

Contents

Preface XIX

List of Contributors XXI

Abbreviations XXV

Part I Fundamentals of Cellular and Molecular Biology 1

- 1 The Cell as the Basic Unit of Life 3
M. Wink
- 2 Structure and Function of Cellular Macromolecules 7
M. Wink
 - 2.1 Structure and Function of Sugars 8
 - 2.2 Structure of Membrane Lipids 10
 - 2.3 Structure and Function of Proteins 14
 - 2.4 Structure of Nucleotides and Nucleic Acids (DNA and RNA) 21
 - 2.5 References 27
- 3 Structure and Functions of a Cell 29
M. Wink
 - 3.1 Structure of a Eukaryotic Cell 29
 - 3.1.1 Structure and Function of the Cytoplasmic Membrane 29
 - 3.1.1.1 Membrane Permeability 30
 - 3.1.1.2 Transport Processes across Biomembranes 31
 - 3.1.1.3 Receptors and Signal Transduction at Biomembranes 33
 - 3.1.2 Endomembrane System in a Eukaryotic Cell 38
 - 3.1.3 Mitochondria and Chloroplasts 40
 - 3.1.4 Cytoplasm 45
 - 3.1.5 Cytoskeleton 47
 - 3.1.6 Cell Walls 49
 - 3.2 Structure of Bacteria 50
 - 3.3 Structure of Viruses 51
 - 3.4 Differentiation of Cells 52
- 4 Biosynthesis and Function of Macromolecules (DNA, RNA, and Proteins) 57
M. Wink
 - 4.1 Genomes, Chromosomes, and Replication 57
 - 4.1.1 Genome Size 57
 - 4.1.2 Composition and Function of Chromosomes 62
 - 4.1.3 Mitosis and Meiosis 64
 - 4.1.4 Replication 66
 - 4.1.5 Mutations and Repair Mechanisms 66

4.2	Transcription: From Gene to Protein	71
4.3	Protein Biosynthesis (Translation)	76
5	Distributing Proteins in the Cell (Protein Sorting)	81
	<i>M. Wink</i>	
5.1	Import and Export of Proteins via the Nuclear Pore	82
5.2	Import of Proteins in Mitochondria and Chloroplasts	83
5.3	Protein Transport into the Endoplasmic Reticulum	85
5.4	Vesicle Transport from the ER via the Golgi Apparatus to the Cytoplasmic Membrane	86
6	Evolution and Diversity of Organisms	91
	<i>M. Wink</i>	
6.1	Prokaryotes	91
6.2	Eukaryotes	91
Part II	Standard Methods in Molecular Biotechnology	99
7	Isolation and Purification of Proteins	101
	<i>T. Wieland, M. Lutz</i>	
7.1	Introduction	101
7.2	Producing a Protein Extract	102
7.3	Gel Electrophoretic Separation Methods	103
7.3.1	Principles of Electrophoresis	103
7.3.2	Native Gel Electrophoresis	104
7.3.3	Discontinuous Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)	104
7.3.4	Two-Dimensional (2D) Gel Electrophoresis, Isoelectric Focusing (IEF)	105
7.3.5	Detecting Proteins in Gels	105
7.4	Methods of Protein Precipitation	106
7.5	Column Chromatography Methods	107
7.5.1	General Principles of Separation	107
7.5.1.1	Size Exclusion Chromatography (Gel Filtration)	107
7.5.1.2	Hydrophobic Interaction Chromatography	108
7.5.1.3	Ion Exchange Chromatography	109
7.5.1.4	Hydroxyapatite Chromatography	110
7.5.2	Group-specific Separation Techniques	110
7.5.2.1	Chromatography on Protein A or Protein G	110
7.5.2.2	Chromatography on Cibacron Blue (Blue Gel)	111
7.5.2.3	Chromatography on Lectins	111
7.5.2.4	Chromatography on Heparin	111
7.5.3	Purification of Recombinant Fusion Proteins	112
7.5.3.1	Chromatography on Chelating Agents	112
7.5.3.2	Chromatography on Glutathione Matrices	112
7.6	Examples	113
7.6.1	Example 1: Purification of Nucleoside Diphosphate Kinase from the Cytosol of Bovine Retina Rod Cells	113
7.6.2	Example 2: Purification of Recombinant His ₆ -RGS16 after Expression in <i>E. coli</i>	114
8	Peptide and Protein Analysis with Electrospray Tandem Mass Spectrometry	115
	<i>A. Schlosser, W. D. Lehmann</i>	
8.1	Introduction	115
8.2	Principles of Mass Spectrometry	115
8.3	Mass Precision, Resolution, and Isotope Distribution	116

