

Table of Contents

1	Characterizing Hearing in Mice	1
	<i>Karen P. Steel</i>	
1.1	Introduction	1
1.2	Behavioral Tests of Hearing	2
1.3	Physiological Tests of Hearing	4
1.4	Anatomy of the Ear	7
1.5	Conclusions	12
	Acknowledgements	13
2	Molecular Phenotyping: Gene Expression Profiling	15
	<i>Johannes Beckers</i>	
2.1	Why this Screen? Medical and Biological Relevance	15
2.2	Examples: Diseases of Mouse and Man	17
2.3	Diagnostic Methods: History and State of the Art	18
2.4	Technical Requirements for Screening Protocols (Short): First and Second Line Approaches	20
2.5	Logistics (Whom, When, How Many, Why)	20
2.5.1	Choice of Platform	20
2.5.2	Biological Samples	22
2.6	Trouble Shooting	23
2.6.1	Preparation of Hybridization Target	24
2.6.2	Critical Issues of Chip Hybridization	27
2.6.3	Image Processing and Array Design	30
2.7	Short-term Outlook	31
3	Screening for Bone and Cartilage Phenotypes in Mice	35
	<i>Helmut Fuchs, Thomas Lisse, Koichiro Abe, and Martin Hrabé de Angelis</i>	
3.1	Introduction	35
3.1.1	The Skeleton	35
3.1.2	Skeletal Development in the Embryo	36
3.1.3	Growth and Maintenance of Bone and Cartilage	36
3.1.4	Diseases Involving Cartilage and Bone	38
3.1.5	The Mouse as a Model for Skeletal Diseases	41
3.2	Screening Protocols	42

3.2.1	Morphological Analysis	42
3.2.1.1	Protocol	43
3.2.2	X-Ray Analysis	44
3.2.2.1	General	44
3.2.2.2	Imaging	45
3.2.2.3	X-Ray Analysis	45
3.2.2.4	Protocol	46
3.2.3	DXA-Analysis	47
3.2.3.1	General	47
3.2.3.2	Advantages	48
3.2.3.3	Disadvantages	48
3.2.3.4	Small Animal Applications	49
3.2.3.5	Precision and Accuracy	49
3.2.3.6	Considerations	50
3.2.3.7	Protocol	51
3.2.4	Biochemical Bone Markers	51
3.2.4.1	Clinical Utility of Biochemical Markers of Bone Turnover in Small Animals	51
3.2.4.2	Mouse Markers of Bone Turnover/Metabolism and Hormonal Regulation	53
3.2.4.3	Variability/Sensitivity/Sample Choice	58
3.2.4.4	Which Markers Should be Used During the Screen?	58
3.2.5	Advanced Small Animal Imaging Techniques	59
3.2.5.1	pQCT	59
3.2.5.2	μ CT	61
3.2.5.3	μ MRI	63
3.2.5.4	Whole-mount Skeletal Preparations	65
3.2.5.5	Histomorphometry	66
3.2.5.6	Miscellaneous	72
3.2.5.7	Order of the Tests	74
3.3	Conclusion	76
	List of Abbreviations	78
	Appendix	85
	μ CT Volumetric Data Processing	85
	MRI Principles	85
4	Clinical Chemical Screen	87
	<i>Martina Klempt, Birgit Rothkolb, Bernhard Aigner, and Eckhard Wolf</i>	
4.1	Introduction	87
4.1.1	Relevance of the Screen	87
4.1.2	Biology and Medical Application	88
4.1.2.1	Biology of Clinical Chemical Parameters	88
4.1.2.2	Medical Application	88
4.2	Diseases in Mouse and Humans	89
4.2.1	Diagnostic Impact of Clinical Chemistry	89
4.2.2	Clinical Chemistry in Selected Disorders	91

