Methods in Molecular Biology™

VOLUME 137

Developmental Biology Protocols

Volume III

Edited by

Rocky S. Tuan Cecilia W. Lo



HUMANA

Developmental Biology Protocols

Volume III



University of Pennsylvania, Philadelphia, PA

To Chuck and our parents

© 2000 Humana Press Inc. 999 Riverview Drive, Suite 208 Totowa, New Jersey 07512

All rights reserved. No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from the Publisher. Methods in Molecular Biology¹⁵ is a trademark of The Humana Press Inc.

All authored papers, comments, opinions, conclusions, or recommendations are those of the author(s), and do not necessarily reflect the views of the publisher.

This publication is printed on acid-free paper.

ANSI Z39.48-1984 (American Standards Institute)
Permanence of Paper for Printed Library Materials.

Cover design by Patricia F. Cleary

Cover illustration taken from Fig. 1 in Chapter 20 of Vol. I in this series. It is a composite three-dimensional view of epifluorescence micrographs of representative stages of the developing rat embryo coated with acridine orange and imaged in toto by confocal laser-scanning microscopy.

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel.: 973-256-1699; Fax: 973-256-8341; E-mail: humana@humanapr.com; or visit our Website: http://humanapress.com

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Humana Press Inc., provided that the base fee of US \$10.00 per copy, plus US \$00.25 per page, is paid directly to the Copyright Clearance Center at 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license from the CCC, a separate system of payment has been arranged and is acceptable to Humana Press Inc. The fee code for users of the Transactional Reporting Service is: [0-89603-854-8'00 (hardcover) or 0-89603-576-X'00 (paper) \$10.00 + \$00.25].

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging in Publication Data

Main entry under title: Developmental biology protocols/edited by Rocky S. Tuan and Cecilia W. Lo.

Methods in molecular biology18.

Developmental biology protocols/edited by Rocky S. Tuan and Cecilia W. Lo.

p. cm.—(Methods in molecular biology; v. 135, 136, 137) Includes bibliographical references and indexes.

ISBN 0-89603-852-1 (v. 1: alk. paper) (hardcover). ISBN 0-89603-574-3 (paper): ISBN 0-89603-853-X (v. 2: alk. paper) (hardcover). ISBN 0-89603-575-1 (paper): ISBN 0-89603-854-8 (v. 3: alk. paper) (hardcover). ISBN 0-89603-576-X (paper); ISBN 0-89603-855-6 (hc: alk paper); ISBN 0-89603-578-6 (comb: alk paper)

Developmental biology—Laboratory manuals.
 Molecular biology—Laboratory manuals.
 Tuan, Rocky S. II. Lo, Cecilia W. III. Series.
 QH491.D4625 2000
 4c21

99-23532 CIP

Preface

Developmental biology is one of the most exciting and fast-growing fields today. In part, this is so because the subject matter deals with the innately fascinating biological events—changes in form, structure, and function of the organism. The other reason for much of the excitement in developmental biology is that the field has truly become the unifying melting pot of biology, and provides a framework that integrates anatomy, physiology, genetics, biochemistry, and cellular and molecular biology, as well as evolutionary biology. No longer is the study of embryonic development merely "embryology." In fact, development biology has produced important paradigms for both basic and clinical biomedical sciences.

Though modern developmental biology has its roots in "experimental embryology" and the even more classical "chemical embryology," the recent explosive and remarkable advances in developmental biology are critically linked to the advent of the "cellular and molecular biology revolution." The impressive arsenal of experimental and analytical tools derived from cell and molecular biology, which promise to continue to expand, together with the exponentially developing sophistication in functional imaging and information technologies, guarantee that the study of the developing embryo will contribute one of the most captivating areas of biological research in the next millennium.

There is a demonstrated need for students of developmental biology to be knowledgeable of the breadth and depth of the available experimental methodologies, by necessity derived from multiple disciplines, which are applicable to the study of the developing embryo. In particular, because developmental biology deals with multiple model systems, from organismal to tissue and cell levels, as well as a wide range of "change"-related biological activities, the investigator is often frustrated as to how his/her findings relate to those obtained in another model system and/or by using different reagents or functional markers. Compared to other more strictly defined fields of biological research, the number of "reference" publications that deal specifically with the practical aspects of experimental developmental biology are, however, relatively scarce.

