doi 10.18699/CRISPR-2025-153

In vitro characterization of a novel type II CRISPR-Cas system KiCas9

M.S. Liashchenko[1], A.A. Vasileva[2], D.A. Kretov[2], M.V. Abramova[2], T.A. Zair-Bek[2], M.A. Khodorkovskii[2], A.N. Arseniev[2]*

[1] *Saint Petersburg State University, St. Petersburg, Russia*

[2] *Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia*

* *arsenievanatoly@gmail.com*

Key words: CRISPR; Cas9; nuclease

CRISPR-Cas systems represent a powerful tool for editing the genomes of various organisms, including humans and animals. However, currently used systems have significant limitations related to the narrow range of recognizable PAM sequences (5'-NGG-3' for SpCas9) and the large size of effector proteins (160 kDa for SpCas9) [1]. To expand the genome editing toolkit, it is critically important to broaden the spectrum of utilized systems by searching for and studying new orthologs of effector proteins.

This study investigates a novel type II CRISPR-Cas system – KiCas9, discovered in bacteria of the genus *Kiritimatiella*. For the functional characterization of the system, the recombinant effector protein KiCas9 (size 120 kDa) was obtained, and crRNA, tracrRNA, and single guide RNA (sgRNA) were designed and synthesized to perform *in vitro* reactions.

The research results confirmed the nuclease activity of the KiCas9 protein *in vitro*. The highest DNA cleavage efficiency was observed when using the engineered sgRNA and a DNA template containing the PAM sequence 5'-TAACAC-3'. The optimal conditions for the nuclease's function were determined: the most effective temperature range is 35–42°C, which is optimal for potential work in mammalian cells. An interesting feature of the KiCas9 PAM sequence is that it can potentially encode stop codons, which theoretically allows the use of the KiCas9 system for the efficient design of expression genetic constructs.

References

1. Jinek M., Chylinski K., Fonfara I. et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337(6096):816-821. doi 10.1126/science.1225829