Genome-Scale CRISPRi in the Rapidly Growing Cyanobacterium *Synechococcus* sp. PCC 7002

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Project Goals: Our objective is to accelerate the engineering of designer organisms with traits amenable to sustainable biochemical production. By devising functional genetic screens using a genome-scale CRISPRi library in the photoautotrophic cyanobacterium *Synechococcus* sp. PCC 7002, we seek to: i) identify gene targets for maximizing short chain alcohol synthesis, ii) enable CRISPR-based trackable genome engineering (CREATE) experiments[1], and iii) uncover light-responsive signaling pathways in a host capable of direct CO_2 capture.

Abstract text: Sustainable bioenergy production in microbes requires deep understanding of a host's native biological functions and the development of tools and design principles necessary to engineer production at sufficient titers. While these objectives have been heavily investigated in model organisms (e.g., E. coli, S. cerevisiae), similar development in chassis organisms capable of efficient conversion of sustainable feedstocks has lagged behind. Synechococcus sp. PCC 7002 (PCC 7002) has emerged as a choice host for biochemical production thanks to its rapid growth rate and direct conversion of CO2 into products through photosynthesis. This work seeks to establish genome-scale CRISPR interference (CRISPRi) in PCC 7002, revealing uncharacterized gene functionalities, validating gRNA activities, and enabling downstream design and engineering of synthetic regulatory networks and hybrid control structures for enhanced production of industrially relevant short chain alcohols. The library will initially be screened across light wavelengths as a means of broadening understanding of light and color perception in PCC 7002, facilitating development of tunable production phenotypes using novel optogenetic control systems active across the light spectrum.

References:

1. Garst, A., Bassalo, M., Pines, G. *et al.* Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering. *Nat Biotechnol* **35,** 48–55 (2017). https://doi.org/10.1038/nbt.3718

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