SHORT PROTOCOLS IN MOLECULAR BIOLOGY

Fourth Edition

EDITED BY FREDERICK M. AUSUBEL
ROGER BRENT
ROBERT E. KINGSTON
DAVID D. MOORE
J. G. SEIDMAN
JOHN A. SMITH
KEVIN STRUHL

A Compendium of Methods from
CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

SHORT PROTOCOLS IN MOLECULAR BIOLOGY

Fourth Edition

A Compendium of Methods from
Current Protocols in Molecular Biology

Published by John Wiley & Sons, Inc.

New York • Chichester • Weinheim • Brisbane • Singapore • Toronto

Cover art by Pauline B. Lim

Library of Congress Cataloging in Publication Data:

Short protocols in molecular biology / edited by Frederick M. Ausubel
...[et al.] —4th ed.
p. cm.
Includes index.
1. Molecular biology—Technique. 2. Molecular biology—Laboratory manuals. I. Ausubel, Frederick M.

QH506.S54 1992
574.8'8'028—dc20

92-6616
CIP

ISBN 0-471-32938-X

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

Preface

This volume presents shortened versions of the methods published in *Current Protocols in Molecular Biology*. Drawing from both the original "core" manual as well as the quarterly update service, this compendium includes all step-by-step descriptions of the principal methods covered in CPMB. Designed for use at the lab bench, it is intended for graduate students and postdoctoral fellows who are familiar with the detailed explanations found in CPMB. However, sufficient detail is provided to allow experienced investigators to use it as a stand-alone bench guide.

Although mastery of the techniques herein will enable the reader to pursue research in molecular biology and related disciplines, the manual is not intended to be a substitute for graduate-level courses in molecular biology or a comprehensive textbook in the field. In addition, we recommend cross-referencing the commentaries and detailed annotations in *Current Protocols in Molecular Biology*. Finally, we strongly recommend that readers obtain first-hand experience in basic techniques and safety procedures by working in a molecular biology laboratory alongside more experienced investigators.

HOW TO USE THIS MANUAL

Organization

This manual is organized by chapters, with individual protocols contained in units. Each unit includes listings of materials, the protocol steps, and references for each technique. Full references for the entire manual can be found in APPENDIX 5. The sequence and organization of material in this manual generally follows that of *Current Protocols in Molecular Biology*. Although the unit numbers may not correspond in all cases, the unit titles are identical in both versions. Thus, users who own both manuals will find it easy and convenient to cross-reference CPMB when more explanatory details are required.

Many reagents and procedures are employed repeatedly throughout the manual. Rather than duplicate this information, cross-references among units are used extensively. Early chapters (and APPENDIX 3A to 3E) describe commonly used techniques such as basic microbiology and basic manipulation of enzymes, DNA, and RNA, while later chapters describe more advanced techniques. Thus, whenever a particular enzyme is used in a protocol, the appropriate unit in Chapter 3—describing reaction conditions for that enzyme—is cross-referenced (e.g., UNIT 3.7 for reverse transcriptase). Similarly, throughout the book readers are referred to UNIT 1.3 for spreading or streaking a plate, UNIT 2.1A for phenol extraction/alcohol precipitation, UNIT 2.5A for agarose gel electrophoresis, and so on. As a result, protocols in the later chapters of the book are not overburdened with steps describing auxiliary procedures required to prepare, purify, and analyze the sample or molecule of interest.

The appendixes provide recipes for reagents and solutions (APPENDIX 1), a list of useful measurements and data (APPENDIX 2), commonly used biochemical techniques (APPENDIX 3), the names and addresses of suppliers (APPENDIX 4), and complete listings for all cited references (APPENDIX 5) .

