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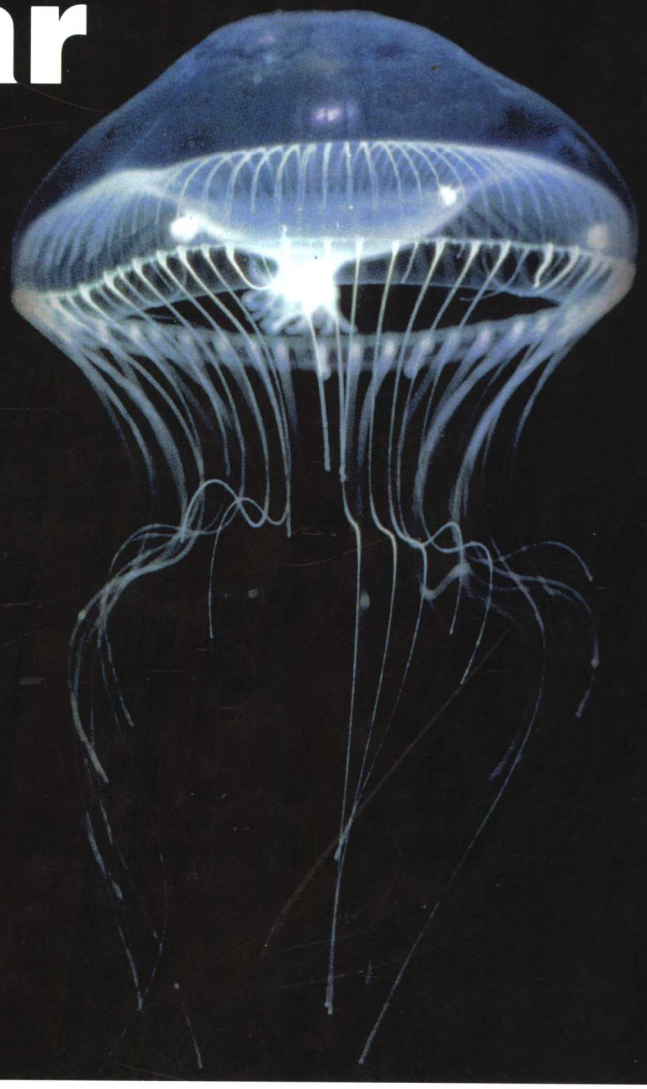
Molecular Cloning

A LABORATORY MANUAL



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A LABORATORY MANUAL

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——实验室手册

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Front cover (paperback): The gene encoding green fluorescent protein was cloned from *Aequorea victoria*, a jellyfish found in abundance in Puget Sound, Washington State. This picture of a 50-mm medusa was taken on color film by flash photography and shows light reflected from various morphological features of the animal. The small bright roundish blobs in the photograph are symbiotic amphipods living on or in the medusa. The bright ragged area in the center is the jellyfish's mouth.

Bioluminescence from *Aequorea* is emitted only from the margins of the medusae and cannot be seen in this image. Bioluminescence of *Aequorea*, as in most species of jellyfish, does not look like a soft overall glow, but occurs only at the rim of the bell and, given the right viewing conditions, would appear as a string of nearly microscopic fusiform green lights. The primary luminescence produced by *Aequorea* is actually bluish in color and is emitted by the protein aequorin. In a living jellyfish, light is emitted via the coupled green fluorescent protein, which causes the luminescence to appear green to the observer.

The figure and legend were kindly provided by Claudia Mills of the University of Washington, Friday Harbor. For further information, please see Mills, C.E. 1999–2000. Bioluminescence of *Aequorea*, a hydromedusa. Electronic Internet document available at http://faculty.washington.edu/cemills/Aequorea.html. Published by the author, web page established June 1999, last updated 23 August 2000.

Back cover (paperback): A portion of a human cDNA array hybridized with a red fluor-tagged experimental sample and a green fluor-tagged reference sample. Please see Appendix 10 for details. (Image provided by Vivek Mittal and Michael Wigler, Cold Spring Harbor Laboratory.)

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Preface

THE FIRST EDITION OF THIS MANUAL was written some 20 years ago, when basic methods in molecular cloning were far from robust and were established in only a few laboratories. The appearance of that book did much to change the picture. The manual's didactic character gave readers confidence to use techniques that still seemed magical during the late 1970s and early 1980s. The second edition, published just 7 years after the first, was smoother in style and far richer in reliable content. By then, individual methods had become more durable and portable, but it was still difficult to string them successfully together into multistep procedures. This new edition, almost certainly the last to appear in book form, reflects a mature discipline working at full power and with high reliability. During the 1990s, many of the drearier, more repetitive techniques of molecular biology have been automated; demanding, multistep procedures have been converted into kits; high-quality genomic and cDNA libraries are now available from the shelves of commercial manufacturers; and all manipulations involving nucleic acids have benefited greatly from improvements in the quality of reagents and enzymes. As a consequence of these and other advances, competent laboratory workers can now easily avoid experimental problems that beset even the best investigators just a few years ago. This is not to say that everything works perfectly all of the time or that no further improvement is possible. However, difficulties now can largely be avoided by careful planning and application of existing knowledge rather than by experimental trial and error.

