B83 Mitochondrial DNA Screening Tests: Issues and Alternatives

Terry Melton, PhD, Charity Holland, MPH, and Kimberlyn Nelson, PhD, Mitotyping Technologies, 2565 Park Center Boulevard, Suite 200, State College, PA 16801*

After attending this presentation, attendees will give careful consider- ation to the appropriateness of doing a full or partial mitochondrial DNA analysis on candidate evidentiary samples, and learn about alternatives for partial screening tests.

This presentation will impact the forensic community and/or humanity by assisting mitochondrial DNA practitioners in being better able to choose probative samples with an understanding of the advantages and disadvantages of performing partial v. full sequencing analyses.

Forensic mtDNA analysis has the reputation of being difficult, costly, and time-consuming. Common evidentiary samples are shed hairs and hair fragments. To save time and money, a particular "theory of the crime" may entice a laboratory to screen hair evidence with a partial SNP-like analysis to attempt to include or exclude a suspect or victim. However, often the suspect *du jour* is replaced in time with equally likely suspects. When a screening approach is used, there is concern that the partial mtDNA profile that eliminates an early suspect may be the only available result on evidence when a new suspect appears months to years later, especially if the hair has been consumed. For this reason, it is most appropriate to gen- erate a full mtDNA sequence profile on highly probative evidence at the time of DNA extraction.

Screening is not necessary in most cases. Because mtDNA is not a unique identifier, sample choice is a critical step in mitochondrial DNA analysis. The screening of dozens of marginally relevant hairs (for example, all the hairs collected from a public restroom floor) may result in the false inclusion of an individual who is unrelated to the crime. Instead, microscopic evaluation combined with careful consideration of the pro- bative value of a sample (for example, the hair found in the victim's hand), will usually minimize the number of samples. Highly probative samples will often match victims, family members and even crime scene personnel, and relate to the crime scene in informative ways. Full profiles on these samples, including those of the known individuals, will be required to confirm these matches. On the other hand, a highly probative hair with a mtDNA sequence that matches no obvious person may eventually take on great significance as the case matures, and its full profile should be developed.

Screening methods such as the Roche Linear Array identify common but not especially informative or unique polymorphisms. When a failure to exclude occurs with these methods, full sequencing of HV1 and HV2 must follow to confirm the match. However, the Roche system requires addi- tional equipment beyond that normally required for a sequencing analysis, and also needs laboratory-specific internal validation studies. The rate-lim- iting steps of a quality mtDNA analysis are extraction and amplification of an individual sample in parallel with its accompanying extraction blanks and PCR controls. Screening methods supplant the sequencing step only, and then only in the approximately 50% of samples that are excluded. As noted below, an alternative to screening with linear arrays is readily available as an intermediate step in a standard sequencing analysis.

In the rare case where sample screening is desired, one may sequence a single amplicon in the specific case. The choice of amplicon is deter- mined by a search for informative, and even lineage-specific, polymor- phisms in the known samples. With this approach, sample extraction, PCR amplification and cycle sequencing of the amplicon occur in a single day. If this initial screen indicates that a full sequence profile is necessary for the sample, the remaining ¾ of the profile can be completed the second day. Keeping mtDNA analysis limited to extraction, amplification, and sequencing obviates the need to validate any alternative system such as the linear array, with its associated equipment. This approach also provides the flexibility to use many different primers, which is especially useful in the event that the mtDNA in the evidence is minimal or degraded.

Using a case example, an attorney requested analysis of multiple hairs collected from a homicide victim's hand. A microscopic analysis had sug- gested that all the hairs had come from the same individual (and might well be the victim's), but the attorney wanted confirmation that no hairs could represent a perpetrator who was not the current suspect. Mitochondrial DNA analysis was performed on the victim and suspect and the HV1 region between 16160-16400 was selected for screening evidence hairs due to several highly informative polymorphisms that characterized and distin- guished the two known individuals. Ten evidentiary hairs were analyzed individually at this region by amplifying and sequencing the same region. Per laboratory protocol, the samples were not batched, and each hair was extracted, amplified, and sequenced in a single day. All hairs were found to match the victim. A database search was then performed to estimate the frequency of the partial type, which was low.

Mitochondrial DNA, DNA Sequencing, Screening Tests

 ** Presenting Author*