4.2.2.1	Hypercholesterolemia	91
4.2.2.2	Albuminuria	91
4.2.2.3	Acute Myeloid Leukemia (AML)	92
4.3	Clinical Chemistry as Diagnostic Tool	93
4.3.1	History	93
4.3.2	State of the Art	94
4.4	Technical Requirements and Screening Protocols	94
4.4.1	Technical Requirements	94
4.4.1.1	Blood Collection	94
4.4.1.2	Sample Preparation	95
4.4.1.3	Sample Analysis	96
4.4.2	Screening Protocols	99
4.4.2.1	Primary Screen	99
4.4.2.2	Secondary Screen	100
4.4.2.3	Tertiary Screen	100
4.5	Logistics of the Screen	102
4.5.1	General Considerations	102
4.5.2	Lessons from ENU Mutants	103
4.6	Trouble Shooting	104
4.6.1	Factors Interfering <i>In Vivo</i>	105
4.6.2	Factors Interfering <i>In Vitro</i>	105
4.7	Short-term Outlook	105
5	Exploration of Metabolic and Endocrine Function in the Mouse	109
	<i>Marie-France Champy, Carmen A. Argmann, Pierre Chambon, and Johan Auwerx</i>	
5.1	General Introduction	109
5.1.1	Investigating a Mouse with Endocrine and Metabolic Dysfunction	109
5.1.2	Principles of Endocrine and Metabolic Testing	110
5.1.3	Strain in Relation to Mouse Models of Metabolic Disease	110
5.2	Evaluation of Energy Homeostasis	112
5.2.1	Body Weight and Food Intake	112
5.2.2	Energy Expenditure by Indirect Calorimetry	114
5.2.3	Cold Test	114
5.2.4	Exercise Test	115
5.2.5	Lean and Fat Composition of the Body	116
5.3	Evaluation of Standard Clinical Chemistry Blood Parameters	117
5.4	Evaluation of Glucose Homeostasis	117
5.4.1	HOMA (Homeostasis Assessment Model)	118
5.4.2	Meal Tolerance Test (MTT)	118
5.4.3	Intra-Peritoneal or Oral Glucose Tolerance Test (IPGTT or OGTT)	118
5.4.4	Intra-Peritoneal Insulin Sensitivity Test (IPIST)	120
5.4.5	Glucose Clamps	120
5.4.6	Utilization of Glucose by Individual Tissues	121
5.4.7	Insulin Secretion Test	122
5.5	Measurement of Serum Lipids and Lipoprotein Parameters	123

5.5.1	Serum Lipid Parameters	124
5.5.2	Isolation of Plasma Lipoprotein	124
5.5.3	Apolipoproteins	125
5.6	Measurement of Hormones	126
5.7	Reproduction and Fertility	128
5.8	Bile Acids	128
5.9	Post-Mortem Analysis and Histology	130
5.10	Molecular Imaging	132
	Acknowledgements	132
6	Behavioral and Neurological Phenotyping in the Mouse	135
	<i>Valter Tucci, Gonzalo Blanco and, Patrick M. Nolan</i>	
6.1	Introduction	135
6.2	Human Neurological and Psychiatric Disorders	136
6.2.1	Neurodegenerative Disorders	137
6.2.2	Mental Retardation Syndromes	138
6.2.3	Disorders Affecting Social Behavior	139
6.2.3.1	Anorexia	139
6.2.3.2	Autism	139
6.2.4	Mood Disorders: Depression, Manias and Schizophrenia	140
6.2.5	Anxiety	141
6.2.6	Neuromuscular Disorders, Myopathies and Neuropathies	142
6.3	Behavioral and Neurological Phenotyping in the Mouse	143
6.3.1	Neurological and Neuromuscular Function	144
6.3.2	Learning and Cognition	145
6.3.3	Social Behavior	146
6.3.4	Emotionality in Mice	147
6.3.5	Processing Sensory Information in Mice	148
6.3.6	Endophenotypes	149
6.4	Behavioral and Neurological Screening Protocols in the Mouse	151
6.4.1	Screens for Neurological and Neuromuscular Function	153
6.4.1.1	Primary Screens	153
6.4.1.2	Secondary Screens	154
6.4.2	Screens for Motor Function	155
6.4.2.1	Primary Screens	155
6.4.2.2	Secondary Screens	156
6.4.3	Screens for Learning and Cognitive Function	157
6.4.3.1	Primary Screens	157
6.4.3.2	Secondary Screens	157
6.4.4	Screens for Social Behavior	158
6.4.4.1	Primary Screens	158
6.4.4.2	Secondary Screens	159
6.4.5	Screens for Emotionality	160
6.4.5.1	Primary Screens	160
6.4.5.2	Secondary Screens	161
6.4.6	Screens for Central Processing of Sensory Information	162

