

96. A novel method to retain viability of obligate anaerobic microorganisms during single cell sorting

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Anaerobic microorganisms are major components in numerous environments ranging from naturally-occurring subsurface ground waters, anaerobic hydrocarbon deposits, and anoxic waters and sediments to man-made environments such as wastewater treatment plants and anaerobic digesters. Defining the community structure and the functional ecology of these microbial communities is a key component of research campaigns within the ENIGMA project. Flow cytometry is a powerful tool for high-throughput single cell analysis and sorting of targeted individual cells or populations, however preserving the viability of anaerobic cells during cell sorting has remained a challenge. We have developed a novel technology, called Live Anaerobic Cell Sorting (LAnCS), to address this challenge. Modifications made to the BD Influx high-speed cell sorter create an enclosed path cell sorter that is capable of maintaining an anoxic environment from sample to sort collection tube. We tested LAnCS on a synthetic anaerobic community composed of two organisms. Here, we demonstrate that LAnCS was successful in preserving the viability of both members of this anaerobic community, *Desulfovibrio vulgaris* Hildenborough (DvH) and *Methanococcus maripaludis* (Mmp), by growing colonies from single-cell sorts of both organisms on agar plates. This technology will provide the ENIGMA team with a novel tool for addressing campaigns such as Evolution and Assembly of Communities (Stahl), Environmental Microbial Characterization (Deutschbauer), Groundwater Microbiome (Hazen), and Time Series (Zhou). For example, LAnCS could help to investigate the role and importance of low abundance organisms that are not recovered in enrichment cultures, develop strategies to generate viable communities by mixing and matching organisms directly from field samples, and targeting functional groups in complex environmental samples for downstream study of viable cells.

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