B41 Using Clonal Massively Parallel Sequencing (MPS) to Characterize Heteroplasmy in the Mitochondrial DNA (mtDNA) of Human Head Hair, Pubic Hair, and Buccal Samples

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After attending this presentation, attendees will better understand how MPS better characterizes heteroplasmy in the mtDNA profiles of head hair, pubic hair, and buccal samples.

This presentation will impact the forensic science community by contributing to an understanding of the heteroplasmy levels that are present in forensically relevant tissues, how heteroplasmy affects the interpretation of mtDNA profiles, and how MPS can be applied to forensic mtDNA analysis.

MtDNA is a useful target for analyzing forensic samples when nuclear DNA (nDNA) profiles are difficult to obtain (for example, when a hair sample does not have follicular tissue attached). Obtaining mtDNA does not often depend on the presence of follicular tissue because mtDNA is present in the hair shaft; however, mtDNA analysis presents challenges that nDNA analysis does not. One such challenge is interpreting heteroplasmy, which is an mtDNA mutation phenomenon that causes more than one mtDNA sequence to be present in an individual. Heteroplasmy has the potential to be useful in forensic mtDNA typing because the presence of heteroplasmy at identical sites in both a casework and a reference sample could not only help confirm a match but also increase the significance of the match.

MPS can overcome the challenges of forensic mtDNA typing. MPS methods are highly sensitive approaches for clonally amplifying and sequencing many samples at once. MPS methods can resolve complex mixtures (from ≥3 contributors) and can also quantify mtDNA mutations. The level of sensitivity achieved with MPS allows for better detection of low-level mutations unobtainable with Sanger sequencing, which is the current forensic mtDNA typing method. The goals of this study were to use an MPS method to better characterize low levels of heteroplasmy across tissues and to compare MPS to Sanger sequencing.

In this study, the Roche® 454 GS Junior MPS platform was used to sequence the mtDNA Hypervariable regions I and II (HVI/HVII) from human head hair, pubic hair, and buccal samples. The MPS sequencing results were compared to the Sanger sequencing results for different tissues from the same sample sets. Point heteroplasmy was detected in more samples when MPS was used than when Sanger sequencing was used. The frequency of heteroplasmy was highest in head hairs, followed by pubic hairs, then by buccal swabs. The 454 was able to quantify levels of heteroplasmy, which cannot be achieved with Sanger sequencing. The 454 detected low levels of heteroplasmy better than Sanger sequencing by reporting levels as low as 1.14% (with at least 500X coverage). With Sanger sequencing, only instances of heteroplasmy at approximately 10% or above could be confidently reported. The 454 also detected more somatic mutations and more heteroplasmy differences within and between different tissue types. Roughly equal numbers of somatic and germ-line heteroplasmy were observed, as well as four heteroplasmic "hot spots" at positions 150, 183, 185, and 189 in the HVII. Finally, there was no significant difference in the frequency of heteroplasmy with an increase in an individual's age.

In conclusion, this study improves understanding of forensic mtDNA sequencing by better characterizing the mutations inherently present in mtDNA. This study also further argues for the use of MPS platforms for forensic mtDNA sequencing.

Massively Parallel Sequencing, Mitochondrial DNA, Heteroplasmy