

User Bulletin

Applied Biosystems 3500/3500xL Genetic Analyzer

June 2011

SUBJECT: Protocols for the analysis of AmpF \mathcal{L} STR ® PCR Amplification Kit PCR products and validation summary

This user bulletin describes protocols for processing AmpF \mathcal{L} STR ® PCR Amplification kit products on the Applied Biosystems 3500 Series Genetic Analyzers, using 3500 Data Collection software Version 1.0 and GeneMapper ® ID-X Software Version 1.2 or later. In addition, comprehensive information regarding the validation of the 3500 Series Genetic Analyzers for use with the AmpF \mathcal{L} STR ® Kits is also included. This user bulletin is intended to be used in conjunction with related 3500 documentation. References to relevant supplemental documentation are provided where appropriate.

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Overview

The 3500 Series Genetic Analyzers for Human Identification Applications are the first capillary electrophoresis (CE) instruments developed specifically to meet the requirements of Human Identification (HID) laboratories. Features of these analyzers include:

- 8-capillary 3500 System and 24-capillary 3500xL System provide higher throughput.
- Advanced thermal system design improves temperature control for more consistent data migration and contributes to reduced run times.
- Reduced signal variation from instrument to instrument, run to run, and capillary to capillary.
- Innovative “snap-in-and-go” consumable design with Radio Frequency Identification (RFID) technology helps to track and record key consumables data.
- HID specific workflow, pre-configured for AmpF \mathcal{L} STR ® kits, simplifies run setup and software navigation.
- Integrated data collection and QC analysis software provides real-time assessment of data quality and re-injection options.
- Solid State laser allows use with standard electrical power configurations and requires no heat removal.



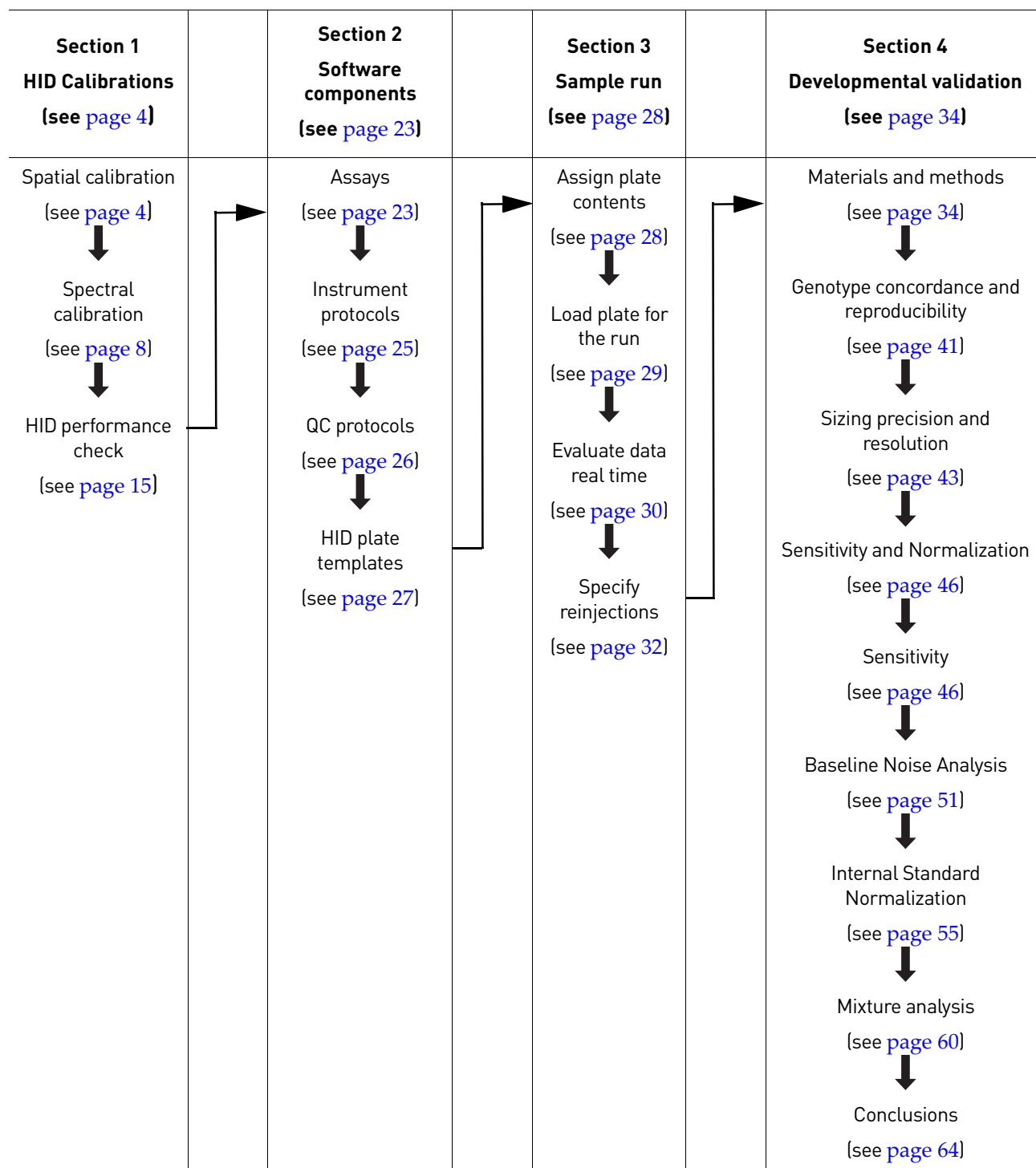
Supplementary references

For more detailed information on the 3500 Series Genetic Analyzers and related procedures, please refer to the following documents:

- *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661)
- *3500 and 3500xL Genetic Analyzers Quick Reference Card* (Part no. 4401662)
- *GeneMapper® ID-X Software Version 1.2 Quick Reference Card* (Part no. 4426482)
- 3500 Data Collection v1.0 Software Release Notes (can be found on the 3500 Data Collection Software CD)
- User Bulletin: Applied Biosystems 3500/3500xL Genetic Analyzer (Part no. 4445098)
- *GeneMapper® ID-X Software v1.2 User Bulletin* (Part no. 4462639)
- Frequently Asked Questions (FAQs) and supplementary information is contained on the Applied Biosystems website, http://marketing.appliedbiosystems.com/mk/get/3500_HID_FAQS.

Note: The information and procedures contained in this user bulletin relate specifically to the HID workflow and analysis of AmpFSTR® PCR Amplification kit products. Some of the recommendations may differ from information contained in the related documentation. Users should follow the specific recommendations contained in this guide unless directed specifically to related documentation.

Document overview



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Section 1: Preparing the Instrument for Human Identification STR Analysis

This section covers:

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Note: To start the 3500 Genetic Analyzer properly, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661). Also, perform any required maintenance tasks as indicated by the Instrument Gauges and Maintenance Notifications on the Data Collection Dashboard Screen described in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Upon installation of a new 3500 series instrument for HID analysis, and prior to performing HID analysis of AmpF^{STR}® PCR Amplification kit products for the first time, the field service engineer will complete the following procedures:

- Spatial calibration
- Spectral calibration
- HID Install Standard performance check

These procedures should be completed by individual laboratories or Life Technologies Field Service Engineers as necessary after installation.

Note: In addition to the guidance in this section, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661) for additional guidance pertaining to the instrument calibrations and performance check.

Save or print calibration reports!

IMPORTANT! The software does not save historical calibration or performance check results. Only the most recent information is maintained in the software; therefore, after performing a calibration, the calibration reports should be saved electronically for record keeping purposes. Follow the instructions for printing calibration records or saving them electronically referenced in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Performing a spatial calibration

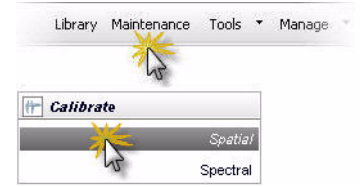
The 3500 Series Data Collection software uses images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where that signal is detected by the CCD camera. A spatial calibration is required to ensure correct alignment of the capillaries and optimal detection of the signal in each capillary.

A new spatial calibration is recommended when:

- A capillary array is installed, replaced or temporarily removed from the detection block
- The instrument is moved
- The detection cell door is opened

Perform a spatial calibration

1. Click **Maintenance** on the Dashboard. Click **Spatial** under the **Calibrate** heading on the left of the navigation pane shown below.
2. Select **No Fill**, or select **Fill** to fill the array with polymer before starting the calibration.



Status: Idle

Capillary	Position (pixels)	Spacing	Intensity
<div style="display: flex; justify-content: space-between;"> <div style="width: 60%;"> <p>Options</p> <p><input type="radio"/> Fill <input checked="" type="radio"/> No-Fill</p> <p><input checked="" type="checkbox"/> Perform QC Checks</p> <p>Start Calibration Stop Calibration</p> <p>Accept Results Reject Results</p> <p>Export Results...</p> </div> <div style="width: 35%;"> <p>Step 2</p> <p>Step 3</p> <p>Step 4</p> </div> </div>			

3. (Optional) Select **Perform QC Checks** if you want the system to check each capillary against the specified range for spacing and intensity. During the calibration, the software calculates:

Attribute	Calculation	Threshold
Average peak height	$\frac{\text{sum of all peak heights}}{\text{number of peaks}}$	<ul style="list-style-type: none"> 8-cap: 6400 RFU 24-cap: 3000 RFU
Uniformity (peak height similarity)	$\frac{\text{standard deviation}}{\text{average peak height}}$	0.2
Capillary spacing	max spacing – min spacing	2 pixels

4. Click **Start Calibration**. The display updates as the run progresses.



Note: If you engaged the optional QC Check, and the average of any of the QC values exceeds the threshold, a Spatial QC Check error message is displayed. Click **OK** and re-run the Spatial Calibration.

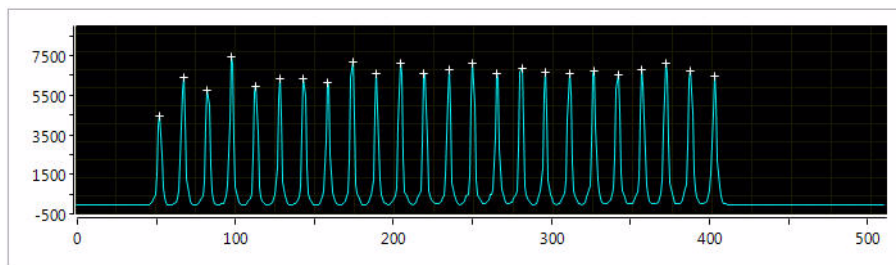
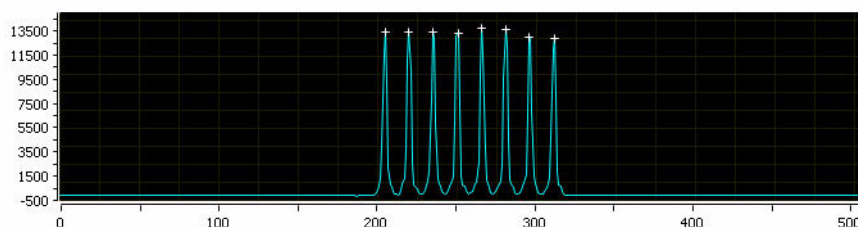
Evaluate the spatial calibration profile

When the run is complete, evaluate the spatial calibration profile to ensure that you see the following:

1. One sharp peak for each capillary. Small shoulders may be acceptable.
2. One marker (+) at the apex of every peak. No off-apex markers.
3. An even peak profile (all peaks about the same height).
4. Spacing should be between 13 and 16.

Note: If any peaks are lower than usual for the instrument or the heights drastically slope up or down, repeat the calibration with a fill. See the following figures for common acceptable Spatial Profiles.

Figure 1 Acceptable spatial profiles (8-capillary: top, 24-capillary: bottom)

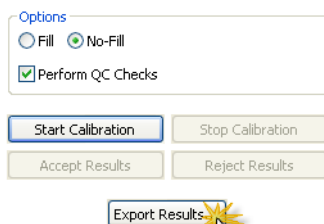


5. If the results meet the criteria above, click **Accept Results**. If the results do not meet the criteria listed above, click **Reject Results**, then refer to “Spatial calibration troubleshooting” in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661) for troubleshooting information.

Export or print spatial calibration results

To export spatial calibration results:

1. Click **Export Results**.



Note: Do not open the instrument door during a spatial calibration run.

2. Enter an export file name.

3. Select the export file type.



4. Click **Save**.

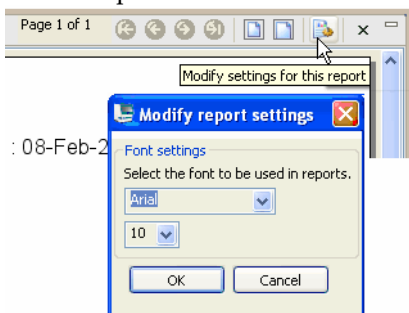
View or print spatial calibration report

Note: Spatial and spectral calibration reports include the date on which a capillary array is installed for the first time on the instrument. Install Standard reports use the most recent install date if a capillary array was removed and re-installed on the instrument.

1. Click **View Spatial Calibration Report**.

2. In the Report screen, click toolbar options to manipulate the report as needed.

Place the mouse pointer over an item for a description of the item.



3. To print the report, click  **Print**.

4. Close the report.



IMPORTANT! After performing a calibration, you can save the calibration report electronically for record keeping. *The software does not save historical calibration results. Only the most recent spatial calibration is maintained in the software.* In the Printer dialog box, select **CutePDF Writer** as the printer to print the report to a PDF file.

For additional information on performing and evaluating a spatial calibration, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Performing a spectral calibration

A spectral calibration creates a matrix that corrects for the overlapping fluorescence emission spectra of the dyes. Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes. The goal of multicomponent analysis is to effectively correct for spectral overlap and minimize the presence of artifacts, such as spectral pull-up, in the data.

A new spectral calibration is recommended when:

- The capillary array is changed or temporarily removed from the detection block.

Note: Better dye separation is achieved when a new spectral calibration is performed after removal or replacement of the capillary array and is therefore highly recommended.

- The instrument is moved.
- The laser or CCD camera has been realigned/replaced by a service engineer.
- An increase in (pull-up and/or pull-down peaks) is observed.
- A new dye set is used on the instrument.
- The capillary array length or polymer type is changed.
- The detection cell door is opened (not required by the software, but recommended).

Prepare the instrument

1. If necessary, perform a spatial calibration as described in the previous section.
2. In the Dashboard, check consumable status and ensure that:
 - Consumables are not expired
 - Adequate injections remain for consumables
3. Ensure that the buffer levels are at the fill lines.
4. Set the oven temperature to 60°C, then click **Start Pre-heat**.

Note: Preheating for approximately 30 minutes helps mitigate subtle first-run migration rate effects. The pre-heat function automatically turns off after 2 hours.

5. Check the pump assembly for bubbles and run the Remove Bubble wizard if needed.

Prepare the standard calibration plate

For all chemicals, read the SDS, and follows the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Choose the appropriate Dye Set and Matrix Standards.

For AmpF ℓ STR $\text{\textcircled{R}}$ kits that use a...	And use...	AmpF ℓ STR $\text{\textcircled{R}}$ kit Examples
5-dye system, which includes the following dyes: <ul style="list-style-type: none"> • 6FAMTM • VIC$\text{\textcircled{R}}$ • NEDTM • PET$\text{\textcircled{R}}$ • LIZ$\text{\textcircled{R}}$ 	Matrix Standard Set DS-33 (Dye Set G5)	<ul style="list-style-type: none"> • Identifiler$\text{\textcircled{R}}$ Plus PCR Amplification Kit • Identifiler$\text{\textcircled{R}}$ PCR Amplification Kit • Identifiler$\text{\textcircled{R}}$ Direct PCR Amplification Kit • NGMTM PCR Amplification Kit • NGM SElectTM PCR Amplification Kit • Yfiler$\text{\textcircled{R}}$ PCR Amplification Kit • SEfiler PlusTM PCR Amplification Kit • Other five-dye AmpFℓSTR$\text{\textcircled{R}}$ kits
4-dye system, which includes the following dyes: <ul style="list-style-type: none"> • 5FAMTM • JOETM • NEDTM • ROXTM 	Matrix Standard Set DS-32 (Dye Set F)	<ul style="list-style-type: none"> • Profiler Plus$\text{\textcircled{R}}$ PCR Amplification Kit • COfiler$\text{\textcircled{R}}$ PCR Amplification Kit • Profiler Plus$\text{\textcircled{R}}$ ID PCR Amplification Kit • SGM Plus$\text{\textcircled{R}}$ PCR Amplification Kit • Other 4-dye AmpFℓSTR$\text{\textcircled{R}}$ kits

2. Thaw and thoroughly vortex the Matrix Standard tube and spin the tube briefly in a microcentrifuge.
3. Prepare the Matrix Standard by combining the following in a labeled 1.5-mL colorless microcentrifuge tube:

Reagent	Matrix Standard Set DS-33 (Dye Set G5)	Matrix Standard Set DS-32 (Dye Set F)
Matrix Standard (Part no. 4345833)	3 μ l	4 μ l
Hi-Di TM Formamide (Part no. 4311320)	297 μ l	246 μ l
Final Volume (1.5mL tube)	300 μ l	250 μ l


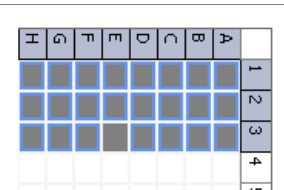
4. Vortex thoroughly and spin the mixture briefly in a microcentrifuge.
5. Heat the tube at 95°C for 5 minutes to denature the sample and immediately place the tube on ice for 3 minutes.

Note: In some instances, if the spectral calibration is of sub-optimal quality as determined by inspection of the calibration data or the presence of excess pull-up in the test sample data, an improved spectral may be obtained by eliminating the chilling step after heating.

6. Load 10 μ L of the spectral calibration mixture into each well.

Note: For a 96-well plate, use 10 μ L of mixture per well. For a 384-well plate, use 5 μ L of mixture per well. Add the mixture to wells A1-H1 on a 3500 instrument or A1-H3 on a 3500xL instrument and load the plate assembly on the instrument.

