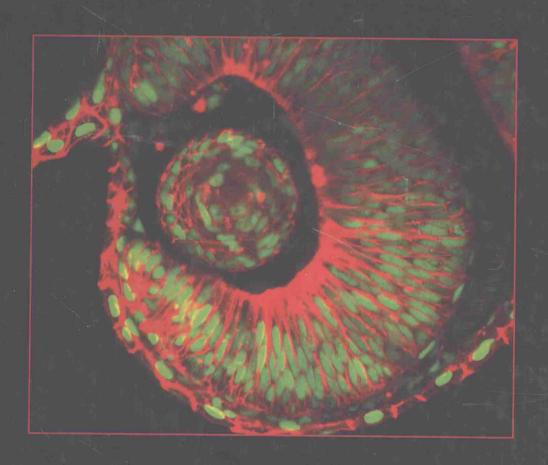


THE ZEBRAFISH: 2ND EDITION CELLULAR AND DEVELOPMENTAL BIOLOGY



Edited by

H. William Detrich, III • Monte Westerfield • Leonard I. Zon

Methods in Cell Biology

VOLUME 76

The Zebrafish: Cellular and Developmental Biology

Edited by

H. William Detrich, III

Department of Biology Northeastern University Boston, Massachusetts

Monte Westerfield

Institute of Neuroscience University of Oregon Eugene, Oregon

Leonard I. Zon

Division of Hematology/Oncology Children's Hospital of Boston Department of Pediatrics and Howard Hughes Medical Institute Boston, Massachusetts



AMSTERDAM • BOSTON • HEIDELBERG • LONDON NEW YORK • OXFORD • PARIS • SAN DIEGO SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO Elsevier Academic Press 525 B Street, Suite 1900, San Diego, California 92101-4495, USA 84 Theobald's Road, London WC1X 8RR, UK

This book is printed on acid-free paper.

Copyright © 2004, Elsevier Inc. All Rights Reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the Publisher.

The appearance of the code at the bottom of the first page of a chapter in this book indicates the Publisher's consent that copies of the chapter may be made for personal or internal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per copy fee through the Copyright Clearance Center, Inc. (www.copyright.com), for copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale. Copy fees for pre-2004 chapters are as shown on the title pages. If no fee code appears on the title page, the copy fee is the same as for current chapters. 0091-679X/2004 \$35.00

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone: (+44) 1865 843830, fax: (+44) 1865 853333, E-mail: permissions@elsevier.com.uk. You may also complete your request on-line via the Elsevier homepage (http://elsevier.com), by selecting "Customer Support" and then "Obtaining Permissions."

For all information on all Academic Press publications visit our Web site at www.books.elsevier.com

ISBN: 0-12-564171-0

PRINTED IN THE UNITED STATES OF AMERICA 04 05 06 07 08 9 8 7 6 5 4 3 2 1

CONTRIBUTORS

Numbers in parenthess indicates the pages on which the authors' contributions begin.

- R. Craig Albertson (437), Department of Cytokine Biology, The Forsyth Institute; and Department of Oral and Developmental Biology, Harvard School of Dental Medicine, Boston, Massachusetts 02115
- James F. Amatruda (109), Division of Hematology-Oncology, Children's Hospital and Dana-Farber Cancer Institute, Boston, Massachusetts 02115
- Nate Anderson (75), Phylonix Pharmaceuticals, Inc., Cambridge, Massachusetts 02139
 Andrei Avanesov (333), Department of Ophthalmology, Harvard Medical School.
 Boston, Massachusetts 02114
- Robert Baker (385), Department of Physiology and Neuroscience, New York University School of Medicine, New York, New York 10016
- Laure Bally-Cuif (163), Zebrafish Neurogenetics Junior Research Group, Institute of Virology, Technical University-Munich, D-81675 Munich, Germany; and GSF-National Research Center for Environment and Health, Institute of Developmental Genetics, D-85764 Neuherberg, Germany
- Serena Banden (261), Department of Biology, Northeastern University, Boston, Massachusetts 02115
- James C. Beck (385), Department of Physiology and Neuroscience, New York University School of Medicine, New York, New York 10016
- Melissa A. Borla (261), Department of Biology, Northeastern University, Boston, Massachusetts 02115
- Robert J. Bryson-Richardson (37), Victor Chang Cardiac Research Institute, Sydney 2010, Australia
- Douglas S. Campbell (13), Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, Utah 84132
- Stephen P. Chambers (593), Molecular Genetics and Development Group, School of Biological Sciences, University of Auckland, Auckland 1001, New Zealand
- Joanne Chan (475), Vascular Biology Program, Children's Hospital of Boston, Boston, Massachusetts 02115
- Prisca Chapouton (163), Zebrafish Neurogenetics Junior Research Group, Institute of Virology, Technical University-Munich, D-81675 Munich, Germany; and GSF-National Research Center for Environment and Health, Institute of Developmental Genetics, D-85764 Neuherberg, Germany
- Keith C. Cheng (555), Jack Gittlen Cancer Research Institute, Department of Pathology and Department of Biochemistry and Molecular Biology, Pennsylvania State College of Medicine, Hersey, Pennsylvania 17033

