Preface

As the major task of sequencing the human genome is near completion and full complement of human genes are catalogued, attention will be focused on the ultimate goal: to understand the normal biological functions of these genes, and how alterations lead to disease states. In this task there is a severe limitation in working with human material, but the mouse has been adopted as the favored animal model because of the available genetic resources and the highly conserved gene conservation linkage organization.

In just of ten years since the first gene-targeting experiments were preformed in embryonic stem (ES) cells and mutations transmitted through the mouse germline, more than a thousand mouse strains have been created. These achievements have been made possible by pioneering work that showed that ES cells derived from preimplantation mouse embryos could be cultured for prolonged periods without differentiation in culture, and that homologous recombination between targeting constructs and endogenous DNA occurred at a frequency sufficient for recombinants to be isolated. In the next few years the mouse genome will be systematically altered, and the techniques for achieving manipulations are constantly being streamlined and improved.

Recently new technologies have developed for inducible gene expression in transgenic mice that in combination with conventional gene targeting can give temporal and tissue-specific expression. These advances have been spurred on by the desire to study the function of genes that show an embryonic-lethal phenotype when deleted or "knocked out". Gene targeting in mice initially concentrated on making gene knockouts or null mutations, but increasingly the technology is being used to create subtle point mutations to simulate human disease states.

Gene Knockout Protocols brings together distinguished contributors with extensive experience in the gene targeting and mouse genetics fields. In line with the successful format of *Methods in Molecular Biology*, the volume contains step-by-step protocols for the design of targeting constructs to protocols for the analysis of the mouse phenotype. Emphasis has been paid to the inclusion of other techniques used in mouse genetics that are relevant to researchers performing gene targeting. These include embryo transplantation, In vitro

ES cell differentiation, creation of aggregation chimeras, mouse pathology, embryo cryopreservation, and transplantation. Issues such as the use of existing mouse mutation resources and the influence of genetic background and epigenetic effects upon phenotype are also covered. We hope that *Gene Knockout Protocols* will be an invaluable source of proven protocols for those just entering the field of gene targeting, but also a valuable reference for researchers in the process of describing the phenotype of mutant mice.

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