DEVELOPMENT OF A NOVEL MULTIPLEXED DNA ANALYSIS SYSTEM FOR HIGHLY DEGRADED DNA SAMPLES

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Retrotransposable elements (REs) consisting of long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) are a group of markers that can be useful for human identity testing. SINEs are a class of REs that are typically less than 500 nucleotides long; while LINEs are typically greater than 500 nucleotides and up to several thousand base pairs in length. The third type of RE is the composite retrotransposon known as SVA (**S**INE/**V**NTR/***A****lu*) elements.

RE's are identical by descent only. In addition, REs do not yield stutter artifacts due to slippage during the PCR. Until now however, due to the inherent size difference (>300bp) associated with insertion and null alleles (or INNULs), the use of REs has not been practical for forensic applications. Although the use of SINEs such as *Alu* in determining human identity has been studied and reported in the literature, the more than 300 bp size difference between the two alleles prevented development of any useful multiplexed system. This is the first time any research on the use of LINEs, SINEs, or SVA element insertions, as a multiplexed system, has been proposed.

To circumvent the allele size disparity, we have developed a primer design methodology that essentially removes the intra-specific locus competition that occurs in heterozygotes. This involves utilization of the direct repeat units that flank an *Alu* element. The novel primer design reduces overall amplicon size among loci as well as the difference in amplicon size between the two allelic states of INNULs. The resulting INNUL allelic amplicons can be designed to differ by as little as one base pair instead of the 300 bp ALU insertion. Additionally, the amplicon size has been reduced substantially, to a size much smaller than currently used STR markers, such that substantially degraded DNA samples can be profiled. Utilizing this primer design, a more simplified, rapid and automated typing technology can be applied to LINE, SINE and SVA insertion polymorphism typing.

The development of 15 RE's and Amelogenin into a single multiplexed amplification system will be presented. The markers and base pair sizes are depicted in Figure 1 below. As is evident, we were successful in significantly reducing the amplicon size to 50-125 bp. The small amplicon sizes result in an extremely sensitive, rapid and useful multiplex for highly degraded forensic samples. When evaluating 3 major North American populations, these markers generally met Hardy-Weinberg expectations and showed little evidence of detectable levels of linkage disequilibrium between the markers tested.

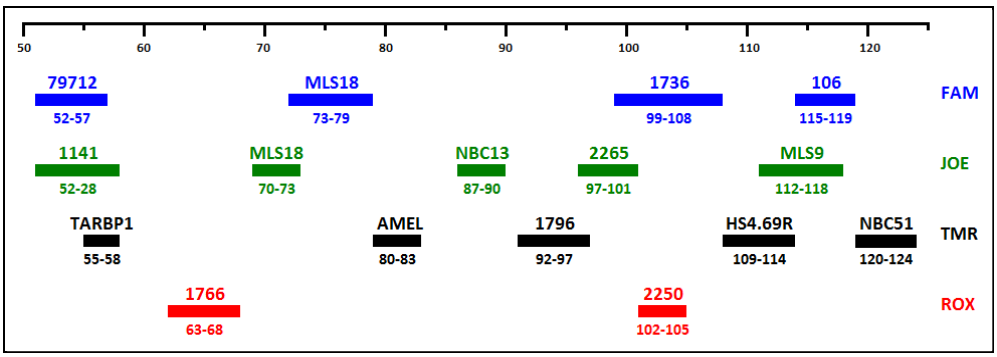

Figure 1. Schematic of the system detailing a multiplex comprised of 16 markers. The X axis depicts the bp sizes and the Y axis depicts the fluorescent dyes used. The locus names are shown at the top of each marker and the bp size range for the alleles is shown below each marker.

Data to be presented will support the usefulness of this system for analyzing highly degraded DNA samples which did not produce usable STR results, but can provide results having high discrimination power, without Mt DNA analysis. Limitations of this system due to the bi-allelic nature, such as mixture interpretation, will be discussed. This system will prove very useful for analyzing single source degraded DNA samples such as those found in mass disasters.

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