

32. Microbial Characterization in ENIGMA: Flexible Tools for Annotating Genes and Pathways in Environmental Bacteria

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Project Goals: The Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) program broadly seeks to understand the interactions between environmentally relevant microorganisms and their environment. One aim of this large interdisciplinary project is to bring environmental bacteria to model-organism status rapidly to enable systems-level investigations into microbial metabolism, regulation, stress response, and interactions under defined laboratory conditions. In addition to developing scalable, multi-scale, and high-throughput characterization techniques, the ENIGMA Microbial Characterization campaign aims to apply these techniques to answer outstanding questions in microbial ecology.

Our understanding of the roles of microbes in important natural processes is hindered by the lack of tools to study their function at the molecular level. A scarcity of functionally well-characterized microbes means that the genomes of many microorganisms, including those with important roles at the Oak Ridge FRC site, remain poorly annotated. To meet this challenge, the ENIGMA Microbial Characterization campaign is developing and applying methods for the flexible and inexpensive characterization of virtually any culturable prokaryote. Here, we present an overview of our technical developments and how we are using these approaches to address ENIGMA science.

As mutant phenotypes provide insight into gene function by providing a direct link between genes and phenotypes, we have developed a next-generation strategy, termed random barcode transposon insertion sequencing (RB-TnSeq), for assaying the mutant fitness of thousands of strains in parallel. RB-TnSeq takes advantage of two recent ENIGMA advances, transposon liquid enrichment sequencing (TnLE-seq; PMID 24077707) for the generation of mutant libraries and DNA barcode sequencing (BarSeq) for assaying mutant fitness. To demonstrate the reproducibility and scalability of RB-TnSeq, we present data from over 200 experiments in the denitrifying environmental isolate *Pseudomonas stutzeri* RCH2. As many single gene mutations do not result in a strong phenotype under laboratory conditions, we are also developing a flexible strategy for screening genetic interactions (double mutants), using the sulfate-reducing bacterium (SRB) *Desulfovibrio vulgaris* Hildenborough as a pilot. Lastly, to complement the high-throughput genetics data, we are pairing mutant fitness assays with untargeted metabolomics to link genes to specific metabolites to physiological role. Using this combined genetics and metabolomics approach, we have identified a novel metabolite (and the encoding genes) required for the SRB *Desulfovibrio alaskensis* G20 to grow under salt stress.

As part of the Microbial Characterization campaign, we are using the developed genetic and metabolomics approaches to address two hypotheses: (1) The function of many genes can only be assessed under conditions that closely mimic the natural environments from which the microorganisms are isolated, and (2) Core species are present across several Oak Ridge FRC sites, regardless of geochemistry, and the

adaptations of these ubiquitous clades to diverse environments are due to differences in gene content, protein activity, novel metabolic pathways, and gene regulation. In addition, we are investigating the molecular basis of key microbial metabolisms at the FRC site, with a current focus on the oxidation of dissolved organic matter (humics). Lastly, the data, tools, and genetic resources generated by the Microbial Characterization campaign are being used in a number of collaborative ENIGMA projects to investigate metal metabolism, microbial interactions, and gene regulatory networks.

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