

## B59 The Correlation of Hair Morphology and Length to Mitochondrial DNA (mtDNA) Massively Parallel Sequencing (MPS) Results

Lauren C. Canale, BS\*, Pennsylvania State University, University Park, PA 16802; Gloria Dimick, MS, Mitotyping Technologies, State College, PA 16801; Jason Beckert, MS, Microtrace, Elgin, IL 60123; Mitchell M. Holland, PhD, Penn State University, University Park, PA 16802

**Learning Overview:** The goal of this presentation is to provide information on how the physical characteristics of a hair shaft, including the length of the tested hair, impact the success of obtaining mtDNA sequence profiles when using an MPS approach. Microscopical analysis of hairs prior to sequencing is typically not performed to assess whether morphological features contribute to the outcome. In addition, forensic laboratories often receive small hair fragments for analysis (<1cm), which limits the amount of testing that can be performed when using the conventional Sanger-Type Sequencing (STS) workflow. Attendees will learn about a newly developed hair extraction protocol which, along with a mitogenome MPS approach, can overcome many of the challenges.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a “morphology calculator” for determining the relative expectations of successfully generating mitogenome sequence data from a given hair.

Hairs are one of the most common evidence types found at crime scenes, and the extraction of both nuclear DNA (nucDNA) and mtDNA has been demonstrated.<sup>1,2</sup> Hairs in the anagen and catagen growth stages present follicular tissue, especially when forcibly removed, making them providers of nucDNA profiles when the roots are present. Telogen hairs are easily shed, and due to keratinization, leading to degradation of cellular organelles and nucleic acids, nucDNA is too degraded for analysis.<sup>2,3</sup> Therefore, mtDNA sequencing is the method of choice when analyzing shed hairs or fragments of hair shafts.

Historically, DNA testing has focused on recovering the control region of the mitogenome using electrophoretic techniques such as STS.<sup>1</sup> Haplotypes generated from the control region are generally good enough for discriminatory purposes; however, expanding to the entire mitogenome increases the discriminatory power.<sup>4</sup> Sequencing the mitogenome using STS is labor-intensive and a low-throughput method. Contrary to this, MPS analysis of the mitogenome can be performed relatively quickly with higher sample throughput. This study considered the effects of physical hair characteristics, including diameter, presence or absence of a medulla, and length tested, on mtDNA recovery and MPS data quality.

Donated hairs, ranging from 1-45 years of age, were sampled (0.5 and 0.1cm cuttings) starting at least 1cm from the root end, with some cuttings taken at various (mostly unknown) lengths for hairs without a root. The fragments were characterized microscopically prior to DNA extraction, which was performed using a modification to a previously published protocol, and copies of mtDNA were quantified using a custom quantitative PCR (qPCR) assay.<sup>3</sup> The PowerSeq™ Whole Mito System prototype kit was used for amplification, followed by preparation of libraries using the Illumina® TruSeq® DNA PCR-Free HT Library Preparation kit. The Illumina® MiSeq® instrument and 600 Cycle MiSeq® Reagent Kit v3 was used for sequencing, and the sequencing data was analyzed using GeneMarker® HTS from SoftGenetics®. A “morphology calculator” was developed for practitioners and researchers to use that will assist in determining the relative success rates for generating complete mitogenome haplotypes from small hair fragments with different physical characteristics.

### Reference(s):

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3. Jamie M. Gallimore, Jennifer A. McElhoe, and Mitchell M. Holland. Assessing heteroplasmic variant drift in the mtDNA control region of human hairs using an MPS approach. *Forensic Science International: Genetics* (January 2018) 32:7-17.
4. Jonathan L. King, Bobby L. LaRue, Nicole M. Novroski, Monika Stoljarova, Seung Bum Seo, Xiangpei Zeng, David H. Warshauer, et al. High-quality and high-throughput massively parallel sequencing of the human mitochondrial genome using the Illumina MiSeq. *Forensic Science International: Genetics* (September 2014) 12:128-135.

### Next Generation Sequencing, Forensic, Hairs