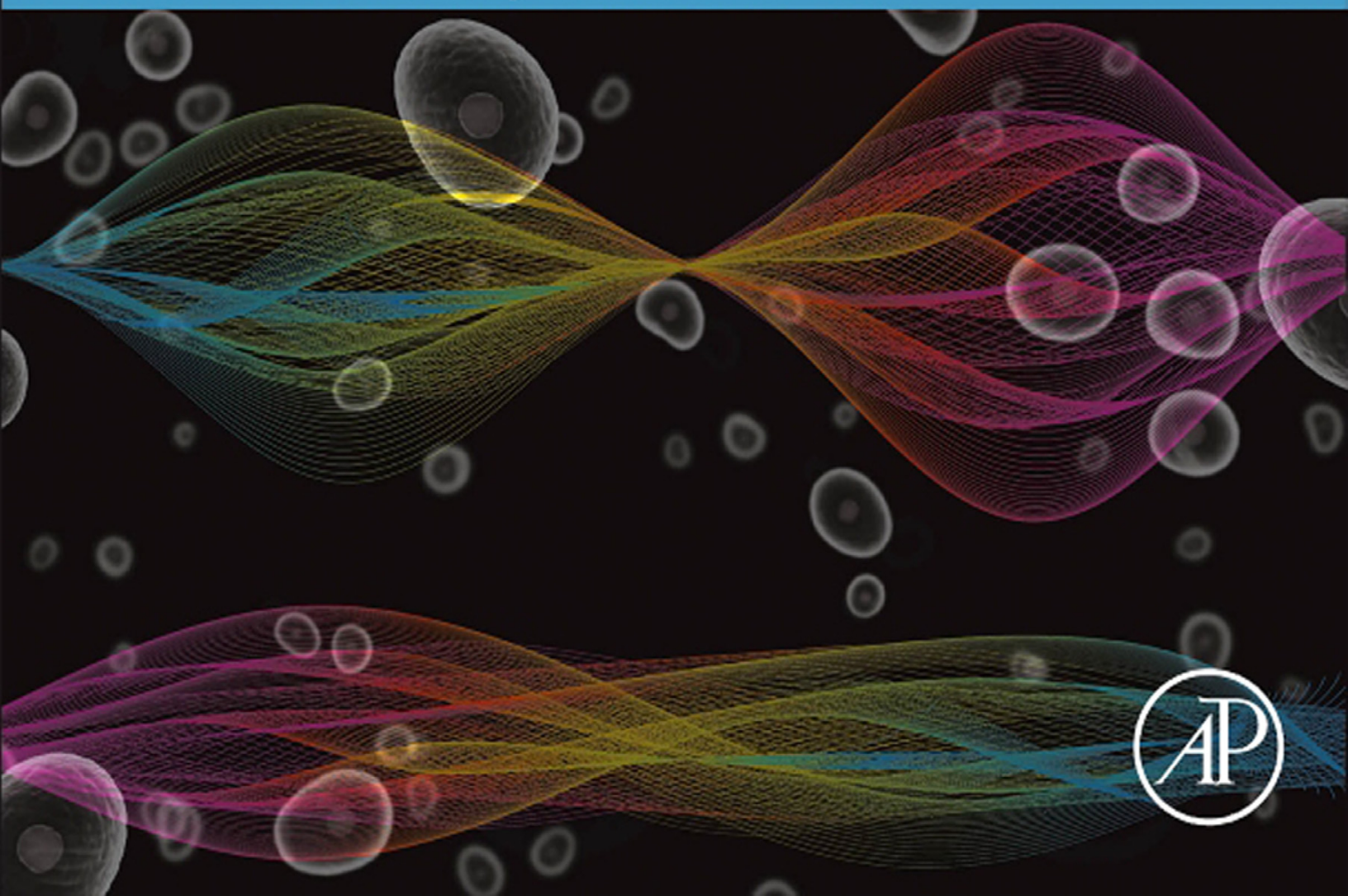
Ashish S. Verma
Anchal Singh

Animal Biotechnology

Models in Discovery and Translation



Animal Biotechnology

Models in Discovery and Translation

Animal Biotechnology

Models in Discovery and Translation

Edited by

Ashish S. Verma
Amity Institute of Biotechnology
Amity University Uttar Pradesh,
NOIDA (UP), India

Anchal Singh
Amity Institute of Biotechnology
Amity University Uttar Pradesh,
NOIDA (UP), India

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD • PARIS
SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK
225 Wyman Street, Waltham, MA 02451, USA

First published 2014

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloguing in Publication Data
A catalogue record for this book is available from the Library of Congress

ISBN: 978-0-12-416002-6

Printed and bound in the United States

14 15 16 17 18 10 9 8 7 6 5 4 3 2 1

Dedication

This book is dedicated in fond memories of
Dr. Har Swarup Verma

(1941–1995)
Loving Father,
Admirable Professor,
&
Compassionate Physician.

Ashish (son) & Anchal

Contents

Contributors xxi
Foreword xxv
Preface xxvii
Acknowledgments xxix

Section I
Human Diseases: In Vivo and In Vitro Models

1. Drosophila: A Model for Biotechnologists

K. Ravi Ram and D. Kar Chowdhuri

Summary 3
What You Can Expect to Know 3
History and Methods 3
Introduction 3
Classical Aspects of *Drosophila melanogaster* 4
History 7
Principle 9
Methodology 10
Culturing of Drosophila 10
Preparation of Drosophila Food Medium 10
Handling of Flies 10
Fly Disposal 10
Egg Collection 10
Dechorination of Eggs 10
Preparation of DNA for Injection 11
Protocols 11
Protocol for Germ-Line Transformation in Drosophila 11
Ethical Issues 12
Translational Significance 13
Drosophila Models of Human Disease 13
Drosophila-Based Models for Understanding Human Neurodegenerative Diseases 13
Drosophila as a Model for Understanding Human Metabolic Disorders 14
Drosophila as a Model for Understanding Nephrolithiasis (Kidney Stones) 15
Drosophila-Based Model for Understanding HIV Pathology 15
Drosophila-Based Therapeutic Peptide Production 15
World Wide Web Resources 17
References 17
Further Reading 18
Glossary 18
Abbreviations 19
Long Answer Questions 19
Short Answer Questions 19
Answers to Short Answer Questions 19

2. Animal Models of Tuberculosis

Devyani Dube, Madhu Gupta, Udita Agrawal and Suresh P. Vyas

Summary 21
What You Can Expect to Know 21
History and Methods 22
Introduction 22
Comparative Pathology of TB in Humans and Animals 22
Pathogen Diversity: Crossing Species Barriers 22
Host Diversity: Fundamental Processes and Fine Tuning 23
Animal Models of TB: Limits and Lessons 24
Various Animal Models 25
Mouse Model 25
Guinea Pig Model 26
Rabbit Model 26
Non-Human Primate Model 26
Cattle Model 28
Ethical Issues 28
Translational Significance 30
World Wide Web Resources 30
Protocols 30
Preparing *M. tuberculosis* Inoculum for Aerosol Exposure 30
Aerosol Infection of Mice Using the Middlebrook Apparatus 30
Aerosol Infection of Guinea Pigs Using a Madison Chamber 31
Bacterial Loading 31

Intravenous Infection of Mice with *M. tuberculosis* 31
Isolation of Samples for Determining *M. tuberculosis* Load by RT-PCR 32
Determination of Bacterial Loads in Target Organs 32
Preparation of Lungs or Other Tissues for Histology 35
Preparation of Lung Cell Suspension 35
References 35
Further Reading 36
Glossary 36
Abbreviations 36
Long Answer Questions 36
Short Answer Questions 36
Answers to Short Answer Questions 36

3. Animal Models for Neurodegenerative Disorders

Hitomi Tsuiji and Koji Yamanaka

Summary 40
What You Can Expect to Know 40
History and Methods 40
Introduction 40
Neurodegenerative Diseases 40
Amyotrophic Lateral Sclerosis (ALS) 40
Spinal Muscular Atrophy (SMA) 41
Spinal and Bulbar Muscular Atrophy (SBMA) 42
Principles 43
Genetics of ALS 43
Genetics of SMA 44
Genetics of SBMA 44
Methodology 44
Generation of Transgenic Mice 45
Cre-loxP Technology 46
ALS Models 47
SMA Models 49
SBMA Models 49
Examples and their Applications 49
SOD1-Linked ALS 50
Other ALS 51
SMA 52
SBMA 52
Ethical Issues 53
Translational Significance 53
World Wide Web Resources 54
References 54
Further Reading 55
Glossary 55
Abbreviations 55
Long Answer Questions 56
Short Answer Questions 56
Answers to Short Answer Questions 56

4. Epigenetics and Animal Models: Applications in Cancer Control and Treatment

Mukesh Verma, Neelesh Agarwal and Mudit Verma

Summary 57
What You Can Expect to Know 57
History and Methods 58
Introduction 58
History: Landscape of Epigenetics 58
Principle 61
Use of Mouse Models in the Epigenetics of Cancer 61
Examples with Applications 61
Methodology 64
Methylation Profiling 64
Histone Profiling 64
Nucleosome Mapping 64
Data Storage and Processing 64
Protocols 65
Some Useful Points to Consider in a Project Involving Epigenetic Profiling 65
Ethical Issues 67
Translational Significance 67
World Wide Web Resources 69
References 70
Further Reading 71
Glossary 71
Abbreviations 72
Long Answer Questions 72
Short Answer Questions 72
Answers to Short Answer Questions 72

5. Development of Mouse Models for Cancer Research

Amrita Datta and Debasis Mondal

Summary 73
What You Can Expect to Know 73
History and Methods 74
Introduction 74
History 75
Principle 75
IACUC Approval 75
IACUC Guidelines 76
Methodology 76
Inbred Mice 76
Examples with Applications 78
Immunocompetent Mice 78
Immunodeficient Mice 81
Checklist for a Successful *In Vivo* Experiment 83

Protocols 85
An Orthotopic Mouse Model of Colorectal Cancer 85
A Xenograft Model of Prostate Cancer Metastasis 87
Ethical Issues 88
Translational Significance 88
World Wide Web Resources 89
References 90
Further Reading 91
Glossary 91
Abbreviations 92
Long Answer Questions 92
Short Answer Questions 92
Answers to Short Answer Questons 93

6. Human Papillomavirus (HPV): Diagnosis and Treatment

Mausumi Bharadwaj, Showket Hussain, Richa Tripathi, Neha Singh and Ravi Mehrotra

Summary 96
What You Can Expect to Know 96
History and Methods 96
Cancer Overview 96
Classification of Cancer 96
Carcinoma 96
Sarcoma 97
Myeloma 97
Leukemia 97
Lymphoma 97
Mixed Types 97
Tumor Grading 97
Cancer Staging 97
Cancer-Causing Agents 98
Oncogenic Viruses 98
Chemicals 98
Radiation 99
Cervical Cancer 99
Anatomy of the Female Pelvis 99
Historical Overview 100
Mistaken Theories of Causation 100
The First Breakthrough 101
Nobel Prize for Discovering HPV 101
Prevalence and Epidemiology of Cervical Cancer 101
Global Scenario 101
Symptoms of Cervical Cancer 102
Types of Cervical Cancer 102
Risk Factors for Cervical Cancer 102
Pre-Cancer Classification 102
Cancer Classification 103
Human Papillomaviruses (HPVs) 104
Ethical Issues 109
Public Health Concerns with Screening Implementation 109
Risk 109
Benefit vs. Cost 110
Patient Autonomy and Coercion 110
Public Health Concerns about Vaccine Implementation 110
Treatment 110
Ablative Techniques 110
Excisional Techniques 110
Follow-Up for Excisional/Ablative Treatment 111
Hysterectomy 111
Therapeutic Interventions 111
Preventive Measures 112
Translational Significance 112
Prophylactic HPV Vaccines 112
Therapeutic HPV Vaccines 113
Genetic-Based DNA Vaccines 113
Issues/Unanswered Questions Associated with HPV Vaccines 113
Vaccine Efficacy 113
Vaccine Protection 113
Who Should be Vaccinated? 114
Future Prospects of HPV with Respect to Biotechnology 114
Conclusion 115
Key Points 115
World Wide Web Resources 116
References 117
Further Reading 117
Glossary 118
Abbreviations 118
Long Answer Questions 119
Short Answer Questions 120
Answers to Short Answer Questions 120

7. Human DNA Tumor Viruses and Oncogenesis

Pravinkumar Purushothaman and Subhash Chandra Verma

Summary 121
What You Can Expect to Know 121
History and Methods 122
Introduction 122
Transformation and Oncogenesis 122
History of Human DNA Tumor Viruses and Cancer 123
Epstein–Barr Virus 125
Kaposi's Sarcoma-Associated Herpesvirus (KSHV) 125
Human Papillomavirus (HPV) 126
Hepatitis B Virus (HBV) 127

Human Polyomaviruses 128
Principle 129
Current Research Perspectives 134
Ethical Issues 135
Translational Significance 135
World Wide Web Resources 135
References 136
Further Reading 136
Glossary 137
Abbreviations 137
Long Answer Questions 137
Short Answer Questions 137
Answers to Short Answer Questions 137

8. Animal Models for Human Disease

Mohammad Reza Khorramizadeh and Farshid Saadat

Summary 139
What You Can Expect to Know 139
History and Methods 140
Introduction 140
Rheumatoid Arthritis 140
Epidemiology and Etiology 140
Pathogenesis 140
Clinical Manifestations 141
Treatment 141
Experimental Models 142
Methodology and Protocols 143
Multiple Sclerosis 144
Epidemiology and Etiology 144
Pathogenesis 145
Clinical Manifestations 145
Treatment 146
Experimental Models 146
Methodology and Protocol 146
Ethical Issues 149
Translational Significance 150
World Wide Web Resources 150
References 150
Further Reading 151
Glossary 151
Abbreviations 153
Long Answer Questions 153
Short Answer Questions 153
Answers to Short Answer Questions 154

9. HIV and Antiretroviral Drugs

Ashish Swarup Verma, Iqram Govind Singh, Ruby Bansal and Anchal Singh

Summary 155
What You Can Expect to Know 155
History and Methods 156
Introduction 156
Discovery and Origin of HIV 156
History of HIV and AIDS 156
Global Disease Burden 157
Clinical Stages of HIV 158
Classification of Clinical Stages 159
Molecular Biology of HIV 160
Envelope (Env) 161
Group Specific Antigen (Gag) 161
Long-Terminal Repeats (LTR) 161
Negative Factor (Nef) 161
Polymerase (Pol) 161
Regulator of Expression of Viral Proteins (Rev) 162
Transactivator of Transcription (Tat) 162
Viral Infectivity Factor (Vif) 162
Viral Protein U (Vpu) 162
Viral Protein R (Vpr) 162
Replication: Steps and Drug Targets 162
Antiretroviral Drugs 164
HIV Resistance and Antiretroviral Treatment 165
Highly Active Antiretroviral Treatment (HAART) 165
Salvage Therapy 165
Drug Holiday 165
New Types of Antiretrovirals 166
Methodology and Principles 167
Growing HIV Stock 167
Assays for Antiretroviral Drugs 168
Monitoring Antiretroviral Drug Toxicity 169
Evaluating Anti-HIV Effects of Antiretroviral Drugs 170
NeuroAIDS: An Emerging Health Concern 171
Bone Marrow Transplantation: A Probable Cure for HIV 172
Ethical Issues 173
Translational Significance 173
World Wide Web Resources 174
References 174
Further Reading 175
Glossary 175
Abbreviations 175
Long Answer Questions 176
Short Answer Questions 176
Answers to Short Answer Questions 176

10. Animal Biotechnology as a Tool to Understand and Fight Aging

Pawan Kumar Maurya

Summary 177
What You Can Expect to Know 178
History and Methods 178
Introduction 178
Theories of Aging 178

Principle 181
Reactive Oxygen Species – Causative Agent of Aging 181
Methodology: Measurement of Free Radicals and Methods to Monitor Aging 183
Protein Oxidation/Protein Carbonyl Content 183
Common Laboratory Animal Experimental Models for Aging Research 184
Mouse 184
Fish 184
Other Models 185
Polyphenols as an Agent to Fight Aging 185
Flavonoids 185
Tea as a Source of Anti-Aging Compounds 185
Types of Teas 186
Tea Catechins 187
Health Benefits of Tea 187
Molecular Mechanisms of Green Tea Effects 187
Green Tea in Aging and Neurodegenerative Diseases 188
Animal Biotechnology as a Tool to Understand and Fight Aging 188
Ethical Issues 188
Translational Significance 189
World Wide Web Resources 189
References 189
Further Reading 190
Glossary 190
Abbreviations 190
Long Answer Questions 190
Short Answer Questions 191
Answers to Short Answer Questions 191

Section II
Animal Biotechnology: Tools and Techniques

11. MultiCellular Spheroid: 3-D Tissue Culture Model for Cancer Research

Suchit Khanna, Anant Narayan Bhatt and Bilikere S. Dwarakanath

Summary 195
What You Can Expect to Know 195
History and Methods 196
Introduction 196
MultiCellular Tumor Spheroids 196
Historical Facts Towards the Development of Tissue Culture Technology from 2-D and 3-D Cultures 199
An Example where 3-D Culture is More Beneficial over 2-D Culture 200
Techniques for the Generation of Spheroids 200
Hanging-Drop Method 200
Liquid Overlay Method 201
Microfabricated Microstructures Method 202
Rotatory Flask Methods 202
Surface Modification-Based Methods 202
Chip-Based Spheroid Generation 202
Protocol for Tumor Spheroid Generation 203
Drug Treatment Protocol 203
Parameters to Monitor Drug Efficacy in 3-D Cultures 203
Radiation Response of Tumor Cells and its Modifications 203
Response to Anticancer Drugs 204
Response to Photodynamic Therapy 205
Response to Anti-Angiogenesis Therapeutics 205
Evaluation of Response to Immunotherapy 206
Application of 3-D Cultures in Other Diseases 207
Conclusions 207
Ethical Issues 207
Translational Significance 207
World Wide Web Resources 208
References 208
Further Reading 209
Glossary 210
Abbreviations 210
Long Answer Questions 210
Short Answer Questions 210
Answers to Short Answer Questions 210

12. Animal Tissue Culture: Principles and Applications

Anju Verma

Summary 212
What You Can Expect to Know 212
History and Methods 212
Introduction 212
Development of Animal Cell Culture 213
Basic Concept of Cell Culture 214
How are Cell Cultures Obtained? 214
Cell Passage and Use of Trypsin 214
Quantitation 215
Reconstruction of Three-Dimensional Structures 215
Types of Cell Culture 215
Primary Cell Culture 215
Secondary Cell Culture 216
Cell Line 216
Finite Cell Lines 216
Indefinite Cell Lines 216
Commonly Used Cell Lines 216
Advantages of Continuous Cell Lines 216
Growth Cycle 217
Phases of the Growth Cycle 217

Monitoring Cell Growth 217
Characteristics of Cell Cultures 218
Cell Viability 218
Cytotoxicity 218
Hayflick's Phenomenon 219
Culture Media 219
Basic Components in Culture Media 220
Serum 220
Serum-Free Media 221
Chemically Defined Media 221
Protein-Free Media 221
Characterization of Cell Lines 221
Identity Testing 221
Purity Testing 222
Stability Testing 222
Viral Testing Assays 222
Advantages of Animal Cell Culture 222
Disadvantages of Animal Cell Culture 222
Ethical Issues 223
Use of Fetal Bovine Serum in Animal Culture Media 223
Translational Significance 223
Anti-Viral Vaccines 223
Viral Particles Production by Cell Culture 223
Production of Virus-Like Particles (VLPs) 224
Recombinant Therapeutic Proteins 225
Main Therapeutic Proteins 225
Gene Therapy 227
Importance of Cell Culture in Gene Therapy 227
Clinical Studies 227
Biopesticides 227
Baculovirus Production in Animal Cell Culture 227
Cell Lines for Biopesticide Production 228
Viral Mutant Formation in Cell Culture 228
Monoclonal Antibodies 228
Stem Cells 228
Culturing Embryonic Stem Cells in the Laboratory 228
World Wide Web Resources 229
References 229
Further Reading 230
Glossary 230
Abbreviations 230
Long Answer Questions 231
Short Answer Questions 231
Answers to Short Answer Questions 231

13. Concepts of Tissue Engineering

Poonam Verma and Vipin Verma

Summary 233
What You Can Expect to Know 233
History and Methods 234
Introduction 234
History 234
Basic Approach to Tissue Engineering: Principles and Methodology 234
Cells 234
Scaffolds 235
Media 235
Bioreactors 235
Methodology 235
Scaffold Design 235
Materials for Scaffolds 237
Scaffold Fabrication Methods 237
Fiber Bonding 237
Solvent Casting and Particulate Leaching 238
Melt Molding 238
Membrane Lamination 239
Phase Separation 239
Gas Foaming 239
Polymer Ceramic Composite Foam 239
Solid Free form Techniques 239
Pressure-Assisted Micro Syringe Method 239
Freeze Drying 240
Examples of Tissue-Engineered Organs 240
Skin 240
Pancreas 240
Liver 240
Kidney 240
Bone/Cartilage 240
Nerves 241
Blood Vessels 241
Tissue Engineering Using Stem Cells 241
Issues and Challenges 241
Ethical Issues 242
Translational Significance 242
World Wide Web Resources 242
References 242
Further Reading 244
Glossary 244
Abbreviations 244
Long Answer Questions 244
Short Answer Questions 244
Answers to Short Answer Questions 244

14. Nanotechnology and Its Applications to Animal Biotechnology

Ashok K. Adya and Elisabetta Canetta

Summary 247
What You Can Expect to Know 247
History and Methods 248
Introduction 248
Methodologies 249
Nanotools and Nanotechniques 249
Chemical Modification of AFM Probes 255

Nano-Structural Features of Animal Cells and Tissues 255
Nanomechanical Properties of Animal Cells and Tissues 255
Nanomanipulation 256
Nanofabrication 256
Examples of Nanotechnology Applications to Animal Biotechnology 256
AFM as a Diagnostic Tool to Identify Orthopoxvirus in Animals 256
Frictional Response of Bovine Articular Cartilage 256
Microstructure and Nanomechanical Properties of Cortical Bone Osteons from Baboons 256
Use of Calf Thymus DNA for Cancer Experiments 257
Characterization of Mitochondria Isolated from Normal and Ischemic Hearts in Rats 257
Polymorphism and Ultrastructural Organization of Prion Protein 257
Ultrastructural Investigation of Animal Spermatozoa Using AFM 257
Multifactor Analysis of Living Animal Cells for Biotechnology and Medicine 258
Ethical Issues 258
Translational Significance 258
World Wide Web Resources 259
References 259
Further Reading 260
Glossary 261
Abbreviations 261
Long Answer Questions 261
Short Answer Questions 261
Answers to Short Answer Questions 262

15. Antibodies: Monoclonal and Polyclonal

Anchal Singh, Sushmita Chaudhary, Ashima Agarwal and Ashish Swarup Verma

Summary 265
What You Can Expect to Know 265
History and Methods 266
Introduction 266
Tiselius and Kabat's Experiment 266
History 266
Elucidation of Immunoglobulin Structure 267
Edelman's Experiment 268
Porter's Experiment 268
Nisonoff's Experiment 268
Conclusion from Papain and Pepsin Digestion 268
Immunoglobulin G: A Prototype for Immunoglobulin 268
Polyclonal Antibody *versus* Monoclonal Antibody 269
Polyclonal Antibodies (PoAb) 269
Monoclonal Antibodies (MoAb) 270
Naming Monoclonal Antibodies 271
Prefix 271
Infix-1 272
Infix-2 272
Additional Words 272
Antibodies as Therapeutics: Adverse Effects 272
Serum Sickness 272
Human Anti-Monoclonal Antibody (HAMA) Response 273
Human Anti-Chimeric Antibody (HACA) Response 273
Human Anti-Humanized Antibody (HAHA) Response 274
Applications of Antibodies 274
Therapeutic Applications 274
Analytical Applications 275
Preparative Applications 276
Methodology, Principles, and Protocols 276
Polyclonal Antibodies 276
Monoclonal Antibodies 278
Antibody Titration 281
Biochemical Pathway: Hybridoma Selection 282
Ethical Issues 283
Translational Significance 284
World Wide Web Resources 284
References 285
Further Reading 285
Glossary 286
Abbreviations 286
Long Answer Questions 286
Short Answer Questions 286
Answers to Short Answer Questions 286

16. Molecular Markers: Tool for Genetic Analysis

Avinash Marwal, Anurag Kumar Sahu and R.K. Gaur

Summary 289
What You Can Expect to Know 289
History and Methods 290
Introduction 290
Methodology 291
Restriction Fragment Length Polymorphism (RFLP) 291
Allele-Specific Oligonucleotide (ASO) 292
Allele-Specific PCR (AS-PCR) 293
Single-Strand Conformation Polymorphism (SSCP) 293

Sequence Tagged Site (STS) 293
Random Amplified Polymorphic DNA (RAPD) 293
Restriction Landmark Genome Scanning (RLGS) 294
Single Nucleotide Polymorphisms (SNP) 294
Amplified Fragment Length Polymorphism (AFLP) 295
Methylation Sensitive Amplification Polymorphism (MSAP) 296
Miniature Inverted-Repeat Transposable Element (MITE) 296
Microsatellites 296
Ethical Issues 299
Translational Significance 301
World Wide Web Resources 301
References 302
Further Reading 303
Glossary 303
Abbreviations 304
Long Answer Questions 304
Short Answer Questions 304
Answers to Short Answer Questions 304

17. Gene Expression: Analysis and Quantitation

Denys V. Volgin

Summary 307
What You Can Expect to Know 307
History and Methods 308
Introduction 308
Principles 308
Methodology 310
Quantitation of mRNA Levels Using RT-PCR 310
Quantitation of Protein Levels 312
Protocols 316
Protocol 1. Parallel Quantitation of mRNA and Protein from Defined Rodent Brain Region 316
Protocol 2. Single-Cell RT-PCR 318
Protocol 3. Semi-Quantitative Immunohistochemistry 319
Examples 320
Prolonged Upregulation of Gene Expression for the Hypothalamic $GABA_A$ Receptors in a Rodent Model of Prenatal Exposure to Alcohol 320
Hypothalamic Orexin System and mRNA Expression Profiling at the Single-Cell Level 321
Ethical Issues 321
Translational Significance 322
World Wide Web Resources 322
References 323
Further Reading 323
Glossary 324
Abbreviations 324
Long Answer Questions 324
Short Answer Questions 324
Answers to Short Answer Questions 325

18. Ribotyping: A Tool for Molecular Taxonomy

S.K. Kashyap, S. Maherchandani and Naveen Kumar

Summary 327
What You Can Expect to Know 327
History and Methods 328
Introduction 328
Historical Developments in Bacterial Taxonomy 328
Typing Methods Used for Bacterial Systematics 329
Phenotypic Typing Methods 330
Genotypic Typing Methods 331
Basis of Using rRNA and rRNA Genes as Taxonomic Tools 331
Organization of the Ribosomal Operon 331
Different Techniques of Ribotyping 332
Conventional Ribotyping 333
Selection of Restriction Endonuclease for Ribotyping by Sequence Analysis (*In Silico*) 333
Automated Ribotyping 335
PCR Ribotyping 335
In Situ Hybridization Targeted to Detect rRNA 338
Limitations of Ribotyping 341
Future Perspectives 341
Ethical Issues 342
Translational Significance 342
World Wide Web Resources 342
References 342
Further Reading 343
Glossary 343
Abbreviations 343
Long Answer Questions 344
Short Answer Questions 344
Answers to Short Answer Questions 344

19. Next Generation Sequencing and Its Applications

Anuj Kumar Gupta and U.D. Gupta

Summary 345
What You Can Expect to Know 346
History and Methods 346
Introduction 346
History of DNA Sequencing 346
Generation of Sequencing Technologies 346
Principle of Sanger Sequencing vs. Next Generation Sequencing 347

Next Generation/Second Generation Technologies 348
Pyrosequencing Technology 348
Reversible Terminator Technology 349
Sequencing by Ligation Technology 349
Third Generation Sequencing Technologies 350
Single Molecule Real-Time Sequencing 350
True Single Molecule Sequencing (tSMS) 350
Ion Semiconductor Sequencing 351
Other/4th Generation Sequencing Technologies 351
Nanopore Sequencing 351
Polony-Based Sequencing Technology 352
DNA Nanoball Sequencing 352
Downstream Bioinformatics 353
De Novo Assembly 353
Mapping 353
General Principles of NGS Methods in Various Applications 354
Whole Genome *De Novo* Sequencing 354
Whole Genome Re-Sequencing 355
Targeted Re-Sequencing 356
Transcriptome Sequencing 356
Amplicon Sequencing 357
ChIP DNA Sequencing 357
Small RNA Sequencing 357
Animal Biotechnology and the Cattle Genome 357
Applications of NGS in Animal Biotechnology 358
Evolutionary Research 358
Epigenetics 359
Metagenome Sequencing 359
Ancient DNA 359
Genomic Variability and SNP/CNV Discovery 360
Beef Cattle Selection 360
Animal Breeding and Improvement of Livestock Productivity and Health 361
Food, Safety, and Nutrition 361
Transgenics 361
Applications of NGS in Human Health 361
Cancer Research 361
Genetic Disorders 362
Human Microbiome 362
Pre- and Post-Natal Diagnosis 362
Infectious Diseases 362
Personalized Medicine 362
Future Perspectives 363
Challenges 363
Ethical Issues 363
Translational Significance 364
World Wide Web Resources 364
References 364
Further Reading 365
Glossary 365
Abbreviations 366
Long Answer Questions 366
Short Answer Questions 366
Answers to Short Answer Questions 366

20. Biomolecular Display Technology: A New Tool for Drug Discovery

Madhu Biyani, Koichi Nishigaki, and Manish Biyani

Summary 369
What You Can Expect to Know 369
History and Methods 370
Introduction 370
Principle 370
Necessity: Small Molecule vs. Biomolecular (Biologics) Drugs 371
Methodology: Biomolecular Display Technologies 373
Phage Display 374
Ribosome Display 376
mRNA Display 377
Other Display Systems 377
A General Method for Discovery of Functional Peptide Aptamers 378
Conclusion and Future Perspectives 380
Ethical Issues 380
Translational Significance 380
References 381
Further Reading 382
Glossary 383
Abbreviations 383
Long Answer Questions 383
Short Anwers Questions 383
Answers to Short Answers Questions 384

21. *In Silico* Models: From Simple Networks to Complex Diseases

Debmalya Barh, Vijender Chaitankar, Eugenia Ch Yiannakopoulou, Emmanuel O Salawu, Sudhir Chowbina, Preetam Ghosh and Vasco Azevedo

Summary 385
What You Can Expect to Know 385
History and Methods 386
Bioinformatics in Animal Biotechnology 386
Bioinformatics and Systems Biology 387
Common Computational Methods in Systems Biology 387
Experimental Methods in Systems Biology 388
Protein–Protein Interactions (PPIs) 388
Transcriptional Control Networks 388
Signal Transduction Networks 388
Mathematical Modeling Techniques 389
The Concept of Modeling 390

***In Silico* Models of Cells** 391
Advantages and Disadvantages of *In Silico* Modeling 392
Applications of *In Silico* Disease Modeling in Practice 392
In Silico Models of Cancer 392
In Silico Models and Inflammatory Response Syndrome in Trauma and Infection 394
Infectious Diseases 394
Neuronal Diseases 397
Conclusion 400
Ethical Issues 400
Translational Significance 400
World Wide Web Resources 401
References 401
Further Reading 403
Glossary 403
Abbreviations 403
Long Answer Questions 403
Short Answer Questions 403
Answers to Short Answer Questions 403

Section III
Animal Biotechnology: Applications and Concerns

22. Transgenic Animals and their Applications

Shet Masih, Pooja Jain, Rasha El Baz and Zafar K. Khan

Summary 407
What You Can Expect to Know 407
Introduction 408
Creating Transgenic Animals 409
Construction of a Transgene 409
Microinjection 409
Embryonic Stem Cell Transfer 409
Retrovirus-Mediated Gene Transfer 411
Gene Knockdown and RNA Interference 412
Screening for Transgenic Positives 412
Transgenic Animals as Disease Models 413
OncoMouse 413
AIDS Mouse 413
Alzheimer's Mouse 413
Parkinson's Fly 414
Transgenic Animals as Biological Models 414
ANDi (Monkey) 414
Doogie (Smart Mouse) 414
Supermouse 414
Youth Mouse 414
Influenza-Resistant Mouse 415
Transgenic Animals as Xenotransplanters 415
Transgenic Animals as Food Sources 415
Superpig 415
Superfish 416
Transgenic Animals for Drug and Industrial Production 416
Transgenic Animals' Impact on the Environment 417
Patenting Transgenic Animals 418
Ethical Issues 419
FDA Guidelines on Genetically Engineered Animals 420
Translational Significance 420
World Wide Web Resources 420
References 421
Further Reading 422
Glossary 422
Abbreviations 423
Long Answer Questions 423
Short Answer Questions 423
Answers to Short Answer Questions 423

23. Stem Cells: A Trek from Laboratory to Clinic to Industry

Bhudev C. Das and Abhishek Tyagi

Summary 426
What You Can Expect to Know 426
History and Methods 426
History of Stem Cell Research 426
What are Stem Cells and Why are they Important? 427
What Makes Stem Cells Special? 428
Differentiation Potential/Potency of Stem Cells 428
Totipotent Stem Cells 428
Pluripotent Stem Cells 429
Multipotent Stem Cells 429
Unipotent Stem Cells 429
Are Stem Cells Immortal? 429
Characteristics of Stem Cells 429
Stem Cell Plasticity 429
Mechanisms of Stem Cell Plasticity 429
Stem Cell Fate 430
Stem Cell Quiescence 430
Where to Find Stem Cells? 430
Embryos 430
Fetal Tissue 430
Amniotic Fluid-Derived Stem Cells (AFSCs) 431
Where do Stem Cells Come From? 431
Embryonic Stem Cells (ESCs) 431
Embryonic Germ Cells (EGCs) 432
Adult Stem Cells (ASCs) 432

How are Stem Cells Identified, Isolated, and Characterized? 432
Embryonic Stem Cell Identification 433
Adult Stem Cell Identification 433
Methods to Identify Stem Cells 433
The Stem Cell Niche 436
What Keeps Stem Cells in their Niche? 436
How Do Stem Cells Get Activated in their Niche? 437
Growing Stem Cells in the Laboratory 437
iPSCS: A Stem Cell Research Breakthrough 437
Cancer Stem Cells (CSCs) 438
Protocols for Hoechst 33342 or DCV Staining and Stem Cell Purification 440
Hoechst or DCV Staining 440
Flow Cytometry Set-Up 440
Tips for Optimal Resolution of SP Cells 440
Ethical Issues 440
Stem Cell Research at the Crossroads of Religion and Social Issues 440
Greek Orthodox and Roman Catholic Churches 441
Protestant Churches 441
Judaism 441
Islamic Countries 441
Hinduism and Buddhism 441
Translational Significance 442
Therapeutic Potential of Stem Cells: A New Age in Health Care 442
Stem Cell Therapy: A Ray of Hope 442
Principles of Stem Cell Therapy 442
How are Stem Cells Used in Cell-Based Regenerative Therapies? 443
Can Stem Cells be used to Find New Medicines? 443
Potential Uses of Stem Cells 443
Damaged Tissue Replacement 444
Human Development Studies 444
New Drug Testing 444
Screening Toxins 444
Testing Gene Therapy Methods 444
Stem Cell Banking 445
What is Stem Cell Banking? 445
Why Bank Stem Cells? 445
Present Scope and Future Possibilities of Stem Cell Banking 445
Stem Cell Banks in India 445
World Wide Web Resources 445
References 447
Further Reading 447
Glossary 447
Abbreviations 448
Long Answer Questions 449
Short Answer Questions 449
Answers to Short Answer Questions 449

24. Role of Cytogenetics and Molecular Genetics in Human Health and Medicine

Madhumita Roy Chowdhury and Sudhisha Dubey

Summary 451
What You Can Expect to Know 451
History and Methods 452
Introduction 452
Cytogenetics: An Overview 452
Chromosome Morphology and Classification 453
Nomenclature 454
Chromosomal Disorders 455
Structural Abnormalities 456
Chromosome Breakage and Fragile Sites 458
Methodology: Application of Different Cytogenetic Techniques in Diagnosis of Genetic Disorders 459
Identification of Chromosomes and Karyotyping 459
Fluorescence *In Situ* Hybridization (FISH) 460
Spectral Karyotyping (SKY) and Multicolor FISH (M-FISH) 460
Comparative Genomic Hybridization (CGH) and array-CGH (aCGH) 461
Principle 462
Molecular Genetics: An Overview 462
Hereditary Material 462
Single-Gene Disorders 465
Multigenic and Multifactorial Disorders 466
Mitochondrial Disorder 466
Acquired Somatic Genetic Disorders 466
Methodology: Application of Different Molecular Techniques for Diagnosis of Genetic Disorders 466
Southern Blotting 467
Polymerase Chain Reaction (PCR) 468
DNA Sequencing 468
Principle 469
Case Study 469
Ethical Issues 470
Translational Significance 471
World Wide Web Resources 471
References 471
Further Reading 472
Glossary 472
Abbreviations 472
Long Answer Questions 472
Short Answer Questions 472
Answers to Short Answer Questions 472

25. Antibodies and their Applications

Fahim Halim Khan

Summary 473
What You Can Expect to Know 473
History and Methods 474
Immunodiagnostics: Role of Antibodies 474
Introduction 474
History 474
Antigens and Antibodies 474
Polyclonal and Monoclonal Antibodies 475
Hybridoma Technology and Methodology 476
Application of Monoclonal Antibodies 478
Antibody Constructs 485
Diabody 485
Human Antibodies from Transgenic Mouse 485
Bispecific Antibodies 486
Ethical Issues 487
Translational Significance 487
World Wide Web Resources 488
Pros 489
Cons 489
Websites 489
References 489
Further Reading 489
Glossary 489
Abbreviations 490
Long Answer Questions 490
Short Answer Questions 490
Answers to Short Answer Questions 490

26. Vaccines: Present Status and Applications

Dinesh K. Yadav, Neelam Yadav and Satyendra Mohan Paul Khurana

Summary 491
What You Can Expect to Know 491
History and Methods 491
Introduction 491
Types of Vaccines 493
Traditional Vaccines 493
Toxoid Vaccines 496
Subunit Vaccines 497
Conjugate Vaccines 498
DNA Vaccines 498
Recombinant Vector Vaccines 500
Molecular Farming using Plants as Bio-Reactors 501
Role of Adjuvants 502
Immune-Stimulating Complexes (ISCOMs) 503
Protocol for the Development of Vaccines 503
Future Challenges in Vaccine Development 504
Foremost Infectious Disease Problems 504
Infectious Disease Threats 504
Ethical Issues 505
Mandates 505
Vaccine Research and Testing 505
Informed Consent 505
Access Issues 506
Translational Significance 506
World Wide Web Resources 506
References 506
Further Reading 507
Glossary 507
Abbreviations 507
Long Answer Questions 507
Short Answer Questions 508
Answers to Short Answer Questions 508

27. Safety Assessment of Food Derived from Genetically Modified Crops

Premendra D. Dwivedi, Mukul Das, Sandeep Kumar and Alok Kumar Verma

Summary 509
What You Can Expect to Know 509
History and Methods 510
Introduction 510
Rationale for the Allergenicity Assessment of GM Foods 511
Mechanism of Food Protein-Induced Allergenicity 511
Simulated Gastric Fluid (SGF) Assay 512
How the SGF Assay Works 512
Components of SGF 514
General Protocol of the SGF Assay 515
Factors Relevant to Gastrointestinal Digestion of Allergens 515
Supportive and Negative Evidence of SGF 516
Simulated Intestinal Fluid (SIF) Assay 516
General Protocol of SIF Assay 516
Effect of Assay Conditions on Protein Stability in the SIF Assay 517
Supportive and Negative Evidences of the SIF Assay 517
Contradictory Results of the SIF Digestibility of Food Proteins 517
Thermal Treatment Assay 518
Mechanism of Thermal Treatment Assay 518
Standard Protocol for Thermal Treatment Assay 519
Functional Stability of Proteins and Importance of Thermal Stability Assay 520
Contradictory Results in Thermal Treatment Procedure 521
Ethical Issues 521
Translational Significance 522
World Wide Web Resources 522

References 522
Further Reading 523
Glossary 523
Abbreviations 523
Long Answer Questions 524
Short Answer Questions 524
Answers to Short Answer Questions 524

28. Nanotechnology and Detection of Microbial Pathogens

Rishi Shanker, Gulshan Singh, Anurag Jyoti, Premendra Dhar Dwivedi and Surinder Pal Singh

