B146 Miniplex Primer Sets: Sensitivity, Peak Balance, Inhibitor and Concordance Studies

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After attending this presentation, attendees will have been presented research on the current development of the Miniplex primer sets for the analysis of degraded DNA.

This presentation will discuss the utility of the Miniplex primer sets in the analysis of degraded DNA. In degraded DNA, only low concentrations of DNA template are generally available and PCR inhibitors may be present. The effect of DNA template concentration on signal intensity and peak balance ratio will be presented. This presentation will also discuss the effect of PCR inhibitors on the amplification efficiency of the Miniplex primer sets. Lastly, a full concordance study of 541 DNA samples as part of the method validation process will be presented.

In DNA recovered from the crime scene or site of disaster, the possibility of finding an intact target DNA sequence is greatly reduced due to extensive fragmentation of the DNA template. Most commercial kits have amplicon products ranging from 100-500 base pairs. Thus, in multiplex typing kits with a wide range of amplicon sizes, the larger PCR amplicons often have lower signal intensity and fall below the detection threshold. Re-designed Miniplex primers are positioned as close as possible to the ends of the stretch of repeats to reduce the amplified product size. However, in situations where DNA is degraded, the DNA template is not only highly fragmented but the concentration of the DNA template recovered is also low. To test the hypothesis that shorter PCR amplicons can improve amplification efficiency, we examined the effect of DNA template concentration on signal intensity and peak balance ratio.

DNA concentrations ranging from 31 pg to 500 pg were amplified with Miniplex 2, Miniplex 4 and Big Mini in 25 µl reaction volume. The Miniplex primer sets were able to successfully amplify DNA targets at these concentrations although these are below the range recommended for commercial sets. For example, amplification at 100 pg/ 25 µl gave signal intensities of 2000 RFU and 800 RFU for Miniplex 2 and Miniplex 4, respectively. For the Big Miniplex, template concentrations greater than 250 pg/ 25 µl were needed to avoid allele dropout. Good peak balance ratio (>0.6) was achieved at these concentrations. Primer concentrations for the Big Miniplex were further tested to improve the sensitivity and peak balance ratio of this multiplex set.

Another challenge associated with forensic samples is the presence of PCR inhibitors. The presence of these compounds can interfere with the amplification process resulting in preferential amplification of one locus, allele drop out, or no amplification at all. The effect on amplification efficiency of the Miniplex primer sets due to PCR inhibitors such as hematin, indigo dye, humic acid, melanin, calcium, and collagen was also investigated and will be presented.

Although all known polymorphisms have been taken into account in the design of the Miniplex primers, primer binding related problems may occur. Only comparison studies can verify the presence of previously undetected polymorphisms. A concordance study of 541 DNA samples was performed to check for the existence of mutations that could lead to allele dropout or low sensitivity of one allele in standard STR typing kits. At the same time, potential point mutations in the Miniplex primer binding region or insertion/deletion between commercial primers and Miniplex primers were also investigated. These samples were obtained from the National Institute of Standards and Technology (NIST) and were previously typed with the commercial kit AmpF*l*STR™ Identifiler.

Overall, these Miniplex primer sets can provide an alternative to standard STR typing kits when allele drop out and low sensitivity of large amplicons becomes a problem due DNA degradation, PCR inhibition or primer binding site mutations.

Degraded DNA, Miniplex, STR

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