## B103 Development of 8 New STR Miniplexes for Their Usage in the Improved Analysis of Degraded DNA Samples

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After attending this presentation, attendees will learn the importance of the development of these 8 new STR miniplexes within the forensic community and their value in the analysis of degraded DNA will be dis- cussed, as well as the approach used in their development.

This presentation will impact the forensic community and/or humanity by enhancing the knowledge of the forensic community in regards to methodologies used in the development of 8 new STR mini- plexes for their usage in the improved analysis of degraded DNA samples.

The loci in each of the 8 miniplexes (3) loci per miniplex for a total of 24 new loci) were chosen based on their size and location on certain chro- mosomes. The candidate loci are all either located on chromosomes that differ from the 13 CODIS core loci or are at least ~ 50 Mb apart from an existing CODIS loci on the same chromosome, and therefore unlinked from that particular marker. New autosomal STR loci are being examined because many of the CODIS core loci have large allele ranges (*e.g.*, D21S11 and FGA) that make it impossible to create small PCR products. A number of studies have demonstrated that successful analysis of degraded DNA samples improves with smaller sized polymerase chain reaction (PCR) products (1). In addition, by moving PCR primers closer to the STR region, it is possible to decrease the possibility of allele or locus- dropout that may occur in degraded samples.

The characterization of the 24 miniSTR loci used in the 8 new miniplexes will be discussed along with the various processes used throughout their development. The first two miniplexes were previously characterized (2). The process for their development and characterization form the basis of the current work. The remaining 18 miniSTR loci to be tested were determined based on certain properties including size and location of the marker (*e.g.* from the literature, Genbank sequences, and human BLAT searches) (3). It was then necessary to design the forward and reverse primers for each locus using Primer3 software. These primers were then tested in the AutoDimer software (4) to determine the compati- bility of the primers used in multiplex. Next, the optimal concentrations of primers must be empirically determined for balanced dye signals, elimi- nating bleed-through from one dye to another. The primers were separated into 6 new multiplexes in addition to the 2 multiplexes that were previously developed and were evaluated across more than 600 samples representing the three major populations in the U.S. Caucasian, African Americans, and Hispanic. Several alleles from each locus were sequenced to define the number of repeats. From this information, bins and panels were created for each locus in the GeneMapper ID, version 3.2 software. The population data was then genotyped, and by using the PowerMarker data analysis software, allele frequencies and population statistics within each locus were determined. Finally, allelic ladders were created for each of the miniplexes using the appropriate population samples.

These 8 new miniplexes with 24 different loci in total have all been designed and are currently being evaluated using population samples.

Eight novel miniplexes have been developed to improve analysis of degraded DNA samples and complex forensic paternity cases where the 13 CODIS loci are insufficient.

**Refrences:**

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2. 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.
3. Schoske, R., Vallone, P.M., Ruitberg, C.M., Butler, J.M. (2003) Multiplex PCR design strategy used for the simultaneous amplification of 10 Y chromosome short tandem repeat (STR) loci. *Anal. Bioanal. Chem.* 375: 333-343.
4. Vallone, P.M., Butler, J.M. (2004) AutoDimer: a screening tool for primer-dimer and hairpin structures. *BioTechniques* 37: 226-231.

**Short Tandem Repeat DNA Typing, Degraded DNA, Reduced Size PCR Products**

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