Guide to Molecular Cloning Techniques

EDITED BY

Shelby L. Berger
NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

Alan R. Kimmel
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

1987

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando San Diego New York Austin
Boston London Sydney Tokyo Toronto
Table of Contents

Contributors to Volume 152 ........................................ xi
Preface ............................................................... xv
Volumes in Series .................................................. xvii
Process Guide ......................................................... xxxi

Section I. Requirements for a Molecular Biology Laboratory

1. Equipping the Laboratory ........................................ Daphne D. Blumberg 3
2. Creating a Ribonuclease-Free Environment .................. Daphne D. Blumberg 20

Section II. General Methods for Isolating and Characterizing Nucleic Acids

4. Large- and Small-Scale Phenol Extractions ............... Donald M. Wallace 33
5. Precipitation of Nucleic Acids ............................... Donald M. Wallace 41
6. Quantifying 32P-Labeled and Unlabeled Nucleic Acids .. Shelby L. Berger 49
7. Autoradiograms: 35S and 32P ............................... William M. Bonner 55
8. Electrophoresis in Agarose and Acrylamide Gels ......... Richard C. Ogden and Deborah A. Adams 61

Section III. Enzymatic Techniques and Recombinant DNA Technology

9. Nick Translation ................................................. Judy L. Meinkoth and Geoffrey M. Wahl 91
10. Enzymes for Modifying and Labeling DNA and RNA .... Fabio Cobianchi and Samuel H. Wilson 94
TABLE OF CONTENTS

Section IV. Restriction Enzymes

11. Properties and Uses of Restriction Endonucleases
   J O A N E . B R O O K S 113

12. Restriction and Modification in Vivo by Escherichia coli K12
   E L I S A B E T H A . R A L E I G H 130

Section V. Growth and Maintenance of Bacteria

13. Practical Aspects of Preparing Phage and Plasmid DNA: Growth, Maintenance, and Storage of Bacteria and Bacteriophage
   H A R V E Y M I L L E R 145

Section VI. Genomic Cloning

14. Host Strains That Alleviate Underrepresentation of Specific Sequences: Overview

15. Isolation of Genomic DNA

16. Digestion of DNA: Size Fractionation
   A N N A - M A R I A F R I S C H A U F 183

17. Construction and Characterization of a Genomic Library in λ
   A N N A - M A R I A F R I S C H A U F 190

18. Cloning Large Segments of Genomic DNA Using Cosmid Vectors

Section VII. Preparation and Characterization of RNA

19. Preparation and Characterization of RNA: Overview
   S H E L B Y L . B E R G E R 215

20. Isolation of RNA Using Guanidinium Salts

21. Isolation of Cytoplasmic RNA: Ribonucleoside–Vanadyl Complexes
   S H E L B Y L . B E R G E R 227

22. Isolation and Analysis of Nuclear RNA
   J O S E P H R . N E V I N S 234

23. Isolation of Messenger RNA from Membrane-Bound Polysomes
   B E R N A R D M . M E C H L E R 241
Enzymes

JOAN E. BROOKS 113
ELISABETH A. RALEIGH 130

Cloning

ARLENE R. WYMAN AND KENNETH F. WERTMAN 173
BERNHARD G. HERRMANN AND ANNA-MARIA FRIECHAUF 180
ANNA-MARIA FRIECHAUF 183
ANNA-MARIA FRIECHAUF 190
ANTHONY G. DILELLA AND SAVIO L. C. WOO 199

Characterization of RNA

SHELBY L. BERGER 215
RAYMOND J. MACDONALD, GALVIN H. SWIFT, ALAN E. PREZYBYLA, AND JOHN M. CHIRGWIN 219
SHELBY L. BERGER 227
JOSEPH R. NEVINS 234
BERNARD M. MECHLER 241

TABLE OF CONTENTS

24. Use of Antibodies to Obtain Specific Polysomes DENNIS C. LYNCH 248
25. Purification and Fractionation of Poly(A)+ RNA ALLAN JACOBSON 254
26. Determination of the Molar Concentration of Messenger RNA MARC S. KRUG AND SHELBY L. BERGER 262
27. Translation in Cell-Free Systems ROSEMARY JAGUS 267
29. Preparation of Oocytes for Microinjection of RNA and DNA CAROL J. MARCUS-SEKURA AND MICHAEL J. M. HITCHCOCK 284
30. Translation of Messenger RNA in Injected Frog Oocytes DOUGLAS A. MELTON 288
31. Characterization of in Vitro Translation Products ROSEMARY JAGUS 296

