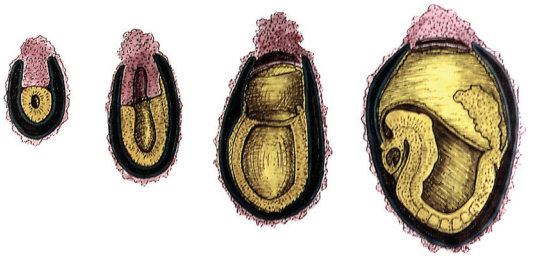
Manipulating the Mouse Embryo

A LABORATORY MANUAL

FOURTH EDITION



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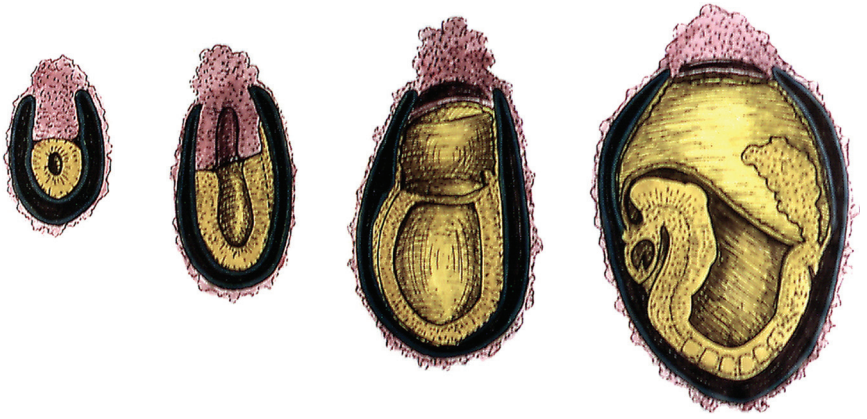

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COLD SPRING HARBOR LABORATORY PRESS
Cold Spring Harbor, New York • www.cshlpress.org

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Printed in the United States of America

Publisher	John Inglis
Acquisition and Managing Editor	Judy Cuddihy
Director of Editorial Development	Jan Argentine
Developmental Editors	Kaaren Janssen, Andrea R. Russo, and Michael Zierler
Project Managers	Mary Cozza and Inez Sialiano
Permissions Coordinator	Carol Brown
Production Editor	Kathleen Bubbeo
Production Manager	Denise Weiss
Cover Designer	Ed Atkeson

Front cover artwork: Mouse embryos at the blastocyst stage, just before implantation, with three distinct cell types: trophectoderm cells, which will form the placenta, in light blue; pluripotent epiblast cells, which will give rise to all cells of the animal body, in navy; and primitive endoderm cells, which will differentiate into the yolk sac, in pink. (Courtesy of Mubeen Goolam and Magdalena Zernicka-Goetz, University of Cambridge, United Kingdom.)

Title page illustration: Early postimplantation stages of mouse embryogenesis. Illustration by Rosa Beddington. (Printed, with permission, from John Skehel, Medical Research Council.)

Library of Congress Cataloging-in-Publication Data
Behringer, Richard.
Manipulating the mouse embryo : a laboratory manual / Richard Behringer, University of Texas, M.D. Anderson Cancer Center [and three others]. -- Fourth edition.
pages cm
Includes bibliographical references and index.
ISBN 978-1-936113-00-2 (hardback) -- ISBN 978-1-936113-01-9 (paper)
1. Mice--Embryos--Laboratory manuals. 2. Mice--Genetic engineering--Laboratory manuals.3. Transgenic mice--Laboratorymanuals. 4. Mice as laboratory animals. I.Title.

QL737.R6M2468 2013
616.02'7333--dc23

2013023915

10 9 8 7 6 5 4 3 2 1

This edition is dedicated to Anne McLaren (1927–2007) who was a pioneer in mouse genetics and embryology. Anne took particular interest in talking with young scientists about their research and providing them with valuable feedback and encouragement. She also said, "always keep in mind that the embryos are so precious. Never waste a single one, there is always another unanswered question it can solve." Years ago we were among those fortunate young scientists who were inspired by Anne.

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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 such 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 3 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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Experimental Animals

Procedures for humane treatment of animals must be observed at all times. Consult your local institutional guidelines.