Summary 525
What You Can Expect to Know 525
History and Methods 526
Introduction 526
Indicators of Microbial Water Quality 526
Need for Detection of Water- and Food-Borne Pathogens 527
Conventional Methods to Detect Fecal Indicator Organisms and Other Pathogenic Bacteria 528
Most Probable Number Method 529
Membrane Filtration Method 529
Defined Substrate Methods 529
Rapid Detection Using Chromogenic Substrates 529
Immunological Methods 529
Molecular Methods Based on Genetic Signature of Target Pathogen 530
Polymerase Chain Reaction Technique and Quantitative PCR 530
Nanotechnology and its Promise 531
Metallic Nanoparticles 532
History 532
Detection Principle 533
Methodology 533
Synthesis of Gold Nanoparticles 533
Computation of ssDNA Sequences for Functionalization of Gold Nanoparticles 534
Functionalization of Gold Nanoparticles with Thiol-Modified DNA 534
Examples 534
Colorimetric Detection of DNA of Shiga Toxin Producing *Escherichia Coli* (Using Bio-Conjugated Gold Nanoparticles) 535
Colorimetric Detection of Enterotoxigenic *Escherichia coli* (ETEC) Gene Using Gold Nanoparticle Probes 535
Clinical Significance of Nanoparticle-Based Detection 536
Ethical Issues 536
Translational Significance 537
Future Approaches 537
World Wide Web Resources 537
References 538
Further Reading 539
Glossary 539
Abbreviations 539
Long Answer Questions 539
Short Answer Questions 539
Answers to Short Answer Questions 539

29. Biotechnological Exploitation of Marine Animals

Surajit Das

Summary 541
What You Can Expect to Know 541
History and Methods 542
Introduction 542
Marine Bioresources and Biotechnology 542
Historical Background 542
Biotechnologically Important Marine Animals 542
Genetic Engineering and its Application in Aquaculture 545
Transgenic Fish 545
Methods of Gene Transfer 545
Chromosome Manipulation 545
Sex Reversal 548
Cryopreservation of Gametes 549
Marine Ornamental Fish Trade and Chromosomal Manipulation 549
Advances in Mariculture 550
Captive Rearing Technology 550
Feed Technology: Microencapsulated, Micro-Coated, and Bio-Encapsulated Feeds 550
Application of Nanotechnology in Aquaculture 550
Marine Genomics 551
Disease Diagnosis of Cultivable Animals 552
Immunodiagnostics 552
DNA-Based Diagnosis 554
Therapeutics from Marine Animals 554
Marine Natural Products of Animal Origin 554
Commercial Bio-Products from Marine Organisms 556
Green Fluorescent Protein from Jelly Fish and its Application 557
Ethical Issues 559
Translational Significance 559
Future Directions 559
World Wide Web Resources 560
References 560
Further Reading 561
Glossary 561

Abbreviations 562
Long Answer Questions 562
Short Answer Questions 562
Answers to Short Answer Questions 562

30. Herbal Medicine and Biotechnology for the Benefit of Human Health

Priyanka Srivastava, Mithilesh Singh, Gautami Devi and Rakhi Chaturvedi

Summary 563
What You Can Expect to Know 563
History and Methods 563
Introduction 563
Traditional Medicine 564
Ancient System of Medicine 565
Methodology 565
Investigation of Medicinal Plants 565
Biotechnological Approaches for Herbal Drug Production 569
Organ Cultures 569
Callus Cultures 570
Suspension Cultures 570
Case Study: *Lantana camara* L 570
Opportunities and Challenges 571
Conclusions and Outlook 573
Ethical Issues 573
Translational Significance 573
World Wide Web Resources 574
References 574
Further Reading 574
Glossary 574
Abbreviations 575
Long Answer Questions 575
Short Answer Questions 575
Answers to Short Answer Questions 575

31. Perspectives on the Human Genome

Aruna Kumar and Kailash C. Upadhyaya

Summary 577
What You Can Expect to Know 577
History and Methods 577
Introduction 577
Human Genome Sequencing Project 578
History 578
Human Genome: Organization and Perspective 579
Complexity of Human Genome 579
Gene Content 579
Principle 586
Methodology 587
Examples with Applications 588
Ethical Issues 589
Translational Significance 590
World Wide Web Resources 591
References 591
Further Reading 592
Abbreviations 592
Glossary 592
Long Answer Questions 593
Short Answer Questions 593
Answers to Short Answer Questions 593

32. Ethical Issues in Animal Biotechnology

Abhik Gupta

Summary 597
What You Can Expect to Know 597
History and Methods 598
Introduction 598
A Brief Overview of Ethical Thoughts and Principles 599
Virtue Ethics 599
Deontological (Duty-Based) Ethics 600
Consequentialist Ethics 600
Principles 600
Methodology 601
Application of Ethics in Animal Biotechnology 601
Ethical Concerns in Animal Biotechnology 602
Intrinsic Concerns 602
Extrinsic Concerns 604
Some Challenging Ethical Issues in Animal Biotechnology 607
Chimeras 607
Animal Biopharming 608
Constitution of Ethics Committees 610
Translational Significance 610
Human Therapeutic Cloning and Other Techniques in Animal Biotechnology 610
Conclusions 611
World Wide Web Resources 611
References 612
Further Reading 612
Glossary 612
Abbreviations 613
Long Answer Questions 613
Short Answer Questions 613
Answers to Short Answer Questions 613

Index 615

Contributors

Ashok K. Adya BIONTHE (Bio- and Nano-technologies for Health & Environment) Centre, Division of Biotechnology & Forensic Sciences, School of Contemporary Sciences, University of Abertay, Dundee, Scotland, UK

Neelesh Agarwal Epidemiology and Genomics Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute, National Institutes of Health (NIH), Bethesda, MD, USA

Ashima Agarwal Amity Institute of Biotechnology, Amity University Uttar Pradesh, NOIDA (UP), India

Udita Agrawal Department of Pharmaceutical Sciences, Dr. Hari Singh Gour University, Sagar, M.P., India

Vasco Azevedo Laboratorio de Genetica Celular eMolecular, Departmento de Biologia Geral, Instituto de Ciencias Biologics, Universidade Federal de Minas Gerais Belo Horizonte, Minas Gerais, Brazil

Ruby Bansal Crosslay Wellness Program, Pushpanjali Crosslay Hospital, Ghaziabad (UP), India

Debmalya Barh Centre for Genomics and Applied Gene Technology, Institute of Integrative Omics and Applied Biotechnology (IIOAB), Nonakuri, Purba Medinipur, India

Mausumi Bharadwaj Division of Molecular Genetics & Biochemistry, Institute of Cytology & Preventive Oncology (ICMR), Noida, Uttar Pradesh, India

Anant Narayan Bhatt Institute of Nuclear Medicine and Allied Sciences, Defense Research Developmental Organization (DRDO), Ministry of Defense, Delhi, India

Manish Biyani Department of Biotechnology, Biyani Group of Colleges, Jaipur, India, Department of Bioengineering, The University of Tokyo, Tokyo, Japan

Madhu Biyani Department of Biotechnology, Biyani Group of Colleges, Jaipur, India, Department of Functional Materials Science, Saitama University, Saitama, Japan

Elisabetta Canetta Cardiff School of Biosciences, Cardiff University, Cardiff, Wales, UK

Vijender Chaitankar Department of Computer Science, Virginia Commonwealth University, Richmond, VA, USA

Rakhi Chaturvedi Department of Biotechnology, Indian Institute of Technology-Guwahati, Guwahati, Assam, India

Sushmita Chaudhary Amity Institute of Biotechnology, Amity University Uttar Pradesh, NOIDA (UP), India

Sudhir Chowbina Advanced Biomedical Computing Center, SAIC-Frederick, Inc., Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, MD, USA

D. Kar Chowdhuri Embryotoxicology, CSIR-Indian Institute of Toxicology Research, Mahatma Gandhi Marg, Lucknow, Uttar Pradesh, India

Madhumita Roy Chowdhury Genetic Unit, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India

Bhudev C. Das Laboratory of Molecular Oncology, Dr. B. R. Ambedkar Center for Biomedical Research (ACBR), University of Delhi, Delhi, India

Mukul Das Food, Drug and Chemical Toxicology Group, CSIR-Indian Institute of Toxicology Research, Lucknow, U.P., India

Surajit Das Department of Life Science, National Institute of Technology, Rourkela, Odisha, India

Amrita Datta Department of Pharmacology, Tulane University Medical Center, New Orleans, LA, USA

Gautami Devi Department of Biotechnology, Indian Institute of Technology-Guwahati, Guwahati, Assam, India

Devyani Dube ISF College of Pharmacy, Moga, Punjab, India

Sudhisha Dubey Department of Genetic Medicine, Sir Ganga Ram Hospital, New Delhi, India

Bilikere S. Dwarakanath Institute of Nuclear Medicine and Allied Sciences, Defense Research Developmental Organization (DRDO), Ministry of Defense, Delhi, India

Premendra D. Dwivedi Food, Drug and Chemical Toxicology Group, CSIR-Indian Institute of Toxicology Research, Lucknow, U.P., India

Rasha El Baz Department of Microbiology and Immunology, Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, Doylestown, PA, USA

R.K. Gaur Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science, Rajasthan, India

Preetam Ghosh Department of Computer Science, Virginia Commonwealth University, Richmond, VA, USA

Madhu Gupta Department of Pharmaceutical Sciences, Dr. Hari Singh Gour University, Sagar, M.P., India

Anuj Kumar Gupta C-11/Y-1, C-Block, Dilshad Garden, Delhi, India

U.D. Gupta National JALMA Institute for Leprosy & Other Mycobacterial Diseases (ICMR), Agra, UP, India

Abhik Gupta Department of Ecology & Environmental Science, Assam University, Silchar, India

Showket Hussain Division of Molecular Genetics & Biochemistry, Institute of Cytology & Preventive Oncology (ICMR), Noida, Uttar Pradesh, India

Pooja Jain Department of Microbiology and Immunology, Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, Doylestown, PA, USA

Anurag Jyoti Nanotherapeutics & Nanomaterial Toxicology Group, CSIR-Indian Institute of Toxicology Research, Lucknow, U.P., India

S.K. Kashyap Department of Vet Microbiology & Biotechnology, Rajasthan University of Veterinary & Animal Sciences, Bikaner, Rajasthan, India

Zafar K. Khan Department of Microbiology and Immunology, Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, Doylestown, PA, USA

Fahim Halim Khan Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, India

Suchit Khanna Institute of Nuclear Medicine and Allied Sciences, Defense Research Developmental Organization (DRDO), Ministry of Defense, Delhi, India

Mohammad Reza Khorramizadeh Endocrinology and Metabolic Research Institute, Tehran University of Medical Sciences, Tehran, Iran and Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

Naveen Kumar Central Institute for Research on Goats, Indian Council of Agricultural Research, Makhdoom, District-Mathura, UP, India

Sandeep Kumar Food, Drug and Chemical Toxicology Group, CSIR-Indian Institute of Toxicology Research, Lucknow, U.P., India

Satyendra Mohan Paul Khurana Amity Institute of Biotechnology, Amity University, Haryana, India

Aruna Kumar Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India

S. Maherchandani Department of Vet Microbiology & Biotechnology, Rajasthan University of Veterinary & Animal Sciences, Bikaner, Rajasthan, India

Avinash Marwal Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science, Rajasthan, India

Shet Masih Department of Microbiology and Immunology, Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, Doylestown, PA, USA

Pawan Kumar Maurya Center for Reproductive Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

Ravi Mehrotra Division of Cytopathology, Institute of Cytology & Preventive Oncology (ICMR), Noida, Uttar Pradesh, India

Debasis Mondal Department of Pharmacology, Tulane University Medical Center, New Orleans, LA, USA

Koichi Nishigaki Department of Functional Materials Science, Saitama University, Saitama, Japan

Pravinkumar Purushothaman Department of Microbiology & Immunology, University of Nevada, Reno, School of Medicine, Center for Molecular Medicine, Reno, NV, USA

K. Ravi Ram Embryotoxicology, CSIR-Indian Institute of Toxicology Research, Mahatma Gandhi Marg, Lucknow, Uttar Pradesh, India

Farshid Saadat Department of Immunology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

Anurag Kumar Sahu Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science, Rajasthan, India

Emmanuel O. Salawu Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu, Taiwan; PhD informatics Program, Taiwan International Graduate Program, Academia Sinica, Taipei, Taiwan; and Institute of Information Science, Academia Sinica, Taipei, Taiwan

Rishi Shanker Nanotherapeutics & Nanomaterial Toxicology Group, CSIR-Indian Institute of Toxicology Research, Lucknow, U.P., India

Anchal Singh Amity Institute of Biotechnology, Amity University Uttar Pradesh, NOIDA (UP), India

Gulshan Singh Nanotherapeutics & Nanomaterial Toxicology Group, CSIR-Indian Institute of Toxicology Research, Lucknow, U.P., India

Iqram Govind Singh Amity Institute of Biotechnology, Amity University Uttar Pradesh, NOIDA (UP), India.

Surinder Pal Singh CSIR-National Physical Laboratory, New Delhi, India

Mithilesh Singh G. B. Plant Institute of Himalayan Environment and Development, Sikkim Unit, Pangthang, Gangtok, Sikkim, India

Neha Singh Department of Biotechnology, Panjab University, Chandigarh, India

Priyanka Srivastava Department of Biotechnology, Indian Institute of Technology-Guwahati, Guwahati, Assam, India.

Richa Tripathi Division of Molecular Genetics & Biochemistry, Institute of Cytology & Preventive Oncology (ICMR), Noida, Uttar Pradesh, India

Hitomi Tsuiji Laboratory for Motor Neuron Disease, RIKEN Brain Science Institute, Saitama, Japan

Abhishek Tyagi Laboratory of Molecular Oncology, Dr. B. R. Ambedkar Center for Biomedical Research (ACBR), University of Delhi, Delhi, India

Kailash C. Upadhyaya Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India

Mukesh Verma Epidemiology and Genomics Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute, National Institutes of Health (NIH), Bethesda, MD, USA

Mudit Verma Epidemiology and Genomics Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute, National Institutes of Health (NIH), Bethesda, MD, USA

Subhash Chandra Verma Department of Microbiology & Immunology, University of Nevada, Reno, School of Medicine, Center for Molecular Medicine, Reno, NV, USA

Ashish S. Verma Amity Institute of Biotechnology, Amity University Uttar Pradesh, NOIDA (UP), India

Anju Verma University of Missouri, Columbia, MO, USA

Poonam Verma Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India

Vipin Verma Corning Life Sciences, Gurgaon, India

Alok Kumar Verma Food, Drug and Chemical Toxicology Group, CSIR-Indian Institute of Toxicology Research, Lucknow, India

Denys V. Volgin Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Suresh P. Vyas Department of Pharmaceutical Sciences, Dr. Hari Singh Gour University, Sagar, India

Dinesh K. Yadav Amity Institute of Biotechnology, Amity University, Gurgaon, Haryana, India

Neelam Yadav Amity Institute of Biotechnology, Amity University, Gurgaon, Haryana, India

Koji Yamanaka Laboratory for Motor Neuron Disease, RIKEN Brain Science Institute, Saitama, Japan; Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

Eugenia Ch Yiannakopoulou Department of Basic Medical Lessons Faculty of Health and Caring Professions, Technological Educational Institute of Athens, Athens, Greece

Foreword

Animal biotechnology is one of the eight disciplines – along with environmental, food, plant, aquaculture, industrial, molecular, and medical studies – of biotechnology. This volume, drawn together by Professor Ashish Verma and Dr. Anchal Singh, is a comprehensive overview of animal biotechnology from a diverse set of perspectives. The volume is comprised of 32 chapters divided into three main sections: (1) *in vivo* and *in vitro* models of human disease, (2) tools and techniques, and (3) applications and concerns.

The term animal biotechnology is broadly applied when the production or the processing of products derived from animals or aquatic species is subjected to a particular set of scientific and engineering principles in order to enhance accessibility and services. Some classic examples are the development of transgenic animals or aquatic species, the use of cloning techniques to generate nearly identical animals, and various gene knockout strategies. Transgenic animals, including cattle, pigs, and poultry, have been developed to enhance the production of human pharmaceuticals and proteins such as enzymes, antibodies, clotting factors, and albumin. Somatic cell nuclear transfer has been used to clone several important mammalian species, including sheep, pigs, goats, cattle, rats, and mice. Because success rates for implanted embryos are often quite low, this offers opportunities for research and development. It is critical that this stimulating and wide-ranging progress be assembled, assessed, and considered in a timely manner, for the development of future initiatives, and to provide appropriate and accessible background for agricultural and health regulators. This treatise does just that. From a societal perspective, there are two main questions: (1) How is animal biotechnology addressing the needs of human agriculture and health? (2) Are products from the technology safe for human consumption and not detrimental to the environment? This volume does not shy away from those tough discussions, and anchors the responses in science.

Section I of the volume offers 10 chapters on *in vivo* and *in vitro* model systems that have been developed for animal biotechnology research. It includes discussions on the applications of *Drosophila*, and the use of animal models for tuberculosis, human neurodegenerative diseases, and aging, as well as work on cancer, HIV and other antiretrovirals, HPV diagnosis, and DNA tumor viruses.

Section II assembles 11 chapters on the basic tools and techniques that are being used in contemporary animal biotechnology. These include the use of multicellular spheroids in cancer research, animal tissue culture and tissue engineering, and the applications of nanotechnology, antibodies, and molecular markers. The techniques and uses of gene expression and ribotyping are discussed, and the future of sequencing strategies presented. Finally, the importance of biomolecular displays and *in silico* modeling of networks and complex diseases in contemporary research are delineated.

Section III, which also consists of 11 chapters, focuses on applications and societal concerns. It provides summaries of the development and applications of transgenic animals, the saga of stem cells in medical research and therapy, the role of cytogenetics in medicine, and the applications of antibodies and vaccines. The importance of safety assessment of crop-derived foods is presented, together with the use of nanotechnology for the detection of pathogens, the development of marine animal biotechnology, and discussions on how the phytochemistry and pharmacology of herbal medicine biotechnology are linked to animal health. Finally, there are two chapters that provide an overview of the human genome and its relationship to animal biotechnology, and a consideration of the ethical issues that are fundamental to many aspects for the future evolution of animal biotechnology.

This volume makes clear both the vibrant diversity of the field of animal biotechnology, and the ethical and societal concerns that must be addressed. It is therefore an important volume for a wide audience, including researchers, veterinarians, physicians, agricultural and developmental economists, and policy regulators. The next few years are likely to see major breakthroughs in this field, which will be necessary to meet the nutritional and health care needs of a burgeoning global society.

Geoffrey A. Cordell, Ph.D.
Professor Emeritus, University of Illinois at Chicago
Adjunct Professor, University of Florida
President, Natural Products Inc.

Preface

Lately, "biotechnology" has become a buzz-word in both the academic arena and in day-to-day life. It is still debatable as to when and where the term originated. Who is its originator? Was biotechnology always known to the world in its present form? The answers to these questions are not known. The scientific literature tells us that Karl Erkey, a Hungarian Engineer, coined the term biotechnology in 1919. The next question is, did nature sire biotechnology or is it human beings that have created it in its present form? Again, it is difficult to come to any conclusion about the current state of knowledge. Let us go back and review the evolution of life from the most primitive form of organisms (i.e. viruses) to the most evolved form of life (i.e. human beings).

Certainly, one of the most important and advanced aspects of biotechnology and biotechnological tools is the manipulation of the genome of an organism. These manipulations can have either good or bad implications, but the answer lies in the final outcome. The most primitive form of life (i.e. viruses: bacteriophages) infects bacteria and replicates in bacterial hosts due to the integration of the viral genome into the bacterial genome. Is it Nature's biotechnological experiment to integrate genomes of two entirely different organisms? It is probably a natural need of life to compete and evolve with selection of better traits to survive against adversaries. It can be easily concluded that the present state of biotechnology has evolved due to the in-depth understanding of some of these natural processes and biological phenomenon.

There is no doubt that the life sciences have seen tremendous improvements by virtue of keen observations and discoveries made by numerous great scientists. Antibiotics and vaccinations are two of the most pronounced examples. During previous years, knowledge gained through various branches of science, namely biochemistry, molecular biology, virology, and recombinant DNA technology, etc., has tempted scientists to imitate Nature's experiments in laboratories. For successful and useful manipulations, there are three essential requirements: (1) to understand the mechanism of the biological process, (2) to replicate the same process exactly in an experimental model, and (3) to have a logical hypothesis. If these manipulations are successful, we may be able to find solutions to many prevailing and unresolved problems, namely famine, malnutrition, infectious diseases, new and emerging infections, genetic disorders, aging, debilitating diseases, etc. No doubt advancements in biotechnology, with reference to the animal sciences, have already provided solutions for some of these issues. Some issues are even partially resolved, while others are still in experimental stages.

The explosion in the knowledge of biotechnology is attributed to two important discoveries: (1) the structure of DNA, and (2) the Polymerase Chain Reaction (PCR). Advancements and applications of biotechnology have become so fascinating that it is almost difficult to confine it to the domain of scientists and high-end laboratories. This information has to be passed to the general public in order to increase awareness and to reap the benefits of these discoveries. With the explosion of biotechnology, numerous large and small companies dealing with the production and commercialization of biotechnology products have come into existence. To survive and thrive in the biotech market, companies are in a perdurable search for trained manpower.

That's how biotechnology as an educational course found its niche in the university curricula. The demand for trained biotechnologists led to the development of undergraduate and postgraduate courses in biotechnology at various universities and academic institutions. Realizing the needs of industry, some institutions developed management courses pertaining to biotechnology. In the last couple of decades it was realized that biotechnology education had to be imparted even to younger students, and that is the reason biotechnology was also included in the curricula of 10th and 12th Standard. Biotechnology itself is an amalgamation of various disciplines in the life sciences. Some of these disciplines are well evolved and have numerous good books to cater to the needs of audiences: biochemistry, molecular biology, genetics, microbiology, etc. However, animal biotechnology as a subject is still in its infancy, and has yet to develop and evolve as a full discipline in academic departments at universities. As such, it is difficult to find books in animal biotechnology that can fulfill the need of biotechnology students.

We teach animal biotechnology to undergraduate and postgraduate students. We have had a tough time teaching this course because of major limitations like an ever-evolving curricula and unavailability of reasonable textbooks on the subject. The only available resources are

research publications and books semi-related to research topics. On the one hand it's hard for students to find a place to start when learning the subject, and on the other hand instructors have a difficult time locating and organizing materials and resources for the classroom. The ultimate resource for instructors and students is the World Wide Web (WWW). In our teaching experience, we come across curious students who ask numerous intelligent questions almost every day. Their quest for information and knowledge remains insatiable due to the limitation of consolidated sources of information. Not only this, but we routinely face questions from students about where they can get more information on a specific subject or topic, and to their utter disappointment, it's hard for us to pinpoint one book or a good resource to answer all their questions. We frequently discuss the issue of the lack of applicable literature, almost every day over coffee with our colleagues. Discussing various options and trying to narrow down our search to fill this void of content in the area of animal biotechnology was not getting us anywhere.

After numerous deliberations, it was Dr. Anchal Singh who came up with the idea to explore the possibility of developing a book on animal biotechnology to partially (if not completely) fill this void. Then we deliberated on our *modus operandi* to develop this book. Finally, we decided to develop a book by inviting chapters from experts in the field who have relevant research experience and an understanding of the intricacies of the subject. We had in mind a book that would help to alleviate most of the worries of both students and instructors. We discussed, argued, and disagreed until we came up with the thought that a resource book would be a reasonable format, as it could provide sufficient information and literature for instructors to teach the subject, while providing students with ample information to gain better insight about the subject. Once we formulated these thoughts to develop a resource book, the ball started rolling, and we identified various experts and convinced them to contribute chapters.

Bringing this book to completion was a joint effort. We could not possibly assemble all subjects together in one book, therefore we tried to bring together some of the important topics that usually interest students and instructors of animal biotechnology. The subject matter of this book varies from the basics of animal biotechnology, to animal tissue culturing, to the production of antibodies against infectious agents like HIV. Included are chapters dealing with animal models of important diseases like cancer and tuberculosis, and also *in silico* models, to emphasize their importance in understanding disease pathogenesis. An attempt was made to include the latest tools and technology related to the subject, namely, ribotyping, epigenetics, cytogenetics, bimolecular display technologies, next generation sequencing, and many more such topics not listed here.

This is our maiden effort to produce a book to help students and instructors of animal biotechnology. We hope that we will get support from the readers of this book. We are always open to criticism, suggestions, and recommendations that can help to improve the content and presentation of the book. Your suggestions and criticisms will give us an opportunity to explore other aspects of animal biotechnology in our future ventures and endeavors.

Ashish S. Verma
Anchal Singh

Acknowledgments

We are grateful to The GOD, because of whom we exist. God has gifted us (Human Beings) with a brain to hypothesize and analyze, courage to dream, and motivation to achieve.

Then we would like to thank Prof. Geoffrey A. Cordell, who agreed to write a Foreword for our book. This turned out to be a power boost for the editors.

Anchal would like to thank her dad, Mr. Kanhaiya Ji Singh, her mom, Ms. Mohini Singh, her brother, Abhisar, and his wife, Meenakshi, for their support, love, and help. Anchal's eight-year-old son, Aviral, was a stress buster whose unstoppable questions and witty answers alleviated the stress and pressure of editing this book.

I (Ashish) would like to express my indebtedness to my mother, Ms. Sushma Saxena. I do exist due to her great efforts to raise and groom me. She has always been the person in my life whom I can bank upon for anything, anytime. My brother, Mr. Saumya Swarup, his wife, Ms. Nimisha Swarup, and their kids, Utkarsh and Shreeparna, have also supported me as and when, I needed them. Similarly, my sisters and their family members always encouraged me to remain focused on this book. It is not only family members who inspired me during the development of this book, but also Anchal's son, Aviral with his inquisitiveness and unending innocent queries, which kept me refreshed. We must admit that it is young kids who are our prime stress relievers.

We are thankful to Dr. A. K. Chauhan, Founder President for his support and encouragement. Our special thanks go to Prof. Ajit Varma, who was always with us when we needed him, with his excellent and practical advices. We would not do justice to this project if we do not acknowledge the role of Prof. Soom Nath Raina, one of our colleagues. For us, Prof. Raina is more than a colleague. His affection, care, and concern made him a part of our extended family. We will always remain thankful for his untiring support, which kept us motivated in this long and sometimes clumsy journey of book editing.

Our students – Priyadarshini Mallick, Shruti Rastogi, Shishir Agrahari, Sneha Saran, Deepak Kushwaha, and Ajay Yadav – contributed both directly and indirectly towards the development of this book. We remain thankful for their support. Some of them provided help and support to organize us better, some of them offered their viewpoints, and some of them did not forget to offer us their critiques. We admire all of them for what they have contributed to this book. We have discovered that the biggest motivations for teachers are always their students and students' needs.

We are deeply indebted to Mr. Dinesh Kumar, who has worked with us since we joined this organization and has always provided crucial secretarial assistance. To Mr. Yogendra Singh, who has worked for a long time as a member of our group, and is always there with freshly brewed coffee to fulfill our caffeine requirements. Mr. Sandeep Kumar, who, though recently joined our group, also contributed with his efforts to this project.

Our special thanks go to Ms. Chirstine A. Minihane, who helped us initiate and develop this book. Last but not least, this book could never have been completed without constant support from dedicated persons at Elsevier: Mr. Unni Kannan (Technical Assessor), Ms. Catherine A. Mullane (Editorial Project Manager), Graham Nisbet (Acquisitions Editor), and Edward Taylor (Production Manager). They all provided the support and motivation to push us through to the completion of this project.

As editors, we would like to express our gratitude and thanks to all the contributing authors who shared their expertise and experience by writing chapters in their respective fields. Finally, as the editors, we would like to convey our heartfelt thanks to everyone who has contributed directly or indirectly towards this book.

Ashish S.Verma
Anchal Singh

Chapter 30

Herbal Medicine and Biotechnology for the Benefit of Human Health

Priyanka Srivastava[*†], Mithilesh Singh[**], Gautami Devi[*] and Rakhi Chaturvedi[*]

**Department of Biotechnology, Indian institute of Technology – Guwahati, Guwahati, Assam, India, **G.B. Plant Institute of Himalyan Environmental and Development, Sikkim Unit, Panthang, Gangtok, Sikkim, India, †Division of Biomedical Sciences, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India*

Chapter Outline

Summary 563
What You Can Expect to Know 563
History and Methods 563
Introduction 563
Traditional Medicine 564
Ancient System of Medicine 565
Methodology 565
Investigation of Medicinal Plants 565
Extraction 566
Chemical Screening 567
Biological Assays 567
Isolation and Characterization of Bioactive Compounds 567
Biotechnological Approaches for Herbal Drug Production 569
Organ Cultures 569
Callus Cultures 570
Suspension Cultures 570
Case Study: *Lantana camara* L 570
Opportunities and Challenges 571
Conclusions and Outlook 573
Ethical Issues 573
Translational Significance 573
World Wide Web Resources 574
References 574
Further Reading 574
Glossary 574
Abbreviations 575
Long Answer Questions 575
Short Answer Questions 575
Answers to Short Answer Questions 575

SUMMARY

The present chapter discusses the importance of plants and their metabolites in herbal medicines. Various examples of biotechnological tools have been highlighted how plants can be exploited commercially without affecting their natural population. Furthermore, the chapter discusses processing plants for herbal medicine and drug discovery from natural products.

WHAT YOU CAN EXPECT TO KNOW

How do herbal medicines compare to conventional forms of medicine? What are their advantages and limitations? Besides this, what are their methods of production from plant sources, and what are the various techniques required to analyse and characterize them?

HISTORY AND METHODS

INTRODUCTION

Herbal medicines refer to the use of plant seeds, berries, roots, leaves, bark, or flowers for medicinal purposes (Figure 30.1). Medicinal plants have been a major source of drugs for thousands of years, and even today they are the basis of systematic traditional medicines in almost all countries of the world. Unani and Ayurveda systems of medicine are two of the classic and oldest examples of this category. Around 80% of the population in developing countries is completely dependent on plants for their primary health care (Bannerman et al., 1983). Even in developed countries, which are enormously advanced in terms of medicinal chemistry, over one-fourth of all prescribed pharmaceuticals originate directly or indirectly from plants

Animal Biotechnology. http://dx.doi.org/10.1016/B978-0-12-416002-6.00030-4

FIGURE 30.1 Herbal medicines. *(Courtesy: Google)*

(Newman et al., 2000). Furthermore, out of 252 drugs considered as indispensable by the World Health Organization (WHO), 11% are mainly derived from flowering plants, and 28% of synthetic drugs are obtained from natural precursors (Namdeo, 2007).

As already mentioned, herbal medicines are derived from plants. Understandably, these pharmaceuticals are produced solely from massive quantities of whole plant parts, which can lead to problems. One problem is that excessive harvesting can diminish local plant populations and erode genetic diversity. A second, but important, concern is inconsistency of the derived products in terms of quality and quantity. The latter can spell trouble in terms of safety, supply, and economic feasibility of these herbal products on a commercial scale. In order to overcome these bottlenecks, domestication and acceptance of good agricultural practices are crucial, especially for revival of diminishing plant populations. However, the conventional methods of plant propagation are lengthy and time consuming. The long cultivation periods between planting and harvesting make the entire process cumbersome and uneconomical, which in turn leads to the high cost of drugs. Moreover, wild populations are susceptible to problems of disease, drought, environmental fluctuations, low rate of fruit set, and poor seed yield, germination, and viability. This vulnerability of plants also affects batch-to-batch consistency of derived metabolites to be used as drugs or drug precursors. Clearly, there is an urgent need of alternative and complimentary methods for uniform production of herbal medicine. In this context, tools and techniques of biotechnology, like *in vitro* plant, cell, tissue, and organ culture, offer solutions in terms of mass propagation of plants in a shortened time span, occupying much less space than wild populations, and uniform production of metabolites all year round, irrespective of seasons and vagaries of climatic conditions. One clarification required at this point is the term "metabolites." The majority of the compounds used as drugs are secondary metabolites (Kubmarawa et al., 2007), whose production is largely affected by environmental fluxes.

Traditional Medicine

The World Health Organization (WHO) defines traditional medicine as being the "sum total of knowledge, skills, and practices based on the theories, beliefs and experiences that are indigenous to different cultures, which are used to maintain health, as well as to prevent, diagnose, improve, or treat physical and mental illnesses." Every early civilization used plants as their main source of medicine, and most of the world's population still relies on them. The first

recorded literature on medicinal plants can be traced back to early human history, the Atharvaveda (2000 B.C.) in India. With time, the original population of an area gained knowledge which plants could be used for certain diseases or states of illness. In addition, they also gained knowledge of the harmful and poisonous plants. It is evident that the modern drug industry has been developed to a considerable degree as a result of plant-based traditional medicines.

There are a few closely related terms in use today, meanings of which should be understood clearly. *Traditional medicine* refers to the following components: acupuncture (China), Ayurveda (India), Unani (Arabic countries), traditional birth attendant's medicines, mental healer's medicines, herbal medicines, and various forms of indigenous medicines. *Complementary or alternative medicine* refers to a broad set of health care practices that are not part of a country's own tradition, and are not integrated into the dominant health care system. Traditional medicine has maintained its popularity in all regions of the developing world, and its use is rapidly spreading in industrialized countries.

Ancient System of Medicine

Ayurveda, perhaps the most ancient of all medicinal traditions, is probably older than traditional Chinese medicine. It is derived from "Ayur" meaning "life," and "Veda," meaning "knowledge." Ayurveda means the "science of life." It takes a holistic view of human beings, their health, and illness. It aims at positive health, which has been defined as a well-balanced metabolism coupled with a healthy state of being. According to Ayurveda, disease can arise from the body and/or mind due to external factors or intrinsic causes. The origin of Ayurveda is lost in prehistoric antiquity, but its characteristic concepts appear to have matured between 2,500 and 500 B.C. in ancient India. The earliest references to drugs and diseases can be found in the Rigveda and Atharvaveda.

Ayurvedic drugs have been found to perform very well against chronic ailments. Today, they are also attracting attention for diseases for which there are no (or inadequate) drugs for treatment in modern medicine, such as metabolic and degenerative disorders. Most of these diseases have multifactorial causation, and there is a growing awareness that in such circumstances, a combination of drugs, acting at a number of targets concurrently, is likely to be more effective than drugs acting at one target. Ayurvedic drugs, which are often multi-component, have a special impact on such conditions. For various reasons, Ayurveda has not included much of modern science/scientific tools. Studies of the biological activity of multicomponent Ayurvedic drugs will bring Ayurveda into the mainstream of scientific investigations.

METHODOLOGY

Investigation of Medicinal Plants

Medicinal plants have formed the basis of health care throughout the world since the earliest days of civilization, and are still widely used, and have noteworthy significance in international trade. Recognition of their clinical, pharmaceutical, and economic value is still growing, although this varies widely between countries. Plants are important for pharmacological studies and drug development, not only when bioactive compounds are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds.

Each plant species has its own specific set of secondary metabolites. Apart from the family Poaceae, which harbors the world's worst weeds but is low in medicinal plants, many of the top twelve weed families are also the ones that are important for medicinals. The ecological and biochemical evidence suggest the preponderance of weeds in medicinal floras. Secondary compounds in plants are involved in the interaction of the plant with its environment and are important for ecological functions such as allelopathy, insect and animal attractants for pollination, seed dispersal, and for chemical defense against microbes, insects, and herbivory (Bourgaud et al., 2001). These compounds do not participate in the vital metabolic processes of the plant system, but are the ones that exhibit bioactivity, and can serve as medicinals for humans. The spectrum of chemical structures synthesized by the plant kingdom is broader than that of perhaps any other group of organisms (Rao and Ravishankar, 2002).

In the present scenario, a large proportion of the drugs used in modern medicine are either directly isolated from plants, or synthetically modified from a lead compound of natural origin. However, rarely is the drug isolated in the pure, usable form. What is initially obtained is the crude extract, which requires stepwise purification to obtain the finished product. The finished product as herbal medicine most of the time is a mixture of several compounds. When each and every component in the mixture is characterized qualitatively and quantitatively, it is called *"characterized extract,"* which is understandably more desirable than the *"uncharacterized extract."* Plant extracts are known to consist of many chemicals, and among them, a few compounds could be acting synergistically. Sometimes, isolation of the compounds from the extract may cause a decrease in desired activity, which underlines the importance of extract screening (Orhan et al., 2009).

Evidence-based studies on the efficacy and safety of traditional Indian medicines are limited. The essential ingredients in most formulations are not precisely defined. This is one of the most important challenges to scientists attempting to identify a single bioactive compound. Therefore, in-depth studies and more stringent conditions should

be followed to make a herbal formulation so that the role of each and every component is known.

Drug discovery is the process by which drugs are discovered or designed. Plants have long been a very important source of drugs, and many plant species have been analyzed to see if they contain substances with therapeutic activity. Many plant drugs of folklore were investigated to determine the active ingredient in the mixture. Several reviews are available in the literature pertaining to approaches for selecting plants as candidates for drug discovery programs.

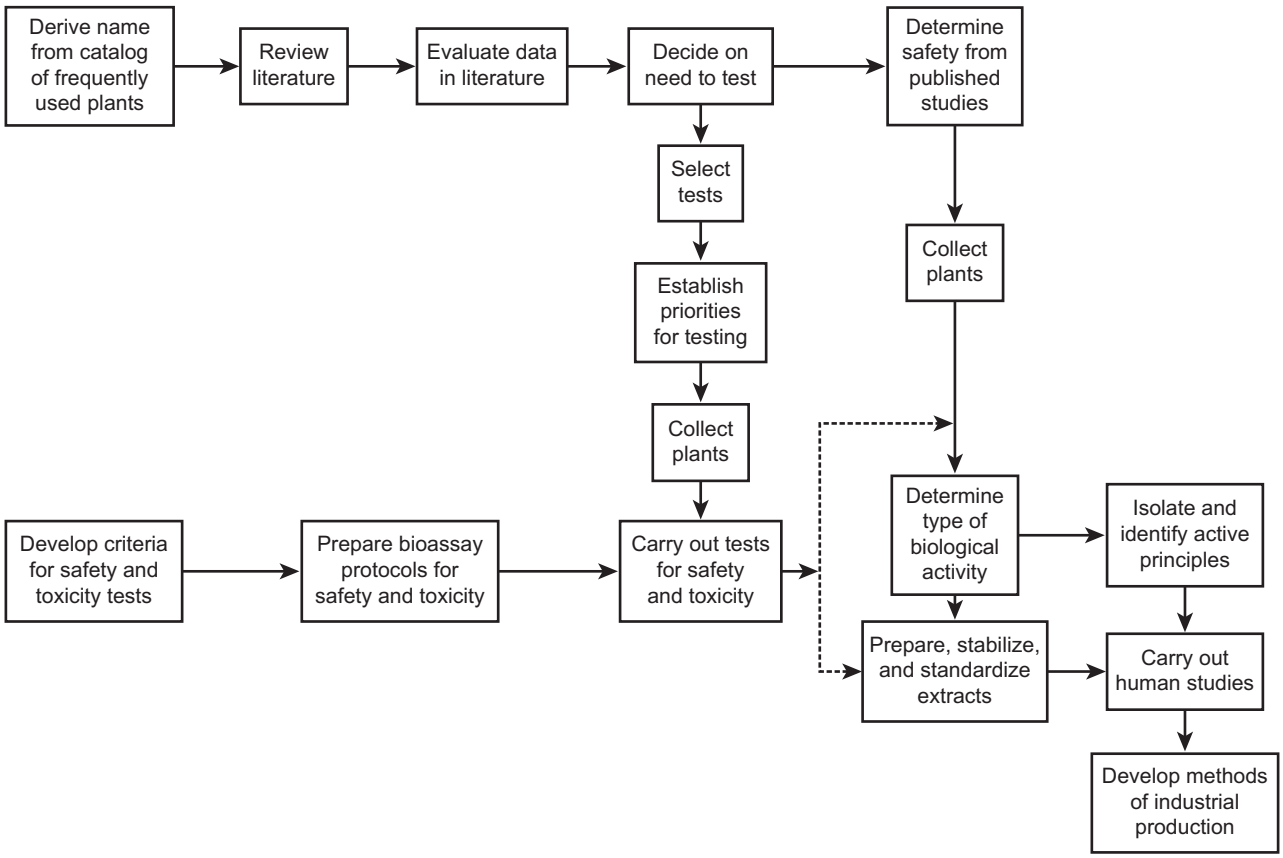
Today, many new chemotherapeutic agents are obtained synthetically, based on "rational" drug design. The study of natural products has many rewards over synthetic drug design. The former leads to materials having new structural features with novel biological activity. In this context not only do plants continue to serve as possible sources for new drugs, but chemicals derived from the various parts of these plants can also be extremely useful as lead structures for synthetic modification and optimization of bioactivity. The starting materials for about one-half of the medicines we use today come from natural sources. There is no doubt that the future of plants as sources of medicinal agents for use in investigation, prevention, and treatment of diseases is very promising.

Drug discovery from natural resources is a very tedious process. It involves identification of plant material, extraction, preliminary phytochemical screening of the crude extract, evaluation of biological activity, isolation of various bioactive compounds, and finally elucidation of structures. If the molecule is appealing, with strong pharmacological properties, then further preclinical studies are conducted on the molecules, such as toxicity, stability, and solubility studies. After undertaking these studies, if it is found that a molecule is substantially more active than the currently used drug, only then are processes developed for its economical and easy isolation from the source so that it can be readily available for therapeutic use.

In the context of isolation and screening of chemicals from plants that may possess medicinal properties, different approaches can be used. The process of obtaining bioactive substances and their chemical characterization can be schematically represented as in Flow Chart 30.1.

