

Development of qPCR Assays for *Vibrio cholerae* Using Insignia, a Novel Comprehensive DNA Signature Discovery System.

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DNA signatures are nucleotide sequences that distinguish a set of genomes from all other genomes. Classically, these signatures are discovered by targeting a specific gene or region of the genome and comparing that gene sequence among organisms. With the availability of hundreds of complete bacterial and viral genome sequences, it is now possible to use computational, whole genome comparison methods to identify signature sequences. We have used Insignia, a novel DNA signature computational system for the rapid identification of signatures in the genomes of bacteria and viruses, to discover signature sequences for *Vibrio cholerae* and have validated those signatures using qPCR assays.

The Insignia computational resource was used to generate all possible 20mers unique to the genome of *V. cholerae* O1 biovar El Tor strain N16961 among a database of approximately 400 bacterial genomes. Fifty TaqMan qPCR assays were designed using the Insignia signature sequence output. To test whether the signature assays were broadly inclusive of *V. cholerae* strains, each assay was tested against a panel of 45 strains of *V. cholerae* comprising a global distribution of both clinical and environmental strains from all major serotypes. To test whether the signature assays excluded non-cholera vibrios, each signature assay was tested against a panel of 23 nearest neighbor species in the family *Vibrionaceae*.

Analysis of the CT values for all qPCR reactions validated 49 of the 50 Insignia signatures. Furthermore, the inclusive assay results showed that some of the validated signatures were unique to the pathogenic serotypes of *V. cholerae* (O1 and O139) and were capable of being distinguished from the non-O1/non-O139 strains (nonpathogenic strains). The exclusive assay results showed that 573 of the 575 tests (99.6%) had CT values > 50, indicating that all the *V. cholerae* signatures are either absent or divergent from other members of the family *Vibrionaceae*. These results indicate that whole genome signature discovery, whereby the entire genomic complement of organisms are compared to identify targets, is a promising new tool for diagnostic assay development.

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