

# CONTENTS

Contributors	xi
Preface	xv

## **PART I Embryological Microsurgery and Tissue Culture Methods**

### **1. Operations on Primitive Streak Stage Avian Embryos**

*Andrea Streit and Claudio D. Stern*

I. Introduction	3
II. Staging of Embryos	4
III. Anatomy of the Primitive Streak Stage Embryo	6
IV. New Culture	8
V. Hensen's Node Grafts and Other Induction Assays	10
VI. Other Types of Graft	14
VII. Fixing Operated Embryos for Analysis	15
References	16

### **2. Quail-Chick Transplantations**

*Nicole Le Douarin, Françoise Dieterlen-Lièvre, Sophie Cruzet, and Marie-Aimée Teillet*

I. Introduction	20
II. Differential Diagnosis of Quail and Chick Cells	23
III. Material and Equipment	27
IV. Egg Holders	28
V. Preparation and Sealing of Eggs	31
VI. Neural Tissue Transplantations	31
VII. Early Transplantations in Blastodiscs	39
VIII. Transplantation of Epiblast or Primitive Streak Fragments	40
IX. Hemopoietic Organ Rudiment Transplantations	40
X. Genetic Manipulation by Electroporation in the Quail-Chick System	47
XI. Results. Discussion. and Perspectives	49
References	52

### 3. Other Chimeras: Quail–Duck and Mouse–Chick

*Peter Y. Lwigale and Richard A. Schneider*

I. Introduction	60
II. Preparation of Quail, Duck, Chick, and Mouse Embryos	62
III. Generation and Analysis of Quail–Duck Chimeras	65
IV. Generation and Analysis of Mouse–Chick Chimeras	67
V. Conclusion	71
References	71

### 4. Manipulations of Neural Crest Cells or Their Migratory Pathways

*Marianne Bronner-Fraser and Martín García-Castro*

I. Introduction	76
II. Preparation of Avian Neural Crest Cultures	76
III. Induction and Specification Assays for Neural Crest Cells	84
IV. Microinjection of Cells and Antibodies into Embryos	88
V. Labeling of Neural Crest Cells <i>In Vivo</i> with Vital Dyes	90
VI. Grafting Techniques	92
VII. Conclusions	94
References	95

### 5. Embryo Slices and Strips: Guidance and Adhesion Assays in the Avian Embryo

*Catherine E. Krull and Kathryn Tosney*

I. Introduction	98
II. Preparing and Culturing Embryo Slices	98
III. Preparing and Culturing Somite Strips	104
IV. Perspectives	108
References	112

### 6. Neural Crest, Sensory Neuron, and Muscle Cultures

*Vivian M. Lee and Peter Y. Lwigale*

I. Introduction	116
II. Materials	117
III. Methods	119
IV. Neural Crest Culture	120
V. Sensory Neuron Culture	122
VI. Pectoral Muscle Culture	126
VII. Results and Discussions	126
References	130

7. Methods in Avian Embryology Experimental and Molecular Manipulation of the Embryonic Chick Limb

*Lee Niswander*

I. Introduction	136
II. Visualization of the Limb Bud and Analysis of the Experimental Outcome	137
III. Proximal–Distal Limb Development	139
IV. Anterior–Posterior Patterning	147
V. Dorsal–Ventral (D–V) Patterning	150
References	150

8. Cell Division, Differentiation, and Death in Avian Embryos

*Sara Ahlgren*

I. Introduction	153
II. Studies in Cell Death	154
III. Studies in Cell Proliferation and Differentiation	155
IV. Selected Protocols	156
References	164

## **PART II Labeling and Transgenesis Approaches**

9. *In Situ* Hybridization Analysis of Chick Embryos in Whole–Mount and Tissue Sections

*Hervé Aclouge, David G. Wilkinson, and M. Angela Nieto*

I. Introduction	170
II. Solutions	171
III. Single and Multiple Detection of RNA in Floating Sections or Whole–Mount Embryos	172
IV. Photography and Sectioning	179
V. Whole–Mount Fluorescent <i>In Situ</i> Hybridization	179
VI. Photography and Sectioning	182
VII. <i>In Situ</i> Hybridization to Tissue Sections	182
References	185

