Functional Characterization of Microbial Genomes by Tagged Transposon Mutagenesis

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A primary goal of the Environmental Stress Pathway Project (ESPP) is a systems-level model of sulfate-reducing bacteria (SRB) metabolism, stress responses, and gene regulation. However, current systems-level analyses of less studied bacteria such as SRBs are limited by the presence of numerous uncharacterized genes and an over reliance on annotations from well studied bacteria such as *E. coli*. Therefore, it is imperative that rapid and quantitative methods are developed to determine microbial gene function in a high-throughput manner. To meet this challenge, we are developing a mutagenesis and phenotyping strategy that is comprehensive across the genome and applicable to any microorganism amenable to transposon mutagenesis. We have cloned and sequence-verified 4280 tag modules into a Gateway entry vector. Each tag module is a 175 base pair element containing two unique 20 base pair sequences, the UPTAG and DOWNTAG, flanked by common PCR priming sites. Each tag module can then be rapidly transferred *in vitro* to any DNA element, such as a transposon, that is made Gateway compatible. Transposon mutants marked by the modules will be sequenced to determine which of the 4280 tag modules was used and which gene was disrupted. Transposon mutants can be rapidly re-arrayed into a single pool containing 4280 uniquely tagged, sequence-verified mutant strains. By sequencing saturating numbers of transposon mutants, we can identify and assay mutants in most nonessential genes in a given genome. The fitness of each mutant in the pool will be monitored by the hybridization of the barcodes to an Affymetrix microarray containing the tag complements in a system identical to that used for the yeast deletion collection. To facilitate both strain construction and mutant pool phenotyping, we have implemented a robotic infrastructure (both aerobic and anaerobic) for assay setup and automated mutant pool growth, collection, and processing. Here we describe the initial application of our approach to the metal-reducing bacterium *Shewanella oneidensis* MR1 and the SRBs *Desulfovibrio desulfuricans* G20 and *Desulfovibrio vulgaris* Hildenborough.

The successful completion of this project will enable the quantitative phenotypic analysis of thousands of mutants across a wide range of conditions. These data will be used to assign gene function on a global scale, aid in the identification of missing metabolic enzymes, provide insight into the functional connectivity of different pathways, and enable the construction of genome-wide models of SRB function and activity.