

Embryonic Stem Cells

METHODS IN MOLECULAR BIOLOGY™

John M. Walker, SERIES EDITOR

200. **DNA Methylation Protocols**, edited by Ken I. Mills and Bernie H. Ramsahoye, 2002
199. **Liposome Methods and Protocols**, edited by Subhash C. Basu and Manju Basu, 2002
198. **Neural Stem Cells: Methods and Protocols**, edited by Tanja Zigova, Juan R. Sanchez-Ramos, and Paul R. Sanberg, 2002
197. **Mitochondrial DNA: Methods and Protocols**, edited by William C. Copeland, 2002
196. **Oxidants and Antioxidants: Ultrastructural and Molecular Biology Protocols**, edited by Donald Armstrong, 2002
195. **Quantitative Trait Loci: Methods and Protocols**, edited by Nicola J. Camp and Angela Cox, 2002
194. **Post-translational Modification Reactions**, edited by Christoph Kannicht, 2002
193. **RT-PCR Protocols**, edited by Joseph O'Connell, 2002
192. **PCR Cloning Protocols, 2nd ed.**, edited by Bing-Yuan Chen and Harry W. Janes, 2002
191. **Telomeres and Telomerase: Methods and Protocols**, edited by John A. Double and Michael J. Thompson, 2002
190. **High Throughput Screening: Methods and Protocols**, edited by William P. Janzen, 2002
189. **GTPase Protocols: The RAS Superfamily**, edited by Edward J. Manser and Thomas Leung, 2002
188. **Epithelial Cell Culture Protocols**, edited by Clare Wise, 2002
187. **PCR Mutation Detection Protocols**, edited by Bimal D. M. Theophilus and Ralph Rapley, 2002
186. **Oxidative Stress and Antioxidant Protocols**, edited by Donald Armstrong, 2002
185. **Embryonic Stem Cells: Methods and Protocols**, edited by Kursad Turksen, 2002
184. **Biostatistical Methods**, edited by Stephen W. Looney, 2002
183. **Green Fluorescent Protein: Applications and Protocols**, edited by Barry W. Hicks, 2002
182. **In Vitro Mutagenesis Protocols, 2nd ed.**, edited by Jeff Braman, 2002
181. **Genomic Imprinting: Methods and Protocols**, edited by Andrew Ward, 2002
180. **Transgenesis Techniques, 2nd ed.: Principles and Protocols**, edited by Alan R. Clarke, 2002
179. **Gene Probes: Principles and Protocols**, edited by Marilena Aquino de Muro and Ralph Rapley, 2002
178. **Antibody Phage Display: Methods and Protocols**, edited by Philippa M. O'Brien and Robert Aitken, 2001
177. **Two-Hybrid Systems: Methods and Protocols**, edited by Paul N. MacDonald, 2001
176. **Steroid Receptor Methods: Protocols and Assays**, edited by Benjamin A. Lieberman, 2001
175. **Genomics Protocols**, edited by Michael P. Starkey and Ramnath Elasarapu, 2001
174. **Epstein-Barr Virus Protocols**, edited by Joanna B. Wilson and Gerhard H. W. May, 2001
173. **Calcium-Binding Protein Protocols, Volume 2: Methods and Techniques**, edited by Hans J. Vogel, 2001
172. **Calcium-Binding Protein Protocols, Volume 1: Reviews and Case Histories**, edited by Hans J. Vogel, 2001
171. **Proteoglycan Protocols**, edited by Renato V. Iozzo, 2001
170. **DNA Arrays: Methods and Protocols**, edited by Jang B. Rampal, 2001
169. **Neurotrophin Protocols**, edited by Robert A. Rush, 2001
168. **Protein Structure, Stability, and Folding**, edited by Kenneth P. Murphy, 2001
167. **DNA Sequencing Protocols, Second Edition**, edited by Colin A. Graham and Alison J. M. Hill, 2001
166. **Immunotoxin Methods and Protocols**, edited by Walter A. Hall, 2001
