SeqStudio™ Genetic Analyzer for HID

New software features, verification studies, and validation studies

Publication Number MAN1001221 Revision A

New features in the SeqStudio™ Data Collection Software

SeqStudio™ Data Collection Software v1.2.5

- **Accept a failed HID Install Run**—If the expected number of alleles and size standard peaks were not found during the HID install run, you can analyze the install standard data files in your secondary analysis software (such as GeneMapper™ *ID-X* Software). If the alleles are properly called in the secondary analysis software, you can accept the install standard results.

  Note: Technical Support may recommend that you run this procedure as part of troubleshooting to verify precision and correct genotyping. If the procedure is recommended, Technical Support will provide instructions.

- **Improved auto-spectral performance**—To improve auto-spectral performance, the analysis parameters have been optimized, including the method used to apply the algorithm. A verification study was done to demonstrate that this update did not affect the amount of pull-up; see “Pull-up study” on page 10.
- **Delete spectral calibration history**—Within the Service tools, the ability to delete all spectral calibration histories has been added.

SeqStudio™ Data Collection Software v1.2.4

- **Export Status screen**—If sample data files (AB1 and FSA) are not exported to the expected save location (Cloud, Network Drive, and/or USB), you can open the **Export Status** screen to view failed exports at the plate- or sample-level. You can also re-export the files from the **Export Status** screen.
- **Security, Auditing, and E-signature (SAE) v2.1 module**—You can select the **Use HTTPS** checkbox to enable a secure version of the HTTP protocol.
- **Wifi Dongle**—The USB Wifi Dongle is supported. A wireless adapter (also referred to as a dongle) is provided with the SeqStudio™ Genetic Analyzer to support a wireless network connection.

SeqStudio™ Data Collection Software v1.2.1

- **Spectral Calibration Run Time**—The run time for manual spectral calibrations has been increased from 330 seconds to 460 seconds. This change allows sufficient time to capture all fragments for Applied Biosystems™ dye sets (including DS-33, DS-36, and DS-37 matrix standards) and other dye sets that may require more time for larger fragments.
- **Pre-run check**—If the Current Check test fails, the system will purge and refresh the polymer up to two additional times.
- **Non-HID related support**—See the *Applied Biosystems™ SeqStudio™ Genetic Analyzer v1.2.1 Release Notes* (Pub. No. 100063138).

SeqStudio™ Data Collection Software v1.2

- **HID install check**—When the instrument is installed, the field service representative will perform an HID install check, including a manual J6 dye calibration. It is not necessary to repeat this procedure after installation; we recommend that each HID laboratory determine the appropriate Quality Control measures to verify correct instrument operation.
- **Resolution algorithm for HID**—During data collection, the algorithm aligns resolution power traditionally generated with a 3500xL Genetic Analyzer/POP-4™ Polymer configuration to the resolution power generated with a SeqStudio™ Genetic Analyzer/POP–1™ Polymer configuration. Using the resolving power of POP–1™ Polymer, the algorithm maintains similar resolution for smaller fragments across the two platforms, while allowing for improved resolution for larger fragments. For optimal alignment, ensure that you select the correct Applied Biosystems™ HID kit when you create or modify a plate setup in the software.

- **Plate setup for HID**—When you create or modify a plate setup in the software, you can select an HID-specific run module, dye set, size standard, and kit.

Category	Option
Run module	HIDAnalysis
Dye set	– G5 (DS-33) – J6 (DS-36) – J6-T (DS-37)
Size standard	– GS500(-250)LIZ[1] – GS600_LIZ_(60-460) – GS600_LIZ_(80-400)
Kit	– GlobalFiler™ [2] – GlobalFiler™ Express – Huaxia™ Platinum – Identifiler™ Plus – MiniFiler™ – NGM Detect™ – NGM SElect™ – Verifiler™ Express – Verifiler™ Plus – Yfiler™ – Yfiler™ Plus **Note:** Selecting **None** disables the Marker-to-Marker pull-up feature ("Kit" is displayed when **None** is selected.)

[1] The GeneScan™ 500 LIZ™ Size Standard was not included in the HID validation of the instrument, but it is a default size standard option in the software.

[2] Select **GlobalFiler**™ for the GlobalFiler™ PCR Amplification Kit and the GlobalFiler™ IQC PCR Amplification Kit.

- **Marker-to-Marker pull-up reduction feature for HID**—This feature is optimized for the markers in each Applied Biosystems™ HID kit. Ensure that you choose the correct Applied Biosystems™ HID kit when you create or modify a plate setup in the software.
- **Security, Auditing, and E-signature (SAE) v2.0 module (not HID-specific)**—Provides the following SAE functionality on the instrument:
  - **System security**—Controls user sign in and access to functions.
  - **Auditing**—Tracks changes and actions performed by users.
  - **E-signature** —Allows users to provide an electronic signature (user name and password) when performing certain functions.

  For more information, see the *SAE Administrator Console v2 User Guide for PCR systems* (Pub. No. MAN0017468).

Performance verification of SeqStudio™ Data Collection Software v1.2.5

Objective of the verification

The objective of this verification is to test the performance of the SeqStudio™ Genetic Analyzer with SeqStudio™ Data Collection Software v1.2.5 for HID.

Performance verification testing includes: Genotyping Concordance, Minimum Detection Threshold, Mixture Analysis, Average Peak Height, and Pull-up.

Equipment and materials used for the verification

Instruments and software

Item	No. of instruments
PCR amplification	
GeneAmp™ PCR System 9700	1
ProFlex™ 96-well PCR System	2
Capillary electrophoresis (CE)	
SeqStudio™ Genetic Analyzer for HID, with: • SeqStudio™ Data Collection Software v1.2.5 • HIDAnalysis run module (see Table 7 on page 13)	2
(For use as a control) SeqStudio™ Genetic Analyzer for HID, with: • SeqStudio™ Data Collection Software v1.2.4 • HIDAnalysis run module (see Table 7 on page 13)	1
Secondary analysis	
GeneMapper™ *ID-X* Software v1.7.2, with SeqStudio_v7x panel, bin, and stutter files You can download the analysis files from **www.thermofisher.com/GMIDXsoftware**.	N/A

Note: CE instrument preparation and analysis settings from the verification were followed. See "Instrument preparation" on page 14 and "Analysis settings" on page 14. For information on using the CE instrument and software, see the *SeqStudio™ Genetic Analyzer Instrument and Software User Guide* (Pub. No. MAN0018646).

PCR amplification kits

Applied Biosystems™ PCR amplification kit	Cat. No.
AmpFℓSTR™ NGM SElect™ PCR Amplification Kit	4472193
GlobalFiler™ IQC PCR Amplification Kit	A43565
Verifiler™ Plus PCR Amplification Kit	A35495

Verification methods and results summary

Samples and methods

Unless otherwise stated, the samples were amplified and run according to the standard protocols, including the DNA input recommended in the applicable kit user guide. Each study was repeated with each kit listed in "PCR amplification kits" on page 5.

Study	Samples[1,2]	Method and analysis	Replicates[3]
Minimum Detection Threshold	No-template control (NTC) samples	Run NTC samples for each kit in 4 wells. Analyze the data with a peak amplitude threshold of 1 RFU. Calculate the baseline noise, limit of detection, and limit of quantification. Determine an appropriate peak amplitude threshold (PAT) per kit and per instrument to be used for the verification studies. Evaluate the NTC samples for contamination.	4 wells × 3 injections on 3 CE instruments
Genotyping Concordance	DNA Control 007 samples at the kit-recommended input amount (0.5 ng or 1 ng)	Amplify the kit positive control and gDNA samples. Compare the genotypes calls across the SeqStudio™ instruments.	4 wells × 3 injections on 3 CE instruments
	Single-source DNA samples, extracted from the blood of 23 donors, at the kit-recommended input amount (0.5 ng or 1 ng)		1 well × 1 injection (per sample) on 3 CE instruments
Mixture Analysis	1:2 or 2:1 mixtures (0.5 ng or 1 ng): • IGT2052:IGT2077 mixtures • DNA Control 007:IB-0996 mixtures	Run each mixture pair at the stated ratios. Evaluate the minor genotypes calls.	3 or 4 wells × 1 injection on 3 CE instruments
	1:3 mixtures (0.5 ng or 1 ng): NIST Comp D mixtures	Run each mixture pair at the stated ratios. Evaluate the minor genotypes calls.	3 wells × 1 injection on 3 CE instruments

(continued)

Study	Samples[1,2]	Method and analysis	Replicates[3]
Mixture Analysis	1:7 and 7:1 mixtures (0.5 ng or 1 ng): DNA Control 007:IB-0996	Run each mixture pair at the stated ratios. Evaluate the minor genotypes calls.	1:7 mixture: 3 wells × 1 injection on 3 CE instruments
			7:1 mixture: 4 wells × 1 injection on 3 CE instruments
Average Peak Height	DNA Control 007 at the kit-recommended input amount (0.5 ng or 1 ng)	Run the samples and export sizing tables. Exclude IQC and any OL/artifact peaks, then calculate the average peak height of each sample.	4 wells × 3 injections on 3 CE instruments
Pull-up	The 23 single-source DNA samples from the Genotyping Concordance study at the kit-recommended input amount (0.5 ng or 1 ng)	Run the samples on the SeqStudio™ instruments. Analyze the data with GeneMapper™ *ID-X* Software v1.7, with pull-up detection disabled.	1 well × 1 injection (per sample) on 3 CE instruments
	DNA Control 007 at the kit-recommended input amount (0.5 ng or 1 ng)		4 wells × 3 injections on 3 CE instruments

[1] GeneScan™ 600 LIZ™ Size Standard v2.0 was used for sizing.

[2] The samples were run with the appropriate allelic ladder (required for genotyping).

[3] The replicates were run on three CE instruments: one instrument with SeqStudio™ Data Collection Software v1.2.4 (for use as a control) and two instruments with SeqStudio™ Data Collection Software v1.2.5. For more information, see "Instruments and software" on page 4.

Verification results summary

Passing results were obtained for all test cases.

Study	Expected result	Result
Minimum Detection Threshold	No allelic data should be observed when using the minimum peak amplitude thresholds (PAT) in the NTC samples.	Pass
Genotyping Concordance	SeqStudio™ Software allele calls should be concordant with genotype results across SeqStudio™ Data Collection Software v1.2.4 and v1.2.5. The DNA Control 007 samples and the 23 single-source samples should generate the correct, full-profile genotyping results. All data should be on-scale.	Pass
Mixture Analysis	1:2 or 2:1 mixtures—A full profile for a minor contributor in a 1:2 mixture sample with 0.5 ng or 1 ng total DNA (0.33 ng:0.67 ng) should be observed when the minor contributor allele calls are a single allele or repeat away from the major contributor alleles.	Pass
	1:3 mixtures (NIST Comp D)—Minor contributor allele calls that are a single allele or repeat away from the major contributor alleles should be resolved comparably to SeqStudio™ Data Collection Software v1.2.4.	Pass

(continued)

Study	Expected result	Result
Mixture Analysis	1:7 and 7:1 mixtures—Full profiles for the minor contributor should be observed at 1:7 and 7:1 mixture ratios when all minor contributor alleles are ≥2 bp from the major alleles.	Pass
Average Peak Height	There should be no significant difference in the average peak heights from SeqStudio™ Data Collection Software v1.2.4.	Pass
Pull-up	≤5% maximum pull-up should be observed for ≥95% of runs. ≤3% mean pull-up should be observed across all detected pull-up peaks in the analyzed data.	Pass

Detailed verification results

Minimum Detection Threshold study

For each kit, an average minimum threshold was calculated for each of the 3 CE instruments per dye and used as the PAT settings in the GeneMapper™ *ID-X* Software. The limit of quantification (LOQ) was rounded to the nearest multiple of 5. See Table 1.

Table 1 Minimum thresholds calculated and used for the PAT settings for each kit in the GeneMapper™ *ID-X* Software

Dye channel	Instrument SSv1.2.5-1			Instrument SSv1.2.5-2			Instrument SSv1.2.4		
	NGM SElect™ kit	Verifiler™ Plus kit	GlobalFiler™ IQC kit	NGM SElect™ kit	Verifiler™ Plus kit	GlobalFiler™ IQC kit	NGM SElect™ kit	Verifiler™ Plus kit	GlobalFiler™ IQC kit
Blue	25	45	35	35	65	40	35	50	50
Green	25	45	45	35	60	55	30	50	75
Yellow	45	35	25	50	35	20	60	35	35
Red	105	40	35	135	50	40	145	60	50
Purple	NA	60	45	NA	60	55	NA	70	70
Orange	35	40	45	40	40	50	45	40	80

Genotyping Concordance study

All runs passed with no quality flags. See Table 2.

Table 2 Genotyping Concordance study results

Kit	% Concordance		
	SSv1.2.5-1	SSv1.2.5-2	SSv1.2.4
GlobalFiler™ IQC	100%	100%	100%
NGM SElect™	100%	100%	100%
Verifiler™ Plus	100%	100%	100%

Mixture Analysis study—1:2 and 2:1 mixtures

In all mixture runs, full profiles were observed when the minor contributor allele calls were a single allele or repeat away from the major contributor alleles. See Table 3.

Table 3 Mixture Analysis study results for 1:2 and 2:1 mixtures

Kit	% Concordance		
	SSv1.2.5-1	SSv1.2.5-2	SSv1.2.4
GlobalFiler™ IQC	100%	100%	100%
NGM SElect™	100%	100%	100%
Verifiler™ Plus	100%	100%	100%

Mixture Analysis study—1:3 mixtures

In all mixture runs, full profiles were obtained for the minor contributor. Minor contributor alleles that were a single allele or repeat away from the major contributor alleles were resolved comparably to SeqStudio™ Data Collection Software v1.2.4. See Table 4 and Figure 1.

