

# Molecular Cloning

A LABORATORY MANUAL

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A LABORATORY MANUAL

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FOURTH EDITION

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# Molecular Cloning

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## Preface

THE READY AVAILABILITY OF THE COMPLETE GENOME SEQUENCES for humans and model organisms has profoundly affected the way in which biologists of every discipline now practice science. Exploration of the vast genomic landscape required the development of a great variety of new experimental techniques and approaches. Inevitably, venerable cloning manuals became outdated and established methods were rendered obsolete. These events provided the major impetus for a thorough revision of *Molecular Cloning*.

In the first stages of *Molecular Cloning 4* (MC4), we undertook an extensive review process to determine what old material should be kept, what new material should be added, and, most difficult of all, what material should be deleted. The names of the many scientists who contributed invaluable advice during the review process are listed on the following acknowledgments page. We are grateful to them all.

It is of course impossible to include in any single laboratory manual all experimental methods used in molecular biology, and so choices, sometimes tough ones, had to be made. Individuals may disagree with some of our choices (and we suspect some will). However, our two guiding principles are as follows. First, *Molecular Cloning* is a “nucleic acid–centric” laboratory manual, and therefore in general we have not included methods that do not directly involve DNA or RNA. So, although MC4 contains a chapter on yeast two-hybrid procedures for analyzing protein–protein interactions, we did not include the many other approaches for studying protein–protein interactions that do not directly involve nucleic acids. Second, we have attempted to include as many as possible of those nucleic acid–based methods that are widely used in molecular and cellular biology laboratories with the hope in the John Lockean sense that we “do the greatest good for the most people.” The harder task for us was to decide which material to delete. However, our task was made somewhat easier by the agreement of our publisher, Cold Spring Harbor Laboratory Press, to make these older methods freely available on the *Cold Spring Harbor Protocols* website ([www.cshprotocols.org](http://www.cshprotocols.org)).

The explosion of new experimental approaches has made it impractical, if not impossible, for any single person (or even two) to write with authority on all the relevant experimental methods. As a result, a major departure from previous editions of *Molecular Cloning* was the commissioning of experts in the field to write specific chapters and to contribute specific protocols. Without the enthusiastic participation of these scientists, MC4 simply would not exist.

Since the last edition of *Molecular Cloning*, there has been a relentless and continuing proliferation of commercial “kits,” which is both a blessing and a curse. On the one hand, kits offer tremendous convenience, particularly for procedures that are not routinely used in an individual laboratory. On the other hand, kits can often be too convenient, enabling users to perform procedures without understanding the underlying principles of the method. Where possible, we have attempted to deal with this dilemma by providing lists of commercially available kits and also describing how they work.

There are many people who played essential roles in the production of MC4 and whom we gratefully acknowledge. Ann Boyle was instrumental in getting MC4 off the ground and played a critical organizational role during the early stages of the project. Subsequently, her responsibilities were taken over by the able assistance of Alex Gann. Sara Deibler contributed at all stages of

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JOSEPH SAMBROOK

## A Note from the Publisher

Readers are encouraged to visit the website [www.molecularcloning.org](http://www.molecularcloning.org) to obtain up-to-date information about all aspects of this book and its contents.



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### General Safety and Hazardous Material Information

This manual should be used by laboratory personnel with experience in laboratory and chemical safety or students under the supervision of suitably trained personnel. The procedures, chemicals, and equipment referenced in this manual are hazardous and can cause serious injury unless performed, handled, and used with care and in a manner consistent with safe laboratory practices. Students and researchers using the procedures in this manual do so at their own risk. It is essential for your safety that you consult the appropriate Material Safety Data Sheets, the manufacturers' manuals accompanying equipment, and your institution's Environmental Health and Safety Office, as well as the General Safety and Disposal Information in Appendix 4 for proper handling of hazardous materials described in this manual. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in this manual and has no liability in connection with the use of these materials.

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