Combinatorial Metabolic Engineering Using an Orthogonal Tri-functional CRISPR System

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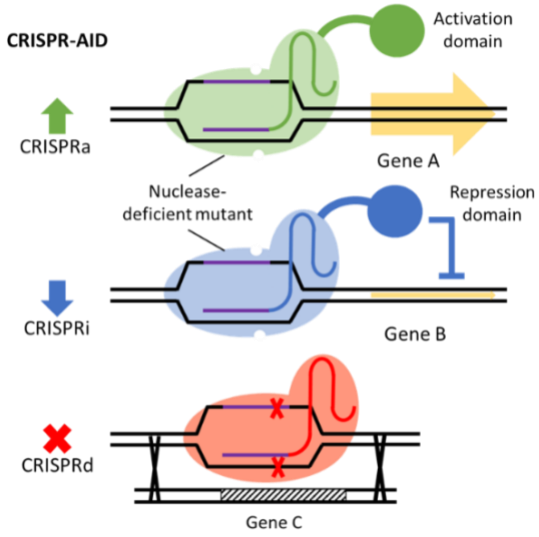
Project Goals: We aim to enable access to a complete range of expression profiles for any gene or combination of genes in the yeast *Saccharomyces cerevisiae* by using a tri-functional CRISPR/Cas system to perform simultaneous, multiplexed gene activation, interference, and deletion. This will enable metabolic engineering to systematically optimize phenotypes of interest through a combination of gain, decrease, and complete loss of functional mutations.

Designing an optimal microbial cell factory typically requires overexpression, knock-down, and knock-out of multiple gene targets. Unfortunately, such rewiring of cellular metabolism is often carried out sequentially and with low throughput. We report a combinatorial metabolic engineering strategy utilizing an orthogonal tri-functional CRISPR system that combines transcriptional activation, transcriptional interference, and gene deletion (CRISPR-AID) in the yeast *Saccharomyces cerevisiae*. This strategy enables multiplexed perturbation of the metabolic and regulatory networks in a modular, parallel, and high throughput manner.



To implement this system, three orthogonal Cas proteins were utilized: dLbCpf1 fused to a transcriptional activator, dSpCas9 fused to a transcriptional repressor, and SaCas9 for gene deletion. Deletion was accomplished by the introduction of a 20 bp frame-shift mutation using a homology donor on the guide RNA expression vector.

As a proof of concept, we demonstrate the application of CRISPR-AID to increase the production of β-carotene by 3-fold in a single step through the simultaneous activation of *HMG1*, interference of *ERG9*, and deletion of *ROX1*. Additionally, we effected a 2.5-fold improvement in the display of an endoglucanase on the yeast surface by optimizing a 15x18x6 matrix of metabolic engineering targets (activation, interference, and deletion, respectively) in a combinatorial manner.

Publications

1. Lian, J., Hamedirad, M., Hu, S., & Zhao, H. Combinatorial metabolic engineering using an orthogonal tri-functional CRISPR system. *Nature Communications* **8**, 1-9 (2017).

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