Center for Experimental Medicine and Systems Biology

Division of Genome Engineering
ゲノム編集研究分野

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Genome engineering technologies such as clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) nucleases (CRISPR-Cas) have been widely used in life sciences and medicine. We have developed a novel genome editing tool, CRISPR-Cas3, to overcome the technical and patent limitations of the CRISPR-Cas9 system. We are analyzing the molecular mechanisms underlying Cas3-mediated genome editing in human cells and improving this tool for translational research, such as gene therapy and viral diagnostics. We are also developing some efficient genome editing strategies using these tools in rodents. These technologies enable easy and flexible gene editing in living organisms.

Type I-E CRISPR-Cas3 for large-scale genomic modifications in mice and rats

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Genome editing technologies are highly effective tools for genetic engineering in various organisms including experimental animals. Type I-E CRISPR-Cas3 uses an RNA-guided multi Cas-protein complex, Cascade, which detects and degrades foreign nucleic acids via the helicase-nuclease Cas3 protein. However, it is unclear whether the system can be used for genome editing in fertilized eggs.

We applied the CRISPR-Cas3 system with several modification to generate genetically modified animals, and could generated knockout mice and rats in several genetic loci with optimizing method for the introduction into embryos even by using electroporation methods. This work with the Type I CRISPR zygote editing system represents a significant leap forward, offering increased flexibility and broader applications in genetic engineering across multiple species.

CRISPR-Cas3-based diagnostics for virus detection and genetic screening

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CRISPR-based diagnostics (CRISPR-dx), including the Cas12-based DETECTR and Cas13-based SHERLOCK Class 2 CRISPRs, have been used to detect the presence of DNA or RNA from pathogens such as the 2009 pandemic influenza A (IVA) virus and the 2019 novel coronavirus SARS-CoV-2. Here, the collateral single-stranded DNA cleavage we observed with class 1 type I CRISPR-Cas3 highlights its potential for development as a Cas3-mediated, rapid (within 40 minutes), low-cost, instrument-free detec-

tion method for SARS-CoV-2. This assay, which we have named Cas3-operated nucleic acid detection (CONAN), not only detects SARS-CoV-2 in clinical samples, but also provides specific detection of single base pair mutations in IVA variants. We are also optimizing protocols for cancer detection by liquid biopsy and genetic screening for inherited diseases such as trinucleotide repeat disorders.

Publications

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