

99. High-throughput genetic characterization of 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 rapidly bring environmental bacteria to model-organism status to enable systems-level investigations into microbial metabolism, regulation, stress response, and interactions under defined laboratory conditions. Here, we describe our development of random barcode transposon site sequencing, a high-throughput tool for screening of bacterial phenotype, and its application for gene function discovery in diverse environmental bacteria.

Transposon mutagenesis with next-generation sequencing (TnSeq) is a powerful approach to annotate gene function in bacteria, but existing protocols for TnSeq require laborious preparation of every sample before sequencing. Thus, the existing protocols are not amenable to the throughput necessary to identify phenotypes and functions for the majority of genes in diverse bacteria. Here we present a method, random barcode transposon-site sequencing (RB-TnSeq), that increases the throughput of mutant fitness profiling by incorporating random DNA barcodes into Tn5 and mariner transposons and by using barcode sequencing (BarSeq) to assay mutant fitness. RB-TnSeq can be used with any transposon and TnSeq is performed once per organism instead of once per sample. Each BarSeq assay requires only a simple PCR and 48-96 samples can be sequenced on one lane of Illumina HiSeq. We demonstrate the reproducibility and biological significance of RB-TnSeq with *Escherichia coli*, *Phaeobacter inhibens*, *Pseudomonas stutzeri* RCH2, *Shewanella amazonensis* SB2B, and *Shewanella oneidensis* MR-1. To demonstrate the increased throughput of RB-TnSeq, we performed 387 successful genome-wide mutant fitness assays and identified 5,196 genes with significant phenotypes across the five bacteria. To support ENIGMA science, we have applied RB-TnSeq to 3 genetically diverse *Pseudomonas* strains isolated from the ORNL iFRC site and identified phenotypes for over 1,000 genes from each isolate. Lastly, in collaboration with ENIGMA researchers, we are applying RB-TnSeq to dissect mechanisms of microbial interactions, identify genetic determinants of metal metabolism, and identify epistatic (genetic) interactions.

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