Embryonic Stem Cell Protocols

Volume 2: Differentiation Models

SECOND EDITION

Edited by

Kursad Turksen

Ottawa Health Research Institute
Ottawa, Ontario, Canada
Preface

The potentials, and hence popularity, of assessing embryonic stem (ES) cells in regenerative medicine applications is no longer a surprise to either scientists or the general public. This is clearly reflected in the ever-increasing publications in which ES cell biology and differentiation along diverse lineages appear in the academic as well as the popular press. It is also reflected in the intense interest in the isolation and characterization of ES cells from other species for preclinical studies. It therefore seemed timely to capture important advances in the field since the publication of the Embryonic Stem Cells: Methods and Protocols volume four years ago.

To provide an update and complement the original mouse ES cell book, I have focused the initial part of the first volume of the new series on ES cells recently isolated from other/nonmouse species. Second, the volumes contain numerous updates, more advanced approaches and completely new protocols for the use of ES cells in studies of diverse cell lineages. I believe that these two volumes will complement and expand the experimental repertoires of both experts and novices in the field. I would therefore like to take this opportunity to thank all of the contributors for their generosity and dedication in putting together their protocols. Without them, these volumes would not exist.

I am grateful to Dr. John Walker for his support and encouragement during the process. I would also like to thank several others at the Humana Press for their support: initially Elyse O’Grady and Craig Adams, and more recently Damien DeFrances. Also, I am grateful to Jennifer Hackworth for her wonderful support during the production of this volume.

I would also like to thank Jane Aubin and N. Urfe for their continuous support and encouragement as well as Tammy Troy who has once again been fantastic in helping to put together these volumes.

Kursad Turksen
Contents

Preface ..............................................................................................................v
Contents of the Companion Volume ............................................................ xi
Contributors .................................................................................................... xv
Guide to the Companion CD ......................................................................... xix

1 Neural Stem Sphere Method: 
   Induction of Neural Stem Cells and Neurons 
   by Astrocyte-Derived Factors in Embryonic Stem Cells In Vitro 
Takashi Nakayama and Nobuo Inoue ................................................... 1

2 Generation and Characterization of Oligodendrocytes 
   From Lineage-Selective Embryonic Stem Cells In Vitro 
Nathalie Billon, Christine Jolicoeur, and Martin Raff ........................... 15

3 Derivation and Characterization of Neural Cells 
   From Embryonic Stem Cells Using Nestin Enhancer 
Nibedita Lenka .................................................................................... 33

4 Optimized Neuronal Differentiation 
   of Murine Embryonic Stem Cells: 
   Role of Cell Density 
Matthew T. Lorincz ............................................................................. 55

5 Generation of Inner Ear Cell Types From Embryonic Stem Cells 
Marcelo N. Rivolta, Huawei Li, and Stefan Heller .............................. 71

6 Derivation of Epidermal Colony-Forming Progenitors 
   From Embryonic Stem Cell Cultures 
Tammy-Claire Troy and Kursad Turksen ............................................ 93

7 Directing Epidermal Fate Selection by a Novel 
   Co-Culture System 
Tammy-Claire Troy and Kursad Turksen .......................................... 105

8 In Vitro Generation of T Lymphocytes 
   From Embryonic Stem Cells 
Renée F. de Pooter, Thomas M. Schmitt, 
   and Juan Carlos Zúñiga-Pflücker .................................................. 113

