Human identification

Boost forensic casework DNA analysis. Increase speed and reliability with the HID NIMBUS systems and Human Identification Professional Services

Keywords
Automated casework workflow, sample preparation, sample purification, quantification setup, normalization, amplification setup

In this application note, we demonstrate the following:

- Achieving consistent outcomes in DNA purification, quantification, normalization, and amplification
- Efficiently processing a wide range of sample types and quantities
- Streamlining the setup and integration of HID NIMBUS systems with the assistance of our Human Identification Professional Services

Optimizing the internal validation process

Thermo Fisher Scientific adheres to best practices and completes thorough developmental validations for all new forensic instruments and analytical methods. This process complies with the DNA Advisory Board (DAB) Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories and the guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM). Additionally, each laboratory must conduct internal validation studies to establish interpretation criteria and demonstrate the solution's suitability for human identification (HID) purposes.

The Human Identification Professional Services (HPS) team designs and performs internal validation studies to assess the functionality of Applied Biosystems™ workflows. The scope of these internal validation tests enables laboratories to establish methods while meeting validation standards and guidelines common to the forensics community. HPS services include a defined scope of testing kits and consumables and onsite validation execution by validation specialists.

During this study, HPS designed and tested various studies tailored to the Applied Biosystems™ HID NIMBUS® system workflows. The studies included in these services (Table 1) align with the internal validation requirements defined in global forensics standards such as the ISO/ICE 17025, QAS, and other applicable standards or guidelines. Using a consultative approach, the HPS team designs services to meet technical and budgetary requirements. These services enable customers to quickly operationalize their HID NIMBUS systems, saving valuable time and resources.

Table 1. Validation studies based on the tested workflow.

Study	Purification	Quantification	Amplification
Contamination study	•	•	•
Standard curve and control metrics study		•	
Sensitivity and stochastic study	•	•	•
Precision study: repeatability and reproducibility	•	•	•
Accuracy study	•	•	•
Known and non-probative sample study	•	•	•
Mixture study		•	•
Assessment of non-allelic peaks			•

This application note demonstrates how the HPS generates data that align with internal validation requirements for the HID NIMBUS systems, streamlining the internal validation process and expediting the time to operation.

Materials and methods

Internal testing that highlights selected studies offered as part of the HPS validation services was conducted using the Applied Biosystems™ HID NIMBUS® Presto QNA System.

The instruments, kits, and software used for the testing are listed in Table 2. The experimental design for this example testing consisted of four runs, summarized in Table 3.

Table 2. Applied Biosystems™ instrumentation, kits, and software used for testing.

Automation	
Robot	HID NIMBUS Presto QNA System
Software	HID NIMBUS® System Software v2.0 with package that includes the following: v3.2.2 extraction workflow; input labware tubes (runs 1–3) and deep-well plate (run 4); output labware tubes (runs 1–3) and plate (run 4); v1.2.2 quantification and amplification workflows
Extraction	
Kit	PrepFiler™ Automated Forensic DNA Extraction Kit
Quantification	
Kit	Quantifiler™ Trio DNA Quantification Kit
Instrument	QuantStudio™ 5 Real-Time PCR System for Human Identification
Software	HID Real-Time PCR Analysis Software v1.4
STR amplification	
Kit	GlobalFiler™ PCR Amplification Kit Yfiler™ Plus PCR Amplification Kit
Thermal cycler	ProFlex™ PCR System
Parameters	GlobalFiler kit—29 cycles; 1 ng target input Yfiler Plus kit—30 cycles; 1 ng target input
Capillary electrophoresis	
Instrument	SeqStudio™ 24 Flex Genetic Analyzer
Software	SeqStudio™ 24 Flex Series Data Collection Software v1.1
Injection conditions	1.2 kV, 24 seconds
Data analysis	
Analysis	GeneMapper™ *ID-X* Software v1.7
Threshold	50 relative fluorescence units (RFU)

Table 3. Sample types included in the validation study.