IMPORTANT! You are not required to create a plate for the calibration. The software uses predetermined positions for the calibration.

8-capillary 96-well plate	A1 through H1	
24-capillary 96-well plate	A1 through H1, A2 through H2, and A3 through H3	

- Centrifuge the plate to ensure that the samples are at the bottom of the wells.
IMPORTANT! If the reagents of any well contain bubbles or are not located at the bottom of the well, briefly centrifuge the plate, remove the plate from the centrifuge, and verify that each sample is positioned correctly in the bottom of its well.

Prepare the plate assembly

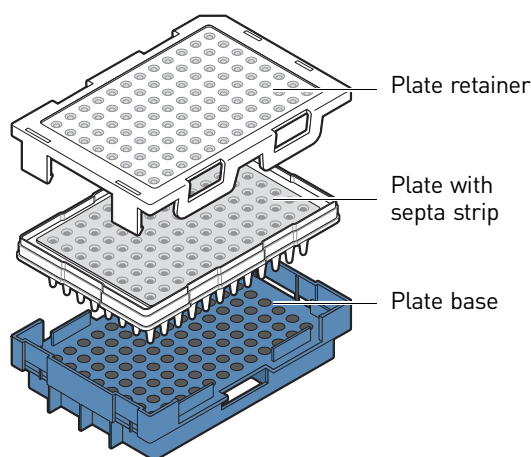
- Place the sample plate into the plate base.
- Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.

IMPORTANT! Make sure to use the correct plate base for standard plates versus 8-tube strips and fast plates. Using the wrong plate base may affect performance.

- Snap the plate retainer (cover) onto the plate, septa, and plate base.

- Verify that the holes of the plate retainer and the septa strip are aligned. If not aligned, re-assemble and then assemble the plate assembly.

IMPORTANT! The array tips will be damaged if the plate retainer and septa strip holes do not align correctly.



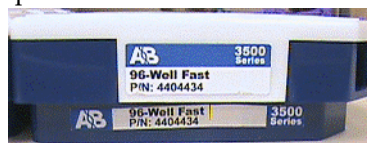
Load the plate in the instrument and initiate the calibration

IMPORTANT! Do not change electronic signature settings during a spectral calibration.

IMPORTANT! If you change polymer type, spectral calibrations for the original polymer type are not retained.

The following are the basic steps to initiate the spectral calibration. Refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661) for more information.

1. Place the plate in the autosampler with the labels facing outward and the notched corner of the plate in the notched corner of the autosampler.



2. Close the instrument door to re-initialize the instrument.
3. Click **Maintenance** on the dashboard.
4. Click on **Spectral** under **Calibrate** in the left navigation pane.
5. Select the number of wells and plate position on the instrument.
6. Select the chemistry standard and dye set.
(Optional) Select **Allow Borrowing** to have a failed capillary use a neighboring capillary's spectral data. Refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661) for more information.

5 — Number of Wells: 96 96-Fast 384

6 — Plate Position: A B

7 — Capillary Run Data

Capillary	1	2	3	4	5	6	7	8
Run 1								
Run 2								
Run 3								
Overall								

Legend: ■ Passed ■ Failed ■ Borrowed ■ Not Calibrated

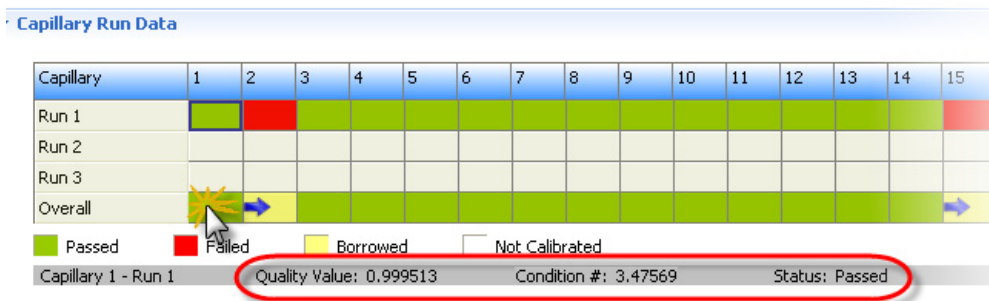
Intensity vs Scan Number

Intensity vs Pixel Number

Accept Reject

7. Click **Start Run**. The following occurs:
 - The system sets up three injections.
 - The Capillary Run Data display updates after each injection is complete.
 - The status bar updates during Run 1.
IMPORTANT! The status bar does not update during Run 2 or Run 3.
 - Passing capillaries are shown in green and failing capillaries are shown in red. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed.

- To display the result for each capillary (spectral data, Quality Value, and Condition Number) below the run results table, click a capillary in the table.



Note: The ranges that the software uses to determine if a capillary passes or fails are:

Dye Set	Quality Value Minimum	Condition Number Maximum
AnyDye	0.8 (default)	20.0 (default)
F	0.95	8.5
G5	0.95	13.5

- If the data for all capillaries meet the acceptance criteria, click the **Accept Results** button.

Note: If the data for all capillaries does not meet the acceptance criteria, click the **Reject Results** button, repeat the analysis and begin the troubleshooting process. For more information on troubleshooting spectral calibrations refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Evaluate the spectral calibration data

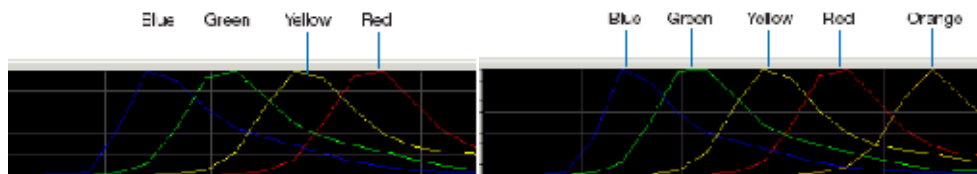
This section contains basic information regarding evaluation of the spectral calibration data. For more information regarding evaluation of spectral calibration data, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Excessive pull-up signal in the analyzed data is the most common manifestation of a poor quality spectral calibration. The following characteristics will assist the user in determining if the spectral calibration may be suitable:

- Order of the peaks in the profile from left to right:
 - F:** Blue, green, yellow, red
 - G5:** Blue, green, yellow, red, orange
- No extraneous peaks in the raw data profile.
- No gross dips, overlaps or other irregular morphology.
- Spectral profile peaks are separate and distinct.

- Ideally, spectral peak heights should be approximately similar to the peak heights typically analyzed by the laboratory.

Figure 2 Order of the 4-dye (left) and 5-dye (right) spectral calibration peaks



A spectral calibration automatically sets up three injections. The number of injections performed depends on the number of capillaries that pass or fail during an injection and whether you select Allow Borrowing.

When borrowing is disabled, all capillaries must meet passing criteria. When borrowing is enabled, all capillaries have to pass within the borrowing limits.

For a successful calibration, there must be at least one passing attempt for each capillary within the three injection profile. A passing attempt is defined within the parameters set regarding borrowing and are described in the following section and in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Spectral calibrations with borrowing disabled

When borrowing is disabled, any failure in the quality value or condition number will prompt a reinjection of the matrix standards and new attempt at obtaining acceptable values. If, after all three injections, no runs are acceptable, the calibration will fail.

Spectral calibrations with borrowing enabled

When borrowing is enabled, the user can define the number of acceptable spectral failures they will allow for a 3500xL (up to 3 maximum). For a 3500 series Genetic Analyzer, only one failure is allowed.

Any failure in the quality value or condition number will prompt a reinjection of the matrix standards and new attempt at obtaining acceptable values. If, after all three injections, no runs are acceptable, the calibration will fail.

The results displayed when you click a borrowed capillary are the passing results borrowed from the adjacent capillary. To determine the reason that a capillary fails, view the spectral calibration report.

Figure 3 Example of borrowing in a 3500 Series Genetic Analyzer

Capillary	1	2	3	4	5	6	7	8
Run 1	Passed	Passed	Passed	Passed	Passed	Passed	Borrowed	Failed
Run 2	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Run 3	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Overall	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed

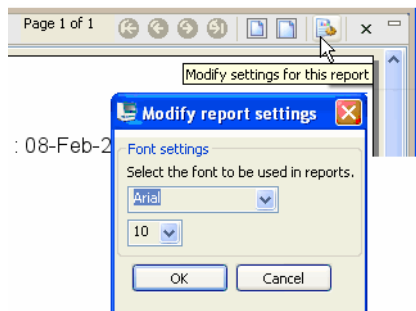
■ Passed
 ■ Failed
 ■ Borrowed
 ■ Not Calibrated

Export or print spectral calibration results

View and print a spectral calibration report

Note: Spatial and spectral calibration reports include the date on which a capillary array is installed for the first time on the instrument. Install Standard reports use the most recent install date if a capillary array was removed and re-installed on the instrument.

1. Click **View Spectral Calibration Report**.
2. In the Report screen, click toolbar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.



3. To print the report, click  **Print**.

4. Close the report.

IMPORTANT! After performing a

calibration, save the calibration report electronically for record keeping. The software does not save historical calibration results. Only the most recent spectral calibration for each dye set is maintained in the software. To save the file electronically as a PDF, in the Printer dialog box, select **CutePDF Writer** as the printer.



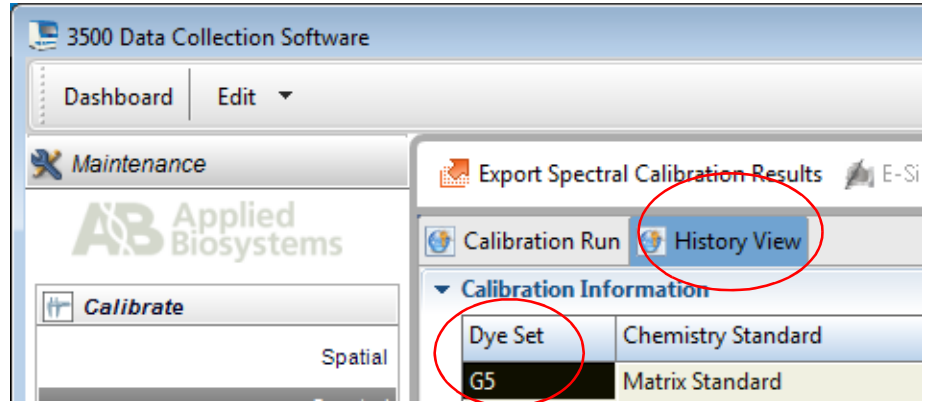
Export spectral calibration results

To export spectral calibration results:

1. Click **Export Spectral Calibration Results**.
2. Specify an export file name and location.
3. Click **Save**.

View the spectral calibration history

Select **History View**, then select a dye set to view the associated calibration history.



Performing a HID Install Standard performance check

This section contains the basic information needed for performing the HID Install Standard Performance Check. For more information on preparing the instrument and running an instrument performance check, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).



Note: While the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661) recommends users perform the HID Install Standard Performance Check monthly, this is not a mandatory requirement for ongoing instrument operation in an HID laboratory. The HID Install Standard Performance Check is intended to be performed at installation by a Life Technologies Field Service Engineer. This procedure may be repeated by the Service Engineer during maintenance or service visits. It is not necessary to repeat this procedure after install and it is recommended that each HID laboratory determine the appropriate Quality Control measures to verify correct instrument operation. These measures may or may not include repeated injections of allelic ladder to verify precision and correct genotyping instead of performing the HID Install Standard Performance Check.

Prepare the reagents

1. Vortex the AmpFSTR® Identifiler®, Identifiler® Direct, or Identifiler® Plus allelic ladder, GeneScan™ 600 LIZ® Size Standard v2.0, and Hi-Di™ Formamide (if stored in aliquoted tubes) and spin each tube briefly in a microcentrifuge.
2. Prepare performance-check chemistry by combining the following in a labeled colorless 1.5-mL microcentrifuge tube:

Reagent	Volume	
	3500	3500xL
Allelic Ladder	8 µL	24 µL
GeneScan™ 600 Liz® Size Standard v2.0	4 µL	12 µL
Hi-Di™ Formamide	68 µL	204 µL

- Denature the sample at 95°C for 3 minutes, then store on ice until ready to use.
- Aliquot 10 µL of the mixture into each appropriate well of the plate as shown below.
- Load the standards in injection position 1 in the plate as shown:
IMPORTANT! You do not create a plate for the performance check. The software uses predetermined positions for the performance check run. You cannot specify standard location on the plate. If you do not place standards in the positions indicated, the calibration will fail.

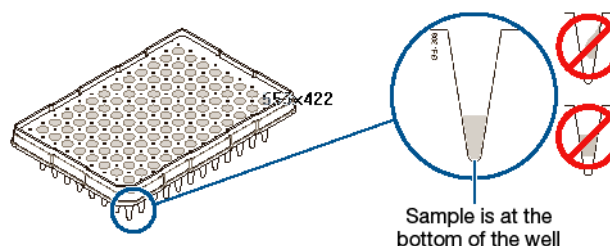
8-capillary 96-well plate	A1 through H1	
24-capillary 96-well plate	A1 through H1, A2 through H2, and A3 through H3	

- Briefly centrifuge the plate containing the standards.

- Verify that each sample is positioned correctly in the bottom of its well.

IMPORTANT! If the reagents of any well contain bubbles or are not located at the bottom of the well,

briefly centrifuge the plate, remove the plate from the centrifuge, and verify that each sample is positioned correctly in the bottom of its well.



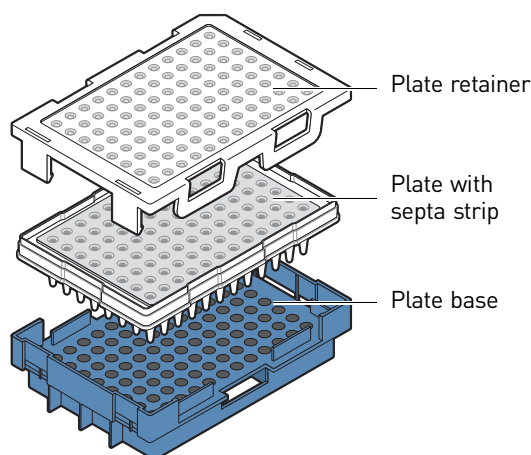
Prepare the plate assembly

- Place the sample plate into the plate base.

- Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.

IMPORTANT! Make sure to use the correct plate base for standard plates versus 8-tube strips and fast plates. Using the wrong plate base may affect performance.

- Snap the plate retainer (cover) onto the plate, septa, and plate base.

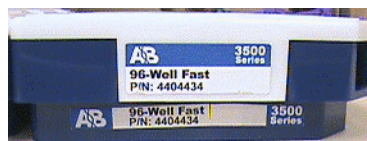


- Verify that the holes of the plate retainer and the septa strip are aligned. If not aligned, re-assemble and then assemble the plate assembly.

IMPORTANT! The array tips will be damaged if the plate retainer and septa strip holes do not align correctly.

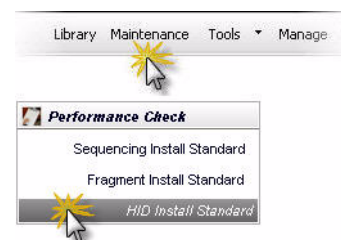
Load the plate in the instrument

- Place the plate in the autosampler with the labels facing you (or the instrument door) and the notched corner of the plate in the notched corner of the autosampler.



- Close the instrument door to re-initialize the instrument.

- Access the HID Install Standard screen: Select **Maintenance**, then select **HID Install Standard** in the navigation pane.



- Select the plate type and plate position in the instrument.

Note: You do not create a plate for the performance check. The software uses predetermined positions for the run. You cannot specify the standard locations on the plate.

- Click **Start Run**.

Step 4 →

Step 5 →

The screenshot shows the software interface with the following sections:

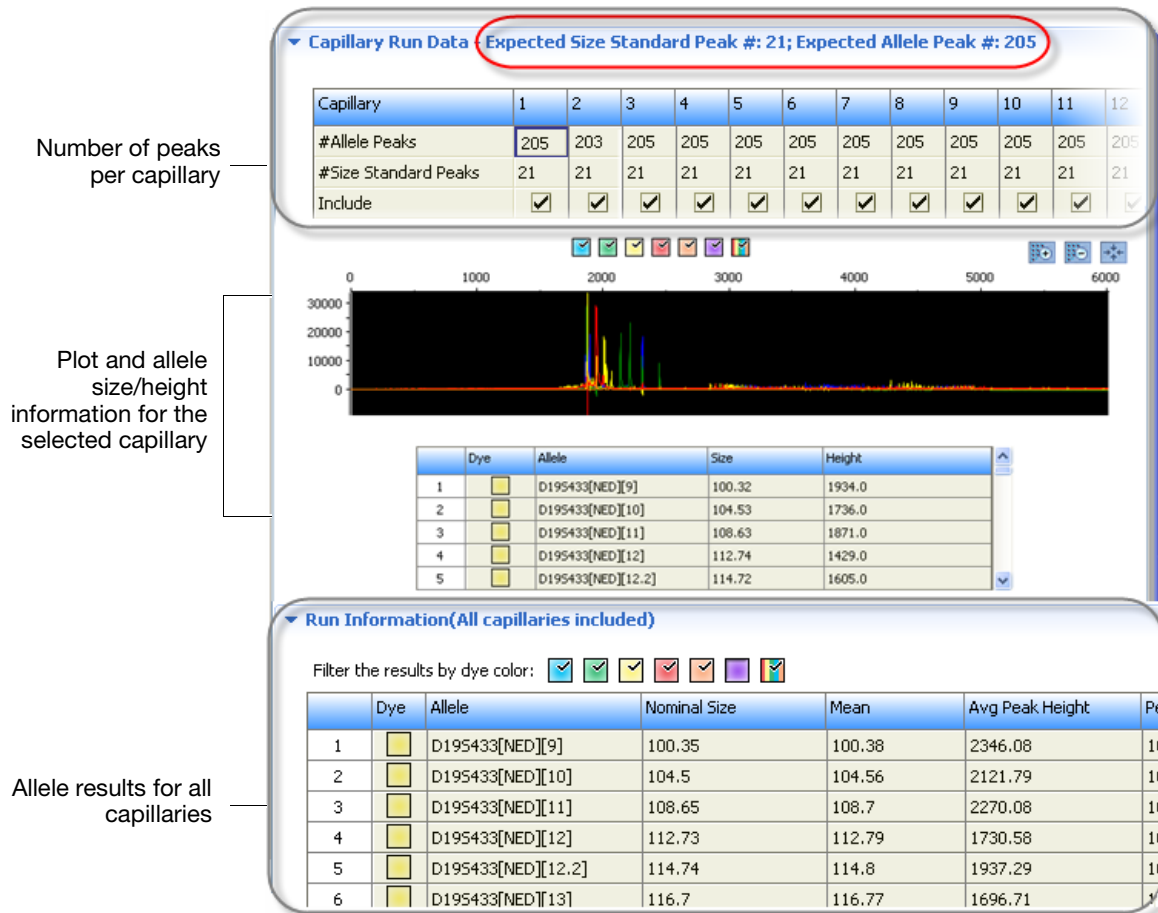
- Calibration Settings:** Number of Wells: 96 96-FastTube 384; Plate Position: A B
- Current Instrument Consumables:** Polymer Type: POP4; Capillary Length: 36cm
- Start Run** button
- Status:** Ready
- Capillary Run Data:** A table with 21 columns (Capillary 1-21) and 3 rows (#Allele Peaks, #Size Standard Peaks, Include). All 'Include' checkboxes are checked.
- Capillary Information:** A graph showing a flat line at 0 on the y-axis (0 to 50000) against a time axis (0 to 32000). Below the graph is a table with 5 rows (Dye 1-5) and 4 columns (Allele, Size, Height).
- Run Information (All capillaries):** Accept Reject buttons

What you see during a run

The system performs one run and indicates the number of observed allele and size standard peaks.

