B114 Mitochondrial Analysis of Challenging Samples Utilizing the ForenSeq™ mtDNA Control Region Solution on the MiSeq® FGx

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Learning Overview: After attending this presentation, attendees will comprehend the application of massively parallel sequencing technologies to mitochondrial control region analysis. Additionally, attendees will be given an overview of the advantages and limitations of massively parallel sequencing as it applies to data analysis and sample throughput for mitochondrial DNA (mtDNA) processing using the ForenSeq™ mtDNA Control Region Solution on a MiSeq® FGx.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by illustrating the advantages of applying massively parallel sequencing to mitochondrial control region analysis. With scalable sample preparation and low limits of detection, this technology could make mtDNA analysis easier to accomplish for current analysts and more accessible to forensic laboratories that wish to expand services.

While Short Tandem Repeat (STR) genotyping is the best practice for human identification, mtDNA sequencing is a supplementary analysis with a great impact on forensic genetics. In some cases, including highly degraded bones, mass disaster remains, or rootless hairs, STR analysis cannot be used to positively identify a sample. This leaves important forensic questions that can be answered with mitochondrial genotyping. While not as discriminatory as STR typing, mtDNA analysis remains a powerful tool for forensic analysts in source attribution and identification. Despite the utility of mtDNA testing, it has been limited to few laboratories because it is labor intensive with high error rates and requires substantial time on valuable capillary electrophoresis instrumentation. Many of the limitations of Sanger sequencing can be overcome through the use of massively parallel sequencing technologies. As this newer instrumentation is implemented in forensic laboratories, it will allow for the expansion of forensic services to include mtDNA analysis of highly challenging samples.

This study utilized the newly released ForenSeq™ mtDNA Control Region Kit for the Illumina® MiSeq® FGx to genotype the mitochondrial control region of typical mock forensic samples, including buccal swabs, challenging bone samples, and rootless hairs. This study also tested the limits of detection for the assay as well as both manufacturer-supported library normalization protocols. This data was analyzed using the integrated ForenSeq™ Universal Analysis Software. Variant calls generated with this assay and software package were confirmed using traditional Sanger sequencing.

This assay is designed around multiple overlapping small amplicons that cover the control region of the mitochondrial genome. This chemistry is scalable for variable sample multiplexing allowing for high or low throughput based on analyst needs. Discriminatory mitochondrial profiles can be generated using as little as 1pg of input DNA, and the system allows for potential advances in the reporting of heteroplasmy in samples. All results obtained with massively parallel sequencing were concordant with those found with Sanger sequencing.

Mitochondrial Control Region, Massively Parallel Sequencing, MiSeq® FGx

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