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A novel DNA microarray system for analysis of limited forensic evidence material

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The ability to match a genetic profile from an evidence material with genetic profiles of suspects makes an important contribution to the traditional criminal investigations. Furthermore, the possibility of analyzing very small amounts of DNA found at the scene of a crime has greatly influenced forensic analysis. Today mitochondrial DNA analysis is widely used on biological samples of limited amounts because of the high copy number of mtDNA. However, its discriminating power is not as high as its analysis of nuclear DNA, wherefore a method that combines mitochondrial and nuclear markers would be a powerful tool to reduce the probability of DNA identity by chance for limited DNA samples. Analysis of DNA from material of bad quality and small amounts is also often time-consuming and difficult to perform. In order to increase the discrimination power and reduce the turnaround time in analysis of samples with minute DNA amounts, we have developed a one-color microarray-based SNP detection system.

The system relies on minisequencing in solution prior to hybridization to universal tag-arrays. The minisequencing reaction is based on the extension of a tagged primer with a chain terminating fluorescent dideoxynucleotide at the position to be interrogated. Our one-color system detects C and T polymorphisms in separate multiplex reactions with the fluorophore TAMRA coupled to the respective dideoxynucleotide. The extended primers are hybridized through its complementary tag sequence on the array and positive signals are detected by a confocal laser scanner (Scan Array 5000, Packard BioScience). The tag-arrays are produced with an in-house robotic workstation (GMS 417 Arrayer, SDS) using silylated slides with aldehyde chemistry. In order to maintain a high sensitivity even for analysis of the nuclear markers, the PCR systems have been designed to amplify very short fragments.

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A first chip to be evaluated contains 13 nuclear and 21 mitochondrial SNP markers. The mitochondrial markers cover polymorphisms within the coding region as well as in the hypervariable HVI and HVII regions of the mitochondrial genome. The markers have been tested and evaluated together on microarrays on a number of control samples with known genotypes as well as on a few forensic samples. This microarray based forensic analysis has proven very reliable, sensitive and rapid. A fully developed system will make it possible to perform large, rapid and cost effective analyses with high discrimination power.