3500 Series Data Collection Software 4 for HID

New Features and Developmental Validation

Publication Number 100075298 **Revision** D

This user bulletin describes new features. For more information on using the software, see *3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3.1 User Guide* (Pub. No. 100031809).

Changes in the 3500 Series Data Collection Software 4 v4.0.1

If you have not previously run the 3500 Series Data Collection Software 4 software, skip this section and proceed to "New features in 3500 Series Data Collection Software 4" on page 2. All changes described in this section have been made throughout the rest of this document.

If you have been running the 3500 Series Data Collection Software 4 software, review the following changes that have been made in the v4.0.1 software patch.

- **Assay library**—The following assays have been removed.
  - Assays with _SO suffix (signal optimization)
  - Legacy assays with the old naming convention (*STR kit*)
- **Instrument protocol library**—Removed instrument protocols with _SO suffix (signal optimization).
- **Run module option**—HID36_POP4(xl)_SO run module (SO=signal optimization) has been removed.

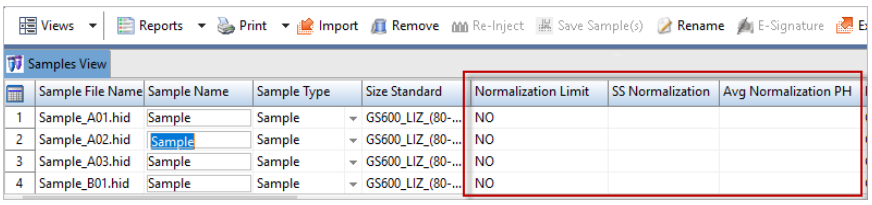
  Note: The HID36_POP4(xl)_SO run module was provided to minimize injection variability between capillaries. The new run module introduced polymer before the injection and set a higher capillary position during injection. However, the introduction of polymer caused artifacts in the data.

  New recommendations for reducing signal variation across capillaries are available in *Technical Bulletin: Updated Recommendations for use of the Signal Optimization Features in 3500 Series Data Collection Software 4 for HID*.

- **Default injection order**—The defect that caused inconsistent default plate injection order has been fixed.
- **Additional minor defect fixes**—See the *3500 Series Data Collection Software 4.0.1 Release Notes* (Pub. No. 100087946).

New features in 3500 Series Data Collection Software 4

New feature	Description
General support	• Windows™ 10, 64-bit operating system (IOT Enterprise) is supported. • No license activation or yearly license renewal is required. The 3500 Series Data Collection Software 4 has been tested with these antivirus software applications: • Symantec Endpoint Protection 12 • McAfee Endpoint Security version 10.5 **IMPORTANT!** McAfee Endpoint Security can block services that are needed to start the Data Collection software. If you observe this issue, disable the Firewall from McAfee Endpoint Security Settings or create a rule to allow traffic for the IP address 192.168.0.1 on the local network.
Data optimization	• "Signal optimization feature" on page 4 • "Off-scale recovery feature" on page 5 • "Pull-up reduction feature" on page 6
Flexible plate loading	The software allows you to load an additional plate to the autosampler at any time during a run. See "Pause a run and load a new plate (flexible plate loading)" on page 10.

New feature	Description
DS-36 install check (6-dye)	A 6-dye **J6** selection is available in the **Chemistry** list in the **Install Check** screen. The components of the install check reaction are as follows: • GeneScan™ 600 LIZ™ Size Standard v2.0 (Cat. No. 4408399) • Hi-Di™ Formamide (Cat. No. 4311320 or 4440753) • GlobalFiler™ Allelic Ladder (from the GlobalFiler™ PCR Amplification Kit, or ordered separately Cat. No. 4476033) The volumes of install check components per reaction are as follows: • Size standard—0.4 µL • Hi-Di™ Formamide—9.6 µL • Allelic ladder—1 µL Pass/fail criteria for HID J6 install check: • 26 size standard peaks • 343 ladder peaks • All markers except TH01: ±0.7 bp of nominal size for the allele. • TH01: – Seven alleles are ±0.7 bp of nominal size for the allele – Three alleles are ±0.5 bp of nominal size for the allele • Minimum peak height >400 RFU
New assays, instrument protocols, QC protocols, and dye sets	Library items have been updated for signal optimization and off-scale data recovery. See "Library enhancements—overview" on page 7 and "Library enhancements—detailed description" on page 12.
Preferences for reagent use	You can set **Instrument Settings** preferences to: • Prevent an instrument run if reagents exceed on-instrument limits or expiration date. • Control the display and timing of warnings that are related to reagent usage limits and expiration. See "Set preferences for reagent use" on page 11.
Size standard normalization information	By default, the Samples view displays the following columns. • Normalization Limit set in the instrument protocol. • Size Standard Normalization Factor calculated by the software. This factor can be applied to data in GeneMapper™ *ID-X* Software by enabling the Normalization checkbox in the GeneMapper™ *ID-X* Software analysis method. • Avg Normalization PH (peak height) of the size standard peaks used to calculate the factor. You can change table settings to hide these columns. 

New feature	Description
Consumables log export	You can export the consumables log in CSV or XLS format. For each consumable, the consumables log contains installation date, instrument serial number, lot, serial number, expiration date, and the name of the user who installed the consumable. 1. In any screen, select **Tools** > **Export Consumables Log**. 2. Select a location and enter a name for the export file. IMPORTANT! In the exported file, the conditioning reagent part description incorrectly lists "Polymer Pouch". However, the lot number that is listed is correct for the conditioning reagent.
Injection list export	You can export the injection list from two locations: • **Preview Run** screen at any time before a run or during a run. • **Samples view** screen when a run is complete. See "Export the injection list from Preview Run or Samples view" on page 10.
Calendar	Tasks have new **Every Two Weeks** repeat setting.
EPT plot for completed or terminated runs	You can view the ElectroPhoresis Telemetry (EPT) plot in the **Monitor Run** screen. The EPT plot shows instrument data conditions for a completed or terminated run until the plate for the run is unlinked. **Note: EP Voltage**, **Laser Power**, **EP Current**, and **Run Temp** are stored in the sample file and is available to view in secondary analysis software.

Signal optimization feature

The signal optimization feature reduces peak height variation across capillaries in an injection. This variation is introduced by the optics of the instrument and the injection conditions used. The signal optimization feature has two components:

- Spatial calibration-dependent signal optimization (24-capillary instruments only, always enabled)
- Capillary-position–dependent signal optimization (8-capillary and 24-capillary instruments, requires autosampler adjustment by field service engineer.)

For maximum signal optimization on 24-capillary instruments, we suggest that you use both components of the signal optimization feature.

Spatial calibration-dependent signal optimization

To enable this feature: 24-capillary instruments only, always enabled. Not available on 8-capillary instruments.

To disable this feature: Cannot be disabled

During spatial calibration, a **Signal Optimization Factor** is calculated for each capillary using a fitted curve method. The fitted curve method minimizes background signal and reduces noise.

The adjusted signal intensity, not the signal intensity displayed for a capillary, is used to calculate the **Signal Optimization Factor** for the capillary. The **Signal Optimization Factor** for each capillary is displayed in the **Spatial Calibration** screen (the adjusted signal intensity is not displayed).

① Signal Optimization Factor

Note: The signal intensity, average peak height, and uniformity displayed for the capillaries are used for the **Perform QC Checks** function in spatial calibration.