Protocols

Many units contain groups of protocols. The *Basic* Protocol is presented first in each unit and is generally the recommended approach. *Alternate* Protocols are provided where (1) different equipment or reagents can be employed to achieve similar ends, (2) the starting material requires a variation in approach, or (3) requirements for the end product differ from those in the Basic Protocol. *Support* Protocols describe additional steps that are required to perform the Basic or Alternate Protocols; these steps are separated from the core protocol because they might be applicable to other uses in the manual or because they are performed in a time frame separate from the Basic Protocol steps.

Reagents and Solutions

Reagents required for a protocol are listed in the Materials list before the procedure begins. As noted, corresponding recipes are listed in APPENDIX 1 except for medium recipes and buffers for restriction endonucleases—the locations of these recipes are cross-referenced parenthetically in the Materials list. It is important to note that the *names* of some of these special solutions might be similar from unit to unit (e.g., hybridization solution, lysis buffer, etc.) while the *recipes* differ; thus, make certain that reagents are prepared from the proper recipes. To avoid confusion, parenthetical listings of the unit or units in which each recipe is used are provided next to the name of each reagent in APPENDIX 1, except in the case of commonly used buffers and solutions—e.g., TE buffer, PBS, and 1 M $CaCl_2$.

NOTE: Deionized, distilled water should be used in all protocols in this manual, and in the preparation of all reagents and solutions.

EQUIPMENT

Standard pieces of equipment in the modern molecular biology laboratory are listed in the accompa-

Special equipment is also itemized in the Materials list of each protocol. We have not attempted to list all items required for each procedure, but rather have noted those items that might not be readily available in the laboratory or that require special preparation. Listed below are standard pieces of equipment in the modern molecular biology laboratory, i.e., items used extensively in this manual and thus not included in the individual materials lists.

Autoclave

Balances analytical and preparative

Bench protectors plastic-backed (including "blue pads")

Centrifuges a low-speed (20,000 rpm) refrigerated centrifuge and an ultracentrifuge (20,000 to 80,000 rpm) are required for many procedures. Vertical ultracentrifuge rotors are very convenient for preparing plasmid DNA. At least one microcentrifuge that holds standard 1.5-ml microcentrifuge tubes is essential. It is also useful to have a large-capacity, low-speed centrifuge for spinning down large bacterial cultures and a tabletop swinging-bucket centrifuge with adapters for spinning 96-well microtiter plates.

Computer and printer

Darkroom and developing tanks or X-Omat automatic X-ray film developer.

Filtration apparatus for collecting acid precipitates on nitrocellulose filters or membrane.

Fraction collector

Freezers and refrigerators for 4°, –20°, and –70°C incubation and storage.

Fume hood

Geiger counter

Gel dryer

Gel electrophoresis equipment at least one full-size horizontal apparatus and one horizontal minigel apparatus, two sequencing gel setups for each person engaged in large-scale sequencing projects, one vertical gel apparatus for polyacrylamide protein gels, and specialized equipment for two-dimensional protein gels as required.

Heating blocks thermostat-controlled metal heating blocks that hold test tubes and/or microcentrifuge tubes are very convenient for carrying out enzymatic reactions.

Ice maker

Incubator (37°C) for growing bacteria. We recommend an incubator large enough to hold a "tissue culture" roller drum that can be used to grow 5-ml cultures in standard 18 × 150–mm test tubes. A convenient and durable tube roller is made by New Brunswick Scientific.

Incubator/shaker(s) an enclosed shaker (such as the New Brunswick Controlled Environment Incubator Shaker) that can spin 4-liter flasks is essential for growing 1-liter *E. coli* cultures. A rotary shaking water bath (New Brunswick R76) is useful for growing smaller cultures in flasks.

Light box for viewing autoradiograms.

Liquid nitrogen

Magnetic stirrers (with heater is useful).

Microcentrifuge Eppendorf-type, maximum speed 12,000 to 14,000 rpm

Microcentrifuge tubes 1.5-ml

Microwave oven to melt agar and agarose.

