- commercial applications of, 412
- L-cysteine, 413–414
- genetic code in **E. coli** and humans, 101
- modified Corynebacterium, 416–417
- mutant proteins with unusual, 156–158
- PEST sequences, 106
- plant nutritional content, 594–595, 596
- proteins and designations, 725
- small biological molecules, 412–417
- L-valine, 414–415
- Amoxicillin, 394
- Ampicillin, 21, 23, 142
- resistance to, 117
- vector coding resistance, 100
- Ampicillin resistance gene, 18
- AmpliChip CYP450 microarray, 208–209, 215, 225

**α-Amylase inhibitor**, protecting plants against insects, 559

**Amyloid plaques**, 492, 645

**Amylose**
- enzymatic hydrolysis of, 446
- portion of chain showing linkages, 605

**Ancestry determination**, 212–213

**Animal and Plant Health Inspection Service**, 605

**Animals**
- genetic improvement, 625–626
- microarrays for determination, 214
- see also **Transgenic livestock; Transgenic mice**

**Anthonomus grandis grandis**, 560

**Anthrax**
- antibodies against, 286–287
- flowchart of monoclonal antibody against, 288

**Antibiotics**
- actinorhodin, 427–429
- altered erythromycin derivatives, 422, 424
- assembly line biosynthesis of erythromycin A, 422, 423
- biosynthesis genes, 420–421
- clinical and agricultural use of, 252–253
- cystic fibrosis, 240, 251–252
- engineering polyketide, 421–423
- gene clusters for biosynthesis of aromatic polyketide, 422, 424
- Helicobacter pylori resistance, 394
- overproducing, 427–429
- penicillin, 418
- recombinant DNA technology, 418–420
- ribocil, 426–427
- small molecules, 418–429
- staphyloxanthin, 424, 425–426
- Streptomyces strains, 419
- undecylprodigiosin, 420, 421
- unique, 424–427

**Amylase**
- enzymatic hydrolysis of, 446
- portion of chain showing linkages, 605

**Amyotrophic lateral sclerosis**, 297

**Anthrax**
- antibodies against, 286–287
- flowchart of monoclonal antibody against, 288

**Antibiotics**
- actinorhodin, 427–429
- altered erythromycin derivatives, 422, 424
- assembly line biosynthesis of erythromycin A, 422, 423
- biosynthesis genes, 420–421
- clinical and agricultural use of, 252–253
- cystic fibrosis, 240, 251–252
- engineering polyketide, 421–423
- gene clusters for biosynthesis of aromatic polyketide, 422, 424
- Helicobacter pylori resistance, 394
- overproducing, 427–429
- penicillin, 418
- recombinant DNA technology, 418–420
- ribocil, 426–427
- small molecules, 418–429
- staphyloxanthin, 424, 425–426
- Streptomyces strains, 419
- undecylprodigiosin, 420, 421
- unique, 424–427
CRISPR (continued)
genome engineering using, 32–35, 88
testing of CRISPR-Cas system, 708
therapeutic agents, 299, 317–318, 338
Crohn disease, treatment for, 255–258, 711
Crops, genetically modified, 687–690
Dementia, 415
Delivery systems.
DDT (dichlorodiphenyltrichloroethane), 551
Cystic fibrosis, 177, 297, 712
Cyanovirin N, 259–260
Cyanophos, 442
Cupriavidus metallidurans, 431–432, 434
Cucurbita pepo, 586
Cryphonectria parasitica
Crops, genetically modified, 687–690
Crohn disease, treatment for, 255–258, 711
CRISPR
Sequencing
therapeutic agents, 299, 317–318, 338
testing of CRISPR-Cas system, 647–651
safety of CRISPR-Cas system, 708
genome engineering using, 32–35, 88
test of CRISPR-Cas system, 708
therapeutic agents, 299, 317–318, 338
Crohn disease, treatment for, 255–258, 711
Crops, genetically modified, 687–690
Dementia, 415
Delivery systems.
DDT (dichlorodiphenyltrichloroethane), 551
Cystic fibrosis, 177, 297, 712
Cyanovirin N, 259–260
Cyanophos, 442
Cupriavidus metallidurans, 431–432, 434
Cucurbita pepo, 586
Cryphonectria parasitica
Crops, genetically modified, 687–690
Crohn disease, treatment for, 255–258, 711
CRISPR
indirect ELISA, 184–185
measuring disease–associated proteins by
sandwich, 186–188
pregnancy test, 186, 187
sandwich ELISAs, 184–186, 187
Enzymes, 240–255
alginate lyase, 242–245
α-antitrypsin, 247–248
DNase I, 240, 241
engineering bacteriophages, 250–253
glycosidases, 248–249
masking nonhuman epitopes, 249–250
phenylalanine ammonia lyase, 245–247
preparing DNA for cloning, 15–16
replacement therapy, 254
targeting mitochondria, 253–255
zinc finger nucleases, 298–299, 315–317
Epigenetic biomarkers, detection of, 209–211
Epis gene, 699
Epstein-Barr virus, 273
Ereky, Karl, 1–2
Epstein-Barr virus, 273
Epsps gene, 699
Epigenetic biomarkers, detection of, 209–211
Epstein-Barr virus, 273
Ereky, Karl, 1–2
Erwinia herbicola, 407, 409
, 407–408
Erwinia, 407, 409
Ereky, Karl, 1–2
Erwinia herbicola, 407, 409
Erythromycin A, as sem bly bio syn the sis
Erythromycin, 394, 421–423
F
Fabry disease, 254
Fed-batch fermentation, 479–480
batch vs., 498–501
Federal Food, Drug and Cosmetics Act, 706, 707, 709
Federal Insecticide, Fungicide, and
Rodenticide Act (FIFRA), 683
Fermentation, 476, 511
batch, 477–479
batch vs. fed-batch, 498–501
bioreactors, 491–494
continuous, 480–481
disrupting microbial cells, 502–504
fed-batch, 479–480
harvest microbial cells, 501–502
high-density cell cultures, 483–484, 488
increasing plasmid stability, 484–485
large-scale systems, 494–501
maximizing efficiency of process, 481–491
protein secretion, 486, 488–489
quiescent E. coli cells, 485–486, 487
reducing acetate, 489–491
scheme for large-scale process, 477
soluble starch by yeast strains, 450
two-stage, in single STRs, 496–498
two-stage, in tandem airlift reactors, 495–496
Ferritin, iron storage protein, 602
Fish, transgenic, 673–675
Flavobacterium, 243, 244, 442
Flavobacterium genomic DNA library, 242
Flavobacterium okanokoites, 316
Flower wilting, 592–594
Fluorescent proteins, 219–221
Folate (vitamin B9), 600–601
Folding chaperones, protein, 103–104
Follicular thyroid cancer, 214–215
Fomivinsen (Vitrawene), for patient with
AIDS, 301
Food and Agriculture Organization of
United Nations, 518
Food plant
genetically modified crops, 518, 687–690
preventing discoloration, 603–605
starch, 605–608
taste and appearance, 603–608
Foot-and-mouth disease virus (FMDV), vac-
cine for, 354–356
Freonilicin, 424
Friends of the Earth, 697
Fruuctose
conversion of glucose to, 453–454
production of alcohol and, 446–448
Fruit ripening, 592–594
Fungal expression systems, recombinant
proteins, 136
Fungus and bacterium resistance
cauliflower mosaic virus 35S promoter, 585
chestnut blight, 586–587
fungal rice blast, 583
Fusarium, 585–586
Pierce disease, 587
salicylic acid, 583–585
transgenic plants, 583–588
Fusarium, pathogenic fungi, 585–586
Fusion proteins
construction of, 104–105
immunoaffinity chromatography, 110,
111, 113
G
Galanthus nivalis, 561
Gammaroviruses, viral delivery, 320–321
Ganciclovir, 336–337
Gardasil, subunit vaccine for HPV, 353–354
Gateway cloning technology, 28
Gaucher disease
gene sequencing, 215
imiglucerase (Cerezyme) for, 711
storage disease, 254
GenBank, 64
Gene cloning, 4
Gene expression
detecting disease-associated changes,
215–216
translation efficiency, 98–100, 102
Gene expression in plants
facilitating protein purification, 539–541
manipulation of, 533–542
plant promoters, 536–538
second generation transient–expression
vector system, 534–536
targeted gene editing, 538–539
transient gene expression, 533–536
Gene expression profiling
cirrhotic liver tissue, 69, 70
DNA microarrays, 67–70
RNA sequencing, 70–72
transcriptomics, 67–72
Genentech, 7, 268, 716–717

