
Contents

Preface	v
Contributors	xv
PART I NUCLEIC ACID EXTRACTION	
1 Isolation of High-Molecular-Weight DNA from Animal Cells	3
<i>Ian Garner</i>	
2 Isolation of mRNA by Affinity Chromatography	9
<i>Sian Bryant and David L. Manning</i>	
3 Isolation and Purification of DNA from Plants	13
<i>Justin Stacey and Peter G. Isaac</i>	
4 Purification of Uncontaminated, Intact Plant RNA	17
<i>Shu-Hua Cheng, Brandon D. Moore, and Jeffrey R. Seemann</i>	
5 An Improved Method to Isolate Mitochondrial RNA from Green Plant Tissue	23
<i>Fei Ye and Ralf Reski</i>	
6 Isolating Chromosomal DNA from Bacteria	29
<i>Elisabeth Chachaty and Patrick Saulnier</i>	
7 Bacterial DNA Extraction for Polymerase Chain Reaction and Pulsed-Field Gel Electrophoresis	33
<i>Elisabeth Chachaty and Patrick Saulnier</i>	
8 Isolation of Fungal Nucleic Acids	37
<i>Surapareddy Sreenivasaprasad</i>	
9 Total RNA Isolation from Bacteria	47
<i>John Heptinstall</i>	
10 Simultaneous RNA and DNA Extraction from Biopsy Material, Culture Cells, Plants, and Bacteria	53
<i>Udo Döbbeling</i>	
11 Spectrophotometric Analysis of Nucleic Acids	57
<i>John Heptinstall and Ralph Rapley</i>	
PART II BASIC SEPARATION AND ANALYSIS OF DNA	
12 Restriction Endonuclease Digestion of DNA	63
<i>Duncan R. Smith</i>	
13 Agarose Gel Electrophoresis of Nucleic Acids	67
<i>D. Ross Williams and Ralph Rapley</i>	

14	Preparation of RNA Dot Blots	71
	Rachel Hodge	
15	Native Polyacrylamide Gel Electrophoresis	73
	Adrian J. Harwood	
16	Southern Blotting of Agarose Gels by Capillary Transfer	77
	Ralph Rapley and Jane Davenport-Jones	
17	Pulsed-Field Gel Electrophoresis	81
	John Maule	
18	HPLC of DNA and PCR Products	105
	Elena D. Katz	
 PART III PROBE DESIGN, SYNTHESIS, AND LABELING		
19	End-Labeling of DNA Fragments.....	117
	Adrian J. Harwood	
20	Nick Translation and Random Hexamer Labeling of DNA	123
	Jane Davenport-Jones	
21	Generation of Labeled Probes by Polymerase Chain Reaction	127
	Y. M. Dennis Lo and Shu F. An	
22	Nonradioactive Oligonucleotide Probe Labeling	135
	Sue Fowler and Ian Durrant	
23	Preparation of Direct, Enzyme-Labeled DNA Probes	145
	Ian Durrant and Timothy Stone	
24	Random Prime Labeling of DNA Probes with Fluorescein-Tagged Nucleotides	149
	Bronwen M. Harvey, Claire B. Wheeler, and Martin W. Cunningham	
25	Hybridization and Detection of Fluorescein-Labeled DNA Probes Using Chemiluminescence	153
	Claire B. Wheeler, Bronwen M. Harvey, and Martin W. Cunningham	
26	Hybridization of Enzyme-Labeled Probes and Detection by Chemiluminescence	157
	Timothy Stone and Ian Durrant	
27	Hybridization and Competition Hybridization of Southern Blots	163
	Rosemary Kelsell	
28	Autoradiography and Fluorography	169
	Eric Quéméneur	
 PART IV RNA ANALYSIS TECHNIQUES		
29	Formaldehyde Gel Electrophoresis of Total RNA	177
	Sian Bryant and David L. Manning	
30	RNA Probes for the Analysis of Gene Expression	181
	Dominique Belin	
31	Primer Extension Analysis of mRNA	195
	Maggie Walmsley, Mark Leonard, and Roger Patient	