165. **SV40 Protocols**, edited by Leda Raptis, 2001
164. **Kinesin Protocols**, edited by Isabelle Vernos, 2001
163. **Capillary Electrophoresis of Nucleic Acids, Volume 2: Practical Applications of Capillary Electrophoresis**, edited by Keith R. Mitchelson and Jing Cheng, 2001
162. **Capillary Electrophoresis of Nucleic Acids, Volume 1: Introduction to the Capillary Electrophoresis of Nucleic Acids**, edited by Keith R. Mitchelson and Jing Cheng, 2001
161. **Cytoskeleton Methods and Protocols**, edited by Ray H. Gavin, 2001
160. **Nuclease Methods and Protocols**, edited by Catherine H. Schein, 2001
159. **Amino Acid Analysis Protocols**, edited by Catherine Cooper, Nicole Packer, and Keith Williams, 2001
158. **Gene Knockout Protocols**, edited by Martin J. Tymms and Ismail Kola, 2001
157. **Mycotoxin Protocols**, edited by Mary W. Trucksess and Albert E. Pohland, 2001
156. **Antigen Processing and Presentation Protocols**, edited by Joyce C. Solheim, 2001
155. **Adipose Tissue Protocols**, edited by Gérard Ailhaud, 2000
154. **Connexin Methods and Protocols**, edited by Roberto Bruzzone and Christian Giaume, 2001
153. **Neuropeptide Y Protocols**, edited by Ambikaipakan Balasubramanian, 2000
152. **DNA Repair Protocols: Prokaryotic Systems**, edited by Patrick Vaughan, 2000
151. **Matrix Metalloproteinase Protocols**, edited by Ian M. Clark, 2001
150. **Complement Methods and Protocols**, edited by B. Paul Morgan, 2000
149. **The ELISA Guidebook**, edited by John R. Crowther, 2000
148. **DNA-Protein Interactions: Principles and Protocols (2nd ed.)**, edited by Tom Moss, 2001
147. **Affinity Chromatography: Methods and Protocols**, edited by Pascal Bailon, George K. Ehrlich, Wen-Jian Fung, and Wolfgang Berthold, 2000
146. **Mass Spectrometry of Proteins and Peptides**, edited by John R. Chapman, 2000
145. **Bacterial Toxins: Methods and Protocols**, edited by Otto Holst, 2000
144. **Calpain Methods and Protocols**, edited by John S. Elce, 2000
143. **Protein Structure Prediction: Methods and Protocols**, edited by David Webster, 2000
142. **Transforming Growth Factor-Beta Protocols**, edited by Philip H. Howe, 2000
141. **Plant Hormone Protocols**, edited by Gregory A. Tucker and Jeremy A. Roberts, 2000
140. **Chaperonin Protocols**, edited by Christine Schneider, 2000
139. **Extracellular Matrix Protocols**, edited by Charles Streuli and Michael Grant, 2000
138. **Chemokine Protocols**, edited by Amanda E. I. Proudfoot, Timothy N. C. Wells, and Christine Power, 2000
137. **Developmental Biology Protocols, Volume III**, edited by Rocky S. Tuan and Cecilia W. Lo, 2000
136. **Developmental Biology Protocols, Volume II**, edited by Rocky S. Tuan and Cecilia W. Lo, 2000
135. **Developmental Biology Protocols, Volume I**, edited by Rocky S. Tuan and Cecilia W. Lo, 2000
134. **T Cell Protocols: Development and Activation**, edited by Kelly P. Kears, 2000
133. **Gene Targeting Protocols**, edited by Eric B. Kmiec, 2000
132. **Bioinformatics Methods and Protocols**, edited by Stephen Misener and Stephen A. Krawetz, 2000
131. **Flavoprotein Protocols**, edited by S. K. Chapman and G. A. Reid, 1999
130. **Transcription Factor Protocols**, edited by Martin J. Tymms, 2000