The **Signal Optimization Factor** range is 0.5–2. Higher or lower values are rounded down or up to bring them within range. Therefore, you may observe two peaks with different intensities but with the same **Signal Optimization Factor**.

Note: On 8-capillary instruments, the **Signal Optimization Factor** field displays 1.0 for all capillaries after a spatial calibration.

The **Signal Optimization Factor** is applied to signal data for each capillary during data collection to minimize optical variation effects and increase signal uniformity between capillaries.

The **Signal Optimization Factor** is exported or printed and included in the spatial calibration report with the other spatial calibration results.

Capillary-position–dependent signal optimization

An injection position higher in the well than the default position can be set by a field service engineer. After this autosampler adjustment, use the default HID36POP4(xl) run module for improved signal optimization.

Off-scale recovery feature

To enable this feature:

- Use Applied Biosystems™ DS-33, DS-36, or DS-37 matrix standards *and*
- Select **G5-OSR** (off-scale recovery), **J6-OSR**, or **J6T-OSR** dye set in spectral calibration *and*
- Use a **G5OSR**, **J6OSR**, or **J6TOSR** assay to run samples

To disable this feature:

- Use a non-Applied Biosystems™ dye set and an **AnyDye** dye set for spectral calibration *or*
- Use an Applied Biosystems™ dye set and a non-OSR or **AnyDye** dye set for spectral calibration

The off-scale recovery (OSR) feature accommodates data that would otherwise saturate the CCD camera. This feature improves the first-pass success rate for high-DNA-input samples in a reference or database workflow.

The OSR feature uses spectral calibration data from Applied Biosystems™ DS-33, DS-36, and DS-37 matrix standards. To enable the OSR feature, you must use one of the matrix standards listed previously, and select the **G5-OSR** (off-scale recovery), **J6-OSR**, or **J6T-OSR** dye set in spectral calibration. Use a **G5 OSR**, **J6 OSR**, or **J6T OSR** assay to run samples.

When the OSR feature is enabled, the CCD camera saturation limit during data collection is extended from ~32K RFU to 65K RFU. When you use GeneMapper™ *ID-X* Software v1.6 to analyze data collected with this feature enabled, off-scale (OS) PQVs are not triggered until the signal is > 65K RFU.

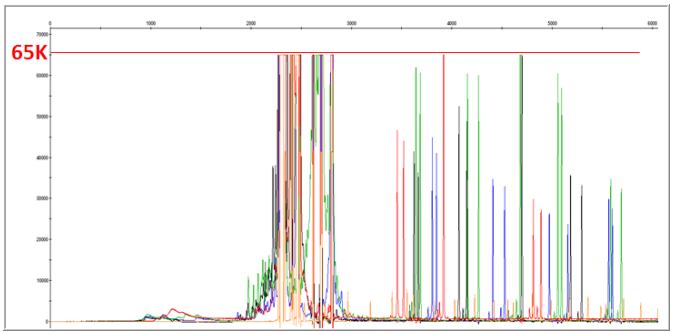


Figure 1 Raw data view of a sample collected with the OSR feature enabled in 3500 Series Data Collection Software 4. Signal is saturated at 65K RFU.

Note: If you use GeneMapper™ *ID-X* Software v1.5 or earlier, the OSR feature *is not* applied (older versions of software cannot interpret the information needed for OSR; the saturation limit is not extended in earlier versions of the software).

Using non-Applied Biosystems™ dye sets

If you use a dye set other than the dye sets used in the DS-33, DS-36, or DS-37 matrix standards, or if you do not use an OSR dye set for spectral calibration, the off-scale recovery feature is not applied.

AnyDye templates are available (**AnyDye**, **Any5dye VB**, or **Any6Dye VB**) to create the appropriate dye set for use in spectral calibration. The **Any5dye VB** and **Any6Dye VB** templates contain variable binning patterns that match the binning patterns in Applied Biosystems™ 5-dye and 6-dye dye sets.

Pull-up reduction feature

To enable this feature:

- Use Applied Biosystems™ DS-33, DS-36, or DS-37 matrix standards *and*
- Select **G5**, **J6**, **J6T**, **G5-OSR** (off-scale recovery), **J6-OSR**, or **J6T-OSR** dye set in spectral calibration
- Use any assay to run samples

To disable this feature:

- Use a non-Applied Biosystems™ dye set and an **AnyDye** dye set for spectral calibration *or*
- Use an Applied Biosystems™ dye set and an **AnyDye** dye set for spectral calibration

The pull-up reduction feature minimizes pull-up when you use Applied Biosystems™ DS-33, DS-36, and DS-37 dye sets. You can use G5, J6, or J6T dye sets (or the OSR versions of the dye sets) for spectral calibration. Any HID G5, J6, or J6T assay can be used to run samples (OSR versions of the assay are not required).

Note: If you use GeneMapper™ *ID-X* Software v1.6 or earlier, the pull-up reduction feature *is* applied.

Using non-Applied Biosystems™ dye sets

If you use a dye set other than the dye sets used in the DS-33, DS-36, or DS-37 matrix standards, the pull-up reduction feature is not applied.

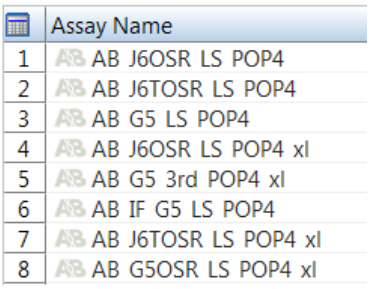
AnyDye templates are available (**AnyDye**, **Any5dye VB**, or **Any6Dye VB**) to create the appropriate dye set for use in spectral calibration. The **Any5dye VB** and **Any6Dye VB** templates contain variable binning patterns that match the binning patterns in Applied Biosystems™ 5-dye and 6-dye dye sets.

Library enhancements—overview

Assay library enhancements

The naming convention for assays has changed from *STR kit* to *dye set name*.

In addition to the dye set name and polymer name, new assay names use the following codes:



	Assay Name
1	AB J6OSR LS POP4
2	AB J6TOSR LS POP4
3	AB G5 LS POP4
4	AB J6OSR LS POP4 xl
5	AB G5 3rd POP4 xl
6	AB IF G5 LS POP4
7	AB J6TOSR LS POP4 xl
8	AB G5OSR LS POP4 xl

- **3rd**—Third-order least-squares curve fitting
- **AB**—For use with Applied Biosystems™ dye sets
- **IF**—Identifiler
- **LS**—Local Southern curve fitting
- **OSR**—Off-Scale Recovery (for use with direct, single-source workflows)
- **xl**—For use on 24-capillary instruments

Note: If your assay library contains assays with the _SO suffix (which indicates that you are not running the latest v4.0.1 version of the software), see *Technical Bulletin: Updated Recommendations for use of the Signal Optimization Features in 3500 Series Data Collection Software 4 for HID* for updated recommendations for the use of _SO assays.