Run	Samples*	Supported studies	HID NIMBUS system workflows
1	• Donor 1 DNA (stock ~200 ng/µL) high sensitivity series: neat, 1:2, 1:4, 1:8, 1:16, 1:32 dilutions • Run in triplicate for quantification and amplification	• Sensitivity and stochastic • Precision	• Quantification • Amplification (includes normalization)
2	• Donor 1 DNA dilution series (2 ng/µL to 0.2 pg/µL) • Run in triplicate for quantification and amplification	• Sensitivity and stochastic • Precision	• Quantification • Amplification (includes normalization)
3	• Donor 1 DNA partial sensitivity series • Run in triplicate for quantification only	• Precision	• Quantification • Amplification (includes normalization)
4	• Blood swabs in the range of 35 µL to 1 µL, run in triplicate for extraction, quantification, and amplification • Extraction blanks run as single samples for extraction and quantification • Samples and controls were organized in a checkerboard pattern	• Sensitivity • Contamination	• Purification • Quantification • Amplification (includes normalization)

* Purified genomic DNA stocks were used to generate sensitivity samples.

Results

Contamination study

The non-template controls, negative controls, and reagent blanks were assessed for quantification results across the four runs. Detectable concentrations of DNA were reported during quantification in 6 of 41 controls (15%), with the highest detectable concentration being 1 pg/µL.

Non-template controls and negative amplification controls from runs 1, 2, and 3 were tested using the GlobalFiler PCR Amplification Kit and the Yfiler Plus PCR Amplification Kit. Of the 21 controls processed with the GlobalFiler kit, two allele calls were reported, with the highest peak height being 92 RFU (Figure 1). The two alleles observed in the negative controls were not consistent with sample profiles processed in the same run. Of the 27 controls processed with the Yfiler Plus kit, two off-ladder peaks and several artifacts outside marker regions were observed, and no alleles were reported (Figure 2).

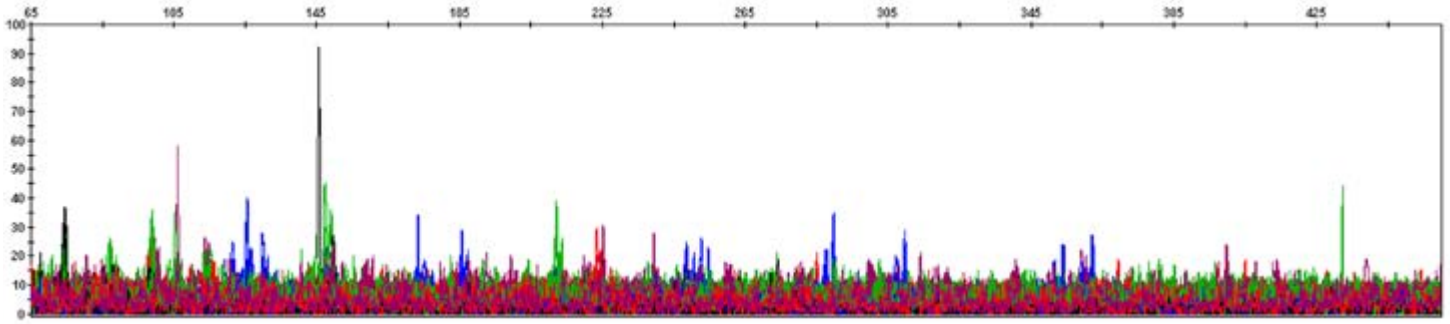

Figure 1. Overlay of all negative controls amplified with the GlobalFiler kit. Data were analyzed with a 50 RFU threshold.