Mortar and pestle

Paper cutter large size, for 46 × 57–cm Whatman sheets.

pH meter

pH paper

Pipettors that use disposable tips and dispense 1 to 1000 µl. It is best to have a set for each full-time researcher.

Polaroid camera and UV transilluminator for taking photographs of stained gels.

Policemen rubber or plastic

Power supplies 300-volt power supplies are sufficient for agarose gels; 2000-volt power supply required for DNA sequencing.

Radiation shield (Lucite or Plexiglas)

Radioactive ink

Radioactive waste container for liquid and solid waste

Refrigerator 4°C

Safety glasses

Scalpels and blades

Scintillation counter

Seal-A-Meal bag sealer or equivalent

Shakers orbital and platform, room temperature or 37°C

Spectrophotometer UV and visible

Speedvac evaporator

Thermal cycler

Tissue culture equipment CO_2 humidified incubator, phase-contrast microscope, liquid nitrogen storage container, and laminar flow hood.

UV cross-linker

UV light sources long- and short-wave

UV transilluminator

Vacuum desiccator/lyophilizer

Vacuum oven

Vortex mixers

Water baths at least two with 80°C capacity

Water purification equipment or glass distillation apparatus to purify all water used in molecular biology experiments.

nying box. These items are used extensively in this manual. The Materials list that precedes each protocol includes only "specialized" items—i.e., items that might not be readily available in the laboratory or that require special preparation.

COMMERCIAL SUPPLIERS

In some instances throughout the manual, we have recommended commercial suppliers of chemicals, biological materials, or equipment. This has been avoided wherever possible because preference for a specific brand is subjective and is generally not based on extensive comparison testing. Our guidelines for recommending a supplier are that (1) the particular brand has actually been found to be of superior quality, or (2) the item is difficult to find in the marketplace. *APPENDIX 4* lists the names, locations, and phone numbers of recommended suppliers, but these are by no means the only vendors of biological supplies. Readers may experiment with substituting their own favorite brands.

SAFETY CONSIDERATIONS

Anyone carrying out these protocols will encounter the following hazardous materials: (1) radioactive substances, (2) toxic chemicals and carcinogenic or teratogenic reagents, (3) pathogens and infectious biological agents, and (4) certain recombinant DNA construct. It is essential that these materials be used in strict accordance with local and national regulations. Cautionary notes are included in many instances throughout the manual, but we emphasize that users must proceed with the prudence and precaution associated with good laboratory practice.

ACKNOWLEDGMENTS

The Current Protocols staff at John Wiley & Sons provided us with the support and assistance needed to bring this project together. Among those who helped us, we are extremely grateful to Virginia Chanda, Alice Ro, Jerri Hurlbutt, Janet Blair, and Kathy Wisch.

We are especially grateful to our co-workers who have helped with the manual by contributing material to it, commenting on the chapters, or field-testing the procedures. To those people—in our own labs and in academic and industrial labs all over the world—we offer our deepest thanks.

Frederick M. Ausubel, Roger Brent, Robert E. Kingston,
David D. Moore, J.G. Seidman, John A. Smith, and
Kevin Struhl

CONTRIBUTORS

Susan M. Abmayr
Pennsylvania State University
University Park, Pennsylvania

Lonnie D. Adams
Upjohn Company
Kalamazoo, Michigan

Lisa M. Albright
Reading, Massachusetts

Alejandro Aruffo
Bristol-Myers Squibb
Seattle, Washington

Frederick M. Ausubel
Massachusetts General Hospital
and Harvard Medical School
Boston, Massachusetts

Omar Bagasra
Thomas Jefferson University
Philadelphia, Pennsylvania

Albert S. Baldwin, Jr.
University of North Carolina
Chapel Hill, North Carolina

Daniel M. Becker
Pennie & Edwards
Menlo Park, California

Claude Besmond
Hôpital Robert Debré
Paris, France

Stephen M. Beverley
Harvard Medical School
Boston, Massachusetts

Kenneth D. Bloch
Massachusetts General Hospital
Boston, Massachusetts

Juan S. Bonifacino
National Institute of Child Health
and Human Development
Bethesda, Maryland

