PCR Applications: Protocols for Functional Genomics

PCR

APPLICATIONS

PROTOCOLS FOR FUNCTIONAL GENOMICS

Edited by

Michael A. Innis
Chiron Corporation, Emeryville, California

David H. Gelfand
Roche Diagnostic Systems, Alameda, California

John J. Sninsky
Roche Molecular Systems, Alameda, California

ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

Front cover photograph (paperback edition only): Analysis of the human breast cancer cell line BT474 using comparative genomic hybridization (CGH). During CGH, green fluorescing tumor DNA and red fluorescing normal reference DNA samples were hybridized (along with excess unlabeled cot-1 DNA) to normal metaphase spreads. The chromosomes were then counterstained with DAPI. Chromosomal regions showing green are increased in copy number in BT474, while regions showing red are relatively decreased in copy number.

This book is printed on acid-free paper. ♾

Academic Press
a division of Harcourt Brace & Company
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.apnet.com

Academic Press
24-28 Oval Road, London NW1 7DX, UK
http://www.hbuk.co.uk/ap/

International Standard Book Number: 0-12-372185-7 (hb)
International Standard Book Number: 0-12-372186-5 (pb)

PRINTED IN THE UNITED STATES OF AMERICA
99 00 01 02 03 04 EB 9 8 7 6 5 4 3 2 1

PCR
APPLICATIONS

PROTOCOLS FOR FUNCTIONAL GENOMICS

CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Richard D. Abramson (33), Applied Biosystems Division-Perkin Elmer, Foster City, California 94404

Ellen M. Beasley (55), Department of Genetics, Stanford Human Genome Center, Stanford University School of Medicine, Stanford, California 94305-5120

Nick Benson (341), Sidney Kimmel Cancer Center, San Diego, California 92121

Barry R. Bloom (49), Department of Microbiology and Immunology, Albert Einstein College of Medicine, Howard Hughes Medical Institute, Bronx, New York 10461

David Botstein (485), Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

Bob Bonner (497), National Institute of Children's Health and Human Diseases, National Institutes of Health, Bethesda, Maryland 20892

Mary Ann D. Brow (537), Third Wave Technologies, Inc., Madison, Wisconsin 53719

Patrick Brown (485), Department of Biochemistry, Stanford University, Stanford, California 94305

Sheng-Yung P. Chang (285), Roche Molecular Systems, Alameda, California 94501

Koei Chin (473), University of California Cancer Center, San Francisco, California 94143-0808

L. A. Christel (105), Cepheid, Sunnyvale, California 94089

Rodrigo F. Chuaqui (497), Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Kristina A. Cole (497), Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

David R. Cox (55), Department of Genetics, Stanford Human Genome Center, Stanford University School of Medicine, Stanford, California 94305-5120

Ronald W. Davis (445), Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, California 94305-5307
Alan D. Dean (95), Gene Check, Inc., Fort Collins, Colorado 80524
Peter A. Doris (231), Institute for Molecular Medicine, University of Texas Health Sciences Center, Houston, Texas 77025
Xiaozhu Duan (297), Chiron Corporation, Emeryville, California 94608-2916
Dwight B. DuBois (197), Cenetron Diagnostics, LLC, Austin, Texas, 78744
Barbara Dunn (485), Department of Genetics, Stanford University School of Medicine, Stanford, California 94305
Michael R. Emmert-Buck (497), Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892
Tracy Ferea (485), Department of Genetics, Stanford University School of Medicine, Stanford, California 94305
David H. Gelfand (3), Roche Diagnostics Systems, Alameda, California 94501
Klaus Giese (297), Chiron Corporation, Emeryville, California 94608-2916
Joe W. Gray (473), University of California Cancer Center, San Francisco, California 94143-0808
Amanda L Hayward (231), Department of Biology, Yale University, New Haven, Connecticut 06520
Mark G. Herrmann (211), Department of Pathology, University of Utah Medical School, Salt Lake City, Utah 84132
Ralf Herwig (457), Max-Planck Institut für Molekulare Genetik, D-14195 Berlin, Germany
Russell Higuchi (263), Roche Molecular Systems, Alameda, California 94501
Cruz A. Hinojos (231), Institute for Molecular Medicine, University of Texas Health Sciences Center, Houston, Texas 77025
Michael J. Holland (429), Department of Biological Chemistry, University of California School of Medicine, Davis, California 95616
Rhonda Honeycutt (341), Phenotypics Corporation, San Diego, California 92121
Mary Huckabee (497), Laboratory of Pathology, National Cancer Institute, National Institute of Health, Bethesda, Maryland 20892
Jim R. Hully (169), PE Biosystems, Foster City, California 94044
Michael A. Innis (3), Chiron Corporation, Emeryville, California 94608-2916

