VALIDATION AND OPTIMIZATION OF THE ABI3100® GENETIC ANALYZER AND BIGDYE VERSION 1.1 FOR PROCESSING MITOCHONDRIAL DNA CASE SAMPLES AT THE ARMED FORCES DNA IDENTIFICATION LABORATORY

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There is a general trend in the forensic field for laboratories to move toward high sample throughput with out losing data quality, therefore, the experiments described below were aimed at validating the ABI 3100® Genetic Analyzer and BigDye version 1.1 (ABI, Foster City CA) for use in processing mtDNA case samples in a more efficient manner. Prior to implementing DNA technologies into a Forensic Laboratory, the Scientific Working Group on DNA Analysis and Methods (SWGDAM) requires all laboratories perform an internal validation on those methods. An essential part of the validation process is identifying the limitations of the reagents, kits or machines being validated, thus this validation included defining the upper and lower detection thresholds for the ABI 3100® Genetic Analyzer and BigDye version 1.1, standardization of the amount of product to be sequenced, purification techniques, and evaluation non-probative case samples.

Initially, the sensitivity of the 3100 Genetic Analyzer and BigDye version 1.1 was tested in duplicate by serially diluting a 100pg positive control DNA amplicon (straight, 1/5, 1/10, 1/20, 1/30, 1/40, 1/50, 1/100), sequencing the dilutions with either full strength or quarter-strength BigDye version 1.1 (BigDye1.1) and dGTP. Sequenced products were then purified using either the Performa DTR 96 well standard filtration plate (standard plate) or the Performa DTR 96 well short plate (short plate) (EDGE Biosystems, Gaithersburg MD). Results for these experiments showed that full strength BigDye generated higher quality data with less shoulder and longer confirmed regions than identical samples sequenced with quarter strength BigDye1.1. In addition, both straight and quarter reaction samples purified using standard plates worked better than the same samples purified using short plates. Interestingly, it was observed that purification through standard plates typically reduced the Relative Fluorescent units (RFU) by half compared to samples purified by the short plate, but in no instance were the RFU's below the minimum RFU threshold of 25. However, the reduction in RFUs was acceptable since standard plate purified samples had lower base line pull up and no dye blobs due to unpurified unincorporated dye molecules, while short plate purified samples had increased base line pull up and dye blobs leading to increased ambiguous bases.

Serially diluted samples were re-sequenced and processed as described above, but with half strength instead of quarter strength BigDye1.1. As above, purification by standard plates was better than by short plates and the quality of data obtained from both the half and full strength reactions were of similar quality. In both cases, full profiles were obtained from a 1/100 dilution of the 100pg amplicon product. For the rest of the experiments described below, samples were sequenced with both straight and half reaction BigDye in duplicate and purified with standard plates.

In an attempt to streamline the sequencing process, the next set of experiments compared serially diluted amplicon products to the 20ng band present in a DNA mass ladder (DNA Ladder II, PGC, Scientifics, Frederick MD). Bands that showed intensities greater than the 20ng DNA Mass ladder band were sequenced with 2µl of product and less intense bands were sequenced with 5µl of product. Results demonstrated that 2µl of undiluted amplicon for either the half or full strength sequencing reactions generated data whose RFU's exceeded the desired maximum RFU value of 3500. However, the rest of the dilutions generated data that fell within AFDIL's preliminary upper (3500 RFU) and lower (25 RFU) RFU cut offs for analyzing mtDNA case samples.

To address samples that were above 3500 RFUs the next set of experiments looked at re-injecting samples for a shorter time verse resuspending purified highly robust amplification (Hypervariable region and mini-primer set) amplicons in 100µl of distilled water instead of the standard 50µl before sequencing. Seven-second injections demonstrated that there was a linear reduction in RFU from the standard 15-

second injection and all of the RFUs were with in the defined parameters. Likewise, diluting the sample two fold also generated results that fell with in the defined RFU parameters.

Finally non-probative case samples that worked with primer-sets and mini-primer sets were reanalyzed using the following guidelines for analyses on the ABI 3100 Genetic Analyzer. Mini-primer set and HV amplicons were resuspended in 100µl of distilled water while primer set amplicons were resuspended in 50µl distilled water. Then two or five microliters was sequenced and purified as described above. Results demonstrated that the 3100 data for these samples were concordant with the 377 results for the same data and of a higher quality better peak height definition and less ambiguous bases.

The results generated from this validation demonstrate that using the modified sequencing procedures described above produces high quality and reproducible sequencing data in a more time effective manner, since only two volumes of DNA are required for sequencing. In addition, the new time effective procedure should help alleviate the sequencing bottleneck to processing mtDNA case samples by lowering the amount of resequencing due to miscalculations in input template DNA as well as reducing the cost of sequencing reactions since half instead of full BigDye version 1.1 reactions can be used.