Validation Report May 2017

Developmental validation of the forensic STAR Q SP/AS workflow

The Investigator® STAR Lyse&Prep Kit is designed for automated purification of total DNA from samples encountered in forensic and human identity applications. The extraction chemistry has been adapted from the QIAsymphony® DNA Investigator Kit. Proven magnetic-particle technology provides high-quality DNA, which is suitable for direct use in downstream applications, such as quantitative PCR amplification reactions or STR analyses, or for storage for later use. Purified DNA is free of proteins, nucleases and inhibitors. The STAR Q SP/AS performs all steps of the sample extraction procedure after lysis, according to the pretreatment protocols. Up to 96 samples can be processed in one run. Extraction protocols for 300 and 500 µl sample lysate volume are available. DNA can be eluted in 50 or 100 µl low TE buffer.

The instrument can set up PCR reactions for quantification and normalized STR. Protocols for setup of Investigator Quantiplex®/Quantiplex HYres products and/or Investigator STR assays for STR typing in human identity and forensics are available.

The performance of the Investigator STAR Lyse&Prep Kit, as well as the performance of the assay setup was evaluated with regard to various sample types and conditions commonly encountered in forensic and parentage laboratories.

The assays make use of well-established methodologies for forensic DNA analysis. The validation of the assays is dealt with in detail in their respective validation report, which can be found on the respective QIAGEN product pages.

Specific issues that can arise during forensic casework were investigated, such as the ability to obtain results from samples that have been subjected to adverse environmental conditions. This was tested using DNA spiked with inhibitors. In addition, cross-contamination was tested, the reproducibility of the results was verified and the performance on typical casework samples was evaluated.

Results of developmental validation

The validation study was performed by the QIAGEN R&D department. Investigator Lyse&Spin baskets were used to remove solid sample material and obtain a cleared lysate where applicable. Quantification and STR PCRs were performed according to the instructions of the corresponding kit handbooks. All of the electropherograms shown were generated on an Applied Biosystems® 3500 Genetic Analyzer. The standard conditions specified in the respective kit handbooks were used for all experimental steps unless stated otherwise. A GeneAmp® PCR System 9700 with Gold-Plated Silver 96-Well Block Module was used for amplification, unless stated otherwise. Data were analyzed using Applied Biosystems GeneMapper® ID-X software (v1.2 or v1.4). Samples used were simulated casework samples where mentioned.

Linearity and sensitivity

In order to determine the range of sample input amounts that can be reliably processed using the Investigator STAR Lyse&Prep Kit, dilution series of saliva and blood samples were performed. Five replicates on four sample volumes were extracted: 10, 1, 0.1 and 0.01 µl. The study was conducted using both, the 300 and 500 µl protocol and DNA was eluted in 50 µl. Samples were quantified using the Investigator Quantiplex HYres Kit on an Applied Biosystems 7500 Real-Time PCR System for Human Identification. The quantification results were loaded into the STAR Q SP/AS instrument for normalized setup of Investigator 24plex QS STR PCR and 500 pg template DNA per reaction was targeted.

The DNA yields obtained increased proportionally to the amount of sample extracted (Figure 1–Figure 2). DNA was efficiently recovered from the lowest volume of 0.01 µl tested. All samples provided consistent full STR profiles (Figure 3–Figure 4). Observed yields were comparable to the QIAsymphony SP with the QIAsymphony DNA Investigator Kit and corresponding protocols (data not shown).