Daniel B. Kainer (231), Institute for Molecular Medicine, University of Texas Health Sciences Center, Houston, Texas 77025
John J. Kang (429), Department of Biological Chemistry, University of California School of Medicine, Davis, California 95616
Steve Kaye (153), Department of Virology, University College London, London W1P 6DB, United Kingdom
Jenny M. Kelley (127), The Institute for Genomic Research, Rockville, Maryland 20850
Frank Kullmann (341), University of Regensburg, 93042 Regensburg, Germany
Laura C. Lazzeroni (55), Department of Genetics and Division of Biostatistics, Stanford University School of Medicine, Stanford, California 94305-5405
Jeffrey Y. Lee (497), Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892
Hans Lehrach (457), Max-Planck Institut für Molekulare Genetik, D-14195 Berlin, Germany
Lance A. Liotta (497), Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892
Emmanuel Liscum (505), Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211
Jörgen Lonngren (521), Professional Genetics Laboratory AB, S-751 83 Uppsala, Sweden
Li Mao (355), Department of Thoracic/Head and Neck Medical Oncology, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030
Françoise Mathieu-Daudé (341), Sidney Kimmel Cancer Center, San Diego, California 92121
Michael McClelland (341), Sidney Kimmel Cancer Center, San Diego, California 92121
W. A. McMillan (105), Cepheid, Sunnyvale, California 94089
Sebastian Meier-Ewert (457), GPC-Aktiengesellschaft, D-82152 Martinsried, Germany
Catherine Moberg (521), Professional Genetics Laboratory AB, S-751 83 Uppsala, Sweden
Thomas W. Myers (141), Program in Core Research, Roche Molecular Systems, Alameda, California 94501
Richard M. Myers (55), Department of Genetics, Stanford Human Genome Center, Stanford University School of Medicine, Stanford, California 94305-5120
Peter S. Nelson (307), Department of Molecular Biotechnology and Medicine, University of Washington, Seattle, Washington 98195

M. A. Northrup (105), Cepheid, Sunnyvale, California 94089
Peter J. Oefner (231), Department of Biochemistry, Stanford University, Stanford, California 94305
Yun Oh (355), Department of Thoracic/Head and Neck Medical Oncology, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030
Brittan L. Pasloske (197), Ambion, Inc., Austin Texas 78744
Tomi Pastinen (521), Department of Human Molecular Genetics, National Public Health Institute, S-00300 Helsinki, Finland
K. Peterson (105), Cepheid, Sunnyvale, California 94089
F. Pourahmadi (105), Cepheid, Sunnyvale, California 94089
John Quackenbush (127), The Institute for Genomic Research, Rockville, Maryland 20850
Filippo Randazzo (393), Chiron Corporation, Emeryville, California 94608
R. Reynolds (73), Roche Molecular Systems, Alameda, California 94501
Anna-Louise Reysenbach (377), Environmental Sciences Department, Portland State University, Portland, Oregon 97207-0751
R. Saiki (73), Roche Molecular Systems, Alameda, California 94501
Mark Schena (445), Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, California 94305-5307
Armin O. Schmitt (457), metaGen Gesellschaft für Genomforschung, D-14195 Berlin, Germany
Todd Seeley (405), Chiron Corporation, Emeryville, California 94608
Chetan Seshadri (497), Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892
Nicole L. Simone (497), Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892
Gisela Sitbon (521), Professional Genetics Laboratory AB, S-751 83 Uppsala, Sweden
James Snider (329), PE Biosystems, Foster City, California 94404
John J. Sninsky (23), Roche Molecular Systems, Alameda, California 94501
James C. Stephans (297), Chiron Corporation, Emeryville, California 94608-2916
Ann-Christine Syvänen (521), Department of Human Molecular Genetics, National Public Health Institute, S-00300 Helsinki, Finland
Ayly L. Tucker (365), Genentech, Inc., San Francisco, California 94080