Table 4 Mixture Analysis study results for 1:3 mixtures

Kit	% Concordance		
	SSv1.2.5-1	SSv1.2.5-2	SSv1.2.4
GlobalFiler™ IQC	100%	100%	100%
NGM SElect™	100%	100%	100%
Verifiler™ Plus	100%	100%	100%

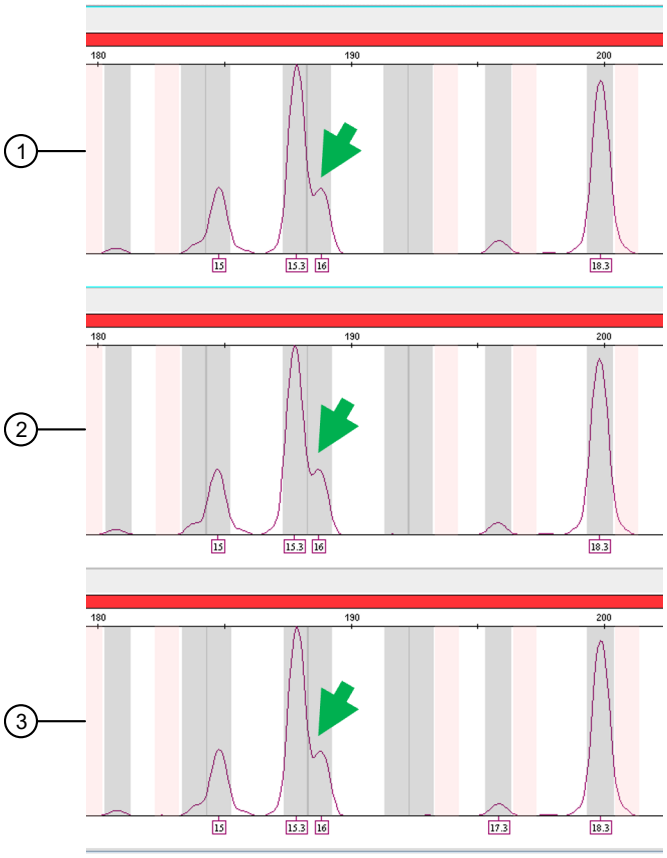

Figure 1 Mixture Analysis study for 1:3 mixtures 1:3 mixtures (NIST Comp D) amplified with the GlobalFiler™ IQC kit and run on 2 SeqStudio™ instruments with SeqStudio™ Data Collection Software v1.2.5 and 1 SeqStudio™ instrument with SeqStudio™ Data Collection Software v1.2.4. Both the v1.2.5 and v1.2.4 software resolved the minor allele at D1S1656.

① SSv1.2.5-1
② SSv1.2.5-2
③ SSv1.2.4

Mixture Analysis study—1:7 and 7:1 mixtures

In all mixture runs, full profiles were obtained for the minor contributors. See Table 5.

Table 5 Mixture Analysis study results for 1:7 and 7:1 mixtures

Kit	% Concordance		
	SSv1.2.5-1	SSv1.2.5-2	SSv1.2.4
GlobalFiler™ IQC	100%	100%	100%
NGM SElect™	100%	100%	100%
Verifiler™ Plus	100%	100%	100%

Average Peak Height

Average peak height results were similar between SeqStudio™ Data Collection Software v1.2.5 and v1.2.4. See Figure 2.

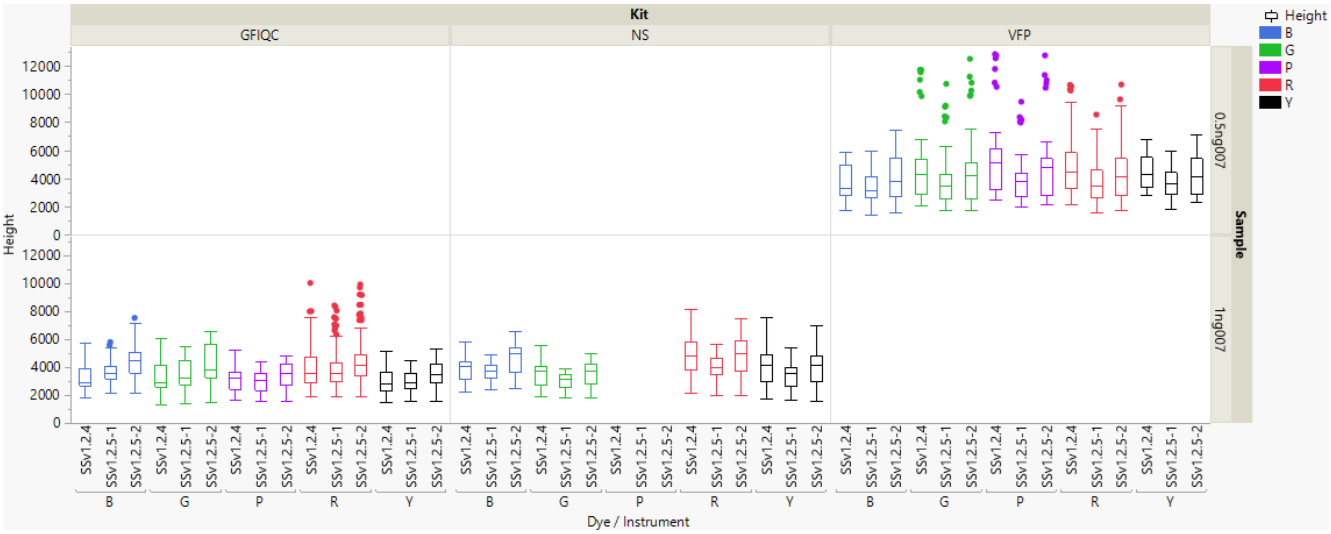

Figure 2 Average Peak Height study—Peak height vs. dye and instrument DNA Control 007 samples amplified with the 3 kits and run on 2 SeqStudio™ instruments with SeqStudio™ Data Collection Software v1.2.5 and 1 SeqStudio™ instrument with SeqStudio™ Data Collection Software v1.2.4. Average peak height results were similar between the v1.2.5 and v1.2.4 software.

Pull-up study

Pull-up results were similar between SeqStudio™ Data Collection Software v1.2.5 and v1.2.4. See Table 6.

Table 6 Pull-up study results

Parameter	Kit					
	GlobalFiler™ IQC		NGM SElect™		Verifiler™	
Instrument	SSv1.2.5[1]	SSv1.2.4	SSv1.2.5[1]	SSv1.2.4	SSv1.2.5[1]	SSv1.2.4
Total no. of peaks	51	82	85	84	119	175
Mean	0.86%	1.13%	2.24%	2.22%	1.38%	1.30%
Percent <5%	100.00%	100.00%	97.02%	97.62%	100.00%	100.00%
Mean peak height of samples	5,016	4,765	4,729	4,644	5,298	5,787

[1] The values provided are the average values for all runs on the 2 CE instruments with SeqStudio™ Data Collection Software v1.2.5: SSv1.2.5-1 and SSv1.2.5-2.

Conclusion

The performance verification testing of SeqStudio™ Data Collection Software v1.2.5 (on the SeqStudio™ Genetic Analyzer for HID) showcased exceptional performance across all tested parameters. The Genotyping Concordance, Minimum Detection Threshold, Mixture Analysis, Average Peak Height, and Pull-up studies all had similar results to SeqStudio™ Data Collection Software v1.2.4.

Performance verification of the SeqStudio™ Data Collection Software v1.2.4 with the GlobalFiler™ IQC kit

Objective of the verification

The objective of this verification is to test the performance of the GlobalFiler™ IQC PCR Amplification Kit (Cat. No. A43565) on the SeqStudio™ Genetic Analyzer with SeqStudio™ Data Collection Software v1.2.4 for HID. Performance testing includes Sizing, Minimum Detection Threshold and Contamination, Genotyping Concordance, and Sensitivity and Dynamic Range.

The GlobalFiler™ IQC kit is a 6-dye, short tandem repeat (STR) multiplex assay for the amplification of human genomic DNA. In addition to amplifying the same set of markers in the GlobalFiler™ PCR Amplification Kit (Cat. No. 4476135), the GlobalFiler™ IQC kit amplifies two Internal Quality Control (IQC) markers. The primers used for the overlapping markers between the GlobalFiler™ kit and the GlobalFiler™ IQC kit are the same.

Equipment and materials used for the verification

Instruments and software

- One SeqStudio™ Genetic Analyzer with SeqStudio™ Data Collection Software v1.2.4 was used for this verification.

  Note: For information on using the instrument and software, see the *SeqStudio™ Genetic Analyzer Instrument and Software User Guide* (Pub. No. MAN0018646).

- The HIDAnalysis run module was used throughout the verification. See Table 7 on page 13.
- STR amplification was performed on a GeneAmp™ PCR System 9700 thermal cycler.
- Instrument preparation and analysis settings from the validation were followed for this verification. See “Instrument preparation” on page 14 and “Analysis settings” on page 14.
- SeqStudio_GFIQC_v2x panel, bin, and stutter files were used for all analysis in GeneMapper™ *ID-X* Software v1.6. You can download the analysis files from **www.thermofisher.com/GMIDXsoftware**.

Samples and methods

All samples were amplified and run according to the standard protocol, including the kit recommended DNA input, in the *GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits User Guide* (Pub. No. MAN0029969). Similar methods and analysis to those described in the validation were used in this verification; see "Validation methods and results summary" on page 17.

Study	Samples	Replicates
Sizing	Allelic ladder	8 wells × 3 injections on 1 instrument
Minimum Detection Threshold and Contamination	No-template controls (NTC)	4 wells × 1 injection on 1 instrument
Genotyping Concordance	DNA Control 007 (1 ng)	4 wells × 1 injection on 1 instrument
Sensitivity and Dynamic Range	DNA Control 007 (125 pg, 4 ng)	4 wells × 1 injection on 1 instrument

Verification results

Passing results were obtained for all test cases, based on the same expected outcomes for applicable studies in the validation; see "Results summary" on page 19.

- The standard deviation in sizing within an injection and across multiple injections was <0.15 bp across one SeqStudio™ Genetic Analyzer. The mean size difference of each allele bin in the ladder was <0.5 bp.
- Minimum threshold values were calculated using the NTC data on one instrument. An average minimum threshold was calculated per dye and used as the peak amplitude threshold (PAT) settings in the GeneMapper™ *ID-X* Software:
  - Blue—25 RFU
  - Green—30 RFU
  - Yellow—20 RFU
  - Red—40 RFU
  - Purple—35 RFU
  - Orange—25 RFU
- DNA Control 007 samples run at 125 pg, 1 ng, 2 ng, and 4 ng inputs on one instrument generated the correct, full-profile genotyping results. All 2 ng and 4 ng data was on-scale.

HID instrument validation of the SeqStudio™ Genetic Analyzer

Objective of the validation

The objective of the validation is to confirm the performance of the SeqStudio™ Genetic Analyzer system with Applied Biosystems™ PCR amplification kits for forensic DNA profiling. System performance includes general instrument operation, consumables, and SeqStudio™ Data Collection Software v1.2.

The validation was performed according to guidelines from the *Scientific Working Group for DNA Analysis Methods* (SWGDAM, December, 2016). Each laboratory should perform internal validation studies to establish appropriate guidelines, protocols, and procedures before implementing a new technology.

Equipment and materials used for the verification

Instruments and software

- Eight SeqStudio™ Genetic Analyzers with SeqStudio™ Data Collection Software v1.2 were used in the validation experiments. Not all instruments were used for every study; however, each instrument was used in at least one study.

  Note: For information on using the instrument and software, see the *SeqStudio™ Genetic Analyzer Instrument and Software User Guide* (Pub. No. MAN0018646).

- The HIDAnalysis run module was used throughout the validation. See Table 7.
- The SeqStudio™ Genetic Analyzer Cartridge v2 was used throughout the validation.
- A 3500xL Genetic Analyzer with 3500 Series Data Collection Software v4 was used for comparative studies, as indicated in "Validation methods and results summary" on page 17.
- All STR amplifications were performed on GeneAmp™ PCR System 9700 thermal cyclers.
- All DNA quantification was performed on a 7500 Real-Time PCR System for Human Identification using the Quantifiler™ Trio DNA Quantification Kit.

Table 7 HIDAnalysis run module.

Description	Default setting
Capillary Temperature	60°C
Pre Run Voltage	13 kV
Pre Run Time	180 s
Injection Voltage[1]	1.2 kV
Injection Time[1]	10 s
Run Voltage[2]	11 kV

Table 7 HIDAnalysis run module. *(continued)*

Description	Default setting
Run Ramp Duration	300 s
Run Time	1,120 s

[1] A single injection voltage and time was utilized throughout the validation for all kits tested. It is up to the laboratory to determine if alternate injection parameters would be better suited based on the complete workflow in use.

[2] The resolution algorithm was developed using an 11kV run voltage. Run voltage has an inverse relationship with peak resolution. Using a run voltage different than 11kV could impact the effectiveness of the resolution algorithm leading to an increase in n±1 peaks.

Instrument preparation

Before validation studies were run, the following steps were performed with passing results on the eight SeqStudio™ Genetic Analyzers.