9 The Role of Hex in Hemangioblast and Hematopoietic 
   Development 
Rebecca J. Chan, Robert Hromas, and Mervin C. Yoder ................. 123
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Generation of Osteoblasts and Chondrocytes</td>
<td>Jitsutaro Kawaguchi</td>
</tr>
<tr>
<td></td>
<td>From Embryonic Stem Cells</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Analysis of Embryonic Stem Cell-Derived Osteogenic Cultures</td>
<td>Nicole L. Woll and Sarah K. Bronson</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Generation of Chondrocytes From Embryonic Stem Cells</td>
<td>Jaspal Singh Khillan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Derivation and Characterization of Chondrocytes</td>
<td>Jan Kramer, Gunnar Hargus, and Jürgen Rohwedel</td>
</tr>
<tr>
<td></td>
<td>From Embryonic Stem Cells In Vitro</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Generation and Characterization of Cardiomyocytes Under Serum-Free</td>
<td>Cornelia Gissel, Michael Xavier Doss, Rita Hippler-Altenburg,</td>
</tr>
<tr>
<td></td>
<td>Conditions</td>
<td>Jürgen Hescheler, and Agapios Sachinidis</td>
</tr>
<tr>
<td>15</td>
<td>Analysis of Arrhythmic Potential of Embryonic Stem Cell-Derived</td>
<td>Lijuan L. Shang, Samuel C. Dudley, Jr., and Arnold E. Pfahnl</td>
</tr>
<tr>
<td></td>
<td>Cardiomyocytes</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Derivation and Characterization of Alveolar Epithelial Cells</td>
<td>Ali Samadikuchaksaraei and Anne E. Bishop</td>
</tr>
<tr>
<td></td>
<td>From Murine Embryonic Stem Cells In Vitro</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Derivation and Characterization of Thyrocyte-Like Cells</td>
<td>Reigh-Yi Lin and Terry F. Davies</td>
</tr>
<tr>
<td></td>
<td>From Embryonic Stem Cells In Vitro</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Derivation and Characterization of Gut-Like Structures</td>
<td>Takatsugu Yamada and Yoshiyuki Nakajima</td>
</tr>
<tr>
<td></td>
<td>From Embryonic Stem Cells</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Formation of Gut-Like Structures In Vitro From Mouse Embryonic Stem</td>
<td>Shigeko Torihashi</td>
</tr>
<tr>
<td></td>
<td>Cells</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>In Vitro Derivation and Expansion of Endothelial Cells</td>
<td>Kara E. McCloskey, Steven L. Stice, and Robert M. Nerem</td>
</tr>
<tr>
<td></td>
<td>From Embryonic Stem Cells</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Differentiation of Mouse Embryonic Stem Cells Into Endothelial Cells</td>
<td>Clotilde Gimond, Sandrine Marchetti, and Gilles Pagès</td>
</tr>
<tr>
<td></td>
<td>Genetic Selection and Potential Use In Vivo</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Integrins and Vascular Development in Differentiated Embryonic Stem</td>
<td>Sheila E. Francis</td>
</tr>
<tr>
<td></td>
<td>Cells In Vitro</td>
<td></td>
</tr>
</tbody>
</table>
23 TGF-β Signaling in Embryonic Stem Cell-Derived Endothelial Cells
   Tetsuro Watabe, Jun K. Yamashita, Koichi Mishima,
   and Kohei Miyazono ................................................................. 341

24 The Role of the Adapter Protein SHB in Embryonic
   Stem Cell Differentiation Into the Pancreatic β-cell
   and Endothelial Lineages
   Johan Saldeen, Nina Ågren, Björn Åkerblom, Lingge Lu,
   Bashir Adem, Łukasz Sędek, and Vitezslav Kriz ...................... 353

25 In Vitro Differentiation of Embryonic Stem Cells Into
   the Pancreatic Lineage
   Przemyslaw Błyszczuk and Anna M. Wobus ......................... 373

26 Derivation and Characterization of Hepatocytes From Embryonic
   Stem Cells In Vitro
   Shigeaki Ishizaka, Yukiteru Ouji, Masahide Yoshikawa,
   and Kazuki Nakatani ................................................................. 387

27 Differentiation of Embryonic Stem Cells to Retinal Cells In Vitro
   Xing Zhao, Jianuo Liu, and Iqbal Ahmad ................................. 401

28 Derivation and Characterization of Lentoid Bodies and Retinal
   Pigment Epithelial Cells From Monkey Embryonic
   Stem Cells In Vitro
   Masayo Takahashi and Masatoshi Haruta .............................. 417