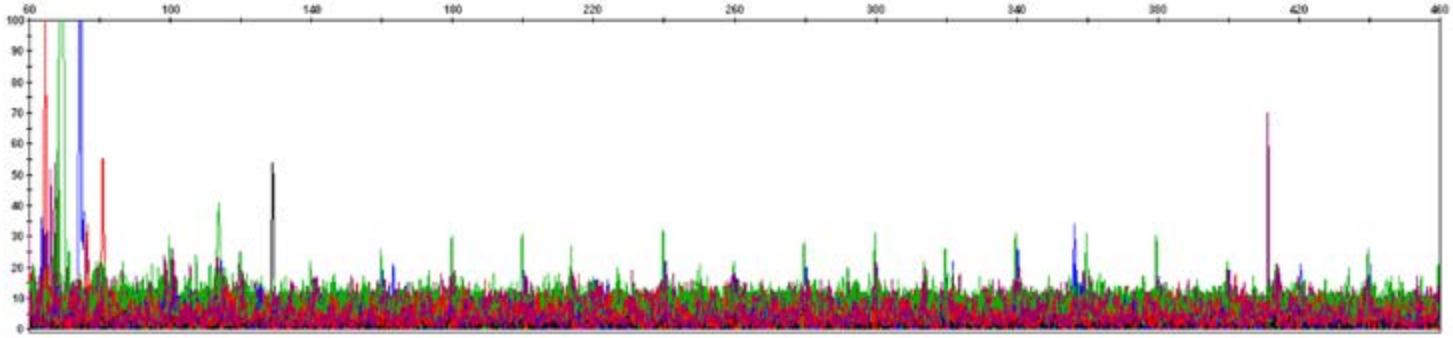

Figure 2. Overlay of all negative controls amplified with the Yfiler Plus kit. Data were analyzed with a 50 RFU threshold.

Sensitivity and stochastic study and precision study: repeatability and reproducibility

Three independent sensitivity series studies were conducted to assess the HID NIMBUS Presto QNA System workflow. Run 1 was performed to evaluate a high-concentration sensitivity series in the quantification, normalization, and amplification (QNA) portion of the workflow, with DNA concentrations ranging from approximately 200 ng/µL to 5 ng/µL. Runs 2 and 3 were performed to assess a sensitivity series with samples ranging from 2 ng/µL down to 0.2 pg/µL in the QNA portion of the workflow. Run 4 involved a whole-blood volumetric series to assess the purification workflow.

Replicate samples within each sensitivity series were evaluated for repeatability, while replicates processed across runs were assessed for reproducibility.

Run 1

The quantification data from the high sensitivity dilution series processed on the HID NIMBUS Presto QNA System showed that the relative standard deviation (RSD) for triplicates ranged from 1.4% to 41%. The 41% RSD was due to an isolated liquid-level detection error associated with low sample volume for the 1:2 dilution, which yielded lower-than-expected quantification results. When the outlier was removed the highest remaining RSD was 4.5%. There is close correlation among the replicates for each input in the series, as shown in Figure 3.

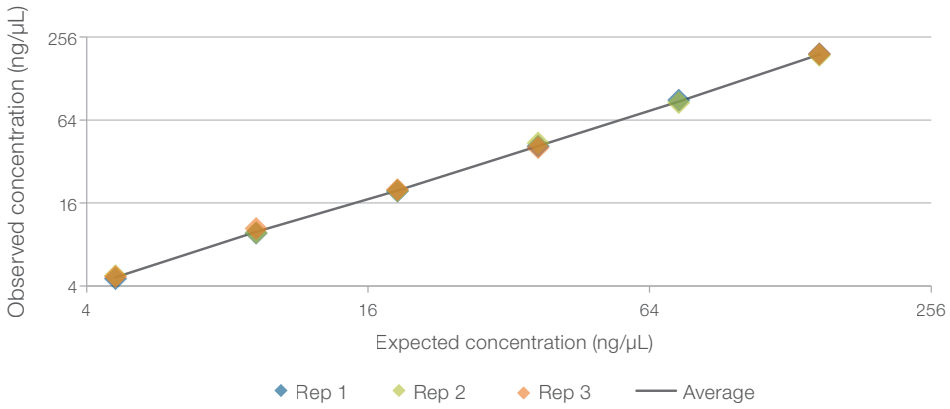

Figure 3. Observed sample replicate concentrations for the high sensitivity dilution series. The outlier was removed from the data set. The y-axis is on a $\log_2$ scale.