- A manual spectral calibration with DS-33, DS-36, and DS-37 Matrix Standards using the default dye sets in the SeqStudio™ Software
- An HID installation check with the GlobalFiler™ PCR Amplification Kit Allelic Ladder and GeneScan™ 600 LIZ™ Size Standard v2.0

Analysis settings

GeneMapper™ *ID-X* Software v1.6 with SeqStudio_v6x panel, bin, and stutter files were used for all analyses with the following analysis settings.

Note: The analysis files are not preloaded in the GeneMapper™ *ID-X* Software. You can download the analysis files from **www.thermofisher.com/GMIDXsoftware** for import into GeneMapper™ *ID-X* Software v1.6.

Parameter	Kit settings		
	G5 dye set (5-dye)	J6 dye set (6-dye)	J6-T dye set (6-dye)
Size Standard	GS600 LIZ (80–400)	GS600 LIZ (60–460)	GS600 LIZ (60–460)
Smoothing	Light	Light	Light
Size Calling Method	MiniFiler: 3rd order Others: Local Southern	Local Southern	Local Southern
Baseline Window	51	33	33
Peak Amplitude Threshold	Kit-specific thresholds are provided in Table 8 on page 27.		
Minimum Peak Half Width	2	2	2
Polynomial Degree	3	3	3
Peak Window Size	15	13	11
Normalization	Unchecked (not applicable to the SeqStudio™ Genetic Analyzer)		

PCR amplification kits

Validation study

Applied Biosystems™ PCR amplification kit	Cat. No.
AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit	4427368
AmpFℓSTR™ MiniFiler™ PCR Amplification Kit	4373872
AmpFℓSTR™ NGM SElect™ PCR Amplification Kit	4472193
AmpFℓSTR™ Yfiler™ PCR Amplification Kit	4359513
GlobalFiler™ Express PCR Amplification Kit	4476609
GlobalFiler™ PCR Amplification Kit	4476135
NGM Detect™ PCR Amplification Kit	A31832
Verifiler™ Express PCR Amplification Kit/Huaxia™ Platinum™ PCR Amplification Kit	A32014/A31323
Verifiler™ Plus PCR Amplification Kit	A35495
Yfiler™ Plus PCR Amplification Kit	4484678

Not all kits were used in every study. Representative kits were used in each study to demonstrate the ability of the SeqStudio™ Genetic Analyzer to achieve accurate results within the parameters of each test. Kit selection was based on dye chemistry, workflow applications, and other similarities among the PCR amplification kits.

Performance verification

Applied Biosystems™ PCR amplification kit	Cat. No.
GlobalFiler™ IQC PCR Amplification Kit	A43565

For more information, see “Performance verification of the SeqStudio™ Data Collection Software v1.2.4 with the GlobalFiler™ IQC kit” on page 11.

Samples

All samples were amplified and run according to the standard protocols, including the kit recommended DNA input, in the applicable user guide. Any deviations from the default conditions are noted.

Study	Samples[1,2]	PCR amplification kit
Sizing	Allelic ladder	Identifiler™ Plus, MiniFiler™, Yfiler™, NGM SElect™, GlobalFiler™, Yfiler™ Plus, Verifiler™ Express/Huaxia™ Platinum, NGM Detect™, Verifiler™ Plus kits
Minimum Detection Threshold and Contamination	No-template controls (NTC)	Identifiler™ Plus, MiniFiler™, Yfiler™, NGM SElect™, GlobalFiler™, GlobalFiler™ Express, Yfiler™ Plus, Verifiler™ Express/Huaxia™ Platinum, NGM Detect™, Verifiler™ Plus kits
Genotyping Concordance	Kit positive control (0.5 ng, 1 ng, 4 ng, 6 ng) 23 gDNA samples (0.5 ng, 1 ng) 10 buccal swabs 10 blood FTA 1.2-mm punches	
Resolution	Data from the Sensitivity and Dynamic Range study and data from a Consumable Stability study that was performed independently from the validation presented here.	Identifiler™ Plus, MiniFiler™, Yfiler™, NGM SElect™, GlobalFiler™, Yfiler™ Plus, NGM Detect™, Verifiler™ Plus kits
Sensitivity and Dynamic Range	Data from the Genotyping Concordance study for positive controls and 5 gDNA samples amplified at the kit recommended input (0.5 ng or 1 ng) Kit positive control and 5 gDNA samples: • 15.5 pg, 125 pg, 0.5 ng, and 1 ng for MiniFiler™, Verifiler™ Plus, and NGM Detect™ kits • 31 pg, 125 pg, 1 ng, and 2 ng for the remaining kits listed	
Mixture Analysis	2 pairs of DNA mixtures at 1:0, 7:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:7, 0:1 (0.5 ng, 1 ng) DNA Control 007/9947A 1:7, 7:1 (0.5 ng, 1 ng)	GlobalFiler™, NGM Detect™ kits
Signal Variability	DNA Control 007 (0.5 ng, 1 ng)	Yfiler™, GlobalFiler™, NGM Detect™ kits

(continued)

Study	Samples[1,2]	PCR amplification kit
Crosstalk	DNA Control 007 (1 ng, 2 ng) Hi-Di™ Formamide	Yfiler™, Yfiler™ Plus, NGM Detect™ kits
Carryover	DNA Control 007 (1 ng, 2 ng) NTC	

[1] GeneScan™ 600 LIZ™ Size Standard v2.0 was used for sizing.

[2] The samples were run with the appropriate allelic ladder (required for genotyping).

Validation methods and results summary

Methods

Study	Method and analysis	Replicates
Sizing	Run allelic ladder (1 µL/well) for each kit in 8 wells. Calculate the sizing precision (standard deviation) of each allele per injection (4 capillaries) and per 6 injections. For all injections, calculate the mean size difference of each allele bin in the allelic ladder. For the Identifiler™ Plus, GlobalFiler™, and NGM Detect™ kit injections, calculate the allelic ladder passing rate.	8 wells × 3 injections per kit on 3 instruments
Minimum Detection Threshold and Contamination	Run NTCs for each kit in 4 wells. Analyze the data with a peak amplitude threshold of 1 RFU. Calculate the baseline noise, limit of detection, and limit of quantification. Determine an appropriate peak amplitude threshold per kit to be used for this validation.[1] Evaluate the NTCs for contamination.	4 wells × 3 injections per kit on 4 instruments

(continued)

Study	Method and analysis	Replicates
Genotyping Concordance	Amplify the kit positive control and gDNA samples at the kit recommended input (0.5 ng or 1 ng). GlobalFiler™ Express kit—Amplify 4 ng of the kit positive control and direct samples. Verifiler™ Express kit—Amplify 6 ng of the kit positive control and direct samples. Run kit positive controls and samples on the SeqStudio™ Genetic Analyzers and a 3500xL Genetic Analyzer. Compare the genotypes calls across the SeqStudio™ instruments and to the 3500xL instrument. Calculate the inter- and intra-color balances for the kit positive control and a single replicate of each sample for each kit on each instrument. Evaluate the direct amplification samples for full, on-scale results.	3 or 4 wells of each control or sample per kit on 4 instruments Inject the same plate setup for each kit on a 3500xL instrument
Resolution	Evaluate single base pair resolution up to 470 bp using a Thermo Fisher Scientific software development tool.	NA
Sensitivity and Dynamic Range	Amplify 15.5 pg or 31 pg, 125 pg, and 1 ng or 2 ng (twice the kit recommended input) of the kit positive control and 5 gDNA samples. Run the alternate input kit positive controls and samples on the SeqStudio™ instruments. Evaluate peak height, peak height ratios, dropout, and artifacts across the sensitivity series, including the Genotyping Concordance study data for the kit recommended input data.	1 or 2 wells of each control or sample at the alternate input amounts per kit on 4 instruments
Mixture Analysis	Run each mixture pair at all ratios on the SeqStudio™ instruments and a 3500xL instrument. Evaluate the minor genotypes calls.	3 or 4 wells of each pair/ratio per kit on 4 instruments Inject the same plate setup for each kit on a 3500xL instrument

(continued)

Study	Method and analysis	Replicates
Signal Variability	Run 0.5 ng or 1 ng (the kit recommended input) of the kit positive controls in 7 wells. Calculate the maximum-to-minimum average peak height for each variability test.	For capillary-to-capillary and injection-to-injection variability: 7 wells × 3 injections per kit on 3 instruments For instrument-to-instrument variability: 7 wells × 3 injections per kit on 3 instruments with the same cartridge For cartridge-to-cartridge variability: 7 wells × 3 injections per kit on 1 instrument with 4 cartridges
Crosstalk	Set up a checkerboard pattern of the kit positive control at twice the kit recommended input and Hi-Di™ Formamide. After each injection containing positive control in 2 of the 4 wells, inject two compete Hi-Di™ Formamide blank injections. Evaluate the Hi-Di™ Formamide blanks in the injections containing positive control for evidence of crosstalk.	2 capillaries per injection group with the kit positive control injected in capillaries 1 and 3 × 2 injections on 4 instruments. 2 capillaries per injection group with the kit positive control injected in capillaries 2 and 4 × 2 injections on 4 instruments.
Carryover	Set up a zebra pattern of kit positive control at twice the kit recommended input and NTC. Evaluate the NTC blanks for evidence of carryover.	4 wells × 2 injections of the kit positive control followed by 4 wells × 2 injections of NTC on 4 instruments.

[1] An average peak amplitude threshold across four SeqStudio™ instruments was used for analysis. Each HID laboratory should determine if an instrument-specific threshold is more appropriate.

Results summary

Study	Test case	Expected outcome	Result[1]
Sizing	Precision	Sizing precision is ≤0.15 bp within an injection or between injections.	Pass
	Accuracy	Size range is less than ±0.5 bp per injection.	Pass
	Ladder Passing Rate	Allelic ladders should pass more than 95% of the time with spike detection enabled in GeneMapper™ *ID-X* Software v1.6.	Pass
Minimum Detection Threshold and Contamination	Thresholds	No allelic data should be observed using minimum peak amplitude thresholds in the NTCs.	Pass
Genotyping Concordance	Concordance	SeqStudio™ Software allele calls are concordant with genotype results on the 3500xL Genetic Analyzer/POP-4™ Polymer configuration.	Pass

(continued)

Study	Test case	Expected outcome	Result[1]
Genotyping Concordance	Color Balance	SeqStudio™ Software inter- and intra-color balance should be consistent with validations of the kits on the 3500xL Genetic Analyzer/POP-4™ Polymer configuration.	Pass
	Direct Amplification Kit Evaluation	Profiles generated from the direct amplification kits should be consistent with validations of the kits on the 3500xL Genetic Analyzer/POP-4™ Polymer configuration.	Pass
Resolution	Resolution	The SeqStudio™ Genetic Analyzer for HID shall reliably detect and resolve alleles that differ in length by a single base pair from 60–470 bp.	Pass
Sensitivity and Dynamic Range	Lower End Limits	Full, on-scale profiles are observed at 125 pg of DNA input.	Pass with comment[2]
		Partial profiles are observed at the lowest amplified input (15.5 pg or 31 pg).	Pass
	Upper End Limits	On-scale heterozygote peak heights are observed when 2 × the kit recommended DNA input is used for all kits tested.	Pass
	Recommended Input	Full, on-scale profiles are observed with average heterozygote peak height ratios (PHR) consistent with validations of the kits on the 3500xL Genetic Analyzer/POP-4™ Polymer configuration.	Pass
	Pull-up	Mean pull-up is <3%.	Pass
	n±1 Artifacts	Resolution of n±1 artifacts observed on the SeqStudio™ Genetic Analyzer are comparable with those resolved on the 3500xL Genetic Analyzer/POP-4™ Polymer configuration.	Pass with exception[3]
Mixture Analysis	Control Mixture	Full profiles for the minor contributor should be observed at 1:7 and 7:1 mixture ratios when all minor contributor alleles are ≥2 bp from the major alleles.	Pass
	gDNA Mixtures	Resolution of minor contributor alleles that are 1 bp from the major alleles should be resolved comparably to the 3500xL Genetic Analyzer/POP-4™ Polymer configuration.	Pass

(continued)

Study	Test case	Expected outcome	Result[1]
Signal Variability	• Capillary-to-capillary • Injection-to-injection • Instrument-to-instrument • Cartridge-to-cartridge	Signal maximum-to-minimum ratios should be ≤1.9.	Pass
Crosstalk and Carryover	Crosstalk	Crosstalk observed should be ≤0.2%.	Pass
	Carryover	Carryover observed should be ≤0.1%.	Pass with exception[4]

[1] For more information on the results and for calculations of minimum threshold values, see "Detailed validation results" on page 22.

[2] Instrument-specific minimum thresholds were calculated for each dye in each kit. A four-instrument average minimum threshold was used for the validation, except where noted. Drop-out of allelic data in Identifiler™ Plus, NGM SElect™, and Yfiler™ Plus samples at 125 pg input was observed using an average PAT; an instrument-specific PAT would have resulted in full profiles for all occurrences. Each HID laboratory should determine the appropriate method for setting analytical thresholds for each instrument or across instruments.

[3] NGM Detect™ and Verifiler™ Plus samples demonstrated more n+1 artifact peaks off of stutter alleles when compared to the same data set on the 3500xL Genetic Analyzer/POP-4™ Polymer configuration.

[4] A single instance of potential carryover, with carryover peaks ~1% of the parent peaks, was observed in a no-template control (NTC) after an injection of a 2 ng DNA Control 007 sample using the Yfiler™ kit. This result was not reproducible.