The Capillary Run Data display updates after the run is complete. The number of observed size standard and allele peaks is shown. Results for each allele are shown at the bottom of the screen in the Run Information table.

Note: The following example is for the HID install standard.



Number of peaks per capillary

Plot and allele size/height information for the selected capillary

Allele results for all capillaries

How the software determines passing and failing capillaries for the fragment/HID performance check

The software evaluates peaks in the data for each capillary. To be identified as a possible allele, peaks must be within the following ranges (nominal allele size, or reference bin size, is hard-coded):

- All markers except TH01: ± 0.7 bp of nominal size for the allele
- TH01: ± 0.5 bp of nominal size for the allele

For all peaks that are within the nominal size range, the software calculates the Average Peak Height and the Sizing Precision. Peaks that meet the following thresholds pass.

Result	Description	Threshold
Avg Peak Height	Average of peak heights for observed allele peaks of the included capillaries.	Fragment: >175 RFU HID: >400 RFU
Sizing Precision	Standard deviation of the observed allele fragment sizes.	≤ 0.15 for expected alleles
Pass/Fail	Alleles with a sizing precision and average peak height that do not meet thresholds fail. Note: Review the data for failed alleles as described below.	

Result	Description
For information only	
Nominal Size	Expected allele fragment peak size (bp).
Mean	Average fragment size for the observed allele peaks.
Peak Height % >Min	Percentage of observed allele peaks with a peak height above the minimum threshold.
Sizing Accuracy	Difference between the allele size and the mean allele size.

Evaluate fragment/HID install standard data

1. Examine the number of size standard and allele peaks found for each capillary.

Note: The number of expected peaks shown below is for the HID install standard.

Expected	Capillary Run Data Expected Size Standard Peak #: 21; Expected Allele Peak #: 205												
Observed	Capillary	1	2	3	4	5	6	7	8	9	10	11	12
	#Allele Peaks	205	203	205	205	205	205	205	205	205	205	205	205
	#Size Standard Peaks	21	21	21	21	21	21	21	21	21	21	21	21
	Include	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

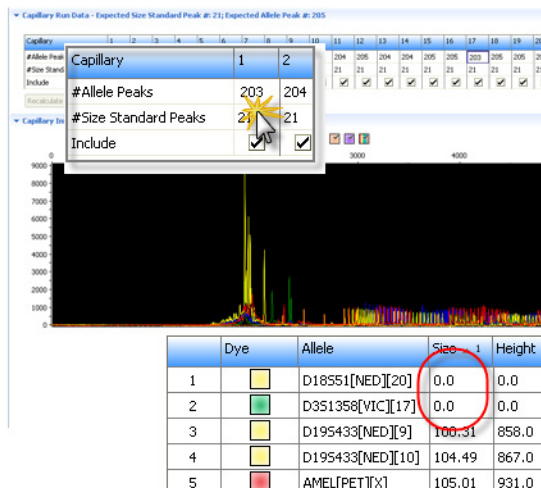
2. If the expected number of alleles and size standard peaks are found, click **Accept Results**.

If the expected number of alleles and size standard peaks are not found, troubleshoot as described below.

Troubleshoot

1. Click a capillary with fewer than the expected number of peaks to display detailed information for each allele in the table below the plot.
2. Double-click the **Size** column to sort results and identify the alleles that were not found.

A “0” Size value indicates that an allele falls outside the expected size window (Nominal Size ± 0.7 bp or ± 0.5 for TH01).

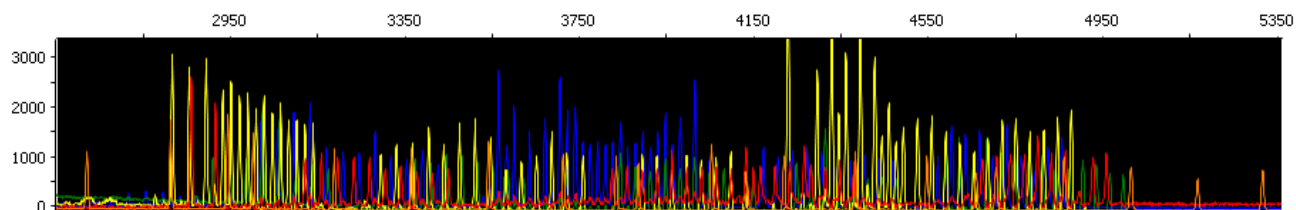


3. Troubleshoot failing data:
 - a. Analyze the install standard data files in your secondary analysis software (GeneMapper® ID-X Software v1.2 or later using Identifiler® kit panels and bins).
 - b. Evaluate the failed data and examine the alleles not found by the 3500 Series Data Collection Software.
 - c. If the alleles are properly called in the secondary analysis software, you can:
 - Deselect the Include checkmark for a capillary.
 - Click **Recalculate**.
 - Accept the install standard results.

Note: The GeneMapper® ID-X Software may identify alleles not identified by the 3500 Series Data Collection Software because of the bin-offsetting feature (which uses the observed alleles in the allelic ladder samples to adjust the reference bin locations for samples).

- d. If the alleles are not properly called:
 - (Optional) Click **View Detail Report** to save a record of the failed run. To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer. For more information, see [“Save historical performance check reports \(.pdf\) for record keeping” on page 21](#).
 - Click **Reject Results**.
 - Rerun the install standard to determine if the problem may be caused by sample preparation, a poor injection, a capillary issue, or a system problem (which may require instrument service). For more information, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661) for fragment/HID install standard troubleshooting information.

Example HID install standard results



View previously run install standards


Select **History View**, then select an install standard to view the associated calibration information.

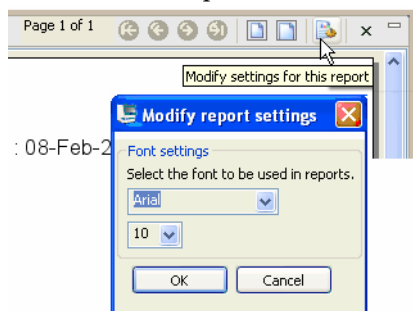
View and print a fragment or HID install standard detail report

IMPORTANT! Ensure that all dyes are selected before viewing the report. The report will contain incomplete data if all dyes are not selected.

Note the following:

- Install standard reports include the most recent install date if a capillary array was removed, then re-installed on the instrument. Spatial and spectral calibration reports include the date on which a capillary array is installed on the instrument for the first time.
- The sorting in the Install Standard screen is not applied to the report.
- To generate a report for a failed installation standard run, you must do so before you click Reject Results.

1. Click  **View Detail Report**.
2. In the Report screen, click toolbar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.



3. To print the report, click  **Print**.
4. Close the report.



Save historical performance check reports (.pdf) for record keeping

IMPORTANT! After performing a performance check, save the performance check report electronically for record keeping. The software does not save historical calibration results. Only the most recent spectral calibration for each dye set is maintained in the software.

1. Click  **View Detail Report**.
2. Click  **Print**.

3. In the Printer dialog box, select **CutePDF Writer** as the printer.
4. Specify a name and location for the report.

Note: While the number of expected allele peaks is 205, the actual number observed may be lower than 205 and may still be acceptable. The reference file for these peaks in the software is a static reference, meaning that no bin offsetting has been applied. Therefore, it is advisable that any questionable data or runs be analyzed in Genemapper® *ID-X* software (v1.2 or later) to determine if the instrument is performing as expected for AmpFSTR® kits. These files are located at *X:\Applied Biosystems\3500\Data\[Run Date]* and stored according to the date the run was performed. The run date can be identified by examining the **History View** tab in the HID Install Standard portion of the Maintenance menu.

Section 2: 3500 Data Collection Software Components

This section highlights the features of the 3500 data collection software that are designed to enhance the human identification laboratory workflow:

Assays	23
Instrument protocols	25
QC protocols	26
HID plate templates	27

For more information regarding basic run set up for the AmpF Φ STR[®] kits, refer to *the Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

The 3500 Data Collection software is designed to minimize the number of steps required to initiate a sample run while maximizing flexibility. The software has been pre-configured to streamline the set up and analysis of the AmpF Φ STR[®] kits. Assays and Plate Templates are the primary information that the data collection software uses when performing HID analyses on the 3500 Series Genetic Analyzers. Users can only enter (or import) sample names and assign an Assay to begin a run. This section contains more information on creating and using these files to optimize workflow efficiency.

Assays

An assay is a configurable component of the software that contains all the information the instrument requires to perform a run.

Each assay contains:

- One or more instrument protocols which define the chemistry or kit being analyzed and the run parameters
- A primary QC analysis protocol

The 3500 data collection software contains a library of assay templates pre-configured to support the AmpF Φ STR[®] kits. New assays can be created and added to the library by the user. For more information on creating assays, refer to *the Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661). The following is an example of the validated configuration for the HID Assay for the Identifiler[®] kit:

Edit Assay Copy Of IF+Norm_POP4_xl

Setup an Assay

* Assay Name: Locked Color:

Application Type:

Protocols

Do you wish to assign multiple instrument protocols to this assay? No Yes

* Instrument Protocol:

* QC Protocol:

GeneMapper IDX Protocol:

The default HID Assay settings contain one instrument protocol, which determines the instrument run parameters, and one QC protocol which assists the user in evaluating the data for reinjections or reanalysis. When the user imports an assay for use with a particular sample run, the option is available to assign multiple instrument protocols to enable subsequent injections of the same sample set to be performed under different conditions. The user is also able to change the QC protocol selected for the sample run.

Instrument protocols

An instrument protocol contains the parameters that control the instrument during data acquisition. An instrument protocol is a required element of an assay for all applications. While defined Instrument Protocol parameters are established for validated protocols, users may create their own protocols to fit laboratory procedures. For more information on creating instrument protocols, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661). The following is an example of a validated HID instrument protocol for the Identifier® kit:

Copy Of IF+Norm_POP4_xl - Edit Instrument Protocol HID36_POP4xl_G5_NT3800

Setup an Instrument Protocol

Application Type: HID Capillary Length: 36 cm Polymer: POP4

Dye Set: G5

Instrument Protocol Properties

* Run Module: HID36_POP4xl

* Protocol Name: HID36_POP4xl_G5_NT3800 Locked

Description:

Oven Temperature (°C): 60 Run Voltage (kVolts): 15.0 PreRun Voltage (kVolts): 15 Injection Voltage (kVolts): 1.2

Run Time (sec.): 1210 PreRun Time (sec.): 180 Injection Time (sec.): 24 Data Delay (sec.): 1

Advanced Options

Following values are not recommended to be changed.

Voltage Tolerance (kVolts): 0.7 Voltage # of Steps (nk): 20 Voltage Step Interval (sec.): 15

First Read Out Time (ms): 160 Second Read Out Time (ms): 160

Normalization Target: 3800.0 Normalization Factor Threshold Min: 0.3 Normalization Factor Threshold Max: 3.0

Close Apply to Assay Save to Library

IMPORTANT! The software allows users to define instrument protocol parameters. Any adjustments made to the validated parameters defined by Applied Biosystems, should be supported by appropriate validation studies. The Normalization Target value should be adjusted according to the individual laboratory's results.

Note: “Validated instrument protocol specifications” on page 67 contains a complete list of all validated preloaded Assays and Instrument Protocols.

QC protocols

A QC protocol is a required component of an assay and determines how primary analysis of the data will occur in the Data Collection software. A QC protocol defines peak detection, threshold, and sizing values applied during primary analysis. This primary analysis allows the user to view sized data in the Data Collection software for completed injections while additional run data is still being collected.

Note: The QC analysis protocol is intended to assist users in performing a basic quality assessment of the data during a run. A more complete assessment of the final data can be performed using GeneMapper® ID-X Software v1.2 or later. To simplify data analysis, it may be advisable for users to align the parameters defined in the QC protocols in the 3500 Data Collection software with those used in GeneMapper® ID-X Software.

For more information on creating QC protocols, please refer to *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

The following is an example of a QC protocol for the Identifier® kit as validated at Applied Biosystems utilizing the Internal Standard Normalization function:

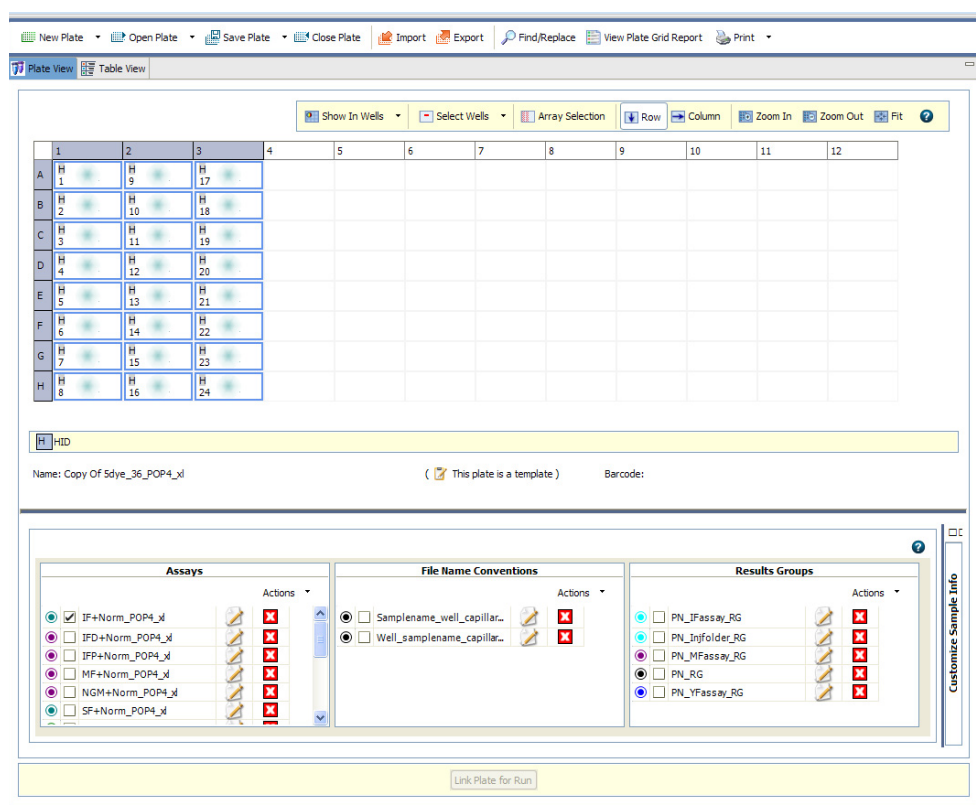
IMPORTANT! Parameters within the QC protocol may be adjusted following appropriate validation by the laboratory. The Peak Amplitude Threshold settings shown above are defined based on developmental validation studies and noise analysis performed by Applied Biosystems. Evaluations in each individual HID laboratory will assist the user in setting appropriate Peak Amplitude Thresholds for their specific workflow.

HID plate templates

The instrument workflow may be further streamlined through the use of plate templates. Users may create their own plate templates, modify user created templates, or customize the factory-provided defaults to fit laboratory specific procedures. For more information on creating a plate from a template, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Templates allow users to easily manage the options available and restrict the choices to simplify the set up of a plate. In the following example, a new template was created to allow the user to select from multiple Identifiler® kit assays, two File Name Conventions, and five Results Groups. This example shows sample names (numbers) pre-filled, but the user may choose to type each name individually or utilize the sample import feature.

Example of a new plate template configured by the user.



Users are provided with multiple options for visualizing the plate layout, as well as assigning sample names, Assays, File Name Conventions, and Results Groups. It is suggested that users name custom Library objects with titles that reflect the important parameters defined within the template.

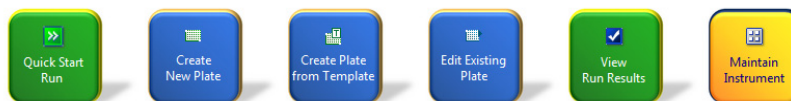
Section 3: Sample Run Workflow and Run Setup

This section covers:

Common operations in the dashboard	28
Assign plate contents	28
Load plates for the run	29
Evaluate data in real time	30
Specify re-injections	32

For a simplified list of steps required for setting up for a run, refer to the *3500 and 3500xL Genetic Analyzers Quick Reference Card* (Part no. 4401662). While many options are available for creating a plate, in this section we illustrate a run set up using the “Create Plate from Template” menu option. For illustration purposes, specific conditions for running the validated parameters for the Identifiler® Plus kit on a 3500xL will be detailed. For more information on creating a plate from a template, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Common operations in the dashboard



1. Define plate properties:
 - a. In the Dashboard, click **Create Plate from Template**, Choose “HID” from the Filter menu, then select the appropriate Plate Name
Note: For the Identifiler® Plus kit, choose “5dye_36_POP4_xl”.
 - b. Click **Open** to define the plate details. Enter the Plate Name in the “Name” field.
Note: A checkbox for **Perform Auto-Analysis** is present here. To perform autoanalysis, a full version of the GeneMapper® ID-X Software v1.2 or later needs to be co-installed with the 3500 Data Collection software which limits the number of clients that can connect to the full install. Therefore auto-analysis is not supported for human identification applications on the 3500 series Genetic Analyzers and is not recommended for HID laboratories.
 - c. Click the **Assign Plate Contents** button at the bottom of the screen.

