INTERNAL VALIDATION STUDY OF THE POWERPLEX Y23 SYSTEM FOR REFERENCE SAMPLES AND DATABASE ANALYSIS

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In order to implement in our lab the new Promega PowerPlex® Y23 System for the data banking purpose, we conducted an internal validation study with the intent to assess if our laboratory's standard practices produce reliable and robust results. All the experiments were performed keeping the cycle number fixed at 26 as the sensitivity study is beyond the scope of the present work. This validation study has been performed accordingly to Promega "Internal Validation of STR Systems" guide (GE053, revised 9/06).

The aim of the first experiment is to determine the stochastic threshold when amplifying and analyzing replicate reactions of serial dilution (from 500pg to 7pg) of the control DNA (2800M) provided in the kit using 26 cycles. Stochastic threshold has been defined as the mass of template DNA amplified below which peaks drop out is visible. In the second and third experiment we have assessed male/female mixtures in order to determine the smallest amount of male DNA still detectable. In one case we kept constant the amount of male DNA (500pg) and increased amounts of female DNA ranging from 0 to 50ng. In the other case we added decreased amount of male DNA from 500pg to 7pg to a constant amount of female DNA (10ng). Finally, we analyzed mixtures of two different male DNA contributors at different ratios. Each mixture corresponds to 500pg total DNA.

The results of this validation study allow us to set the Stochastic Threshold at less than 250pg (total mass of DNA template) when using PCR settings suitable for reference samples, that is 26 cycles. True sensitivity studies should involve amplification with increased PCR cycle numbers (work still in progress). All the experiments performed so far showed no cross-amplification with female DNA. The mixtures study has indicated that it's possible to get a full profile of the minor contributor when the ratio is below 1:4. We conclude that the PowerPlex® Y23 System fulfills our needs for reference/databasing analysis. Further testing and validation analysis is required for casework samples and is actually ongoing in our lab. ⌘