Developmental Biology Protocols grows out of the need for a comprehensive laboratory manual that provides the readers the principles, background, rationale, as well as the practical protocols, for studying and analyzing the events of embryonic development. This three-volume set, consisting of 142 chapters, is intentionally broad in scope, because of the nature of modern developmental biology. Information is grouped into the following topics: (1) systems—production, culture, and storage; (2) developmental pattern and morphogenesis; (3) embryo structure and function; (4) cell lineage analysis; (5) chimeras; (6) experimental manipulation of embryos; (7) application of viral vectors; (8) organogenesis; (9) abnormal development and teratology; (10) screening and mapping of novel genes and mutations; (11) transgenesis production and gene knockout; (12) manipulation of developmental gene expression and func-

tion; (13) analysis of gene expression; (14) models of morphogenesis and development; and (15) in vitro models and analysis of differentiation and development.

Throughout *Developmental Biology Protocols*, the authors have consistently striven for a balanced presentation of both background information and actual laboratory details. It is believed that this highly practical format will permit readers to bring the concepts and principles we present into their personal research practices in a most efficient manner. Specifically, the wide range of model systems and multidisciplinary experimental techniques presented here should lower the "activation energy" for the student of developmental biology to become a contributing member of this exciting scientific discipline. In addition, teachers of developmental biology at all levels should also readily find relevant and useful information to enrich the experience of their students.

The practice of developmental biology is currently in a state of constant change, reflecting the close relationship of the field to other rapidly developing fields of biological research, particularly cell and molecular biology, and imaging and information technology. The materials presented in this three-volume set are therefore the beginning of a project that will involve continuous update and upgrade to reach and enhance the scientific endeavors of developmental biologists at large.

The production of *Developmental Biology Protocols* would not have been possible without the outstanding work of the contributing authors who share here with the readers the hands-on wisdom they have earned in the laboratory. We are grateful for their intellectual contributions as well as their remarkable tolerance to our constant reminders. Tom Lanigan and his staff at Humana Press worked diligently on the project to ensure a final product of the highest quality. Chuck, our young son, persevered throughout the gestation period of the project, and constantly demonstrated to us the meaning of "developmental biology."

Our heartfelt thanks go to Lynn Stierle, who expertly and single-handedly maintained the massive organization of the manuscripts and the correspondence (snailmail and e-mail), as well as the sanity of the editors! Michelle Levinski also provided valuable assistance in proofreading the final production.

Finally, we hope that these volumes will find their place on the laboratory shelves, with their pages well soiled and their contents tried and tested, and prove their utility as an everyday resource for the students of developmental biology, the most exciting discipline of biology for many decades to come!

Rocky S. Tuan, PhD Cecilia W. Lo, PhD

List of Color Plates

Color plates 1–12 appear as an insert following p. 262.

- Plate 1 (Fig. 1 from Chapter 9). (A) Uninfected term human placentae. (B) Infected term human placentae. (C) Uninfected third trimester human placental explant. (D) Infected third trimester human explant.
- Plate 2 (Fig. 1 from Chapter 11). Construction of a calibration curve from gelatin calibration spots.
- Plate 3 (Fig. 2 from Chapter 11). (A) Optical density of a section of a 13-d-old rat embryo hybridized for SERCA2a. (B) and (C) "Bright-field" and "dark-field" colored cpm representations of image in (A).
- **Plate 4 (Fig. 2 from Chapter 10).** Radioactive *in situ* hybridization of ³⁵S-labeled probes to transverse sections of paraffin-embedded E13.5 mouse embryos.
- Plate 5 (Fig. 1 from Chapter 13). An example of a two-color WMISH. The E8.5 embryo has been hybridized with a DIG-labeled probe to the Paraxis gene and a fluorescein-labeled probe to the Brachyury gene.
- Plate 6 (Fig. 1 from Chapter 14). (A) Blastoderm stage *Drosophila* embryo stained first in blue for expression of *sloppy-paired*, then in red for expression of *empty-spiracles*, and finally in purple for *fushi tarazu* transcripts. (B) Whole-mount of the head of a 27-hpf zebrafish embryo stained by a combination of *in situ* hybridization and immunostaining.
- Plates 7 and 8 (Fig. 1 from Chapter 20). Photomicrographs of a chicken embryo cultured on the agar—albumin substratum described in the chapter.
- Plate 9 (Fig. 4 from Chapter 25). Living stage 17 chick embryo viewed through a window cut in the egg shell.
- Plate 10 (Fig. 2 from Chapter 30). Detection of (A) prostate-specific antigen (PSA) and (B) a membrane protein (connexin-43) in archival paraffin sections of human prostate tumors.
- Plate 11 (Fig. 1 from Chapter 37). Red blood cell formation within chicken blastoderm aggregates. Aggregate culture was visualized using differential interference contrast optics.