A major goal of all three editions of *Molecular Cloning* has been to provide researchers with up-to-date protocols that work reproducibly. Users of the previous editions will recognize many of the organizational features in the experimental sections of this book. Nevertheless, the revision of the text has been extensive and detailed. Ancient protocols have been modernized, while new protocols have been added to reflect the continuing penetration of molecular cloning into almost all areas of biomedical research. Of equal importance has been our desire to explain how and why particular methods work, and with reasoned arguments for choosing between alternative procedures. This edition therefore contains not only annotations at crucial points in the protocols, but also an abundance of material in the form of Information Panels, which are placed at the end of the chapters as well as in Appendix 9. We hope that these 115 panels spread throughout the three volumes of the book will provide clear insights into the reasons why methods are carried out in a certain manner and how techniques have progressively evolved. Finally, we have provided extensive references to the scientific literature so that curious readers can trace methods and ideas to their roots. Few will read this book from beginning to end. But we hope that the community of cloners will find in these pages much to stimulate the mind and to facilitate the work of their hands.

As might be imagined of a book that has been long in the making, scores of individuals have provided material. We particularly thank Erica Golemis and her colleagues for Chapter 18 on

"Protein Interaction Techniques," a large and rapidly changing field that neither of us felt comfortable covering. The people listed on the facing page have all made valuable contributions — some verbal, some written, and some corrective — that we gratefully acknowledge. Other colleagues have provided, sometimes unwittingly, critical insights into problems of both style and substance. In addition, the chocolate-loving editorial and production staff at Cold Spring Harbor Laboratory Press have spent thousands of hours meticulously checking references, facts, and grammar and producing, we think, a book of harmonious and elegant design. Without the enduring efforts, diligence, and cheerful dedication of Maryliz Dickerson, Inez Sialiano, Joan Ebert, Mary Cozza, Dorothy Brown, Susan Schaefer, Danny deBruin, Nora McInerny, and Denise Weiss, this book, if it existed at all, would be an embarrassment. The manual could not have been completed without the patient understanding and speedy responses of the librarians at Cold Spring Harbor Laboratory, The University of Texas Southwestern Medical Center at Dallas, and the Peter MacCallum Cancer Institute, Melbourne.

We owe deep debts to our Associate Authors, Kaaren Janssen and Nina Irwin, who have given us unstinting support, expert work, clarifying ideas, and dedicated and unflagging optimism. Siân Curtis and Michael Zierler ironed out scientific problems in the protocols, and Siân also assembled the appendices. Mark Curtis converted rough drafts drawn on scraps of paper into elegant and intelligent illustrations. All of these people came up with many good suggestions. Foolishly, perhaps, we did not accept them all, so any remaining errors of fact or interpretation are ours alone.

Personal debts can never be adequately acknowledged. Jan Argentine, our Managing Editor, has given us support in more ways than we can list here. She has given ungrudgingly of her time and has brought common sense, order, civility, and timeliness to a process that sometimes threatened to fall out of control. No writers could have received greater help and friendship. We owe special thanks to Daphne Davis, who cheerfully provided answers to many questions concerning experimental details. We have also benefited from the encouragement and sustaining enthusiasm of many others — in particular, John Inglis and Jim Watson at Cold Spring Harbor, Nancy Ford, who worked with us during the early stages of this project, and Rose Williams in Melbourne. Kate Simpson, a person of rare charm and intelligence, worked on the manual for a few months but did not live long enough to see the project completed. We hope that some of her lively grace shines through these pages. Finally, we owe an unquantifiable debt to our families, who have seen these three volumes built sentence by sentence and whose encouragement has never flagged.

Joe Sambrook
David Russell

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THIS PRINT EDITION OF *MOLECULAR CLONING* is associated with a Web Site (www.MolecularCloning.com) that is evolving into an on-line laboratory manual.

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The public pages of the site contain answers to FAQs about the registration procedure and a demonstration of the functions available to registered users. For additional assistance with registration, and for all other inquiries about the *Molecular Cloning* Web Site, please e-mail molecular.cloning@cshl.org or call 1-800-843-4388 (in the continental U.S. and Canada) or 516-349-1930 (all other locations) between 8:00 A.M. and 5:00 P.M. Eastern U.S. time.

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Cometh al this newe science that men lere.
GEOFFREY CHAUCER

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