VALIDATION AND IMPLEMENTATION OF MITOCHONDRIAL DNA WITH MASSIVELY PARALLEL SEQUENCING

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Mitochondrial DNA (mtDNA) is typically utilized for the identification of ancient, disaster victim, missing person, and heavily degraded samples. Sanger Sequencing has been the validated gold standard in analyzing said mtDNA samples, however it is a long and laborious process. With the release of the Precision ID mtDNA Control Region Panel (mtDNA CR Panel) and Converge™, laboratories can process large numbers of mtDNA samples simultaneously. However, implementation requires a laboratory validation.

A validation was developed and executed for the Precision ID System, mtDNA CR Panel, and Converge. Currently published guidelines were still used, even though they are not NGS-specific. Data was generated for the following standard studies: DNA Amplification Sensitivity, Baseline/Stochastic, Accuracy, Precision Repeatability & Reproducibility, Mixture, and Known & Non-Probative. Additionally, 2 new studies unique to NGS, were developed: Contamination and Sequencing Sensitivity studies. A Contamination study is needed due to a combination of high mtDNA copy numbers and sensitivity of NGS systems. The lower baseline of NGS systems also affect contamination detection. Sequencing sensitivity is assessed because template preparation is a form of PCR where input library concentration may be low due to low amplification DNA input. Results from all the studies aligned with expected trends and concordance.

The results and execution of the laboratory validation demonstrated that NGS is making headway in the forensic market. The adoption of the NGS system is no longer a daunting hurdle because the same mtDNA laboratory and interpretation guidelines can be followed.

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