G25 Methodologies for Heteroplasmy Identification

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After attending this presentation, attendees will receive information about the detection and study of heteroplasmy in the forensic field.

This presentation will impact the forensic community because it shows two caseworks in which different techniques were implemented to achieve good results.

Mitocondrial DNA (mtDNA) sequencing has been considered a useful tool for forensic analysis, and it is typed routinely in forensic analyses to assist in determining the source of old bones, teeth, hair shafts, and other biological samples where nuclear DNA content is too low or degraded to genotype by analyzing autosomal short tandem repeat (STR) loci.

Typically, forensic mtDNA data are obtained by sequencing (i.e. Sanger method, followed by electrophoresis and fluorescent detection) the two hypervariable regions (HV1 and HV2) of the noncoding control region of the human mtDNA genome. Traditionally, sequencing has been the method of choice because all polymorphisms contained within the amplified fragment can be detected. The definition of heteroplasmy is the existence of two types of mtDNA within an individual. It is known that the sensitivity of heteroplasmy detection is method-dependent, and the most fundamental approach to sampling the individual mtDNA present in an individual is achieved through cloning.

The most common form of heteroplasmy observed in the mtDNA control region is length heteroplasmy. Depending on its extent, length heteroplasmy may result in an inability to read or interpret sequence data and must be compensated for with alternative sequencing strategies. There are different methods to evaluate the mutation load of defective mtDNAs: primer extension, TTGE, RT-PCR, restriction fragment analysis and SSCP. In addition heteroplasmy using SSO typing, DHPLC/nuclear loci and DGGE can be detected. These methods are highly sensitive and can detect and sometimes quantitate heteroplasmy at levels lower than 1%. In this study, information regarding the management of different cases using clonage and DHPLC respectively will be presented. In both cases heteroplasmies were present. The aim of this work is to demonstrate the utility of these two techniques showing the main indications and advantages for the forensic community.

mtDNA, Heteroplasmy, Clonage vs. DHPLC

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