A98 Comparison of a Commercial mtDNA Sequencing Kit to Standard mtDNA Sequencing Protocols

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After attending this presentation, attendees will have gained knowledge of the typical sequence coverage, cost, and ease of use of a commercial mtDNA sequencing kit as compared to the standard sequencing protocol used for mitochondrial DNA (mtDNA) population databasing at the Armed Forces DNA Identification Laboratory (AFDIL) in Rockville, MD.

This presentation will impact the forensic community by providing information relevant to practical considerations for the implementation of mtDNA sequencing in forensic laboratories.

Over the past eight years, the Armed Forces DNA Identification Laboratory has sequenced greater than 15,000 entire mtDNA control regions (CRs) and 500 entire mtDNA genomes as a part of the laboratory's grant-funded databasing projects. Sample processing typically utilizes protocols optimized for high quality samples, and is performed in a high-throughput, 96-well format from extraction through sequence detection. Robust and reliable sequence data is assured by redundant sequence coverage, particularly in regions prone to length heteroplasmy (LHP), and through additional safeguards in data analysis and data transfer.[1, 2]

New commercial mtDNA sequencing kits for the mtDNA CR and entire mitochondrial genome are marketed as an out-of-box alternative to standard sequencing methods for the detection of sequence variation. In contrast to most PCR-based methods which use a large number of sequencing primers to ensure complete sequence coverage, these commercial kits instead utilize a greater than usual number of amplicons along with M13 primers for universal sequencing.

A comparison of the commercial mtDNA CR and entire mitochondrial genome sequencing kits to AFDIL's standard mtDNA sequencing protocols for population databasing will be reported. The sequence coverage obtained by each method on replicates of positive control DNA and on high-quality population samples, particularly in the hypervariable regions (HV1, HV2, and HV3) of the mtDNA CR was investigated. The AFDIL and commercial sequencing protocols use a similar number of sequences to cover the mtDNA CR (16-17 and 18 sequences, respectively), and a similar percentage of nucleotide bases with double-stranded sequence coverage was obtained using the two protocols. However, the results of the study indicate that significantly greater re-processing would be required in order to obtain complete double-stranded coverage with the commercial CR kit as compared to the AFDIL protocol. This result was due in part to sequence coverage differences between the kits in regions where LHP occurs frequently. When LHP is encountered in a sample, any additional sequence failures or poor quality sequence data can easily result in gaps in double-stranded coverage if there is insufficient redundancy in the sequencing strategy. Given that approximately 50% of all individuals will have at least one LHP in the CR,[3] frequent gaps in double-stranded coverage when LHP is encountered may substantially increase sample processing and analysis time and costs for the commercial kit relative to the AFDIL protocol.

These comparisons of sequence coverage, as well as considerations related to the ease of laboratory processing and sample processing costs for both methods, will be presented.

Reference:

1 Brandstätter A, Peterson C, Irwin J, Mpoke S, Koech W, Parson W, Parsons TJ. Int J Legal Med 2004; 118:294-306.

2 Irwin J, Saunier J, Strouss K, Sturk K, Diegoli T, Just R, Coble M, Parsons W, Parsons TJ. Forensic Sci Int Genet 2007; 1:154-157.

3 Irwin JA, Saunier JL, Strouss KM, Sturk KS, Diegoli TM, Brandstätter A, Parson W, Parsons TJ. *Submitted.*

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