8.4	Principles of ESI	116
8.5	Tandem Mass Spectrometers	117
8.5.1	Mass Analyzers	117
8.5.2	Triple Quadrupole	118
8.5.3	Linear Trap Quadrupole (LTQ) and LTQ Orbitrap	118
8.5.4	Q-TOF	119
8.5.5	Q-FT-ICR	119
8.6	Peptide Sequencing with MS/MS	119
8.7	Identifying Proteins with MS/MS Data and Protein Databases	120
8.7.1	Database Search with MS/MS Raw Data	120
8.8	Determining Protein Molecular Mass	121
8.9	Analysis of Covalent Protein Modification	122
8.10	Relative and Absolute Quantification	123
9	Isolation of DNA and RNA	125
	<i>H. Weiher, R. Zwacka, I. Herr</i>	
9.1	Introduction	125
9.2	DNA Isolation	125
9.3	RNA Isolation	127
9.3.1	Enrichment of mRNA	127
10	Chromatography and Electrophoresis of Nucleic Acids	129
	<i>H. Weiher, R. Zwacka, I. Herr</i>	
10.1	Introduction	129
10.2	Chromatographic Separation of Nucleic Acids	129
10.3	Electrophoresis	130
10.3.1	Agarose Gel Electrophoresis: Submarine Electrophoresis	130
10.3.2	Pulsed Field Agarose Gel Electrophoresis	131
10.3.3	Polyacrylamide Gel Electrophoresis (PAGE)	131
11	Hybridization of Nucleic Acids	133
	<i>H. Weiher, R. Zwacka, I. Herr</i>	
11.1	Significance of Base Pairing	133
11.2	Experimental Hybridization: Kinetic and Thermodynamic Control	133
11.3	Analytical Techniques	134
11.3.1	Clone Detection, Southern Blotting, Northern Blotting, and Gene Diagnosis	134
11.3.2	Systematic Gene Diagnosis and Expression Screening based on Gene Arrays	135
11.3.3	<i>In Situ</i> Hybridization	135
12	Use of Enzymes in the Modification of Nucleic Acids	137
	<i>A. Groth, R. Zwacka, H. Weiher, I. Herr</i>	
12.1	Restriction Enzymes (Restriction Endonucleases)	137
12.2	Ligases	139
12.3	Methyltransferases	139
12.4	DNA Polymerases	140
12.5	RNA Polymerases and Reverse Transcriptase	141
12.6	Nucleases	141
12.7	T4 Polynucleotide Kinase	141
12.8	Phosphatases	142
13	Polymerase Chain Reaction	143
	<i>A. Mohr, H. Weiher, I. Herr, R. Zwacka</i>	
13.1	Introduction	143
13.2	Techniques	143

13.2.1	Standard PCR	143
13.2.2	RT-PCR	144
13.2.3	Quantitative/Real-Time PCR	145
13.2.4	Rapid Amplification of cDNA Ends (RACE)	146
13.3	Areas of Application	146
13.3.1	Genome Analysis	146
13.3.2	Cloning Techniques	147
13.3.3	Expression Studies	147
14	DNA Sequencing	149
	<i>R. Zwacka, A. Mohr, I. Herr, H. Weiher</i>	
14.1	Introduction	149
14.2	DNA Sequencing Methods	149
14.2.1	Chemical Sequencing Method (MaxamGilbert Method)	150
14.2.2	Enzymatic Sequencing (SangerCoulson Method)	150
14.2.3	Pyrosequencing	151
14.3	Strategies for Sequencing the Human Genome	151
14.4	Practical Significance of DNA	152
15	Cloning Procedures	153
	<i>T. Wieland, S. Lutz</i>	
15.1	Introduction	153
15.2	Construction of Recombinant Vectors	153
15.2.1	Insert	154
15.2.2	Vector	156
15.2.3	Essential Components of Vectors	156
15.2.3.1	Bacterial Origin of Replication (<i>ori</i>)	156
15.2.3.2	Antibiotic Resistance	156
15.2.3.3	Polylinkers	157
15.2.4	Cloning Using Recombination Systems	157
15.2.5	Further Components of Vectors for Prokaryotic Expression Systems	158
15.2.5.1	Promoter	158
15.2.5.2	Ribosome-Binding Site	159
15.2.5.3	Termination Sequence	159
15.2.5.4	Fusion Sequence	159
15.2.6	Further Components of Eukaryotic Expression Vectors	159
15.2.6.1	Eukaryotic Expression Vectors: Yeast	160
15.2.6.2	Eukaryotic Expression Vectors for Mammal Cells	161
15.2.6.3	Viral Expression Systems for Mammalian Cells	163
15.2.7	Nonviral Introduction of Heterologous DNA to Host Organisms (Transformation, Transfection)	165
15.2.7.1	Transformation of Prokaryotes	165
15.2.7.2	Transformation of Yeast Cells	166
15.2.7.3	Transfection of Mammal Cells	166
16	Expression of Recombinant Proteins	169
	<i>T. Wieland, S. Lutz</i>	
16.1	Introduction	169
16.2	Expression of Recombinant Proteins in Host Organisms	170
16.2.1	Expression in <i>E. coli</i>	173
16.2.2	Expression in Yeasts	174
16.2.3	Expression in Insect Cells	176
16.2.3.1	Expression Based on Recombinant Baculoviruses	176
16.2.3.2	Expression of Proteins in Stably Transfected Insect Cells	177
16.2.4	Expression of Proteins in Mammalian Cells	177
16.3	Expression in Cell-Free Systems	178