Assign plate contents

1. Enter sample names:
 - a. Click the **Plate Map** or **Table View** tab, then in the plate view, click a well to select it.

-
- b. Type a sample name directly into the field, then press **Enter**.
 - c. (*Optional*) You can right-click and use the Fill Series options to populate all sample names on the plate.

Note: Users may import sample names by using the “Import” option at the top of the window. Users are advised to manually create a desired plate first and select “Export” to define a standardized and compatible Import file template. This file may then be used as the plate import template, streamlining the import of plates in future runs.

IMPORTANT! Use of Microsoft Excel templates, or .txt files generated from Microsoft Excel worksheets, may contain hidden characters that render the format of the file unsuitable for sample import. Users may choose to use .csv files for sample import to allow editing in Excel while preserving the proper format. Some versions of Microsoft Excel or laboratory generated worksheets may be incompatible for use for any file import template editing.

- d. To select the Assay, File Name Conventions and Results Groups, select the wells you want to change, then select the check box(es) of interest. The assay is the only mandatory selection.

Note: For the Identifiler® Plus kit, choose “IFP+Norm_POP4_x1”.

IMPORTANT! To utilize internal standard normalization, select an assay that contains a sizecalling protocol or a QC protocol that specifies an internal standard normalization size standard (e.g. GeneScan™ 600 LIZ® Size Standard v2.0).

- e. (*Optional*) In the Customize Sample Info pane, select the sample type (Sample, Positive Control, Negative Control, Allelic Ladder, Hi-Di™, or No Data).
- f. Save the changes made by selecting **Save Plate**, then **Save**, **Save As**, or **Save as Template**.
- g. Click **Link Plate for Run** in the Assign Plates for Run screen or **Load Plates for Run** in the navigation pane to assign the plate and specify the position of the plate in the autosampler.

Load plates for the run

1. Inspect the information presented on the screen. Confirm that the linked plate is in the correct position of the autosampler.
2. Click **Start Run** to begin the run immediately, or review the injection list by clicking **Create Injection List** in the Load Plates for Run screen.

Note: In the **Preview Run screen**, users may rearrange the injection list or assign duplicate injections. The re-inject option is available upon completion of the first injection and prior to cessation of all runs in the injection list.

3. Click **Start Run** if the **Create Injection List** option was chosen.

Evaluate data in real time

Review results for the currently running plate

After each injection, click **View Fragment/HID Results in Review Results**. If you access the View Fragment/HID Results screen while an instrument run is in progress, the samples table lists results for completed injections in the current run.

Select one or more samples in the samples table to display their data in the plot view and sizing table view.

The screenshot displays the 'Samples View' window. At the top, there are tabs for 'HID Samples' and 'Fragment Samples', and a 'Show/Hide Samples' dropdown set to 'Show All'. Below this is a table with columns: Sample Name, Sample Type, Size Standard, Assay Name, Offscale, and Sizing Quality. Three samples are listed, all with 'Pass' status. Below the table are icons for 'Pass', 'Fail', and 'Check'. The main area contains four chromatograms labeled '1ng-6-B03.fsa', '1ng-7-C01.fsa', '1ng-8-C02.fsa', and '1ng-9-C03.fsa', each showing a peak at approximately 325-327. The bottom section is the 'Sizing Table View' with columns: Dye Color, Dye/Sample Peak, Sample File Name, Size, Height, and Area in Point. It lists six peaks with their respective data.

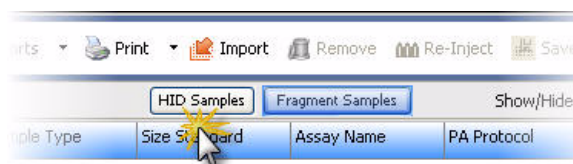
	Dye Color	Dye/Sample Peak	Sample File Name	Size	Height	Area in Point
49	Blue	B, 52	1ng-7-C01.fsa	327.21	1246	7962
50	Blue	B, 55	1ng-9-C03.fsa	327.18	1369	8360
51	Blue	B, 55	1ng-6-B03.fsa	327.16	1218	7966
52	Yellow	Y, 68	1ng-8-C02.fsa	325.75	59	424
53	Green	G, 57	1ng-6-B03.fsa	325.5	1190	8002
54	Green	G, 57	1ng-8-C02.fsa	325.49	1230	8275
55	Green	G, 57	1ng-7-C01.fsa	325.45	1224	8036

Review previously run samples

If you access the View Fragment/HID Results screen when no run is in progress and no plate is linked, no samples are listed. (If the plate from the most recent run is linked, the results from that plate are displayed.)

To view results for samples other than those from the most recent run, click **Import**, then select the samples to review.

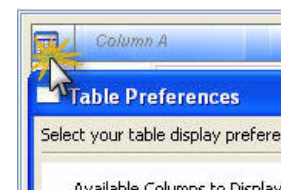
Note: By default, the Fragment Samples view is selected. If you are importing HID files, click **HID Samples**.



Review sample quality

1. In the samples view, click the **Table Settings** button, then specify the columns to show or hide.
2. Double-click **Offscale**, **Pull-Up** (fragment), **Broad Peak** (HID), and SQ columns to sort suspect and failing flags to the top of the table.

Multi-column sorting is supported.



Offscale	Spectral PullUp	Sizing Quality 1
■	▲	✓

Flag/Symbols	Description
Offscale 	At least one data point in the analysis range has saturated the CCD camera. Note: In the Monitor Run screen, an offscale sample is flagged with .
Spectral Pull-Up (fragment analysis only) 	At least one peak contains a pull-up peak. A pull-up peak is identified when the peak height of the minor peak is $\leq X\%$ of and within $\pm Y$ data point of the major peak, where X and Y are values you specify.
Broad Peak (HID analysis only) 	At least one peak exceeds the Broad Peak threshold. Broad peaks affect Sizing Quality. Note: The value displayed when you place the mouse pointer over a Broad Peak flag is an internal value and does not reflect the peak width.
Normalization Limit 	<ul style="list-style-type: none"> • – Sample was collected with a normalization size standard, sample Normalization Factor is within range. • – Sample was collected with a normalization size standard, sample Normalization Factor is not within range. • No Data – Normalization is enabled, but Sizing Quality is . • NO – Sample was not collected with a normalization size standard. • N/A – Sample was not collected on a 3500 Dx Series Genetic Analyzer CS2 instrument. Note: If the Sizing Quality is , normalization is not applied, even if the Normalization Factor is within the normalization range.
Sizing Quality Note: If the Sizing Quality is , normalization is not applied, even if the Normalization Factor is within the normalization range.	The Sizing Quality is in the Fail or Suspect range. Place the mouse pointer over a flag to display the Sizing Quality value for the sample.

3. Click a flag in the samples table, or select samples in the samples table to display the associated data in the Plot View and Sizing Table View.
4. (Optional) Modify the sample view:
 - Right-click the **Size Standard** field to view the size standard for a sample.
 - Click **Minimize** and **Restore** to collapse and expand the samples table.





For more information, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).




Specify re-injections

You can specify a re-injection before the run completes. A re-injection physically re-injects all samples in the capillary array. You can specify whether to collect data for all or only selected samples in the array.

1. Select the injections or wells to re-inject:

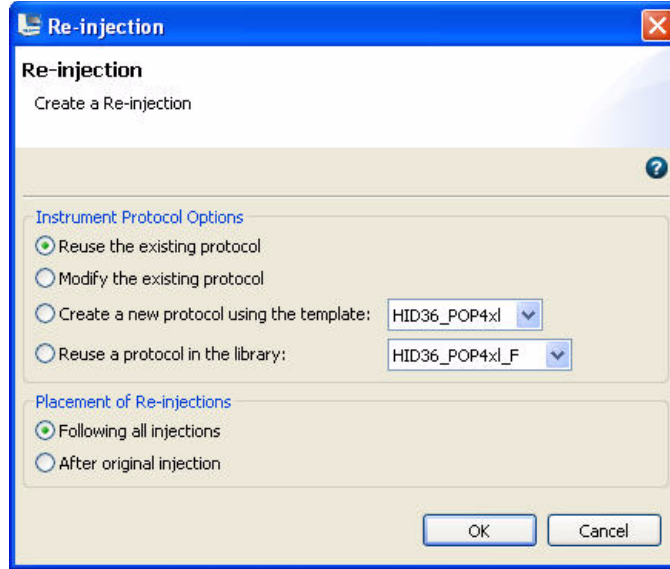
Note:  Re-inject is grayed if you select an injection that contains more than one results group, or if you select flags in the flags table that correspond to samples with different results groups. To enable  Re-inject, select samples that specify the same results group.

IMPORTANT! Re-injection wells cannot be selected by clicking on the plate map. The user should select samples for reinjection by clicking on the samples in the Instrument Run Views and Flags section. Samples or flags can be used to specify reinjections.

Goal	Action
To collect data for all wells in an injection	<ol style="list-style-type: none"> 1. Select the injection in the injection list. 2. Click  Re-inject.
To collect data for only specific wells (Samples with assays that specify more than one instrument protocol are listed one time in the injection list for each instrument protocol.) Note: You can also specify re-injections for specific samples in Review Results.	<ol style="list-style-type: none"> 1. Select the injection. 2. Select in the array view the capillary that corresponds to the well or sample of interest. 3. Click  Re-inject.
To collect data for only samples that contain flags	<ol style="list-style-type: none"> 1. Select the samples in the flag table. 2. Click  Re-inject.

2. In the Re-injection dialog box, make selections for the following options, then click **OK**:
 - The protocol to use for the re-injection: original, modified, new, or one from the library
 - When to make the re-injection

Note: Sample data files for each re-injection can be saved in a separate folder in the results group folder if specified in the results group.



If you select a protocol other than the original

If you select a protocol other than the original, the software:

- Creates a copy of the assay specified for the re-injected well (Original_Assay-1).
- Adds the new or modified instrument protocol to Original_Assay-1.
- Assigns Original_Assay-1 to the re-injected well only.
- Saves the plate (the software does not save the copy of the assay to the library).

How re-injections are displayed in the plate view

If the Injection Number attribute is selected for display in the plate view, the number of the original injection and the re-injection are shown.

	1	2	
A	sample 1 1, 2		Sample 1 selected for re-injection
B	sample 2 1, 2		Re-injection number listed for all samples in the re-injection
C	sample 3		

Note: If you select only specific wells for the re-injection (which physically re-injects all samples for the capillary array but collects data only for the selected wells), the re-injection number is displayed for all samples in the re-injection, not just the samples selected for data collection.

Section 4: Developmental Validation

This section covers:

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Sensitivity.....	46
Baseline Noise Analysis	51
Internal Standard Normalization (ISN).....	55
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Overview

The Applied Biosystems Human Identification Product Assurance Group validated the use of the 3500 series Genetic Analyzers (3500 and 3500xL) with the AmpF ℓ STR® PCR Amplification Kits.

The validation experiments were performed according to the DNA Advisory Board (DAB) Quality Assurance Standards, effective October 1, 1998 (DNA Advisory Board, 1998), and the revised guidelines from the Scientific Working Group on DNA Analysis Methods [Revised Validation Guidelines-Scientific Working Group on DNA Analysis Methods (SWGDM)]. *Forensic Sci. Communications* (July 2004) Volume 6 (3).

The studies performed include:

- Genotype concordance and reproducibility (page 41)
- Sizing precision and resolution (page 43)
- Sensitivity and Normalization (page 46)
 - Sensitivity (page 46)
 - Baseline Noise Analysis (page 51)
 - Internal Standard Normalization (ISN) (page 55)
- Mixture Analysis with GeneMapper® ID-X Software Version 1.2 (page 60)

IMPORTANT! Each laboratory should perform internal validation studies to establish appropriate guidelines, protocols, and procedures prior to implementing a new technology.

Materials and methods

Kits The following AmpF ℓ STR® kits were tested during this validation study:

- MiniFiler™ PCR Amplification Kit
- Yfiler® PCR Amplification Kit Yfiler

-
- Profiler Plus® PCR Amplification Kit
 - Cofiler® PCR Amplification Kit
 - Identifiler® PCR Amplification Kit
 - Identifiler® Direct PCR Amplification Kit
 - Identifiler® Plus PCR Amplification Kit
 - NGM™ PCR Amplification Kit
 - SGM Plus® PCR Amplification Kit
 - SEfiler Plus™ PCR Amplification Kit
 - Sinofiler™ PCR Amplification Kit

Not every AmpFSTR® kit was utilized in every study. Representative kits were used in each study to demonstrate the ability of the 3500 series instruments to achieve accurate results within the parameters of each test. Selection of representative kits for each study was based on factors including dye chemistry, use of a particular size standard, and other similarities among the AmpFSTR® kits.

Instrumentation

Three 24-capillary instruments (3500xL) and three 8-capillary instruments (3500) were used in the validation experiments. Each instrument was installed and calibrated by field service engineers prior to the experiments. The spectral calibrations described in [“Performing a spectral calibration” on page 8](#) were performed on all six instruments with both DS33 (G5) and DS32 (F) matrix standards. The HID performance check was performed on all six instruments with the HID Install Standard and GeneScan™ 600 LIZ® v2 size standard. All of the instruments met all specifications for sizing precision and all spectral calibration specifications including peak height (see [“Evaluate fragment/HID install standard data” on page 19](#)).

Quantification of the DNA samples was performed using the Quantifiler® or Quantifiler® Duo DNA Quantification kits on a 7500 Real-Time PCR System (Applied Biosystems) to determine input DNA concentrations, and using GeneAmp PCR System 9700 thermal cyclers equipped with gold-plated blocks for PCR amplification. Where required, 3130xl instruments were used with the same sample set for genotype concordance and overall STR profile comparison.

Factory-provided AmpFSTR® assays containing the recommended instrument protocol and run information were utilized for all studies. For information on each assay and specific run conditions see [“Validated instrument protocol specifications” on page 67](#).

The AmpFSTR® kits were validated on instruments using the consumables listed in the following table. Anode buffer, cathode buffer and polymer were changed according to the recommended maintenance schedule described in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Consumable	Part Number
POP-4 [®] Polymer 960 Samples	4393710
Cathode Buffer Container	4408256
Anode Buffer Container	4393927
GeneScan [™] 600 LIZ [®] v2 Size Standard	4408399 (kit) 4408293 (tube)
GeneScan [™] 500 ROX [™] Size Standard	4310361 (9600 reactions) 401734 (800 reactions)
Hi-Di [™] Formamide, 5 mL bottle	4401457
3500 36cm Capillary Array, 8 capillary	4404683
3500xL Genetic Analyzer Capillary Array, 24 capillary, 36 cm	4404687

Samples The DNA samples tested in each experiment are listed in the following table. GeneScan™ 600 LIZ® v2 size standard was used with the 5-dye chemistries and GeneScan™ 500 ROX™ size standard was used with the 4-dye chemistries.

Table 1 Studies performed, kits tested, and DNA inputs

Study	Samples	PCR amplification kits tested	DNA input per reaction	Replicates per instrument
Genotype Concordance and Reproducibility	<ul style="list-style-type: none"> 40 male and 42 female extracted gDNA samples†. 1 positive control, 1 NTC, and allelic ladder Identifiler® Direct: 84 (1.2 mm) punches of blood stained FTA paper 	Identifiler®, Identifiler® Direct, Identifiler® Plus, MiniFiler™, NGM™, SGM Plus®, Yfiler®, SEfiler Plus™, Profiler Plus®, Cofiler®, and Sinofiler™	0.5-2 ng total DNA	1 replicate per sample 2 injections
Sizing Precision and Accuracy	Allelic ladder§	Identifiler®, Identifiler® Direct, Identifiler® Plus, MiniFiler™, NGM™, Yfiler®, SEfiler Plus™, Sinofiler™, SGM Plus®, Profiler Plus®, and Cofiler®	1 µl per well	24 replicates 4 injections
Sensitivity	007 control DNA, 3 gDNA samples, NTC and allelic ladder	Identifiler®, Identifiler Plus, NGM™, SGM Plus®, MiniFiler™	Identifiler® (3 lots GS600 v2) and SGM Plus® (GS 500 ROX): 0.125 ng, 0.5ng, 1 ng, 3 ng; MiniFiler™ (2 lots GS600): 0.125 ng, 0.25 ng, 0.5 ng, 0.75 ng	5 replicate DNA samples 3 replicate NTC 4 injections
Internal Standard Normalization (ISN)			Identifiler® Plus, NGM™: 0.062 ng, 0.125 ng, 0.5 ng, 1 ng, and 1.5 ng	
Mixture Analysis	4 pairs of male and female gDNA, each with mixture ratio of 1:0, 1:1, 1:3, 1:5, 1:9, and 0:1; 1 positive control 1 NTC and allelic ladder	Identifiler®, SGM Plus®, Profiler Plus®, Cofiler®	Identifiler®, SGM Plus®: total input 1 ng Profiler Plus®, Cofiler®: 2 ng	1 replicate per sample 1 injection
Resolution	SEfiler Plus™ allelic ladder	SEfiler Plus™	1 µl per well	24 replicates 1 injection

† Includes African American, Caucasian, Hispanic, and Asian donors.

§ Identifiler® Direct, Identifiler® Plus, Identifiler®, NGM™, MiniFiler™, SGM Plus®, Yfiler®, Sinofiler™, and SEfiler Plus™ Kits with GeneScan™ 600 LIZ® Size Standard v2.0 and Profiler Plus®, Cofiler® Kits using GeneScan™ 500 ROX™ Size Standard®.

