Abstract
The goal of this project is to characterize and evaluate UM431 which is tetracycline inducible Orn that can be used as a selection tool to identify ligand dependent mutants from a large DNA library with the end goal of generating a synthetic auxotroph E. coli strain. Experimental results demonstrate that the expression of wild-type Orn rescues cell survival while the mutant Orn does not, with 500 fold difference in the survival rate which proves the suitability of the strain for selection.

Introduction
As the application for synthetic biology is growing, the concern about the environmental containment of engineered microorganisms used in this field is growing as well. E. coli is one of the most widely used experimental microorganisms in the field of synthetic biology, therefore, the environmental containment regarding an engineered E. coli is warranted and that motivates the synthetic biologist to create a biosafety strain that its survival is conditional and dependent to some inexpensive chemical ligands.

Designing a Biosafety Strain
Creating a biosafety strain can be achieved by engineering on a homodimeric essential genes in E. coli by mutation in proteins that cannot survive without adding small molecules. A mutant homodimeric essential gene can only dimerize in the presence of synthetic ligands. The specific gene that we work with is oligoribonuclease, which is a very important factor in regulation of gene expression by degrading mRNA.

Experimental Approach
The conditional E. coli Orn mutant (strain UM341) has an inducible promoter that uses tetracycline to express Orn which helps it to survive. This strain can be used to identify the ligand dependent Orn plasmids. My hypothesis is that the wild-type Orn plasmid will rescue UM431 leading to survival in the absence of tetracycline, but a non-functional mutant Orn will not.

Experimental Result
Both wild-type and mutant plasmids were transformed into E. coli cell and after the colony PCR and gene sequencing confirmed the right sequence of the plasmids, they were transformed into UM431 strain.

Transformation into Bactria Cell:

Transformation into UM431:

Conclusion
As a result, UM431 strain is suitable for selection as wild-type Orn rescues cell survival and mutant Orn does not in the absence of tetracycline.

Reference:

Support Information
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