Validation Report October 2018

Developmental validation of sample purification using Investigator® STAR Lyse&Prep chemistry on the Tecan Freedom EVO® automated platform

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 was adapted from the QIAsymphony® DNA Investigator Kit. With either kit, proven magnetic-particle technology provides high-quality DNA, which is suitable for direct use in downstream applications, such as quantitative PCR amplification or STR analyses, or for storage for later use. Purified DNA is free of proteins, nucleases and inhibitors.

The Tecan Freedom EVO automated platform can perform 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 volumes are available, and DNA can be eluted in 30 to 100 µl low TE buffer.

Performance of the Investigator STAR Lyse&Prep Kit was evaluated on the Tecan Freedom EVO platform with regard to various sample types and conditions commonly encountered in forensic and parentage laboratories. Specific issues that can arise during forensic casework were investigated, such as the ability to obtain results from samples subjected to adverse environmental conditions. These conditions were tested using DNA spiked with inhibitors. In addition, cross-contamination was tested; reproducibility of the results was verified; and performance was evaluated using various typical sample types.

Results of developmental validation

The validation study was performed by the QIAGEN HID and Forensics Department. Quantification and STR PCRs were performed according to instructions in the corresponding kit handbooks. All of the electropherograms shown were generated on an Applied Biosystems® 3500 Genetic Analyzer. Standard conditions specified in the respective kit handbooks were used for all experimental steps unless otherwise stated. A GeneAmp® PCR System 9700 with Gold-Plated Silver 96-Well Block Module was used for amplification unless otherwise stated. Data were analyzed using Applied Biosystems GeneMapper® ID-X software v1.2.

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, a 1/10 dilution series was used, consisting of 5 concentrations of an individual's blood, saliva and semen samples. Samples were extracted and quantified in replicates of 5 to assess the quality of the results for each sample type. The study was conducted using the 300 µl tube-to-plate protocol, and DNA was eluted in 50 µl. Samples were quantified using the Investigator Quantiplex® Pro RGQ Kit on a QIAGEN® Rotor-Gene® Q real-time PCR cycler. The quantification results were analyzed using the Quantiplex Pro Quant Assay Data Handling Tool v3.3.

DNA yields increased in proportion to the amount of sample extracted (Figure 1–Figure 3), and DNA was efficiently recovered from the lowest concentration replicate tested. Observed yields were comparable to those obtained using the QIAsymphony DNA Investigator Kit with corresponding protocols on the QIAsymphony SP.

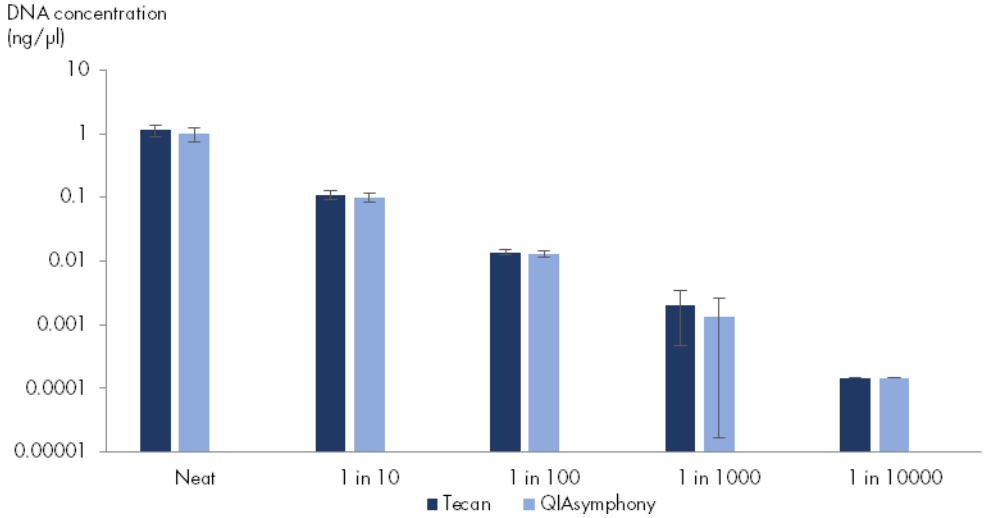

Figure 1. Sensitivity and linearity study for blood. Results obtained were comparable between protocols run on Tecan Freedom EVO and QIAsymphony SP instruments.