Assay name first characters[1]	For use with AmpFℓSTR™ kit
AB_G5_3rd_POP4...	MiniFiler™
AB_ G5_LS_POP4...	Identifiler™ Plus, NGM™, NGM SElect™, Yfiler™
AB_G5OSR_LS_POP4...	Identifiler™ Direct, NGM SElect™ Express, Yfiler™ Direct
AB_IF...	Identifiler™
AB_J6...	GlobalFiler™, GlobalFiler™ IQC, Yfiler™ Plus

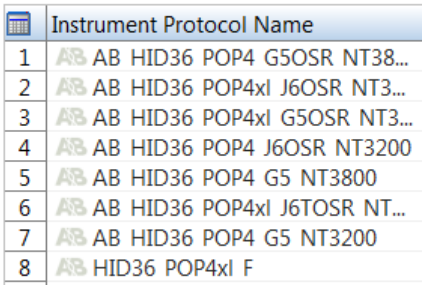
Assay name first characters[1]	For use with AmpFℓSTR™ kit
AB_J6OSR...	GlobalFiler™ Express, VeriFiler™ Express, Huaxia™ Platinum
AB_J6T...	NGM Detect™, VeriFiler™ Plus

[1] Additional assays with these starting characters are listed in the software. The additional assays may include optimization features or may be for use with 24-capillary instruments.

For a comprehensive list of changes, see "Assay library changes" on page 14.

Instrument protocol library enhancements

In addition to the dye set name and polymer name, new instrument protocol names use the following codes:



- **AB**—For use with Applied Biosystems™ dye sets
- **HID36**—HID use with a 36-cm capillary array
- **NT3200**— Normalization Target setting = 3200
- **NT3800**— Normalization Target setting = 3800
- **OSR**—Off-Scale Recovery
- **xl**—For use on 24-capillary instruments

Note: If your instrument protocol library contains protocols with the _SO suffix (which indicates that you are not running the latest v4.0.1 version of the software), see *Technical Bulletin: Updated Recommendations for use of the Signal Optimization Features in 3500 Series Data Collection Software 4 for HID* for updated recommendations for the use of _SO protocols.

Instrument protocol name first characters[1]	Suggested for use with AmpFℓSTR™ kit
AB_HID36_POP4_G5_NT3200...	MiniFiler™, Identifiler™ Plus, SEfiler Plus™, NGM™, NGM SElect™, Yfiler™
AB_HID36_POP4_G5OSR_NT3200...	Identifiler™ Direct, NGM SElect™ Express, Yfiler™ Direct
AB_HID36_POP4_G5_NT3800...	Identifiler™
AB_HID36_POP4_J6_NT3200...	GlobalFiler™, GlobalFiler™ IQC,Yfiler™ Plus
AB_HID36_POP4_J6OSR_NT3200...	GlobalFiler™ Express, VeriFiler™ Express, Huaxia™ Platinum
AB_HID36_POP4xl_J6T_NT3200...	NGM Detect™, VeriFiler™ Plus

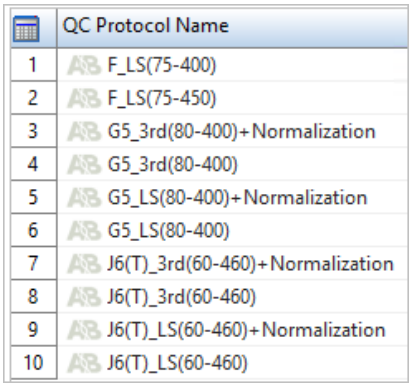
[1] Additional protocols with these starting characters are listed in the software. The additional protocols may include optimization features or may be for use with 24-capillary instruments.

For a comprehensive list of changes, see "Instrument protocol library changes" on page 16.

QC protocol library enhancements

In addition to the dye set name, new QC protocol names use the following codes:

- **3rd**—Third-order curve fitting
- **LS**—Local Southern curve fitting
- **(starting bp-ending bp)**—Sizing range
- **Normalization**—Use if data is collected with Size Standard Normalization enabled



	QC Protocol Name
1	F_LS(75-400)
2	F_LS(75-450)
3	G5_3rd(80-400)+Normalization
4	G5_3rd(80-400)
5	G5_LS(80-400)+Normalization
6	G5_LS(80-400)
7	J6(T)_3rd(60-460)+Normalization
8	J6(T)_3rd(60-460)
9	J6(T)_LS(60-460)+Normalization
10	J6(T)_LS(60-460)

QC protocol name first characters[1]	Suggested for use with kit
F_...	4-dye
G5...	5-dye
J6(T)...	6-dye (J6 or J6-T)

[1] Additional protocols with these starting characters are listed in the software. The additional protocols may include optimization features or may be for use with 24-capillary instruments.

For a comprehensive list of changes, see "QC protocol library changes" on page 19.

Dye set library enhancements

In addition to the dye set name, new dye set names use the following codes:

- **OSR**—Off-Scale Recovery
- **VB**—Variable Binning

Dye set names	Application
G5	DNA sizing for Applied Biosystems™ 5-dye chemistry
G5-OSR	DNA sizing for Applied Biosystems™ 5-dye chemistry, off-scale recovery (databasing applications)
J6, J6T	DNA sizing for Applied Biosystems™ 6-dye chemistry
J6-OSR, J6T-OSR	DNA sizing for Applied Biosystems™ 6-dye chemistry, off-scale recovery (databasing applications)
F	DNA sizing for Applied Biosystems™ 4-dye chemistry
AnyDye	DNA sizing with standard binning
Any5Dye VB	DNA sizing with G5 variable binning pattern
Any6Dye VB	DNA sizing with J6(T) variable binning pattern

For a comprehensive list of changes, see "Dye set library changes" on page 18.

Export the injection list from **Preview Run** or Samples view

You can export the injection list from two screens.

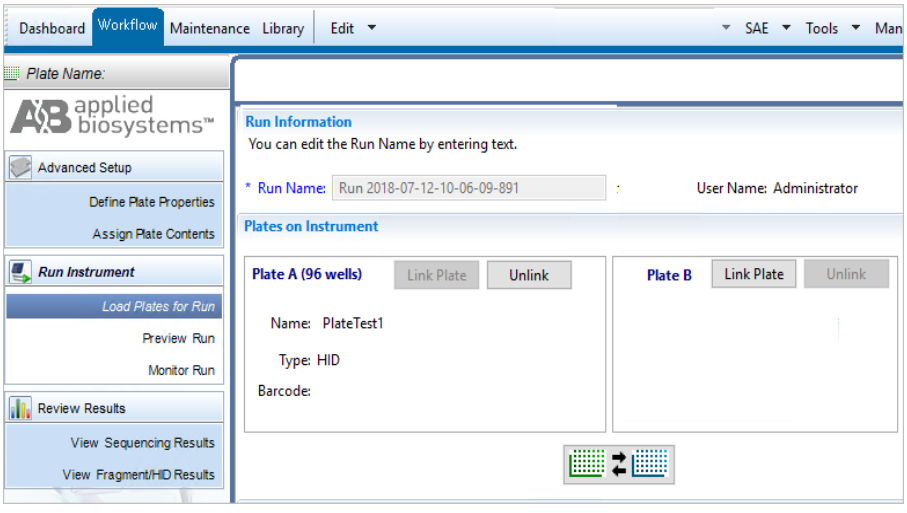
Screen	Procedure
Preview Run Provides a CSV, XLS, or TXT export file that lists injections in the order in which they are displayed on the screen.	1. Select **Preview Run** in the left pane. 2. Click **Export**.
Samples view Provides a CSV, XLS, or TXT export file that lists samples in the order in which they are displayed on the screen.	1. Select **View Fragment/HID results** in the left pane. 2. Create a table setting that includes **Injection Start Date** column. 3. Sort the table by sample file name, then by **Injection Start Date** column (which also includes the time of the injection). 4. Click **Export**.