General Electric Corporation, 719
Generally recognized as safe (GRAS), 124
General secretory pathway (Gsp), 108, 109
Gene silencing, 311
Gene therapy, 297–299, 335–338
protoprotocol for creating XylS protein, 439, 440
manipulation by transfer of plasmids, 436–445
degradation of organophosphorus pesticides, 442–443
manipulation by gene alteration, 438–445
manipulation by transfer of plasmids, 437–438
protocol for creating XylS protein, 439, 440
tolerance- and xylene-degrading plasmid (pWWO), 438, 439
Genetic engineering of plants
Agrobacterium tumefaciens, 516, 518–522, 546–547
chloroplast engineering, 527–530
facilitating protein purification, 539–541
manipulation of gene expression, 533–542
microprojectile bombardment, 526–527
pharmaceutical proteins in transgenic plants, 516
plant promoters, 536–538
plant transformation with Ti plasmid of A. tumefaciens, 516, 518–522
production of marker-free transgenic plants, 542–546
protein glycosylation, 541–542
reporter genes in transformed plant cells, 532–533
strategy for overproduction of lysin protein, 530, 531
targeted gene editing, 538–539
Ti plasmid-derived vector systems, 522–526
transgenic tobacco plants, 530, 531
transient gene expression, 533–536
very high level protein expression, 529–530
Genetic immunization
advantages of, 364
delivery of, 364–370
DNA vaccines, 363–372
survival of DNA-immunized mice, 365
Genetic testing, 709–711
Genome engineering
bacterial CRISPR-Cas system, 33–34
CRISPR-Cas system, 34–35
using CRISPR technology, 32–35
Genomes
genetic disease risk, 214–215
Genome 10K project, 67
sequencing whole, 59–64, 214–215
synthetic, 43
Genomic DNA libraries, 30–32
cellulase genes, 457
construction of, 31
construction of metagenomic libraries, 65
DNA hybridization screening of, 459–461
generation of clusters of sequencing templates, 62
metagenomes, 64, 65
preparation of, sequencing libraries, 60–61
prey library, 83
screening of, 32, 33
Genomics, 64–87
DNA microarrays, 67–70
genome annotation, 66
metabolomics, 85–87
proteomics, 72–85
RNA sequencing, 70–72
testing, 709–711
transcriptomics, 67–72
see also Proteomics
Gentamicin, 142
German measles, vaccine for, 346
Ghrelin, antiobesity antibody, 289–290
Gilbert, W., 53
Gilead Sciences, 7
GlaxoSmithKline, 7
Glioma cells, insulin-like growth factor 1 mRNA, 299, 301
Global Alliance for Vaccines and Immunization, 345
Glomeromycota, 585
Gluconobacter, 407
Glucose
conversion to fructose, 453–454
pathways for metabolism in E. coli strain, 490
β-Glucosidase, 457, 459
Gluten, plant nutritional content, 602–603
GlycoDelete, 542
Glycosidases, 248–249
Glyphosate (Roundup), 579, 580, 705
Golden Rice, vitamin A, 599–600, 695
Gonsalves, Dennis, 575
Green fluorescent protein (GFP), 151–153
Griseusin, 424
Group B Streptococcus, subunit vaccine for, 356–357
Guillain-Barré syndrome, 707

