Single-cell Analysis Platforms for Genomic Analysis of Uncultivable Environmental Microbes

Suzan Yilmaz[1*] (syilmaz@sandia.gov), Peng Liu[1], Robert J. Meagher[1], Yooli Light[1], Adam P. Arkin[2], Terry C. Hazen[3], **Anup K. Singh**[1] and Paul D. Adams[2]

[1] Sandia National Laboratories, Livermore, California; [2] Lawrence Berkeley National Laboratory, Berkeley, California; [3] University of Tennessee, Knoxville, Tennessee

http://enigma.lbl.gov

Project Goals: We are developing a pipeline for single cell genomics that 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 from single cells for genome sequencing. This pipeline is being used for a number of collaborative projects in ENIGMA.

Current metagenomic techniques (e.g., microarray or 16S rRNA sequencing) relying on pooled nucleic acids from lysed bacteria can independently measure metabolic activity and the species present, but cannot link the activity deterministically to species. We are developing high-throughput tools for studying bacteria one cell at a time, allowing us to unravel the complex dynamics of population, gene expression, and metabolic function in mixed microbial communities. Our approach includes FISH-based identification of desired species, enrichment by cell sorting, followed by single-cell encapsulation, whole genome amplification and sequencing. Encapsulation of bacteria in nanoliter plugs in particular allows us to scale down conventional (microliter-volume) assays, such as WGA, into much smaller reaction volumes better suited to the size of an individual microbe. We are using this pipeline to analyze water samples from DOE bioremediation sites (e.g., Hanford) to identify keystone organisms and link their functions to species. Furthermore, we are also using our single-cell genomics pipeline to complement the metagenomic sequencing efforts in ENIGMA. Metagenomic sequencing typically fails to achieve complete assembly and metabolic reconstruction of individual genomes in a complex community. Single-cell sequencing, together with metagenomics, makes it possible to assemble genomes of novel uncultivated organisms.

This work conducted by ENIGMA was supported by the Office of Science, Office of Biological and Environmental Research, of the U. S. Department of Energy under Contract No. DE-AC02-05CH11231.