Pause a run and load a new plate (flexible plate loading)

The software allows you to load an additional plate in the instrument at any time during a run, and move injections from the new plate to the top of the injection list.

1. In the **Workflow** tab, click **Monitor Run** in the left pane.
2. In the **Monitor Run** screen, click **Pause Run**.

3. Click **OK** to accept the message that indicates that the instrument will pause after completing the current injection.
4. When the run pauses, click **Load Plate for Run** in the left pane.
5. If both plate positions are filled in the **Load Plate for Run** screen, click **Unlink** for one of the positions, then remove the plate.



6. Install the new plate, click **Link Plate**, then select the plate you want to load.

7. At the bottom of the **Load Plate for Run** screen, click **Create Injection List**.
8. Click **Monitor Run** in the left pane.
9. As needed, change the injection order in the **Monitor Run** screen.
   You can move injections from the newly loaded plate up in the injection list to inject from the newly loaded plate before the first plate has finished running.
10. Click **Resume Run**.

Set preferences for reagent use

1. Select **Preferences** in the toolbar.
2. Click **Instrument Settings**, then set the desired options.

Options	Description
Allow runs with reagents that exceed limits (Figure 2)	By default, the software allows use of reagents that are expired or exceed on-instrument limits. This setting allows a user to dismiss expiration or limit warnings, then continue the run. An Administrator can prevent the use of reagents that are expired or exceed on-instrument limits by deselecting reagents in the **Instrument Settings** screen. By default, all reagents are selected, which means that a user can dismiss a reagent usage limits and/or expiration warning and continue to run. Deselect the reagents that will not allow runs unless they are within on-instrument limits (to set a "hard stop"). If you do not have Administrator role, these options are not active.
Enable warning messages (Figure 3)	You can control the display and timing of warnings that are related to reagent usage limits and expiration. • Expiration warnings are displayed when a reagent is expired or exceeds on-instrument limits. • Pre-expiration warnings are displayed the following number of days before a reagent is expired: – ABC and CBC: 7 days – Array and polymer: 14 days

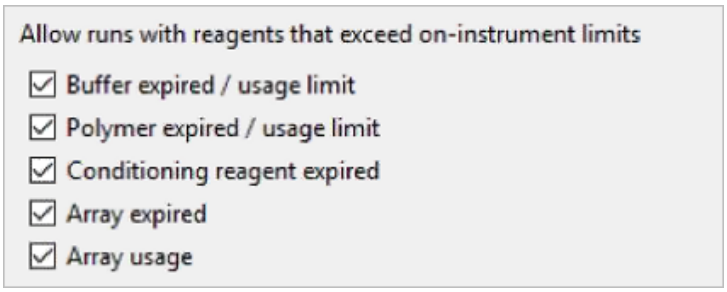


Figure 2 Allow runs with reagents that exceed limits

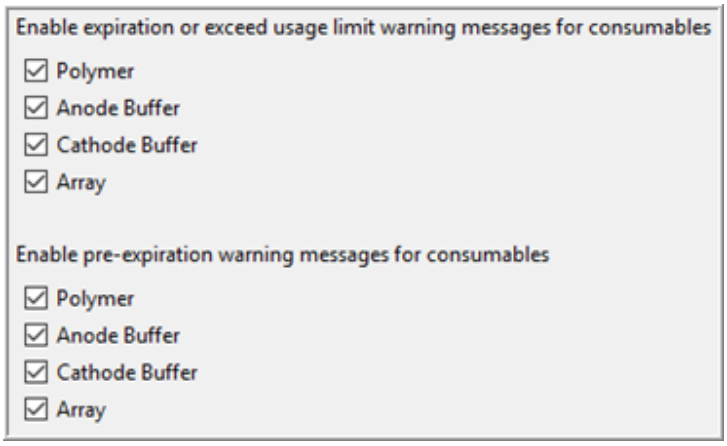


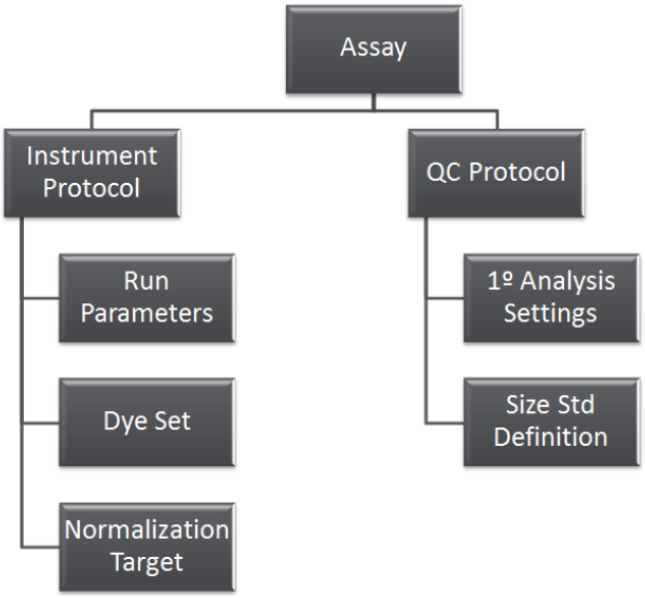
Figure 3 Enable warning messages

3. Click **OK**.

Library enhancements—detailed description

Data Collection Software library overview

The library structure and elements are unchanged in the 3500 Series Data Collection Software 4. The changes to each library are described in the following sections.



- **Assay**—Contains one or more instrument protocols needed to collect data and a QC protocol for primary analysis.
  - **Instrument protocol**— Contains:
    - **Dye set**—Defines the dye colors, order of the dye peaks in the dye standard, and spectral analysis parameters.
    - **Run module**—Specifies instrument control parameters.
    - **Normalization target**—Sets the expected average RFU peak height when using the size standard normalization feature.
  - **QC protocol**—Contains:
    - **Primary analysis parameters—**Defines peak detection, sizing, and quality values.
    - **Size standard definition**—Defines the sizes of known fragments and is used to determine the sizing of unknown samples.

Assay library changes

- The naming convention for assays has changed from *STR kit* to *dye set name*. For more information on naming conventions, see “Assay library enhancements“ on page 7.
- Assays that contained the following items have been removed:
  - QC protocols with size standard normalization
  - Instrument protocols with the F dye set

The following table lists only the items for Applied Biosystems™ 5-dye and 6-dye STR kits. No changes have been made to other items.

Use items with the "AB_" prefix when you use Applied Biosystems™ dye sets to take advantage of the new data optimization features. Use items without the "AB_" prefix when you use non-Applied Biosystems™ dye sets.

Note: If your assay library contains assays with the _SO suffix (which indicates that you are not running the latest v4.0.1 version of the software), see *Technical Bulletin: Updated Recommendations for use of the Signal Optimization Features in 3500 Series Data Collection Software 4 for HID* for updated recommendations for the use of _SO assays.