Extraction

Extraction involves the separation of medicinally active fractions of plant from inactive or inert components by using selective solvents through extraction procedures. The products so obtained from plants are relatively complex



FLOW CHART 30.1 Flow chart of sequence for the study of plants used in traditional medicine. *(Adapted from Fabricant and Farnsworth, 2001.)*

mixtures of metabolites in liquid, semi-solid, or (after removing the solvent) dry powder form. This is the critical first step in the investigation of medicinal plants.

The selection of a solvent system mainly depends on the exact nature of the bioactive compounds being targeted because during the extraction process, solvents diffuse into the solid plant material and solubilize compounds of similar polarity. The extraction of hydrophilic compounds uses polar solvents, such as methanol, ethanol, or ethyl acetate. For extraction of more lipophilic compounds, dichloromethane is used. In a few cases, extraction with hexane is used to eliminate chlorophyll and oil.

As the target compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction must be well thought out. Different methods, such as sonication, heating under reflux, soxhlet extraction, and others, are commonly used for plant sample extraction. Additionally, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems.

Other modern extraction techniques include solid-phase micro-extraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, solid-phase extraction, and surfactant-mediated techniques, which possess certain advantages.

Chemical Screening

This technique is also known as phytochemical screening. In this method, aqueous and organic extracts are prepared from those plant samples that are the reservoir of secondary metabolites, such as leaves, stems, roots, or bark. The plant extracts are then analyzed for the presence of secondary metabolites like alkaloids, terpenes, and flavonoids. Standard tests are available in the literature for each class of compounds to be analyzed. Following this, a simple separation technique like thin-layer chromatography (TLC) is generally used to analyze the number and type of components present in the mixture. In TLC, the extracts are loaded in a glass coated with silica gel or other adsorbent, which is then kept in a chromatographic chamber containing a suitable running solvent. This technique mainly consists of a mobile phase and a stationary phase, whereby the compounds are separated based on their polarity. Sometimes a developing solvent might also be used after the plate has been taken out of the chromatographic chamber to detect the chemicals. This approach has been used in the past, and is still being used in developing countries. Since the isolation of pure bioactive components is a long and tedious process, this procedure enables the early recognition of known metabolites in the extracts, and is thus economically viable. The tests are simple to perform, however, it is not suitable for the efficient separation of metabolites, and has low selectivity and sensitivity of detection, which makes it difficult to detect traces of components in the sample.

Biological Assays

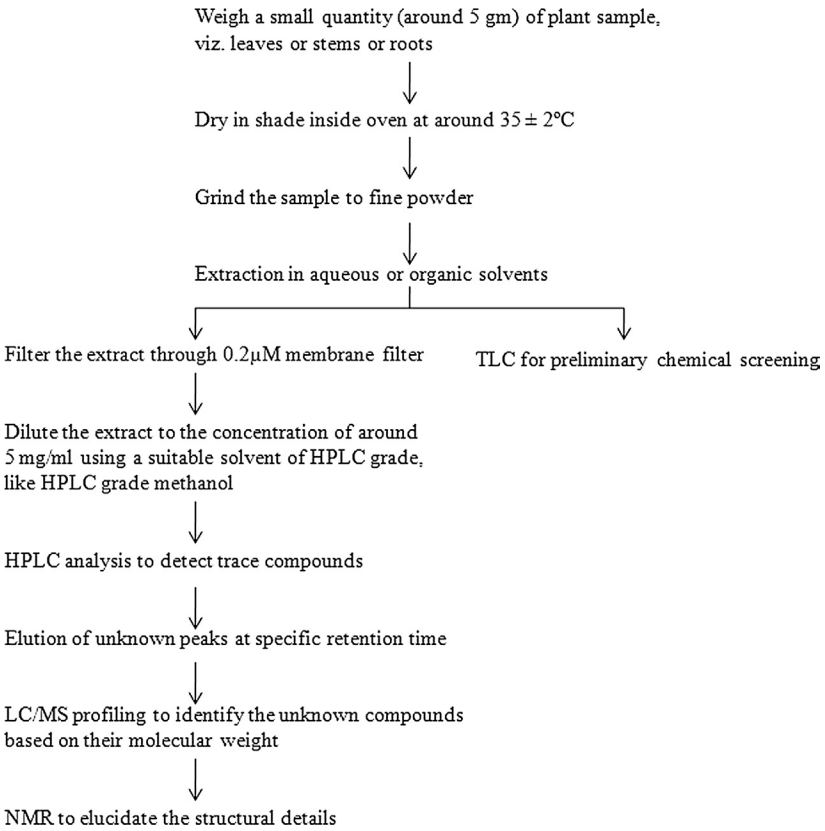
Plant extracts have served as an important source of bioactive compounds for many drug discovery programs, and several important drugs have been isolated and identified from plants. In any isolation program in which the end product is a drug or lead compound, some type of bioassay screening or pharmacological evaluation must necessarily be used to guide the isolation process towards the pure bioactive component.

The selection of the biological assay to be adopted usually depends on the target syndrome as well as on the available information about the plant to be studied. For instance, if a plant has an ethanopharmacological history of use against a particular disease, then one would rationally use a specific bioassay technique that can predict the reputed therapeutic activity in order to isolate the lead that is responsible for that biological activity.

In the past, the extracts from plants were mainly evaluated in experimental animals, primarily mice and rats. Currently, anti-microbial assay by the disk diffusion method is in practice. However, this technique had several disadvantages. Firstly, the phytochemical extracts are highly heterogeneous due to the presence of a mixture of different bioactive components. A desired biological response may not be due to a single bioactive compound, but to a mixture of several bioactive compounds. Moreover, although several new bioassay techniques have been developed, at present these techniques are still expensive, time-consuming, and technologically complicated. The major disadvantage of bioassay techniques is the use of biological organisms, particularly mice and rats, which is not practical as these living organisms most often have to be sacrificed. Lastly, isolation, screening, and quantification of a specific bioactive compound are difficult using biological assays. Hence, this technique is losing popularity.

Isolation and Characterization of Bioactive Compounds

Due to the fact that plant extracts usually contain various types of compounds with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. Apart from this, there are always chances of wide variations with respect to their chemical content in crude drugs/raw materials of plant origin due to varied reasons such as climatic conditions, geographical distribution, source and season of collection, and lack of scientific methods of post-harvest processing, storage, and preservation. Therefore, identification and quantification of bioactive compounds are essential prerequisites for herbal drug development (Flow Chart 30.2). Thin-layer chromatography is a powerful and simple analytical tool used for this purpose. However,



FLOW CHART 30.2 Schematic representation showing the process of chemical screening, isolation and characterization of bioactive substances from plants.

there are situations where this tool does not give satisfactory results because of its own limitations. High-pressure liquid chromatography (HPLC), liquid chromatography/mass spectrometry (LC/MS), nuclear magnetic resonance (NMR), etc., are well-suited quantitative and qualitative analytical methods of choice to control the quality of phytopharmaceuticals.

High-pressure liquid chromatography, also called as high-performance liquid chromatography (HPLC), is an important analytical tool for the efficient localization and rapid characterization of natural products. It involves the injection of a small volume of liquid sample into a tube packed with porous particles (stationary phase), and the individual components of the sample are pulled along the packed tube (column) by a solvent (mobile phase) moved by gravity. A pump forces the liquid through the column at a specific flow rate and generates high pressure. The column packing separates the components of the sample by various physical and chemical interactions between the molecules and the packing material. The separated components get collected at the exit of the column and are detected by several techniques like UV, fluorescence detection, diode array detection, etc. Data is generated in the form of chromatograms, where individual components show peaks at specific retention times at which the component was eluted. Since, HPLC has a high resolution and is very sensitive, this technique is suitable for the detection of trace components whose concentration in the sample is very low.

The processing of a plant crude extract to provide a sample suitable for HPLC analysis, as well as the selection of solvent for sample reconstitution, can have a significant bearing on the overall success of natural product isolation and identification. The source material (e.g. dried powdered plant) will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. This is where an efficient extraction protocol becomes important. An organic solvent may be used for extraction, and then solid material is removed by centrifugation and filtration of the extract. The filtrate is then concentrated and injected into an HPLC instrument for separation. Use of guard columns is necessary in the analysis of crude extract. Many natural product materials contain significant levels of strongly binding components such as chlorophyll and other endogenous materials that may in the long term compromise the performance of analytical columns.

Liquid chromatography coupled to mass spectrometry (LC/MS) is a newer technique, and is one of the most

sensitive methods of molecular analysis. It yields information on the molecular weight and structure of the analytes. A component showing a specific retention time in HPLC can be eluted out at that particular retention time, and its mass spectral analysis can be done to get more details about its molecular weight and structure. An MS detector senses a compound eluting from the HPLC column first by ionizing it, and then by measuring its mass or by fragmenting the molecule into smaller pieces that are unique to the compound. The MS detector can sometimes directly identify the compound since every compound has its own unique mass spectrum and acts as a fingerprint for that particular compound.

Nuclear magnetic resonance (NMR) is another important analytical tool that helps in elucidation of the structural details of bioactive compounds. NMR has the ability to provide a detailed picture of molecules. Even the conformational space of molecules can be studied in great detail using this tool. This technique probes the magnetic properties of nuclei induced by their spin states. Almost every element has an isotope that is magnetically active, and their magnetic vectors align in an external field either parallel or anti-parallel to the field. There is always a small energy difference associated with the parallel and anti-parallel orientations, and the difference in energy can be visualized by irradiation with proper radio frequencies. The amount of splitting of energy levels is different for different nuclei, and is linearly dependent on the magnetic field. Therefore, different nuclei can be observed at different radio frequencies, and hence, each radio frequency becomes unique for a particular nucleus and can be easily identified.

Gas chromatography/mass spectrometry (GC/MS) is based upon the partitioning of compounds between a liquid and a gas phase. This technique is widely used for the qualitative and quantitative analysis of a large number of herbal drugs because it has high sensitivity, reproducibility, and speed of resolution. It has proved to be most valuable for the separation of volatile, non-polar, and semi-polar bioactive compounds. In GC/MS, the sample is injected into a long tubular column, the chromatography column, which has a high boiling point stationary phase, such as silicon grease. The basis of the separation is the difference in the partition coefficients of volatilized compounds between the liquid and gas phase as the plant metabolites are carried through the column by the inert carrier gas (e.g. nitrogen, helium, or argon). The time taken by the sample to pass through the length of the column is referred to as its retention time (RT). The RT for a given sample is an identifying characteristic. The detector for the GC is the mass spectrometry (MS) detector. As a sample exits the end of the GC column, it is fragmented by ionization, and the fragments are sorted by mass to form a fragmentation pattern.

BIOTECHNOLOGICAL APPROACHES FOR HERBAL DRUG PRODUCTION

Intact plants in the field or wild habitats produce high-value bioactive compounds. However, the quantity and availability of these economic products from natural resources restrict their maximized uses for the benefit of humankind. For the last few years, as the demand for bioactive compounds has increased, exploitation of medicinal plants has also increased. Hence, there is an urgent need to develop an alternative method for the large-scale production of metabolites and quality plants. In this respect, biotechnology put forward an attractive alternative to whole-plant extraction for homogeneous, controlled production, especially, when we take the commercial demand into picture. It also results in more consistent yield and quality of the products, irrespective of the seasons and the regions. Biotechnology offers an opportunity to exploit plant cells, tissues, organs, or entire organisms by growing them *in vitro* and genetically manipulating them to get desired compounds (Rao and Ravishankar, 2002). Many biotechnological strategies, such as embryogenesis, organogenesis, screening of cell lines, media optimization, and elicitation, can be carried out for enhanced production of secondary metabolites from medicinal plants. The subsequent sections briefly discuss the different *in vitro* culture techniques that can be used for herbal drug production.

Organ Cultures

The selection of an appropriate technique depends on the results that one wants. In plants where molecules of interest are localized in specialized cells, dedifferentiated cultures are not desirable. Therefore, establishment of organogenic cultures would be advantageous. Under *in vitro* conditions, redifferentiation is generally associated with an improved synthesis of secondary metabolites (Collin 2001). This is probably due to the appearance of complex cells and tissues that are metabolically more proficient. In all redifferentiated cell lines, along with the shoot-forming nodules, non-morphogenic cell masses are also present, which though non-morphogenic, might have a certain degree of differentiation at the cellular stage, and due to co-evolution, imitate the biochemistry of redifferentiated cells (Brown et al., 1986). The reports on *Artemisia annua* and *Azadirachta indica* stated that artemisinin and azadirachtin production, respectively, were very poor in dedifferentiated callus cultures, and a certain degree of redifferentiation was obligatory for compound production. Organogenesis was also found to be an essential prerequisite for steroidal saponin production in *Ruscus aculeatus*. Similar observations were made for the biosynthesis of picroside in *Picrorhiza kurroa,* wherein the metabolite did not accumulate in the dedifferentiated callus cultures, but occurred specifically in

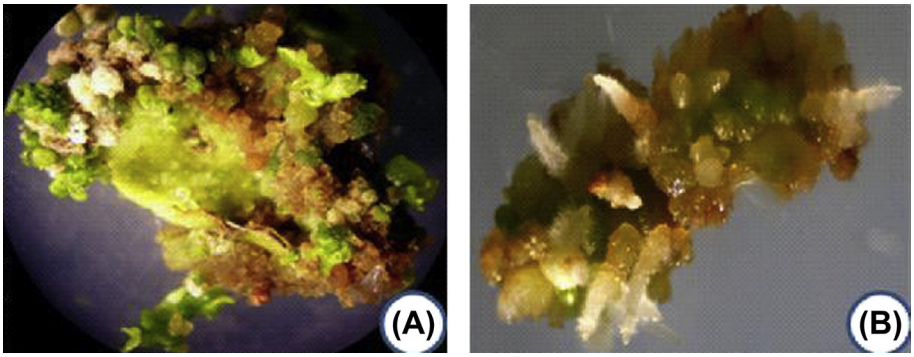


FIGURE 30 2 Neem organogenesis from leaf explants indirectly via callusing: (A) Shoot differentiation. (B) Root differentiation

the redifferentiated cultures. Berkov et al. (2010) also demonstrated that alkaloid synthesis in *Pancratium maritimum* is closely related to tissue differentiation.

Since it was observed that production of bioactive compounds is generally higher in organized plant tissues; there are attempts to regenerate whole plant organs (i.e. shoots or roots) under *in vitro* conditions, either directly from explants, or indirectly via an intervening callus phase (Figure 30.2). As expected, such regenerating cultures produce patterns of secondary metabolites that are similar to the field-grown parent plant, with the added advantage of improved production of metabolites. Another advantage of using the organized cultures is that they are relatively more stable in the production of secondary metabolites than cultures of undifferentiated cells, such as cells in callus or suspension cultures (Rao and Ravishankar, 2002).

Callus Cultures

Callus culture is the culture of dedifferentiated plant cells induced on media usually containing relatively high auxin concentrations or a combination of auxin and cytokinin under *in vitro* conditions. In plants, where sought after metabolites are present in leaves, establishing *in vitro* cultures from leaves and using them for the extraction of compounds would be an ideal alternative. Callus cultures containing the bioactive substances are collected at a specific stage (usually during the stationary phase of their growth cycle, since secondary metabolite production is greater during the stationary phase), dried, extracted, and the extract then taken for identification and quantification of the desired medicinal compound using HPLC, LC-MS, etc. The further scale-up and yield enhancement studies of the compound are performed by raising the callus in suspension, first in a shake-flask culture, and then in a suitably designed bioreactor, to maximize its production.

Suspension Cultures

A breakthrough in cell-culture methodology occurred with the successful establishment of cell lines capable of producing high yields of secondary compounds in cell suspension cultures (Zenk, 1978). During the past decades, this approach of metabolite production has attracted much academic and industrial interest. The technique of using plant cell suspension cultures for secondary metabolite production is based on the concept of biosynthetic totipotency of plant cells, which means that each cell in the culture retains the complete genetic information for production of the range of compounds found in the whole plant. Cell suspension cultures are initiated from established callus cultures by inoculating them into liquid media. The cultures are then kept in glass flasks under continual agitation on horizontal or rotating shakers; they can eventually be transferred to a specialized bioreactor. Cells in suspension cultures grow much better than in semi-solid media because of better mixing of oxygen and nutrients during shaking conditions.

Productivity of suspension cultures is critical to the practical application of this cell technology for bioactive compound production. To improve the production of secondary metabolites in *in vitro* cultures, various strategies such as the manipulation of parameters of the environment and medium, selection of high-yielding cell clones, precursor feeding, and elicitation can be opted for.

Case Study: *Lantana camara* L

This example using *Lantana camara* L. shows how plant tissues can be employed in tissue culture and further in biochemical studies.

Lantana camara L. (Sage (English) or Caturang (Hindi)) is an aromatic, evergreen shrub belonging to the family Verbenaceae. It is a reservoir of several important bioactive molecules. It has been listed as one of the important medicinal plants in the world (Sharma et al., 2000). For many years, natural products from *Lantana* have been used in the prevention and cure of many serious diseases, including cancers. The most significant bioactive molecules of this plant are shown in Figure 30.3.

For establishing tissue cultures, the first prerequisite is the selection of healthy plant material. Thus, for this study, leaves from *Lantana* plants bearing pink-yellow flowers were

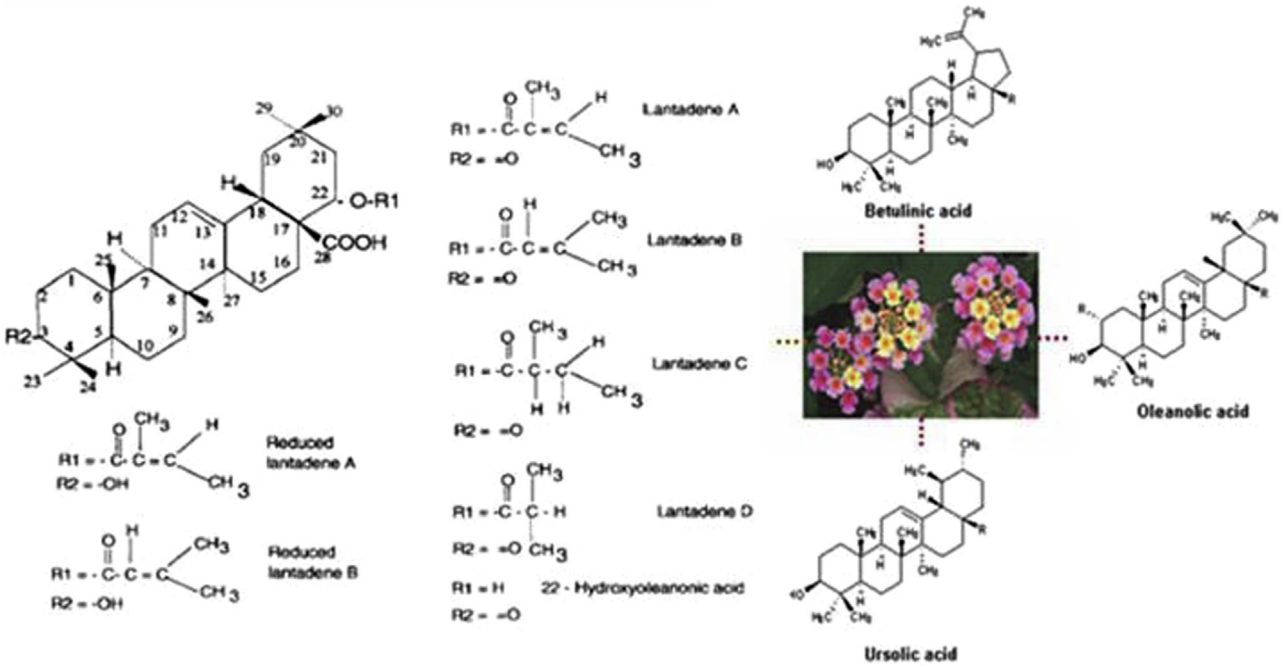


FIGURE 30.3 Bioactive compounds of *Lantana*.

picked. Leaves were disinfected using 1% (v/v) Tween-20 and 0.1% (w/v) mercuric chloride, followed by three rinses in sterile distilled water after each step. The leaf disk explants were prepared using a cork borer of 5 mm diameter. The basal media used in all the experiments related to callus induction and proliferation consisted of MS (Murashige and Skoog, 1962) medium enriched with 30 g/L sucrose and solidified with 0.8% agar (HiMedia Laboratories, Mumbai, India). The pH of the media was adjusted to 5.8 before autoclaving at 1.06 kg cm^{-2} and 121°C for 15 min. The media was supplemented with different plant growth regulators (auxins and cytokinins) at defined concentrations. Remaining steps are explicitly described in Figure 30.4.

OPPORTUNITIES AND CHALLENGES

The consumption of herbal medicines and the importance of the herbal medical industry are fast growing and widespread. According to estimates of the World Health Organization, more than 80% of the world's population depends primarily on herbal medicines. The ancient art of herbal medicine is fast developing today, and is undergoing something of a renaissance all over the world, particularly in developed countries. Most of the ingredients used in herbal medicines are taken from wild plants, and the increasing demand for medicinal plants, along with habitat loss, is putting pressure on many species. Indiscriminate harvesting from the wild has led to loss of genetic diversity, diminishing populations, local extinctions, and habitat destruction. This has raised the ire of plant conservationists.

Domestic cultivation of medicinal plants offers a viable conservation strategy, and also eliminates the problems that are generally faced in herbal extracts, such as misidentification, genetic and phenotypic variability, extract variability and instability, toxic components, and contamination. Optimized yield and uniform high-quality product can also be achieved through cultivation. However, in a rapidly shifting and fashion-prone market, the cultivator has to make the difficult decision of which particular species to grow. Therefore, the difficulty in predicting which extracts will remain marketable is another serious obstacle in bringing medicinal plants into successful commercial cultivation.

Although a large number of plant species used in herbal medicine are cultivated, a great majority of them are still utilized from the wild population. There are certain difficulties faced by growers in the cultivation of herbal plants because of low germination rates or specific ecological requirements. Lack of knowledge about the specific requirements for pollination, seed germination, and growth are the main hindrances in the cultivation of herbal plants. Fungal infection or mechanical damage frequently results in low germination rates that can be easily overcome by improved seed treatments and by ensuring optimal storage conditions. Moreover, difficult-to-grow herbal plants can be easily cultivated on a commercial scale by using controlled environments, including hydroponic systems.

Another major challenge faced in the production of herbal medicines is that the main bioactive component, which is the major ingredient in the herbal medicine, is synthesized in a very small quantity in the specific plant. This is obvious, as the bioactive components are mainly produced as secondary

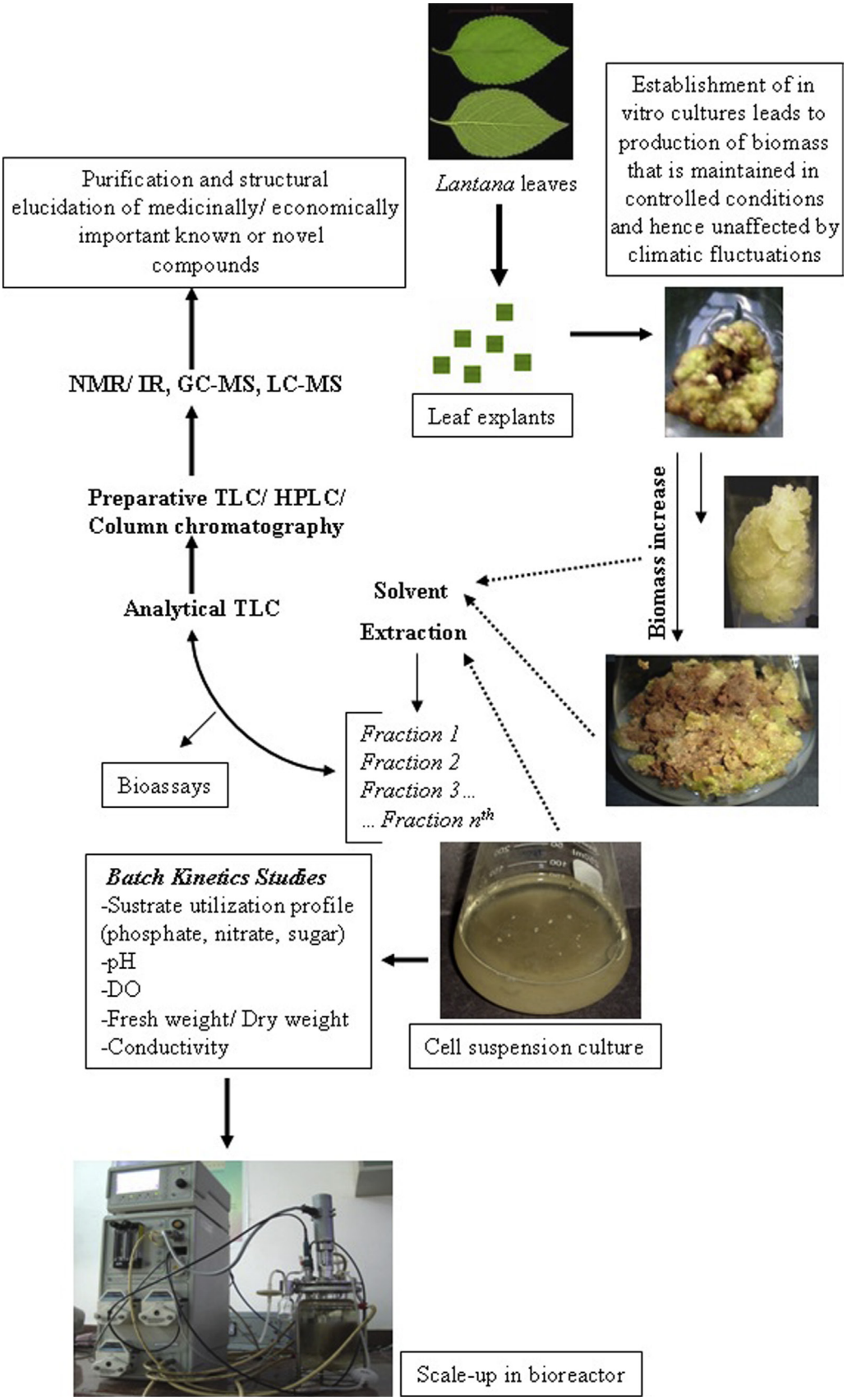


FIGURE 30.4 Isolation of bioactive compounds from *Lantana camara*, a medicinal plant.

metabolites in plant cells that are produced in small quantities. This leads to cutting down of a large number of herbal plants for producing a single drug. However, by the use of modern tissue culture techniques and genetic transformation that alters the pathways for the biosynthesis of target metabolites, today this wasteful harvesting technique can be easily overcome.

Together with supporting the use of herbal medicines, it is high time for everyone, herbalist and conservationist alike, to reduce the overexploitation of the world's wild plants. In the modern world, the trade in medicinal plants is ever-increasing, but largely unmonitored. At the moment, many harvesting practices are unsustainable, which is threatening populations of medicinal plants and their habitats, and also the livelihoods of those people engaged in their collection. It is time for the conservationists, the government, and each and every one of us to find workable global solutions.

CONCLUSIONS AND OUTLOOK

Medicinal plants are widely used by the people living in both rural and urban areas. Globalization has greatly renewed the interest in herbal medicines, and today most people prefer to take herbal medicines as an alternative therapy. This resurgence in plant remedies has mainly resulted from the following factors:

1. Herbal medicines are found to be highly effective in curing diseases.
2. Most modern drugs have one or more side effects.
3. Development of science and technology.

In addition to these factors, economic advantages also contribute to their ever-increasing popularity. Development of modern science and technology, and further studies into traditional plant medicines conducted with modern theories and techniques have greatly enriched the use of herbal medicines by absorbing new ideas and concepts from traditional plant medicine from all over the world. This has led to the tremendous expansion of the herbal medicine industry in the last few decades, and has paved the route for employment of millions of unemployed persons. Looking at all these factors, we can say that the in the not-too-distant future, traditional plant medicine will become an area of major importance in the health care system. However, efforts should be made to achieve sustainable harvesting of medicinal plants so that they are not overexploited. Also, in order to utilize the available resources of medicinal plants to their full extent, social, cultural, and economic problems, lack of well-planned and integrated strategies, and poor access to scientific information must be dealt with first.

ETHICAL ISSUES

Although approximately 80% of people today depend upon herbal medicine as a component of their primary health care, there is still concern about the safety and efficacy of herbal drugs. Despite the fact that herbal medicine can potentially contribute to the improvement of health care, many major challenges must be overcome prior to the successful incorporation of herbal remedies into medicine. Beneficence, non-malfeasance, patient autonomy, justice, and public accountability are the pillars of bioethical principles, which are religiously followed in conventional medicine. They guide the clinicians such that the patients' interests are best served. As the use of complementary medicine (including herbal medicines) becomes increasingly popular, it is becoming apparent that the same bioethical principles are applicable to these alternate forms of health care (Kemper and Cohen, 2004). Beneficence is the principle that says it is a clinician's responsibility to promote a patient's well-being; clinicians must take appropriate measures to ensure that some positive outcome will occur. Non-malfeasance is the responsibility to not hurt others. This ethical principle is almost the same as beneficence, but with important distinctions, as one's duty to prevent harm is not the same as the duty to promote well-being (Beauchamp and Childress, 2009). Patient autonomy is a foundation of conventional medicine that is pertinent to the use of herbal medicines too. In most parts of the world, consumer access to herbal medicines is controlled by prescription, thus allowing for extensive use. With self-care as one component of patient autonomy, another key element is that the patient has sound information to make an informed treatment decision (Ernst and Cohen, 2001). Time and again researchers come across cases where a patient has gathered information about herbal medicines from relatives, friends, magazines, and the Internet (Gardiner and Riley, 2007; Khader et al., 2008; Low, 2009), all of which are perceived as less reputable than official sources (Health Canada and Reid, 2005).

TRANSLATIONAL SIGNIFICANCE

Animal models are used in the study of human diseases because both animals and humans are similar in genetics, anatomy, and physiological aspects. Also, animal models are often preferable because of their easy and abundant supply and ease of manipulation. Also, for statistical analysis, a sufficient number of specimens must be used for a particular experiment. Therefore, scientists cannot conduct research on just one animal or human, and it is easier for scientists to use sufficiently large numbers of animals instead of humans to get reliable results. Only in cases of advanced clinical trials are humans used for investigations. Otherwise, animals like mice, rats, monkeys, dogs, and several fungal, bacterial, and plant species, are used as model organisms for such studies. However, even with the evident similarities between animal models and humans, only about 1% of drugs reach the last phase of clinical trials. As far as herbal medicines are concerned, the chemical constituents present

in them are a part of the physiological functions of living plants, and therefore they have better compatibility with the human body. However, scientific proof of this statement is not sufficient, and this is therefore one major area where research can be carried out.

WORLD WIDE WEB RESOURCES

One of the first steps in the use of herbal medicine is to find out the best source for complete information about herbs and/or derivatives. At present, the Web is the most powerful (and perhaps most familiar) tool, but the Internet, like other resources, has its own strengths and weaknesses.

The major strength of the Internet is that it is an especially valuable research tool when looking for information that is current and frequently updated. It is also quick to access.

As far as weaknesses go, the Internet is not the best place to find established viewpoints in their original form since it is often the case that information is changed from its original source. Information on the Internet is often second-, third-, or even fourth-hand. Published books remain the safest place to get established facts and opinions, especially when looking for traditional ideas.

However, the following web sites do provide comprehensive information on herbal medicines:

http://ethnomedicinetomodern.blogspot.in/
http://www.umm.edu/altmed/articles/herbal-medicine-000351.htm
http://www.nlm.nih.gov/medlineplus/herbalmedicine.html

REFERENCES

Bannerman, R. H. (1983). The role of traditional medicine in primary health care, traditional medicine and health care coverage. *World Health Organization, Geneva*, 318–327.

Beauchamp, T. L., & Childress, J. F. (Eds.), (2009). *Principles of Biomedical Ethics* (6th edn.). New York: Oxford University Press.

Berkov, S., Pavlov, A., Georgiev, V., Weber, J., Bley, T., Viladomat, F., Bastida, J., & Codina, C. (2010). Changes in apolar metabolites during *in vitro* organogenesis of *Pancratium maritimum. Plant Physiology and Biochemistry*, *48*, 827–835.

Bourgaud, F., Gravot, A., Milesi, S., & Gontier, E. (2001). Production of plant secondary metabolites: a historical perspective. *Plant Science*, *161*, 839–851.

Brown, J. T., & Charlwood, B. V. (1986). Differentiation and monoterpene biosynthesis in plant cell cultures. In P. Morris, A. H. Scragg, A. Stafford & M. W. Fowler (Eds.), *Secondary Metabolism in Plant Cell Cultures* (pp. 68–74). Cambridge: Press Syndicate of the University of Cambridge.

Collin, H. A. (2001). Secondary product formation in plant tissue cultures. *Plant Growth Regulation*, *34*, 119–134.

Ernst, E., & Cohen, M. H. (2001). Informed consent in complementary and alternative medicine. *Archives of Internal Medicine*, *161*, 2288–2292.

Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, *109*, 69–75.

Gardiner, P., & Riley, D. S. (2007). Herbs to homeopathy – medicinal products for children. *Pediatric Clinics of North America*, *54*, 859–874.

Canada, Health, & Reid, I. (2005). *Baseline natural health products survey among consumers*.

Kemper, K. J., & Cohen, M. (2004). Ethics meet complementary and alternative medicine: New light on old principles. *Contemporary Pediatrics*, *21*, 61–67.

Khader, Y., Sawair, F. A., Ayoub, A., Ayoub, N., Burgan, S. Z., & Amarin, Z. (2008). Knowledge and attitudes of lay public, pharmacists, and physicians toward the use of herbal products in north Jordan. *Journal of Alternative and Complementary Medicine*, *14*, 1186–1187.

Kubmarawa, D., Ajoku, G. A., Enwerem, N. M., & Okorie, D. A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology*, *6*, 1690–1696.

Low, D. T. (2009). The use of botanicals during pregnancy and lactation. *Alternative Therapies in Health and Medicine*, *15*, 54–58.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol. Plant*, *15*, 473–497.

Namdeo, A. G. (2007). Plant cell elicitation for production of secondary metabolites: a review. *Pharmacology Reviews*, *1*, 69–79.

Newman, D. J., Cragg, G. M., & Snader, K. M. (2000). The influence of natural products upon drug discovery. *Natural Product Reports*, *17*, 215–234.

Orhan, I., Deliorman, O. D., & Özçelik, B. (2009). Antiviral activity and cytotoxicity of the lipophilic extracts of various edible plants and their fatty acids. *Food Chemistry*, *115*, 701–705.

Rao, R. S., & Ravishankar, G. A. (2002). Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnology Advances*, *20*, 101–153.

Zenk, M. H. (1978). The impact of plant cell culture on industry. In T. A. Thorpe (ed.), *Frontiers of Plant Tissue Culture*. Calgary: IAPTC.

FURTHER READING

Hostettmann, K. (1998). Strategy for the biological and chemical evaluation of plant extracts. *Pure and Applied Chemistry*, *70*, 1–9.

Klefenz, H. (2002). *Industrial Pharmaceutical Biotechnology*. New Delhi: Business Horizons Pharmaceutical Publishers.

Kohli, J. P. S. (2009). *Dictionary of Pharmaceuticals and Biotechnology*. New Delhi: Business Horizons Pharmaceutical Publishers.

Makkar, H. P. S., Sidhuraju, P., & Becker, K. (2010). *Plant Secondary Metabolites*. New York: Humana Press.

Verpoorte, R. (2000). *Metabolic Engineering of Plant Secondary Metabolism*. Springer Verlag.

GLOSSARY

Bioactivity Specific effect on, or a reaction in, a living being upon exposure to a substance.

Biosynthetic Totipotency The inherent potentiality of a plant cell to give rise to a whole plant.

Dedifferentiation The phenomenon of a mature cell reverting to its meristematic state and forming undifferentiated callus tissue.

Plant Metabolite The intermediates and products of metabolism. Usually restricted to small molecules of a plant.

Morphogenic The development of form and structure during growth.

Redifferentiation The phenomenon of whole-plant formation from undifferentiated callus tissue.

Secondary Metabolite Organic compounds that are not directly involved in the normal growth, development, or reproduction of a plant, but often have an ecological role, such as attractant of pollinators and chemical defense against microorganisms. Humans use secondary metabolites as medicines, flavorings, and recreational drugs.

Traditional Medicine (TM) Refers to the knowledge, skills, and practices based on the theories, beliefs, and experiences, used in the maintenance of health, and in the prevention, diagnosis, improvement, or treatment of physical and mental illness.

Natural Product A chemical compound or substance produced by a living organism. A natural product often has pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. A natural product can be considered as such even if it can be prepared by total synthesis.

ABBREVIATIONS

GC/MS Gas Chromatography/Mass Spectrometry
HPLC High-Performance Liquid Chromatography
LC/MS Liquid Chromatography/Mass Spectrometry
MS Mass Spectrometry
NMR Nuclear Magnetic Resonance
RT Retention Time
TLC Thin-Layer Chromatography
WHO World Health Organization

LONG ANSWER QUESTIONS

1. Write an essay on plant secondary metabolites.
2. Elucidate various steps for the study of plants in traditional medicine.
3. What is drug discovery? What are different ways for drug discovery from natural products?
4. Write a detailed account of the tools and techniques of plant tissue culture and highlight the importance of each.
5. Enlist and describe in detail important analytical techniques associated with characterization of medicinal metabolites.

SHORT ANSWER QUESTIONS

1. Define the term "secondary metabolites."
2. What is ethnobotany?
3. Differentiate between *characterized* and *uncharacterized* plant extracts.
4. Give the names of three solvents that can be used for the extraction of hydrophilic compounds?
5. Which analytical technique can be used for the separation and identification of volatile compounds?

ANSWERS TO SHORT ANSWER QUESTIONS

1. Secondary metabolites are compounds that are not directly involved in primary metabolic processes of an organism. They generally defend the organisms from environmental stresses and predators.
2. Ethnobotany is the study of how people of a particular region relate to the plants of their environment.
3. Characterized extracts are ones where each component, its concentration, and function, are known; for uncharacterized extracts, the entire components of the mixture and the role they play are not known.
4. Methanol, ethanol, and acetone.
5. GC-MS.

Index

Note: Page numbers followed by "f" denote figures; "t" tables.