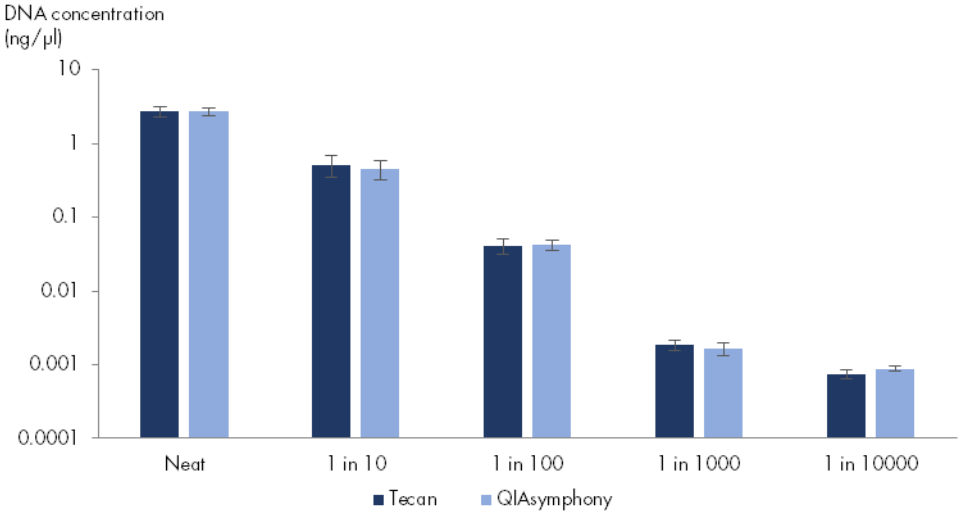

Figure 2. Sensitivity and linearity study for saliva. Results obtained were comparable between protocols run on Tecan Freedom EVO and QIAsymphony SP instruments.

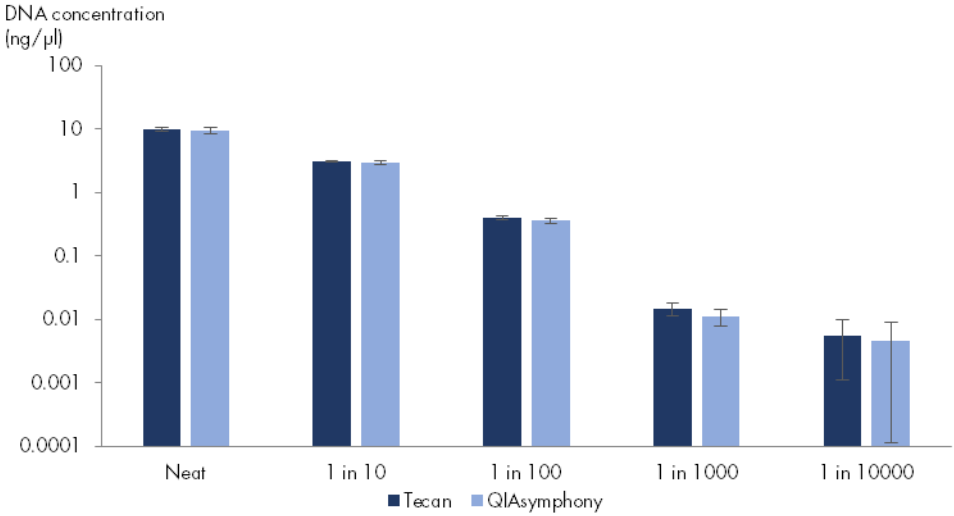

Figure 3. Sensitivity and linearity study for semen. Results obtained were comparable between protocols run on Tecan Freedom EVO and QIAsymphony SP instruments.