When the high sensitivity series was normalized to the 1 ng target and amplified with the GlobalFiler kit, the average heterozygous peak height across all the dilution inputs was 14,611 RFU (Figure 4). The RSD for replicates at each dilution ranged from 2% to 35%. Complete and concordant profiles were reported for the sensitivity samples across all inputs.

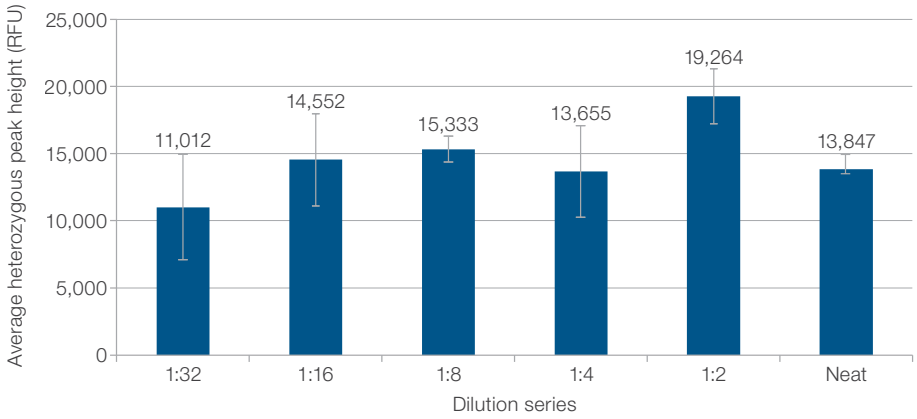

Figure 4. Average heterozygous peak heights of replicate samples when a range of DNA inputs (approximately 200 ng/µL to 5 ng/µL) was normalized to a target input of 1 ng and amplified with the GlobalFiler kit. Error bars are ±1 standard deviation.

Run 2

The quantification results for the sensitivity series samples processed on the HID NIMBUS Presto QNA System showed relative standard deviation ranging from 1.8% to 16.1% for concentrations down to 0.97 pg/µL. The lowest two points in the series, which were in the sub-picogram range, exhibited increased variability due to stochastic amplification effects (Figure 5).

The relative standard deviation for the average heterozygous peak height of sample replicates across the sensitivity series ranged from 4% to 22%, with the highest variation observed at the lowest DNA inputs (data not shown). The lowest five inputs in the sensitivity series (quantification values ranging from 3.1 pg/µL to 0.4 pg/µL) were amplified with maximum DNA volumes rather than the sensitivity series inputs. Complete concordant profiles were reported for all samples with DNA input equal to or greater than 63 pg. Refer to Figure 6 for the average peak heights obtained for these samples. Concordant partial profiles were reported for samples with inputs of 36 pg or less. Samples with sub-picogram concentrations and less than 10 pg of total amplified DNA exhibited allelic dropout. Allele recovery for the two lowest inputs was between 12 and 23 of the expected 42 alleles recovered. Allelic dropout is shown in Figure 7.

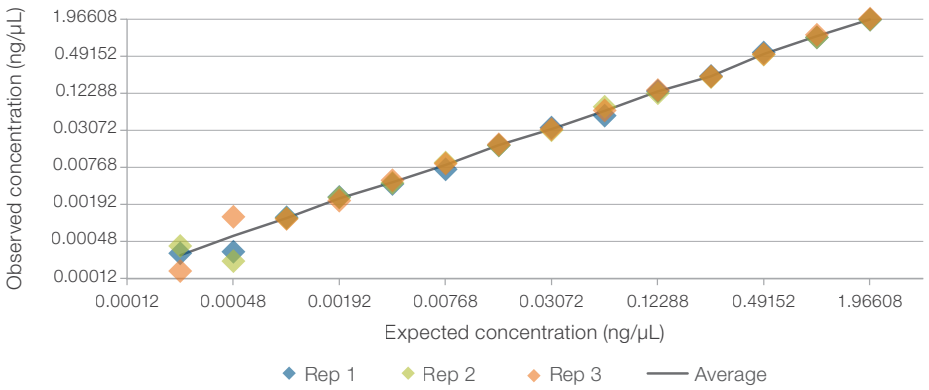

Figure 5. Observed sample replicate concentrations for the sensitivity dilution series. The y-axis is on a $\log_2$ scale.

