Assays for spatial structure and transdomain dynamics in environmental communities

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Project Goals: Our group focuses on biotechnology development that moves the field of microbial ecology toward complete genomic awareness. Specifically, we use bulk water-in-oil emulsion droplets combined with tailored molecular biology to provide a more comprehensive microscale view of transdomain ecological players and their functional capacity within complex environments.

The bulk activity of microbial communities is composed of the additive effect of microscale interactions between bacteria, viruses, and eukaryotes coexisting within a dynamic environment. These microscale competitive or mutualistic exchanges bridge between the foundational principles of ecology and the global activity of microbial communities that we observe in bulk assays. Before we are able to understand, model, or perturb systems at the macroscopic scale, we need improved methods at the resolution of individual cells. Our group recently developed an emulsion-based droplet assay termed epicPCR (emulsion, paired isolation, and concatenation PCR) to physically link functional genes with phylogenetic indicators within single cells. Here we expand upon this platform to map the physical associations of bacteria with each other and with eukaryotic hosts.

We are beginning to assay biofilm and host-prey structures by capturing small aggregates of cells in nanoliter droplets, then physically linking segments of the 16S rRNA gene between cells. In biofilms a preliminary untargeted assay, trying to link every cell with every other adjacent cell, highly favored only the most abundant strains present. We've now redesigned primer sets that anneal to specific phyla of interest and their physical partners, a semi-targeted version of the assay. With this approach we've recovered library contructs enriched for cells as rare as 1 in 10,000 within complex biofilms. In parallel to assays of bacterial proximity, we've refined the same droplet methodology to capture eukaryotes with their bacterial symbionts and prey. Sequenced co-aggregations between eukaryotes and bacteria in both wastewater and lake water are enriched for predators, heterotrophs, and known symbionts. We plan to apply this approach to Oak Ridge FRC samples, and in preparation we've completed standard eukaryotic and bacterial sequencing from control wells. These structural assays in combination will provide novel microscale data about the complex interchanges connecting bacteria with each other and their broader ecological context.

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