Stability

Forensic casework samples are frequently associated with potential inhibitors of PCR. These inhibitors must be efficiently removed during extraction to prevent any negative impact on the analysis. The Investigator STAR Lyse&Prep Kit was tested for removal of inhibitors from samples spiked with known inhibitors. Humic acid (1000 ng/µl) and hematin (3500µM) were spiked into samples containing saliva. The study was conducted using the 300 µl protocol, and DNA was eluted in 50 µl. Samples were quantified using the Investigator Quantiplex Pro RGQ Kit on a Rotor-Gene Q, and 500 pg template DNA per reaction was used for Investigator 24plex QS STR PCR.

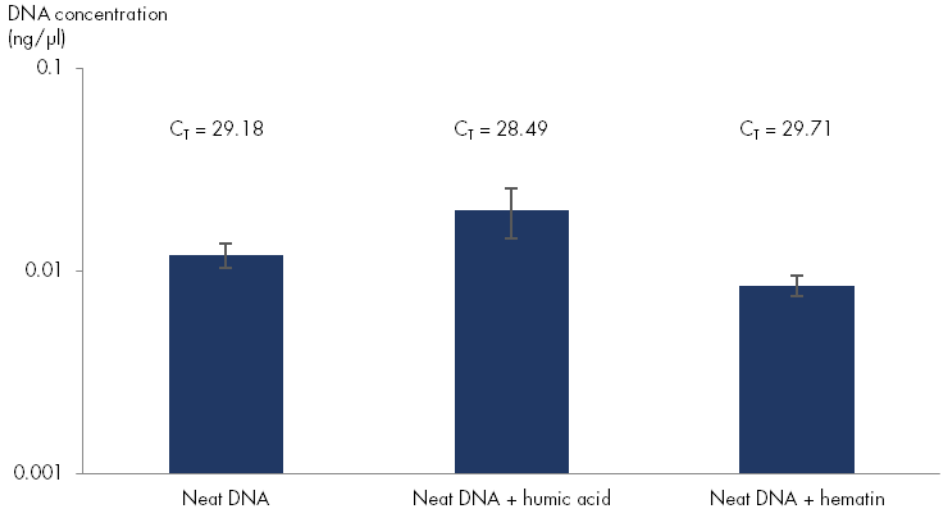

Figure 4. Performance of inhibited samples in the 300 µl protocol. Humic acid (1000 ng/µl) and hematin (3500 µM) were spiked into neat saliva. Samples were processed in 5 replicates. DNA yield and the C_T values of the internal control (IC) of the Investigator Quantiplex Pro RGQ Kit are shown.

No change in amplification of the Investigator Quantiplex Pro RGQ Kit internal control was observed for any of the samples, which indicates that no inhibition was present (Figure 4 and Table 1–Table 2). 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 5, page 8 as an example).

Table 1. Performance of inhibited samples in the 300 µl protocol – DNA yield and C_T values

	Human		Human degradation		IC	Male	
Sample name*	**C_T**	**Quantity**	**C_T**	**Quantity**	**C_T**	**C_T**	**Quantity**
TV_INH_N1	28.975	0.0137	29.761	0.0062	16.566	29.759	0.0079
TV_INH_N2	29.060	0.0129	29.875	0.0058	16.486	29.968	0.0068
TV_INH_N3	29.187	0.0118	29.531	0.0073	16.425	30.137	0.0061
TV_INH_N4	29.153	0.0121	29.395	0.0080	16.265	30.069	0.0064
TV_INH_N5	29.515	0.0093	30.166	0.0047	16.504	30.075	0.0063
TV_INH_HA1	27.921	0.0289	28.746	0.0124	16.362	28.364	0.0205
TV_INH_HA2	28.328	0.0217	28.948	0.0108	16.477	28.652	0.0168
TV_INH_HA3	28.629	0.0175	29.555	0.0072	16.358	28.323	0.0211
TV_INH_HA4	28.798	0.0155	29.826	0.0060	16.450	29.223	0.0114
TV_INH_HA5	28.761	0.0159	29.576	0.0071	16.413	28.719	0.0161
TV_INH_H1	29.651	0.0085	29.780	0.0062	16.606	30.002	0.0066
TV_INH_H2	29.720	0.0081	30.038	0.0052	16.251	29.938	0.0069
TV_INH_H3	29.493	0.0095	30.084	0.0050	16.494	30.139	0.0061
TV_INH_H4	29.904	0.0071	30.606	0.0035	16.380	30.287	0.0055

