

Manipulating the Mouse Embryo

**A LABORATORY MANUAL
Second Edition**

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Manipulating the Mouse Embryo

A Laboratory Manual

Second Edition

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Front cover: Whole mount in situ hybridization of HNF-3 β expression in 9.0-day p.c. embryo (see Sasaki and Hogan 1993). (Photo courtesy of H. Sasaki, HHMI, Vanderbilt Medical School.)

Back cover: Whole mount in situ hybridization of follistatin expression in early somite stage embryos. (Photo courtesy of R. Avkell, National Institute for Medical Research.)

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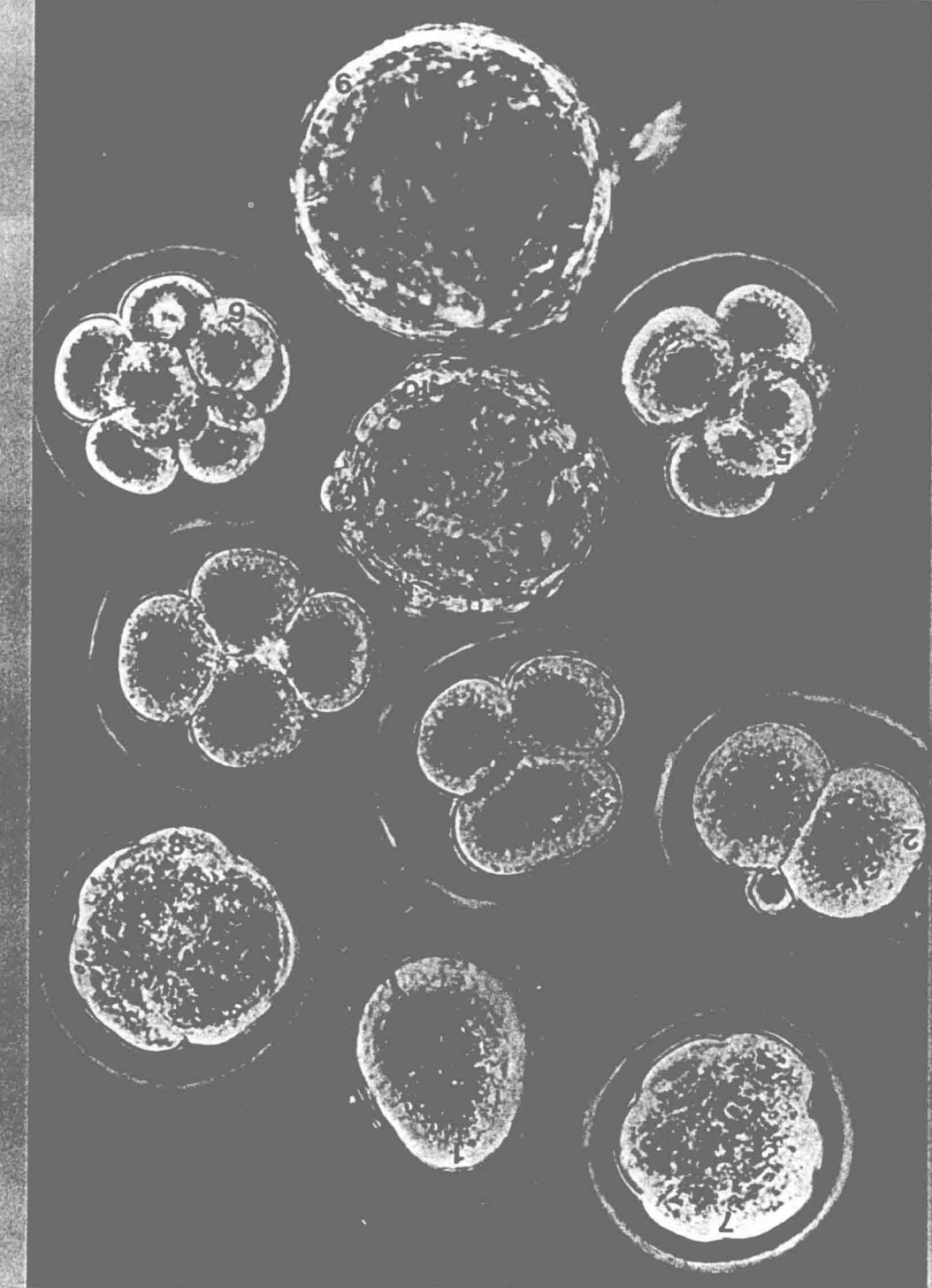
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Frontispiece: Preimplantation stages in mouse embryogenesis. (1) Fertilized egg. Note the sperm within the zona pellucida. (2) Two-cell stage. A polar body is still visible. (3) Three-cell stage. Cleavage of the blastomeres is asynchronous. (4) Four-cell stage. (5) Five-cell stage. (6) Eight-cell uncompact morula. (7) Compacted morula. (8) Early blastocyst. A small blastocoel cavity is present. (9) Expanded blastocyst. (10) Hatched blastocyst. Prior to hatching the blastocoel cavity expands and contracts, possibly because the permeability seal created by the zonular apical tight junctions between the trophoblast cells is temporarily broken when these cells divide. (Photograph provided by Dr. P.R. Braude, Department of Obstetrics and Gynecology, Cambridge University.)