METHODS IN MOLECULAR BIOLOGY™


Embryonic Stem Cells

Methods and Protocols

Edited by

Kursad Turksen

Ottawa Health Research Institute, Ottawa, Ontario, Canada

Humana Press  Totowa, New Jersey

© 2002 Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

humanapress.com

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 Biology™ is a trademark of The Humana Press Inc.

The content and opinions expressed in this book are the sole work of the authors and editors, who have warranted due diligence in the creation and issuance of their work. The publisher, editors, and authors are not responsible for errors or omissions or for any consequences arising from the information or opinions presented in this book and make no warranty, express or implied, with respect to its contents.

This publication is printed on acid-free paper. ∞
ANSI Z39.48-1984 (American Standards Institute)
Permanence of Paper for Printed Library Materials.

Production Editor: Diana Mezzina

Cover design by Patricia F. Cleary.

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: www.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: [0-89603-881-5/02 (hardcover) \$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

Embryonic Stem Cells: methods and protocols / edited by Kursad Turksen.

p. cm. -- (Methods in molecular biology ; v. 185)

Includes bibliographical references and index.

ISBN 0-89603-881-5 (alk. paper)

1. Embryonic Stem Cells--Laboratory manuals. I. Turksen, Kursad. II. Series.

QH440.5 .E43 2002
612'.0181--dc21

2001026459

Preface

It is fair to say that embryonic stem (ES) cells have taken their place beside the human genome project as one of the most discussed biomedical issues of the day. It also seems certain that as this millennium unfolds we will see an increase in scientific and ethical debate about their potential utility in society.

On the scientific front, it is clear that work on ES cells has already generated new possibilities and stimulated development of new strategies for increasing our understanding of cell lineages and differentiation. It is not naïve to think that, within a decade or so, our overall understanding of stem cell biology will be as revolutionized as it was when the pioneering hemopoietic stem cell studies of Till and McCulloch in Toronto captured our imaginations in 1961. With it will come better methods for ES and lineage-specific stem cell identification, maintenance, and controlled fate selection. Clearly, ES cell models are already providing opportunities for the establishment of limitless sources of specific cell populations. In recognition of the growing excitement and potential of ES cells as models for both the advancement of basic science and future clinical applications, I felt it timely to edit this collection of protocols (*Embryonic Stem Cells*) in which forefront investigators would provide detailed methods for use of ES cells to study various lineages and tissue types.

We are pleased to provide *Embryonic Stem Cells: Methods and Protocols*, a broad-scaled work of 35 chapters containing step-by-step protocols suitable for use by both experienced investigators and novices in various ES cell technologies. In the first section of the volume, there are chapters with detailed protocols for ES cell isolation, maintenance, modulation of gene expression, and studies of ES cell cycle and apoptosis. *Embryonic Stem Cells* also includes chapters with protocols for the use of ES cells to generate diverse cell and tissue types, including blood, endothelium, adipocytes, skeletal muscle, cardiac muscle, neurons, osteoclasts, melanocytes, keratinocytes, and hair follicle cells. The second part of the volume contains a series of cutting edge techniques that have already been shown to have, or will soon have, tremendous utility with ES cells and their differentiated progeny. These chapters include the use of cDNA arrays in gene expression analysis, phage display antibody libraries to generate antibodies against very rare antigens, and phage display libraries to identify and characterize protein and protein interactions, to name a few. Collectively, these protocols should prove a useful resource not only to those who are using or wish to use ES cells to study fate choices and specific lineages, but also to those interested in cell and developmental biology more generally. We hope that this book will also serve as a catalyst spurring others to use ES cells for lineages not yet being widely studied with this model and to develop new methodologies that would contribute to both the fundamental understanding of stem cells and their potential utility.

Embryonic Stem Cells would not have materialized at all had the contributors not recognized the special value of disseminating their protocols and hard-won expertise. I am extremely grateful to them for their commitment, dedication, and promptness with submissions! I am also grateful to Dr. John Walker for having faith in and supporting me throughout this project. I wish also to acknowledge the great support provided by many at Humana Press, specifically Elyse O'Grady, Craig Adams, Diana Mezzina, and Tom Lanigan. A special thank you goes to my dedicated coworker, Tammy-Claire Troy, who, with her infectious optimism and tireless commitment, became a crucial factor in the editing and completion of the volume.

