

Contents

	Preface	ix
CHAPTER 1	Overview of CRISPR–Cas9 Biology	
	INTRODUCTION	
	Overview of CRISPR–Cas9 Biology	1
	<i>Hannah K. Ratner, Timothy R. Sampson, and David S. Weiss</i>	
CHAPTER 2	Guide RNAs: A Glimpse at the Sequences that Drive CRISPR–Cas Systems	
	INTRODUCTION	
	Guide RNAs: A Glimpse at the Sequences that Drive CRISPR–Cas Systems	17
	<i>Alexandra E. Briner and Rudolphe Barrangou</i>	
	PROTOCOL	
	1 Prediction and Validation of Native and Engineered Cas9 Guide Sequences	24
	<i>Alexandra E. Briner, Emily D. Henriksen, and Rodolphe Barrangou</i>	
CHAPTER 3	Characterization of Cas9–Guide RNA Orthologs	
	INTRODUCTION	
	Characterization of Cas9–Guide RNA Orthologs	31
	<i>Jonathan L. Braff, Stephanie J. Yaung, Kevin M. Esvelt, and George M. Church</i>	
	PROTOCOL	
	1 Characterizing Cas9 Protospacer-Adjacent Motifs with High-Throughput Sequencing of Library Depletion Experiments	35
	<i>Jonathan L. Braff, Stephanie J. Yaung, Kevin M. Esvelt, and George M. Church</i>	
CHAPTER 4	Large-Scale Single Guide RNA Library Construction and Use for CRISPR–Cas9-Based Genetic Screens	
	INTRODUCTION	
	Large-Scale Single Guide RNA Library Construction and Use for CRISPR–Cas9-Based Genetic Screens	39
	<i>Tim Wang, Eric S. Lander, and David M. Sabatini</i>	
	PROTOCOLS	
	1 Single Guide RNA Library Design and Construction	43
	<i>Tim Wang, Eric S. Lander, and David M. Sabatini</i>	

- 2 Viral Packaging and Cell Culture for CRISPR-Based Screens 49
Tim Wang, Eric S. Lander, and David M. Sabatini

CHAPTER 5 Adeno-Associated Virus–Mediated Delivery of CRISPR–Cas Systems for Genome Engineering in Mammalian Cells

PROTOCOL

- 1 Adeno-Associated Virus–Mediated Delivery of CRISPR–Cas Systems for Genome Engineering in Mammalian Cells 57
Thomas Gaj and David V. Schaffer

CHAPTER 6 Detecting Single-Nucleotide Substitutions Induced by Genome Editing

INTRODUCTION

- Detecting Single-Nucleotide Substitutions Induced by Genome Editing 69
Yuichiro Miyaoka, Amanda H. Chan, and Bruce R. Conklin

PROTOCOL

- 1 Using Digital Polymerase Chain Reaction to Detect Single-Nucleotide Substitutions Induced by Genome Editing 73
Yuichiro Miyaoka, Amanda H. Chan, and Bruce R. Conklin

CHAPTER 7 CRISPR–Cas9 Genome Engineering in *Saccharomyces cerevisiae* Cells

PROTOCOL

- 1 CRISPR–Cas9 Genome Engineering in *Saccharomyces cerevisiae* Cells 79
Owen W. Ryan, Snigdha Poddar, and Jamie H.D. Cate

CHAPTER 8 Cas9-Mediated Genome Engineering in *Drosophila melanogaster*

INTRODUCTION

- Cas9-Mediated Genome Engineering in *Drosophila melanogaster* 89
Benjamin E. Housden and Norbert Perrimon

PROTOCOLS

- 1 Design and Generation of Donor Constructs for Genome Engineering in *Drosophila* 93
Benjamin E. Housden and Norbert Perrimon
- 2 Detection of Indel Mutations in *Drosophila* by High-Resolution Melt Analysis (HRMA) 98
Benjamin E. Housden and Norbert Perrimon

3	Design and Generation of <i>Drosophila</i> Single Guide RNA Expression Constructs	106
	<i>Benjamin E. Housden, Yanhui Hu, and Norbert Perrimon</i>	

CHAPTER 9 Optimization Strategies for the CRISPR–Cas9 Genome-Editing System

INTRODUCTION

Optimization Strategies for the CRISPR–Cas9 Genome-Editing System	113
<i>Charles E. Vejnár, Miguel Moreno-Mateos, Daniel Cifuentes, Ariel A. Bazzini, and Antonio J. Giraldez</i>	

PROTOCOL

1 Optimized CRISPR–Cas9 System for Genome Editing in Zebrafish	117
<i>Charles E. Vejnár, Miguel Moreno-Mateos, Daniel Cifuentes, Ariel A. Bazzini, and Antonio J. Giraldez</i>	

CHAPTER 10 Editing the Mouse Genome Using the CRISPR–Cas9 System

INTRODUCTION

Editing the Mouse Genome Using the CRISPR–Cas9 System	133
<i>Adam Williams, Jorge Henao-Mejia, and Richard A. Flavell</i>	

PROTOCOL

1 Generation of Genetically Modified Mice Using the CRISPR–Cas9 Genome-Editing System	139
<i>Jorge Henao-Mejia, Adam Williams, Anthony Rongvaux, Judith Stein, Cynthia Hughes, and Richard A. Flavell</i>	

CHAPTER 11 Genome Editing in Human Pluripotent Stem Cells

INTRODUCTION

Genome Editing in Human Pluripotent Stem Cells	149
<i>Cory Smith, Zhaohui Ye, and Linzhao Cheng</i>	

PROTOCOL

1 A Method for Genome Editing in Human Pluripotent Stem Cells	153
<i>Cory Smith, Zhaohui Ye, and Linzhao Cheng</i>	

CHAPTER 12 An Introduction to CRISPR Technology for Genome Activation and Repression in Mammalian Cells

INTRODUCTION

An Introduction to CRISPR Technology for Genome Activation and Repression in Mammalian Cells	159
<i>Dan Du and Lei S. Qi</i>	

PROTOCOL

- 1 CRISPR Technology for Genome Activation and Repression in Mammalian Cells 163
Dan Du and Lei S. Qi

- APPENDIX General Safety and Hazardous Material Information 173
-

- INDEX 179
-