Plate 12 (Fig. 1 from Chapter 42). The *Splotch* mouse. (A) E 11.5 wild-type (left) and homozygous *Splotch* (right) embryos are shown. Note the patent neural tube of the *Splotch* mouse in the lumbosacral region (arrow). (B) Transverse section through an E 11.5 homozygous *Splotch* embryo heart reveals persistent truncus arteriosus. (C) Wild-type and (D) homozygous *Splotch* embryos reveal *Pax3* expression in the dorsal neural tube, the dorsal root ganglia (arrow, C), and the dermamyotome (arrowhead). (E) E 11.5 wild-type and (F) *Splotch* embryos at the forelimb level shows a complete absence of *MyoD* expression in the limb (lb) of *Splotch* embryos.

Contributors

- Peter G. Alexander Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- JOSEPH C. Ayoob Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA
- George L. Barnes Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- Sona Bartunkova Beth Israel Deaconess Medical Center, Boston, MA
- Vahe Bedian Pfizer Central Research, Groton, CT
- VICKIE D. Bennett (Deceased) Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- Anne-Gaelle Borycki Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA
- Peter C. Brooks Department of Biochemistry and Molecular Biology, University of Southern California School of Medicine, Los Angeles, CA
- Belinda M. Chapman Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS
- Allen W. Clark Department of Anatomy, University of Wisconsin–Madison, Madison, WI
- Ronald A. Conlon Department of Genetics, Case Western Reserve University, Cleveland, OH
- Christopher Cox Department of Anatomy and Cell Biology, State University of New York Health Science Center, Syracuse, NY
- BARBARA A. DANOWSKI Department of Biology, Union College, Schenectady, NY
- PIET A. J. De Boer Department of Anatomy and Embryology, Academic Medical Center, Amsterdam, The Netherlands
- Anthony M. DeLise Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- Camille Dilulo Department of Anatomy, Philadelphia College of Osteopathic Medicine, Philadelphia, PA
- Maria P. De Miguel Department of Microbiology and Immunology, Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, PA
- Peter J. Donovan Department of Microbiology and Immunology, Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, PA
- Carol A. Eisenberg Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC
- Charles P. Emerson, Jr. Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA

xxii Contributors

JONATHAN A. EPSTEIN • Departments of Medicine and Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA

- John F. Fallon Department of Anatomy, University of Wisconsin-Madison, Madison, WI
- Diego Franco Department of Anatomy and Embryology, Academic Medical Center, Amsterdam, The Netherlands
- Silvio Garofalo Centro Biotecnologie Avanzate, Instituto Nazionale Ricera per la Ricerca sul Cancro, Genova, Italy
- Pamela Gehron Robey Craniofacial and Skeletal Diseases Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD
- MINDY GEORGE-WEINSTEIN Department of Anatomy, Philadelphia College of Osteopathic Medicine, Philadelphia, PA
- Jacquelyn Gerhart Department of Anatomy, Philadelphia College of Osteopathic Medicine, Philadelphia, PA
- THOMAS GERSTER Biozentrum der Universität Basel, Basel, Switzerland
- TROY A. GIAMBERNARDI Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX
- ROBERT M. GREENE Department of Biological and Biophysical Sciences, School of Dentistry, University of Louisville, Louisville, KY
- Andrew R. Haas Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- Brian K. Hall Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada
- GISELBERT HAUPTMANN Institut für Biologie I (Zoologie), Albert-Ludwigs-Universität Freiburg, Freiburg, Germany
- Jaco Hagoort Department of Anatomy and Embryology, Academic Medical Center, Amsterdam, The Netherlands
- Susan Heyner Center for Research in Reproduction and Women's Health, University of Pennsylvania, Philadelphia, PA
- WILLIAM A. HORTON Research Department, Shriners Hospital for Children, Portland, OR
- OLENA JACENKO Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA
- Brian Johnstone Department of Orthopaedics, Case Western Reserve University, Cleveland, OH
- Daniel S. Kessler Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA
- ROBERT J. KLEBE Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX
- Karen A. Knudsen Department of Biochemistry and Molecular Pharmacology, Lankenau Medical Research Center, Wynnewood, PA
- MICHAEL R. KUEHN Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD

