

Contents

Preface XXI

List of Contributors XXIII

Abbreviations XXVII

Colour Plates XXXVII

Part I Fundamentals of Cellular and Molecular Biology

1 The Cell as the Basic Unit of Life 3

M. Wink

2 Structure and Function of Cellular Macromolecules 7

M. Wink

2.1 Introduction 7

2.2 Structure and Function of Sugars 9

2.3 Structure of Membrane Lipids 12

2.4 Structure and Function of Proteins 17

2.5 Structure of Nucleotides and Nucleic Acids (DNA and RNA) 29

3 Structure and Function of a Cell 39

M. Wink

3.1 The Structure of a Eukaryotic Cell 40

3.1.1 The Structure and Function of the Cytoplasmic Membrane 40

3.1.1.1 Membrane Permeability 41

3.1.1.2 Transport Processes Across Biomembranes 43

3.1.1.3 Receptors and Signal Transduction at Biomembranes 47

3.1.2 The Endomembrane System in a Eukaryotic Cell 52

3.1.3 Mitochondria and Chloroplasts 55

3.1.4 Cytoplasm 62

3.1.5 Cytoskeleton 65

3.1.6 Cell Walls 68

3.2	The Structure of Bacteria	69
3.3	The Structure of Viruses	70
3.4	The Differentiation of Cells	72
4	Biosynthesis and Function of Macromolecules (DNA, RNA, and Proteins)	77
	<i>M. Wink</i>	
4.1	Genomes, Chromosomes, and Replication	77
4.1.1	Genome Size	78
4.1.2	Composition and Function of Chromosomes	83
4.1.3	Mitosis and Meiosis	86
4.1.4	Replication	89
4.1.5	Mutations and Repair Mechanisms	91
4.2	Transcription: From Gene to Protein	96
4.3	Protein Biosynthesis (Translation)	102
5	Distributing Proteins in the Cell (Protein Sorting)	109
	<i>M. Wink</i>	
5.1	Import and Export of Proteins via the Nuclear Pore	111
5.2	Import of Proteins in Mitochondria and Chloroplasts	112
5.3	Protein Transport in the Endoplasmic Reticulum	114
5.4	Vesicle Transport from the ER via the Golgi Apparatus to the Cytoplasmic Membrane	116
6	Diversity of Organisms	121
	<i>M. Wink</i>	
6.1	Prokaryotes	121
6.2	Eukaryotes	122
	Further Reading for Chapters 1–6	126
Part II	Standard Methods in Molecular Biotechnology	
7	Isolation and Purification of Proteins	131
	<i>T. Wieland, S. Lutz</i>	
7.1	Introduction	131
7.2	Producing a Protein Extract	133
7.3	Gel Electrophoretic Separation Methods	134
7.3.1	Principles of Electrophoresis	134
7.3.2	Native Gel Electrophoresis	134
7.3.3	Discontinuous Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)	135
7.3.4	Two-Dimensional (2D) Gel Electrophoresis, Isoelectric Focussing (IEF)	136
7.3.5	Detecting Proteins in Gels	137
7.4	Methods of Protein Precipitation	137

