THE DETECTION OF HETEROPLASMY FROM LIMITED AMOUNT OF MITOCHONDRIAL DNA

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In forensic mitochondrial DNA (mtDNA) analysis for personal identification, there are some issues including the problem of heteroplasmy which is the state when two or more different sequences coexist in a single individual and sometimes confuse the decision of sequence. In this experiment, we examined the situation of heteroplasmy detection when strict amount of DNA was amplified and sequenced. From fresh blood of three individuals, from whom heteroplasmy was detected in our previous experiment, DNA was extracted and diluted. Using a Nested-PCR technique for amplification of limited amount of mtDNA, Hyper Variable region of amplified products were sequenced. As a result, when more than 1,000 copies of mtDNA were amplified the ratio of heteroplasmy among examinations from the same individual was not remarkably different while various degrees of heteroplasmies or even the replacement of the nucleotides were detected when less than 100 copies of them were amplified. And more, we diluted the mtDNA of an individual from whom any heteroplasmy was not detected by our regular analysis protocols and reduced template DNA was amplified using Nested-PCR. As a result of sequence analysis of the products, heteroplasmies were newly detected at some positions when less than 100 copies of mtDNA were analyzed. The results of this experiment suggest that sufficient amount of DNA should be offered for forensic mtDNA analysis in order to obtain a reliable result. Otherwise we need to interpret the detected result carefully when we can use only very limited amount of samples, especially it potentially contains the heteroplasmy.