THE UTILIZATION OF PRESSURE SYCLING TECHNOLOGY TO ENHANCE DNA EXTRACTION FROM LOW TEMPLATE SAMPLES

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Low template DNA analysis has become a primary focus of forensic DNA laboratories as scientists increasingly attempt to develop DNA profiles from smaller quantities of DNA and to purify DNA deposited on challenging substrates. Low template analysis techniques have been developed to increase sensitivity and elucidate profiles from these low level samples. However, most of these methods involve post-extraction enhancements which currently prevent the resulting profiles from being uploaded to NDIS. Allelic peak imbalance, drop-out, drop-in, and increased stutter peaks are commonly observed when low template DNA is STR amplified and analyzed. The development of techniques that increase DNA extraction efficiencies from low template samples may eliminate the necessity for post-extraction procedures to obtain meaningful profiles from low level samples. Pressure cycling technology (PCT), the application of rapid cycles of ultrahigh hydrostatic and ambient pressure to biomolecular samples, was evaluated as a method to enhance DNA extraction from low template samples when used in conjunction with current extraction techniques. The extraction procedure utilized for these experiments was the DNA IQ™ System on the Biomek® NX automation platform. DNA was extracted from swabs containing neat blood diluted to low template levels. Average DNA quantitation values obtained from PCT-treated samples were compared to control sample values and evaluated for statistical significance. Preliminary testing indicated that pre-treatment of swabs with PCT prior to DNA IQ™ System purification on the Biomek® NX platform increased DNA yields. STR profiles will be examined to determine if PCT treatment and the associated increase in DNA yield decreased stochastic effects including allelic drop-out, allelic drop-in, homozygote peaks below stochastic threshold, and peak height imbalance percentages. In addition, a contamination study will be conducted, and PCT parameters including buffer, cycle number, and temperature will be optimized. Finally, the optimized procedure will be applied to low template mock case samples.