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Genome-wide gene deletions in Streptococcus sanguinis by high throughput PCR.

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Abstract

Transposon mutagenesis and single-gene deletion are two methods applied in genome-wide gene knockout in bacteria (1,2). Although transposon mutagenesis is less time consuming, less costly, and does not require completed genome information, there are two weaknesses in this method: (1) the possibility of a disparate mutants in the mixed mutant library that counter-selects mutants with decreased competition; and (2) the possibility of partial gene inactivation whereby genes do not entirely lose their function following the insertion of a transposon. Single-gene deletion analysis may compensate for the drawbacks associated with transposon mutagenesis. To improve the efficiency of genome-wide single gene deletion, we attempt to establish a high-throughput technique for genome-wide single gene deletion using Streptococcus sanguinis as a model organism. Each gene deletion construct in S. sanguinis genome is designed to comprise 1-kb upstream of the targeted gene, the aphA-3 gene, encoding kanamycin resistance protein, and 1-kb downstream of the targeted gene. Three sets of primers F1/R1, F2/R2, and F3/R3, respectively, are designed and synthesized in a 96-well plate format for PCR-amplifications of those three components of each deletion construct. Primers R1 and F3 contain 25-bp sequences that are complementary to regions of the aphA-3 gene at their 5' end. A large scale PCR amplification of the aphA-3 gene is performed once for creating all single-gene deletion constructs. The promoter of aphA-3 gene is initially excluded to minimize the potential polar effect of kanamycin cassette. To create the gene deletion constructs, high-throughput PCR amplification and purification are performed in a 96-well plate format. A linear recombinant PCR amplicon for each gene deletion will be made up through four PCR reactions using high-fidelity DNA polymerase. The initial exponential growth phase of S. sanguinis cultured in Todd Hewitt broth supplemented with 2.5% inactivated horse serum is used to increase competence for the transformation of PCR-recombinant constructs. Under this condition, up to 20% of S. sanguinis cells can be transformed using ~50 ng of DNA. Based on this approach, 2,048 mutants with single-gene deletion were ultimately obtained from the 2,270 genes in S. sanguinis excluding four gene ORFs contained entirely within other ORFs in S. sanguinis SK36 and 218 potential essential genes. The technique on creating gene deletion constructs is high throughput and could be easy to use in genome-wide single gene deletions for any transformable bacteria.

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