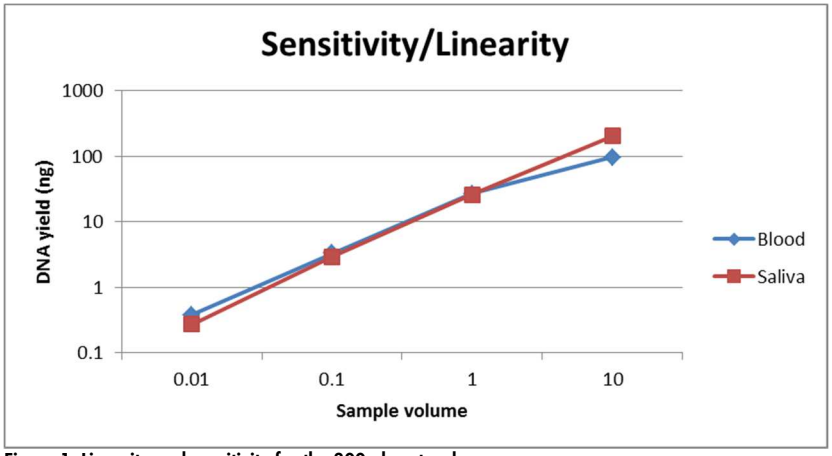


Figure 1. Linearity and sensitivity for the 300 µl protocol

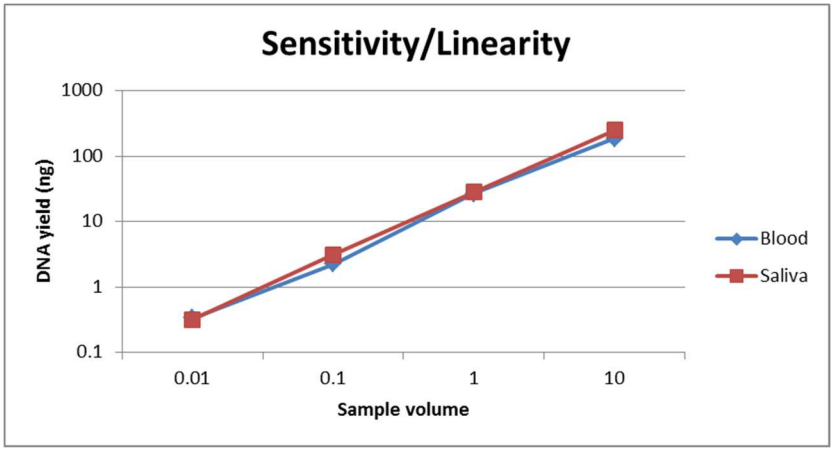


Figure 2. Linearity and sensitivity for the 500 µl protocol

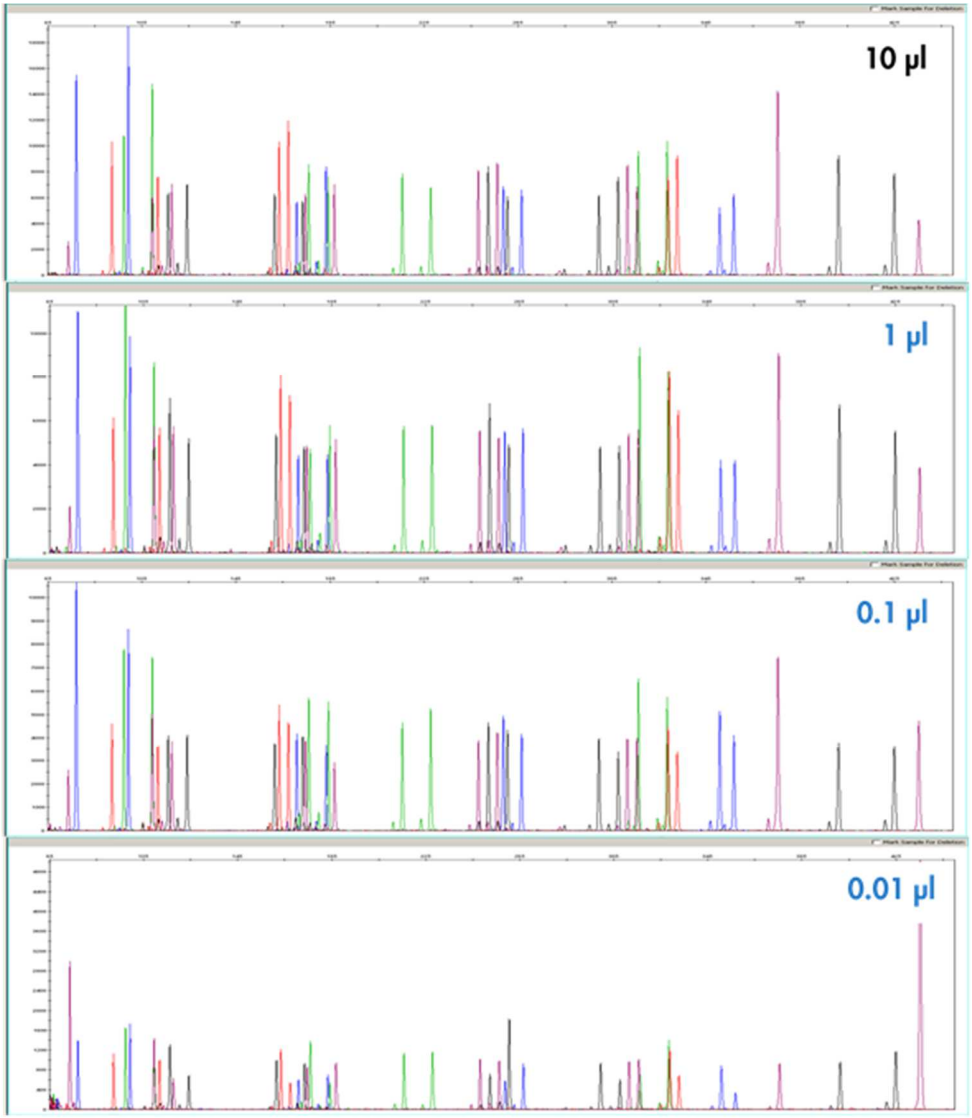


Figure 3. Linearity and sensitivity for the 300 µl protocol. Example electropherograms of saliva samples are shown.

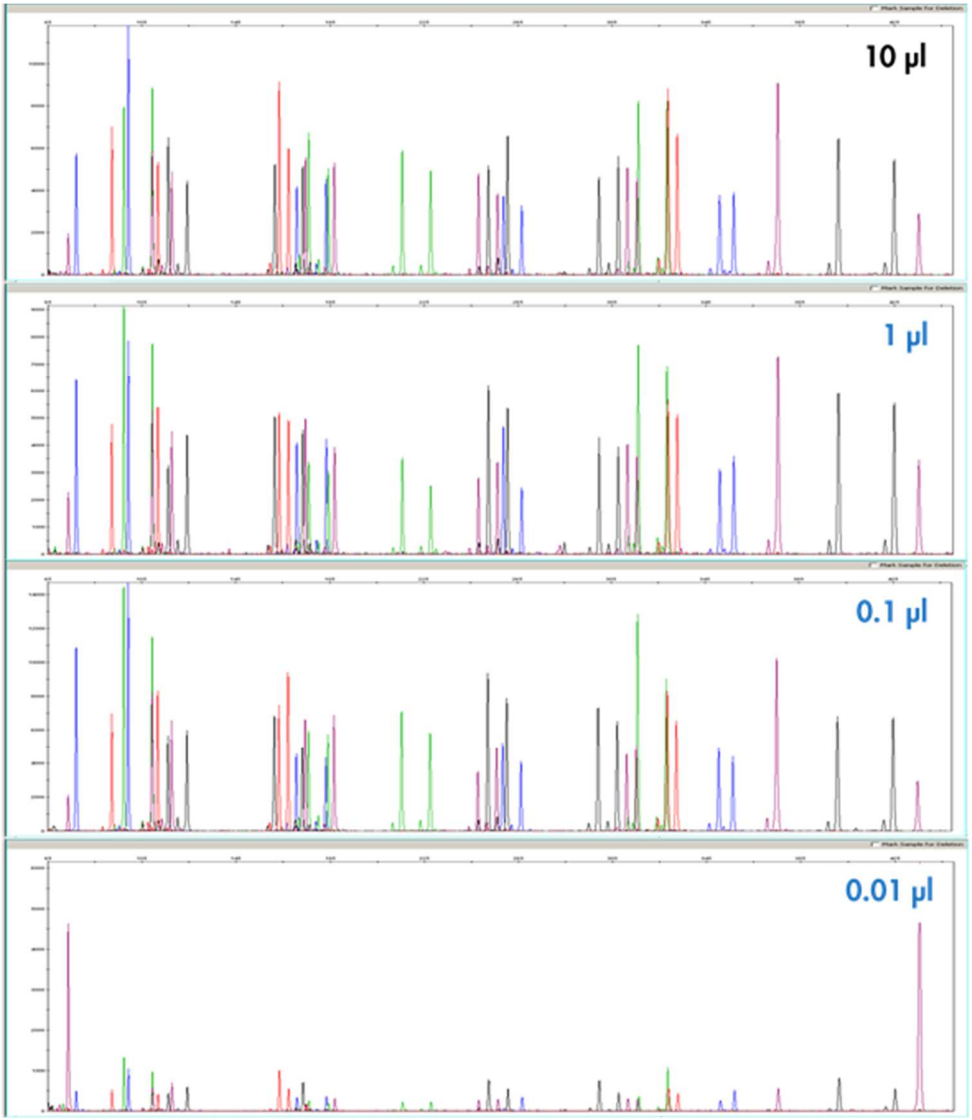


Figure 4. Linearity and sensitivity for the 500 µl protocol. Example electropherograms of saliva samples are shown.

Stability

Forensic casework samples are frequently associated with potential inhibitors of PCR reactions. These inhibitors have to be removed efficiently during extraction to prevent any negative impact on the analysis. The Investigator STAR Lyse&Prep Kit was tested for removal of inhibitors using mocked casework samples. Used engine oil was applied to pieces of fabric, and slurries of soil or sand to cotton swabs to mimic forensic sample substrates. Samples were completed by addition of 1 µl of blood and dried before starting extraction. The study was conducted using both, the 300 and 500 µl protocol and DNA was eluted in 50 µl. Samples were quantified using the Investigator Quantiplex HYres Kit on an Applied Biosystems 7500 Real-Time PCR System for Human Identification and 500 pg template DNA per reaction was used for Investigator 24plex QS STR PCR.

