

PCR Sequencing Protocols

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Edited by

Ralph Rapley

Coventry University, Coventry, UK

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Preface

Advances in bioscience research usually arise as a result of the continuing refinement of existing technologies. However, there are a number of occasions where newly developed methodologies have a profound effect on nearly all areas of research. Frequently these are techniques that are elegantly simple in concept and require minimal technical manipulation. Two of these revolutionary techniques are the focus of *PCR Sequencing Protocols*. The first such technique is enzymatic chain termination sequencing developed by Sanger and his co-workers in Cambridge and reported in 1977. This essentially brought the possibility of deriving nucleotide sequence information in a very short time scale and has been widely accepted in many laboratories as a routine molecular biological research tool. Furthermore, it has not only led to the sequencing of many genes and gene fragments, but has also allowed the technical means of sequencing the human genome.

The second technique that has found widespread acceptance in basic applied research and many routine applications is the polymerase chain reaction. This technique, first reported in 1985 by Mullis and his colleagues, provides the means to amplify nucleic acid sequence, which immediately proved invaluable in nearly all fields of biological laboratory research. Here, as with enzymatic DNA sequencing, is a very simple concept that relies on minimal information to prepare short oligonucleotide primers that direct the synthesis of a specified fragment of DNA in the presence of a thermostable DNA polymerase.

The fact that these two methods lend themselves well to automation has contributed to their widespread acceptance. Together these two techniques provide the most rapid way of generating nucleic acid sequence information and consequently numerous variations and refinements of methods have been developed. The aim of *PCR Sequencing Protocols* is to bring many of the accepted protocols for PCR sequencing together. Essentially the volume focuses on three broad areas of PCR sequencing that are not mutually exclusive, but overlap to a certain extent. Many of the direct PCR sequencing protocols deal with the problem of the rapid reassociation of amplified complementary strands by modifying the sequencing reactions. This is detailed in the earlier chapters, in addition to PCR purification, primer labeling, and preparation of sequencing gels. This leads to more complex methods of primer manipulation to enable

affinity purification or transcript production providing homogeneous single strands. The latter chapters deal with more conventional means of cloning PCR products into vectors and their subsequent sequencing. More extensive protocols for conventional cloning and sequencing may be found in *DNA Sequencing Protocols (Methods in Molecular Biology, vol. 23)*.

PCR Sequencing Protocols has been prepared in the same format as other volumes in this series in that it not only provides easy and clear instructions on the protocols presented, but also important practical points or notes on the individual intricacies of the methods. These are frequently small, but important, details that lead to a successful outcome with a particular protocol. I would like to thank all those involved in the preparation of this volume, my colleagues for helpful suggestions and Professor John Walker, the series editor, for all his help and encouragement.

Ralph Rapley

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Contributors

- BENTLEY A. ATCHISON • *Victorian Institute of Forensic Pathology, Monash University, South Melbourne, Australia*
- BARBARA BACHMANN • *Deutsches Primatenzentrum, Abteilung für Virologie und Immunologie, Göttingen, Germany*
- PETER B. BECKER • *Gene Expression Programme, European Molecular Biology Laboratory, Heidelberg, Germany*
- NEIL BREWIS • *Department of Pathology, School of Medicine, University of California, San Diego, La Jolla, CA*
- FRANK C. BROSIUS III • *Departments of Internal Medicine and Nephrology, University of Michigan Medical School, Ann Arbor, MI*
- XINAN CAO • *Ann Arbor Veterans Administration Hospital, Ann Arbor, MI*
- JEAN-LAURENT CASANOVA • *Developpement Normal et Pathologique, INSERM U132, Hopital Necker, Paris, France*
- C. THOMAS CASKEY • *Merck Research Labs, Merck and Co. Inc., West Point, PA*
- ALISON COFFEY • *The Sanger Centre, Cambridge, UK*
- SUSAN E. DANIELS • *Wellcome Trust Centre for Human Genetics, Oxford, UK*
- ALBERT B. DEISSEROTH • *Department of Neuro-oncology and Department of Haematology, MD Anderson Cancer Center, The University of Texas, Houston, TX*
- ANDREA M. DOUGLAS • *Bone Marrow Research Laboratories, Royal Melbourne Hospital, Melbourne, Australia*
- IAN DUNHAM • *The Sanger Centre, Cambridge, UK*
- RUPERT EGENSERGER • *Laboratory of Molecular Neuropathology, Ludwig-Maximilians-University, Munich, Germany*
- MORTIMER M. ELKIND • *Deptment of Radiological Health Sciences, Colorado State University, Fort Collins, CO*
- MANUEL B. GRAEBER • *Laboratory of Molecular Neuropathology, Ludwig-Maximilians-University, Munich, Germany*
- BARBARA ANNE HALES • *Division of Biological Sciences, School of Natural Sciences, Coventry University, Coventry, UK*
- HOLLY A. HAMMOND • *Merck Research Labs, Merck and Co. Inc., West Point, PA*

