
Essential Stem Cell Methods

Reliable Lab Solutions

Edited by

Dr. Robert Lanza

Advanced Cell Technology
Worcester, Massachusetts

Dr. Irina Klimanskaya

Advanced Cell Technology
Worcester, Massachusetts



ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier



CONTENTS

Contributors	xiii
Preface	xix

SECTION I Organ-Derived Stem Cells

1. Neural Stem Cells: Isolation and Characterization

Rodney L. Rietze and Brent A. Reynolds

1. Introduction	4
2. Reagents and Instrumentation	5
3. Methods	8
References	21

2. Neural Stem Cells and Their Manipulation

Prithi Rajan and Evan Snyder

1. Introduction	24
2. Adult Niches for Stem Cells <i>In Vivo</i>	25
3. <i>In Vitro</i> Manipulation of NSCs	27
4. Conclusions and Projections	39
5. Sample Protocols for the Culture and Characterization of NSCs	40
6. Culture of Rodent/Murine NSCs	41
7. Culture of human NSCs	43
8. ICC of markers to identify stem cells and differentiation products	45
References	45

3. Retinal Stem Cells

Thomas A. Reh, Joseph A. Brzezinski IV, and Andy J. Fischer

1. Introduction	54
2. Materials and methods	60
3. Overview	68
References	69

4. Dental Pulp Stem Cells

He Liu, Stan Gronthos, and Songtao Shi

1. Introduction	74
2. Identification of Dental Pulp Stem Cells (DPSCs)	75
3. Isolation of DPSCs	77
4. Differentiation of DPSCs	79
References	83

5. Mouse Spermatogonial Stem Cells: Culture and Transplantation

Jon M. Oatley and Ralph L. Brinster

1. Introduction	88
2. Spermatogonial Stem Cells (SSCs)	89
3. SSC Transplantation	91
4. SSC Culture	98
5. siRNA Transfection of Cultured Mouse SSCs	105
6. Implications	109
References	110

6. Stem Cells in the Adult Lung

Xiaoming Liu, Ryan R. Driskell, and John F. Engelhardt

1. Introduction	114
2. Anatomical and Cellular Diversity of the Adult Lung	115
3. Stem Cell Phenotypes and Niches in the Adult Lung	117
4. <i>In Vivo</i> Injury Models of the Lung	121
5. <i>Ex Vivo</i> Epithelial Tracheal Xenograft Model to Study Stem Cells Expansion in the Proximal Airway	128
6. <i>In Vitro</i> Colony-Forming Efficiency (CFE) Assay to Characterize Stem/Progenitor Cell Populations in Conducting Airway Epithelium	136
7. Models to Study Stem/Progenitor Cells of Airway SMG	141
References	143

7. Pancreatic Cells and Their Progenitors

Seth J. Salpeter and Yuval Dor

1. Introduction	150
2. Pancreas Development	151
3. Origins of Beta Cells During Postnatal Life	153
4. Preexisting Beta Cells	154
5. Ducts	155
6. Acini	155
7. Bone Marrow Cells	156
8. Adult Stem Cells	156

9. Dedifferentiation of Beta Cells	157
10. Summary and Perspective	157
11. Methods: Design of a Lineage Tracing Experiment in Mice	158
References	160
8. Pluripotent Stem Cells from Germ Cells: Derivation and Maintenance	
<i>Candace L. Kerr, Michael J. Shamblott, and John D. Gearhart</i>	
1. Introduction	166
2. Germ Cell Development	167
3. EGC Derivation	168
4. Characterization of EG Cultures	177
5. EB Formation and Analysis	182
6. EB-Derived Cell Formation	183
References	185
9. Pluripotent Stem Cells from Amniotic Fluid and Placenta	
<i>Dawn M. Delo, Paolo DeCoppi, Anna Milanese, Minhaj Siddiqui, and Anthony Atala</i>	
1. Introduction	192
2. Amniotic Fluid and Placenta in Developmental Biology	192
3. Amniotic and Placental Cells for Therapy—A New Connection	193
4. Isolation and Characterization of Progenitor Cells	194
5. Differentiation of Amniotic and Placental-Derived Progenitor Cells	195
6. Conclusion	199
References	199
10. Hematopoietic Stem and Progenitor Cells from Cord Blood	
<i>Hal E. Broxmeyer, Edward Srour, Christie Orschell, David A. Ingram, Scott Cooper, P. Artur Plett, Laura E. Mead, and Mervin C. Yoder</i>	
1. Introduction: Cord Blood Transplantation	204
2. Methodologies for Assessing Hematopoietic Progenitor and Stem Cells, as Well as EPC, Present in Human Cord Blood	211
3. Uses of Above Assays	227
References	229
11. Hematopoietic Stem Cells from Bone Marrow: Purification and Functional Analysis	
<i>K. K. Lin and M. A. Goodell</i>	
1. Introduction	238
2. Other HSC Surface Markers: Tie-2, Endoglin, and the SLAM Family Receptors	239
3. Fluorescent Dye Efflux in HSCs and the SP ^{KLS}	239
4. Characteristics of HSCs from Different Purification Schemes	242