6.4.6.1	Primary Screens	162
6.4.6.2	Secondary Screens	163
6.4.7	Supportive Screens	164
6.4.7.1	Biochemical Measurements	164
6.4.7.2	Histopathology	164
6.4.7.3	Re-testing of Aged Mice	165
6.5	Implementation of Behavioral and Neurological Phenotypic Analysis	165
6.5.1	Gene-driven Approach (Reverse Genetics)	165
6.5.2	Phenotype-driven Approach (Forward Genetics)	166
6.5.3	Phenotype-driven Screens: A Short Guideline	166
6.5.4	Environmental and Genetic Influences on Mutant Behavior	167
6.5.5	Standardization of Screening	168
6.6	Outlook	168
6.6.1	Use of Imaging Technology	169
6.6.2	Investigation of Complex Traits in Compound Mutants: Sensitized Screens	169
6.6.3	Use of Reporter Strains	170
7	Cardiovascular Disorders: Insights into <i>In Vivo</i> Cardiovascular Phenotyping	177
	<i>Laurent Monassier and André Constantinesco</i>	
7.1	Introduction	177
7.2	<i>In Vivo</i> Imaging for Mouse Cardiovascular Phenotyping: Interests and Limits	178
7.2.1	Echography	178
7.2.2	Magnetic Resonance Imaging (MRI)	178
7.2.3	Single Photon Emission Computed Tomography (SPECT)	182
7.2.4	Positron Emission Tomography (PET) Imaging	183
7.2.5	X-Ray Computed Tomography (CT)	184
7.2.6	Limitations in Studies Using Contrast Agents: Particular Aspects of Nuclear-based Imaging	185
7.3	Exploring the Heart in Living Animals	187
7.3.1	Anatomy	187
7.3.1.1	Systolic Function and Hemodynamics	188
7.3.1.2	Global Systolic Function	188
7.3.1.3	Regional Function	189
7.3.1.4	Diastolic Function	189
7.3.1.5	Impaired Myocardial Relaxation Pattern	190
7.3.1.6	Restrictive Filling Pattern	190
7.3.1.7	Myocardial Perfusion, Metabolism and Gene Expression Imaging	190
7.3.1.8	Cardiac Conduction and Arrhythmias	191
7.3.1.9	Exploring the Great Arteries	193
7.3.1.10	Exploring Microvessels	194
7.4	A Scheme for Identifying the Main Cardiovascular Disorders in Genetically-modified Mice	194

8	Phenotyping of Host-Pathogen Interactions in Mice	201
	<i>Andreas Lengeling, Werner Müller, and Rudi Balling</i>	
8.1	Introduction	201
8.2	Looking Back and Forward: History and State-of-the-Art of Mouse Infection Phenotyping and Studies of Genetic Infection Susceptibility	202
8.3	The Impact of Mouse Genetics on the Understanding of Human Infectious Diseases	205
8.4	Phenotyping at the GBF-Mouse Infection Challenge Platform (ICP)	207
8.4.1	Screening Protocols	208
8.4.1.1	Infection with <i>Listeria monocytogenes</i>	208
8.5	Practical Guidelines	212
8.5.1	Growing Log-phase Cultures of <i>Listeria monocytogenes</i> EGD for Mouse Infection	212
8.5.2	Infection of Mice with <i>Listeria monocytogenes</i> EGD	212
8.5.3	Quantification of Bacterial Growth in Spleen and Liver after <i>L. monocytogenes</i> Infection	213
8.5.4	Troubleshooting	214
8.6	Outlook	214
	Acknowledgement	215
9	Animal Models of Nociception	221
	<i>Ildikó Rácz and Andreas Zimmer</i>	
9.1	Introduction	221
9.2	Ethical Considerations	222
9.3	General Considerations	223
9.4	Assays for Acute Pain Thresholds	224
9.4.1	Thermal Stimuli	224
9.4.1.1	Tail-flick Test	224
9.4.1.2	Hargreaves Test	225
9.4.1.3	Hot-plate Test	226
9.4.2	Mechanical Stimuli	227
9.4.2.1	The Tail- and Paw-pressure Test	227
9.4.2.2	Von Frey Filament Test	227
9.5	Tonic and Visceral Pain Models	228
9.5.1	The Writhing Test	228
9.5.2	The Formalin Test	229
9.6	Hyperalgesia and Allodynia	230
9.6.1	Hyperalgesia and Allodynia Caused by Neuropathic Pain	230
9.6.1.1	Chronic Constriction Injury Model	231
9.6.1.2	Segmental Spinal Nerve Ligation Model	231
9.6.2	Hyperalgesia and Allodynia Caused by Tissue Inflammation	232
9.6.2.1	Determination of Mechanical Allodynia and Thermal Hyperalgesia	232
9.7	Stress-induced Analgesia	233

