

Contents

Foreword	xi
Foreword to the First Edition	xiii
Acknowledgments	xv
1 What Is Molecular Biology?	1
1.1 The Substrate of Molecular Biology: The Molecular World for Beginners	2
1.2 What Is Required for This Work?	7
1.3 Safety in the Laboratory	8
2 Fundamental Methods	11
2.1 Differences in Nucleic Acids	11
2.2 Precipitation and Concentration of Nucleic Acids	13
2.2.1 Alcohol Precipitation	13
2.2.2 Concentrators	16
2.2.3 Savant Speed Vac	17
2.2.4 Salting Out	17
2.3 Purification of Nucleic Acids	17
2.3.1 Phenol-Chloroform Extraction	17
2.3.2 Precipitation Using Polyethylene Glycol	19
2.3.3 Protein-Binding Filter Membranes	19
2.3.4 Anion-Exchange Columns	19
2.3.5 Glass Milk	20
2.3.6 Cesium Chloride Density Gradient	21
2.3.7 Dialysis	22
2.4 Determining the Concentration of Nucleic Acid Solutions	22
2.4.1 Optical Density Measurements with the Aid of Absorption Spectrometry	22
2.4.2 Determination of Concentration by Means of Agarose Gels	24
2.4.3 Dot Quantification	24
2.4.4 Fluorometric Determination	25
2.4.5 Nucleic Acid Dipsticks	25
2.4.6 Enzymatic Evidence	25
2.5 Methods of DNA Preparation	26
2.5.1 Preparation of Plasmid DNA on a Small Scale	26
2.5.2 Preparation of Plasmid DNA on a Large Scale	28

2.5.3	Bacterial Media	31
2.5.4	Preparation of Phage DNA	31
2.5.5	Preparation of Single-Stranded DNA with the Aid of Helper Phages	35
2.5.6	Preparation of Genomic DNA	35
3	The Tools	37
3.1	Restriction Enzymes	37
3.1.1	Nomenclature	37
3.1.2	The Activity Test	39
3.1.3	Making a Restriction Digestion	40
3.1.4	Difficulties Associated with Restriction Digestion	41
3.1.5	Works of Reference for Restriction Digestion	44
3.2	Gels	45
3.2.1	Agarose Gels	45
3.2.2	Isolating DNA Fragments from Agarose Gels	50
3.2.3	Polyacrylamide Gels	53
3.2.4	Isolating DNA Fragments from Polyacrylamide Gels	55
3.2.5	Pulse-Field Gel Electrophoresis	56
3.2.6	Capillary Electrophoresis	56
3.3	Blotting	57
3.3.1	Southern Blots	57
3.3.2	Northern Blots	60
3.3.3	Dot Blots and Slot Blots	62
4	The Polymerase Chain Reaction	65
4.1	Standard Polymerase Chain Reaction	65
4.2	Suggestions for Improving the Polymerase Chain Reaction	74
4.2.1	Nested Polymerase Chain Reaction	76
4.2.2	Multiplex Polymerase Chain Reaction	76
4.2.3	Amplification of Longer DNA Fragments	77
4.3	PCR Applications	78
4.3.1	Reverse Transcription-Polymerase Chain Reaction	78
4.3.2	Rapid Amplification of cDNA Ends	79
4.3.3	Amplification of Coincidental Products	80
4.3.4	Classic Quantitative Polymerase Chain Reaction	82
4.3.5	Real-Time Quantitative Polymerase Chain Reaction	84
4.3.6	Inverse Polymerase Chain Reaction	89
4.3.7	Biotin-RAGE Method and Supported Polymerase Chain Reaction	89
4.3.8	Mutagenesis with Modified Primers	90
4.3.9	Amplification Refractory Mutation System	91
4.3.10	In Situ Polymerase Chain Reaction	92
4.3.11	Cycle Sequencing	92
4.3.12	cDNA Synthesis	93
4.3.13	Single-Cell Polymerase Chain Reaction	94

