

METHODS IN MOLECULAR BIOLOGY™

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Zebrafish

Methods and Protocols

Edited by

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Preface

In the last 20 years, research activity using the zebrafish *Danio rerio* has increased dramatically. Their contribution to modern genetic and molecular research originates with their use as a vehicle for testing ideas concerning the genetic basis of vertebrate brain formation and function at the University of Oregon's Institute for Neuroscience. Their research use has expanded into their becoming a leading model system for understanding the basic genetics, cell biology, and physiology of vertebrate development and human disease states in hundreds of labs around the world. It has been a heady time for the little fish! There are good reasons for this rapid rise of popularity, both practical and technical. Practically, zebrafish are easy and inexpensive to keep, breed, and raise, and—similar to yeast, mice, and fruit flies—zebrafish like being around humans. Technically, the genetic tractability, embryonic accessibility, and imaging potential of the zebrafish are, in our opinion, the features that have tempted so many people to push the boundaries of zebrafish research so far in such a short time. Although each model organism has its strengths and weaknesses, we now regard zebrafish as sitting alongside mouse, worm and fruit fly as key animal model systems in modern biology.

There are already a number of excellent books and papers dealing with zebrafish experimental techniques, which begs the question—why another one? In choosing the contributions to this book, we were guided by three principles as we sought to make sure that this volume made a useful contribution to the field. First, because of the rapid development of techniques and reagents, we looked for material that was not yet well known or widely distributed. Second, we sought experience from newer labs with approaches that had not received exposure. Third, we tried to avoid duplicating familiar, well tested, and trusted material. The material in this volume is organized loosely along three strengths of the zebrafish: genetic modification, accessibility for manipulation, and ease of *in vivo* live imaging.

With a nearly complete sequenced genome, with significant genetic homology to that of humans, and with ease of mutagenesis and housing of sufficient numbers to enable forward genetic screens, the zebrafish is a natural candidate for genetic analysis of biologic processes. Chapters 1 and 2 describe dense chemical and retroviral mutagenesis, Chapter 3 covers resource-efficient haploid screening, and Chapter 4 discusses effective cryopreservation of zebrafish sperm for the precious mutants harvested from these techniques.

External fertilization and the production of large numbers of embryos from each mother have made practical the microinjection of lineage dyes, mRNA for protein overexpression, and DNA for transgenesis, as well as the transplantation of cells for genetic cell-autonomy studies. It has also made possible large-scale screens for gene expression using *in situ* hybridization, and enhancer traps. Part II of this volume develops these themes, describing the use of transposons in Chapters 5 and 6, or homologous recombination in bacterial artificial chromosomes in Chapter 7 to modify zebrafish chromosomal DNA for transgenic analysis of gene expression, as well as efficient single-copy transgenesis in

Chapter 8. Having thus created reporter strains of zebrafish with fluorescently-labeled cells, a novel method of ablating these cells specifically with nitroreductase allows their role in the organism to be tested, and is discussed in Chapter 9. Such cellular-level precision is also found in Chapter 10, which focuses on the focal electroporation of dyes or DNA into cells deep within the fish. However, sometimes a slightly larger specific region of the embryo must be manipulated, and zebrafish surgical techniques along the lines of those utilized in chick experimental embryology are presented in Chapter 11. Having plentiful embryonic material also facilitates the use of microarrays to analyze mRNA expression. Chapters 12 and 13 describe their synthesis and use for the zebrafish. The recent emergence and importance of microRNA biology has been underscored by pioneering work in the zebrafish; Chapter 14 outlines methods for validating microRNA targets *in vivo*.

It is perhaps the optical transparency of the zebrafish embryo that has most tipped the balance in its favor. In this volume, we included chapters showcasing methods that most labs with access to the equipment of a modern biology department can use. Chapter 15 describes a protocol for following tissue-scale morphogenesis simultaneously in multiple embryos that allows for the estimation of precision and variability. The striking beauty and power of single-cell resolution in the living zebrafish is seen in Chapters 16 and 17, which focus on imaging the early immune system using laser confocal scanning microscopy and the deeper cells of the gastrula using two-photon. The significant technical challenges of imaging the late-developing gut are tackled in Chapter 18 with a range of methods that include principles with application to other larval organ systems. Finally, Chapter 19 presents methods for achieving the lofty goal of following every cell in an organ, or indeed an entire organism, during development.

We hope that these chapters not only meet experimental needs that already exist, but also that they might inspire approaches that were not previously considered, and finally that they might give close insight and perspective into the emerging literature. The editors would like to thank John Walker and the staff at Humana Press and Springer for their continuous assistance, and the authors for their hard work and flexibility.

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Dresden, Germany
Mishima, Japan

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