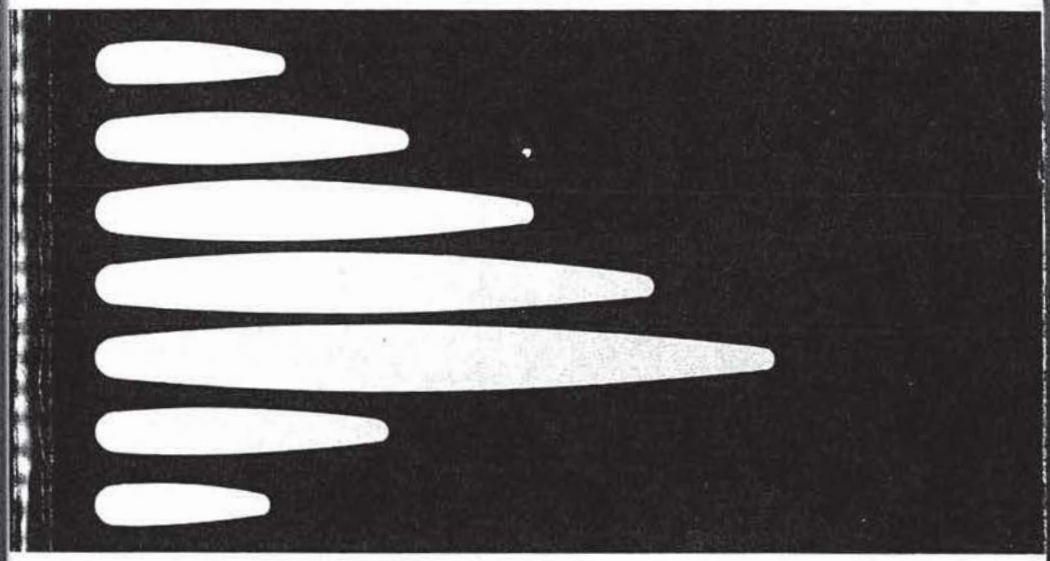
A Biologist's Guide to Principles and Techniques of Practical Biochemistry

Third Edition

Edited by
Keith Wilson and Kenneth H. Goulding



Contemporary Biology

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Contemporary Biology

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Edward Arnold
A division of Hodder & Stoughton
LONDON NEW YORK MELBOURNE AUCKLAND

First published in Great Britain 1975
Reprinted 1976, 1979
Second edition 1981
Reprinted with corrections 1983
Reprinted 1984
Third edition 1986
Reprinted 1988

Distributed in the USA by Routledge, Chapman and Hall, Inc.
29 West 35th Street, New York, NY 10001

British Library Cataloguing in Publication Data

A Biologist's guide to principles and
techniques of practical biochemistry.—
3rd ed.—(Contemporary biology)
1. Biological chemistry—Technique
I. Wilson, Keith, *1936–* II. Goulding,
Kenneth H. III. Series
576.19'2'028 QP519.7

ISBN 0-7131-2942-5

Typeset in 10/11pt Times Compugraphic by Colset Private Ltd, Singapore. Printed and bound in Great Britain for Edward Arnold, the educational, academic and medical publishing division of Hodder and Stoughton Limited, 41 Bedford Square, London WC1B 3DQ by Richard Clay Ltd., Bungay, Suffolk.

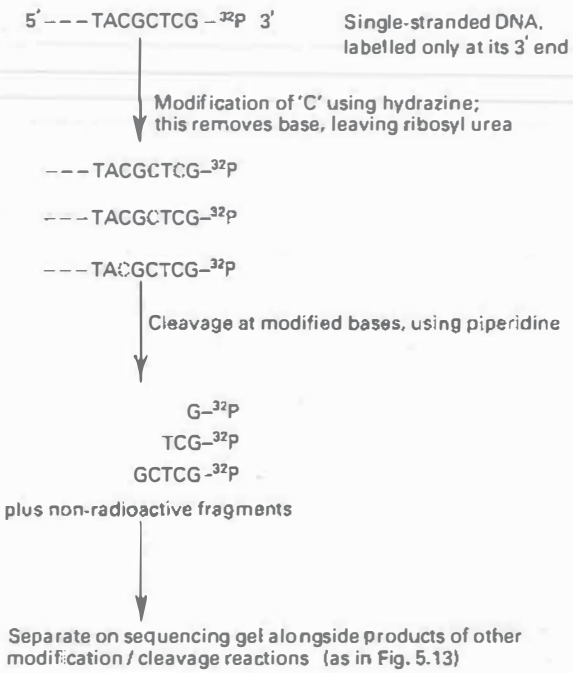

Fig. 5.14 Maxam and Gilbert sequencing of DNA. Only modification and cleavage of deoxycytidine is shown, but three more aliquots of the end-labelled DNA would be modified and cleaved at G, G + A, and T + C, and the products would be separated on the sequencing gel alongside those from the 'C' reactions.

to that produced by the Sanger method, since each sample now contains radioactive molecules of various lengths, all with one end in common (the labelled end), and with the other end cut at the same type of base. Analysis of the reaction products by electrophoresis is as described for the Sanger method.

Because the Sanger method produces oligonucleotides which are radioactively labelled throughout their lengths, rather than only at one end, the molecules can be made a lot more radioactive, and therefore easier to detect; so less DNA is needed for sequencing. Once M13 cloning has been set up in a laboratory, it provides a very convenient and rapid way to obtain single-stranded DNA. For these reasons, dideoxy sequencing of M13-cloned DNA is probably the most commonly used sequencing method, though the chemical procedure is still used by many laboratories.

5.5.3 Protein sequencing

Although protein sequencing may seem out of place in a section dealing with the analysis of DNA, the molecular biologist can often make use of a

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