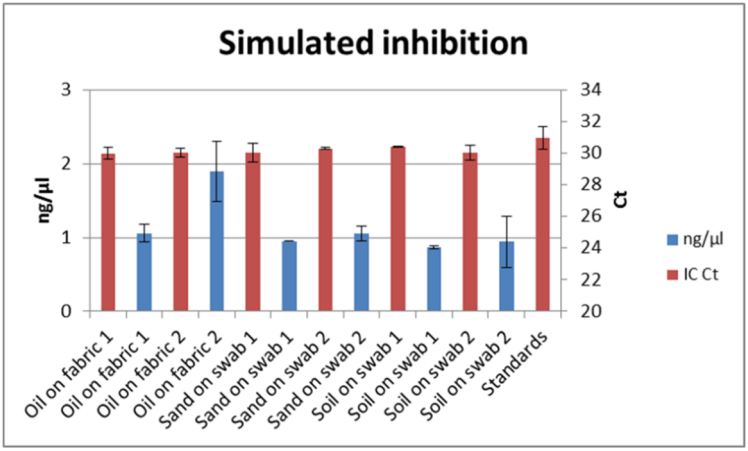


Figure 5. Performance on inhibited samples for the 300 µl protocol. Blood from two donors (1 and 2) was used. Oil on fabric samples were processed in four replicates, swab samples in duplicates. DNA yield and the C_T values of the internal control (IC) of the Investigator Quantiplex HYres Kit are shown. The IC C_T values of the quantification standards serve as a non-inhibited reference.

No change of amplification of the Investigator Quantiplex HYres Kit internal control was observed for any of the samples indicating no inhibition was present (Figure 5–Figure 6). Furthermore, all samples provided full STR profiles without any indication of inhibition. These

findings were supported by balanced amplification of the Investigator 24plex Quality Sensor™ (see Figure 7, as an example).

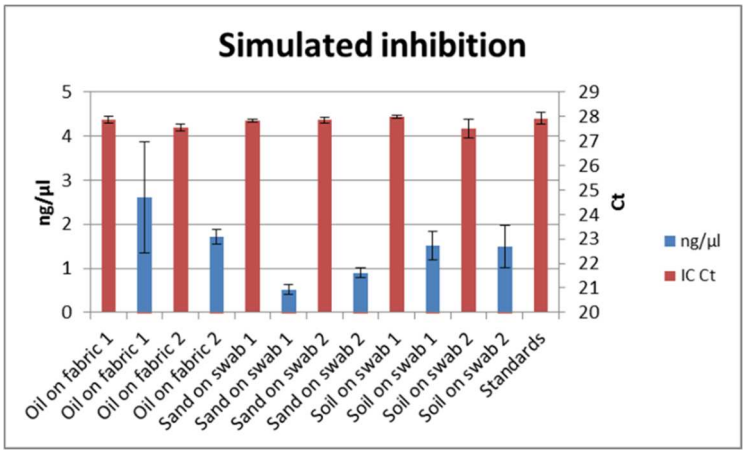


Figure 6. Performance on inhibited samples for the 500 µl protocol. Blood from two donors (1 and 2) was used. Oil on fabric samples were processed in four replicates, swab samples in duplicates. DNA yield and the C_T values of the internal control (IC) of the Investigator Quantiplex HYres Kit are shown. The IC C_T values of the quantification standards serve as a non-inhibited reference.

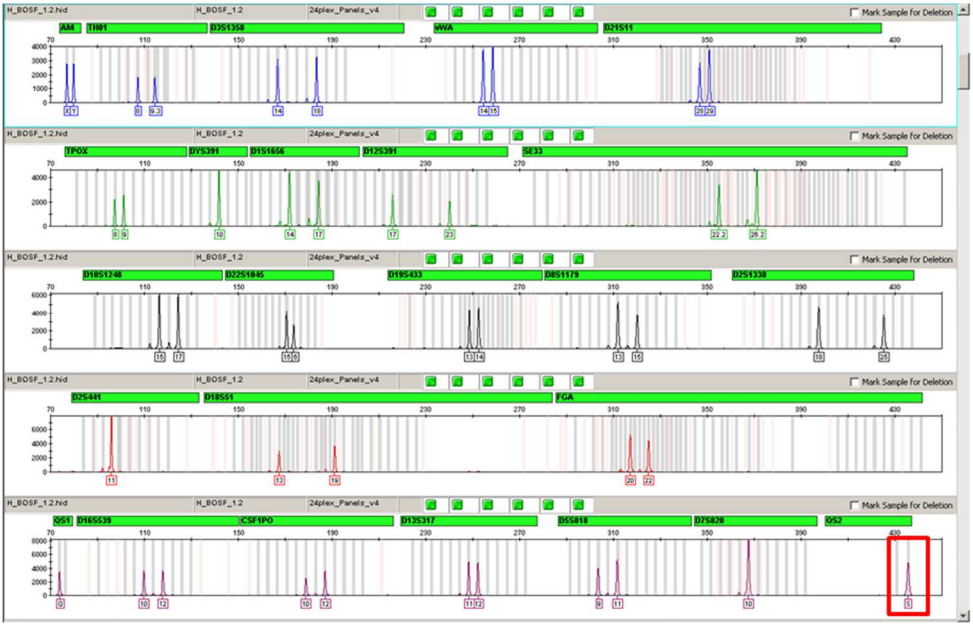


Figure 7. Example electropherogram for oil on fabric sample. Note that full amplification of the large Quality Sensor fragment QS2 (labeled with a red box) indicates no inhibition was present.

Another typical problem encountered in casework analysis is degradation of DNA due to adverse environmental conditions. The protocols have been optimized for efficient recovery of DNA fragments that can still be used to create an STR profile. To simulate degraded DNA, defined DNA ladders were used as spiked samples. Ladders were analyzed on a QIAxcel® instrument and individual fragments quantified (Figure 8). Full recovery was observed for the 200 bp fragment of the ladder.

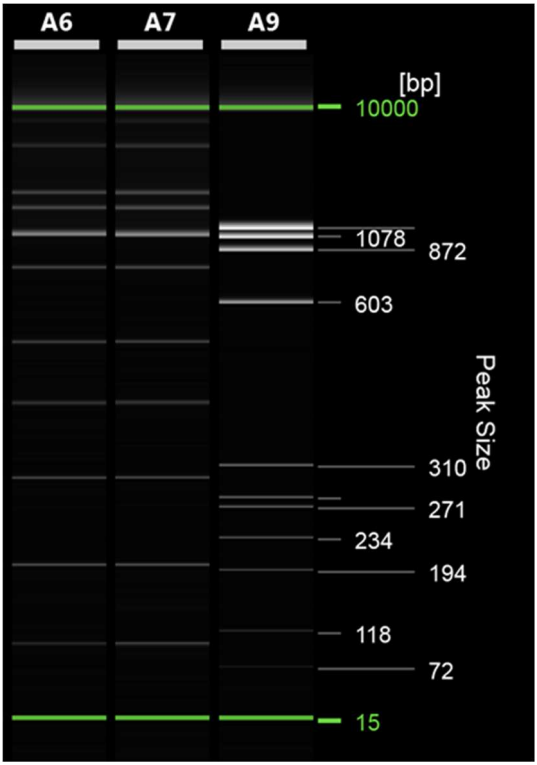


Figure 8. Recovery of small DNA fragments. A6: Ladder after purification using the 300 µl protocol. **A7:** Equivalent amount before extraction. **A9:** QIAxcel DNA size standard.

Reproducibility

To test reproducibility of the extraction, recovery of DNA was determined in three runs performed on different days (Figure 9–Figure 10). In each run, 24 samples were used, with an expected yield of 10 ng.