A

AAALAC. *See* Association for Assessment and Accreditation of Laboratory Animal Care
Ab. *See* Antibody
ABC. *See* ATP-binding cassette
ABCG2 marker, 434
Abdominal-A (AbdA), 6–7
ABI SOLiD, 349–350
Ablative techniques, 110
Absorption, distribution, metabolism and excretion (ADME), 88–89, 400
Abstraction, 390
ABTS. *See* 2,2-azobis-3-ethylbenzthiazoline-6-sulfonic acid
ACE2. *See* Angiotensin converting enzyme-2
aCGH. *See* Array-comparative genomic hybridization
AcMNPV. *See Autographa California* nucleopolyhedroviruses
Acquired Immunodeficiency Disease (AID), 156–157
Acquired immunodeficiency syndrome (AIDS), 15, 156
Acrocentric chromosomes, 453
ACSF. *See* Artificial cerebrospinal fluid
Activated T cells, 493–494
Activator Protein-1 (AP-1), 115
Acute lymphoblastic leukemias (ALL), 62–63
Acute myelocytic leukemia (AML), 97, 173
AD. *See* Alzheimer's disease
Adaptive immunity, 474
ADCC. *See* Antibody-dependent cell-mediated cytotoxicity
Adenine, 462
Adenine/thymidine (AT), 459
Adenocarcinoma, 96–97, 102
Adenoma polyposis coli (APC), 79–80
Adenosine triphosphate (ATP), 181
Adenoviruses (ADV), 206–207
ADEPT. *See* Antibody Directed Enzyme Prodrug Therapy
Adequate pressure driving, 33f
Adherent cell trypsinization, 215f
Adipokinetic hormone (AKH), 14–15
Adjuvants, 502–503
ADME. *See* Absorption, distribution, metabolism and excretion
Adult human GI tract, 512–514
Adult stem cells (ASCs), 432, 433t
  identification, 433
  single mature cell type, 428
ADV. *See* Adenoviruses
Aequorea victoria, 557
Affinity screening. *See* Biopanning
Aflatoxin B1 toxin, 98
AFLP. *See* Amplified fragment length polymorphism
AFM. *See* Atomic Force Microscope
AFM force mapping (AFM–FM), 251
AFM force spectroscopy (AFM–FS), 251, 251f
AFSCs. *See* Amniotic Fluid-Derived Stem Cells
8–AG. *See* 8–azoguanine
Agglutination reactions, 281
Aggregation, 533
Aging, 178
  animal experimental models, 184
    fish, 184–185
    mouse, 184
  biotechnology, 188
  ethical issues, 188–189
  polyphenols, 185–188, 185f
  protein oxidation, 183–184
  ROS, 178f, 181–183
  theories, 178
    cellular theories, 179–180
    CR theory, 180–181
    evolutionary theories, 178–179
    FRTA, 180
    molecular theories, 179
    system-based theories, 180
  translational significance, 189
  WWW resources, 189
Agrobacterium tumefaciens, 510
AGUS. *See* Atypical glandular cells of undetermined significance
AHR. *See* Aryl hydrocarbon receptor
AI. *See* Artificial insemination
AID. *See* Acquired Immunodeficiency Disease
AIDS. *See* Acquired immunodeficiency syndrome
AKH. *See* Adipokinetic hormone
Alemtuzumab, 480
Alkoxyl radicals (RO·), 182
Alkyl peroxides (ROOH), 182
Alkyl radicals (R·), 182
ALL. *See* Acute lymphoblastic leukemias
All-Steps-All-Combinations (ASAC), 378
Allele-specific oligonucleotide (ASO), 292–293
Allele-specific PCR (AS-PCR), 293
Allele(s), 294–295
Allelic heterogeneity, 465
Allen Brain Atlas, 322–323
Allergenic proteins, 511–512
Allergenicity, 511
Allergens, 511, 517, 520
  cross-linking of, 512
Allergic reactions, 511
Allograft transplants, 81–82. *See also* Xenograft transplants
  cancerous cells or solid tumors, 81–82
  generation of, 81f
  PyMT tumors, 82
Alpha-1-antitrypsin, 608–609
α1-PI. *See* α1-proteinase inhibitor
α1-proteinase inhibitor (α1-PI), 416–417
ALS. *See* Amyotrophic lateral sclerosis
Alternative medicine. *See* Complementary medicine
Alu family, 584
Alzheimer's disease (AD), 13, 40, 398–399
Alzheimer's mouse, 413–414
AMA. *See* American Medical Association
American Medical Association (AMA), 284
American Public Health Association (APHA), 526
American Veterinary Medical Association (AVMA), 76
Ames test, 98
Amino acids, 519
AML. *See* Acute myelocytic leukemia
Amniotic Fluid-Derived Stem Cells (AFSCs), 431
Amplicon Sequencing, 357
Amplified fragment length polymorphism (AFLP), 289–290, 295, 331
  applications of, 296
  steps in analysis, 295–296
Amplified ribosomal DNA restriction analysis (ARDRA), 336
Amyloid precursor protein (APP), 13, 40, 148–149
Amyloid β (Aβ), 13
Amyotrophic lateral sclerosis (ALS), 40–41
  adult motor neuron disease, 40–41
  causative genes, 42t
  genes implication in RNA metabolism, 43–44
  pathomechanisms, 41
  Sod1-ALS, 43
  SOD1-linked ALS
    in vitro, 51
    mis-folded mutant SOD1 protein, 50
    non-cell autonomous neurodegeneration, 51
    SOD1 mouse models, 51f
    SOD1 mutations, 50
    $SOD1^{G93A}$ mice, 50, 50f

Amyotrophic lateral sclerosis (ALS) (*Continued*)
TDP-43 wild-type, 51–52
transgenic mice
$SOD1^{G37R}$, 47–48, 48f
$SOD1^{G93A}$, 48
$SOD1^{WT}$, 49
Analysis of variance (ANOVA), 84–85
Analytical step-by-step model, 601
Anaphylaxis, 512, 555
Anchorage-dependent/adherent cells, 216
Anchorage-independent/suspension cells, 216
Ancient DNA, 359–360
Ancient Greek moral theories, 599–600
Androgen receptor (AR), 44, 49, 52–53
Androgenesis, 548, 548f
Aneuploidy, 455
Angiotensin converting enzyme-2 (ACE2), 374f
Animal biopharming, 598, 608–609
escape of, 609
ethical issues in, 609
food chain contamination, 609
horizontal gene transfer, 609
risks to human health, 609
welfare issues, 609
Animal biotechnology, 357, 386, 590–591
ancient DNA, 359–360
anthropocentrism, 598
applications, 358, 358f, 601–602
beef cattle selection, 360–361
bioinformatics in, 386–387
biopharming, 598
challenging ethical issues in, 607–610
animal biopharming, 608–609
chimeras, 607–608
constitution of ethics committees, 610
concerns
over animal biotechnology, 598
for human health, 598
consequentialist ethics, 600
deontological
approaches, 597
ethics, 600
epigenetics, 359
ethical concerns in, 602
ethical
debates, 599, 602–607
theories, 597
evolutionary research, 358–359
extrinsic concerns
animal welfare, 604–606
environmental concerns, 607
human health, 606–607
precautionary principle, 607
food, safety, and nutrition, 361
gene-based technologies, 598
genomic variability, 360
human therapeutic cloning, 610–611
Internet Encyclopedia of Philosophy, 598–599
intrinsic concerns, 602–603
hESC religious critique, 603
human reproductive cloning religious critique, 603–604
IVF religious critique, 604
religious intrinsic critique, 603–604
livestock productivity and health improvement, 361
for medical purposes, 598
metagenome sequencing, 359
methodological approach, 601, 602f
physical and mental health, 598
pluripotent stem cells, 598
principles, 598, 600–601
public health ethics, 606–607
public perceptions, 604
secular intrinsic objections, 604
SNP/CNV discovery, 360
transgenics, 361
virtue ethics, 599–600
WWW resources, 611–612
Animal breeding, 361
Animal cell culture, 214
advantages of, 222
anti-viral vaccines, 223–225
baculovirus production, 227–228
cell growth, 217
cell line, 216
cell passage, 214–215
cell viability, 218–219
culture media, 219–221
development of, 213
disadvantages of, 222
ethical issues, 223
FBS use of, 223
gene therapy, 227
historical events in, 213t
in vitro maintenance, 214
monolayer cultures, 214
organ culture, 214
organotypic and histotypic cultures, 214
primary, 215–216
advantages and disadvantages, 215–216
anchorage-dependent/adherent cells, 216
anchorage-independent/suspension cells, 216
quantitation, 215
secondary, 216
suspension cultures, 214
3D structure reconstruction, 215
translational significance, 223
trypsin use, 214–215
types, 214–216
variety of research efforts, 212
viral mutant formation, 228
viral vaccines, 212
WWW resources, 229
Animal models, 573–574
Animal rights, 601
Animal vaccines, 492
Animal welfare, 601–602, 604–605
animal biotechnology, 605
animal treatment by humans, 605–606
freedoms, 605
gene knockout technology, 606
rDNA, 605
technological application, 606
Animal Welfare Act (AWA), 420
ANOVA. *See* Analysis of variance
Antennapedia (ANTP), 6
Anthropocentrism, 598
Anti-angiogenesis therapeutics, response to, 205–206
Anti-microbial assay, 567
Anti-viral vaccines
DNA technology, 223
HBV, 223
recombinant hepatitis B vaccines, 223t
viral particle production, 223–224
stages, 224
VLPs, 224–225
Antibodies, 265–266
adverse effects, 272–274, 273t
analytical applications, 275–276
ethical issues, 283
functions, 266
hybridoma selection, 282–283, 283f
immunoglobulins, 266
structure elucidation, 267–268, 267f, 269f
methodology, principles, and protocols, 276–282
preparative applications, 276
therapeutic applications, 274–275, 275t
Tiselius and Kabat's experiment, 266
translational significance, 284
WWW resources, 284
Antibody (Ab), 473, 553
class and function, 476t
drugs by FDA, 482t
ethical issues, 487
immunodiagnostics, 474–487, 488t
production, 265–266
titration, 277, 281–282, 282f
translational significance, 487–488
WWW resources, 488
Antibody Constructs, 485
Antibody Directed Enzyme Prodrug Therapy (ADEPT), 274–275
Antibody-dependent cell-mediated cytotoxicity (ADCC), 480
Anticipation, 582
Antigen (Ag), 474–475, 553
determinants, 474
epitopes, 497
molecules, 497
preparation, 277
Antigen binding fragment. *See* Fragment antigen binding (Fab)
Antigen-presenting cells (APC), 493–494, 512
Antigenic determinants, 511
Antioxidant capacity, 184
Antiretroviral drugs, 164–165, 165t
assays, 168–169
Antiretroviral Treatment (ART), 164
Antisense oligonucleotide (AS-ODN), 114–115
Antiserum, 272
ANTP. *See* Antennapedia
AP-1. *See* Activator Protein-1
APC. *See* Adenoma polyposis coli; Antigen-presenting cells
APHA. *See* American Public Health Association
APP. *See* Amyloid precursor protein
Applied ethics, 597, 599
Approach cycle, 251

Aquaculture, 552
chromosome manipulation, 545–548
fish culture, 545
gametes cryopreservation, 549
gene transfer methods, 545
nanotechnology application, 550–551
sex reversal, 548–549
transgenic fish technology, 545
AR. *See* Androgen receptor
Ara-C. *See* Arabinosyl cytosine
Arabidopsis, 583
Arabinosyl cytosine (Ara-C), 556
ARACNE, 388
ARDRA. *See* Amplified ribosomal DNA restriction analysis
Area under the curve (AUC), 25–26
Array-comparative genomic hybridization (aCGH), 459, 461, 461f
Arsenic, 60–61
ART. *See* Antiretroviral Treatment; Assisted reproductive technology
Arthropods, 544
Artificial cerebrospinal fluid (ACSF), 316
Artificial insemination (AI), 601–602
Aryl hydrocarbon receptor (AHR), 78
AS-ODN. *See* Antisense oligonucleotide
AS-PCR. *See* Allele-specific PCR
ASAC. *See* All-Steps-All-Combinations
ASC. *See* Atypical Squamous Cell
ASCs. *See* Adult stem cells
ASCUS. *See* Atypical squamous cells of undetermined significance
ASO. *See* Allele-specific oligonucleotide
Aspirin, 370
Assisted reproductive technology (ART), 604
Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), 301, 420
Astrocytes, 51
Asymmetric stem cell division, 430f
AT. *See* Adenine/thymidine
Atlantic cod (*Gadus morhua*), 295
Atlantic Ocean, 542
Atomic Force Microscope (AFM), 248–250
advantage, 250
applications, 251
force mapping mode, 251, 251f
force spectroscopy mode, 251
imaging capabilities of, 250
operating principle of, 250f
orthopoxvirus in animals, 256
probe, 250
protocol for, 251, 252f
Raman confocal hybrid systems, 254–255
ATP. *See* Adenosine triphosphate
ATP-binding cassette (ABC), 434
Attenuated bacteria, 500
Atypical glandular cells of undetermined significance (AGUS), 103
Atypical Squamous Cell (ASC), 103
Atypical squamous cells of undetermined significance (ASCUS), 103
AUC. *See* Area under the curve
Autographa California nucleopolyhedroviruses (AcMNPV), 228
Automated capillary sequencing, 338
Automated ribotyping, 335
Autonomous retrotransposons, 584
Autonomous transposons, 583
Autonomy, 110, 600
Autopap, 108
Autosomal dominant trait, 465
Autosomal recessive disorders, 465
Autosomes, 453–454
Avascular tumor growth, 389–390
AVMA. *See* American Veterinary Medical Association
AWA. *See* Animal Welfare Act
Ayurvedic drugs, 565
5-Aza-2-deoxycytidine, 67–68
5-azacytidine, 61, 67–68
AZF. *See* Azoospermia
Azido group, 164
2,2-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 184
Azidothimidine (Azt), 157
8–azoguanine (8–AG), 282–283
Azoospermia (AZF), 293
Azt. *See* Azidothimidine
Aβ. *See* Amyloid β

B

B-cell receptor (BCR), 132
Baby hamster kidney (BHK), 228
BAC. *See* Bacterial artificial chromosome
Bacillus thuringiensis, 510
Bacterial artificial chromosome (BAC), 134, 357–358, 551–552, 578
Bacterial loading, 31, 33f
Bacterial products, 502–503
Bacterial systematics
genotypic typing methods, 331
phenotypic typing methods, 330–331
sources of information, 329–330
Bacterial taxonomy, 328–329
BAL. *See* Bioartificial liver
Bam A. *See* BamHI A region
BamHI A region (Bam A), 132
BamHI A rightward transcripts (BARTs), 132
Barrett's esophagus, 62
BARTs. *See* BamHI A rightward transcripts
Bayesian network analysis, 389
BBB. *See* Blood–brain barrier
BCR. *See* B-cell receptor
BCRP1 marker. *See* ABCG2 marker
BDNF. *See* Brain-derived neurotrophic factor
Beef cattle selection, 360–361
Beneficence, 600
Berlin Man, 172
Betaine homocysteine-S-methyltransferase (BHMT), 63
Bethesda System, 102–103
BHK. *See* Baby hamster kidney
BHMT. *See* Betaine homocysteine-S-methyltransferase
Bi-thorax complex (BX-C), 6–7
Bicoid, 6
"Big data" problem, 68
Bio-encapsulated feeds, 550
Bio-Safety Level-3 (BSL-3), 167
Bioactive compounds
GC/MS, 569
HPLC, 568
LC/MS, 568–569
natural product materials, 568
NMR, 569
thin-layer chromatography, 567–568
Bioartificial liver (BAL), 202
Biochemical
markers, 290
networks, 389
Biocompatibility, 235–237
Bioinformatics
in animal biotechnology, 386–387
enrichment tools, 387
and systems biology, 387
Bioinformatics Resource Centers (BRCs), 401
Biological assays, 567
Biomolecular display technologies, 373, 375
display module in, 373f
ethical issues, 380
illustration and comparison, 374f
mRNA display, 377
phage display, 373–375
ribosome display, 376–377
shortcomings, 377–378
therapeutic peptides, 381t
translational significance, 380
Biomolecules, 532
Biopanning, 375, 484–485
Biopesticides, 227
baculovirus production, 227–228
cell lines for, 228
DIP mutations, 228
factors, 228
in vitro production process, 227–228
insect baculovirus–cell system, 228
viral mutant formation, 228
Biopharm animals, 609
Biopharming. *See* Animal biopharming
Biopsy, 107
Bioreactors, 235
Biotechnology, 363, 386, 545, 598
religious intrinsic critique, 603–604
secular intrinsic objections to, 604
BIS. *See* Bureau of Indian Standards
Bispecific antibodies, 486–487
Bivalves, 543–544
BK virus (BKV), 128
BL cells. *See* Burkitt lymphoma cells
Black Tea, 186
Blackwith-Wiedemann syndrome (BWS), 60
Blastocyst, 426, 431–432
Blastomas, 97
Blood typing reagent, 479
Blood vessels, 241
Blood–brain barrier (BBB), 172
Blue biotechnology, 559
Blue fluorescent protein (EBFP), 557–558
BMS. *See* Bristol Meyers Squibb
BMT. *See* Bone marrow transplantation
Body cavity lymphoma. *See* Primary Effusion Lymphoma (PEL)
Bone marrow transplantation (BMT), 130–131
Bone tissue, 240

Bottom–up approach, 249–250. *See also* Top–down approach
Bovine articular cartilage, 256
Bovine serum albumin (BSA), 488
bovine somatotropin (bST), 606
Bovine sponigioform encephalopathy (BSE), 609
Brain, 308
Brain cancer, 61
Brain-derived neurotrophic factor (BDNF), 435
BRCs. *See* Bioinformatics Resource Centers
Brdu. *See* Bromo-deoxy-uridine
Breast cancer, 61
Bristol Meyers Squibb (BMS), 166–167
Brittle stars, 544
Broad-range PCR ribotyping, 337
Broiler poultry, 605
Bromo-deoxy-uridine (Brdu), 203, 436
Bryostatins, 556
BSA. *See* Bovine serum albumin
BSE. *See* Bovine sponigioform encephalopathy
BSL-3. *See* Bio-Safety Level-3
bST. *See* bovine somatotropin
Buddhism, 603–604
Bugula neritina, 556
Bureau of Indian Standards (BIS), 526
Burkitt lymphoma cells (BL cells), 130–131, 133
BWS. *See* Blackwith-Wiedemann syndrome
BX-C. *See* Bi-thorax complex

C

c-Myc gene, 79
C9orf72 gene, 44
CA. *See* Capsid protein
Cadmium exposure, 60–61
CAH. *See* Congenital Adrenal Hyperplasia
CAID. *See* Community Acquired Immuno Dysfunction
CALCA. *See* Calcitonin
Calcitonin (CALCA), 590
Calcium oxalate (CaOx), 15
California Institute of Regenerative Medicine (CIRM), 446
Callus cultures, 570
Caloric Restriction theory (CR theory), 180–181
Calorie-restricted monkeys, 180–181
CAM. *See* Cell adhesion molecule
Camellia sinensis (*C. sinensis*), 185–186
Campylobacter jejuni, 528
Canadian Council on Animal Care (CCAC), 301–302
Canadian Institutes of Health Research (CIHR), 301–302
Cancer, 58, 96
  cervical cancer, 99–101
  classification, 96–98, 103–104
  continuum models, 393
  development, 96
  diagnostic applications in, 480
  discrete models, 393
  hybrid models, 393–394
  in silico models, 392–394
  research, 361–362
  staging, 97–98
  therapeutic applications in, 480–481
Cancer Genetic Markers of Susceptibility (CGEMS), 589
Cancer stem cells (CSC), 115, 426
  cancer stem cell targeting, 439f
  cell surface CD markers, 438–439, 439t
  embryonal rest theory, 438
  isolation, 434f
  teratocarcinomas, 438
Cancer-causing agents, 98
  chemicals, 98–99
  oncogenic viruses, 98, 98t
  radiation, 99
CaOx. *See* Calcium oxalate
Capsid protein (CA), 161
Capsomeres, 105
Captive rearing technology, 550
Carbonyl group content, 183
Carcinogenesis, 122
Carcinoma, 96–97
Carcinoma *in situ* (CIS), 102
CARD. *See* Catalyzed reported deposition
CARD Fish, 340
Cartagena Protocol on biosafety, 53
Caseous necrosis, 23
Catalyzed reported deposition (CARD), 340
Catholic Church, 604
Cattle genome, 357–358
Cattle model, 28. *See also* Guinea pig models
Causation mistaken theories, 100–101
CBER-OCTGT. *See* Center for Biologics Evaluation and Research, Office of Cellular, Tissue, and Gene Therapies
CCAC. *See* Canadian Council on Animal Care
CCD. *See* Charge-coupled device
CCR5. *See* Chemokines receptors
CD. *See* Cluster of differentiation
CD133 marker, 434
CD34 marker, 434
CDC. *See* Centers for Disease Control and Prevention
CDI. *See* Cumulative disease index
CDK. *See* Cyclin-dependent kinase
Cdkn2a gene, 63
CDMS. *See* Clinically definite MS
cDNA. *See* complementary DNA
CDR. *See* Complementarity determining region
Cell adhesion molecule (CAM), 235–237
Cell growth monitoring, 217–218
  cell culture characteristics, 218
Cell line, 217t
  advantages of, 216
  for biopesticide production, 228
  characterization of, 221–222
  finite cell lines, 216
  indefinite cell lines, 216
  therapeutic proteins production, 226t
  viral vaccine used, 224t
Cell viability, 218
  cytotoxicity, 218–219
  dead cell, 219
  Hayflick's phenomenon, 219
  LDH release, 219, 219f
  luminescent protease release assay, 219f
  MTT assay, 219f
  viability assays, 218f
Cell-based therapy, 442
Cell-cycle models, 389
Cell-cycle regulators, 589
Cell-mediated immunity (CMI), 25, 474–475
Cell(s), 234–235, 391
  culture, 459
  death, 235
  passaging, 214–215
Cellphone base stations, 599
Cellular Senescence Theory, 179–180
Cellular theories, 179–180. *See also* Molecular theories
Center for Biologics Evaluation and Research, Office of Cellular, Tissue, and Gene Therapies (CBER-OCTGT), 446
Centers for Disease Control and Prevention (CDC), 156, 158
Central nervous system (CNS), 128, 139, 144, 172, 435
Centromere, 453
Cephalopods, 544
Cephalothorax, 544
Ceramics, 237
Cervical cancer
  biological behavior of HPV infection, 100f
  causation mistaken theories, 100–101
  female pelvis anatomy, 99–100
  historical overview, 100
  noble prize for discovering HPV, 101
  prevalence and epidemiology
    cancer classification, 103–104
    global scenario, 101–102
    HPV, 104–109
    pre-cancer classification, 102–103
    risk factors for, 102, 102t
    symptoms, 102
    types, 102
Cervical dysplasia, 102
Cervical intraepithelial neoplasia (CIN), 100, 102
Cervicography, 108
CFA. *See* Complete Freunds' adjuvant
CFM. *See* Chemical Force Microscopy
CFUs. *See* Colony-forming units
CGD. *See* Chronic granulomatous disease
CGEMS. *See* Cancer Genetic Markers of Susceptibility
CGH. *See* Comparative genomic hybridization
CH. *See* Congenital hypothyroidism
Chamomile tea, 186
Characterized extract, 565
Chargaff's rule, 462
Charge-coupled device (CCD), 10–11
Chemical Force Microscopy (CFM), 250–251
Chemical screening, 567
Chemically defined media, 221
Chemo-labeled antibodies, 481
Chemokines receptors (CCR5), 166
Chicken RBCs (CRBC), 277
Chimeras, 607–608
Chimeric antibodies, 483
Chimerism, 458
Chinese hamster ovary (CHO), 228
ChIP. *See* Chromatin immunoprecipitation
ChIP DNA sequencing, 357
Chip-based spheroid generation, 202–203

ChIP-on-chip method, 64
ChIP-seq, 66
CHO. *See* Chinese hamster ovary
Choice of Linkers, 481–483
Cholera toxin B subunit (CTB subunit), 502–503
Chordates, 544
Chromatids, 453
Chromatin immunoprecipitation (ChIP), 66, 357
Chromogenic substrates, 529
Chromosomal disorders
  aneuploidy, 455
  chromosome breakage syndrome, 458–459
  down syndrome, 455
  fragile sites, 458–459
  monosomy, 456
  polyploid cells, 456
  structural abnormalities, 456–457
    balanced translocation, 457f
    chimera and mosaic, 458
    deletion, 457, 457f
    down syndrome patient, 456f
    isochromosome, 457, 458f
    mosaicism, 458
    paracentric and pericentric inversion, 458f
    ring chromosome, 457f
    turner syndrome patient and karyotype, 456f
  trisomy, 455
Chromosomal fragile sites, 458–459
Chromosome, 451–453, 453f
  banding methods, 452–453
  breakage syndrome, 458–459
  genetic(s)
  component, 452
  and biochemistry, 452
  in medicine, 452
  laws of, 452
  medical genetics field, 452
  morphology and classification
    autosomes and sex chromosomes, 453–454
    centromere, 453
    chromosomes, 453, 453f
    descending order of size and position, 454t
    identical strands, 453
    karyotype, 454, 454f
    mitosis and meiosis, 453
    nomenclature, 454–455, 455t
    telomeres, 453
  transformation process, 452
Chromosome 21, 578
Chromosome manipulation, 545–546, 549
  androgenesis, 548, 548f
  gynogenesis, 547–548, 547f
  inducing process, 546f
  polyploidy, 546–547
Chronic granulomatous disease (CGD), 225–226
Chronic lymphocytic leukemia (CLL), 63, 97, 480
Chronic myelocytic leukemia (CML), 97
CIA. *See* Collagen-induced arthritis
CIHR. *See* Canadian Institutes of Health Research
CII. *See* Collagen type II
CIN. *See* Cervical intraepithelial neoplasia
Cinnamon tea, 186
CIRM. *See* California Institute of Regenerative Medicine
CIS. *See* Carcinoma *in situ*
CKC. *See* Cold-knife conization
Cladistic approach, 328
Clams (*Macoma, Mercenaria*), 543–544
Class Bivalvia. *See* Bivalves
Class Cephalopoda. *See* Cephalopods
Class Gastropoda. *See* Gastropods
Clawless spiny lobster (*Panulirus*), 544
Clinical diagnostic testing, 466
Clinically definite MS (CDMS), 145–146
CLL. *See* Chronic lymphocytic leukemia
Clone-FISH technique, 339
Clones
  expansion, 280
  selection, 280
Cloves tea, 186
Cluster of differentiation (CD), 438–439
CMI. *See* Cell-mediated immunity
CML. *See* Chronic myelocytic leukemia
CN. *See* Copy number
Cnidocysts. *See* Nematocysts
CNS. *See* Central nervous system
CNV. *See* Copy number variation
CO. *See* Cytochrome oxidase
COBRA. *See* Combined bisulfate restriction analysis
Cold-knife conization (CKC), 111
Collagen type II (CII), 143
Collagen-induced arthritis (CIA), 142
Colloidal metal nanoparticles, 532
Colony-forming units (CFUs), 25
Colorectal cancer, 61–62
Colorimetric detection
  E. coli, 535
  ETEC gene, 535–536
  UV–Vis spectra, 536f
Colposcopy, 107
Column-based kit, 316
Combined bisulfate restriction analysis (COBRA), 66
Commercial bio-products
  amino acid sequences, 557t
  Ara-C, 556
  enzyme inhibitors, 556
  in marine biotechnology, 556–557
Community Acquired Immuno Dysfunction (CAID), 156–157
Comparative genomic hybridization (CGH), 461
Comparative Genomics, 590
Complementarity determining region (CDR), 274, 475, 483
complementary DNA (cDNA), 163–164
Complementary medicine, 565
Complete Freunds' adjuvant (CFA), 142–143
Complete Genomics, 352–353
Complex diseases, 577–578
Conalbumin, 517
Concerns over animal biotechnology, 598
Cone snails, 556
Confidentiality, 601
Confocal Raman Spectroscopy (CRS), 253–254
Congenic strains, 76–77
Congenital Adrenal Hyperplasia (CAH), 468
Congenital hypothyroidism (CH), 467
Conjugate vaccines, 498, 498f
Conopeptides, 556
Conotoxins, 556
Consequentialist ethics, 600
Consomic strains, 76–77
Continuum models, 393
Conus peptides, 556
Conventional ribotyping, 333, 334f
Conventional techniques, 528–529
Copaxone®. *See* Glatiramer acetate
Copy number (CN), 360
Copy number variation (CNV), 360
Cord blood, 445
Core-like particle (CPL), 224
Cornybacterium diphtheriae (*C. diphtheriae*), 274
Cortisol-bindng protein. *See* Transcortin
COX-2. *See* Cycloxygenase-2
CPE. *See* Cytopathic effect
CpG islands, 583
CPL. *See* Core-like particle
CR theory. *See* Caloric Restriction theory
CRBC. *See* Chicken RBCs
Cre recombinase, 46–47
CRE-Lox recombinase system, 75
Cre-loxP system, 46–47, 47f
Cre/Lox system, 80f
  Cre-recombinase enzyme, 80
  gene expression, 80
  human colorectal cancers, 80
CRS. *See* Confocal Raman Spectroscopy
Crustaceans. *See* Transgenic fish
Cryopreservation, 549
Cryoprotectants, 549
Cryotherapy, 110
CSC. *See* Cancer stem cells
CTB subunit. *See* Cholera toxin B subunit
CTCL. *See* Cutaneous T-cell lymphoma
CTL. *See* Cytotoxic T lymphocytes
CTL cells. *See* Cytotoxic T cells
Cultivable animals
  disease diagnosis, 552
  DNA-based diagnosis, 554
    oyster, 554
    shrimp, 554
  immunodiagnostics, 552
    enzyme immunoassay, 552–553
    latex agglutination test, 553
    monoclonal antibodies, 553–554
Culture media, 219
  artificial media, 220
  chemically defined media, 221
  components, 220
  natural media, 220
  protein-free media, 221
  serum, 221
  serum-free media, 221
    advantages of, 221
    disadvantages of, 221
Culture-based methods, 528–529
Cumulative disease index (CDI), 147
Cutaneous T-cell lymphoma (CTCL), 480
Cuttlefish ink, 555

Cuttlefishes (*Sepia*), 544, 555
Cyan fluorescent protein (ECFP), 557–558
Cyclin-dependent kinase (CDK), 389
Cyclo-(L-Pro-L-Phe), 555
Cycloxygenase-2 (COX-2), 62
Cyp-450. *See* Cytochrome P450
Cytidine deaminase, 62
Cytochrome oxidase (CO), 529
Cytochrome P450 (Cyp-450), 89, 182
Cytogenetics, 452–453, 459
Cytopathic effect (CPE), 123–125, 222
Cytosar-UP, 556
Cytosine, 462
Cytotoxic T cells (CTL cells), 493–494, 499
Cytotoxic T lymphocytes (CTL), 206–207
Cytotoxicity, 218

D

Dark-staining regions (G-dark regions), 459
Data storage and processing technology, 64–65
Data-driven modeling approaches, 389
DBT. *See* Department of Biotechnology
DCs. *See* Dendritic cells
DCV staining, 440
ddNTPs. *See* dideoxy nucleotides
De novo assembly, 353
De novo pathway, 282
De-differentiation, 429–430
Dead cell, 219
Declaration of Helsinki, 610
Deductive models, 390
2-DG. *See* 2-deoxy-D-glucose
Dehydroepiandrosterone, 180
Delayed apoptosis, 203–204
Delayed type hypersensitivity (DTH), 26
Δ32 deletion, 172
Demyelination, 146
Dendritic cells (DCs), 512
Deontological ethics, 600
2-deoxy-D-glucose (2-DG), 204
deoxynucleoside triphosphate (dNTP), 348
Deoxyribonucleic acid (DNA), 8–9, 462
  chemical components, 462
  chromosome, 463
  cytometry, 108
  DMD and alpha thalassemia, 464f
  double helix structure, 463, 463f
  dynamic mutations, 465
  fragments, 467–468
  frameshift mutation, 464–465, 464f
  genetic disorders, 465
  genetic polymorphism, 465
  human gene structure, 463f
  immunization, 500
  insertion, 465, 465f
  introns, 463
  methylation, 58–59
  missense mutation, 464, 464f
  modifying genes, 463
  molecule, 462–463
  mutation, 464
  nano-vaccines, 550–551
  non-sense mutation, 464, 464f
  non-synonymous mutation, 464
  polynucleotide strand, 463
  sequencing, 346, 468, 577–578
    technologies generation, 346–347
    timeline of, 347f
  stop codons, 463
  synonymous mutation, 464
  transposons, 584
  tumor virus infection, 122–123, 123f
  vaccines, 498, 499f
    advantages, 500
    construction, 498–499
    disadvantages, 500
    future of, 500
    mechanisms, 499, 500f
    steps in development, 499f
Deoxyribose sugar, 462
Department of Biotechnology (DBT), 445–446
DES. *See* Diethylstilbestrol
Diabetes, 240
Diabody, 485
Dicer, 581
dideoxy nucleotides (ddNTPs), 347, 468
Dietary supplementation, 548
Diethylstilbestrol (DES), 98–99
Digital transcriptome subtraction (DTS), 128
2,4-dinitrophenylhydrazine (DNPH), 183
Dinucleotide CpG, 583
Dinucleotides (dNTPs), 468
1,2-dimethylhydrazine (DMH), 61–62
1,1-diphenyl-2-picrylhydrazyl free radical (DPPH·), 184
Diphtheria bacteria, 492
DIPs-defective interfering particles, 228
Direct detection methods, 531
Direct RNA Sequencing (DRS™), 357
Discrete models, 393
Disease-modifying anti-rheumatic drug (DMARD), 141–142
Disease-related genes, 588–589
Disposable Soma Theory of aging, 178–179
disulfide-linked variable fragment (dsFv), 473, 485
DMARD. *See* Disease-modifying anti-rheumatic drug
DMD. *See* Duchenne Muscular Dystrophy
DMH. *See* 1,2-dimethylhydrazine
DNA. *See* Deoxyribonucleic acid
DNA methyl transferaes (DNMTs), 60
DNA-based diagnosis, 554
  oyster, 554
  shrimp, 554
DNA-based markers, 578, 586
DNA-binding domain, 115
DNMTs. *See* DNA methyl transferaes
DNPH. *See* 2,4-dinitrophenylhydrazine
dNTP. *See* deoxynucleoside triphosphate
dNTPs. *See* Dinucleotides
DOE. *See* United States Department of Energy
Doogie mouse, 414
Dopamine receptor, D1-α (DRD1), 590
Dot blot technique, 317
Dot immunobinding assay, 553
dot-ELISA, 553
Down syndrome, 452, 455
Downey cells, 133
Downstream bioinformatics, 353
  de novo assembly, 353
  mapping, 353–354
DPPH·. *See* 1,1-diphenyl-2-picrylhydrazyl free radical
DRD1. *See* Dopamine receptor, D1-α
Dried powdered plant, 568
Drosophila busckii (*D. busckii*), 8–9
Drosophila melanogaster (*D. melanogaster*), 3–4, 558
  biological processes, 9–10
  biotechnology research, 9
  culturing, 10
  development, 4
  DNA preparation for Injection, 11
  egg collection, 10
  eggs dechorination, 10–11
  embryogenesis in, 4–5
  ethical issues, 12–13
  fly disposal, 10
  food medium, 10
  genetic manipulation, 3–4
  genome, 7
  handling of flies, 10
  HIV pathology, 15
  homeotic genes in, 6–7
  human disease models, 13
  human metabolic disorders, 14–15
  human neurodegenerative diseases, 13–14
  life cycle, 4, 5f
  mutations, 9
  nephrolithiasis, 15
  pattern formation in, 5–6, 7f
  physical appearance, 4
  plant models, 7
  polytene chromosomes, 8–9
  protocol for germ-line transformation in, 11–12
  sex-limited trait, 7–8
  sex-linked inheritance, 8f
  species, 4
  therapeutic peptide production, 15–17
  translational significance, 13
  WWW resources, 17
    network resources, 16t
    physical resources, 17t
DRS™. *See* Direct RNA Sequencing
Drug discovery and development, 369–370, 566. *See also* Biomolecular display technology
  from natural resources, 566
  paradigm shift in, 370f
  principle, 370–371
  small molecule *vs*. biomolecular drugs, 371–372
  stages and timeline, 370–371
  therapeutics comparison, 372f
Drug holiday, 166
"Drug re-purposing" approach, 400–401
Drug treatment protocol, 203
dsFv. *See* disulfide-linked variable fragment
DTaP vaccine, 492
DTH. *See* Delayed type hypersensitivity
DTS. *See* Digital transcriptome subtraction
Duchenne Muscular Dystrophy (DMD), 466
  patient affected with, 469f

Duty-based ethics. *See* Deontological ethics
Dwarf octopus (*Octopus joubini*), 544
Dye efflux property, 435–436, 435f
Dynamic mutations, 465
Dynamical systems theory, 385–386
Dystrophin, 579–580

E

e-infrastructure, 551–552
E6-associated protein (E6AP), 106
E6AP. *See* E6-associated protein
EAE. *See* Experimental allergic encephalomyelitis
EBER. *See* EBV-encoded RNA
EBFP. *See* Blue fluorescent protein
EBNA-LP protein, 131–132
EBNA1 DNA-binding protein, 131
EBNA3 family, 131
EBV. *See* Epstein–Barr virus; Estimated breeding value
EBV lymphoblastoid cell lines (EBV-LCLs), 134
EBV-encoded RNA (EBER), 132
EBV-LCLs. *See* EBV lymphoblastoid cell lines
ECFP. *See* Cyan fluorescent protein
ECG. *See* Epicatechin-3-gallate
Echinacea tea, 186
Echinoderms, 544
ECM. *See* Extracellular matrix
Ectodomain, 161
Edelman's experiment, 268
Edible vaccines, 501–502
EDS. *See* Energy Dispersive Spectroscopy
EFSA. *See* European Food Safety Authority
EGCG. *See* Epigallocatechin-3-gallate
EGCs. *See* Embryonic germ cells
EGE. *See* European Group on Ethics
EGF. *See* Epidermal growth factor
EGFR. *See* Epidermal growth factor receptor
Egg collection, 10
Eggs dechorination, 10–11
EHEC. *See* Enterohemorrhagic *E. coli*
EIA. *See* Enzyme immunoassay
Electromagnetic radiation, 99
Electronic counting, 215
electronic Models Information, Communication, and Education (eMICE), 90
Electroporation, 545
Electrosurgical Excision (LEEP), 110
ELISA. *See* Enzyme-linked immunosorbant assay
ELS. *See* Essential life span
ELSI. *See* Ethical, legal, and social issues
Embryo development process, 426
Embryonal rest theory, 438
Embryonic germ cells (EGCs), 430, 432
Embryonic stem cells (ESC), 75, 78–79, 205, 241, 408, 430–432, 432t–433t, 610–611
  markers, 433
  transfer, 409–411, 411f
Embryos, 430
Embyronic stem cell identification, 433
EMEA. *See* European Medicines Agency
eMICE. *See* electronic Models Information, Communication, and Education
Encode Project, 590
Enderling's earlier model, 389
Endogenous retroviruse (ERV), 584
Endoplasmic reticulum (ER), 225, 499
Energy Dispersive Spectroscopy (EDS), 249
Enterohemorrhagic *E. coli* (EHEC), 527–528, 534
Enterotoxigenic *Escherichia coli* (ETEC), 527–528
Env. *See* Envelope
Envelope (Env), 161
Environment Scanning Electron Microscope (ESEM), 249
Environmental concerns. *See also* Intrinsic concerns
  ethical positions, 607
  by transgenic technology, 607
Environmentally benign nature, 601
Enzyme immunoassay (EIA), 132–133, 552
  dot immunobinding assay, 553
  ELISA, 552–553, 553f
  western blotting, 553
Enzyme inhibitors, 556
Enzyme-linked immunosorbant assay (ELISA), 149, 275–276, 488f, 552–553, 553f
(−)-epicatechin (EC), 187
Epicatechin-3-gallate (ECG), 187
Epidermal growth factor (EGF), 220, 226
Epidermal growth factor receptor (EGFR), 486
(−)-epigallocatechin (EGC), 187
Epigallocatechin-3-gallate (EGCG), 187
Epigenetics, 57, 359, 584
  applications, 61–64
  arsenic, 60–61
  biomarkers of exposure, 60
  chromatin structure, 59
  CpG in promoter, 60
  DNA methylation, 58–59
  DNMT activity, 60
  ethical issues, 67
  gene expression, 58f
  gene imprinting, 60
  genetic alterations, 58
  histone analysis, 70f
  histone octamer, 59
  inhibitors, 68t
  liver cancer patients, 59
  MBP, 59
  methodology, 64–65
  methylation profiling, 69f
  miRNAs, 59–60
  MLL, 59
  using mouse models, 61
  protocols, 65–67
  translational significance, 67–69
  WWW resources, 69
Epigenome, 58
Epitopes. *See* Antigenic determinants
EPO. *See* European Patent Office
Epstein–Barr virus (EBV), 121–125
  clinical symptoms and diagnosis, 132–134
  entry into cell, 129–130
  ethical issues, 135
  genome structure, 129
  latency, 130–131
  latent genes, 131
  lifecycle stages, 129f
  lytic replication, 130
  research methods and protocols, 134
  translational significance, 135
  WWW resources, 135–136
Equality, 601
ER. *See* Endoplasmic reticulum
eRAPANSY. *See* Evolutionary Rapid Panning Analysis System
ERBs. *See* Ethical review boards
ERV. *See* Endogenous retroviruse
ES cell. *See* Embryonic stem cell
ES cells. *See* Embryonic stem cells (ESC)
ESC. *See* Embryonic stem cells
ESCC. *See* Esophageal squamous cell carcinoma
ESEM. *See* Environment Scanning Electron Microscope
Esophageal cancer, 62
Esophageal squamous cell carcinoma (ESCC), 62
Essential life span (ELS), 178
EST. *See* Expressed sequence tag
Estimated breeding value (EBV), 361
ETEC. *See* Enterotoxigenic *Escherichia coli*
Ethical, legal, and social issues (ELSI), 589
Ethical review boards (ERBs), 610
Ethical theories, 597
Ethicos, 598–599
Ethics, 305
  committee constitution, 610
Ethos. *See* Ethicos
Etoposide, 481
Euchromatin, 59, 459, 579
Eudaimonia, 599–600
European Commission, 611
European Food Safety Authority (EFSA), 606
European Group on Ethics (EGE), 300
European Medicines Agency (EMEA), 609
European Patent Office (EPO), 419
Evolutionary Rapid Panning Analysis System (eRAPANSY), 378
Evolutionary research, 358–359
Evolutionary systematics. *See* Synthetic systematics
Evolutionary theories, 178–179
Experimental allergic encephalomyelitis (EAE), 139, 146
Expressed sequence tag (EST), 357–358, 586
Extensively drug resistant (XDR), 22
Extracellular matrix (ECM), 234
Extraction, 566–567
Extranodal lymphomas, 97
Extrinsic concerns. *See also* Environmental concerns
  animal welfare, 604–606
  human health, 606
  precautionary principle, 607
  by transgenic technology, 607
Extrinsic value, 601

F

FA. *See* Folic acid
Fab. *See* Fragment antigen binding
FACS. *See* Fluorescent associated cell sorting

