Public Abstract
First Name:Samuel
Middle Name:Ronald
Last Name:Fels
Adviser's First Name:Judy
Adviser's Last Name:Wall
Co-Adviser's First Name:
Co-Adviser's Last Name:
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Illumina sequencing and other massively-parallel, short read sequencing technologies have become prevalent methods for querying the genetic systems of organisms. This trend was initially driven by the demand for general de novo and resequencing applications in eukaryotes, but uses for these instruments have recently grown in scope to also 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 obligate anaerobe will ensure their extension to other bacteria outside the easily-manipulated groups 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 analyses of whether the absence of each gene product alters the 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, and also reduces barriers to application of the technique in microbes lacking a facile genetic system. The second assay provides a means of mapping 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 determining 5’ start sites of transcripts. Together these facilitate the application of current sequencing technology to a wider array of microbes and a new type of biologically relevant genetic data.