CRISPR/Cas9-Mediated Genome Editing via Homologous Recombination in a Centric Diatom *Chaetoceros muelleri*

Wenxiu Yin and Hanhua Hu*

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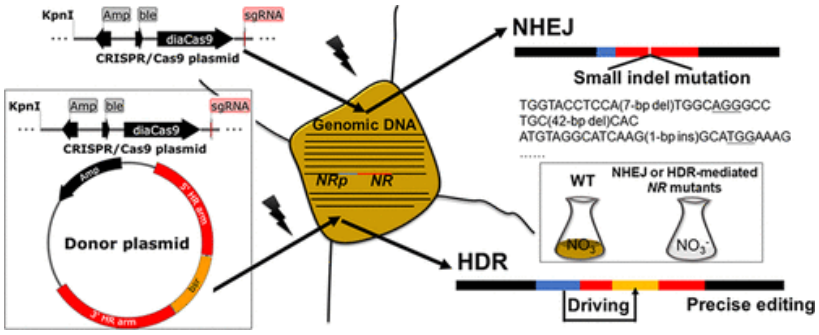
Abstract



Chaetoceros, the most abundant genus of marine planktonic diatoms, can be used in mariculture. An effective genetic transformation system with a short transformation period was established in *Chaetoceros muelleri* by electroporation in our previous study. In this study, a sequence-specific clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 vector applicable for *C. muelleri* was constructed, and the expressions of sgRNA, resistance gene, and *Cas9* gene were driven by the endogenous promoters *U6*, *acetyl-CoA acetyltransferase,* and *fucoxanthin chlorophyll a/c binding protein*, respectively, in the vector. *Nitrate reductase* (*NR*) and *urease* (*URE*) genes were edited in *C. muelleri*, and the *NR* knockout and *NR*/*URE* double-knockout lines displayed the strict auxotrophic phenotype. In addition, the DNA double-strand break was repaired by homologous recombination when a donor DNA was introduced. CRISPR/Cas9 technology was successfully applied to *C. muelleri* with an editing efficiency of up to 86%, providing a molecular tool for the study of basic biology in *C. muelleri* and its synthetic biology applications.

KEYWORDS: Chaetoceros muelleri, diatom, CRISPR/Cas9, nitrate reductase, homologous recombination

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssynbio.3c00051.

- Identities of required gene sequences between *P. tricornutum* and *C. muelleri* in this study; sequence information of required genetic components in this study; primers used in this study; sequence information of plasmids constructed in this study; positions of sgRNAs and detection primers in *NR* and *URE* in *C. muelleri*; editing of the *C. muelleriurease* gene (*URE*) via CRISPR/Cas9-mediated NHEJ; growth of colonies on solid media with different sole nitrogen sources; and editing of the *C. muelleri* nitrate reductase gene (*NR*) via CRISPR/Cas9-mediated HDR (PDF)

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Washington, DC 20036