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TECHNICAL NOTE

Validation of the PrepFiler™ Automated Forensic DNA Extraction Kit on the Hamilton ID NIMBUS® Presto for DNA Extraction of Human Identification Sample Types

Introduction

A reliable extraction method that facilitates cell lysis and DNA purification across various sample and substrate combinations is critical to any forensic DNA casework workflow. Traditionally DNA extraction methods were performed manually and required significant hands-on time to complete. Although robotic methods have minimized hands-on time, multiple purification steps are required to clean forensic casework samples which remains time-consuming.

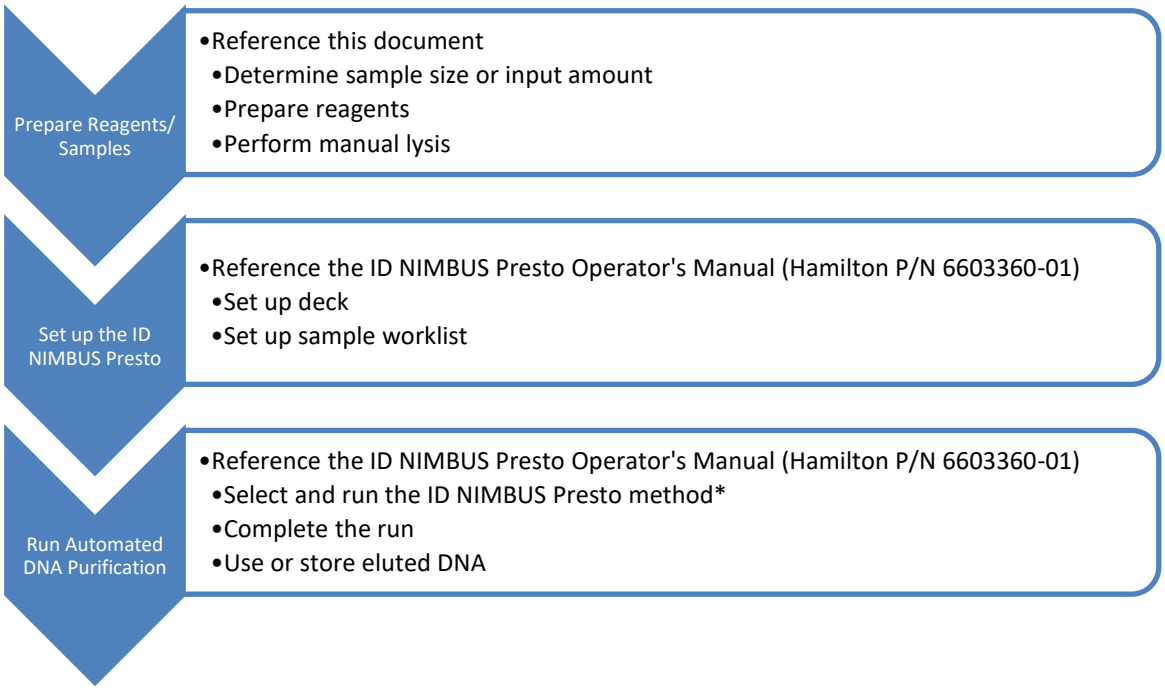
The Thermo Scientific™ KingFisher™ Presto is an automated purification system that, when coupled with PrepFiler extraction chemistry, promotes binding of DNA to magnetic particles, removal of cellular debris/inhibitors and elution of bound DNA through the movement of the magnetic particles across reagent plates. Add Hamilton's Microlab® NIMBUS® liquid handler for filling of the reagent plates, and the result is the ID NIMBUS® Presto, a hands-free purification system that can purify up to 96 samples in less than an hour using the PrepFiler™ Automated Forensic DNA Extraction Kit.

Objective

In conjunction with Hamilton Robotics, ThermoFisher Scientific has performed validation studies to demonstrate that the PrepFiler Automated Forensic DNA Extraction Kit can be used on the ID NIMBUS Presto with the associated Hamilton-developed robotic script for purification of common human identification sample types.

This technical note reviews the protocols used and presents the results of the validation studies. It is intended to provide a reference for laboratories interested in or planning to use the PrepFiler Automated Forensic DNA Extraction Kit on the ID NIMBUS Presto for extraction of human identification samples. Each laboratory should perform internal validation studies in accordance with laboratory protocols and appropriate quality standards. For information related to operation, maintenance, and troubleshooting of the ID NIMBUS Presto system, please refer to the ID NIMBUS Presto Operator's Manual (Hamilton P/N 6603360-01).

Workflow for Manual Lysis and Automated Purification



*The script starts with lysate in a KingFisher deep-well 96 plate (P/N 95040450) and finishes with elution into a second KingFisher deep-well 96 plate (P/N 95040450).

Protocol for Reagent and Sample Preparation

Determine Sample Size or Input Amount

Determine the appropriate sample size or input amount for each sample. Examples of recommended sample types and inputs are listed in Table 1.

Table 1: Example sample types and inputs

Example Sample Type	Example Sample Input
Liquid samples (blood, saliva)	Up to 40 µL
Body fluids on FTA™ paper or fabric	Up to 25-mm^2 (cutting or punch)
Body fluids on swabs	Up to one swab
Hair root	Up to 5 mm cutting from root

Prepare Reagents / Reagent Considerations

1. Before each use, examine the PrepFiler™ Magnetic Particles for precipitation. If precipitate is present, incubate the magnetic particles at 37°C for 10 minutes. Vortex at medium

speed or manually invert the tubes until the particles are completely resuspended. Quick spin to remove bubbles from the top of the tubes.

a. The ID NIMBUS Presto requires four tubes of Magnetic Particles, regardless of the number of samples extracted.
b. The ID NIMBUS Presto requires a dead space volume in each Magnetic Particles tube of at least 40 µL.
    i. When this dead space limit is reached, a new tube should be used or contents from multiple tubes of the same Magnetic Particles lot can be combined to achieve a volume greater than 40 µL.
c. Magnetic particles should be kept in their original tubes, with caps removed, during ID NIMBUS Presto processing.
    i. Do not transfer to a secondary container.
    ii. Once the ID NIMBUS Presto run is complete, immediately recap the remaining Magnetic Particles to prevent evaporation.

2. If the PrepFiler™ Lysis Buffer contains precipitate, heat the buffer solution to 37°C for 15 minutes, then vortex the bottle for 5 seconds.

3. Prepare PrepFiler™ Wash Buffers A and B before first use:
    a. Mix 260 mL of PrepFiler™ Wash Buffer A Concentrate with 740 mL of freshly opened 95% ethanol in a separate, clean container to prepare a 1× solution.
    b. Mix 200 mL of PrepFiler™ Wash Buffer B Concentrate with 300 mL of freshly opened 95% ethanol in a separate, clean container to prepare a 1× solution.

4. Thaw or prepare a fresh 1.0 M solution of DL-dithiothreitol (DTT) in molecular-biology grade DNase-free water.

Manual Lysis Protocol

1. Bring a thermal shaker to 70°C.

2. Insert a PrepFiler™ LySep™ Column (P/N 4468323) into a hingeless sample tube (Phenix P/N MH-815NC) (together called the "column/tube assembly"), then carefully transfer the sample into the LySep column.
    a. For lysis using PrepFiler™ Spin Tubes and Filter Columns (P/N A36853) or PrepFiler™ 96-Well Spin Plates and Filter Plates (P/N 4476031), refer to the PrepFiler™ Automated Forensic DNA Extraction Kit User Guide (P/N 4463349).

3. Prepare a fresh lysis solution. Each sample requires:
    a. 300* µL PrepFiler Lysis Buffer
    b. 3 µL freshly prepared 1.0 M DTT (5 µL may be used for samples containing semen)

4. Add 300* µL of freshly prepared lysis solution to the column/tube assembly.

*Note: The ID NIMBUS Presto PrepFiler method can accommodate both a 300 µL (standard lysis protocol) and 500 µL (large volume lysis protocol) lysate input. Only the 300 µL input volume has been validated and is described in this technical note.

5. Tightly close the lid of the column/tube assembly. Do not place labels on tube caps; doing so can cause leakage.

6. Place the column/tube assembly in a thermal shaker. Incubate at 70°C and 750-900 rpm for 40 minutes. The validation studies referenced in this document were completed at a 900-rpm shaking speed.

7. Centrifuge the column/tube assembly for 2 minutes at 10,000 × g to transfer the lysate to the sample tube.

8. Carefully remove the LySep column from the sample tube. If there is clear lysate remaining in the LySep column, transfer the lysate to the sample tube.
    a. Properly dispose of the LySep column. Used LySep columns are potentially biohazardous.
    b. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new sample tube.
    c. If any salt precipitation is observed, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. Do not load any sample tube that contains precipitate on the ID NIMBUS Presto. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.

9. Transfer the sample lysate from the sample tubes to individual wells of a KingFisher Deep-Well 96 Plate (P/N 95040450).

10. Proceed directly to automated purification on the ID NIMBUS Presto using the ID NIMBUS Presto Operator's Manual (Hamilton P/N 6603360-01) for reference.
    a. To avoid precipitation, do not chill the sample lysate after lysis.

Validation Studies – Materials & General Methods

Lysis and Purification		
Material	Manufacturer	Manufacturer Catalog or P/N
PrepFiler Automated Forensic DNA Extraction Kit	ThermoFisher Scientific	4463353
Microlab NIMBUS liquid handler	Hamilton	Contact Account Manager
KingFisher Presto	ThermoFisher Scientific	
Ethanol (Molecular biology grade; 95% or 190 proof)	Sigma-Aldrich	E7148
Isopropanol (2-Propanol, ACS reagent grade, ≥99.5%)	Sigma-Aldrich	190764
DL-dithiothreitol (Molecular biology grade; ≥98% (HPLC), ≥99% (titration))	Sigma-Aldrich	D9779
PrepFiler LySep Columns	ThermoFisher Scientific	4468323

1.5 mL Capless Microtubes	Phenix Research Products	MH-815NC
KingFisher Deep-Well 96 Plate, V-bottom, polypropylene	ThermoFisher Scientific	95040450
KingFisher 96 Tip Comb for DW Magnets	ThermoFisher Scientific	97002534
Tips, Conductive Non-sterile Filter, 1000 µL	Hamilton	235905
Tips, Conductive Non-sterile Filter, 300 µL	Hamilton	235903
50 mL Reagent Reservoir with V-bottom	Hamilton	187297
200 mL Reagent Reservoir with flat bottom	Hamilton	56695-01
General purpose shaking incubator capable of temperatures up to 70°C		
Quantification		
Material	Manufacturer	Manufacturer Catalog or P/N
Quantifiler™ Trio DNA Quantification Kit	ThermoFisher Scientific	4482910
7500 Real-Time PCR System for Human Identification	ThermoFisher Scientific	4366605
HID Real-Time PCR Analysis Software v1.2	ThermoFisher Scientific	A24664
Amplification		
Material	Manufacturer	Manufacturer Catalog or P/N
GlobalFiler™ PCR Amplification Kit	ThermoFisher Scientific	4476135
ProFlex™ 96-well PCR System	ThermoFisher Scientific	4484075
DNA Suspension Buffer (Tris 10 mM, EDTA 0.1 mM) pH 8.0	Teknova	T0227
Capillary Electrophoresis and Data Analysis		
Material	Manufacturer	Manufacturer Catalog or P/N
3500xL Genetic Analyzer for Human Identification w/ Data Collection v3.1	ThermoFisher Scientific	4406016
GeneMapper™ ID-X Software v1.6	ThermoFisher Scientific	A39975

*Other standard laboratory equipment/consumables not listed

The following validation studies were completed to evaluate and demonstrate the performance of the PrepFiler Automated Forensic DNA Extraction Kit for extracting common human identification sample types on the ID NIMBUS Presto automated extraction platform:

- Contamination
- Accuracy
- Sensitivity
- Repeatability & Reproducibility
- Case-type Samples

These experiments supplement the developmental validation studies, described in the PrepFiler™ and PrepFiler™ BTA Forensic DNA Extraction Kits User Guide (P/N 4463348), that were performed to validate the chemistry used by both the PrepFiler™ Forensic DNA Extraction and PrepFiler Automated Forensic DNA Extraction Kits. A performance comparison to an already established automated extraction protocol was also completed for yield and quality correlation.

