B87 Acoustic Differential Extraction: A Novel Alternative to Conventional Differential Extraction

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The goal of this presentation is to introduce a novel means for analysis of sexual assault evidence. In addition, the audience will be introduced to a unique aspect of microchip technology for forensic DNA analysis.

This presentation will impact the forensic community and/or humanity by demonstrating the acoustic differential extraction method pre- sented has the potential to significantly alter the means by which sexual assault evidence is processed in crime laboratories. This method is one step of a totally integrated, automated microchip format for forensic DNA analysis.

Differential extraction, the conventional method for isolating male and female fractions of DNA, is a time-consuming sample preparation step in the forensic DNA analysis of rape kit evidence. In addition, it is not easily amenable to automation. The development of a novel alternative for isolating male and female fractions of biological material from sexual assault evidence through the use of acoustic forces in microfabricated devices, termed acoustic differential extraction (ADE) has been completed. Since differential extraction is only one processing step of forensic DNA analysis, replacing it with a microdevice method provides a distinct advantage with the possible integration of several sample preparation steps, including DNA extraction, DNA quantitation, PCR amplification, and sep- aration of PCR products on a single device. In addition, the method pre- sented here will be shown to be versatile (accommodating microliters to milliliters of sample), automatable, and highly amenable to multiplexing.

This novel method for obtaining isolated male and female fractions of DNA on a microfabricated device uses a mild lysis buffer, as in the con- ventional differential extraction method, to selectively lyse the epithelial cells. The sperm cells are then selectively captured from the cell lysate, using acoustic forces to trap the sperm in the microchannel, while free DNA (including that from the lysed epithelial or other cells) passes through the trap and is directed to an outlet reservoir for collection and analysis. The trapped sperm cells are then released and directed down another microchannel for recovery and subsequent analysis of the sperm cell fraction.

ADE utilizes a glass microfabricated device with defined microfluidic channels in contact with a piezoelectric transducer to form an ultrasonic resonator. Upon generation of ultrasonic waves by activation of the piezo- electric transducer, a standing ultrasonic wave is generated in the microchannel, and cells are trapped in the standing wave depending on cell type and the separation medium, see equation 1. The force on the cell, F_r, is dependent on the amplitude of the applied acoustic pressure, P_0, the volume of the cell, V_c, and the compressibility and density of both the cell and the separation medium. Hence, the force acting on the sperm cells is much greater than that on free DNA, resulting in the trapping of sperm in the acoustic wave, while free DNA is carried with the fluid.

$$F_r = -\left(\frac{\pi P_0^2 V_c \beta_w}{2\lambda}\right) \cdot \Phi(\beta, \rho) \cdot \sin\left(\frac{4\pi z}{\lambda}\right) \qquad (1)$$

Digital video microscopy was utilized to visualize the cell trapping and demonstrate the purity and efficiency of the process. The optimized frequency and amplitude of the ultrasound were determined for most efficiently trapping the sperm cells. Using mock sexual assault vaginal swabs, the cell separation product obtained on the microdevice resulted in a clean sperm cell fraction. DNA from the isolated cells was extracted with a commercial extraction kit, amplified with a Profiler® PCR kit, and analyzed on an ABI 310 commercial CE, yielding the profile of the male sperm donor. Efficiency and purity measurements were also obtained using real time PCR.

Acoustic Trapping, Differential Extraction, Microchip Technology

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