DEVELOPMENT OF GENOME-WIDE GENETIC ASSAYS IN *DESULFOVIBRIO VULGARIS* HILDENBOROUGH

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ABSTRACT

Illumina® and other massively-parallel sequencing technologies have become prevalent methods for querying genetic systems. This trend was initially driven by the demand for sequencing eukaryotic genomes, but uses for these instruments have recently grown to include more specific assays and methods intended for use in prokaryotic systems. This thesis seeks to develop two such assays for use in the sulfate reducing bacteria type species *Desulfovibrio vulgaris* Hildenborough. These novel assays continue advances made in other bacteria, and the use of this environmentally relevant anaerobe will ensure their extension to other bacteria outside the few studied previously. The first assay is a variation on existing transposon sequencing (Tn-seq) assays, which seek to determine gene fitness profiles and essential genes by simultaneous correlation of loss of each gene product with altered growth kinetics of the bacterium. Here we modify the standard Tn-seq procedure by including delivery of the transposon through conjugation and liquid culture enrichment of the mutant pool, creating transposon liquid enrichment sequencing (TnLE-seq). This simplifies and shortens the process, while reducing barriers to application of the technique in microbes lacking a facile genetic system. The second assay maps consensus 3' end sites of RNA transcripts across a reference genome. This method is known as 3' RNA-seq and compliments an established technique for mapping 5' start sites. Together these facilitate the application of current sequencing technology to a wider array of microbes and a new type of biologically relevant genetic data.