H
Haemophilus influenzae, 5, 59
Hansenula polymorpha, 134, 135
Haplotypes, 212, 213
Hawaiian papaya industry, 575, 705
Heart disease, protein microarray, 190–191
Hedén, Carl Göran, 2
Helicobacter pylori, antigen delivery system, 394–395
Helicobacter virescens, 558
Helicobacter zea, 556
Helling, R. B., 3
Hemagglutinin, 219, 220
Hemagglutinin, 396, 397
Hemicecellulose. See Cellulose and hemicellulose
Hemicelluloses, 455, 456
Hemophilia, 297
Hepatic cancer, interfering RNAs, 314
Hepatitis B virus, interfering RNAs, 314
Hepatitis C virus, interfering RNAs, 314
Herculean resistance
elements of gene-based, 579
transgenic plants, 578–583
Hereceptin (trastuzumab), 229, 264
Herpes simplex virus, 24
Herpes simplex virus 1 (HSV-1) antibodies against, 284
attenuated vaccines for, 372–373
nucleic acid-based test for, 710
organization and structure of, 324
ribozyme inhibiting expression of, 309
subunit vaccine for, 348–349
viral delivery, 320, 324–325
Herpes simplex virus 2 (HSV-2) antibodies against, 284
attenuated vaccines for, 372–373
Leader peptide, 107
Legionella pneumophila, 194
Leishmania species, attenuated vaccines, 378, 379
Leucoma minor, 541, 542
Lentiviruses, viral delivery, 320, 321
Leptin, 258
Lignin, 455, 456
biosynthesis of, 615–616
content in plant yield, 615–617
Lignocellulose, 471
Lignocellulosics, 455–457
Listeria monocytogenes
Lipoamide dehydrogenase (LAD), mitochondrial, 245–255
Lipids
chemical structures of, 597
from cyanobacteria, 467–468
nonviral delivery, 327–328
plant nutritional content, 595–599
Lipoamide dehydrogenase (LAD), mitochondrial, 245–255
Listeria monocytogenes, 329
Listeria monocytogenes, 329
Liver, 225
Liposomes, 226
Liver, 225
Living organisms, patenting, 719
Livestock
growth hormone, 236
Macaca fascicularis, growth hormone, 236
Magnetic resonance imaging (MRI), 87
Mammalian cell expression systems, 143–153
chromosomal integration and environment, 151–153
engineering hosts for productivity, 148–151
expression vectors, 146–147
selectable markers for vectors, 146, 148
strategies increasing expression, 151–153
strategy increasing yields of recombinant cells, 149
strategy increasing yields of recombinant proteins from, 150
vector design, 144–146
MammaPrint diagnostic array, 215–216
Manduca sexta, 556
Manuscripts, vaccines, 347
Marker gene, removal of, 119–120
Marker gene systems, mammalian cells, 146, 148
Mass spectrometry (MS), matrix-assisted laser desorption ionization-time of flight (MALDI-TOF), 74, 75, 76
Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, proteins, 74, 75, 76
Maxam, A. M., 53
MDM2 (mouse double mutant 2 protein), engineering productivity, 148, 149
Medaka, transgenic, 674–675
Medical biotechnology products
economic issues, 711–714
genetic and genomic testing, 709–711
new biological drugs, 706–709
regulation and safety of, 706–714
Melanoma, 214
BRAF gene, 203, 204
MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke), 254
Merck, 7
Messenger ribonucleic acid (mRNA), 11
second structure of, 99
Metabolomics, 85–87, 89
Metagenomes, sequencing, 64, 65
Metastatic Ewing sarcoma, interfering RNAs, 314
Methanococcus jannaschii, mutant proteins of, 156–157
Methicillin-resistant Staphylococcus aureus (MRSA), 204–205
nucleic acid-based test for, 710
vaccine for, 351
Methylase, protecting DNA, 404
Metronidazole, 394
Microarrays, 224
ancestry determination, 212–213
animal species determination, 214
deoxyribonucleic acid (DNA), 67–70, 208–214, 224–225
detecting disease–associated gene expression changes, 215–216
detection of epigenetic markers, 209–211
detection of multiple alleles, 208–209
Microbial degradation
detoxification of organophosphate pesticides, 442–443
genetic engineering of biodegradative pathways, 436–445
manipulation by gene alteration, 438–445
manipulation by transfer of plasmids, 437–438
nitroaromatic compounds, 442–443, 444
organic compounds, 437
organic contaminants, 435–436
of xenobiotics, 434–445
Microbial growth
disrupting microbial cells, 502–504
downstream processing, 504–508
harvesting cells, 501–502
pattern of, in batch fermenter, 478
principles of, 476–481, 511
see also Fermentation
Microbiology, 6
Microinjection of DNA, transgenic mice, 627–629
Microprojectile bombardment, 526–527
MicroRNAs
detection of miRNA signatures of cancers, 217–219
production and activity of, 218
virus resistance, 576, 577
Microsatellite DNA pattern, forensic analysis of, 206, 207
Milk
production in transgenic livestock, 658–660
protein composition of, 626
quality improvement, 664–665
Minimalistic immunogenically defined gene expression (MIDGE) vectors, 369–370
Mipomersen, cholesterol-reducing drug, 301
Mitochondria, targeting proteins to, 253–255
Modified vaccinia Ankara virus (MVA), 385–386
Molecular beacons, 198–200, 201
Molecular biology, 6
Molecular biotechnology
bioreactors, 475–476
commercialization, 4–7
concerns and consequences, 7–9
emergence of, 1–3
history of, 5
scientific disciplines of, 6
Molecular cloning, 11–32
eukaryotic genes, 24, 26–28
insertion of target DNA into plasmid vector, 16–20
preparation of DNA for, 11–16
recombinational cloning, 28–30
transformation and selection of cloned DNA in bacterial host, 20–24
Molecular diagnostics, 177
biofluorescent and bioluminescent systems, 219–224
detecting RNA signatures of disease, 215–219
DNA-based approaches, 192–215
immunological approaches for protein biomarkers, 178–192
see also Immunological approaches for diagnostics
Monoclonal antibodies, 180–183
antibody fragments, 270–274
anticancer, 281–284
enhancing half-life of, 290–291
human, 268–269
hybrid human-mouse, 264–268
non-Hodgkin lymphoma treatment, 265
production of, 182
targeting tumor cells for destruction by,
281–284
targets for, 183
trastuzumab for breast cancer, 264
see also Antibodies
Monsanto, 7, 703
*Mortierella alpina*, 599
Mucosal immunity, DNA vaccines, 367
Mullis, Kary, 36
Multiple alleles, detection of, 208–209
Multiple-cloning site, 18
Multiple endocrinopathy, 254
Multiple sclerosis, 712
Mung bean nuclease, 15
Muscular dystrophies, direct injection for,
326
Mutagenesis
directed, 154–158
methodology of mutations, insertions or
deletions, 156
mutant proteins with unusual amino
acids, 156–158
random, 158–162
see also Protein engineering
Mutant protein p53, aptamer against,
305–307
*Mycobacterium bovis*, 389
*Mycobacterium tuberculosis*
anti-bacterial resistance of, 216–217
antibiotics for, 389
low dose antibiotics, 713
*Mycoplasma genitalium*, 43
*Mycoplasma mycoides*, 43
Myeloma-spleen fusion cells, 181
Myopathy (muscle disease), 254
Myriad Genetics Inc., 717, 718

