

Contents

1	Generation of Transgenic Animals Using Lentiviral Vectors	1
	<i>Carlos Lois</i>	
1.1	Introduction	1
1.2	The Production of Lentiviral Vectors	3
1.2.1	Design and Construction of the Lentiviral Transfer Vector.....	3
1.2.2	Promoters	4
1.2.3	Reporters	4
1.2.4	Plasmid Preparation	6
1.2.5	Preparation of 293T Cells.....	6
1.2.6	Transfection.....	8
1.2.7	Viral Concentration	10
1.2.8	Virus Titration	12
1.3	Production of Transgenic Mice and Rats.....	12
1.3.1	Superovulation and Embryo Collection	13
1.3.2	Delivery of Lentiviruses to Single-Cell Embryos	14
1.3.3	Transfer of Embryos into Recipient Females	17
1.4	Establishment of Stable Strains from Lentiviral Founder Animals	18
1.4.1	Mating of Founder Animals	18
1.5	Safety Guidelines for Pseudotyped Retroviruses	19
	References	21
2	Intracytoplasmic Sperm Injection (ICSI) in the Mouse	23
	<i>Ming-Wen Li, K.C. Kent Lloyd</i>	
2.1	General Description of Intracytoplasmic Sperm Injection (ICSI)	23
2.2	Principles of ICSI.....	24
2.3	Quality and Treatment of Sperm Used for ICSI.....	25
2.4	ICSI Instrumentation	26
2.5	Preparation of Microtools	27
2.6	Culture Media	28
2.7	ICSI Procedure	29

2.7.1	Preparation of Sperm	29
2.7.2	Preparation of Oocytes	29
2.7.3	Injection Procedure	30
2.7.4	Troubleshooting	32
2.7.5	Embryo Transfer	35
2.8	Transgenesis by ICSI	37
2.9	Reasonable Cautions and Concerns Regarding ICSI	38
	References	38
3	Generation of Embryonic Stem (ES) Cell-Derived Embryos and Mice by Tetraploid-Embryo Complementation	41
	<i>Kevin Eggan, Rudolf Jaenisch</i>	
3.1	Introduction	41
3.1.1	Advantages of Using Tetraploid Embryo Complementation	44
3.2	ES Cell Culture	48
3.2.1	Derivation, Culture and in vitro Gene Targeting of F1 ES Cells	48
3.2.2	Sub-cloning of ES Cells to Identify 39X0 Derivatives of Targeted Cell Lines	50
3.2.3	Y Chromosome Genotyping by Southern Hybridization	50
3.2.4	Identification of 39X0 Subclones by PCR	51
3.2.5	Karyotyping of ES Cell Lines during Serial Gene Targeting	52
3.3	Production of Tetraploid Embryos	52
3.3.1	Isolation and In Vitro Culture of Preimplantation Embryos	53
3.3.2	Electrofusion of Two-cell Embryos	54
3.3.3	Electrofusion by AC Alignment and DC Pulse	56
3.3.4	Manual Alignment and DC Fusion of Two-cell Embryos	57
3.3.5	Culture of Tetraploid Embryos to Blastocyst Stage	58
3.3.6	Microscope Set-up for Microinjection	58
3.3.7	Preparation of Microinjection Instruments for Piezo Microinjection	60
3.3.8	Piezo-Micromanipulator Injection of Tetraploid Blastocysts with ES Cells	62
3.3.9	Embryo Transfer to Recipient Females	64
3.3.10	Cesarian Section and Cross Fostering of ES Cell Tetraploid Mice	66
3.4	Final Words	66
	References	67

4 Cloning The Laboratory Mouse by Nuclear Transfer	69
<i>Kevin Eggan, Rudolf Jaenisch</i>	
4.1 Introduction	69
4.2 Factors Influencing Cloning Success	71
4.2.1 Cell Cycle Status of the Donor Cell	72
4.2.2 Genetic Influences on the Cloning Process.....	72
4.2.3 Intrinsic Developmental Potential of the Donor Cell ...	74
4.2.4 Cellular Identity of the Donor Cell	74
4.2.5 Epigenetic Reprogramming after Nuclear Transfer	75
4.3 Methods, Equipment and Techniques	76
4.3.1 Embryo Culture Media and Common Stock Solutions	76
4.3.2 Mouse Strains and Animal Husbandry.....	77
4.3.3 Preparation of Cumulus Cells for Nuclear Transfer	77
4.3.4 Preparation of Tail-tip Cells for Nuclear Transfer.....	77
4.3.5 Culture and Preparation of ES donor cells for Nuclear Transfer	78
4.3.6 Microscope Set-up	78
4.3.7 Micromanipulation Instruments for Nuclear Transfer	78
4.3.8 Isolation of Metaphase II Oocytes for Nuclear Transfer	79
4.3.9 Enucleation of MII Oocytes.....	80
4.3.10 Nuclear Transfer.....	80
4.3.11 Oocyte Activation and Subsequent Culture of Cloned Embryos	80
4.3.12 Derivation of Nuclear Transfer ES Cells.....	83
4.3.13 Embryo Transfer of Cloned Embryos.....	84
4.3.14 Cesarean Section and Cross Fostering of Cloned Animals	85
4.4 Protocol for Direct Injection Nuclear Transfer.....	85
4.4.1 Production of Embryo Culture Medium, Reagents and Mice	85
4.4.2 Oocyte (Egg) Collection	88
4.4.3 Enucleation	90
4.4.4 Donor Nucleus Isolation	92
4.4.5 Nuclear Transfer.....	93
4.4.6 Oocyte Activation and Long-Term Culture	94
References	94

