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COLD SPRING HARBOR LABORATORY PRESS
Cold Spring Harbor, New York • www.cshlpress.org

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Publisher	John Inglis
Acquisition Editor	Richard Sever
Managing Editor	Maria Smit
Director of Editorial Services	Jan Argentine
Project Manager	Maryliz Dickerson
Permissions Coordinator	Carol Brown
Production Editor	Joanne McFadden
Production Manager	Denise Weiss
Director of Product Development & Marketing	Wayne Manos
Cover Designer	Denise Weiss

Cover art: CRISPR–Cas adaptive immunity in bacteria and archaea relies on the capture of invading foreign DNA and its subsequent integration into the CRISPR locus to elicit memory of the infection. The RNA products of newly captured DNA, called spacers, are used by Cas proteins, such as Cas9, as guides for destruction of complementary foreign DNA sequences. The image depicts the process of capturing foreign DNA, catalyzed by the proteins Cas1 and Cas2, in preparation for integration into the host's CRISPR locus. Credit: Megan Riel-Mehan and Graham Johnson (UCSF).

Library of Congress Cataloging-in-Publication Data

Names: Doudna, Jennifer A., editor. | Mali, Prashant, editor.
Title: CRISPR-Cas: a laboratory manual/edited by Jennifer Doudna,
Prashant
Mali.
Description: Cold Spring Harbor, New York: Cold Spring Harbor Laboratory
Press, [2016] | Includes bibliographical references and index.
Identifiers: LCCN 2015050261 | ISBN 9781621821304 (hardcover: alk.
paper) |
ISBN 9781621821311 (pbk. : alk. paper)
Subjects: | MESH: CRISPR-Cas Systems | CRISPR-Associated Proteins |
Genetic
Techniques | Laboratory Manuals
Classification: LCC QP623 | NLM QU 25 | DDC 572.8/8078--dc23
LC record available at http://lccn.loc.gov/2015050261

10 9 8 7 6 5 4 3 2 1

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Preface

Genomes encode the rules for life-forms. Differences in genomes underlie most organismal diversity, and aberrations in genomes underlie many disease states. With the rapid advances in DNA sequencing, we now have near-complete genomes for a range of organisms and a fairly comprehensive catalog of human germline and somatic variants, as well as rich annotations of functional genomic elements. The next frontier in the field is to obtain a complete functional annotation of genetic variants and genomic elements at the cellular and organismal levels. Such an understanding, especially in the human context, will not only pave the way for a deeper understanding of the genomic code but will also power therapeutic interventions directed at both effecting cures and eventually also engineering disease resistance. Consequently as we move from reading genomes to interpreting genomes and ultimately engineering genomes, technologies to directly and precisely perturb genomic elements and combinations thereof will be a most critical toolset in these basic science cum engineering endeavors.

In this regard, the recent advent of RNA-guided effectors derived from clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated systems (Cas) has dramatically transformed our ability to engineer the genomes of diverse organisms. As unique factors capable of colocalizing RNA, DNA, and protein, tools and techniques based on CRISPR–Cas are paving the way for unprecedented control over cellular organization, regulation, and behavior.

Notably, CRISPR–Cas systems evolved as adaptive immune defenses of bacteria and archaea and use short RNA to direct degradation of foreign nucleic acids. They provide immunity by incorporating fragments of invading phage and plasmid DNA into CRISPR loci and using the corresponding CRISPR RNAs (crRNAs) to guide the degradation of homologous sequences. Each CRISPR locus encodes acquired "spacers" that are typically separated by repeat sequences. Transcription of the locus yields a pre-crRNA, which is processed to yield crRNAs that guide effector nuclease complexes to disrupt sequences complementary to the spacer. CRISPR systems are thus readily retargeted by expressing or delivering appropriate crRNAs, and progressive mechanistic insights into these fundamental processes thus paved the way for their recent engineering into a range of prokaryotic and eukaryotic organisms.

In considering the developments in this rapidly evolving field and its applications for understanding basic biology and engineering of new therapeutic paradigms, our goal in developing this book was to highlight the major advances that have been made that have led to the current state of research, while also providing a guide for implementation of these approaches. As such, the book is divided into multiple parts and, focusing specifically on the CRISPR–Cas9 targeting methodology, it details protocols for applications in a range of species and in ex vivo cum in vivo genome targeting scenarios. We begin with an overview of CRISPR–Cas9 biology, followed by computational and experimental protocols for prediction and validation of native and engineered Cas9 orthologs and guide sequences. Toward harnessing the massively multiplexable and scalable genome engineering enabled by this platform, we next detail protocols for constructing CRISPR libraries for effecting large-scale genetic screens in human cell lines. Given the impending applications of CRISPR–Cas in engineering therapeutics, protocols on establishing an adeno-associated virus–based delivery system into cells and mice are provided next. High-resolution assaying of genomic changes induced by this platform are critical for effectively implementing this approach, and thus we also detail highly sensitive polymerase chain reaction (PCR)-based assays to quantify genome-editing events. We follow this with a collection of protocols for precision genome engineering in a range of organisms including yeast, fruit flies, zebrafish, and mice, as well as human induced pluripotent stem cells. We conclude by detailing protocols to enable targeted genome regulation using

the CRISPR–Cas9 platform. These chapters provide a comprehensive, in-depth overview of the experimental procedures prevalent in the field. Looking forward, we anticipate the versatility and ease of use afforded by CRISPR–Cas effectors, coupled with their singular ability to bring together RNA, DNA, and protein in a fully programmable fashion, to form the basis of a progressively expanding experimental toolset for the perturbation, regulation, and monitoring of complex biological systems.

We would like to thank the many scientists who have contributed to this book. We are very grateful for their enthusiasm, hard work, and attention to detail in preparing this book, which can serve as a broad resource for technicians, graduate students, postdocs, and any investigator engaged in genetic studies. Special thanks also go to Maryliz Dickerson at Cold Spring Harbor Laboratory Press for helping make this book a reality.

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