VALIDATION OF THE PROMEGA POWERPLEX® ESX17 SYSTEM WITH A 5X AMPSOLUTION™ REAGENT ON ABI 3500 SERIES GENETIC ANALYSER FOR REFERENCE SAMPLE ANALYSIS

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In-house validation was conducted on the Promega PowerPlex® ESX 17 System, a human identification PCR kit that amplifies 17 markers in a multiplex reaction. The current study uses FTA reference samples which can be stored long term at room temperature. However, the FTA paper also contains PCR-inhibiting chemicals which need to be removed via a pre-amplification processing step with FTA wash buffers. The amplified products were injected and detected on the ABI 3500 series genetic analysers. The samples were analysed for allele peak heights, heterozygous peak height ratios, n-1 repeat stutter percentages and commonly encountered amplification artefacts at respective loci. In addition, the analytical threshold was determined using data collected from amplification negative controls. Allelic ladder data were used to demonstrate sizing precision.

The Promega 5x AmpSolution™ Reagent was developed for direct amplification of FTA samples without the need to perform the pre-amplification processing step. The solution is added to the PCR reaction mix prior to amplification. The allele peak heights, heterozygous peak height ratios and n-1 repeat stutter percentages obtained from direct amplification with the use of the 5x AmpSolution™ Reagent was compared with results from the above validation study. No significant difference was detected, indicating that direct amplification of FTA samples through the addition of the Promega 5x AmpSolution™ Reagent is as robust as the previously validated method. Thus, the Promega PowerPlex® ESX 17 System with 5x AmpSolution™ Reagent is validated and suitable for direct amplification of FTA reference samples.