29 Differentiation of Rhesus Monkey Embryonic Stem Cells
   in Three-Dimensional Collagen Matrix
   Silvia Sihui Chen, Roberto P. Revoltella, Joshua Zimmerberg,
   and Leonid Margolis ................................................................. 431

Index ........................................................................................... 445
CONTENTS OF THE COMPANION VOLUME
Volume 1: Isolation and Characterization

PART I. ISOLATION AND MAINTENANCE
1 Isolation and Differentiation of Medaka Embryonic Stem Cells
   Yunhan Hong and Manfred Schartl
2 Maintenance of Chicken Embryonic Stem Cells In Vitro
   Hiroyuki Horiuchi, Shuichi Furusawa, and Haruo Matsuda
3 Derivation and Culture of Mouse Trophoblast Stem Cells In Vitro
   Satoshi Tanaka
4 Derivation, Maintenance, and Characterization of Rat Embryonic Stem Cells In Vitro
   Maren Schulze, Hendrik Ungefroren, Michael Bader, and Fred Fändrich
5 Derivation, Maintenance, and Induction of the Differentiation In Vitro of Equine Embryonic Stem Cells
   Shigeo Saito, Ken Sawai, Arika Minamihashi, Hideyo Ugai, Takehide Murata, and Kazunari K. Yokoyama
6 Generation and Characterization of Monkey Embryonic Stem Cells
   Hirofumi Suemori and Norio Nakatsuji
7 Derivation and Propagation of Embryonic Stem Cells in Serum- and Feeder-Free Culture
   Jennifer Nichols and Qi-Long Ying

PART II. SIGNALING IN EMBRYONIC STEM CELL DIFFERENTIATION
8 Internal Standards in Differentiating Embryonic Stem Cells In Vitro
   Christopher L. Murphy
9 Matrix Assembly, Cell Polarization, and Cell Survival: Analysis of Peri-Implantation Development With Cultured Embryonic Stem Cells
   Shaohua Li and Peter D. Yurchenco
10 Phosphoinositides, Inositol Phosphates, and Phospholipase C in Embryonic Stem Cells
   Leo R. Quinlan
11 Cripto Signaling in Differentiating Embryonic Stem Cells
   Gabriella Minchiotti, Silvia Parisi, and M. Graziella Persico

12 The Use of Embryonic Stem Cells to Study Hedgehog Signaling
   Sandy Becker and Laura Grabel

13 Transfection and Promoter Analysis in Embryonic Stem Cells
   Sangmi Chung and Kwang-Soo Kim

14 SAGE Analysis to Identify Embryonic Stem Cell-Predominant Transcripts
   Kenneth R. Boheler and Kirill V. Tarasov

15 Utilization of Digital Differential Display to Identify Novel Targets of Oct3/4
   Yoshimi Tokuzawa, Masayoshi Maruyama, and Shinya Yamanaka

16 Gene Silencing Using RNA Interference in Embryonic Stem Cells
   J. Matthew Velkey, Nicole A. Slawny, Theresa E. Gratsch, and K. Sue O’Shea

PART III. GENETIC MANIPULATION OF EMBRYONIC STEM CELLS

17 Efficient Transfer of HSV-1 Amplicon Vectors Into Embryonic Stem Cells and Their Derivatives
   Dieter Riethmacher, Filip Lim, and Thomas Schimmang

18 Lentiviral Vector-Mediated Gene Transfer in Embryonic Stem Cells
   Masahiro Oka, Lung-Ji Chang, Frank Costantini, and Naohiro Terada

19 Use of the Cytomegalovirus Promoter for Transient and Stable Transgene Expression in Mouse Embryonic Stem Cells
   Katie M. Barrow, Flor M. Perez-Campo, and Christopher M. Ward

20 Use of Simian Immunodeficiency Virus Vectors for Simian Embryonic Stem Cells
   Takayuki Asano, Hiroaki Shibata, and Yutaka Hanazono