16.3.1	Expression of Proteins in Reticulocyte Lysates	179
16.3.2	Protein Expression Using <i>E. coli</i> Extracts	179
17	Patch Clamp Method	181
	<i>R. Kraft</i>	
17.1	Biological Membranes and Ion Channels	181
17.2	Physical Foundations of the Patch Clamp Method	182
17.3	Patch Clamp Configurations	182
17.4	Applications of the Patch Clamp Method	184
18	Cell Cycle Analysis	187
	<i>S. Wölfl, A. Kitanovic</i>	
18.1	Analyzing the Cell Cycle	187
18.2	Experimental Analysis of the Cell Cycle	189
18.2.1	Preparing Synchronized Cell Cultures of <i>S. cerevisiae</i>	190
18.2.1.1	Centrifugal Elutriation	190
18.2.1.2	Cell Cycle Arrest Using α -Factor	191
18.2.2	Identification of Cell Cycle Stages	191
18.2.2.1	Budding Index	192
18.2.2.2	Fluorescent Staining of the Nucleus	192
19	Microscopic Techniques	197
	<i>S. Diekmann</i>	
19.1	Electron Microscopy	197
19.1.1	Cryo-electron Microscopy	199
19.1.2	Electron Tomography	200
19.2	Atomic or Scanning Force Microscopy	200
19.2.1	Force Spectroscopy	201
19.2.2	Advantages and Disadvantages	201
19.3	Light Microscopy	202
19.3.1	Deconvolution	203
19.3.2	Confocal Microscopy	203
19.3.3	Why Fluorescence?	204
19.3.4	Nanoscopy	204
19.4	Microscopy in the Living Cell	206
19.4.1	Analysis of Fluorescently Labeled Proteins <i>In Vivo</i>	207
19.4.2	Fluorescence Recovery after Photobleaching	208
19.4.3	Fluorescence Correlation Spectroscopy	208
19.4.4	Förster Resonance Energy Transfer and Fluorescence Lifetime Imaging Microscopy	209
19.4.5	Single-Molecule Fluorescence	209
20	Laser Applications	211
	<i>M. Vogel, R. Fink</i>	
20.1	Principles of Laser Technology	211
20.2	Properties of Laser Radiation	213
20.3	Types of Lasers and Setups	213
20.4	Applications	214
20.4.1	Laser Scanning Microscopy	214
20.4.2	Optical Tweezers	215
20.4.3	Laser Microdissection	215

Part III Key Topics 217

21 Genomics and Functional Genomics 219

S. Wiemann, M. Frohme

21.1 Introduction 219

21.2 Technological Developments in DNA Sequencing 221

21.3 Genome Sequencing 222

21.3.1 Mapping 222

21.3.1.1 Restriction Mapping and Restriction Fingerprinting 224

21.3.1.2 BAC End Sequencing 224

21.3.1.3 Genetic Mapping 226

21.3.1.4 Radiation Hybrid Mapping 227

21.3.1.5 HAPPY Mapping 228

21.3.1.6 Mapping Through Hybridization 228

21.3.1.7 Sequence Tagged Sites, Expressed Sequence Tags, Single Nucleotide Polymorphisms, and Sequence Length Polymorphisms (Amplified Fragment Length Polymorphisms) 231

