B164 Allele Drop Out at Locus D5S818 Caused by a Single Nucleotide Polymorphism at a Primer Binding Site

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After attending this presentation, attendees will understand the reason for the loss of allele D5S818*10 when using PowerPlex®16 kit, approaches to overcome this and similar problems, and the impact and consequences for data bases.

This presentation will impact the forensic community and/or humanity by providing the forensic community with the knowledge of the nature and the frequency of a primer binding site mutation and its impact on multiplex PCR analyses. Moreover, information is provided how to overcome the problem of allele drop out in general.

The goal of this presentation is to clarify the reason for loss of allele *10 at locus D5S818 when using PowerPlex® 16 kit.

Outcome: The authors present data about a single nucleotide polymorphism (SNP) at STR locus D5S818 including association of the mutant with the STR allele *10 and strategies to overcome the problem of allele drop out.

Three SNPs were localized in close proximity to the D5S818 STR region at positions –13 (C/T), +4 (G/T) and +36 (T/C). The reverse primer for amplification of this locus in the PowerPlex® 16 kit binds in the region of the +36T/C SNP. In order to investigate allele frequencies of the +36T/C SNP the authors established an allele-specific PCR method using sequencespecific primers (PCR-SSP). The PCR-SSP method was used for genotyping 288 samples from a DNA archive of blood donors from Southwest Germany. Four of the samples revealed a heterozygous genotype of the +36T/C SNP, thus, the frequency of the +36C-allele was 0.69% in this population. According to the Hardy-Weinberg-Equilibrium the heterozygous +36T/C genotype occurred at a frequency of 1.4%, whereas the homozygous +36C/C type may be observed in 1:20,000 individuals only. Sequence analysis of the D5S818 locus in the 4 individuals with the heterozygous +36T/C genotype demonstrated a linkage of the +36C-allele with the STR allele *10 in all cases. In the multiplex STR-analysis using PowerPlex® 16 kit the samples showed homozygous phenotypes at locus D5S818. The same samples were investigated further using the same primers for amplification of the D5S818 locus but in monoplex PCR. The monoplex analysis revealed heterozygous genotypes for all samples with unambigous amplification of allele *10 in addition to other alleles. In order to solve the problem of allele drop out in multiplex STR analysis the authors investigated the use of a PCR primer containing a wobble base at the corresponding position of the +36T/C SNP. The standard reverse primer for amplification of the D5S818 locus was replaced by the wobblebase primer in the multiplex assay. DNA samples with known STRand +36T/C SNP-types were analyzed by using the modified primer mix. The STR allele *10 could be clearly detected even in samples with a heterozygous +36T/C genotype, indicating that the wobble primer is suitable to overcome the problem of allele drop out. On the other hand the modified primer led to an increased background, i.e., higher numbers of unspecific amplification products. Modification of the PCR program should overcome this problem. In cases of a homozygous D5S818 phenotype the authors would suggest the use of one of the following strategies: 1) Decreasing the annealing temperature; 2) Retyping of the samples using D5S818 monoplex PCR; 3) Genotyping of the +36T/C SNP using the PCR-SSP approach; 4) Use of a D5S818 wobble-base primer in multiplex STR-analysis.

STR Locus D5S818, Aallele Drop Out, Single Nucleotide Polymorphism (SNP)

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