Optimization of template DNA volumes in PowerPlex® 16 amplification reactions when DNA concentrations fall below the QuantiBlot® detection threshold.

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In-house experiments conducted to determine sensitivity1 and acceptable stochastic limits2 to optimize the amount of template DNA in Polymerase Chain Reactions using Promega PowerPlex® 16 multiplex Short Tandem Repeat amplification kit have often produced apparently inconsistent results with reasonable predictability. Estimation of optimal DNA template volumes at the lower end of the DNA detection threshold using Applied Biosystem QuantiBlot® have proved to be particularly challenging in this respect. This apparent anomaly presents itself as follows: Not infrequently, complete failure of the PowerPlex® 16 kit to amplify any DNA and yield even a partial genetic profile is observed when 19.2 ul, the maximum volume that can be added in a 25 ul amplification reaction, of the low concentration DNA (below Quantiblot® detection levels) is used. However, if the volume of the same DNA extract is reduced to 10 ul or 7 ul and amplification performed again under identical cycling conditions, a full genetic profile with acceptable inter-locus and intra-locus peak height ratios is often obtained. This indicates that the reason for the failed amplification is neither lack of nor insufficient DNA template but rather a property of the amplification system itself. The PowerPlex® 16 system is very likely sensitive to overloading with regard to template volume and / or its concentration. Note: This observation is restricted to the efficiency of the amplification. It does not, in any way affect the quality, reproducibility and / or reliability of the genetic profile, if one is obtained. (b) Comparable amplification results were noted using the 480 as well as the 9700 Thermal Cyclers. Our observations indicate that the potential for erroneously assuming that a sample has insufficient DNA to yield a genetic profile when, in fact, the 'false negative' may be a property of PCR kit amplification dynamics. We will present data which illustrate our observations and discuss strategies we have employed at our facility to enhance data collection from samples that were previously considered to have insufficient DNA for generating usable genetic profiles. Current experiments are focused on

determining if Real Time Quantitation-PCR can alleviate this issue of template target estimation in future amplification set-ups. (1 Sensitivity= Minimum template concentration required to produce a complete genetic profile at the 15 marker loci) (2 Acceptable stochastic limits= DNA concentration which produces balanced intra-locus and inter-locus peak height ratios)