

## Genome remodeling to control the persistence of engineered functions in soil microbes

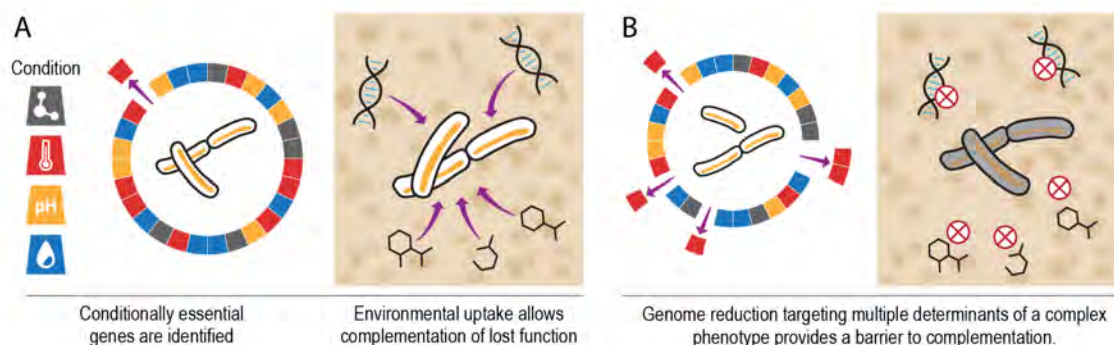
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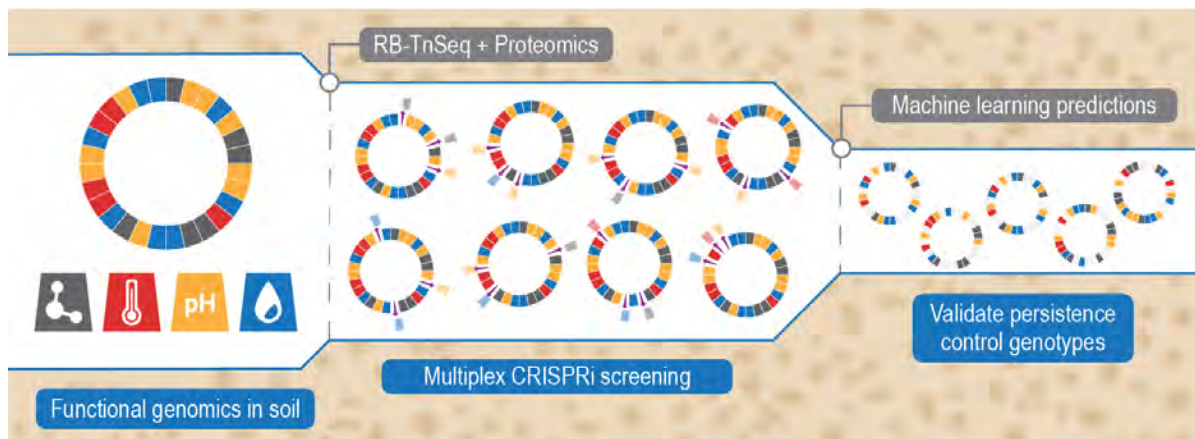
**Project Goals: Our overarching vision is to develop fundamental understanding of what controls the persistence of engineered microbial functions in complex environments. Complex traits are often governed by multiple genes, but no methods exist to predict how remodeling a genome will impact cellular function or govern the conditions under which a microbe will persist. We are developing a data-driven genome reduction strategy to control persistence across environmental variations such as temperature or pH, focusing on two closely related bacteria: *Pseudomonas putida*, which is of high value to industrial biotechnology; and *Pseudomonas fluorescens*, a plant growth promoting bacterium.**

Prediction and control of the persistence of recombinant microorganisms in the environment are key to threat assessment across the biodefense enterprise. Effective biocontainment strategies based on this fundamental understanding will enable DOE Biosystems Design research to advance from the laboratory to the field. Key knowledge gaps in controlling microbial persistence in soil are how genes function in those conditions and how gene networks contribute to complex phenotypes<sup>1</sup>. Our team's recent research is beginning to address these gaps through high-throughput genetics<sup>2</sup>, synthetic biology chassis development<sup>3,4</sup>, multi-omics characterization of soil communities<sup>5</sup>, and machine learning applied to biological systems design. We have also recently elucidated the impacts of genome reduction on the persistence of violacein bioproduction in *E. coli* and are poised to extend this research to new microbes and new environments. This work



**Figure 1.** Developing fundamental understanding of how disparate genes contribute to complex phenotypes will enable effective control of persistence. (A) Existing biocontainment strategies rely on loss of function phenotypes that can be bypassed by environmental uptake of complementing DNA or metabolites. (B) A fundamental understanding of the genetic determinants of persistence will enable effective, condition-specific biocontainment through genome reduction. Removing multiple genes of environmental importance are less likely to be overcome by complementation.

leverages expertise from the PNNL Soil Microbiome Scientific Focus Area (SFA), which is funded to model and predict microbial dynamics in grassland soils.



**Figure 2.** This work applies expertise in functional genomics, global proteomics, synthetic biology, and machine learning to enable validation of genome reduction as an effective strategy to control persistence. Gene-level conditional essentiality scores based on random-barcode transposon sequencing drive the design of pooled combinatorial multi-gene CRISPR-Cas repression assays. These assays and data-driven machine learning inferences will guide the engineering of conditional persistence phenotypes in soil environments.

This project integrates tools from functional genomics, global proteomics, and synthetic biology with machine learning to identify and validate the genetic determinants of microbial persistence in soil. The primary outcomes of this project will be (1) to elucidate gene function across environmental perturbations in soil via high-throughput genetics and proteomics, (2) to develop high-throughput genome editing tools for a plant growth-promoting bacterium, and (3) to create machine learning approaches that synthesize genome remodeling designs from multi-omics inputs. Ultimately, these efforts will advance science critical to engineering enhanced stability, resilience, and controlled performance in DOE-relevant biological systems.

*This work is funded as a pilot project through the Secure Biosystems Design program within the Office of Biological and Environmental Research of the Department of Energy (proposal #0000246830).*

## References

1. Venturelli, O. S., Egbert, R. G., & Arkin, A. P. (2016). Towards engineering biological systems in a broader context. *Journal of molecular biology*, 428(5), 928-944.
2. Price, M. N., Wetmore, K. M., Waters, R. J., Callaghan, M., Ray, J., Liu, H., ... & Carlson, H. K. (2018). Mutant phenotypes for thousands of bacterial genes of unknown function. *Nature*, 557(7706), 503.
3. Egbert, R. G., Rishi, H. S., Adler, B. A., McCormick, D. M., Toro, E., Gill, R. T., & Arkin, A. P. (2019). A versatile platform strain for high-fidelity multiplex genome editing. *Nucleic acids research*, 47(6), 3244-3256.
4. Elmore, J. R., Furches, A., Wolff, G. N., Gorday, K., & Guss, A. M. (2017). Development of a high efficiency integration system and promoter library for rapid modification of *Pseudomonas putida* KT2440. *Metabolic engineering communications*, 5, 1-8.
5. Hultman, J., Waldrop, M. P., Mackelprang, R., David, M. M., McFarland, J., Blazewicz, S. J., Harden, M.R., Turetsky, A.D., McGuire, M.B., Shah, N.C., VerBerkmoes, N. C., Ho Lee, L., Mavrommatis, K., Jansson J.K. (2015). Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes. *Nature*, 521(7551), 208.