Table 1 Recommended assays for Applied Biosystems™ 5-dye and 6-dye STR kits

Assay name[1]	Instrument protocol name (dye set is indicated in protocol name)	QC protocol name/Size standard name	Supports data optimization feature	For use with kit	Changes required before use with kit
AB_J6T_LS_POP4	AB_HID36_POP4_J6T_ NT3200	J6(T)_LS(60-460)/ GS600_LIZ _(60-460)	Pull-up reduction	NGM Detect™ VeriFiler™ Plus	NGM Detect™, change injection times: 3500: 11 sec 3500*xl*: 20 sec
AB_J6TOSR_LS_POP4	AB_HID36_POP4_ J6TOSR_ NT3200		Pull-up reduction Off-scale recovery	NGM Detect™ (direct workflow)	—
AB_J6_LS_POP4	AB_HID36_POP4_J6_ NT3200		Pull-up reduction	GlobalFiler™ GlobalFiler™ IQC Yfiler™ Plus	Yfiler™ Plus kit, change injection time: 3500: 16 sec
AB_J6OSR_LS_POP4	AB_HID36_POP4_ J6OSR_ NT3200		Pull-up reduction Off-scale recovery	GlobalFiler™ Express VeriFiler™ Express Huaxia™ Platinum	—
AB_IF_G5_LS_POP4	AB_HID36_POP4_G5_ NT3800	G5_LS(80-400)/ GS600_LIZ_(80-400)	Pull-up reduction	Identifiler™	—
AB_ G5_LS_POP4	AB_HID36_POP4_G5_ NT3200		Pull-up reduction	Identifiler™ Plus NGM™ NGM SElect™ Yfiler™	—
AB_G5OSR_LS_POP4	AB_HID36_POP4_ G5OSR_ NT3200		Pull-up reduction Off-scale recovery	Identifiler™ Direct NGM SElect™ Express Yfiler™ Direct	—
AB_G5_3rd_POP4	AB_HID36_POP4_G5_ NT3200	G5_3rd(80-400)/ GS600_LIZ_(80-400)	Pull-up reduction	MiniFiler™	—

[1] Items for 8-capillary array instruments are listed. Items for 24-capillary array instruments assays are also provided in the library with XL identifiers.

Instrument protocol library changes

- The naming convention for instrument protocols has changed. For more information on naming conventions, see "Instrument protocol library enhancements" on page 8.
- Instrument protocols are provided that support the new off-scale recovery (OSR) features.

The following table lists only the items for Applied Biosystems™ 5-dye and 6-dye STR kits. No changes have been made to other items.

Use items with the "AB_" prefix when you use Applied Biosystems™ dye sets to take advantage of the new data optimization features. Use items without the "AB_" prefix when you use non-Applied Biosystems™ dye sets.

Note: If your instrument protocol library contains protocols with the _SO suffix (which indicates that you are not running the latest v4.0.1 version of the software), see *Technical Bulletin: Updated Recommendations for use of the Signal Optimization Features in 3500 Series Data Collection Software 4 for HID* for updated recommendations for the use of _SO protocols.

Table 2 Recommended instrument protocols for Applied Biosystems™ 5-dye and 6-dye STR kits

Instrument protocol name[1]	Run module name[2]	Dye set name	Supports data optimization feature	For use with kit	Changes required before use
AB_HID36_POP4_J6T_ NT3200	HID36_POP4	J6T	Pull-up reduction	NGM Detect™ VeriFiler™ Plus	NGM Detect™ kit, change injection times: 3500: 11 sec 3500*xl*: 20 sec
AB_HID36_POP4_ J6TOSR_ NT3200	HID36_POP4	J6T-OSR	Pull-up reduction Off-scale recovery	NGM Detect™ (direct amplification workflow)	—
AB_HID36_POP4_J6_ NT3200	HID36_POP4	J6	Pull-up reduction	GlobalFiler™ GlobalFiler™ IQC Yfiler™ Plus	Yfiler™ Plus , change injection time: 3500: 16 sec
AB_HID36_POP4_ J6OSR_ NT3200	HID36_POP4	J6-OSR	Pull-up reduction Off-scale recovery	GlobalFiler™ Express VeriFiler™ Express Huaxia™ Platinum	—

Instrument protocol name[1]	Run module name[2]	Dye set name	Supports data optimization feature	For use with kit	Changes required before use
AB_HID36_POP4_G5_ NT3200	HID36_POP4	G5	Pull-up reduction	MiniFiler™ Identifiler™ Plus NGM™ NGM SElect™ Yfiler™	—
AB_HID36_POP4_ G5OSR_ NT3200	HID36_POP4	G5-OSR	Pull-up reduction Off-scale recovery	Identifiler™ Direct, NGM SElect™ Express Yfiler™ Direct	—
AB_HID36_POP4_G5_ NT3800	HID36_POP4	G5	Pull-up reduction	Identifiler™	The Identifiler™ kit uses Normalization Target of 3800 and uses a different instrument protocol than other G5 kits.
AB_HID36_POP4_ G5OSR_ NT3800	HID36_POP4	G5-OSR	Pull-up reduction Off-scale recovery	Identifiler™ (direct amplification workflow)	

[1] Items for 8-capillary array instruments are listed. Items for 24-capillary array instruments assays are also provided in the library with XL identifiers.

[2] Run modules are also available when creating a new instrument protocol.

Dye set library changes

Dye sets and templates are provided that support the new off-scale recovery (OSR) and variable binning (VB) features. The following table lists only the items for 5-dye and 6-dye STR kits. No changes have been made to other items.

If you are using non-Applied Biosystems™ dye sets, use AnyDye dye sets.

Note: The Any5Dye VB and Any6Dye VB dye sets use the same variable binning pattern as the G5 or J6 binning.

Table 3 Dye sets for Applied Biosystems™ 5-dye and 6-dye STR kits

Dye set name	Dye set template name[1]	Variable binning pattern	Supports new feature	For use with kit
J6T	J6T Template	J6	Pull-up reduction	Applied Biosystems™ 6-dye: GlobalFiler™ GlobalFiler™ IQC Yfiler™ Plus NGM Detect™ VeriFiler™ Plus
J6	J6 Template			
Any6Dye VB	Any6Dye VB Template		NA	Non-Applied Biosystems™ 6-dye
J6T-OSR	J6T-OSR Template	J6-OSR	Pull-up reduction Off-scale recovery	Applied Biosystems™ 6-dye (direct amplification workflow): GlobalFiler™ Express VeriFiler™ Express
J6-OSR	J6-OSR Template		Pull-up reduction Off-scale recovery	
G5	G5 Template	G5	Pull-up reduction	Applied Biosystems™ 5-dye: Identifiler™ Identifiler™ Plus MiniFiler™ NGM™ NGM SElect™ Yfiler™

Dye set name	Dye set template name[1]	Variable binning pattern	Supports new feature	For use with kit
Any5Dye VB	Any5Dye VB Template	G5	NA	Non-Applied Biosystems™ 5-dye
G5-OSR	G5-OSR Template	G5-OSR	Pull-up reduction Off-scale recovery	Applied Biosystems™ 5-dye (direct amplification workflow): Identifiler™ Direct NGM SElect™ Express
AnyDye	AnyDye Template	Standard	N/A	Non-Applied Biosystems™

[1] Templates also available when creating a new 5- or 6-dye dye set.

QC protocol library changes

- The naming convention for QC protocols has changed. For more information on naming conventions, see "QC protocol library enhancements" on page 9.
- QC protocols are provided that support the J6-T dye set.