Ann Boyle
Current Protocols
Madison, Connecticut

Allan R. Brasier
Univerity of Texas
Galveston, Texas

Michael Brenowitz
Albert Einstein College
Bronx, New York

Roger Brent
Molecular Sciences Institute
Berkeley, California

Terry Brown
University of Manchester
Institute of Science and Technology
Manchester, England

Bernard H. Brownstein
Howard Hughes Medical Institute
Washington University
School of Medicine
St. Louis, Missouri

Kim Budelier
QIAGEN, Inc.
Valencia, California

William Buikema
University of Chicago
Chicago, Illinois

Linda Buonocore
Yale University School of Medicine
New Haven, Connecticut

Stephen Buratowski
Harvard Medical School
Boston, Massachusetts

Miles W. Carroll
Oxford BioMedica
Oxford, United Kingdom

A. Nigel Carter
The Salk Institute for Biological Studies
La Jolla, California

Anthony Celeste
Genetics Institute
Cambridge, Massachusetts

Constance Cepko
Harvard Medical School
Boston, Massachusetts

David D. Chaplin
Howard Hughes Medical Institute
Washington University
School of Medicine
St. Louis, Missouri

Claudia A. Chen
National Institute of Mental Health
Bethesda, Maryland

Jin-Long Chen
Tularik, Inc.
South San Francisco, California

J. Michael Cherry
Stanford University
Palo Alto, California

Jonathan D. Chesnut
Invitrogen Corporation
Carlsbad, California

Lewis A. Chodosh
University of Pennsylvania
Philadelphia, Pennsylvania

Piotr Chomczynski
University of Cincinnati College
of Medicine
Cincinnati, Ohio

Joanne Chory
The Salk Institute
La Jolla, California

Donald M. Coen
Harvard Medical School
Boston, Massachusetts

Martine A. Collart
Harvard Medical School
Boston, Massachusetts

James F. Collawn
The Salk Institute
La Jolla, California

Helen M. Cooper
Melbourne Hospital
Victoria, Australia

Norman Cooper
National Institute of Allergy &
Infectious Diseases
Bethesda, Maryland

Lynn M. Corcoran
Walter & Eliza Hall Institute
Victoria, Australia

Brendan Cormack
Harvard Medical School
Boston, Massachusetts

Robert L. Dorit
Yale University
New Haven, Connecticut

Allan D. Duby
Medical City
Dallas, Texas

Barbara Dunn
GenPharm International
Mountain View, California

Patricia L. Earl
National Institute of Allergy &
Infectious Diseases
Bethesda, Maryland

Richard L. Eckert
Case Western Reserve School
of Medicine
Cleveland, Ohio

Karen L. Elbing
Fish and Richardson
Boston, Massachusetts

Elaine Elion
Harvard Medical School
Boston, Massachusetts

Andrew Ellington
University of Texas
Austin, Texas

Orna Elroy-Stein
Tel Aviv University
Tel Aviv, Israel

JoAnne Engebrecht
State University of New York
Stony Brook, New York

Michael J. Evelegh
ADI Diagnostics
Rexdale, Ontario

Rhonda Feinbaum
Massachusttes General Hospital
Boston, Massachusetts

Russell L. Finley, Jr.
Wayne State University
School of Medicine
Detroit, Michigan

Michael Finney
MJ Research
Watertown, Massachusetts

John J. Fortin
Tropix, Inc.
Bedford, Massachusetts

Verna Frasca
Amersham Pharmacia Biotech
Piscataway, New Jersey

Steven A. Fuller
Univax Biologics
Rockville, Maryland

Sean R. Gallagher
Hoefer Scientific Instruments
San Francisco, California

Subinay Ganguly
SmithKline Beecham
King of Prussia, Pennsylvania

Paul A. Garrity
University of California Los Angeles
Los Angeles, California

David H. Gelfand
Roche Molecular Systems
Alameda, California

Michael Gilman
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Erica A. Golemis
Fox Chase Cancer Center
Philadelphia, Pennsylvania

