Title: Development of a Multiplex Bio-barcode DNA Biosensor for Bacillus anthracis Phase II

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Abstract: The long-term goal of this project was to develop a DNA-based detection device that will not require PCR amplification and still retain PCR sensitivity. For this particular project, the deliverables include: (1) Synthesis and characterization of the DNA probe-coated and bio-barcoded goldnanoparticles and DNA probe-coated magnetic nanoparticles; (2) Assay development for Bacillus anthracis DNA recognition and bio-barcode release; and (3) Fabrication of the bio-barcode DNA biosensor and evaluating its sensitivity performance. We are progressing well on these deliverables.However, one of the main issues of PCR-less assays is target DNA preparation and amplification without the use of a themocycler. This is very important if we are to transfer molecular-based technologies from the lab to the field. We are therefore requesting a Phase II of this project to address this issue. We will explore two main approaches: (1) the use of isothermal amplication, and (2) fragmentation of the genomic DNA. For the isothermal amplification approach, we will evaluate thermophilic helicase-dependent isothermal amplification (HDA). The HDA technique utilizes a DNA helicase to generate single-stranded templates of the target for primer hybridization and subsequent primer extension by a DNA polymerase without thermocycling.This technique offers a simple reaction scheme that can be performed at one temperature for the entire process. In case of unforseen constraints on the HDA approach, we will use other methods, such as isothermal and chimeric primer-initiated amplification of nucleic acids. This method allows for the amplification of target DNA under isothermal conditions (around 55 C) using only a pair of 5'-DNA-RNA-3' chimeric primers, a thermostable RNaseH and a DNA polymerase with strong strand-displacing activity. The second proposed approach is the introduction of fragmented and preheated bacterial DNA directly into the probe hybridization without any previous amplification. This method will allow for direct detection with minimum sample preparation. Specificity and sensitivity evaluations of both isothermal and fragmented alternatives will determine the most appropriate procedure for the bacterial DNA preparation before detection. Success of any or both approaches will allow for the further development of the present project as well as for the development of other simple portable DNA detection/diagnostic devices for field use at potentially lower cost and ease of use.

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