Contents

Preface

IT HAS BEEN MORE THAN 10 YEARS SINCE THE PUBLICATION of the third edition of *Manipulating the Mouse Embryo*. Ten years ago, many of the basic methods of mouse embryology and genetic manipulation were routine, including zygote injection, embryonic stem (ES) cell culture, homologous recombination, and blastocyst injection to generate chimeras. Indeed many of these methodologies had been relegated to institutional core facilities. Many of the "important" genes had been knocked out and their phenotypes characterized. The mouse genome (C57BL/6 J) had been sequenced, assembled, and annotated, and scientists could order cDNA and genomic clones without having to screen libraries. Fluorescent proteins of many different colors were shining in the cells of transgenic mouse embryos and tissues and dancing in time-lapse movies. Much was known about the molecular embryology of the mouse. At the time, it seemed there would only be small incremental advances in new embryological and genetic methods to manipulate the mouse. Since then, new stem cell lines were derived, including epiblast stem and XEN cells, creating new cellular resources to understand pluripotency and differentiation. Large-scale ethylnitrosourea (ENU) mutagenesis programs created many new alleles in the mouse identified by phenotype. This was succeeded by the International Knockout Mouse Consortium (IKMC), which aims to mutate all protein-coding genes in the mouse genome by gene trapping or gene targeting. Scientists can now order ES cells with mutations for their favorite gene and have their core facilities generate mutant mice. Many of these mutant ES cell lines are now being turned into mice for standardized phenotyping by the International Mouse Phenotyping Consortium (IMPC).

Certainly the advances in DNA sequencing technologies have facilitated many experiments and opened up new opportunities for genome analysis. However, the biggest breakthrough during the previous decade was the derivation of induced pluripotent stem (iPS) cells by the expression of a discrete set of transcription factors in somatic cells. Here again, the mouse led the way because of the foundation of knowledge of ES cells—their culture, genetic manipulation, and assays of pluripotency. The ability to reprogram somatic cells to a state of pluripotency has made a tremendous impact on our concepts of stem cells and differentiation, including their relevance to cellular therapies for a variety of human diseases. As we complete this fourth edition, new targeted gene manipulation technologies have been reported that may supplant ES cells as vehicles to generate mutant mice because RNA/DNA constructs can be injected into zygotes to achieve gene targeting, bypassing the need to generate chimeras. In 1989, Brinster et al. reported achieving homologous recombination by zygote injection of a gene-targeting construct; however, the efficiency was prohibitively low. The new TALEN and CRISPR/Cas methods exploit the basic biology of plants and bacteria for very efficient gene targeting in mice by expression in zygotes achieved by microinjection. Once again, there seem to be no limits for manipulating the mouse embryo to address fundamental biological questions and provide novel biomedical insights for human biology and disease.

As the field of mouse developmental genetics progresses, the "Mouse Manual" continues to evolve. The fourth edition is built upon the foundation of the previous editions—notably the efforts of the original editors, Brigid Hogan, Frank Costantini, Elizabeth Lacy, and subsequently Rosa Beddington. This new edition has been updated, incorporating many new methods since the publication of the third edition in 2003. New chapters and protocols have been added, including the most up-to-date assisted reproduction techniques for sperm and embryo cryopreservation; generation of induced pluripotent stem cells; isolation, generation, and transplantation of spermatogonial

stem cell lines; in utero electroporation of gene constructs into postimplantation embryos; vibratome sectioning of live and fixed tissues for imaging thick tissue sections; and whole-mount fluorescent staining methods for three-dimensional visualization.

We are very grateful to the many people who generously helped us to produce the present edition. They provided new and updated protocols, figures, and images and served as an incredibly helpful source of expert information. We thank (in alphabetical order): Vernadeth Alarcon, Wojtek Auerbach, Ralph Brinster, Gabrielle Brons, Jorge Cabezas, Abel Carcagno, Tracy Carroll, Andrew Corso, Thomas DeChiara, Michael Dewey, Amanda Duselis, Gabriel Gonzalez, Shaun Goodyear, Mubeen Goolam, Anna-Katerina Hadjantonakis, Cheng-Chiu Huang, Scott Hutton, Kimberly Inman, Angelo Iulianella, Kenneth Jones, Min Kang, Yusuke Marikawa, Maria Mileikovskaia, Keiji Mochida, Claudio Monetti, Lluis Montoliu, Naomi Nakagata, Jennifer Nichols, Mark Nolte, Jon Oatley, Jan Parker-Thornburg, Shirley Pease, Jaime Rivera-Pérez, Larysa Pevny, Peter Rugg-Gunn, Nestor Saiz, Lisa Sandell, Thomas Saunders, Jillian Shaw, Allison Stewart, Robert Taft, Patrick Tam, Paul Tesar, Peter Tonge, Paul Trainor, Balázs Varga, Monkia Veres, Paul Vrana, Knut Woltjen, Yojiro Yamanaka, and Magda Zernicka-Goetz.

We also thank the many individuals at Cold Spring Harbor Laboratory Press who have helped to make this new edition a reality, including John Inglis, Jan Argentine, Denise Weiss, Kaaren Janssen, Michael Zierler, Andrea R. Russo, Inez Sialiano, and Kathleen Bubbeo. We especially thank our editor Judy Cuddihy for her enthusiasm, endless patience, friendly encouragement, and creative insights. Finally, as with the third edition, it is our hope that the newest edition of this manual will help train the future leaders and innovators of the mouse developmental genetics and molecular embryology fields.

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