Preface

In 1981, the production of homozygous clones of zebrafish was reported by Dr. George Streisinger and his colleagues. This achievement launched zebrafish as a new laboratory model system to study vertebrate genetics and development. By the 1990s, zebrafish large-scale husbandry and chemical mutagenesis had been developed, leading to large-scale vertebrate genetic screens and the successful identification of embryonic development mutants by the Wolfgang Driever and Christiane Nüsslein-Volhard laboratories in a special issue of *Development* in 1996. Since then, zebrafish has established its status as a major model animal and has been widely used for research of various aspects of biological and biomedical sciences. Accordingly, researchers have enthusiastically developed new methods for genetics, genomics, molecular biology, cell biology, tissue manipulation, and imaging analyses in zebrafish. By using these methods, important biological findings and discoveries have been achieved. Needless to say, development and rapid dissemination of such important methods should accelerate progress of the zebrafish research.

Previously, a method book *Zebrafish: Methods and Protocols* (edited by Graham J. Lieschke, Andrew C. Oates and Koichi Kawakami) of this series (*Methods in Molecular Biology*) was published in 2009. The book had five sections ("Mutagenesis and Mutants," "Transgenesis," "Tissue-Specific Manipulations," "Analyzing Gene Expression," and "Imaging") and contains basic techniques and protocols related to these section titles.

This book is the successor of the first version, including three new focus areas to include methods for what have become some of the most active areas of zebrafish research. We hope it will serve as a useful complement to the first book to the new and experienced zebrafish researcher alike.

Part I is the "Genetics and Genomics" section. This section comprises cutting-edge techniques for genetic and genomic analyses. Chapter 1 describes chemical genetics that enables phenotype-based (forward) screening of small molecules, leading to identification of new cellular pathways and drug discoveries. For the last several years, the Talen and CRISPR/Cas9 techniques have been developed and revolutionized genetic studies in every organism including zebrafish. Applications of these methods for mutagenesis and genome editing in zebrafish are extensively described in Chaps. 2–5. Chapter 6 describes targeted transgenesis using the PhiC31 system that ensures reliable transgene expression, and Chap. 7 describes the application of GFP-expressing transgenic fish that have been generated by numerous zebrafish laboratories to FACS sorting. Finally, Chap. 8 describes for the first time the construction of zebrafish inbred strains.

In Part II, we present techniques for developing and analyzing zebrafish disease models. We now know that over 80 % of human disease genes are conserved in zebrafish, and when combined with genomic editing and live imaging, zebrafish have emerged as a leading preclinical model system for new mechanistic insights into disease and in the development, discovery, and application of new therapies. Experimental techniques and analysis for cancer models, including genetic as well as xenotransplantation models, are described in Chaps. 9–12. Chapter 13 describes the analytical methods for the zebrafish hematopoietic system, including stem cells and progenitor populations. In Chap. 14, techniques for live

imaging of infection and host-pathogen interactions are described, leading to powerful understanding of how pathogens invade and survive in the host. Chapter 15 describes cardiac injury and regeneration and highlights the power of zebrafish as a model system to study regeneration. Finally, Chap. 16 describes techniques for the metabolism and transport of lipids, underscoring the value of zebrafish as a system to study metabolic disease.

Part III comprises methods for neuroscience. In particular, it covers techniques for studying the structure and function of neural circuits and their role in generating behavior, an area that has recently seen rapid growth within the zebrafish community. The first three chapters cover methods that allow the targeted labeling, manipulation, and interrogation of specific neurons in the small brain of the zebrafish. Chapter 17 describes the use of electroporation to deliver DNA constructs and other reagents to specific neurons in larval and adult zebrafish. Chapter 18 shows how laser microsurgery can be used to study regeneration in sensory axons, and Chap. 19 describes methods for making in vivo patch clamp recordings. Zebrafish are increasingly used to study complex social behavior, and Chap. 20 provides methods for quantifying aggressive encounters. There has been great progress recently in the use of optical approaches to record activity from the whole larval brain. Chapter 21 describes methods for relating neuronal calcium signals to behavior. Chapter 22 explains how to build a light-sheet microscope for fast volumetric imaging, and Chap. 23 gives a protocol for imaging from freely swimming fish. Methods to manipulate activity are essential to establish causal relationships between neuronal firing and behavior. Chapters 24 and 25 describe ways to specifically perturb or isolate genetically identified neuronal populations via optogenetics, ablations, and the expression of toxin-insensitive channels.

We hope this method book will be of help to all zebrafish researchers, especially scientists newly entering this ever-growing field.

We wish you all the best in your work with this truly exciting vertebrate animal system that continues to push forward the boundaries of science.

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