21 Generation of Green Fluorescent Protein-Expressing Monkey Embryonic Stem Cells
   Tatsuyuki Takada, Yutaka Suzuki, Nae Kadota, Yasushi Kondo, and Ryuzo Torii
22 DNA Damage Response and Mutagenesis in Mouse Embryonic Stem Cells
   *Yiling Hong, Rachel B. Cervantes, and Peter J. Stambrook*

23 Ultraviolet-Induced Apoptosis in Embryonic Stem Cells In Vitro
   *Dakang Xu, Trevor J. Wilson, and Paul J. Hertzog*

**PART IV. USE OF EMBRYONIC STEM CELLS IN PHARMACOLOGICAL AND TOXICOLOGICAL SCREENS**

24 Use of Differentiating Embryonic Stem Cells in Pharmacological Studies
   *Brigitte Wdziekonski, Phi Villageois, Cécile Vernochet, Blaine Phillips, and Christian Dani*

25 Embryonic Stem Cells as a Source of Differentiated Neural Cells for Pharmacological Screens
   *Patrick J. Mee, Carmel M. O’Brien, Hazel Thomson, Sjaak van der Sar, Viktor Lakics, and Timothy E. Allsopp*

26 Use of Murine Embryonic Stem Cells in Embryotoxicity Assays: The Embryonic Stem Cell Test
   *Andrea E. M. Seiler, Roland Buesen, Anke Visan, and Horst Spielmann*

27 Use of Chemical Mutagenesis in Mouse Embryonic Stem Cells
   *Sonja Becker, Martin Hrabé de Angelis, and Johannes Beckers*

**PART V. EPIGENETIC ANALYSIS OF EMBRYONIC STEM CELLS**

28 Nuclear Reprogramming of Somatic Nucleus Hybridized With Embryonic Stem Cells by Electrofusion
   *Masako Tada and Takashi Tada*

29 Methylation in Embryonic Stem Cells In Vitro
   *Koichiro Nishino, Jun Ohgane, Masako Suzuki, Naka Hattori, and Kunio Shiota*

**PART VI. TUMOR-LIKE PROPERTIES**

30 Identification of Genes Involved in Tumor-Like Properties of Embryonic Stem Cells
   *Kazutoshi Takahashi, Tomoko Ichisaka, and Shinya Yamanaka*

31 In Vivo Tumor Formation From Primate Embryonic Stem Cells
   *Takayuki Asano, Kyoko Sasaki, Yoshihiro Kitano, Keiji Terao, and Yutaka Hanazono*
PART VII. ANIMAL MODELS AND THERAPY

32 Directed Differentiation and Characterization of Genetically Modified Embryonic Stem Cells for Therapy
   Adeline A. Lau, Kim M. Hemsley, Adrian Meedeniya, Aaron J. Robinson, and John J. Hopwood

33 Use of Differentiating Embryonic Stem Cells in the Parkinsonian Mouse Model
   Fumihiko Nishimura, Hayato Toriumi, Shigeaki Ishizaka, Toshisuke Sakaki, and Masahide Yoshikawa
Contributors

BASHIR ADEM • Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

NINA ÅGREN • Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

IQBAL AHMAD • Department of Ophthalmology, University of Nebraska Medical Center, Omaha, NE

BJÖRN ÅKERBLOM • Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

NATHALIE BILLON • Laboratoire "cellules souches et différenciation," UMR 6543 CNRS, centre de biochimie, Faculté des Sciences Valrose, Nice, France

ANNE E. BISHOP • Tissue Engineering and Regenerative Medicine, Center Faculty of Medicine, Imperial College, and Westminster Hospital, London, UK

PRZEMYSLAW BLYSZCZUK • In Vitro Differentiation Group Institute, Plant Genetics and Crop Plant Research, Gatersleben, Germany

SARAH K. BRONSON • Department of Cellular and Molecular Physiology, The Penn State College of Medicine, Milton S. Hershey Medical Center, Hershey, PA

REBECCA J. CHAN • Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN

SILVIA SIHUI CHEN • NASA/NIH Center for Three Dimensional Tissue Culture, Laboratory of Cellular and Molecular Biophysics, Bethesda MD; and Wyle Laboratories, Life Sciences, Systems, and Service, Houston, TX