xiv Contributors

Chi-Bin Chien (13), Department of Neurobiology and Anatomy, University of Utah Medical Center, Salt Lake City, Utah 84132

- Paul Collodi (151), Department of Animal Sciences, Purdue University, West Lafayette, Indiana 47907
- Jennifer Crodian (151), Department of Animal Sciences, Purdue University, West Lafayette, Indiana 47907
- Peter D. Currie (37), Victor Chang Cardiac Research Institute, Sydney 2010, Australia Matthew Cykowski (489), Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229
- Robert A. DeRose (87), Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107
- Yun Deng (87), Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107
- H. William Detrich III (261), Department of Biology, Northeastern University, Boston, Massachusetts 02115
- Andrew Dodd (593), Molecular Genetics and Development Group, School of Biological Sciences, University of Auckland, Auckland 1001, New Zealand
- **John E. Dowling** (315), Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138
- **Wolfgang Driever** (531), Developmental Biology, University of Freiburg, D-79104 Freiburg, Germany
- Iain A. Drummond (501), Department of Medicine, Harvard Medical School and Renal Unit, Massachusetts General Hospital, Charlestown, Massachusetts 02129
- James M. Fadool (315), Department of Biological Science, Florida State University, Tallahassee, Florida 32306
- Lianchun Fan (151), Department of Animal Sciences, Purdue University, West Lafayette, Indiana 47907
- Steven A. Farber* (87), Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107
- Mark C. Fishman (569), Novartis Institutes for Biomedical Research, Cambridge, Massachusetts 02139
- Scott E. Fraser (207), Biological Imaging Center, Beckman Institute, California Institute of Technology, Pasadena, California 91125
- Ethan Gahtan (261), Department of Biology, Northeastern University, Boston, Massachusetts 02115
- Erin E. Gestl (555), Jack Gittlen Cancer Research Institute, Department of Pathology and Department of Biochemistry and Molecular Biology, Pennsylvania State College of Medicine, Hersey, Pennsylvania 17033
- **Edwin Gilland** (385), Department of Physiology and Neuroscience, New York University School of Medicine, New York, New York 10016
- Lara Gnügge (531), Developmental Biology, University of Freiburg, D-79104
 Freiburg, Germany
- *Current address: Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210

- Paul D. Henion (237), Center for Molecular Neurobiology and Department of Neuroscience, Ohio State University, Columbus, Ohio 43210
- Shiu-Ying Ho (87), Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107
- Lara D. Hutson (13), Department of Biology, Williams College, Williamstown, Massachusetts 01267
- Sumio Isogai (51), Department of 1st Anatomy, School of Medicine, Iwate Medical University, Morioka 020-8505, Japan
- Pudur Jagadeeswaran (489), Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229
- Yashar Javidan (415), Department of Developmental and Cell Biology, University of California, Irvine, Irvine, California 92697
- Makoto Kamei (51), Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892
- John P. Kanki (237), Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115
- Reinhard W. Köster (207), GSF-National Research Centre for Environment and Health, Institute of Developmental Genetics, 85764 Neuherberg, Germany
- Charles A. Lessman* (285), Department of Microbiology & Molecular Cell Sciences, The University of Memphis, Memphis, Tennessee 38152
- A. Thomas Look (237), Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115
- Donald R. Love (593), Molecular Genetics and Development Group, School of Biological Sciences, University of Auckland, Auckland 1001, New Zealand
- Jarema Malicki (333), Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts 02114
- Patricia McGrath (75), Phylonix Pharmaceuticals, Inc., Cambridge, Massachusetts 02139
- **Dirk Meyer** (531), Developmental Biology, University of Freiburg, D-79104 Freiburg, Germany
- Jessica L. Moore[†] (555), Jake Gittlen Cancer Research Institute, Department of Pathology, Pennsylvania State College of Medicine, Hershey, Pennsylvania 17033
- Peter E. Nielsen (593), Center of Biomolecular Recognition, Department of Medical Biochemistry and Genetics, University of Copenhagen, Copenhagen 2200, Denmark
- **Donald M. O'Malley** (261), Department of Biology, Northeastern University, Boston, Massachusetts 02115
- Sandra Parker (261), Department of Biology, Northeastern University, Boston, Massachusetts 02115
- Chuenlei Parng (75), Phylonix Pharmaceuticals, Inc., Cambridge, Massachusetts 02139