False building blocks, 164
Familial disorders, 465
FAO. *See* Food and Agriculture Organization
Farm Animal Welfare Advisory Committee, 605
Farm animals, 386
FASD. *See* Fetal alcohol spectrum disorders
FBS. *See* Fetal bovine serum
FC. *See* Fecal coliforms
Fc epsilon receptor 1 (FcεR1), 512
FCA. *See* Freund's Complete Adjuvant
FcεR1. *See* Fc epsilon receptor 1
F–d curve. *See* Force–distance curve
FDA. *See* Food and Drug Administration; U.S. Food and Drug Administration
FDCA. *See* Food, Drug, and Cosmetic Act
Fecal coliforms (FC), 527
Fecal indicator bacteria (FIB), 526–527
Fecal indicator organism detection
  using chromogenic substrates, 529
  conventional techniques, 528–529
  immunological methods, 529–530
  MF method, 529
  MPN method, 529
  pathogen detection methods, 529f
  substrate methods, 529
Fecal wastes, 526
Female triploids, 546
Ferric Reducing Ability of Plasma (FRAP), 184, 184f
Fetal alcohol spectrum disorders (FASD), 322
Fetal bovine serum (FBS), 87, 223
Fetal tissue, 430
FIA. *See* Freund's Incomplete Adjuvant
FIB. *See* Fecal indicator bacteria
Fiber bonding, 237–238
FIGO system. *See* International Federation of Gynaecology and Obstetrics system
FISH. *See* Fluorescence *in situ* hybridization
(F)ISH procedure, 339
Fish biotechnology, 545
Flavonoids, 185, 186f
Flow cytometry set-up, 440
Fluid Force microscopy (FluidFM), 250, 252
FluidFM. *See* Fluid Force microscopy
Fluorescence *in situ* hybridization (FISH), 459–460, 460f, 467, 530
Fluorescence labeling, 531
Fluorescence-based detection systems, 531
Fluorescent associated cell sorting (FACS), 275–276, 435–436
Fluorescent dyes, 65–66
Fluorescent protein imaging (FPI), 85–86
5-fluorouracil (5-FU), 199
Fly morgue, 10
FOCM. *See* Folic acid one carbon metabolism
Folic acid (FA), 61–62, 69
Folic acid one carbon metabolism (FOCM), 69
Food, Drug, and Cosmetic Act (FDCA), 420
Food allergy, 511
Food and Agriculture Organization (FAO), 290, 511
Food chain contamination, 609
Food protein-induced allergenicity mechanism, 511–512
Food proteins, 511–512
Food-borne pathogens detection, 527–528
Force mapping mode, 251
Force spectroscopy mode, 251
Force–distance curve (F–d curve), 251
Foreign protein, 499
454 sequencing, 348
Fourier transform infrared spectroscopy (FTIR), 330
4th generation sequencing technologies, 351
  DNA nanoball sequencing, 352–353
  nanopore sequencing, 351–352
  polony-based sequencing technology, 352
Fp-few polyhedral, 228
FPI. *See* Fluorescent protein imaging
Fractional vaccines, 495
Fragile X syndrome, 459, 485
Fragment antigen binding (Fab), 268
Frameshift mutation, 464–465, 464f
FRAP. *See* Ferric Reducing Ability of Plasma
Free Radical Theory of Aging (FRTA), 180
Free radicals, 181
Freemartinism, 299
Freeze drying, 240
Freund's Complete Adjuvant (FCA), 277, 487
Freund's Incomplete Adjuvant (FIA), 277, 487
Frontotemporal lobar degeneration (FTLD), 40
FRTA. *See* Free Radical Theory of Aging
Fruit fly study (*Drosophila melanogaster*), 452
FTIR. *See* Fourier transform infrared spectroscopy
FTLD. *See* Frontotemporal lobar degeneration
5-FU. *See* 5-fluorouracil
Functional peptide aptamers. *See also* Biomolecular display technologies
  eRAPANSY, 378
  PLM, 378, 380f
    ASAC and selection, 378–379
    method for *in vitro* evolution, 379f
    p&p, 379–380
    YLBS and selection, 378
  shortcomings for drug discovery, 377–378
Fused deposition modeling, 239
Fused in sarcoma (FUS), 43–44
Fusion of cells, 280

G

G-dark regions. *See* Dark-staining regions
G-light regions. *See* Light-staining regions
GABA. *See* Gamma-amino-butyric acid
Gag. *See* Group Specific Antigen
GAIN. *See* Genetic Association Information Network
Galleria mellonella nucleopolyhedroviruses (GmMNPV), 228
Gamma-amino-butyric acid (GABA), 320
GAP. *See* Great Ape Project
Gas chromatography/mass spectrometry (GC/MS), 569
Gas foaming, 239
Gastric cancer, 62
Gastrointestinal tract (GI tract), 511
Gastropods, 543
Gastrulation, 4–5
GATU. *See* Genome Annotation Transfer Utility
Gay Compromise Syndrome (GCS), 156–157
Gay Related Immune Deficiency (GRID), 156–157
GC-rich regions, 579–580
GC/MS. *See* Gas chromatography/mass spectrometry
GCP. *See* Good clinical practice
GCS. *See* Gay Compromise Syndrome
GCs. *See* Germinal centers
Gel electrophoresis, 292
GEM. *See* Genetically engineered mouse
GenBank®, 322
Gene, 463
  delivery, 551
  discovery, 290–291
  imprinting, 60
  knockout technology, 606
  transfer methods, 545
Gene content, 579
  biological insights, 582
  CpG islands, 583
  DNA transposons, 584
  dystrophin, 579–580
  ensemble database version 68. 37, 580t
  epigenetic mechanisms, 584
  genes and diseases, 582
  goals of HGP, 586
  LINE, 584
  long-range variation, 582–583
  LTR retrotransposons, 584
  miRNAs, 581
  mitochondrial genome, 585
  mobile elements, 583t
  mtDNA, 585–586
  non-coding genes, 580
  non-coding RNA types, 582t
  polygenic disorders, 582
  protein-coding genes, 579
  pseudogenes, 584–585
  repeat content, 583
  rRNAs, 581
  segmental duplications, 585
  SINE, 584
  small nuclear RNAs, 581
  snoRNAs, 581
  snRNAs, 581
  SSRs, 585
  structure of chromosomes, 582
  transposable element-derived repeats, 583
  tRNAs, 581
  X chromosome, 579
Gene expression, 308
  ethical issues, 321
  hypothalamic orexin system, 321
  mRNA expression profiling, 321–322
  mRNA quantitation, 310–312
  principles, 308–310
  prolonged upregulation of, 320–321
  protein quantitation, 312–316
  protocols, 316
    mRNA and protein quantitation, 316–318
    semi-quantitative immunohistochemistry, 319–320
    single-cell RT-PCR, 318–319
    Western blot technique, 316

quantitative analysis of, 307–308
quantitative real-time PCR, 309f
translational significance, 322
WWW resources, 322–323
Gene Knockdown, 412
Gene regulation theory of aging, 179
Gene therapy, 227
method testing, 444
Gene-Ontology (GO), 387
GeneNetWeaver tool (GNW tool), 390
Generation, 346
Genetic
biomarkers, 299–300
code, 463
diseases, 577–578
disorders, 362, 465
engineering methods, 510
heterogeneity, 465
monitoring, 298
polymorphism, 465
signature sequences, 530
Genetic Association Information Network (GAIN), 589
Genetic disorder diagnosis
CGH and aCGH, 461, 461f
chromosomes and karyotyping identification, 459–460
cytogenetic and molecular techniques, 459
electropherogram, 469f–470f
ethical issues, 470–471
FISH, 460, 460f
human lymphocyte culture, 459f
MLPA picture, 470f
molecular diagnostic technologies, 466–469
multiplex PCR amplification, 469f
patient affected with DMD, 469f
principle, 462
SKY and M-FISH, 460–461, 461f
translational significance, 471
WWW resources, 471
Genetic Information Non-Discrimination Act (GINA), 589
Genetically engineered mouse (GEM), 73–75
breast cancers, 79
gastrointestinal cancer, 79–80
homologous recombination, 78–79
in immunocompetent mice, 79f
knock in models of cancer, 79
Genetically engineered vaccines, 497
Genetically modified crops (GM crops), 510
accumulated hectare, 510
allergenicity, 511
allergens, 511
ethical issues, 521–522
food allergens stability, 513t
food protein-induced allergenicity mechanism, 511–512
safety assessment and regulation, 510–511
SGF assay, 512–516
simulated intestinal fluid assay, 516–518
translational significance, 522
WWW resources, 522
Genome Annotation Transfer Utility (GATU), 401
Genome of interest (GoI), 388
Genome-wide association studies (GWAS), 361–362, 589–590
Genome-wide gene expression analysis, 199
Genomic information, 329–330
Genomic variability, 360
Genomics, 386, 470
Genotype, 463
Genotypic typing methods, 331
GEObs. *See* Global Ethics Observatory
Germ stem cells (GSCs), 436–437
Germ-line transformation, 11–12
Germinal centers (GCs), 493–494
GFP. *See* Green fluorescence protein
GI tract. *See* Gastrointestinal tract
Giant clam (*Tridacna*), 543–544
Giant squid (*Architeuthis*), 544
Giemsa banding, 459
Giemsa reagent, 459
GINA. *See* Genetic Information Non-Discrimination Act
Ginger tea, 186–187. *See also* Peppermint tea
Ginkgo tea, 187
Ginseng tea, 187
Glass-Col apparatus, 31f
Glatiramer acetate, 146
Glaxo-SmithKline (GSK), 112
Gliosis, 51
Global Ethics Observatory (GEObs), 611
Global marine aquarium trade, 549t
GLP. *See* Good laboratory practices
Glycophorin A (GPA), 435
Glycoprotein gp120, 161
GM crops. *See* Genetically modified crops
GM foods, allergenicity assessment, 511
GM-CSF. *See* Granulocyte macrophage colony stimulating factor
GmMNPV. *See Galleria mellonella* nucleopolyhedroviruses
GMP. *See* Guanosine monophosphate
GNPs. *See* Gold nanoparticles
GNW tool. *See* GeneNetWeaver tool
GO. *See* Gene-Ontology
GoI. *See* Genome of interest
Gold nanoparticles (GNPs), 525–526, 532, 535
high surface-to-volume ratio, 532
rich surface chemistry, 532
synthesis, 533–534, 534f
with thiol-modified DNA functionalization, 534
Golgi complex, 164
Gompertz function, 197–198
Gonadal ridge, 432
Good clinical practice (GCP), 445–446
Good laboratory practices (GLP), 445–446
GPA. *See* Glycophorin A
Granulocyte macrophage colony stimulating factor (GM-CSF), 141
Granulomas, 23
Great Ape Project (GAP), 301
Green fluorescence protein (GFP), 414, 554–555, 557
D. melanogaster, 558
marine by-products, 558t
mutants of, 557–558
Green tea, 186. *See also* Black Tea
in aging and neurodegenerative diseases, 188
molecular mechanisms, 187–188
GRID. *See* Gay Related Immune Deficiency
Group Specific Antigen (Gag), 161
Growth cycle, 217
characteristic growth pattern
lag phase, 217
log phase, 217
plateau phase, 217
GSCs. *See* Germ stem cells
GSK. *See* Glaxo-SmithKline
Guanine, 462
Guanosine monophosphate (GMP), 179
Guinea pig models
immunologic reagents for, 26
TB transmission, 26
GWAS. *See* Genome-wide association studies
Gynogenesis, 547–548, 547f

H

H-strand, 585
H_3PO_4. *See* Phosphoric acid
HAART. *See* Highly Active Antiretroviral Treatment
HACA response. *See* Human anti–chimeric antibody response
HAD. *See* HIV-associated dementia
hADSCs. *See* human adipose-derived stem cells
Haemophilis influenzae A (HiA), 497
Haemophilus influenza genome, 587
Haemophilus influenza type b vaccines (Hib vaccines), 492
Haemophilus influenzae strain Rd, 333–334
in silico analysis, 334–335
HAHA response. *See* Human anti–humanized antibody response
HAMA. *See* Human anti-mouse antibody
HAMA response. *See* Human anti–monoclonal antibody response
Hanging-drop method, 200–201
Hank's-buffered saline solution (HBBS), 87
Haplosporidium nelsoni, 554
HaSNPV. *See Helicoverpa armigera* nucleopolyhedroviruses
HAT. *See* Hypoxanthine, Aminopterin, and Thymidine
Hayflick limit, 429
Hayflick's phenomenon, 219
Hazelnut allergens, 517–518
HBBS. *See* Hank's-buffered saline solution
HBcAg. *See* Hepatitis B core antigen
HBeAg. *See* Hepatitis B early antigen
HBsAg. *See* Hepatitis B surface antigen
HBV. *See* Hepatitis B virus
HC. *See* Hemorrhagic colitis
HCC. *See* Hepatocellular carcinoma; Human hepatocellular carcinoma
HCG. *See* Human chorionic gonadotropin
HCT/Ps. *See* Human cells, tissues, and cellular and tissue-based products
HCV. *See* Hepatitis C virus
HD. *See* Huntington's disease
HDMs. *See* Histone demethylases

Head and neck squamous cell carcinoma (HNSCC), 62
Head cancer, 62
Heat shock protein 27 (HSP27), 205
Heat treatment, 518–519
Heat-labile enterotoxin (LTI), 527–528
Hedgehog gene, 9
HEK. *See* Human embryonic kidney
HeLa. *See* Henrietta Lacks
Helicobacter pylori (*H. pylori*), 62
Helicos biosciences, 350
Helicoverpa armigera nucleopolyhedroviruses (HaSNPV), 228
Hemagglutination, 277, 281
Hematopoietic stem cells (HSCs), 434
  cord blood and, 445
  markers, 434
Hemocytometer, 215
Hemolytic-uremic syndrome (HUS), 534
Hemorrhagic colitis (HC), 534
Henrietta Lacks (HeLa), 199–200
Hepatitis B core antigen (HBcAg), 127–128
Hepatitis B early antigen (HBeAg), 127–128
Hepatitis B surface antigen (HBsAg), 127–128, 497
Hepatitis B vaccines, 497
Hepatitis B virus (HBV), 121–125, 223
  inflammatory disease of liver, 127
  non-HCC tissues, 128
  viral cause, 127–128
Hepatitis C virus (HCV), 121–122, 127, 377
Hepatocellular carcinoma (HCC), 63
Hepatocytes, 235, 236f
HER2. *See* Human epidermal growth factor receptor
Herbal drug production, biotechnological approaches for
  callus cultures, 570
  high-value bioactive compounds, 569
  neem organogenesis, 570f
  organ cultures, 569–570
  suspension cultures, 570
Herbal medicines, 563–564, 564f
  ancient system, 565
  ethical issues, 573
  using *Lantana camara* L., 570–571
  medicinal plants investigation, 565–569
  opportunities and challenges, 571–573
  pharmaceuticals, 564
  traditional medicine, 564–565
  translational significance, 573–574
  WWW resources, 574
Herceptin, 83
Herpes viruses, 101
hES. *See* Human embryonic stem cells (hESCs)
hESCs. *See* Human embryonic stem cells
Heterochromatin, 59, 459, 579
HGP. *See* Human Genome Project
HGPRT. *See* Hypoxanthine-guanine phosphoribosyl transferase
HGSIL. *See* High-grade squamous intraepithelial lesions
hHeps. *See* human hepatocytes
HHV-6. *See* Human herpes virus 6
HiA. *See* Haemophilis influenzae A
Hib vaccines. *See* Haemophilus influenza type b vaccines
High Squamous Intraepithelial Lesions (HSIL), 103
High-grade squamous intraepithelial lesions (HGSIL), 111
High-performance liquid chromatography (HPLC), 567–568
High-pressure liquid chromatography. *See* High-performance liquid chromatography (HPLC)
High-temperature requirement protein A2 (HTRA2), 13–14
Highly Active Antiretroviral Treatment (HAART), 165
Hinduism, 603–604
Histidine-proline-glutamine (HPQ), 377
Histocompatibility complex (HLA), 150
Histocompatibility testing, 467
Histone analysis, 70f
"Histone code" hypothesis, 59
Histone deacetylase inhibitor. *See* Suberoylanilide hydroxamic acid (SAHA)
Histone demethylases (HDMs), 59
Histone profiling, 64
HIV. *See* Human immunodeficiency virus
HIV-associated dementia (HAD), 171
HIV-associated encelopathy (HIVE), 171
HIV-associated minor cognitive and motor disorders (MCMD), 171
HIVE. *See* HIV-associated encelopathy
HLA. *See* Histocompatibility complex; Human leukocyte antigen
HLA-A. *See* Human leukocyte antigen (HLA)
HMP. *See* Human Microbiome Project
HNF. *See* Holographic notch filter
HNSCC. *See* Head and neck squamous cell carcinoma
Hodgkin/Reed–Sternberg cells (HRS cells), 133
Hodgkin's lymphoma, 97, 133
Hoechst 33342 dye staining, 435–436
Holographic notch filter (HNF), 253
Holothurin, 556
Homeostasis, 140, 428
Homing, 436–437
Homoplasy, 299
Horizontal gene transfer, 609
Horse Radish Peroxidase (HRPO), 280–281
Horseradish peroxidase (HRP), 340
Horseshoe Crabs, 544
Host–pathogen/agent equilibrium, 396
HPA axis. *See* Hypothalamo-pituitary-adrenal axis
HPETE. *See* Hydroperoxyeico-satetraenoic acid
HPLC. *See* High-performance liquid chromatography
HPQ. *See* Histidine-proline-glutamine
HPRT. *See* Hypoxanthine phosphoribosyltransferase
HPV. *See* Human papillomavirus
HPV16 E6/E7 transcription, 115
HRP. *See* Horseradish peroxidase
HRPO. *See* Horse Radish Peroxidase
HRS cells. *See* Hodgkin/Reed–Sternberg cells
HSCs. *See* Hematopoietic stem cells
HSE. *See* Human Skin Equivalent
HSIL. *See* High Squamous Intraepithelial Lesions
HSP27. *See* Heat shock protein 27
HTLV-1. *See* Human T lymphotropic virus
HTRA2. *See* High-temperature requirement protein A2
human adipose-derived stem cells (hADSCs), 207
Human anti-mouse antibody (HAMA), 483
Human antibody transgene, 486
Human anti–chimeric antibody response (HACA response), 273–274
Human anti–humanized antibody response (HAHA response), 274
Human anti–monoclonal antibody response (HAMA response), 273
Human cells, tissues, and cellular and tissue-based products (HCT/Ps), 446
Human chorionic gonadotropin (HCG), 40–41
Human chromosome nomenclature systems, 454–455, 455t
Human development studies, 444
Human dignity, 601, 606
Human disease for animal models
  blood circulatory system, 140
  disease mechanisms, 140
  ethical issues, 149–150
  homeostasis, 140
  MS, 144–149
  RA, 140–144
  translational significance, 150
  WWW resources, 150
Human DNA tumor viruses, 123–125, 124t
  EBV, 125
  KS, 126
  KSHV, 125–126
  PEL, 126
Human embryonic kidney (HEK), 227–228
Human embryonic stem cells (hESCs), 228, 440–441, 603
Human epidermal growth factor receptor (HER2), 206–207
Human genome, 463
Human Genome Project (HGP), 370, 578
  sequencing, 579
Human genome sequencing project, 577–578
  complexity, 579
  DNA sequences, 577–578
  ethical issues, 589
  examples with applications, 588–589
  full-scale sequencing, 578
  gene content, 579–586
  high-density genetic and physical maps, 578
  methodology, 587–588
  organization and perspective, 579
  principle, 586–587, 587f
  translational significance, 590–591
  whole genome shotgun sequencing approach, 588f
  WWW resources, 591
Human health
  animal biotechnology, 606–607
  nature of risk, 606
  public health ethics, 606–607

Human hepatocellular carcinoma (HCC), 122–125, 127
human hepatocytes (hHeps), 207
Human herpes virus 6 (HHV-6), 144–145
Human herpesvirus 4. *See* Epstein–Barr virus (EBV)
Human herpesvirus 8 (HHV8). *See* Kaposi sarcoma-associated herpesvirus (KSHV)
Human immunodeficiency virus (HIV), 15, 156–157
  antiretroviral
    drugs, 164–165
    types, 166–167
  bone marrow transplantation, 172–173
  clinical stages, 158–160
    asymptomatic stage, 159–160
    persistent generalized lymphadenopathy, 160
    seroconversion stage, 159
    symptomatic stage, 160
    WHO classification, 159t
  ethical issues, 173
  genome, 161f
  global disease burden, 157–158
  history, 156–157
  methodology and principles, 167
    anti-HIV effects evaluation, 170–171
    antiretroviral drugs assays, 168–169
    growing HIV stock, 167–168, 168f
    monitoring antiretroviral drug toxicity, 169–170, 169f
  molecular biology, 160–162
  NeuroAIDS, 171–172
  replication, 162–164, 163f
  resistance and antiretroviral treatment, 165
    drug holiday, 166
    HAART therapy, 165
    salvage therapy, 165
  structure, 160f
  translational significance, 173–174
  types, 156
  WWW resources, 174
Human JC virus (JCV), 128
Human leukocyte antigen (HLA), 140, 179
Human lymphocyte culture, 459, 459f
Human metabolic disorders, 14–15
Human microbiome, 362
Human Microbiome Project (HMP), 362
Human neurodegenerative diseases, 13–14
Human papillomavirus (HPV), 96, 114t, 121–122, 126–127, 230
  cervical cancer diagnostic methodologies, 107–109
  DNA testing, 108f
  effects and diseases, 104t
  genetic-based DNA vaccines, 113
  genomic organization, 105
  genotypes, 104
  inactivation and degradation of p53, 106–107
  infections, 104, 126–127
  onco-proteins E6 and E7, 106
  prophylactic HPV vaccines, 112–113
  replication, 106
  therapeutic HPV vaccines, 113
  transcriptional regulation, 105–106
  vaccines, 497
  viral proteins, 127
Human pathogens, 505
Human polyomaviruses, 128
  MCPyV, 128–129
Human repeat sequences, 583
Human reproductive cloning, 603–604
Human Skin Equivalent (HSE), 240
Human T lymphotropic virus (HTLV-1), 121–122, 157
Human-to-animal chimeras, 607–608
Humanized antibody, 274
Humanized mice, 135
Humoral immunity, 474–475
Huntington's disease (HD), 40
HUS. *See* Hemolytic-uremic syndrome
Hybrid cell selection, 280
Hybrid models, 393–394
Hybrid protein, 503
Hybridoma cell lines, 553–554
Hybridoma selection, 282–283, 283f
Hydrogen Peroxide (H_2O_2), 182
Hydroperoxyeico-satetraenoic acid (HPETE), 182–183
Hydroxyl Radical (·OH), 182
5-hydroxymethyl cytosine, 64
Hyper–immune serum, 273
Hypothalamic orexin system, 321
Hypothalamo-pituitary-adrenal axis (HPA axis), 180
Hypothesis, 390
Hypoxanthine, Aminopterin, and Thymidine (HAT), 280
Hypoxanthine phosphoribosyltransferase (HPRT), 179
Hypoxanthine-guanine phosphoribosyl transferase (HGPRT), 179, 282–283, 476–478
Hypoxia, 205
Hypoxic fraction, 203–204
Hysterectomy, 111

I

IACUC. *See* Institutional Animal Care and Use Committees
IAP. *See* Inhibitor of apoptosis
IBC. *See* International Bioethics Committee
ICGC. *See* International Cancer Genome Consortium
ICMR. *See* Indian Council for Medical Research
ICR. *See* Imprinting control regions
Identity testing, 467
Ideogram, 454
IEC. *See* Independent Ethics Committee
IFA. *See* Immunofluorescence assay
IFBC. *See* International Food Biotechnology Council
IFN. *See* Interferon
IGBC. *See* Intergovernmental Bioethics Committee
IgE. *See* Immunoglobulin E
IGF. *See* Insulin-like growth factor
IGF1R. *See* Insulin-like growth factor-1 receptor
IgG. *See* Immunoglobulin–G
IHC. *See* Immunohistochemistry
IHGSC. *See* International Human Genome Sequencing Consortium
IIS. *See* Insulin-like growth factor-1 signaling
IL-10. *See* Interleukin-10
IL-2. *See* Interleukin
Illumina Solexa, 349
ILSI. *See* International Life Sciences Institute
IM. *See* Infectious mononucleosis
ImageJ, 322
Immersion technique, 548
Immortal DNA strand hypothesis, 429
Immune antibody libraries, 485
Immune system, 491–492
Immune-stimulating complexes (ISCOMs), 503
Immune–purification, 276
Immunis, 491–492
Immunitas, 491–492
Immunization, 277
Immunoblotting, 309–310, 316
Immunocompetent mice, 78–80
Immunodeficient mice, 81–83
Immunodiagnostics, 552
  enzyme immunoassay, 552–553
  latex agglutination test, 553
  monoclonal antibodies, 553–554
Immuno–enrichment. *See* Immune–purification
Immunofluorescence assay (IFA), 132–133
Immunogens. *See* Antigens
Immunoglobulin E (IgE), 512
  IgE-binding epitopes, 519
  molecules, 512
Immunoglobulin–G (IgG), 268–269
Immunohistochemistry (IHC), 275–276, 309–310
  semi-quantitative, 319–320
Immunological methods, 532–533
  antibody-based detection, 529–530
  IMS method, 530
  rapid culture-based methods, 530
Immunomagnetic separation (IMS), 530
Immunosorbent chromatography, 479
Immunotoxins, 274–275, 481
IMP. *See* Ionosine ionophosphate
Imprinting control regions (ICR), 60
IMS. *See* Immunomagnetic separation
IMS/culture methods, 530
IMSR. *See* International Mouse Strain Resource
IMV. *See* Intracellular mature virus
In silico models, 385–386
  advantages and disadvantages, 392
  applications, 392
  bioinformatics
    in animal biotechnology, 386–387
    and systems biology, 387
  cancer, 392–394
    in silico model types, 393–394
  of cells, 391–392
  ethical issues, 400
  infectious diseases, 394
    approach/scenario, 395–396
    bacterial and viral dynamics model, 397
    challenges in, 397
    examples, 396–397
    parameters, 395
    triad of, 394–395

In silico models (*Continued*)
and inflammatory response syndrome, 394
mathematical modeling
concept, 390–391
techniques, 389–390
neuronal diseases, 397
Alzheimer's disease model, 399
approach/scenario, 398
examples, 398–399
limitations, 399
parameters, 398
pathophysiology of, 397–398
PPIS, 388
signal transduction networks, 388–389
systems biology
computational methods in, 387–388
experimental methods in, 388
transcriptional control networks, 388
translational significance, 400–401
WWW resources, 401
In situ hybridization, 338–341, 339f
CARD Fish, 340
clone-FISH technique, 339
PNA probes, 341
RING FISH, 340
stable isotope-labeled probing, 340–341
In vitro 2-D cultures, 199–200
In vitro assays, 88–89
In vitro cell cultures, 196
In vitro compartmentalization (IVC), 373–374
In vitro fertilization (IVF), 470, 604
public perceptions, 604
secular intrinsic objections to biotechnology, 604
Inactivated whole virus vaccines, 495, 495f
advantages, 495–496
bacterial and viral vaccines, 496t
comparison, 496t
disadvantages, 496
methodology, 495
Inbred mice methodology, 77f
BALB/c inbred mouse strain, 76
cancer investigations, 76, 77t
Consomic strains, 76–77
DBA strain, 76
Inbreeding, 294
Independent Ethics Committee (IEC), 207, 610
Indian Council for Medical Research (ICMR), 445–446, 470
Indicator organisms, 526
Indicators, 527
induced pluripotent stem cells (iPSCs), 51, 426, 432, 437
generation, 438f
reprogramming factors, 438
somatic cell, 437
Inductive models, 390
Infectious disease, 362, 493
problems, 504
threats, 504–505
triad of, 394–395
Infectious disease *in silico* models, 394
approach/scenario, 395–396
bacterial and viral dynamics model, 397
challenges in, 397
examples, 396–397
parameters, 395
Infectious mononucleosis (IM), 125, 133
Infix-1, 272
Infix-2, 272
Influenza Research Database (IRD), 401
Influenza vaccine, 492
Influenza-Resistant Mouse, 415
Information, 378
Informed choice, 600
Informed consent, 600
Inhibitor of apoptosis (IAP), 375
InIs. *See* Integrase Inhibitors
Injection, 548
INN. *See* International Non-Proprietary Name
Institute of Nanotechnology (IoN), 259
Institutional Animal Care and Use Committees (IACUC), 67, 75, 321–322, 409
approval, 75–76
guidelines, 76
Institutional Review Board (IRB), 207, 610
Insulin, 14
Insulin resistance, 14
Insulin-like growth factor (IGF), 226
Insulin-like growth factor-1 receptor (IGF1R), 590
Insulin-like growth factor-1 signaling (IIS), 14
Intact food allergens, 512
Integrase Inhibitors (InIs), 165
Integrase inhibitors, 164
Inter simple sequence repeats (ISSR), 297
Interferon (IFN), 106, 140, 225
applications of, 225–226
Interferon regulatory factor (IRF), 106
Intergovernmental Bioethics Committee (IGBC), 611
Interleukin (IL-2), 134
Interleukin-10 (IL-10), 140
Internal repeat sequences (IRs), 129
Internal ribosomal entry site (IRES), 377
Internal spacer regions (ISRs), 332
length heterogeneity of, 335f
International Bioethics Committee (IBC), 611
International Cancer Genome Consortium (ICGC), 361–362
International Federation of Gynaecology and Obstetrics system (FIGO system), 103, 103t
International Food Biotechnology Council (IFBC), 510–511
International HapMap project, 589
International Human Genome Sequencing Consortium (IHGSC), 578
International Life Sciences Institute (ILSI), 510–511
International Mouse Strain Resource (IMSR), 54
International Non-Proprietary Name (INN), 271
International Organization for Standardization (ISO), 530–531
International Sheep Genomics Consortium (ISGC), 358
International Society for Stem Cell Research (ISSCR), 446
Interspersed repeats. *See* Transposable element-derived repeats
Intervening sequences (IVS), 337–338
Intestinal epithelium, 512
Intracellular mature virus (IMV), 256
Intrinsic concerns, 602–603. *See also* Extrinsic concerns
IVF, 604
religious intrinsic critique, 603–604
Intrinsic value, 601
Introns, 463
Inversion, 458
Ioinizing radiation, 99
IoN. *See* Institute of Nanotechnology
Ion Semiconductor Sequencing, 351
Ion Torrent technology, 351
Ionizing radiation, 203–204
Ionosine ionophosphate (IMP), 179
IonTorrent, 347
iPSCs. *See* induced pluripotent stem cells
IRB. *See* Institutional Review Board
IRD. *See* Influenza Research Database
IRES. *See* Internal ribosomal entry site
IRF. *See* Interferon regulatory factor
IRMS. *See* Isotope ratio mass spectrometry
IRs. *See* Internal repeat sequences
ISCOMs. *See* Immune-stimulating complexes
ISGC. *See* International Sheep Genomics Consortium
ISO. *See* International Organization for Standardization
Isochromosome, 457, 458f
Isoschizomers, 303
Isotope ratio mass spectrometry (IRMS), 341
ISRs. *See* Internal spacer regions
ISSCR. *See* International Society for Stem Cell Research
ISSR. *See* Inter simple sequence repeats
IVC. *See In vitro* compartmentalization
IVF. *See In vitro* fertilization
IVS. *See* Intervening sequences

J

JCV. *See* Human JC virus
Jellies. *See* Jellyfish
Jellyfish, 542–543, 554
Judaism, 603
Jumonji AT-rich interactive domain (JARID1), 59
Justice, 601

K

Kaposi sarcoma-associated herpesvirus (KSHV), 121–125
Kaposi's sarcoma (KS), 126
Kaposi's Sarcoma and Opportunistic Infections (KSOI), 156–157
Karyotype, 454
Kautilya's Arthasastra, 599–600
KDR. *See* Kinase domain-containing receptor
Kennedy-Alter-Sung disease. *See* Spinal and bulbar muscular atrophy (SBMA)
Ketamine–xylazine–acepromazine (KXA), 86
Keyhole limpet hemocyanin (KLH), 552
Kidney, 240
Kinase domain-containing receptor (KDR), 375
Kissing disease. *See* Infectious Mononucleosis (IM)

KLF4. *See* Kuppel-like factor-4
KLH. *See* Keyhole limpet hemocyanin
KS. *See* Kaposi's sarcoma
KSHV. *See* Kaposi sarcoma-associated herpesvirus
KSOI. *See* Kaposi's Sarcoma and Opportunistic Infections
Kugerberg-Welander disease, 41–42
Kuppel-like factor-4 (KLF4), 61
KXA. *See* Ketamine–xylazine–acepromazine

L

L-strand, 585
LAD. *See* Liver assist devices
Lag phase, 217
LAIV. *See* Live, attenuated influenza vaccine
LAK. *See* Lymphokine activated killer cells
LANA. *See* Latency-associated nuclear antigen
Lantana camara L., 570–571, 571f–572f
Laparotomy, 86
Large-insert clones, 588
Laser ablation, 110
Laser conization, 110–111
Latency-associated nuclear antigen (LANA), 125–126
Latent infection, 164
Latent-membrane proteins (LMP), 130–131
Latex agglutination test, 553
LAV. *See* Lymphadenopathy Associated Virus
LC/MS. *See* Liquid chromatography/mass spectrometry
LCLs. *See* Lymphoblastoid cell lines
LCR. *See* Long control region
Lead identification, 370–371
Least harm, 600
LEEP. *See* Electrosurgical Excision
Leptin receptor (LEPR), 293
Leucine-rich-repeat kinase 2 (LRRK2), 13–14
Leukemia, 62–63, 97
Leukotrienes, 182–183
Leuprorelin, 54
LGSIL. *See* Low-grade squamous intraepithelial lesion
LHRH. *See* Luteinizing hormone-releasing hormone
Licorice tea, 187
LifeCell, 445
Ligation technology, 349–350
Light-staining regions (G-light regions), 459
LINE. *See* Long Interspersed Nuclear Elements
Lineage labeling assay, 436
Linear epitopes, 519
Linnaeus's plant taxonomy, 328–329
Lipopolysaccharide (LPS), 142
Lipoxygenase, 182–183
Liquid cancers, 97
Liquid chromatography/mass spectrometry (LC/MS), 567–569
Liquid overlay method, 201–202, 203f
Live, attenuated influenza vaccine (LAIV), 494f, 497
  advantages, 495
  disadvantages, 495
  methodology, 493–495
Live typhoid vaccine (Ty21a), 497
Liver, 240
Liver assist devices (LAD), 240
Liver cancer, 63
Livestock productivity and health improvement, 361
Living modified organism (LMO), 53
LLNA. *See* Local lymph node assay
LMO. *See* Living modified organism
LMP. *See* Latent-membrane proteins
LMP1 protein, 132
LMW. *See* Low-molecular-weight
LNGFR. *See* Low-Affinity Nerve Growth Factor Receptor
Lobsters (*Homarus*), 544
Local lymph node assay (LLNA), 207, 400
Locus, 463
Locus heterogeneity, 465
Log phase, 217
LOI. *See* Loss of imprinting
Long control region (LCR), 105
Long Interspersed Nuclear Elements (LINE), 58–61, 584
Long PCR ribotyping, 337
Long terminal repeat (LTR), 155, 160–161
  retrotransposons, 584
Loss of imprinting (LOI), 60
Low Squamous Intraepithelial Lesions (LSIL), 103
Low-Affinity Nerve Growth Factor Receptor (LNGFR), 435
Low-grade squamous intraepithelial lesion (LGSIL), 103
Low-molecular-weight (LMW), 399
"Lower motor" neurons, 40. *See also* "Upper motor" neurons
LPS. *See* Lipopolysaccharide
LRRK2. *See* Leucine-rich-repeat kinase 2
LSIL. *See* Low Squamous Intraepithelial Lesions
LTI. *See* Heat-labile enterotoxin
LTR. *See* Long terminal repeat
Lung cancer, 62
Lung cell suspension, 35, 35f
Luteinizing hormone-releasing hormone (LHRH), 54
Lymphadenopathy Associated Virus (LAV), 157
Lymphoblastoid cell lines (LCLs), 125, 129–130
Lymphokine activated killer cells (LAK), 206
Lymphoma, 62–63, 97
Lyophilizer, 240
Lytic origin of replication (ori-lyt), 130

M

M-FISH. *See* Multicolor FISH
MA. *See* Matrix protein
mAB. *See* Monoclonal antibodies (MoAb)
Macrophage inflammatory protein 1 (MIP-1), 141
Madison chamber, 31
  exterior and interior, 32f
  photohelic meter for, 32f
Madison guinea pig aerosol chamber, 32f
MAG. *See* Myelin-associated glycoprotein
Magnetic resonance imaging (MRI), 85–86, 141
Maillard reaction, 519, 519f
Maine animals, 542
Major histocompatibility complex (MHC), 140–141, 474–475
MALDI-TOF. *See* Matrix-assisted laser desorption ionization time-of-flight
MALDI-TOF MS. *See* Matrix-assisted laser desorption/ionization-time of flight mass spectrometry
Male triploids, 546
Mammalian cell line characterization
  identity testing, 221
  purity testing, 222
  stability testing, 222
  viral testing assays, 222
Mammalian ion channels, 556
Mammalian-wide interspersed repeat (MIR), 584
Mapping, 353–354
Mariculture, 550, 559
  captive rearing technology, 550
  feed technology, 550
  nanotechnology application in aquaculture, 550–551
Marine animals
  arthropods, 544
  bivalves, 543–544
  cephalopods, 544
  chordates, 544
  crustaceans, 544
  cuttlefishes, 544
  echinoderms, 544
  gastropods, 543
  Horseshoe Crabs, 544
  jellyfish, 542–543
  sponges, 542
  squids, 544
  therapeutics from
    commercial bio-products, 556–557
    ethical issues, 559
    future directions, 559
    green fluorescent protein, 557–558
    marine by-products, 558t
    marine natural products, 554–556
    translational significance, 559
    valuable medical products, 554
    WWW resources, 560
  WWW resources, 560
Marine bioresources
  earth's surface, 542
  knowledge of ocean, 542
  marine animals, 542–544
Marine biotechnology, 542, 559
  animal phyla characteristics, 543t
  archaeologists, 542
  average depths and areas, 542t
  earth's surface, 542
Marine character, 551
Marine genomics, 551
  BAC, 551–552
  genetic analysis, 551
  marine organisms, 552
  novel proteins, 551
Marine natural products
  Conus peptides, 556
  Conus spp., 556
  cuttlefish and squid, 555
  E. Conus, 556

Marine natural products (*Continued*)
GFP, 554–555
jellyfish, 554
using nematocysts, 555
scyphozoan jellyfish stings, 555
sea snake, 555–556
snake venoms, 556
sponges, 555
squid ink from *Loligo* spp., 555
Marine organisms, 541–542
Marine ornamental fishery, 549
Marine sponges, 555
Mass spectrometry (MS), 569
Master Cell, 428
Mate tea, 187
Mathematical abstraction, 391
Matrix metalloproteinase-7 (MMP-7), 62
Matrix metalloproteinases (MMPs), 134–135, 141
Matrix protein (MA), 161
Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), 67
Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), 331
MBP. *See* Methylated binding proteins; Myelin basic protein
MCA. *See* Methylated CpG island amplification
MCBD2. *See* Methyl CpG binding domain 2
MCC. *See* Merkel cell carcinoma
MCD. *See* Multi-centric Castleman's disease
MCMD. *See* HIV-associated minor cognitive and motor disorders
MCP-1. *See* Monocyte chemoattractant protein 1
MCPyV. *See* Merkel cell polyomavirus
MCSs. *See* Multicellular spheroids
MCTS. *See* Multi-cellular tumor spheroids
MDP. *See* Muramyle dipeptide
MDR. *See* Multi-drug resistance
Measles vaccine, 492
Media, 235
Medicinal plants, 563–564
biological assays, 567
chemical screening, 567, 568f
domestic cultivation, 571
drug discovery, 566
extraction, 566–567
investigation, 565
isolation and characterization, 567–569
in modern medicine, 565
plant species, 565
rational drug design, 566
in traditional medicine, 566f
MedlinePlus, 150
Medulloblastoma, 61
MEF. *See* Mouse embryonic fibroblast feeder layers
Mega-HAART. *See* Salvage therapy
Meiosis, 453
Meiosis I triploidy, 546–547
Meiosis II triploid larvae, 546–547
Meiotic gynogenesis, 547
MELAS. *See* Mitochondrial encephalopathy with lactic acidosis and stroke
Melatonin receptor 1a (MTNR1A), 293
Melt molding, 238
Melt spinning, 237–238
Membrane filter technique (MF technique), 529
Membrane lamination, 239
Mendelian disorders, 588–589
Mendelian inheritance, 299
Merkel cell carcinoma (MCC), 128–129
Merkel cell polyomavirus (MCPyV), 121–125, 128
MERRF. *See* Myoclonic epilepsy with ragged red fibers
Mesenchymal stem cells (MSCs), 241
messenger RNA (mRNA), 463
Meta-proteomics, 551–552
Meta-transcriptomics, 551–552
Metabolic engineering, 552
Metaethics, 597–599
Metagenome sequencing, 359
Metagenomics, 551–552
Metallic nanoparticles, 532
Methyl CpG binding domain 2 (MCBD2), 66
5-methyl cytosine, 64
methyl DIP. *See* methylated DNA immunoprecipitation
Methyl-seq, 66
Methylated binding proteins (MBP), 59
Methylated CpG island amplification (MCA), 65–66
Methylated CpG island recovery assay (MIRA), 66
methylated DNA immunoprecipitation (methyl DIP), 63–64
Methylation biomarkers, 62
Methylation profiling, 69f. *See also* Histone profiling
bisulfite treatment, 64
DNA, 64
genome-wide profiling techniques, 64
QMSP, 64
Methylation sensitive amplification polymorphism (MSAP), 296
Methylation-sensitive single-strand conformational analysis (MS-SSCA), 67
Methylene tetrahydrofolate reductase (MTHFR), 62
MethylLight technology, 64
MethylScope, 65–66
MF technique. *See* Membrane filter technique
MGI. *See* Mouse Genome Informatics
MHC. *See* Major histocompatibility complex
Micro-coated feeds, 550
Microarray, 468
Microbial pathogens
detection principle, 532–533
EHEC, 534–535
ethical issues, 536–537
fecal indicator organisms detection, 528–530
future approaches, 537
GNPs synthesis, 533–534, 534f
methodology, 535f
microbial water quality indicators, 526–527
nanoparticle-based detection, 536
nanotechnology, 531–532
ssDNA sequences, 534, 535t
target pathogen genetic signature, 530–531
thiol-modified DNA, 534
translational significance, 537
water quality, 526
water-and food-borne pathogens, 527–528
waterborne disease, 526
WWW resources, 537–538
Microbial systematics, 329
Microbial taxonomy, 327–328, 341–342
Microbial water quality indicators
environmental indicators, 527
FIB, 526–527
indicator organisms, 527f
microbiological indicators, 528t
types of, 527
Microbiology, 328–329
Microdeletion syndrome, 461
Microduplication syndrome, 461
Microencapsulated feeds, 550
Microenvironment-tissue level models, 393
Microfabricated microstructures method, 202
Microglia, 51
Microinjection, 409, 411f, 545
Microinvasive carcinoma treatment, 111f
microRNA (miRNA), 59–60, 132, 357, 580–581
Microsatellites, 296–297
advantages and disadvantages, 299
applications of, 299
detection, 298, 299f
examples, 298, 299f
ISSR, 297
markers, 289–290
SSLP, 298
SSRs, STRs and SSTR, 297
STMS, 298
VNTRs, 297–298
Microscopic polymeric particles, 553
Microsphere-based diagnostic tests, 553
Microvessel density (MVD), 85–86
MIDAS. *See* Models of Infectious Disease Agent Study
Middlebrook airborne infection apparatus, 30–31, 31f
Mini-satellites, 297–298
Miniature inverted-repeat transposable element (MITE), 296, 301
MIP-1. *See* Macrophage inflammatory protein 1
MIPs. *See* Molecular inversion probes
MIR. *See* Mammalian-wide interspersed repeat
MIRA. *See* Methylated CpG island recovery assay
miRNA. *See* microRNA
Missense mutation, 464, 464f
MITE. *See* Miniature inverted-repeat transposable element
Mitochondrial disorder, 466
mitochondrial DNA (mtDNA), 585–586
Mitochondrial encephalopathy with lactic acidosis and stroke (MELAS), 466
Mitochondrial genome, 585
Mitochondrial myopathies, 585–586
Mitosis, 453
Mitotic gynogenesis, 547–548
Mixed lineage leukemia (MLL), 59
MLL. *See* Mixed lineage leukemia