Extraction, qPCR, PCR, CE and Analysis Protocols

1. Per the protocol described above, lysis was performed manually in Lysep Columns with PrepFiler Lysis Buffer from the PrepFiler Automated Forensic DNA Extraction kit.

2. DNA purification and elution into a final elution volume of 50 µL were completed with the relevant PrepFiler Automated kit reagents on the ID NIMBUS Presto following the protocol listed in the ID NIMBUS Presto Operator's Manual (Hamilton P/N 6603360-01).

   At the start of each PrepFiler extraction method on the ID NIMBUS Presto, Users must enter the desired elution volume**, ranging from 35 µL to 150 µL.

   **Note: It was identified during our internal validation that the volume entered into the ID NIMBUS Presto PrepFiler method should be about 15 µL higher than the desired final elution volume. For example, to obtain a 50 µL final elution volume, users should enter 65 µL into the method dialog box. It is recommended that this be evaluated during internal validation studies to achieve optimal eluent volume.

3. Quantification through qPCR was performed using the Quantifiler™ Trio DNA Quantification Kit and associated protocol listed in the Quantifiler™ HP and Trio DNA Quantification Kits User Guide (P/N 4485354).

4. STR PCR amplification was completed targeting 1 ng total DNA input using the GlobalFiler PCR Amplification Kit and the 29-cycle protocol listed in the GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits User Guide (P/N 4477604).

   Samples with insufficient concentrations of DNA to target 1 ng total input were amplified at the maximum input obtained using 15 µL of the DNA extract.

5. Capillary electrophoresis was performed on a 3500xL Genetic Analyzer with Data Collection v3.1 and data analysis utilized GeneMapper™ *ID-X* v1.6. Both steps in the workflow were carried out as per the GlobalFiler and GlobalFiler IQC PCR Amplification Kits User Guide (P/N 4477604). The following key parameters were used:
   a. HID36_POP4xl run model
      i. 1.2 kV, 24 second injection for contamination study
      ii. 1.2 kV, 18 second injections for all other studies
   b. Peak Amplitude Threshold (PAT)
      i. 50 RFU for all dye channels for contamination study
      ii. 175 RFU for all dye channels for all other studies

Validation Studies – Methods & Results

Contamination & Accuracy

Method

A checkerboard pattern consisting of the following samples and extraction reagent blanks was utilized to confirm that the ID NIMBUS Presto does not introduce cross-contamination during automated extraction. The NIST standard was used to assess the accuracy of the process.

- 19 Neat Blood (NB) swabs

    - 20 µL liquid blood from donor A dried on separate Puritan cotton swabs
    - 1 whole swab used for each replicate
- 14 Neat Saliva (NS) swabs
    - 20 µL liquid saliva from donor B dried on separate Puritan cotton swabs
    - 1 whole swab used for each replicate
- 1 NIST Standard Reference Material 23901c Component F (NIST F) – single source male cells on 903 paper
- 34 Extraction Reagent Blanks (RB)

Results

- As per Figure 1, Quantifiler Trio was used to evaluate all 34 extraction reagent blanks for indications of human DNA contamination.
    - 33 reagent blanks produced an undetected result (Threshold Cycle or C_T > 40) for all targets.
    - 1 reagent blank produced a Small Autosomal C_T of 39.8 (0.2 pg/µL) that did not generate a downstream STR profile in GlobalFiler.

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1 50	STD 1 50	NB1	RB5	NB9	RB13	NS1	RB21	NS9	RB29	NB19	
B	STD 2 5	STD 2 5	RB1	NB5	RB9	NB13	RB17	NS5	RB25	NS13	RB33	
C	STD 3 0.5	STD 3 0.5	NB2	RB6	NB10	RB14	NS2	RB22	NS10	RB30	NIST F	
D	STD 4 0.05	STD 4 0.05	RB2	NB6	RB10	NB14	RB18	NS6	RB26	NS14	RB34	
E	STD 5 0.005	STD 5 0.005	NB3	RB7	NB11	RB15	NS3	RB23	NS11	RB31		
F	NTC	NTC	RB3	NB7	RB11	NB15	RB19	NS7	RB27	NB17		
G			NB4	RB8	NB12	RB16	NS4	RB24	NS12	RB32		
H			RB4	NB8	RB12	NB16	RB20	NS8	RB28	NB18		

Figure 1: Quantifiler Trio plate layout for Contamination and Accuracy studies. Reagent blanks are indicated with yellow shading and were placed in a checkerboard pattern with Neat Saliva (NS) or Neat Blood (NB) samples. The NIST F standard was in well 11C.

- The Quantifiler Trio IPC C_T values for the contamination study samples were compared to determine the presence or absence of detectable PCR inhibitors in DNA extracted using the ID NIMBUS Presto System.
    - As per Figure 2, the IPC C_T values for the blood and saliva samples were within ±1 C_T unit of the Quantifiler Trio standards 3-5 and the quantification Negative Template Controls (NTCs), indicating that PCR inhibitors were effectively removed during extraction.
    - The IPC CT values for the extraction blanks were also within ±1 C_T unit, indicating that the system consumables did not introduce PCR inhibitors into the samples.

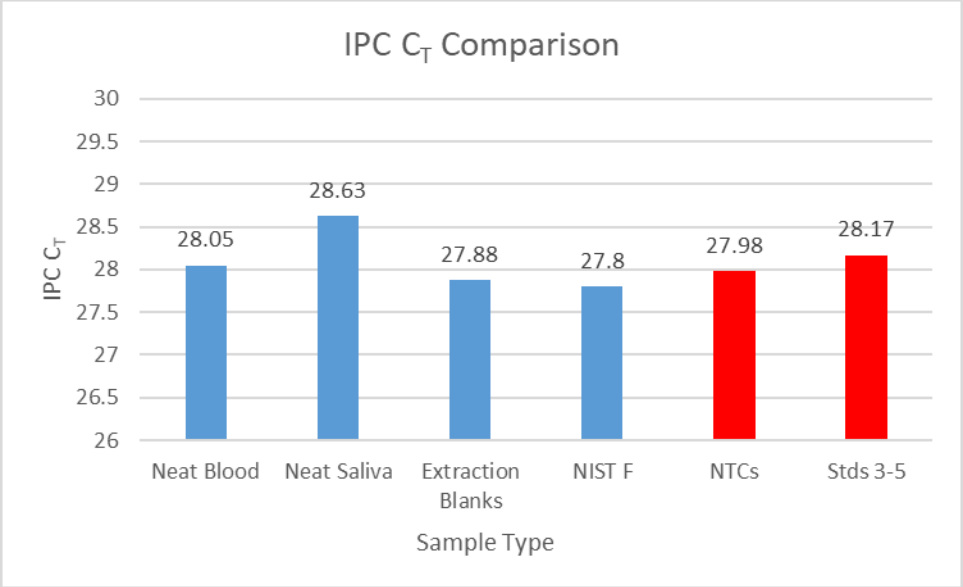


Figure 2: Quantifiler Trio IPC C_T values obtained from the samples included in the contamination study. The IPC results were within ±1 C_T, indicating that the ID NIMBUS Presto system effectively removes inhibitors from common sample types and does not introduce inhibitors.

- The 34 extraction reagent blanks were then amplified in GlobalFiler as another method to detect potential human DNA contamination. The data was generated using a 1.2 kV, 24 second injection time on a 3500xL and was analyzed using a 50 RFU PAT for detectable and reproducible allele activity.
    - All 34 reagent blanks displayed no allele activity above 50 RFU. See Figure 3 for a representative electropherogram overlay of the reagent blanks.

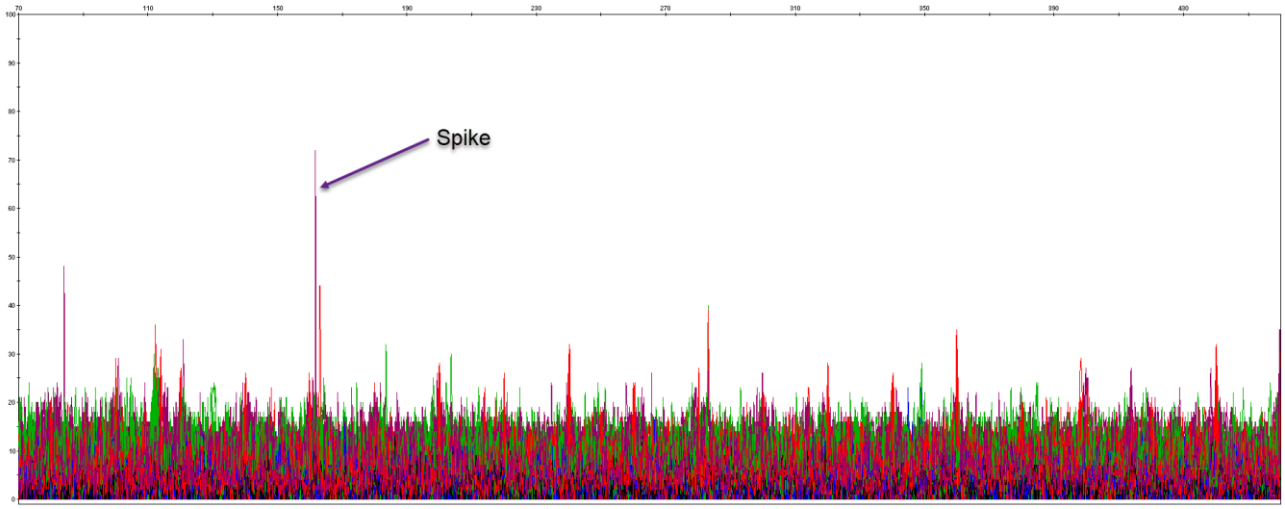


Figure 3: Electropherogram overlay of all 34 extraction reagent blanks, displaying all dye channels, extracted on the ID NIMBUS Presto system and amplified with 15 µL of eluent in GlobalFiler at 29 cycles. The Y-axis is scaled to 100 RFU, showing no reproducible alleles above a 50 RFU PAT. One spike is present in the purple dye channel above 50 RFU and the "chatter" in red is pullup from the GeneScan™ 600 LIZ™ dye size standard.

