

IDENTIFICATION OF POLYMORPHIC NUCLEOTIDES THAT AFFECT THE PERFORMANCE OF MULTIPLEX STR SYSTEMS EMPLOYED FOR CODIS PROFILING

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The analysis of criminal offender specimens destined for entry into the CODIS database revealed samples that displayed variability in the amplitudes of fragments generated for heterozygous alleles in the markers FGA and vWA when repeatedly processed using the Profiler Plus STR multiplex system. The extreme variability in the ratios of fragment amplitudes in these specimens interfered with the consistent accurate designation of STR alleles. Nucleotide sequencing showed that these specimens contained polymorphic base pairs located nearby the problematic STR repeat regions. Presumably, the variability in fragment amplitudes in these samples occurs because these polymorphisms localize to the PCR primer sites. The effects of these polymorphisms were overcome by using thermocycling profiles with reduced annealing temperatures. When the recommended annealing temperature of 59°C was reduced to either 55°C or 50°C, the markers containing polymorphisms reliably produced even fragment amplitudes. Also, no other problems with PCR amplification were identified in samples processed using reduced annealing temperatures. Additional polymorphisms surrounding the FGA and vWA loci were identified by nucleotide sequencing DNA isolated from a genetically diverse population selected by SNP haplotype-pair analysis. A comparison between STR typing results obtained with samples containing these polymorphisms using a variety of commercially available multiplex systems will also be presented.

These results demonstrate that SNPs can impact the data quality and the accuracy of allele designations in STR systems employed to process samples for the CODIS database. A protocol that used reduced annealing temperatures to diminish the competitive disadvantage during amplification at the mismatched allele improved data quality and may have general application to samples suspected to contain polymorphisms. Also, data from a general search for polymorphisms at the FGA and vWA loci provide direct information regarding the extent of this problem. These results have implications regarding the concordance of STR allele determinations made between laboratories and between STR systems.