N
Nanoparticles, therapeutic agents, 319
Nanopatch, vaccine delivery device,
357–359
Nanoyzymes, therapeutic agents, 318–319
National Association of Boards of
Pharmacies, 713
*Nature* (journal), 680, 702
*Neisseria gonorrhoeae*, 194, 710
Neoschizomers, 14, 15
Nicotiana benthamiana, 575
Nicotiana plumbaginifolia, 541
Nicotine, biosynthesis of, 619
Nocardia argentinensis, 15
Nocardia corallina, 15
Non-Hodgkin lymphoma, therapeutic
monoclonal antibodies, 265, 268
Nonhomologous random recombination,
162, 163
Nonviral delivery systems
antibodies, 332
aptamers, 332–334
bacteria, 328–331
dendrimers, 331, 332
direct injection, 325–327
lipids, 327–328
transposons, 334–335
*Nocardia corallina*, 15
*Nocardia argentinensis*, 15
Nicotiana plumbaginifolia
Nicotiana benthamiana, 575
Neoschizomers, 14, 15
*Nocardi a corallina*, 15
*Neisseria gonorrhoeae*, 194, 710
*Nontovus*, 14, 15
*Nopallone, chemical structure of*, 522
*Nostoc ellipsosporum*, 259
Novartis, 7
Nuclear magnetic resonance (NMR) spec-
troscopy, 86, 87
Nucleic acids as therapeutics
antisense ribonucleic acid (RNA),
299–302
aptamers, 302–307
CRISPR-Cas system, 317–318
DNAzymes, 309–310
FDA-approved tests, 710
gene therapy, 335–338
interfering RNA, 311–315
nanoparticles, 319
nanozymes, 318–319
nonviral delivery systems, 325–335
ribozymes, 307–309
RNA-based drugs in clinical trials, 315
direct injection, 319–325
zinc finger nucleases, 298–301, 315–317
see also Nonviral delivery systems
Nucleic acid sequences, patenting, 717–719
Nutritional content of food, alteration of,
692–695
Nutritional content of plants
amino acids, 594–595, 596, 620
follate (vitamin B9), 600–601
gluten, 602–603
iron, 601–602
lipids, 596–599
vitamin A, 599–600
vitamins, 599–601
O
Obesity, antibodies against, 287–290
Obinutuzumab, non-Hodgkin lymphoma
treatment, 265, 268
Octopine, chemical structure of, 522
Octopine synthase expression, 519
*Oleispira antarctica*, 103
*Oleosins*, 540
Oligonucleotide ligation assay (OLA),
197–198
Oligonucleotide primers, random mutagen-
esis with degenerate, 160, 161
Oligonucleotides
assembling into genes, 48–49
assembly and in vitro enzymatic DNA
synthesis of gene, 49
flowchart for chemical synthesis of DNA,
44
synthesis of, 42–48, 88
yields of chemically synthesized, 48
Oligosaccharides
glycosylations, 122
N-linked, 122, 123
O-linked, 122
Oncomouse, 719, 720
Online Mendelian Inheritance in Man, 214
Organic molecules, biodegradation of,
435–436
Organization for Economic Co-operation
and Development, 688
Organophosphate pesticides, detoxification of,
442–443
Orphan drugs, 712, 713
*Oryza sativa*, vitamin A in rice, 599
Osmoprotectants, 588
Ovarian cancer
monoclonal antibodies, 282–283, 284
sandwich ELISA, 186–188
Oxygen content, plant yield, 618–619
P
Padlock probes, 198, 199
*Panicum virgatum*, 617
*Pantoea agglomerans*, 418
Papaya ringspot virus (PRSV), 575
Paraoxon, 442
Parathion, methyl and ethyl, 442
Parkinson’s disease, 191, 214, 415
Pasteur, Louis, 719
Patenting biotechnology, 714–721, 722
categories of patents, 714–715
criteria for, 715–716
in different countries, 716–717
fundamental research and, 720–721
living organisms, 719
nucleic acid sequences, 717–719
OncoMouse, 720
Pathogenesis-related (PR) proteins, 583–585
*Pectinaphora gossypii*, 569
Pegaptanib, aptamer targeting vascular
endothelial growth factor (VEGF),
305
Penicillin, 418
discovery of, 250–251
*Penicillium*, 136
*Penicillium chrysogenum* strains, 427
Peptide vaccines
malaria, 359–362
see also Vaccines
Pfizer, 7
Pharmaceutical companies, 229, 712–713
Pharmaceutical proteins, transgenic plants,
516
Pharmaceuticals
clinical trials, 234
human growth hormone, 235–237
human interferons, 231–233, 235
production using transgenic livestock,
658–660
protein therapeutics, 230–240
tumor necrosis factor alpha (TNF),
237–238
*Phaseolus vulgaris*, 541, 559
identification of proteins, 73–74, 76
peptide mass fingerprinting, 74, 75
protein expression profiling, 76–79
protein-protein interactions, 80–85
two-dimensional polyacrylamide gel electrophoresis (2D PAGE), 73, 74
Prototeras adjacent motif (PAM), 34
Protospacers, 33
*Pseudomonas*, 410
*Pseudomonas aeruginosa*
alginate excretion, 242–243, 245
cystic fibrosis, 251–252
*Pseudomonas alcaligenes*, 442
*Pseudomonas diminuta* MG, 442
*Pseudomonas exotoxin A*, 272
*Pseudomonas fluorescens*, 93
*bio luminescence*, 222
*Pseudomonas olieverae*, 433
*Pseudomonas plasmids*, degradation and sizes, 435
*Pseudomonas pseudalcaligenes*, 444–445
*Pseudomonas putida*, 416
PSMA (prostate specific membrane anti-gens), prostate cancer, 307
Public Health Service Act, 707, 709
pUC19 cloning vector
*E. coli* host cells, 21, 22–23
plasmid, 18–20
Purification tags, recombinant proteins, 110–115
Pyrosequencing, deoxyribonucleic acid (DNA), 55–57

