PREFACE

The availability of whole-genome sequencing data for large numbers and types of organisms including humans holds exciting promise for advancing both research and healthcare. A major challenge has been understanding and using genomic data through targeted manipulation of cellular DNA. Ever since the discovery of DNA structure in the 1950s, researchers and clinicians have been contemplating the possibility of making site-specific changes to the genomes of cells and organisms. Many of the earliest approaches to what has become known as genome editing relied on the principle of site-specific recognition of DNA sequences. The study of natural DNA repair pathways in bacteria and yeast, as well as the mechanisms of DNA recombination, showed that cells have endogenous machinery to repair DNA double-strand breaks that would otherwise be lethal. Thus, methods for introducing precise breaks in the DNA at desired editing sites were recognized as a valuable strategy for targeted genomic engineering.

Although some isolated successes were achieved using oligonucleotides or small molecules to localize DNA-cleaving activities to specific sequences, proteins that could be programmed to bind and cleave particular DNA sites proved more broadly useful. Modular DNA recognition proteins, when coupled to the sequence-independent nuclease domain of the restriction enzyme FokI, could function as site-specific nucleases. When designed to recognize a chromosomal sequence, such zinc-finger nucleases and TAL effector nucleases can be effective at inducing genomic sequence changes in both animal and plant cells. Difficulties of protein design, synthesis, and validation have limited widespread adoption of these engineered nucleases for routine use, paving the way for the CRISPR/Cas9 system in which a natural protein with double-stranded DNA-cleaving activity can be programmed with a short RNA sequence to recognize and cut DNA sites of interest. The ease of use, efficiency, and multiplexing capabilities of this technology have enabled rapid adoption for many different genome engineering applications.

In this volume, we provide readers with a collection of protocols for the major protein-based genome editing techniques, with a particular emphasis

on the more recently developed CRISPR/Cas9 approaches. As these systems are used more widely and for ever-increasing types of projects, we anticipate that the facility of genome manipulation for application in human health and biotechnology will continue to expand.

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