- To evaluate accuracy of the extraction and purification process, the extracted NIST F standard was amplified in GlobalFiler, with the resulting STR profile compared to the expected genotype.
    - The NIST F profile generated was 100% concordant to the expected genotype.

Sensitivity

Methods

Blood and saliva serial dilution series were utilized to determine the range of biological sample amounts that can be reliably extracted using the ID NIMBUS Presto system.

- Using PBS buffer, serial dilution series consisting of the following dilutions were created each for blood and saliva.
    - Saliva: neat, 1:3, 1:9, 1:27, 1:81, 1:243
    - Blood: neat, 1:3, 1:9, 1:27, 1:81, 1:243, 1:729
- 40 µL of each dilution from each dilution series (10 µL dilution + 30 µL PBS buffer) were added to separate Puritan cotton swabs and allowed to air dry before proceeding through extraction using 1 whole swab for each extraction. Each dilution point was extracted in triplicate.
    - Neat saliva was depleted and unavailable for this study.

Results

- Quantifiler Trio was used to evaluate the DNA concentration obtained from each extraction dilution point and the linearity of concentrations from each dilution series (see Figure 4).
    - Sensitivity for saliva swabs was demonstrated down to ~1 pg/µL.
    - Sensitivity for blood swabs was demonstrated down to ~2 pg/µL.
    - The DNA yield was proportional to sample input.

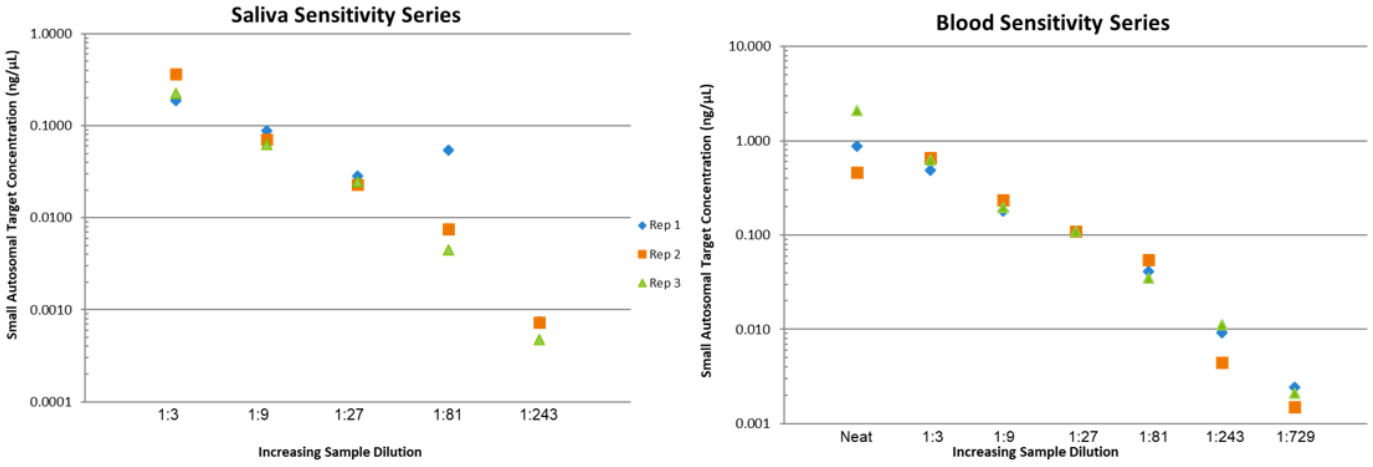


Figure 4: Sensitivity serial dilution series DNA concentration results for both saliva and blood ranging from ~0.26 ng/µL to ~0.001 ng/µL for saliva (1:3 to 1:243 dilution) and from ~1.14 ng/µL to ~0.002 ng/µL for blood (neat to 1:729 dilution). Replicate 1 of the 1:243 saliva dilution is masked by replicate 2.

- The quality of the DNA extract obtained from the ID NIMBUS Presto system was further evaluated by examining the GlobalFiler STR profiles. Figure 5 illustrates the average heterozygous peak heights from each dilution point. See Appendix A for representative STR profiles.
    - Full balanced profiles were obtained from saliva DNA extracts out to a 1:81 dilution and from blood DNA extracts out to a 1:243 dilution.

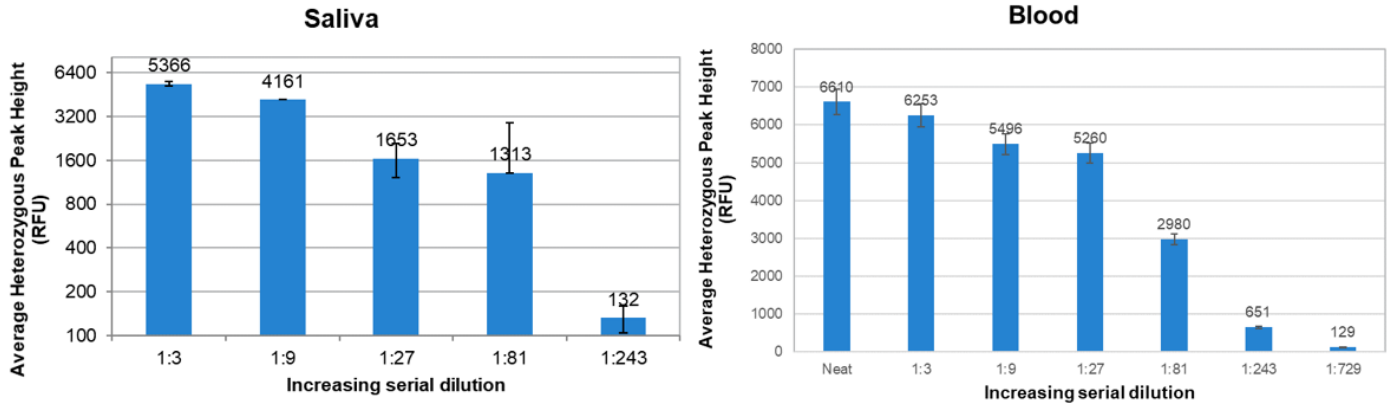


Figure 5: Average heterozygous peak heights obtained from each serial dilution point for saliva (1:3, 1:9, 1:27, 1:81, 1:243) and blood (neat, 1:3, 1:9, 1:27, 1:81, 1:243, 1:729) when amplified with either 1 ng total DNA input or at max input using 15 µL of DNA extract.

Repeatability & Reproducibility

Methods

Quantifiler Trio Small Autosomal C_T data from the sensitivity study (blood and saliva dilution series extracted and processed in triplicate) was used to evaluate repeatability of the extraction and purification process. To assess reproducibility of the process, an additional 3 replicate swabs of each blood dilution series were extracted on a second extraction plate for a total of 6 blood swabs per dilution.

Results

- As per Figure 6, consistent DNA concentrations, represented by C_T values, were obtained for each sample type and range of dilutions.
    - The variation in concentrations is within the expected variation introduced through the extraction and quantitation procedures.

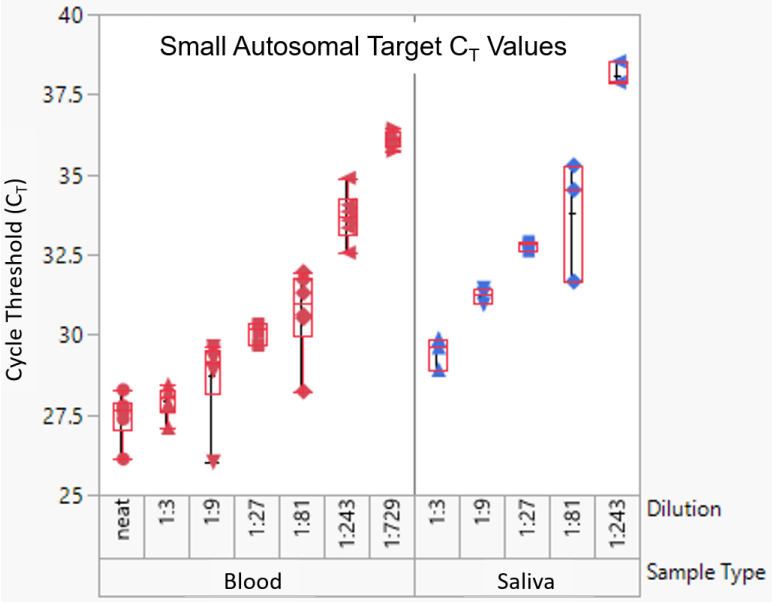


Figure 6: Quantifiler Trio Small Autosomal C_T values obtained from each serial dilution point for saliva (1:3, 1:9, 1:27, 1:81, 1:243) and blood (neat, 1:3, 1:9, 1:27, 1:81, 1:243, 1:729). The variation in concentrations is within the expected variation introduced through the extraction and quantitation procedures.

Correlation

Methods

For performance comparison to an already established automated extraction system, replicate swabs of the blood dilution series were also extracted in triplicate on an AutoMate *Express*™ Forensic DNA Extraction System. The Quantifiler Trio kit was then used to compare the yield and quality (presence or absence of inhibition) of the DNA obtained from each extraction system.

Results

- As per Figure 7, the DNA concentration obtained from samples extracted with the ID NIMBUS Presto system was comparable to or higher than that of the Automate Express system, particularly for lower DNA inputs. With the highest DNA inputs, the Automate Express performed slightly better but the difference may be exaggerated by a small sample size.

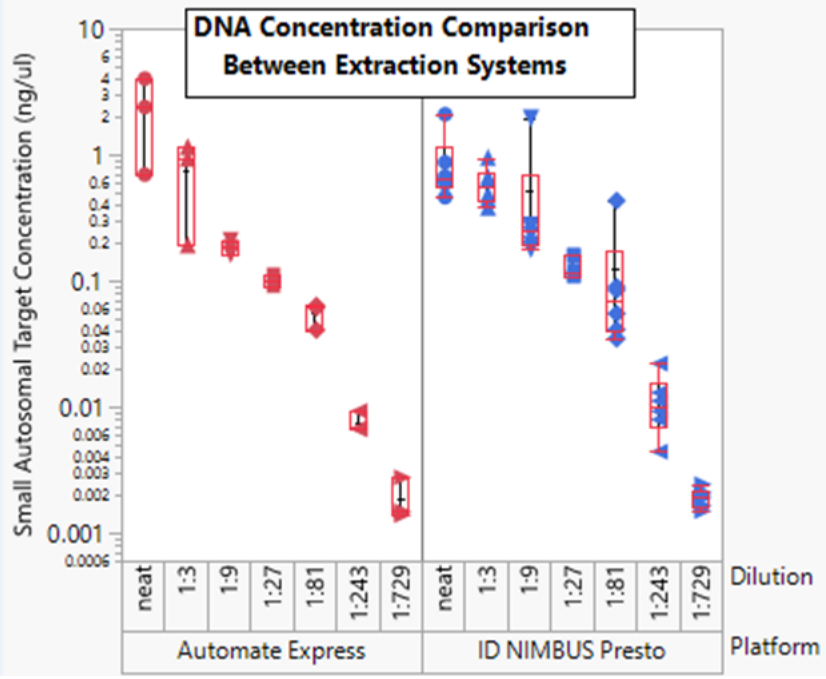


Figure 7: Comparison of the concentration of DNA obtained from each blood dilution extracted on both an ID NIMBUS Presto system (N=6 per dilution) and an Automate Express system (N=3 per dilution).