5	Large Insert Transgenesis	97
	<i>Shiaoching Gong, Nat Heintz</i>	
5.1	Introduction	97
5.2	Highly Efficient BAC Modification Based on the R6K γ Origin of Replication.....	99
5.3	An Approach to High Throughput Studies.....	101
5.3.1	A Precisely Modified BAC Clone for Use in the Production of Transgenic Mice (Protocol 1)	101
5.3.2	Preparation of BAC DNA by Double Acetate Precipitation and CsCl Gradient Centrifugation (Protocol 2).....	105
5.3.3	Injection of BAC DNA	108
5.4	Tools and Materials	108
5.4.1	Materials	108
5.4.2	Solutions	109
	References	109
6	Regional and Temporal Control of Genetic Manipulation in the Mouse	111
	<i>Mansuo L. Hayashi, Shigemi Hayashi</i>	
6.1	Introduction	111
6.2	The Cre- <i>loxP</i> System	111
6.2.1	Principles	111
6.2.2	Applications	112
6.2.3	Generating Cre lines	114
6.2.4	Genotyping Cre Lines.....	115
6.2.5	Screening Cre Lines.....	115
6.2.6	Generating Floxed Lines	118
6.2.7	RNA in situ Hybridization	119
6.2.8	Tyramide Signal Amplification (TSA) for Fluorescence Immunostaining	122
6.2.9	The Temporally Controllable Cre- <i>loxP</i> System	122
6.3	The Tetracycline Regulatory System.....	123
6.3.1	Principles	123
6.3.2	Applications	124
6.3.3	Generating tTA/rtTA Regulator Lines.....	125
6.3.4	Genotyping tTA/rtTA Regulator Lines.....	126
6.3.5	Screening tTA/rtTA Regulator Lines	126
6.3.6	Generating tet-Responsive Lines	127
6.3.7	Genotyping tet-Responsive Lines	127
6.3.8	Screening tet-Responsive Lines	127

6.4	New Directions for Regional and Temporal Gene Manipulation	128
	References	129
7	High Resolution Gene Expression Analysis Using Reporter Genes	131
	<i>Niels C. Adams, Nicholas W. Gale</i>	
7.1	Introduction	131
7.2	Reporter Genes.....	133
7.2.1	Selection of the Appropriate Reporter Gene.....	133
7.2.2	Placental Alkaline Phosphatase.....	136
7.2.3	<i>LacZ</i> (β -Gal).....	138
7.3	Reporter Constructs.....	141
7.3.1	Position Effects	141
7.4	Protocols	142
7.4.1	Silicone Plates	142
7.4.2	Dissection of Embryos for Whole-mount Staining for β -Gal or PLAP visualization	142
7.4.3	Whole-mount Staining of Embryos for <i>LacZ</i>	145
7.4.4	Whole-mount PLAP Staining of Embryos.....	147
7.4.5	Dissection of Adult Tissue.....	148
7.4.6	Preparing Embryos and Adult Tissues for Cryo-Sectioning	151
7.4.7	Cryo-sectioning	153
7.4.8	Whole-mount Adult <i>LacZ</i> Staining.....	155
7.4.9	Enhancements to the Standard <i>LacZ</i> Staining Protocol.....	156
7.4.10	Stock Solutions	157
	References	159
	Color Plates	161
8	Nuclear Transfer in the Cow	173
	<i>William A. Ritchie</i>	
8.1	Introduction	173
8.2	Tools and Equipment	174
8.2.1	Micromanipulation.....	174
8.2.2	Microtools.....	175
8.2.3	Manipulation Chamber	176
8.2.4	Oocyte Handling	176
8.2.5	Cell Fusion	177
8.2.6	Embryo Culture.....	177
8.2.7	Supplies	177
8.3	Media and Solutions.....	178

