B51 9-plex MiniSTR Assay to Increase Successful Human Identification From Compromised Samples

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After attending this presentation, attendees will learn about the devel- opment of a new multiplex STR assay for degraded and inhibited DNA samples.

This presentation will impact the forensic community and/or humanity by presenting results which will provide additional information to forensic scientists on the development of a mini-STR multiplex assay that can be utilized as valuable tool for analysis of compromised DNA samples. The goal of this presentation is to share preliminary results on the development of a mini STR multiplex assay containing 8 STR loci and the sex-determining locus Amelogenin.

Multiplex short tandem repeat (STR) genotyping assays using fluo- rescent detection and capillary electrophoresis represent the most popular method of human identification due to the highly polymorphic nature of STRs and their small fragment size. Although the STRs are relatively small (~100-500 bp), DNA degradation may occur as the result of sample decom- position due to environmental exposure producing DNA templates that are highly fragmented. This leads to a reduction in the yield of intact target fragments resulting in genetic profiles with allele and/or complete locus dropout. The problem is exacerbated when large multiplex STR reactions are used due to the wide fragment size range of the amplified PCR products e.g. the largest STR loci fall below the detection limit due to preferential amplification of the smaller loci.

To solve this problem and recover information from degraded and/or inhibited DNA samples, the amplicon size of the largest eight STR loci in the AmpFlSTR® Identifiler® PCR Amplification Kit (D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, FGA) were reduced by moving primers closer to the STR repeat region. Five of these loci (D16S539, D21S11, D2S1338, D18S51 and FGA) also represent five of the largest loci in the AmpFlSTR SGM Plus® kit. Size reduction of the STR amplicons ranged from 33 to 208 bp. However, reducing the amplicon size caused an overlap of the STR loci, which prevented simultaneous separation by capillary electrophoresis (CE) and limited the number of loci that could be simultaneously multiplexed using 5-dye labeling tech- nology. To resolve this problem mobility modifiers were coupled to the dye-labeled primers used in the PCR. The sizes of selected amplified frag- ments were increased using these mobility modifiers. This technology enabled simultaneous CE separation of DNA fragments of similar length and creation of a larger miniplex. In this presentation preliminary results of a miniplex assay containing 8 STR loci and the sex-determining locus Amelogenin will be described. The miniplex assay was compared to current commercially available STR kits for sensitivity, genotype concor- dance, and performance with simulated inhibited and degraded DNA samples.

The results presented will provide additional information to forensic scientists on the development of a mini-STR multiplex assay that can be utilized as valuable tool for analysis of compromised DNA samples.

miniSTRs, Multiplex PCR, Genotyping

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