21.3.1.8 Fluorescence *In Situ* Hybridization, Fiber Fish, Optical Mapping, and Comparative Genome Hybridization 232

21.3.2 Timeline of Genome Sequencing 233

21.3.3 Genome Sequencing Strategies 234

21.3.3.1 Conventional Approach: Random Shotgun Strategy 234

21.3.3.2 Whole-Genome Shotgun Strategy 235

21.3.3.3 Sequencing of the Human Genome 237

21.3.4 Outlook for Genome Sequencing 238

21.4 cDNA Projects 238

21.4.1 cDNA Libraries Represent the Cell's mRNA 238

21.4.2 Production of cDNA Libraries 240

21.4.3 EST Projects for Gene Identification 243

21.4.3.1 What is an EST? 243

21.4.4 Full-length Projects for the Production of Resources for Functional Genomics 245

21.5 Functional Genomics 246

21.6 Identification and Analysis of Individual Genes 248

21.6.1 Positional Cloning 248

21.6.2 Gene Trap 251

21.6.3 DNA/RNA *In Situ* Hybridization 251

21.6.4 Tissue Arrays 252

21.7 Investigation of Transcriptional Activity 253

21.7.1 Serial Analysis of Gene Expression 253

21.7.2 Subtractive Hybridization 254

21.7.3 RNA Fingerprinting 257

21.7.4 Array-based Techniques 258

21.7.4.1 Macroarrays 261

21.7.4.2 Microarrays 262

21.7.4.3 Global and Specific Arrays 264

21.7.5 Specificity and Sensitivity 265

21.8 Cell-based Methods 266

21.8.1 Green Fluorescence Protein Techniques 266

21.8.2 Alternatives to Green Fluorescence Protein 267

21.8.3 Fluorescence Resonance Energy Transfer 268

21.8.4 Fluorescence Recovery After Photobleaching 269

21.8.5 Cell-based Assays 269

21.8.5.1 Assay Design 270

21.8.5.2 Pipetting Systems 270

21.8.5.3 Reading and Recording of Data 270

21.8.5.4 Data Analysis 271

21.9	Functional Analysis of Entire Genomes	272
21.9.1	Genotypic Screening in Yeast	272
21.9.2	Phenotypic Screening in the Mouse	273
22	Bioinformatics	275
	<i>B. Brors, K. Fellenberg</i>	
22.1	Introduction	275
22.2	Data Sources	276
22.2.1	Primary Databases: EMBL/GenBank/DDBJ, PIR, Swiss-Prot	276
22.2.2	Genome Databases: Ensembl, GoldenPath	276
22.2.3	Genome Databases: Ensembl, GoldenPath	277
22.2.4	Molecular Structure Databases: PDB, SCOP	277
22.2.5	Transcriptome Databases: SAGE, ArrayExpress, GEO	277
22.2.6	Reference Databases: PubMed, OMIM, GeneCards	278
22.2.7	Pathway Databases and Gene Ontology	278
22.3	Sequence Analysis	279
22.3.1	Kyte-Doolittle Plot, Helical Wheel Analysis, Signal Sequence Analysis	279
22.3.2	Pairwise Alignment	280
22.3.2.1	Local/Global	281
22.3.2.2	Optimal/Heuristic	281
22.3.3	Alignment Statistics	282
22.3.4	Multiple Alignment	282
22.4	Evolutionary Bioinformatics	283
22.4.1	Statistical Models of Evolution	284
22.4.2	Relation to Score Matrices	285
22.4.3	Phylogenetic Analysis	285
22.5	Gene Prediction	287
22.5.1	Neural Networks or HMMs Based on Hexanucleotide Composition	287
22.5.2	Comparison with Expressed Sequence Tags or other Genomes (<i>Fugu</i> , Mouse)	288
22.6	Bioinformatics in Transcriptome and Proteome Analysis	288
22.6.1	Preprocessing, Normalization	288
22.6.2	Feature Selection	290
22.6.3	Similarity Measures: Euclidean Distance, Correlation, Manhattan Distance, Mahalanobis Distance, Entropy Measures	290
22.6.4	Unsupervised Learning Procedures: Clustering, Principal Component Analysis, Multidimensional Scaling, Correspondence Analysis	291
22.6.5	Supervised Learning Procedures: Linear Discriminant Analysis, Decision Trees, Support Vector Machines, ANNs	291
22.6.6	Analysis of Over-Representation of Functional Categories	293
22.7	Bioinformatic Software	293
23	Cellular Systems Biology	295
	<i>H. Schmidt-Gienewinkel, S. Legewie, B. Brors, R. König</i>	
23.1	Introduction	295
23.2	Analysis of Cellular Networks by Top-Down Approaches	296
23.2.1	Motivation	296
23.2.2	Definitions and Reconstruction of the Networks	296
23.2.3	Gene Set Enrichment Tests	297
23.2.4	Network Descriptors	298
23.2.4.1	Scale-Free Networks	299
23.2.4.2	Triangle Motifs in Networks	299
23.2.4.3	Centrality and Further Topology Features	300