- As per Figure 8, the IPC C_T values for all blood samples were within ±1 C_T unit of the Quantifiler Trio standards 3-5 and the quantification Negative Template Controls (NTCs), indicating that PCR inhibitors were effectively removed during extraction, regardless of extraction system used.

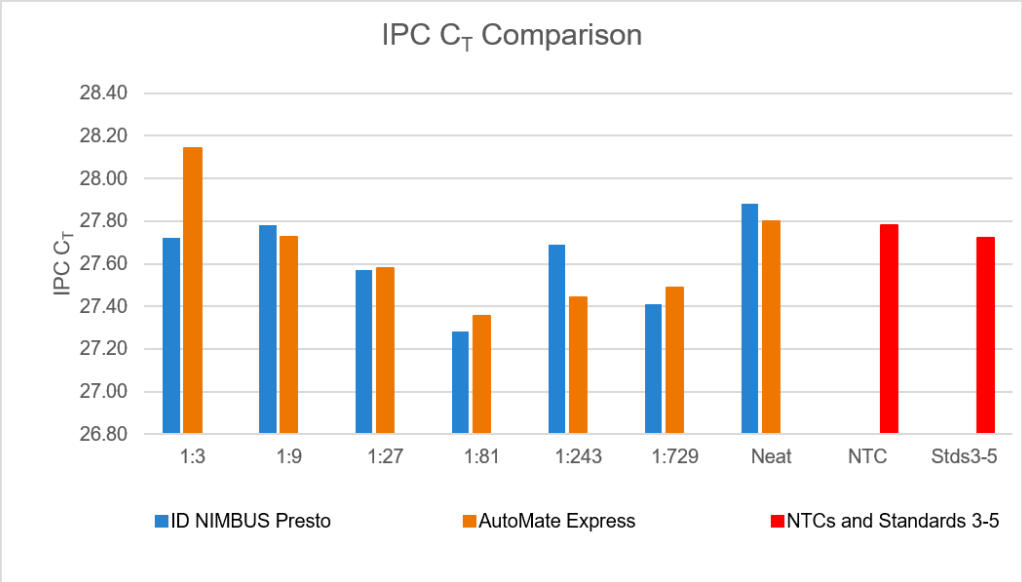


Figure 8: Comparison of the Quantifiler Trio IPC C_T values obtained from a blood serial dilution series extracted on both an ID NIMBUS Presto system and an Automate Express system.

Case-type Samples

Methods

Figure 9 illustrates the 45 known and non-probative case-type samples used to evaluate the ability of the ID NIMBUS Presto system to extract inhibitor-free DNA of the highest quantity and quality possible from common human identification sample types.

Sample Identifier	Sample Description
NP1	Swab from drink can
NP2	Swab from drink can
NP3	Swab from cell phone
NP4	Swab from cell phone
NP5	Cigarette butt
NP6	Cigarette butt
NP7	Chewing gum
NP8	Chewing gum
NP9	Semen 1:10
NP10	Semen 1:10
NP11	Semen 1:10
NP12	Semen 1:100
NP13	Semen 1:100
NP14	Semen 1:100
NP15	Swab from coffee cup
NP16	Swab from coffee cup
NP17	Swab from coffee cup
NP18	Swab from coffee cup
NP19	Swab from coffee cup
NP20	Buccal swab
NP21	Buccal swab
NP22	Buccal swab
NP23	Buccal swab

Sample Identifier	Sample Description
NP24	Swab from baseball hat
NP25	Swab from baseball hat
NP26	Blood on denim
NP27	Blood on denim
NP28	Blood on denim
NP29	Blood on denim
NP30	Blood on cotton
NP31	Blood on cotton
NP32	Blood mixture 1:10
NP33	Blood mixture 1:10
NP34	Blood mixture 1:1
NP35	Blood mixture 1:1
NP36	Hair root x3
NP37	Hair root x2
NP38	Saliva on 3mm FTA punch
NP39	Saliva on 3mm FTA punch
NP40	Saliva on 3mm FTA punch
NP41	Saliva on 3mm FTA punch
NP42	Saliva on 3mm FTA punch
NP43	Saliva on 3mm FTA punch
NP44	Saliva on 3mm FTA punch
NP45	Saliva on 3mm FTA punch

Figure 9: List of 45 known and non-probative case-type samples extracted on the ID NIMBUS Presto system for the case-type study.

Results

- Figure 10 lists the DNA concentration and Figure 11 lists the IPC C_T value obtained from each sample using Quantifiler Trio.
    - The DNA concentration obtained from the sample types tested ranged from 33 ng/µL to 0.005 ng/µL.
    - Due to sample collection failure, one of the coffee cup swabs (NP16) produced no detectable DNA.
    - Variation in DNA concentrations between samples was expected due to the variation in the amount of biological material present in different samples from different donors and different body fluids.

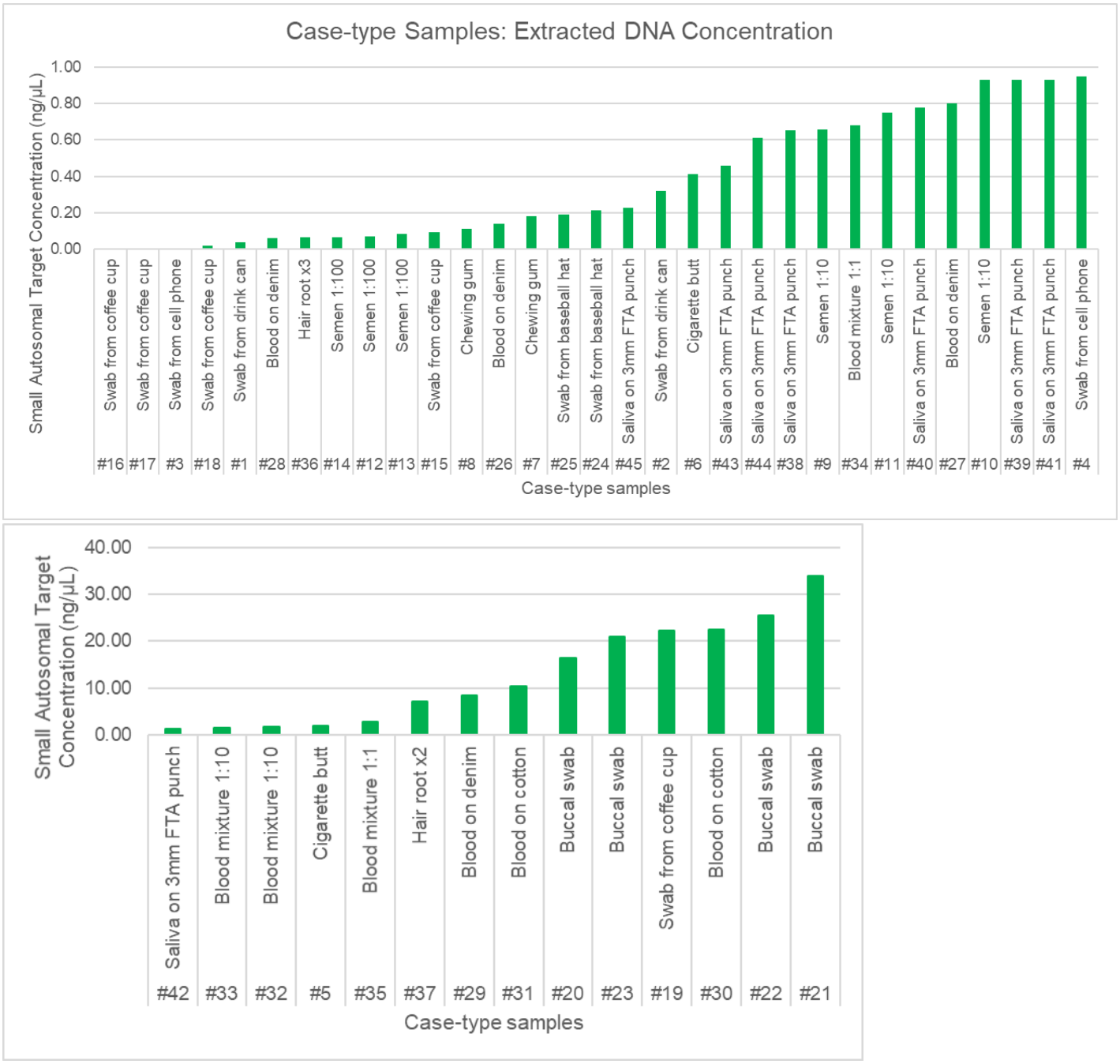


Figure 10: List of 45 known and non-probative samples and their resulting DNA concentrations extracted for the case-type study using the ID NIMBUS Presto system.

- As per Figure 11, the IPC C_T values for all case-type samples were within ±2 C_T units of the Quantifiler Trio standards 3-5 and the quantification Negative Template Controls (NTCs), indicating that PCR inhibitors were effectively removed during extraction.

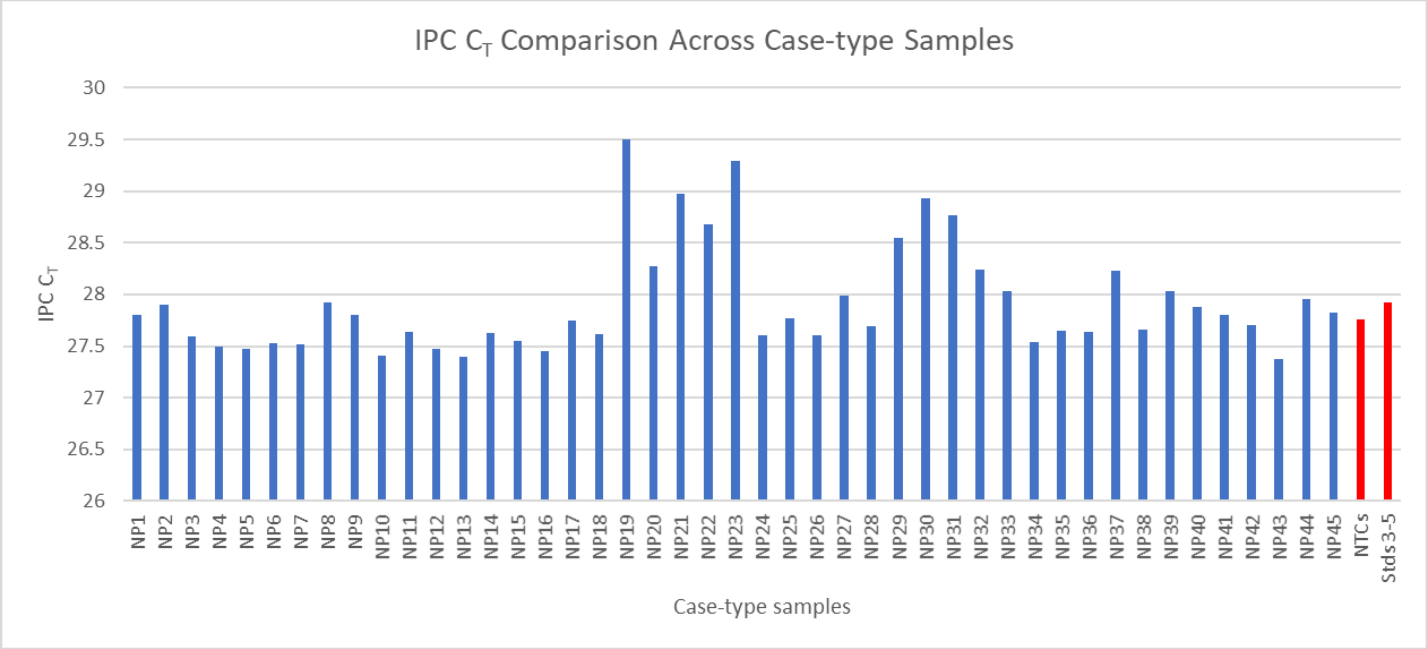


Figure 11: Quantifiler Trio IPC C_T comparison of all case-type samples and the Quantifiler NTCs and Standards 3-5, showing inhibitors were effectively removed by the ID NIMBUS Presto extraction system.