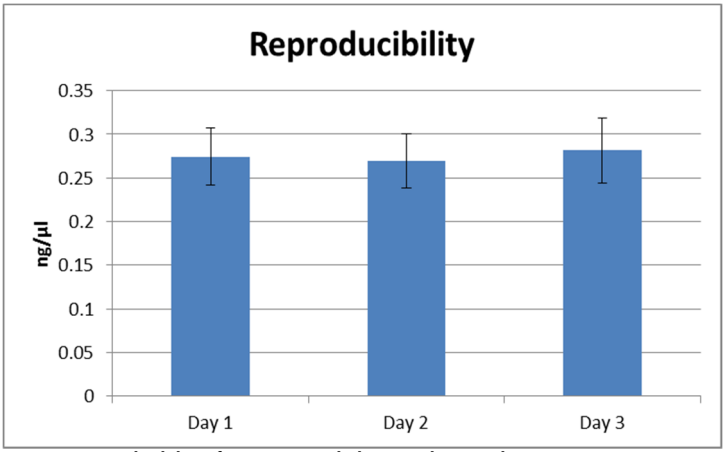


Figure 9. Reproducibility of extraction with the 300 µl protocol.

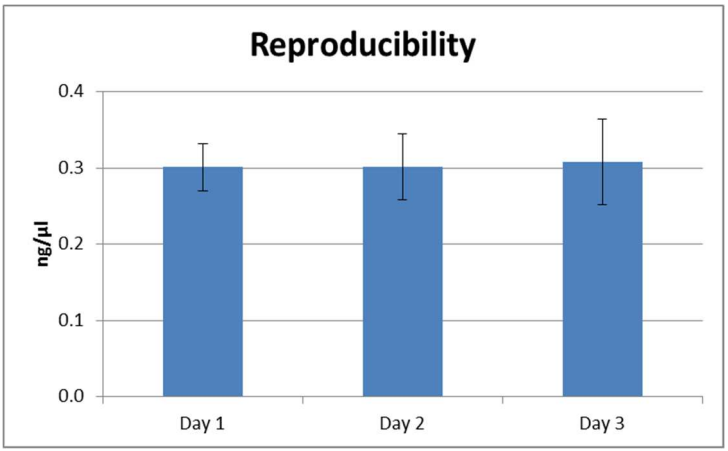


Figure 10. Reproducibility of extraction with the 500 µl protocol.

Cross-contamination

A contamination study was performed for the 300 µl protocol using buccal swabs from different donors arranged in checker board patterns, with alternating negative extraction controls. The average concentration of buccal swab eluate was 67ng/µl. None of the negative samples were detected (Figure 11). Negative samples were amplified with the Investigator

24plex QS Kit using 15 µl as template. For samples showing spurious peaks above 50 RFU, analysis was repeated. No peak was verified.

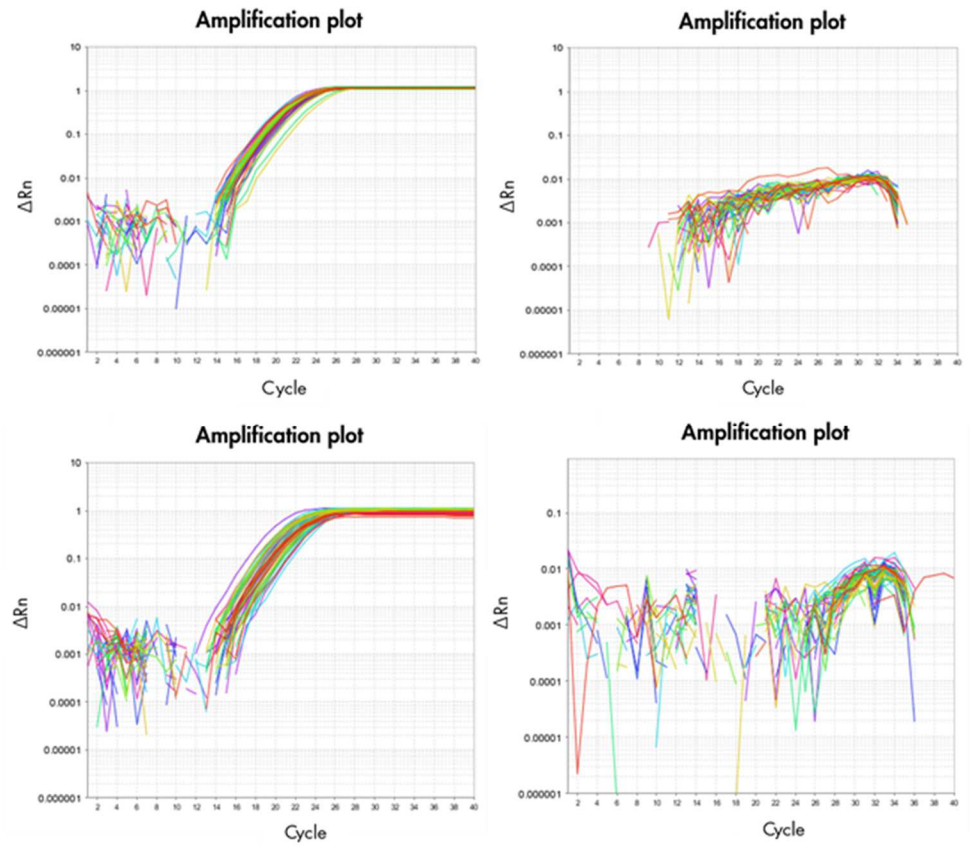


Figure 11. Contamination study with the 500 µl protocol. Eluates from buccal swab samples (left) and negative samples (right) were quantified using the Investigator Quantiplex HYres Kit on an Applied Biosystems 7500 Real-Time PCR System for Human Identification.

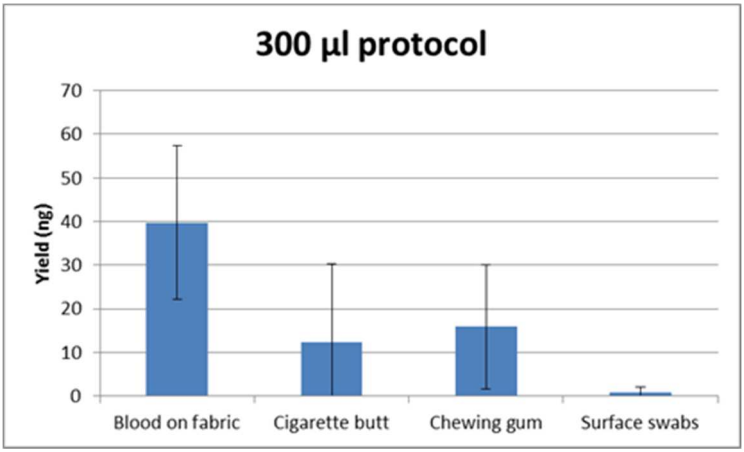
Case-type samples

A selection of mocked casework samples was processed: Blood on fabric, chewing gum, cigarette butts and surface swabs. Samples were extracted using both the 300 µl and the 500 µl protocol with 50 µl elution (Figure 12). Samples were quantified using the Investigator Quantiplex HYres Kit on an Applied Biosystems 7500 Real-Time PCR System for Human Identification. STR profiles were generated with the Investigator 24plex QS Kit.

