**Benchmark Papers in Microbiology / 15**

**A BENCHMARK ® Books Series**

# RECOMBINANT DNA

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STROUDSBURG, PENNSYLVANIA

Benchmark Papers in Microbiology, Volume 15
Library of Congress Catalog Card Number: 80-14100
ISBN: 0-87933-378-2



83 82 81 1 2 3 4 5
Manufactured in the United States of America.

LIBRARY OF CONGRESS CATALOGING IN PUBLICATION DATA
Main entry under title:
Recombinant DNA.
(Benchmark papers in microbiology; v. 15)
Includes bibliographical references and index.
1. Recombinant DNA—Addresses, essays, lectures. I. Denniston, K. J.
II. Enquist, Lynn W.
[DNLM: 1. DNA, Recombinant. W1 BE517M v. 15 / QU58 R311]
QH442. R37 574.87'3282 80-14100
ISBN 0-87933-378-2

Distributed world wide by Academic Press,
a subsidiary of Harcourt Brace Jovanovich,
Publishers.

# Benchmark Papers in Microbiology

**Series Editor: Wayne W. Umbreit**
**Rutgers—The State University**

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# SERIES EDITOR'S FOREWORD

The decade of the seventies witnessed such remarkable advances not only in our understanding of at least the prokaryotic gene but also, and perhaps more important, in our ability to manipulate and characterize such genetic elements. A major part of these new developments is the concept and technology of "recombinant DNA." Even individuals not working in the field (which constitutes the vast majority of biological scientists) have been markedly influenced by recombinant DNA developments, and new horizons have opened before us. Drs. K. J. Denniston and L. W. Enquist, as active contributors to this field, have collected for us the papers that describe the concepts, the methods, and the opportunities of this new area. One gets a sense of the excitement of the new developments, and their discussions generate a desire to understand these achievements in which we ourselves are not engaged and in which the terminology and even the methodology seem incomprehensible. How, indeed, is one to join DNA molecules, and then prove that one has joined them? How is one to recognize a plasmid? How can a phage carry a "foreign" DNA? How can one identify specific DNA fragments? How can one separate a specific gene from the thousands of others with which it occurs? How can one control what is obtained, lest one make an organism carrying genes that might destroy us? How can one insert an isolated or reconstructed gene into a living organism and have it develop therein? All these questions (and many more) were essentially unanswerable in 1970; but by 1980 there were clear, definitive, positive answers, surely progress that should not go unnoted.

The developments under the general title "recombinant DNA" are known to a relatively few workers in this area of knowledge, but increasingly to a wider group not specifically concerned with microbial and eukaryotic genetics. It is to this group (with a minimum background in genetics) that this volume will be especially useful. These individuals do not have either the time or the knowledge to search the scattered literature and are not likely to respond to "Nonchromosomal Antibiotic Resistance in Bacteria: Genetic Transformation of *Escherichia coli* by R-Factor DNA" (Paper 7), for example. Yet when selected by people who do know its significance and who explain just what its significance was (and is), such a paper is not only read but becomes an important living

part of the basic structure of a science. Of course, in almost any vital development, there are many papers on a given facet of the problem. One relies on the editor to select from among them, and, of course, the selections reflect the sometimes subjective judgments of the editors. Needless to say, other papers could have been selected, and an essentially similar pattern would have emerged. There is, however, a new difficulty that has arisen in that certain journal editors will not allow the reproduction of such material (even for a fee), evidently regarding it as a personal possession rather than as a part of science. Authors who publish in such journals face the restriction that their work may never be known to a larger public because other papers will be selected for such reproduction simply because of editorial policy. Editors of Benchmark and similar series are thus not necessarily responsible for the precise papers selected and might have, on occasions, preferred papers whose inclusion was not possible because of the inability to obtain permission for reprinting. This is clearly a restriction on the free flow of scientific information.

