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Robust STR multiplexes for challenging casework samples

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Abstract. The presence of amplification inhibitors and degraded DNA in many World Trade Center (WTC) victim bone fragment samples motivated us to create STR multiplex sets displaying more robust amplification with challenging casework samples. We developed multiplex sets that co-amplify smaller versions of the larger amplicons present in commercial sets. They generate well-balanced profiles with at least five-fold the typical signal using 0.25 ng of human DNA template and generated three times the genetic information derived from commercial kits using the more challenging WTC samples. Sensitivity of the systems approaches detection of individual DNA fragment templates, requiring implementation of appropriate interpretation guidelines for this low copy number, high throughput environment.

Keywords: Short tandem repeat; PCR; World Trade Center; Low copy number

1. Introduction

Many World Trade Center (WTC) disaster bone samples had been exposed to extensive moisture in combination with substantial heating for periods of up to 2 months. Limited or degraded DNA and amplification inhibitors in some samples lead to limited profile determination using commercial multiplex systems. Here, we describe the design, development and application of new multiplex sets that generated substantially more robust genotype determination with degraded DNA, low copy number samples and samples containing amplification inhibitors.

2. Results and discussion

We processed 12 392 bone samples during the first phase of analysis of World Trade Center remains. Using the QIAamp 96 DNA Blood Kit DNA extraction method [1] in combination with Profiler Plus and COfiler multiplex systems, we generated full 13-locus profiles with 27% of samples. Another 17% produced 7–12 loci, while 22% revealed 1–7

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loci and 34% of samples provided negative results. We initiated two projects to improve genotyping success rates. We previously reported development of a modified bone sample DNA extraction procedure [1], and here describe the design, development, testing, manufacture and validation of new multiplex sets providing more robust genotype determination with challenged samples.

We reasoned that re-design of the larger loci of Profiler Plus and COfiler into smaller amplicons in two multiplex sets, BodePlex 1 and BodePlex 2 (Fig. 1) and modifying protocol and primer mix parameters to optimize performance at reduced DNA templates would alleviate limitations due to DNA degradation or inhibition. In development, we demanded robust amplification with 0.25 ng template or less, complete polymerase-directed non-templated addition and no inappropriate primer-generated products.

We used some previously described primer sequences [2], but found about half the sequences required re-design to meet the demands of high product yield in a low template environment. Evaluation of primer set combinations necessitated additional primer design. In all, more than 100 different primer sequences from six different oligonucleotide vendors were evaluated in dozens of combinations to generate operationally useful multiplex sets. The extremely successful performance of the first two systems encouraged us to complete the set of the 13 core STR loci of the CODIS system by developing a third multiplex (data not shown). We employed previously identified primers [3] for the smaller loci of the PowerPlex 16 system and modified only the vWA locus primers of this set.

Fig. 1 displays the locus combinations and typical profiles generated with 0.25 ng template for the BodePlex 1 and BodePlex 2 systems, respectively. Note that the average peak height displayed in each case is similar to a typical amplification of 1–2 ng template using standard commercial multiplex systems. This increased product yield is due not only to modified primer selection, but also to protocol modifications such as increased enzyme concentration, prolonged elongation times and 32 cycles of amplification.

Primer-generated artifacts were not detected. However, low-RFU dye artifacts could not be completely eliminated despite vendor selection based primarily on this trait. On the

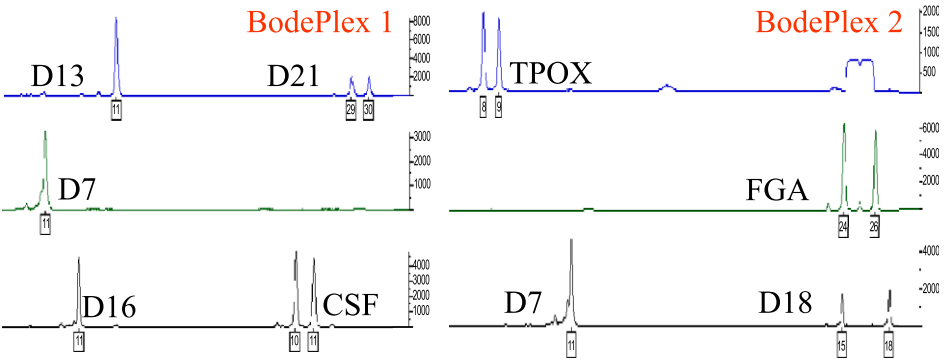

Fig. 1. BodePlex 1 and BodePlex 2 contain smaller versions of the large amplicon loci of Ampl*F*lSTR Profiler Plus and COfiler. The amplified product of 0.25 ng DNA template is shown for each multiplex, respectively. Fluorescein-labeled (top), HEX-labeled (middle) and NED-labeled (bottom) loci are indicated.

positive side, these artifacts have low RFU values and can be readily discounted in profile determination since they are reproducibly present even in negative controls.

Validation of all three BodePlex systems was conducted according to United States DNA Advisory Board guidelines, including demonstration of accuracy, precision, reproducibility and concordance with other systems. Species-specificity and performance with non-probative samples and mixed DNA samples was characterized. Sensitivity experiments indicate alleles are generated with as little as one to three copies of DNA (data not shown). With such low copy number (LCN) situations, stochastic processes often generate allele imbalance or allele dropout. Therefore, our interpretation guidelines include a required minimum 400 RFU for homozygote calls and 100 RFU for individual allele calls. When one allele between 100 and 400 RFU is observed at a locus, it is called but the presence or absence of a second allele is considered unknown.

LCN projects with other systems have revealed "allele drop-in" in negative controls and samples [4]. This phenomenon is distinguished from general contamination in that only one or possibly two foreign fragments are observed rather than a full or substantially full profile. The alleles observed are random in nature and are not derived from the analyst performing the work. With BodePlex systems, we observe allele drop-in in 1–2% of negative samples. However, to insure proper assignment, allele designations are made only if the same allele is observed in two separate amplification reactions of the sample. This requirement for redundancy limits the number of identified alleles, but insures the highest quality of the accepted data in LCN situations.

In summary, these multiplex sets support high throughput, low copy number casework with extremely challenged samples.

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