

Methods in
ENZYMOLOGY

Volume 477

Guide to Techniques in
Mouse Development, Part B

Mouse Molecular Genetics
2nd Edition

Edited by

Paul M. Wassarman
and
Philippe M. Soriano



CONTENTS

<i>Contributors</i>	<i>xiii</i>
<i>Preface</i>	<i>xix</i>
<i>Volumes in Series</i>	<i>xxi</i>

Section I. Transgenesis	1
1. Lentivirus Transgenesis	3
Alexander Pfeifer, Tiongti Lim, and Katrin Zimmermann	
1. Introduction	4
2. Generation of Lentiviral Vectors	5
3. Generation of Transgenic Animals with Lentiviral Vectors	8
4. Characterization of Transgenic Animals	11
5. Summary	13
Acknowledgments	13
References	13
2. Germline Modification Using Mouse Spermatogonial Stem Cells	17
Mito Kanatsu-Shinohara and Takashi Shinohara	
1. Introduction	18
2. Establishing and Maintaining a GS Cell Culture	19
3. Gene Transduction and Genetic Selection of GS Cells	27
4. Spermatogonial Transplantation and Offspring Production	29
References	34
3. Embryonic <i>In Vivo</i> Electroporation in the Mouse	37
Tetsuichiro Saito	
1. Introduction	38
2. Materials	40
3. <i>In Utero</i> Electroporation	43
4. <i>Exo Utero</i> Electroporation	47
5. Analysis of Electroporated Mice	49
Acknowledgments	49
References	49

Section II. Transposons	51
4. Current Applications of Transposons in Mouse Genetics	53
Adam J. Dupuy	
1. Introduction	54
2. Molecular Characteristics of TEs with Activity in Mice	54
3. Applications of TEs in Mouse Genetics	61
4. The Future of TEs in Mouse Genetics	67
References	67
5. Functional Genomics in the Mouse using the Sleeping Beauty Transposon System	71
Kyoji Horie, Chikara Kokubu, and Junji Takeda	
1. Introduction	72
2. Genome-Wide Germline Mutagenesis with the SB Transgenic Approach	74
3. Region-Specific Chromosome Engineering with the SB Knock-in Approach	82
4. Concluding Remarks	87
Acknowledgments	88
References	88
6. The Use of DNA Transposons for Cancer Gene Discovery in Mice	91
George Vassiliou, Roland Rad, and Allan Bradley	
1. Introduction	92
2. Choice of Transposon	93
3. Transposon Design for Cancer Screens	95
4. Whole-Body (Constitutive) Screens	98
5. Tissue-Specific Mutagenesis	98
6. Inducible Transposase Expression	100
7. Mapping of Transposon Integration Sites	100
8. Statistical Mining of Recurrent Integration Sites	101
9. Validation of Putative Cancer Genes	101
10. Idiosyncrasies of Transposons as Cancer Gene Discovery Tools	102
11. Concluding Remarks	103
References	103

Section III. Recombinases	107
7. A Practical Summary of Site-Specific Recombination, Conditional Mutagenesis, and Tamoxifen Induction of CreERT2	109
Konstantinos Anastasiadis, Stefan Glaser, Andrea Kranz, Kaj Berhardt, and A. Francis Stewart	
1. Introduction	110
2. Recombinase Target Sites	110
3. Applications	112
4. Allele Design	112
5. Recombinase Properties	114
6. Tamoxifen Administration in Mice and Cultured Cells	117
7. Problems	118
8. Concluding Remarks	119
Acknowledgments	119
References	119
8. A Recombineering Pipeline to Make Conditional Targeting Constructs	125
Jun Fu, Madeleine Teucher, Konstantinos Anastasiadis, William Skarnes, and A. Francis Stewart	
1. Introduction	126
2. Recombineering	126
3. Methods	127
4. Standard Recombineering Electroporation Protocol	140
5. Concluding Remarks	142
Acknowledgments	142
References	142
9. Confirmation of Recombination Site Functionality in Gene Targeting Vectors using Recombinase-Expressing Bacteria	145
M. David Stewart and Richard R. Behringer	
1. Introduction	146
2. Materials	147
3. Method	147
4. Example of Results	148
5. Summary	150
Acknowledgments	150
References	150

