SINGLE NUCLEOTIDE POLYMORPHISMS THAT IMPACT THE RELIABILITY OF MULTIPLEX STR SYSTEMS FOR CODIS PROFILING

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Criminal offender specimens analyzed for STRs and destined for entry into the CODIS database revealed samples that exhibit variability in the amplitudes for the signals of heterozygous alleles for the markers D8S1179 and D13S317 using the Profiler Plus™ STR multiplex system. Such variability can result in inaccurate allele designations that may weaken the reliability of identification by DNA genotyping analysis. If a polymorphic nucleotide resides in the priming sequence for a given marker, the polymorphic allele may be placed at a competitive disadvantage in PCR amplification. To ascertain the source of this variability, the samples were sequenced, and polymorphic nucleotides were found in the loci of both markers. In an attempt to recover the signal for the allele with the reduced amplitude, these samples were processed with the Profiler Plus™ STR multiplex system using annealing temperatures (55°C and 50°C) lower than that recommended by the manufacturer (59°C). PCR with lower annealing temperatures increased the amplitudes of the weaker allele in both markers. A comparison of two commercially available STR multiplex systems performed on the anomalous samples demonstrated variability in only one of the systems likely because of different primer locations between the two systems.

This study demonstrates that rare SNPs within the loci used in CODIS profiling can impact the reliability of allele designations for specimens submitted to the CODIS database. Improved data quality was obtained for samples containing SNPs by employing reduced annealing temperatures in the PCR or by using an alternative multiplex system that presumably contains primers that anneal elsewhere in the STR loci. To better understand the genetic variation in these regions, 96 genetically diverse DNAs were sequenced and analyzed for polymorphisms at both loci. No additional polymorphisms were found in the D8S1179 locus, and one polymorphism was found in the D13S317 locus. Also, variation was found within the STR repeats for both markers. This study demonstrates the potential for ambiguities in allele determinations made between laboratories and between STR systems. It also suggests a possible method of ensuring concordance among samples suspected to contain polymorphisms.