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Development of the Cas12a-based microdeletion and microinsertion detection system

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CRISPR/Cas-based systems are widely used as genome editing systems, nucleic acid detection systems and molecular visualization instruments [1]. In our laboratory using CRISPR/Cas9 technology we have obtained several KO mouse lines harboring deletions ranging from 2 to 20 base pairs. While 20 bp deletions are easily PCR-detected, when it comes to 2 bp deletions it is essential to genotype numerous mice using time-consuming Sanger sequencing. We propose a microdeletion/microinsertion detection system based on Cas12a nuclease from *Lachnospiraceae* bacterium (LbCas12a). Its active complex consists of the Cas12a enzyme and one crisprRNA [2]. A special sequence called protospacer adjacent motif (PAM) is required for target recognition by LbCas12a. In our laboratory we have discovered new PAM TTAA recognized by LbCas12a [3]. Via agarose electrophoresis and fluorescent analysis using FAM-labeled probes we have shown that new PAM allowed detection of 1 bp substitutions in target DNA *in vitro*. Also we have tested different FAM-labeled probes and have shown that AT-rich probes longer than 10 bp are cleaved most effectively. Finally we have used our system for detecting 2 bp deletions in Pde6b-KO mice and Grin3A-KO mice and successfully distinguished these mice from wild type mice.

In conclusion, new PAM TTAA greatly increases specificity of DNA cleavage allowing to use this system as an instrument for rapid detection if microdeletions in mice.

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