

HIGH THROUGHPUT PROCESSING OF FAMILY REFERENCE SAMPLES FOR MISSING PERSONS PROGRAMS: THE USE OF ROBOTICS IN EXTRACTION AND AMPLIFICATION SETUP FOR STR AND mtDNA ANALYSIS

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The University of North Texas (UNT) System Center for Human Identification is a partner in the National DNA Index System database for missing persons. In order for the missing persons program to be successful, building a family reference database for comparison to recovered remains is critical. Since the DNA from many of the recovered remains is highly degraded, both nuclear DNA and mtDNA results are evaluated. The UNT Center for Human Identification anticipates its program growing to over 20,000 family reference samples per year. With the incorporation of multi-capillary instruments speeding up the data acquisition, bottlenecks in sample processing exist in DNA extraction and PCR amplification setup. Many robotics systems are used in forensic and vendor laboratories to process convicted offender samples worldwide. However, the use of robotics to extract DNA for both nuclear DNA and mtDNA testing with fixed pipette tips is not commonplace. Our laboratory has adapted a method using the Slicprep™ 96 Device and DNA IQ™ System from Promega Corporation in conjunction with the Tecan Freedom EVO® 100 robot using fixed tips. The Slicprep™ 96 Device is used to pre-process the buccal swab samples submitted in the Family Reference Sample collection kits. Buccal swab heads arranged in batch format are incubated in lysis buffer and then centrifuged using the collar expander of the Slicprep™ 96 Device. The 2.2 mL deep-well collection plate from the Slicprep™ 96 Device is conveniently placed on the Tecan Freedom EVO® 100 deck. The Tecan Freedom EVO® 100 robot is configured with 8 fixed liquid-sensing tips, a magnetic block, and heat block. The fixed tip format was selected to reduce the cost and storage requirements involved with disposable tips. Using a paramagnetic resin and magnetic block, the DNA IQ™ System eliminates the need for any hands-on manipulation. Scripts and procedures provided by Promega Corporation for the DNA IQ™ System using the Tecan were modified to reduce the number of transfers made between plates, to reduce the amount of sample lysate and resin used, and to introduce multiple wash procedures for use with a fixed-tip system. Since the Tecan Freedom EVO® 100 can be programmed to independently control each tip, well positions on the plate can be skipped to allow for addition of controls and/or ladders later in the testing process. This procedure insures that the same plate layout can be used throughout the entire testing process with no waste of additional reagents. Studies were performed to reduce the amount of DNA IQ™ Resin used in the extraction procedure. Even with this reduced amount of DNA IQ™ Resin, consistent amounts of extracted DNA were obtained. Cross-contamination studies were performed for both STRs and mtDNA using checkerboard and zebra-stripe patterns. Cleaning procedures were developed for the fixed-tip platform, eliminating the impact of any inadvertent well to well crosstalk originally detected through mtDNA sequencing. Following DNA extraction, STR and mtDNA amplification setup is performed by a Tecan MiniPrep 75 Sample Processor. Together, the overall process incorporating Slicprep™ 96 pre-

processing, DNA IQ™ extraction on the Tecan Freedom EVO® 100, and amplification setup on the Tecan MiniPrep 75 has resulted in consistency in sample yield, elimination of cross-contamination effects, and reproducibility for both STR and mtDNA analysis.