^{*}Current address: Department of Biology, The University of Memphis, Memphis, Tennessee 38152

[†]Current address: Department of Biology, University of South Florida, Tampa, Florida 33620

Xvi Contributors

Brian D. Perkins[‡] (315), Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138

- Randall T. Peterson (569), Developmental Biology Laboratory, Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, Massachusetts 02129
- Kathleen L. Pfaff (109), Division of Hematology-Oncology, Children's Hospital and Dana-Farber Cancer Institute, Boston, Massachusetts 02115
- Nagarajan S. Sankrithi (261), Department of Biology, Northeastern University, Boston, Massachusetts 02115
- Evelyn Santana (87), Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107
- **Thomas F. Schilling** (415), Department of Developmental and Cell Biology, University of California, Irvine, Irvine, California 92697
- Fabrizio C. Serluca (475), Novartis Institutes for Biomedical Research, Cambridge, Massachusetts 02139
- Jennifer L. Shepard (109), Division of Hematology-Oncology, Children's Hospital and Dana-Farber Cancer Institute, Boston, Massachusetts 02115
- **Didier Y. R. Stainier** (455), Department of Biochemistry and Biophysics, Programs in Developmental Biology, Genetics, and Human Genetics, University of California, San Francisco, San Francisco, California 94143-0448
- Howard M. Stern (109), Division of Hematology-Oncology, Children's Hospital and Dana-Farber Cancer Institute, Boston, Massachusetts 02115; and Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115
- Rodney A. Stewart (237), Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115
- David W. Tank (385), Departments of Physics and Molecular Biology, Princeton University, Princeton, New Jersey 08544
- **Bijoy Thattaliyath** (489), Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229
- Juanita L. Thorpe (87), Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107
- Christopher Ton (75), Phylonix Pharmaceuticals, Inc., Cambridge, Massachusetts 02139
- David Traver (127), Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, California 92093
- **Le A. Trinh** (455), Department of Biochemistry and Biophysics, Programs in Developmental Biology, Genetics, and Human Genetics, University of California, San Francisco, San Francisco, California 94143-0448
- Brant M. Weinstein (51), Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

[‡]Current address: Department of Biology, Texas A & M University, College Station, Texas 77843

Contributors

Pamela C. Yelick (437), Department of Cytokine Biology, The Forsyth Institute; and Department of Oral and Developmental Biology, Harvard School of Dental Medicine, Boston, Massachusetts 02115

- Hao Zhu (3), Division of Hematology/Oncology, Children's Hospital of Boston, Department of Pediatrics and Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02115
- Leonard I. Zon (3), Division of Hematology/Oncology, Children's Hospital of Boston, Department of Pediatrics and Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02115

PREFACE

Research Vessel Nathaniel B. Palmer Southern Atlantic Ocean, 54° 47′ S, 59° 15′ W On the Burdwood Banks 20 May 2004

Monte, Len, and I welcome you to two new volumes of *Methods in Cell Biology* devoted to *The Zebrafish: Cellular and Developmental Biology* and *Genetics, Genomics, and Informatics.* In the five years since publication of the first pair of volumes, *The Zebrafish: Biology* (Vol. 59) and *The Zebrafish: Genetics and Genomics* (Vol. 60), revolutionary advances in techniques have greatly increased the versatility of this system. At the Fifth Conference on *Zebrafish Development and Genetics*, held at the University of Wisconsin in 2003, it was clear that many new and compelling methods were maturing and justified the creation of the present volumes. The zebrafish community responded enthusiastically to our request for contributions, and we thank them for their tremendous efforts.