Detailed validation results

Sizing study: Precision and Accuracy

The standard deviation in sizing within an injection and across multiple injections was <0.15 bp across three SeqStudio™ Genetic Analyzers tested for each kit. The mean size difference of each allele bin in the ladder was <0.5 bp. Figure 3, Figure 4, and Figure 5 show the data distribution for sizing precision of all alleles from wells containing Identifiler™ Plus, GlobalFiler™, and NGM Detect™ allelic ladders. Comparable results were observed for MiniFiler™, NGM SElect™, Verifiler™/Huaxia™ Platinum™, Verifiler™ Plus, Yfiler™, and Yfiler™ Plus allelic ladders.

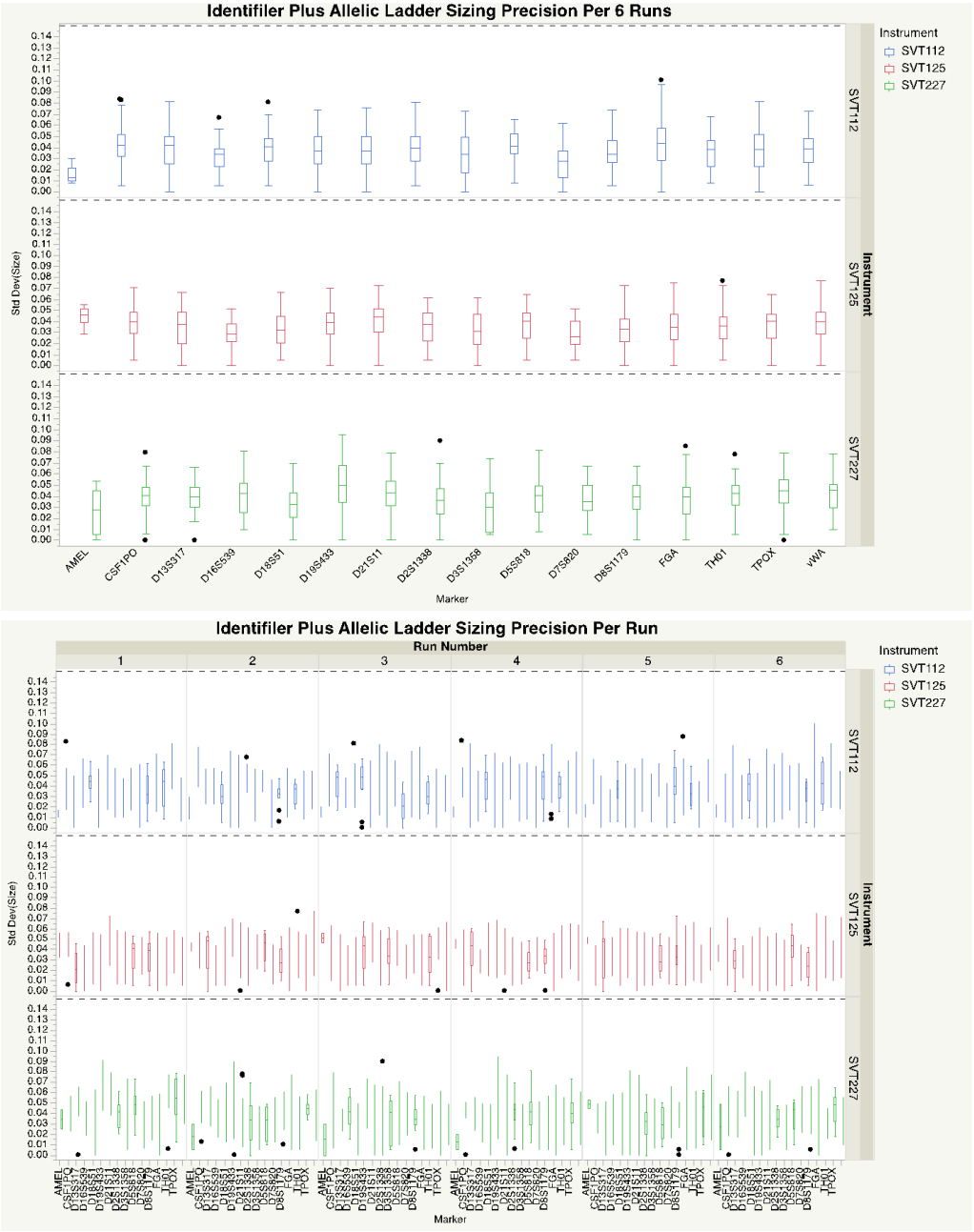

Figure 3 Sizing precision of the Identifiler™ Plus allelic ladder. The allelic ladder is in eight wells, each injected three times for a total of 24 data points across six total injections. The top image shows precision per all six injections and the bottom image shows precision per injection for each marker. The three instruments are represented by blue, red, and green color panes. Dashed lines in each pane are at 0.15 bp.

The data is displayed using Tukey outlier box plots to show distribution of results and outliers.

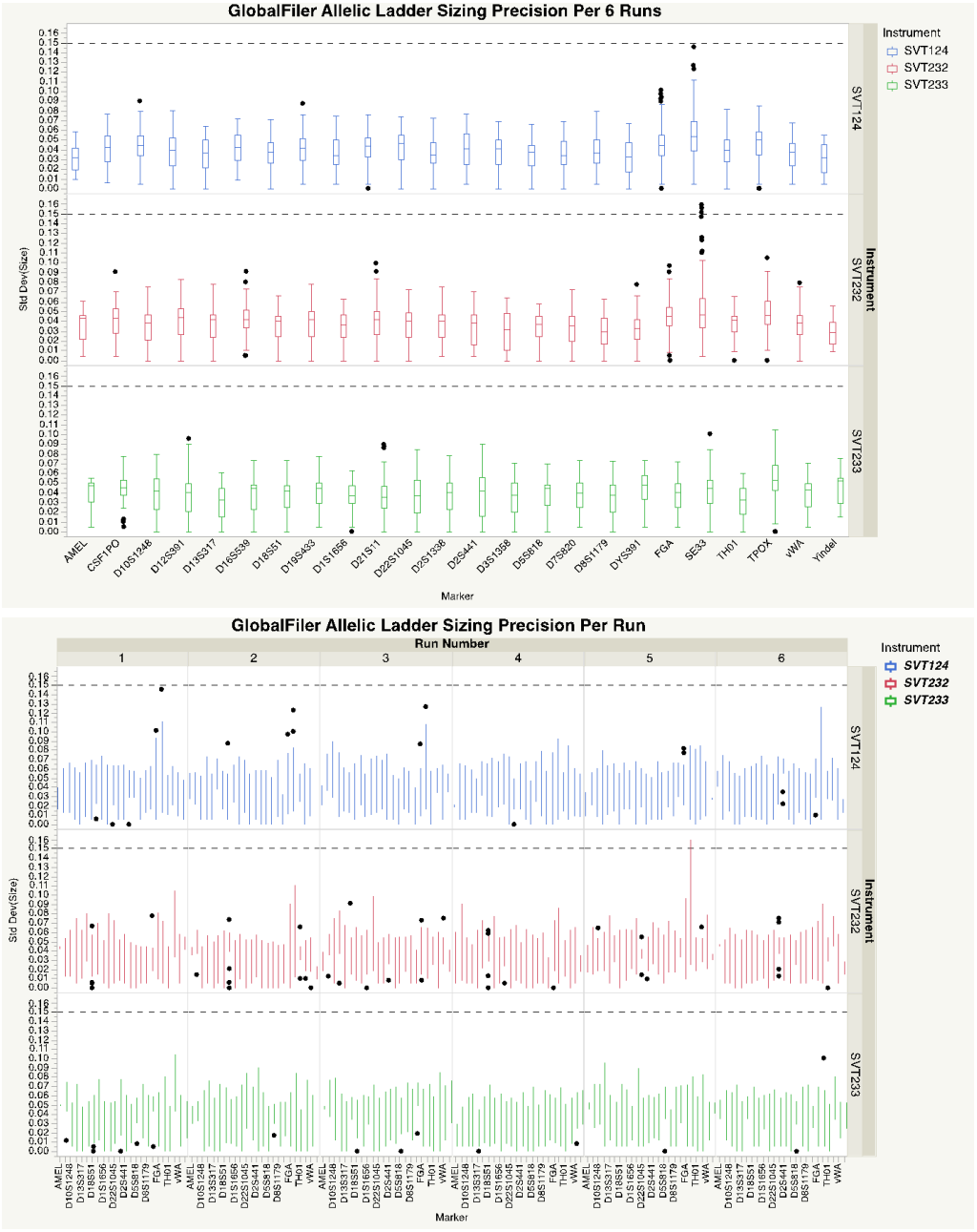

Figure 4 Sizing precision of the GlobalFiler™ allelic ladder. The allelic ladder is in eight wells, each injected three times for a total of 24 data points across six total injections. The top image shows precision per all six injections and the bottom image shows precision per injection for each marker. The three instruments are represented by blue, red, and green color panes. Dashed lines in each pane are at 0.15 bp.

The data is displayed using Tukey outlier box plots to show distribution of results and outliers.

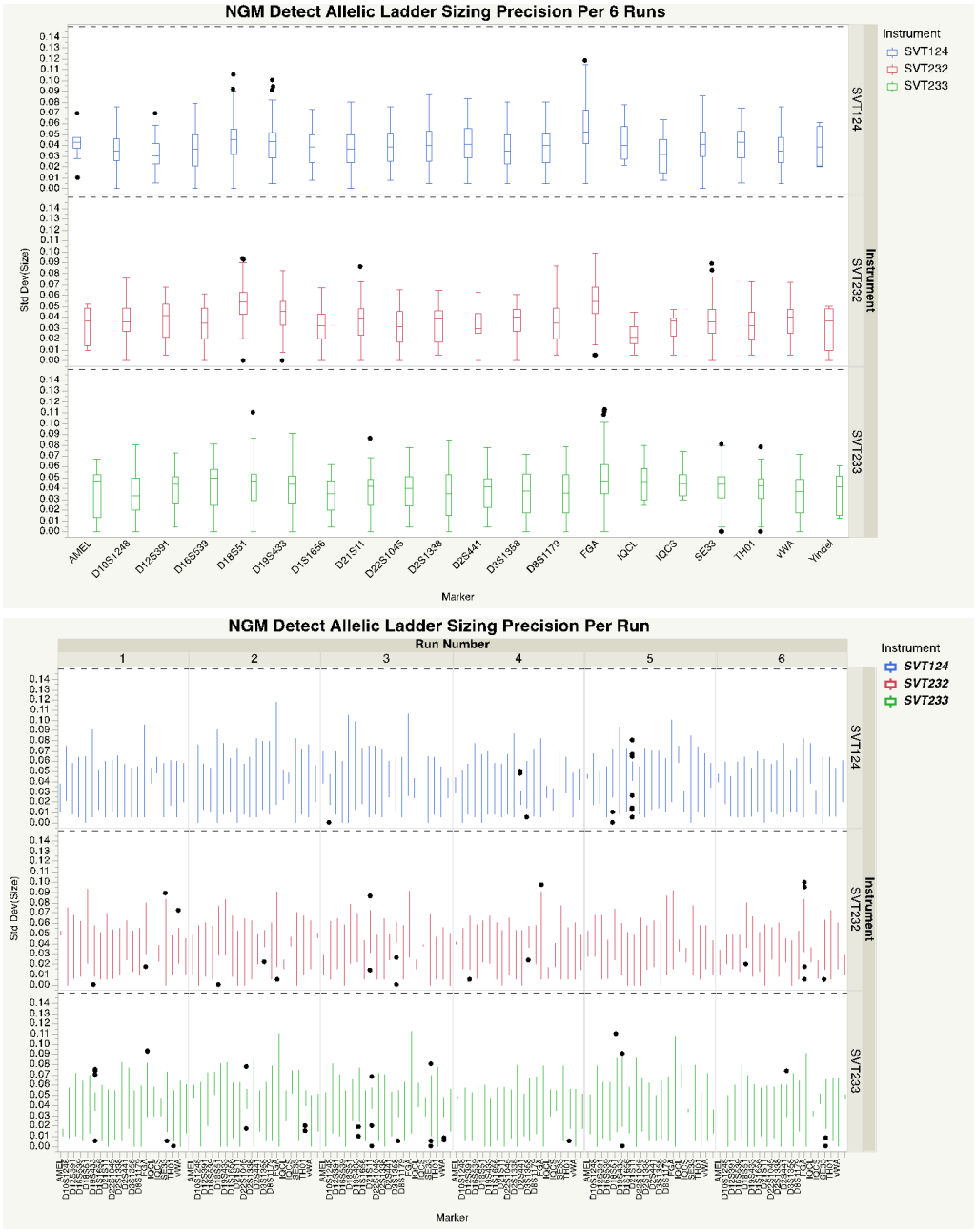

Figure 5 Sizing precision of the NGM Detect™ allelic ladder. The allelic ladder is in eight wells, each injected three times for a total of 24 data points across six total injections. The top image shows precision per all six injections and the bottom image shows precision per injection for each marker. The three instruments are represented by blue, red, and green color panes. Dashed lines in each pane are at 0.15 bp.

The data is displayed using Tukey outlier box plots to show distribution of results and outliers.

Minimum Detection Threshold and Contamination study

Minimum threshold values are established based on the baseline noise of the instrument and the kit. To calculate these values, no-template control (NTC) data for each kit on four instruments was analyzed at 1 RFU. Calculations were performed for limit of detection (LOD), limit of quantification (LOQ), and minimum threshold (LOQ rounded to the multiple of five). Unless otherwise noted, an average minimum threshold was calculated across four instruments per dye for each kit and used as the peak amplitude threshold (PAT) settings in the GeneMapper™ *ID-X* Software. Each HID laboratory should evaluate background noise for each instrument based on internal validation studies and determine whether variable thresholds should be considered based on factors including instrument and/or dye set.

