Effectiveness of STR Quality Sensors to Inform Rework Strategies for Challenging Database and Casework Samples using a Semi-Automated Workflow

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INTRODUCTION

Automation and direct PCR for database and reference samples has increased throughput within forensic DNA laboratories 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 with casework samples, it may not be a reliable or accurate representation of the true inhibition level due to the relatively small input volume. In addition, inhibitors may 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. In these cases in particular, Internal quality sensors (QS) included in STR reactions can provide useful information. 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 (1).

MATERIALS AND METHODS

Sample Preparation and DNA Purification:

- Database-like samples were exposed to various insults and processed as shown in Figure 1.

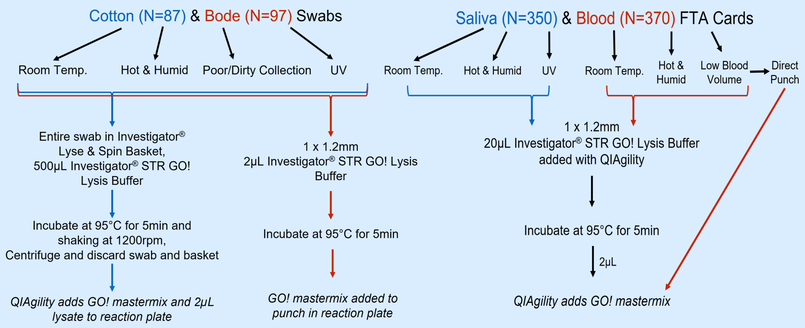


Figure 1 – Outline of insults and preparation steps for each databasing sample group.

- Single-source casework quality samples included aged biological stains, extracts previously identified as low template, inhibited, or degraded, and control DNA spiked with common PCR inhibitors.
- Aged stains were simulated by depositing 500 µL of saliva (n=5) and blood (n=5) on bed sheets before storing in a humid oven at 37 °C for 16 weeks.
- Low template samples included touch DNA collected from handled rifle magazines using cotton CEP® Swabs (n=12) (FITZCO, Spring Park, Minnesota) and nylon FLOQSwabs™ (n=12) from Copan (Murrieta, California, USA) and extracted using the QIAamp DNA Investigator kit (QIAGEN) on the QIAcube (QIAGEN).
- Low template and/or degraded skeletal samples (n=20) extracted using a previously published total demineralization protocol (2) were also included.
- Inhibited (n=11) and degraded (n=10) sample extracts were from cadaver muscle biopsies preserved Tent buffer (10mM Tris, 10mM EDTA, 1M NaCl, 2% Tween 20; 100 mL, pH 8.0), or using nylon swabs.
- Finally, control DNA (n=12) was spiked with hematin (500-1500 ng/µL), melanin (25-60 ng/µL), or humic acid (100-400 µM).

DNA Quantification and STR Analysis:

- DNA extracts were quantified with the Investigator Quantiplex Pro RGQ kit and amplified with either the Investigator 24plex QS (casework samples) or Investigator 24plex GO! (databasing samples) kit (QIAGEN).
- Data analysis was performed with the QIAGEN Data Handling Tool and in-house Excel book.
- Electropherograms (EPGs) with QS markers redacted (Fig. 2) were provided to DNA analysts from a crime laboratory to identify rework strategies based on STR profile quality alone.
- Samples were then reworked based on; 1) the strategy identified by the analyst without the QS marker and 2) STR quality and QS information (if different) to determine any improvement in allele recovery and/or profile quality.

RESULTS AND DISCUSSION

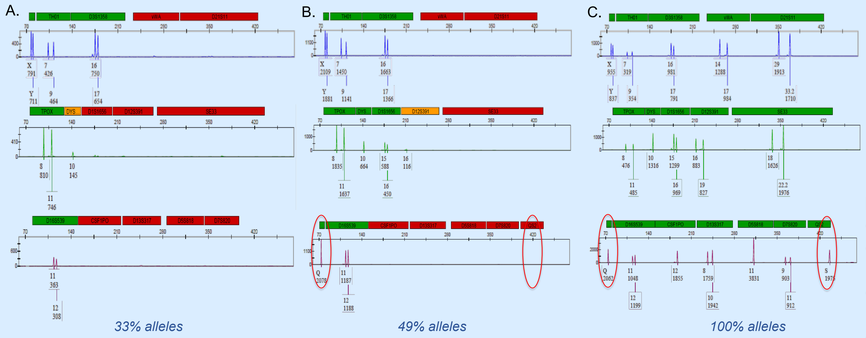


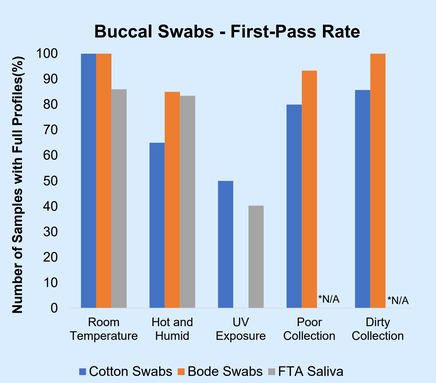
Figure 2 – Examples (partial EPGs) of: A) an EPG provided to an analyst with the QS markers removed; B) EPG after sample was reworked according to strategy without QS information (process new punch) and C) EPG after sample was reworked guided by the QS markers (dilution and reamplification).

Databasing

- As expected, the Investigator 24plex GO! Kit performed well with complete profiles for room temperature controls. However, with saliva on FTA - two donor cards had low amounts of DNA.
- Overall, challenged samples had reduced allele recovery (Fig. 3).
- 21 out of 38 reworked database samples improved based on STR profile alone (Fig. 4).
- More alleles were recovered in 10 additional samples when the rework strategy was based on the QS markers compared to reworks performed without QS information (Fig. 2 and Fig. 4).

