

# **PCR Protocols**

**A Guide to Methods and Applications**

**Edited by**

**Michael A. Innis  
David H. Gelfand  
John J. Sninsky  
Thomas J. White**

# PCR

# PROTOCOLS

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**Michael A. Innis,**  
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*Cetus Corporation, Emeryville, California*

**Thomas J. White**

*Hoffmann-La Roche, Inc., Emeryville, California*



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# CONTRIBUTORS

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

- Mark A. Abbott** (325), Regional Oncology Center, State University of New York, Upstate Medical Center, Syracuse, New York 13210
- Ronald M. Atlas** (399), Department of Biology, University of Louisville, Louisville, Kentucky 40292
- Isabele Baginski** (348), INSERM, F69424 Lyons, Cedex 03, France
- Asim K. Bej** (399), Department of Biology, University of Louisville, Louisville, Kentucky 40292
- Cynthia D. Bottema** (197), Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, Minnesota 55905
- Mary Ann D. Brow** (189), University of Wisconsin, McArdle Laboratory for Cancer Research, Madison, Wisconsin 53706
- Tom Bruns** (315), Department of Plant Pathology, University of California, Berkeley, California 94720
- Jean-Marie Buerstedde** (197), Basel Institute of Immunology, Basel, Switzerland
- Teodorica L. Bugawan** (261), Cetus Corporation, Emeryville, California 94608
- H. Franklin Bunn** (60), Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115
- C. Thomas Caskey** (46, 272), Institute for Molecular Genetics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030
- Joslyn Cassady** (197), Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, Minnesota 55905
- Jeffrey S. Chamberlain** (272), Institute for Molecular Genetics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030
- Chu-an Chang** (99), Department of Nucleic Acid Chemistry, Chiron Corporation, Emeryville, California 94608
- D. Chappel** (442), Royal Postgraduate Medical School, Hammer-smith Hospital, London W12 ONN, United Kingdom

- H. L. Chappelle** (245), The Salk Institute Biotechnology/Industrial Associates, Inc., San Diego, California 92138, and SSKA Diagnostics, Inc., P.O. Box 85453, San Diego, California 92138-9216
- Teresa Compton** (39), Research Institute of Scripps Clinic, Department of Immunology, La Jolla, California 92037
- David R. Cox** (205), Departments of Psychiatry, Biochemistry, and Biophysics, The University of California, San Francisco, California 94143
- Marco Crescenzi** (391), Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892
- N. C. P. Cross** (442), Royal Postgraduate Medical School, Hammer-smith Hospital, London W12 ONN, United Kingdom
- G. R. Davis** (245), The Salk Institute Biotechnology/Industrial Associates, Inc., San Diego, California 92138, and SSKA Diagnostics, Inc., San Diego, California 92138-9216
- Peter Denton** (435), Department of Pathology, University of North Carolina, Chapel Hill, North Carolina 27599
- L. J. DiMichele** (245), The Salk Institute Biotechnology/Industrial Associates, Inc., San Diego, California 92138, and SSKA Diagnostics, Inc., San Diego, California 92138-9216
- Garth D. Ehrlich** (325), Regional Oncology Center, State University of New York, Upstate Medical Center, Syracuse, New York 13210
- Henry A. Erlich** (261), Cetus Corporation, Emeryville, California 94608
- Fred Faloona** (378), Cetus Corporation, Emeryville, California 94608
- Andrew Ferrie** (348), Digeme, University of Maryland, College Park, Maryland 20742
- Kenneth A. Fleming** (113), University of Oxford, Nuffield Department of Pathology, John Radcliffe Hospital, Oxford OX3 9DU, England
- N. S. Foulkes** (442), Royal Postgraduate Medical School, Hammer-smith Hospital, London W12 ONN, United Kingdom
- Kenneth D. Friedman** (253), The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin 53233
- Michael A. Frohman** (28, 227), Department of Anatomy, University of California, San Francisco, California 94143
- Dan Garza** (219), Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110
- David H. Gelfand** (3, 129), Cetus Corporation, Emeryville, California 94608
- Richard A. Gibbs** (272), Institute for Molecular Genetics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030

