

## 105. Towards Rapid Characterization of Microbial Communities, Interactions, and Community Assembly

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**Project Goals:** Our major goals are 1) the development of a high-throughput method to accurately measure the absolute abundances of multiple species in microbial assemblages, 2) the identification of microbial interactions amongst cultivated FRC isolates, 3) the demarcation of the fundamental niche of these isolates across multiple environmental dimensions, and 4) a predictive understanding of interactions and niche behavior based on genetic elements.

**Abstract:** Microbial communities are shaped by a complex network of interaction between different species (e.g., competition or mutualism) as well as selection from the environment (e.g., geochemical parameters). Understanding how these forces influence community assembly remains a difficult task—limiting our ability to predict community response to perturbation. Towards this end, we are developing a high-throughput assay to rapidly generate time-resolved measures of absolute abundance of multiple species in co-culture. The method is based on 1) a set of dual-indexed primers amplifying variable regions of the 16S rRNA, and 2) application of a “spike-in” standard organism and species-specific standard curves which allow quantitation of the number of cells of a given organism within each assemblage. The primers used amplify a ~450 bp variable region of the 16S rRNA gene (V4/V5) and contain 5’ adapter sequences with everything necessary for binding and sequencing on Illumina MiSeq and HiSeq platforms. We present a general overview of the efficacy of this approach and the efforts that have been made to reduce both random and systematic bias from the measurement. We further present a demonstration of the method in the context of several co-culture experiments using different numbers of organisms within different environments. Together with time-resolved population measurements, we applied exometabolomic footprinting to explore the mechanisms of interspecific interactions and degrees of resource utilization overlap. Ultimately, we aim to use exometabolomic footprinting and high-throughput genetics together with precise population tracking to understand the mechanisms and principles that shape the ecology of complex microbial assemblages across an increasingly realistic set of controlled environmental parameter regimes.

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