23.2.5	Detecting Essential Enzymes with a Machine Learning Approach	301
23.2.6	Elementary Flux Modes	301
23.2.7	Inference of Regulatory Networks: Boolean and Bayesian Networks	303
23.3	Overview of Bottom-Up Modeling of Biochemical Networks	304
23.3.1	Motivation	304
23.3.2	Choosing Model Complexity	305
23.3.3	Model Construction	305
23.3.4	Model Simulation	306
23.3.5	Model Calibration	307
23.3.6	Model Verification and Analysis	309
23.4	Biological Examples	309
24	Protein-Protein and Protein-DNA Interaction	315
	<i>P. Uetz, E. Pohl</i>	
24.1	Protein-Protein Interactions	315
24.1.1	Classification and Specificity: Protein Domains	316
24.1.2	Protein Networks and Complexes	316
24.1.3	Structural Properties of Interacting Proteins	318
24.1.4	Which Forces Mediate Protein-Protein Interactions?	319
24.1.4.1	Thermodynamics	319
24.1.4.2	Energetics	320
24.1.5	Methods to Examine Protein-Protein Interactions	320
24.1.6	Regulation of Protein-Protein Interactions	322
24.1.7	Theoretical Prediction of Protein-Protein Interactions	323
24.1.7.1	Predicting Interacting Proteins by their Genome Sequence	323
24.1.7.2	Phylogenetic Profiles	324
24.1.8	Biotechnological and Medical Applications of Protein-Protein Interactions	324
24.2	Protein-DNA Interactions	324
24.2.1	Sequence-Specific DNA Binding	324
24.2.2	Thermodynamic Considerations Regarding Protein-DNA Complexes	325
24.2.3	Methods to Study Protein-DNA Interactions	325
24.2.3.1	Structural Classification of Protein-DNA Complexes	326
24.2.4	Regulatory Networks and Systems Biology	327
24.2.4.1	Medical Relevance of Protein-DNA Interactions	328
24.2.5	Biotechnological Applications of Protein-DNA Interactions	328
24.2.5.1	Synthetic Biology	328
25	Drug Research	331
	<i>M. Kogel, R. Tolle, U. Deuschle, C. Kremoser</i>	
25.1	Introduction	331
25.2	Active Compounds and their Targets	331
25.2.1	Identification of Potential Targets in the Human Genome	332
25.2.2	Comparative Genome Analysis	334
25.2.3	Experimental Target Identification: <i>In Vitro</i> Methods	334
25.2.4	Experimental Identification of Targets: Model Organisms	335
25.2.5	Experimental Target Identification in Humans	336
25.2.6	Difference between Target Candidates and Genuine Targets	337
25.2.7	Biologicals	337
25.2.8	DNA and RNA in New Therapeutic Approaches	339
25.2.9	Patent Protection for Targets	339
25.2.10	Compound Libraries as a Source of Drug Discovery	340
25.2.11	High-Throughput Screening	341
25.2.12	High-Quality Paramounts in Screening Assays	343

