Possible differences in efficiency of guide RNA with different spacer sequence in CRISPR knock-down or knock-out of particular gene

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Abstract

Cluster regularly interspersed short palindromic repeats (CRISPR) mediated genome editing has emerged as the dominant technique for modulating the expression of target genes. Specifically, when coupled with different effectors, CRISPR could be utilized to either activate or repress gene expression. Specificity of the CRISPR gene editing method arises from the unique spacer sequence in guide RNA that mediates the specific localization of Cas9 endonuclease to particular stretches of DNA. However, complementary base pairing between the guide RNA and template DNA depends critically on existence of protospacer adjacent motif (PAM) sequence immediately downstream of the spacer sequence. Such three nucleotide PAM sequence could be present at multiple loci in a given gene, which meant that different spacer sequence could be incorporated in guide RNA design to target the same gene. Given that different spacer sequences have different binding affinities to template DNA, differences could exist in the efficiency in which CRISPR-Cas9 could be guided to generate a double strand break in a particular gene locus. Using green fluorescent protein (GFP) reporter gene expressed in recombinant *Escherichia coli* as experimental system, this study sought to understand if differences in targeting efficiency exist between guide RNA with different spacer sequence that could target the same gene. Fluorescent intensity of cells at the population level would serve as readout of the targeting efficiency. For example, spacer sequence in guide RNA that could better activate the endonuclease activity of Cas9 would result in lower fluorescent intensity of GFP. To check for the effect of expression mode on targeting efficiency of guide RNA, GFP gene would be expressed on a plasmid in *E. coli* as well as integrated into the genome of the bacterium. Doing so would provide critical information on whether the CRISPR-Cas9 system has differentiated efficacy in generating double strand breaks in genomic versus plasmid DNA. Such information would inform future experimental design involving CRISPR-Cas9 genome editing technology as well as hold implications on how CRISPR evolved as an adaptive immune system in defending bacterial cells against foreign DNA. Given the goal of the study to understand the relative extent in which a target gene would be disrupted by CRISPR-Cas9 guided by different spacer sequence on guide RNA, no repair module for the target gene would be provided. Collectively, multiple occurrence of PAM sequence in a target gene meant that different spacer sequences could be used in CRISPR-Cas9 to downregulate gene expression. Relative efficacies of different spacer sequence in guide RNA in achieving targeted gene inactivation remain poorly understood and constitutes the basis of this study, which hopefully would provide guidance on the selection of specific spacer sequence that would yield the most efficacious disruption of gene expression at the genome and plasmid level.

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Conflicts of interest

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