5. Protocol of HSC Sorting with Hoechst 33342 Staining (SP Population)	243
References	247
12. Microarray Analysis of Stem Cells and Their Differentiation	
<i>Howard Y. Chang, James A. Thomson, and Xin Chen</i>	
1. Introduction	250
2. Overview of Microarray Technology	250
3. Experimental Design	253
4. Confirmation Studies	270
5. Examples of Microarray Experiments for Stem Cell Biology and Differentiation	271
6. Identification of “Stemness”	271
7. Differentiation	272
8. Stem Cell Niches	273
9. Future Directions	275
References	275
13. Tissue Engineering Using Adult Stem Cells	
<i>Daniel Eberli and Anthony Atala</i>	
1. Introduction	282
2. Biomaterials	284
3. Angiogenic Factors	286
4. Adult Stem Cells for Tissue Engineering	287
5. Conclusion	292
References	292
14. Tissue Engineering Using Mesenchymal Stem Cells	
<i>Jeremy J. Mao and Nicholas W. Marion</i>	
1. MSCs: Definition and Therapeutic Promise	298
2. Isolation and Expansion of MSCs	300
3. Multilineage Differentiation of MSCs	303
4. Clinical Translation of MSC-Based Therapies	310
5. Conclusions	312
References	313

SECTION II Embryonic Stem Cells and Their Derivatives

15. Mouse Embryonic Stem Cells	
<i>Andras Nagy and Kristina Vintersten</i>	
1. Historical Overview	322
2. Factors Affecting the Efficiency of Mouse ES Cell Establishment	324

3. Factors Affecting the Contribution of Mouse ES Cell to Chimeric Embryos	325
4. Critical Events During Mouse ES Cell Establishment	326
5. Freezing of ES Cell Lines	330
6. Characterization	330
7. Protocols	332
References	337
16. Human Embryo Culture	
<i>Amparo Mercader, Diana Valbuena, and Carlos Simón</i>	
1. Introduction	342
2. Human Embryo Development	342
3. Embryo Biopsy	345
4. Human Embryo Culture	347
5. Results	351
References	354
17. Human Embryonic Stem Cells: Derivation and Maintenance	
<i>Hidenori Akutsu, Chad A. Cowan, and Douglas Melton</i>	
1. Introduction	360
2. Derivation of hES Cell Lines	360
3. Maintenance of hES Cells	365
4. Conclusion	368
References	369
18. Human Embryonic Stem Cells: Characterization and Evaluation	
<i>Chunhui Xu</i>	
1. Introduction	374
2. Characterization of Undifferentiated hESCs	375
3. Conclusion	389
References	389
19. Human Embryonic Stem Cells: Feeder-Free Culture	
<i>Michal Amit and Joseph Itskovitz-Eldor</i>	
1. Introduction	396
2. Methods for Feeder Layer-Free Culture of hESCs	401
References	404

20. Neural Stem Cells, Neurons, and Glia from Embryonic Stem Cells	
<i>Steven M. Pollard, Alex Benhoua, and Sally Lowell</i>	
1. Introduction	408
2. Protocols	409
3. Summary	421
4. Media and Reagents	422
References	424
21. Hematopoietic Cell Differentiation from Embryonic Stem Cells	
<i>Malcolm A. S. Moore, Jae-Hung Shieh, and Gabsang Lee</i>	
1. Introduction	426
2. Methods	436
References	451
22. Cardiomyocyte Differentiation from Embryonic Stem Cells	
<i>X. Yang, X.-M. Guo, C.-Y. Wang, and X. Cindy Tian</i>	
1. Introduction	460
2. Embryoid Body Generation	461
3. Cardiomyocyte Differentiation	470
4. Enrichment of Cardiomyocytes	471
5. Conclusion	473
References	474
23. Insulin-Producing Cells from Mouse Embryonic Stem Cells	
<i>Insa S. Schroeder, Sabine Sulzbacher, Thuy T. Truong, Przemyslaw Blyszczuk, Gabriela Kania, and Anna M. Wobus</i>	
1. Introduction	478
2. Materials and Methods	485
3. Results	493
4. Summary	494
References	496
24. Transgene Expression and RNA Interference in Embryonic Stem Cells	
<i>Holm Zaehres and George Q. Daley</i>	
1. Retrovirus Expression Vectors and ESCs	502
2. RNA Interference and ESCs	503
3. siRNA Expression Vector Design	504
4. Retrovirus Production	505

5. Retroviral and Lentiviral Gene Transfer into Mouse and Human ESCs	506
6. Transgene and siRNA Expression in Mouse and hESCs	507
7. Biotechnological and Medical Applications	511
References	512
25. Lentiviral Vector-Mediated Gene Delivery into Human Embryonic Stem Cells	
<i>Michal Gropp and Benjamin Reubinoff</i>	
1. Introduction	519
2. Design of HIV-1 Based Vectors for Transduction of hESCs	523
3. Generation of Recombinant Viral Particles	527
4. Transduction of hESCs	529
5. Measurement of Transduction Efficiency	532
6. Enrichment for Transduced hESCs Expressing High Levels of the Transgene	533
7. Determination of Viral Titer	535
References	536
26. Engineering Embryonic Stem Cells with Recombinase Systems	
<i>Frank Schnütgen, A. Francis Stewart, Harald von Melchner, and Konstantinos Anastasiadis</i>	
1. Introduction	542
2. Site-Specific Recombination	543
3. Designing Substrates for Site-Specific Recombination	547
4. Generation of Conditional Alleles	553
5. Recombinase Mediated Cassette Exchange (RMCE)	558
6. Molecular Switches	559
7. Protocols	562
References	572
27. Tissue Engineering Using Embryonic Stem Cells	
<i>Shahar Cohen, Lucy Leshanski, and Joseph Itskovitz-Eldor</i>	
1. Introduction	580
2. Special Considerations when using hESCs as the Cell Source for TE	581
3. Growing hESCs in Defined Animal-free Conditions	581
4. Obtaining the Desired Cell Population	582
5. Choosing the Right Scaffold	583
6. Scaling-Up a Regulable Bioprocess	583
7. hESC-Derived Connective Tissue Progenitors for TE	584
8. Culture and Maintenance of hESC on MEF Feeders	584
9. Harvesting Samples for Analyses	598
References	593
Index	595