W. W. UMBREIT

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## PART III: BASIC TECHNIQUES

# INTRODUCTION

A fusion of ideas and techniques gave rise in the early seventies rather explosively to a new era in molecular biology. Daniel Nathans (1979), in his Nobel address, called this era "the new genetics." It is now possible for scientists to manipulate DNA molecules, reintroduce them into cells, and occasionally cross major species barriers. Genes and control elements of higher organisms, once seemingly intractable to molecular methods, can now be analyzed in detail, often at the level of DNA sequence. An integral part of the new genetics is the technology of "recombinant DNA": the joining together of diverse DNA molecules. This terminology, that prior to 1972 would have meant almost nothing, is now a part of the lexicon of the scientific and popular press. Several other terms are often interchanged when describing this technology, including "cloning" and "genetic engineering." Molecular cloning is a fairly accurate description of what happens in recombinant DNA technology in that a single DNA fragment is joined to a self-replicating unit (a "vector," see Part II), and many copies of the original molecule are produced. Unfortunately, the conceptual leap from cloning molecules to cloning individuals is made too easily, and the ethical problems of this latter technology are transferred to the former. At the moment, there is a vast expanse between the two concepts of cloning. Similarly, "genetic engineering" is an accurate, if somewhat imaginative, description of the art of joining DNA molecules together. One need only listen to a discussion of recombinant DNA experiments among a group of scientists using phrases like "blunt-end joining," "trimming back," "filling in," "hooking up," or "flipping around" to visualize some kind of microscopic construction work.

Recombinant DNA experiments gave rise to many other new, nonscientific entities, including: abbreviations, bureaucracy and multiauthored papers. RAC (Recombinant DNA Advisory Committee), ORDA (Office of Recombinant DNA Activities), MUA

(memorandum of understanding and agreement), EK1–EK2 (levels of biological containment), P1-P4 (levels of physical containment) and so forth, all are entrenched in the rules and regulations engendered by studies on recombinant DNA.

Recombinant DNA research is not one study or discipline but rather a variety of techniques that can be used for many kinds of experiments in molecular biology. This collection of papers is our attempt to define some of the critical work that created this remarkable technology. Some of the discoveries that form the foundation of this field have already been recognized: witness the 1978 Nobel prizes in Physiology and Medicine to Drs. Werner Arber, Hamilton Smith, and Daniel Nathans and the 1980 Nobel prizes in Chemistry to Drs. Paul Berg, Walter Gilbert, and Frederick Sanger. The field continues to expand rapidly, but the basic concepts set down by the papers contained here remain the backbone of recombinant DNA experiments. Some of the papers are reports of original work that opened up new areas; others are excellent reviews or elegant utilization of the technology. We sought to include those papers that are cited often, even though their methodology may be outdated, simply because they establish valuable technical ideas.

The papers included are for the most part devoted to work done using *Escherichia coli*, its phages and plasmids. Two papers are included describing the development of a recombinant DNA system in yeast. Certainly other systems are available including *Bacillus* systems (Young et al. 1977) and animal virus systems like SV40 (Ganem et al. 1976; Goff and Berg 1976, 1979; Hamer 1977). We chose to emphasize *E. coli* simply because of its central role in the development of recombinant DNA technology. We apologize for the omission of many papers obviously significant or incisive, but the final choice was limited primarily by space and inability to obtain permission for reprinting of some papers. Therefore, the final selection was influenced by our own prejudices.

The commentaries preceding each section are intended to provide continuity in the ideas and to give some historical perspective. Where possible, references to current reviews or recent papers are included, but the literature review is not intended to be exhaustive.

The papers are arranged in five sections. There is considerable overlap between sections, but the organization enables the reader to examine first those investigations that formed the foundations of recombinant DNA technology, to read those papers that detail the concepts and construction of vectors, to study the vari-

ety of methods evolving for use of such vectors in recombinant DNA studies, to see how these methods were used to analyze specific genes of complex organisms and, in the last section, to see one of the applications of this technology, namely, the expression of eukaryotic genes in *E. coli*.

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