The new volumes present the post-2000 advances in molecular, cellular, and embryological techniques (Vol. 76) and in genetic, genomic, and bioinformatic methods (Vol. 77) for the zebrafish, *Danio rerio*. The latter volume also contains a section devoted to critical infrastructure issues. Overlap with the prior volumes has been minimized intentionally.

The first volume, Cellular and Developmental Biology, is divided into three sections: Cell Biology, Developmental and Neural Biology, and Disease Models. The first section focuses on microscopy and cell culture methodologies. New microscopic modalities and fluorescent reporters are described, the cell cycle and lipid metabolism in embryos are discussed, apoptosis assays are outlined, and the isolation and culture of stem cells are presented. The second section covers development of the nervous system, techniques for analysis of behavior and for screening for behavioral mutants, and methods applicable to the study of major organ systems. The volume concludes with a section on use of the zebrafish as a model for several diseases.

The second volume, *Genetics, Genomics, and Informatics*, contains five sections: Forward and Reverse Genetics, The Zebrafish Genome and Mapping Technologies, Transgenesis, Informatics and Comparative Genomics, and Infrastructure. In the first, forward-genetic (insertional mutagenesis, maternal-effects screening), reverse-genetic (antisense morpholino oligonucleotide and peptide nucleic acid gene knockdown strategies, photoactivation of caged mRNAs), and hybrid (target-selected screening for ENU-induced point mutations) technologies are

described. Genetic applications of transposon-mediated transgenesis of zebrafish are presented, and the status of the genetics and genomics of *Medaka*, the honorary zebrafish, is updated. Section 2 covers the zebrafish genome project, the cytogenetics of zebrafish chromosomes, several methods for mapping zebrafish genes and mutations, and the recovery of mutated genes via positional cloning. The third section presents multiple methods for transgenesis in zebrafish and describes the application of nuclear transfer for cloning of zebrafish. Section 4 describes bioinformatic analysis of the zebrafish genome and of microarray data, and emphasizes the importance of comparative analysis of genomes in gene discovery and in the elucidation of gene regulatory elements. The final section provides important, but difficult to find, information on small- and large-scale infrastructure available to the zebrafish biologist.

The attentive reader will have noticed that this Preface was drafted by the first editor, Bill Detrich, while he (I) was at sea leading the sub-Antarctic ICEFISH Cruise (International Collaborative Expedition to collect and study Fish Indigenous to Sub-antarctic Habitats; visit www.icefish.neu.edu). Wearing my second biological hat, I study the adaptational biology of Antarctic fish and use them as a system for comparative discovery of erythropoietic genes. Antarctic fish embryos generally hatch after six months of development, and they reach sexual maturity only after several years. Imagine attempting genetic studies on these organisms! My point is that the zebrafish system and its many advantages greatly inform my research on Antarctic fish, while at the same time I can move genes discovered by study of the naturally evolved, but very unusual, phenotypes of Antarctic fish into the zebrafish for functional analysis. We the editors emphasize that comparative strategies applied to multiple organisms, including the diverse fish taxa, are destined to play an increasing role in our understanding of vertebrate development.

We wish to express our gratitude to the series editors, Leslie Wilson and Paul Matsudaira, and the staff of Elsevier/Academic Press, especially Kristi Savino, for their diligent help, great patience, and strong encouragement as we developed these volumes.

H. William Detrich, III Monte Westerfield Leonard I. Zon These volumes are dedicated to Jose Campos-Ortega and Nigel Holder, departed colleagues whose wisdom and friendship will be missed by the zebrafish community