I am grateful to N. Urfe, P. Kael, and M. Chambers for their unintentional “awesome” contributions.

Finally, I hope that the volume will achieve the intent that I had originally imagined: that it will prove a volume with something for both experts and novices alike, that it will serve as a launching point for further developments in stem cells, and that we will all-too-soon wish to expand and update it with other emerging concepts, insights and methods!

Kursad Turksen

Contents

Preface	v
Contributors	xi
Color Plates	xv
1 Methods for the Isolation and Maintenance of Murine Embryonic Stem Cells <i>Marsha L. Roach and John D. McNeish</i>	1
2 The Use of Chemically Defined Media for the Analyses of Early Development in ES Cells and Mouse Embryos <i>Gabriele Proetzel and Michael V. Wiles</i>	17
3 Analysis of the Cell Cycle in Mouse Embryonic Stem Cells <i>Pierre Savatier, Hélène Lapillonne, Ludmila Jirmanova, Luigi Vitelli, and Jacques Samarut</i>	27
4 Murine Embryonic Stem Cells as a Model for Stress Proteins and Apoptosis During Differentiation <i>André-Patrick Arrigo and Patrick Mehlen</i>	35
5 Effects of Altered Gene Expression on ES Cell Differentiation <i>Yong Fan and J. Richard Chaillet</i>	45
6 Hypoxic Gene Regulation in Differentiating ES Cells <i>David M. Adelman and M. Celeste Simon</i>	55
7 Regulation of Gap Junction Protein (Connexin) Genes and Function in Differentiating ES Cells <i>Masahito Oyamada, Yumiko Oyamada, Tomoyuki Kaneko, and Tetsuro Takamatsu</i>	63
8 Embryonic Stem Cell Differentiation as a Model to Study Hematopoietic and Endothelial Cell Development <i>Stuart T. Fraser, Minetaro Ogawa, Satomi Nishikawa, and Shin-Ichi Nishikawa</i>	71
9 Analysis of <i>Bcr-Abl</i> Function Using an In Vitro Embryonic Stem Cell Differentiation System <i>Takumi Era, Stephane Wong, and Owen N. Witte</i>	83
10 Embryonic Stem Cells as a Model for Studying Osteoclast Lineage Development <i>Toshiyuki Yamane, Takahiro Kunisada, and Shin-Ichi Hayashi</i>	97

11	Differentiation of Embryonic Stem Cells as a Model to Study Gene Function During the Development of Adipose Cells Christian Dani	107
12	Embryonic Stem Cell Differentiation and the Vascular Lineage Victoria L. Bautch	117
13	Embryonic Stem Cells as a Model to Study Cardiac, Skeletal Muscle, and Vascular Smooth Muscle Cell Differentiation Anna M. Wobus, Kaomei Guan, Huang-Tian Yang, and Kenneth R. Boheler	127
14	Cardiomyocyte Enrichment in Differentiating ES Cell Cultures: Strategies and Applications Kishore B. S. Pasumarthi and Loren J. Field	157
15	Embryonic Stem Cells as a Model for the Physiological Analysis of the Cardiovascular System Jürgen Hescheler, Maria Wartenberg, Bernd K. Fleischmann, Kathrin Banach, Helmut Acker, and Heinrich Sauer	169
16	Isolation of Lineage-Restricted Neural Precursors from Cultured ES Cells Tahmina Mujtaba and Mahendra S. Rao	189
17	Lineage Selection for Generation and Amplification of Neural Precursor Cells Meng Li	205
18	Selective Neural Induction from ES Cells by Stromal Cell-Derived Inducing Activity and Its Potential Therapeutic Application in Parkinson's Disease Hiroshi Kawasaki, Kenji Mizuseki, and Yoshiki Sasai	217
19	Epidermal Lineage Tammy-Claire Troy and Kursad Turksen	229
20	ES Cell Differentiation Into the Hair Follicle Lineage In Vitro Tammy-Claire Troy and Kursad Turksen	255
21	Embryonic Stem Cells as a Model for Studying Melanocyte Development Toshiyuki Yamane, Shin-Ichi Hayashi, and Takahiro Kunisada	261
22	Using Progenitor Cells and Gene Chips to Define Genetic Pathways S. Steven Potter, M. Todd Valerius, and Eric W. Brunskill	269
23	ES Cell-Mediated Conditional Transgenesis Marina Gertsenstein, Corrinne Lobe and Andras Nagy	285
24	Switching on Lineage Tracers Using Site-Specific Recombination Susan M. Dymecki, Carolyn I. Rodriguez, and Rajeshwar B. Awatramani	309

