Targeted DNA Editing Within Microbial Communities

Benjamin E. Rubin[1](brubin@berkeley.edu), Spencer Diamond[1], Brady Cress[1], Trenton K. Owens[3], Christine He[1], Alex Crits-Christoph[1], Zeyi Zhou[1], Michael Xu[1], Kimberly Tang[1], Dylan C. Smock[1], N. Louise Glass[3], Rodolphe Barrangou[2], **Jillian Banfield[1], Jennifer A. Doudna[1],** Adam M. Deutschbauer[3], and Trent R. Northen[3]

[1]University of California, Berkeley; [2]North Carolina State University, Raleigh; [3]Lawrence Berkeley National Laboratory, Berkeley.

http://mCAFEs.lbl.gov

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.

Our functional understanding of microbial DNA is predominantly founded on the principles of isolate genetics, where the effects of genetic manipulations on cultivable organisms are observed in isolation. Unfortunately, this provides limited insight into the workings of genes in the complex and societally relevant microbial communities that exist in nature. In order to move beyond the paradigm of manipulating microbes in confinement, we have created a generalizable toolset for targeted genome editing of individual organisms within complex microbial communities. First, we have developed environmental transformation sequencing (ET-Seq) to determine *in situ* which microbes within a community can be edited by untargeted transposases, and with what efficiency. Second, we have repurposed RNA-guided CRISPR-Cas transposases to paste customized DNA into unique target sites within the genomes of specific microbes in a community. Third, we have applied these technologies to track the fitness effects of genetic mutants in communities, as well as for enrichment and isolation of edited organisms. The ability to make organism- and locus-specific changes within microbiomes will lead to improved understanding of microbial communities and enable us to effect meaningful changes within them.

Funding statement.
This material by m-CAFEs Microbial Community Analysis & Functional Evaluation in Soils, (m-CAFEs@lbl.gov) an SFA led by Lawrence Berkeley National Laboratory is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological & Environmental Research under contract number DE-AC02-05CH11231