The following table lists only new QC protocols. No other changes have been made to the QC protocols for 4- or 5- dye Applied Biosystems™ kits.

Table 4 New QC protocols for Applied Biosystems™ 6-dye STR kits

QC protocol name	Size standard	Size calling method/ Size range: 60–460 bp	For use with kit
J6(T)_3rd(60-460)+Normalization	GS600_LIZ+Normalization_(60-460)	3rd order	GlobalFiler™ GlobalFiler™ IQC GlobalFiler™ Express Yfiler™ Plus NGM Detect™ VeriFiler™ Plus VeriFiler™ Express
J6(T)_3rd(60-460)	GS600_LIZ _(60-460)		
J6(T)_LS(60-460)+Normalization	GS600_LIZ+Normalization_(60-460)	Local Southern	
J6(T)_LS(60-460)	GS600_LIZ _(60-460)		

Size standard library changes

No changes have been made to the size standard library.

This library includes GeneScan™ 500 ROX™ Size Standard for 4-dye Applied Biosystems™ STR kits and GeneScan™ 500 LIZ™ Size Standard and GeneScan™–600 LIZ™ Size Standard for 5- and 6-dye Applied Biosystems™ STR kits.

3500 Series Data Collection Software 4 v4.0.1 verification

The goal of this verification is to:

- Confirm that the updates included in 3500 Series Data Collection Software 4 v4.0.1 function as expected.
- Ensure that the upgrade from v4 to v4.0.1 does not affect functional performance or data quality.

Table 5 Instruments and software

Instrument	Make/model	Operating system (OS)/Service pack (SP)	Data Collection Software
3500	Dell OptiplexXE2 7480	Windows™ 10, 64-bit operating system (IOT Enterprise)	v4 upgraded to v4.0.1
3500xL			

All data was analyzed in GeneMapper™ *ID-X* Software v1.6 using the default analysis settings that are recommended for the GlobalFiler™ PCR Amplification Kit with a 50 RFU peak amplitude threshold.

After the upgrade, spatial and spectral calibrations and an HID 6-dye install run were performed. All runs passed.

In addition to the results of the studies listed below, the pull-up reduction and off-scale data recovery algorithms were confirmed to be applied to the data and performed as expected.

Table 6 Results

Study	Samples	Expected Outcome	Result
Precision	GlobalFiler™ Allelic Ladder: • 3500xL: 24 wells × 4 injections • 3500: 8 wells ×4 injections	Standard Deviation of mean size of each allele in allelic ladder <0.15 bp	Pass
Genotyping	DNA Control 007, 1 ng: 3 wells ×1 injection	Expected genotype obtained	Pass
Sensitivity	DNA Control 007 125 pg: 3 wells × 1 injection	Full profile obtained	Pass
Dynamic range	DNA Control 007, 4 ng: • J6 run module: 3 wells × 1 injection • J6OSR run module: 3 wells × 1 injection	• J6 run module: OS peaks capped at ~32,000 RFU • J6OSR run module: OS peaks recovered up to 65,000 RFU	Pass
Contam-ination	3 wells ×1 injection	No typeable alleles	Pass

3500 Series Data Collection Software 4 validation

Objective of the validation

The objective of the validation is to confirm that the performance of the 3500 Series Data Collection Software 4 meets the following requirements:

- Operational accuracy for instrument control, calibration, run set up, and data collection with legacy and current 5 and 6-dye Applied Biosystems™ PCR amplification kits.
- Data collection accuracy compared to 3500 Series Data Collection Software 3 v3.1.2. The studies in this validation assessed sizing precision, accuracy, resolution, and genotyping concordance of genomic DNA samples with representative Applied Biosystems™ PCR amplification kits.
- Reduction of pull-up peaks and reduction of off-scale data in single-source samples with representative Applied Biosystems™ PCR amplification kits.
- Improvement in peak height variation across capillaries in an injection.
- Free of any critical limitations or defects in the general user interface and workflow.

The validation was performed according to the guidelines from the *Scientific Working Group on DNA Analysis Methods* (SWGDAM, December 2016).

Materials and samples

Instrumentation and computers

Three 24-capillary instruments (3500xL) and two 8-capillary instruments (3500) were used in the validation experiments.

Instrument	Make/model	Operating system (OS)/Service pack (SP)	Data Collection Software
3500	Dell OptiplexXE2 7480	Windows™ 10 , 64-bit operating system (IOT Enterprise)	v4.0
3500	Dell OptiplexXE2 7480		v4.0
3500xL	Dell OptiplexXE2 7480		v4.0
		Windows™ 7 Professional/SP1, 32-bit	v3.1.2[1]
3500xL	Dell OptiplexXE2 7480	Windows™ 10 , 64-bit operating system (IOT Enterprise)	v4.0
3500xL	Dell OptiplexXE2 7480		v4.0

[1] 3500 Series Data Collection Software 3 v3.1.2 includes a signal optimization feature. For validation information for this feature, see "3500 Series Data Collection Software 3 v3.1.2 signal optimization validation" on page 36.

All instruments were not used in every study. However, each instrument was used in at least one study.

A new SO (signal optimization) run module was introduced in v4 and was used for all runs.

Note: The SO (signal optimization) run module was removed in the v4.0.1 software. For more information, see *Technical Bulletin: Updated Recommendations for use of the Signal Optimization Features in 3500 Series Data Collection Software 4 for HID*.

Before validation studies were run, the following steps were performed on all instruments:

- Spatial calibration
- Spectral calibration with DS-33, DS-36, and DS-37 matrix standards using the standard and OSR dye sets (all instruments). For more information, see page 18 and page 9.
- HID install check using the GlobalFiler™ kit allelic ladder (6-dye) and GeneScan™ 600 LIZ™ Size Standard v2.0 (all instruments).
- HID install check using the Identifiler™ kit allelic ladder (5-dye) and GeneScan™ 600 LIZ™ Size Standard v2.0 (one 3500 instrument and one 3500xL instrument).

Software

The following software was used in this validation:

- 3500 Series Data Collection Software 4 and 3500 Series Data Collection Software 3 v3.1.2
- GeneMapper™ *ID-X* Software was used for all analysis with the analysis settings listed in the following table.
  - v1.6 was used for all studies.
  - v1.5 was used for the following studies: Sizing precision, concordance, off-scale recovery.

Table 7 GeneMapper™ *ID-X* Software Analysis Settings

Parameter	5-dye kit setting	6-dye kit setting
Size standard	**GS600_LIZ_(80-400)**	**GS600_LIZ_(60-460)**
Smoothing	**Light**	**Light**
Size Calling Method	**Local Southern**	**Local Southern**
Baseline Window	**51**	33
Peak Amplitude Threshold	**BGPO**=50; **Y**=70; **R**=100	**BGYROP**=50
Minimum Peak Half Width	**2**	**2**
Polynomial Degree	**3**	**3**
Peak Window Size	**15**	**13**
Normalization	Not selected.	

Kits

All DNA quantification was performed on a 7500 Real-Time PCR System for Human Identification using the Quantifiler™ Trio DNA Quantification Kit.