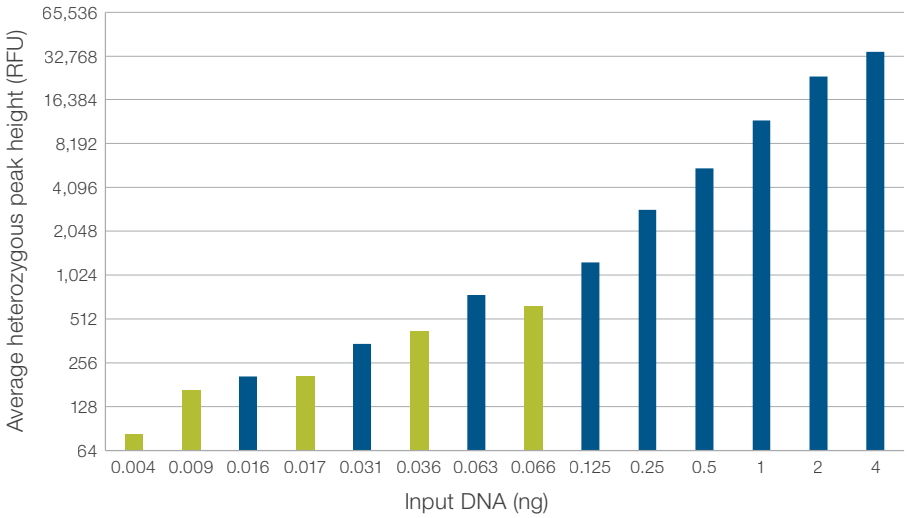

Figure 6. Blue bars represent sensitivity samples amplified with the GlobalFiler kit using approximately 2 µL of DNA extract, while green bars represent samples amplified using 15 µL of DNA extract. The y-axis is on a $\log_2$ scale.

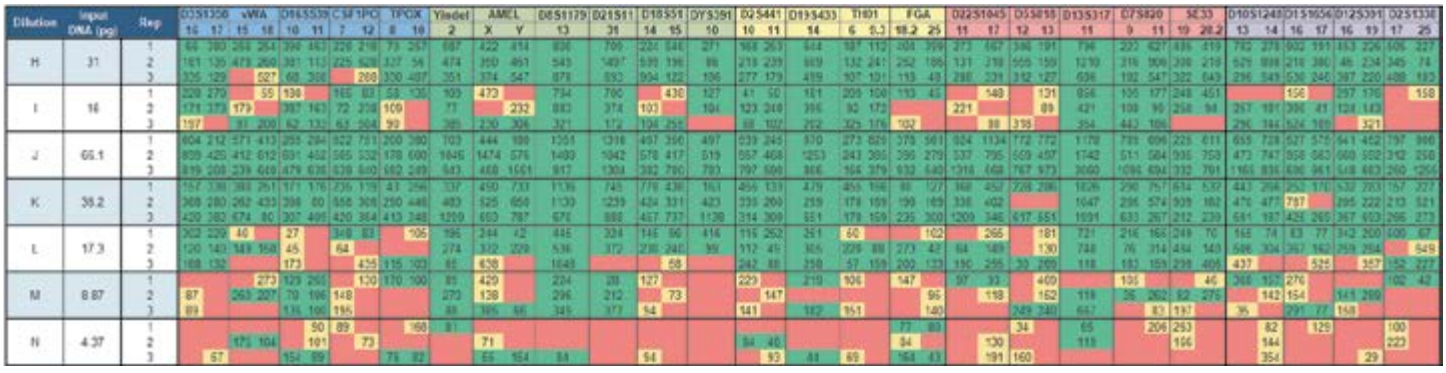

Figure 7. Peak heights for samples with allelic dropout observed. Inputs ranged from 31 to 4.37 pg of total DNA. Red indicates dropouts and yellow indicates surviving sister alleles.