MLPA. *See* Multiplex ligation-dependent probe amplification
MMAE. *See* Monomethyl auristatin E
MMPs. *See* Matrix metalloproteinases
MoAb. *See* Monoclonal antibodies
Mobile DNA sequences, 583
Models of Infectious Disease Agent Study (MIDAS), 401
Modern extraction techniques, 567
Modulated Raman Spectroscopy (MRS), 253–254
MOF. *See* Multiple organ failure
MOG. *See* Myelin oligodendrocyte glycoprotein
MOI. *See* Multiplicity of Infection
Molecular diagnostic technologies, 466
  case study, 469
  clinical diagnostic testing, 466
  congenital hypothyroidism, 467
  DNA segment, 467
  DNA sequencing, 468
  histocompatibility testing, 467
  identity testing, 467
  infectious diseases, 466
  monitoring response to therapy, 467
  PCR, 468
  pharmacogenomics, 466–467
  populations screening, 467
  principle, 469
  southern blotting, 467–468
Molecular farming, 501–502, 501f
Molecular genetics, 462
  acquired somatic genetic disorders, 466
  Chargaff's rule, 462
  hereditary material
    DNA molecules, 462
    DNA structure, 462–465
  mitochondrial disorder, 466
  multigenic and multifactorial disorders, 466
  nuclein, 462
  single-gene disorders, 465–466
Molecular inversion probes (MIPs), 356
Molecular markers, 289–290
  AFLP analysis, 295–296
  AS-PCR, 293
  ASO, 292–293
  ethical issues, 299–301
  for genetic analysis of animals, 291t
  microsatellites, 296–299
  MITE, 296
  MSAP, 296
  RAPD analysis, 293–294
  RFLP analysis, 291–292
  RLGS, 294
  SNP, 294–295
  SSCP, 293
  STS, 293
  three-fold applications, 291
  translational significance, 301
  WWW resources, 301–302
Molecular techniques, 459
Molecular theories, 179
Mollusks. *See* Transgenic fish
Monitoring antiretroviral drug toxicity, 169–170, 169f
Monoclonal antibodies (MoAb), 228, 270, 553–554
  additional words, 272
  advantages, 478
  application, 478–485, 481f
  development, 271
  disadvantages, 478
  hybridoma technology, 278
  methodology and rationale, 279–281
  naming system, 271, 271t
  organizations, 271
  production of, 279f, 281, 281f, 477f
Monocyte chemoattractant protein 1 (MCP-1), 141
Monogenic diseases, 582
Monomethyl auristatin E (MMAE), 481
Monosomy, 456
Monozygotic twins, 299
Moral philosophy. *See* Ethics
Morphological markers, 290
Mosaicism, 458
Most probable number technique (MPN technique), 529
Motor neuron diseases, 40
Mouse chimeras, 608
Mouse embryonic fibroblast feeder layers (MEF), 228
Mouse Genome Informatics (MGI), 90
Mouse immunization, 279
Mouse model
  advantages and limitations, 74t
  using aerosolized microorganisms, 25
  applications
    immunocompetent mice, 78–80
    immunodeficient mice, 81–83
  cancer research, 73–74
  ethical issues, 88
  genetic inheritance, 75
  genetic variation, 25
  H37Rv strain, 25
  in vitro testing, 25–26
  in vivo experiment, 83–85
  inbred mice methodology, 76–77
  mycobacterial infections, 26
  protocols, 85–88
  TB infection, 25
  transgenic technologies, 75
  translational significance, 88–89
  tumorigenesis, 74
  WWW resources, 89–90
Mouse Phenome Database, 90
Mouse Tumor Biology Database (MTBD), 90
MPN technique. *See* Most probable number technique
MRI. *See* Magnetic resonance imaging
mRNA. *See* messenger RNA
mRNA expression profiling, 321
mRNA quantitation, 310. *See also* Protein quantitation
  brain tissue sampling procedure, 311f
  immunocytochemical
    identification design, 312f
    identified neuron, 313f
  qPCR calibration, 317
  reverse-transcribed total RNA, 311
  RNA extraction, 316–317
  RT-qPCR, 316–317
  single-cell RT-PCR protocol, 310–311
  tissue extraction, 316
  using two-round PCR amplification, 312
MRS. *See* Modulated Raman Spectroscopy
MS. *See* Mass spectrometry; Multiple sclerosis
MS-SSCA. *See* Methylation-sensitive single-strand conformational analysis
MSAP. *See* Methylation sensitive amplification polymorphism
MSCs. *See* Mesenchymal stem cells
MTBD. *See* Mouse Tumor Biology Database
mtDNA. *See* mitochondrial DNA
MTHFR. *See* Methylene tetrahydrofolate reductase
MTNR1A. *See* Melatonin receptor 1a
μ-conotoxins, 556
Multi-cellular tumor spheroids (MCTS), 195–196, 197f
  cancer research, 196
  diffusion-limited tissue, 198–199
  drug treatment protocol, 203
  ethical issues, 207
  genome-wide gene expression analysis, 199
  Gompertz function, 197–198
  human glioma cell line, 199
  in vitro 2-D cultures, 199–200, 200t
  in vitro cell cultures, 196
  in vivo response, 198f
  protocol, 203
  radiation response, 203–204
  in solid tumors, 196
  spheroids generation techniques, 200–203, 201t
  3-D culture, 200, 200t, 207
  translational significance, 207–208
  treatment modalities, 198
  WWW resources, 208
Multi-centric Castleman's disease (MCD), 125–126
Multi-drug resistance (MDR), 22, 434
Multi-laboratory protocol, 514
Multicellular spheroids (MCSs), 205
Multicolor FISH (M-FISH), 460–461, 461f
Multifactorial disorders, 466
Multiple organ failure (MOF), 394
Multiple sclerosis (MS), 139, 144
  clinical manifestations, 145–146
  epidemiology and etiology, 144–145
  experimental models
    induced models, 146
    spontaneous models, 146
  methodology and protocol, 146–147
    clinical evaluation, 147
    EAE induction, 147f
    ELISA, 149
    histology, 147–148
    immunohistochemistry, 148–149
    real-time RT-PCR, 149
  pathogenesis, 145
  treatment, 146
Multiplex ligation-dependent probe amplification (MLPA), 468
Multiplicity of Infection (MOI), 168, 228

Multipotent stem cells, 429, 432
Municipal water, 526
Muramyle dipeptide (MDP), 142
Mutation Accumulation Theory of Aging, 178
Mutations, 9, 464
  acquired, 466
Muzzle soft tissue, 605
MVD. *See* Microvessel density
Mycobacterium tuberculosis (*M. tuberculosis*), 22–23. *See also* Tuberculosis (TB)
  bacterial loading, 31
  bacterial loads in target organs, 32, 34f
  experimental infection, 29t
  inoculum for aerosol exposure, 30, 31f
  intravenous infection of mice, 31, 33f
  lung cell suspension, 35, 35f
  lungs preparation, 34f, 35
  Madison chamber, 31
  Middlebrook airborne infection apparatus, 30–31
  PCR analysis, 33f
  RT-PCR, 32
Mycobacterium tuberculosis (*M. tuberculosis*), 277, 396
Myelin basic protein (MBP), 149
Myelin oligodendrocyte glycoprotein (MOG), 147
Myelin-associated glycoprotein (MAG), 146
Myeloma, 97
Myoclonic epilepsy with ragged red fibers (MERRF), 466

N

NA. *See* Nucleocapsid
NaBU. *See* Sodium butyrate
nAChR. *See* nicotinic acetylcholine receptor
Naked DNA vaccines, 499
Nano–bio interface, 533
Nanobiotechnology, 248
Nanocapsules, 550–551
Nano–coumarin (NC), 257
Nanofabrication, 256
Nanofluidics, 252
Nanomanipulation, 256
Nanoparticle-based detection, 536
Nanoparticles (NP), 257
Nanopore sequencing, 351–352
Nanoscale processes, 248
Nanotechniques, 249
Nanotechnology, 247, 531
  AFM probes chemical modification, 255
  animal cells and tissues
    nanomechanical properties, 255–256
    nano–structural features, 255
  application in aquaculture, 550–551
  applications to animal biotechnology, 256–258
  cell's environment, 248
  ethical issues, 258
  interfacial and quantization effects, 248–249
  macroscopic objects, 249
  metallic nanoparticles, 532
  microscopes, 249
  nanobiotechnology, 248
  nanofabrication, 249
  nanomanipulatations, 248
  nanomaterial applications, 532f
  nanomaterial components, 532f
  nanoparticle functionalization, 531f
  nanoscale processes, 248
  nanotools and nanotechniques, 249–255
  surface functionalization, 531
  translational significance, 258–259
  WWW resources, 259
Nanotechnology-based approaches, 531
Nanotools, 249
NASA. *See* National Aeronautics and Space Administration
Nasopharyngeal Cancer (NPC), 133
Natalizumab, 145
National Aeronautics and Space Administration (NASA), 202
National Bureau of Animal Genetic Resources (NBAGR), 290
National Cancer Institute (NCI), 157
National Center for Biotechnology Information (NCBI), 174, 189, 284
National Heart, Lung, and Blood Institute (NHLBI), 401
National Human Genome Research Institute (NHGRI), 578
National Institute of Allergy and Infectious Diseases (NIAID), 401
National Institutes of Health (NIH), 68, 150, 165, 284, 401, 578
Natural killer cells (NK cells), 206
Natural product materials, 568
Natural Sciences and Engineering Research Council of Canada (NSERC), 301–302
NBAGR. *See* National Bureau of Animal Genetic Resources
NC. *See* Nano–coumarin
NCBI. *See* National Center for Biotechnology Information
NCC. *See* Norwegian coastal cod
NCI. *See* National Cancer Institute; US National Cancer Institute
ncRNA. *See* non-coding RNA
NCSCs. *See* Neural crest stem cells
ND. *See* Neurodegenerative diseases
NEAC. *See* North-East Arctic cod
Near–field Scanning Optical Microscopy (NSOM), 250
  advantage, 252–253
  non–destructive microscopy technique, 253
Neck cancer, 62
Negative Factor (Nef), 161
Negative selection, 479
Nematocysts, 555
Nephrolithiasis, 15
Nerve growth factor (NGF), 435
Nerves, 241
  development, 213
Nestin, 435
Neural crest stem cells (NCSCs), 435
Neural stem cells (NSCs), 428
  markers, 435
NeuroAIDS
  AZT, 171
  CNS, 172
  efficacy of drugs, 172
  neuronal damage, 172
  neuropsychiatric complications, 171, 171t
Neurodegenerative diseases (ND), 13, 40
  AD, 40
  animal models
    ALS, 40–41
    applications, 49–53
    ethical issues, 53
    methodology, 44–49
    neurodegenerative diseases, 40
    principles, 43–44
    translational significance, 53–54
    WWW resources, 54
  characteristics, 40
  motor neuron diseases, 40
  representative, 41t
Neuroendocrine theory, 180
Neuromuscular diseases, 582
Neuronal disease *in silico* models, 397
  Alzheimer's disease model, 399
  approach/scenario, 398
  examples, 398–399
  limitations, 399
  parameters, 398
  pathophysiology of, 397–398
Neurotrophin-3 (NT-3), 435
New drug testing, 444
Next generation sequencing (NGS), 346
    See also DNA sequencing; Third generation sequencing technologies
  Amplicon Sequencing, 357
  animal biotechnology, 357–358
  applications and sequencing methods, 354f
  applications in human health, 361
    cancer research, 361–362
    genetic disorders, 362
    human microbiome, 362
    infectious diseases, 362
    personalized medicine, 362–363
    pre-and post-natal diagnosis, 362
  cattle genome, 357–358
  challenges, 363
  ChIP DNA sequencing, 357
  comparison, 353t
  ethical issues, 363
  experiment scheme, 348f
  future perspectives, 363
  by ligation technology, 349–350
  principle of Sanger sequencing *vs*., 347
  principles, 354
  protocols, 355f
  pyrosequencing technology, 348–349
  reversible terminator technology, 349
  small RNA sequencing, 357
  targeted re-sequencing, 356
  techniques, 338
  transcriptome sequencing, 356–357
  translational significance, 364
  whole genome *de novo* sequencing, 354–355
  whole genome re-sequencing, 355–356
  WWW resources, 364
Next-next generation sequencing. *See* Third generation sequencing
NF-kB. *See* Nuclear Factor-kB

NGF. *See* Nerve growth factor
NGS. *See* Next generation sequencing
NHGRI. *See* National Human Genome Research Institute
NHLBI. *See* National Heart, Lung, and Blood Institute
NIAID. *See* National Institute of Allergy and Infectious Diseases
Nickel, 60–61
nicotinic acetylcholine receptor (nAChR), 556
NIH. *See* National Institutes of Health
Nineteen allergenic proteins, 517
Nisonoff's Experiment, 268
Nitric oxide (NO·), 181–182
Nitrogen-containing organic bases, 462
Nitrotyrosine, 182
NK cells. *See* Natural killer cells
NMR. *See* Nuclear magnetic resonance
NNRTIs. *See* Non-Nucleoside Reverse Transcriptase Inhibitors
NOD. *See* Non-obese diabetics
Non-allergenic food proteins, 517
non-coding RNA (ncRNA), 308, 580, 582t
Non-cytolysis mechanisms, 499
Non-disjunction, 455
Non-Hodgkin's lymphoma, 97
Non-Human primate model, 26–28
Non-immune antibody libraries, 485
Non-LTR retrotransposons, 583
Non-maleficence, 600
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), 165
Non-obese diabetics (NOD), 81
Non-replicating vaccines, 497
Non-sense mutation, 464, 464f
Non-steroidal anti-inflammatory drugs (NSAIDs), 141–142
Non-synonymous mutation, 464
Non-viral delivery systems, 551
North-East Arctic cod (NEAC), 295
Northern Hemisphere, 542
Norwegian coastal cod (NCC), 295
Novel food proteins, 521
Novel protein, 516
NP. *See* Nanoparticles
NPC. *See* Nasopharyngeal Cancer
NRTIs. *See* Nucleoside Reverse Transcriptase Inhibitors
NSAIDs. *See* Non-steroidal anti-inflammatory drugs
NSCB. *See* U.S. National Stem Cell Bank
NSCs. *See* Neural stem cells
NSERC. *See* Natural Sciences and Engineering Research Council of Canada
NSOM. *See* Near–field Scanning Optical Microscopy
NT-3. *See* Neurotrophin-3
NtRTIs. *See* Nucleotide Reverse Transcriptase Inhibitors
Nuclear Factor-kB (NF-kB), 115
Nuclear magnetic resonance (NMR), 567–569
Nuclear reprogramming, 432
Nucleic acid sequences, 530
Nuclein, 462
Nucleocapsid (NA), 161
Nucleoside Reverse Transcriptase Inhibitors (NRTIs), 164
Nucleosome mapping, 64
Nucleotide Reverse Transcriptase Inhibitors (NtRTIs), 164
Nucleotide synthesis, 282
Numerical taxonomy. *See* Phenetics
Nuremberg Code, 610

O

o-phenylenediamine (OPD), 553
Ocean, 542
Octamer-binding transcription factor 4 (Oct-4), 433
Octopuses (*Octopus*), 544
ODD. *See* Ouchterlony's Double Diffusion
ODE. *See* Ordinary differential equations
OECD. *See* Organization for Economic Co-operation and Development
Oligonucleotides, 339, 412
ω-conotoxins, 556
OMIM. *See* Online Mendelian Inheritance in Man
Oncogenic viruses, 98, 98t
OncoMouse, 413
Online Mendelian Inheritance in Man (OMIM), 54, 582
$ONOO^-$. *See* Peroxynitrite
OPD. *See* o-phenylenediamine
Open reading frames (ORFs), 105, 125–126, 129, 463
Oral polio vaccine, 492
Ordinary differential equations (ODE), 394
ORFs. *See* Open reading frames
Organ cultures, 569–570
Organ transplantation, 415
Organization for Economic Co-operation and Development (OECD), 510–511
Organogenesis, 569–570
ori-lyt. *See* Lytic origin of replication
ori-P. *See* Plasmid DNA replication origin
Ornamental coral reef fishery, 549
Oropharyngeal cancer (OSCC), 126–127
Orthotopic Intra-Tibial Model, 87
Orthotopic mouse model
  of colorectal cancer, 85–86
  design and execution, 86–87
  interpretation of results, 87
OSCC. *See* Oropharyngeal cancer
Osteoporosis, 256–257
Ouchterlony's Double Diffusion (ODD), 275–277
Ovulated eggs, 547
Oxford Nanopore, 351–352
Oxygen, 218
Oxygen-centered radicals, 181
Oysters (*Crassostrea*), 543–544, 554

P

p&p. *See* pair peptide library
*p*53 tumor suppressor gene, 106
p75 neurotrophin receptor. *See* Low-Affinity Nerve Growth Factor Receptor (LNGFR)
Pacific biosciences, 350
PAGE. *See* Polyacrylamide gel electrophoresis
pair peptide library (p&p), 379–380
PAMPs. *See* Pathogen-Associated Molecular Patterns
Pancreas, 240
Pap smear test, 107
Papain digestion, 268
"Paper smear" method, 109
Papnet, 108
PAR4. *See* Protease activated receptor 4
Parkinson's disease (PD), 13–14, 40
Parkinson's Fly, 414
Pars intercerebralis (PI), 14–15
Partial differential equations (PDE), 394
Pathogen detection methods, 529f
Pathogen-Associated Molecular Patterns (PAMPs), 474
Pattern recognition receptors (PRRs), 474
PBMCs. *See* Peripheral blood mononuclear cell
PBPK. *See* Physiologically based rapid pharmacokinetic
PBS. *See* Phosphate buffered saline
PCP. *See* Pneumocystis carinii pneumonia
PCR. *See* Polymerase chain reaction
PCR ribotyping, 327–328
  ARDRA, 336–337
  broad-range, 337
  and endonuclease subtyping, 336
  followed by sequencing of ISR, 336
  limitations of, 337–338
  long, 337
  ribosomal DNA sequence analysis, 338
  terminal restriction fragment length polymorphism, 337
PCR-SSCP. *See* Polymerase chain reaction single-strand conformation polymorphism
PCs. *See* Plasma cells
PD. *See* Parkinson's disease; Pharmacodynamics; Photodetection
PDE. *See* Partial differential equations
PDGF. *See* Platelet-derived growth factor
PDI. *See* Protein disulfide isomerase
PDMS. *See* Polydimethylsiloxane
PDT. *See* Photodynamic therapy
Peanut allergens, 517–518
Pearl oysters (*Pinctada*), 543–544
PEG. *See* Polyethylene glycol
PEL. *See* Primary effusion lymphoma
Penaeid shrimps, 546–547
Penicillin, 370
Pentose-reducing sugars, 519
Peppermint tea, 187
Pepsin, 512–514
  concentration, 515
  digestion, 268
  enzymatic action, 514f
  mechanism, 515f
  resistance, 511
Pepsin-resistant protein-induced food allergies, 512, 513t
Peptide nucleic acid probes (PNA probes), 341
Peptides, 14–15
  aptamers, 369, 372
  for mRNA display, 377
  for phage display, 375
  for ribosome display, 376–377

Perfluorooctane sulfonate (PFOS), 60–61
Pericentric inversions, 458
Perifornical region (PF region), 320
Peripheral blood lymphocyte cultures, 459
Peripheral blood mononuclear cell (PBMCs), 134, 167
Peripheral nervous system (PNS), 172
Peroxisomes, 181
Peroxyl radicals (ROO·), 182
Peroxynitrite ($ONOO^-$), 182
Personal Genome Machine (PGM), 351
Personalized genomic sequencing, 590
Personalized medicine, 362–363, 466–467, 470
PF region. *See* Perifornical region
PFOS. *See* Perfluorooctane sulfonate
PG. *See* Pharmacogenomics
PGM. *See* Personal Genome Machine
pH, 218
Phage display, 373–375. *See also* Ribosome display
  antibody library, 483–484
  peptide selection, 375
  steps, 375
  technology, 483–485, 484f
Pharmaceutical industries, 552
Pharmaceutical Technology Journal, 229
Pharmaceuticals, 564
Pharmacodynamics (PD), 74, 88
Pharmacogenomics (PG), 74, 303, 466–467, 590
Pharmacokinetics (PK), 74, 88
PHEMA. *See* Poly (2-hydroxyethl methacrylate)
Phenetics, 328
Phenotype, 329–330, 463
Phenotypic typing methods, 330–331
Phosphatase and tensin homologue (PTEN), 13–14
Phosphatase and tensin homologue induced kinase 1 (PINK1), 13–14
Phosphate, 462
Phosphate buffered saline (PBS), 86, 147–148
Phosphinothricin acetyltransferase (PAT), 521
Phosphoric acid (H_3PO_4), 462
Photodetection (PD), 205
Photodynamic therapy (PDT), 205
  response to, 205
PHRED software package, 587–588
PHS. *See* Public Health Services
Phylum Arthropoda. *See* Arthropods
Phylum Chordata, 544
Physiological shock, 547
Physiologically based rapid pharmacokinetic (PBPK), 400
Phytochemical screening. *See* Chemical screening
PI. *See* Pars intercerebralis; Propidium Iodide
PIC. *See* Pre-integration complex
PINK1. *See* Phosphatase and tensin homologue induced kinase 1
piRNA. *See* piwi-interacting RNA
PIs. *See* Protease Inhibitors
piwi-interacting RNA (piRNA), 357
PK. *See* Pharmacokinetics
PKC. *See* Protein kinase C
PKCα. *See* Protein kinase Cα
Placental tissue, 431
Plants, 501
  cells, 570
  extracts, 567
  metabolites, 569
  plant-derived vaccine antigens, 501–502
  species, 565
Plasma cells (PCs), 493–494
Plasmid DNA replication origin (ori-P), 130
Plateau phase, 217
Platelet-derived growth factor (PDGF), 220
Platinum (Pt), 532
Pleiotropy, 304
PLGA. *See* Poly-lactide-co-glycolide acid
PLM. *See* Progressive library method
Pluripotent stem cells, 429, 431–432, 598
PML. *See* Progressive multifocal leukoencephalopathy
PMSG. *See* Pregnant mare's serum gonadotropin
PNA probes. *See* Peptide nucleic acid probes
Pneumocystis carinii pneumonia (PCP), 156
PNI-PAAm. *See* Poly-N-isopropyl acrylamide
PNS. *See* Peripheral nervous system
PoAb. *See* Polyclonal antibodies
Pol. *See* Polymerase
Polio virus, 492
Polony-based sequencing technology, 352
Poly (2-hydroxyethl methacrylate) (PHEMA), 201–202
Poly (glycolic acid), 237
Poly (lactic acid), 237
Poly (ortho esters), 237
Poly (α-hydroxy ester), 239
Poly (ε-caprolactones), 237, 239
Poly-lactide-co-glycolide acid (PLGA), 238, 550–551
Poly-N-isopropyl acrylamide (PNI-PAAm), 202
Poly-*N*-*p*-vinylbenzyl-D-lactonamide, 201–202
Poly-sialic acid (PSA), 435
Polyacrylamide gel electrophoresis (PAGE), 312–314
Polyclonal antibodies (PoAb), 265–266, 269–270, 270t, 475–476
  B-cells, 476
  crucial steps for, 278
  disadvantages, 478
  methodology, 277–278
  principle, 276
Polyclonals, 478
Polycomb-group proteins, 60
Polycyclic aromatic hydrocarbons, 98
Polydimethylsiloxane (PDMS), 202, 207
Polyethylene glycol (PEG), 202, 476–478
Polygenic disorders, 582
Polyglutamine diseases, 53
Polymer ceramic composite foam, 239
Polymerase (Pol), 161–162
Polymerase chain reaction (PCR), 298, 408, 467–468, 530–531, 554, 578
Polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP), 293
Polymorphisms, 290
Polyomavirus nephropathy (PVN), 128
Polyomaviruses, 128
Polyphenols, 185–188, 185f
Polyploid cells, 456
Polyploidy, 546–547, 546f
Polysialic Acid-Neural Cell Adhesion Molecule (PSA-NCAM), 435
Polystyrene (PS), 553
Polytene chromosomes, 8–9
  puffing on, 9f
  salivary gland of third instar larva, 8f
Polyunsaturated fatty acids (PUFA), 182–183
Polyvinylpyrrolidone (PVP), 205
Population genetics, 304
Populations screening, 467
Porogen, 238
Porter's experiment, 268
Position sensitive detector (PSD), 250
Positive selection, 479–480
Potable water, 526
POU5F1. *See* Octamer-binding transcription factor 4 (Oct-4)
PP. *See* Precautionary principle
PPIS. *See* Protein–protein interactions
PPMS. *See* Primary progressive MS
PPs. *See* Processed pseudogenes
Pre-and post-natal diagnosis, 362
Pre-cancer classification, 102–103
Pre-integration complex (PIC), 162
pre-miRNA. *See* Precursor miRNA
Precautionary principle (PP), 599, 601, 607
Precursor miRNA (pre-miRNA), 581
Prefix, 271–272
Pregnant mare's serum gonadotropin (PMSG), 46
Pressure-assisted micro syringe method, 239–240
Primary effusion lymphoma (PEL), 125–126
Primary library construction, 378
Primary progressive MS (PPMS), 145
Prion protein (PrP), 257
Pristane, 487
Pro-oxidative enzymes, 182–183
Pro-viral DNA, 163–164
Processed pseudogenes (PPs), 584–585
Progressive library method (PLM), 378
  ASAC and selection, 378–379
  method for *in vitro* evolution, 379f
  p&p, 379–380
  YLBS and selection, 378
Progressive multifocal leukoencephalopathy (PML), 128
Prokaryotic protein-coding genes, 579
Proof-of-concept studies, 533
Propidium Iodide (PI), 440
Proportionality, 607
Prostate cancer, 63
Prostate-specific antigen (PSA), 376–377
Protease activated receptor 4 (PAR4), 62
Protease Inhibitors (PIs), 165
Protein disulfide isomerase (PDI), 166–167
Protein kinase C (PKC), 556
Protein kinase Cα (PKCα), 375
Protein post-translation modifications (PTM), 225
Protein quantitation, 312–314
  in brain tissue micropunches, 314f
  detection chemistry, 314–316
  dot blot technique, 317
  neutral red-stained section, 315f
  Western blot technique, 317

Protein tyrosine kinases (PTKs), 132
Protein-coding genes, 579
Protein-free media, 221
Protein–protein interactions (PPIS), 388, 396
Proteins, 511–512
  conformation, 520–521
  oxidation, 183–184, 183f
Proto-oncogenes, 98
PrP. *See* Prion protein
PRRs. *See* Pattern recognition receptors
Prugosenes, 555
PS. *See* Polystyrene
PSA. *See* Poly-sialic acid; Prostate-specific antigen
PSA-NCAM. *See* Polysialic Acid-Neural Cell Adhesion Molecule
PSD. *See* Position sensitive detector
Pseudogenes, 584–585
PTEN. *See* Phosphatase and tensin homologue
PTKs. *See* Protein tyrosine kinases
PTM. *See* Protein post-translation modifications
Public Health Services (PHS), 67, 606–607
PubMed, 150
PUFA. *See* Polyunsaturated fatty acids
Purified antigens, 530
Puromycin, 377
PVN. *See* Polyomavirus nephropathy
PVP. *See* Polyvinylpyrrolidone
Pyrazinamide (PZA), 25
Pyrolysis mass spectrophotometry, 330
Pyrosequencing technology, 65–66, 348–349
PZA. *See* Pyrazinamide

Q

Q-bands, 452–453
QIAquick Gel Extraction Kit (QIAGEN), 317
Quadroma technology, 486
Quantitative fluorescent PCR (QF PCR), 468
Quantitative methylation specific polymerase chain reaction (QMSP), 64
quantitative PCR (qPCR), 530–533
Quantitative structure–activity relationship (QSAR), 400
Quantitative structure–property relationship (QSPR), 400
Quantitative trait loci (QTL), 299
Quantum dots (QDs), 536
Quinacrine mustard (QM), 452–453

R

R-promoter (Rp), 130
RA. *See* Rheumatoid arthritis
Rabbit model, 26
Rabbit papillomavirus (RPV), 100–101
Radial immunodiffusion (RID), 275–277
Radio–immunotherapy, 274–275
Radioisotopes, 481
Radiolabeled monoclonals, 481
Raman imaging (RI), 253, 254f
Raman shift, 253
Raman spectroscopy (RS), 253
Random amplified polymorphic DNA (RAPD), 289–290, 293–294, 331, 530
RAPD. *See* Random amplified polymorphic DNA
Rapid culture-based methods, 530
RARB. *See* Retinoic acid receptor beta
Rat Genome Database (RGD), 401
Rationality, 600
RDA. *See* Representational differential analysis
rDNA, stable isotope-labeled probing, 340–341
rDNA. *See* recombinant DNA
Reaction–diffusion equation, 390
Reactive nitrogen species (RNS), 183
Reactive oxygen species (ROS), 180, 181t
Real-time (RT), 222
Real-Time PCR. *See* Quantitative PCR (qPCR)
Recognition of Individual Gene (RING), 340
recombinant DNA (rDNA), 605
Recombinant human ErbB-2 tyrosine kinase receptor, 375
Recombinant therapeutic proteins
  antibodies, 227
  blood coagulation factors, 227
  cytokines, 225
  groups, 225–227
  growth factors, 226
  hormones, 226
  PTM, 225
  recombinant α-galactosidase A, 226–227
  therapeutic enzymes, 226
Recombinant vector vaccines, 500–501
Red light, 533
Reduction, 53
Reduction, Refinement, and Replacement (3Rs), 53
Refinement, 53
Regulator of Expression of Viral Proteins (Rev), 162
Relapse remitting MS (RRMS), 145
Religious critique
  hESCs, 603
  human reproductive cloning, 603–604
  IVF, 604
Religious intrinsic critique
  human reproductive cloning, 603–604
  human stem cell research, 603
  in vitro fertilization, 604
    public perceptions, 604
    secular intrinsic objections to biotechnology, 604
Replacement, 53
Replication, 463
Replicative cell senescence, 429
Representational differential analysis (RDA), 65–66
Reserpine transporter inhibitors, 435–436
Response evaluation to immunotherapy, 206–207
Response to anticancer drugs, 204–205
Restriction endonuclease selection, 333–335
Restriction enzymes, 292, 304
Restriction fragment length polymorphism (RFLP), 291, 331, 530, 586
  applications of, 292
  steps in analysis, 291–292, 292f
Restriction landmark genome scanning (RLGS), 65, 294
Retention time (RT), 569
Retinoic acid receptor beta (RARB), 293
Retract cycle, 251
Retrovirus-Mediated Gene Transfer, 411–412, 412f
Rev. *See* Regulator of Expression of Viral Proteins
Reverse engineering, 388
Reverse transcriptase (RT), 160, 162–163, 309
Reverse transcriptase-polymerase chain reaction (RT-PCR), 310–311
  mRNA level quantitation, 310–312
  single-cell, 318–319
Reversible terminator technology, 349
RFLP. *See* Restriction fragment length polymorphism
RGD. *See* Rat Genome Database
Rheumatoid arthritis (RA), 139–140
  clinical manifestations, 141
  epidemiology and etiology, 140
  experimental models
    induced models, 142–143
    spontaneous models, 142
  methodology and protocols, 143
    CIA clinical assessment, 143–144, 144t
    CIA induction, 143, 143f
    histological assessment, 144, 144f, 144t
    radiographic evaluation, 144, 144t
  pathogenesis, 140–141
  treatment, 141–142
RI. *See* Raman imaging
Ribonucleic acid (RNA), 331
Ribosomal genes, 581
Ribosomal operon organization, 331–332, 332f
ribosomal RNAs (rRNAs), 581
Ribosome, 331
  moieties, 376–377
Ribosome display, 376
  peptide selection, 376–377
  steps, 376
Ribotyping, 327–328, 332
  automated, 335
  conventional, 333, 334f
  ethical issues, 342
  future perspectives, 341–342
  in situ hybridization, 338–341, 339f
    CARD Fish, 340
    clone-FISH technique, 339
    PNA probes, 341
    RING FISH, 340
    stable isotope-labeled probing, 340–341
  ISR length heterogeneity, 335f
  limitations of, 341
  PCR ribotyping, 335–338
    ARDRA, 336–337
    broad-range, 337
    and endonuclease subtyping, 336
    followed by sequencing of ISR, 336
    limitations of, 337–338
    long, 337
    ribosomal DNA sequence analysis, 338
    terminal restriction fragment length polymorphism, 337
  restriction endonuclease selection, 333–335
  techniques of, 332
  translational significance, 342
  WWW resources, 342
RID. *See* Radial immunodiffusion

Rifapentine, 25–26
RING. *See* Recognition of Individual Gene
Ring chromosome, 457, 457f
RISC. *See* RNA-induced silencing complex
Rituximab, 480
RLGS. *See* Restriction landmark genome scanning
RNA. *See* Ribonucleic acid
RNA aptamer-based drug, 371–372
RNA codons, 463
RNA Interference (RNAi), 412
RNA recognition motifs (RRM), 43
RNA-induced silencing complex (RISC), 412, 581
RNAi. *See* RNA Interference
RNS. *See* Reactive nitrogen species
RO·. *See* Alkoxyl radicals
Roadmap Epigenomics Program, 68
Roche-454 GS FLX, 348
Roche/454 FLX pyrosequencer, 586
ROO·. *See* Peroxyl radicals
ROOH. *See* Alkyl peroxides
ROS. *See* Reactive oxygen species
Rosehip tea, 187
Rotatory Flask Methods, 202
Rp. *See* R-promoter
RPV. *See* Rabbit papillomavirus
RRM. *See* RNA recognition motifs
RRMS. *See* Relapse remitting MS
rRNA
  genes, 579
  stable isotope-labeled probing, 340–341
  as taxonomic tools, 331
rRNAs. *See* ribosomal RNAs
RS. *See* Raman spectroscopy
RT. *See* Real-time; Retention time; Reverse transcriptase
RT-PCR. *See* Reverse transcriptase-polymerase chain reaction

S

Safety assessment approaches, 511
SAHA. *See* Suberoylanilide hydroxamic acid
Salmonella, 528
Salvage therapy, 165
Sanger's method, 578
SAP. *See* SLAM-associated protein
Sarcoma, 97
SARS. *See* Severe acute respiratory syndrome
SBMA. *See* Spinal and bulbar muscular atrophy
SC. *See* Synthesized natural coumarin
Scaffold design, 235–237
Scaffold fabrication methods, 237–238, 238t
Scaffolds, 235
Scaffolds materials, 237t
Scallops (*Pecten*), 543–544
Scanning Electron Microscopy (SEM), 249
Scanning Probe Microscopy (SPM), 248–249, 256
  AFM, 250
  animal biotechnology techniques, 250
  CFM, 251
  FluidFM, 252
  NSOM, 252–253
Scanning Tunneling Microscope (STM), 248–249
SCD. *See* Spinocerebellar degeneration
scFv. *See* single chain variable fragment
SCID. *See* Severely compromised immunodeficient
SCJ. *See* Squamocolumnar junction
Sclerose en plaques, 144
SCNT. *See* Somatic cell nuclear transfer
Screening, 109
Screening toxins, 444
Scyphozoan jellyfish stings, 555
SDS. *See* Sodium dodecyl sulfate
SE. *See* Standard error
Sea cucumbers, 544
Sea jellies. *See* Jellyfish
Sea snake, 555–556
Sea stars, 544
Sea urchins, 544, 556–557
Second generation sequencing. *See* Next generation sequencing (NGS)
Secondary library construction, 378–379
Secondary progressive MS (SPMS), 145
Segmental duplications, 585
Selective estrogen receptor modulators (SERMs), 78
Selective LASER sintering, 239
SELEX. *See* Systematic Evolution of Ligands by Exponential Enrichment
Self-renewal assay, 436
SEM. *See* Scanning Electron Microscopy
Semi-conservative method, 463
Semiconductor quantum dots, 531
Sense strand, 463
Sepia, 555
Sequence assembly, 354–355
Sequence tagged site (STS), 293, 586
Sequence-tagged microsatellites (STMS), 298
SERMs. *See* Selective estrogen receptor modulators
Seroconversion, 159
SERS. *See* Surface–Enhanced Raman Spectroscopy
Serum, 220
  advantages of, 220–221
  components, 220t
  disadvantages of, 221
  sickness, 272–273
Serum-free media, 221
Severe acute respiratory syndrome (SARS), 13
Severely compromised immunodeficient (SCID), 82
Sex chromosomes, 453–454
Sex reversal, 548–549
Sex-linked disorders, 465–466
SFC. *See* Sphere-forming cell
SGF assay. *See* Simulated gastric fluid assay
"sham" reverse transcription reaction, 317
Sheep Red Blood Cells (SRBC), 277, 282
Short Interspersed Nuclear Elements (SINE), 58–61, 584
Short tandem repeats (STRs), 297
Shrimp, 554
shRNA. *See* small hairpin RNA
Sialomucin. *See* CD34 marker
Sickle-cell anemia, 370
Side Population (SP), 434–436
SIF assay. *See* Simulated intestinal fluid assay
Signal Transducer and Activator of Transcription (STAT), 115
Signature labels, 327–328
Signature sequence, 333
SIL. *See* Squamous intraepithelial lesion
Silver-Russell Syndrome (SRS), 60
Simian viral immunodeficiency (SIV), 26–28
Simple sequence length polymorphisms (SSLP), 298, 586
Simple sequence repeats (SSR), 289–290, 297, 585
Simple sequence tandem repeats (SSTR), 297
Simulated gastric fluid assay (SGF assay), 512
  components, 514–515
  correlation between, 512
  factors relevant to GI digestion, 515–516
  protocol, 515, 515f, 516t
  supportive and negative evidence, 516
  works, 512–514
Simulated intestinal fluid assay (SIF assay), 516. *See also* Thermal treatment assay
  assay conditions effect, 517
  contradictory results, 517–518
  enzyme/test protein ratios, 518t
  protocol of, 516–517, 517f
  supportive and negative evidences, 517
SINE. *See* Short Interspersed Nuclear Elements
single chain variable fragment (scFv), 473, 484–485
Single Molecule Real-Time Sequencing (SMRT), 350
Single nucleotide polymorphisms (SNPs), 61, 294–295, 360, 469, 589
Single-cell RT-PCR, 318
  brain cells labeling, 318
  cell dissociation, 318
  cell harvesting and, 318–319
Single-gene disorders, 465–466
Single-nucleotide polymorphism (SNP), 356
Single-strand conformation polymorphism (SSCP), 293
single-stranded DNA (ssDNA), 533–534
Singlet oxygen, 182
SIP. *See* Stable Isotope Probing
siRNAs. *See* small interfering RNAs
SIRS. *See* Systemic inflammatory response syndrome
Sister-chromatids, 453
SIV. *See* Simian viral immunodeficiency
16S ribosomal RNA (16S rRNA), 530
  pseudomonads, 341
  terminal restriction fragment length polymorphism, 337
16S ribotype, 333
Skeletal stem cells, 241
Skin, 240
SKY. *See* Spectral karyotyping
SLAM-associated protein (SAP), 133–134
Slow-cycling population assay, 436