10	Mouse Phenotyping: Immunology	237
	<i>Svetoslav Kalaydjiev, Tobias J. Franz, and Dirk H. Busch</i>	
10.1	Introduction	237
10.2	Diagnostic Methods	239
10.2.1	Antibody-based Techniques	239
10.2.2	Cellular Immunity Techniques	240
10.2.3	Molecular Genetic Techniques	241
10.3	Immunological Phenotyping at the German Mouse Clinic	242
10.4	Screening Protocols	245
10.4.1	FACS for Leukocyte Subpopulations	246
10.4.1.1	Reagents and Equipment	246
10.4.1.2	Procedure	246
10.4.2	Bead Array for Immunoglobulin Concentrations	247
10.4.2.1	Reagents and Equipment	247
10.4.2.2	Procedure	247
10.4.3	ELISA for Autoantibodies	248
10.4.3.1	Reagents and Equipment	248
10.4.3.2	Procedure	248
10.5	Troubleshooting	249
10.6	Outlook	249
11	Phenotyping Allergy in the Laboratory Mouse	253
	<i>Thilo Jakob, Francesca Alessandrini, Jan Gutermuth, Gabriele Köllisch, Anahita Javaheri, Antonio Aguilar, Martin Mempel, Johannes Ring, Markus Ollert, and Heidrun Behrendt</i>	
11.1	Introduction	253
11.2	Phenotyping Different Forms of Allergic (Hypersensitivity) Reactions	255
11.2.1	Immediate Type Hypersensitivity (Type I)	256
11.2.1.1	Total IgE Baseline Levels in Laboratory Mice	256
11.2.1.2	Allergen-specific IgE	257
11.2.1.3	Passive Cutaneous Anaphylaxis (PCA)	259
11.2.1.4	Allergic Airway Inflammation, BAL, Body Plethysmography	262
11.2.1.5	Impact of Different Sensitization and Challenge Protocols on Parameters of Allergen-induced Airway Inflammation	265
11.2.2	Immune Complex Mediated Hypersensitivity (Type III, Arthus Reaction)	267
11.2.2.1	Reverse Passive Cutaneous Arthus Reaction	268
11.2.3	Delayed Type Hypersensitivity (Type IV)	270
11.2.3.1	Local Lymph Node Assay	270
11.2.3.2	Contact Hypersensitivity Assay	273
11.2.4	Granulomatous Hypersensitivity (Type V)	275
11.2.4.1	Experimental Protocol for Type V Hypersensitivity	275
11.3	General Considerations, Logistics and Outlook	276

12	Eye Disorders	283
	<i>Claudia Dalke, Oliver Puk, Angelika Neuhäuser-Klaus, Jack Favor, and Jochen Graw</i>	
12.1	Introduction	283
12.2	Medical and Biological Relevance of Eye Disorders	283
12.3	Eye Disorders in Mouse and Man	284
12.3.1	Mutations Affecting Early Eye Development Leading to Anophthalmia or Microphthalmia	284
12.3.2	Cataracts	285
12.3.3	Retinal Dysfunction and Degeneration	286
12.3.4	Glaucoma	286
12.4	Diagnostic Methods	287
12.4.1	History	287
12.4.2	Routine Methods	288
12.4.2.1	Fundoscopy	289
12.4.2.2	Electroretinography (ERG)	289
12.4.2.3	The Visual Tracking Drum or Optomotor Drum	291
12.4.2.4	Measurement of Intraocular Pressure	292
12.4.2.5	Histological Analysis	292
12.4.3	Methods in Development	292
12.4.3.1	The Scheimpflug Camera	292
12.4.3.2	Length of the Axis	293
12.4.3.3	Measurement of Intraocular Pressure	295
12.4.4	Future Combinations of First and Secondary Screens for Vision Phenotyping	296
12.5	Screening Protocols	296
12.5.1	Slit Lamp	297
12.5.2	Funduscopy Using an Ophthalmoscope	297
12.5.3	Electroretinography (ERG)	298
12.5.4	The Optokinetic Drum	300
12.6	Logistics	301
12.6.1	Slit Lamp	301
12.6.2	Ophthalmoscope	302
12.6.3	ERG	302
12.6.4	The Optokinetic Drum	302
12.7	Troubleshooting	302
12.7.1	Slit Lamp	302
12.7.2	Ophthalmoscope	303
12.7.3	ERG	303
12.7.4	The Optokinetic Drum	304
12.8	Outlook	304
	Acknowledgments	304

13	EUMORPHIA and the European Mouse Phenotyping Resource for Standardized Screens (EMPreSS)	309
	<i>Steve Brown, Heena Lad, Eain Green, Georgios Gkoutos, Hilary Gates, Martin Hrabé de Angelis, and members of the EUMORPHIA consortium</i>	
13.1	Introduction	309
13.2	The EUMORPHIA Project	309
13.2.1	Project Structure	310
13.3	Using Mouse Models	311
13.4	European Mouse Phenotyping Resource for Standardized Screens (EMPreSS)	312
13.4.1	Development of the SOPs	312
13.4.2	Review	313
13.4.3	Validation of SOPs	314
13.5	Ontologies and Structure of the Empress Resource	315
13.6	The EMPreSS Browser	316
13.7	Future Work	317
	Acknowledgments	317
Index		321