VALIDATION AND OPTIMIZATION OF A ROBOTIC SYSTEM USED TO FULLY AUTOMATE FORENSIC DNA EXTRACTION, QUANTIFICATION, DNA NORMALIZATION AND STR AMPLIFICATION ANALYSIS

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In recent years the Centre of Forensic Sciences (CFS) in Toronto has expanded its services to include “break and enter” and other high volume type cases. To accommodate the increased DNA caseload, several recently improved DNA profiling methods were put into practice. To facilitate their execution and further enhance throughput, two MultiPROBE II PLUS HT EX robotic liquid handling systems from Perkin Elmer were used to automate the new procedures. We report here on the validation and optimization of a set of protocols for the fully automated processing of forensic casework DNA samples in 96-well format. The two workstations are being used to fully automate a robust process that includes (1) DNA isolation using the Promega DNA IQ™ System, (2) DNA quantification using real-time quantitative PCR (QPCR) analysis, (3) DNA normalization and dilution over a 10,000 fold range and (4) STR-typing amplification setup.

One of the MultiPROBE II systems is dedicated to extracting DNA using a modified Promega DNA IQ™ System protocol [1], which relies on a paramagnetic particle separation strategy. Validation of the casework DNA isolation method is ongoing using heterogeneous samples - processed at the same time with the same procedure. Samples include blood stained paper and cloth, cigarette butts, gum, FTA punches and swab swipes. The robotic system carries out automated extraction, binding, washing and elution steps using a fully integrated shaker and dual-temperature heater controller option.

The second MultiPROBE II system is devoted to running the QPCR setup and STR amplification setup methods. An in-house QPCR assay was previously validated for forensic casework using an ABI 7900HT SDS platform [2]. For automated QPCR setup, isolated DNA was used to generate two sample plates (neat, 5X dilution) before adding custom designed TaqMan–MGB sequence specific probes (CFS-HumRT). Output sample concentration files from the ABI SDS software are uploaded into a Visual Basic macro that directs the robotic system to perform DNA normalization and dilution to achieve a target template concentration of 0.167 ng/ul. Finally, ABI AmpF*l*STR Profiler Plus™ or COfiler™ PCR Master Mix is added for STR amplification on an ABI 9700 thermal cycler.

Validation followed SWGDAM [3] guidelines and demonstrated the robotic systems to be accurate, precise, reproducible, contamination-free, and successful in processing non-probative casework. The effect has been to virtually eliminate manual liquid handling, resulting in higher sample throughput and reduced turnaround time. It is anticipated that with all systems in place, the DNA unit will process the same number of samples it processes today in 60% of the time required and with only 15% of the staff required.

[1] Komonski D, Marignani A, Richard ML, Frappier RH, Newman JC. Validation of the DNA IQ System for use in the DNA extraction of high volume forensic casework. Canadian Society of Forensic Science Journal 2004; in press.

[2] Richard ML, Frappier RH, Newman JC. Developmental Validation of a Real-Time Quantitative PCR Assay for Automated Quantification of Human DNA. Journal of Forensic Science 2003;48 (5):1041-6.

[3] Scientific working group on DNA analysis methods (SWGDAM): Guidelines for a Quality Assurance Program for DNA analysis. Jan 2004; Draft.