nature immunology

Making the most of mouse models

Sharing insight into esoteric techniques and ideas for manipulating the mouse genome fosters the production of better *in vivo* immunological systems.

To assess the physiological relevance of an experimental finding—whether the function of a newly identified surface receptor in host defense, or the developmental consequences of perturbing a signaling pathway or removing a transcription factor—immunologists often head to the mouse facility. Other organisms, such as fruit flies and zebrafish, also have qualities that make them very useful in certain types of experiments. In addition, as immunological processes in experimental organisms are not always analogous to those in human beings, a justifiably strong push to directly 'query' the human immune system is underway. Nevertheless, as an experimental system, the mouse remains an extremely powerful and popular model of choice for immunologists.

The creation of small alterations in the mouse genome, at random or in specific regions, holds the potential to return enormous insight into the underpinnings of immunological processes. However, manipulation of the mouse genome requires an equally enormous investment of financial and personnel resources and thus should be undertaken only with carefully considered experimental strategies. New techniques to modify the mouse genome have often been developed in one or two laboratories or institutes. Consequently, if they are not in close collaboration with those responsible for developing the technique, other investigators attempting to adapt or modify the new technique for their own immunological purposes can remain unaware of pitfalls and problems encountered during the design and optimization of the technique. Obviously, after a genetically modified mouse is produced is not the best time to realize that a crucial control or important consideration was overlooked!

Unlike the detailed set of instructions accompanying reagents purchased from a commercial vendor, techniques designed by an independent laboratory or institute often have less explicit protocols that are often disseminated informally at conferences or reproduced incompletely in scientific publications. In this issue of *Nature Immunology*, leading immunologists responsible for pioneering some of the widely used techniques for manipulating the mouse genome provide their own insights gained over the years. We hope that these discussions will serve as a central repository of information about these techniques that are less codified in formal protocols than are other well known procedures.

Bruce Beutler and colleagues compare the strategies of forward and reverse genetics (the introduction of undefined and defined mutations in the mouse genome, respectively) and discuss which strategy is best suited to answer particular immunological questions, emphasizing the possibility that forward genetics might someday be used to actually spark new immunological questions. The prospect of a sifting through thousands of mice generated during a forward genetics experiment, each with of an unidentified (and potentially unimportant) randomly induced mutation, is understandably daunting. Helpfully, Beutler and colleagues share their tried-and-tested strategies for rendering the process more tractable and efficient in time, cost and scale.

Klaus Rajewsky and Mark Schmidt-Supprian highlight considerations important for those researchers who want to use reverse genetics to conditionally ablate or modify the expression of a particular gene. The first experiments showing that Cre recombinase derived from the *Escherichia coli* bacteriophage P1 can be expressed in mice in almost any tissue- or a time-specific way to excise genes flanked by *lox*P sites in surrounding DNA made the deletion or modification any gene at any time in any cell type seem possible. Only after years of experimental trials and tribulations have researchers realized that Cre recombinase is not as selective in its target sites or as resistant to epigenetic effects as the initial experiments had indicated. Rajewsky and Schmidt-Supprian highlight key control experiments that when done correctly can greatly increase confidence that the phenotype demonstrated after Cre expression is the direct result of the complete excision of the gene of interest in the cell type of interest rather than of the absence of the gene in another cell type, incomplete or temporally inappropriate gene deletion or other nonspecific effects of Cre.

Finally, Linda Wicker, along with William Ridgway and colleagues, manages to refine the somewhat murky issue of genetic 'background' into the defined parameter of 'flanking' genes. These authors provide examples of cases in which phenotypes attributed to a genetic lesion in one gene are actually the result of the presence of mouse strain–specific sequence differences in nearby genes. The examination of flanking gene sequences is a crucial component of strategies designed to use forward genetics to 'map' a phenotype to a gene or to use reverse genetics to examine the phenotypic consequences of ablating or inducing expression of a gene in specific inbred mouse strains. Freely available computational tools that incorporate annotation of the genomes of commonly used inbred mouse strains and are thus able to render this flanking-gene problem more tractable are also presented and discussed.

We realize that this set of commentaries does not constitute an all-encompassing manual on the genetic manipulation of the mouse genome. Instead, we hope that by collecting and distributing the experimental insights made by these and other experts, the techniques described here will become a little less mysterious to more scientists, which may help to prevent the more common pitfalls when planning future experiments.