H114 Evaluation of the Ion Torrent™ PGM™ for Use With Low-Copy and Degraded Whole Mitochondrial Genome

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After attending this presentation, attendees will understand the applications of next generation sequencing in forensic DNA analysis and, more specifically, its use with whole mitochondrial genome sequencing and human identification.

This presentation will impact the forensic science community by determining the threshold of sensitivity of the technology in the realm of human identification and to showcase the performance of Next Generation Sequencing (NGS) systems on compromised forensic samples.

The field of forensic DNA analysis, since the beginning of DNA fingerprinting assays, has continued to grow alongside newer and more sensitive technologies in the past decades. The use of NGS has found its niche among disease diagnosis/research, genetic research, genomic studies, and is beginning to delve into the world of forensics; however, this technology has yet to find its permanent place inside the forensic science toolbox as the community tends to adhere to more tested and well-used technologies that have undergone rigorous forensic optimization.

A continuing area of research in forensics is the analysis of mitochondrial DNA (mtDNA) for use with degraded or otherwise compromised samples where nuclear DNA is not present or is too damaged for Short Tandem Repeat (STR) analysis. Though the copy number may vary by tissue type, typically mtDNA is approximately 500 times more abundant than nuclear DNA; this overwhelming majority of mtDNA along with its circular form may allow for some copies to remain undamaged or whol, unlike nuclear DNA.

In many cases, the only evidence recoverable from a crime scene may be teeth, bones, or a shaft of hair, none of which may have useable nuclear DNA to generate an STR profile. Typically, the HV1 and HV2 control regions of the mitochondrial genome (mtGenome) are analyzed for human identification purposes, yet the HV1/2 regions have a lower power of discrimination compared to traditional STR methods; however, the entire mtGenome holds much more data than the HV1/2 regions alone and could offer increased intelligence in human identification. The prospect of sequencing the whole genome offers a potentially higher power of discrimination, especially when databases become more robust and more studies are performed to better understand variation within the mtGenome.

Recently, the Research, Development, Test, & Evaluation (RDT&E) laboratory at the University of North Texas-Health Science Center has developed a high-throughput protocol for mtGenome sequencing. This study evaluates the performance of the Ion Torrent™ PGM™ with whole mitochondrial DNA sequencing under three phases. The initial phase tested the mtGenome sequencing protocol at the Defense Forensic Science Center with pristine reference samples. The second phase tested the method under more stressful conditions with low-copy samples. A dilution series of DNA concentrations used in similar studies was used to determine the threshold at which "useable" analytical data can be obtained for forensic human identification purposes with the Ion Torrent™ PGM™.[1] The final phase tested the system with degraded samples that are representative of real-world evidentiary samples. Samples were enzymatically degraded to generate varying lengths of fragmented DNA. Since the mtDNA sequencing protocol begins with a long-PCR reaction requiring longer, intact DNA, the method needed to be altered. A custom RNA-capture reaction was used to preferentially enrich for mtDNA within the degraded sample in place of PCR.[2]

The combination of data from all three phases of this study will allow for a greater understanding of the application of this instrument and system for human identification. By testing the performance of the Ion Torrent™ PGM™ protocol with low-copy and degraded samples, forensic laboratories can begin to develop methods by which this technology can be incorporated into the field and provide the most useful data possible.

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References:

1. Ballantyne, K., van Oorschot, R., and Mitchell, R. (2007). Comparison of two whole genome amplification methods for STR genotyping of LCN and degraded DNA samples. *Forensic Science International*, 166, 35-41.
2. Carpenter, M., Pettener, D., Theodossiev, N., Dimitrova, D., Leshtakov, K., Buenrostro, J., et al. (2013). Pulling out the 1%: Whole-Genome Capture for the Targeted Enrichment of Ancient DNA Sequencing Libraries. The American Journal of Human Genetics, 93(5), 852-864.

Next Generation Sequencing, Mitochondrial, Whole-Genome Sequencing