- Wouter H. Lamers Department of Anatomy and Embryology, Academic Medical Center, Amsterdam, The Netherlands
- PASCALE LEGOUX Sanofi Recherche de Labe 'ge, Labe 'ge Cedex, France
- YI-HSIN LIU Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angeles, CA
- CECILIA W. Lo Department of Biology, University of Pennsylvania, Philadelphia, PA
- Linda A. Lowe Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD
- Adesola Majolagbe Craniofacial and Skeletal Diseases Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD
- ROBERT E. MAXSON, Jr. Department of Biochemistry and Molecular Biology, Kenneth R. Norris Cancer Hospital and Institute, Los Angeles, CA
- PARMENDER P. Mehta Department of Medicine, Sylvester Comprehensive Cancer Center, University of Miami School of Medicine; and Geriatric Research, Education, and Clinical Center and Research Service, VAMC, Miami, FL
- Maria Alice Mello Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- RICHARD K. MILLER Department of Obstetrics and Gynecology, University of Rochester Medical Center, Rochester, NY
- Antoon F. M. Moorman Department of Anatomy and Embryology, Academic Medical Center, Amsterdam, The Netherlands
- Mark Murphy The Murdoch Institute, Embryology Laboratory, Royal Children's Hospital, Parkville, Victoria, Australia
- Mehrdad Nadji Department of Pathology, Sylvester Comprehensive Cancer Center, University of Miami School of Medicine and Geriatric Research Education and Clinical Center, Miami, FL
- Donald F. Newgreen The Murdoch Institute, Embryology Laboratory, Royal Children's Hospital, Parkville, Victoria, Australia
- CHRISTOF NIEHRS Deutsches Krebsforschungzentrum, Heidelberg, Germany
- Steven A. Oberlender Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- David S. Packard, Jr. Department of Anatomy and Cell Biology, State University of New York Health Science Center, Syracuse, NY
- Carlos Perez-Stable Department of Medicine, Sylvester Comprehensive Cancer Center, University of Miami School of Medicine; and Geriatric Research, Education, and Clinical Center and Research Service, VAMC, Miami, FL
- THOMAS J. Peters Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS
- ERIC PETITCLERC Department of Biochemistry and Molecular Biology, University of Southern California School of Medicine, Los Angeles, CA
- M. Michele Pisano Department of Biological and Biophysical Sciences, School of Dentistry, University of Louisville, Louisville, KY

xxiv Contributors

Bruno M. Polliotti • Department of Obstetrics and Gynecology, University of Rochester Medical Center, Rochester, NY