25.2.13	Virtual Ligand Screening	343
25.2.14	Activity of Drugs Described in Terms of Efficacy and Potency	344
25.2.15	Chemical Optimization of Lead Structures	344
25.3	Preclinical Pharmacology and Toxicology	344
25.4	Clinical Development	346
25.5	Clinical Testing	346
26	Drug Targeting and Prodrugs	349
	<i>G. Fricker</i>	
26.1	Drug Targeting	349
26.1.1	Passive Targeting by Exploiting Special Physiological Properties of the Target Tissue	350
26.1.2	Physical Targeting	350
26.1.3	Active Targeting	351
26.1.4	Cellular Carrier Systems	354
26.2	Prodrugs	355
26.2.1	Prodrugs to Improve Drug Solubility	355
26.2.2	Prodrugs to Increase Stability	355
26.3	Penetration of Drugs through Biological Membranes	356
26.4	Prodrugs to Extend Duration of Effect	357
26.5	Prodrugs for the Targeted Release of a Drug	357
26.6	Prodrugs to Minimize Side Effects	358
27	Molecular Diagnostics in Medicine	359
	<i>S. Wölff, R. Gessner</i>	
27.1	Uses of Molecular Diagnostics	359
27.1.1	Introduction	359
27.1.2	Monogenic and Polygenic Diseases	359
27.1.3	Individual Variability in the Genome: Forensics	362
27.1.4	Individual Variability in the Genome: HLA Typing	362
27.1.5	Individual Variability in the Genome: Pharmacogenomics	362
27.1.6	Individual Variability in the Genome: Susceptibility to Infectious Diseases	363
27.1.7	Viral Diagnosis	363
27.1.8	Microbial Diagnosis and Resistance Diagnosis	364
27.2	Which Molecular Variations Should be Detected	364
27.2.1	Point Mutations	365
27.2.2	Insertions and Deletions	366
27.2.3	Nucleotide Repeats	366
27.2.4	Deletion or Duplication of Genes	366
27.2.5	Recombination between Chromosomes	366
27.2.6	Heading3	367
27.3	Molecular Diagnostic Methods	367
27.3.1	DNA/RNA Purification	367
27.3.2	Determination of Known Sequence Variations	368
27.3.2.1	Length Polymorphism	368
27.3.2.2	Restriction Fragment Length Polymorphism (RFLP)	368
27.3.2.3	Amplification-Created Restriction Sites (ACRS)	369
27.3.2.4	Amplification Refractory Mutation System (ARMS)	369
27.3.2.5	Mutationally Separated (MS)-PCR	369
27.3.2.6	Allele-Specific Hybridization	369
27.3.2.7	Ligase Chain Reaction (LCR)	371
27.3.2.8	Minisequencing	371
27.3.2.9	Pyrosequencing	371
27.3.2.10	Quantitative PCR	371
27.3.2.11	Chip Technology	372

27.3.2.12	Production and Manufacture of Microarrays	373
27.3.2.13	Determination of Unknown Mutations	374
27.4	Outlook	375
28	Recombinant Antibodies and Phage Display	377
	<i>S. Dübel</i>	
28.1	Introduction	377
28.2	Why Recombinant Antibodies?	379
28.2.1	Recombinant Antibodies are Available <i>In Vitro</i> without Immunization	379
28.2.2	Antibodies with New Characteristics Can Be Created	379
28.3	Obtaining Specific Recombinant Antibodies	379
28.3.1	Preparation of the Variety of Antibody Genes	380
28.3.2	Selection Systems for Recombinant Antibodies	380
28.3.2.1	Transgenic Mice	380
28.3.2.2	<i>In Vitro</i> Selection Systems	382
28.4	Production of Recombinant Antibodies	384
28.4.1	Recombinant Production Systems	384
28.4.2	Purification of Recombinant Antibodies and their Fragments	385
28.5	Formats for Recombinant Antibodies	386
28.5.1	Monospecific Antibody Fragments	386
28.5.1.1	Fab Fragments	388
28.5.1.2	Fv Fragments	388
28.5.1.3	Single-Chain Antibody Fragments (scFv)	388
28.5.1.4	Single-Chain Fab Fragments (scFab)	389
28.5.1.5	Disulfide-Stabilized Fv Fragments (dsFv)	389
28.5.1.6	V _H and Camel Antibodies	389
28.5.2	Multivalent Antibody Fragments	389
28.5.2.1	Bifunctional Antibodies	390
28.5.2.2	Bispecific Antibodies	390
28.6	Applications of Recombinant Antibodies	392
28.6.1	Clinical Applications	392
28.6.2	Applications in Research and <i>In Vitro</i> Diagnostics	392
28.6.2.1	Recombinant Antibodies Selected to Avoid Cross-Reactivity	393
28.6.2.2	Intracellular Antibodies	394
28.6.2.3	Recombinant Antibodies as Binding Molecules for Arrays	394
28.7	Outlook	394
29	Transgenic and Gene-Targeted Mice and their Impact in Medical Research	395
	<i>R. Sprengel</i>	
29.1	Overview	395
29.2	Transgenic Mice	395
29.2.1	Retroviral Infection	396
29.2.2	Pronuclear Injection	397
29.3	Homologous Recombination: knock-out (-in) mice	398
29.4	Conditionally Regulated Gene Expression	399
29.5	Impact of Genetically Modified Mice in Biomedicine	400
29.5.1	Alzheimer's Disease	401
29.5.2	Amyotrophic Lateral Sclerosis (ALS)	401
29.5.3	Psychological Disorders	402
29.6	Outlook	402
30	Gene Therapy: Strategies and Vectors	403
	<i>A. Groth, I. Herr</i>	
30.1	Introduction	403
30.2	Principles of Somatic Gene Therapy	404