SMA. *See* Spinal muscular atrophy
small hairpin RNA (shRNA), 412
small interfering RNAs (siRNAs), 114–115, 357, 412
small nuclear ribonucleoprotein (snRNP), 44
Small nuclear RNAs, 581
Small Nucleolar RNA (snoRNAs), 581
Small RNA sequencing, 357
Small tandem repeat (STR), 468
Smithburn neurotropic strain (SNS), 224
SMN. *See* Survival of motor neuron
SMRT. *See* Single Molecule Real-Time Sequencing
Snake venoms, 556
snoRNAs. *See* Small Nucleolar RNA
SNP. *See* Single-nucleotide polymorphism
SNPs. *See* Single nucleotide polymorphisms
snRNAs. *See* Spliceosomal Small Nuclear RNAs
snRNP. *See* small nuclear ribonucleoprotein
SNS. *See* Smithburn neurotropic strain
Social justice, 607
Social utility, 606
SOD1. *See* Superoxide dismutase 1
$SOD1^{G37R}$ transgenic mice, 47–48, 48f
$SOD1^{G93A}$ transgenic mice, 48
$SOD1^{WT}$ transgenic mice, 49
Sodium bisulfate treatment, 64
Sodium butyrate (NaBU), 61–62
Sodium dodecyl sulfate (SDS), 317
Software packages, 578
Solid free form techniques, 239
Solvent casting, 238
Somatic cell nuclear transfer (SCNT), 437, 440–441
Somatic genetic disorders, acquired, 466
Sorting. *See* Biopanning
Southern blotting, 467–468
SP. *See* Side Population
Species-specific signature band, 334–335
Spectral karyotyping (SKY), 460–461, 461f
Speculoscopy, 107
Sperm morphology, 257–258
Sphere-forming cell (SFC), 115
Spheres, 436
Spheroids, 235
Spinal and bulbar muscular atrophy (SBMA), 40
  abnormal androgen receptor protein, 43
  androgen hormone and mutant AR receptor, 53
  AR-97Q and AR-24Q transgenic mice, 49, 49f
  AR-97Q mice, 52–53
  genetics of, 44
  X-linked recessive inheritance, 42–43
Spinal muscular atrophy (SMA), 40
  Cre-loxP systems, 52
  genetics, 44, 45f
  human SMN2 transgenic mice, 52
  severe SMA mice, 49
  SMA Type II mice, 49
  SMN, 41–42
  type 1, 2, and 3, 42
Spinner flask method, 202
Spinocerebellar degeneration (SCD), 40
Splenocytes preparation, 280
Spliceosomal Small Nuclear RNAs (snRNAs), 581
SPM. *See* Scanning Probe Microscopy
SPMS. *See* Secondary progressive MS
Spontaneous tumor models
  carcinogens, 78, 78f
  mouse-inbred strains, 78
SPR. *See* Surface Plasmon Resonance
Squamocolumnar junction (SCJ), 99–100
Squamous cell carcinoma, 102
Squamous intraepithelial lesion (SIL), 100, 103
Squid ink, 555
Squids (*Loligo*), 544, 555
SRBC. *See* Sheep Red Blood Cells
SRS. *See* Silver-Russell Syndrome
SSCP. *See* Single-strand conformation polymorphism
ssDNA. *See* single-stranded DNA
SSEA. *See* Stage-Specific Embryonic Antigen
SSLP. *See* Simple sequence length polymorphisms
SSR. *See* Simple sequence repeats
SSTR. *See* Simple sequence tandem repeats
Stable Isotope Probing (SIP), 340–341
Stable isotope-labeled probing, 340–341
Stage-Specific Embryonic Antigen (SSEA), 434
Standard error (SE), 84–85
STAT. *See* Signal Transducer and Activator of Transcription
Statins, 370
Stem cell(s), 228, 241, 426–427, 431f
  banking, 445
    biological resource, 445
    cord blood, 445
    in India, 445
    life-threatening diseases, 445
    scope and future possibilities, 445
    treatment, 445
  banking, 445
  in cell-based regenerative therapies, 443
  classes, 428t
  culturing embryonic, 228–229
  differentiation potential, 428–429
  embryos, 430
  features, 428
  fetal tissue, 430
  Hoechst 33342 or DCV staining, 440
  immortality, 429
  laboratory studies, 427
  methods, 433–436
  new medicines, 443
  niche, 436–437
  plasticity, 429
  potential uses, 443–444, 444f
  principles, 442
  properties, 428f
  regulatory framework, 446t
  religion and social issues, 440
    Buddhism, 441–442
    Greek Orthodox and Roman Catholic Churches, 441
    Hinduism, 441–442
    Islamic countries, 441
    Judaism, 441
    Protestant Churches, 441
  research, 426, 443f
  therapy, 442
  translational significance, 442
  WWW resources, 445–446
Step-by-step approach, 509–510
Stereolithography, 239
Stirring, 520–521
STM. *See* Scanning Tunneling Microscope
STMS. *See* Sequence-tagged microsatellites
Stone formation, 15
Stop codons, 463
STR. *See* Small tandem repeat
STRO-1 marker, 435
STRs. *See* Short tandem repeats
STS. *See* Sequence tagged site
Suberoylanilide hydroxamic acid (SAHA), 67–68
Sublimation, 240
Submetacentric chromosome, 453
Subphylum Crustacea. *See* Crustaceans
Substrate methods, 529
Subunit vaccines, 497–498, 497f
Superfish, 408, 416
Supermouse, 414
Superoxide Anion ($\cdot O_2^-$), 182
Superoxide dismutase 1 (SOD1), 43, 182
Superpig, 415–416
Surface charge properties, 533
Surface Modification Based Methods, 202
Surface Plasmon Resonance (SPR), 531, 533
Surface water, 526
Surface–Enhanced Raman Spectroscopy (SERS), 253–254
Survival of motor neuron (SMN), 41–42
Symmetric stem cell division, 430f
Synonymous mutation, 464
Synovial pannus, 141
Synthesized natural coumarin (SC), 257
Synthetic polymers, 237
Synthetic systematics, 328
System-based theories, 180
Systematic Evolution of Ligands by Exponential Enrichment (SELEX), 371–372
Systemic inflammatory response syndrome (SIRS), 394
Systems biology, 387
  bioinformatics and, 387
  computational methods in, 387–388
  experimental methods in, 388
  integrative approach, 390

T

T cell receptor complex (TCR), 493–494
T-helper cells, 163
Tailoring care issue, 370
TAM. *See* Tumor-associated macrophages
TAR-DNA binding protein 43 (TDP-43), 43
Target identification, 370–371
Targeted re-sequencing, 356
Tat. *See* Transactivator of Transcription
Taxon, 304

Taxonomic profiling, 328, 338
TB. *See* Tuberculosis
TBS. *See* Tris-buffered saline
TC. *See* Total coliforms
TCA. *See* Tricloroacetic acid
TCR. *See* T cell receptor complex
TD. *See* Transposon Display
TDM. *See* Trehalosedimycolate
TDP-43. *See* TAR-DNA binding protein 43
Tea, 185–186
  catechins, 187, 187f
  health benefits, 187
Telocentric chromosomes, 453
Telomeres, 453
Temperature, 218
Template genome (TG), 388
Template network (TN), 388
Template-based transcriptional control network reconstruction method, 388
Teratocarcinomas, 438
Terminal repeat (TR), 129
Terminal restriction fragment length polymorphism, 337
TERS. *See* Tip–Enhanced Raman Spectroscopy
Tetrachloroauric acid ($HAuCl_4$), 533–534
Tetradecanoyl phorbol acetate (TPA), 98–99, 134
Tetramethyl benzedine (TMB), 553
TG. *See* Template genome
TGF. *See* Transforming growth factor
TGF-B. *See* Transforming growth factor beta
The Institute of Genomic Research (TIGR), 346
Therapeutic nucleic acids (TNAs), 114–115
Thermal treatment assay, 518. *See also* Simulated gastric fluid assay (SGF assay)
  contradictory results in, 521
  Maillard reaction and relation, 519f
  mechanism of, 518–519
  protein allergenicity, 521f
  proteins functional stability, 520–521
  standard protocol for, 519–520, 520f
Thin Prep Pap Test, 107
Thin-layer chromatography (TLC), 567–568
Third generation sequencing technologies, 347. *See also* 4th generation sequencing technologies
  ion semiconductor sequencing, 351
  SMRT, 350
  tSMS, 350–351
Third library construction, 379–380
1000 Genomes Project, 590
3D printing, 239
3Rs. *See* Reduction, Refinement, and Replacement
Thymine, 462
TIGR. *See* The Institute of Genomic Research
Tip–Enhanced Raman Spectroscopy (TERS), 253–254
Tirapazamine (TPZ), 199
Tissue culture, 570–571
Tissue engineering, 233, 236f
  bioreactors, 235
  cells, 234–235
  ethical issues, 242
  issues and challenges, 241
  media, 235
  methodology, 235
  scaffold, 235
    design, 235–237
    materials, 237t
    scaffold fabrication methods, 237–238, 238t
  using stem cells, 241
  tissue-engineered organs, 240–241
  translational significance, 242
  WWW resources, 242
tissue plasminogen activator (tPA), 226
Tissue replacement, damaged, 444
Titanium (Ti), 532
*TKTL*1. *See* Transkelolase-like 1
TLC. *See* Thin-layer chromatography
TLS protein. *See* Translocated in liposarcoma protein
5-TM. *See* 5-transmembrane
TMB. *See* Tetramethyl benzedine
TN. *See* Template network
TNAs. *See* Therapeutic nucleic acids
TNF. *See* Tumor necrosis factor
TNFR. *See* Tumor necrosis factor receptor
TNM. *See* Tumor, node, metastases
Top–down approach, 249–250
Total coliforms (TC), 527
Totipotent stem cells, 428–429, 431–432
Toxoid vaccines, 496–497, 496f
TPA. *See* Tetradecanoyl phorbol acetate
tPA. *See* tissue plasminogen activator
TPZ. *See* Tirapazamine
TR. *See* Terminal repeat; Translational research
Traditional medicine, 564–565
Traditional vaccines, 493
  inactivated whole virus vaccines, 495–496, 495f
  live, attenuated vaccines, 493–495
TRAMP. *See* Transgenic adenoma of mouse prostate
Trans-differentiation, 429–430
Transactivation domain. *See* DNA-binding domain
Transactivator of Transcription (Tat), 162
Transcortin, 479
Transcriptome sequencing, 356–357
transfer RNAs (tRNAs), 581
Transforming growth factor (TGF), 226
Transforming growth factor beta (TGF-B), 220
Transgene construction, 409, 410f
Transgenic adenoma of mouse prostate (TRAMP), 63
Transgenic animals, 407
  animal's physiological processes, 408
  biological models, 414–415
  categories, 408
  creation, 409–413
  disease models, 413–414
  drug and industrial production, 416–417, 417t
  environmental impact, 417–418
  ES cell manipulation, 408
  ethical issues, 419–420
  FDA guidelines, 420
  food sources, 415–416
  novel gene combinations, 408
  patenting transgenic animals, 418–419
  production, 409
  recombinant DNA, 408
  regulatory authorities, 409
  translational significance, 420
  WWW resources, 420
  xenotransplanters, 415
Transgenic Atlantic salmon, 607
Transgenic crops, 520
Transgenic fish, 544
Transgenic fish technology, 545
Transgenic mice generation, 45, 45f
  founder mice screening, 46
  harvesting donor eggs, 46
  microinjected egg implantation, 46
  preparation and purification, 45–46
  stable transgenic line establishment, 46
  transgene microinjection, 46
Transgenic positives screening, 412–413
Transgenic salmon, 418
Transgenic sheep, 416–417
Transgenic technology, 607
Transgenics, 361
Transkelolase-like 1 (*TKTL*1), 62
Translation process, 463
Translational research (TR), 471, 610
Translocated in liposarcoma protein (TLS protein), 43–44. *See* Fused in sarcoma (FUS)
Translocation, 456–457
5-transmembrane (5-TM), 434
Transmissible spongioform encephalopathies (TSEs), 609
Transpharmers, 408
Transposable element-derived repeats, 583
Transposon Display (TD), 296
Trastuzumab™. *See* Herceptin
Trehalosedimycolate (TDM), 142
Triad of infectious diseases, 394–395
Tricloroacetic acid (TCA), 183
Triploid fish, 546
Tris-buffered saline (TBS), 317
Trisomy, 455
tRNAs. *See* transfer RNAs
Trojan Horse Hypothesis, 172
true Single Molecule Sequencing (tSMS), 350–351
TSEs. *See* Transmissible spongioform encephalopathies
tSMS. *See* true Single Molecule Sequencing
Tuberculosis (TB), 22
  animal models, 22, 24–25, 24t
  comparative pathology in humans and animals, 22
  crossing species barriers, 22–23
  ethical issues, 28–30
  experimental models, 28t
  host diversity, 23–24
  immunological life cycle stages, 27f
  pathogenesis in animal species, 23t
  re-exposure to, 22

translational significance, 30
WWW resources, 30
Tumor, node, metastases (TNM), 97
Tumor grading, 97
Tumor monitoring, 84
Tumor necrosis factor (TNF), 140, 435
Tumor necrosis factor receptor (TNFR), 132
Tumor suppressor genes, 98
Tumor-associated macrophages (TAM), 206
Tumorigenesis, 57, 74
Twin zygosity, 299
Ty21a. *See* Live typhoid vaccine
Tysabri®. *See* Natalizumab

U

U-rich small nuclear RNA (U snRNA), 44
U.S. Department of Agriculture (USDA), 420
U.S. Food and Drug Administration (FDA), 107, 157, 229, 407–408, 606
U.S. National Stem Cell Bank (NSCB), 445
Ubiquitin carboxy-terminal hydrolase L1 (UCHL1), 13–14
Ubx. *See* Ultrabithorax
UCBSC. *See* Umbilical Cord Blood Stem Cell
UCHL1. *See* Ubiquitin carboxy-terminal hydrolase L1
UFAW. *See* Universities Federation for Animal Welfare
Ultrabithorax (Ubx), 6–7
Ultraviolet radiation (UV radiation), 99
Umbilical Cord Blood Stem Cell (UCBSC), 431
Uncharacterized extract, 565
UNESCO. *See* United Nations Educational, Scientific, and Cultural Organization
Unipotent stem cells, 429
United Nations Educational, Scientific, and Cultural Organization (UNESCO), 611
United States Adopted Names Council (USANC), 271
United States Department of Agriculture (USDA), 284
United States Department of Energy (DOE), 578
United States Environmental Protection Agency (USEPA), 526
United States Food and Drug Administration (USFDA), 510
Universities Federation for Animal Welfare (UFAW), 301
3′-untranslated regions (3′-UTRs), 581
Upper motor neurons, 40
Upstream Regulatory Region (URR), 105, 115
Urine-based non-invasive HPV DNA detection method, 108–109
URR. *See* Upstream Regulatory Region
US National Cancer Institute (NCI), 90
USANC. *See* United States Adopted Names Council
USDA. *See* U. S. Department of Agriculture; United States Department of Agriculture
USEPA. *See* United States Environmental Protection Agency
USFDA. *See* United States Food and Drug Administration
Utilitarian ethics, 597
3′-UTRs. *See* 3′-untranslated regions
UV radiation. *See* Ultraviolet radiation

V

Vaccination, 493
Vaccine Information Statement (VIS), 505
Vaccines, 492
access issues, 506
adjuvants, 502–503
challenges
foremost infectious disease problems, 504
infectious disease threats, 504–505
vaccination methods, 504
conjugate vaccines, 498, 498f
DNA vaccines, 498–500
ethical issues, 505
foreign pathogenic bacteria or viruses, 493
infectious agents, 504t
informed consent, 505
ISCOMs, 503
key milestones, 492
mandates, 505
molecular farming using plants, 501–502, 501f
plant-derived vaccines, 502f
properties of, 493f
protocol for development, 503–504
recombinant vector vaccines, 500–501
research and testing, 505
subunit vaccines, 497–498, 497f
toxoid vaccines, 496–497, 496f
traditional vaccines, 493
inactivated whole virus vaccines, 495–496
live, attenuated vaccines, 493–495
translational significance, 506
WWW resources, 506
Vaccinia virus (VACV), 256
VACV. *See* Vaccinia virus
Variable number tandem repeats (VNTR), 297–298
Variant Creutzfeldt–Jakob disease (vCJD), 609
Variolation technique, 491–492
Vascular endothelial growth factor (VEGF), 88, 205, 227
VBNC. *See* Viable but non-culturable
vCJD. *See* Variant Creutzfeldt–Jakob disease
VEGF. *See* Vascular endothelial growth factor
Ventrolateral preoptic (VLPO), 320
Very Late Antigen-4 (VLA-4), 146
vGPCR. *See* viral G-protein-coupled receptor
VHL. *See* Von Hippel–Lindau
VIA. *See* Visual inspection with acetic acid
Viable but non-culturable (VBNC), 530
Vibrio cholerae (*V. cholerae*), 528
Vibrio parahaemolyticus, 528
Vif. *See* Viral Infectivity Factor
VILI. *See* Visual inspection with Lugol's iodine
Vinegar-loving fly. *See* Drosophila
ViPR. *See* Virus Pathogen Database and Analysis Resource
viral G-protein-coupled receptor (vGPCR), 134–135
Viral infections, 495
Viral Infectivity Factor (Vif), 162
Viral mutant formation, 228
Viral oncoproteins, 124t
Viral Protein R (Vpr), 162
Viral Protein U (Vpu), 162
Virtue ethics, 599–600
Virus Pathogen Database and Analysis Resource (ViPR), 401
Virus-like-particles (VLPs), 112
advantage, 224
HPV vaccine, 224
Mammalian and Baculo cell lines, 225t
production, 224
vaccines based, 224
VIS. *See* Vaccine Information Statement
Visual inspection with acetic acid (VIA), 107
Visual inspection with Lugol's iodine (VILI), 107
VLA-4. *See* Very Late Antigen-4
VLPO. *See* Ventrolateral preoptic
VLPs. *See* Virus-like-particles
VNTR. *See* Variable number tandem repeats
Von Hippel–Lindau (VHL), 122–123
von Willebrand factor (VWF), 556
Vpr. *See* Viral Protein R
Vpr-binding protein, 161
Vpu. *See* Viral Protein U
VWF. *See* von Willebrand factor

W

Water quality, 526
Water-borne pathogens detection, 527–528
Waterborne disease, 526
Weight-of-evidence approach, 520
Wellcome Trust Sanger Institute, 401
Werdnig-Hoffmann syndrome, 41–42
Western blot technique, 316–317, 553
Wet spinning, 237–238
WGA. *See* Whole genome amplification
White spot syndrome virus (WSSV), 554
WHO. *See* World Health Organization
Whole cell metabolism, 391–392
Whole genome amplification (WGA), 66
Whole genome *de novo* sequencing, 354–355
Whole genome re-sequencing, 355–356
Whole Genome Shotgun Sequencing, 588, 588f
Whole inactivated bacterial vaccines, 495
Whole-cell pertusis vaccine, 506
Whole-pathogen vaccines, 495
Wikepedia, 69
WMA. *See* World Medical Association
World Health Organization (WHO), 14
classification, 102
consultation committee, 511
EBV infection and tumorigenesis, 135
genetics, 470
HIV infection, 158
immunization programs, 493
MoAb, 271
potable water, 526
traditional medicine, 564–565
World Medical Association (WMA), 610
World Wide Web (WWW), 69
WSSV. *See* White spot syndrome virus

X

X chromosome, 579
X-linked agammaglobulinemia (XLA), 130–131
X-linked lymphoproliferative disease (XLP disease), 133–134, 588–589
XDR. *See* Extensively drug resistant
Xenograft model
  design and execution, 87–88
  interpretation of results, 88
  of prostate cancer metastasis, 87
Xenograft transplants
  chemoendocrine therapy, 82–83
  herceptin, 83
  human tumor cells, 82
  subcutaneous prostate tumor xenografts, 82
  tumor-medium mixture, 82
Xenotransplanters, 415

Y

Y-ligation-based block shuffling (YLBS), 379–380
Yellow fluorescent protein derivatives (YFP derivatives), 557–558
Youth Mouse, 414–415

Z

ZEBRA response element (ZRE), 130
Zero Mode Waveguide (ZMW), 350
Zygote, 431–432

Color Plates

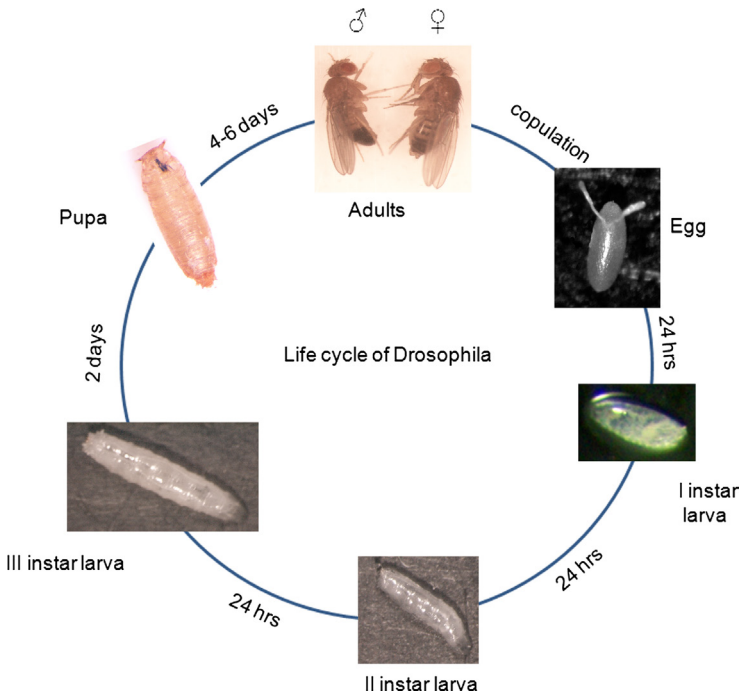


FIGURE 1.1 Life cycle stages of *Drosophila melanogaster*.

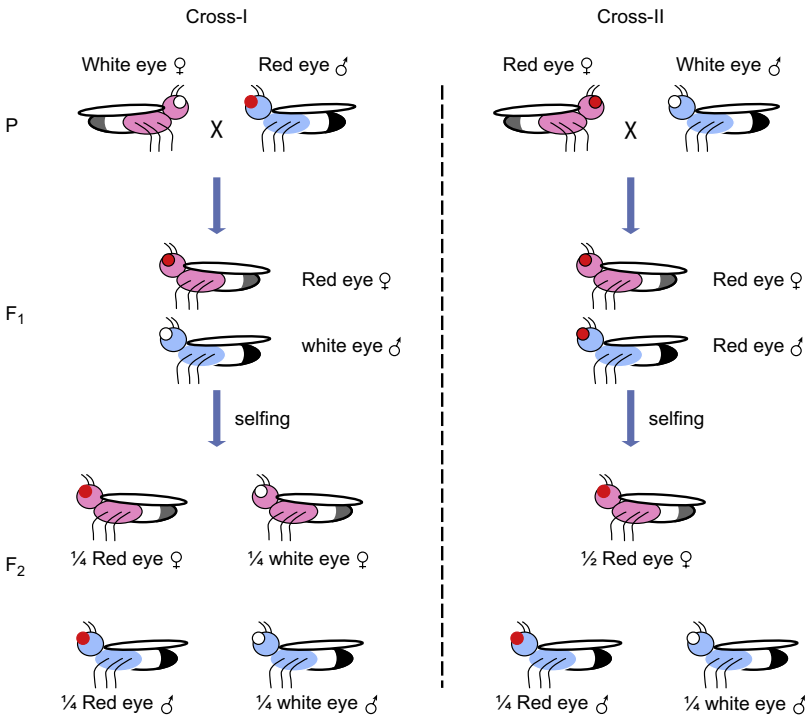


FIGURE 1.2 Schematic depiction of classes of genes associated with pattern formation in *Drosophila melanogaster*.

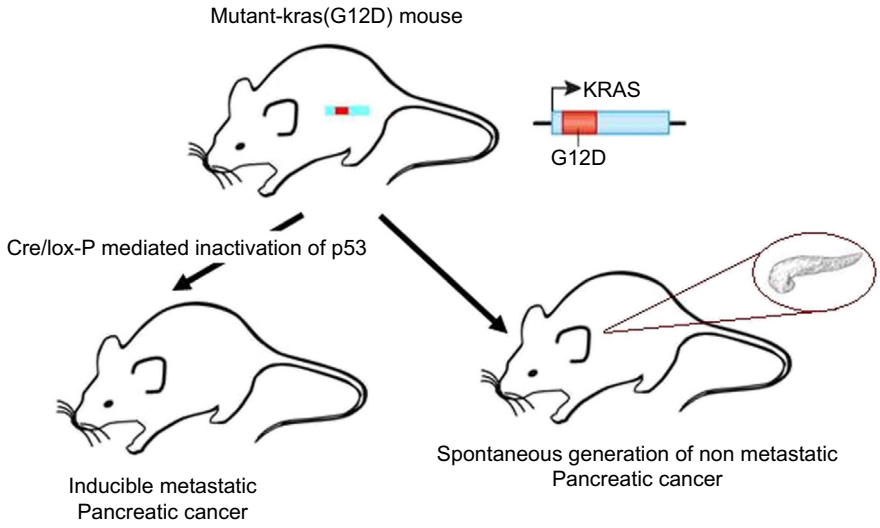


FIGURE 5.2 Generation of spontaneous tumor models for carcinogen studies.

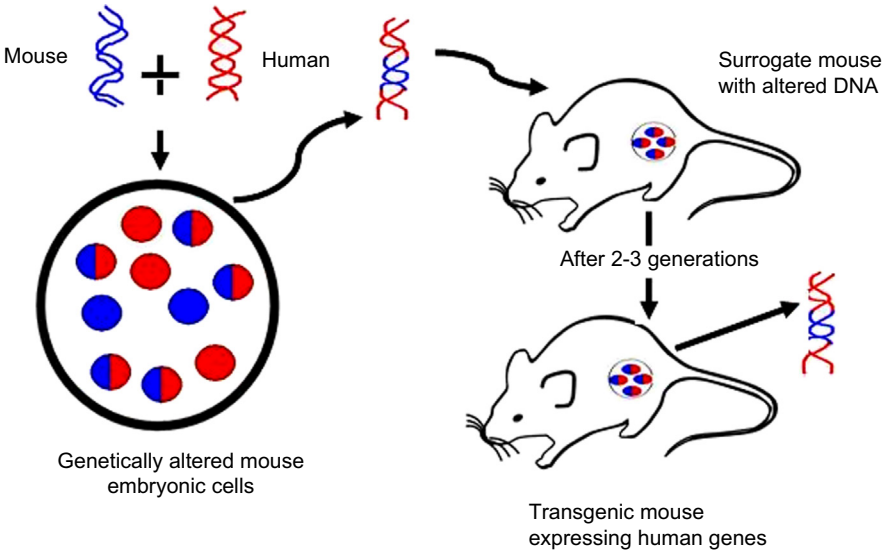


FIGURE 5.3 Generation of GEM models in immunocompetent mice.

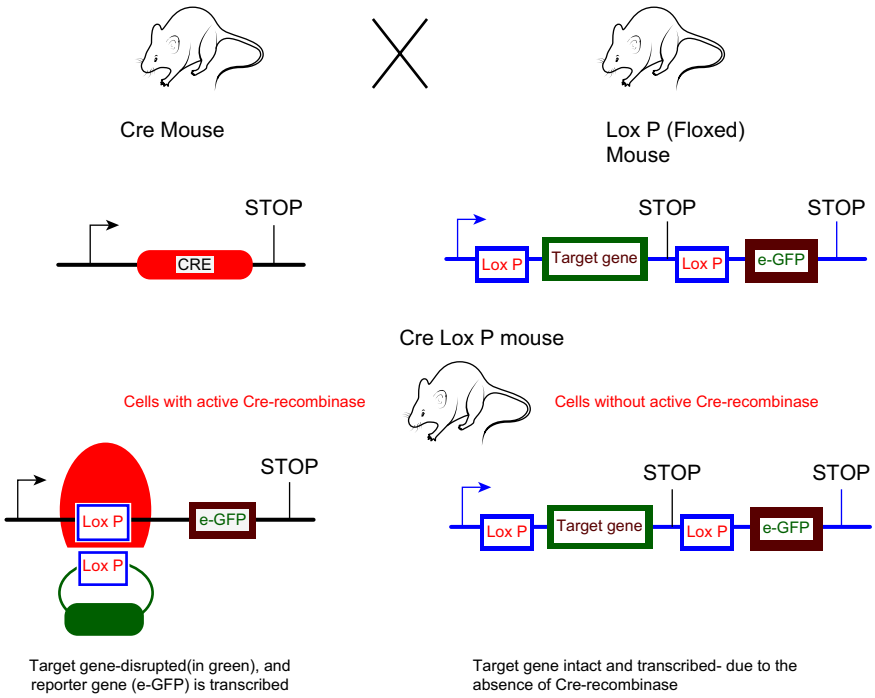


FIGURE 5.4 Generation of the Cre/Lox mouse model.

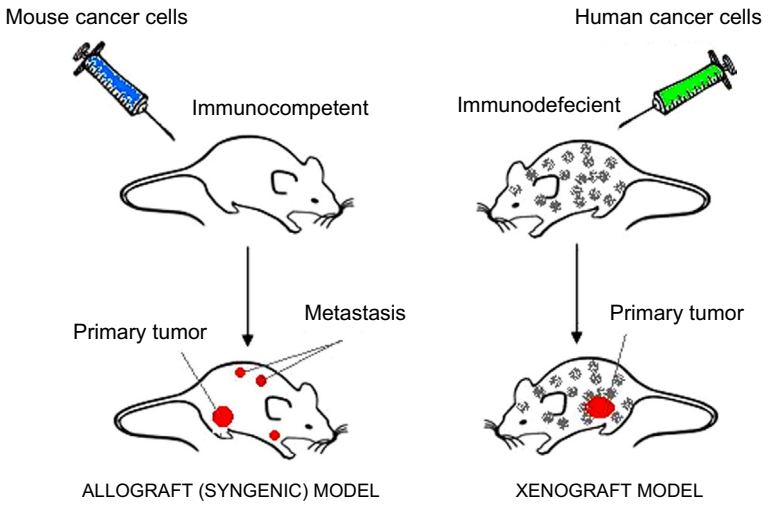


FIGURE 5.5 Generation of allograft and xenograft tumor models.

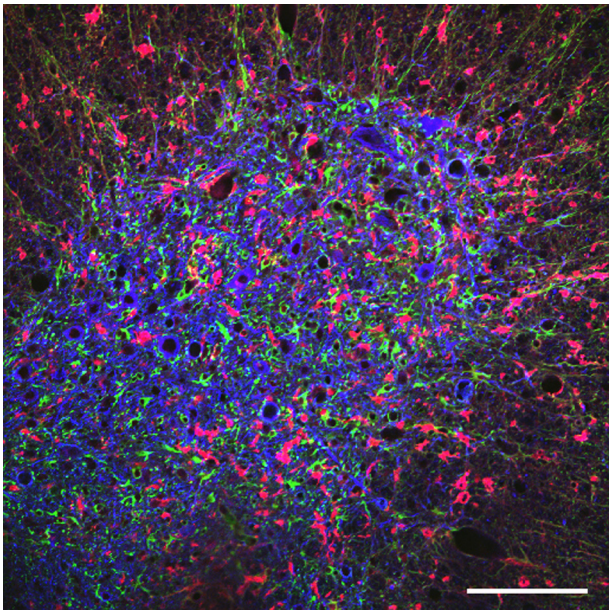


FIGURE 3.6 Activated Microglia and Astrocytes in Lumbar Spinal Cord of Symptomatic Mutant SOD1 Mice. Red: microglia stained with anti-Mac2 antibody, Green: astrocytes stained with anti-GFAP antibody, Blue: motor neurons stained with anti-neurofilament H antibody. Bar: 100 μm.

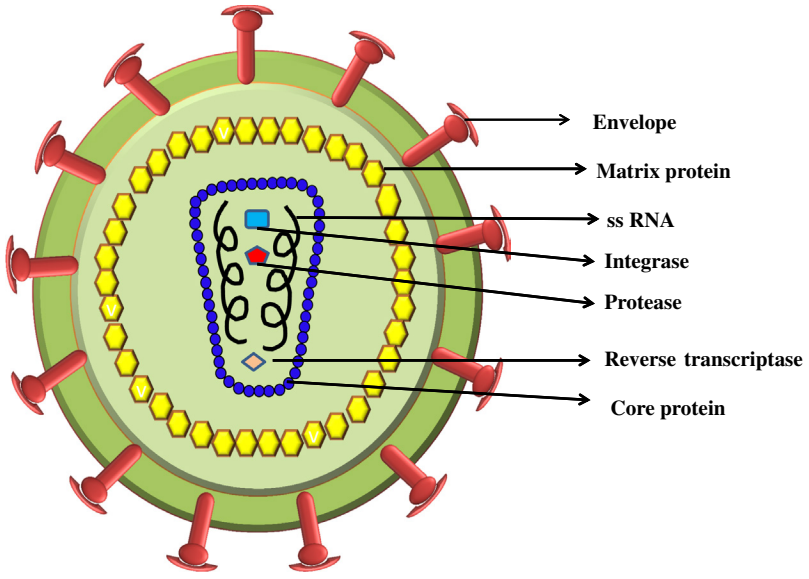


FIGURE 9.1 Structure of HIV. Graphical representation of cross-section of HIV. Envelope is the outermost layer; it consists of a lipid bilayer. The envelope layer is comprised of gp120 and gp41. The layer next to the envelope is the matrix protein. The matrix layer is followed by the core protein. At the center of the virion, two molecules of single-stranded RNA (ssRNA) and other enzymes are present. These enzymes are protease, integrase, and reverse transcriptase. Reverse transcriptase also contains RNase H. The location of each individual protein and RNA is shown in the figure and the molecular mass of the protein is shown in brackets. (Polymerase is not shown; it contains integrase, protease, reverse transcriptase, and RNAse H).

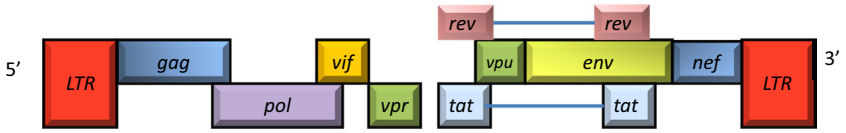


FIGURE 9.2 HIV Genome. Schematic representation of the HIV genome. The genome is 9.8 kB in size, and consists of 9 genes, which are flanked by LTRs on either side of the genome. These 9 genes finally produce 15 proteins: *env*, envelope; *gag*, group specific antigen; *LTR*, Long Terminal Repeat; *nef*, negative factor; *pol*, polymerase; *rev*, regulator of expression of viral proteins; *tat*, transactivator of transcription; *vif*, viral infectivity factor; *vpr*, viral protein R; *vpu*, viral protein U.

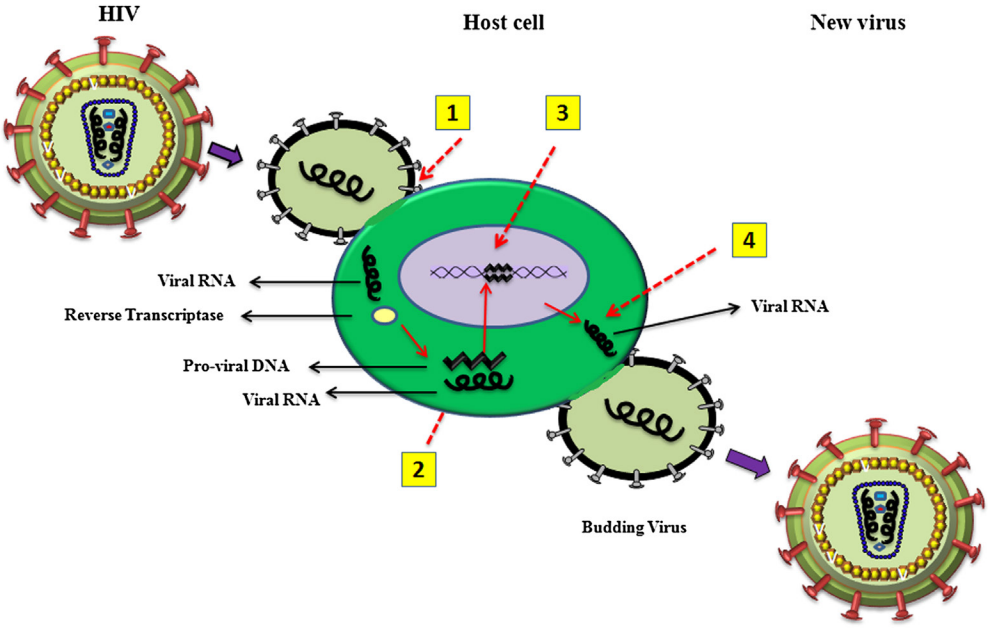


FIGURE 9.3 HIV Replication Steps and Drug Targets. Schematic representation of HIV replication along with the stages where different groups of antiretrovirals work. During infections, HIV attaches to the cell surface of target cells and fuses with the cells to release viral RNA and other proteins. Reverse transcriptase produces pro-viral RNA in the cytoplasm. Pro-viral RNA moves to the nucleus and integrates with the host cell genome with the activity of the integrase. After integration of pro-viral DNA into the host DNA, it gives rise to mRNA, which finally translates into different proteins required for synthesis of new virions. These proteins get cleaved by proteases to get assembled into new virions; new virions are released into circulation due to budding from the cells. Steps for drug targets are mentioned in numerals in blocks: Step 1 is the target for fusion inhibitors, Step 2 is the target for reverse transcriptase inhibitors, Step 3 is the target for integrase inhibitors, and Step 4 is the target for protease inhibitors. : Viral RNA; : Pro-viral DNA.

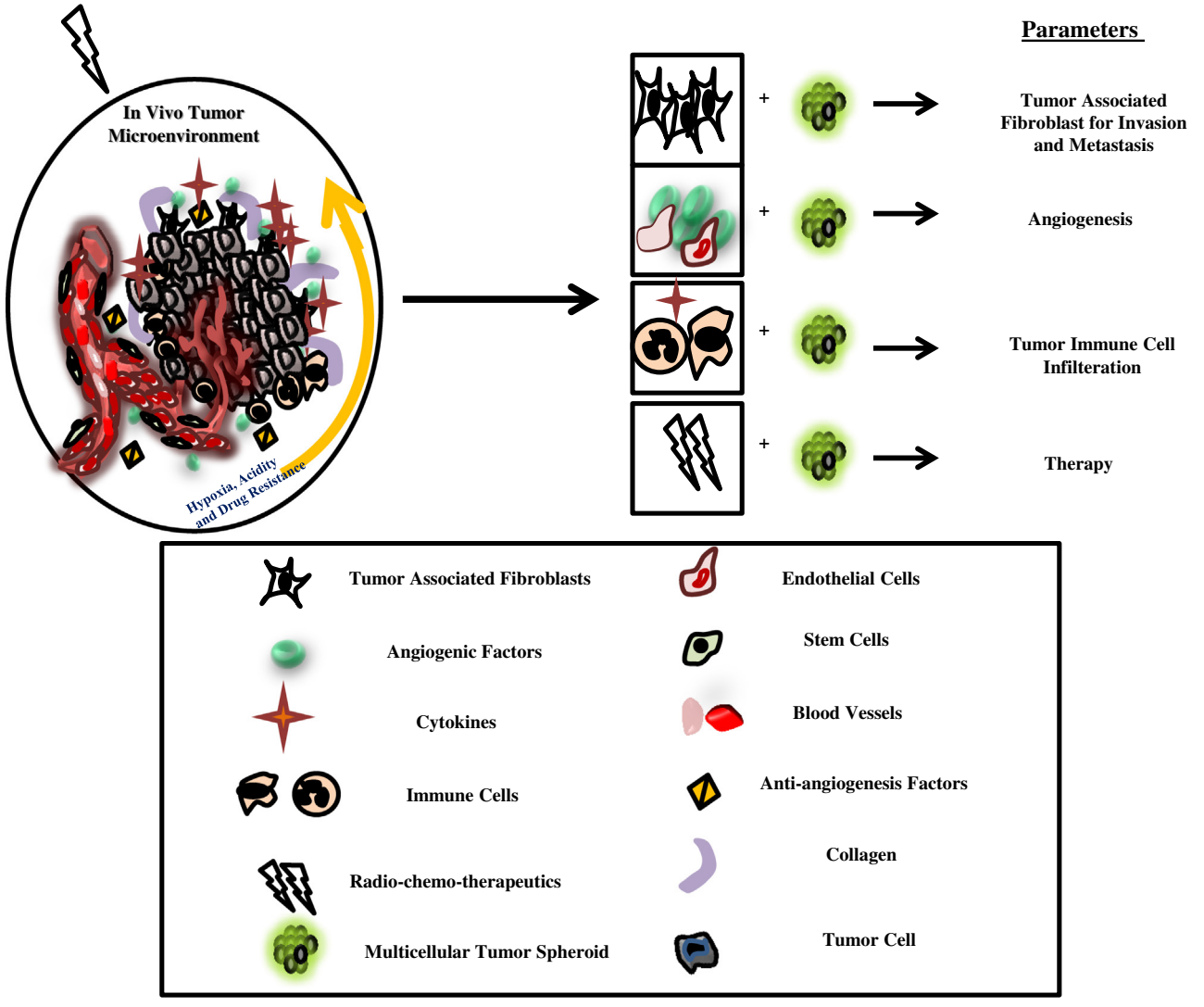


FIGURE 11.2 Approaches for studying the effects of tumor associated parameters on the *in vivo* response of tumors using multicellular tumor spheroid (MCTS).

