Genetic Tools for Photosynthetic Microbes; Toward Genome-scale Engineering

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Project Goals: Expand recombineering techniques into model cyanobacteria, and explore recoding and pooled screening using these techniques. Explore new photosynthetic microbial systems and work to characterize new genetic tools.

Tremendous genetic variation both exists in nature and is designed by genome refactoring and recoding efforts, but our ability to create and characterize genetic variants *in vivo* remains far more limited in scale. Facile genetic editing along with pooled screening approaches enable characterization of millions of genetic loci[1], but have yet to be applied more broadly outside of our best-characterized prokaryotic hosts. One focus of our efforts to deploy these tools more broadly are photosynthetic microbes, which serve both as capable models for understanding photosynthesis, and as a photosynthetic bioenergy chassis.

To explore recombineering approaches in photosynthetic organisms, we are focusing on UTEX2973 as a fast-growing and well-characterized host organism[2]. By searching both curated protein databases and metagenome datasets, we are able to locate phage recombination systems (similar to the canonical lambda-RED used in *E. coli*) within phage predicted to infect cyanobacteria, overcoming the host-range limitations of these systems[3-5]. We are testing these recombination systems in UTEX2973 to improve the efficacy of genome engineering and enable ssDNA recombineering approaches in cyanobacteria for the first time.

In tandem, we were inspired by other work isolating novel, fast-growing cyanobacterial models[6-8] to prospect for new photosynthetic microbes growing quickly in lab conditions. We have isolated several such new organisms and are further characterizing their genomes and phenotypes. This work can synergize with more detailed exploration of existing isolates, and to this end we are working with the Pakrasi and Wangikar labs to identify elements of interest in fast-growing cyanobacterial isolates, including novel retrons and episomes.

This work aims to pave the way for pooled screening in phototrophs, recoding efforts in phototrophs, and to inform future efforts to bring recombineering techniques into new prokaryotic hosts.

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