
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 Bedington. 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.

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