

# ***In silico* experiments validate that twenty nucleotide spacer sequence provides precise targeting of bacterial genes in CRISPR-Cas9**

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## **Abstract**

As a genome editing tool useful for modulating the expression of different genes, CRISPR-Cas9 is known for its precision in targeting specific genes. To do this, CRISPR-Cas9 utilizes a guide RNA for guiding the Cas9 endonuclease to specific stretches of the DNA for genome editing or modulation of gene expression. Guide RNA comprises a spacer sequence and a protospacer adjacent motif (PAM) sequence. Both components work together to help target Cas9 to a specific stretch of DNA within a gene. In particular, spacer sequence provides a unique address for localizing Cas9 to specific stretch of DNA. But, possibility exists that there could be off-target effects for particular spacer sequence used in guide RNA. Specifically, spacer sequence might engage in complementary base pairing with other stretches of DNA in the bacterial genome, and result in additional genome editing or modulation of gene expression at genes that are not targeted. Results from an *in silico* experiment conducted with the *rpoH* gene of *Pseudomonas aeruginosa* PAO1 revealed that all spacer sequences derived from different stretches of the *rpoH* gene did not elicit off-target effects in the genome of the bacterium. This concurs with theoretical predictions that the probability of off-target effects from a 20 nucleotide long spacer region is vanishingly small. Hence, a 20 nucleotide spacer sequence in guide RNA should provide a unique DNA address for precise targeting of specific gene in the genome of a bacterium. Collectively, off-target effects of CRISPR-Cas9 is a valid concern for both genetic engineering and genome editing applications as targeting of additional genes from the desired one would result in unpredictable physiological and biochemical impacts on the cell. Using the *rpoH* gene of *Pseudomonas aeruginosa* PAO1 as example, results from an *in silico* experiment examining possible off-target effects of different 20 nucleotide spacer sequence able to target the sense and antisense strand of the gene revealed no off-target effects. Specifically, each spacer sequence used could only target the intended *rpoH* gene, which concurs with theoretical predictions of vanishingly small possibility of off-target effects on a bacterial genome from a 20 nucleotide spacer sequence. Overall, the results highlight that use of a 20 nucleotide spacer sequence in guide RNA could offer precise targeting of specific gene in a bacterium.

**Keywords:** *Pseudomonas aeruginosa* PAO1, *rpoH*, off-target effects, protospacer adjacent motif, spacer sequence, guide RNA, precise targeting, unique address, CRISPR-Cas9, endonuclease,

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### **Conflicts of interest**

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