Run 2 (continued)

The 4 ng to 16 pg inputs for the run 2 sensitivity series were also processed with the Yfiler Plus kit. Observed peak heights and variations were similar to the data from the GlobalFiler kit. The comparison of results is shown in Figure 8. Relative standard deviation of replicates at each input for the Yfiler Plus samples ranged from 1% to 24% (data not shown).

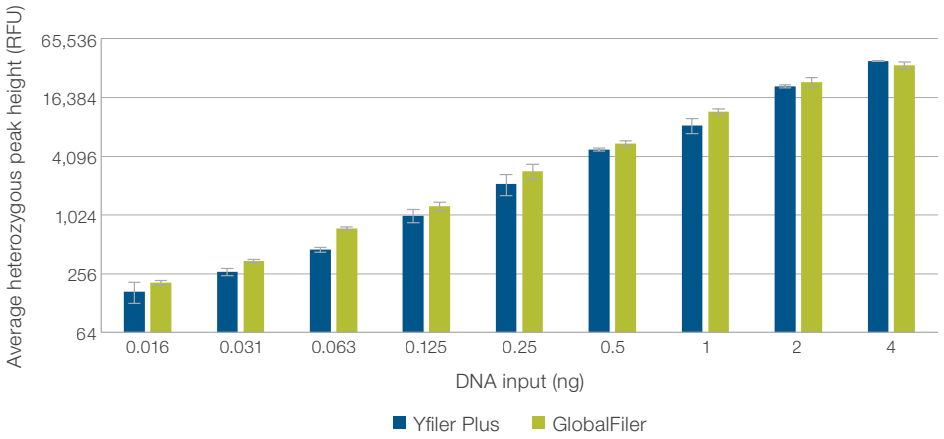

Figure 8. Average heterozygous peak heights for the sensitivity series samples amplified with the Yfiler Plus and GlobalFiler kits. Error bars represent ±1 standard deviation. The y-axis is on a $\log_2$ scale.

Run 3

A subset of the sensitivity series with DNA inputs ranging from 2 ng to 0.25 ng was amplified with the GlobalFiler kit and compared to the results from run 2. The average heterozygous peak height and RSD for replicates at each input were similar to those obtained in run 2, demonstrating the system's reproducibility (Figure 9).

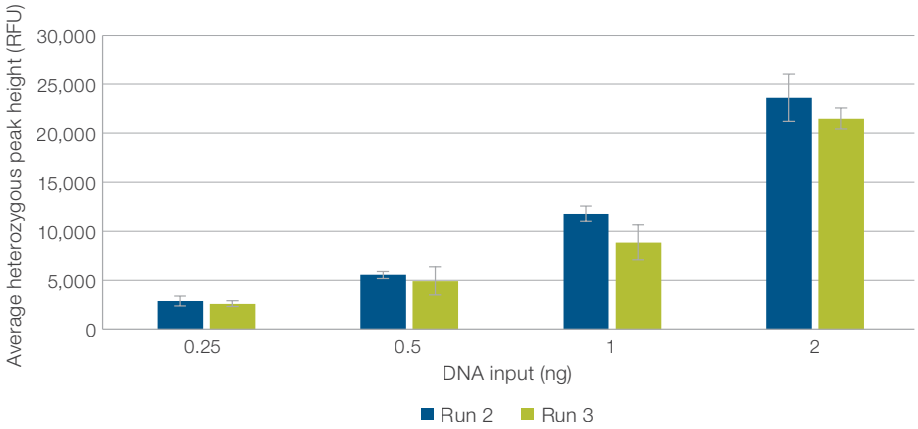

Figure 9. Reproducibility of sensitivity series samples processed with the GlobalFiler kit on runs 2 and 3.

