

## QUANTIFICATION AND SEX DETERMINATION OF FORENSIC EVIDENCE MATERIALS

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A majority of the forensic evidence materials found at a scene of a crime contain minute amounts of DNA. Such evidence materials are saliva stains, shed hairs, skin cells and fingerprints on various items. We have developed a method to quantify the DNA amounts in various evidence materials.

The quantification is based on a real-time 5' exonuclease detection assay (TaqMan) assay using the ABI Prism® 3700 instrument. Both a nuclear and a mitochondrial DNA target system have been developed to allow DNA copy number determinations of very small amounts. The nuclear marker is based on quantification of the Rb1 gene and the mitochondrial target is a tRNA gene in the coding region. The assay will give an estimate of the total number of cells as well as the total number of mitochondrial DNA molecules in a particular evidence sample simultaneously. Based on these results a proper selection of DNA analysis method (nuclear or mitochondrial DNA analysis) can be made without excessive waste of valuable DNA material. The method is optimised and tested on a number of different evidence materials as well as in casework analysis.

Furthermore, a system with simultaneous human sex determination and quantification of evidence materials has been developed. The human enamel protein gene amelogenin has a three base pair deletion on the X chromosome previously used as a sex determination marker in forensic analysis. Increase in fluorescence caused by the binding of SYBR® Green to double-stranded DNA is detected during the PCR. The deletion is detected in a dissociation diagram showing the melting temperatures during the PCR cycles. This system has been tested on control samples, mixed samples and casework examples.

Both systems have proven to be very reliable, reproducible and very useful in our routine forensic DNA analysis. We use one of these methods to pre-screen all the evidence materials to be further analysed in order to not only make a proper choice of analysis method, (nuclear markers, mtDNA, multiplex or single PCR) but also to estimate the minimum amount of the DNA extract to use in the PCR.