- LAWRENCE B. HOLZMAN • *Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI*
- GERHARD HUNSMANN • *Deutsches Primatenzentrum, Abteilung für Virologie und Immunologie, Göttingen, Germany*
- GABOR L. IGLOI • *Institut für Biologie, University of Freiberg, Germany*
- BERNHARD KALTENBOECK • *Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL*
- SIEGFRIED KÖSEL • *Laboratory of Molecular Neuropathology, Ludwig-Maximilians-University, Munich, Germany*
- KONSTANTIN G. KOUSOULAS • *School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA*
- TAMMY LIND • *Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN*
- JINGMEI LIU • *Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM*
- CHRISTOPH B. LÜCKING • *Laboratory of Molecular Neuropathology, Ludwig-Maximilians-University, Munich, Germany*
- WOLFGANG LÜKE • *Deutsches Primatenzentrum, Abteilung für Virologie und Immunologie, Göttingen, Germany*
- JOAKIM LUNDEBERG • *Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden*
- IVOR J. MASON • *Division of Anatomy and Cell Biology, UMDS Guy's Hospital, London, UK*
- GEORGES-RAOUL MAZARS • *Ludwig Institute for Cancer Research, University College, London, UK*
- MARCIA A. MCALEER • *Department of Molecular Biology, Yamanouchi Research Institute, Oxford, UK*
- MARY I. COOLBAUGH MURPHY • *University of Texas Health Science Center, Texas Medical Center, Houston, TX*
- HARALD PETRY • *Deutsches Primatenzentrum, Abteilung für Virologie und Immunologie, Göttingen, Germany*
- BERTIL PETTERSSON • *Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden*
- ERAN PICHESKY • *Department of Biology, University of Michigan, Ann Arbor, MI*
- JEAN-PIERRE QUIVY • *Gene Expression Programme, European Molecular Biology Laboratory, Heidelberg, Germany*
- RALPH RAPLEY • *School of Natural Sciences, Coventry University, Coventry, UK*
- ZHIYUAN SHEN • *Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM*

- STEVE S. SOMMER • *Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN*
- ANU SUOMALAINEN • *Department of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland*
- ANN-CHRISTINE SYVÄNEN • *Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden*
- CHARLES THEILLET • *Ludwig Institute for Cancer Research, University College, London, UK*
- BIMAL D. M. THEOPHILUS • *Department of Haematology, The Birmingham Children's Hospital, Birmingham, UK*
- ERIK C. THORLAND • *Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN*
- MATHIAS UHLÉN • *Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden*
- ALISON WADE-EVANS • *Institute of Animal Health, Pirbright, Woking, Surrey, UK*
- DAVID WALSH • *Department of Molecular Genetics, School of Biological Sciences, University of Auckland, New Zealand*
- ROBERT L. WELLS • *Deptment of Radiological Health Sciences, Colorado State University, Fort Collins, CO*
- CRAIG WINSTANLEY • *Division of Biological Sciences, School of Natural Sciences, Coventry University, Coventry, UK*
- WEI ZHANG • *Department of Neuro-oncology and Department of Haematology, MD Anderson Cancer Center, The University of Texas, Houston, TX*