All STR amplification was performed on GeneAmp™ PCR System 9700 thermal cyclers. The following amplification kits were used in this validation study:

- GlobalFiler™ PCR Amplification Kit
- GlobalFiler™ Express PCR Amplification Kit
- NGM Detect™ PCR Amplification Kit
- Yfiler™ Plus PCR Amplification Kit

- VeriFiler™ Plus PCR Amplification Kit
- VeriFiler™ Express PCR Amplification Kit
- Identifiler™ Plus PCR Amplification Kit
- Identifiler™ Direct PCR Amplification Kit

Note: For validation information for the GlobalFiler™ IQC PCR Amplification Kit, see the *GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits User Guide* (4477604 Rev. F or later).

All Applied Biosystems™ PCR Amplification kits were not used 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, workflow application, and other similarities among the PCR Amplification kits.

Samples

All samples in this study were amplified and run according to the standard protocols in the applicable User Guide, unless otherwise noted.

Study	Samples	PCR Amplification kit tested[1]	Dye set (in instrument method)
Sizing precision and accuracy	Allelic ladder (1 µL/well)	GlobalFiler™ NGM Detect™ VeriFiler™ Express	J6, J6-T
Signal optimization	Uses the data from the sizing precision and accuracy study.		
Genotyping concordance	• 22 gDNA samples (1–2ng)[2] • 22 blood FTA card samples (1.2 mm punch) • Kit positive controls (2 ng) • Kit allelic ladder[3]	GlobalFiler™ Yfiler™ Plus NGM Detect™ Identifiler™ Plus GlobalFiler™ Express VeriFiler™ Express	J6, J6-T, G5
Sensitivity	• Kit positive controls (0.125 ng) • Kit allelic ladder[3]	GlobalFiler™ Yfiler™ Plus Identifiler™ Plus	J6, G5
Resolution	• 22 gDNA samples (1–2ng)[2] • 22 blood FTA card samples (1.2 mm punch) • 23 Kit positive controls (125 pg) • Kit allelic ladder[3]	GlobalFiler™ Yfiler™ Plus NGM Detect™ Identifiler™ Plus GlobalFiler™ Express VeriFiler™ Express	J6, J6-T, G5

Study	Samples	PCR Amplification kit tested[1]	Dye set (in instrument method)
Pull-up reduction	• 22 gDNA samples (1–2ng) • 22 blood FTA card samples (1.2 mm punch) • Kit positive controls (2 ng) • Kit allelic ladder[3] • Control DNA 007/9947A 1:7 mixture (1 ng)	GlobalFiler™ Yfiler™ Plus NGM Detect™ Identifiler™ Plus GlobalFiler™ Express VeriFiler™ Express	J6, J6-T, G5
Off-scale Recovery	• Kit positive controls (3–6 ng) • Kit allelic ladder[3]	GlobalFiler™ Express VeriFiler™ Express Identifiler™ Direct	J6, G5

[1] GeneScan™ 600 LIZ™ Size Standard v2.0 was used for sizing.
[2] Blood extracts from male and female donors.
[3] The appropriate allelic ladder was run with samples (required for genotyping).

Methods and study summary

Study	Method and analysis	Replicates
Sizing precision and accuracy	• Run allelic ladders (1 µL/) from well, NGM Detect™, and VeriFiler™ Express kits in all capillaries, 24 wells per kit. • On one 3500xL instrument: Perform 4 consecutive runs with the OSR dye set. • On a different 3500xL instrument and on one 3500 instrument: Perform 4 consecutive runs with the standard dye set. • Analyze data in GeneMapper™ *ID-X* Software v1.6 and v1.5 with the analysis settings listed in Table 7. • For each run: Calculate the standard deviation and maximum–minimum of the mean size of each allele in the ladder.	24 wells × 4 injections per kit on each instrument
Signal optimization	Calculate the following results from the sizing precision and accuracy study data: • Average peak height for each capillary in each run. • Maximum-to-minimum peak height ratio of the average peak height for all capillaries in each run.	—

Study	Method and analysis	Replicates
Genotyping concordance	• Amplify: – 22 gDNA samples (1–2ng), the kit positive controls (2 ng), with GlobalFiler™, NGM Detect™, Identifiler™ Plus, and Yfiler™ Plus (male samples only) kits. – 22 blood FTA samples (1.2 mm punches) with GlobalFiler™ Express and VeriFiler™ Express kits. • On one 3500xL instrument: Perform the runs with the OSR dye set. • On a different 3500xL instrument and on one 3500 instrument: Perform runs with the standard dye set. • Repeat the runs on the 3500xL instrument that is running 3500 Series Data Collection Software 3 v3.1.2. • Analyze data in GeneMapper™ *ID-X* Software v1.6 and v1.5 with the analysis settings listed in Table 7. • Compare allele call results between 3500 Series Data Collection Software versions and GeneMapper™ *ID-X* Software versions.	1 reaction per sample per kit 1 well × 2 injections per sample on each instrument with 3500 Series Data Collection Software 4 1 well × 1 injection per sample on instrument 3500 Series Data Collection Software 3 v3.1.2
Sensitivity	• Amplify 23 kit positive controls (0.125 ng) with GlobalFiler™, Identifiler™ Plus, and Yfiler™ Plus kits. • On one 3500xL instrument: Perform the runs with the OSR dye set. • On a different 3500xL instrument and on one 3500 instrument: Perform 4 consecutive runs with the standard dye set • Analyze with GeneMapper™ *ID-X* Software v1.6 with the analysis settings listed in Table 7. • Evaluate the allele call results.	23 wells × 2 injections per kit on each instrument
Resolution	In the genotype concordance and sensitivity study data, evaluate single base-pair resolution up to 465 bp.	—
Pull-up reduction	For pull-up reduction: • Disable the pull-up reduction feature in 3500 Series Data Collection Software 4 (an internal Thermo Fisher Scientific software development tool is required). • Reprocess the genotype concordance study data. • In the original and the reprocessed genotype concordance study data, count the total number of pull-up peaks. • Calculate the absolute percent value of each pull-up incidence, then compare the results.	—

Study	Method and analysis	Replicates
Pull-up reduction	For minor contributor peak: • Amplify a 1:7 mixture of control DNA 007 and control DNA 9947A (1 ng) with the GlobalFiler™ kit. • Run the mixture samples on one 3500 instrument and one 3500xL instrument using the standard dye set. • Disable the pull-up reduction feature in 3500 Series Data Collection Software 4 (we used an internal Thermo Fisher Scientific software development tool). • Reprocess the data. • Compare the original and reprocessed data to evaluate effects of pull-up reduction on the minor contributor peak in the mixture.	22 wells × 1 injection
Off-scale recovery	• Amplify kit positive controls with the following kits: – GlobalFiler™ Express kit (3 ng and 6 ng) – VeriFiler™ Express kit (3 ng and 6 ng) – Identifiler™ Direct kit (4 ng and 6 ng) • Run the samples on one 3500 instrument and two 3500xL instruments using the OSR dye sets. • Inject the samples three times with different injection voltages and times to generate peak heights (heterozygote alleles) of <32,000 RFU, 32,000–65,000 RFU, and >65,000 RFU. • Analyze data in GeneMapper™ *ID-X* Software with the analysis settings listed in Table 7. – **v1.6 and v1.5**: To test how the OSR feature affects pull-up: For the 32,000–65,000 RFU data set: Disable the off-scale recovery feature in 3500 Series Data Collection Software 4 with an internal Thermo Fisher Scientific software development tool, reprocess, then compare pull-up peaks between the data sets. – **v1.6**: To test how the pull-up reduction feature affects pull-up peaks in high-signal samples: For the 32,000–65,000 RFU data set: Disable the pull-up reduction feature in 3500 Series Data Collection Software 4 with an internal Thermo Fisher Scientific software development tool. Leave the off-scale recovery feature enabled. Reprocess, then compare pull up peaks between the data sets.	23 wells × 3 injections per kit on each instrument