Samples processed:

- 16 samples of blood on fabric, 4 replicates from 4 different donors
- 12 cigarette butts in duplicates, ¼ paper per sample. 8 fresh samples, 4 exposed to the environment
- 6 chewing gum samples in duplicates, approximately 30 mg per sample. 4 fresh samples, 2 exposed to the environment
- 12 surface swabs, each taken from: A Computer keyboard, a computer mouse and the inner part of gloves

Consistent full profiles were obtained for all blood on fabric samples, fresh chewing gum and cigarette butts. Chewing gum and cigarette butts exposed to environmental conditions frequently showed drop-outs of high-molecular-weight markers due to DNA degradation (Figure 13–Figure 20). As expected, surface swabs showed considerable sample-to-sample variation in terms of quantity and quality of DNA, resulting in partial profiles and mixtures. Please note that samples were collected independently from different sources, and extracted after differing time-intervals post-collection for the two protocols tested. Absolute yields cannot, therefore, be directly compared and differences are no indication of overall performance. Please refer to the sensitivity section for data based on equivalent sample material.



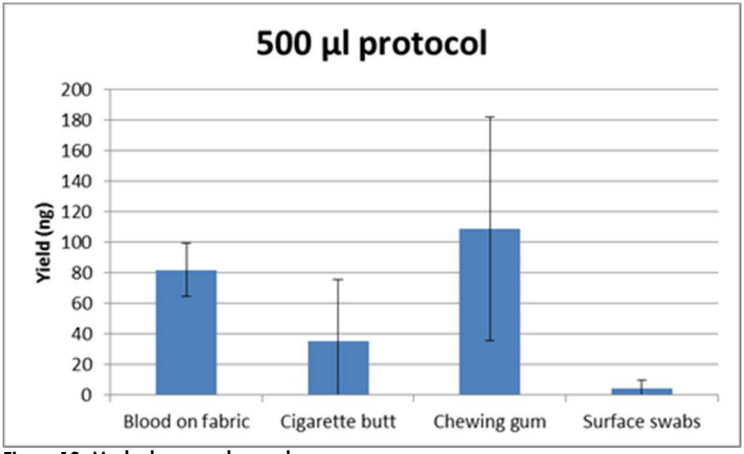


Figure 12. Mocked casework samples.

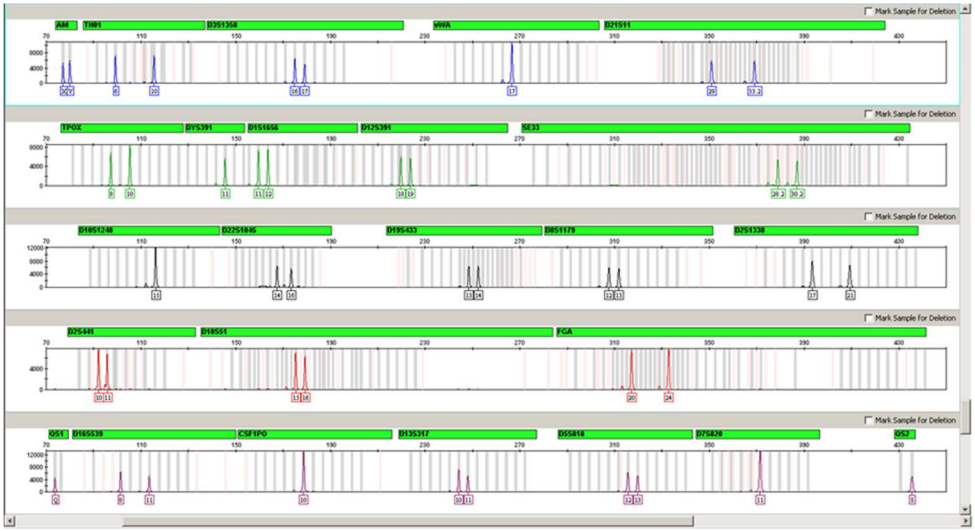


Figure 13. Example electropherogram for blood on fabric extracted with the 300 µl protocol.

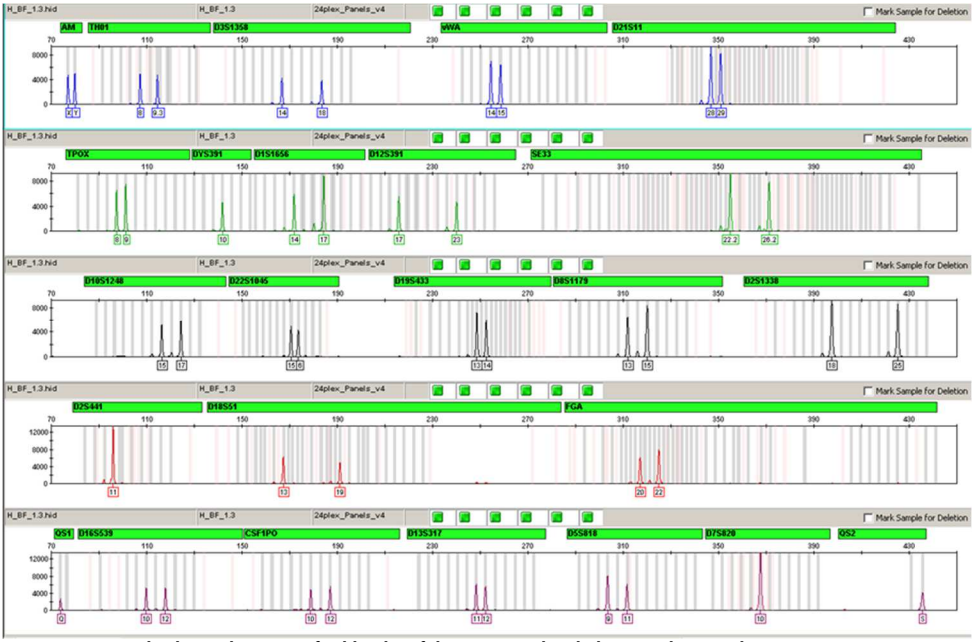


Figure 14. Example electropherogram for blood on fabric extracted with the 500 µl protocol.