- THOMAS J. POOLE Department of Anatomy and Cell Biology, State University of New York Health Science Center, Syracuse, NY
- Jenny E. Rooke Department of Genetics, Yale University School of Medicine, New Haven. CT
- Bernard A. Roos Department of Medicine, Sylvester Comprehensive Cancer Center, University of Miami School of Medicine; and Geriatric Research, Education, and Clinical Center and Research Service, VAMC, Miami, FL
- Maria A. Ros Departamento de Anatomía y Biología Celular, Universidad de Cantabria, Santande, Spain
- JAN M. Ruijter Department of Anatomy and Embryology, University of Amsterdam, Amsterdam, The Netherlands
- JEAN M. SANGER Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA
- JOSEPH W. SANGER Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA
- Thomas N. Sato The University of Texas Southwestern Medical Center, Dallas. TX
- Asad U. Sheikh Department of Obstetrics and Gynecology, University of Rochester Medical Center, Rochester, NY
- David Shire Sanofi Recherche de Labe 'ge, Labe 'ge Cedex, France
- B. Kay Simandl Department of Anatomy, University of Wisconsin–Madison, Madison, WI
- MALCOLM L. SNEAD Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angeles, CA
- MICHAEL J. SOARES Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS
- Alejandro Peralta Soler Department of Biochemistry and Molecular Pharmacology, Lankenau Medical Research Center, Wynnewood, PA
- Emanuela Stringa Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- NICOLE A. THEODOSIOU Department of Genetics, Yale University School of Medicine, New Haven, CT
- ROCKY S. TUAN Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- MICHAEL J. TUCKER Reproductive Biology Associates, Atlanta, GA
- Charles B. Underhill Department of Cell Biology, Georgetown Medical Center, Washington, DC
- Tami von Schalscha Department of Immunology, The Scripps Research Institute, San Diego, CA
- Stefan Wawersik Department of Medicine, Brigham and Women's Hospital, Boston, MA

Contributors

ELIZABETH L. WILDER • Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA

- Wendy A. Woodward Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
- Tian Xu Department of Genetics, Yale University School of Medicine, New Haven, CT
- JIE YAO Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA
- Jung Yoo Department of Medicine, Case Western Reserve University, Cleveland, OH
- Lurong Zhang Department of Cell Biology, Georgetown Medical Center. Washington, DC

Contents

PA	RT I INTRODUCTION
1	Developmental Biology Protocols: Overview III Rocky S. Tuan and Cecilia W. Lo
Pa	RT II MANIPULATION OF DEVELOPMENTAL GENE EXPRESSION AND FUNCTION
2	Ectopic Expression in <i>Drosophila</i> Elizabeth L. Wilder
3	Clonal Analysis in the Examination of Gene Function in <i>Drosophila</i> Jenny E. Rooke, Nicole A. Theodosiou, and Tian Xu
4	Application of Antisense Oligodeoxynucleotides in Developing Chick Embryos
	Peter G. Alexander, George L. Barnes, and Rocky S. Tuan
5	Application of Functional Blocking Antibodies: N-Cadherin and Chick Embryonic Limb Development
	Steven A. Oberlender and Rocky S. Tuan 37
Par	RT III ANALYSIS OF GENE EXPRESSION
6	Gene Expression Analyzed by Ribonuclease Protection Assay Vickie D. Bennett
7	Relative Reverse Transcription-Polymerase Chain Reaction Troy A. Giambernardi and Robert J. Klebe
8	Gene Expression Analysis Using Quantitative Reverse Transcription- Polymerase Chain Reaction and a Multispecific Internal Control
	David Shire and Pascale Legoux 59
9	In Situ PCR Detection of HIV Expression in the Human Placenta Asad U. Sheikh, Bruno M. Polliotti, and Richard K. Miller
10	Gene Expression Analysis by <i>In Situ</i> Hybridization: Radioactive Probes
	Stefan Wawersik and Jonathan A. Epstein 87
11	Radio-Isotopic In Situ Hybridization on Tissue Sections: Practical Aspects and Quantification
	Antoon F. M. Moorman, Piet A. J. De Boer, Jan M. Ruijter, Jaco Hagoort, Diego Franco, and Wouter H. Lamers

