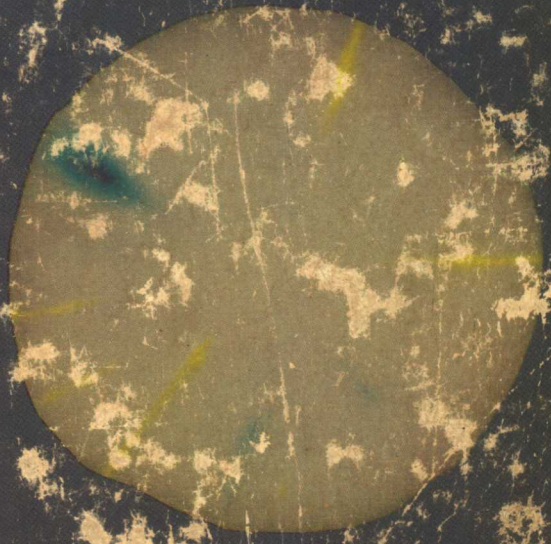
Experiments in Molecular Genetics



Jeffrey H. Miller

Experiments in Molecular Genetics

Jeffrey H. Miller

Society of Fellows, Harvard University
and
Département de Biologie Moléculaire
Université de Genève

Cold Spring Harbor Laboratory 1972

EXPERIMENTS IN MOLECULAR GENETICS

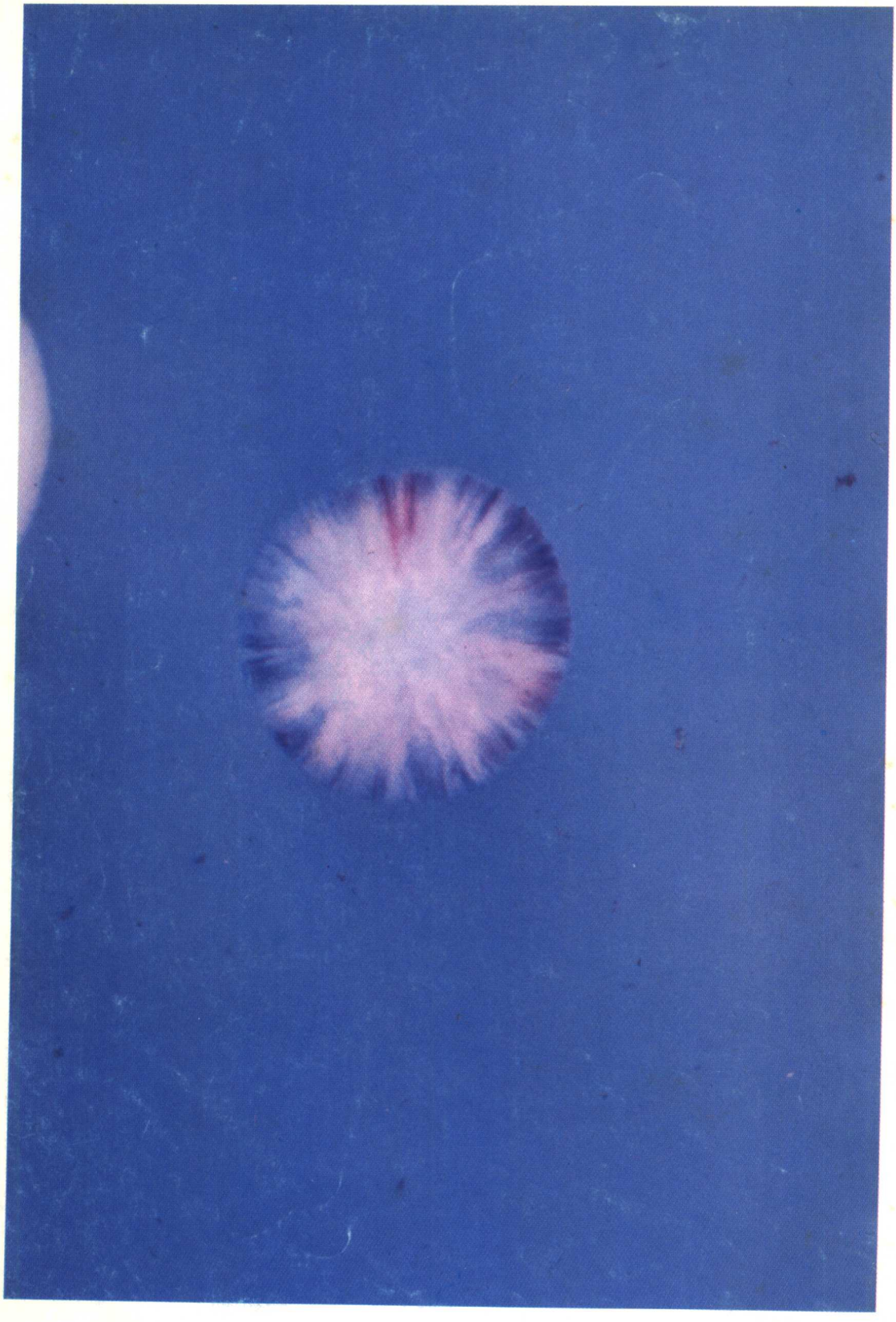
International Standard Book Number: 0-87969-106-9

