

---

# Contents

<i>Preface</i> . . . . .	<i>v</i>
<i>Contributors</i> . . . . .	<i>ix</i>
PART I UNDERSTANDING MOUSE SKELETAL DEVELOPMENT AND REPAIR	
1 Overview of Skeletal Development . . . . .	3
<i>Tatsuya Kobayashi and Henry M. Kronenberg</i>	
2 Overview of Skeletal Repair (Fracture Healing and Its Assessment) . . . . .	13
<i>Elise F. Morgan, Anthony De Giacomo, and Louis C. Gerstenfeld</i>	
PART II SKELETAL REPAIR, TRANSPLANTATIONS, AND ORGAN CULTURES	
3 Generation of Closed Transverse Fractures in Small Animals . . . . .	35
<i>Anthony De Giacomo, Elise F. Morgan, and Louis C. Gerstenfeld</i>	
4 The Murine Femoral Bone Graft Model and a Semiautomated Histomorphometric Analysis Tool . . . . .	45
<i>Robinder S. Dhillon, Longze Zhang, Edward M. Schwarz, Brendan F. Boyce, and Chao Xie</i>	
5 Surgical Induction of Posttraumatic Osteoarthritis in the Mouse . . . . .	61
<i>Daisuke Hamada, Erik R. Sampson, Robert D. Maynard, and Michael J. Zuscik</i>	
6 A Mouse Model of Flexor Tendon Repair . . . . .	73
<i>Sys Hasslund, Regis J. O’Keefe, and Hani A. Awad</i>	
7 Cartilage Explant Cultures . . . . .	89
<i>Manuela Wuelling and Andrea Vortkamp</i>	
8 Renal Capsule Transplantations to Assay Skeletal Angiogenesis . . . . .	99
<i>Rana Abou-Khalil and Céline Colnot</i>	
PART III HISTOLOGICAL AND MOLECULAR ANALYSES ON WHOLE TISSUES AND TISSUE SECTIONS	
9 Whole-Mount Skeletal Staining . . . . .	113
<i>Diana Rigueur and Karen M. Lyons</i>	
10 Tips and Techniques for Processing and Sectioning Undecalcified Murine Bone Specimens . . . . .	123
<i>Thomas B. Bemenderfer, Jonathan S. Harris, Keith W. Condon, and Melissa A. Kacena</i>	

11	Preparation of Thin Frozen Sections from Nonfixed and Undecalcified Hard Tissues Using Kawamot's Film Method (2012) . . . . .	149
	<i>Tadafumi Kawamoto and Komei Kawamoto</i>	
12	Demineralized Murine Skeletal Histology . . . . .	165
	<i>Sarah A. Mack, Kathleen M. Maltby, and Matthew J. Hilton</i>	
13	Beta-Galactosidase Staining in the Skeleton . . . . .	185
	<i>X.L. Han and Jian Q. Feng</i>	
14	Whole-Mount In Situ Hybridization on Murine Skeletogenic Tissues . . . . .	193
	<i>Timothy Rutkowski, Deepika Sharma, and Matthew J. Hilton</i>	
15	Nonradioactive In Situ Hybridization on Skeletal Tissue Sections . . . . .	203
	<i>Yulia Shwartz and Elazar Zelzer</i>	
16	Radioactive In Situ Hybridization to Detect Gene Expression in Skeletal Tissue Sections . . . . .	217
	<i>Kyu Sang Joeng, Jenna Regan, and Fanxin Long</i>	
17	Proliferation Assays (BrdU and EdU) on Skeletal Tissue Sections . . . . .	233
	<i>Timothy J. Mead and Véronique Lefebvre</i>	
18	TUNEL Assay on Skeletal Tissue Sections to Detect Cell Death . . . . .	245
	<i>Laura Mangiavini and Ernestina Schipani</i>	
PART IV PRIMARY CELL ISOLATIONS, CULTURES, AND ASSAYS		
19	Analysis of Chondrogenesis Using Micromass Cultures of Limb Mesenchyme . . . . .	251
	<i>T. Michael Underhill, Helen J. Dranse, and Lisa M. Hoffman</i>	
20	Isolation and Culture of Murine Primary Chondrocytes . . . . .	267
	<i>Anthony J. Mirando, Yufeng Dong, Jinsil Kim, and Matthew J. Hilton</i>	
21	Bone Marrow Stromal Cell Assays: In Vitro and In Vivo . . . . .	279
	<i>Pamela Gebron Robey, Sergei A. Kuznetsov, Mara Riminucci, and Paolo Bianco</i>	
22	Isolation and Culture of Neonatal Mouse Calvarial Osteoblasts . . . . .	295
	<i>Jennifer H. Jonason and Regis J. O'Keefe</i>	
23	RANKL-Based Osteoclastogenic Assays from Murine Bone Marrow Cells . . . . .	307
	<i>Lianping Xing and Brendan F. Boyce</i>	
24	Hematopoietic Stem Cell Cultures and Assays . . . . .	315
	<i>Benjamin J. Frisch and Laura M. Calvi</i>	
	<i>Index</i> . . . . .	325

**Skeletal Development and Repair**

**Methods and Protocols**

Hilton, M.J. (Ed.)

2014, X, 328 p. 78 illus., 40 illus. in color., Hardcover

ISBN: 978-1-62703-988-8

A product of Humana Press