32	S1 Mapping Using Single-Stranded DNA Probes	201
	Stéphane Viville and Roberto Mantovani	
33	Measurements of Rate of Transcription in Isolated Nuclei by Nuclear "Run-Off" Assay	207
	Rai Ajit K. Srivastava and Gustav Schonfeld	
34	One-Tube RT-PCR with Sequence-Specific RT Primers	213
	Ulrich Pfeffer and Paola Ferro	
35	Characterization of RNA Using Continuous RT-PCR Coupled with ELOSA	219
	François Mallet	
36	Quantitative Analysis of RNA Species by Polymerase Chain Reaction and Solid-Phase Minisequencing	229
	Anu Suomalainen and Ann-Christine Syvänen	
37	Nonradioactive Northern Blotting of RNA	239
	Rainer Löw	
38	Analysis of RNA by Northern Blotting Using Riboprobes	249
	Rai Ajit K. Srivastava	
PART V GENE LIBRARY CONSTRUCTION AND SCREENING		
39	Production of Double-Stranded cDNA for Gene Library Synthesis	261
	Jane Kirk and Steve Mayall	
40	Using Rapid Amplification of cDNA Ends (RACE) to Obtain Full-Length cDNAs	267
	Yue Zhang and Michael A. Frohman	
41	cDNA Library Construction Using Streptavidin-Paramagnetic Beads and PCR	289
	Kris N. Lambert and Valerie M. Williamson	
42	Rapid (Ligase-Free) Subcloning of Polymerase Chain Reaction Products	295
	Alan R. Shuldiner and Keith Tanner	
43	Subtraction Hybridization cDNA Libraries	305
	Clifford W. Schweinfest, Peter S. Nelson, Michael W. Graber, Rita I. Demopoulos, and Takis S. Papas	
44	Cloning Polymerase Chain Reaction Products Utilizing the T/A Overhang and a Kit	319
	Melissa Lail-Trecker	
45	Extraction and Purification of Plasmid DNA	327
	Craig Winstanley and Ralph Rapley	
46	Biotinylated Probes in Colony Hybridization	333
	Michael J. Haas	
47	Cloning Long Polymerase Chain Reaction Products	339
	Songrong Ren and J. Michael Ruppert	
48	Cloning DNA Fragments in M13 Vectors	347
	David Walsh	

49	cDNA Library Construction for the Lambda ZAP®-Based Vectors	355
	<i>Marjory A. Snead, Michelle A. Alting-Mees, and Jay M. Short</i>	
50	Expression and Preparation of Fusion Proteins from Recombinant λ gt11 Phages	367
	<i>Sheng-He Huang and Ambrose Jong</i>	
51	Antibody Screening of Bacteriophage λ gt11 DNA Expression Libraries	373
	<i>Peter Jones</i>	
52	Screening cDNA Libraries by Hybridization with Double-Stranded DNA Probes and Oligonucleotides	381
	<i>Caroline A. Austin</i>	
53	cDNA Library Screening with the Tetramethylammonium Chloride (TMAC) Technique Using Highly Degenerate Oligonucleotide Probes	389
	<i>Bent Honoré and Peder Madsen</i>	
54	Screening Recombinant Libraries by Polymerase Chain Reaction	397
	<i>Michael W. King</i>	
55	Construction and Screening of Cosmid Libraries	405
	<i>Jens Hanke and Jörg D. Hoheisel</i>	
56	Generation of Large Insert YAC Libraries	415
	<i>Zoia Larin, Anthony P. Monaco, and Hans Lehrach</i>	
57	YAC Library Storage and Transport	425
	<i>John E. Collins, Sheila Hassock, and Ian Dunham</i>	
58	YAC Library Screening: <i>Preparation of Hybridization Filters and Polymerase Chain Reaction Pools</i>	431
	<i>Charlotte G. Cole, John E. Collins, and Ian Dunham</i>	
59	YAC Library Screening: <i>Hybridization and PCR-Based Screening Protocols</i>	437
	<i>Charlotte G. Cole, John E. Collins, and Ian Dunham</i>	
60	Phage-Display Libraries of Murine and Human Antibody Fab Fragments	449
	<i>Jan Engberg, Lene K. Johansen, Michelle Westengaard-Hildinge, Erik S. Riise, and Bjarne Albrechtsen</i>	
 PART VI DNA SEQUENCING		
61	Preparation and Analysis of DNA Sequencing Gels	481
	<i>Bimal D. M. Theophilus</i>	
62	DNA Sequencing of Plasmids	489
	<i>George Murphy</i>	
63	Sequencing DNA Fragments Cloned into M13 and Phagemid Vectors	493
	<i>Neil Brewis</i>	
64	Direct cDNA Sequencing Using Sequential Linear/Asymmetric Polymerase Chain Reaction	499
	<i>Ivor J. Mason</i>	