7.5	Column Chromatography Methods	138
7.5.1	General Principles of Separation	138
7.5.1.1	Size Exclusion Chromatography (Gel Filtration)	139
7.5.1.2	Hydrophobic Interaction Chromatography (HIC)	141
7.5.1.3	Ion Exchange Chromatography	141
7.5.1.4	Hydroxyapatite Chromatography	143
7.5.2	Group-specific Separation Techniques	143
7.5.2.1	Chromatography on Protein A or Protein G	143
7.5.2.2	Chromatography on Cibacron Blue (Blue Gel)	144
7.5.2.3	Chromatography on Lectins	144
7.5.2.4	Chromatography on Heparin	145
7.5.3	Purification of Recombinant Fusion Proteins	145
7.5.3.1	Chromatography on Chelating Agents	145
7.5.3.2	Chromatography on Glutathion-Matrices	146
7.6	Examples	146
7.6.1	Example 1: Purification of Nucleoside Diphosphate Kinase from the Cytosol of Bovine Retina Rod Cells	146
7.6.2	Example 2: Purification of Recombinant His ₆ -RGS16 after Expression in <i>E. coli</i>	148
	Further Reading	149
8	Peptide and Protein Analysis with Electrospray Tandem Mass Spectrometry	151
	<i>A. Schlosser, W. D. Lehmann</i>	
8.1	Introduction	151
8.2	Principles of Mass Spectrometry	151
8.3	Mass Precision, Resolution, and Isotope Distribution	152
8.4	Principles of Electrospray Ionization	153
8.5	Tandem Mass Spectrometers	154
8.5.1	Mass Analyzers	154
8.5.2	Triple Quadrupole	155
8.5.3	Ion Trap (Paul Trap)	156
8.5.4	Q-TOF	156
8.5.5	Q-FT-ICR	157
8.6	Peptide Sequencing with MS/MS	157
8.7	Identifying Proteins with MS/MS-Data and Protein Databases	158
8.7.1	Databank Search with Sequence Data	158
8.7.2	Databank Search with MS/MS Raw Data	159
8.8	Determining Protein Molecular Mass	160
8.9	Analysis of Covalent Protein Modification	162
	Further Reading	164
9	Isolation of DNA and RNA	165
	<i>H. Weiher, R. Zwacka, I. Herr</i>	
9.1	Introduction	165

9.2	DNA Isolation	166
9.3	RNA Isolation	168
9.3.1	Enrichment of Messenger RNA (mRNA)	169
	References	169
10	Chromatography and Electrophoresis of Nucleic Acids	171
	<i>H. Weiher, R. Zwacka, I. Herr</i>	
10.1	Introduction	171
10.2	Chromatographic Separation of Nucleic Acids	171
10.3	Electrophoresis	172
10.3.1	Agarose Gel Electrophoresis: Submarine Electrophoresis	173
10.3.2	Pulsed Field Agarose Gel Electrophoresis	174
10.3.3	Polyacrylamide Gel Electrophoresis (PAGE)	174
	Further Reading	174
11	Hybridization of Nucleic Acids	175
	<i>H. Weiher, R. Zwacka, I. Herr</i>	
11.1	The Significance of Base Pairing	175
11.2	Experimental Hybridization, Kinetic, and Thermodynamic Control	176
11.3	Analytical Techniques	176
11.3.1	Clone Detection, Southern Blotting, Northern Blotting, and Gene Diagnosis	176
11.3.2	Expression Screening	178
11.3.3	<i>In Situ</i> Hybridization	179
	Further Reading	180
12	The Use of Enzymes in the Modification of Nucleic Acids	181
	<i>I. Herr, H. Weiher, R. Zwacka</i>	
12.1	Restriction Enzymes (Restriction Endonucleases)	181
12.2	Ligases	183
12.3	Methylases	184
12.4	DNA Polymerases	185
12.5	Nucleases	186
12.6	T4 Polynucleotide Kinase	187
12.7	Calf Intestinal Phosphatase	187
	Further Reading	187
13	Polymerase Chain Reaction (PCR)	189
	<i>A. Mohr, H. Weiher, I. Herr, R. Zwacka</i>	
13.1	Introduction	189
13.2	Techniques	190
13.2.1	Standard PCR	190
13.2.2	RT-PCR	192
13.2.3	Quantitative/Real Time PCR	193
13.2.4	Rapid Amplification of cDNA Ends (RACE)	194

