Title: Genome-scale CRISPRi Enables Functional Annotation of the *Zymomomas mobilis* Genome

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Project Goals: Enable rational engineering of the promising biofuel producer *Zymomonas mobilis* by using genome-wide CRISPR interference (CRISPRi) screens for gene function discovery and annotation, including for essential and/or biofuel-relevant genes.

Abstract: The emerging model bacterium, *Zymomonas mobilis*, is a promising biofuel producer, but rational engineering of the genome to improve biofuel yields relies on gene function information that is currently lacking. To probe gene function in *Z. mobilis*, we previously developed a CRISPRi (clustered regularly interspaced short palindromic repeats interference) system that caused robust gene knockdown and enabled phenotyping of metabolic and stress genes. Here, we utilize *Z. mobilis* CRISPRi for systematic gene phenotyping at the genome scale and identify genes that are conditionally essential for growth in aerobic or anaerobic conditions. Our screen uncovered expected (e.g., superoxide dismutase) and unexpected (e.g, ssDNA-specific exonuclease RecJ) players in oxygen tolerance. Further, we found a surprising role for the ATP synthase in maintaining the electrochemical gradient during anaerobic growth. Future work will focus on genes that are crucial for growth in the presence of biofuel-relevant stresses such as plant-derived toxins and accumulation of fermentation products. Identification of bioenergy-relevant genes will enable informed genetic engineering of stress-tolerant *Z. mobilis* strains with increased yields, closing the economic gap between biofuels and fossil fuels and paving the way toward mitigation of climate change.

References/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

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