Michael E. Greenberg
Harvard Medical School
Boston, Massachusetts

John M. Greene
National Institutes of Health
Bethesda, Maryland

David Greenstein
Massachusetts General Hospital
Boston, Massachusetts

Mitchell S. Gross
SmithKline Beecham
King of Prussia, Pennsylvania

Barbara Grossman
Amersham Life Sciences, Inc.
Cleveland, Ohio

Stefan Grünwald
Pharmingen
San Diego, California

Tod Gulick
Massachusetts General Hospital
Charlestown, Massachusetts

Jeno Gyuris
Mitotix, Inc.
Cambridge, Massachusetts

Lars Hagel
Amersham Pharmacia Biotech AB
Uppsala, Sweden

John Hanson
MJ Research
Watertown, Massachusetts

Grant Hartzog
Harvard Medical School
Boston, Massachusetts

Joseph S. Heilig
University of Colorado
Boulder, Colorado

Peter Heinrich
Consortium für Elektrochemische
Industrie
Munich, Germany

William J. Henzel
Genentech, Inc.
South San Francisco, California

David E. Hill
Applied Biotechnology
Cambridge, Massachusetts

James P. Hoeffler
Invitrogen Corporation
Carlsbad, California

Charles S. Hoffman
Boston College
Chestnut Hill, Massachusetts

Peter Hornbeck
University of Maryland
Baltimore, Maryland

John G.R. Hurrell
Boehringer Mannheim Biochemicals
Indianapolis, Indiana

Charles B.-C. Hwang
Harvard Medical School
Boston, Massachusetts

Nina Irwin
Harvard University
Cambridge, Massachusetts

Kenneth A. Jacobs
Genetics Institute
Cambridge, Massachusetts

Kaaren Janssen
Current Protocols
Guilford, Connecticut

Stephen Albert Johnston
University of Texas Southwestern
Medical Center
Dallas, Texas

Jingyue Ju
Incyte Pharmaceutical
Palo Alto, California

Mustak A. Kaderbhai
University College of Wales
Penglais, Aberystwyth, United Kingdom

James T. Kadonaga
University of California San Diego
La Jolla, California

Steven R. Kain
Clontech Laboratories
Palo Alto, California

Randal J. Kaufman
University of Michigan
Ann Arbor, Michigan

Leslie A. Kerrigan
University of California San Diego
La Jolla, California

Robert E. Kingston
Massachusetts General Hospital
and Harvard Medical School
Boston, Massachusetts

Carol M. Kissinger
Millipore
Burlington, Massachusetts

Lloyd B. Klickstein
Brigham and Women's Hospital
Boston, Massachusetts

Mikhail G. Kolonin
Wayne State University
School of Medicine
Detroit, Michigan

Martha F. Kramer
Harvard Medical School
Boston, Massachusetts

Thomas A. Kunkel
National Institute of Environmental
Health Sciences
Research Triangle Park, North Carolina

Edward R. LaVallie
Genetics Institute
Cambridge, Massachusetts

Peng Liang
The Vanderbilt Cancer Center
Nashville, Tennessee

Peter Lichter
Deutsches Krebforschungzentrum
Heidelberg, Germany

Victoria Lundblad
Baylor College of Medicine
Houston, Texas

Thomas L. Madden
National Center for Biotechnology Information
Bethesda, Maryland

Chris S. Martin
Tropix, Inc.
Bedford, Massachusetts

John M. McCoy
Genetics Institute
Cambridge, Massachusetts

Jill Meisenhelder
The Salk Institute
La Jolla, California

David D. Moore
Baylor College of Medicine
Houston, Texas

Malcolm Moos, Jr.
Center for Biologics Evaluation & Research
Food and Drug Administration
Bethesda, Maryland

