
Foreword

The establishment of microinjection protocols about 20 years ago for cultured cells and shortly thereafter for the generation of transgenic mice by microinjection of DNA into fertilized mouse eggs greatly influenced many fields of biology. Not only have the data generated using these approaches contributed to a large extent to our present understanding of gene regulation and cellular function of higher eukaryotic cells, but current knowledge and future developments in this area will certainly have a great impact on basic and applied research for many years to come.

This laboratory manual describes the current state of the art in this research area and focuses primarily on both the experimental strategies with an extensive bibliography and the detailed procedures. A large number of studies are presently being performed and a great variety of new experimental designs are rapidly being developed. The book contains protocols on injection of somatic cells as well as on injection of embryos, the use of similar equipment being a common feature. In the articles dedicated to somatic cells, full descriptions of the manual and automatic injection systems are given as well as the methods for the analysis of injected cells by video-microscopy, electron microscopy or in situ hybridizations. In addition, comprehensive protocols are given for injection experiments with very different purposes, such as to study signal transduction or microtubule dynamics. A large section deals with the principles, the new strategies and various applications of transgenic technology primarily employing mice, but also using rabbits and *Drosophila*.

Following an introduction to mouse husbandry with a description of the requirements for transgenic mouse studies, we learn about the culture of male germ cells and the attempts to transfer sperm-mediated genes. The different strategies for generating germline chimaeras, as well as the production of YAC transgenic mice are outlined, and the numerous possible applications for using the mouse embryo as a „test tube“ to

identify gene activities are described. Finally, the various ways to apply histological markers, such as *lacZ*, for multiple purposes are presented and an overview is given on the application of transgenic mouse studies to analyze the immune system and on virus research. No book on transgenic approaches can now be published without reviewing the possibilities and strategies for conditionally altering gene activities using inducible site-specific recombinases. On the other hand, the cloning of new genes from transgenic insertion sites still appears to provide a valuable tool for mouse geneticists for the study of developmental control genes.

The book contains a large amount of useful information. The advantages of microinjection protocols as well as their limitations are extensively discussed and I am convinced that the reader of this book will find useful suggestions, novel ideas as well as surprising facts on a relatively new but fast-growing research area. The numerous novel approaches in transgenic research with the constantly improving techniques, such as the transfer of nuclei from differentiated cells into enucleated sheep oocytes, together with the advancement of better cryopreservation techniques for mouse sperm and fertilized eggs, will indeed revolutionize modern biology. Some of the excitement of those working in the field is described here and is passed on to the reader of the book.

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Erwin F. Wagner

Preface

"... Even granting that the genius subjected to the test of critical inspection emerges free from all error, we should consider that everything he has discovered in a given domain is almost nothing in comparison with what is left to be discovered"

Santiago Ramón y Cajal (1825–1934)
*Precepts and Counsels on Scientific
Investigation: Stimulants of the spirit, 1951*

The interest of man in manipulating and modifying everything he discovers must obey an ancestral instinct which is indispensable for evolution. Once the adequate tools were available such interest focused on the functional unit of living organisms, the cell.

Despite the enormous complexity of their molecular organization, it is possible to microinject eukaryotic cells so that they recover perfectly from the perturbations caused by the manipulation. Indeed, careful intranuclear and/or intracytoplasmic microinjections neither alter significantly the proliferation capacity of somatic cells in culture, nor interfere with the development of fertilized eggs. This fact, together with the advantages of the direct manipulation of cells, have made microinjection the technique of choice for single cell experiments in vitro and for producing genetically modified animals.

This manual is intended to provide the reader with the techniques and methods needed to perform experiments using microinjection in studies on single cells or on transgenic embryos and animals, in particular mice, but also rabbits and flies. We are aware of the fact that the success of such experiments relies strongly on their feasibility, the proper design and correct interpretation of results. Therefore, special emphasis has been placed on providing a comprehensive introduction to each strategy described, besides the necessary working protocols. In addition, extensive references provide greater detail or alternative methods. In order to accomplish these goals within the limited space of a manual, repetition of earlier books has been carefully avoided wherever possible without affecting the comprehensiveness of the protocols.

Basically the same equipment is required for microinjection of somatic cells in culture and of oocytes and fertilized eggs. For this reason, both methodologies are described in this book. Complete microinjection setups for manual/automatic and computer assisted microinjection are described in Part I. The first 10 chapters include not only some complementary techniques that must often be combined with microin-

jection for single cell experiments, but also a compilation of representative applications of these techniques to cell biology, gene expression and signal transduction studies. Part II, consisting of the remaining 20 chapters, deals with a great variety of aspects ranging from mouse husbandry to the production of transgenic animals and the targeted inactivation of genes using the emerging techniques of site-specific recombination. These chapters have been arranged according to subject following ontogenic criteria: germ cells, oocytes, pre-implantation embryos, post-implantation embryos, targeting inactivated mutants and transgenics. All chapters deal with techniques using mice, except for Chapters 29 and 30 which describe transgenic rabbits and the microinjection of *Drosophila* embryos, respectively.

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