Table 2 Sample sources and kits and instruments used for preparation and amplification

Study	Sample source	Extraction	Quantitation	Amplification [‡]	CE [§]
Genotype Concordance and Reproducibility	Commercially sourced material and kit-specific positive control	BloodPrep™ Chemistry on the ABI PRISM™ 6100 Nucleic Acid PrepStation	Quantifiler® Human on 7500	9700 gold block	3130x/ and 3500
Sizing Precision and Accuracy	Identifiler® allelic ladder	N/A	N/A	N/A	3500
Sensitivity	Commercially sourced material and 007	BloodPrep™ Chemistry on the ABI PRISM™ 6100 Nucleic Acid PrepStation	Quantifiler® Duo on 7500	9700 gold developmental validation block	3500
Internal Standard Normalization (ISN)					
Mixture Analysis	SERACARE	DNA	Quant Duo on 7500	9700 gold block	3500
Resolution	SEfiler Plus™, Identifiler®, and research methods	N/A	N/A	N/A	3500

‡ On a GeneAmp® PCR System 9700 with gold block in 9600 emulation mode. Amplifications used 25 µl reaction volumes for the 5 dye chemistries and 50µl reaction volumes for the 4 dye chemistries.

§ A mixture of Hi-D1™ Formamide: Size Standard:PCR product was prepared for each sample, control or allelic ladder according to a 8.5:0.5: 1 ratio, respectively.

Data Analysis

All data were analyzed using GeneMapper® ID-X v1.2 software with internal standard normalization enabled or disabled in the Analysis Method. The following kit standard panels, bins, and stutter files were used for data analysis at the settings shown in the table:

- MiniFiler™ PCR Amplification Kit
- Yfiler® PCR Amplification Kit
- Profiler Plus® PCR Amplification Kit
- Cofiler® PCR Amplification Kit
- Identifiler® PCR Amplification Kit
- SGM Plus® PCR Amplification Kit
- SEfiler Plus™ PCR Amplification Kit
- Identifiler® Direct PCR Amplification Kit (v1X)
- Identifiler® Plus PCR Amplification Kit (v1X)
- NGM™ PCR Amplification Kit (v1X)
- Sinofiler™ PCR Amplification Kit (v2X)

Table 3 Analysis settings

Size Standard	GeneScan™ 500 ROX™ †or GeneScan™ 600 LIZ® v2§
Peak Detection Mode	Advanced
Smoothing	Light
Size Calling Method	Local Southern, 3 rd Order Least Squares (MiniFiler™ only)
Baseline Window Size	51
Minimum Peak Half Width	2
Polynomial Degree	3
Peak Window Size	15

† CE_G5_HID_GS500 size standard peaks defined: 75.0, 100.0, 139.0, 150.0, 160.0, 200.0, 300.0, 340.0, 350.0, 400.0, 450.0

§ GS600_LIZ size standard peaks defined: 80.0, 100.0, 114.0, 120.0, 140.0, 160.0, 180.0, 200.0, 214.0, 220.0, 240.0, 250.0, 260.0, 280.0, 300.0, 314.0, 320.0, 340.0, 360.0, 380.0, 400.0, 414.0, 420.0, 440.0, 460.0

Additional parameters were examined according to set criteria. The following table describes the type of study and the associated criteria.

Table 4 Study criteria matrix

Study type	Test cases	Test method	Passing criteria
Genotyping	Concordance	Confirm that the STR profiles are complete and accurate by comparison to known STR profiles and data generated from 31xx runs using GeneMapper® <i>ID-X</i> v1.2.	All 3500/3500xL allele calls are 100% concordant with the 31xx genotype results for all the kits tested.
	Reproducibility	Confirm that the STR profiles generated on different 3500 series instruments and different injections are complete and accurate using GeneMapper® <i>ID-X</i> v1.2.	STR profiles from the same samples or controls run on different instrument and across injections are 100% concordant.

Study type <i>(continued)</i>	Test cases	Test method	Passing criteria
Sizing	Precision	Calculate the standard deviation of the mean base pair size for each allele in the allelic ladder within an injection and across a plate.	The sizing precision does not exceed a standard deviation of 0.15 bp within an injection (8- or 24-cap) or across a plate (96 wells) for all alleles in all kits tested.
	Accuracy	Calculate the size range (max-min) for each allele in the allelic ladder samples across all samples per injection and per plate.	The size range does not exceed 0.5 bp per injection (88 or 24-cap) for all alleles in all kits tested.
Sensitivity	Minimum DNA input	Determine the lowest DNA input amount per reaction that can generate a full STR profile above the GeneMapper® ID-X peak amplitude threshold [‡] .	Kit dependent.
	Maximum recommended DNA input	Determine the highest DNA input amount per reaction that can generate a full STR profile without camera saturation (offscale peaks).	No allele dropout or off-scale data for kit recommended range.
	Artifacts	Identify reproducible artifacts.	No new reproducible artifacts within the read region.
	Pull-up	Calculate the maximum and mean pull-up for each peak in each color for on-scale data.	Mean pull-up is ≤ 5%.
Internal Standard Normalization (ISN)	Internal Size Standard average peak height and Normalization Factor	<p>Determine the Normalization Factor by dividing the average peak height of the ISN peaks by the internal standard normalization threshold setting.</p> <p>ISN thresholds setting[§]:</p> <ul style="list-style-type: none"> • 3200 RFU for Identifiler® Direct, Identifiler® Plus, MiniFiler™, Yfiler®, NGM™ • 3800 for Identifiler® and Sinofiler 	Normalization Factors are between 0.3 and 3.

Study type <i>(continued)</i>	Test cases	Test method	Passing criteria
Mixture Analysis	Full profile for minor contributor	The highest mixture ratio that can generate detectable alleles for the minor contributor.	Detection of minor contributor peaks at 1:9 ratio with total DNA input of 1ng. Concordance between mixture analysis values between different versions of GeneMapper® <i>ID-X</i> software.

‡ Values determined for instruments used during the developmental validation. Each laboratory should perform their own studies to determine the appropriate settings for their instruments.

§ Variation among instruments, among injections, and among capillaries was also examined.

Genotype concordance and reproducibility

The genotype concordance and reproducibility study was conducted to demonstrate that the STR profiles of the kits are complete and accurate, by comparing known STR profiles to kit data generated by runs on the 3500 and 3500xL instruments using GeneMapper® *ID-X* v1.2 and the 3130xL instrument.

Experimental design

For details of study parameters, see [Table 1, “Studies performed, kits tested, and DNA inputs,”](#) on page 37, [Table 2, “Sample sources and kits and instruments used for preparation and amplification,”](#) on page 38, and [Table 4, “Study criteria matrix,”](#) on page 39.

The data were analyzed using GeneMapper® *ID-X* v1.2 software with a peak detection threshold of 175 RFU (see [“Baseline noise and threshold determination for the G5 and F Dye Sets”](#) on page 51 for more information on the 175 RFU threshold setting). Samples were analyzed with the ISN feature enabled and disabled in GeneMapper® *ID-X* v1.2 software and with a kit-specific ISN threshold as defined in the validated instrument protocols in [“Internal Standard Normalization \(ISN\)”](#) on page 55. STR profiles generated on the 3500 and 3500xL instruments were compared to data previously generated on the 3130xL Genetic Analyzer to confirm genotype concordance.

Results For all the kits tested, the results demonstrated that the genotype profiles from six 3500 or 3500xL instruments were 100% concordant with each other and with the 3130xl results. Also, the genotype profiles showed 100% concordance between the analysis methods with ISN activated and ISN deactivated.

Table 5 Concordance results (82 samples per run)

Kit	Concordance	Internal Standard Normalization
Identifiler [®]	100%	Enabled
	100%	Disabled
Identifiler [®] Direct	100%	Enabled
	100%	Disabled
Identifiler [®] Plus	100%	Enabled
	100%	Disabled
MiniFiler [™]	100%	Enabled
	100%	Disabled
NGM [™]	100%	Enabled
	100%	Disabled
SGM Plus [®]	100%	Enabled
	100%	Disabled
Yfiler [®]	100%	Enabled
	100%	Disabled
SEfiler Plus [™]	100%	Enabled
	100%	Disabled
Profiler Plus [®]	100%	Enabled
	100%	Disabled
Cofiler [®]	100%	Enabled
	100%	Disabled
Sinofiler [™]	100%	Enabled
	100%	Disabled

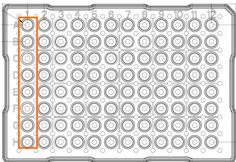
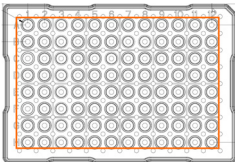
Sizing precision and resolution

To assess the ability of the 3500 instrument to reproducibly measure the size of an amplified DNA fragment, allelic ladder aliquots were injected multiple times on three 3500 and three 3500xL instruments.

Experimental design

For details of study parameters, see [Table 1, “Studies performed, kits tested, and DNA inputs,”](#) on page 37, [Table 2, “Sample sources and kits and instruments used for preparation and amplification,”](#) on page 38, and [Table 4, “Study criteria matrix,”](#) on page 39.

Precision was expressed as a standard deviation (SD) of the mean base pair size calculated for each allele within each locus across one injection and across multiple injections. The SD of the mean size of each allele within a run was calculated and plotted by marker. The graph below demonstrates how the two different types of analyses were performed on a 3500 and 3500xL; however, both single and multiple injections were performed on both instrument types.

One injection (3500 instrument)	Multiple injections (3500xL instrument)
	
<ul style="list-style-type: none"> • 8 data points per mean • 12 injections per plate • 12 columns • 3 Instruments 	<ul style="list-style-type: none"> • 96 data points per mean • 4 injections per plate • 3 Instruments

Results

The SD in sizing within an injection and across multiple injections was less than 0.15 bp on both instruments for all kits tested using a full plate of 96 samples. The median standard deviation is similar regardless of STR fragment size and capillary array type. Sample allele sizing error rarely exceeds ± 0.5 nt when the SD is ≤ 0.15 bp (see Smith, et al. 1995, *Biotechniques* 18(1):122-8.)

[Figures 4](#) and [5](#) show the data distribution of all alleles from wells containing Identifiler® Allelic Ladder. Comparable results were observed for all kits. The box portion of the box plots represent the data that fell within the 75th percentile, with the median indicated by a line in the center of the box plot. The whisker lines protruding from the boxes extend to the 90th percentile, and the individual outliers beyond the whiskers represent the small percentage of data that were not in the 90th percentile.

Figure 4 Standard Deviation of Mean Size for Identifiler® Allelic Ladder: within one injection on 3500 (8 capillaries) and 3500xL (24 capillaries)

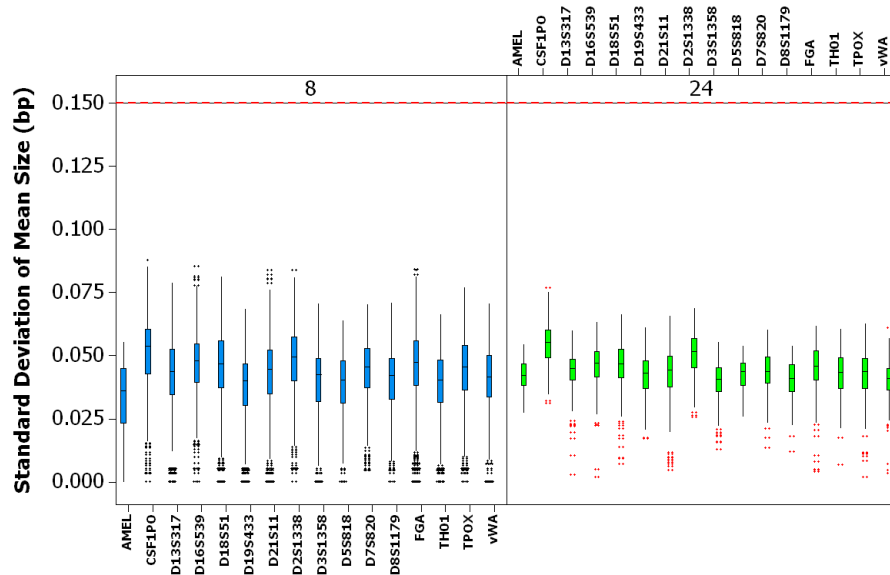
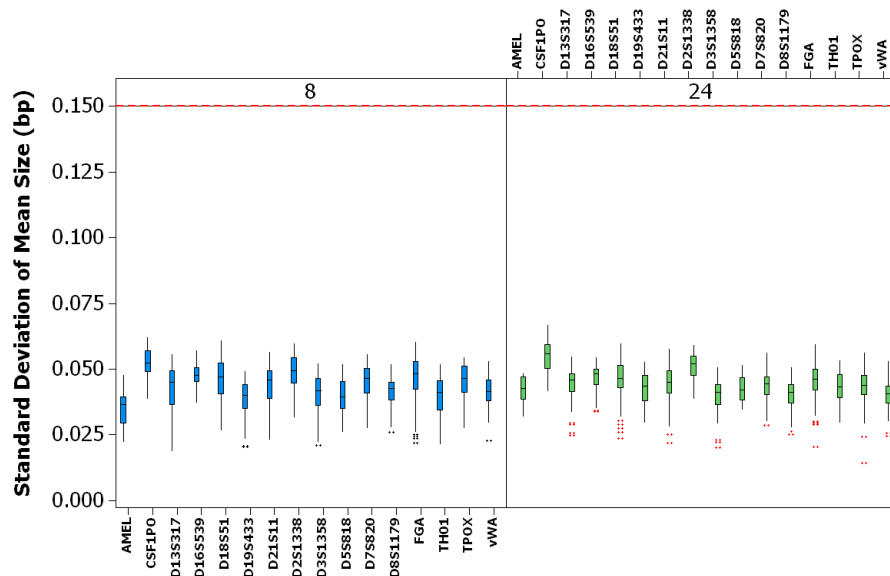


Figure 5 Standard Deviation of Mean Size for Identifiler® Allelic Ladder: across multiple injections (entire 96-well plate) on 3500 (8 capillaries) and 3500xL (24 capillaries)



Resolution

The study tested the ability of the system to reliably detect and resolve alleles that differ in length by a single base pair (bp). In addition, research methods were developed to evaluate larger fragments (>300 bp) differing in size by 1 bp (data not shown).

Experimental design

For details of study parameters, see [Table 1, “Studies performed, kits tested, and DNA inputs,”](#) on page 37, [Table 2, “Sample sources and kits and instruments used for preparation and amplification,”](#) on page 38, and [Table 4, “Study criteria matrix,”](#) on page 39.

A minimum of 160 injections of the SEfiler Plus™ Kit Allelic Ladder (containing SE-33 alleles 21, 21.1, 21.2 separated by a single base pair) and the Identifier® Kit Allelic Ladder (containing TH01 alleles 9.3,10) were performed on each of three 3500 and three 3500xL instruments.

Results

The SEfiler Plus™ SE-33-marker allele triplet (21, 21.1, and 21.2) and the Identifier® TH01 marker alleles (9.3 and 10), were resolved for all samples tested. Research methods were used to confirm single base-pair resolution up to 400 bp.

Figure 6 Representative data for the SE-33 marker on a 3500xL instrument

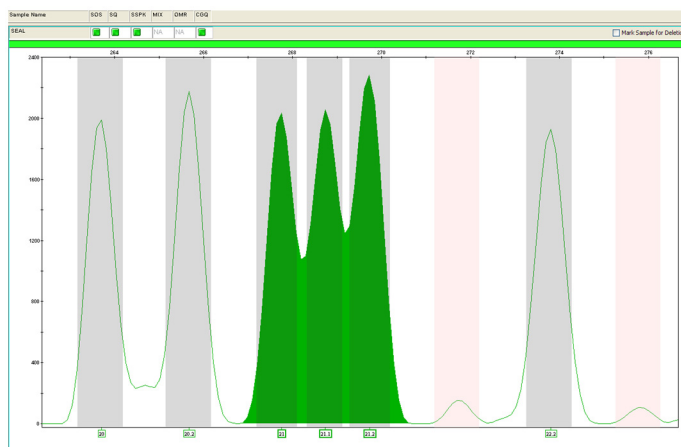
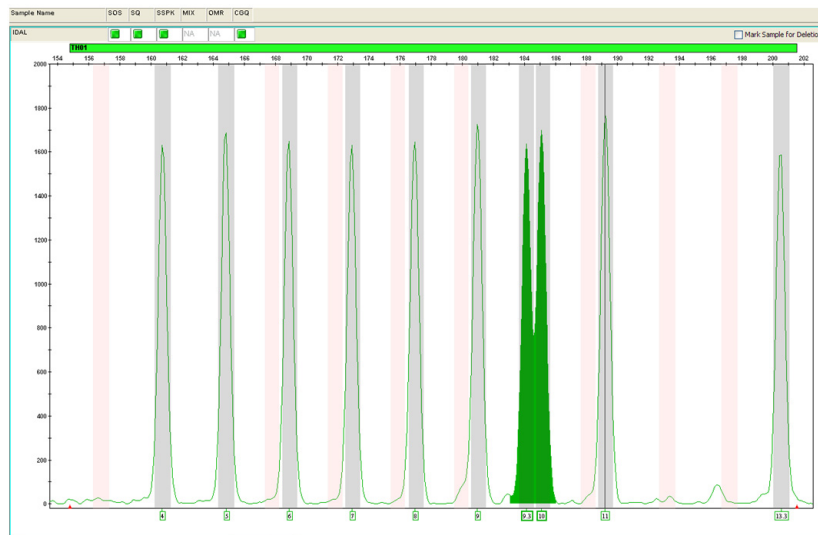


Figure 7 Representative data for the TH01 marker on a 3500xL instrument



Sensitivity and Normalization

Sensitivity

The study tested the ability of the systems to generate a full and accurate STR profile with given amounts of DNA input.

Each laboratory should perform their own evaluation to determine appropriate peak amplitude settings for their instruments, laboratory protocols, and chemistry.

Experimental design

For details of study parameters, see [Table 1, “Studies performed, kits tested, and DNA inputs,”](#) on page 37, [Table 2, “Sample sources and kits and instruments used for preparation and amplification,”](#) on page 38, and [Table 4, “Study criteria matrix,”](#) on page 39. POP-4[®] polymer was used for all instrument runs. Sensitivity study of the Identifiler[®] Direct kit was not applicable because it uses FTA paper punches.