Table 8 Minimum thresholds calculated and used for the peak amplitude thresholds in for each kit in the GeneMapper™ *ID-X* Software.

Dye channel	Kit									
	NGM SElect™	Yfiler™	Identifiler™ Plus	MiniFiler™	Yfiler™ Plus	GlobalFiler™ Express	Verifiler™ Express	GlobalFiler™	Verifiler™ Plus	NGM Detect™
Blue	55	40	30	55	70	75	65	65	75	75
Green	60	45	50	75	100	80	80	95	65	60
Yellow	95	45	70	90	55	40	45	45	35	30
Red	210	95	135	160	50	45	45	40	50	30
Purple	NA	NA	NA	NA	80	70	70	70	65	50
Orange	110	155	75	150	125	145	115	140	170	120

Genotyping Concordance study

For all kits tested, the results demonstrated that the genotype profiles from four SeqStudio™ Genetic Analyzers were 100% concordant with each other and with the 3500xL Genetic Analyzer results for the following test cases: Concordance, Color Balance, and Direct Amplification Kit Evaluation.

Concordance results

Table 9 lists concordance results for the following kits (3 or 4 injections per instrument):

- Eight casework kits—Concordance results for the positive control and 23 gDNA samples at the kit recommended input of 0.5 or 1 ng
- Two direct kits—Concordance results for the positive control and 20 buccal and blood samples at the kit recommended inputs of 4 ng (GlobalFiler™ Express kit) and 6 ng (Verifiler™ Express kit)

Table 9 Concordance results: 3500xL Genetic Analyzer to SeqStudio™ Genetic Analyzer.

Kit	% Concordance			
	SeqStudio™ instrument 1	SeqStudio™ instrument 2	SeqStudio™ instrument 3	SeqStudio™ instrument 4
NGM SElect™	100%	100%	100%	100%
Yfiler™	100%	100%	100%	100%
Identifiler™ Plus	100%	100%	100%	100%
MiniFiler™	100%	100%	100%	100%
Yfiler™ Plus	100%	100%	100%	100%
GlobalFiler™ Express	100%	100%	100%	100%
Verifiler™ Express	100%	100%	100%	100%
GlobalFiler™	100%	100%	100%	100%
Verifiler™ Plus	100%	100%	100%	100%
NGM Detect™	100%	100%	100%	100%

Color Balance

Inter-color balance is the ratio of the average peak heights between dyes. Intra-color balance is the ratio of the lowest peak height for any allele by the highest peak height for any allele within a dye channel.

The inter- and intra-color balances were within expected ranges based on the validation data for these kits on the 3500xL Genetic Analyzer. All autosomal STR and Amelogenin peak heights were normalized: homozygous alleles were divided by 2 and heterozygote peak heights were averaged; native peak heights were used for all Y-markers.

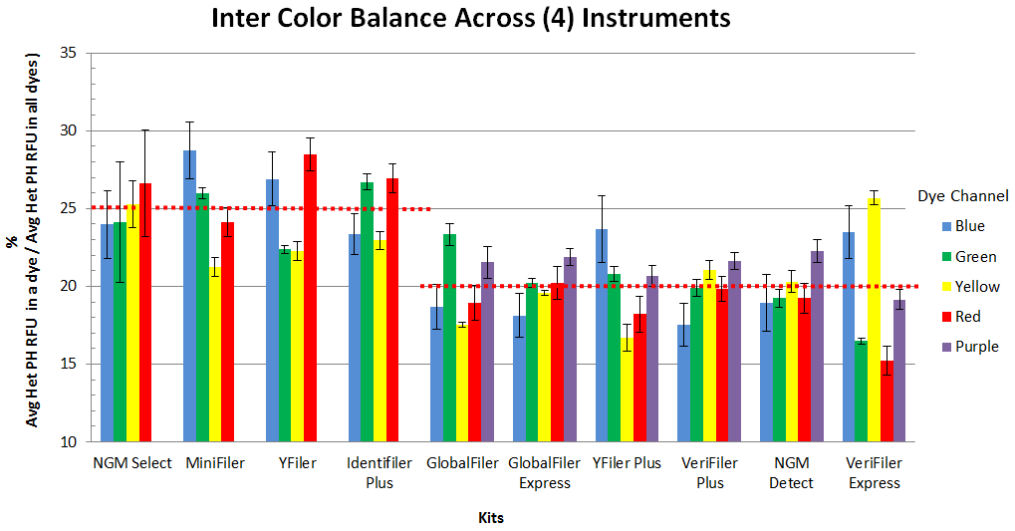

Figure 6 Inter-color balance averaged across four instruments for each of the ten kits tested. Equal dye contribution in a 5-dye kit results in 25% contribution (red dashed line) from the blue, green, yellow, and red dyes channels. Equal dye contribution in a 6-dye kit results in 20% contribution (red dashed line) from the blue, green, yellow, red, and purple dyes channels. N=94 samples (injections) per casework kit; N=86 samples (injections) per direct kit.

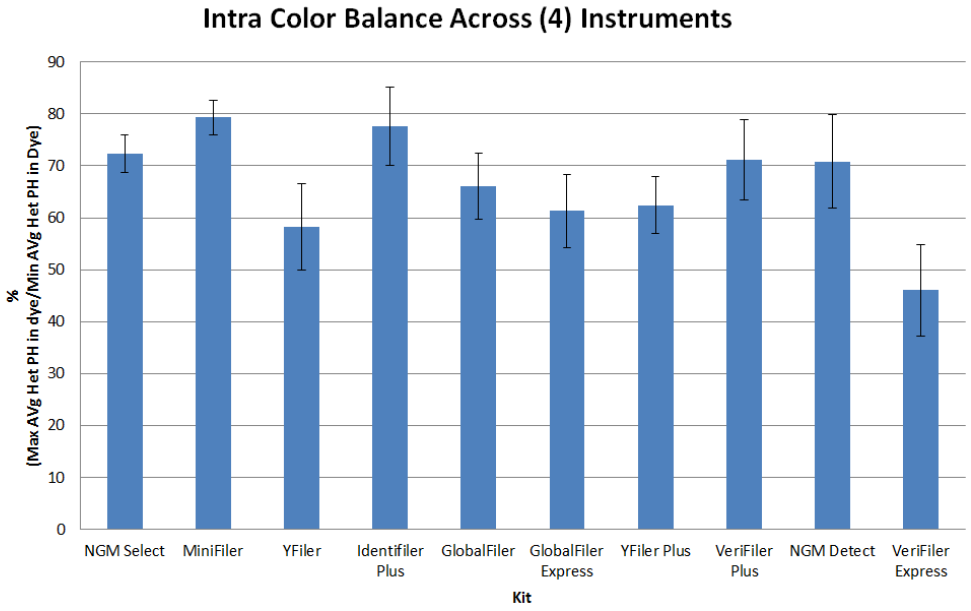

Figure 7 Intra-color balance averaged across four instruments for each of the ten kits tested. N=94 samples (injections) per casework kit; N=86 samples (injections) per direct kit.

Direct Amplification Kit Evaluation

Data quality was examined for the two direct kits to ensure quality performance expectations related to allelic drop-out and off-scale peaks. Control DNA and buccal and blood samples were amplified with the GlobalFiler™ Express and Verifiler™ Express kits using the kit recommended conditions. The samples were injected on four instruments; N=328 samples (injections) per kit. Laboratories should carefully optimize cycle number as described in the kit protocols to account for sample to sample variation in peak heights.

- GlobalFiler™ Express kit—94.8% of the data generated full, on-scale profiles. The samples (injections) that did not meet these metrics had homozygote peaks that were off-scale.
- Verifiler™ Express kit—98.8% of the data generated full, on-scale profiles. Four samples (injections) had size quality flags. Three of the four samples generated usable data when the size quality was addressed and one sample required a re-injection.

Resolution study

The ability of the system to reliably detect and resolve alleles that differ in length by a single base pair was analyzed in two independent sets of data. The resolution of the size standard peaks co-injected with samples amplified with eight casework kits up to 470 bp was reviewed. This study also included testing with GlobalFiler™, NGM Detect™, and NGM SElect™ kit data when the cathode buffer was newly installed and on Day 14. In all cases, more than 3,000 injections across all kits, single-base resolution was achieved between 60–470 bp more than 99% of the time.

Using data from the Sizing Precision and Accuracy study for the Identifiler™ Plus, GlobalFiler™, and NGM Detect™ kits, results from the Ladder Passing Rate test case demonstrated the ability of the system to resolve peaks separated by a single base pair in the allelic ladders. The following marker alleles were resolved for all samples tested (72 allelic ladders per kit across three instruments):

- Identifiler™ Plus kit—TH01
- GlobalFiler™ kit—TH01, D12S391, D1S1656, and D2S441
- NGM Detect™ kit—TH01, D12S391, D1S1656, and D2S441

Representative data is shown in Figure 8.

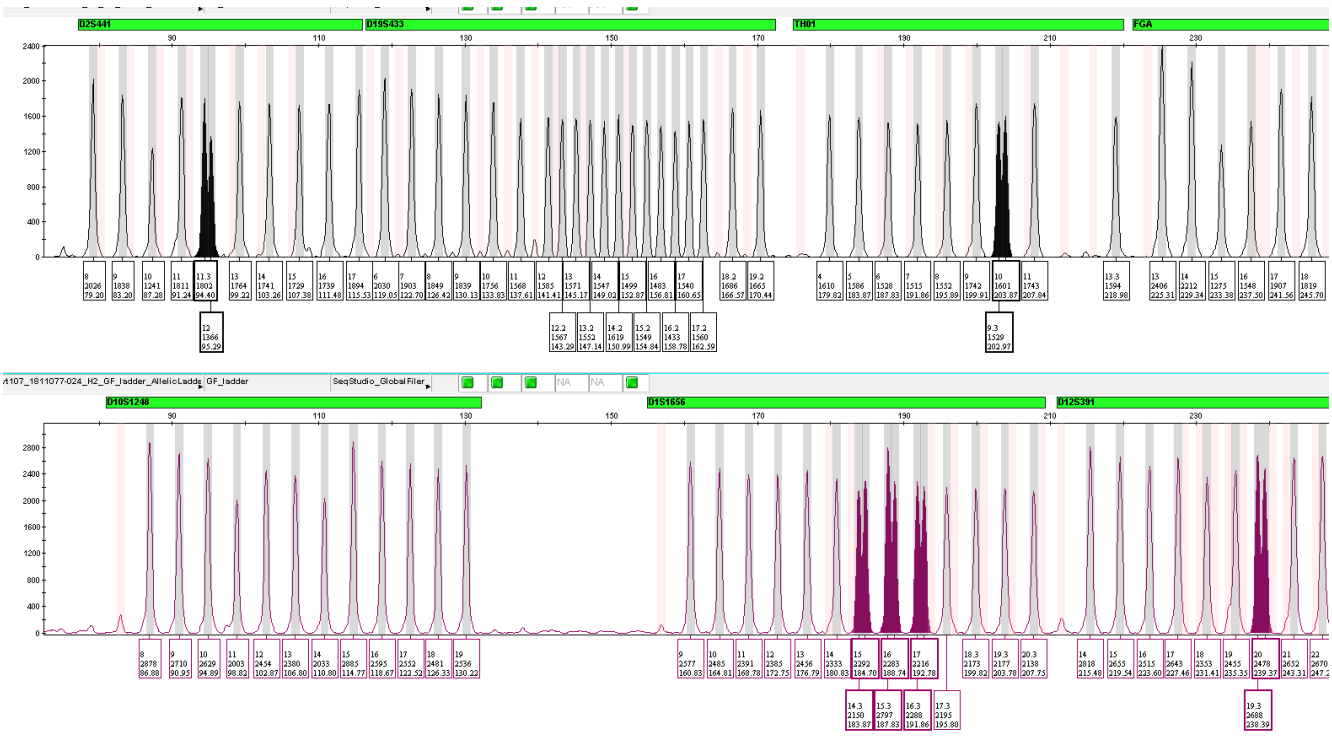

Figure 8 Representative data for the GlobalFiler™ kit—TH01, D12S391, D1S1656, and D2S441 marker alleles. Highlighted alleles are those separated by a single base pair.

Sensitivity and Dynamic Range study

Inputs

Data from four DNA input amounts were evaluated for the positive control and 5 gDNA samples amplified with each of the eight casework kits. Input amounts ranged from below stochastic to two times the kit recommended input.

Data generated from samples at the kit recommended input (0.5 ng or 1 ng) generated full, on-scale profiles with average heterozygote peak height ratios (PHR), within expected ranges based on the validation data for these kits on the 3500xL Genetic Analyzer.

The majority of samples tested for all kits produced full profiles at 125 pg of DNA input, with the following exceptions:

- Drop-out of allelic data in the Identifiler™ Plus, NGM SElect™, and Yfiler™ Plus kit samples at 125 pg input was observed using the average thresholds described above. An instrument-specific threshold would have resulted in full profiles for all of these occurrences.
- Partial profiles were observed for samples at 15.5 pg (MiniFiler™, NGM Detect™, and Verifiler™ Plus).
- Partial profiles were observed for samples at 31 pg (Identifiler™ Plus, NGM SElect™, Yfiler™, GlobalFiler™, and Yfiler™ Plus).