**R**
*Ralstonia eutropha*, protein secretion yields, 107
Random mutagenesis
amino acid changes, 161
with degenerate oligonucleotide primers, 160, 161
deletion, 158–160
DNA shuffling, 160–162
error–prone PCR, 158
insertion, 158–160
nonhomologous random recombination, 162, 163
*see also* Protein engineering
*Raphanus sativus*, 586
Reactors. *See Bioreactors*
Recombinant antibodies
antibodies against diseases, 284–287
antibody fragments, 270–274
anticancer antibodies, 281–284
chimeric antibody, 265, 266
combinatorial libraries of antibody fragments, 274–277
combinatorial library of full-length antibodies, 277, 278
complementarity-determining regions (CDRs) of mouse, 265, 266, 267
dual-variable-domain antibodies, 280
genetically engineered antibodies, 266
humanized antibody, 265, 266, 267, 267–268
human monoclonal antibodies, 268–269
hybrid human-mouse monoclonal anti-bodies, 264–268
shuffling CDR sequences, 278–280
therapeutic monoclonal antibodies, 263
therapy, 261, 263–264
Recombinant DNA Molecule Program Advisory Committee (RAC), 681
Recombinant DNA molecules, potential biohazards of, 680
Recombinant DNA research, development of guidelines for, 680–681
Recombinant DNA technology, 2, 3–4, 9
cellulose and hemicellulose, 454–467
hydrogen production, 468–470
lignocelluloses, 455–457
lipids from cyanobacteria, 467–468
microbial degradation of xenobiotics, 434–445
new generation of vaccines, 346–347, 397
restriction endonucleases for, 403–405
small biological molecules, 405–434
starch and sugars, 445–454
*see also* Small biological molecules
Recombinant human proteins, production, 94
Recombinant mammalian cells, strategy for increasing yields, 148, 149
Recombinant proteins
examples for human use, 230
fungal expression systems, 136
*Saccharomyces cerevisiae* expression systems, 125
Recombinational cloning, 28–30
Red fluorescent protein (RFP), 220–221
Regulations
genetically modified microorganisms, 683–684
medical biotechnology products, 706–714
Reporter genes, in transformed plant cells, 532–533
Respiratory syncytial virus, interfering RNAs, 314
Restriction endonuclease 11–13, 87–88
DNA cleavage by, 12
ligation of DNA fragments, 17
name of, 13
recognition sequences of, 12
recombinant DNA technology, 403–405
strategy for isolating genes for, 404–405
Retroviral RNA, reverse transcription of, 301–302
Retroviral vector method, transgenic mice, 629–631, 632
Retromiridae, 320
Retroviruses, antisense RNA, 301–302
Reversible chain terminators, DNA sequencing with, 57, 58
Rheumatoid arthritis
adalimumab (Humira), 229
antibodies against, 285–286
indirect ELISA, 189
*Rhizobium etli*, 489, 490
*Rhizopus*, 136
Rhizosecretion, transgenic plants, 540–541
*Rhodospirillum toruloides*, 247
Riboflavin (vitamin B2), 426–427
Ribonucleic acid (RNA)
antibiotic resistance in bacteria, 216–217
antisense, 299–302
detecting RNA signatures of disease, 215–219
detection of miRNA signatures of can-cers, 217–219
gene knockdown by RNA interference, 643, 644
insect resistance, 562–564
interfering, 311–315
sequencing, 70–72
short hairpin (shRNAs), 312, 314
small interfering (siRNAs), 311–315, 327–328
*see also* MicroRNAs; Transfer RNAs (tRNAs)
Ribosomal RNA (rRNA), 24
Ribozymes, 298, 307–309
Rice
genetically modified crops, 518
vitamin A, 599–600
*Rickettsia rickettsii*, 389
Rituximab (Rituxan), 229
anticancer antibody, 281
non-Hodgkin lymphoma treatment, 265, 268
RNA interference (RNAi), 311–315
Roundup (glyphosate), 579, 580
*Saccharomyces cerevisiae*, 43, 223, 417
alcohol production, 449, 452, 465
expression of cloned eukaryotic genes, 124
expression vectors, 125–126
integration of DNA in, 127
secretion of heterologous proteins by, 126–131
yeast expression, 124
yeast strains for HPV vaccine, 353
*Saccharomyces diastaticus*, 450
*Saccharomyces fibuliger*, 461
*Saccharopolyspora erythraea*, 422, 484
Safety, medical biotechnology products, 706–714
Salmon
nutritional content comparison, 694
transgenic fish, 625, 675–675
*Salmoneella*, 329, 330
antigen delivery system, 392–394
attenuated vaccines, 375–378
deleted genes and functions in attenuated strains, 377
*Salmoneella enterica*, 41
Salmonella enterica serovar Typhi, 194, 367
Salmonella typhimurium, 392
Salt and drought stress, transgenic plants, 588–592
Sanger, Frederick, 50, 53
Science (journal), 680
Scrapie, disease resistant livestock, 662
SELEX (systematic evolution of ligands by exponential enrichment), selecting aptamers, 303–304
Self-cleaving protein, purification tags, 7–11
Sequencing whole genomes, 59–64
High-throughput next-generation sequencing, 61–62
Metagenomes, 64
Preparation of genomic DNA sequencing libraries, 60–61
Serratia fonticola, 15
Severe acute respiratory syndrome (SARS), 15
Severe acute respiratory syndrome coronavirus (SARS-CoV), 142
Severe acute respiratory syndrome (SARS), subunit vaccine for, 350–351
Shiga toxin, 613–614
Shigella flexneri
Small interfering RNAs (siRNAs), 311–315, 327–328
Smallpox vaccine, 346
Sodium bisulfite, cytosine to uracil, 210
Somatostatin, 297–298
Somatic cell nuclear transfer, cloning livestock, 656–658
Spacers, 33
Spleen-myeloma fusion cells, 181
Spondyloptosis frugiperda, 137, 142
Stabilizing and antirepressor (STAR) elements, insertion, 152–153
Staphylococcus aureus, 318, 323, 330
Development of vaccine against, 352
disease resistant livestock, 663
methylcillin-resistant (MRSA), 204–205, 351
protein A, 277, 278
subunit vaccine for, 351–353
Staphylococcus carnosus, protein secretion yields, 107
Staphyloxanthin, 424–426
Starch
Crop plants, 605–608
Reactions in biosynthesis of, 606
Starch and sugars
Alcohol tolerance, 451–453
Amylose, 445–446
Commercial production of fructose and alcohol, 446–448
Improving fructose production, 453–454
Increasing alcohol production, 448–453
Utilization of, 445–445
StarLink corn, 697–698
Streptococcus
Subunit vaccines, 357–359
Streptococcus agalactiae, 356–357, 530
Streptococcus mutans, cause of dental caries, 370–372
Streptococcus pneumoniae, 34, 317, 323
Streptococcus sobrinus, cause of dental caries, 370–372
Streptokinase, protease sensitivity, 170–171
Streptomyces, antibiotics, 419–421, 427–429
Streptomyces lividans, protein secretion yields, 107
Streptomyacin, 23
Discovery of, 250–251
Subtilisins, modifying, 167–169
Subunit vaccines, 347–359
Cholera, 350
Delivery of, 357–359
Foot-and-mouth disease, 354–356
Herpes simplex virus (HSV), 348–349
Human papillomavirus (HPV), 353–354
Injection techniques, 358
Tetracycline, 23, 142
Tetrahydrofolate, 600–601
Tetraploid wheat, 412–413
Tetrahydropalmatine, 424
Thymidine kinase gene, 626
Thermus thermophilus, 454, 607
Thermus thermophilus, 454
Thermus thermophilus, 454
Tobacco, transgenic plants, 530, 531
Toxic Substances Control Act (TSCA), 683
Toxins, potential for introducing into food, 696–698
Trade, impact of genetically modified organisms, 705–706
Transcription, regulation of, 94–98
Transcription activator-like effector nucleases (TALEN), 668, 669, 688
Transcriptional activator of transcription (TAT) peptide, 253, 254
Transcriptomics, 67–72, 89
DNA microarrays, 67–70
RNA sequencing, 70–72
Transfer RNAs (tRNAs), 25
Mutant proteins, 156–157
Production of, 99–100, 102
Transgenes, potential for transferring from food to humans, 698–699
Transgenesis, 626
Animals, 625, 676
Engineering, chickens, 670–672
Transgenic crops, 687–690
Impact on biodiversity, 700–703
Nutritional content, 692–695
Transgenic fish, 673–675
Transgenic livestock, 656, 676