Data were analyzed with the ISN targets set at 3800 RFU for Identifiler[®] kit and Sinofiler[®] kit data and 3200 RFU for all other 5-dye kits. The ISN target, which is the expected average signal of the ISN peaks from the GeneScan[™] 600 LIZ[®] Size Standard v2.0, was defined based on analysis of data across multiple 3500 instruments. A peak amplitude threshold setting of 175 RFU was used.

Each replicate was injected twice on each instrument at sample well locations selected such that each sample would be injected in different capillaries across the array. A total of 10 data points were collected for each sample and concentration on each instrument. For the kits that used multiple lots of GeneScan[™] 600 LIZ[®] Size Standard v2.0 size standard, the CE runs were repeated for each lot. Three injections on each instrument for each sample were performed with the Identifiler[®] Plus and NGM[™] kits, resulting in a total of 12 replicates for each sample at each concentration.

Six CE plates (one for each instrument) were prepared according to the following procedure:

1. Prepare a master mix of the size standard and Hi-Di™ Formamide for all plates using 0.5 µL of size standard and 8.5 µL of Hi-Di™ Formamide for each well
2. Pipet 9 µL of the master mix into each well. Pipet 1 µL of each sample from the PCR plates or the allelic ladder to the CE plates. Denature the plates at 95°C for 3 minutes and cool on ice until use.
3. Run the CE plates on each instrument with 2 or 3 injections (kit dependent).

The following table shows an example of the plate layouts used for 007 Control DNA (columns 1– 3) and genomic DNA samples H1126 (columns 4– 6), H1152 (columns 7– 9), and H1161 (columns 10– 12) amplified at input amounts of 125 – 750 pg across the plate.

Table 6 Plate layout for MiniFiler™ kit sensitivity study

	1	2	3	4	5	6	7	8	9	10	11	12
A	007 0.125 ng	007 0.25 ng	007 0.5 ng	H1126 0.125 ng	H1126 0.25 ng	H1126 0.5 ng	H1152 0.125 ng	H1152 0.25 ng	H1152 0.5 ng	H1161 0.125 ng	H1161 0.25 ng	H1161 0.5 ng
B	007 0.75 ng	007 0.125 ng	007 0.25 ng	H1126 0.75 ng	H1126 0.125 ng	H1126 0.25 ng	H1152 0.75 ng	H1152 0.125 ng	H1152 0.25 ng	H1161 0.75 ng	H1161 0.125 ng	H1161 0.25 ng
C	007 0.5 ng	007 0.75 ng	007 0.125 ng	H1126 0.5 ng	H1126 0.75 ng	H1126 0.125 ng	H1152 0.5 ng	H1152 0.75 ng	H1152 0.125 ng	H1161 0.5 ng	H1161 0.75 ng	H1161 0.125 ng
D	007 0.25 ng	007 0.5 ng	007 0.75 ng	H1126 0.25 ng	H1126 0.5 ng	H1126 0.75 ng	H1152 0.25 ng	H1152 0.5 ng	H1152 0.75 ng	H1161 0.25 ng	H1161 0.5 ng	H1161 0.75 ng
E	007 0.125 ng	007 0.25 ng	007 0.5 ng	H1126 0.125 ng	H1126 0.25 ng	H1126 0.5 ng	H1152 0.125 ng	H1152 0.25 ng	H1152 0.5 ng	H1161 0.125 ng	H1161 0.25 ng	H1161 0.5 ng
F	007 0.75 ng	007 0.125 ng	007 0.25 ng	H1126 0.75 ng	H1126 0.125 ng	H1126 0.25 ng	H1152 0.75 ng	H1152 0.125 ng	H1152 0.25 ng	H1161 0.75 ng	H1161 0.125 ng	H1161 0.25 ng
G	007 0.5 ng	007 0.75 ng	NTC	H1126 0.5 ng	H1126 0.75 ng	NTC	H1152 0.5 ng	H1152 0.75 ng	NTC	H1161 0.5 ng	H1161 0.75 ng	NTC
H	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL

Results The sensitivity study evaluated allelic dropout, absolute peak height, peak height versus concentration, inter-color and intra-locus balance, and heterozygote peak height ratio. The data were also used to evaluate noise and spectral pull-up.

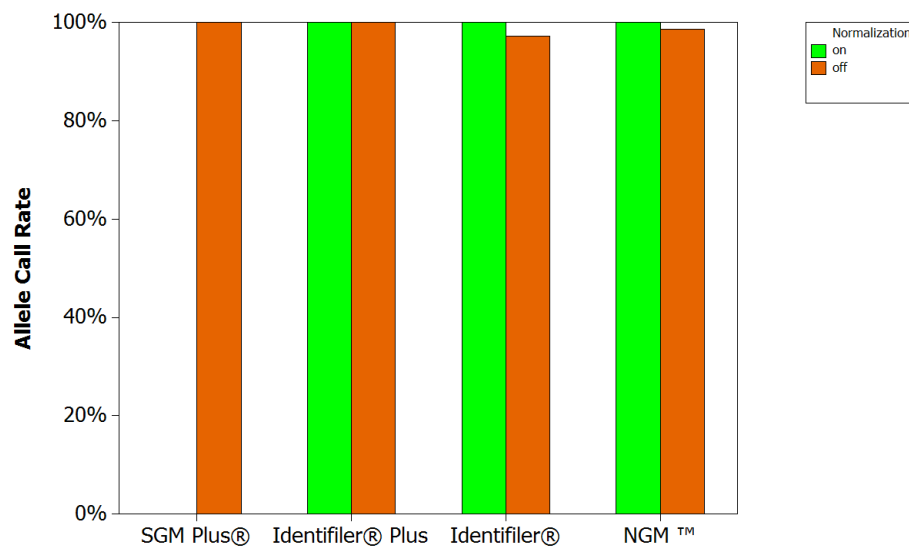
Allelic dropout

With few exceptions, the majority of samples tested for all kits produced a full profile at 0.125 ng of input DNA using a 175 RFU peak detection threshold. The rate of allelic dropout was sample-dependent and varied based on the input DNA concentration, amplification kit chemistry, sensitivity of individual instruments and whether ISN was enabled or disabled.

The allele call rate was highest for the SGM Plus® (99.97%), Identifiler® Plus (99.95%), NGM™ (98.65%), and Identifiler® (97.24%) kits. In this study, the allele call rate was 100% for all three kits that utilize ISN.

Figure 8 shows the allele call rate (the ratio of number of alleles observed:number of alleles in a full profile). The data incorporates all four different DNA sources and all six instruments. The SGM Plus® kit does not use ISN.

Figure 8 Allele call rate by kit with 0.125 ng DNA input. (ISN enabled: green; disabled: red)

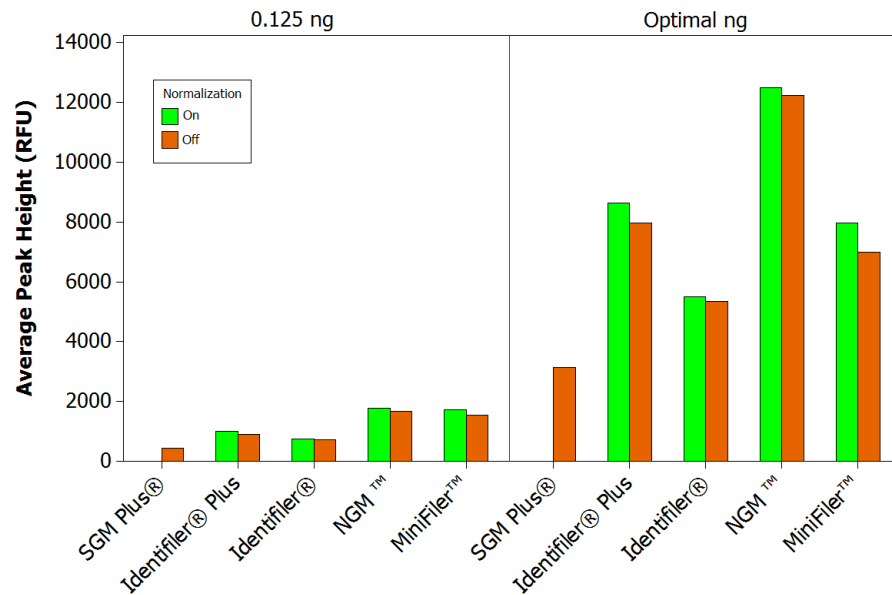


Peak Height vs. Concentration

Figure 9 shows the average peak height with standard error bars for five AmpFSTR® kits. The graph includes data for two of the four concentrations used for each kit, 0.125 ng and the optimal concentration, which is 0.5 ng for MiniFiler™ and 1.0 ng for all other kits tested. Each bar represents the average of all dye colors, all replicates, and 6 instruments for the 007 Control DNA. The other DNA samples produced similar results.

The graph includes data analyzed with ISN enabled and disabled. The SGM Plus® kit does not use ISN.

Figure 9 Average Peak Heights of Five AmpF/STR® Kits for 007 Control DNA at two concentrations (ISN enabled: green; disabled: red)



The results show the expected peak heights for the relative DNA concentrations. Peak heights increased slightly with ISN enabled, demonstrating that the Internal Standard Normalization Targets used were appropriate for this set of six instruments. Each laboratory should perform a similar analysis on their individual instruments when evaluating Normalization Targets.

Intra-color balance and inter-color balance

Intra-color balance for a dye is calculated by:

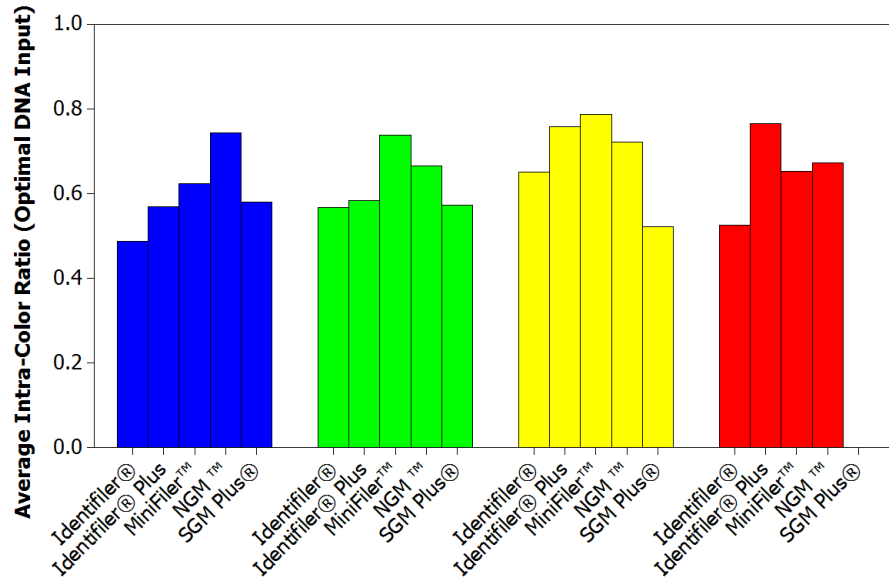
1. Dividing the peak heights of homozygous alleles by 2 and averaging the peak heights of heterozygous alleles
2. For the resulting peak heights at all loci within a color, dividing the lowest peak height for any allele by the highest peak height for any allele

Inter-color balance is the ratio of average peak heights between dyes.

The bars in the graph below represent the intra-color peak balance ratio for five AmpF/STR® kits separated by dye color to also show inter-color balance. All alleles from markers of the same dye color were averaged for each replicate of 007 Control DNA and all instruments. Other DNAs that were tested produced similar results.

Note: The data below represents commonly observed intra-color ratios for pristine samples. Degraded, inhibited, compromised samples, or samples using modified amplification procedures may produce different results.

Figure 10 Intra-color ratio for 007 Control DNA at recommended concentration



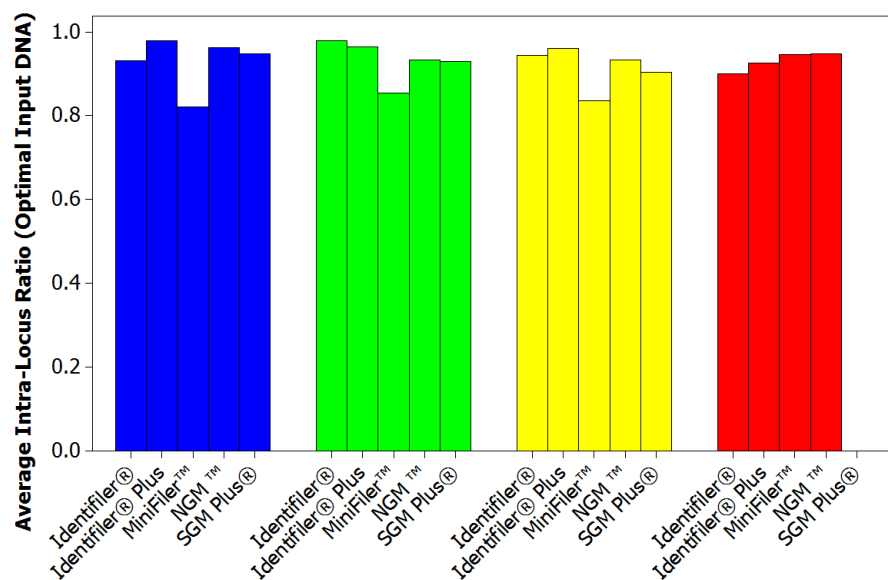
Intra-locus balance/Heterozygote Peak Height Ratio (PHR)

Intra-locus balance is the peak height of the lower of two alleles in a heterozygous pair divided by the peak height of its partner allele. This balance is generally impacted more by factors related to amplification, such as DNA input amount, than by factors relating to capillary electrophoresis.

The 3500 systems exhibited the intra-locus balance behavior that was expected from validations of this kit with Applied Biosystems 310 and 31xx genetic analyzers. These data represent 007 Control DNA at nominal concentration, although the other DNAs tested performed similarly. All six instruments were utilized for this testing.

Note: The data that follows represents commonly observed intra-locus ratios for pristine samples. Degraded, inhibited, compromised samples, or samples using modified amplification procedures may produce different results.

Figure 11 Average intra-locus peak balance (PHR) for 007 Control DNA at recommended concentration



Baseline Noise Analysis

Background noise can be defined as any signal which does not represent the signal of interest. All biochemical detection systems have a level of inherent noise that should be evaluated to distinguish true signal from background noise. Peak heights of artifacts, anomalies, and other sources of baseline noise vary depending on the sensitivity of each instrument, the type of chemistry used, and in most cases, the sample injection parameters and the amount of amplicon loaded onto the instrument.

Refer to the appropriate kit User Manual or User Bulletins for information on known chemistry-related artifacts.

Baseline noise and threshold determination for the G5 and F Dye Sets

Noise in a CE system can be generated inherently from the hardware components or as a result of the introduction of chemicals or dye-labeled fragments into the system.

Background capillary electrophoresis instrument signal may be attributed to electrical noise from the instrument electronics or background Raman signal from the water in the polymer. Because of these factors, it is impossible to eliminate all the baseline noise in a detection system. To analyze the baseline noise coming from the dye sets used in the multiplex STR chemistries (G5 and F), experiments were conducted to estimate signal to noise levels of each and to identify optimal peak amplitude threshold settings for data analysis.

Non-normalized data from five instruments and normalized data from four instruments were used to examine the noise in each color of the G5 dye set. Four instrument runs from two instruments were used to investigate the noise level in dye set F.

The DS33 Installation Standard was used to evaluate default noise levels for the G5 dye set. The noise level for each dye was analyzed individually. The default 175 RFU peak amplitude threshold was established by taking 10X the average standard deviation (SD) of the noise in the G5 dye set for each color. The DS33 Installation Standard represents the G5 dye set but contains few fragments, allowing noise measurements to be made between peaks.

Note: Calculating 10X the SD of the baseline noise translates to a 99.999999999% confidence that true peaks can be distinguished from peaks due to instrument noise as shown in the table below.

Table 7 Confidence interval distribution by Standard Deviation (SD)

SD	Percentage
1	68.27%
2	95.45%
3	99.73%

Figure 12 Noise from each dye in the G5 dye set (Y = 10Xaverage SD)

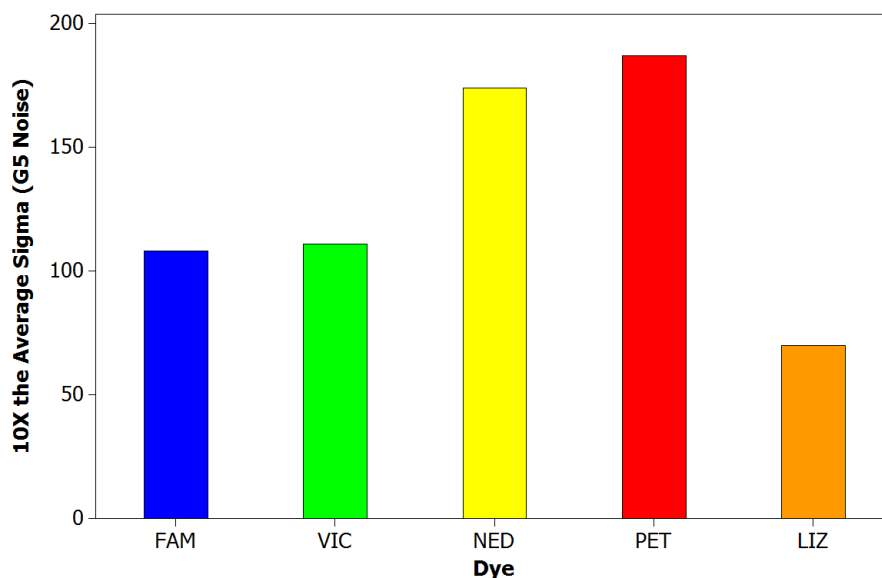
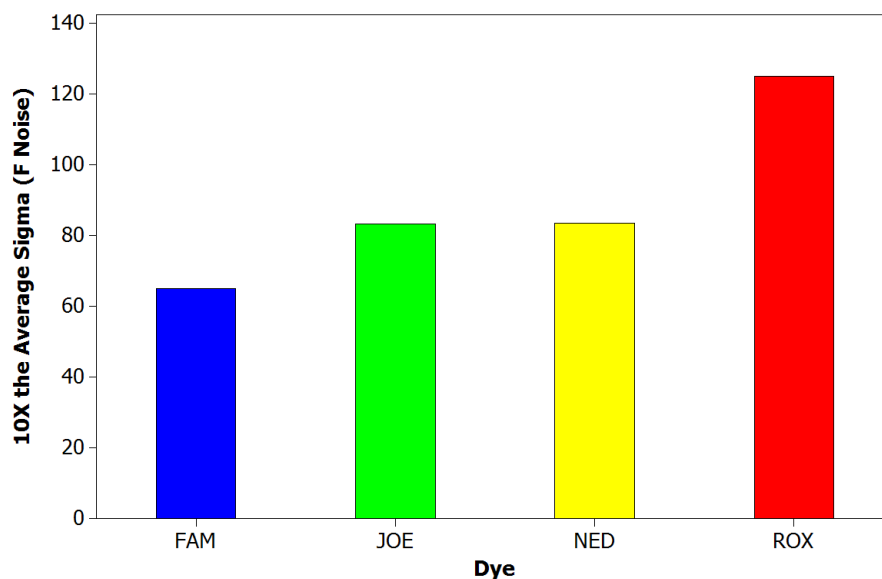


Figure 13 Noise from each dye in the F dye set (Y = 10Xaverage SD)



As shown, for the G5 dye set, the highest values were observed in the NED and PET dye channels. Figures 12 and 13 demonstrate that for a 10 X SD analysis, a threshold of 175 RFU should be sufficient to capture the noise generated in the system. Each lab should evaluate background noise for each instrument based on internal validation studies.