Data generated from samples at two times the kit recommended input generated average normalized peak heights within the saturation limits of the system.

Table 10 Average peak heights for each of the DNA inputs evaluated and average peak height ratio (PHR) for the kit recommended input samples across four instruments.

N=92 samples (injections) per kit for the kit recommended input of 0.5 ng (MiniFiler™, NGM Detect™, and Verifiler™ Plus kits) or 1 ng (all other kits). N=48 samples (injections) per kit for two times the kit recommended input. N=48 samples (injections) per kit for inputs of 15.5, 31, and 125 pg.

Kit	DNA input and average normalized peak height				Average PHR
	Recommended	2 × recommended	125 pg	15.5 or 31 pg	
NGM SElect™	4,832	8,947	560	148	91.5%
Yfiler™	7,284	13,487	725	179	NA
Identifiler™ Plus	3,217	5,867	347	90	92.4%
MiniFiler™	7,119	9,832	1,864	148	90.1%
Yfiler™ Plus	4,765	8,257	431	140	NA
GlobalFiler™	5,140	9,491	506	140	92.5%
Verifiler™ Plus	6,194	12,151	1,539	171	91.2%
NGM Detect™	7,013	13,055	2,069	200	90.9%

Artifacts: Pull-up and n±1

Spectral pull-up was analyzed in samples amplified at the kit recommended input for each of the eight casework kits across four instruments. The percentage of pull-up was determined by dividing the peak height of the pull-up peak by the peak height of the parent peak. The results demonstrate the performance of spectral deconvolution using both the auto-spectral and marker-to-marker pull-up reduction features optimized in SeqStudio™ Data Collection Software v1.2.

There were 947 pull-up peaks identified in 730 injections (across all kits on all four instruments). The average pull-up percentage for all pull-up peaks observed across the kits was <3%. In all kits, the majority of the pull-up peaks were ≤3% with only two kits showing any pull-up >5%. The highest incidence of pull-up was in an Identifiler™ Plus kit sample at 7.01%. All pull-up >5% seen in the Identifiler™ Plus kit samples was observed in a single capillary on one instrument.

Table 11 Pull-up details for the eight kits tested across four instruments.

For all kits tested: N=730 samples (injections) of positive control and 5 gDNA samples amplified at the kit recommended input.

Kit	Pull-up peaks						Average heterozygote peaks heights
	Total	Average/sample (injection)	Mean	Maximum	≤3%	≤5%	
MiniFiler™	33	0.36	1.65%	3.27%	81.8%	100%	7,119 RFU
Identifiler™ Plus	22	0.24	1.94%	7.01%	63.6%	68.2%	3,217 RFU
NGM SElect™	100	1.09	2.76%	6.72%	72%	91%	4,832 RFU
Yfiler™	38	0.41	1.19%	2.93%	100%	100%	7,284 RFU

Table 11 Pull-up details for the eight kits tested across four instruments. *(continued)*

Kit	Pull-up peaks						Average heterozygote peaks heights
	Total	Average/sample (injection)	Mean	Maximum	≤3%	≤5%	
GlobalFiler™	49	0.53	1.37%	4.10%	89.8%	100%	5,140 RFU
Yfiler™ Plus	21	0.23	1.89%	3.03%	97.4%	100%	4,765 RFU
NGM Detect™	400	4.65	0.87%	3.58%	99.5%	100%	7,013 RFU
Verifiler™ Plus	284	3.09	1.15%	3.36%	98.6%	100%	6,194 RFU

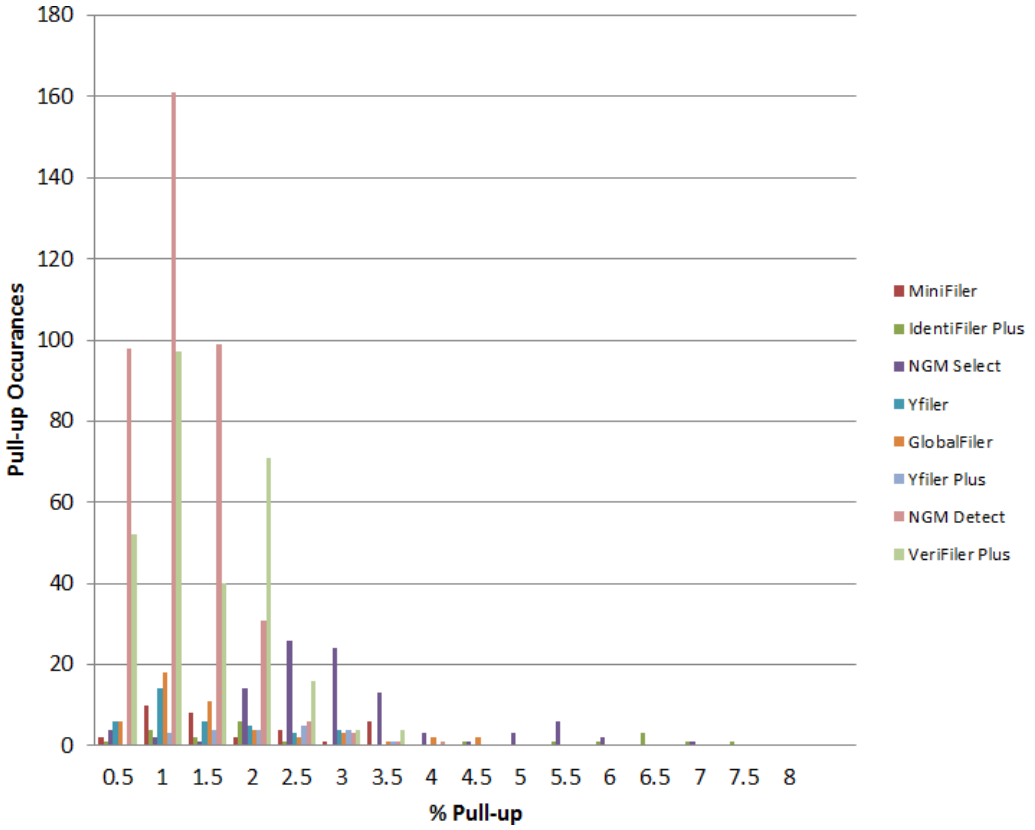

Figure 9 Frequency distribution of pull-up occurrences plotted for each kit.

To align results generated in traditional POP-4™ Polymer workflows with data from POP–1™ Polymer, SeqStudio™ Data Collection Software v1.2 was validated for HID applications on the SeqStudio™ Genetic Analyzer.

SeqStudio™ Data Collection Software v1.2 includes a unique resolution algorithm. If STR data from HID kits are run on the SeqStudio™ Genetic Analyzer without this algorithm, higher resolution of STR fragment peaks will be observed. However, artifact peaks in the n ±1 position to the main or stutter allele and intrinsic to the amplification process may interfere with data analysis and interpretation. As amplification artifacts, these peaks may be in samples run with POP-4™ Polymer, but are not seen as frequently as with POP–1™ Polymer (without the resolution algorithm) because of limitations in the resolving power for POP-4™ Polymer.

The results demonstrate the expected occurrence of n±1 artifacts across the eight casework kits tested on the SeqStudio™ Genetic Analyzer/POP–1™ Polymer configuration, and provide a comparison of the same data set run with the 3500xL Genetic Analyzer/POP-4™ Polymer configuration. Using minimum thresholds, there were no shoulder peaks resolved from the main peaks in samples amplified at the kit recommended input with Identifiler™ Plus, GlobalFiler™, or Yfiler™ Plus kit data run on four SeqStudio™ Genetic Analyzers [a total of 92 samples (injections) per kit on four instruments].

Approximately 2.4% of samples (injections) from the MiniFiler™, NGM SElect™, and Yfiler™ kit data sets had n±1 peaks resolved above minimum thresholds. The same samples injected on the 3500xL Genetic Analyzer/POP-4™ Polymer configuration did not show resolution of these n±1 peaks. Figure 10 shows an example of this comparison data.

NGM Detect™ and Verifiler™ Plus kit samples run on the SeqStudio™ Genetic Analyzer/POP–1™ Polymer configuration demonstrated more n+1 artifact peaks off of stutter alleles when compared to the same data set run on the 3500xL Genetic Analyzer/POP-4™ Polymer configuration. Figure 11 shows an example of this comparison data; Table 12 details the observances of these artifacts. In all cases, the n+1 peaks resolved above threshold were shoulders off of stutter. In NGM Detect™ kit data for the positive control and 5 gDNA samples, the artifacts were seen in only the vWA, D1S1656, and TH01 markers. In Verifiler™ Plus kit data for the positive control and 5 gDNA samples, the artifacts were seen in only the D1S1656 and D2S441 markers. These results indicate that when interpreting data from these kits, it is important to recognize these artifacts in n+1 stutter positions, especially during mixture interpretation.

Table 12 n+1 details for all kits tested across four instruments.

N=92 samples (injections) of positive control and 5 gDNA samples amplified at the kit recommended input for all kits except the NGM Detect™ kit. N=86 samples (injections) for the NGM Detect™ kit because of failed injections that were excluded from analysis.

Kit	Markers with observed n+1 peaks above the minimum threshold	Description of artifact peak	Samples (injections) with n+1 peaks above minimum threshold by marker
Identifiler™ Plus	None		
GlobalFiler™	None		
Yfiler™ Plus	None		
MiniFiler™	D7S820	n–1 off of stutter	1.1%
NGM SElect™	TH01	n+1 off main allele	1.1%
Yfiler™	YGATAH4	n+1 off of stutter	2.2%
	DYS385	n–1 off of stutter	3.3%
NGM Detect™	vWA	n+1 off of stutter	33.7%
	D1S1656		14%
	TH01		22.1%
Verifiler™ Plus	D1S1656	n+1 off of stutter	4.3%
	D2S441		34.8%

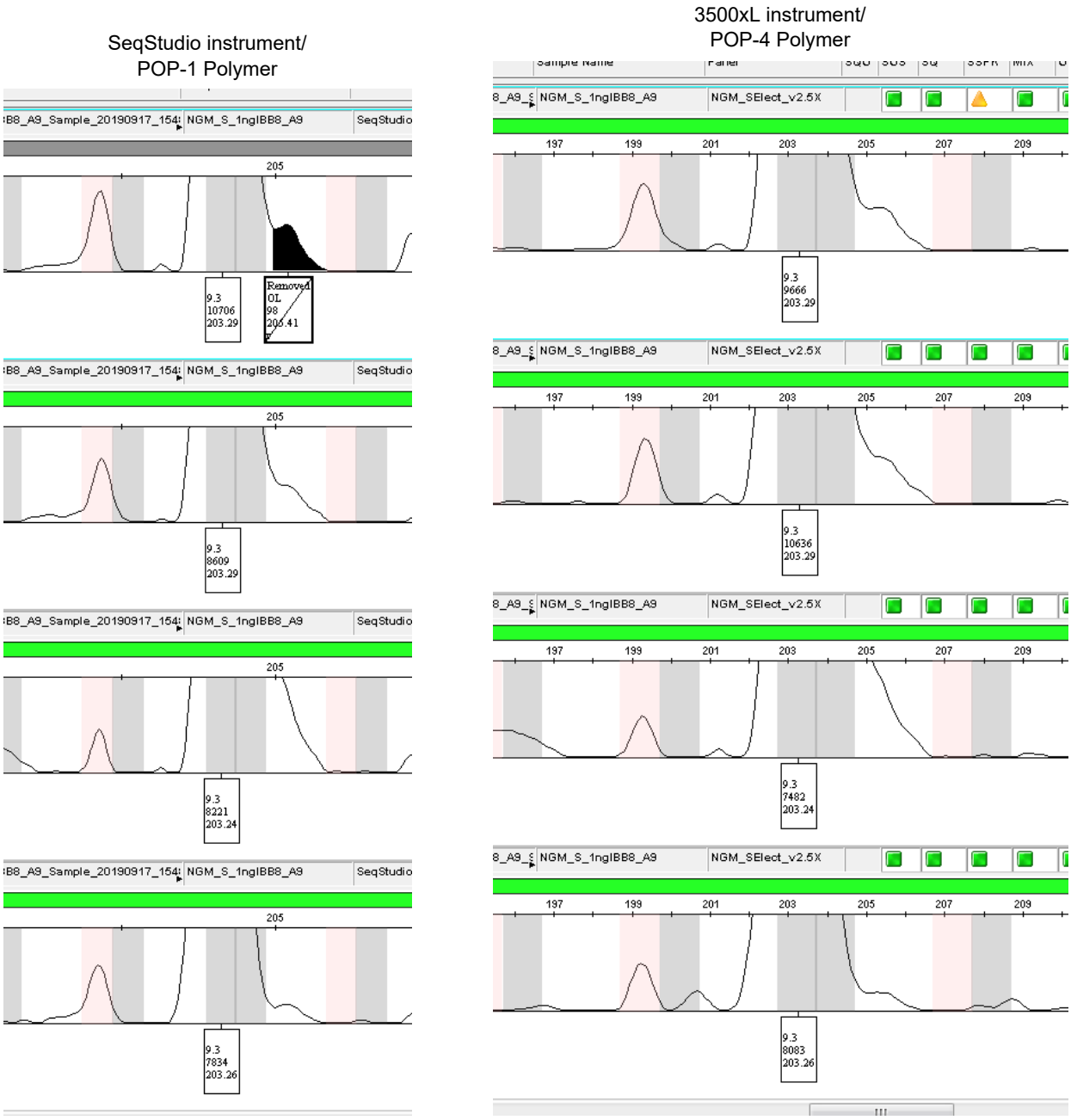

Figure 10 NGM SElect™ kit comparison: 1 ng of gDNA sample injected four times on each instrument. The left image shows four injections on one SeqStudio™ instrument. In 1 of 16 injections, an OL peak is resolved from the 9.3 parent peak at TH01 and is above the minimum threshold. The right images shows the same amplicon injected four times on the 3500xL instrument; the POP-4™ Polymer does not resolve this OL peak from the parent peak or the shoulder peak is below threshold. The Y-axis is zoomed to 200 RFU and a 95 RFU peak amplitude threshold was used with the suggested GeneMapper™ *ID-X* Software analysis settings for NGM SElect™ kits.