* N1–N5: Neat saliva
HA1–HA5: Neat saliva + humic acid
H1–H4: Neat saliva + hematin

Table 2. Performance of inhibited samples in the 300 µl protocol – quality assessment

	Quality assessment using the Quantiplex Pro Quant Assay Data Handling Tool v3.3					
Sample name*	**Mixture index**	**Mixture threshold**	**Degradation index**	**Degradation threshold**	**Inhibition index**	**Inhibition threshold**
TV_INH_N1	1.74	Below threshold	2.20	Below threshold	–0.20	Below threshold
TV_INH_N2	1.90	Below threshold	2.23	Below threshold	–0.12	Below threshold
TV_INH_N3	1.95	Below threshold	1.62	Below threshold	–0.06	Below threshold
TV_INH_N4	1.90	Below threshold	1.51	Below threshold	0.10	Below threshold
TV_INH_N5	1.48	Below threshold	1.97	Below threshold	–0.14	Below threshold
TV_INH_HA1	1.41	Below threshold	2.32	Below threshold	0.01	Below threshold
TV_INH_HA2	1.29	Below threshold	2.00	Below threshold	–0.11	Below threshold
TV_INH_HA3	0.83	Below threshold	2.44	Below threshold	0.01	Below threshold
TV_INH_HA4	1.37	Below threshold	2.60	Below threshold	–0.08	Below threshold
TV_INH_HA5	0.99	Below threshold	2.25	Below threshold	–0.04	Below threshold
TV_INH_H1	1.28	Below threshold	1.38	Below threshold	–0.24	Below threshold
TV_INH_H2	1.16	Below threshold	1.56	Below threshold	0.12	Below threshold
TV_INH_H3	1.57	Below threshold	1.90	Below threshold	–0.13	Below threshold
TV_INH_H4	1.30	Below threshold	2.02	Below threshold	–0.01	Below threshold

* N1–N5: Neat saliva
HA1–HA5: Neat saliva + humic acid
H1–H4: Neat saliva + hematin

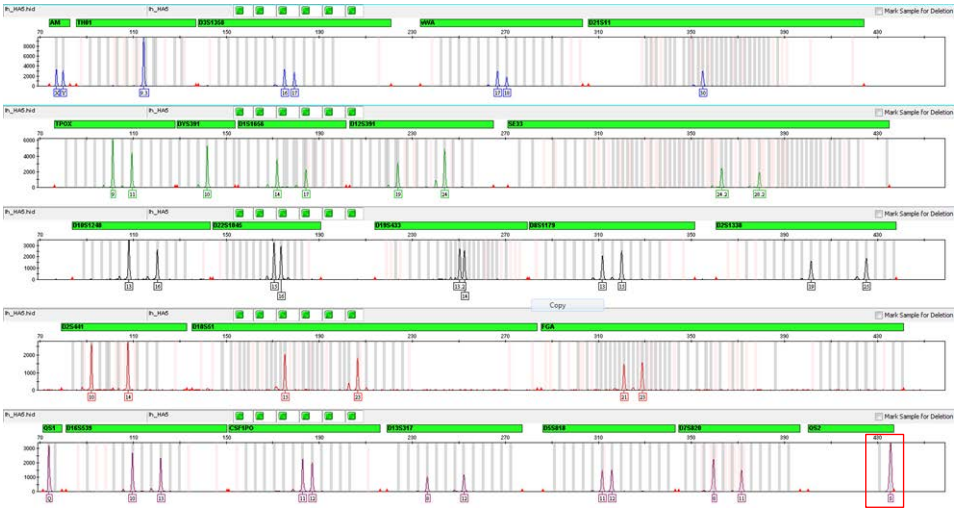

Figure 5. Example electropherogram for a sample spiked with humic acid. Note that full amplification of the large Quality Sensor fragment QS2 (labeled with a red box) indicates that no inhibition was present.