Run 4

DNA was extracted from a volumetric blood sensitivity series using the PrepFiler Automated Forensic DNA Extraction Kit, with sample purification performed on the HID NIMBUS Presto QNA System. Samples were quantified using the Quantifiler Trio DNA Quantification Kit, and amplified with the GlobalFiler and Yfiler Plus kits.

When DNA was amplified with the GlobalFiler kit, complete concordant profiles were obtained for all samples across the blood volume inputs. Average heterozygous peak heights for samples normalized to a 1 ng target input were consistent with those observed for the run 1 high sensitivity samples normalized to 1 ng, as well as the 1 ng target input replicates processed on runs 2 and 3. Peak height ratios were greater than 50% for all samples (data not shown). Interlocus balance was consistent across the blood series (Figure 10).

When DNA from the same extracted blood samples was amplified with the Yfiler Plus kit, complete concordant profiles were obtained for all samples across the blood volume inputs. When normalized to a 1 ng target input, the average peak heights were consistent with those observed for the 1 ng target input replicates processed on runs 2 and 3. Interlocus balance was similar across the blood series (Figure 11).

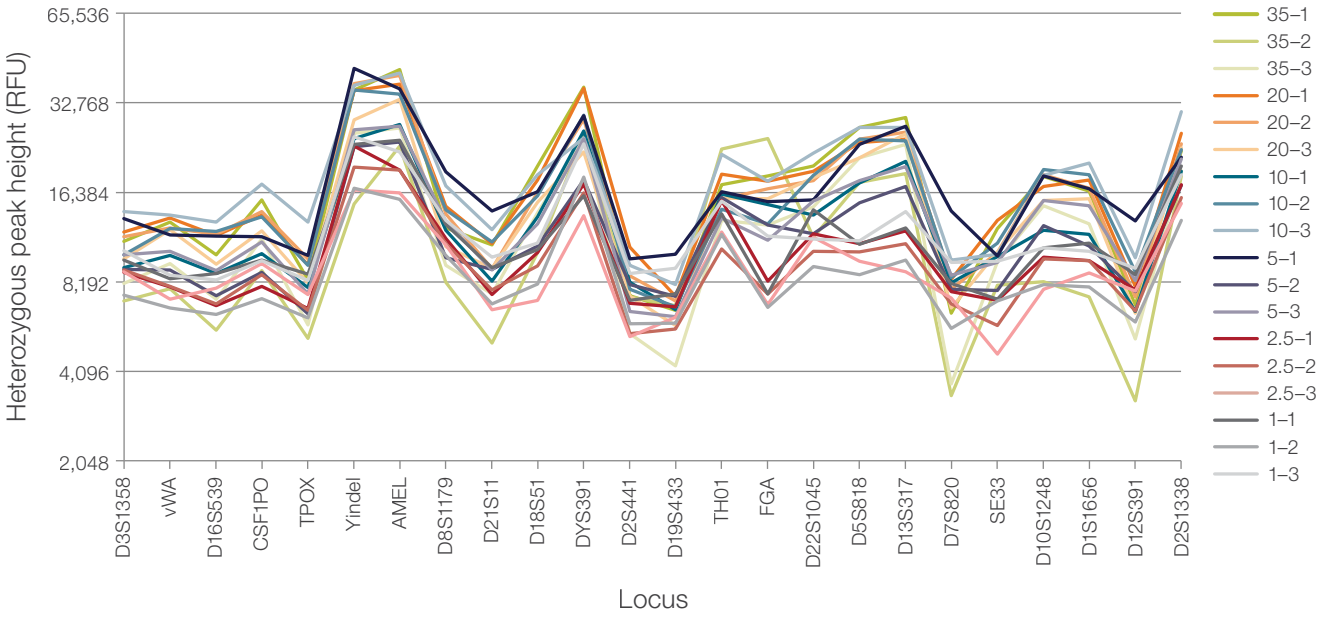

Figure 10. Heterozygous peak heights of blood dilution samples amplified with the GlobalFiler kit. The y-axis is on a $\log_2$ scale.

