From Sequence to Cell to Population: Secure and Robust Biosystems Design for Environmental Microorganisms

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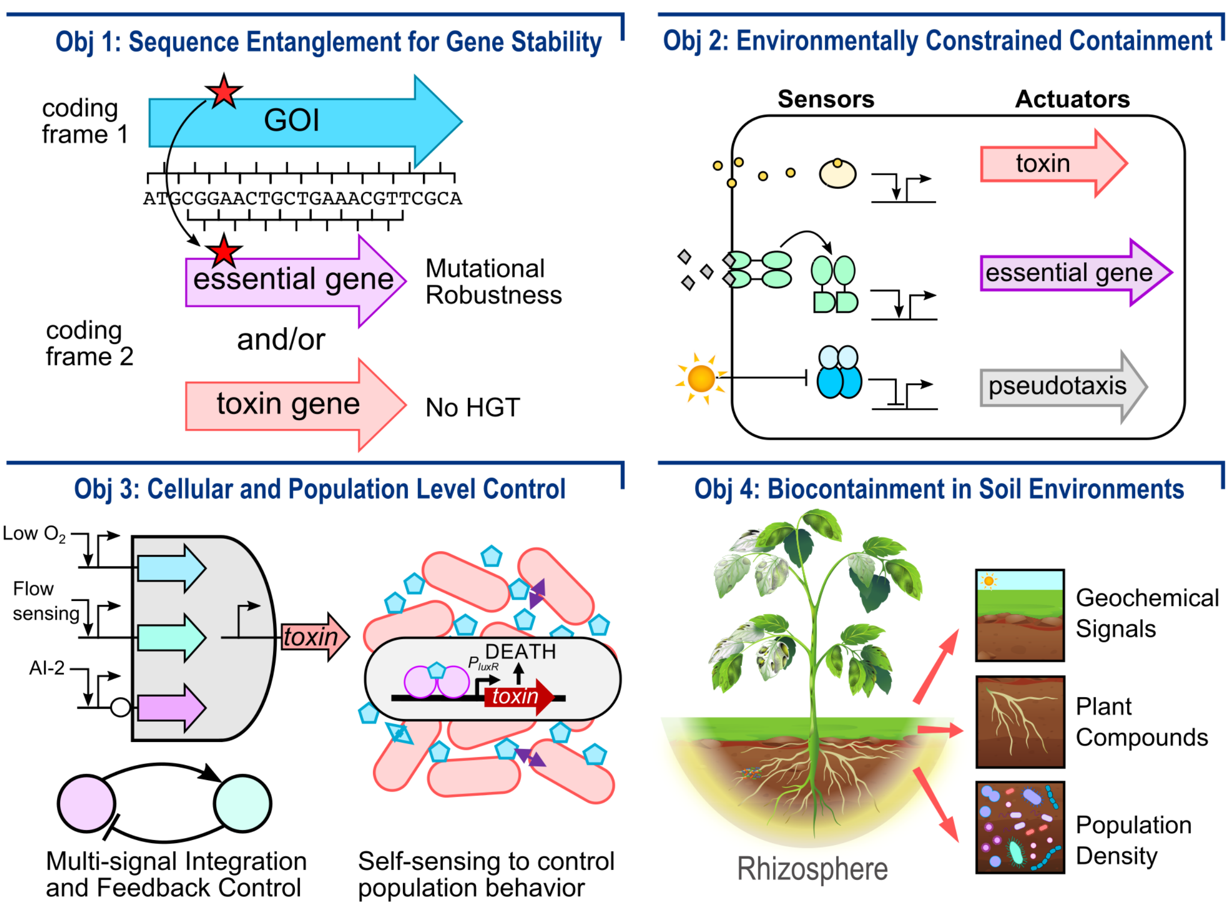
https://genomicscience.energy.gov/research/sfas/llnlseqcellpop.shtml

Project Goals:

Establish robust, generalizable biocontainment strategies in environmentally relevant soil microbes at the sequence, cellular, and population levels.

1) Establish sequence entanglement as a generalizable strategy to improve genetic stability and to prevent horizontal gene transfer
2) Build robust containment circuits that are resistant to mutation and responsive to *in situ* environmental cues
3) Implement cellular- and population-level containment mechanisms that are robust to environmental perturbation
4) Gain a system-level understanding of the genomic and phenotypic response of secure engineered organisms in soil and rhizosphere environments



Abstract: Genetically engineered microorganisms (GEMs) hold significant promise for establishing a sustainable bioeconomy. However, the lack of robust and generalizable biocontainment strategies hinders technology adoption and public trust. To reduce the risk of unintended ecological consequences from environmentally deployed GEMs, built-in security mechanisms are needed to ensure that GEMs function where and when needed without proliferating beyond target conditions. Here, we develop robust, generalizable biocontainment strategies in environmentally relevant soil microbes at the sequence, cellular, and population levels. Leveraging Lawrence Livermore National Laboratory's high-performance computing (HPC) and high-throughput gene-editing capabilities, we are advancing a synthetic gene entanglement concept for containment. Here, two genes are encoded within different coding frames of the same sequence space to protect engineered functions against mutational inactivation and/or to mitigate the horizontal transfer of potentially invasive genes. Building on this layer of sequence stability, we design sense-and-respond circuits that constrain the survival and function of plant-benefiting microorganisms to their target application environments. Control strategies, such as sequestration-based feedback control, multi-signal integration, pseudotaxis, and quorum sensing-based population coordination will be incorporated to increase the overall system robustness to environmental fluctuations that are expected in environmental applications. We leverage LLNL's rich experience in soil microbial ecology to evaluate the ecological effects of these containment mechanisms in soil and rhizosphere environments. Ultimately, our results will yield safeguard mechanisms that control the niche-specific function of GEMs and prevent the transfer of potentially "invasive traits" to neighboring native microorganisms, thereby enabling safer and more effective use of GEMs in environmental applications.

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