EFFECTIVENESS OF STR QUALITY SENSORS TO INFORM REWORK STRATEGIES FOR CHALLENGING DATABASE AND CASEWORK SAMPLES USING A SEMI-AUTOMATED WORKFLOW

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Forensic DNA laboratories process 100,000s of samples each year. Automation and direct PCR for database and reference samples has increased laboratory throughput and bypasses DNA extraction and quantification processes. Although full STR profiles are generated from the majority of these samples, the lack of purification can leave some vulnerable to PCR inhibition, while other complicating factors such as DNA degradation and low amounts of DNA can also affect downstream STR success.

While quantification provides an indication of inhibition, it may not be a reliable or accurate representation of the true inhibition level due to the relatively small input volume, or if inhibitors differentially affect quantification and STR chemistries. For databasing samples, the quantification step is bypassed altogether and therefore DNA quantity and quality is unknown prior to amplification. Internal quality sensors (QS) included in STR reactions can provide useful information for these types of samples. The presence, absence, or relative amplification of these QS markers can assist the interpretation of STR profiles and direct analysts towards more effective rework strategies to improve the STR profile and/or avoid unnecessary or multiple rework attempts.

In this study we subjected database-like samples (e.g. buccal swabs and blood or saliva on FTA cards) to various challenging conditions including hot and humid storage, UV exposure, and poor collection techniques such as a single swipe for buccal swabs and low blood volumes in EDTA tubes before transferring to FTA cards. Additionally, mock casework samples identified as low-template, degraded, or inhibited, as well as simulated sexual assault and authentic post-coital samples were included. All samples were processed with a semi-automated workflow for DNA lysis, quantification (when applicable) and STR amplification.

All database and single-source casework samples that generated less than 90% reported alleles in the first amplification round were reworked. To assess the value of the QS markers included in these STR kits, samples were then reworked based on the quality of the electropherogram 1) with the QS markers redacted, and 2) in conjunction with the QS markers. Results from each of the rework approaches were compared to determine which strategy, if any, improved the profile quality and the number of STR alleles reported. Overall, the most notable improvement in STR completeness was observed in inhibited samples that were reworked based on the information provided by the STR quality sensors.