- Using GlobalFiler at 29 cycles and an 18 second injection time on the 3500xL, average heterozygous peak heights for all case-type samples ranged from ~267 RFU (NP17 – coffee cup swab) to ~11,000 RFU (NP27 – blood on denim), relative to the corresponding DNA input for each sample.
    - Partial or full profiles were obtained from all sample types except for one coffee cup swab (NP16) that was likely due to sample collection error. See Appendix B for each STR profile.

Conclusions

Validation studies confirmed that the ID NIMBUS Presto system using the PrepFiler Automated Forensic DNA Extraction Kit provides robust and reliable results in obtaining genomic DNA from forensic biological samples for downstream applications such as real-time quantitative PCR and PCR for STR profiling:

- The ID NIMBUS Presto extraction system provides reliable and accurate results at different DNA input amounts and is effective in maximizing the amount of DNA obtained from samples that contain both small and large quantities of biological material.
- The utility of the extraction method in forensic DNA analysis was demonstrated using forensic-type samples.
- The DNA that was extracted was free of PCR inhibitors as determined by the IPC C_T values using the Quantifiler Trio DNA Quantification Kit.
- The reagents and operations of the ID NIMBUS Presto extraction system exhibited clean operations and did not introduce any detectable cross contamination of human DNA.

- The performance of the ID NIMBUS Presto extraction system for extracting DNA from human identification samples is comparable to the AutoMate Express extraction system.

Appendix A

Representative 3500xL electropherograms of the saliva and blood serial dilution series, extracted on the ID NIMBUS Presto and amplified with the GlobalFiler PCR Amplification Kit using either 1 ng input or max input with 15 µL of DNA extract.

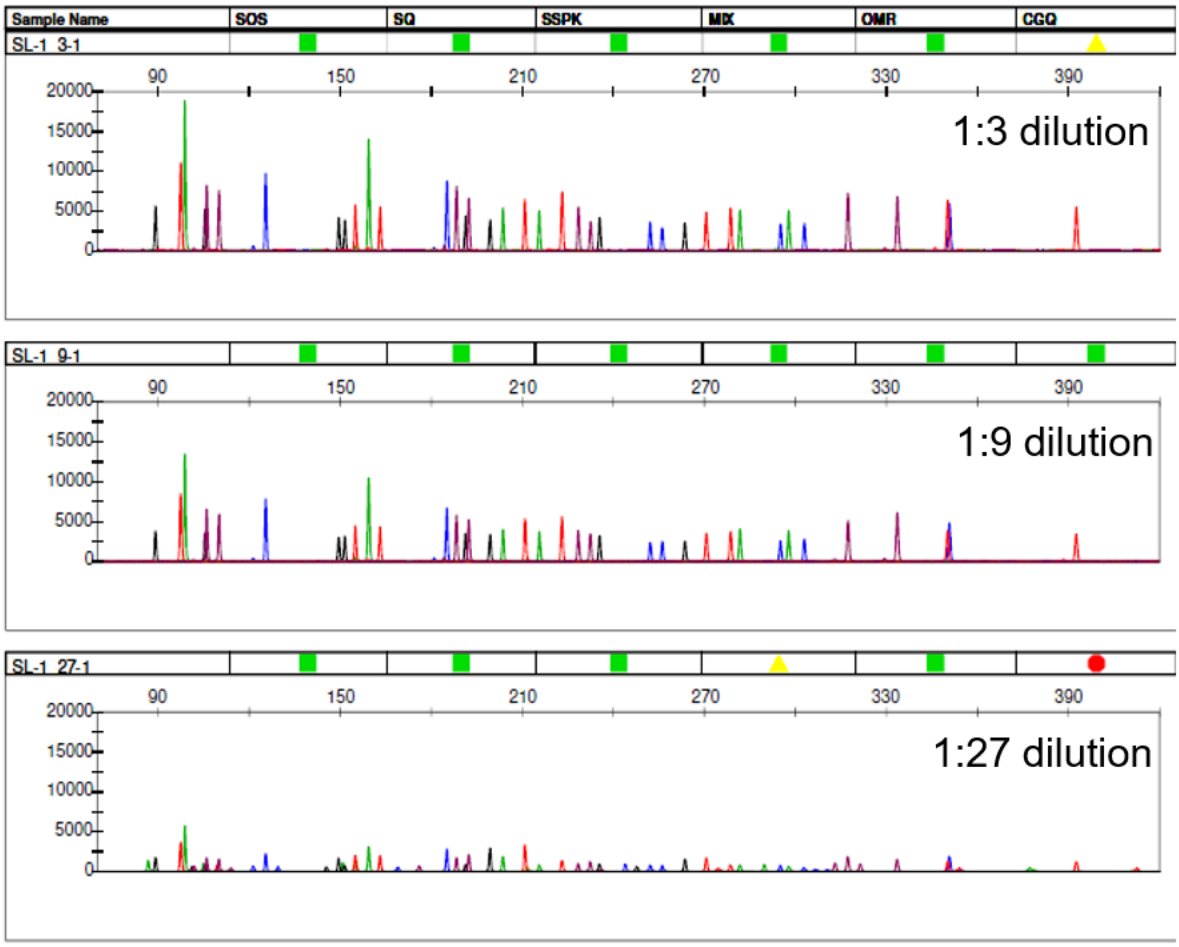


Figure 1: 3500xL electropherograms of one replicate of the saliva dilution series, showing all dye channels for the 1:3 to 1:27 dilutions, amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is scaled to 20,000 RFU.

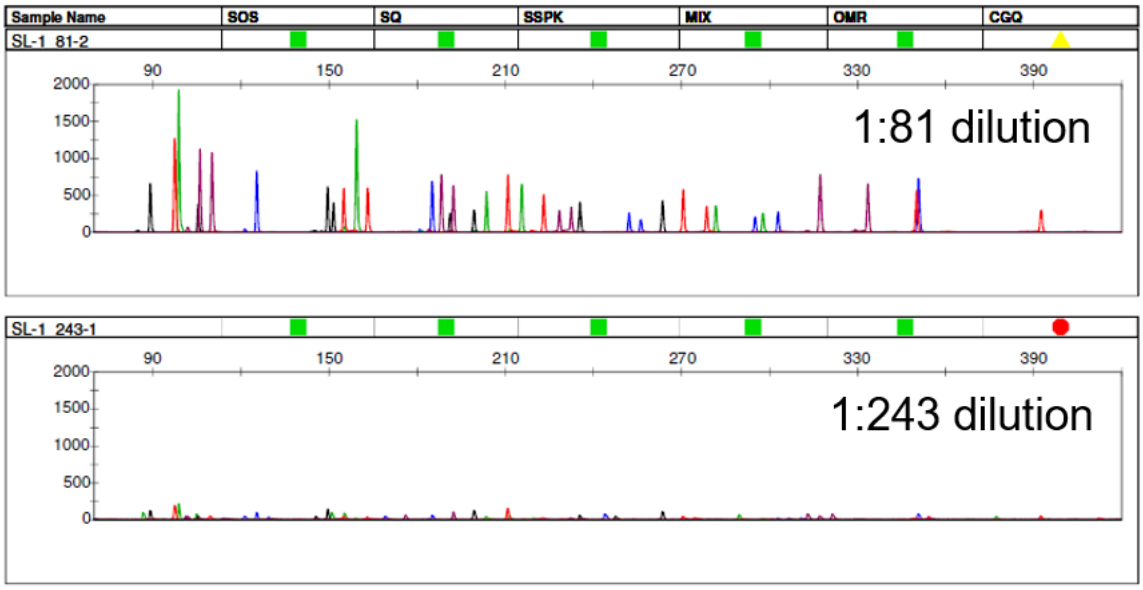


Figure 2: Continuation of the 3500xL electropherograms of one replicate of the saliva dilution series, showing all dye channels for the 1:81 and 1:243 dilutions, amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is scaled to 2,000 RFU.

