IMAGINE BioSecurity: Biocontainment Efficacy of Toxin-Antitoxin Cassettes in Laboratory and Industrial *S. Cerevisiae*

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https://genomicscience-qa.ornl.gov/research/sfas/nrelimagine.shtml

Project Goals: The IMAGINE BioSecurity initiative aims to develop genome-scale engineering tools to test fundamental principles that drive biological systems, with the specific goal of conferring enhanced stability, resilience, and controlled performance in DOE-relevant plant and microbial systems. Specifically, this task will design and develop a library of biocontainment modules in *Saccharomyces cerevisiae* and experimentally analyze the growth, escape frequency, and bioproductivity of the engineered strains using high-throughput screening analyses in laboratory and environmental settings.

Abstract: Genetically modified organisms are widely used to produce a variety of bioproducts and fuels. With the increasingly sophisticated genetic engineering used to produce these genetically modified organisms comes the elevated risk of environmental escape. To establish a secure bioeconomy, new biocontainment strategies must be developed and deployed to maintain optimal microbial fitness and production while minimizing the risk of escape. *Saccharomyces cerevisiae,* often referred to as budding yeast, are a highly studied model organism commonly used in the industrial production of various fuels and bioproducts. In order to establish secure biocontainment designs in *S. cerevisiae*, the IMAGINE BioSecurity SFA is pursuing the high-throughput design and screening of a library of toxin-antitoxin biocontainment modules to determine how they affect fitness, productivity, and escape frequency in laboratory (BY4171) and Brazilian (PE2) industrial *S. cerevisiae* strains to inform and improve future biocontainment designs. We hypothesize stacking biocontainment modules will increase efficacy while providing insights into the mechanisms governing biocontainment. Here we report the effects of biocontainment copy number on efficacy and bioproduction finding a two layered system provides longer term biocontainment while not altering ethanol production.

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