8.3.1	Aspiration Medium	178
8.3.2	Dissection Medium (50 ml)	178
8.3.3	Maturation Medium.....	178
8.3.4	Stock solution B (250 mM NaHCO ₃)	178
8.3.5	Stock solution C (33 mM pyruvate)	179
8.3.6	Stock solution D (171 mM CaCl ₂ · 2H ₂ O)	179
8.3.7	Stock solution G (60 mM Glucose).....	179
8.3.8	Stock solution GLN (10 mM L-Glutamine).....	179
8.3.9	Stock solution H (250 mM Hepes)	180
8.3.10	Stock solution K (kanamycin sulphate)	180
8.3.11	Stock solution L (330 mM Na lactate).....	180
8.3.12	Stock solution M (MgCl ₂ · 6H ₂ O).....	180
8.3.13	Stock solution S2	181
8.3.14	Hepes synthetic oviduct fluid (hSOF) +Ca	181
8.3.15	Hepes SOF (hSOF) –Ca.....	182
8.3.16	Culture Medium (SOFaBSA)	182
8.3.17	Fusion Medium	183
8.4	Procedure	183
8.4.1	Oocyte Maturation	184
8.4.2	Enucleation of Oocytes.....	185
8.4.3	Cell Preparation	188
8.4.4	Injection of cells	188
8.4.5	Electrofusion	189
8.4.6	Activation.....	190
8.4.7	Culture.....	191
8.5	Troubleshooting	191
8.6	Discussion	192
	References	192
9	Production of Transgenic Pigs by DNA Microinjection	195
	<i>Robert M. Petters, Rebecca L. Krisher</i>	
9.1	Introduction	195
9.1.1	In Vivo Produced Embryos	195
9.1.2	In Vitro Produced Embryos.....	196
9.2	Protocol for In Vitro Production of Pig Embryos	197
9.2.1	Media, Solutions and Reagents	197
9.2.2	Timeline.....	204
9.2.3	Oocyte Collection and In Vitro Maturation (Day 1)	204
9.2.4	In Vitro Fertilization	207
9.2.5	In Vitro Culture.....	211
9.3	Protocols for In Vivo Production of Pig Embryos	212

9.3.1	Media, Solutions and Reagents	212
9.3.2	Timeline.....	212
9.3.3	Synchronization and Superovulation of Embryo Donors	212
9.3.4	Embryo Recovery	213
9.4	Production of Transgenic Pigs	215
9.4.1	Centrifugation and Microinjection of Embryos	215
9.4.2	Embryo Recipient Selection and Embryo Transfer	217
9.4.3	Methods for Animal Identification	218
	References	219
10	Production of Transgenic Birds Using Lentiviral Vectors	221
	<i>Benjamin B. Scott, Carlos Lois</i>	
10.1	Introduction	221
10.2	Overview of the Strategy.....	222
10.3	Design of Lentiviral Vectors for Transgene Expression.....	222
10.3.1	Promoters	222
10.3.2	Transgenes	224
10.4	Production of Transgenic Birds.....	225
10.4.1	Egg Preparation	225
10.4.2	Injection for the Production of Mosaic Founders (F0)	226
10.4.3	Transgenic Offspring.....	228
10.4.4	Husbandry	228
	References	229
11	Ancillary Techniques	231
	<i>Shirley Pease</i>	
11.1	Introduction	231
11.2	Getting Started	231
11.2.1	Microinjection Equipment	232
11.2.2	Mouse Stocks for Embryo Production and Implantation.....	233
11.2.3	Rat Stocks for Embryo Production and Implantation ..	236
11.3	Embryo Production in Rats and Mice.....	237
11.3.1	Preparation of Hormones and Enzymes	240
11.3.2	Hormone Priming of Mice	240
11.3.3	Hormone Priming of Rats	241
11.3.4	Embryo Collection and Culture in Mice	243
11.3.5	Embryo Collection and Culture in Rats	246
11.4	Transfer of Mouse and Rat Embryos	247

11.4.1 Synchronization and Implantation of Recipient Mice.....	247
11.4.2 Oviduct Transfers in Mice: Unilateral, Infundibulum or Ampulla	249
11.4.3 Uterine Transfers in Mice.....	254
11.4.4 Synchronization and Embryo Transfer in Recipient Rats	256
11.4.5 Oviduct Implants in Rats, by Infundibulum or Ampulla.....	257
11.5 Murine ES Cells	258
11.5.1 Commonly used ES Cell Lines	258
11.5.2 Elementary Karyotyping.....	263
11.6 The Establishment of Stable Strains from Genetically Altered Animals	267
11.6.1 Breeding from Chimeric Mice	267
11.6.2 Breeding from Lentiviral Founder Animals.....	272
References	274
Subject Index	277



<http://www.springer.com/978-3-540-28415-4>

Mammalian and Avian Transgenesis - New Approaches

Pease, S.; Lois, C. (Eds.)

2006, XX, 281 p. 48 illus., 12 illus. in color., Hardcover

ISBN: 978-3-540-28415-4