B60 Development of a New Autosomal STR Multiplex With Additional Loci to Benefit Human Identity Testing

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After attending this presentation, attendees will gain knowledge of a new autosomal STR multiplex assay with non-CODIS loci.

This presentation will impact the forensic community by introducing a new DNA test for human identification purposes.

Learning Objective and Outcome: The importance of the development of this new autosomal STR multiplex for its use within the forensic community and its value for rapid reference sample typing will be discussed, as well as the approach used in its development.

A total of 26 additional STR markers spanning unused chromosomal locations across the 22 autosomes have been characterized[1, 2] so that they may be combined without conflicting with the current 13 CODIS core loci that are widely used in U.S. DNA databases. These 26 STRs were originally developed as reduced size 3plex miniSTRs with product sizes below 140 bp for recovery of information from degraded DNA that can come from missing persons or mass disaster samples.[3] A single amplification, five-dye multiplex has been designed and developed to combine all of these new loci in one reaction to enable rapid analysis of reference samples. These new STR loci have been examined in U.S. population samples and sequenced for calibration of allele nomenclature in standard samples.[1, 2] In addition, a concordance evaluation was performed with the multiplex to compare genotypes obtained with the previously characterized miniSTR loci to determine if there are any null alleles present with the newly designed primer sets. The multiplex was also run across father/son plates to determine the individual mutation rates of each STR locus characterized.

Methods and Materials: The development of the new autosomal STR multiplex will be discussed along with the accompanying concordance evaluation and mutation rate study performed with its use. The 26 miniSTR loci were previously characterized[1, 2] and redesigned to be combined in a single amplification five-dye reaction. The primers for these loci were all designed using Primer3 software. These primers were then screened using the AutoDimer software[4] and BLAST searches were performed to determine the compatibility of the primers used in multiplex. Four separate dyes were then assigned to the forward primers (6-FAM, VIC, NED, and PET) according to the multiplex design. The fifth dye channel (LIZ) was reserved for the appropriate size standard. The primers were quantified and mixed together to be run with previously characterized samples. Problematic loci causing artifacts were identified and redesigned. Once all of the adjustments were made optimization of the multiplex ensued, such as empirically determining primer concentrations for balanced dye signals and dye-blob removal with post- amplification filters. SRM 2391b was genotyped using the multiplex and from this information, bins and panels were created for each locus in the GeneMapper ID, version 3.2.1 software. The multiplex was then evaluated across more than 600 samples representing the three major populations in the U.S.: Caucasian, African Americans, and Hispanic. The population data were genotyped and a concordance study was performed comparing these types to those previously determined from the miniSTR loci[1, 2] to identify any null alleles from either dataset. The multiplex was also examined across approximately 400 father/son paired samples and mutation rates from father to son were calculated.

Summary of Results: The new STR multiplex with 26 different loci and Amelogenin has been designed and evaluated for concordance with non-overlapping PCR primers using >600 U.S. population samples. Mutation rates were also determined using father/son samples.

Conclusions: A new STR multiplex has been developed in a single amplification reaction for rapid reference sample typing.

References:

1 Coble, M.D., Butler, J.M. (2005) Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA. *J. Forensic Sci. 50(1): 43-53.*

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3 Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci.* 48(5): 1054-1064.

4 Vallone, P.M. and Butler, J.M. (2004) AutoDimer: a screening tool for primer-dimer and hairpin structures. Biotechniques 37(2): 226-231.

Short Tandem Repeat DNA Typing, Autosomal STR Multiplex, Mutation Rates

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