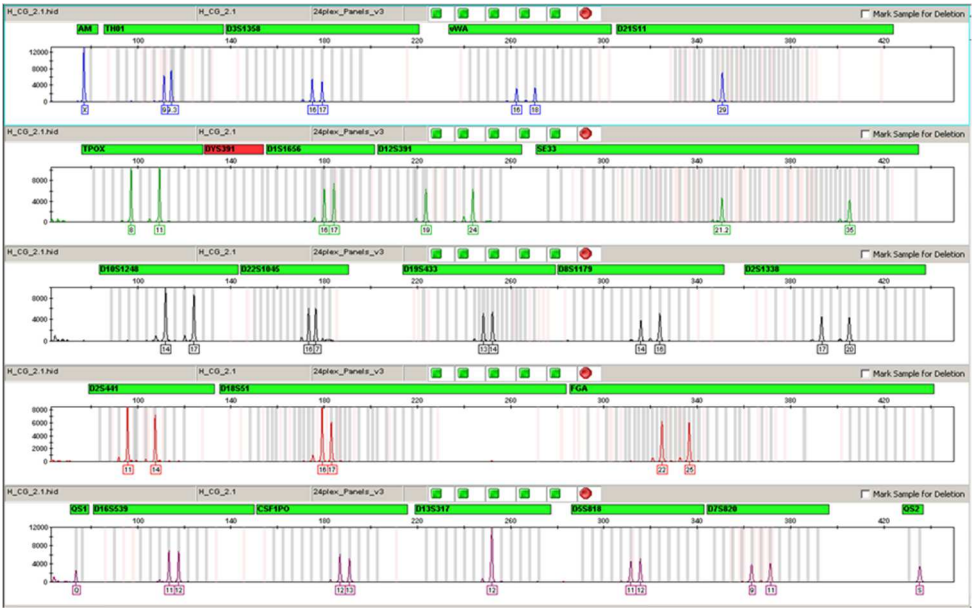


Figure 15. Example electropherogram for chewing gum extracted with the 300 µl protocol. A female profile is shown, which explains the red flag for the missing marker DYS391.

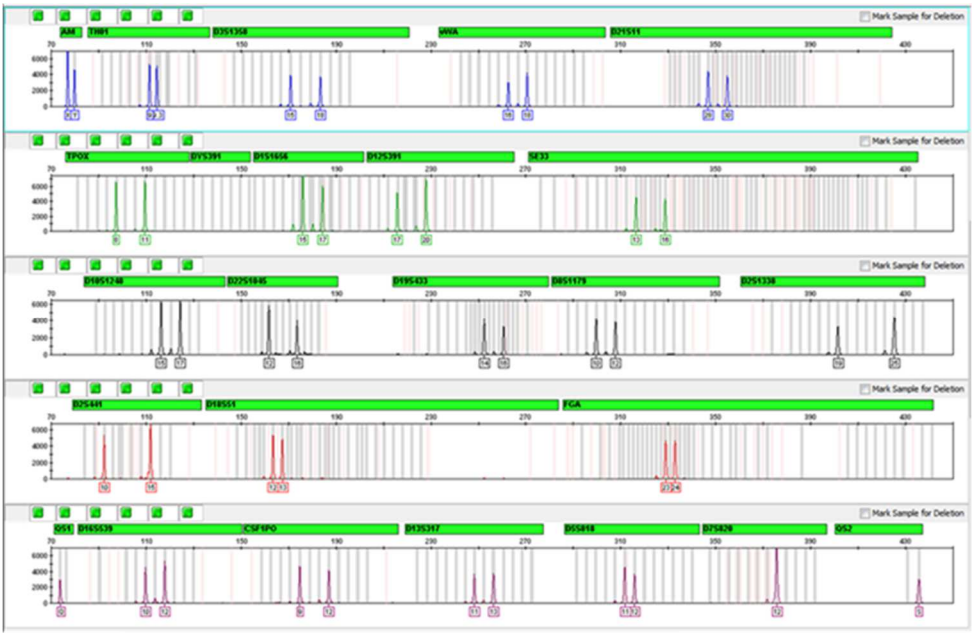


Figure 16. Example electropherogram for chewing gum extracted with the 500 µl protocol.

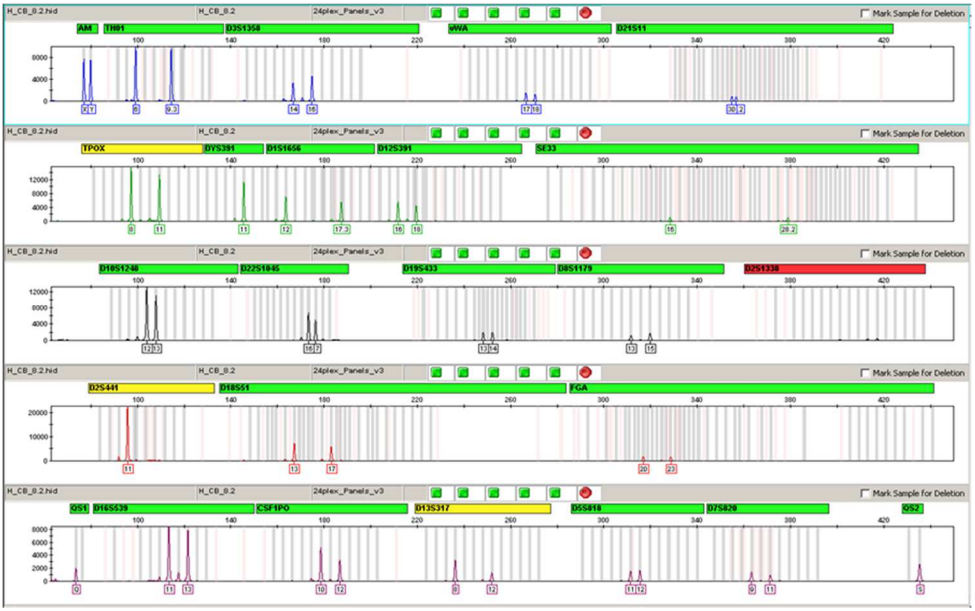


Figure 17. Example electropherogram for a cigarette butt exposed to environmental conditions extracted with the 300 µl protocol. Note that the presence of Quality Sensor fragment 2 excludes inhibition as a reason for the ski slope profile and drop-out of D2S1338. This is proof that the poor sample quality is caused by DNA degradation, as expected.

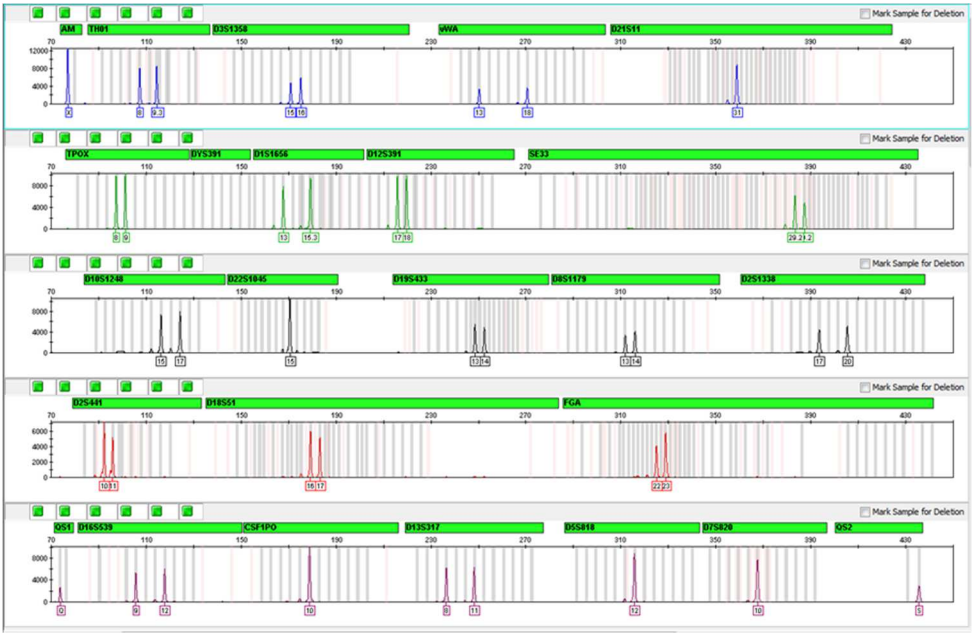


Figure 18. Example electropherogram for a cigarette butt extracted with the 500 µl protocol.

