**Programming high-order combinatorial genetics with Cas9-mediated gene drive approach for cellular engineering**

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**Project Goals: Deciphering combinatorial genetic modifications required for host chassis optimization is key to creating robust and economical systems to realize the promise of metabolic engineering. Our goal is to develop a high-throughput microbial platform that enables rapid generation of targeted, high-order genetic alterations coupled to single-cell mapping of combinatorial genotypes to identify host genome modifications giving rise to enhanced productions of desired biomolecules.**

Current apporaches to cellular engineering rely on introducing a single genetic alteration into a genome one at a time and then studying its effects of metabolic output. This "one gene at a time" approach for discovering which combination of mutations are best able to increase metabolic output is not only time-consuming and labor-intensive but also restricted in the number and order of genetic combinations that can be tested. Here, we introduce a Cas9-based gene drive framework for constructing pools of cells with combinatorial genotypes in which each cell is characterized by *N* combinations of defined genetic alteractions, along with a high-throughput method for genotype-phenotype mapping. We demonstrate the utility of our approach in rapidly swapping promoters of 19 target genes with 7 promoters representing a continuum of gene expression level and identifying all the combination of genetic manipulations that will result in high-level production of the carotenoid lycopene. Our strategy allows high-order combinatorial genetics to be explored in a high-throughput targeted manner, and greatly speed up the rate at which we are able to optimize cellular chassis to produce valuable metabolites for use in consumer, biomedical and industrial applications.

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