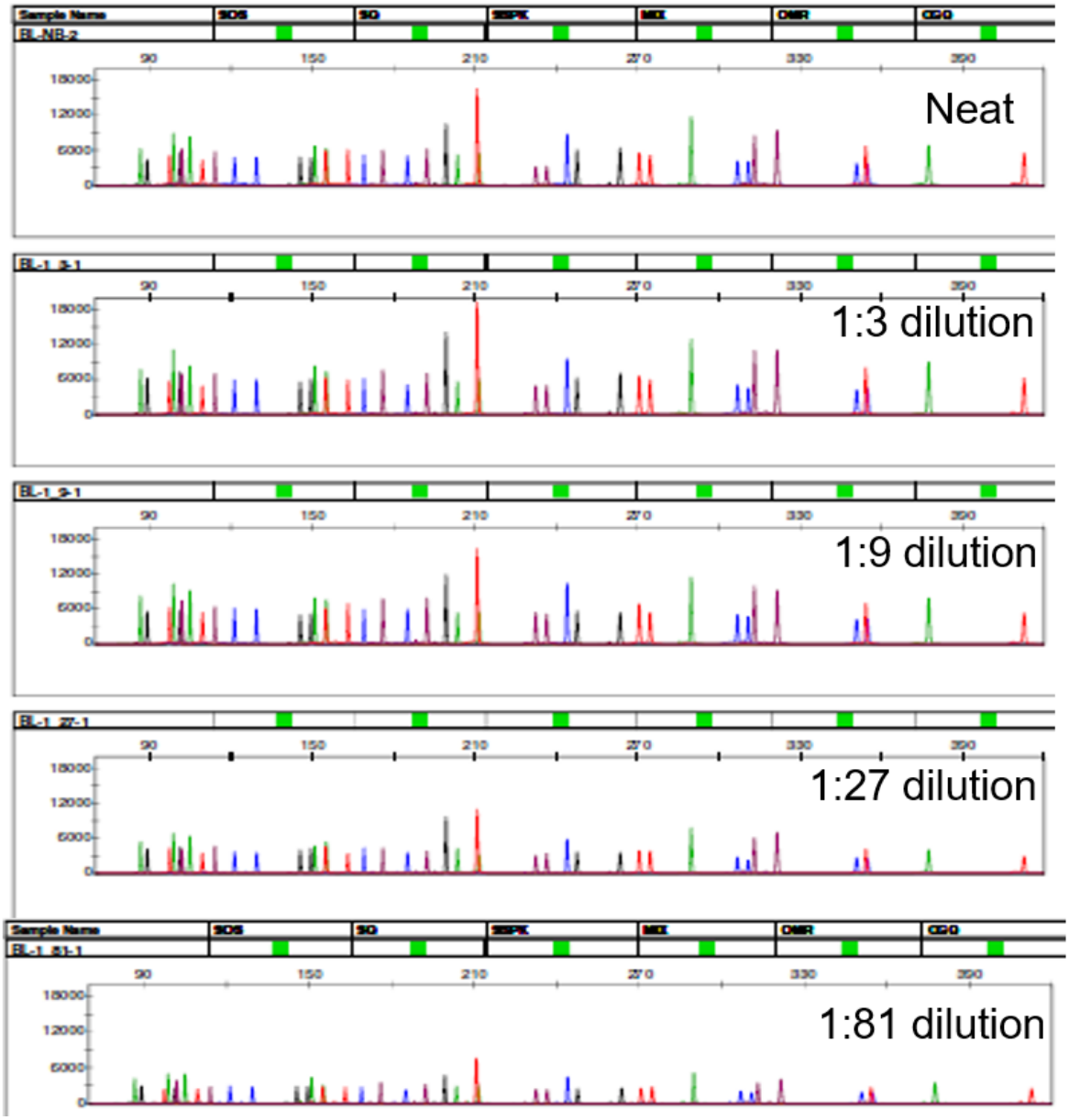


Figure 3: 3500xL electropherograms of one replicate of the blood dilution series, showing all dye channels for the neat to 1:81 dilutions, amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is scaled to 20,000 RFU.

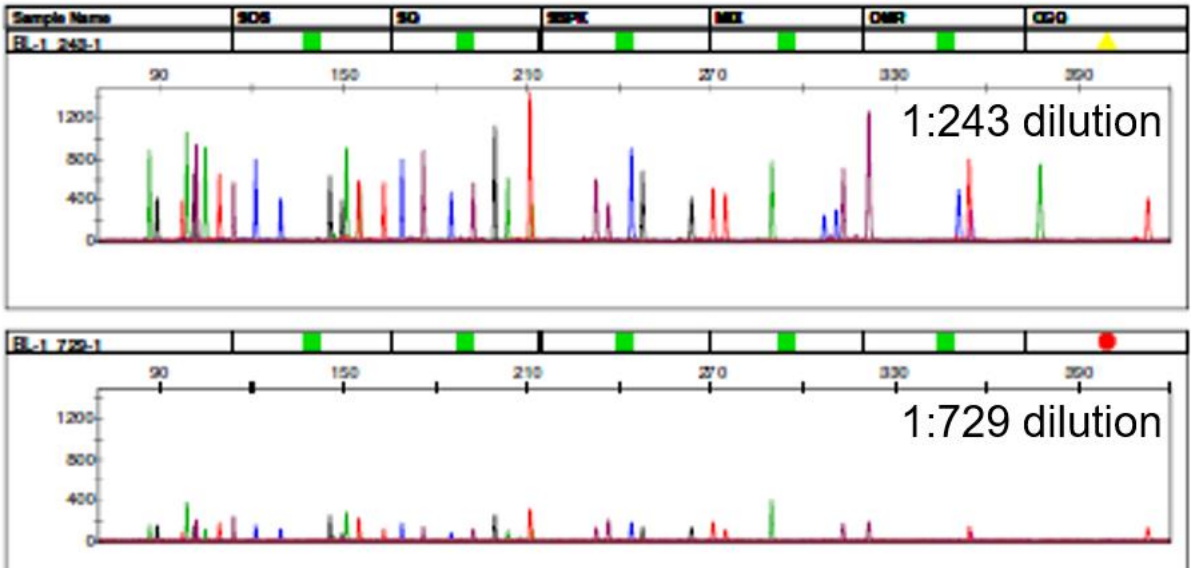


Figure 4: Continuation of the 3500xL electropherograms of one replicate of the blood dilution series, showing all dye channels for the 1:243 and 1:729 dilutions, amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is scaled to 1500 RFU.

Appendix B

3500xL electropherograms of each non-probative case-type sample, extracted on the ID NIMBUS Presto and amplified with the GlobalFiler PCR Amplification Kit using either 1 ng input or max input with 15 µL of DNA extract.

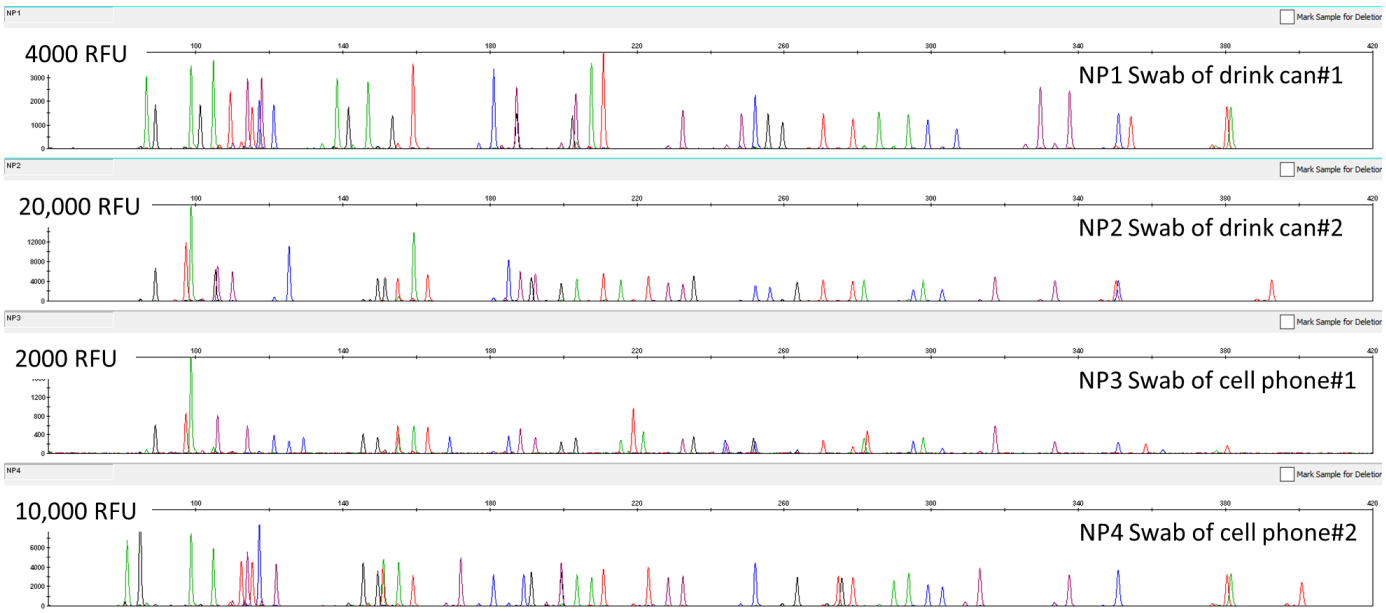


Figure 5: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP1-NP4 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

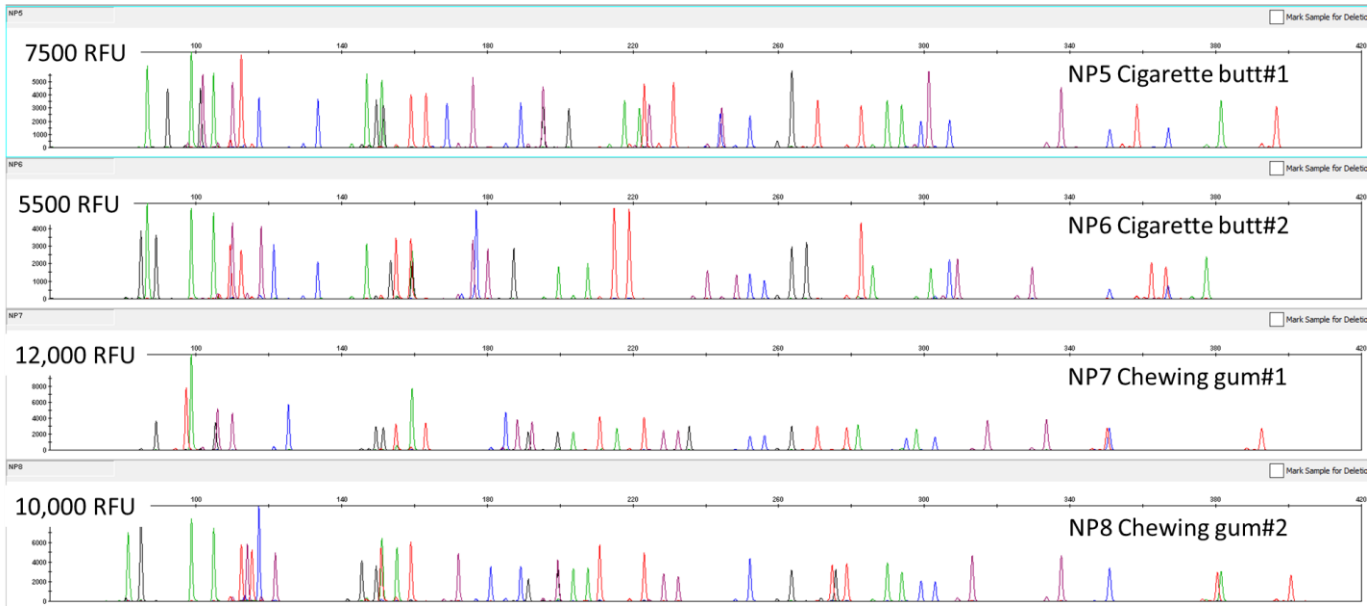


Figure 6: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP5-NP8 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

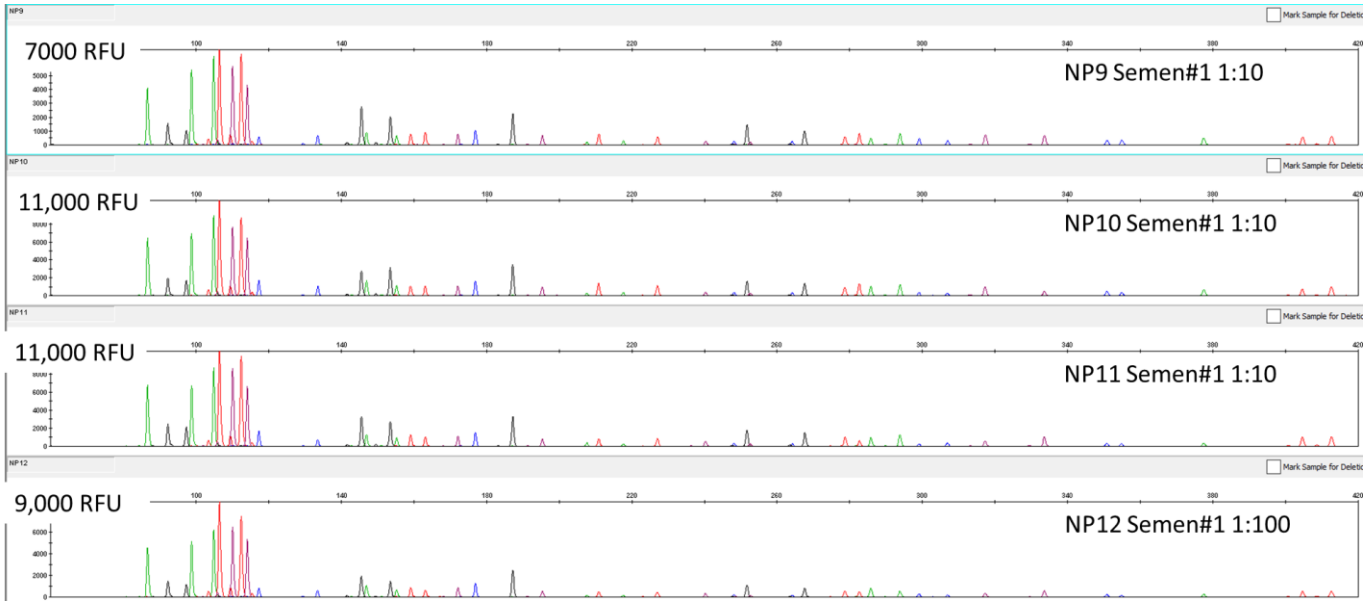


Figure 7: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP9-NP12 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