Repeatability and reproducibility

To test repeatability and reproducibility of the extraction, recovery of DNA was determined in 8 runs performed over different days (Figure 6). In each run, 8 samples were used, for a total of 64 samples. The study was conducted using the 300 µl protocol, and DNA was eluted in 50 µl. Samples were quantified using the Investigator Quantiplex Pro RGQ Kit on a Rotor-Gene Q.

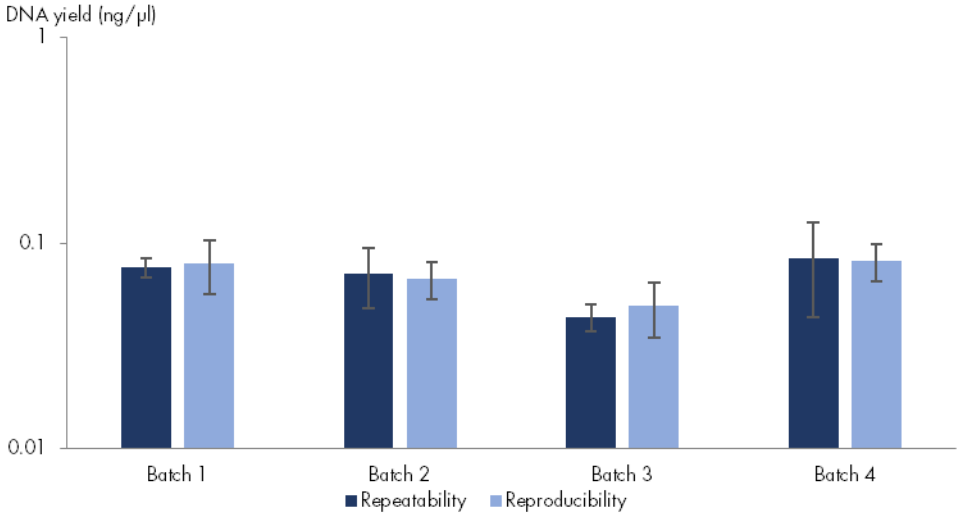

Figure 6. Comparable repeatability and reproducibility. Results were comparable between and within groups, demonstrating reliability of the Investigator STAR Lyse&Prep chemistry to consistently provide high-quality eluates of DNA. Eight samples were processed in 8 replicates for a total of 64 samples.

Cross-contamination and plate homogeneity

A contamination and plate-homogeneity study was performed for the 300 µl protocol using neat saliva arranged in a checkerboard pattern with alternating negative extraction controls. The average concentration of neat saliva eluate was 4.7 ng/µl. Negative samples were amplified using the Investigator 24plex QS Kit with 15 µl as template. Analysis was repeated for samples showing spurious peaks above 50 RFU. No spurious peak was verified.

