

352g Multiple Biospecies Detection in an Integrated Pcr-Electrochemical Microdevice Mediated by Magnetic Particles for Genome Separation and Sequence-Specific Detection

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Decentralized monitoring of pathogens is one of the crucial aspects in hygiene control and anti-epidemic outbreak, and requires fast, accurate, and portable instruments to fulfill the task. Nucleic acid-based analytical systems have been proven to be highly sensitive and selective for pathogen identification, however, simple yet multiplex on-chip detection platform, together with upstream sample preparation, are still under intensive investigation. In this work, the development of an integrated DNA extraction-polymerase chain reaction (PCR)-electrochemical (EC) microdevice for the simultaneous detection of two species (*E. coli* and *Bacillus subtilis*) in a single chamber is demonstrated. The microdevice has a reaction chamber formed in a silicon substrate, with platinum heaters and temperature sensors patterned on top of the chamber for rapid thermal cycling of the PCR. The silicon chamber is sealed by bonding with a glass substrate, which serves also as an EC detection platform. The glass has four working (WE, made of indium tin oxide, ITO), one counter (CE, platinum) and one pseudo reference (RE, platinum) electrodes patterned on top for the sequence-specific EC detection of multiple PCR amplicons inside the PCR microchamber. The assay protocol starts with thermal cell lysis, then the hybridization between genome and the DNA probe takes place to immobilize the target genome from crude cell sample to magnetic particle surfaces via the biotinylated probe. After washing, multiplex and asymmetric PCR is carried out to amplify two specific sequences from the captured genome. Immediately after the thermal cycling, these single-stranded rich amplicons hybridize to the corresponding WE modified with specific detection probe, which is formed by coelectropolymerization of pyrrole and pyrrole-functionalized probes. EC transduction of the hybridization events is based upon the binding of gold nanoparticle via biotin-streptavidin interaction, followed by signal amplification with the electrocatalytic silver deposition onto the gold label, and finally potentiometric stripping of the deposited silver. The combination of PCR and Ag-on-Au renders high sensitivity, while the utilization of individually addressable capture probe-modified electrode arrays offers high specificity as well as multiplexity. The integration of the genomic DNA capturing, DNA amplification and detection functionalities in a single microchamber reduces the design complexity of the microchip.