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CRISPR-Cas9-assisted native end-joining editing offers a simple strategy for efficient genetic engineering in *Escherichia coli*

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ABSTRACT

Unlike eukaryotes, prokaryotes are less proficient in homologous recombination (HR) and non-homologous end-joining (NHEJ). All existing genomic editing methods for *Escherichia coli* (*E. coli*) rely on exogenous HR or NHEJ systems to repair DNA double-strand breaks (DSBs). Although an *E. coli* native end-joining (ENEJ) system has been reported, its potential in genetic engineering has not yet been explored. Here, we present a CRISPR-Cas9-assisted native end-joining editing and show that ENEJ-dependent DNA repair can be used to conduct rapid and efficient deletion of chromosome fragments up to 83 kb or gene inactivation. Moreover, the positive rate and editing efficiency is independent of high-efficiency competent cells. The method requires neither exogenous DNA repair systems nor introduced editing template. The Cas9-sgRNA complex is the only foreign element in this method. This study is the first successful engineering effort to utilize ENEJ mechanism in genomic editing and provides an effective strategy for genetic engineering in bacteria that are inefficient in HR and NHEJ.

METHODS

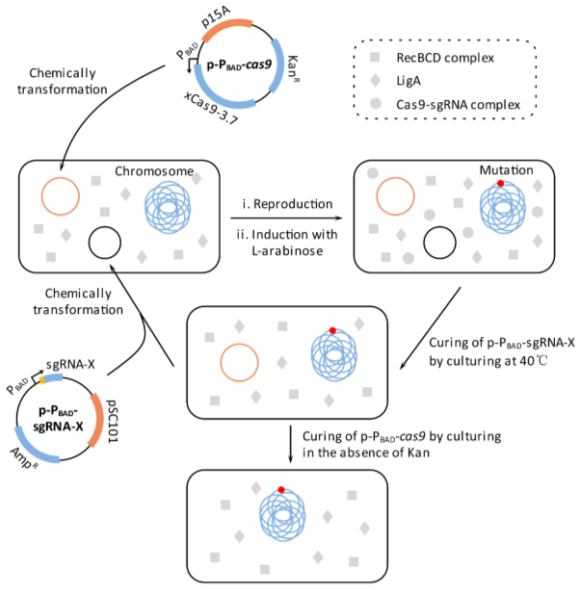

Fig. 2 The schematic procedures of CNEE

RESULTS

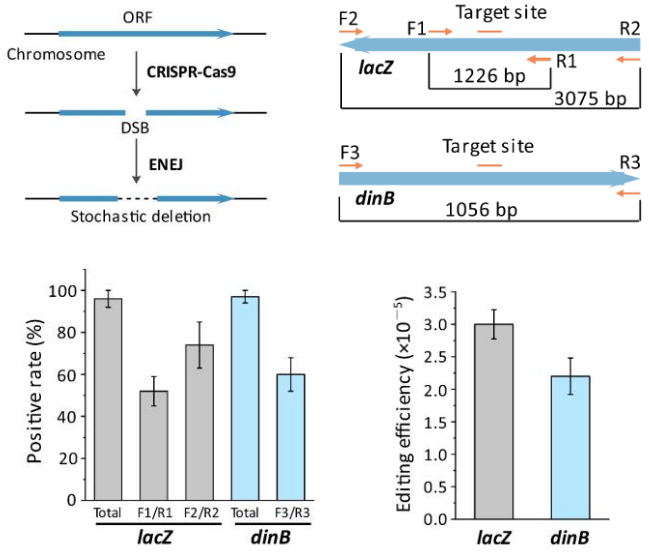

Fig. 3 Gene knockout by CNEE

RESULTS

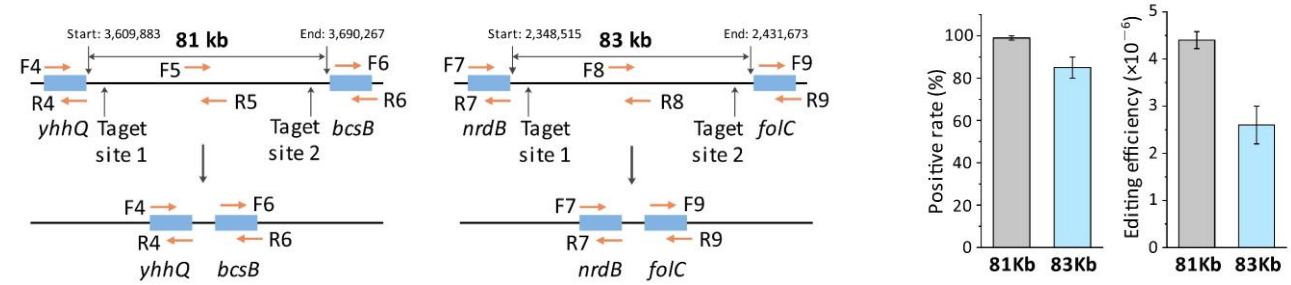

Fig. 4 Large-fragment deletion by CNEE

CONCLUSION

In this study, we developed the CNEE for genomic editing. The method utilizes ENEJ to repair DSBs, thus is independent of exogenous DNA repair systems and editing template. To our knowledge, this is the first successful engineering effort to utilize ENEJ mechanism in genomic editing. Our method has three advantages compared to the existing methods: (i) it requires neither a DNA template nor an exogenous DSBs repair system, (ii) it is simple and efficient in gene inactivation and large-fragment deletion, (iii) it is independent of high-efficiency competent cells.

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