T
T4 DNA polymerase, 159
T4 polynucleotide kinase, 15, 16
Tandem affinity purification tag procedure, protein interactions, 83–85
T-DNA (transferred DNA)
Crown gall formation, 518–522
Expression of B. thuringiensis protoxin, 555–556
Tetanus, vaccine for, 346
Tetracycline, 424
Tetracycline, 23
Tetrahydrofolate, 600–601
Tobacco, transgenic plants, 530, 531
Toxic Substances Control Act (TSCA), 683
Toxins, potential for introducing into food, 696–698
Trade, impact of genetically modified organisms, 705–706
Transcription, regulation of, 94–98
Transcription activator-like effector nucleases (TALEN), 668, 669, 688
Transcriptional activator of transcription (TAT) peptide, 253, 254
Transcriptomics, 67–72, 89
DNA microarrays, 67–70
RNA sequencing, 70–72
Transfer RNAs (tRNAs), 25
Mutant proteins, 156–157
Production of, 99–100, 102
Transgenes, potential for transferring from food to humans, 698–699
Transgenesis, 626
Animals, 625, 676
Engineering, chickens, 670–672
Transgenic crops, 687–690
Impact on biodiversity, 700–703
Nutritional content, 692–695
Transgenic fish, 673–675
Transgenic livestock, 656, 676
animal production traits, 665–669
cloning by somatic cell nuclear transfer, 656–658
cloning sheep by nuclear transfer, 656, 657
disease resistant livestock, 661–664
donor organ production, 660–661
milk quality, 664–665
MSTN gene of sheep, 668–669
milk quality, 664–665

