28. Single Cell Genomics Applications in ENIGMA

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Project Goals: We are developing a versatile single cell genomics pipeline that can respond to the needs of a multi-institutional program like ENIGMA. Our pipeline utilizes FISH (fluorescence *in situ* hybridization) for targeting species of interest, FACS (fluorescence activated cell sorting) for high throughput isolation of single cells, and MDA (multiple displacement amplification) for production of sufficient DNA for genome sequencing. At present, this pipeline is being used for a number of collaborative projects in ENIGMA.

Single cell sequencing is a powerful tool for the analysis of uncultivated microorganisms. Current culture-independent, population based techniques (i.e., metagenomics) relying on pooled nucleic acids from communities of microorganisms can independently measure metabolic activity and the species present, but cannot link the activity deterministically to the species. In an attempt to unravel the complex dynamics of population, gene expression, and metabolic function in mixed microbial communities, we developed a high-throughput approach to study uncultivable microorganisms one cell at a time. Our approach includes isolation of individual cells by cell sorting, followed by whole genome amplification and sequencing. This pipeline is being utilized to analyze groundwater samples from DOE bioremediation sites (e.g., Hanford 100H, Oak Ridge FRC) to identify keystone organisms and link their functions to species as well as to estimate the level of horizontal gene transfer within the community; to isolate and identify viruses in deep subsurface groundwater, and investigate their role in microbial community structure and function; to assess the composition of bioaggregates in environmental samples with the ultimate goal of verifying the stereotypical configurations of microorganisms.

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