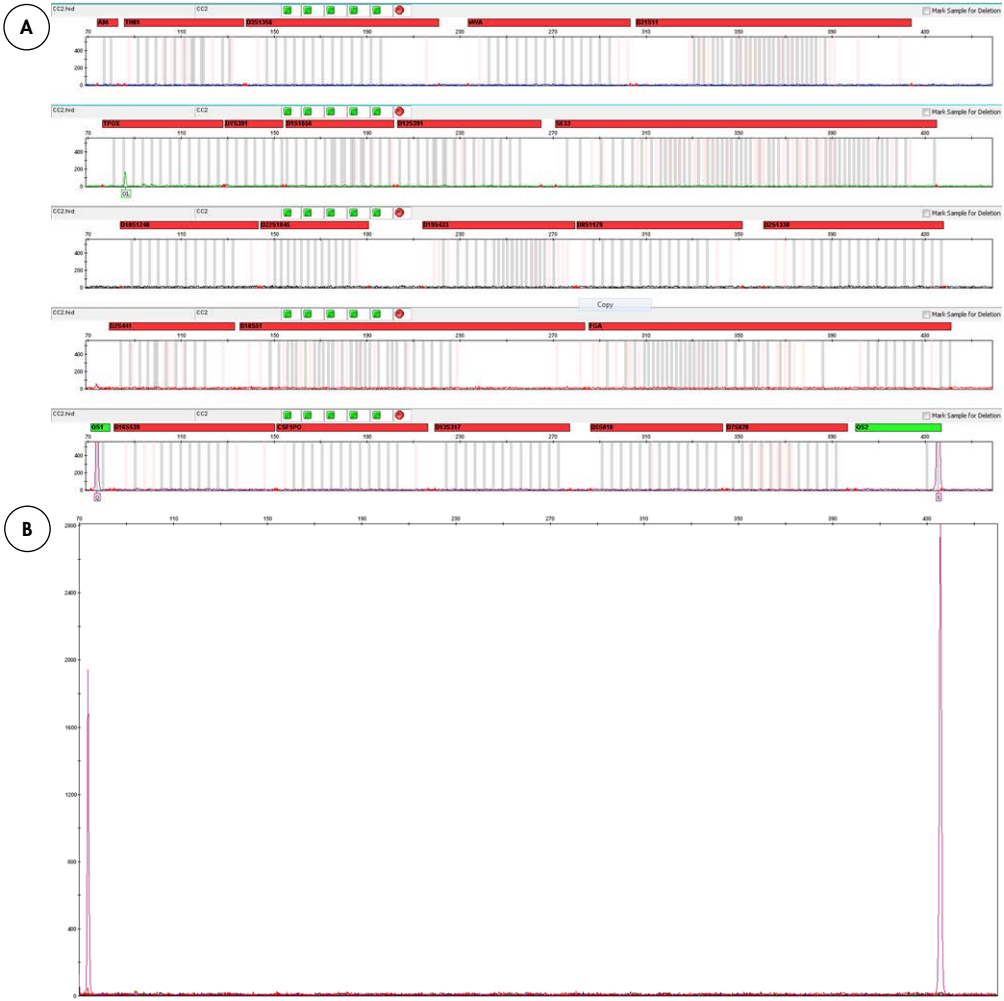

Figure 7. Example electropherogram for a negative sample. Note that there was no amplification of any allelic peaks throughout all colors while showing balanced amplification of both Quality Sensor fragments. These results indicate successful amplification.

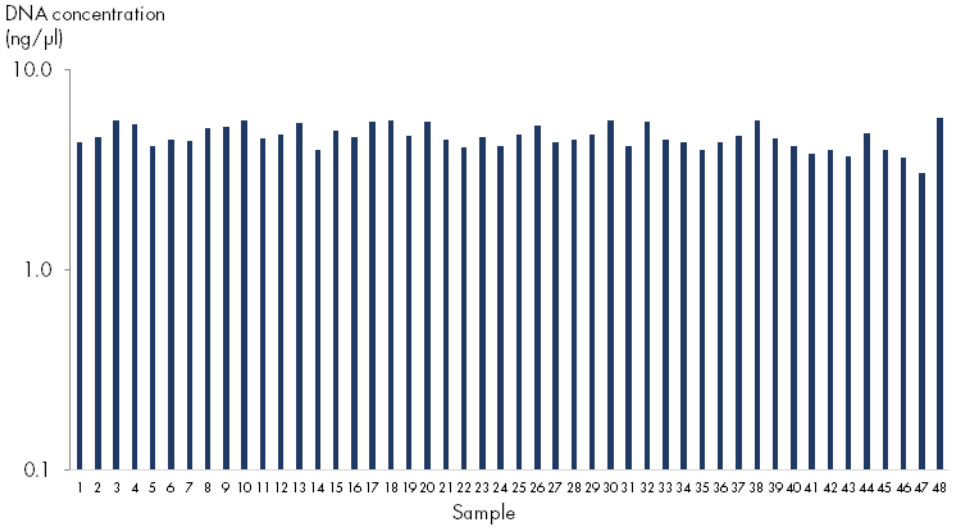

Figure 8. Plate homogeneity. Consistent DNA yields were obtained for each sample across the 96-well plate, indicating plate homogeneity and no bias for any particular position.

