m-CAFEs Phage Engineering for Targeted Editing of Microbial Communities

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Project Goals: Understanding the interactions, localization, and dynamics of grass rhizosphere communities at the molecular level (genes, proteins, metabolites) to predict responses to perturbations and understand the persistence and fate of engineered genes and microbes for secure biosystems design. To do this, advanced fabricated ecosystems are used in combination with gene editing technologies such as CRISPR-Cas and bacterial virus (phage)-based approaches for interrogating gene and microbial functions *in situ*—addressing key challenges highlighted in recent DOE reports. This work is integrated with the development of predictive computational models that are iteratively refined through simulations and experimentation to gain critical insights into the functions of engineered genes and interactions of microbes within soil microbiomes as well as the biology and ecology of uncultivated microbes. Together, these efforts lay a critical foundation for developing secure biosystems design strategies, harnessing beneficial microbiomes to support sustainable bioenergy, and improving our understanding of nutrient cycling in the rhizosphere.

Abstract: Investigation of microbial gene function is essential to elucidation of the ecological roles and complex genetic interactions that take place in microbial communities. While microbiome investigations have increased in prevalence, the lack of viable *in situ* editing strategies impedes experimental design and progress, hindering genetic manipulation of microbial communities and genetic discovery in community contexts. Here, we demonstrate the utility of phage-delivered CRISPR-Cas payloads to perform targeted genetic manipulation within a community context deploying a fabricated ecosystem (EcoFAB) as an analog for the soil microbiome. We provide a roadmap for engineering phages T7 and λ for community editing using Cas9-mediated recombination in non-essential genes. We further engineer λ to deliver

antibiotic resistance and fluorescent genes to an *Escherichia coli* host. Expanding on this platform, we engineer λ to express an APOBEC-1-based cytidine base editor (CBE), which we leverage to perform C to T point mutations guided by a nuclease-deficient Cas9 (dCas9). We strategically introduced these base substitutions to create premature stop codons in-frame, inactivating both chromosomal (*lacZ*) and plasmid-encoded genes (mCherry and ampicillin resistance) without perturbing the surrounding genomic regions and maintaining host viability. Further, using a multi-genera synthetic soil community, we employed phage-assisted base editing to induce host-specific phenotypic alterations in a community context both *in vitro* and within the EcoFAB, observing editing efficiencies from 10% to 28% across the entire bacterial population. Future work aims to harness the enzymatic diversity of CRISPR-Cas systems to expedite and innovate on phage engineering workflows. The concurrent use of Cas nucleases, a synthetic microbial community, soil matrix, and EcoFAB device provides a controlled and reproducible model to more closely approximate *in situ* editing of the soil microbiome.

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