Expanding the Safety, Specificty, and Targeting Range of the CRISPR-Cas Genome Editing Toolbox

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Abstract

The easy reprogrammability of the Cas9 RNA-guided endonuclease has made it one of the most versatile tools for genome editing. The protein's targeting activity occurs in three phases. First, it complexes with a guide RNA (gRNA), comprising of a spacer sequence and a double hairpin. Second, it scans DNA for a short Protospacer Adjacent Motif (PAM) sequence, which is specified by the protein itself. Third, when a PAM is found, Cas9 checks the gRNA spacer sequence for complementarity against the adjacent genomic DNA. If there is a match, Cas9 will cleave the DNA at that location. Though changing the spacer sequence on the gRNA reprograms the sequence specificity of Cas9, there is a natural limitation on its set of genomic targets due to the PAM sequence requirement. The canonical Cas9 used by most scientists, the *S. pyogenes* Cas9 (SpCas9), has a PAM sequence of NGG, limiting it to ≈1 in 8 loci in the human genome. We recently characterized the PAM specificity of evolved Cas9 variants, as well as the *S. aureus* Cas9 (Kleinstiver *et al.* 2015), showing that that Cas9 proteins from orthogonal bacterial strains can indeed be characterized. Here, we propose to attempt to characterize the PAM specificities of 5 more Cas9 proteins from a variety of bacterial strains.

Contents

To the family, friends, and food that sustained me through this process.

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People are amazing, I will acknowledge them here.

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Introduction

Much of the recent history of genome editing consists of building the site-specificity of a nuclease through engineered protein-DNA interactions. Though this technique has proven effective and useful, as seen in the examples of Zinc-Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs), these platforms are difficult to use because of the complexity of programming protein-DNA specificity. In recent years, the CRISPR-Cas bacterial immune system has been extensively used as a genome engineering alternative that relies on RNA-DNA complementarity as the homing interaction that guides the specificity of the Cas9 nuclease. The ease of using such a platform to cleave specific sites in the genome simply by choosing a guide RNA sequence complementary to the

target sequence has lead to an explosion in its use, with enormous amounts of interest and capital being contributed to the investigation of further development and application of this technology (Doudna and Charpentier *et al.* 2014).

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