Mock Casework

- 100% alleles recovered for all aged stain samples; variable success for rest of casework samples.
- For casework samples selected for reworking (<90% alleles), 12% were identified as having no template/failed amp when QS marker information was not included (Fig. 5)
- QS markers were able to resolve failed profiles as inhibited and confirm ambiguous low quality profiles as low template and/or highly degraded samples for targeted (or avoided rework) (Fig. 5).
- 14/20 known inhibited casework samples failed to amplify. Reworking with STR profile alone improved allele recovery for 18/20 samples, and QS markers were able to confirm 1 additional failed profile as inhibited rather than having no template (Fig. 6).



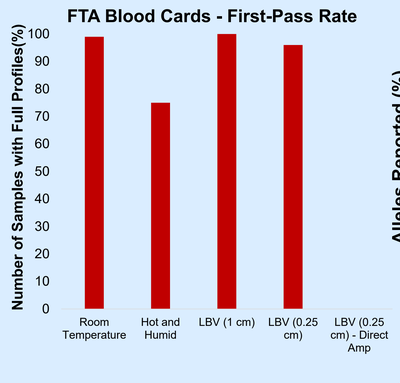


Figure 3 – Percentage of databasing samples that had full STR profiles during first-pass analyses according to sample substrate and challenging insult. LBV = Low Blood Volume. *N/A – not performed.

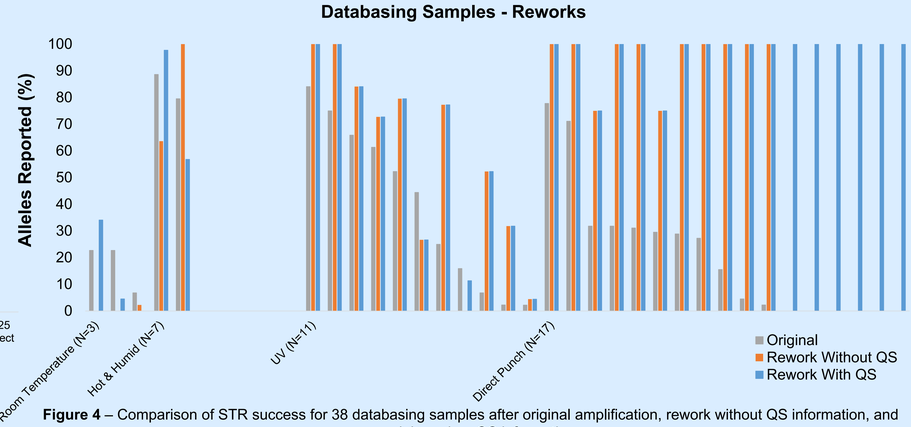
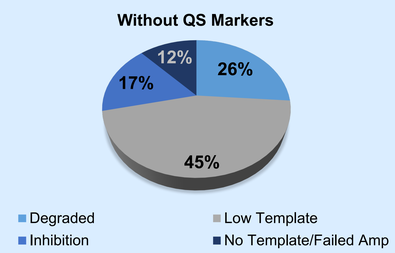


Figure 4 – Comparison of STR success for 38 databasing samples after original amplification, rework without QS information, and rework based on QS information.



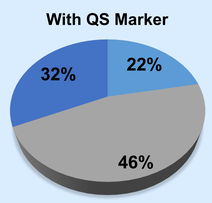


Figure 5 – Breakdown of categories that single-source casework samples were designated as based on STR profile quality alone (left) and when the QS marker information was also included (right).

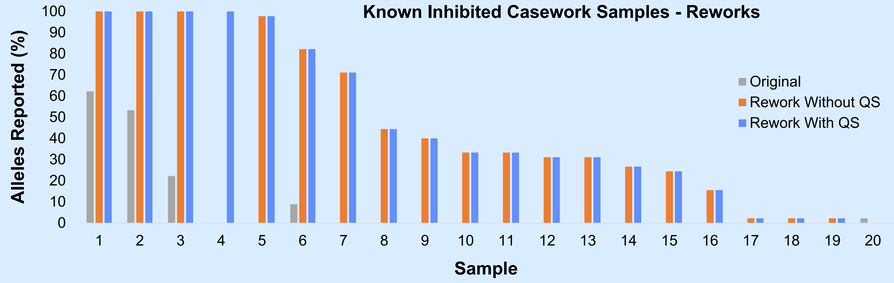


Figure 6 – Comparison of STR success for 20 known inhibited casework samples after original amplification, rework without QS information, and rework based on QS information. Rework was a 1:3 dilution prior to re-amplification.

Overall, this research shows that QS sensors in the Investigator 24plex QS and GO! Kits can provide analysts with a more straightforward interpretation of sample quality during analyses of ambiguous and/or failed STR profiles to determine the most appropriate and effective rework strategy. The most notable improvement in STR completeness was observed in inhibited samples that were reworked based on information provided by the QS markers in conjunction with overall STR quality.

REFERENCES

1. Kraemer M, Prochnow A, Bussmann M, Scherer M, Peist R., and Steffen C. Developmental validation of QIAGEN Investigator 24plex QS Kit and Investigator 24plex GO! Kit: Two 6-dye multiplex assays for the extended CODIS core loci. Forensic Sci Int Genet. 2017; 29: 9–20.
2. Loreille OM, Parr RL, McGregor KA, Fitzpatrick CM, Lyon C, Yang DY, et al. Integrated DNA and Fingerprint Analyses in the Identification of 60-Year-Old Mummified Human Remains Discovered in an Alaskan Glacier. J Forensic Sci. 2010; 55(3):813-8.

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