13.3	Areas of Application	195
13.3.1	Genome Analysis	195
13.3.2	Cloning Technology	195
13.3.3	Expression Studies	196
	Further Reading	196
14	DNA Sequencing	197
	<i>R. Zwacka, A. Mohr, I. Herr, H. Weiher</i>	
14.1	Introduction	197
14.2	DNA Sequencing Methods	198
14.2.1	Chemical Sequencing Method (Maxam-Gilbert Method)	198
14.2.2	Enzymatic Sequencing (Sanger-Coulson Method)	198
14.3	Strategies for Sequencing the Human Genome	200
14.4	The Practical Significance of DNA Sequencing	201
	Further Reading	201
15	Cloning Procedures	203
	<i>T. Wieland, S. Lutz</i>	
15.1	Introduction	203
15.2	The Production of Recombinant Vectors	204
15.2.1	The Insert	204
15.2.2	The Vector	208
15.2.3	Essential Components of Vectors	208
15.2.3.1	The Bacterial Origin of Replication (<i>ori</i>)	208
15.2.3.2	Antibiotic Resistance	209
15.2.3.3	Polylinkers	209
15.2.4	Cloning Using Recombination Systems	209
15.2.5	Further Components of Vectors for Prokaryotic Expression Systems	211
15.2.5.1	The Promoter	211
15.2.5.2	The Ribosome Binding Site	211
15.2.5.3	The Termination Sequence	212
15.2.5.4	The Fusion Sequence	212
15.2.6	Further Components of Eukaryotic Expression Vectors	213
15.2.6.1	Eukaryotic Expression Vectors: Yeast	213
15.2.6.2	Eukaryotic Expression Vectors for Mammal Cells	214
15.2.6.3	Viral Expression Systems for Mammalian Cells	218
15.2.7	Nonviral Introduction of Heterologous DNA to Host Organisms (Transformation, Transfection)	220
15.2.7.1	Transformation of Prokaryotes	220
15.2.7.2	Transformation of Yeast Cells	221
15.2.7.3	Transfection of Mammal Cells	221
	Further Reading	222

16	Expression of Recombinant Proteins	223
	<i>T. Wieland, S. Lutz</i>	
16.1	Introduction	223
16.2	Expression of Recombinant Proteins in Host Organisms	224
16.2.1	Expression in <i>E. coli</i>	228
16.2.2	Expression in Yeasts	230
16.2.3	Expression in Insect Cells	232
16.2.3.1	Expression Based on Recombinant Baculoviruses	232
16.2.3.2	Expression of Proteins in Stably Transfected Insect Cells	234
16.2.4	Expression of Proteins in Mammalian Cells	235
16.3	Expression in Cell-Free Systems	236
16.3.1	Expression of Proteins in Reticulocyte Lysates	237
16.3.2	Protein Expression Using <i>E. coli</i> Extracts	237
	Further Reading	238
17	The Patch Clamp Method	239
	<i>R. Kraft, S. Patt</i>	
17.1	Biological Membranes and Ion Channels	239
17.2	Physical Foundations of the Patch Clamp Method	240
17.3	Patch Clamp Configurations	241
17.4	Heterologous Expression Systems and Section Preparations	244
	Further Reading	245
18	Cell Cycle Analysis	247
	<i>S. Wöfl, A. Kitanovic</i>	
18.1	Analyzing the Cell Cycle	247
18.2	Experimental Analysis of the Cell Cycle	249
18.2.1	Preparing Synchronized Cell Cultures of <i>Saccharomyces cerevisiae</i>	250
18.2.2	Centrifugal Elutriation	250
18.2.3	Cell Cycle Arrest Using the Alpha Factor	251
18.2.4	The Identification of Cell Cycle Stages	252
18.2.4.1	The Budding Index	252
18.2.4.2	Fluorescent Staining of the Nucleus	252
	Further Reading	256
19	Techniques in Microscopy	257
	<i>G. Fricker</i>	
19.1	Light Microscopy	257
19.2	Phase Contrast Light Microscopy	257
19.3	Darkfield Microscopy	259
19.4	Polarization and Interference Microscopy	259
19.5	Fluorescence Microscopy	260
19.6	Confocal Fluorescence Microscopy	261
19.7	Electron Microscopy	262
	Further Reading	264

