ESTABLISHMENT OF A STR-DUPLEX REAL TIME PCR FOR DETECTION AND SELECTION OF FORENSIC SAMPLES CONTAINING LOW COPY NUMBER DNA

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With the high sensitivity of the PCR reaction it is possible to obtain STR profiles from forensic samples containing very small amounts of DNA. Increasing the number of amplification cycles improves the amplification yield from samples containing picogram levels of DNA. Methods have been established and evaluated for the forensic analysis of very low copy number DNA (<100 pg, LCN-Analysis). Trace amounts of human material on the evidence are prepared under microscopic control, DNA is extracted and analysed using either singleplex STR reactions or commercially available multiplex STR-kits. Many samples of a crime scene need to be analysed. These samples often provide important evidence linking a perpetrator to a crime. With methods becoming more and more sensitive it is possible to analyse samples that haven't been before and more evidence is collected at crime scenes. Taken together the forensic analysts are faced with an increasing number of samples with low copy numbers of DNA to be analysed. In order to facilitate this challenge, we have developed a sensitive, real time PCR approach. Monitoring of the PCR reaction by the inclusion of the fluorescent label SYBR Green is used to select the samples containing DNA that can be amplified from the negative ones directly after the amplification. Furthermore the semiquantitative approach allows estimating the amount of DNA in the sample in order to decide if LCN analysis is necessary to provide results. A duplex STR system was established comprising the STR Systems TH01 and D3S1358. Amplicons generated by the real time PCR can be analysed by gel electrophoresis and the profiles generated can be analysed applying the rules of the LCN analysis.