Fully Automated DNA Extraction and Isolation from Small Batches of Forensic Casework Samples as Part of a Complete Automation Scheme

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Automated forensic liquid handling workstations have proved invaluable for processing large numbers of DNA database samples, significantly reducing backlogs for many crime labs. However, for casework samples obtained from crime scenes, the limited number and wide variation in sample type and amount introduce new constraints that affect automated sample processing schemes. An even greater challenge for automation in small crime labs is the need to accomplish full DNA processing - without delay - of small sample batches. Two distinct MultiPROBE II 4-Tip Forensic Workstation configurations from PerkinElmer Life and Analytical Sciences are being used to develop rapid and fully automated DNA processing of small numbers of crime scene samples. One system, with a 32-plate deck capacity and gripper, heater and shaker options, is a dedicated DNA extraction and purification system. The second system, with a smaller 16-plate capacity deck and low volume dispense option, is used to carry out downstream analyses steps. The dual workstations are able to effectively implement a strategy processing batches of up to 24 samples that employs four automated protocols: DNA extraction & isolation using the Promega DNA IQ System, QPCR setup, DNA normalization and dilution, and lastly, STR PCR setup. The 4-Tip systems were chosen because (1) small batch sample processing does not require the throughput needs of larger scale sample processing, (2) they use identical software and hardware, and (3) the cost of two such systems together is similar to a single 8-Tip system. Casework DNA samples (dried blood, sperm and saliva on cloth) were purified in batches of up to 12 samples using the DNA IQ System kit and a robot-directed extraction protocol. No prior sample preparation was performed preceding placement of dry samples in tubes on the deck. Two variations of the DNA IQ kit chemistry were compared: Proteinase K-SDS extraction at 56C method versus direct treatment with Lysis Buffer at 70C. The MultiPROBE II DNA extraction system has the following features: • Automated extraction of samples in 1.5 mL microfuge tubes with parallel shaking and heating using a new 24-tube heat transfer block and on-deck heater tile assembly. • Automated reformatting of tube samples into plates for normal DNA IQ processing (binding, washing and elution), followed by automated transfer of purified DNA samples back into tubes. • Increased throughput of up to 10 fold compared to manual processing. Because crime scene samples are from a variety of biological substrates and display broad concentration ranges, they require accurate DNA quantification. A WinPREP protocol was developed to perform a QPCR setup assay using two ABI Quantifiler Quantification kits in a single PCR plate (manuscript in preparation). One kit was specific for total human DNA and the other for Y human male DNA. A DNA standards dilution series was included for both kits in the QPCR setup protocol. Other fully automated small batch casework forensic sample DNA protocols being optimized include DNA normalization and dilution, PCR master mix creation and PCR setup for STR-based typing assays. We will present experimental details, performance data and analysis results showing that the two MultiPROBE II robotic systems successfully carried out the DNA IQ isolation steps needed for a walk-away small batch casework forensic DNA processing. The two MultiPROBE II 4-Tip robotic systems feature rapid sample turnaround time using the dual robots in parallel. Deck layouts used to carry out the four separate procedures are permanent – minimal or no support tile changes required. The two systems are appropriate for processing small mixed casework samples but scalable for use with reference and database forensic samples, if needed.