A96 High–Throughput Processing of Mitochondrial DNA Analysis Using Robotics

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The goal of this presentation is to inform the forensic community as to the uses and applications of robotics for high - throughput processing of DNA samples for mitochondrial DNA testing.

This presentation will impact the forensic community by offering alternatives to sample processing through robotics in order to increase efficiency and sample throughput, resulting in additional time for analysts to perform more complex tasks and analysis.

The adoption of multi-capillary instrumentation in the forensic community has resulted in the transition from single tube testing to a 96- well plate format as seen in real-time PCR, thermal cycler, and genetic analyzer sample layouts. The identifications of thousands of deceased individuals from the World Trade Center terrorist attack on September 11, 2001 have launched the use of robotics to processing samples using these 96-well plate formats. The data generated using automated systems suggests that robotics is amenable to forensic applications.

Several companies have generated robotic systems that are capable of automating key steps in processing forensic samples. Robots can be configured with fixed single and multiple pipetting tips or disposible tips, and accessorized with shakers, heaters, cooling systems, vacuums, grippers, and plate stackers. In addition, robots can be coupled to quantification equipment such as a luminometer or fluorometer, as well as thermal cyclers used for amplification and sequencing. There are limitations and advantages for every robot. Many laboratories face financial constraints, and are unable to purchase high end robots. Fixed format robots are typically more affordable than robotics amenable to multiple accessories. Because the features of each robot are different, automation script designs must be written to accommodate the robot used for its specific application.

An ideal forensic pre-PCR robotic system could incorporate DNA extraction, quantification and normalization of DNA, and PCR reaction setup, all within a 96-well plate layout. Preferred post-PCR robotics would consist of PCR quantification and normalization, product purification, and preparation of sequencing reactions. The University of North Texas Center for Human Identification houses a Freedom EVO® 100 (Tecan Group Ltd., Männedorf, Switzerland) and two MiniPrep 75 Sample Processors (Tecan Group Ltd.). The Freedom EVO® 100 is used for extraction of reference DNA samples associated with the Missing Persons Program, using the DNA IQ™ System (Promega Corporation, Madison, Wisconsin). We use a modified script design to decrease the number of plate transfers and introduce bleach washes. It was necessary to introduce the bleach washes since the extracted DNA was being used for not only STR, but also the highly sensitive mitochondrial DNA (mtDNA) testing.

Scripts for two MiniPrep 75 Sample Processors (MiniPrep) have recently been developed, which are fixed liquid handling format robots, for the amplification, post-amplification clean-up, and cycle sequencing reactions for the high-throughput sample processing of mtDNA samples. Each MiniPrep is housed in a specific laboratory, either pre-PCR or post- PCR. Robotic automation scripts for the MiniPrep were designed for amplifying the hypervariable regions (i.e., HV1 and HV2) of mtDNA utilized in forensic sciences. The 96-well template DNA plate is placed onto the pre-PCR MiniPrep, along with two empty 96-well plates for reaction setup. The script was designed to aliquot the HV1 and HV2 master mixes into the respective 96-well plates. Template DNA is then transferred to each of the plates containing master mixes. Upon completion, the HV1 and HV2 plates are sealed and placed on the thermal cycler for amplification.

Three separate scripts were created for the post-PCR MiniPrep. The first script was designed to perform enzymatic post-amplification cleanup using ExoSAP-IT® (USB Corp., Cleveland, OH). The two 96-well plates containing PCR products are added to the MiniPrep. The single-tip pipette transfers ExoSAP-IT® from a tube into a clean 96-well plate. The fixed 8-tip pipette then transfers the ExoSAP-IT® into the plate containing the PCR product, introducing a bleach wash before each addition. The sample plates are then sealed and placed on the thermal cycler. A second post-PCR script performs cycle sequencing setup using the ABI PRISM® BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The plates of purified PCR product are placed on the MiniPrep. Sequencing master mix is aliquoted into each well of a new 96-well plate using the single-fixed pipette tip. Purified PCR product is then transferred to the respective plates. Plates are then sealed and placed on the thermal cycler. The third script performs sequence cleanup using Edge Performa® Plates (Edge Biosystems, Gaithersburg, MD). The plates of cycle-sequenced product are placed on the MiniPrep. The 8-tip arm then transfers the entire sample to the column plate. The plates are then removed from the MiniPrep and centrifuged and the purified product retained. The plates are ready to be directly placed on the genetic analyzer for capillary electrophoresis.

This research has shown that successful amplification can be set up twith ExoSAP-IT® purification cycle

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sequencing reactions, and post- sequencing clean-up on the MiniPrep 75 Sample Processor robot. Sequence data obtained displays minimal background noise, and to date, no contamination has been detected from any of our samples. Automated robotic systems can reliably be used to process forensic reference samples for sequencing.

Robotics, Automation, Mitochondrial DNA

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