X

12	mRNA and Protein Co-Localization on Tissue Sections by Sequential, Colorimetric <i>In Situ</i> Hybridization and Immunohistochemistry	
	Rocky S. Tuan	117
13	Whole-Mount <i>In Situ</i> Hybridization to Study Gene Expression During Mouse Development	
	Linda A. Lowe and Michael R. Kuehn	125
14	Multicolor Whole-Mount In Situ Hybridization	
	Giselbert Hauptmann and Thomas Gerster	139
15	Methods for Double Detection of Gene Expression: Combined In Situ Hybridization and Immunocytochemistry or Histochemistry	
	Ronald A. Conion	149
16	Visualization of the Expression of Green Fluorescent Protein (GFP)- Linked Proteins	
	Joseph C. Ayoob, Jean M. Sanger, and Joseph W. Sanger	153
17	Monoclonal Antibodies in the Analysis of Embryonic Development	
	Vahe Bedian	159
Pai	RT IV MODELS OF MORPHOGENESIS AND DEVELOPMENT	
18	Mesoderm Induction in Xenopus: Oocyte Expression System and Animal Cap Assay	
	Jie Yao and Daniel S. Kessler	169
19	Amphibian Organizer Activity	
	Christof Niehrs	179
20	Improved Techniques for Avian Embryo Culture, Somite Cell Culture, and Microsurgery	
21	David S. Packard, Jr., Christopher Cox, and Thomas J. Poole	
	Donald F. Newgreen and Mark Murphy	201
22	The Chimeric Human/Mouse Model of Angiogenesis Eric Petitclerc, Tami von Schalscha, and Peter C. Brooks	212
00		213
23	Analysis of Embryonic Vascular Morphogenesis Thomas N. Sato and Sona Bartunkova	223
24	Epithelial—Mesenchymal Interactions	220
24	Brian K. Hall	235
25	Methods for Manipulating the Chick Limb Bud to Study Gene Expression, Tissue Interactions, and Patterning	
	Maria A. Ros, B. Kay Simandl, Allen W. Clark, and John F. Fallon	245
26	Palate Development: In Vitro Procedures	
	M. Michele Pisano and Robert M. Greene	267
Paf	RT V IN VITRO MODELS AND ANALYSIS OF DIFFERENTIATION AND DEVELOPMENT	
27	In Vitro Fertilization	
	Susan Hevner and Michael J. Tucker	277

Contents

	28	Trophoblast Differentiation: An In Vitro Model for Trophoblast Giant Cell Development	
·		Thomas J. Peters, Belinda M. Chapman, and Michael J. Soares	301
	29	Bone Marrow-Derived Mesenchymal Progenitor Cells Brian Johnstone and Jung Yoo	
	30	Identification, Characterization, and Differentiation of Human Prostate Cells Parmender P. Mehta, Carlos Perez-Stable, Bernard A. Roos, and Mehrdad Nadji	
	31	Preparation of Chick Striated Muscle Cultures Camille DiLullo, Jacquelyn Gerhart, and Mindy George-Weinstein	
	32	Study of Skeletal Myogenesis in Cultures of Unsegmented Paraxial Mesoderm	
		Anne-Gaelle Borycki and Charles P. Emerson, Jr	351
	33	Embryonic Limb Mesenchyme Micromass Culture as an In Vitro Model for Chondrogenesis and Cartilage Maturation Anthony M. DeLise, Emanuela Stringa,	
		Wendy A. Woodward, Maria Alice Mello, and Rocky S. Tuan	359
	34	Electroporation-Mediated DNA Transfection of Embryonic Chick Limb Mesenchymal Cells	
		Anthony M. DeLise and Rocky S. Tuan	377
	35	Murine C3H10T1/2 Multipotential Cells as an In Vitro Model of Mesenchymal Chondrogenesis	
		Andrew R. Haas and Rocky S. Tuan	383
	36	Skeletogenesis: In Vitro Analysis of Bone Cell Differentiation	
	07	Adesola Majolagbe and Pamela Gehron Robey	391
	37	Studying Early Hematopoiesis Using Avian Blastoderm Cultures Carol A. Eisenberg	200
	38	Isolation and Culture of Mouse Germ Cells	333
	50	Maria P. De Miguel and Peter J. Donovan	403
	39	Cadherin-Mediated Cell-Cell Interactions	
		Karen A. Knudsen and Alejandro Peralta Soler	409
	40	Analysis of Hyaluronan Using Biotinylated Hyaluronan-Binding Proteins Charles B. Underhill and Lurong Zhang	441
	41	Microinjection of Fluorescently Labeled α-Actinin into Living Cells Jean M. Sanger, Barbara A. Danowski, and Joseph W. Sanger	
	PAR	T VI DEVELOPMENTAL MODELS OF DISEASES	
	42	Pax3 and Vertebrate Development	
	-	Jonathan A. Epstein	459
	43	Genetic-Engineered Models of Skeletal Diseases I: Collagen Type X Olena Jacenko	

1

INTRODUCTION

一此为试读,需要完整PDF请访问: www.ertongbook.com