25	From ES Cells to Mice: <i>The Gene Trap Approach</i> Francesco Cecconi and Peter Gruss	335
26	Functional Genomics by Gene-Trapping in Embryonic Stem Cells Thomas Floss and Wolfgang Wurst	347
27	Phage-Displayed Antibodies to Detect Cell Markers Jun Lu and Steven R. Sloan	381
28	Gene Transfer Using Targeted Filamentous Bacteriophage David Larocca, Kristen Jensen-Pergakes, Michael A. Burg, and Andrew Baird	393
29	Single-Cell PCR Methods for Studying Stem Cells and Progenitors Jane E. Aubin, Fina Liu, and G. Antonio Candelieri	403
30	Nonradioactive Labeling and Detection of mRNAs Hybridized onto Nucleic Acid cDNA Arrays Thorsten Hoevel and Manfred Kubbies	417
31	Expression Profiling Using Quantitative Hybridization on Macroarrays Geneviève Piétu and Charles Decraene	425
32	Isolation of Antigen-Specific Intracellular Antibody Fragments as Single Chain Fv for Use in Mammalian Cells Eric Tse, Grace Chung, and Terence H. Rabbitts	433
33	Detection and Visualization of Protein Interactions with Protein Fragment Complementation Assays Ingrid Remy, André Galarneau, and Stephen W. Michnick	447
34	Direct Selection of cDNAs by Phage Display Reto Cramer, Gernot Achatz, Michael Weichel, and Claudio Rhyner	461
35	Screening for Protein–Protein Interactions in the Yeast Two-Hybrid System in Embryonic Stem Cells R. Daniel Gietz and Robin A. Woods	471
	<i>Index</i>	487

Contributors

GERNOT ACHATZ • *Department of Genetics, University of Salzburg, Hellbrunnerstrasse Salzburg, Australia*

HELMUT ACKER • *Institute of Neurophysiology, University of Cologne, Koln, Germany*

DAVID M. ADELMAN • *Abramson Research Institute, Department of Cancer Biology, University of Pennsylvania Cancer Center, Philadelphia, PA*

ANDRÉ-PATRICK ARRIGO • *Laboratoire du Stress Oxydant, Chaperons et Apoptose, Center de Genetique Moleculaire et Cellulaire, University Claude Bernard Lyon-I, Villeurbanne, France*

JANE E. AUBIN • *Department of Anatomy and Cell Biology, University of Toronto, Toronto, Ontario, Canada*

RAJESHWAR B. AWATRAMANI • *Department of Genetics, Harvard Medical School, Boston, MA*

ANDREW BAIRD • *Selective Genetics Inc., San Diego, CA*

KATHRIN BANACH • *Institute of Neurophysiology, University of Cologne, Koln, Germany*

VICTORIA L. BAUTCH • *Department of Biology, The University of North Carolina at Chapel Hill, Chapel Hill, NC*

KENNETH R. BOHELER • *In Vitro Differentiation Group, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*

ERIC W. BRUNSKILL • *Division of Developmental Biology, Children's Hospital Medical Center, Cincinnati, OH*

MICHAEL A. BURG • *Selective Genetics Inc., San Diego, CA*

G. ANTONIO CANDELIERE • *Department of Anatomy and Cell Biology, University of Toronto, Toronto, Ontario, Canada*

FRANCESCO CECCONI • *Department of Biology, University of Rome Tor Vergata, Roma, Italy*

J. RICHARD CHAILLET • *Department of Pediatrics University of Pittsburgh, School of Medicine, Children's Hospital of Pittsburgh, PA*