10. Genetic Fate Mapping Using Site-Specific Recombinases	153
Emilie Legué and Alexandra L. Joyner	
1. Principles Behind Genetic Fate Mapping	154
2. Genetic Fate Mapping Technique	156
3. Future Applications: Combining Genetic Fate Mapping with Mutant Analysis	177
References	178
11. Mapping Cell Fate and Function Using Recombinase-Based Intersectional Strategies	183
Susan M. Dymecki, Russell S. Ray, and Jun C. Kim	
1. Introduction	184
2. Accessing Mouse Embryonic Cells <i>In Utero</i> for Tracer Molecule “Delivery” Using Transgenesis and Site-Specific DNA Recombination	185
3. Improving Cell-Subtype Selectivity in Genetic Fate Maps Using a Dual-Recombinase Intersectional Method	189
4. Transgenes Enabling Subtractive as well as Intersectional Genetic Fate Mapping	193
5. Exploiting Different Reporter Molecules to Reveal Different Features of Mapped Cell Populations	195
6. 3 for 1	196
7. Intersectional Transgene Activation Reaches from Mapping Cell Fate to Mapping Cell Function	196
8. Methods and Materials	199
9. Concluding Remarks	208
Acknowledgments	209
References	209
Section IV. Mutagenesis	215
12. Genome-Wide Forward Genetic Screens in Mouse ES Cells	217
Meng Amy Li, Stephen J. Pettitt, Kosuke Yusa, and Allan Bradley	
1. Introduction	218
2. Strategies for Genome-Wide Mutagenesis	220
3. Using <i>Blm</i> -Deficient ES Cells to Conduct Recessive Genetic Screens	229
4. Mutant Validation	232
5. Concluding Remarks	238
Acknowledgments	239
References	240

13. Gene Trap Mutagenesis in the Mouse	243
Roland H. Friedel and Philippe Soriano	
1. Introduction	244
2. Gene Trapping Strategies	244
3. Design of the Gene Trap Vector	253
4. Gene Trapping Protocol for Retroviral Vectors	255
5. Gene Trapping Protocol for Transposon Vectors	258
6. Identification of Trap Insertion Sites by Splinkerette PCR	259
7. Ordering and Handling of Gene Trap Clones from Consortia	261
8. Outlook	264
Acknowledgments	264
References	264
14. A Wider Context for Gene Trap Mutagenesis	271
Joshua M. Brickman, Anestis Tsakiridis, Christine To, and William L. Stanford	
1. Introduction	272
2. Gene Trap Vectors	273
3. Random Integration as a Means to Generate New Mutations	278
4. Targeted Trapping	279
5. Public Resources of Mutagenized ES Cell Clones	280
6. Gene Discovery and Annotation	285
7. Hypothesis-Driven Screens	287
8. Protocols	288
Acknowledgments	291
References	291
15. Mouse Mutagenesis with the Chemical Supermutagen ENU	297
Frank J. Probst and Monica J. Justice	
1. Introduction	298
2. Materials and Methods	304
3. Conclusion	308
Acknowledgments	310
References	310

