

Molecular Cloning

A LABORATORY MANUAL

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Cold Spring Harbor Laboratory

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Front cover: The electron micrograph of bacteriophage λ particles stained with uranyl acetate was digitized and assigned false color by computer. *Thomas R. Broker, Louise T. Chow, and James I. Garrels*

Back cover: *E. coli* VL361 with fimbriae was negatively stained with phosphotungstic acid and the electron micrograph was digitized and assigned false color by computer. *Jeffrey A. Engler, Thomas R. Broker, and James I. Garrels*

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Preface

This manual began as a collection of laboratory protocols that were used during the 1980 Cold Spring Harbor course on the Molecular Cloning of Eukaryotic Genes. These procedures had been in use in our laboratories at that time but were scattered throughout the notebooks of many different people. In 1981 we decided to produce a more complete and up-to-date manual not only for use in the next Cold Spring Harbor course, but also for eventual publication. Out of the many permutations of the methods being used, we assembled a set of "consensus protocols," which were photocopied and widely distributed to many laboratories even as the 1981 course was underway. Then in the winter of 1981-1982, the manual was substantially rewritten, and new or revised protocols and figures, as well as entirely new chapters, were added.

Even since this last rewriting, however, the field has progressed: New methods are constantly being invented and existing techniques are altered in response to changing needs. Although we have included in this manual only those protocols that have been thoroughly tested and used successfully in our laboratories, we make no claim that they are inviolable or perfect. We would welcome suggestions for improvements, and we would be grateful to be told about any new procedures that are devised.

The evolution of protocols poses the difficult problem of attribution. We have tried to give credit at appropriate places in the text to the people who originally developed the procedures presented here, but in many cases tracing a particular method to its undisputed roots has proved to be impossible. We therefore wish to apologize—and to express gratitude—to those we have been unable to acknowledge for an idea, procedure, or recipe. Our major function has been to compile, to verify, and, we hope, to clarify; less frequently we have introduced modifications, and only in rare instances have we devised new protocols. In large part, then, the manual is based on procedures developed by others, and it is to them that any credit belongs.

Because the manual was originally written to serve as a guide to those who had little experience in molecular cloning, it contains much basic material. However, the current version also deals in detail with almost every laboratory task currently used in molecular cloning. We therefore hope that newcomers to cloning and veterans alike will find material of value in this book.

Although molecular cloning seems straightforward on paper, it is more difficult to put into practice. Most protocols involve a large number of individual steps and a problem with any one of them can lead the experimenter into difficulty. To deal with these problems, a well-founded understanding of the principles underlying each procedure is essential. We have therefore provided background information and references that may be useful if trou-

ble should arise. We also suggest that, as a matter of course, the products of each step in a protocol be tested to verify that the reaction was successful.

This manual could not have been written without the help and advice of members of our laboratories and contributions from many others. We therefore wish to thank John Fiddes, Mary-Jane Gething, David Goldberg, Steve Hughes, David Ish-Horowicz, Mike Mathews, Patty Reichel, Joe Sorge, Jim Stringer, Richard Treisman, and Nigel Whittle. We wish particularly to thank Arg Efstratiadis for his helpful discussions and criticisms of Chapter 7; Brian Seed for permission to include a description of his unpublished procedure for screening libraries by recombination (Chapter 10) and many other useful suggestions; Doug Hanahan for advice on transformation (Chapter 8); Bryan Roberts for suggestions on methods of hybrid-selection and cDNA cloning; Doug Melton for providing a protocol for injection of *Xenopus* oocytes; Ronni Greene for suggesting improvements to many protocols; Nina Irwin for providing a critical anthology of methods available for expressing eukaryotic proteins in bacteria (Chapter 12); Rich Roberts for supplying the computer analysis of the sequence of pBR322; Barbara Bachmann for reviewing and correcting the list of *E. coli* strains; and Tom Broker, Louise Chow, Jeff Engler, and Jim Garrels for producing the elegant photographs used for the front and back covers.

We also thank all those who participated in the Cold Spring Harbor Molecular Cloning courses of 1980 and 1981. They were an excellent group of students, who struggled through the first two drafts of the manual and made many useful suggestions. We also thank Nancy Hopkins, who helped us to teach the course the first year and convinced us that producing a manual would be a worthwhile task. In 1981 Doug Engel helped teach the course and suggested many improvements to the manual. Contributing to the success of both courses were the efforts of the teaching assistants, who were Catherine O'Connell and Helen Doris Keller in the summer of 1980 and Susan Vandewoude, Paul Bates, and Michael Weiss in 1981.

We wish to thank Patti Barkley and Marilyn Goodwin for their cheerfulness and forbearance during the typing of successive revisions of the manuscript. Our artists, Fran Cefalu and Mike Ockler, worked with great dedication and perseverance to produce the drawings for the manual. Joan Ebert kept track of the many references added to and deleted from the text and assembled the reference list. We are also grateful to Nancy Ford, Director of Publications, Cold Spring Harbor Laboratory, for her encouragement and support. Finally, without the patience, skill, and diplomacy of Doug Owen, who prepared the manuscript for the printer and helped us in many other ways, this book would not exist.

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Joe Sambrook

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