GRACE CHUNG • *Division of Protein and Nucleic Acid Chemistry, Cambridge, Medical Research Council Laboratory of Molecular Biology, UK*

RETO CRAMERI • *Swiss Institute of Allergy and Asthma Research, Davos, Switzerland*

CHRISTIAN DANI • *Institute of Signaling, Developmental Biology, and Cancer Research, Centre de Biochimie, Nice, France*

CHARLES DECRAENE • *CEA Service de Genomique Fontionnelle, Batiment Genopole, Evry, France*

SUSAN M. DYMECKI • *Department of Genetics, Harvard Medical School, Boston, MA*

TAKUMI ERA • *Howard Hughes Medical Institute, University of California, Los Angeles, CA*

- YONG FAN • *Department of Pediatrics University of Pittsburgh, School of Medicine, Children's Hospital of Pittsburgh, PA*
- LOREN J. FIELD • *Herman B. Wells Center for Pediatric Research, James Whitcombe Riley Hospital for Children, Indianapolis, IN*
- BERND K. FLEISHMANN • *Institute of Neurophysiology, University of Cologne, Koln, Germany*
- THOMAS FLOSS • *GSF-Institute of Mammalian Genetics, Neuherberg, Germany*
- STUART T. FRASER • *Department of Molecular Genetics, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan*
- ANDRÉ GALARNEAU • *Department of Biochemistry, University of Montréal, Québec, Canada*
- MARINA GERTSENSTEIN • *Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada*
- R. DANIEL GIETZ • *Department of Human Genetics, University of Manitoba, Winnipeg, Manitoba, Canada*
- PETER GRUSS • *Department of Molecular Cell Biology, Max-Planck-Institute of Biophysical Chemistry, Göttingen, Germany*
- KAOMEI GUAN • *In Vitro Differentiation Group, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*
- SHIN-ICHI HAYASHI • *Department of Immunology, School of Life Science, Faculty of Medicine, Tottori University, Yonago, Japan*
- JÜRGEN HESCHELER • *Institute of Neurophysiology, University of Cologne, Koln, Germany*
- THORSTEN HOEVEL • *Department of Cell Analytics, Roche Pharmaceutical Research, Roche Diagnostics GmbH, Penzberg, Germany*
- KRISTEN JENSEN-PERGAKES • *Selective Genetics Inc., San Diego, CA*
- LUDMILA JIRMANOVA • *Laboratoire de Biologie Moléculaire de Cellulaire de l'Ecole Normale Supérieure de Lyon, Lyon, France*
- TOMOYUKI KANEKO • *Department of Pathology and Cell Regulation, Kyoto Prefectural University of Medicine, Kyoto, Japan*
- HIROSHI KAWASAKI • *Department of Medical Embryology and Neurobiology, Institute for Frontier Medical Sciences, Kyoto University*
- MANFRED KUBBIES • *Department of Cell Analytics, Roche Pharmaceutical Research, Roche Diagnostics GmbH, Penzberg, Germany*
- TAKAHIRO KUNISADA • *Department of Hygiene, Faculty of Medicine, Gifu University, Gifu, Japan*
- HÉLÈNE LAPILLONE • *Laboratoire de Biologie Moléculaire de Cellulaire de l'Ecole Normale Supérieure de Lyon, Lyon, France*
- DAVID LAROCCA • *Selective Genetics Inc., San Diego, CA*
- MENG LI • *Center for Genome Research, University of Edinburgh, Edinburgh, UK*
- FINA LIU • *INSERM, Hôpital Edouard Herriot, Lyon, France*
- JUN LU • *Department of Laboratory Medicine and Joint Program in Transfusion Medicine, Children's Hospital, Harvard Medical School, Boston, MA*