donor or gan production, 660–661
disease resistant livestock, 661–664
donor organ production, 660–661
milk quality, 664–665
MSTN gene of sheep, 668–669
milk quality, 664–665

Transgenic mice
conditional control of cell death, 654–655
cell death, 654–655
cell death, 654–655
cell death, 654–655
control of transgene expression, 651–654
dna microinjection method, 627–629
DNA microinjection method, 627–629
dna microinjection method, 627–629
expressed in chicken cells, 626
expression of herpes thymidine kinase in, 626
gene knockdown by RNA interference, 643, 644
gene knockdown by RNA interference, 643, 644
genome editing with CRISPR-Cas system, 641–643
methodology, 626–644
model for Alzheimer disease, 644–647
patenting, 719, 720
positive-negative selection procedure, 634, 635
retroviral vector method, 629–631, 632
as test systems, 647–651

Transgenic plants
as bioreactors, 608–611, 620
chloroplast engineering, 527–530
edible vaccines, 611–615
facilitating protein purification, 539–541
food plant taste and appearance, 603–608
fruit ripening and flower wilting, 592–594
fungus and bacterium resistance, 583–588
herbicide resistance, 578–583
insect resistance, 551–569
lignin content and yield, 615–617
nutritional content, 594–603
oleosins, 540
oxygen content and yield, 618–619
pharmaceutical proteins, 516
plant yield, 615–619
production of marker–free, 542–546
rhizoserection, 540–541
salt and drought stress, 588–592
therapeutic antibodies and fragments, 517
tumor-inducing (Ti) plasmid of Agrobacterium tumefaciens, 516, 518–522
tumor–inducing plasmid of Agrobacterium tumefaciens, 516, 518–522
tumor–inducing plasmid of Agrobacterium tumefaciens, 516, 518–522
tumor–inducing plasmid of Agrobacterium tumefaciens, 516, 518–522
vaccine antigens, 517
viral coat protein–mediated protection, 570–574
virus resistance, 570–577
Transgenic poultry, 669–672, 673
transgenic plants, 592
vaccines against HPV, 353–354
U.S. National Institutes of Health (NIH), 680–681
U.S. Office of Technology Assessment, 8
U.S. Orphan Drug Act in 1983, 712
U.S. Patent and Trademark Office (PTO), 715, 717–718