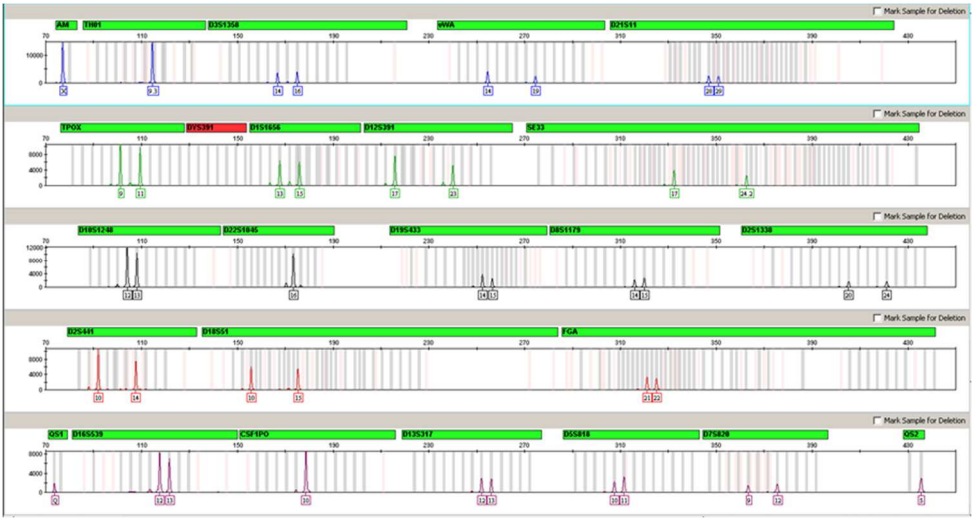


Figure 19. Example electropherogram for touch DNA extracted with the 300 µl protocol. A female profile is shown, which explains the red flag for the missing marker DYS391. The sample was taken from a computer keyboard used by one person only.

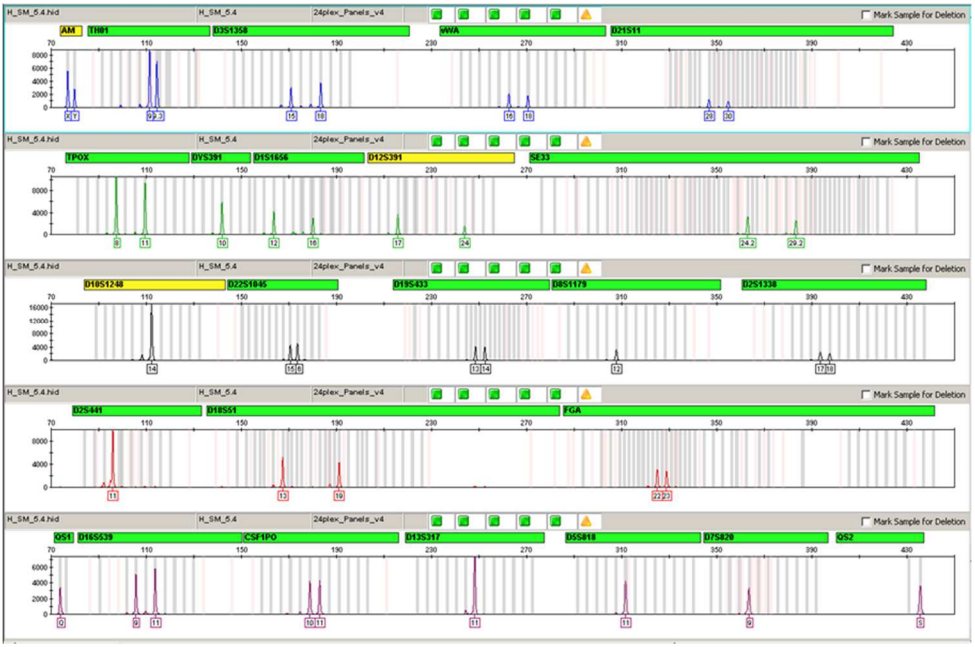


Figure 20. Example electropherogram for touch DNA extracted with the 500 µl protocol. The sample was taken from a computer mouse used by one person only.

Furthermore, samples containing semen were extracted using the STAR Q Lyse&Prep Kit chemistry. In one part of the study, mixtures of semen and epithelial cells were created and applied to swabs to mimic sexual assault samples. Mocked samples were split 1:1 and DNA was extracted from both, the semen and epithelial fraction following a differential extraction protocol. One half was extracted using the EZ1® DNA Investigator Kit, for comparison. Both methods resulted in very similar yields (Figure 21). In a second part of the study, sperm or mixed sperm and saliva samples were applied to different substrates (Figure 22). All samples were typed successfully.

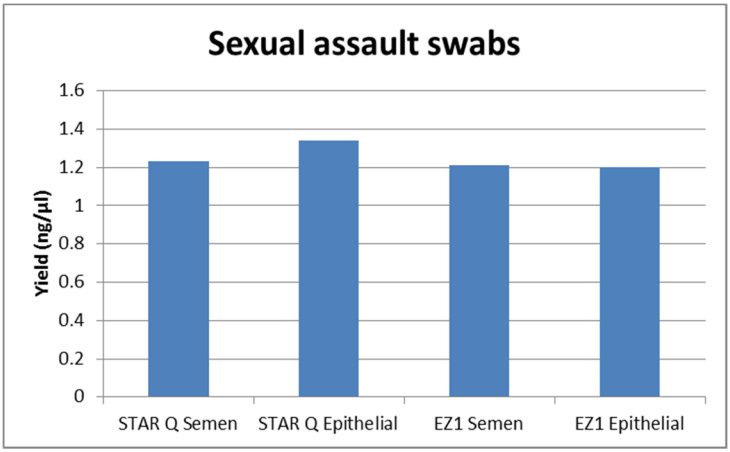


Figure 21. Mocked sexual assault samples. Swabs containing mixtures of sperm and epithelial cells were extracted using differential extraction protocols. The STAR Q chemistry was compared to the EZ1 DNA Investigator Kit.

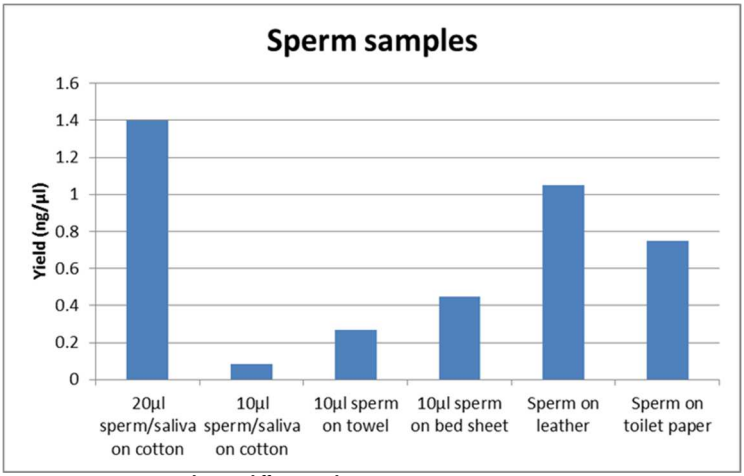


Figure 22. Sperm samples on different substrates.

