B147 Development of New miniSTR Loci for Improved Analysis of Degraded DNA Samples

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An approach to developing new DNA typing markers for improved analysis of degraded DNA samples will be described.

The community will learn about new DNA tests that our group is developing to improve analysis of degraded DNA samples. This will be of interest to DNA analysts doing casework or thinking about handling samples from mass disasters.

A number of studies have demonstrated that successful analysis of degraded DNA specimens from mass disasters or forensic evidence improves with smaller sized polymerase chain reaction (PCR) products (1). Forensic DNA analysts often perform short tandem repeat (STR) typing on highly degraded biological material and then turn to mitochondrial DNA testing, which is less variable but more likely to obtain a result due to higher copy numbers in cells, if many or all of the STRs fail. The commercially available kits for multiplex amplification of the 13 CODIS (FBI's COmbined DNA Index System) STR loci usually exhibit allele or locus-dropout for larger sized loci with degraded DNA or samples containing PCR inhibitors.

By moving PCR primers closer to the STR repeat region, we have demonstrated that it is possible to obtain fully concordant results to the commercial kits while improving successful analysis of degraded DNA with smaller PCR products or miniSTRs (1). However, many of the CODIS core loci have large allele ranges (e.g., D21S11 and FGA) that make it impossible to create small PCR products. Thus, we are going beyond the CODIS core loci and examining a battery of new potential STR loci that can be made less than 100 bp in size and would therefore be helpful in testing highly degraded DNA samples. These new STR loci are being put together into novel DNA testing assays and evaluated across more than 600 samples representing the three largest populations in the U.S.: Caucasian, African American, and Hispanic.

Methods and Materials: Following selection of potential new STR loci, PCR primers were designed using a standardized approach that has been described previously (2). For the miniSTR loci, a single locus is placed into each dye color in order to keep the size ranges less than 100 bp where possible (1). Candidate loci were selected from STRs located on chromosomes that did not possess any CODIS STRs (i.e., chromosomes 1, 6, 9, 10, 14, 15, 17, 19, 20, 22, and X) so that results could be treated as unlinked and the product rule employed in any statistical analysis between the CODIS loci and new ones.

Summary of Results: New PCR primers were designed and tested for a dozen new STR loci. All loci show a moderate degree of polymorphism in the U.S. population samples tested and compared well to results from the CODIS loci in the same sample set.

Conclusions: The selection of STR loci that have a narrow allele size (e.g., less than 50 bp) and can be made smaller than 100 bp works well with degraded DNA samples. In addition, these new STR loci that are under development will be helpful in analysis of casework involving close relatives including complicated forensic paternity cases (e.g., incest) where the 13 CODIS loci are not enough.

Refrences:

(1) 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.*, in press.

(2) 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.

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

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