65	Purification and Enzymatic Sequencing of Polymerase Chain Reaction Products	505
	Frank C. Brosius III, Lawrence B. Holzman, and Xinan Cao	
66	Direct Polymerase Chain Reaction Sequencing with Denaturants	515
	Wei Zhang and Albert B. Deisseroth	
67	Direct DNA Sequencing of Polymerase Chain Reaction Products Using Magnetic Beads	523
	Joakim Lundeberg, Bertil Pettersson, and Mathias Uhlén	
68	Polymerase Chain Reaction Cycle Sequencing with Degenerate Primers	533
	Zhiyuan Shen, Jingmei Liu, Robert L. Wells, and Mortimer M. Elkind	
69	Direct Automated Cycle Sequencing of Polymerase Chain Reaction Products	541
	Susan E. Daniels	
70	Affinity-Capture and Solid-Phase Sequencing of Biotinylated Polymerase Chain Reaction Products	547
	Anu Suomalainen and Ann-Christine Syvänen	
71	DNA Sequencing by the Chemical Method	553
	Eran Pichersky	
72	One-Step One-Lane Chemical Sequencing of DNA	557
	Giovanna Costanzo, Ernesto Di Mauro, and Rodolfo Negri	
PART VII BASIC POLYMERASE CHAIN REACTION METHODS		
73	Polymerase Chain Reaction: <i>Basic Principles and Routine Practice</i>	569
	Lori A. Kolmodin and J. Fenton Williams	
74	Primer Selection and Design for Polymerase Chain Reaction	581
	Wojciech Rychlik	
75	One-Step Optimization Using Touchdown and Stepdown Polymerase Chain Reaction	589
	Kenneth H. Roux and Karl H. Hecker	
76	Cloning Gene Family Members Using Polymerase Chain Reaction with Degenerate Oligonucleotide Primers	595
	Gregory M. Preston	
77	Construction of Synthetic Genes by Polymerase Chain Reaction	609
	Patrick J. Dillon and Craig A. Rosen	
78	Rapid Amplification of cDNA Ends	613
	David Bertioli	
79	Multiplex Polymerase Chain Reaction	619
	Jerald Radich	
80	Inverse Polymerase Chain Reaction	625
	Sheng-He Huang	

81	Long Range Polymerase Chain Reaction	633
	William Waggott	
PART VIII ANALYZING GENES, MUTATIONS, AND PROTEIN INTERACTIONS		
82	Nonradioactive Differential Display of Messenger RNA	645
	Thomas C. G. Bosch and Jan U. Lohmann	
83	Gene Isolation by Exon Trapping	653
	David B. Krizman	
84	DNA Rescue by the Vectorsette Method	667
	Marcia A. McAleer, Alison Coffey, and Ian Dunham	
85	Random Amplified Polymorphic DNA (RAPDs)	675
	Scott Tingey	
86	Restriction Fragment Length Polymorphism	679
	Mohammad S. Enayat	
87	Detection of Mutations in DNA and RNA by Chemical Cleavage	685
	Richard G. H. Cotton	
88	Mutation Screening Using PCR-SSCP: <i>Silver Staining and Isotopic Protocols</i>	695
	Philip J. Saker	
89	Detecting Point Mutations by Denaturing-Gradient Gel Electrophoresis	705
	Stephen R. Dlouhy, Patricia Wheeler, James A. Trofater, Peter J. Stambrook, and Jay A. Tischfield	
90	Analysis of Nucleotide Sequence Variation by Solid-Phase Minisequencing	717
	Anu Suomalainen and Ann-Christine Syvänen	
91	The Amplification Refractory Mutation System	723
	John M. Old	
92	DNase I Footprinting	729
	Benoît Leblanc and Tom Moss	
93	Identification of Protein–DNA Contacts with Dimethyl Sulfate: <i>Methylation Protection and Methylation Interference</i>	737
	Peter E. Shaw and A. Francis Stewart	
94	The Gel Shift Assay for the Analysis of DNA–Protein Interactions	745
	John D. Taylor, Alison J. Ackroyd, and Stephen E. Halford	
95	Yeast Two-Hybrid Library Screening	757
	Ian G. Cowell	
96	The Southwestern Assay	773
	Jacques Philippe	
97	Nonradioactive Methods for the Detection of RNA–Protein Interaction	783
	Asier Echarri, María Eugenia González, Iván Ventoso, and Luis Carrasco	