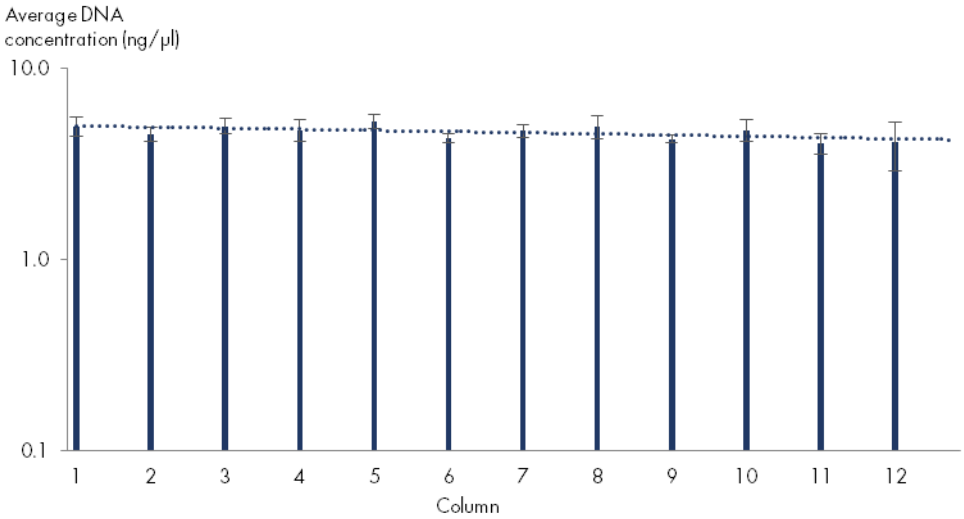

Figure 9. Plate homogeneity grouped by column. Consistent DNA yields were obtained for each column across the 96-well plate, indicating plate homogeneity and no bias for any particular position. Samples were grouped by column; each column was processed at the same time by the Tecan Freedom EVO platform's 8 pipetting channels.

Script verification

To that ensure that all protocols perform equivalently, a script verification study was performed using neat saliva for the 300 and 500 µl protocols with tubes or plates as the input or output plasticware. DNA was eluted in 50 µl. Samples were quantified using the Investigator Quantiplex Pro RGQ Kit on a Rotor-Gene Q.

The average concentration of neat saliva eluate was 3.25 ng/µl.

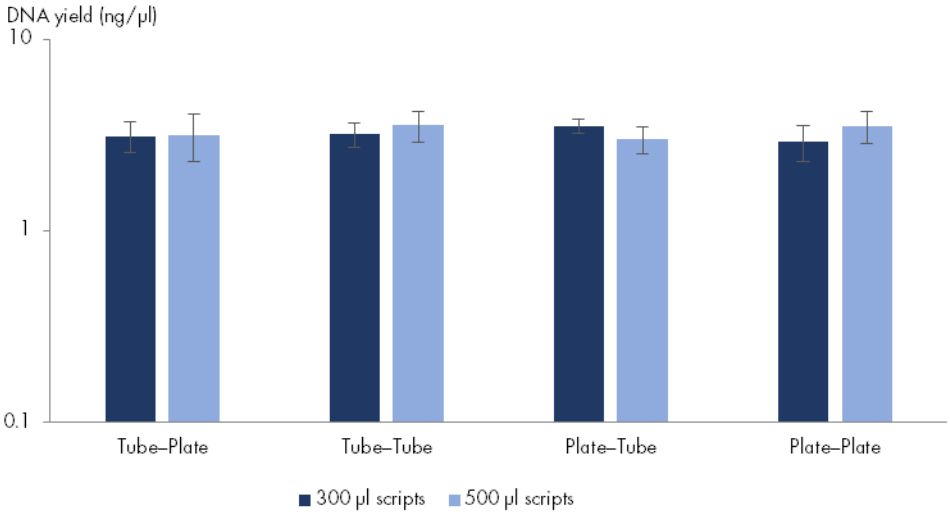

Figure 10. Script verification. Consistent DNA yields were obtained for each protocol. Samples were processed in 5 replicates for each script version.

Ordering Information

Product	Contents	Cat. no.
Investigator STAR Lyse&Prep Kit (400)	For 400 preps of 300 µl each from casework and reference samples: Buffer ATL, Buffer QSL3, Buffer QSW1, Buffer QSW2, Bead Suspension G, Buffer ATE, Proteinase K, Carrier RNA, Q-Card, Handbook	931447
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN RotorGene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316
Rotor-Gene Q 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9001660

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.