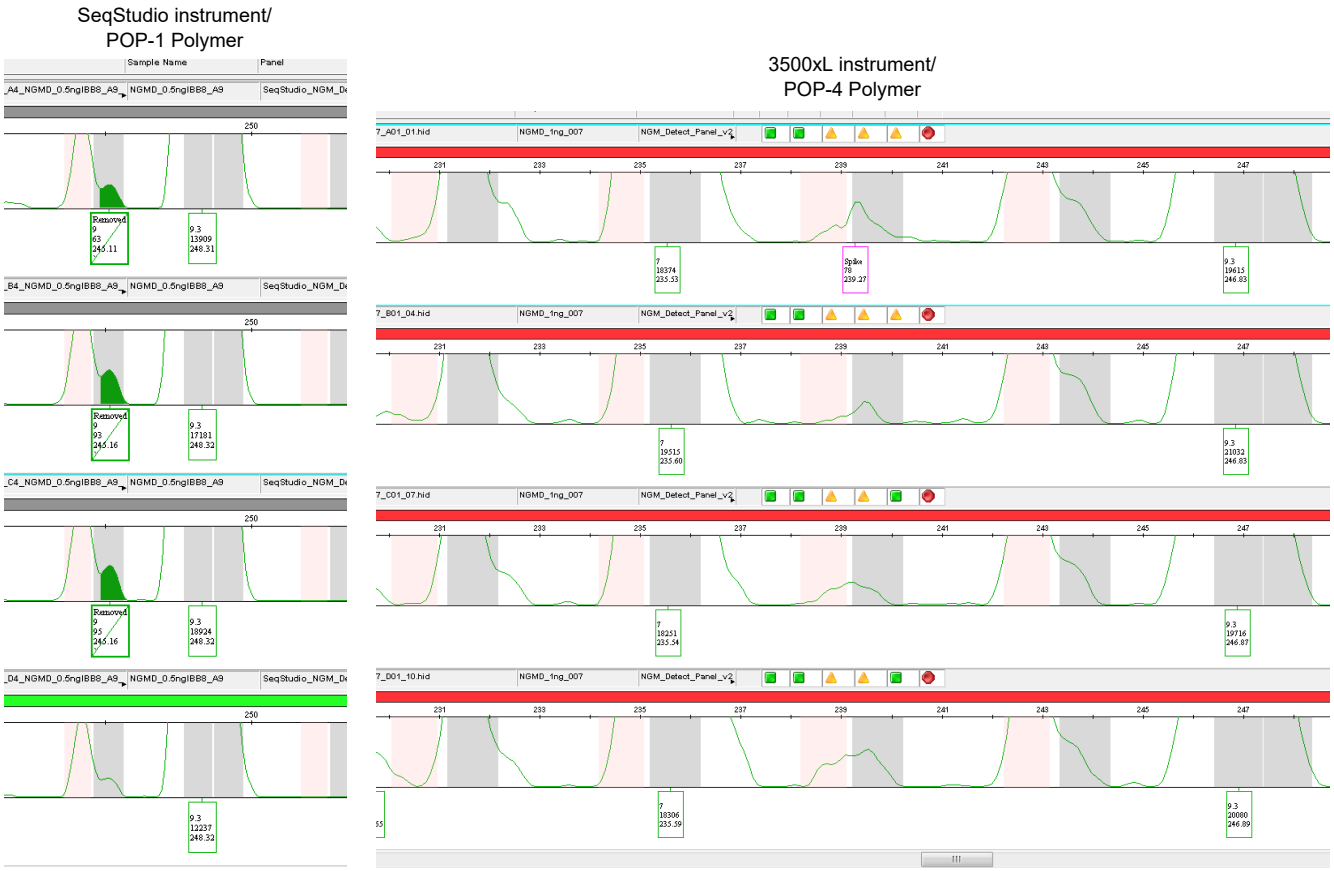

Figure 11 NGM Detect™ kit comparison: 0.5 ng of gDNA sample injected four times on each instrument. The left image shows four injections on one SeqStudio™ instrument. In 11 of 16 injections, a 9 allele is resolved from the 8.3 stutter peak at TH01 and is above the minimum threshold. The right images shows the same amplicon injected four times on the 3500xL instrument; the POP-4™ Polymer does not resolve this 9 allele peak from the parent peak, or the shoulder peak is below threshold. The Y-axis is zoomed to 200 RFU and a 60 RFU peak amplitude threshold was used with the suggested GeneMapper™ *ID-X* Software analysis settings for NGM Detect™ kits.

Mixture Analysis study

Two test cases were used to evaluate the performance of the SeqStudio™ Genetic Analyzer running DNA samples with more than one contributor: Control Mixture and gDNA Mixture.

The Control Mixture test case used control DNA sources 007 and 9947A at two mixture ratios of 1:7 and 7:1. The kit recommended input for each kit was amplified for each reaction: 1 ng for the GlobalFiler™ kit and 0.5 ng for the NGM Detect™ kit. Therefore, the contribution from the minor contributor was 125 pg in the GlobalFiler™ kit reactions and 62.5 pg in the NGM Detect™ kit reactions. For all injections across four instruments, full profiles were generated for the minor contributor; N=4 samples (injections) per mixture ratio for each kit on each instrument.

The gDNA Mixture test case used two pairs of gDNA samples (G1 and G2, G3 and G4) at seven mixture ratios of 1:7, 1:3, 1:2, 1:1, 2:1, 3:1, and 7:1. Table 13 details the total DNA input and minor contribution for each kit and ratio. The gDNA samples were chosen and used for mixture pairs because at least one marker in each pair contained major and minor alleles that were a single base pair apart; the expected genotypes of these markers are shown in Table 14. The goal of this test case was to demonstrate the performance of the SeqStudio™ Genetic Analyzer with the resolution algorithm in SeqStudio™ Data

Collection Software v1.2 and the algorithm's effect on minor contributor detection. A direct comparison was made with the same data set run on a 3500xL Genetic Analyzer.

Table 13 Major-to-minor contributor input amounts for the analyzed mixture ratios.

Mixture ratios depend on the total DNA input of 1 ng for the GlobalFiler™ kit samples or 0.5 ng for NGM Detect™ kit samples.

Mixture ratio	GlobalFiler™ kit (1 ng total input)		NGM Detect™ kit (0.5 ng total input)	
	Major input	Minor input	Major input	Minor input
7:1	0.875 ng	0.125 ng	0.4375 ng	0.625 ng
3:1	0.750 ng	0.250 ng	0.375 ng	0.125 ng
2:1	0.667 ng	0.333 ng	0.334 ng	0.167 ng
1:1	0.500 ng	0.550 ng	0.25 ng	0.25 ng

Table 14 Expected genotypes for the two mixture pairs at markers that contain major and minor alleles that are only a single base pair apart.

Mixture pair	gDNA source	Kit	Marker	Genotype
G1:G2	G1	GlobalFiler™	D7S820	9, 12
	G2			9.1, 12
	G1	GlobalFiler™	D1S1656	11, 16.3
	G2	NGM Detect™		14, 17
G3:G4	G3	GlobalFiler™ NGM Detect™	D2S441	11, 13
	G4			12.3, 14

Table 13 and Table 14 show that the SeqStudio™ Genetic Analyzer/POP–1™ Polymer configuration and the resolution algorithm demonstrated similar performance overall to the 3500xL Genetic Analyzer/POP-4™ Polymer configuration related to minor contributor resolution for the GlobalFiler™ and NGM Detect™ kits across all injections.

In the GlobalFiler™ kit data set, the 3500xL Genetic Analyzer/POP-4™ Polymer configuration was able to resolve the minor 16.3 or 17 alleles at D1S1656 in the G1:G2 mixtures of 1:7 and 7:1 in all injections. The SeqStudio™ Genetic Analyzer/POP–1™ Polymer configuration could not resolve the 16.3 minor in 12 of 12 injections of the 1:2 G1:G2 mixture and resolved the 17 minor in 9 of 16 injections of the 2:1 G1:G2 mixture (across four SeqStudio™ instruments).

Improved ability was observed for the SeqStudio™ Genetic Analyzer/POP–1™ Polymer configuration in the following instances:

- GlobalFiler™ kit data set—The SeqStudio™ Genetic Analyzer/POP–1™ Polymer configuration resolved the minor 12.3 allele from the major 13 allele in the 2:1 G3:G4 mixture at D2S441 peak in 9 of 12 injections. The 3500xL Genetic Analyzer/POP-4™ Polymer configuration could not resolve this minor peak in 3 of 3 injections.
- NGM Detect™ kit data set—The SeqStudio™ Genetic Analyzer/POP–1™ Polymer configuration resolved the minor 17 allele from the major 16.3 allele in the 2:1 G1:G2 mixture at D1S165 in 12 of 12 injections. The 3500xL Genetic Analyzer/POP-4™ Polymer configuration could not resolve this minor peak in 3 of 3 injections
- NGM Detect™ kit data set—The SeqStudio™ Genetic Analyzer/POP–1™ Polymer configuration resolved the minor 13 allele from the major 12.3 allele in the 1:2 G3:G4 mixture set at D2S441 in 6 of 12 injections. The 3500xL Genetic Analyzer/POP-4™ Polymer configuration could not resolve this minor peak in 3 of 3 injections

Table 15 GlobalFiler™ kit gDNA mixture analysis summary.

Results are compared across four SeqStudio™ Genetic Analyzers to the same data set run on a 3500xL Genetic Analyzer. Bold text indicates subtle differences in performance.

Mixture pair/marker	Instrument	Status of the minor genotype[1]						
		1:1	1:2	1:3	1:7	2:1	3:1	7:1
G1:G2/ D7S820	SeqStudio™	R	R	U	U	R	R	U
	3500xL	R	R	U	U	R	R	U
G1:G2/ D1S1656	SeqStudio™	R	R	R	**U**	R	R	**U**
	3500xL	R	R	R	**R**	R	R	**R**
G3:G4/ D2S441	SeqStudio™	R	R	U	U	U	U	U
	3500xL	R	R	U	U	U	U	U

[1] R=Resolved minor peak in an n±1 position in all injections. U=Unresolved minor peak in an n±1 position in ≥1 replicate.

Table 16 NGM Detect™ kit gDNA mixture analysis summary.

Results are compared across four SeqStudio™ Genetic Analyzers to the same data set run on a 3500xL Genetic Analyzer. Bold text indicates subtle differences in performance.

Mixture pair/marker	Instrument	Status of the minor genotype[1]						
		1:1	1:2	1:3	1:7	2:1	3:1	7:1
G1:G2/ D1S1656	SeqStudio™	R	R	**R**	U	**R**	**R**	U
	3500xL	R	R	**U**	U	**U**	**U**	U
G3:G4/ D2S441	SeqStudio™	U	U	U	U	U	U	U
	3500xL	U	U	U	U	U	U	U

[1] R=Resolved minor peak in an n±1 position in all injections. U=Unresolved minor peak in an n±1 position in ≥1 replicate.

Signal Variability study

Peak height variation across capillaries, injections, instruments, and cartridges was evaluated. An example of the plate layout that was used for each of these test cases is shown in Table 17. Table 18 to Table 21 detail the results of each evaluation.

Table 17 Example plate layout for evaluating capillary, injection, instrument, and cartridge signal variation.

One injection group for one kit consists of four wells. Each injection group was injected three times for a total of six continuous injections for each kit. Allelic ladder data was not included as part of the evaluation. Failed injections were also excluded from analysis. This plate setup was used for one, three, or four instruments, depending on the test case.

Capillary	GlobalFiler™ kit	NGM Detect™ kit	Yfiler™ kit
Injection group 1			
1	007 1 ng	007 0.5 ng	007 1 ng
2	007 1 ng	007 0.5 ng	007 1 ng
3	007 1 ng	007 0.5 ng	007 1 ng
4	007 1 ng	007 0.5 ng	007 1 ng
Injection group 2			
1	007 1 ng	007 0.5 ng	007 1 ng
2	007 1 ng	007 0.5 ng	007 1 ng
3	007 1 ng	007 0.5 ng	007 1 ng
4	Allelic ladder	Allelic ladder	Allelic ladder

Capillary-to-capillary and injection-to-injection variation were evaluated on three instruments, each with a different cartridge and cathode buffer lot. The maximum-to-minimum ratios for the average peak heights across three (capillary 4) or six injections (capillaries 1–3) per capillary were ≤1.8 for all kits tested. Capillary 3 consistently resulted in the lowest average peak height compared to the other capillaries. The maximum-to-minimum ratios for the average peak heights of all capillaries in an injection group across six injections were ≤1.2 for all kits tested.

Instrument-to-instrument variation was evaluated on four instruments, each with the same cartridge and cathode buffer consumables transferred from instrument to instrument. The maximum-to-minimum ratios for the average peak heights across all capillaries for all injections for each instrument were ≤1.3 for all kits tested.