20	Laser Applications	265
	<i>M. Vogel, R. Fink</i>	
20.1	Principles of Laser Technology	265
20.2	Properties of Laser Radiation	267
20.3	Types of Lasers and Their Setups	268
20.4	Applications	269
20.4.1	Confocal and Multiphoton Microscopy	269
20.4.2	Optical Tweezers	270
20.4.3	Laser Microdissection	270
	Further Reading	270
Part III	Key Topics	
21	Genomics	273
	<i>M. Frohme, St. Wiemann</i>	
21.1	Introduction	273
21.2	Technological Developments in Sequencing	276
21.2.1	Sequencing Technology	276
21.2.2	Biochemistry	276
21.2.3	Equipment	279
21.2.4	Software and Informatics for Sequencing	280
21.3	Genome Sequencing	281
21.3.1	Mapping	281
21.3.1.1	Restriction Mapping and Restriction Fingerprinting	284
21.3.1.2	BAC-End Sequencing	285
21.3.1.3	Genetic Mapping	285
21.3.1.4	Radiation Hybrid Mapping	288
21.3.1.5	HAPPY Mapping	289
21.3.1.6	Mapping by Hybridization	290
21.3.1.7	STS, ESTs, SNPs, and Sequencing Length Polymorphisms (AFLPs, RAPD)	292
21.3.1.8	FISH, Fibre Fish, Optical Mapping, and CGH	295
21.3.2	Time-line of Genome Sequencing	296
21.3.3	Genome Sequencing Strategies	296
21.3.3.1	Conventional Approach: Random Shotgun Strategy	296
21.3.3.2	The Whole-Genome Shotgun Strategy	300
21.3.3.3	Sequencing of the Human Genome	302
21.3.4	Outlook for Genome Sequencing	303
21.4	cDNA Projects	304
21.4.1	cDNAs Represent the Cell's mRNA	304
21.4.2	Production of cDNA Libraries	306
21.4.3	EST-Projects for Gene Identification	310
21.4.3.1	What is an EST?	310
21.4.3.2	IMAGE Consortium: CGAP	313
21.4.3.3	UniGene	314

- 21.4.4 Full-length Project for the Production of Resources for Functional Genomics 316
- Further Reading 318

22 Functional Genomics 321

M. Frohme, St. Wiemann

- 22.1 Introduction 321
- 22.2 The Identification and Analysis of Individual Genes 324
 - 22.2.1 Positional Cloning 325
 - 22.2.2 Gene Trap 328
 - 22.2.3 DNA/RNA *In Situ* Hybridization 329
 - 22.2.4 Tissue Arrays 330
- 22.3 The Investigation of Transcriptional Activity 331
 - 22.3.1 SAGE (Serial Analysis of Gene Expression) 332
 - 22.3.2 Subtractive Hybridization 334
 - 22.3.3 RNA Fingerprinting 337
 - 22.3.4 Array-based Techniques 340
 - 22.3.4.1 Macroarrays 343
 - 22.3.4.2 Microarrays 345
 - 22.3.4.3 Global and Specific Arrays 347
 - 22.3.5 Specificity and Sensitivity 349
- 22.4 Cell-based Methods 350
 - 22.4.1 GFP Techniques 350
 - 22.4.2 Alternatives to GFP 351
 - 22.4.3 Fluorescence Resonance Energy Transfer (FRET) 352
 - 22.4.4 Fluorescence Recovery After Photobleaching (FRAP) 354
 - 22.4.5 Cell-based Assays 355
 - 22.4.5.1 Assay Design 356
 - 22.4.5.2 Pipetting Systems 357
 - 22.4.5.3 Reading and Recording of Data 358
 - 22.4.5.4 Data Analysis 359
- 22.5 Functional Analysis of Entire Genomes 360
 - 22.5.1 Genotypic Screening in Yeast 360
 - 22.5.2 Phenotypic Screening in the Mouse 361
- Further Reading 363

23 Protein-Protein and Protein-DNA Interaction 365

P. Uetz, E. Pohl

- 23.1 Protein-Protein Interactions 366
 - 23.1.1 Classification and Specificity: Protein Domains 366
 - 23.1.2 Protein Networks and Complexes 367
 - 23.1.3 Structural Properties of Interacting Proteins 370
 - 23.1.4 Which Forces Mediate Protein-Protein Interactions? 371
 - 23.1.4.1 Thermodynamics 371
 - 23.1.4.2 Energetics 372