10. Vital Labeling of Embryonic Cells Using Fluorescent Dyes and Proteins

*Sujata Bhattacharyya, Paul M. Kulesa, and Scott E. Fraser*

I. Introduction	188
II. Iontophoretic Microinjection of Lineage Tracers	189

III. Iontophoretic Application of DiI	198
IV. Relative Advantages of Dextran and DiI	200
V. Photoactivation of Fluorescent Proteins in Single Cells	200
VI. Conclusions and Emerging Technologies	208
References	208
<b>11. Time-Lapse Imaging of the Early Avian Embryo</b>	
<i>Max Ezin and Scott Fraser</i>	
I. Introduction	212
II. The Technology of Time-Lapse Imaging	214
III. Considerations Before Time-Lapse Imaging	218
IV. Methods	222
References	233
<b>12. Gain- and Loss-of-Function Approaches in the Chick Embryo</b>	
<i>Tatjana Sauka-Spengler and Meyer Barembaum</i>	
I. Gain-of-Function by Electroporation	239
II. Loss-of-Function by Electroporation	243
III. Retrovirus-Mediated Protein Expression	251
References	254
<b>13. Manipulation and Electroporation of the Avian Segmental Plate and Somites <i>In Vitro</i></b>	
<i>Tadahiro Iimura and Olivier Pourquié</i>	
I. Introduction	258
II. Rationale	259
III. Methods	260
IV. Materials	266
V. Discussion	267
VI. Summary	269
References	269
<b>14. Transposon-Mediated Stable Integration and Tetracycline-Inducible Expression of Electroporated Transgenes in Chicken Embryos</b>	
<i>Yoshiko Takahashi, Tadayoshi Watanabe, Shinichi Nakagawa, Koichi Kawakami, and Yuki Sato</i>	
I. Introduction	272
II. Tet-Inducible Expression of Electroporated Transgenes	272

III. Stable Integration of Electroporated Transgenes	276
IV. Stage-Specific Manipulation of Stably Integrated Transgenes	278
V. Summary	278
References	280

15. Generating Transgenic Quail using Lentiviruses	
<i>Greg Poynter and Rusty Lansford</i>	
I. Introduction	282
II. Experimental Procedures	284
III. Concluding Remarks	292
References	292

### **PART III Functional Genomics**

16. Gene Discovery: Macroarrays and Microarrays	
<i>Laura S. Gammill and Vivian M. Lee</i>	
I. Introduction	298
II. Chicken as a Model System: Integrating Embryology and Genomics	298
III. The Array: Choosing a Platform	299
IV. Strategy: Devising the Best Screen Possible	302
V. Analysis: Sorting through Several Thousand Data Points	303
VI. Protocol: Macroarray Screening	304
References	311
17. Dissection of Chick Genomic Regulatory Regions	
<i>Hisato Kondoh and Masanori Uchikawa</i>	
I. Introduction	314
II. Rationale	315
III. Methods	319
IV. Discussion	329
References	335
18. Computational Approaches to Finding and Analyzing <i>cis</i> -Regulatory Elements	
<i>C. Titus Brown</i>	
I. Structure and Function of <i>cis</i> -Regulatory Elements	338
II. Effective <i>cis</i> -Regulatory Sequence Analysis	339

III. Approaches and Tools for Finding Conserved Sequence Elements	342
IV. Identifying Transcription Factor Binding Sites Computationally	352
References	363
<b>19. Investigating Regulatory Factors and Their DNA Binding Affinities Through Real Time Quantitative PCR (RT-QPCR) and Chromatin Immunoprecipitation (ChIP) Assays</b>	
<i>Lisa A. Taneyhill and Meghan S. Adams</i>	
I. Introduction	368
II. Materials for RT-QPCR	373
III. General Principles and Definitions	375
IV. Types of Assays	376
V. Methods of Analysis	378
VI. Assay Setup	380
VII. ChIP and RT-QPCR	381
VIII. Conclusions and Perspectives	387
References	389
Index	391
Volumes in Series	401