Costantino Vetriani (377), Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, New Jersey 08901-8521
Robert Wagner (95), Gene Check, Inc., Fort Collins, Colorado 80524
Cindy R. WalkerPeach (197), Cenetron Diagnostics, LLC, Austin, Texas, 78744
Robert Watson (263), Roche Molecular Systems, Alameda, California 94501
John Welsh (341), Sidney Kimmel Cancer Center, San Diego, California 92121
L. Western (105), Cepheid, Sunnyvale, California 94089
P. Mickey Williams (365), Genentech, Inc., San Francisco, California 94080
Matthew M. Winkler (197), Ambion, Inc., Austin Texas 78744
Carl T. Wittwer (211), Department of Pathology, University of Utah Medical School, Salt Lake City, Utah 84132
Hong Xin (297), Chiron Corporation, Emeryville, California 94608-2916
S. Young (105), Cepheid, Sunnyvale, California 94089
G. Zangenberg (73), Roche Molecular Systems, Alameda, California 94501

PREFACE

When approached about the possibility of editing another PCR volume, I thought the timing was appropriate given the explosion of PCR applications for mRNA quantitation, diagnosis, gene discovery, genomic analysis, and expression profiling. Also, although the newest crop of molecular biologists grew up using PCR routinely, I still find myself devoting significant time teaching first principles of PCR to help young investigators troubleshoot specific applications and to guide them through a confusing maze of choices as to which enzyme, buffer, cycling condition, etc., to use for which purpose.

Our objective in compiling *PCR Applications: Protocols for Functional Genomics* was to combine expert advice with contemporary protocols and perhaps a few personal reflections. *PCR Applications* makes a perfect companion to the earlier sourcebooks, *PCR Protocols* and *PCR Strategies*, because completely new chapters update the reader with the latest practical advice, theoretical information, and protocols without repeating the well-established PCR protocols in the earlier books.

This book, which follows the protocol format of the previous volumes, is divided into four sections. The first section, Key Concepts for PCR, contains chapters that provide practical and theoretical information needed to effectively navigate through the explosion of PCR-based techniques. The second section, Quantitative PCR, provides examples and protocols for real-time quantitative PCR and procedures for measuring gene expression. The last two sections, Gene Discovery, and Genomics and Expression Profiling, contain chapters of unique interest in the postgenomic scientific era.

We hope that, like *PCR Protocols* and *PCR Strategies*, *PCR Applications* will serve as a sourcebook and a practical tool for a wide range of research applications. If it causes you to think differently, then we will have succeeded.

The editors thank all the authors who contributed to this volume. We especially extend our thanks and appreciation to Emelyn

Eldredge, Acquisitions Editor at Academic Press, who inspired us to work together on *PCR Applications*. This book would not have been completed without her encouragement, dedication, and hard work.

Michael A. Innis (for the editors)

CONTENTS

Part Three
GENE DISCOVERY

Part Four
GENOMICS AND EXPRESSION PROFILING

Part One

KEY CONCEPTS FOR PCR