V
Vaccination, 343–345
Vaccines
antigens in plants, 517
attenuated, 372–378
cholera, 350
current and future, 345–347
delivery of DNA vaccines, 364–370
delivery of subunit, 357–359
diseases in Canada before and after, 343, 344
edible, 611–615
foot-and-mouth disease, 354–356
ge netic immunization with DNA vacci nes, 363–372
herpes simplex virus (HSV-1 and HSV-2), 348–349
inactivated and attenuated forms of, 345
infections without, 344
influenza virus, 396–397
malaria, 359–362
manufacturers, 347
monoclonal antibody passive immunity, 396–397
peptide, 359–362
Saccharomyces cerevisiae expression systems, 125
severe acute respiratory syndrome (SARS), 350–351
Staphylococcus aureus, 351–353
Streptococcus, 356–357
subunit, 347–359
see also Attenuated vaccines; Subunit vacci nes; Vector vaccines
Vaccinia virus
advantages of live over killed virus, 382–383
characterization of, 378–379
gene integration of, 379–380
mass vaccination campaigns, 384–385
modified vaccinia Ankara virus (MVA), 385–386
resistance of, 383
thyminde kinase–negative mutants of, 381
vector vaccine against, 378–386
vector vaccines constructed from, 381–382
veterinary vaccines, 383–384
L-Valine, biosynthesis of, 414–415
Vascular endothelial growth factor (VEGF), pegaptanib targeting, 305
Vector design, mammalian cells, 144–146

Two-dimensional differential in-gel electrophoresis, proteins, 76
Two-dimensional polyacrylamide gel electrophoresis (2D PAGE), proteins, 73, 74, 76
Two gene expression vector, mammalian cells, 146–147
Type I diabetes
insulin, 260–261
protein microarrays, 190
Type II restriction endonucleases, 11, 13–14

U
Ulcerative colitis, treatment for, 255–258
Undecylprodigiosin biosynthesis of, 420
structure of, 421
United Nations Food and Agriculture Program, 600
U.S. Centers for Disease Control and Prevention, 344, 355, 707
U.S. Department of Agriculture (USDA), 688
U.S. Food and Drug Administration, 9, 105, 124, 188, 225
genetically engineered food, 625
genetically engineered livestock, 690–692
genetically modified foods, 684–692
human growth hormone, 235
new biological drugs, 706–708
nucleic acid–based genetic tests, 710
orphan drugs, 713

Index
Vector vaccines
   bacteria as antigen delivery system, 392–395
   cholera, 392–394
dengue virus, 386, 387
   directed against bacteria, 388–392
directed against viruses, 378–388
   Ebola virus, 386–388
   Helicobacter pylori, 394–395
   live vaccinia virus vaccine, 378–386
tuberculosis, 389–392
   see also Vaccines
Vegetative insecticidal toxins, 560–561
Vemurafenib, 203
Vibrio cholerae
   cholera causative agent, 350
   cholera vaccine, 374–375, 376
   use in humans, 367
   *Vibrio fischeri*, 222
   Viral coat protein-mediated protection, 570–574
   Viral delivery systems, 319–325
   adenovirus-associated virus (AAV), 320, 321–323
   adenoviruses, 320, 323–324
   gammaretroviruses, 320–321
   herpes simplex virus 1 (HSV-1), 320, 324–325
   human immunodeficiency virus type 1 (HIV-1), 321
   lentiviruses, 320, 321
   mouse RNA virus, 320
   Virions, 136–137
Viruses, vector vaccines against, 378–388
   Virus resistance
   CRISPR-Cas system, 577, 578
   micro-RNAs, 576, 577
   protection by gene expression, 574–577
   single-chain antibodies, 574–576
   transgenic plants, 570–577
   viral coat protein-mediated protection, 570–574
   Vitamin A, plant nutritional content, 599–600
   Vitrawene (fomivirsen), for patient with AIDS, 301
   *Vitreoscilla*, 429, 476, 484, 618–619
   W
   Wambugu, Florence, 518
   Watermelon mosaic virus 2 (WMV), 573, 574
   Wet milling, disrupting microbial cells, 503, 504
   Whooping cough, vaccine for, 346
   World Health Organization, 350, 386, 601, 691
   World Intellectual Property Organization, 716
   X
   Xanthan gum, 429–431
   *Xanthomonas campestris*, production of xanthan gum, 429–431
   Xenobiotics, microbial degradation of, 434–445
   XenoMouse, generation of, 268–269
   X-ray crystallography, DNase I, 240
   XTEN unstructured protein, 239, 290
   *Xylella fastidiosa*, 587, 588
   Xylose, schematic of engineered assimilation of, 467
   D-Xylose, chemical structure of, 465
   Xylose/glucose isomerases, glucose to fructose, 453–454
   Y
   *Yarrowia lipolytica*, 134, 135, 418
   Yeast artificial chromosome (YAC), 122, 125
   cloning system, 126, 128
   human genes cloned onto, 268
   selectable markers, 527
   Yeast expression systems, 124–126
   Arxula adeninivorans, 134–135
   Hansenula polymorpha, 134–135
   Pichia pastoris, 131–135, 172
   *Saccharomyces cerevisiae* expression vectors, 125–126
   secretion of heterologous proteins by *S. cerevisiae*, 126–131
   Yarrowia lipolytica, 134–135
   Z
   Zeocin, 151
   Zinc finger nucleases, 298–299, 315–317
   Zucchini yellow mosaic virus, 573, 574
   Zymomonas-<i>E. coli</i> shuttle vector, 465–466
   *Zymomonas mobilis*, 464–467