23.1.5	Methods to Examine Protein–Protein Interactions	373
23.1.6	Regulation of Protein–Protein Interactions	374
23.1.7	Theoretical Prediction of Protein–Protein Interactions	375
23.1.8	Biotechnological and Medical Applications of Protein–Protein Interactions	376
23.2	Protein–DNA Interactions	378
23.2.1	Sequence-specific DNA-binding	378
23.2.2	Thermodynamic Considerations Regarding Protein–DNA Complexes	379
23.2.3	Methods to Study Protein–DNA Interactions	379
23.2.4	Medical Relevance of Protein–DNA Interactions	384
23.2.5	Biotechnological Applications of Protein–DNA Interactions	385
	Further Reading	385
24	Bioinformatics	387
	<i>B. Brors, K. Fellenberg</i>	
24.1	Introduction	387
24.2	Data Sources	388
24.2.1	Primary Databanks: EMBL/GenBank/DDBJ, PIR, SwissProt	388
24.2.2	Motif Databanks: BLOCKS, Prosite, PFAM, ProDom, SMART	389
24.2.3	Molecular Structure Databanks: PDB, SCOP	389
24.2.4	Transcriptome Databanks: SAGE, ArrayExpress, GEO	390
24.2.5	Reference Databanks: PubMed, OMIM, GeneCards	390
24.3	Sequence Analysis	391
24.3.1	Kyte–Doolittle, Helical Wheel, Signal Sequence Analysis	391
24.3.2	Pair Alignment	393
24.3.2.1	Local/Global	394
24.3.2.2	Optimal/Heuristic	394
24.3.3	Alignment Statistics	394
24.3.4	Multiple Alignment	395
24.3.5	Phylogenetic Analysis	396
24.4	Gene Prediction	398
24.4.1	Neuronal Networks or Hidden Markov Models Based on Hexanucleotide Composition	399
24.4.2	Comparison with ESTs or Other Genomes (<i>Fugu</i> , Mouse)	400
24.5	Bioinformatics in Transcriptome and Proteome Analysis	402
24.5.1	Pre-processing, Normalization	402
24.5.2	Character Selection	404
24.5.3	Similarity Measures: Euclidean Distance, Correlation, Manhattan Distance, Mahalanobis Distance, Entropy Measures	405
24.5.4	Unsupervised Learning Procedures: Clustering, Main Component Analysis, Multidimensional Scaling, Correspondence Analysis	406
24.5.5	Supervised Learning Procedures: Linear Discriminant Analysis, Choice Trees, Support Vector Machines, Artificial Neuronal Networks	407

24.6	Systems Biology	408
24.6.1	Networks: Boolean Networks and Bayesian Networks	410
24.6.2	Deterministic Descriptions: Common and Partial Differential Equations	410
24.6.3	Nondeterministic Description: Stochastic Simulation	411
	Further Reading	411
25	Drug Research	413
	<i>U. Denschle, M. Kögl, R. Tolle</i>	
25.1	Introduction	413
25.2	Active Compounds and Their Targets	414
25.2.1	Genomic Methods for the Identification of Targets	415
25.2.2	Bioinformatic Identification of Targets	416
25.2.3	Comparative Genome Analysis	417
25.2.4	Experimental Target Identification: <i>In Vitro</i> Method	418
25.2.5	Experimental Identification of Targets: Model Organisms	418
25.2.6	Experimental Target Identification in Humans	419
25.2.7	The Difference Between Target Candidates and Genuine Targets	420
25.2.8	Biologicals	422
25.2.9	DNA and RNA in New Therapeutic Approaches	424
25.2.10	Patent Protection for Targets	424
25.2.11	Compound Libraries	425
25.2.12	High-Throughput Screening	426
25.2.13	High Quality Paramounts in Screening Assays	430
25.2.14	Virtual Ligand Screening	431
25.2.15	The Activity of Agents Described in Terms of Efficiency and Potency	431
25.2.16	Chemical Optimization of Lead Structures	432
25.3	Pre-Clinical Pharmacology and Toxicology	432
25.4	Clinical Development	433
25.5	Clinical Testing	434
	Further Reading	435
26	Drug Targeting and Prodrugs	437
	<i>G. Fricker</i>	
26.1	Drug Targeting	437
26.1.1	Passive Targeting by Exploiting Special Physiological Properties of the Target Tissue	438
26.1.2	Physical Targeting	439
26.1.3	Active Targeting	440
26.1.4	Cellular Carrier Systems	444
26.2	Prodrugs	445
26.2.1	Prodrugs to Improve Drug Solubility	445
26.2.2	Prodrugs to Increase Stability	446