Library of Congress Catalog Card Number: 72-78914

Printed in the United States of America

Cover photos: Mutator colonies stained for both constitutive β-galactosidase (blue) and constitutive alkaline phosphatase (yellow). For description of methods see Experiment 3. Photograph courtesy of G. Hombrecher from the laboratory of W. Vielmetter, Institut Genetik, Universität Köln.

Frontispiece: Mutator colony stained with Giemsa stain (see Experiment 3). Photograph courtesy of G. Hombrecher from the laboratory of W. Vielmetter, Institut Genetik, Universität Köln.



Foreword

One of the more obvious conclusions from the biology of the past twenty-five years is that biochemistry moves faster when it can utilize genetic methods. Likewise, much of what we now call genetics would not have been discovered had not biochemical methods been introduced. Though today many of the practitioners who merge the biochemical and genetic approaches call themselves molecular biologists, there are many other scientists who see no reason to change their names, seeing nothing dishonorable with the terms biochemist and geneticist. But no matter what we call ourselves today, almost no one argues for a purest approach which sees virtue in solving a problem solely by genetic crosses or by massive numbers of postdoctoral biochemists. Of course, there still exist many important problems where now only one of these approaches can be applied. Increasingly, however, we find we can remove this limitation by choosing a more appropriate organism to work with.

Now there is hardly any discussion as to the organism of first choice for probing a fundamental biological problem. *E. coli* stands so far in the forefront that it frequently provokes boredom on the part of the uninitiated, who may have started reading about it in high school and who in college found it hard to take a biology or biochemistry course where it did not sneak in. Thus, it is easy to say that the days of *E. coli*'s domination must soon pass, and so on to embryology and those organisms which have nuceli, mitochondria, and perhaps a number of chloroplasts.

But sighting a new frontier does not always mean that the time is ripe for everyone to open it up. Over and over the past decade has produced examples of key problems where the use of mutants was indispensible to their solution. This situation is still true today. Virtually each new issue of the *Proceedings of the National Academy of Science* contains one or more incisive articles whose conclusions are based on the use of specific *E. coli* mutants.

So knowledge of how to work with *E. coli* as a genetic system is likely to remain

a key ingredient in the biology curriculum for many years to come. Teaching bacterial genetics, however, as a purely formal subject without integration into the mainstream of modern biochemistry and molecular biology would be a very dull job. It would fail to convey the excitement of current genetic research and leave the impression that it is an esoteric topic best suited for those who only live genetics. Instead we believe the *E. coli* genetics is best taught in the context of contemporary research where mutants are vital for solving fundamental biological dilemmas.

This is the way the bacterial genetics course at Cold Spring Harbor has been taught since its inception the summer of 1950. First taught by M. Demerec, E. Witkin, and V. Bryson, it has been given here each succeeding summer to ten to twenty students of highly diverse backgrounds, ranging from the pure physical scientist to the applied microbiologist. By now some 250 people have come here for this specific purpose. Many have been strongly influenced by this experience and quickly settled on subsequent careers in molecular genetics.