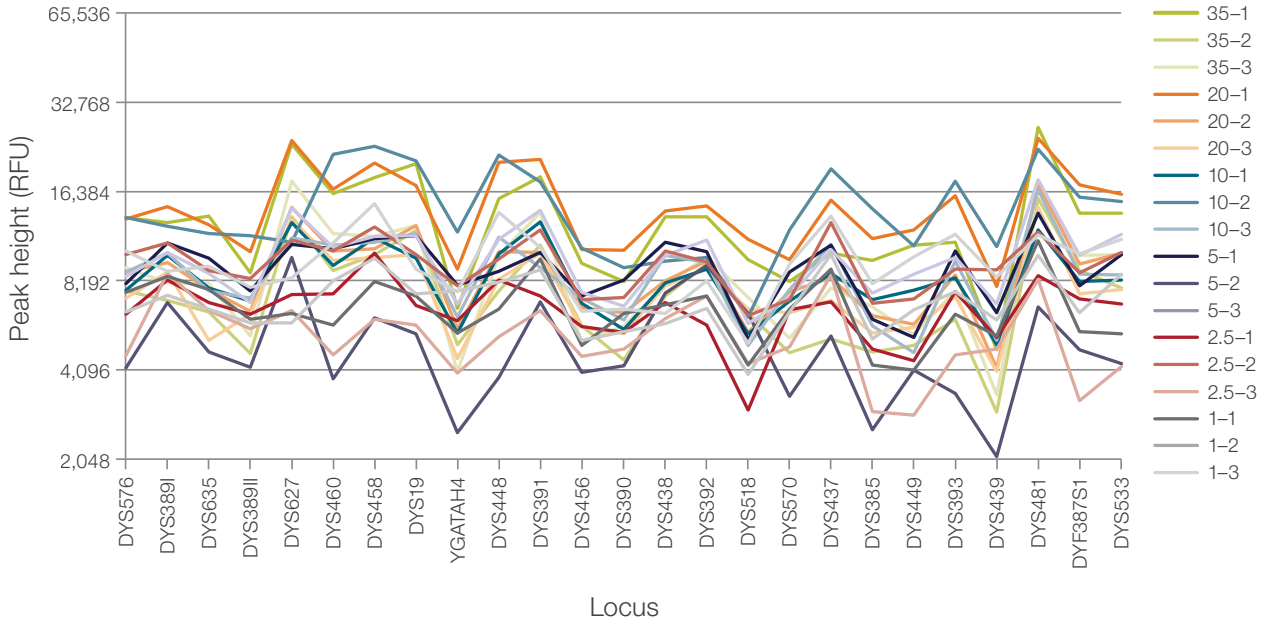

Figure 11. Peak heights of blood dilution samples amplified with the Yfiler Plus kit. The y-axis is on a $\log_2$ scale.

Conclusions

In this study, we assessed the purification, quantification setup, and amplification setup workflows on the HID NIMBUS Presto QNA System. Consistent results were observed within the expected parameters. The data showed no indications of systemic contamination in the negative control samples, and DNA from the extracted samples did not demonstrate signs of inhibition or degradation. Processing on the HID NIMBUS Presto QNA System generated repeatable quantification results from the samples. Further processing yielded consistent peak heights after normalization and amplification, resulting in concordant profiles.

The Human Identification Professional Services team at Thermo Fisher Scientific is committed to helping ensure a seamless and efficient onboarding process for new technologies. With global expertise from completing over 2,000 validation and integration services in over 60 countries, our team of validation specialists provides forensic laboratories with comprehensive validation and implementation support. This support is essential to successfully bringing new technologies into operation.

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