103. Screening for Genetic Interactions in Desulfovibrio vulgaris Hildenborough

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Project Goals: The Biotechnology component of ENIGMA seeks to develop and apply a suite of technological tools in order to expand our capability to obtain systems-levels insights into microbial activity. Such insights will form the foundation for predictive models of key microorganisms from a single environment that will serve as valuable resources for assessing ecological questions relevant to microbial community structure and function. Using environmentally relevant metal and sulfate-reducing bacteria, ENIGMA researchers have developed tools which enable evidence-based annotation of gene function using high-throughput mutagenesis and extensive phenotyping of mutants under stress conditions; evidence-based annotation of transcripts using tiling microarrays and RNAseq; protein complex isolation and identification; mass spectrometry-based proteomics and metabolomics analysis; and high resolution imaging. Here we expand our capabilities by applying a recently developed sequencing based fitness profiling approach (RB-TnSeq) to rapidly screen for genetic interactions (fitness changes in double mutant strains) across multiple growth conditions.

Gene fitness profiling of single mutant strains can routinely identify a statistically significant phenotype for many genes. However, many mutants display only subtle phenotypes which are hard to interpret. This phenomenon is likely attributable to partial functional redundancy between genes in different pathways masking effects of single mutations. Genetic Interactions (GI) describe epistatic relationships between genes and are the basis for functional redundancy between specific pathways and processes within a cell. A common way to screen for genetic interactions is to introduce a second mutation into a collection of single mutants and to assess the compounded effect of the second mutation. Combinations of mutations resulting in a greater or reduced fitness defect compared to either single mutation alone are genetically interacting. Genetic interactions can therefore not only result in stronger, more specific fitness defects due to removal of redundant processes, but may provide more detailed information about gene function such as the order in which genes function in a pathway. We are utilizing a TnSeq-based procedure in which each transposon contains a unique 20 nt molecular barcode (RB-TnSeq; A. Deutschbauer). When applied to strains containing mutations, this approach will allow us to generate double mutant libraries rapidly and detect both synthetic lethal and conditional GI by monitoring the abundance of barcodes under stress conditions. Initial experiments with Desulfovibrio vulgaris Hildenborough (DvH) wild type have successfully resulted in a library with 329,755 transposon insertions across 181,020 distinct locations. A total of 303 genes were found to have no transposon insertion within 5-85% of their coding sequence and were considered as potentially essential genes. We will present a comparison of these data with results from previous methods (Fels et al. AEM 2013). The DvH genome encodes at least three formate dehydrogenase (FDH) isozymes (DVU0587-0588, DVU2481-2482 and DVU2811-2812) and FDH activity is essential for cells to grow using formate as a primary carbon source. A pilot study is ongoing using strains harboring unmarked deletions (JW710 ∆upp background) in these FDH enzyme-encoding genes clusters. We have performed preliminary experiments to generate double mutant libraries and have observed synthetic lethal GI based on the differences observed between DvH wild-type and JW710 strains. Indeed, we have been unable to interrupt a number of genes annotated as being involved in de novo synthesis of UMP in JW710-based strains. The inviability of these double mutants is likely to be the result of synthetic lethal GI between these genes and upp which encodes uracil phosphoribosyltransferase,
a key enzyme in the salvage pathway for UMP synthesis from uracil. This provides a gold standard, positive control for further GI screening in the FDH mutant background and additional markerless deletion mutants.

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