B65 Robust STR Multiplexes for Challenged Casework Samples

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The goals of this presentation are to teach superior methods to produce STR profiles with challenged samples.

Many casework samples contain amplification inhibitors or degraded DNA and sometimes both. This condition has been particularly common with World Trade Center bone fragment samples that were exposed to high levels of moisture and extreme heat for several weeks prior to processing. Work with these samples motivated the authors to create STR multiplexes displaying more robust results under these trying casework conditions. The successful development and implementation of these systems will be described.

Commercial providers of STR multiplex systems have been motivated to develop systems that generate some alleles greater than 350 or even 400 bases. With the selection of 13 CODIS STR loci, the numerous alleles of each locus, the attempt to include as many loci as possible in each multiplex, and the limitation of the number of instrument-compatible dyes allowing clean color separation, these large amplification products are required.

Researchers observed that locus dropout occurs predominantly with the larger amplicons whether the cause is degradation or inhibition. K. Yoshida et al. (1997) and P. Wiegand et al. (2001) have described improved monoplex STR amplification of degraded DNA using primers that generate shorter amplicons. The multiplex design was built upon the efforts of J. Butler et al. (personal communication) to generate smaller amplification products for loci that were originally designed to generate large amplicons.

Developed were 3 multiplexes. In general, they generate no STR products greater than 210 bases long. The single exception is the FGA locus. The largest alleles of FGA can be 206 bases long in the repeat regions themselves. With additional size required for primer lengths and for quality hybridization site selection, products at this locus can sometimes be larger than 210 bases.

Another goal of the multiplex design was to complement the larger CODIS loci of all commonly employed commercial multiplexes. All the large loci of Profiler Plus, COfiler, and Identifiler are contained in BodePlex 1 and BodePlex 2. The combination of BodePlex 2 and BodePlex 3 contain all the large CODIS loci of the PowerPlex 16 and PowerPlex 16BIO multiplex.

Primary developmental concern was multiplex performance under extreme conditions. The systems were developed to generate approximately ten times the normal signal using 0.25 ng of human DNA template. Great care was taken to adjust primer melting temperature, primer concentration, and primer sequence selection to provide robust amplification while avoiding or limiting artifacts in the multiprimer environment.

The reasoned design and robust nature of the product transferred to success with analysis of the World Trade Center samples. In an initial attempt with these samples, 45% of previously poor quality profiles were improved to the point that they could be accepted for use in the identification project. Additional attributes of validation and implementation of these systems will be discussed.

STR, Small Amplicons, World Trade Center

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