Uncovering spatial taxonomic structures of synthetic microbial communities using subcellular RNA sequencing.

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Project Goals:

This study aims to develop methods for characterizing microbial communities with single-cell and spatial resolution. We are employing fluorescent *in situ* sequencing (FISSEQ) technology for acquiring genomic and transcriptomic information to better understand the spatial arrangement of microbes in natural and synthetic microbial communities.

Most investigations reduce microbial physiology to monoculture conditions, which does not consider their abundant interactions in natural environments. Indeed, much of our understanding of microbes stems from gene deletions, heterologous expression, and *in vitro* enzyme characterization. Microbes need to be studied *in situ* where their spatial organization holds biological importance for responding to the environment (*e.g.*, polymicrobial metabolism, biofilms, horizontal gene transfer). However, *in situ* characterizations of these complex phenotypes in natural environments are generally not feasible at scale and remain largely unknown.

Here, we describe our recent developments for the taxonomic identification of microbes with single-cell and spatial resolution. We have established *in situ* hybridization (ISH)-based probes that can distinguish between sequences with as little as one nucleotide difference and with unique barcodes (i.e., unique molecular identifier [UMI]) for highly-multiplexed *in situ* sequencing readouts. For method development, we use a synthetic community of 3 microbes and target a conserved region of the small ribosomal subunit that differs by a single nucleotide. We find that anchoring probes to a hydrogel matrix provides superior nucleotide specificity compared to *in situ* crosslinking for maintaining spatial localization of signal. Furthermore, we extend this method from requiring target-specific reverse transcription primers to a mixture of randomers that achieves the same signal and specificity but reduces the cost of probe synthesis by half. We also demonstrate simultaneous identification of each community member with a unique barcode, which we readout with sequential nucleotide labelling using fluorescent reversible terminators. Finally, we have created software for the automated design of probes for unbiased taxon discrimination at any given taxonomic level and apply this for discrimination on the Domain and Class level for the tree-of-life, including discriminating mitochondria from bacteria and bacterial classes. This work forms the framework for investigating the taxonomic structure of natural microbial communities with unknown compositions, and forms the foundation for future whole-transcriptome sequencing of microbes *in situ*.

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