xxi

CONTENTS

Contributors	X111
Preface	xix
Dedication	xxi
PART I Cell Biology	
1. Use of the DsRed Fluorescent Reporter in Zebrafish	
Hao Zhu and Leonard I. Zon	
I. IntroductionII. DsRed: History and PropertiesIII. DsRed in Transgenic ZebrafishIV. Use of Multiple Fluorescent Reporters in Transplantation Assays	3 4 5 7
V. Fusion Protein Reporters VI. Conclusion	10 11
References	11
2. Analyzing Axon Guidance in the Zebrafish Retinotectal System	
Lara D. Hutson, Douglas S. Campbell, and Chi-Bin Chien	
I. Introduction II. Retinotectal Mutants	13 14
III. Labeling the Retinotectal System	16
IV. Perturbing the Retinotectal System V. Future Directions	27
References	31 32
3. Optical Projection Tomography for Spatio-Temporal Analysis in the Zebrafish	
Robert J. Bryson-Richardson and Peter D. Currie	
I. Introduction II. The Principle of Tomography III. Problems for OPT in Zebrafish	38 39 41

vi

	IV. Materials	42			
	V. Methods	42			
	VI. Sample Preparation	43			
	VII. Scanning	44			
	VIII. Reconstruction	45 46			
	IX. Presentation of Reconstructions X. Discussion	49			
	XI. The Future of OPT	49			
	XII. OPT Equipment	50			
	References	50			
4.	Imaging Blood Vessels in the Zebrafish				
	Makoto Kamei, Sumio Isogai, and Brant M. Weinstein				
	I. Introduction	51			
	II. Imaging Vascular Gene Expression	53			
	III. Nonvital Blood Vessel Imaging	54			
	IV. Vital Imaging of Blood Vessels	63			
	V. Conclusion References	73 73			
5.	Zebrafish Apoptosis Assays for Drug Discovery				
	Chuenlei Parng, Nate Anderson, Christopher Ton, and Patricia McGrath				
	I. Introduction	75			
	II. Zebrafish Apoptosis Genes	76			
	III. Caspase Substrate Recognition in Zebrafish	77			
	IV. Detection of Apoptosis In Situ	77			
	V. Perspectives References	82 83			
	References	03			
6.	Lipid Metabolism in Zebrafish				
	Shiu-Ying Ho, Juanita L. Thorpe, Yun Deng, Evelyn Santana, Robert A. DeRose, and Steven A. Farber				
	I. Introduction	87			
	II. Genetic Screen—Forward, Reverse, and Targeted-Mutagenesis	88			
	III. Pharmacological Studies Using Zebrafish	93			
	IV. Mechanism of Action Studies Using Zebrafish:				
	The Annexin2-Caveolin1 Complex	99			
	V. Gene-Specific Studies: The Acyl-CoA Synthetase Enzymes	102			

Contents

Contents	V11

VI. Summary References	104 104
 Analysis of the Cell Cycle in Zebrafish Embryos <i>Jennifer L. Shepard, Howard M. Stern, Kathleen L. Pfaff, and James F. Amatruda</i> I. Introduction: The Cell Cycle in Zebrafish II. Zebrafish Embryo Cell Cycle Protocols III. Screening for Mutations Affecting the Zebrafish Cell Cycle IV. Screening for Chemical Suppressors of Zebrafish Cell Cycle Mutants V. Conclusions VI. Reagents and Supplies	109 111 118 119 122 123 123
8. Cellular Dissection of Zebrafish Hematopoiesis David Traver I. Introduction II. Zebrafish Hematopoiesis III. Hematopoietic Cell Transplantation IV. Enrichment of Hematopoietic Stem Cells V. Conclusions References	127 128 136 144 147 147
9. Culture of Embryonic Stem Cell Lines from Zebrafish Lianchun Fan, Jennifer Crodian, and Paul Collodi I. Introduction II. Methods III. Materials References	151 152 158 160
10. Neurogenesis Prisca Chapouton and Laure Bally-Cuif I. Introduction II. The Primary Neuronal Scaffold III. Early Development of the Zebrafish Neural Plate	164 164 167