TERRY F. DAVIES • Division of Endocrinology, VA Medical Center, Bronx, NY

RENÉE F. DE POOTER • Department of Immunology, University of Toronto Sunnybrook, and Women’s College Health Sciences Centre, Toronto, Ontario, Canada

MICHAEL XAVIER DOSS • Center of Physiology and Pathophysiology, Department of Neurophysiology, University of Cologne, Cologne, Germany

SAMUEL C. DUDLEY, JR. • Division of Cardiology, Emory University, Decatur, GA

SHEILA E. FRANCIS • Cardiovascular Research Unit, School of Medicine and Biomedical Sciences, University of Sheffield, 7 Sheffield, UK
CLOTILDE GIMOND • CNRS UMR 6543, Institute of Signalling Development Biology and Cancer, Nice, France
CORNELIA GISSEL • Center of Physiology and Pathophysiology, Department of Neurophysiology, University of Cologne, Cologne, Germany
GUNNAR HARGUS • Department of Medical Molecular Biology, University of Lübeck, Lübeck, Germany
MASATOSHI HARUTA • Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, Kyoto, Japan
STEFAN HELLER • Department of Otolaryngology and Program in Neuroscience, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA
JÜRGEN HESCHELER • Center of Physiology and Pathophysiology, Department of Neurophysiology, University of Cologne, Cologne, Germany
RITA HIPPLER-ALTENBURG • Center of Physiology and Pathophysiology, Department of Neurophysiology, University of Cologne, Cologne, Germany
ROBERT HROMAS • Cancer Research and Treatment Center, University of New Mexico, Albuquerque, NM
NOBUO INOUE • Laboratory of Regenerative Neurosciences, Faculty of Health Sciences, Tokyo Metropolitan, Tokyo, Japan
SHIGEAKI ISHIZAKA • Program in Tissue Engineering, Department of Parasitology, Nara Medical University, Nara, Japan
CHRISTINE JOLICOEUR • Laboratoire Biologie du Développement du tissu Adipeux, Cellules Souches et Différenciation, Centre de Biochimie Parc Valrose, Nice, France
JITSUTARO KAWAGUCHI • Centre Development in Stem Biology, Institute for Stem Cell Research, School of Biological Sciences, University of Edinburgh, Edinburgh, UK
JASPAL SINGH KHILLAN • Department of Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA
JAN KRAMER • Department of Medical Molecular Biology, University of Lübeck, Lübeck, Germany
VITEZSLAV KRIZ • Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
NIBEDITA LENKA • National Center for Cell Science, Ganeshkhind, Pune, Maharashtra, India
HUAYEI LI • Department of Otolaryngology and Program in Neuroscience, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA
REIGH-YI LIN • Division of Endocrinology Diabetes and Bone Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY
Contributors

