

Contents

LIST OF CONTRIBUTORS	xiii
ABOUT THE EDITORS	xv
PREFACE	xvii

Chapter 1 Introduction to Human Genetics.....	1
<i>Jennifer E. Posey and Katherina Walz</i>	

1.1 Introduction.....	1
1.2 Current Status of Discovery	1
1.2.1 From 0 to 60: Linking Human Genetic and Genomic Variation to Human Disease.....	1
1.2.2 Mendelian Conditions	3
1.2.3 Common Disease	6
1.3 Establishing a Link Between Genotype and Phenotype.....	8
1.4 Model Organisms	11
References	13

Chapter 2 Disease in a Dish: Cellular Models to Understand Human Conditions	19
<i>Andrew M. Tidball</i>	

2.1 A Brief History of Cell Culture Development.....	19
2.1.1 Growing Cells Outside the Body	19
2.1.2 Discovery of Embryonic Stem Cells	21
2.1.3 The Rise of Cellular Reprogramming	22
2.2 Cell Culture Studies of Human Genetic Disease.....	23
2.2.1 Early Days of Heterologous Expression Systems.....	23
2.2.2 Disease Modeling With Embryonic Stem Cells	25
2.2.3 Disease Modeling With Induced Pluripotent Stem Cells	26
2.2.4 Advances in Induced Pluripotent Stem Cell Generation...	26
2.2.5 Human Pluripotent Stem Cell Differentiation	27
2.2.6 Disease Modeling in Disease-Relevant Cell Types	29
2.3 Organoids: Cell Culture in Three-Dimensions	30

2.4	Genome Engineering in Cell Culture.....	32
2.4.1	Cut: Sequence-Specific Nucleases.....	32
2.4.2	Paste: Homology-Directed Repair.....	33
2.4.3	Genome Editing in Pluripotent Stem Cells.....	34
2.5	Limitations to Human Pluripotent Stem Cell Models.....	36
2.5.1	Cell Line Identification.....	36
2.5.2	Validation of Genomic Integrity.....	37
2.5.3	Epigenetic Variability.....	38
2.5.4	X-Chromosome Erosion.....	39
2.5.5	Aging.....	39
2.5.6	Ethical Considerations.....	40
2.6	Conclusions.....	41
	References.....	41

Chapter 3 Tiny Models to Answer Big Questions: The Worm and the Yeast as Tools in Human Genetics Research 49
Patricia S. Pardo and Katherina Walz

3.1	Introduction.....	49
3.2	The Worm.....	49
3.3	General Characteristics and Important Tools.....	49
3.4	Examples of Methods and Approaches to Understand Gene Function and Genetic Variation With <i>Caenorhabditis elegans</i>	52
3.5	Utilizing <i>Caenorhabditis elegans</i> in Drug Screenings.....	54
3.6	Summary.....	54
3.7	The Baker's and Fission Yeasts: Differences and Similarities.....	54
3.8	Yeasts in Functional Genomics, Important Tools and Approaches.....	57
3.9	Yeasts as Tools for Drug Testing.....	62
3.10	Summary.....	63
3.11	Conclusions.....	63
	Acknowledgment.....	63
	References.....	63

Chapter 4 Understanding Human Genetic Disease With the Fly..... 69
Kevin A. Hope and Lawrence T. Reiter

4.1	Introduction.....	69
4.2	Part I: <i>Drosophila</i> Tools, Techniques, and Resources.....	70
4.2.1	GAL4/Upstream Activator Sequence.....	70
4.2.2	Upstream Activator Sequence—RNA Interference Transgenic RNAi Project Lines.....	71
4.2.3	Trojan-GAL4.....	72
4.2.4	<i>Drosophila</i> Genetic Reference Panel.....	73
4.2.5	FlyORF and Overexpression Constructs.....	73
4.2.6	Bloomington <i>Drosophila</i> Stock Center.....	74

4.2.7	Generating Transgenic <i>Drosophila</i> Lines If Stocks Are Not Publicly Available.....	74
4.2.8	A MARRVELous Database to Identify <i>Drosophila</i> Orthologs	75
4.2.9	Selecting the Right Tools for the Job.....	75
4.3	Part II: Examples of the Successful Use of <i>Drosophila</i> to Investigate Human Disease	78
4.3.1	Using Flies to Evaluate Disease-Associated Variants.....	78
4.3.2	Seeing Disease Through a Fly's Eye	80
4.3.3	Copy Number Variant Disorders.....	82
4.3.4	Metformin and Fragile X.....	83
4.4	Concluding Remarks.....	84
	References	84