26.3	Penetration of Drugs through Biological Membranes	446
26.4	Prodrugs to Extend Duration of Effect	448
26.5	Prodrugs for the Targeted Release of a Drug	449
26.6	Prodrugs to Minimize Side Effects	451
	Further Reading	451
27	Molecular Diagnostics in Medicine	455
	<i>S. Wöfl, R. Gessner</i>	
27.1	Uses of Molecular Diagnostics	456
27.1.1	Introduction	456
27.1.2	Monogenic and Polygenic Diseases	456
27.1.3	Individual Variability in the Genome: Forensics	459
27.1.4	Individual Variability in the Genome: HLA Typing	459
27.1.5	Individual Variability in the Genome: Pharmacogenomics	459
27.1.6	Individual Variability in the Genome: Susceptibility to Infectious Diseases	460
27.1.7	Viral Diagnosis	460
27.1.8	Microbial Diagnosis and Resistance Diagnosis	461
27.2	Which Molecular Variations should be detected?	462
27.2.1	Point Mutations	462
27.2.2	Insertions and Deletions	464
27.2.3	Nucleotide Repeats	464
27.2.4	Deletion or Duplication of Genes	465
27.2.5	Recombination Between Chromosomes	465
27.2.6	Epigenetic Changes	466
27.3	Molecular Diagnostic Methods	466
27.3.1	DNA/RNA Purification	467
27.3.2	Determination of Known Sequence Variations	467
27.3.2.1	Direct Length Polymorphism	467
27.3.2.2	RFLP	467
27.3.2.3	ACRS	469
27.3.2.4	ARMS	469
27.3.2.5	MS-PCR	469
27.3.2.6	Allele-specific Hybridization	470
27.3.2.7	LCR	470
27.3.2.8	Minisequencing	470
27.3.2.9	Pyrosequencing	472
27.3.2.10	Quantitative PCR	472
27.3.2.11	Chip Technology	473
27.3.2.12	Production and Manufacture of Microarrays	474
27.3.2.13	Determination of Unknown Mutations	476
27.4	Outlook	477
	Further Reading	477

28	Recombinant Antibodies and Phage Display	479
	<i>S. Dübel</i>	
28.1	Introduction	479
28.2	Why Recombinant Antibodies?	481
28.2.1	Recombinant Antibodies are Available <i>In Vitro</i> without Immunization	481
28.2.2	Antibodies with New Characteristics Can Be Created	482
28.3	Obtaining Specific Recombinant Antibodies	483
28.3.1	Preparation of the Variety of Antibody Genes	483
28.3.2	Selection Systems for Recombinant Antibodies	485
28.3.2.1	Transgenic Mice	485
28.3.2.2	<i>In Vitro</i> Selection Systems	486
28.4	Production of Recombinant Antibodies	489
28.4.1	Recombinant Production Systems	489
28.4.2	Purification of Recombinant Antibodies and their Fragments	491
28.5	Formats for Recombinant Antibodies	491
28.5.1	Monospecific Antibody Fragments	493
28.5.1.1	Fab Fragments	493
28.5.1.2	Fv Fragments	493
28.5.1.3	Single Chain Antibody Fragments (scFv)	494
28.5.1.4	Disulphide Stabilized Fv Fragments (dsFv)	494
28.5.1.5	V _H and Camel Antibodies	495
28.5.2	Multivalent Antibody Fragments	495
28.5.2.1	Bifunctional Antibodies	496
28.5.2.2	Bispecific Antibodies	496
28.6	Applications of Recombinant Antibodies	499
28.6.1	Clinical Applications	499
28.6.2	Applications in Research and <i>In Vitro</i> Diagnostics	500
28.6.2.1	Recombinant Antibodies in Laboratory Diagnostics	501
28.6.2.2	Intracellular Antibodies	501
28.6.2.3	Recombinant Antibodies as Binding Molecules for Arrays	501
28.7	Outlook	502
	Further Reading	502
29	Genetically Modified Mice (Transgenic and Knockout) and their Impact on Medicine	511
	<i>R. Sprengel</i>	
29.1	Overview	511
29.2	Transgenic Mice	514
29.2.1	Retroviral Infection of the Transgenic Mouse	515
29.2.2	Injection into the Pronucleus for the Production of Transgenic Mice	515
29.2.3	Homologous Recombination for the Production of Transgenic Mice	517
29.3	The Impact of Genetically Modified Mice in Biomedicine	521
	Further Reading	522

