

Nucleic Acids-Based Methods with Enhanced Sensitivity and High Sample Throughput

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Molecular methods for identifying biological agents of concern are of interest in many areas including biodefense, water and food industries, and in clinical diagnostics. Nucleic acids-based methods are receiving special attention because they are sensitive, specific, rugged, quantitative, rapid, and cost-effective. Development of such methods requires integration and optimization of several steps including gene sequence mining and analyses, probe/primer design, validation, target enrichment and amplification, quantification of abundance, data and statistical analyses. Both microfluidic in situ microarrays and nanoliter volume quantitative PCR chips are being used to develop such tests. In one such study using a 10,000 probe microarrays targeting over 500 virulence and marker genes (VMGs), we have observed that i) using multiple signatures per VMG increases confidence in positive calls and the variation in signal intensity between targeted probes can be neglected, ii) thermodynamic parameters are key in finding 18-mer oligonucleotide probes with high selectivity and sensitivity, and iii) modeled association kinetics with the microarray is comparable to experimental results. In another related work using a commercially available on-chip real time PCR equipment, we evaluated the ability to amplify more than 200 VMGs from 30 pathogenic microorganisms in dozens of samples simultaneously. Under the conditions used in this study, the environmental backgrounds used (DNA extracted from river water, tap-water, and tertiary effluent) had a negligible influence on PCR specificity and efficiency. A high correlation existed between predicted and experimental threshold cycle based on primers and genomic characteristics of targeted organisms. In addition, by targeting multiple VMGs per pathogen, only minimal validation was necessary for new primers. Thus by combining the power of real time PCR with arrays to analyze multiple samples for multiple targets, the cost of environmental screening can be substantially reduced. Translating the power of these methods to point-of-use hand-held devices may further enhance the application of such tools.