Zarmik Moqtaderi
Harvard Medical School
Boston, Massachusetts

Richard Mortensen
Brigham and Women's Hospital
Boston, Massachusetts

Bernard Moss
National Institute of Allergy & Infectious Diseases
Bethesda, Maryland

Paul R. Mueller
California Institute of Technology
Pasadena, California

Cheryl Isaac Murphy
Aquila Biopharmaceuticals
Worcester, Massachusetts

Richard M. Myers
Stanford University School of Medicine
Stanford, California

Robert J. Nelson
Molecular Dynamics
Sunnyvale, California

Rachael L. Neve
McLean Hospital
Belmont, Massachusetts

B. Tracy Nixon
Pennsylvania State University
University Park, Pennsylvania

Marjorie Oettinger
Massachusetts General Hospital
Boston, Massachusetts

Osamu Ohara
Shionogi Research Laboratories
Osaka, Japan

Hiroto Okayama
Osaka University
Osaka, Japan

Salvatore Oliviero
Harvard Medical School
Boston, Massachusetts

Arthur B. Pardee
Dana-Farber Cancer Institute
Boston, Massachusetts

Yvonne Paterson
University of Pennsylvania
Philadelphia, Pennsylvania

Warren Pear
University of Pennsylvania
Philadelphia, Pennsylvania

Heather Perry-O'Keefe
Perceptive Biosystems
Framingham, Massachusetts

Kevin Petty
University of Texas Southwestern Medical Center
Dallas, Texas

Mary C. Phelan
Greenwood Genetic Center
Greenwood, South Carolina

Helen Piwnica-Worms
Washington University School of Medicine
St. Louis, Missouri

Jack D. Pollard, Jr.
Massachusetts General Hospital and Harvard Medical School
Boston, Massachusetts

Roy M. Pollock
Ariad Pharmaceuticals
Cambridge, Massachusetts

Roger Pomterantz
Thomas Jefferson University
Philadelphia, Pennsylvania

Huntington Potter
Harvard Medical School
Boston, Massachusetts

Thomas Quertermous
Massachusetts General Hospital
Boston, Massachusetts

Elisabeth Raleigh
New England Biolabs
Beverly, Massachusetts

K.J. Reddy
State University of New York
Binghamton, New York

Ann Reynolds
University of Washington
Seattle, Washington

Randall K. Ribaudo
National Institute of Allergy & Infectious Diseases
Bethesda, Maryland

Eric J. Richards
Washington University
St. Louis, Missouri

Paul Riggs
New England Biolabs
Beverly, Massachusetts

Phil Robakiewicz
Worcester Polytechnic Institute
Worcester, Massachusetts

Melissa Rogers
Harvard Medical School and Dana-Farber Cancer Institute
Boston, Massachusetts

William G. Romanow
Corning Costar
Portsmith, New Hampshire

John K. Rose
Yale University School of Medicine
New Haven, Connecticut

Martin Rosenberg
Smithkline Beecham
King of Prussia, Pennsylvania

Elizabeth F. Ryder
Worcester Polytechnic Institute
Worcester, Massachusetts

Nicoletta Sacchi
National Cancer Institute
Frederick, Maryland

Thomas P. St. John
ICOS Corporation
Bothwell, Washington

Joachim Sasse
Shriners Hospital for Crippled Children
Tampa, Florida

Stephen J. Scharf
Cetus Corporation
Emeryville, California

David Schatz
Howard Hughes Medical Institute & Yale University School of Medicine
New Haven, Connecticut

Paul Schendel
Genetics Institute
Cambridge, Massachusetts

Paul Schendel
Genetics Institute
Cambridge, Massachusetts

Joachim Schorr
QIAGEN GmbH
Hilden, Germany

Robert Scopes
La Trobe University
Bundoora, Australia

Brian Seed
Massachusetts General Hospital
and Harvard Medical School
Boston, Massachusetts