Additionally, these data demonstrate the difference among dyes within a dye set. Laboratories may wish to use this information to establish variable RFU thresholds based on dye color.

The 3500 series instrument software operates on a RFU scale similar to the 37xx series instruments, which is approximately three to four times the scale of the 31xx or 310 instruments. While each instrument and laboratory will perform their own evaluation, taking into account the difference in scale between platforms, the baseline noise level of the 3500 data in this study is comparable to the relative baseline noise seen with the 31xx platform.

Additionally, no reproducible dye artifacts specific to analysis performed on the 3500 instruments were observed in the read region of any marker, as compared to analysis on the 31xx instruments.

Using SGM Plus[®] samples for the F dye set, we also confirmed that a 175 RFU threshold is sufficient to capture noise levels in the F dye set, and that the overall noise signal in the F dye set is lower than in the G5 dye set.

Note: Some methods for customer analysis of Analytical Thresholds may vary from the engineering-based methods used here. Please contact your Life Technologies HID Support representative for more information.

Spectral pull-up

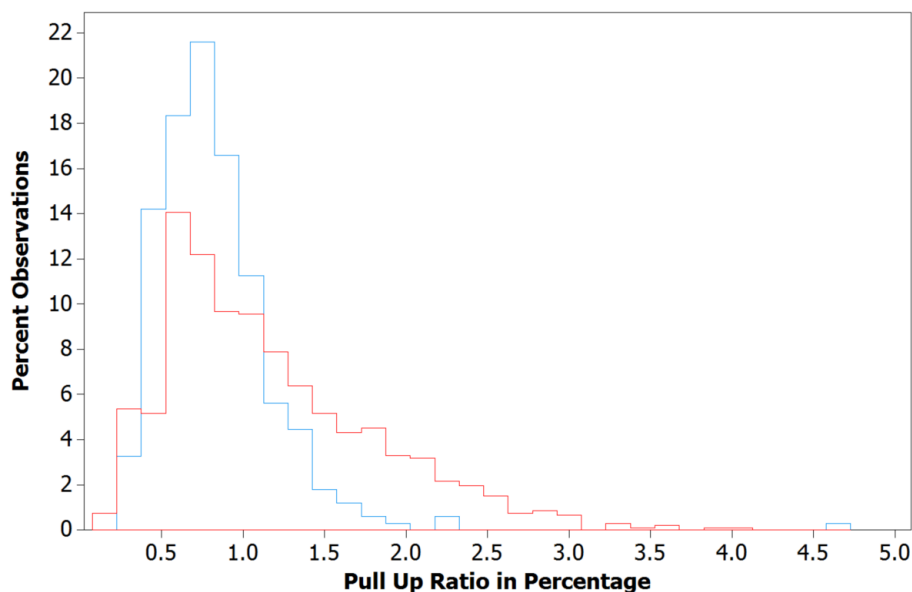
Spectral pull-up occurs when spectral calibration does not completely eliminate signal in one dye that is due to signal in a second dye from the same sample. Spectral pull-up was examined by visual inspection of the Identifiler[®], MiniFiler[™], and SGM Plus[®] kit gDNA samples used in the sensitivity study. In addition, extensive analysis was performed on the Identifiler[®] Plus kit data and is presented here.

The study analyzed data from runs on three 3500 instruments and three 3500xL instruments using the Identifiler® Plus kit with 1.5 ng input DNA. The tabular data was exported and pull-up peaks were screened using spreadsheet analysis. A 50 RFU threshold was used for capturing a full complement of observed pull-up peaks, including low level pull-up in addition to higher signal pull-up.

The percentage of pull-up was determined by dividing the peak height of the pull-up peak (daughter peak) by the peak height of the parent peak. This study was performed with Internal Standard Normalization off.

The data below represent 338 incidences of pull-up on the three 3500 series instruments and 1067 observations of pull-up on the 3500xL instruments.

Figure 14 Average, minimum, and maximum pull-up observed for Identifiler® Plus at 1.5 ng template DNA on six instruments (3500: blue, 3500xL: red)



Platform	Mean	SD	Median	Min	Max	N
3500	0.83	0.38	0.77	0.27	4.58	338
3500xL	1.17	0.65	1.01	0.18	4.02	1067

The average observed pull-up was 0.83% (SD 0.38%) for the 3500 instruments and 1.17% (SD 0.65%) for the 3500xL instruments.

In total, 3.2% of pull-up peaks observed with the 3500xL and 2.7% of those observed with the 3500 instrument exceeded a baseline threshold of 175 RFU. In addition, 0.93% of 3500xL and 0.09% of 3500 data indicated pull-up percentages of >3%.

The highest pull-up peak observed on the 3500 instruments had a peak height 4.58% of its parent peak, which was 7743 RFU. For the 3500xL instruments, the highest pull-up peak was 4.02% of a parent that was 18,073 RFU.

These data align with the expected performance of the 3500 series genetic analyzers and indicate pull-up percentages comparable to other capillary electrophoresis platforms from Applied Biosystems.

Pull-up percentage levels vary from injection to injection, dye to dye, and capillary to capillary. Different kits and dye sets also affect the level of pull-up observed. Users should perform a full analysis of the pull-up on their system, as described in this document. A complete understanding of the pull-up characteristics of the instrument assists the user in setting proper thresholds for analysis. Users are cautioned against relying on individual data points to make assessments of pull-up. Instead, a comprehensive analysis such as the one performed here provides a complete assessment of the pull-up in the system.

Note: Off-scale data (as determined in the Raw Data or Off Scale Flags) does not produce reliable peak heights and therefore is not suitable for the evaluation of pull-up percentages.

Internal Standard Normalization (ISN)

Size Standard Normalization is a feature on 3500 systems that is designed to reduce peak height variation across instruments. This feature attenuates signal variations across instruments that may be due to variations in instrument hardware, capillary array, sample salt load, and injection. For fragment analysis and HID applications, the 3500 Series Data Collection Software includes an Internal Standard Normalization (ISN) feature for use with the GeneScan™ 600 LIZ® Size Standard v2.0 (GS600 LIZ v2). For more information on internal standard normalization, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Experimental design

For details of study parameters, see [Table 1, “Studies performed, kits tested, and DNA inputs,” on page 37](#), [Table 2, “Sample sources and kits and instruments used for preparation and amplification,” on page 38](#), and [Table 4, “Study criteria matrix,” on page 39](#).

The sensitivity sample runs for the Identifiler®, MiniFiler™, Identifiler® Plus, and NGM™ kits were used to test the ISN feature. All samples were run with ISN activated in the 3500 data collection software. The data were analyzed with the ISN option enabled and separately with the ISN option disabled in the GeneMapper® ID-X v1.2 software.

See [Table 6, “Plate layout for MiniFiler™ kit sensitivity study,” on page 47](#) for the plate layout for the four samples and four DNA input amounts used for the MiniFiler™ ISN test. Two lots of size standard were used and samples were run separately with ISN enabled and disabled.

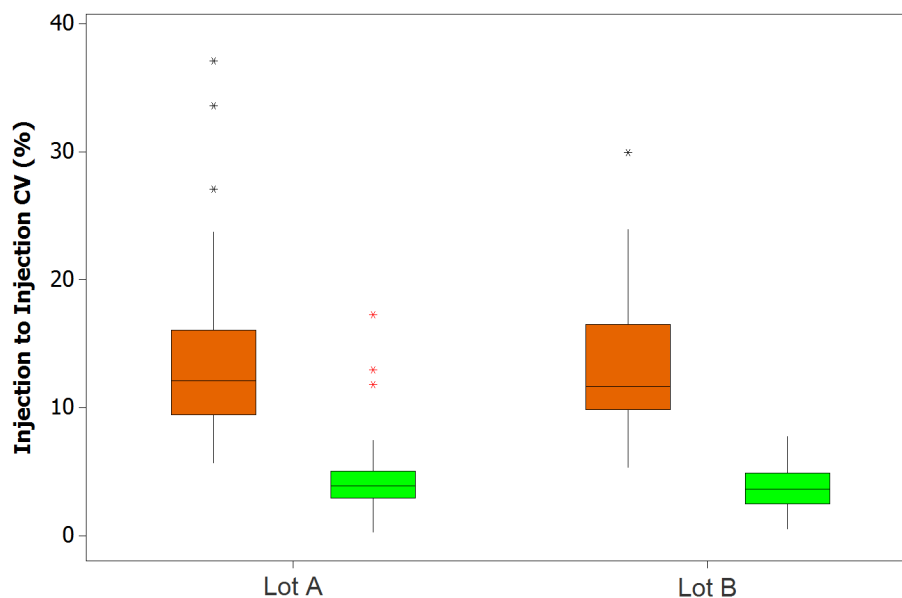
The ISN performance was evaluated by comparing the amount of peak height variation observed between injections and between instruments with the ISN feature enabled and disabled. In addition, lot-to-lot variation in GeneScan™ 600 LIZ® Size Standard v2.0 peak heights was evaluated with Identifiler® and MiniFiler™ kit data.

After the peak height analysis was complete, ISN Targets were determined based on the median peak heights obtained across all six instruments for each AmpFSTR® kit chemistry, using the median of the average peak heights of the internal standard normalization peaks in the GeneScan™ 600 LIZ® Size Standard v2.0 size standard (200, 220, 240, 260, 280, 300, 314, 320, 340, 360, 400) with both PCR reaction mix and amplified DNA. Results showed similar average peak heights for the size standard.

Results The results of this study are expressed as Coefficients of Variation (CVs). The CV of peak heights for an injection is $100 \times [\text{SD of peak heights} / \text{Mean peak height}]$. The study measured injection-to-injection, capillary-to-capillary, and run-to-run variation.

The injection-to-injection variation results are shown in the following figure. Each data point in the graph represents the average height of all the peaks in an injection profile of a sample at a particular concentration on a particular instrument. Each box plot contains 96 data points (4 samples \times 4 concentrations \times 6 instruments). Five capillaries at locations distributed across the array were each injected two times, for a total of ten injections.

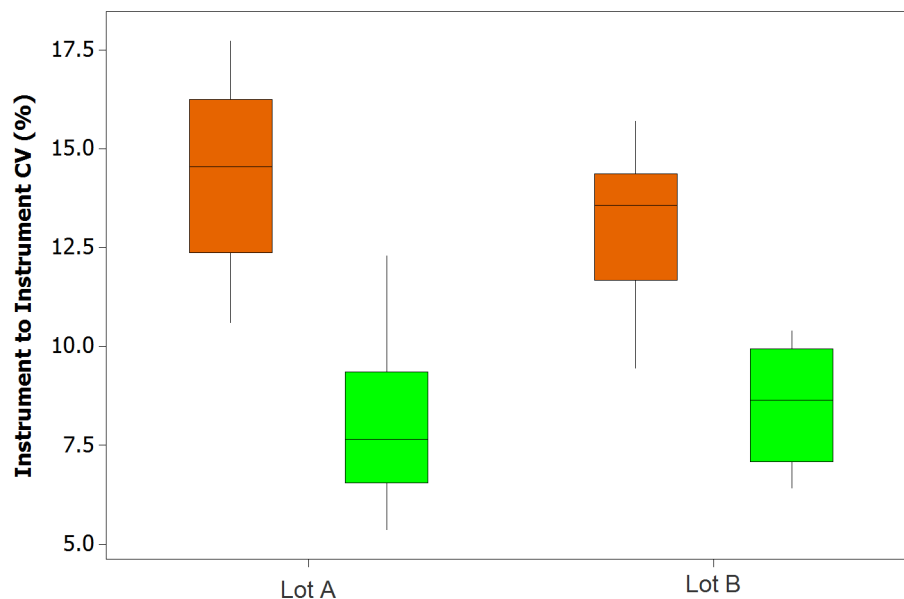
Figure 15 Injection-to-injection Coefficient of Variation of the peak heights for MiniFiler™ Kit samples vs. two lots of size standard



These data demonstrate that the performance for ISN on the 3500 instruments was as expected. When the ISN feature was disabled, the average injection-to-injection CV, which indicates the approximate inherent variation, was approximately 12% for the MiniFiler™ kit. When ISN was enabled, the average injection-to-injection CV was reduced significantly to less than 5%.

The following figure shows instrument-to-instrument CV results for MiniFiler™ kit data plotted against two lots of the GeneScan™ 600 LIZ® Size Standard v2.0, with ISN enabled or disabled. Each box plot contains 16 data points (4 samples \times 4 concentrations). Each point represents the CV across 6 instruments using the average height of peaks from the same sample at the same concentration analyzed on the same instrument.

Figure 16 GeneScan™ 600 LIZ® Size Standard v2.0 instrument-to-instrument variation

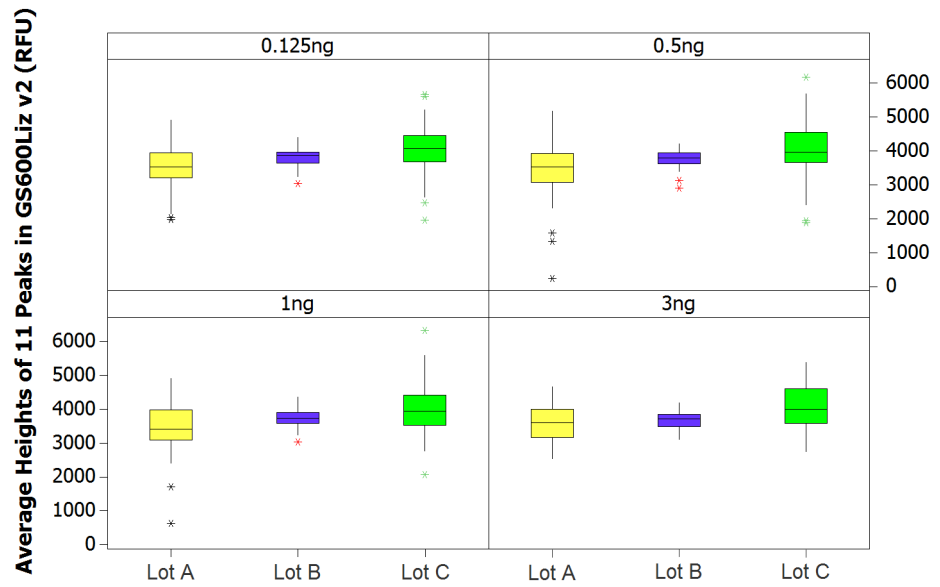


When the ISN was disabled, the average instrument-to-instrument CV was approximately 14% for MiniFiler™ kit data in this study. With ISN enabled, the average instrument-to-instrument CV was reduced significantly to approximately 8%.

The data also demonstrate that different lots of the GeneScan™ 600 LIZ® Size Standard v2.0 gave similar results for the instrument-to-instrument CV, indicating that the performance of the two lots of size standard were consistent. This is also supported by the following analysis showing the average peak height of the size standard over multiple manufacturing lots with Identifiler® kit data.

The following figure shows median peak heights for GeneScan™ 600 LIZ® Size Standard v2.0 analyzed in a background of Identifiler® Sensitivity Samples and three lots of size standard. Each box contains 192 data points from all instruments, all samples, and all concentrations run with each lot of the standard.

Figure 17 GeneScan™ 600 LIZ® Size Standard v2.0 median peak heights: Identifier® samples

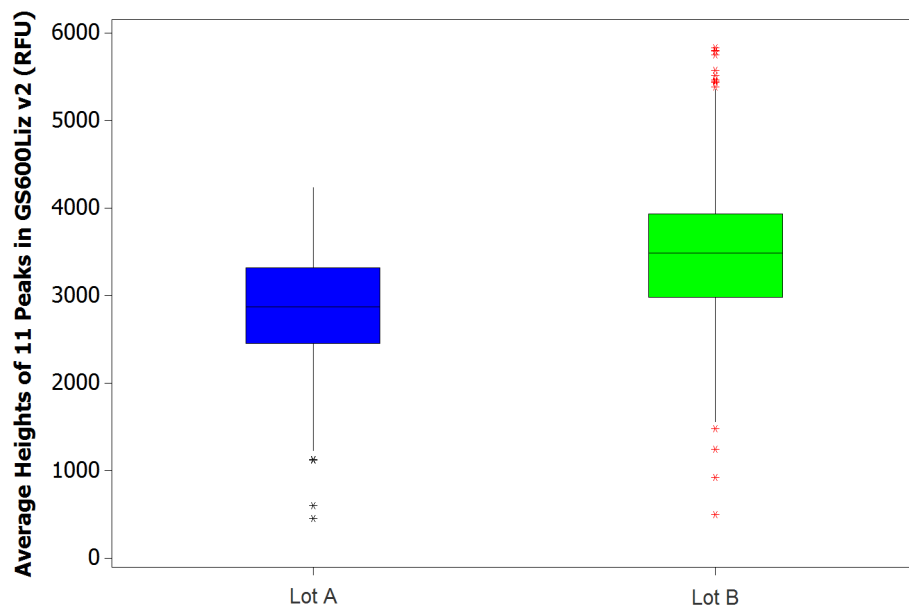


This data also indicates that the GeneScan™ 600 LIZ® Size Standard v2.0 peak heights were not impacted by the PCR input DNA concentration.