30	Gene Therapy: Strategies and Vectors	523
	<i>I. Herr</i>	
30.1	Introduction	523
30.2	Principles of Somatic Gene Therapy	525
30.3	Germ Line Therapy	527
30.4	Setbacks in Gene Therapy	528
30.5	Vectors for Gene Therapy	530
30.5.1	Retroviral Vectors	531
30.5.2	Adenoviral Vectors	535
30.5.3	Adeno-Associated Virus (AAV)	538
30.5.4	Other Viral Vectors	540
30.6	Specific Expression	541
	Further Reading	542
31	Modified DNA, PNA, and Applications in Medicine and Biotechnology	543
	<i>N. Metzler-Nolte</i>	
31.1	Introduction	543
31.2	Modified Nucleic Acids	544
31.2.1	Phosphorthioate	545
31.2.2	Methylphosphonate	547
31.2.3	Peptide Nucleic Acids (PNA)	548
31.3	Interactions of DNA Analogs with Complementary DNA and RNA	549
31.3.1	Melting Temperature	549
31.3.2	Mismatch Sensitivity	551
31.4	Applications	552
31.4.1	Antisense Technique	552
31.4.2	Other Applications for PNA	554
	Further Reading	556
32	Plant Biotechnology	557
	<i>R. Hell, H. Hillebrand</i>	
32.1	Introduction	557
32.1.1	The Green Genetic Engineering – A New Method on the Way to Traditional Goals	557
32.1.2	Special Aspects of Plant Biotechnology	559
32.2	Development of Transgenic Plants	559
32.2.1	Transformation Through DNA Transfer	560
32.2.1.1	<i>Agrobacterium</i> as a Natural Transformation System	560
32.2.1.2	Biolistic Method: Gene Gun	563
32.2.1.3	Other Physical Transformation Systems	565
32.2.1.4	Plastid Transformation	565
32.2.1.5	Viral Systems	567
32.3	Selection of Transformed Plant Cells	568

32.3.1	Requirements for an Optimal Selection Marker System	568
32.3.2	Negative Selection Marker Systems	570
32.3.3	Positive Selection Marker System	571
32.3.4	Counter Selection	572
32.3.5	Visual Markers	573
32.3.6	Selection Systems Genetic Techniques, Safety, and Marker-free Plants	573
32.4	Regeneration of Transgene Plants	575
32.4.1	Regeneration Procedures	575
32.4.2	Compounds of Regeneration Media	576
32.5	Plant Analysis: Identification and Characterization of Genetically Engineered Plants	577
32.5.1	DNA and RNA Verification	577
32.5.2	Protein Analysis	579
32.5.3	Genetic and Molecular Maps	579
32.5.4	Stability of Transgene Plants	580
	Further Reading	581
33	Biocatalysis in the Chemical Industry	583
	<i>B. Hauer, M. Breuer</i>	
33.1	Introduction	583
33.2	Bioconversion/Enzymatic Procedures	590
33.3	Development of an Enzyme for the Industrial Biocatalysis	593
33.3.1	Identification of Novel Biocatalysts	593
33.3.2	Improvement of Biocatalysts	594
33.3.3	Production of Biocatalysts	596
33.3.4	Outlook	596
33.3.5	Case Example 1: Screening for New Nitrilases	597
33.3.6	Case Example 2: Use of Known Enzymes for New Reactions: Lipases for the Production of Optically Active Amines and Alcohols	598
33.3.7	Case Example 3: Enzyme Optimization with Rational and Evolutive Methods	600
33.4	Fermentative Procedures	601
33.4.1	Improvement of Fermentation Processes	601
33.4.2	Classical Strain Optimization	602
33.4.3	Metabolic Engineering	603
33.4.4	Case Example 4: Production of Glutamine Acid with <i>Corynebacterium glutamicum</i>	604
33.4.4.1	Molecular Mechanism of Glutamate Overproduction	605
33.4.5	Case Example 5: Production of Lysine with <i>Corynebacterium glutamicum</i>	606
33.4.5.1	Molecular Mechanism of Leucine Biosynthesis	607
33.4.5.2	Deregulation of the Key Enzyme Aspartate Kinase	608
33.4.6	Genomic Research and Functional Genomics	609
33.4.7	Case Example 6: Fermentative Penicillin Production	610