- Gary Gilliland** (60), Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115
- Thomas R. Gingeras** (245), The Salk Institute Biotechnology/Industrial Associates, Inc., San Diego, California 92138, and SISKADiagnostics, Inc., San Diego, California 92138-9216
- Jane Gitschier** (288), Howard Hughes Medical Institute, University of California, San Francisco, California 94143
- Steven Greenburg** (325), Regional Oncology Center, State University of New York Upstate Medical Center, Syracuse, New York 13210
- Ulf Gyllensten** (300), Department of Medical Genetics, Biomedical Center 23, Uppsala, Sweden
- Daniel L. Hartl** (219), Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110
- Rhea Helmuth** (119), Cetus Corporation, Emeryville, California 94608
- Russell Higuchi** (177), Cetus Corporation, Emeryville, California 94608
- Michael J. Holland** (169), MSIA, Department of Biological Chemistry, University of California, Davis, California 95616
- Leroy Hood** (92), California Institute of Technology, Division of Biology, Pasadena, California 91125
- Michael A. Innis** (3, 54, 169), Cetus Corporation, Emeryville, California 94608
- Robert Kaiser** (92), California Institute of Technology, Division of Biology, Pasadena, California 91125
- Ernest Kawasaki** (21, 146), Cetus Corporation, Emeryville, California 94608
- David E. Kellogg** (337), Cetus Corporation, Emeryville, California 94608
- Mary Claire King** (416), School of Public Health, University of California, Berkeley, California 94720
- Dwight D. Koeberl** (197), Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, Minnesota 55905
- Scott C. Kogan** (288), Howard Hughes Medical Institute, University of California, San Francisco, California 94143
- Barbara Krummel** (184), Department of Biochemistry, University of California, Berkeley, California 94720
- D. Y. Kwoh** (245), The Salk Institute Biotechnology/Industrial Associates, Inc., San Diego, California 92138, and SISKADiagnostics, Inc., San Diego, California 92138-9216
- Shirley Kwok** (142, 337), Cetus Corporation, Emeryville, California 94608

- Oh-Sik Kwon** (378), Cetus Corporation, Emeryville, California 94608
- Ulf Landegren** (92), Department of Medical Genetics, Biomedical Center, S751 #23 Uppsala, Sweden
- Cheng Chi Lee** (46), Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030
- Steven B. Lee** (282, 315), Department of Plant Biology, University of California, Berkeley, California 94720
- Corey Levenson** (99), Cetus Corporation, Emeryville, California 94608
- Y.-M. Dennis Lo** (113), University of Oxford, Nuffield Department of Pathology, John Radcliffe Hospital, Oxford OX3 9DU, England
- L. Luzzatto** (442), Royal Postgraduate Medical School, Hammer-smith Hospital, London W12 0NN, United Kingdom
- John Lyons** (386), Cetus Corporation, Emeryville, California 94608
- David Mack** (348, 378), Department of Molecular Genetics and Cellular Biology, University of Chicago, Cummings Life Science Center, Chicago, Illinois 60637
- Roberta Madej** (455), Cetus Corporation, Emeryville, California 94608
- Michele Manos** (153, 356), Cetus Corporation, Emeryville, California 94608
- David F. Mark** (70), Department of Microbial Chemotherapeutics and Molecular Biology, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065-0900
- Gail R. Martin** (228), Department of Anatomy, University of California, San Francisco, California 94143
- Peter C. McCabe** (76), Cetus Corporation, Emeryville, California 94608
- J. McDonnell** (442), Royal Postgraduate Medical School, Hammer-smith Hospital, London W12 0NN, United Kingdom
- Meetha M. Medhora** (219), Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110
- Wajahat Z. Mehal** (113), University of Oxford, Nuffield Department of Pathology, John Radcliffe Hospital, Oxford OX3 9DU, England
- Robert R. Montgomery** (253), The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin 53233
- Richard M. Myers** (206), Departments of Physiology and Biochemistry and Biophysics, University of California, San Francisco, California 94143
- Peter J. Newman** (253), The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin 53233
- Howard Ochman** (219), Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110

- Christián Orrego**<sup>1</sup> (416, 447) Department of Biochemistry, University of California, Berkeley, California 94720.
- Svante Pääbo** (159), Department of Molecular, Cellular and Developmental Biology, University of California, Berkeley, California 94720
- Sang-Ho Park** (407), Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 01239
- Steven Perrin** (60), Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115
- Lyn M. Powell** (237), Cardiovascular Research, Genentech, South San Francisco, California 94080
- Joel E. Ranier** (272), Institute for Molecular Genetics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030
- H. Reisner** (442), Department of Pathology, University of North Carolina, Chapel Hill, North Carolina 27599
- Nancy L. Rosen** (253), The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin 53233
- Harley A. Rotbart** (372), University of Colorado Health Sciences Center, Denver, Colorado 80262
- Randall K. Saiki** (13), Cetus Corporation, Emeryville, California 94608
- Gobinda Sarkar** (197), Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, Minnesota 55905
- Stephen J. Scharf** (84, 455), Cetus Corporation, Emeryville, California 94608
- David B. Schowalter** (197), Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, Minnesota 55905
- Val C. Sheffield** (206), Department of Pediatrics, The University of California, San Francisco, California 94143
- Darryl Shibata** (368), Los Angeles County-USC Medical Center, Los Angeles, California 90033
- Mitchell L. Sogin** (307), Division of Molecular and Cellular Biology, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, Colorado 80206

