



## A High-Efficacy CRISPRi System And Strong Synthetic Promoters For Alphaproteobacteria And Gammaproteobacteria

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Inventors: Jason Peters, Amy Banta

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### The Invention

The present invention from UW-Madison innovators is a CRISPRi system for studying the genetics of *Z. mobilis* and lac-regulated synthetic *Z. mobilis* promoters that are used in the CRISPRi system but also may be research tools for studying gene expression in *Z. mobilis*. The inventors' *Z. mobilis* CRISPRi vectors cause either strong knockdowns (>100-fold) using single guide RNA (sgRNA) spacers that perfectly match target genes, or partial knockdowns using spacers with mismatches. The inventors had to use a specific type of inactive Cas9 protein, had to swap the fluorescent reporter protein they were using with a super folding green fluorescent protein, had to optimize the sgRNAs and had to develop Lac-regulated synthetic promoters improve knockdown efficiency while preventing leaky expression of the CRISPRi components in order to get the controlled gene knockdowns they desired. The innovators demonstrated the efficacy of *Z. mobilis* CRISPRi by targeting essential genes that are universally conserved in bacteria, key to the efficient metabolism of *Z. mobilis*, or underlie alcohol tolerance.

### Additional Information

#### For More Information About the Inventors

- [Jason Peters](#)

#### Tech Fields

- [Clean Technology: Biobased & renewable chemicals & fuels](#)

For current licensing status, please contact Jennifer Gottwald at [jennifer@warf.org](mailto:jennifer@warf.org) or 608-960-9854