Bartholomew M. Sefton
The Salk Institute
San Diego, California

Christine E. Seidman
Harvard Medical School
Boston, Massachusetts

J.G. Seidman
Harvard Medical School
Boston, Massachusetts

Richard F. Selden
TKT Inc.
Cambridge, Massachusetts

Kentaro Semba
The Salk Institute
La Jolla, California

Donald F. Senear
University of California
Irvine, California

Ilya Serebriiskii
Fox Chase Cancer Center
Philadelphia, Pennsylvania

Thikkavarapu Seshamma
Thomas Jefferson University
Philadelphia, Pennsylvania

Raj Shankarappa
Biogenex
San Ramon, California

Jen Sheen
Massachusetts General Hospital
and Harvard Medical School
Boston, Massachusetts

Shirish Shenolikar
Duke University Medical Center
Durham, North Carolina

Penny Shockett
Yale University School of Medicine
New Haven, Connecticut

Michael H. Simonian
Beckman Instruments, Inc.
Fullerton, California

Harinder Singh
University of Chicago
Chicago, Illinois

Barton E. Slatko
New England Biolabs
Beverly, Massachusetts

Alan Smith
Beckman Center, Stanford University
Stanford, CA

Donald B. Smith
University of Edinburgh
Edinburgh, Scotland

John A. Smith
University of Alabama at Birmingham
Birmingham, Alabama

Timothy A. Springer
Center for Blood Research
Boston, Massachusetts

Julie M. Stone
University of Missouri, Columbia
Columbia, Missouri

William M. Strauss
Whitehead Institute
Cambridge, Massachusetts

Kevin Struhl
Harvard Medical School
Boston, Massachusetts

John T. Stults
Genentech, Inc.
South San Francisco, California

Jonathan C. Swaffield
University of Texas Southwestern
Medical Center
Dallas, Texas

Stanley Tabor
Harvard Medical School
Boston, Massachusetts

Miyoko Takahashi
Spectral Diagnostics, Inc.
Toronto, Ontario

Douglas A. Treco
TKT Inc.
Cambridge, Massachusetts

Steven J. Triezenberg
Michigan State University
East Lansing, Michigan

Baruch Velan
Israel Institute of Biological Research
Ness Ziona, Israel

Daniel Voytas
Iowa State University
Ames, Iowa

Simon Watkins
University of Pittsburgh Medical School
Pittsburgh, Pennsylvania

John H. Weis
University of Utah School of Medicine
Salt Lake City, Utah

Michael Whitt
University of Tennessee
Memphis, Tennessee

Alan Williams
Amersham Pharmacia Biotech
Piscataway, New Jersey

Kate Wilson
Wye College
Wye, England

Fred Winston
Harvard Medical School
Boston, Massachusetts

Scott E. Winston
Univax Biologics
Rockville, Maryland

C. Richard Wobbe
Harvard Medical School
Boston, Massachusetts

Barbara Wold
California Institute of Technology
Pasadena, California

Tyra G. Wolfsberg
National Center for Biotechnology
Information
Bethesda, Maryland

Jerry L. Workman
Pennsylvania State University
University Park, Pennsylvania

Linda S. Wyatt
National Institute of Allergy &
Infectious Diseases
Bethesda, Maryland

Wayne M. Yokoyama
University of California
School of Medicine
San Francisco, California

Rolf Zeller
Harvard Medical School
Boston, Massachusetts

Ning Zhang
Tularik, Inc.
South San Francisco, California

Louis Zumstein
Baylor College of Medicine
Houston, Texas

CONTENTS

6 SCREENING OF RECOMBINANT DNA LIBRARIES

7 DNA SEQUENCING

8 MUTAGENESIS OF CLONED DNA