5 RNA	95
5.1 Inactivating RNases	95
5.2 Methods of RNA Isolation	96
5.2.1 Single-Step Method	96
5.2.2 Lysis with Nonidet P40	97
5.2.3 General Information	97
5.2.4 Determination of the RNA Concentration	98
5.3 Methods of mRNA Isolation	98
5.3.1 Purchasing RNA	99
5.4 Reverse Transcription: cDNA Synthesis	99
5.5 In Vitro Transcription: RNA Synthesis	100
5.6 RNA Interference	102
6 Cloning DNA Fragments	105
6.1 The Basics of Cloning	105
6.1.1 The Vector	106
6.1.2 The DNA Fragment	107
6.1.3 Fill-in Reaction	108
6.1.4 DNA Quantities	109
6.1.5 The Ligation	110
6.1.6 Cloning Polymerase Chain Reaction Products	111
6.1.7 Cloning with Recombinase Systems	113
6.2 Choosing Vectors for Cloning	116
6.2.1 Plasmids	116
6.2.2 Phages	118
6.2.3 Cosmids	119
6.2.4 P1 Artificial Chromosomes and Bacterial Artificial Chromosomes	120
6.2.5 Yeast Artificial Chromosomes	121
6.3 Choosing the Bacteria	122
6.4 Construction of Competent Cells and Their Transformation	122
6.4.1 Calcium Chloride Method	123
6.4.2 Rubidium Chloride Method	124
6.4.3 TSS Method	124
6.4.4 Electroporation	124
6.4.5 Testing the Competency of Bacteria	126
6.5 Problems Associated with Cloning	127
6.6 Storage of Clones	128
7 Hybridization: How to Track Down DNA	131
7.1 Production of Probes	131
7.1.1 Methods for the Production of Labeled Probes	132

7.2	Hybridization	136
7.2.1	The Hybridization Buffer	136
7.2.2	The Hybridization Vessels	137
7.2.3	The Hybridization Temperature	137
7.2.4	Washing	138
7.3	Verification of Labeled DNA	138
7.3.1	Autoradiography	138
7.3.2	Nonradioactive Methods of Detection	140
7.4	Screening of Recombinant DNA Banks	143
7.4.1	Plating Out the Bank	143
7.4.2	Filter Transfer	143
7.4.3	Filter Hybridization	144
7.4.4	Filter Exposure	144
7.4.5	Clone Detection	145
7.4.6	Bank Screening in the Future	146
7.5	Two-Hybrid System	146
8	DNA Analysis	151
8.1	Sequencing	151
8.1.1	Radioactive Sequencing	153
8.1.2	Nonradioactive Sequencing and Automatic Sequencing Units	154
8.1.3	Minisequencing	156
8.1.4	Pyrosequencing	157
8.1.5	Idiosyncrasies in Sequencing: Octamers	159
8.2	Methods of Analyzing DNA for Mutations	160
8.2.1	Restriction Fragment Length Polymorphism	160
8.2.2	Single-Strand Conformation Polymorphism	161
8.2.3	Denaturing Gradient Gel Electrophoresis	162
8.2.4	Temporal Temperature Gradient Electrophoresis	164
8.2.5	Heteroduplex Analysis	165
8.2.6	Amplification Refractory Mutation System	165
8.2.7	Enzyme Mismatch Cleavage	165
8.2.8	Protein Truncation Test	167
9	Investigating the Function of DNA Sequences	169
9.1	Investigating Transcription in Tissues	170
9.1.1	Ribonuclease Protection Assay	170
9.1.2	Real-Time Quantitative Polymerase Chain Reaction	170
9.1.3	In Situ Hybridization	171
9.1.4	Fluorescence In Situ Hybridization of Chromosomes	173
9.1.5	In Situ Polymerase Chain Reaction	175
9.1.6	Microarrays	175
9.2	Mutagenesis	180

9.3	In Vitro Translation	189
9.4	Expression Systems	190
9.4.1	Bacterial Expression Systems	191
9.4.2	Baculoviral Expression Systems	191
9.4.3	Additional Expression Systems	193
9.4.4	Heterologous Expression in Mammalian Cells	194
9.4.5	Transfection Methods	195
9.4.6	Cotransfection of Several Genes	202
9.4.7	Transient and Stable Transfections	203
9.4.8	Reporter Genes	204
9.5	Transgenic Mice	209
9.5.1	Methods of Gene Transfer	210
9.6	Regulation of Transgenic Expression	215
9.6.1	The Tet System	215
9.6.2	The Ecdysone System	216
9.7	Gene Therapy	217
9.8	Genomics	219
10	Using Computers	223
10.1	Something Quite Earnest	223
10.2	A Matter of Practice	225
11	Suggestions for Career Planning: The Machiavelli Short Course for Young Researchers	227
12	Concluding Thoughts	233
Appendix 1		235
Useful Figures and Tables		235
Standard Solutions and Bacterial Media		235
Solutions		235
Bacterial Media		236
Glossary		237
Appendix 2		241
Suppliers		241
Recommended Literature		244
Reading Material for Leisure Time		245
Index		247