OPTIMIZATION OF POWERPLEX®16 FOR AMPLIFICATION OF LOW COPY NUMBER DNA SAMPLES

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Previous research in our laboratory indicates that amplification with PowerPlex 16® (Promega) for 35 cycles produces database eligible profiles from as little as 10 pg of DNA. On average, one spurious allele or drop-in occurred per amplification. Therefore, in order to improve the efficiency of this process, we attempted to optimize cycling conditions and reaction components as well as the separation and analysis parameters.

To increase the sensitivity and specificity of the amplification, various conditions were tested. Specifically, several known PCR enhancers such as BSA, Triton X-100, Tween 20, NP40, and formamide, were added to the Gold Star buffer included in the PowerPlex 16® kit. Taq Gold was titrated for the kit conditions, and alternative enzymes were tested with their respective buffers. In addition, we attempted to optimize the concentration of dNTPs and $MgCl_2$, with the PowerPlex 16® primers. The advantages of increasing the annealing and extension times as well as halving the reaction volume, will also be discussed. Our work suggests that the recommended reagents for the PowerPlex 16® primer sets are optimal for low copy number samples.

According to our low copy number protocol, 2 μL of amplified product is prepared for injection at 3 kV for 20 seconds on the ABI 3100 Prism® Genetic Analyzer with a collection threshold of 75 RFUs. Increasing the amount of sample, the injection voltage or time individually, did not improve allelic calling. However, an increase is seen using 4 μL of product and adjusting the injection conditions to 7 kV and 30 seconds. Samples amplified with 32 cycles also produced more allelic calls using these parameters, although more drop-ins were apparent. Therefore, optimal sensitivity and specificity were achieved by amplification with 35 cycles and injection of samples prepared with 2 μL at 3 kV for 20 seconds.

With these modifications to the PowerPlex16® protocol, coupled with a customized macro developed by Promega, and a general filter of 10%, reliable profiles can be obtained from 10 pg of DNA. Although drop-ins may occur, they can be removed by only calling alleles that appear consecutively in two amplifications. Alternatively, we are currently exploring whether automated allelic calling generates more accurate DNA profiles.