- CORRINNE LOBE • *Cancer Research Division, Sunnybrook and Women's College Health Science Center, Toronto, Ontario, Canada*
- PATRICK MEHLEN • *Laboratoire Différenciation et Apoptose, CNRS, Université Claude Bernard Lyon-I, France*
- JOHN D. McNEISH • *Genetic Technologies, Pfizer Global Research and Development, Groton, CT*
- STEPHEN W. MICHNICK • *Department of Biochemistry, University of Montréal, Québec, Canada*
- KENJI MIZUSEKI • *Department of Medical Embryology and Neurobiology, Institute for Frontier Medical Sciences, Kyoto University*
- TAHMINA MUJTABA • *Department of Neurobiology and Anatomy, University of Utah Medical School, Salt Lake City, UT*
- ANDRAS NAGY • *Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada*
- SATOMI NISHIKAWA • *Department of Molecular Genetics, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan*
- SHIN-ICHI NISHIKAWA • *Department of Molecular Genetics, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan*
- MINETARO OGAWA • *Department of Molecular Genetics, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan*
- MASAHITO OYAMADA • *Department of Pathology and Cell Regulation, Kyoto Prefectural University of Medicine, Kyoto, Japan*
- YUMIKO OYAMADA • *Department of Pathology and Cell Regulation, Kyoto Prefectural University of Medicine, Kyoto, Japan*
- KISHORE B.S. PASUMARTHI • *Herman B. Wells Center for Pediatric Research, James Whitcomb Riley Hospital for Children, Indianapolis, IN*
- GENEVIÈVE PIÉTU • *CEA Service de Genomique Fontionnelle, Batiment Genopole, Evry, France*
- S. STEVEN POTTER • *Division of Developmental Biology, Children's Hospital Medical Center, Cincinnati, OH*
- GABRIELE PROETZEL • *Deltagen Inc., Menlo Park, CA*
- TERENCE H. RABBITS • *Division of Protein and Nucleic Acid Chemistry, Medical Research Council Laboratory of Molecular Biology, Cambridge, UK*
- MAHENDRA S. RAO • *Department of Neurobiology and Anatomy, University of Utah Medical School, Salt Lake City, UT*
- INGRID REMY • *Department of Biochemistry, University of Montréal, Québec, Canada*
- CLAUDIO RHYNER • *Swiss Institute of Allergy and Asthma Research, Davos, Switzerland*
- MARSHA L. ROACH • *Genetic Technologies, Pfizer Global Research and Development, Groton, CT*
- CAROLYN I. RODRIGUEZ • *Department of Genetics, Harvard Medical School, Boston, MA*
- JACQUES SAMARUT • *Laboratoire de Biologie Moléculaire de Cellulaire de l'Ecole Normale Supérieure de Lyon, Lyon, France*

- YOSHIKI SASAI • *Department of Medical Embryology and Neurobiology, Institute for Frontier Medical Sciences, Kyoto University*
- HEINRICH SAUER • *Institute of Neurophysiology, University of Cologne, Koln, Germany*
- PIERRE SAVATIER • *Laboratoire de Biologie Moleculaire de Cellulaire de l'Ecole Normale Supérieure de Lyon, Lyon, France*
- M. CELESTE SIMON • *Abramson Research Institute, Department of Cancer Biology, University of Pennsylvania Cancer Center, Philadelphia, PA*
- STEVEN R. SLOAN • *Department of Laboratory Medicine and Joint Program in Transfusion Medicine, Children's Hospital, Harvard Medical School, Boston, MA*
- TETSURO TAKAMATSU • *Department of Pathology and Cell Regulation, Kyoto Prefectural University of Medicine, Kyoto, Japan*
- TAMMY-CLAIRE TROY • *Ottawa Health Research Institute, Ottawa, Ontario, Canada*
- ERIC TSE • *Medical Research Council Laboratory of Molecular Biology, Division of Protein and Nucleic Acid Chemistry, Cambridge, UK*
- KURSA D TURKSEN • *Ottawa Health Research Institute, Ottawa, Ontario, Canada*
- M. TODD VALERIUS • *Department of Molecular Cell Biology, Harvard University, Cambridge, MA*
- LUIGI VITELLI • *Laboratoire de Biologie Moleculaire de Cellulaire de l'Ecole Normale Supérieure de Lyon, Lyon, France*
- MARIA WARTENBURG • *Institute of Neurophysiology, University of Cologne, Koln, Germany*
- MICHAEL WEICHEL • *Swiss Institute of Allergy and Asthma Research, Davos, Switzerland*
- MICHAEL V. WILES • *Deltagen Inc., Menlo Park, CA*
- OWEN N. WITTE • *Howard Hughes Medical Institute, University of California, Los Angeles, CA*
- ANNA M. WOBUS • *In Vitro Differentiation Group, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*
- ANNA M. WOBUS • *In Vitro Differentiation Group, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*
- STEPHANE WONG • *Howard Hughes Medical Institute, University of California, Los Angeles, CA*
- ROBIN A. WOODS • *Department of Biology, University of Winnipeg, Winnipeg, Manitoba, Canada*
- WOLFGANG WURST • *Clinical Neurogenetics, Max-Planck Institute of Psychiatry, Munich, Germany*
- TOSHIYUKI YAMANE • *Department of Immunology, School of Life Science, Faculty of Medicine, Tottori University, Yonago, Japan*
- HUANG-TIAN YANG • *In Vitro Differentiation Group, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*