Normalization Factor

The following figure shows median peak heights for GeneScan™ 600 LIZ® Size Standard v2.0 analyzed using MiniFiler™ sensitivity samples. This data represents two lots of size standard. The peak heights of the size standard were as expected, and resulted in NF values of approximately 1.0.

Figure 18 GeneScan™ 600 LIZ® Size Standard v2.0 median peak heights: MiniFiler™ samples



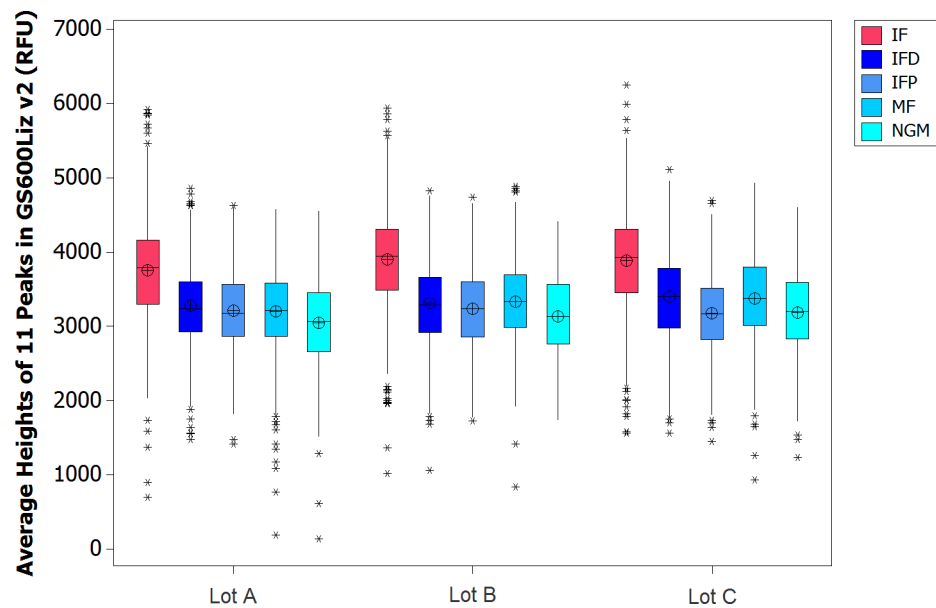
If the Internal Standard Normalization Targets (NT) are set appropriately, the Normalization Factor (NF) should not vary greatly from 1.0 (that is, the average observed value from the sample size standard equals the NT). Therefore, users should set the NT at approximately the median of the peak heights observed during INS validation.

If the NF is consistently closer to 0.3 or 3.0 than to 1.0, users should review the sample setup conditions and begin troubleshooting the source of the variation in peak heights of the size standard or re-evaluate the internal standard normalization target.

Normalization Targets

The Normalization Targets were established by reviewing the GeneScan™ 600 LIZ® Size Standard v2.0 peak height data for all kits tested and determining the median peak height for the 11 peaks used during ISN. The following graph represents the median peak heights of the GeneScan™ 600 LIZ® Size Standard v2.0 size standard when analyzed in the presence of PCR product from five different AmpFSTR® chemistries. The Internal Standard Normalization Target was chosen such that the intended Normalization Factor on subsequent data would be approximately 1.0.

Figure 19 GS600 size standard peak heights injected with Identifiler® (IF), Identifiler® Direct (IFD), Identifiler® Plus (IFP), MiniFiler™ (MF), and NGM™ kit samples



This exemplar data demonstrate that the average peak height for GS600 when coinjected with Identifiler® kit samples is higher than the size standard peak heights observed after coinjection with other chemistries. From this data and other analyses, ISN Target values of 3800 RFU were established for Identifiler® and Sinofiler kit data, and 3200 for Identifiler® Plus, Identifiler® Direct, MiniFiler™, Yfiler®, and NGM™ kit data.

Mixture Analysis with GeneMapper® ID-X Software Version 1.2

To evaluate the ability of the 3500 systems to analyze DNA samples with more than one contributor, a range of mixed DNA samples were analyzed. The default peak amplitude threshold of 175 RFU was used to analyze the samples using GeneMapper® ID-X Software v1.2.

For details of study parameters, see [Table 1, “Studies performed, kits tested, and DNA inputs,”](#) on page 37, [Table 2, “Sample sources and kits and instruments used for preparation and amplification,”](#) on page 38, and [Table 4, “Study criteria matrix,”](#) on page 39.

Experimental design

For all samples in a project, the GeneMapper ID-X Mixture Analysis Tool categorizes and segregates eligible samples according to the minimum number of contributors contained in each sample (1, 2, or 3 or more contributors), separates contributor profiles into individual major and minor genotype combinations, and calculates population statistics. For more information on the Mixture Analysis tool, see the *GeneMapper® ID-X Software Version 1.1 (Mixture Analysis Tool) Getting Started Guide* (Part no. 4396773).

For this study, four pairs of genomic DNA samples were created with seven mixture ratios of 1:0, 1:1, 1:2, 1:3, 1:5, 1:9, and 0:1 (28 total samples). The total DNA input amount in each reaction was the suggested DNA input amount for each kit: 1 ng for Identifiler® and 2 ng for SGM Plus®, Profiler Plus®, and Cofiler®. The percentage of DNA from each contributor therefore varied accordingly depending on the dilution. For example, 1 ng DNA input for a 1:9 mixture results in 0.9 ng of one sample and 0.1 ng of the second target DNA for each sample in the mixture.

The allele dropout rates were measured for each mixture ratio in each kit. The genotyping data were then imported into the Mixture Analysis tool in the software.

Dilution series The number of observed alleles in each mixture profile was tabulated and compared to the expected number of alleles from the known profile.

The percent allele call rates are shown in Table 8 for Identifiler®, SGM Plus®, Profiler Plus®, and Cofiler® respectively. A high percentage of the expected alleles were detected through the 1:9 dilution series utilizing a 175 RFU threshold.

Table 8 Allele call rates for all dilutions using recommended template DNA input amounts, 5 AmpFSTR® kits

Mix ratio	Allele call rate				
	Identifiler® Norm-On	Identifiler® Norm-Off	SGM Plus®	Profiler Plus®	Cofiler®
1:1	100.0%	100.0%	100.0%	100.0%	100.0%
1:2	100.0%	100.0%	100.0%	100.0%	100.0%
1:3	100.0%	100.0%	100.0%	100.0%	100.0%
1:5	99.3%	98.4%	99.2%	99.9%	98.6%
1:9	98.4%	96.5%	89.0%	98.6%	98.2%

.hid file processing

Samples were imported into the Mixture Analysis tool in the GeneMapper® ID-X v1.2 software to demonstrate successful processing of .hid files. The GeneMapper® ID-X v1.2 software validation was described previously in the *GeneMapper® ID-X Software Version 1.2 User Bulletin* (Part no. 4462639). Five analysis categories were used for mixture analysis depending on the kit used and the Mixture Analysis method employed.

- MIT_175

- 175 RFU for the mixture interpretation threshold (MIT).

- Mixture Analysis software default peak height ratio (PHR) thresholds.

Note: RFU values less than 175 are included for illustrative purposes only. Data for RFU values less than 175 was not analyzed.

- PHR_175

- 175 RFU for the mixture interpretation threshold (MIT).

–Customized PHR thresholds and peak height ranges set according to the intra-locus peak height ratios of the single source samples (1:0 and 0:1 mixtures) in the data set of each kit tested.

Table 9 Mixture analysis methods used with defined MIT and PHR thresholds

Kits	MA method	MIT (rfu)	PHR Threshold (rfu)			
			50-150	151-300	301-1000	1001-99999
MA Default	MIT_175	175	50-150	151-300	301-1000	1001-99999
			0.4	0.54	0.63	0.75
Identifiler®	PHR_175 Norm Off	175	175-5500		5501-99999	
			0.67		0.77	
	PHR_175 Norm On	175	175-8500		8501-99999	
			0.67		0.77	
SGM Plus®	PHR_175	175	175-99999			
			0.75			
Profiler Plus®	PHR_175	175	175-3540		3541-99999	
Cofiler®	PHR_175	175	0.685		0.82	

For the Identifiler® samples, the data were analyzed with the ISN option both enabled and disabled. Since ISN changes the absolute peak heights, the peak height range for each PHR threshold was set accordingly.

During mixture analysis, the samples were segregated into two categories, 1-contributor and 2-contributor, according to the MIT and PHR settings in each MA method. The samples under the 2-contributor category were then deconvoluted to give the potential genotype profiles of both contributors according to the PHR and residual thresholds.

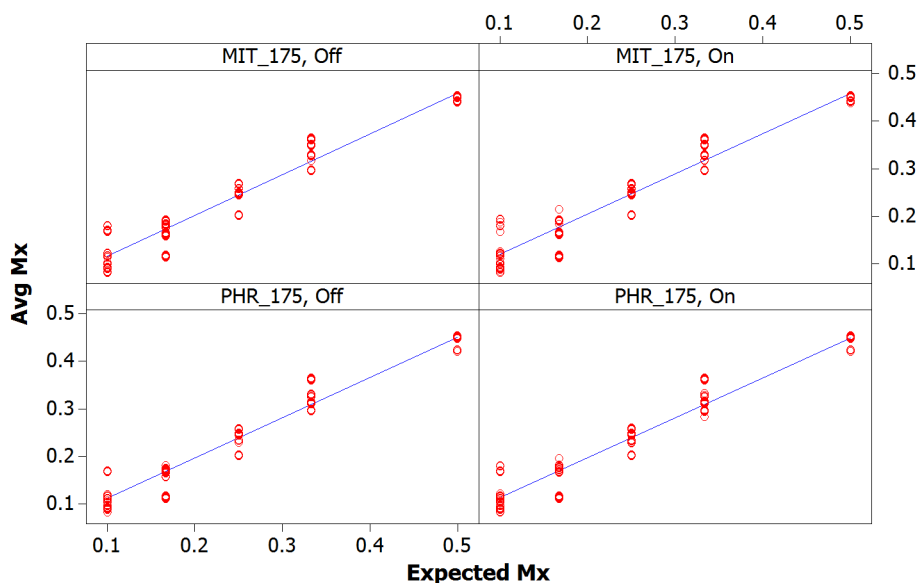
No significant difference in sample segregation and deconvolution was observed between the ISN options

Mixture Ratio prediction

The MA tool in GeneMapper® ID-X v1.2 software calculates the Average Mx value as an estimation of the minor contributor proportion of the sample. Samples used in this test were prepared for the study with known estimated ratios, and were used to evaluate how well the MA calculations fit the expected values.

The Average Mx values were plotted against the expected minor contributor proportions of all samples analyzed using different MA methods and ISN options. The expected minor contributor proportions were calculated as the ratio of the minor contributor DNA concentration to the total DNA concentration for the sample sets. A linear regression fit line was plotted for each group of data.

Figure 20 Example: Regression of average vs. expected minor contributor proportions for Identifiler® samples



The regression lines indicated a good fit of the curves to the data and low variation among the individual data points. The regression line slopes were very similar to results from 3130xL testing with the same DNA inputs and mixture ratios, indicating that the two platforms demonstrated similar performance with mixed samples. The data also demonstrate no statistical difference between the default and customized MA methods, and between the ISN options.

Table 10 Slopes of regression lines for average minus expected Mx ratios

Kits	Internal Standard Normalization	Slope with MIT_175	Slope with PHR_175
Identifiler®	On	0.844	0.8419
	Off	0.8549	0.8473
SGM Plus®	N/A	0.8292	0.87
Profiler Plus®	N/A	0.9278	0.9003
Cofiler®	N/A	0.8978	0.8807

Conclusions

- High-quality data were obtained on the 3500 and 3500xL Genetic Analyzers with the AmpFSTR® PCR Amplification Kits.
- Accurate genotyping results were obtained using GeneMapper® ID-X v1.2 software.
- Resolution estimations confirm single base pair analysis up to 400 base pairs.
- Sizing precision, measured by calculating the standard deviation in the size values obtained for an allele within an injection was <0.15bp for all injections on both the 3500 and 3500xL instruments.
- Noise levels and pull up percentages, when adjusted for the relative RFU scaling of the 3500 instrument, are similar to existing platforms such as 31xx.

Ordering information

Consumables

Note: For reagent or consumable shelf-life expiration date, see the package label.

Description	Part Number
Matrix Standard Set DS-33 3500 Series (Dye Set G5)	4345833
Matrix Standard Set DS-32 (Dye Set F)	4345831
3500 Capillary Array (36 cm)	4404683
3500xL Capillary Array (36 cm)	4404687
Anode Buffer Container (ABC) 3500 Series	4393927
Cathode Buffer Container (CBC) 3500 Series	4408256
Hi-Di™ Formamide	4401457
GeneScan™ 600 LIZ® Size Standard v2.0 Size Standard 3500 Series	4408399
3500 POP-4® Polymer, 384 samples	4393715
3500 POP-4® Polymer, 960 samples	4393710
Conditioning Reagent 3500 Series	4393718
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
GeneScan™ 500 ROX™ Size Standard	401734

HID analysis dye sets

The following dye sets are supported with the HID Fragment Analysis 36_POP4 run module:

Dye set F (4-dye):

- COfiler®
- Profiler Plus®
- Profiler Plus® *ID*
- SGM Plus®
- Other 4-dye kits

Dye set G5 (5-dye)

- Identifiler®
- MiniFiler™
- SEfiler™ Plus
- SinoFiler™
- Yfiler®
- Other 5-dye kits

Validated instrument protocol specifications

The kits were validated by the assays shown in the following table, using POP-4[®] polymer in a 36-cm capillary.

Kit	Assay	Instrument protocol	Primary analysis protocol	Size standard
AmpF [®] STR [®] COfiler [®] PCR Amplification Kit	CO_POP4	HID36_POP4_F	F_LS(75-400)	CE_F_HID_GS500(75-400)
	CO_POP4_xl	HID36_POP4xl_F	F_LS(75-400)	CE_F_HID_GS500(75-400)
AmpF [®] STR [®] Identifiler [®] PCR Amplification Kit	IF_POP4	HID36_POP4_G5_NT3800	G5_LS(80-400)	GS600_LIZ_(80-400)
	IF_POP4_xl	HID36_POP4xl_G5_NT3800	G5_LS(80-400)	GS600_LIZ_(80-400)
	IF+Norm_POP4	HID36_POP4_G5_NT3800	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
	IF+Norm_POP4_xl	HID36_POP4xl_G5_NT3800	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
AmpF [®] STR [®] Identifiler [®] Direct PCR Amplification Kit	IFD_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	IFD_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	IFD+Norm_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
	IFD+Norm_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
AmpF [®] STR [®] Identifiler [®] Plus PCR Amplification Kit	IFP_+Norm_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
	IFP_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	IFP_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	IFP+Norm_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
AmpF [®] STR [®] MiniFiler [™] PCR Amplification Kit	MF_POP4	HID36_POP4_G5_NT3200	G5_3rd(80-400)	GS600_LIZ_(80-400)
	MF_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	MF+Norm_POP4	HID36_POP4_G5_NT3200	G5_3rd(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
	MF+Norm_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)

Kit	Assay	Instrument protocol	Primary analysis protocol	Size standard
AmpF λ STR [®] NGM™ PCR Amplification Kit	NGM_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	NGM_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	NGM+Norm_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
	NGM+Norm_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
AmpF λ STR [®] Profiler Plus [®] ID PCR Amplification Kit	PP_POP4	HID36_POP4_F	F_LS(75-450)	CE_F_HID_GS500(75-450)
	PP_POP4_xl	HID36_POP4xl_F	F_LS(75-450)	CE_F_HID_GS500(75-450)
AmpF λ STR [®] SEfiler Plus™ PCR Amplification Kit	SEfP_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	SEfP_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	SEfP+Norm_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
	SEfP+Norm_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
AmpF λ STR [®] Sinofiler™ PCR Amplification Kit	SF_POP4	HID36_POP4_G5_NT3800	G5_LS(80-400)	GS600_LIZ_(80-400)
	SF_POP4_xl	HID36_POP4xl_G5_NT3800	G5_LS(80-400)	GS600_LIZ_(80-400)
	SF+Norm_POP4	HID36_POP4_G5_NT3800	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
	SF+Norm_POP4_xl	HID36_POP4xl_G5_NT3800	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
AmpF λ STR [®] SGM Plus [®] PCR Amplification Kit	SGM_POP4	HID36_POP4_F	F_LS(75-450)	CE_F_HID_GS500(75-450)
	SGM_POP4_xl	HID36_POP4xl_F	F_LS(75-450)	CE_F_HID_GS500(75-450)
AmpF λ STR [®] Yfiler [®] PCR Amplification Kit	YF_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	YF_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)	GS600_LIZ_(80-400)
	YF+Norm_POP4	HID36_POP4_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)
	YF+Norm_POP4_xl	HID36_POP4xl_G5_NT3200	G5_LS(80-400)+Normalization	GS600_LIZ+Normaliza tion_(80-400)



Documentation

For more detailed information on the 3500 Series Genetic Analyzers and related procedures, refer to:

- *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661)
- *3500 and 3500xL Genetic Analyzers Quick Reference Card* (Part no. 4401662)
- *GeneMapper® ID-X Software Version 1.2 Quick Reference Card* (Part no. 4426482)
- *3500 Data Collection V1.0 Software Release Notes* (on the 3500 Data Collection Software CD)
- *User Bulletin: Applied Biosystems 3500/3500xL Genetic Analyzer* (Part no. 4445098)
- *GeneMapper® ID-X Software Version 1.2 User Bulletin* (Part no. 4462639)

Frequently Asked Questions (FAQs) and supplementary information are available at the Applied Biosystems website, <http://www.appliedbiosystems.com/3500hid>.

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