A High-Efficacy CRISPR Interference System for Gene Function Discovery in *Zymomonas mobilis*

Amy B. Banta[1,2], Amy L. Enright[1,2*] (alenright@wisc.edu), Cheta Siletti[1†], **Jason M. Peters**[1,2,3,4]

[1]DOE Great Lakes Bioenergy Research Center, [2]School of Pharmacy, [3]Department of Bacteriology, and [4]Department of Medical Microbiology & Immunology, University of Wisconsin-Madison; [†]Present address: Department of Food Science, University of Wisconsin-Madison

Project Goals: Enable rational engineering of the promising biofuel producer *Zymomonas mobilis* by establishing CRISPR interference (CRISPRi) for gene function discovery in this non-model organism.

The Alphaproteobacterium *Zymomonas mobilis* is a promising biofuel producer due to its streamlined glycolytic conversion of sugar to ethanol. However, rational engineering of *Z. mobilis* for production of advanced biofuels, such as isobutanol, is hindered by an incomplete understanding of *Z. mobilis* gene functions, particularly those involved in metabolism and alcohol stress. Furthermore, essential genes are linked to many of the industrially desirable attributes of *Z. mobilis*, requiring the use of advanced genetic techniques to preserve cell viability upon manipulation of such genes. CRISPR interference (CRISPRi) is a method which allows for assessment of all genes, including essential genes. In CRISPRi, precise gene knockdown is achieved by a programmable guide RNA complexed with a catalytically inactive Cas9 protein to block transcription of a complementary DNA target. CRISPRi is both inducible and titratable, enabling assessment of essential genes via temporal regulation and precise control over knockdown strength to reveal phenotypes while maintaining viability. Here, we report a high-efficacy *Z. mobilis* CRISPRi system and demonstrate its utility through knockdown of genes that are essential for growth, required for the uniquely efficient ethanologenic metabolism of *Z. mobilis*, or involved in isobutanol tolerance. Our *Z. mobilis* CRISPRi system paves a straightforward path to gene function discovery which can be used to improve rational engineering efforts for increased biofuel production by this non-model organism.

Publications

1. Banta AB, Enright AL, Siletti C, Peters JM. A High-Efficacy CRISPR Interference System for Gene Function Discovery in Zymomonas mobilis. *Appl Environ Microbiol*. 2020;86(23):e01621-20. Published 2020 Nov 10. doi:10.1128/AEM.01621-20

This work was supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Great Lakes Bioenergy Research Center under Award Number DE-SC0018409.