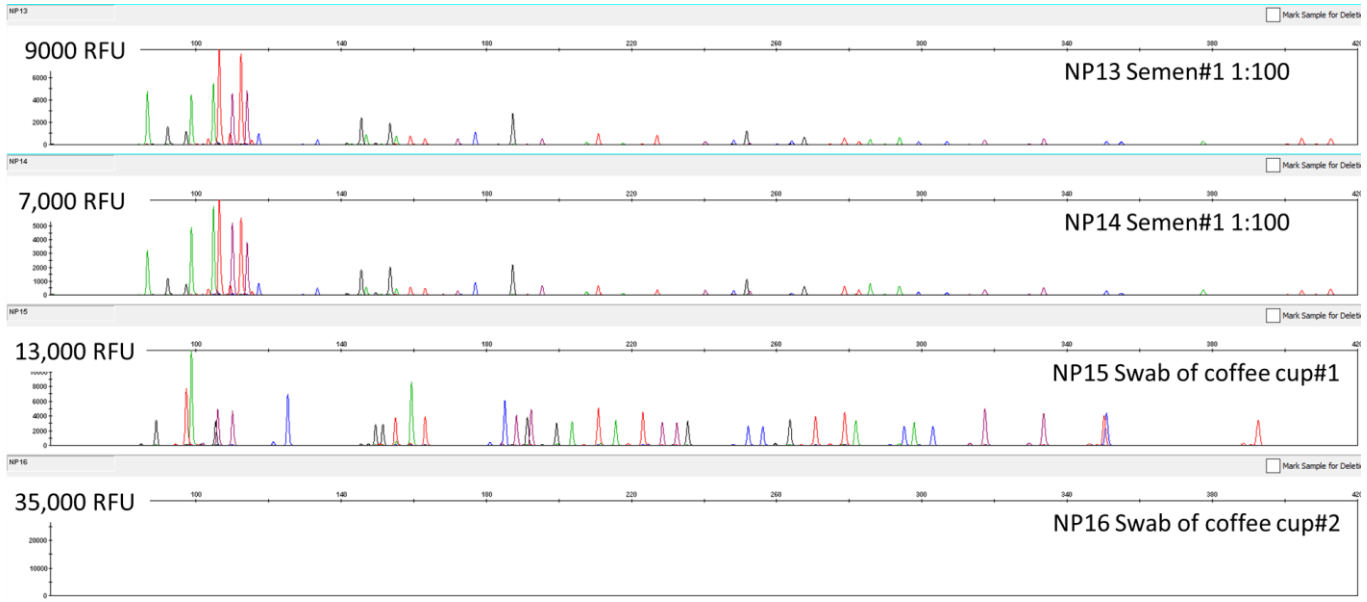


Figure 8: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP13-NP16 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

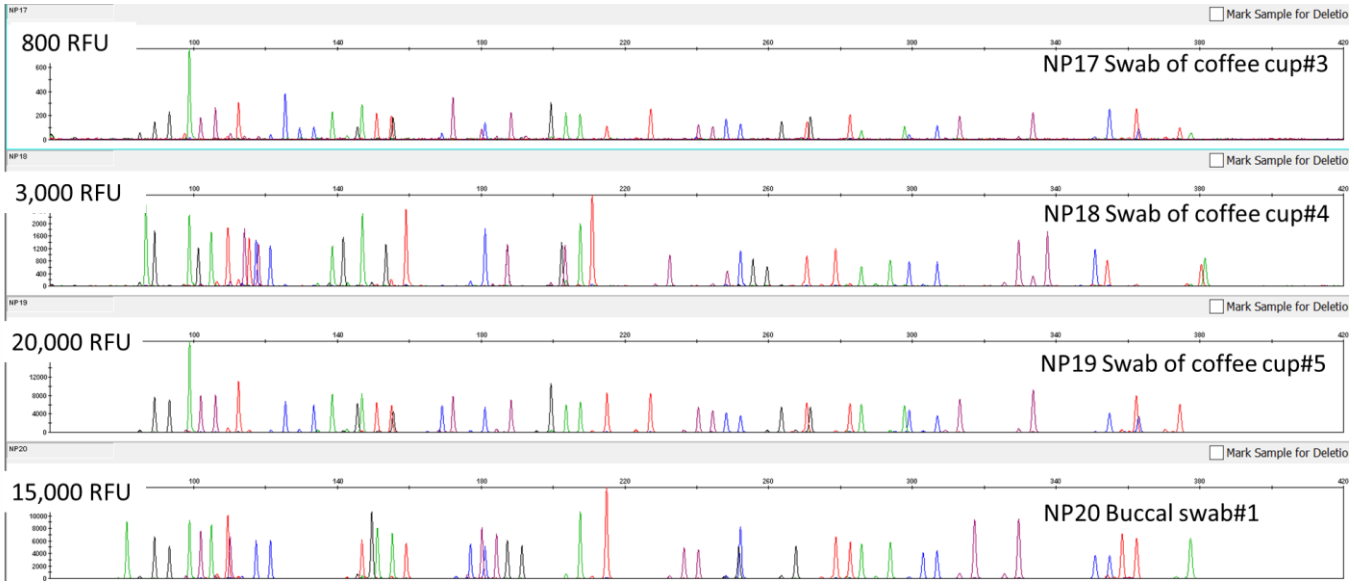


Figure 9: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP17-NP20 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

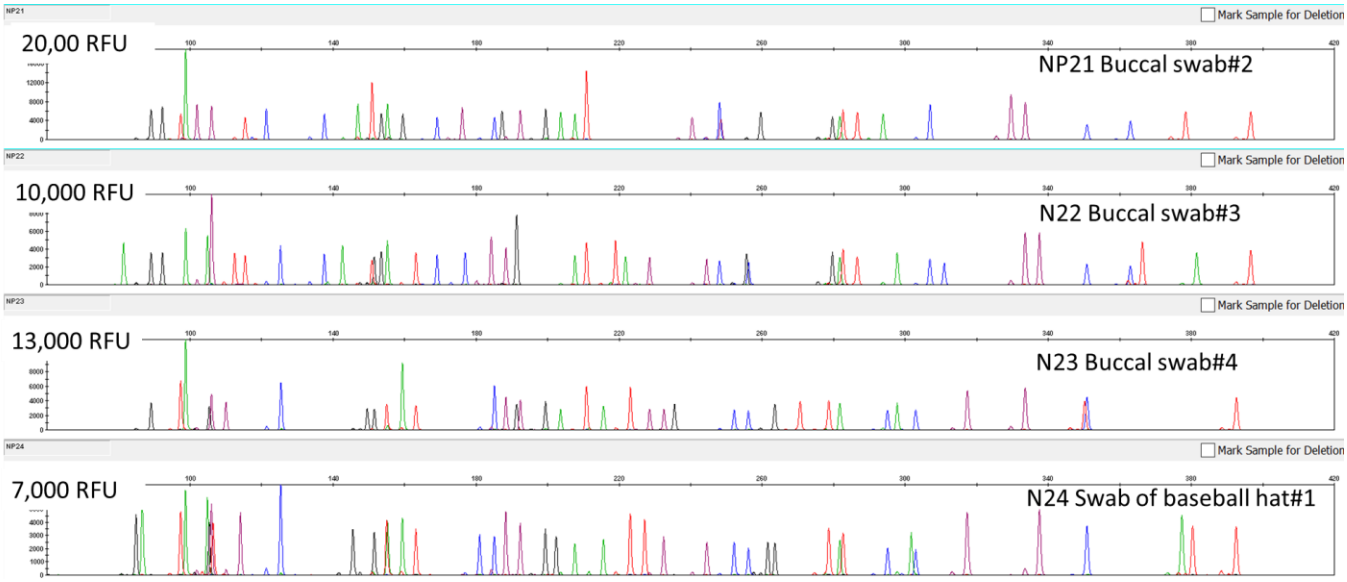


Figure 10: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP21-NP24 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

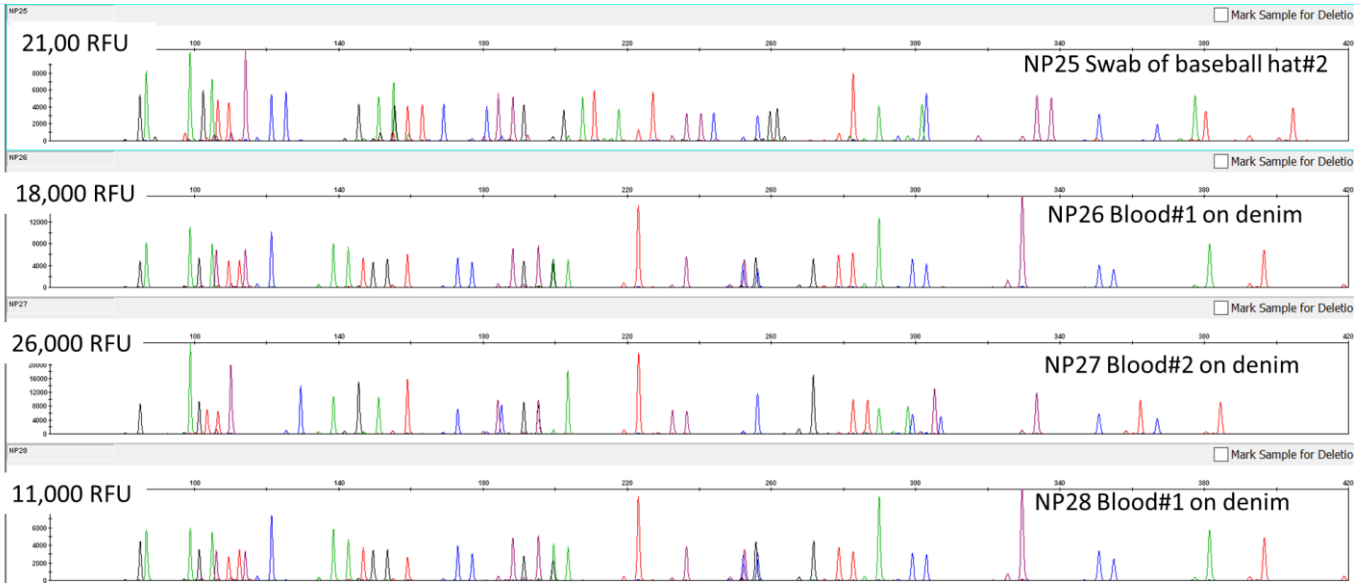


Figure 11: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP25-NP28 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