- 33.4.8 Case Example 7: Vitamin B2 Production 610
- 33.4.8.1 Riboflavin Biosynthesis 611
- 33.4.8.2 Classical Strain Development 611
- Further Reading 612

Part IV Biotechnology in Industry

- 34 **Industrial Application
(Biotech Industry, Markets and Opportunities) 615**
J. Schüler
 - 34.1 Historical Overview and Definitions of Concepts 615
 - 34.2 Areas of Industrial Application of Molecular Biotechnology 617
 - 34.2.1 Red Biotechnology 617
 - 34.2.1.1 Biopharmaceutical Drug Development 617
 - 34.2.1.2 Drug Delivery 620
 - 34.2.1.3 Cell and Gene Therapy 621
 - 34.2.1.4 Tissue Engineering and Regenerative Medicine 622
 - 34.2.1.5 Pharmacogenomics, Personalized Medicine,
and System Biology 623
 - 34.2.1.6 Systems Biology 624
 - 34.2.1.7 Molecular Diagnostics 624
 - 34.2.2 Green Biotechnology 625
 - 34.2.2.1 Transgenic Plants 626
 - 34.2.2.2 Genomic Approaches in Green Biotechnology 626
 - 34.2.2.3 Novel Food and Functional Food 627
 - 34.2.2.4 Livestock Breeding 627
 - 34.2.3 Grey/White Biotechnology 627
 - 34.2.3.1 Technical Enzymes 627
 - 34.2.3.2 The Environment 628
 - 34.3 The Status Quo of the Biotech Industry Worldwide 628
 - 34.3.1 Global Overview 628
 - 34.3.2 USA 629
 - 34.3.3 Europe 629
 - Further Reading 629
- 35 **Patents in the Pharmaceutical Biotechnology Industry:
Legal and Ethical Issues 631**
David B. Resnik
 - 35.1 Patent Law 631
 - 35.1.1 What is a Patent? 631
 - 35.1.2 How Does One Obtain a Patent? 633
 - 35.1.3 What is the Proper Subject Matter for a Patent? 634
 - 35.1.4 Types of Patents in Pharmaceutical Biotechnology 635
 - 35.1.5 Patent Infringement 635
 - 35.1.6 International Patent Law 636

35.2	Ethical and Policy Issues in Biotechnology Patents	637
35.2.1	No Patents on Nature	637
35.2.2	Threats to Human Dignity	638
35.2.3	Access to Technology	640
35.2.4	Benefit Sharing	642
35.3	Conclusion	644
	Further Reading	644
36	Drug Approval in the European Union and United States	647
	<i>Gary Walsh</i>	
36.1	Introduction	647
36.2	Regulation within the European Union	648
36.2.1	The EU Regulatory Framework	648
36.2.2	The EMEA	649
36.2.3	New Drug Approval Routes	650
36.2.3.1	The Centralized Procedure	650
36.2.3.2	Mutual Recognition	653
36.3	Regulation in the USA	653
36.3.1	CDER and CBER	654
36.3.2	The Approvals Procedure	655
36.4	International Regulatory Harmonization	657
	Further Reading	658
37	The Emergence of a Biotechnology Industry	659
	<i>C. Kremoser</i>	
	Further Reading	669
38	The 101 of Founding a Biotech Company	671
	<i>C. Kremoser</i>	
38.1	The First Steps Towards Your Own Company	671
38.2	Employees: Recruitment, Remuneration, Participation	677
39	Marketing	683
	<i>C. Kremoser</i>	
39.1	Introduction	683
39.2	What Types of Deals are Possible?	685
39.2.1	What Milestone or License Fees Are Effectively Paid in a Biotech/Pharma Cooperation?	685
39.3	Public Relations (PR) and Investor Relations (IR) in Biotech Companies	687
	Further Reading	688
Glossary		689
	<i>M. Wink</i>	
Subject Index		733