Chapter 5 Studying Human Genetic Variation in Zebrafish 89

Paola Lepanto, Flavio R. Zolessi and Jose L. Badano

5.1	Introduction	89
5.1.1	Genetics in the Era of Genomics	89
5.2	Zebrafish: General Characteristics	90
5.3	The Zebrafish Genome: Difficulties and Opportunities.....	91
5.4	The Zebrafish Toolkit: Modulating Gene Expression and Function	93
5.5	Forward Genetics.....	93
5.6	Reverse Genetics	94
5.7	The Zebrafish Toolkit: In-Depth Phenotyping in Zebrafish	97
5.8	Microscopy.....	97
5.9	Using Zebrafish to Interpret Human Variation.....	100
5.10	Studying Variants in Coding Regions	100
5.11	Assessing Pathogenicity of Variants.....	103
5.12	Targeting Multiple Genes: Genetic Interactions.....	104
5.13	Targeting Multiple Genes: Dissection of Drivers and Dosage Sensors in Copy Number Variants	107
5.14	Assigning Function to Variants in Non-Coding Regions.....	109
5.15	Concluding Remarks	110
	Acknowledgments.....	111
	References.....	112

Chapter 6 The Mouse, a Model Organism for Biomedical Research..... 119

Cesar P. Canales and Katherina Walz

6.1	Introduction.....	119
6.2	The Mouse: General Information, Tools, and Resources.....	119
6.2.1	Genetic Background.....	120
6.2.2	The Mouse Genome	120
6.2.3	Genetic Modifications, Mutant Collections, and Large-Scale Phenotypic Efforts in Mice	121

6.2.4	Mutant Mouse Lines and Efforts Toward Functional Annotation of the Mouse Genome.....	12
6.3	Approaches to Modeling Human Genetic Conditions in Mice.....	12
6.3.1	Variant Present in a Protein-Coding Region.....	12
6.3.2	Modeling Single-Gene Disorders.....	12
6.3.3	Recessive Mutations.....	12
6.3.4	Dominant, Gain of Function Mutations.....	12
6.3.5	Copy Number Variation.....	12
6.3.6	Complex and Polygenic Disorders.....	12
6.3.7	The Genetic Variant Is Found in Noncoding Genetic or Regulatory Elements.....	12
6.3.8	Moving From Founder to Phenotypic Characterization.....	12
6.4	Dissecting Molecular Mechanisms and Identifying Therapy Opportunities for Human Genetic Conditions With the Mouse Aid.....	12
6.4.1	Conditionals, a Powerful Tool to Understand Reversibility and Prevention of Genetic Disorders.....	12
6.4.2	Mouse Models and Drug Development.....	12
6.5	Conclusion.....	12
	Acknowledgments.....	12
	References.....	12

Chapter 7 CRISPR-Cas Technology as a Tool to Create Animal Models for Biomedical Research..... 14

Abhiraami Kannan Sundhari, Shalini Kamu Reddy, Katherina Walz, Channabasavaiah B. Gurumurthy and Rolan M. Quadros

7.1	A Brief History of Modifying Animal Genomes.....	14
7.1.1	Early Mutagenesis Methods.....	14
7.2	Programmable Nucleases.....	14
7.3	Zinc-Finger Nucleases.....	14
7.4	Transcription Activator-Like Effector Nucleases.....	14
7.5	CRISPR-Cas9.....	14
7.6	The CRISPR-Cas9 System and the Generation of Genetically Modified Organisms.....	14
7.7	CRISPR-Cas9 based Genome Editing in Cells.....	14
7.8	CRISPR-Cas9 based Genome Editing in Lower Organisms: Worms, Zebrafish and Flies.....	14
7.9	CRISPR-Cas9 based Genome Editing in Rodents.....	14
7.10	CRISPR-Cas9 based Genome Editing in Higher Organisms.....	14
7.11	Challenges and Future Directions.....	14
	References.....	15
	Further Reading.....	15

Chapter 8	Integrative Modeling and Novel Technologies in Human Genomics	155
	<i>Paulina Carmona-Mora and Juan I. Young</i>	
8.1	DNA Sequencing Technologies and Associated Techniques	155
8.1.1	Common Sequencing Platforms	156
8.1.2	Common Sequencing Applications	156
8.2	Classification of Genomic Elements	164
8.3	Genetic Loci That Determine Quantitative Traits: quantitative Trait Loci	168
8.4	Human Genomics, the Molecular Characterization of Healthy and Disease States	170
8.5	The Era of Shared Data: Common Human Genomics Databases	173
8.6	Integrative Analyses in Human Genomics: Predicting Candidate Genes	176
	References	181
Chapter 9	Models to Understand Human Genomics, Final Considerations.....	191
	<i>Katherina Walz and Juan I. Young</i>	
9.1	What is a Model and What to Expect From It	191
9.2	Ethical and Statistical Considerations	194
9.3	Future Directions	196
	Acknowledgments.....	197
	References.....	197
INDEX		199