Developmental Validation of a Multiplex PCR Assay for the Direct Amplification of STRs from Single Source Blood or Buccal Cell Samples

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Short Tandem Repeat (STR) analysis using multiplex PCR provides the most discriminating assay for human identification applications. Blood or buccal cells are the most common biological samples received by DNA database laboratories for STR analysis. Storage media such as FTA® paper allow for archiving blood or buccal samples at room temperature and easy re-interrogation of the DNA profiles at any time. FTA® paper is treated with proprietary chemicals and nucleases that lyse cells, inactivate pathogens, and inhibit bacterial growth. The FTA® paper enables safe storage of biological material but the chemicals in the FTA® paper, along with the endogenous constituents of blood or buccal cells, are inhibitory to PCR so a purification step is necessary to remove these inhibitors prior to PCR amplification. The FTA® purification procedure takes approximately 2-3 hours or more depending on the number of samples being processed. It is laborious, time-consuming, and increases the chance for cross contamination.

The AmpFℓSTR® Identifier® Direct PCR Amplification Kit is a multiplex STR assay that has been developed for the direct amplification of single source blood and buccal cell samples on FTA® paper. The primer sequences in the AmpFℓSTR® Identifiler® Kit are maintained in the AmpFℓSTR® Identifiler® Direct Kit. Along with an optimized PCR buffer formulation and an improved thermal cycling protocol, the AmpFℓSTR® Identifiler® Direct assay improves the databasing workflow by eliminating tedious purification steps and minimizing sampling handling. The simple “punch & go” protocol significantly expands throughput capabilities while simplifying automation requirements and maximizing quality assurance for the entire process.

Data will be presented demonstrating the performance of this assay. Experiments performed for the developmental validation study includes: a) reproducibility within samples, b) reproducibility between CE platforms, c) human specificity (non-human DNA analysis), d) FTA® sample stability, e) assay sensitivity, f) concordance with AmpFℓSTR® Identifiler and g) impact of FTA disc size variation.