MODIFICATION OF AMPLIFICATION AND STR DETECTION CONDITIONS TO ENHANCE SENSITIVITY OF THE IDENTIFILER® (ABI) AND POWERPLEX® 16 (PROMEGA) KITS FOR LOW COPY NUMBER DNA SAMPLES.

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Our research demonstrates improvements to the amplification and STR detection of Low Copy Number (LCN) DNA samples, employing two megaplex kits, PowerPlex®16 (Promega) and Identifiler® (ABI). Amplification conditions, reaction components, post-amplification purification, separation and analysis parameters were adjusted for samples as low as 6 pg of DNA. Conclusions were drawn from comparisons of the percentage of allelic determinations, the occurrence of spurious alleles, the peak heights, and the allelic imbalance.

Since the manufacturer recommended cycle numbers were 28 and 28 - 32 cycles for Identifiler® and PowerPlex® 16 respectively, samples were initially amplified for 28 cycles with reagents of the former kit and for 32 cycles with the latter. In order to enhance sensitivity while decreasing the peak height ratios and drop ins, different permutations of reduced reaction volumes, higher concentrations of primers, decreased annealing temperatures, longer annealing and extensions times, reduced reaction volumes and additional cycles were examined. For Identiflier®, reducing the annealing temperature one degree increased allelic determinations and improved allelic imbalance. Doubling the annealing and extension times, also improved these parameters. However, increasing the primer concentration by twenty percent was not advantageous.

Regarding PowerPlex® 16, similar modifications did not prove productive. For example, doubling the extension time moderately enhanced peak heights, but did not affect allelic imbalance. For both kits, as expected, reducing the reaction volume significantly raised peak heights and allelic determinations. Surprisingly, peak height ratios were also more balanced. Furthermore, amplification for 28 cycles with Identifiler® reagents did not produce as many allelic calls as amplification for 32 cycles with PowerPlex® 16 components. However, cycling the former 31 times approximated results from cycling the latter 32 and even 35 times and generated slightly better allelic detection and higher peak heights.

In addition, various injection times and voltage settings of the ABI 3100 Prism® Genetic Analyzer with a collection threshold of 75 RFUs and a general filter of 10% were examined. At the same time, the sensitivity and specificity were evaluated for different volumes of PCR product that were injected directly or following Microcon purification. According to our LCN protocol, 4μL of amplified product was injected at 3 kV for 20 seconds. This produced almost 100% correct allelic calls for 25 pg of DNA. High injection parameters with 6 μL amplicon at 6 kV and 30 seconds were applied to lower amounts, which significantly increased the peak heights, maintained a low number of drop ins but did not affect the peak imbalance.

Overall, amplifying LCN DNA in half reaction conditions and injecting 4 μL of the product at 3 kV 20 seconds produces a satisfactory allelic determinations and peak height imbalance. Nevertheless, allelic imbalance appears to be inherent to amplification of LCN DNA in a megaplex.