

Efficient genome editing in *Zymomonas mobilis* through exploiting an endogenous Type I-F CRISPR-Cas system

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Abstract

Establishment of production platform organisms through prokaryotic engineering represents an efficient means to generate alternatives for yielding renewable biochemicals and biofuels from sustainable resources. *Zymomonas mobilis*, a natural facultative anaerobic ethanologen, possesses many attractive physiological attributes, making it an important industrial microorganism. To facilitate the broad applications of this strain for biorefinery, a genome editing toolkit for *Z. mobilis* was established by repurposing an endogenous Type I-F CRISPR-Cas system upon its functional characterization, which includes a series of genome engineering plasmids, each carrying an artificial self-targeting CRISPR and a donor DNA for the recovery of recombinants. With the toolkit, various genome editing purposes were efficiently achieved, including gene knockout (100% efficiency), deletion of a genomic fragment of >10 kb (50%), gene replacement (100%), *in situ* gene modification including nucleotide substitution (100%) and His-tagging (100%), and multiplex gene deletion (18.75%). This work established thus far the most efficient, straightforward and convenient genome manipulation toolkit for *Z. mobilis*, and laid a foundation for further native CRISPRi studies in *Z. mobilis*. The method extended the application scope of CRISPR-based technologies, and could also be applied to other industrial microorganisms with unexploited endogenous CRISPR-Cas systems.

Brief Biography

Assoc. Prof. Wenfang Peng has about two decades of experience in CRISPR-Cas research. His research is mainly focusing on dissecting mechanisms of CRISPR-Cas antiviral defense and exploiting native CRISPR-Cas systems as toolkits for prokaryotic engineering. Since he started his career at Hubei University in 2015, he has published several SCI papers in reputational journals, such as *Nucleic Acids Research*, *Journal of Biological Chemistry*, *RNA Biology*, *et al.* and has applied for 6 invention patents.

Brief CV

Wenfang Peng, Ph.D.

School of Life Sciences, Hubei University

Education:

B.S. in Agronomy, Yangtze University, China, 2005

M.S. in Crop Genetics and Breeding, Chinese Academy of Agricultural Sciences, China, 2008

Ph.D. in Microbiology, Huazhong Agricultural University, China, 2015

Ph.D. in Biology, University of Copenhagen, Denmark, 2015

Professional Career:

2008-2010: Oil Crops Research Institute, CAAS, China, Research Assistant.

2015-present: Hubei University, China, Associate Professor

Research Interests:

1. CRISPR-Cas antiviral defense mechanisms
2. CRISPR-based technologies

Selected publications

1. Peng, W.* et al. *BioRxiv*, 2019, doi: 10.1101/576355
2. Peng, W. et al. *Nucleic Acids Research*, 2017, 45(4):1902-1913.
3. Peng, W. et al. *Nucleic Acids Research*, 2017, 45(4):1983-1993.
4. Peng, W. et al. *Nucleic Acids Research*, 2017, 45(15):8978-8992.
5. Peng, W. et al. *Nucleic Acids Research*, 2017, 45(4):1983-1993.
6. Peng, W. et al. *Journal of Biological Chemistry*, 2017, 292(50):20707-20719.
7. Peng, W. et al. *Nucleic Acids Research*, 2015, 43(1):406-417.
8. Peng, W. et al. *RAN Biology*, 2013, 10(5):738-748.