BRC Science Highlight
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PNA-Assisted DNAzymes to Cleave Double-Stranded DNA for Genetic Engineering with High Sequence Fidelity

Background/objective

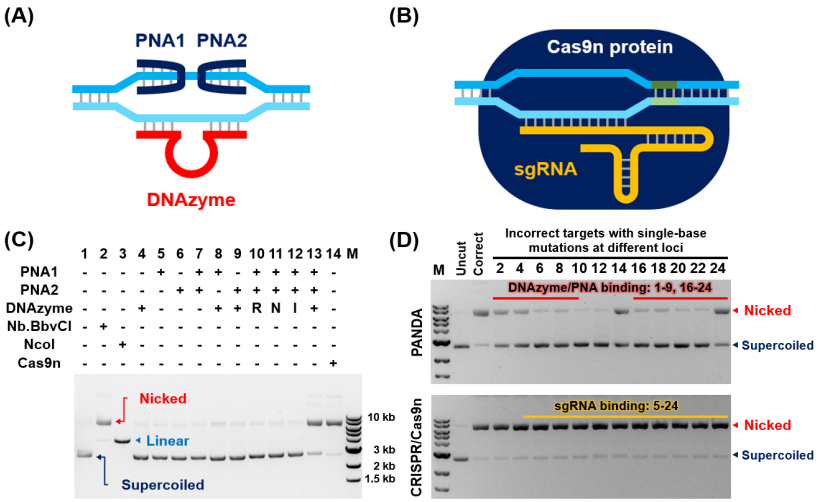
The success of genetic engineering tools such as CRISPR/Cas has revolutionized biology. Despite the progress, sequence fidelity is still a major issue, which can cause off-target effects. DNAzymes are DNA molecules capable of performing enzymatic activities. They can catalyze a wide variety of reactions, especially on nucleic acid substrates. In comparison with ribozymes and protein enzymes, DNAzymes are more stable and cost-effective. However, they have rarely been used for genetic engineering because their substrate scope is mostly limited to single-stranded DNA or RNA, whereas genetic information is stored mostly in double-stranded DNA (dsDNA). To overcome this limitation, we demonstrate a novel system named PNA-Assisted Double-Stranded DNA Nicking by DNAzymes (PANDA), wherein a ssDNA-cleaving DNAzyme has been shown to nick dsDNA with the help from PNA. Such a system shows higher sequence fidelity than CRISPR/Cas.

Approach

- To demonstrate that a DNAzyme can recognize and cleave the ssDNA region on PNA-invaded dsDNA, a proof-of-concept PANDA system was built based on PNA "openers", which invade the dsDNA and expose a single-stranded region, and a DNA-cleaving DNAzyme, which bind and cleave the exposed region.
- To investigate the sequence specificity of PANDA on its target, its nicking activity was tested on mutated targets containing single-base mismatches within the PANDA recognition site.
- To demonstrate the potential of PANDA as genetic engineering tools, PANDA was reprogrammed to target different DNA sequences. Moreover, the ability of PANDA to replace restriction enzymes in generating recombinant DNA was tested.

Results

- PANDA is efficient in nicking or causing double strand breaks on target dsDNA, which mimics protein nickases or restriction enzymes.
- PANDA has a higher sequence fidelity compared with CRISPR/Cas under the condition tested.
- PANDA can be customized to target different sequences and used in the standard molecular cloning method to generate recombinant DNA.



Schematic representations of PANDA (A) and CRISPR/Cas9n (B); gel analysis of PANDA activity (C) and single-base mismatch specificity of PANDA and CRISPR/Cas9n (D).

Significance

The PANDA system is a novel artificial nuclease for genetic engineering and other biochemical applications. Being smaller, more stable, and having higher sequence fidelity, the PANDA system will expand the genetic engineering toolbox to be used for bioenergy research.

Lyu, M., et al. 2021. "PNA-Assisted DNAzymes to Cleave Double-Stranded DNA for Genetic Engineering with High Sequence Fidelity." Journal of the American Chemical Society. DOI: 10.1021/jacs.1c03129.

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