30.3	Germ Line Therapy	405
30.4	Setbacks in Gene Therapy	406
30.5	Vectors for Gene Therapy	406
30.5.1	Retroviral Vectors	407
30.5.2	Adenoviral Vectors	410
30.5.3	Adeno-associated Virus (AAV)	411
30.5.4	Other Viral Vectors	413
30.6	Specific Expression	413
31	RNA Interference, Modified DNA, Peptide Nucleic Acid, and Applications in Medicine and Biotechnology	415
	<i>N. Metzler-Nolte, A. Sosniak</i>	
31.1	Introduction	415
31.2	Modified Nucleic Acids	416
31.2.1	Phosphorothioate	416
31.2.2	Methylphosphonate	417
31.2.3	Peptide Nucleic Acids (PNAs)	418
31.3	Interactions of DNA Analogs with Complementary DNA and RNA	419
31.3.1	Melting Temperature	419
31.3.2	Mismatch Sensitivity	421
31.4	RNAi	421
31.4.1	Biogenesis of Small RNAs	422
31.4.1.1	Biogenesis of siRNAs	422
31.4.1.2	Biogenesis of miRNAs	422
31.4.2	Incorporation into RISC	423
31.4.3	Posttranscriptional Repression by miRNA und siRNA	424
31.5	Applications	424
31.5.1	Antisense Technology with DNA Analogs	424
31.5.2	siRNA in Biotechnological Applications	426
31.5.2.1	Design of siRNAs	426
31.5.2.2	Nonvectorial Applications	427
31.5.2.3	Vectorial Applications	427
31.5.2.4	Other Applications for PNA	428
31.5.3	Comparison of RNAi with DNA Analogs for Antisense Applications	429
32	Plant Biotechnology	431
	<i>H. Hillebrand, R. Hell</i>	
32.1	Introduction	431
32.1.1	Green Genetic Engineering – A New Method Towards Traditional Goals	431
32.1.2	Challenges in Plant Biotechnology	432
32.2	Gene Expression Control	433
32.3	Production of Transgenic Plants	434
32.3.1	Transformation Systems	434
32.3.1.1	<i>Agrobacterium</i> as a Natural Transformation System	435
32.3.1.2	Biolistic Method: Gene Gun	436
32.3.1.3	Plastid Transformation	438
32.3.1.4	Viral Systems	439
32.4	Selection of Transformed Plant Cells	439
32.4.1	Requirements for an Optimal Selection Marker System	440
32.4.2	Negative Selection Marker Systems	441
32.4.3	Positive Selection Marker Systems	442
32.4.4	Counter-Selection using Bifunctional Marker Genes	443
32.4.5	Visual Markers	443

