A "Marionette" *S. cerevisiae* Strain to Control Metabolic Pathways

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Project Goals: Optimizing metabolic networks often requires fine-tuning of gene expression levels to minimize buildup of toxic intermediates while maximizing productivity. Inducible promoters are a straight-forward strategy to systematically test different expression levels, providing levers to independently control targeted genes. However, the limited availability of orthogonal transcriptional sensors in the yeast, *Saccharomyces cerevisiae*, hinders their use to optimize an engineered biosynthetic pathway. Our objective is to expand the set of inducible promoters and develop a "Marionette" yeast strain, containing a genome integrated array of optimized sensors.

We have taken steps towards this "Marionette" strain by constructing and testing an initial set of 4 orthogonal sensors, engineered by placing bacterial operator elements into yeast core promoters. We then demonstrate "Marionette" in yeast by tuning a toxic metabolic pathway to produce the monoterpene Linalool, a valuable fragrance and fuel additive. Initially, a two-level factorial experiment was performed to uncover expression rules of the targeted genes. By incorporating these rules, we performed a second optimization round. Overall, this pilot test of expression profiles allowed us to explore the equivalent of ~300 kb of pathway variant constructs with a single genetic design. Finally, we also demonstrate staging order of operations on the controlled genes.

The ability to establish a synthetic metabolic pathway control to independently tune component genes will accelerate metabolic engineering cycles in yeast, enabling rapid testing of multiple expression levels that ultimately can be used to train learning algorithms and uncover rules for optimal pathway flux.

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