J Forensic Sci, July 2006, Vol. 51, No. 4
doi: 10.1111/j.1556-4029.2006.00198.x
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Commentary on: Divne A-M, Nilsson M, Calloway C, Reynolds R, Erlich H, Allen M. Forensic Casework Analysis Using the HVI/HVII mtDNA Linear Array Assay. J Forensic Sci 2005;50:548–54.

Sir:

We read with interest Divne et al.'s "Forensic casework analysis using the HVI/HVII mitochondrial DNA (mtDNA) linear array assay" (1). As caseworking mtDNA forensic examiners with hundreds of mtDNA cases behind us, including experience with hundreds of biological specimens of all kinds (2–4), we urge caution in the use of any mtDNA screening method like the linear array assay that develops only a partial profile on evidentiary material. One of us (T. M.) also has had extensive experience with the precursor technology of linear arrays, SSO typing [see, for example, (5)] and has a good idea of its limitations, especially the potential for a high frequency of "null" or "blank" results caused by polymorphisms that block hybridization, a problem which is barely mentioned by Divne et al. Our forensic concerns are in these general areas:

(1) Cases never end. Although the authors state that screening to eliminate evidentiary samples is useful in a case with many questioned samples, we have found that mtDNA hypervariable region sequences, or as much of HV1 and HV2 as can be developed, are required on most probative evidentiary samples. In an unsolved homicide, the suspect eliminated today by screening evidence with a partial profile will not be the suspect of next year. Investigators are very interested in sourcing extremely probative hairs, even if they do not include the suspect *du jour*. Many hairs that do not match suspects need to be attributed to victims, their family members, and even crime scene personnel, to understand how relevant hairs were left at a scene. To decide *a priori* which suspect should be "matched" to hairs does not give full consideration to alternative theories of the crime. When a new suspect is developed months to years later, no full-length mtDNA sequence will be available from important evidence, the original evidence sample may have been consumed in the screening process, and the long-term stability of the extracted mtDNA is unknown. The argument that the linear array PCR product will last, will be available, or can even be accounted for many years later is not logical. We are strong advocates for development of a full mtDNA sequence profile at the time when the newly extracted DNA sample is optimal for amplification and sequencing. Because a large number of old cases are now in postconviction review and undergoing retesting, these concerns are legitimate.

(2) While linear array strips have been promoted as more time saving than DNA sequencing, the rate-limiting parts of mtDNA analysis are extraction and PCR amplification. Our practice is to perform DNA extraction on individual samples to optimize mtDNA yield and minimize the likelihood of contamination. In general, we spend 2 days in the laboratory analysis of a single hair, where most of the hands-on time is accounted for by extraction and PCR amplification, including running of yield gels. Extraction, amplification, and running of yield gels (or other quantification methods) are also steps of the linear array-screening assay. Sequencing, once PCR product is available, is rapid, straightforward, and technically simple, and the sequencing run on the genetic analyzer is hands-off overnight. In the rare case where careful consideration of the needs of testing determines that screening will not compromise future handling of irreplaceable evidence, the screening method we would recommend would be to target and sequence a single informative amplicon in questioned samples. The choice of amplicon is determined by a search for particularly informative polymorphisms in the known samples, for which full mtDNA profiles are developed beforehand. With this approach, DNA extraction of a questioned sample takes place on day 1, the PCR amplification of the chosen amplicon is performed, and sequence data on this amplicon is available the next morning. If the sequence of this amplicon, when compared to the known samples' mtDNA sequences, indicates that a full sequence profile is necessary for the sample, the remaining three amplicons can be completed by the end of the second day.

(3) Linear arrays are promoted as cost saving compared with sequencing. However, a cost comparison of using four linear array strips (one each for the sample, reagent blank, PCR negative, and PCR positive) to develop a screening profile shows that the arrays would cost a total of $124 (four at $31 each), whereas amplification and sequencing of a single amplicon (sample, reagent blank, negative, and positive) cost approximately $60, bearing in mind that extraction costs are equal. Our $60 includes one PCR amplification, yield gel, half-strength sequencing reactions, PCR and sequencing column clean-ups of all reactions, and pro-rated maintenance of an ABI 310. It appears that keeping mtDNA analysis limited to extraction, amplification, and sequencing may obviate the need to purchase and validate any alternative system such as the linear array.

(4) We agree that the need to perform DNA sequencing may prohibit the average lab from undertaking mtDNA analysis. It is unlikely, however, that extracted DNA and/or PCR products generated by a lab that performs screening with the linear array will be acceptable to a majority of sequencing labs in the event of a failure to exclude with the array. For example, in our lab, we cannot accept DNA extraction products unless a coextracted reagent blank control is provided to us. We analyze this reagent blank to determine that no contamination is present before proceeding with the sample. This requirement means that any screening lab must produce a single reagent blank control with each sample DNA to provide sufficient reagent blank product to carry through all sequencing. If PCR product is submitted for sequencing, PCR positive and negative controls must be submitted for tandem processing.

(5) Although screening methods have been proposed in mass casualties, the use of linear arrays for this purpose is a very inefficient approach because every sample will eventually need full length sequencing to identify the low-frequency polymorphisms/haplotypes that link individuals to their families. As noted above, a single sequenced amplicon will start the process of matching victims to families, if time is of the essence. Screening methods could become an expensive and time-consuming digression in a mass disaster.

Screening methods have utility in special situations. Although we believe that they should not be used on limited and irreplaceable evidentiary materials, we would support their use in cases

where many known individuals must be excluded as donors of a particular sample. This is because these known samples are usually abundant and replaceable for many years.

Over the past 7 years of casework, the number of mtDNA cases in which "everything but the kitchen sink" is submitted for testing has decreased. The reasons for this are not completely clear. However, we believe that the thoughtful selection of samples may have become paramount for crime scene investigators as they realize the innate limitations of mtDNA as a nonunique DNA marker. In guiding our clients to evaluate what should be submitted and providing comprehensive and complete sequencing analysis, we avoid revisiting old samples as we continue to work with investigators often many years after initial testing commences.

References

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