PCR Sequencing Protocols

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Humana Press  **Totowa, New Jersey**

999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

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ANSI Z39.48-1984 (American Standards Institute) Permanence of Paper for Printed Library Materials.

Cover illustration: Fig. 1 from Chapter 8, "Direct DNA Sequencing of PCR Products Using Magnetic Beads," by Joakim Lundeberg, Bertil Pettersson, and Mathias Uhlén.

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging-in-Publication Data

Main entry under title:

Methods in molecular biology™.

PCR sequencing protocols / edited by Ralph Rapley.
p. cm. — (Methods in molecular biology ; v. 65)
Includes index.
ISBN 0-89603-344-9 (alk. paper)
1. Polymerase chain reaction—Laboratory manuals. I. Rapley, Ralph. II. Series: Methods in molecular biology (Totowa, NJ) ; v. 65.
QP606.D46P365 1996 96-27724
574.87'3283—dc20 CIP

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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