Matt Shirley

Biochemistry
Year in School: Senior
Hometown: Gladstone, Mo.
Faculty Mentor: Dr. Judy Wall, Biochemistry
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Rapid automated characterization of transposon insertion mutants in Desulfovibrio vulgaris Hildenborough by srnPCR

Matthew D. Shirley, Barbara J. Giles, Joseph A. Ringbauer, & Judy D. Wall

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 (Fig. 2). When the insertion position is in a coding sequence or regulatory region of DNA, the insertion results in a mutation. The plasmid pRL27 (a generous gift from Bill Metcalf) encodes a mini-Tn5 transposon, Tn5 transposase, and kanamycin resistance (neo), 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 locate the insertion site of the transposon, in theory, one should be able to directly sequence from the transposon into chromosomal DNA (Fig. 3.1) and identify the mutation site by comparison with the known genome BLAST. Unlike sequencing of plasmid DNA or PCR products, direct genomic sequencing has a limited success rate. Therefore, a method of enriching the transposon-flanking sequence is needed. Nested semi-random PCR (Fig. 3.2) is an efficient and cost effective enrichment method. Sequencing these enriched products allows us to identify the transposon insertion site. The factors that influence characterization success rate are: frequency and location of priming sites, reaction volume, and reaction conditions (annealing temperature, extension time, etc.). By varying these factors, we have developed an efficient and reliable method for characterizing transposon insertion mutants. Utilizing high-throughput robotics and nested semi-random PCR, we have generated single gene mutants that may provide valuable biological data.