Evaluation of Pressure Cycling Treatment on Barocycler® NEP3229 for Extraction of Low-Template Forensic DNA Samples.

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This presentation will summarize our efforts to increase recovery of DNA from low-template samples though the use of a pressure cycle treatment (PCT) using the Barocycler® NEP3229 (Pressure BioSciences). Specific goals, including simultaneous incubation of samples, maximizing DNA yield, and evaluation of instrument parameters and consumables for the target sample, were addressed. An existing QIAGEN protocol was compared to a modified version incorporating pressure cycling pretreatment.

Technological advancements have greatly increased the sensitivity of DNA testing in recent years. Techniques for low-template autosomal DNA testing focus, primarily, on extending amplification cycles or increasing detection of the amplified products on capillary electrophoresis via reduction of salts and unincorporated primers. The class of low-template samples initially targeted for this study, those recovered from touched items, are generally collected on a cotton swab substrate. Labs have long been challenged with more efficient removal of DNA sample from this substrate. Swabbing solutions, substrate material, and incubation methods have been examined in efforts to improve recovery.

Pressure cycling has recently emerged as a technique with the potential to improve DNA yield prior to amplification, with or without adjustments to amplification or clean-up of amplification products. High pressure treatment of samples prior to extraction has been hypothesized to compromise plasma membranes leading to an increase in permeability of extraction reagents and to more efficient cell lysis. In addition, cyclical exposure of samples to alternating high and ambient pressures may improve the removal of cells and cell-free DNA from the substrate. Within the FT-500ND PULSE tubes, a ram generates a pressure differential inside a tube, forcing the buffer through the swab substrate. This action may improve DNA release from the substrate and/or cell lysis in some fashion.

The present study was undertaken to evaluate a pre-amplification technique with the potential to improve recovery of DNA from a commonly used swab substrate and subsequent extraction. Various parameters were evaluated including incubation method, cycle number and time at maximum pressure. Samples were prepared by adding 1000pg, 500pg, 250pg, and 100pg of DNA from a calibrated solution of diluted human saliva to one-half of a cotton swab and drying overnight. Incubation was performed in PULSE tubes at 56° C within the Barocycler® NEP3229 chamber and under a series of experimental conditions including 20, 40, 60, or 80 cycles alternating between 20, 40, 60, or 80 seconds at 35k psi and 10 seconds at ambient pressure. Samples were quantified using Quantifiler® Duo (Applied BioSystems) and amplified using AmpF*l*STR® Identifiler® Plus (Applied BioSystems) reagents at 28 cycles. The amplicons were then injected onto a 3130*xl* genetic analyzer (Applied BioSystems).

Results from the various Barocycler® NEP3229 run parameters tested were varied depending on the specific target amount of DNA in the sample. Optimal run parameters were selected based on an improvement in performance across all low template DNA targets. For the DNA amounts tested, 1000pg, 500pg, 250pg, and 100pg, runs of 20 cycles with 20 second intervals at maximum pressure recovered more DNA than the existing method across all four DNA target amounts with 22.8% (1000pg), 50.5% (500pg), 59.3% (250pg), and 56.2% (100pg) improvements. At 1000pg and 500pg amounts of target DNA, the 80 cycle 20 second method performed slightly better, with additional 23.0% (1000pg)

and 16.8% (500pg) increases. However, this method did not have the same affect at the 250pg and 100pg target amounts of DNA, recovering 45.8% (250pg) and 79.8% (100pg) less DNA than the 20 cycle 20 second method. These data agree with recoveries from 1uL of human saliva. Samples with higher amounts of DNA do benefit from increasing the number of PCT cycles; however, samples containing lower amounts of DNA appear to require fewer cycles to remove the sample from the substrate perhaps preventing re-adherence to fibers of the cotton swab.

Key words: low-template DNA, pressure cycling, extraction