32.4.6	Selection Systems, Genetic Engineering Safety, and Marker-Free Plants	443
32.5	Regeneration of Transgenic Plants	445
32.5.1	Regeneration Procedures	445
32.5.2	Composition of Regeneration Media	446
32.6	Plant Analysis: Identification and Characterization of Genetically Engineered Plants	446
32.6.1	DNA and RNA Verification	446
32.6.2	Protein Analysis	448
32.6.3	Genetic and Molecular Maps	448
32.6.4	Stability of Transgenic Plants	449
33	Biocatalysis in the Chemical Industry	451
	<i>M. Breuer, B. Hauer</i>	
33.1	Introduction	451
33.2	Bioconversion/Enzymatic Procedures	454
33.3	Development of an Enzyme for Industrial Biocatalysis	456
33.3.1	Identification of Novel Biocatalysts	456
33.3.2	Improvement of Biocatalysts	458
33.3.3	Production of Biocatalysts	458
33.3.4	Outlook	459
33.3.5	Case Study 1: Screening for New Nitrilases	459
33.3.6	Case Study 2: Use of Known Enzymes for New Reactions: Lipases for the Production of Optically Active Amines and Alcohols	460
33.3.7	Case Study 3: Enzyme Optimization with Rational and Evolutive Methods	461
33.4	Fermentative Procedures	462
33.4.1	Improvement of Fermentation Processes	462
33.4.2	Classical Strain Optimization	463
33.4.3	Metabolic Engineering	464
33.4.4	Case Study 4: Fermentative Production of <i>n</i> -Butanol	465
33.4.5	Case Study 5: Production of Glutamic Acid with <i>C. glutamicum</i>	466
33.4.5.1	Molecular Mechanism of Glutamate Overproduction	467
33.4.6	Case Study 6: Production of Lysine with <i>C. glutamicum</i>	468
33.4.6.1	Molecular Mechanism of Lysine Biosynthesis	468
33.4.6.2	Deregulation of the Key Enzyme Aspartate Kinase	469
33.4.7	Genomic Research and Functional Genomics	470
33.4.8	Case Study 7: Fermentative Penicillin Production	470
33.4.9	Case Study 8: Vitamin B ₂ Production	471
33.4.9.1	Riboflavin Biosynthesis	471
33.4.9.2	Classical Strain Development	472
Part IV	Biotechnology in Industry	473
34	Industrial Application: Biotech Industry, Markets, and Opportunities	475
	<i>J. Schüler</i>	
34.1	Historical Overview and Definitions of Concepts	475
34.2	Areas of Industrial Application of Molecular Biotechnology	476
34.2.1	Red Biotechnology	477
34.2.1.1	Biopharmaceutical Drug Development	477
34.2.1.2	Drug Delivery	479
34.2.1.3	Cell and Gene Therapy	479
34.2.1.4	Tissue Engineering/Regenerative Medicine	481
34.2.1.5	Pharmacogenomics and Personalized Medicine	481
34.2.1.6	Molecular Diagnostic Agents	482

34.2.1.7	Systems Biology	483
34.2.2	Green Biotechnology	483
34.2.2.1	Transgenic Plants	483
34.2.2.2	Genomic Approaches in Green Biotechnology	484
34.2.2.3	Novel Food and Functional Food	484
34.2.2.4	Livestock Breeding	484
34.2.3	White and Gray Biotechnology	485
34.3	Status Quo of the Biotech Industry World-Wide	485
34.3.1	Global Overview	485
34.3.2	United States	486
34.3.3	Europe	486
35	Patents in the Molecular Biotechnology Industry: Legal and Ethical Issues	487
	<i>David B. Resnik</i>	
35.1	Patent Law	487
35.1.1	What is a Patent?	487
35.1.2	How Does One Obtain a Patent?	488
35.1.3	What is the Proper Subject Matter for a Patent?	489
35.1.4	Types of Patents in Pharmaceutical and Molecular Biotechnology	490
35.1.5	Patent Infringement	490
35.1.6	International Patent Law	491
35.2	Ethical and Policy Issues in Biotechnology Patents	492
35.2.1	No Patents on Nature	492
35.2.2	Threats to Human Dignity	493
35.2.3	Problems with Access to Technology	494
35.2.4	Benefit Sharing	497
35.3	Conclusions	498
36	Drug Approval in the European Union and United States	499
	<i>G. Walsh</i>	
36.1	Introduction	499
36.2	Regulation within the European Union	499
36.2.1	EU Regulatory Framework	499
36.2.2	EMA	500
36.2.3	New Drug Approval Routes	501
36.2.3.1	Centralized Procedure	502
36.2.3.2	Mutual Recognition	503
36.3	Regulation in the United States	503
36.3.1	CDER and CBER	504
36.3.2	Approval Procedure	504
36.4	Advent and Regulation of Biosimilars	506
36.5	International Regulatory Harmonization	506
37	Emergence of a Biotechnology Industry	509
	<i>C. Kremoser</i>	
38	The 101 of Founding a Biotech Company	517
	<i>C. Kremoser</i>	
38.1	First Steps Towards Your Own Company	517
38.2	Employees: Recruitment, Remuneration, Participation	522

39	Marketing	527
	<i>C. Kremoser</i>	
39.1	Introduction	527
39.2	What Types of Deals are Possible?	528
39.3	What Milestone or License Fees are Effectively Paid in a Biotech/ Pharma Cooperation?	529
39.4	PR and IR in Biotech Companies	530
	Appendix	533
	Further Reading	535
	Glossary	551
	<i>M. Wink</i>	
	Subject Index	587