Now we have to face the fact, however, that the three-week interval in which our summer courses must be given is only sufficient for a small fraction of the experiments that make up bacterial genetics. Inevitably, the experiments which are chosen for a given summer must reflect the specific research interests of the instructors of that year. Furthermore, the number of people who need to be familiar with current tricks for doing bacterial genetics greatly exceeds those that can come here to take our course or go for a learning period to a lab that specializes on this topic. Yet the intelligent novice should have at his disposal a way to become familiar with all the new procedures, if not the "lore" that he might someday need.

The moment thus seems propitious to bring forth an all-inclusive manual where "everything you need to know" about bacterial genetics can be found. In getting Jeffrey Miller to do this job, we have been most fortunate. He is old enough already to be a master in this field, yet, when he started writing he was too young to know how much work is necessary to turn out a good book. The final result, I believe, is a superb job; one, I hope, that will find widescale use for many years to come.

Cold Spring Harbor
March, 1972

J. D. Watson

Preface

Why do we still study *E. coli*? One attraction of working with such cells is that they represent a simplified system. The possibility of harvesting a large number of cells in a short time, and the advantage offered by a haploid organism containing only one chromosome and which can double every 20 minutes, have prompted many investigators to use *E. coli* for genetics research. Their work has considerably increased our knowledge of this organism and has resulted in the development of numerous specialized techniques, many of which we use in this manual.

Most importantly, there remain a vast number of basic problems in the field of cell biology which are as yet poorly understood in even a relatively simple system like *E. coli*. DNA replication, recombination, and repair are examples. Much about the mechanism of transcriptional (mRNA) control is unclear, for instance, positive control. The question of how the synthesis of ribosomal and transfer RNA is controlled is still largely unanswered. Many details of protein synthesis have not yet been elucidated, and the DNA and RNA sequences coding for the initiation and termination of transcription and translation are just now being deduced. The problem of controlled degradation of mRNA and of proteins still is mostly unsolved, and the study of membranes and transport may also be dependent on systems such as *E. coli*. Finally, there remain many unresolved aspects of intermediary metabolism.

How can bacterial genetics help solve these problems? The answer to this question is the subject of this manual. The basic approach that this field offers is the isolation of mutants. The discovery of new control systems, the tailoring of enzymes by genetic manipulation, and the definition of genes involved in biochemical pathways and processes are all direct results of this approach. Therefore, much of the text describes methods for the induction, isolation, characterization, and mapping of different types of mutations.

To facilitate the teaching of experimental molecular biology, we have compiled a series of experiments which can be done on a class basis and which cover many of the areas of modern bacterial genetics. Many of these experiments have been performed by student groups, such as the summer Bacterial Genetics Courses at Cold Spring Harbor. We use the *lac* operon for illustration often, and a review text, *The Lactose Operon* (Cold Spring Harbor Laboratory, 1970) has recently been published which provides a valuable summary of work on this basic system. We were fortunate to able to draw on the excellent experimental manual by Clowes and Hayes (John Wiley and Sons, Inc., 1968) which served as a model for much of this book.

We have also tried to bring together into one volume as many recipes and methods as possible to enable investigators to use this text as a research handbook. Although the *lac* system is used for demonstrative purposes throughout part of the manual, almost all of the methods and techniques described are general. Thus, the description of indicator plates, mutagenesis, Hfr crosses, strain construction, hybridization, and enzyme assays are applicable to a wide variety of systems. Also, we hope that compiling these techniques will enable investigators not thoroughly acquainted with genetic manipulations in *E. coli* to form strategies for building strains and isolating mutants. One of the recent advances in bacterial genetics has been the development of techniques for incorporating bacterial genes into the DNA of certain phages. These specialized transducing phage are then used to provide DNA greatly enriched for the specific gene of interest. Experiments utilizing current methods for isolating these phage are presented in detail in this manual.