Section VIII. Preparation of cDNA and the Generation of cDNA Libraries

33. First-Strand cDNA Synthesis Primed with Oligo(dT) MARC S. KRUG AND SHELBY L. BERGER 316
34. Second-Strand cDNA Synthesis: Classical Method UELI GUBLER 325
35. Second-Strand cDNA Synthesis: mRNA Fragments as Primers UELI GUBLER 330
36. Purification of Large Double-Stranded cDNA Fragments WILLIAM H. ESCHENFELDT AND SHELBY L. BERGER 335
37. Homopolymeric Tailing WILLIAM H. ESCHENFELDT, ROBERT S. PUSKAS, AND SHELBY L. BERGER 337
38. Adaptors, Linkers, and Methylation RAY WU, TIYUN WU, AND ANURADHA RAY 343
39. Directional cDNA Cloning in Plasmid Vectors by Sequential Addition of Oligonucleotide Linkers DAVID M. HELFMAN, JOHN C. FITDES, AND DOUGLAS HANAHAN 349
40. Cloning cDNA into λgt10 and λgt11 JERRY JENDRISAK, RICHARD A. YOUNG, AND JAMES DOUGLAS ENGEL 359
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section IX. Selection of Clones from Libraries</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>42. Selection of Clones from Libraries: Overview</td>
<td>ALAN R. KIMMEL</td>
<td>393</td>
</tr>
<tr>
<td>44. Amplification, Storage, and Replication of Libraries</td>
<td>G. VOGEI AND P. S. KAYTES</td>
<td>407</td>
</tr>
<tr>
<td>45. Screening Colonies or Plaques with Radioactive Nucleic Acid Probes</td>
<td>GEOFFREY M. WAHL AND SHELBY L. BERGER</td>
<td>415</td>
</tr>
<tr>
<td>46. Isolation of Differentially Expressed Genes</td>
<td>THOMAS D. SARGENT</td>
<td>423</td>
</tr>
<tr>
<td>47. Oligonucleotide Probes for the Screening of Recombinant DNA Libraries</td>
<td>R. BRUCE WALLACE AND C. GARRETT MIYADA</td>
<td>432</td>
</tr>
<tr>
<td>48. Gene Cloning Based on Long Oligonucleotide Probes</td>
<td>WILLIAM I. WOOD</td>
<td>443</td>
</tr>
<tr>
<td>49. Hybridization of Genomic DNA to Oligonucleotide Probes in the Presence of Tetramethylammonium Chloride</td>
<td>ANTHONY G. DI.LELLA AND SAVIO L. C. WOO</td>
<td>447</td>
</tr>
<tr>
<td>50. Use of Antibodies to Screen cDNA Expression Libraries Prepared in Plasmid Vectors</td>
<td>DAVID M. HELFMAN AND STEPHEN H. HUGHES</td>
<td>451</td>
</tr>
<tr>
<td>51. Gene Isolation by Screening λgt11 Libraries with Antibodies</td>
<td>ROBERT C. MIERENDORF, CHRIS PERCY, AND RICHARD A. YOUNG</td>
<td>458</td>
</tr>
<tr>
<td>52. Gene Isolation by Retroviral Tagging</td>
<td>STEPHEN P. GOFF</td>
<td>469</td>
</tr>
<tr>
<td>53. Isolation of Genes by Complementation in Yeast</td>
<td>MARK D. ROSE</td>
<td>481</td>
</tr>
</tbody>
</table>

# Section X. Identification and Characterization of Specific Clones

<p>| | | |
| | | |
| 54. Identification and Characterization of Specific Clones: Strategy for Confirming the Validity of Presumptive Clones | ALAN R. KIMMEL | 507 |
| 55. Subcloning | JONATHAN R. GREENE AND LEONARD GUARENTE | 512 |</p>
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Authors</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td>Interaction of Protein with DNA in Vitro</td>
<td>Lothar Hennighausen and Henryk Lubon</td>
<td>721</td>
</tr>
<tr>
<td>74</td>
<td>In Vivo Footprinting of Specific Protein–DNA Interactions</td>
<td>P. David Jackson and Gary Felsenfeld</td>
<td>735</td>
</tr>
</tbody>
</table>

Author Index ................................................................. 757
Subject Index ................................................................. 783