JIANUO LIU • Department of Ophthalmology, University of Nebraska Medical Center, Omaha, NE
MATTHEW T. LORINCZ • Department of Neurology, University of Michigan Health Systems, Ann Arbor, MI
LINGGE LU • Department of Medicine/Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA
SANDRINE MARCHETTI • U 526, Activation des Cellules Hematopoietiques et infections virales, Nice, France
LEONID MARGOLIS • Laboratory of Cellular and Molecular Biophysics, NASA/NIH Center for Three Dimensional Tissue Culture, NICHD NIH, Bethesda, MD
KARA E. MCCLOSKEY • School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA
KOICHI MISHIMA • Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
KOHEI MIYAZONO • Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
YOSHIYUKI NAKAJIMA • Department of Surgery, Nara Medical University, Nara, Japan
KAZUKI NAKATANI • Department of Anatomy, Osaka City University, Medical School, Osaka, Japan
TAKASHI NAKAYAMA • Department of Biochemistry, Yokohama City University School of Medicine, Yokohama, Japan
ROBERT M. NEREM • School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA
YUKITERU OUJI • Program in Tissue Engineering, Department of Parasitology, Nara Medical University, Nara, Japan
GILLES PAGÈS • MCU Université de Nice, Sophia-Antipolis, France
ARNOLD E. PFANHNL • Division of Cardiology, Emory University, Decatur, GA
MARTIN RAFF • Medical Research Council, Laboratory for Molecular Cell Biology, Cell Biology Unit, University College London, London, UK
ROBERTO P. REVOLTELLA • CNR Institute of Biomedical Technologies, Unit of Immunobiology and Cell Differentiation, Via Moruzzi 1, 56100 Pisa, Italy
MARCELO N. RIVOLTA • Department of Otolaryngology and Program in Neuroscience, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA
JÜRGEN ROHWEDEL • Department of Medical Molecular Biology, University of Lübeck, Lübeck, Germany
AGAPIOS SACHINIDIS • Center of Physiology and Pathophysiology, Department of Neurophysiology, University of Cologne, Cologne, Germany
JOHAN SALDEEN • Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
ALI SAMADIKUCHAKSARAEI • Tissue Engineering and Regenerative Medicine Center, Faculty of Medicine, Imperial College, London, UK
THOMAS M. SCHMITT • Department of Immunology, University of Toronto Sunnybrook and Women’s College Health Sciences Centre, Toronto, Ontario, Canada
ŁUKASZ SĘDEK • Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
LIJUAN L. SHANG • Division of Cardiology Atlanta, Emory University, Decatur, GA
STEVEN L. STICE • School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA
MASAYO TAKAHASHI • Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, Kyoto, Japan
SHIGEKO TORIHASHI • Department of Anatomy and Molecular Cell Biology, Nagoya University Graduate School of Medicine, Nagoya, Japan
TAMMY-CLAIRE TROY • Development Program, Ottawa Health Research Institute, Ottawa, Ontario, Canada
KURSAĐ TURKSEN • Development Program, Ottawa Health Research Institute, Ottawa, Ontario, Canada
TETSURO WATABE • Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
ANNA M. WOBUS • In Vitro Differentiation Group, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
NICOLE L. WOLL • Department of Cellular and Molecular Physiology, The Penn State College of Medicine, Milton S. Hershey Medical Center, Hershey, PA
TAKATSUGU YAMADA • Department of Surgery, Nara Medical University, Nara, Japan
JUN K. YAMASHITA • Laboratory of Stem Cell Differentiation, Stem Cell Research Center, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan
Mervin C. Yoder • Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN
MASAHIDE YOSHIKAWA • Division of Developmental Biology, Department of Parasitology, Nara Medical University, Nara, Japan
Xing Zhao • Department of Ophthalmology, University of Nebraska Medical Center, Omaha, NE

Joshua Zimmerberg • Laboratory of Cellular and Molecular Biophysics, NASA/NIH Center for Three Dimensional Tissue Culture, NICHD NIH, Bethesda, MD

Juan Carlos Zúñiga-Pflücker • Department of Immunology, University of Toronto Sunnybrook and Women’s College Health Sciences Centre, Toronto, Ontario, Canada
COMPANION CD


All of the electronic versions of illustrations in this book may be found on the Companion CD attached to the inside back cover. The image files are organized into folders by chapter number and are viewable in most Web browsers. The number following “f” at the end of the file name identifies the corresponding figure in the text. The CD is compatible with both Mac and PC operating systems.

COLOR FIGURES

Chapter 3 Figs. 3, 5
Chapter 4 Fig. 1
Chapter 6 Figs. 1, 2
Chapter 9 Figs. 2, 3
Chapter 10 Fig. 1
Chapter 11 Fig. 1
Chapter 13 Figs. 1, 3
Chapter 14 Figs. 1–3

Chapter 18 Figs. 1, 2, 4, 5
Chapter 20 Figs. 1–3
Chapter 23 Fig. 2
Chapter 26 Fig. 2, 3
Chapter 27 Figs. 2–5
Chapter 28 Figs. 1, 5
Chapter 29 Figs. 1–3