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Impedimetric Label-Free Sensing of DNA Hybridization in Real Time for Rapid, PCR-Based Detection of Microorganisms in the Environment

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A novel, impedance-based electronic sensor format from Sharp Laboratories of America (SLA) was tested for label-free, real-time detection of microbial DNA on oligonucleotide probe arrays. Major advantages of the SLA platform over other described impedance sensors used for DNA assays are i) a newly-established technique for industrial mass-manufacturing at low cost; and ii) rapid label-free measurement of long target DNA fragments, which may facilitate integration with PCR amplification. Detection limits of 5 – 10 nM were achieved for single-stranded, PCR-amplified DNA targets. Hybridization selectivity yielded 9- to 12-fold signal increases for specific targets, and the sensor arrays were re-used multiple times without significant signal degradation. We tested the next-generation SLA sensor array – improvements include reduced scan time (0.25-0.5 sec per scan) and an increased number of sensing elements (5 electrodes per cell, and 3 cells for a total of 15 microelectrodes). The new generation impedimetric reader device also performs continuous stimulation of all electrodes on the array. The new data analysis software package allows for efficient noise filtration, automatic subtraction of the baseline, and extraction of the exponential component of the signal for fully automated quantification of the sensor response. The upgraded sensor was used to evaluate improvements in protocols for the preparation of SS DNA targets and the use of blocking agents to decrease nonspecific hybridization. It was additionally used to calibrate the sensor response signal using quantitative PCR, and to test the signal response to DS DNA targets. The simple design of the SLA sensor array, and its ability to acquire continuous measurements of DNA as it accumulates on the array surface, make it an attractive biosensor platform for field detection and monitoring of sentinel and/or pathogenic microorganisms.

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