We have attempted to arrange these experiments in order of increasing difficulty and have tried to introduce the concepts of some of the later experiments in earlier sections. For instance, Experiment 42 (The Isolation of *trp-lac* Fusion Strains) is a series of steps, each of which has been covered previously. The object of the experiment is to isolate strains in which the *lac* genes are under the control of the *trp* operon. First, phage-resistant mutants are isolated and examined on lactose indicator plates. Recombination and complementation tests are then used to determine the end points of the deletions. Finally, Hfr crosses and F′ transfers are employed to prepare and test *trpR* derivatives of the fusion strains. (This experiment was successfully performed by different groups of 20 students at Cold Spring Harbor in 1969 and 1970.) We would like to emphasize, however, that there is no mandatory order to these experiments. We have included many more experiments in this manual than could possibly be accomplished in a one-semester course. This gives the students and instructors a large freedom of choice in selecting experiments and planning courses. For instance, Unit VI is certainly optional since this requires special equipment which may be unavailable in some laboratories.

In order to facilitate the use of this manual, we have made available strain kits containing the 79 strains described here and 5 lysates in small, 1-dram, agar-filled stab bottles. We also include in each kit a precision-made device, described in the Appendix, which is used for interrupted matings. Kits can be obtained from Cold Spring Harbor Laboratory for $100 to cover costs of handling, packaging, and mailing.

Acknowledgments

It is a pleasure to acknowledge the help of many people without whom this manual could never have been compiled. First of all I am indebted to Jim Watson, who conceived of the idea to produce this manual, who encouraged and advised me throughout the preparation of the book, and who spent many hours reading the final manuscript and the proofs.

This book is a collection of experiments written by several authors. I wrote the introductory material, Units I–V, Unit IX through the end of Experiment 59, and part of Unit VII. Terry Platt wrote Unit VIII, Appendix VI, and parts of Unit VII; Bill Haseltine contributed Experiments 44–47 in Unit VI; Jack Greenblatt prepared Experiments 60–62 in Unit IX; and Benno Müller-Hill wrote Experiment 43 in Unit VI and parts of Unit VII. Also, Larry Taylor and Brooks Low contributed Appendices IV and II, respectively, and Ernesto Bade wrote part of Experiment 47. I have reviewed and edited all of this material to put together the final manual, and I am solely responsible for any errors which are present.

Significant parts of this manuscript were read by Charles Yanofsky, Bob Weisberg, Frank Stahl, Joel Kirschbaum, Don Ganem, John Scaife, Larry Taylor, Ray Gesteland, Nancy Hopkins, Geoffrey Zubay, and Terry Platt. I am grateful for their comments. I am particularly indebted to David Botstein, Brooks Low, and Bill Reznikoff who read most of the first draft in detail and suggested many revisions, and also to David Zipser, Ahmad Bukhari, and Ernesto Bade for reading parts of the proofs.

During the initial preparation of the experimental protocols I benefited from the advice of many people. In particular I would like to thank Ed Lin, Bob Weisberg, Joel Kirschbaum, Don Ganem, Larry Taylor, Brooks Low, Max Gottesman, Jon Beckwith, Ethan Signer, Walter Gilbert, Dan Morse, Julian and Marilyn Gross, Lucien Caro, Klaus Weber, David Zipser, Ahmad Bukhari, Jürgen Schrenk, Mike Malamy, Ekke and Linde Bautz, Marc Van Montagu, and David Dressler. Several of these experiments were retested specifically for this manual by Joel Kirschbaum and Don Ganem. I am grateful for their help.

I would also like to thank Madeline Szadkowski for typing the final manuscript, Judy Gordon for doing the copy editing and proof reading, and Glen Lyle and Judy Gordon for preparing the design of the book. Hanna Neubauer and F. W. Taylor, Co., Northport, N.Y., contributed the art work.

I wrote this manual while a junior fellow of the Society of Fellows of Harvard University, and I am most grateful for their support. I also used the facilities of the Harvard Biological Laboratories (in the Watson-Gilbert group), the Genetics Institute of the University of Cologne (in the laboratory of B. Müller-Hill), and the Cold Spring Harbor Laboratories during this period, and am indebted to these institutions for their support.

Finally, I would like to thank all of the people in the Cold Spring Harbor community who made my past two summers at Cold Spring Harbor (where most of this manual was prepared) enjoyable. In particular I am indebted to Elfie, John, and all the Cairns; Liz and Jim Watson; Frauka, Henry, and the Westphals; and Marianne, Walter, and the Kellers.

Cold Spring Harbor
January, 1972

Jeffrey H. Miller

Table of Contents

INTRODUCTION