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Characterization of transposon insertion mutants in *desulfovibrio vulgaris* hilderborough by sequencing genomic DNA

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Tn5 transposon mutagenesis occurs by a mechanism in which a segment of DNA (transposon) encoded in a plasmid is inserted into genomic DNA (the target) by a conservative (cut-and-paste) mechanism. When the insertion position is in a coding sequence or regulatory region of DNA, the insertion results in a mutation. The plasmid pRL27 encodes a mini-Tn5 transposon, Tn5 transposase, and kanamycin resistance, (Metcalf, William W. et al, 2002 Arch Microbiol 178 :193–201) and was used to transform *Desulfovibrio vulgaris* Hildenborough by electroporation. Transposon insertion mutants were identified by their ability to grow in the presence of kanamycin. To identify the insertion sites of the transposons, in theory one should be able to sequence from the transposon into chromosomal DNA and identify the mutation site by comparison with the known genome. Unlike sequencing of plasmid DNA or PCR products, direct genomic sequencing has a limited success rate. Direct genomic sequencing is sensitive to DNA quality, interference of secondary DNA structures, salt concentration, and the availability of primer binding sites. Because of these difficulties, in our attempts to identify insertion sites of mini-Tn5, we examined template DNA quality as well as modifying sequencing reaction conditions. Our objective is to develop an effective, reliable method for sequencing genomic DNA to identify where transposon insertion sites have occurred in each mutant.