ROBUST STR MULTIPLEXES FOR CHALLENGING CASEWORK SAMPLES

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Casework samples often contain amplification inhibitors or limited amounts of DNA and sometimes both. In our recent work, we have developed multiplex STR sets that co-amplify CODIS loci, even when samples contain degraded DNA, amplification inhibitors, or very few copies of target material. The creation, characterization, and applications of these sets relied upon optimization of several amplification parameters to provide robust systems for challenging casework conditions.

Our experiences in generating DNA profiles with World Trade Center bone fragment samples motivated us to develop better tools for analyzing degraded DNA samples and those containing very few DNA copies. These tools initially consisted of two multiplex sets, but more recently we have developed a third multiplex completing the complement of CODIS STR loci. The distribution of loci in the three multiplexes is summarized here:

Multiplex 1: CSF1PO, D7S820, D13S317, D16S539, and D21S11
Multiplex 2: D7S820, D18S51, FGA, and TPOX
Multiplex 3: D3S1358, D5S818, D8S1179, D13S317, TH01, vWA

Note that the D7S820 locus is common to Multiplex 1 and Multiplex 2, while the D13S317 locus is common to Multiplexes 1 and 3. This feature was included in the design to limit the potential for undetected sample mix-up. In general, the new multiplexes generate no STR products longer than 210 bases. However, the largest D8S1179 and FGA alleles can generate fragments up to 250 and 270 bases, respectively. With the FGA repeat unit itself comprising 206 bases in the longest allele, this is not surprising.

Generating smaller amplified products of the CODIS loci than available with most STR multiplex sets was just one critical component of improved performance. Practical application with challenged samples was achieved by taking great care to design compatible primer sequences while adjusting primer melting temperatures, primer concentrations, and several facets of the amplification protocol to provide robust amplification while avoiding or limiting artifacts in the multi-primer environment.

The systems were designed to generate well-balanced profiles with 5 to 10 times the amount of amplification product observed with commercial multiplex sets when using 0.25 ng of human DNA template. This represents about 75 genomes of template, an amount safely above that which might cause stochastic effects of allele imbalance or allele dropout.

However, it has become clear that the greatest value of these sets comes from their remarkable sensitivity. As few as one to three DNA molecules can be detected in some cases. Of course, allele imbalance and allele dropout are common occurrences at these detection levels. Thus, customized allele-calling interpretation guidelines were developed and reviewed by outside parties prior to their implementation. The systems are currently being used for low copy number genotype determinations to assist with World Trade Center identifications that failed using commercially available kits. Approximately three times the genotype determinations are obtained with the new sets when compared with other multiplexes used on the same challenging sample sets. Recent successes with increased genotype determination for at least some mock evidence samples, such as handled trash bags or handled paper, indicates these multiplexes may have much wider application with common casework items.