FORENSIC CASEWORK ANALYSIS USING THE HVI/HVII MTDNA LINEAR ARRAYS

M. Allen¹, C. Calloway², M. Nilsson¹, A-M. Divne¹ and H. Erlich²

¹Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden ²Roche molecular Systems, Alameda, CA



The D-loop of the human mitochondrial genome, in particular the hypervariable regions I and II (HVI/HVII), has proven to be a useful target for the analysis of forensic materials, in which the amount of DNA is limited or highly degraded. Although the discrimination power of mtDNA analysis is substantially lower than the power obtained by analyzing several unlinked nuclear markers, it is valuable in many cases. Several technologies to target these regions are used by forensic laboratories, including sequence-based methods (dye terminators and Pyrosequencing) and probe-based assays (LINEAR ARRAYs and Luminex). Conventional analysis of mtDNA by sequencing can be time-consuming and expensive, limitations that can be minimized using a faster and less expensive typing assay. Over the past 5 years we have used various versions of an immobilized SSO probe assay on more than 400 forensic samples. Most recently, we have evaluated a combined HVI-HVII mtDNA-typing immobilized SSO probe assay (linear array) in 16 forensic cases, comprising 89 samples. Using the HVI/HVII mtDNA linear array, 57% of the samples were excluded and thus only 43% of the samples required further sequencing due to a match or inconclusive results. A 20 % higher exclusion rate was observed in these cases with the HVI probe panel in addition to the HVII probes on the linear arrays. Of the 89 samples compiled for the evaluation of the HVI-II linear array, 38 samples showed inclusions compared to 26 samples in the final sequencing analysis. Thus, only 14% of the samples could not be excluded using the linear array probe assay. These results were also compared to analyses using the earlier HVII linear array on a collection of 143 samples from 19 forensic cases. In this data set 47% of the samples were excluded using the HVII probe panel. Using the LINEAR ARRAY HVI/HVII Region-Sequence Typing kit, we demonstrate the possibility to decrease sequencing efforts substantially and thereby increase the turn-around time in casework analysis. A large number of crime scene samples can be screened simultaneously for inclusion or exclusion to suspects and thereby identify the samples of most interest for further investigation. Furthermore. as the PCR is performed in a duplex reaction and remaining PCR-products can be sequenced directly, valuable evidence material is saved. Overall, we found that the linear HVI-HVII linear array assay is a robust, rapid, accurate and sensitive method with a high potential to discriminate between different mtDNA types. The use of the mtDNA linear arrays in our laboratory has served as a valuable pre-screening method and demonstrates a potential to reduce the sequencing efforts by more than half. Address correspondence to: Dr. Marie Allen, Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden, E-mail: marie.allen@genpat.uu.se