<sup>1</sup>Present address: Evolutionary Genetics Laboratory, Museum of Vertebrate Zoology, University of California, Berkeley, California 94720.



**Steve S. Sommer** (197), Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, Minnesota 55905

**J. Taylor** (315, 282), Department of Plant Biology, University of California, Berkeley, California 94720

**Yi Ting** (355), Cetus Corporation, Emeryville, California 94608

**Alice M. Wang** (70), Cetus Corporation, Emeryville, California 94608

**Robert Watson** (348, 429), Cetus Corporation, Emeryville, California 94608

**Thomas J. White** (129, 315), Hoffmann-La Roche, Emeryville, California 94608

**K. M. Whitfield** (245), The Salk Institute Biotechnology/Industrial Associates, Inc., San Diego, California 92138, and SISK A Diagnostics, Inc., San Diego, California 92138-9216

**Deann K. Wright** (153), Cetus Corporation, Emeryville, California 94608

# PREFACE

Since the unveiling of the polymerase chain reaction (PCR) method of DNA amplification at the American Society of Human Genetics Conference in October 1985, more than 600 publications involving the use of PCR have appeared in the scientific literature. Numerous modifications, improvements, and novel applications of PCR have been devised, yet there has been no source to which scientists could turn for basic instruction in the PCR method that is most suitable for the experimental problem at hand. Furthermore, there is no single set of instructions that works in every situation, even though some authors have drawn definitive conclusions from a single system about the importance or dispensability of certain parameters. This book is a collection of protocols for basic PCR methods which have been repeatedly tested in the authors' laboratories. It is intended to serve as an introduction to PCR for molecular biologists at the graduate level and beyond who are using the method for the first time, and to serve as a resource on novel variations and applications of PCR for scientists who may have considerable experience with the basic method. We have also included chapters for scientists in those fields (e.g., zoology, botany, and ecology) in which there may be little or no familiarity with molecular biological techniques. Our intent is to encourage scientists in these fields to utilize the *in vitro* PCR method to complement or circumvent more complex recombinant DNA methods.

The book has five sections. The first section, on basic methodology, contains chapters that provide protocols for many variations of PCR, a brief theoretical basis for each procedure, a comparison to other techniques, and helpful or cautionary notes on optimizing the procedure and avoiding pitfalls. These chapters contain the latest improvements to PCR and have been extensively tested for general applicability. The chapters in the subsequent sections were selected because they describe specific research and/or diagnostic applications of PCR and have been shown to be reliable procedures in the authors' laboratories. In some instances, the latter chapters may

contain a procedure that the editors regard as suboptimal or in conflict with current information from more detailed studies; in these instances, cross-references to other chapters or editorial notes have been provided. The editors feel that this approach is preferable to altering the protocol, since untested revisions might cause the PCR to fail in the author's specific system.

The second section of the book addresses particular applications of PCR in basic research (sequencing, mutagenesis, etc.) and contains protocols and variations that complement and extend those of the first section. The third section addresses procedures that are useful for genetic analyses, diagnosis of inherited disorders and susceptibility to disease, and evolutionary analyses. The fourth section covers applications of PCR to specific diagnostic tests for infectious diseases and cancer and to forensic tests. Our intent is to provide medical scientists with procedures that can be useful for research on the epidemiology of infectious diseases and cancer as well as on methods for individual identification. The final section gives basic information on the equipment and reagents needed to perform the polymerase chain reaction and includes plans for several inexpensive devices for thermal cycling.

The editors extend their thanks and appreciation for the invaluable and patient efforts of Judy Davis, who formatted and copyedited the chapters for the publisher. They also thank Cetus Corporation, Hoffmann-La Roche, and Academic Press for their encouragement and support of the effort required to produce this book.

Thomas J. White (for the editors)

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