Preface to First Edition

These are exciting times for mammalian embryology. The revolution of recombinant DNA technology has made possible experiments only dreamt of by the pioneers of the field—the ability to isolate and sequence genes, engineer them in specific ways, monitor their expression by *in situ* hybridization, and rapidly map them by means of restriction fragment length polymorphisms. These feats have been matched by equally impressive advances in techniques for introducing foreign DNA into the germ line of mice and larger animals. Microinjection of DNA into the fertilized egg, retroviral infection, and transformation of pluripotential embryonic stem cell lines have all opened up ingenious new ways of studying the control of gene expression during development and of following the consequences of altering or blocking specific gene products. Dazzling though these technologies are, they are worth very little unless applied to projects designed to ask fundamental questions about embryonic development. It is here that the molecular biologist can look for inspiration to an impressive tradition in mammalian embryology that has identified and described the major events that need to be understood in molecular terms. The most obvious are cell diversification and differentiation. But we also know very little about the various morphogenetic processes which, for example, bring about changes in tissue organization, direct the migration of the germ cells and neural crest, and establish specific nerve connections.

Classical mammalian embryology has also identified many areas highly relevant to human reproduction that are ripe for molecular studies, including sex determination, germ cell maturation, implantation, maternal-fetal interaction, and placental function. At another practical level, there are now possibilities for increasing the yield and potential uses of agricultural animals and for understanding and rectifying inherited defects and childhood cancers.

It is to help catalyze the interaction between molecular biology and mammalian embryology that this manual has been written. It grew out of the teaching material for two practical courses in the Molecular Embryology of the Mouse held at Cold Spring Harbor Laboratory in 1983 and 1984. These courses, in turn, could never have been organized without the foresight and enthusiasm of James Watson, and we should like to thank him for so much support and inspiration over the last 3 years. It is indeed fitting that Cold Spring Harbor Laboratory was the first to hold courses of this kind. Not only does the Laboratory have a great tradition in molecular biology, but also, as readers of our introduction will learn, it was the birthplace of some of the first inbred mouse strains and the site of pioneering work in mouse genetics.

Many people generously and enthusiastically contributed their hard-won ex-

v

pertise to the courses and the manual. In particular, we should like to express our gratitude to Anne McLaren, who not only proved to be an almost inexhaustible source of information on all aspects of reproductive biology, but also gave gentle sympathy and encouragement to students searching in vain for embryos and oviducts! We also owe special thanks to Christopher Graham, who initially taught us many of the techniques in this manual, and who provided valuable comments on the manual at various stages of its preparation. Lee Silver and Douglas Hanahan generously helped us to organize the animal breeding, laboratory space, and other facilities necessary to run the course and also provided many helpful comments on the manuscript. We wish to thank the following people for demonstrating techniques in the course and/or contributing information that has been incorporated into the text: Eileen Adamson, Lynne and Bob Angerer, Helene Axelrad, Alan Bernstein, Ralph Brinster, Bruce Cattnach, Verne Chapman, Ted Evans, Susan Howlett, Rudolf Jaenisch, M.H. Kaufman, Robb Krumlauf, Cecelia Lo, James McGrath, Hester Pratt, Liz Robertson, Michael Rosenberg, Janet Rossant, Davor Solter, and Heidi Stuhlman. We also thank Denise Barlow, Kiran Chada, Kathie Raphael, and Liz Robertson, who served as assistant instructors in the course.

Finally, we could not have produced this manual without the skill and expertise of the Cold Spring Harbor Laboratory Publications Department, headed by Nancy Ford. Our editor Judy Cuddihy, in particular, organized much of the production and guided us through times when it seemed that the Manual would never be finished.

**B.L.M.H.
F.C.
E.L.**

Preface to Second Edition

More than a decade has passed since the inaugural Molecular Embryology of the Mouse Course was held at Cold Spring Harbor Laboratory, catalyzing the production of the first edition of this manual. During that time, mouse developmental genetics and molecular embryology have emerged as two of the most exciting and fast-moving fields in current biology. Many different factors have contributed to this spectacular growth, including the realization that genes and mechanisms controlling embryonic development have been conserved during evolution, so that findings in *Drosophila* and *Caenorhabditis elegans* can be quickly applied to the mouse embryo. This has led to many productive and enjoyable collaborations between developmental biologists studying different organisms. Technical and conceptual advances in mouse genetics have also been crucially important, especially the ability to mutate genes selectively by homologous recombination in embryonic stem (ES) cells. In recognition of these and many other seminal innovations, we have made a number of significant additions to the second edition of *Manipulating the Mouse Embryo*. For example, the section describing the early embryology of the mouse has been enlarged and updated, and information has been added about access to new genetic databases. Detailed protocols have been provided concerning the culture and manipulation of embryonic stem cells, and more extensive discussion has been provided of methods for analyzing gene expression, in particular whole mount in situ hybridization and immunocytochemistry.

Many people are responsible for helping us to compile all this new information and to revise previous descriptions. We especially thank, in alphabetic order, Alex Joyner, Nils Lonberg, Andy McMahon, Jill McMahon, Jeff Mann, Andras Nagy, Janet Rossant, Gary Schoenwolf, and Patrick Tam. On occasion we have severely tried their patience in the quest for information, and we hope that the final result will repay our debt. We also thank all our immediate colleagues, graduate students, and postdocs, who went over protocols and criticized their organization so effectively. Production of this Second Edition was, once again, the responsibility of many talented and dedicated people in the Cold Spring Harbor Laboratory Press. They have learned over the years how to interact so successfully with compulsive and busy scientists scattered across the globe. In particular, Mary Cozza, Dorothy Brown, and Christy Kuret, under the guidance of Managing Editor, Nancy Ford, and Executive Director, John Inglis, are to be congratulated for bringing to your laboratory bench yet another superbly produced, and hopefully indispensable, Cold Spring Harbor manual. Finally, we dedicate this Second Edition to the memory of Françoise Kelly, a kind and generous colleague and friend who, like us all, delighted in the study of that beautiful organism—the mouse embryo.

B.L.M.H.
R.S.P.B.
F.C.
E.L.

Manipulating the Mouse Embryo

**A LABORATORY MANUAL
Second Edition**

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