Cartridge-to-cartridge variation was evaluated on a single instrument with four different cartridges and cathode buffer lots. The maximum-to-minimum ratios for the average peak heights across all capillaries for all injections for each cartridge/buffer lot were ≤1.3 for all kits tested.

Table 18 Capillary-to-capillary signal variation results expressed as a maximum:minimum ratio of the average peak heights in each capillary across continuous injections.

Capillary 3 consistently resulted in the lowest average peak height compared to the other capillaries.

Instrument	Capillary	GlobalFiler™ kit		NGM Detect™ kit		Yfiler™ kit	
		Average peak height	Max:Min	Average peak height	Max:Min	Average peak height	Max:Min
1	1	4,283.0	1.5	6,047.3	1.5	6,074.9	1.4
	2	4,453.4		6,078.0		5,701.5	
	3	3,028.5		4,169.4		4,445.3	
	4	3,965.9		6,071.4		5,968.4	
2	1	6,923.1	1.5	9,950.6	1.5	8,863.9	1.4
	2	5,273.9		7,732.1		7,226.5	
	3	4,695.7		6,657.3		6,402.5	
	4	4,864.9		8,177.0		7,860.1	
3	1	5,031.5	1.8	7,206.6	1.8	6,985.3	1.7
	2	5,006.9		7,016.3		6,956.0	
	3	2,780.0		4,029.1		4,063.0	
	4	4,546.9		6,580.1		6,265.2	

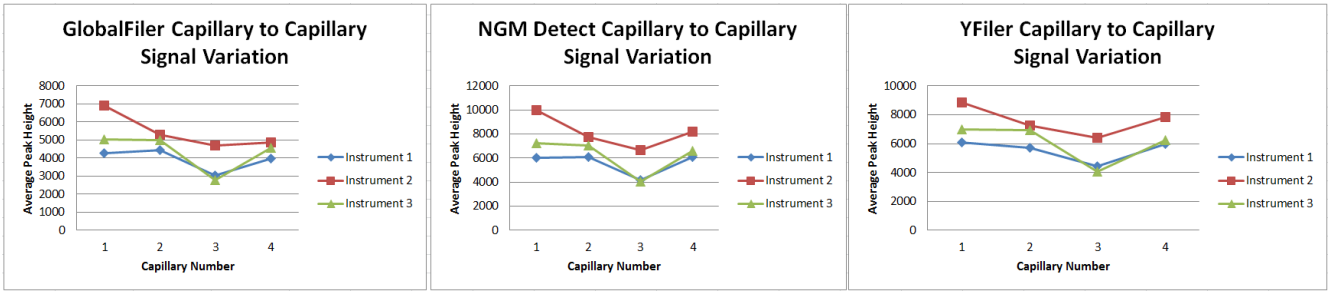

Figure 12 Capillary-to-capillary signal variation results.

Table 19 Injection-to-injection signal variation results expressed as a maximum:minimum ratio of the average peak heights across all capillaries in each injection group.

Instrument	Injection	GlobalFiler™ kit		NGM Detect™ kit		Yfiler™ kit	
		Average peak height	Max:Min	Average peak height	Max:Min	Average peak height	Max:Min
1	1	4,191.2	1.1	5,675.0	1.1	6,164.9	1.2
	2	3,859.5		5,622.7		5,441.4	
	3	3,910.2		5,614.4		5,358.1	
	4	3,966.3		5,375.9		5,629.2	

Table 19 Injection-to-injection signal variation results expressed as a maximum:minimum ratio of the average peak heights across all capillaries in each injection group. *(continued)*

Instrument	Injection	GlobalFiler™ kit		NGM Detect™ kit		Yfiler™ kit	
		Average peak height	Max:Min	Average peak height	Max:Min	Average peak height	Max:Min
1	5	3,808.7	1.1	5,402.5	1.1	5,114.2	1.2
	6	3,772.6		5,333.0		5,048.9	
2	1	5,830.8	1.2	8,405.5	1.1	7,769.2	1.1
	2	5,380.3		8,208.9		7,673.5	
	3	4,968.2		7,997.9		7,664.5	
	4	5,846.9		8,387.5		7,538.7	
	5	5,782.1		7,945.9		7,416.7	
	6	5,448.8		7,707.2		7,080.8	
3	1	4,369.1	1.1	6,317.3	1.1	6,136.4	1.1
	2	4,351.1		6,335.6		6,115.3	
	3	4,381.9		6,154.8		6,118.4	
	4	4,423.2		6,071.1		6,070.7	
	5	4,120.9		6,043.5		5,912.6	
	6	4,170.1		5,892.6		5,797.1	

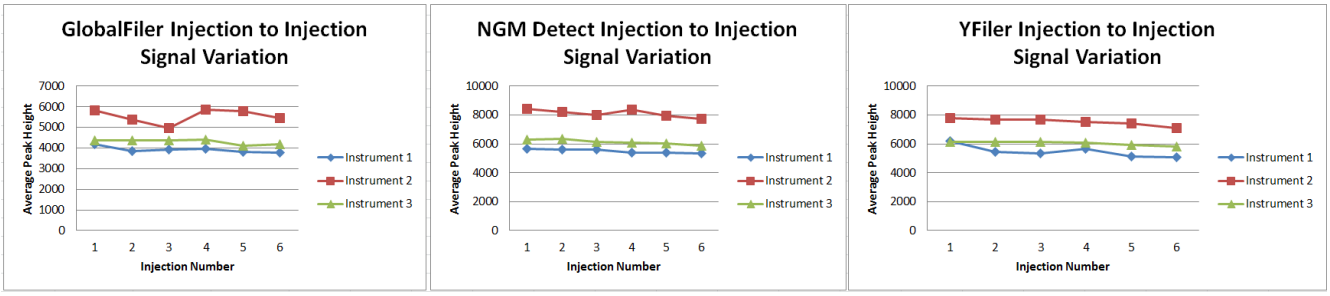

Figure 13 Injection-to-injection signal variation results.

Table 20 Instrument-to-instrument signal variation results expressed as a maximum:minimum ratio of the average peak heights across all capillaries and injections on each instrument.

Instrument	GlobalFiler™ kit		NGM Detect™ kit		Yfiler™ kit	
	Average peak height	Max:Min	Average peak height	Max:Min	Average peak height	Max:Min
1	4,524.2	1.3	6,749.6	1.2	6,808.5	1.3
2	4,868.9		7,383.9		6,975.9	
3	5,722.6		8,133.4		8,346.1	
4	4,614.2		6,693.5		6,644.7	

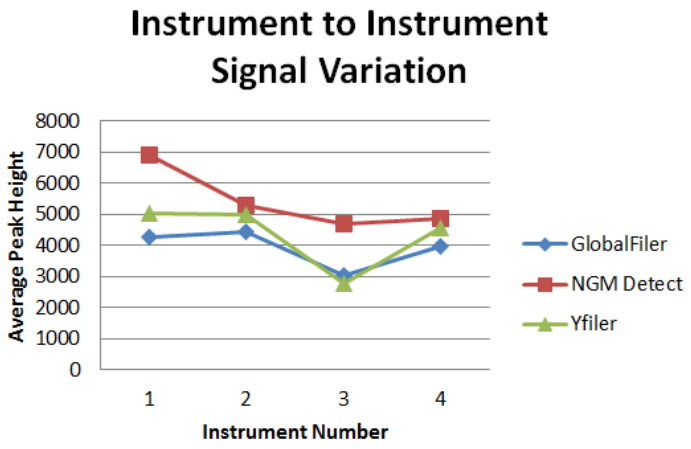

Figure 14 Instrument-to-instrument signal variation results.

Table 21 Cartridge-to-cartridge signal variation results expressed as a maximum:minimum ratio of the average peak heights across all capillaries and injections for each cartridge on a single instrument.

Cartridge	GlobalFiler™ kit		NGM Detect™ kit		Yfiler™ kit	
	Average peak height	Max:Min	Average peak height	Max:Min	Average peak height	Max:Min
1	5830.3	1.2	8610.8	1.2	8708.9	1.3
2	5030.8		7383.9		6975.9	
3	5234.0		7591.2		7620.4	
4	5019.6		7328.8		6923.4	

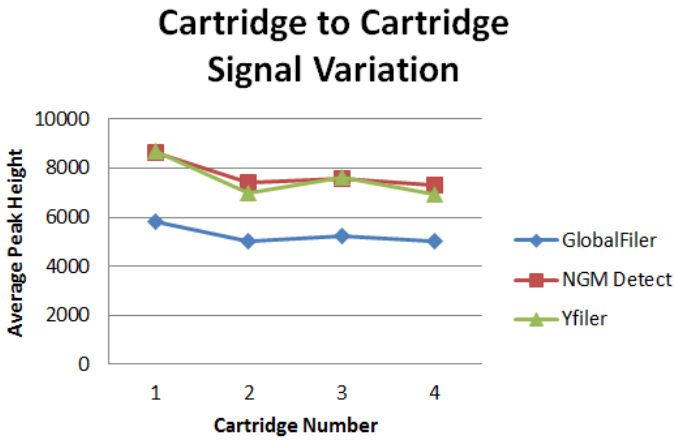

Figure 15 Cartridge-to-cartridge signal variation results.

Crosstalk and Carryover study

Crosstalk

Crosstalk is signal detection from adjacent wells in an injection. To evaluate crosstalk, a checkerboard pattern of DNA Control 007 (at two times the kit recommended input) and Hi-Di™ Formamide was set up for two injections on four instruments.

There were no peaks attributed to signal crosstalk observed on any of the four instruments for any of the kits tested: NGM Detect™, Yfiler™, and Yfiler™ Plus kits.

Carryover

Carryover is a physical transfer of DNA from well to well between injections. To evaluate carryover, a zebra pattern of DNA Control 007 (at two times the kit recommended input) and no-template controls (NTC) was set up for two injections on four instruments.

In 15 of 16 injections, there was no carryover observed in the NTC injection.

In a single injection, there was one instance of potential carryover observed on one instrument in the Yfiler™ kit data set. Peaks observed in the NTC injections corresponded to the allelic peaks from the previous DNA injection in the same capillary and were ~1% of the sample peak heights.

A repeat of the experiment resulted in no carryover observations. It is possible that the original instance was caused by a setup error rather than a physical transfer of DNA from well-to-well during injection.

Guidelines for installing the GeneMapper™ *ID-X* Software on a co-located computer

Co-located means that the computer and SeqStudio™ Genetic Analyzer are directly connected through a network cable.

If you are installing the GeneMapper™ *ID-X* Software on a computer that will be co-located with the SeqStudio™ Genetic Analyzer, follow these guidelines to ensure that the software properly opens.

- Ensure that the computer is not connected to the SeqStudio™ Genetic Analyzer.
- Ensure that the computer is not connected to any network, either with an Ethernet cable or wireless connection.
- Install the GeneMapper™ *ID-X* Software on the computer, then open the software and sign in.
- Co-locate the instrument: With the software open, connect the computer to the SeqStudio™ Genetic Analyzer with an Ethernet cable.

Note: If the computer and SeqStudio™ Genetic Analyzer were already co-located when you installed the GeneMapper™ *ID-X* Software: Disconnect the network cable between the computer and the instrument, uninstall the software, then re-install it following these guidelines.

Conclusion

The SeqStudio™ Genetic Analyzer for HID generates high-quality data with Applied Biosystems™ PCR amplification kits analyzed with GeneMapper™ *ID-X* Software v1.6 that is comparable to existing platforms such as the 3500xL Genetic Analyzer.

General operations of the SeqStudio™ Genetic Analyzer for HID functioned properly for instrument control, calibration, run setup, and data collection.

The resolution algorithm used to align the capabilities of POP–1™ Polymer and POP-4™ Polymer performed successfully across the majority of the HID kits tested. The NGM Detect™ and Verifiler™ Plus kit samples demonstrated more n+1 artifact peaks off of stutter alleles when compared to the same data set on the 3500xL Genetic Analyzer/POP-4™ Polymer configuration. These results indicate that when interpreting data from these kits, it is important to recognize these artifacts in n+1 stutter positions, especially during mixture interpretation. HID laboratories should evaluate analysis settings (including GeneMapper™ *ID-X* Software filtering options and peak amplitude thresholds) during their internal validation. The use of minimum thresholds in this validation aimed to ensure a complete understanding of the differences between POP–1™ Polymer and POP-4™ Polymer resolution across all validated kits.

Capillary-to-capillary signal variation results demonstrated that signal in capillary 3 was consistently lower compared to the other capillaries. Capillary 3 performance across all instruments tested in the Sensitivity study produced results similar to the other capillaries: partial profiles were obtained down to 15.5 or 31 pg for each of the eight casework kits and full profiles were obtained for 125 pg samples using instrument-specific minimum thresholds. HID laboratories should consider signal variation when establishing run parameters and analytical thresholds during their internal validation.

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Revision history: MAN1001221 A (English)

Revision	Date	Description
A	22 January 2025	Changed the publication number to MAN1001221 from 100086084. Changes were added for SeqStudio™ Data Collection Software v1.2.5.
D	14 July 2022	Changes were added for SeqStudio™ Data Collection Software v1.2.4.
C	18 March 2020	Changes were added for SeqStudio™ Data Collection Software v1.2.1.
B	3 February 2020	Performance verification with the GlobalFiler™ IQC PCR Amplification Kit was added.
A	13 November 2019	New user bulletin for SeqStudio™ Data Collection Software v1.2.

The information in this guide is subject to change without notice.

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