Defining New Microfluidic Methodologies for Forensic DNA Sample Preparation: Harnessing the Power of Magnetic and Acoustic Forces for DNA Analysis

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There is little doubt that STR typing has become the accepted gold standard for human identification over the past two decades, now successfully employed in paternity testing, criminal casework, and missing person cases, as well as for databasing efforts. Although highly successful and reliable, current methodologies require 8-10 hours to complete under routine conditions, use large sample volumes, costly reagents, and are labor-intensive. Additionally, samples are open to the environment at multiple points during processing, making them susceptible to contamination. This has driven an aggressive effort from multiple entities to create a miniaturized and integrated microfluidic system (e.g., Easley et al. [1]) that will provide rapid generation of STR profiles from buccal swabs for databasing, and eventually from more complex samples (blood, stains) pertinent to casework. While microfluidic extraction of DNA from forensic samples has been described extensively in the literature [2], as has the targeted PCR amplification of loci associated with commercial STR kits [3], limited, if any, progress has been made on other aspects of forensic DNA analysis.

We describe two new technologies that present unique opportunities for enhancing forensic DNA analysis. The first involves exploiting magnetic fields and Magnesil beads in a new approach to quantifying DNA pre-PCR that we have coined the 'pinwheel assay'. The mechanism for analysis involves the visual detection of DNA via macroscopic aggregate formation facilitated by Magnesil beads – a phenomenon visible on-chip to the naked eye with picogram levels of DNA. We demonstrate that this be a **quantitative** DNA assay when the pinwheel formation is imaged and Image-J software utilized to analyze the image. With this method, a quantitative relationship results from the degree of aggregation (% dark area) and the mass of DNA present. Further, we show the relationship between the DNA fragment length and the degree of aggregation, the effect of bead size/character on the sensitivity (low picogram range), as well as the ability to quantify DNA directly from raw samples. Finally, we will describe the first results indicating that the pinwheel assay can quantify DNA in a human-specific manner. Together, these results suggest the pinwheel assay as a method capable of label-free analysis with unparalleled capabilities.

The second technology involves exploitation of acoustic energy to isolate cells in a selective and size-specific manner. Actuating a transducer under a microfluidic channel sets up a 'trapping node' in 3D fluidic space that can be tuned to a frequency that allows for trapping of cells of a particular size. We have shown that this can be used to trap sperm cells in the presence of epithelial cell lysate, providing an alternative to traditional differential extraction. We present data showing the effectiveness of this approach for separating intact sperm cells from the female fraction in mock vaginal swabs. In addition, we present advances in this technology for yielding male DNA-enhanced fraction from vaginal swabs associated with rape kits.

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