16. Phenotype-Driven Mouse ENU Mutagenesis Screens	313
Tamara Caspary	
1. Introduction	314
2. Screen Design	315
3. Screen Execution	316
4. Gene Identification	323
5. The Future for Mouse Forward Genetics	326
References	326
17. Using ENU Mutagenesis for Phenotype-Driven Analysis of the Mouse	329
Rolf W. Stottmann and David R. Beier	
1. Introduction	330
2. ENU Screen Design	331
3. ENU Treatment	336
4. Mutant Ascertainment	339
5. Mutation Identification	340
6. Mutation Validation	343
7. Summary	345
References	345
Section V. Gene Knockdowns	349
18. Exploration of Self-Renewal and Pluripotency in ES Cells Using RNAi	351
Christoph Schaniel, Dung-Fang Lee, and Ihor R. Lemischka	
1. Introduction	352
2. Maintenance of Mouse Embryonic Stem Cells	353
3. siRNA-Mediated Gene Knockdown	354
4. Lentivirus-Based shRNA Knockdown System	358
5. Concluding Remarks	364
References	364
19. Transgenic RNAi Applications in the Mouse	367
Jost Seibler and Frieder Schwenk	
1. Introduction	368
2. Short Interfering RNAs	369
3. Short Hairpin RNA Expression Vectors	370
4. Transgenic shRNA Expression <i>In Vivo</i>	371
5. Conditional RNAi	373
6. Experimental Protocols	376
References	382

20. Gene Knockdown in the Mouse Through RNAi	387
Aljoscha Kleinhammer, Wolfgang Wurst, and Ralf Kühn	
1. Introduction	388
2. Principle of the Approach	389
3. Materials	398
4. Methods	399
5. Concluding Remarks	412
Acknowledgments	413
References	413
21. <i>In Vivo</i> Analysis of Gene Knockdown in Tetracycline-Inducible shRNA Mice	415
Christopher S. Raymond, Lei Zhu, Thomas F. Vogt, and Myung K. Shin	
1. Introduction	416
2. Methods	419
3. Notes	426
References	426
22. The Power of Reversibility: Regulating Gene Activities via Tetracycline-Controlled Transcription	429
Kai Schönig, Hermann Bujard, and Manfred Gossen	
1. Introduction	430
2. The Tet Regulatory Systems	431
3. Tet-Transgenic Mice Available from Repositories	434
4. Tet-Controlled Transgenes to Study Learning and Memory	439
5. Tet-Transgenic Animals in Cancer Research	439
6. Secondary iPSC Technology	443
7. Transgenic Rats	444
8. Future Perspectives	444
References	447
Section VI. Gene Expression Profiling	455
23. Gene Expression Profiling of Mouse Oocytes and Preimplantation Embryos	457
Francesca E. Duncan and Richard M. Schultz	
1. Introduction	458
2. Experimental Design Considerations	463

3. RNA Isolation and cRNA Target Preparation Protocol (Based on the Affymetrix Protocol for Eukaryotic Small Sample Target Labeling Assay Version II)	464
4. Quality Control Assessment of cRNA	473
5. Methods for Microarray Data Analysis	474
6. Validation Methods	476
7. Archiving Results	476
8. Future Directions	476
Acknowledgments	478
References	479
24. Interrogating the Transcriptome of Oocytes and Preimplantation Embryos	481
Anne E. Peaston, Joel H. Graber, Barbara B. Knowles, and Wilhelmine N. de Vries	
1. Introduction	482
2. RNA Relative Quantification in Oocytes and Preimplantation Embryos	487
3. Comparative Analysis of Large-Scale Expression Analyses	495
4. Taking Isoform-Specific Changes into Account	500
5. Concluding Remarks	502
Acknowledgments	503
References	506
25. Gene Expression Profiling of Mouse Embryos with Microarrays	511
Alexei A. Sharov, Yulan Piao, and Minoru S. H. Ko	
1. Introduction	512
2. Considerations for Methods of Gene Expression Profiling	513
3. Experimental Strategies	515
4. Expression Profiling of Small Amounts of RNAs	520
5. QC of Microarray Results	526
6. Analysis and Interpretation of Microarray Data	531
7. Submitting the Data to the Public Database	536
Acknowledgments	537
References	537
<i>Author Index</i>	543
<i>Subject Index</i>	571