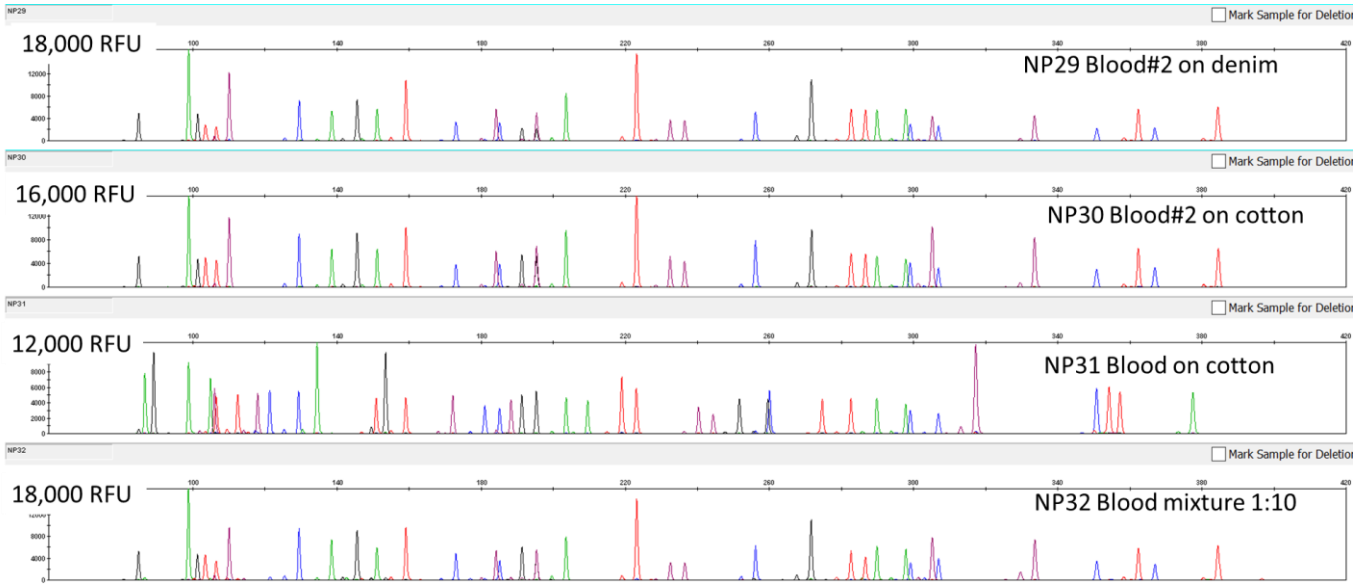


Figure 12: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP29-NP32 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

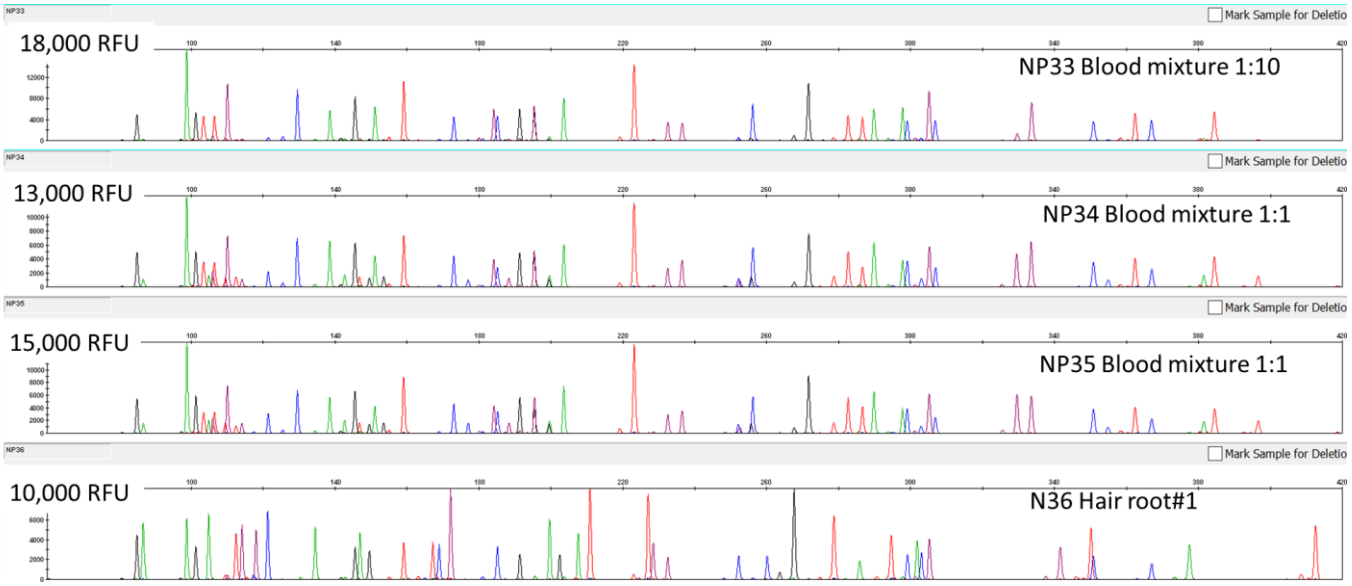


Figure 13: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP33-NP36 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

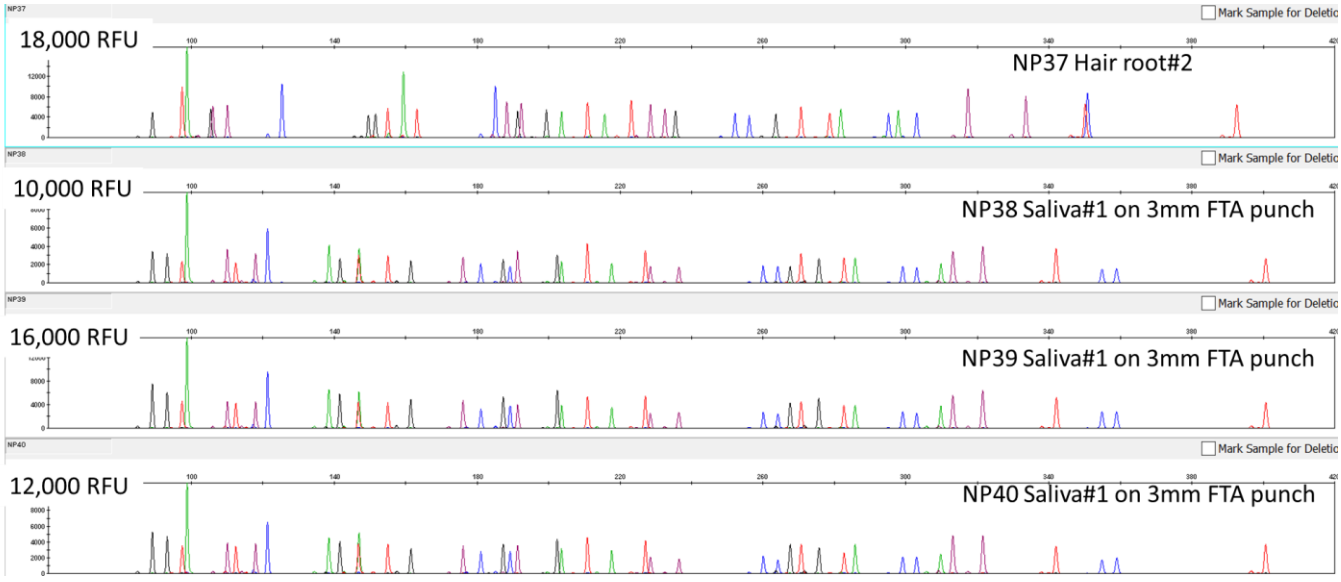


Figure 14: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP37-NP40 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

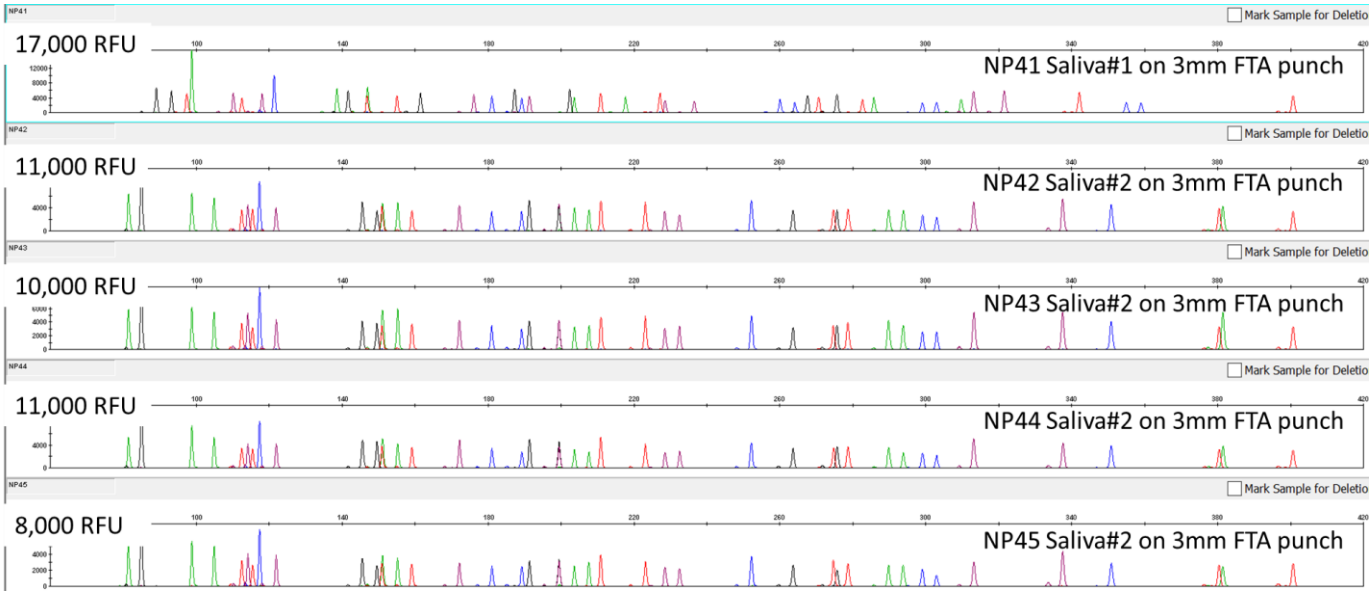


Figure 15: 3500xL electropherograms, showing an overlay of all dye channels, of case-type samples NP41-NP45 amplified with the GlobalFiler PCR Amplification Kit. The Y-axis is adjusted individually per sample as indicated.

Revision History

Revision	Date	Description
A	31-Oct-2019	Initial publication