A46 Evaluation of Automated Systems in a Mitochondrial DNA Unit

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After attending this presentation, attendees will learn how implementation of two automated systems, the Qiagen EZ1® Advanced and Agilent 2100 Bioanalyzer, will benefit forensic mitochondrial DNA units by limiting user handling, decreasing lab time, and allowing for prediction of length heteroplasmy prior to sequencing.

This presentation will impact the forensic science community by understanding how implementing these automated systems into a mitochondrial DNA unit allows for faster analysis by the analyst. Limited user handling of these systems results in decreased risk of contamination, while elimination of analyst interpretation of agarose gel bands allows for more accurate quantitation. Contamination thresholds were set and heteroplasmy may be screened to better prepare for downstream data.

Mitochondrial DNA (mtDNA) analysis is a prominent tool in forensic laboratories for missing persons, unidentified remains, and analysis of samples from which nuclear DNA (nuDNA) is difficult. mtDNA analysis is both time consuming and sensitive to contamination and would therefore benefit greatly from automated systems. The goal of this research was to evaluate two automated robotic platforms, the Qiagen EZ1® Advanced and the Agilent 2100 Bioanalyzer for their suitability for implementation into a mitochondrial DNA unit. The Qiagen EZ1® Advanced would allow for faster DNA extraction with no detectable contamination with limited analyst intervention. The Agilent 2100 Bioanalyzer would allow for more accurate quantitation than currently implemented gel electrophoretic methods as well as indication of heteroplasmy and contamination.

The Qiagen EZ1® Advanced robotic platform allows for the automated extraction and purification of six samples simultaneously. This system was evaluated for concordance by comparison to known mtDNA profiles from both buccal swabs and blood cards as well as resistance to cross-contamination between samples and runs. The ability for the Qiagen EZ1® Advanced to sufficiently extract mtDNA for sequencing from alternate knowns (toothbrush bristles and razors) and nuDNA from all samples used above was demonstrated. The Qiagen EZ1® Advanced DNA extracts were concordant with known mtDNA profiles. Cross-contamination was not observed in studies running alternating samples and blanks. The Qiagen EZ1® Advanced was able to produce full mtDNA and nuDNA profiles from all samples.

The Agilent 2100 Bioanalyzer allows for automated DNA quantitation through separation of nucleic acids into microfabricated channels with fluorescent detection of post-PCR products. This system was evaluated for detection sensitivity with a dilution series of a 200bp fragment, known buccal swabs, and HL60 DNA. Data was compared to determine the optimal amount of DNA required for sequencing. Reproducibility was also shown with these dilution series by running the samples in triplicate on three chips. The 200bp fragment was quantitated with agarose gel electrophoresis to evaluate accuracy between the two methods. The Agilent 2100 Bioanalyzer was also run with known samples containing length heteroplasmy, to determine whether these length variants could be detected prior to sequencing. A contamination threshold, similar to that set by local FBI laboratories, was also determined by examining low concentration samples where DNA was detected but did not sequence. The Agilent 2100 Bioanalyzer has been shown to accurately quantitate samples below 20ng/µl. The Agilent 2100 Bioanalyzer was able to reproduce quantitation values on three separate chips. The instrument was able to correctly predict length heteroplasmy before sequencing by observance of split peaks within the electropherogram.

Qiagen EZ1® Advanced, Agilent 2100 Bioanalyzer, Mitochondrial DNA

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