VALIDATION OF THE TECAN GENESIS ROBOTIC SAMPLE PROCESSOR FOR AUTOMATED CYCLE SEQUENCING OF mtDNA DATABASE SAMPLES

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The Armed Forces DNA Identification Laboratory (AFDIL) aids the Central Identification Laboratory (CILHI) in identification of skeletal remains recovered from previous war conflicts by conducting mitochondrial DNA (mtDNA) testing. The scientists generate sequences from the skeletal remains recovered by CILHI and from maternal relatives of those lost for comparison. The Family Reference Section of AFDIL receives approximately thirty blood references monthly, which equates to about 1,500 samples per year. The Family Reference Section sequences the entire mtDNA control region for these samples, and then enters the data into the Family Reference Database. Currently, the Family Reference Section uses a semi-automated system in which 92 blood samples are punched into a 96-well plate and extracted on a Qiagen 9604 robot (Qiagen, Gaithersburg, MD). Manual manipulations amplify the mtDNA control region (nt15971-599) and purify the amplicon using ExoSAP-IT™. By hand, the use of seven overlapping primers enable sequencing the purified amplicon, resulting in a total of seven 96-well sequencing plates from two separate extractions per sample (a grand total of 14 sequencing plates per set of Family Reference samples). The sequencing phase of this laboratory process requires 12.5 man-hours and 64 machine hours. Since the cycle sequencing and post-cycle sequencing purification steps consume a great deal of time and labor from the scientists, AFDIL preferred a method to increase efficiency through cycle sequencing automation.

Robotic systems suitably designed and thoroughly tested potentially reduce the time scientists apply along with latent human error. The experiments and results described below detail AFDIL's validation for implementing automated cycle sequencing and purification of mtDNA Family Reference samples utilizing the Tecan Genesis Robotic Sampler Processor (Tecan, Research Triangle Park, NC). The Tecan Robot reduced the grueling number of man-hours per Family Reference set of 92 samples from 12.5 to 6.75, but increased the machine hours from 64 to 65.75 hours. This increase in machine hours proved inconsequential because the net five-hour reduction in man-hours enables the scientists to focus on data analysis, the bottleneck of any high throughput methodology. Also, the automation sanctions the efficacy of operation of the Tecan overnight, adding yet another increase in efficiency.

Validation of the Tecan Genesis Robot for high throughput cycle sequencing consisted of the following experiments: liquid-handling accuracy, cross-contamination, and reproducibility. The outcome of the liquid-handling and contamination study demonstrated that the robotic system could precisely and accurately pipette amounts of liquid in various quantities through conducting sequencing reactions with different amounts of amplified DNA. Negative controls were sequenced on the Tecan using both 11 µL and 2 µL of the amplified product. In neither case was significant contamination detected with zero negative controls showing interpretable mtDNA sequence. To test the efficiency of the pipetting of the Tecan, blue dye used in a specific pattern across a 96-well plate with water in wells not containing the dye tested the pipetting abilities of the Tecan, showing that the Tecan pipettes the samples into the correct wells.

AFDIL sought not only to increase time efficiency, but also intensify data efficiency. Therefore, to validate the Tecan control region and hypervariable region programs, a set of 92 Family Reference samples, previously sequenced manually, tested the reproducibility and reliability of the Tecan Robot. AFDIL programmed the Tecan Robot to sequence either the two hypervariable regions (4 primers) or sequence the control region (7 primers). Validation of the hypervariable region consisted of 712 sequencing reactions, out of which nine failed, resulting in a 1.3% failure rate. Control Region sequencing consisted of 1,633 sequencing reactions with 44 failures. This yielded a 2.6% failure rate. Of the specimens sequenced, all of them produced consistent sequence with that generated manually. There was

conversely one negative out of 46 that produced readable sequence. The sequence of this negative control was not consistent with the processing scientist or any samples processed. This negative control and the occurring failures could not be attributed to the Tecan robot. At the time of these tests, the cycle sequencing purification was still done manually, leaving room for human error. Further validation was conducted using the same methodology to automate the cycle sequencing purification, in which there were no failures or negative controls producing sequence. The Tecan Genesis Robotic Sampler Processor proved efficient, reliable, precise, and accurate in automating the cycle sequencing and purification of AFDIL's Family Reference samples.