Precision quantification setup

Reaction setup for the Investigator Quantiplex HYres Kit was performed in three individual runs. In each run, serial dilutions of DNA Z1 were done from the stock provided with the kit. Final concentrations of the standard series were 20, 5, 1.25, 0.31, 0.08, 0.02 and 0.005 ng/µl (Figure 23). For reaction setup, 2 µl standard DNA was added to 18 µl master mix. PCR was performed on an Applied Biosystems 7500 Real-Time PCR System for Human Identification. R2 values observed for the human quantification target were ≥ 0.998 and slopes between –3.354 and –3.379 (Figure 24).

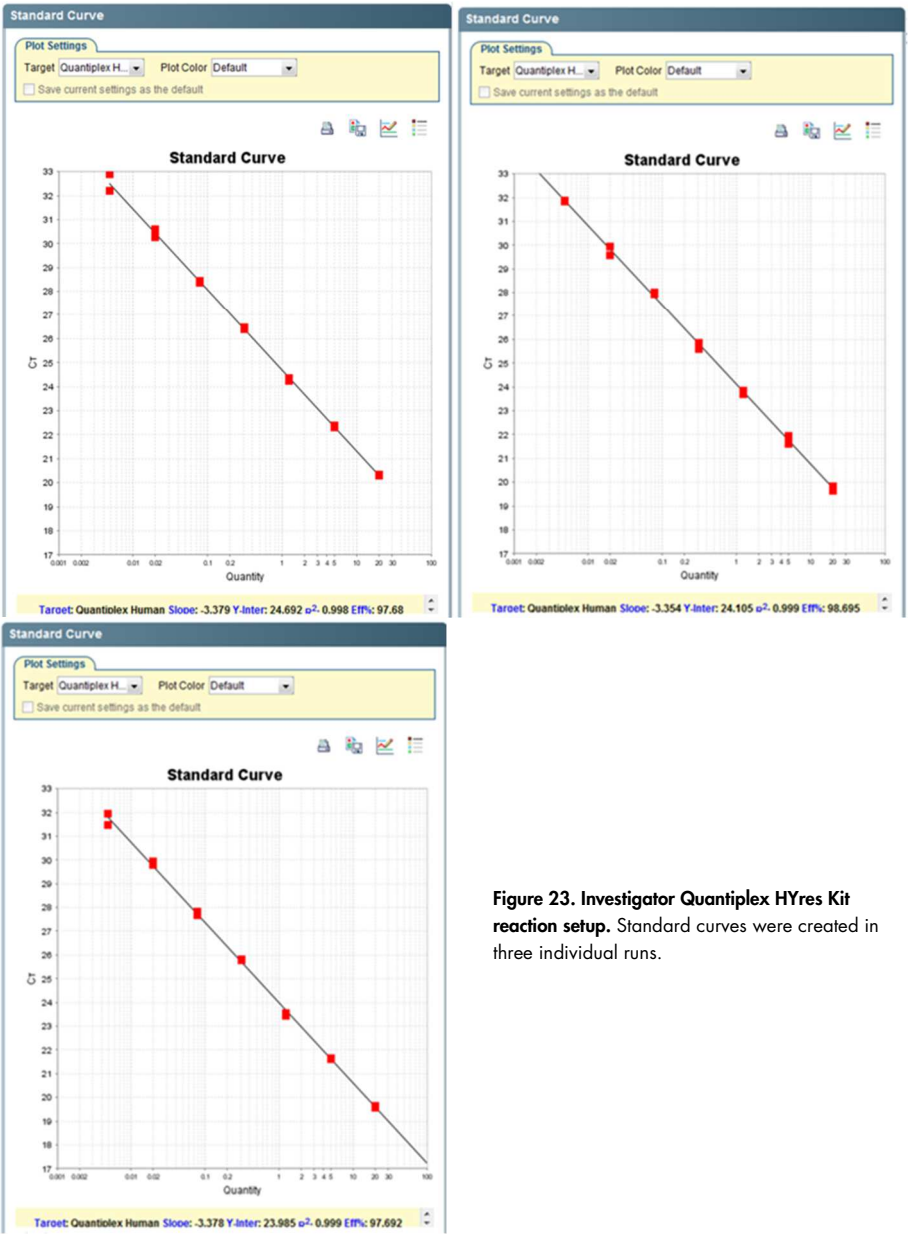


Figure 23. Investigator Quantiplex HYres Kit reaction setup. Standard curves were created in three individual runs.

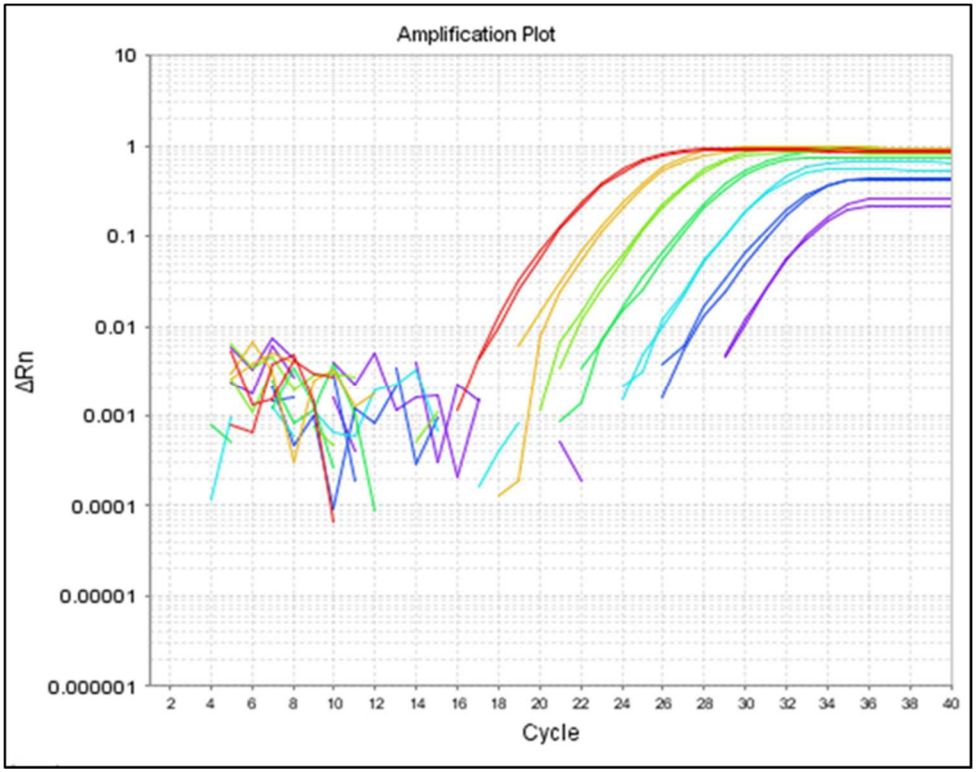


Figure 24. Example of an amplification plot for the human target of a standard curve for the Investigator Quantiplex HYres Kit.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Service or your local distributor.

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