IDENTIFICATION OF A D8S1179 PRIMER BINDING SITE MUTATION AND THE ADDITION OF A PRIMER DESIGNED TO RECOVER NULL ALLELES

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A population study of Chamorros and Filipinos using short tandem repeat (STR) loci amplified with the AmpF*l*STR® Profiler Plus™ kit demonstrated an excess of observed homozygosity at the D8S1179 locus. The use of a different set of D8S1179 primers with the same samples did not demonstrate an excess of homozygosity. A primer binding site mutation was identified as the cause of allele dropout when using the AmpF*l*STR® Profiler Plus™ Primer Set. All samples that exhibited allele dropout at the D8S1179 locus contained a G-to-A transition at position # 143620 of the GenBank sequence accession AF216671.

Variants in the primer binding site that affect amplification of an allele have been reported and are to be expected. Typically, one would expect variants that significantly affect amplification to reside at or near the 3' end of the primer. However, the substitution causing allele dropout at the D8S1179 locus is 16 nucleotides downstream from the 3' end of the reverse primer. The data show that this particular primer binding site mutation resulting in an A•C single base pair mismatch does have a significant effect on T_m.

A second D8S1179 reverse primer specific for the mutation was constructed and included in the newly developed AmpF*l*STR® Profiler Plus™ PCR Amplification Kit. Thus, both the original D8S1179 reverse primer and the newly constructed primer are used simultaneously in this multiplex PCR amplification. The null alleles were recovered in all samples previously determined to carry the primer biding site mutation. No deleterious effects or non-specific peaks were observed in validation experiments evaluating primer concentration, Mg^{2+} concentration, annealing temperature and population samples.