ANALYSIS OF FORENSIC SAMPLES USING THE LINEAR ARRAYS OF IMMOBILIZED SEQUENCE-SPECIFIC OLIGONUCLEOTIDE PROBES AND mtDNA SEQUENCING

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Typing mtDNA using linear arrays of immobilized sequence-specific oligonucleotide (SSO) probes provides a rapid method for comparing hairs without the need to do an extensive microscopic comparison. MtDNA typing using SSO probes also can be used for other types of samples such as blood, semen, and saliva. If needed, sequencing may be performed when the information that is provided by SSO typing is not sufficient to exclude individuals as the source of biological sample.

To investigate the value of linear arrays as a screening tool, a variety of biological samples from adjudicated cases were typed. Hairs from sexual assault cases were used to make eight mock cases. Three questioned pubic hairs, a known victim bloodstain, and a known suspect bloodstain were provided for each case. The linear arrays used to type these samples detect polymorphisms in 10 segments of the mtDNA control region, spanning both hypervariable regions I and II.

In the majority of the cases, the SSO typing was sufficient for determining whether the suspect could be the source of the hairs. As in all mtDNA testing, exclusion can be determined with certainty using SSO typing, while identical SSO types indicate the mtDNA originated from someone within the maternal lineage or someone who has the same SSO type. Of the eight cases, there were three clear exclusions of the suspect, four cases in which the questioned the hairs could have originated from either the suspect or the victim and one case in which the SSO typing could not distinguish between the victim or suspect. Out of these eight mock cases, it was only necessary to sequence the samples from one case in order to distinguish between the victim and suspect. The sequencing of the HVI and HVII regions did provide discriminatory information in each case that was inconclusive using the SSO probe linear arrays.

Evidence from 11 cases already analyzed using STR typing at the Georgia Bureau of Investigation was used for SSO typing and sequencing. The evidence from these cases included sexual assault samples, as well as a semen stain, bloodstains, and saliva stains. As with the samples used above, two hypervariable segments of the mtDNA control region were amplified and typed using the linear arrays of SSO probes. The amplified products were sequenced to compare to the results of the linear array typing. SSO probe typing of the mixed samples from the sexual assault cases proved uninformative since the sperm mtDNA, located within the neckpiece, is co-extracted with the vaginal cell DNA. In those cases the mtDNA originating from the victim overwhelmed any signal from the sperm. Results concordant with those obtained from the prior STR testing were obtained in all of the other cases, indicating the linear arrays of SSO probes do have the ability to quickly and easily exclude individuals as the source of the biological specimen.

Typing of the samples with the SSO probe linear arrays provided a rapid screening method to quickly eliminate or include a suspect as the possible source of the hairs. With this technique up to 40 samples could be typed at one time. When sequence analysis is required to obtain more discriminatory information, the same amplified product used for the SSO probe typing can be used so that no additional aliquots of the extracted sample need to be consumed. The linear arrays of SSO probes provided a rapid, viable method of obtaining discriminatory information from samples that prove unsuitable for STR analysis. Through the use of these linear arrays as a screening tool when performing mtDNA typing, the overall amount of sequence analysis to be performed should be reduced by approximately 50% as seen in these studies.