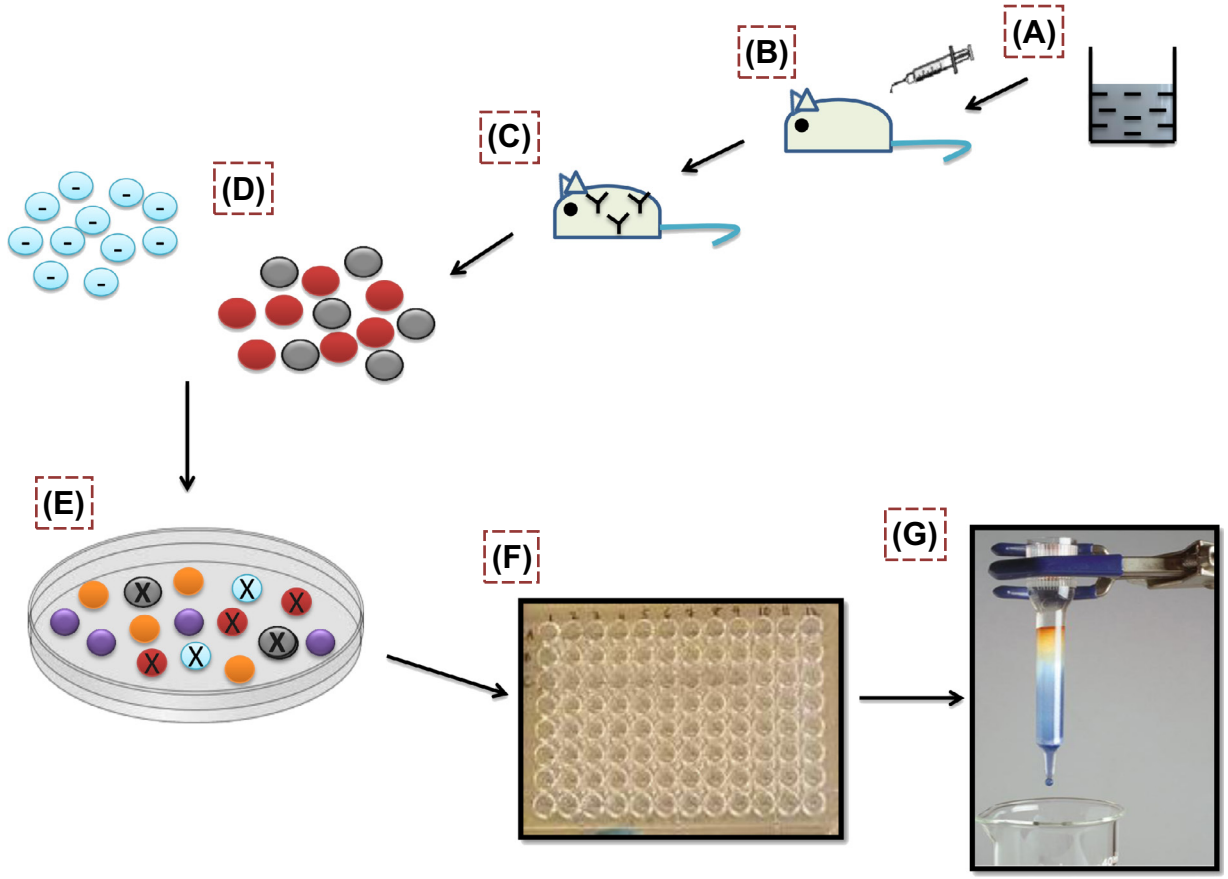


FIGURE 15.3 Schematic Representation of Production of Monoclonal Antibodies. This figure represents different crucial steps in the production of monoclonal antibodies. **(A) Antigen Preparations:** Antigen has to be prepared with Freund's Complete Adjuvant or Freund's Incomplete Adjuvant. **(B &C) Immunization:** Mice have to be immunized by injecting antigen prepared with adjuvant; they also have to be given booster doses. **(D) Preparation of Splenocytes and Fusion with Myeloma:** The spleen has to be removed from immunized mice and a single-cell suspension of splenocytes has to be prepared. Then splenocytes have to be fused with myeloma cells in the presence of fusogenic agents. **(E) Selection of Hybridoma:** After fusion, a hybridoma has to be selected from the cell population mixture. Cells have to be grown in HAT selection medium so that after selection only hybridoma cells can survive, while B-lymphocytes and un-fused myeloma cells will die. **(F) Screening of Clone:** After selection of hybridoma cells, a specific clone has to be selected. Different hybridoma cells are diluted in 96-well plates, and after a period of time each clone has to be tested for specificity against the antigen. **(G) Purification of Monoclonal Antibodies:** After selection of a specific hybridoma clone, monoclonal antibodies can be purified. If downstream application requires purification of monoclonal antibodies, then the clone can be expanded and appropriate methods can be applied for purification of monoclonal antibodies. ( ), myeloma cells; (,), lymphocytes; and (,), hybridomas. Cells marked with an "X" represent cell death in the selection medium.

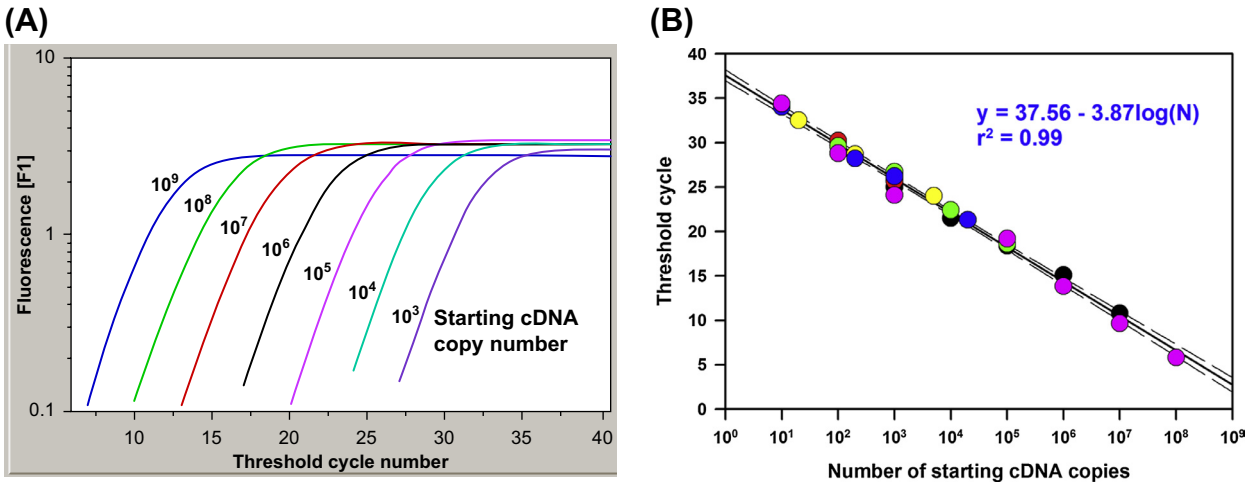


FIGURE 17.1 Example of a quantitative real-time PCR with the primers for α-tubulin. (A) Tubulin cDNA real-time amplification curves for samples containing standard dilutions of the purified target cDNA. (B) Tubulin qPCR calibration curve generated by amplifying known copy numbers of the target cDNA; circles of different colors correspond to separate calibration runs conducted during a 2-year period; dashed lines show 95% confidence intervals for the regression line across all data points.

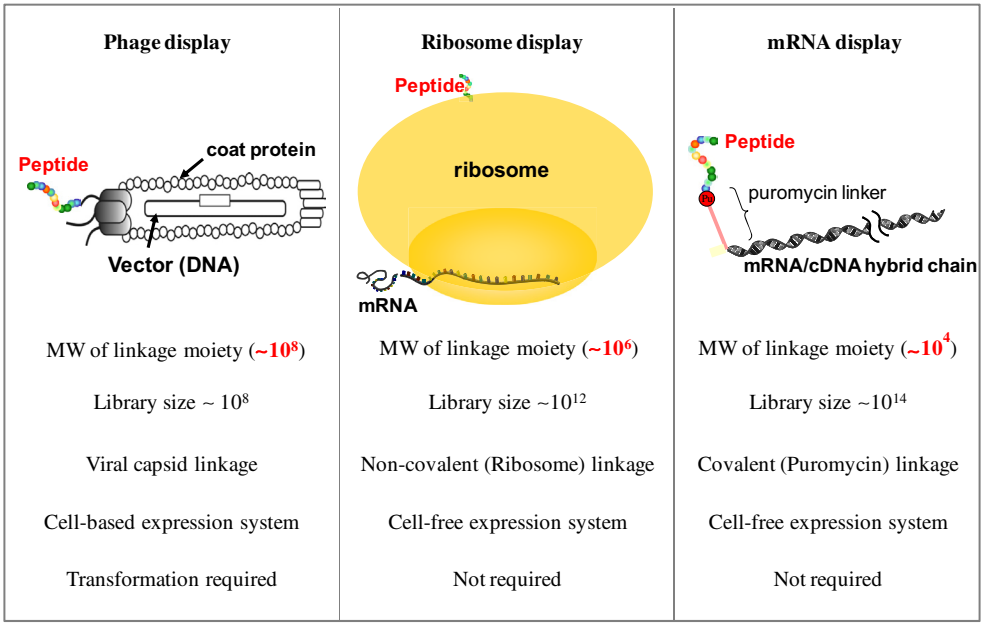


FIGURE 20.5 Illustration and comparison of the most common biomolecular display technologies. In phage display, an indirect linkage (physical) between the gene and gene product is provided by the viral capsid. In ribosome display, a non-covalent linkage is achieved by producing ternary complexes of RNA, ribosomes, and associated nascent peptides. In the mRNA display system, a covalent linkage is generated through a puromycin molecule attached to the encoding mRNA via a short DNA linker molecule.

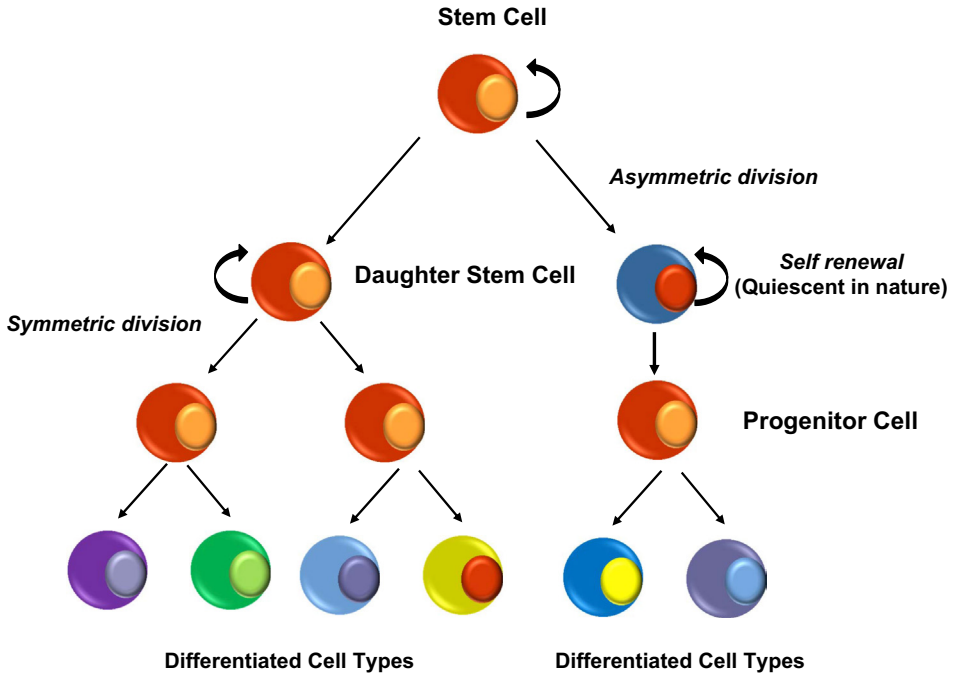


FIGURE 23.2 Asymmetric and symmetric stem cell division.

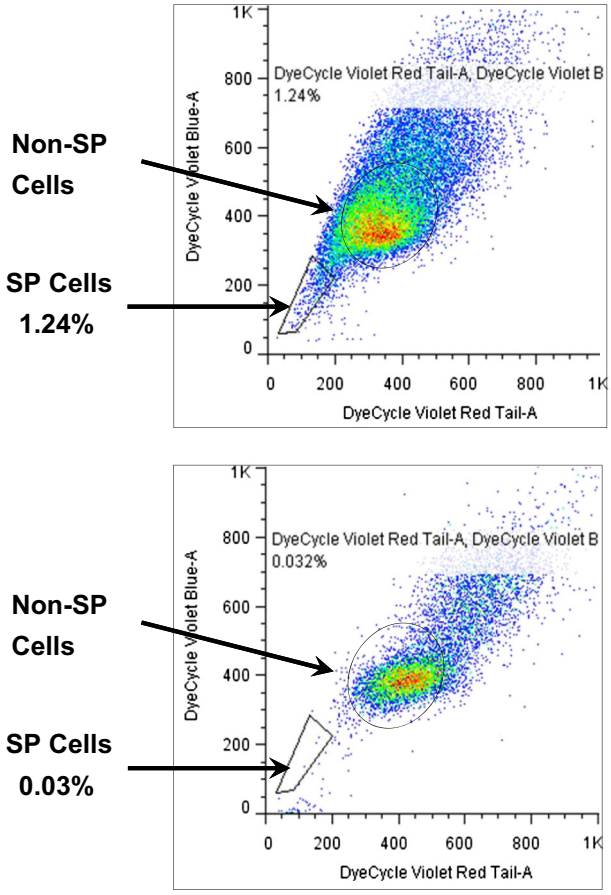


FIGURE 23.4 Side population identification based on DCV dye efflux in HPV16+ve Cervical Cancer Cell Line (SiHa).

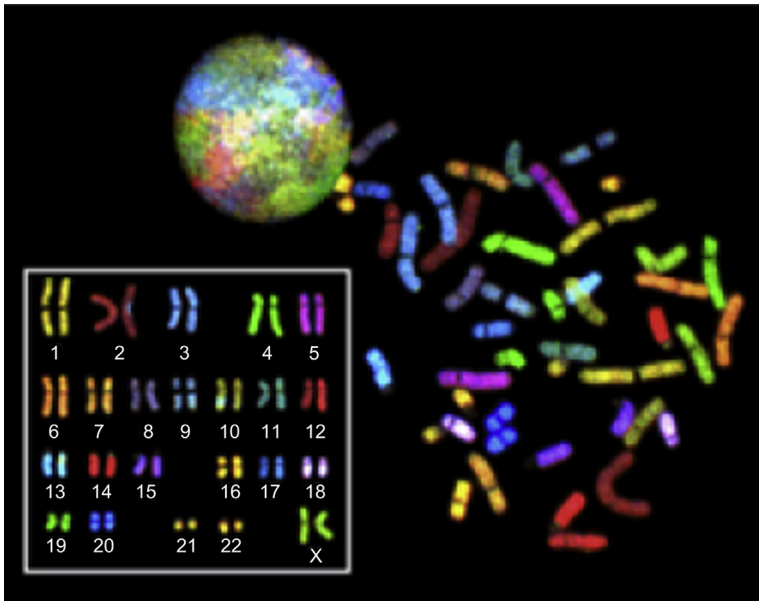


FIGURE 24.5 SKY image showing metaphase chromosomes labeled with different fluorochromes.

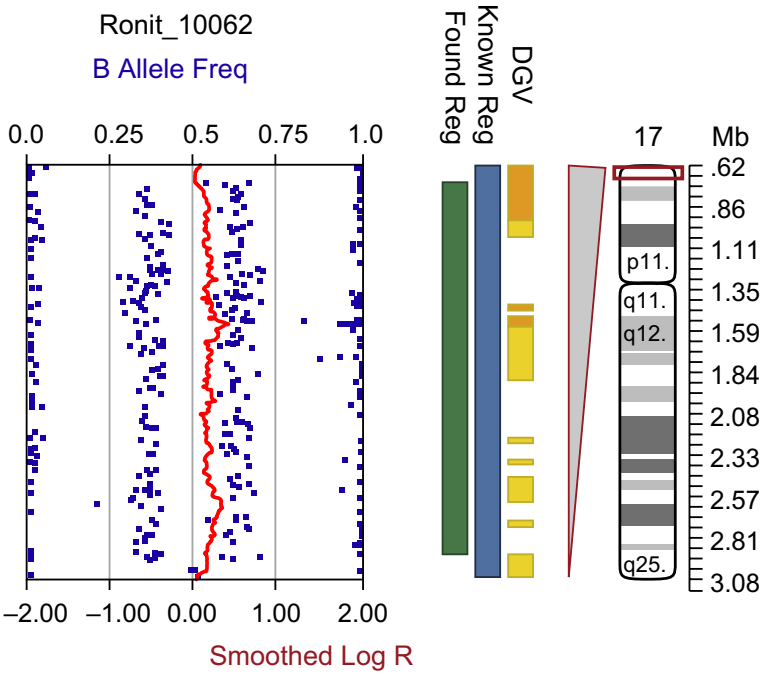


FIGURE 24.6 Array-CGH image showing 2.2 Mb duplication on chromosome 17q13.3.

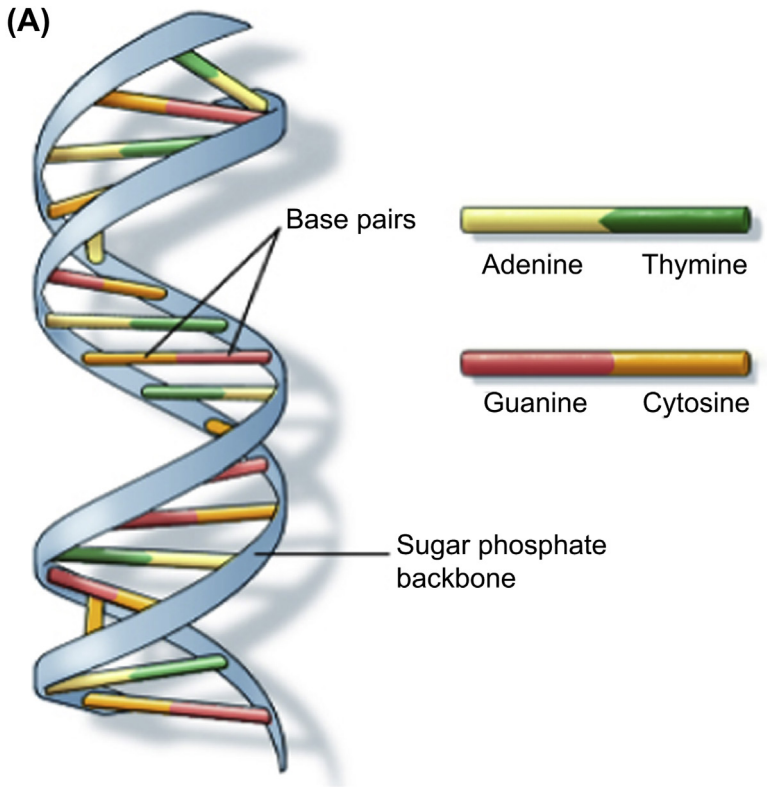


FIGURE 24.7A Double helix structure of DNA. *(Courtesy: U.S. National Library of Medicine.)*

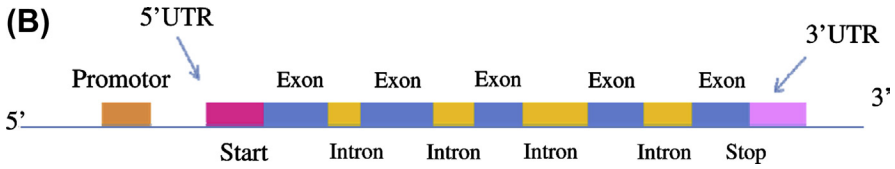


FIGURE 24.7B Structure of a typical human gene.

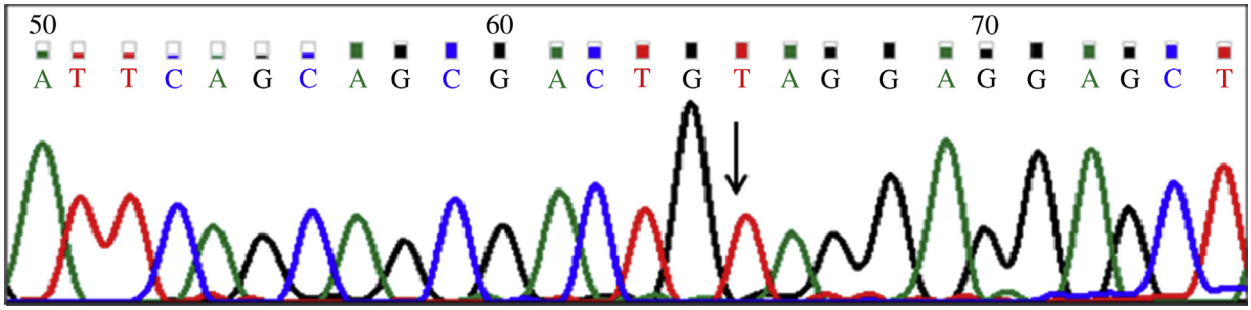


FIGURE 24.8 Electropherogram showing mutation. An arrow shows C to T substitution in codon 318 (CAG to TAG) that results in change of Gutamine (Q) coded by CAG to stop codon TAG, denoted as X.

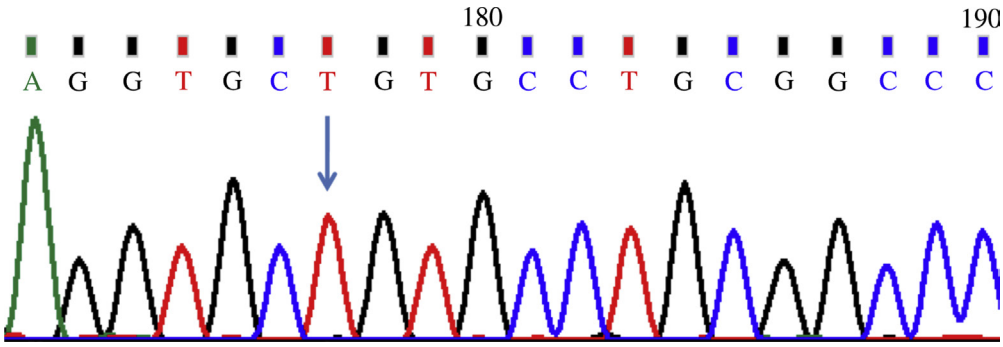


FIGURE 24.9D Partial electropherogram of DMD gene showing C to T change (shown by arrow).

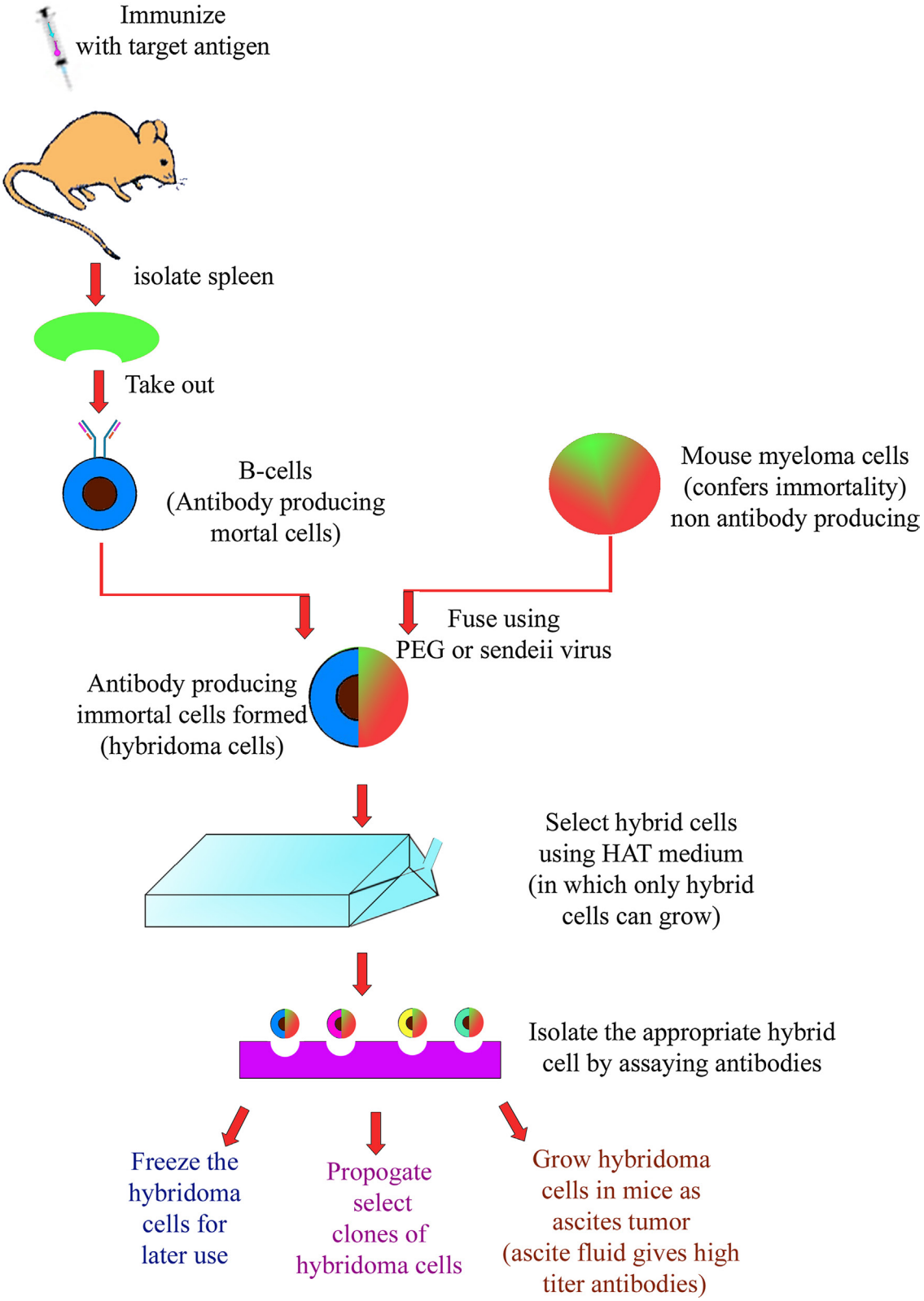


FIGURE 25.1 Production of monoclonal antibodies (a simplified overview).

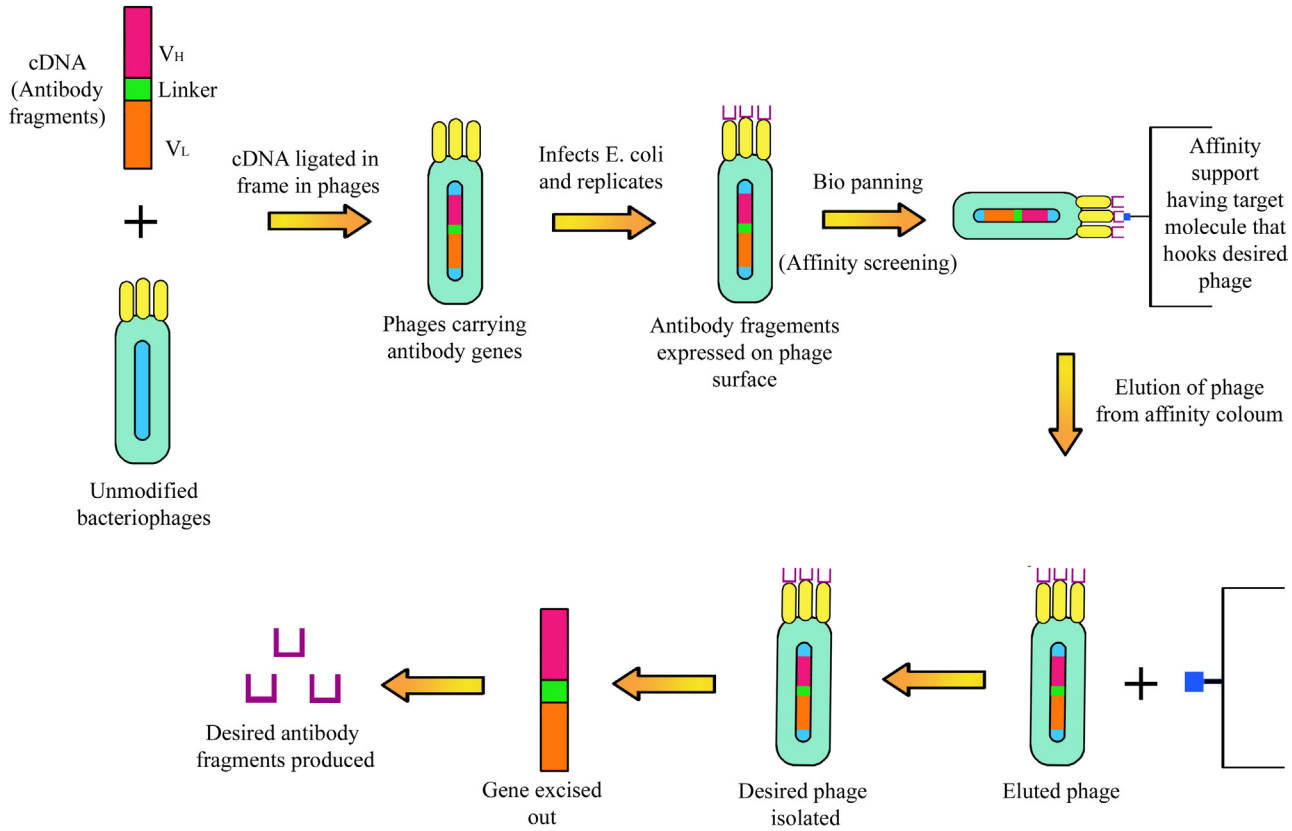


FIGURE 25.3 Phage display technology for antibody screening.

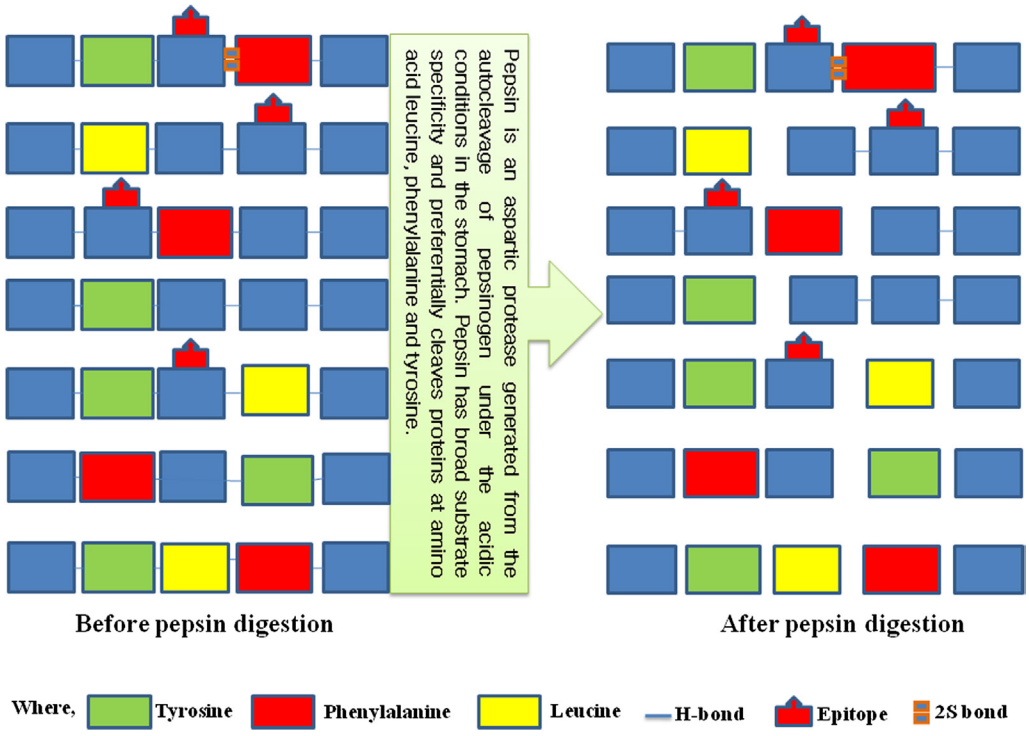


FIGURE 27.2 Mechanism of action of pepsin on polypeptides.

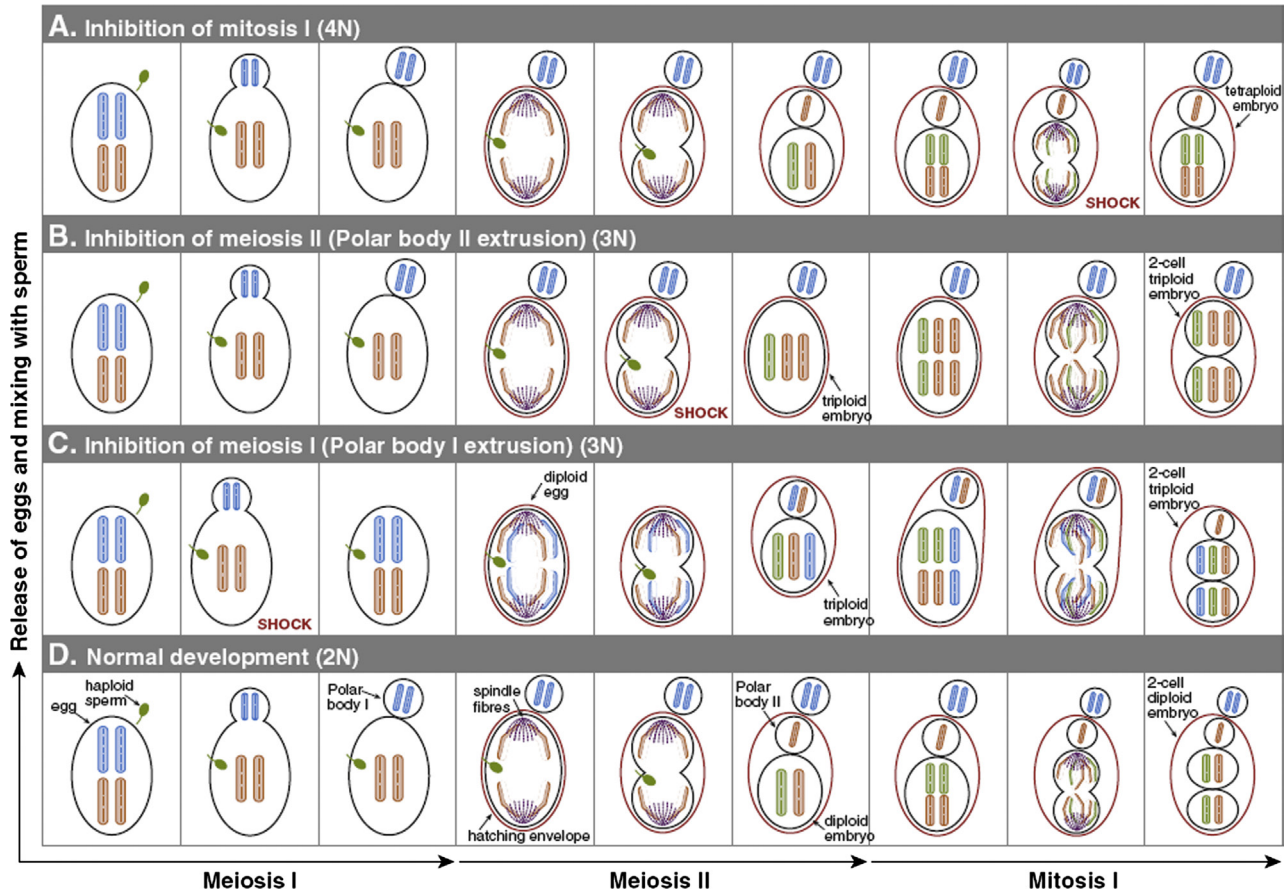


FIGURE 29.1 Process of inducing (A) Meiosis I triploidy, (B) Meiosis II triploidy and (C) Mitotic tetraploidy, and (D) Normal development in penaeid shrimp. *(Source: Sellars et al., 2010)*

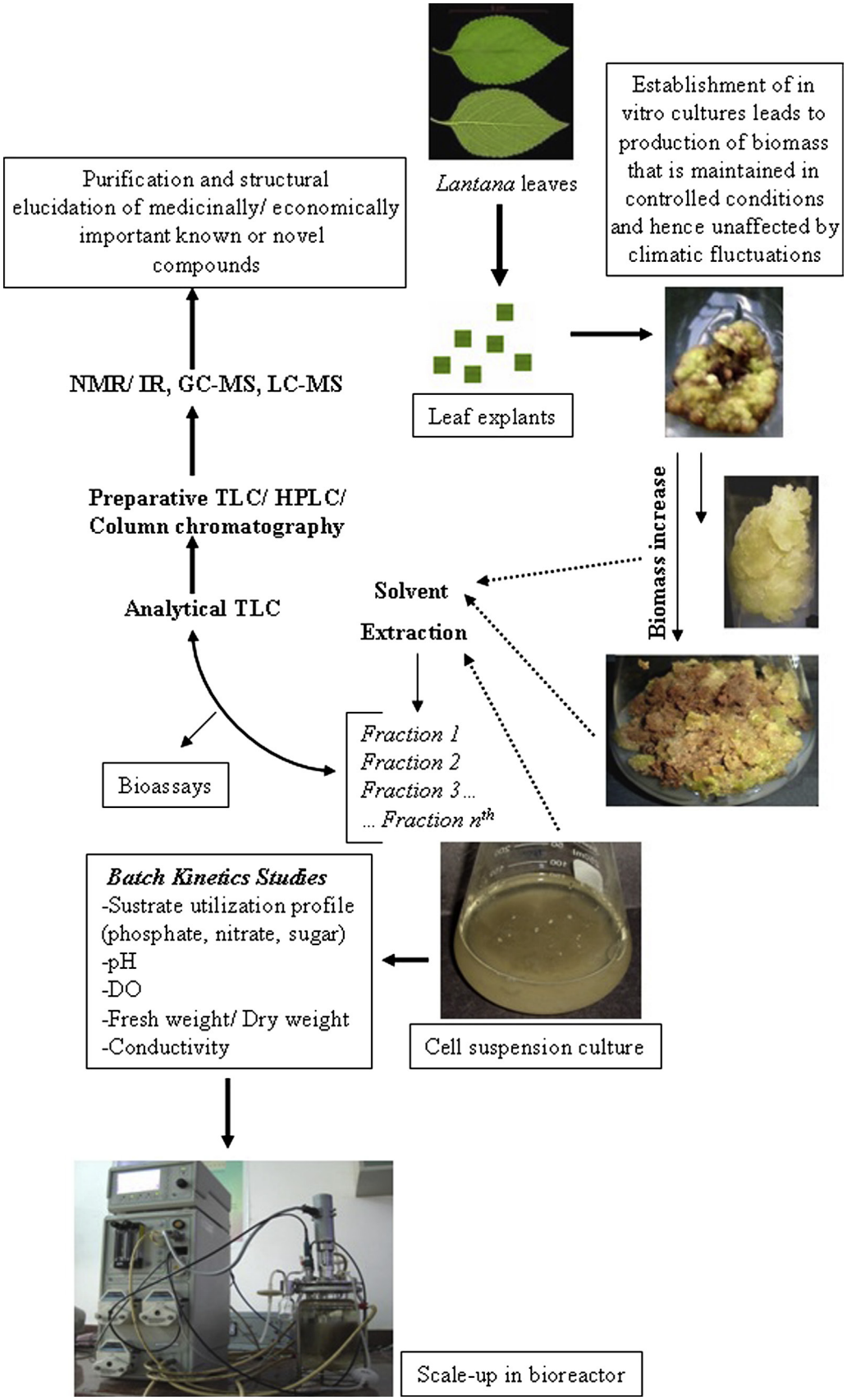


FIGURE 30.4 Isolation of bioactive compounds from *Lantana camara*, a medicinal plant.