B141 Real-Time Mitochondrial and Nuclear DNA Quantification of Forensic Evidence Materials

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The attendee will learn about a new quantification technology based on real-time 5´exonuclease detection, TaqMan. The system has been used to determine the amount of DNA found in various evidence materials. This technology can be used to choose the optimal target (mtDNA or nDNA) and to avoid waste of valuable DNA material.

This TaqMan technology is a highly sensitive method that can estimate the copy numbers of both mtDNA and nDNA simultaneously. It is a quick and simple method that enables valuable DNA found in evidence materials not to be wasted.

Biological evidence material, found at a scene of a crime, often contains limited amounts of DNA. Samples with scarce DNA amounts are often analyzed by sequencing of mitochondrial DNA, due to its high copy number per cell. Higher discrimination power is however obtained by analyzing nuclear STR-markers, which is preferable when possible. Since the choice of analysis method will be influenced by the amounts of available DNA in a sample, a sensitive and accurate DNA quantification assay is essential in forensic DNA analysis. Moreover, DNA quantification results can be used to estimate the optimal DNA amount to be used in different experiments to ensure successful amplification and avoid allelic dropout or preferential amplification.

We have developed a highly sensitive, rapid and reliable system for quantification of nuclear and mitochondrial DNA copy numbers that consumes a minimum of the valuable DNA sample. The system is based on the real-time 5'exonuclease detection assay, using the ABI PRISM® 7700 instrument (TaqMan). Two specific probes, labeled with different dyes, enables simultaneous quantification of the nuclear Retinoblastoma 1 gene and the mitochondrial tRNA Lys gene.

The quantification system has furthermore been used to determine the DNA copy numbers available in a number of different evidence materials frequently found at the scene of a crime. Analysis of cell debris from different accessories, such as rings, watches and necklaces showed large differences in DNA quantity. Shed hairs, roots from plucked hairs and body hairs have also been quantified successfully using the quantification assay. Since shed hairs are common as evidence materials, differences in DNA quantity in the root part between hairs as well as within single hairs at different lengths were studied. In addition, the high sensitivity and short amplicon length in the assay will allow DNA quantification of degraded and ancient DNA. To evaluate the assay performance on ancient DNA it has been used for quantification of mitochondrial DNA extracted from ancient bone remains found in a grave from the 1000-century in Sigtuna in mid Sweden.

This DNA quantification assay and the evaluation of DNA content in different types of forensic materials have proven very useful in forensic analysis. Moreover, it has been used to determine the minimal amount of DNA required in several different DNA typing systems.

DNA Quantification, TaqMan Technology, Real-Time PCR

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