Color Plates

- Plate 1 Fig. 1. (A-F) Hematopoiesis of in vitro ES cell differentiation with M-CSF-deficient OP9 stromal cells.
(See full caption and discussion on p. 84, Chapter 9.)
- Plate 2 Fig. 5. (A-D) Effect of *Bcr-Abl* expression on d 8 and d 15 hematopoietic cells.
(See full caption and discussion on p. 92, Chapter 9.)
- Plate 3 Fig. 2. (A-E) Schematic diagram of the genetic enrichment program.
(See full caption and discussion on p. 160, Chapter 14.)
- Plate 4 Fig. 3. (A-C) PAS staining provides rapid assessment of cardiomyocyte yield in differentiating cells.
(See full caption and discussion on p. 163, Chapter 14.)
- Plate 5 Fig. 4. (A, B) Genetically enriched cardiomyocytes form stable intracardiac grafts.
(See full caption and discussion on p. 164, Chapter 14.)
- Plate 6 Fig. 5. Use of the ES-derived cardiomyocyte colony growth assay to monitor the effects of gene transfer on cardiomyocyte proliferation.
(See full caption and discussion on p. 166, Chapter 14.)
- Plate 7 Fig. 3. A flowchart summarizing the process of magnetic bean sorting.
(See full caption and discussion on p. 198, Chapter 16.)
- Plate 8 Fig. 1. (A, B) Neural stem cell selection strategy.
(See full caption and discussion on p. 206, Chapter 17.)

- Plate 9 Fig. 2. ES cell-derived neurons and glia following *Sox2* selection.
(See full caption and discussion on p. 207, Chapter 17.)
- Plate 10 Fig. 1. (A-H) EPC plated at high density (10^6 cells/35-mm dish) and assayed after 10 and 12 d for hair follicle markers.
(See full caption and discussion on p. 258, Chapter 20.)
- Plate 11 Fig. 1. (A, B) Transduction of mammalian cells by ligand-targeted phage.
(See full caption and discussion on p. 394, Chapter 28.)
- Plate 12 Fig. 1. (A, B) Diagram illustrating the strategy for the selection of specific intracellular antibodies.
(See full caption and discussion on p. 435, Chapter 32.)
- Plate 13 Fig. 2. Diagram showing the restriction maps and polylinker sequences of the yeast expression vectors, (A) pBTM116 and (B) pVP16.
(See full caption and discussion on p. 437, Chapter 32.)
- Plate 14 Fig. 1. (A, B) Two alternative strategies to achieve complementation.
(See full caption and discussion on p. 448, Chapter 33.)
- Plate 15 Fig. 2. (A-H) Applications of the DHFR PCA to detecting the localization of protein complexes and quantitating protein interactions.
(See full caption and discussion on p. 451, Chapter 33.)
- Plate 16 Fig. 3. (A-C) β -Lactamase PCA using the fluorescent substrate CCF2/AM.
(See full caption and discussion on p. 455, Chapter 33.)