		Contents
	IV. Lateral Inhibition and the Neurogenesis Cascade	174
	V. Establishment of Neuronal Identity	176
	VI. Aspects of Neuronal Differentiation	179
	VII. Useful Tools for the Study of Zebrafish Neurogenesis	181
	VIII. Conclusion	190
	References	190
11.	Time-Lapse Microscopy of Brain Development	
	Reinhard W. Köster and Scott E. Fraser	
	I. Introduction—Why and When to Use Intravital Imaging	208
	II. Techniques for Vital Staining of the Nervous System	209
	III. Preparation of the Zebrafish Specimen	218
	IV. The Microscopic System	221
	V. Data Recording	222
	VI. Data Analysis	225
	VII. Pitfalls to Avoid	229
	VIII. Summary	231
	References	232
	Further Reading	235
12.	Development of the Peripheral Sympathetic Nervous System in Zebrafish	
	Rodney A. Stewart, A. Thomas Look, John P. Kanki, and Paul D. Henion	
	I. Introduction	237
	II. The Peripheral Autonomic Nervous System	238
	III. The Zebrafish as a Model System for Studying PSNS Development	242
	IV. Zebrafish as a Novel Model for Studying Neuroblastoma	254
	V. Conclusion and Future Directions	255
	References	256
13.	Optical Physiology and Locomotor Behaviors of Wild-Type and Nacre Zebrafis	h
	Donald M. O'Malley, Nagarajan S. Sankrithi, Melissa A. Borla, Sandra Parker, Serena Banden, Ethan Gahtan, and H. William Detrich III	
	I. Introduction	262
	II. Optical Physiology—Basics	263
	III. Optical Physiology—Alternative Approaches	267
	IV. Options for Improving the Transparency of Larval Zebrafish	272
	V. Behavioral Perturbation Studies	276
	VI. Conclusions	280
	References	280

14.	Computer-Aided Screening for Zebrafish Embryonic Motility Mutants	
	Charles A. Lessman	
	I. Introduction	286
	II. Flatbed Transparency Scanners and Computers	288
	III. Software	290
	IV. Embryos and Larva: Physiological Considerations	291
	V. Data Acquisition	294
	VI. Data Analysis	294
	VII. Potential Problems and Remedies	305
	VIII. Conclusions	311
	References	312
15.	Photoreceptor Structure and Development: Analyses Using GFP Transgenes	
	Brian D. Perkins, James M. Fadool, and John E. Dowling	
	I. Introduction	316
	II. Photoreceptor Development	318
	III. Transgenic Zebrafish Expressing Photoreceptor-Specific Reporter Genes	320
	IV. Developmental Studies of Photoreceptors Using Transgenic Zebrafish	322
	V. Conclusions	327
	References	328
16.	Approaches to Study Neurogenesis in the Zebrafish Retina	
	Andrei Avanesov and Jarema Malicki	
	I. Introduction	333
	II. Development of the Zebrafish Retina	335
	III. Analysis of Wild-Type and Mutant Zebrafish Visual Systems	341
	IV. Genetic Analysis of the Zebrafish Retina	359
	V. Summary	374
	References	375
17.	Instrumentation for Measuring Oculomotor Performance and Plasticity in	
	Larval Organisms	
	James C. Beck, Edwin Gilland, Robert Baker, and David W. Tank	
	I. Introduction	386
	II. Methods	389
	III. Results and Discussion	395
	IV. Conclusion	407
	V. Supplemental Movie Descriptions	407
	References	411

18.	Development of Cartilage and Bone	
	Yashar Javidan and Thomas F. Schilling	
	I. Introduction	415
	II. Cartilage Visualization Techniques	419
	III. Bone Visualization Techniques	427
	IV. Molecular Markers of Skeletal Precursors	431
	V. Strategy and Potential of Future Screens for Skeletal Mutants	431
	References	433
19.	Morphogenesis of the Jaw: Development Beyond the Embryo	
	R. Craig Albertson and Pamela C. Yelick	
	I. Larval Zebrafish Craniofacial Cartilage Development	437
	II. Analysis of Craniofacial Skeletal and Replacement Tooth Development	439
	III. Conclusion	451
	References	451
20.	Cardiac Development	
	Le A. Trinh and Didier Y. R. Stainier	
	I. Introduction	455
	II. Stages of Heart Tube Morphogenesis	456
	III. Gene Expression	465
	IV. Conclusion and Future Directions	469
	References	470
24		
21.	Chemical Approaches to Angiogenesis	
	Joanne Chan and Fabrizio C. Serluca	
	I. Introduction	475
	II. Screening Tools	479
	III. Chemical Screening	482
	IV. Sensitized Screens	485
	References	485
22.	Vascular Occlusion and Thrombosis in Zebrafish	
	Pudur Jagadeeswaran, Matthew Cykowski, and Bijoy Thattaliyath	
	I. Introduction	489
	II. Vascular Occlusion	491