98 Transcriptional Activation Analysis by the Chloramphenicol Acetyl Transferase (CAT) Enzyme Assay	793
David R. Hodge, Delores M. Thompson, Alexandra Panayiotakis, and Arun Seth	
PART IX MUTAGENESIS, TRANSCRIPTION, AND TRANSLATION IN VITRO	
99 Generating Nested Deletions with Exonuclease III	807
George Murphy	
100 Primer-Directed Site-Specific Mutagenesis	815
Michael J. O'Donohue and G. Geoff Kneale	
101 Site-Directed Mutagenesis Using a Uracil-Containing Phagemid Template	827
Christian Hagemeier	
102 Site-Directed Mutagenesis Using Double-Stranded Plasmid DNA Templates	835
Jeffrey Bramam, Carol Papworth, and Alan Greener	
103 Site-Directed Mutagenesis with LA-PCR™ Technology	845
Atsushi Shimada and Osamu Takeda	
104 Recombination and Mutagenesis by Overlap Extension PCR	857
Robert J. Pogulis, Abbe N. Vallejo, and Larry R. Pease	
105 Site-Directed Mutagenesis and Gene Fusion by Megaprimer PCR	865
Sailen Barik	
106 Transcription In Vitro Using Bacteriophage RNA Polymerases	875
Elaine T. Schenborn	
107 In Vitro Translation of mRNA in a Rabbit Reticuloctye Lysate Cell-Free System	885
Louise Olliver and Charles D. Boyd	
108 In Vitro Translation of mRNA in a Wheat Germ Extract Cell-Free System	891
Louise Olliver, Anne Grobler-Rabie, and Charles D. Boyd	
109 The <i>Xenopus</i> Egg Extract Translation System	895
Glenn M. Matthews and Alan Colman	
110 Manipulation of Baculovirus Vectors	907
Claire L. Merrington, Mark J. Bailey, and Robert D. Possee	
111 Procedures for the Analysis and Purification of His-Tagged Proteins	921
Richard E. Kneusel, Joanne Crowe, Melanie Wulbeck, and Joachim Ribbe	
112 Detection and Immobilization of Proteins Containing the 6xHis Tag	935
Richard E. Kneusel, Melanie Wulbeck, and Joachim Ribbe	

113 Expression and Purification of Recombinant Proteins Using the pET System	947
Robert C. Mierendorf, Barbara B. Morris, Beth Hammer, and Robert E. Novy	
PART X GENE LOCALIZATION, MAPPING <i>IN SITU</i> , AND BIOINFORMATICS	
114 Preparation of Tissue Sections and Slides for mRNA Hybridization	981
Giorgio Terenghi	
115 Use of Digoxigenin-Labeled Probes on Tissue Sections	985
Giorgio Terenghi	
116 Gene Mapping by FISH	991
Rafael Espinosa III and Michelle M. Le Beau	
117 Oligonucleotide PRINS DNA Synthesis	1011
John R. Gosden and Diane Lawson	
118 Chromosome-Specific PRINS	1017
Jean-Paul Charlieu and Frank Pellestor	
119 <i>In Situ</i> PCR Amplification of Intracellular mRNA	1023
Raymond H. Chen and Susan V. Fuggle	
120 An Introduction to Bioinformatics	1031
Henry Brzeski	
Index	1045



<http://www.springer.com/978-0-89603-459-4>

The Nucleic Acid Protocols Handbook

Rapley, R. (Ed.)

2000, DLXXII, 478 p., Hardcover

ISBN: 978-0-89603-459-4

A product of Humana Press