Results summary

Study	Expected outcome	Result
Sizing precision and accuracy	For fragments sizes 60–465 bp: • Standard deviation of the mean size is ≤0.15 bp. • Observed size is within ≤0.5 bp of expected size. • Results are concordant for data analyzed in GeneMapper™ *ID-X* Software v1.5 and v1.6.	Pass

Study	Expected outcome	Result
Signal optimization	Maximum-to-minimum ratio of the average peak heights is ≤1.9:1 across all capillaries in each run.	Pass See "Signal optimization study results" on page 28 for more information.
Genotyping concordance	Genotyping results are concordant between: • 3500 Series Data Collection Software 4 and 3500 Series Data Collection Software 3 v3.1.2. • GeneMapper™ *ID-X* Software v1.5 and v1.6.	Pass See "Genotyping concordance study results" on page 29 for more information.
Sensitivity	Full STR profile is generated from the kit positive controls at 125 pg.	Pass
Resolution	Single base-pair resolution is observed between 60–465 bp in all capillaries.	Pass
Pull-up reduction	• Pull-up peaks ≥5% are not observed in ≥95% of on-scale samples. • Fewer pull-up peaks above the peak amplitude threshold are observed. • The minor contributor peak (125 pg) is not impacted by pull-up reduction.	Pass See "Pull-up reduction study results" on page 31 for more information.
Off-scale recovery	• GeneMapper™ *ID-X* Software v1.6: – Signal ≥65,000 RFU: Peaks are flat-topped at 65,000 RFU and are flagged as off-scale (OS). – Signal <65,000 RFU: Peaks are on-scale with expected peak morphology. – 100% genotype concordance between data sets with signal <32,000 RFU and signal 32–65,000 RFU. – Intra-locus balance is within 10% between data sets with signal <32,000 RFU and signal 32–65,000 RFU analyzed in GeneMapper™ *ID-X* Software v1.5. • GeneMapper™ *ID-X* Software v1.5: Signal ~32,000 RFU: Peaks are capped and flagged as off-scale (OS). • GeneMapper™ *ID-X* Software v1.6 and v1.5— Fewer pull-up peaks are observed above the peak amplitude threshold with off-scale recovery enabled.	Pass See "Off-scale recovery study results" on page 34 for more information.

Signal optimization study results

The signal optimization study evaluated the effect of the spatial-calibration–dependant signal optimization factor and the run-module–dependant signal optimization on peak height variation across capillaries in an injection.

Kits tested: GlobalFiler™, NGM Detect™, and VeriFiler™ Express kits

Software versions tested: 3500 Series Data Collection Software 4, GeneMapper™ *ID-X* Software v1.6, and GeneMapper™ *ID-X* Software v1.5

Note: The signal optimization factor and the signal optimization (SO) run modules were validated separately. See "3500 Series Data Collection Software 3 v3.1.2 signal optimization validation" on page 36.

The maximum-to-minimum ratios of the average peak heights of the allelic ladder across all the capillaries in each run was ≤1.9:1.

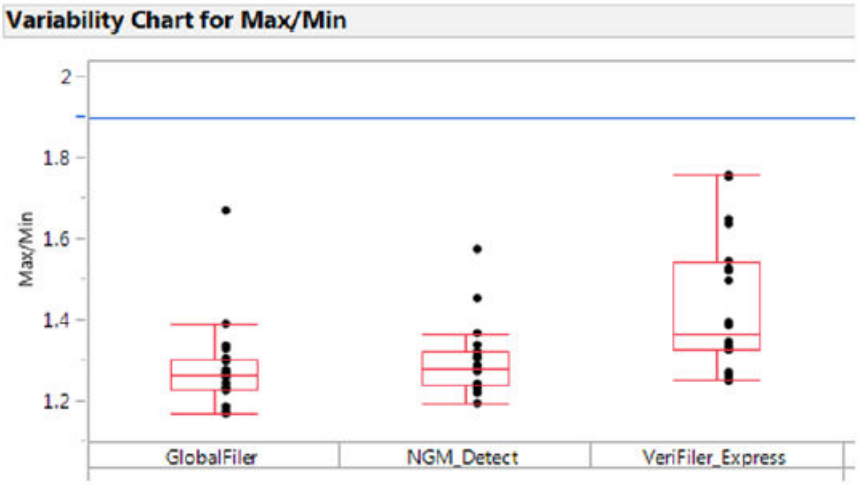


Figure 4 Maximum-to-minimum ratios of the average peak heights of kit allelic ladder (blue line=1.9:1 ratio)

Note: The spatial-calibration-dependent signal optimization factor and the signal optimization run modules were validated in the 3500 Series Data Collection Software 3 v3.1.2 validation study (see "3500 Series Data Collection Software 3 v3.1.2 signal optimization validation" on page 36). During the validation of 3500 Series Data Collection Software 3 v3.1.2, it was observed that the signal optimization factor had no to minimal impact to improving signal optimization on the 8-capillary 3500. During 3500 Series Data Collection Software 4 validation studies, the signal optimization factor calculation was enabled for both the 3500 and 3500xL instruments. However, based on earlier 3500 Series Data Collection Software 3 v3.1.2 validation results, this feature is not available on 3500 instruments using 3500 Series Data Collection Software 4.

Genotyping concordance study results

The genotyping concordance study evaluated the following results:

- Genotype concordance for data collected in 3500 Series Data Collection Software 4 and in 3500 Series Data Collection Software 3 v3.1.2.
- Average peak height for data collected in the two versions of data collection software and analyzed in GeneMapper™ *ID-X* Software v1.6 and GeneMapper™ *ID-X* Software v1.5.
- Concordance of peak height, peak area, data point, size, and PQV results between data collected in 3500 Series Data Collection Software 4 and analyzed in the two versions of GeneMapper™ *ID-X* Software.

Kits tested: GlobalFiler™, NGM Detect™, Identifiler™ Plus, Yfiler™ Plus, GlobalFiler™ Express, and VeriFiler™ Express kits

Software versions tested: 3500 Series Data Collection Software 4, 3500 Series Data Collection Software 3 v3.1.2, GeneMapper™ *ID-X* Software v1.6, and GeneMapper™ *ID-X* Software v1.5

There was 100% genotype concordance for all kits tested between the data collected on the two versions of data collection software.

- The average peak heights for each marker in each kit were comparable between the two versions of data collection software and between the two versions of GeneMapper™ *ID-X* Software.
- Peak size and data points were 100% concordant between the two versions of GeneMapper™ *ID-X* Software.
- Peak area, peak height, and PQV scores showed some expected discordance due to the update of the peak height detection algorithm. The results were consistent with the results observed during the validation of the GeneMapper™ *ID-X* Software v1.6.

Note: For information on the GeneMapper™ *ID-X* Software v1.6 peak height detection enhancement and developmental validation results, see the *GeneMapper™ ID-X Software v1.6 New Features and Software Verification User Bulletin* (Pub. No. 100073905).

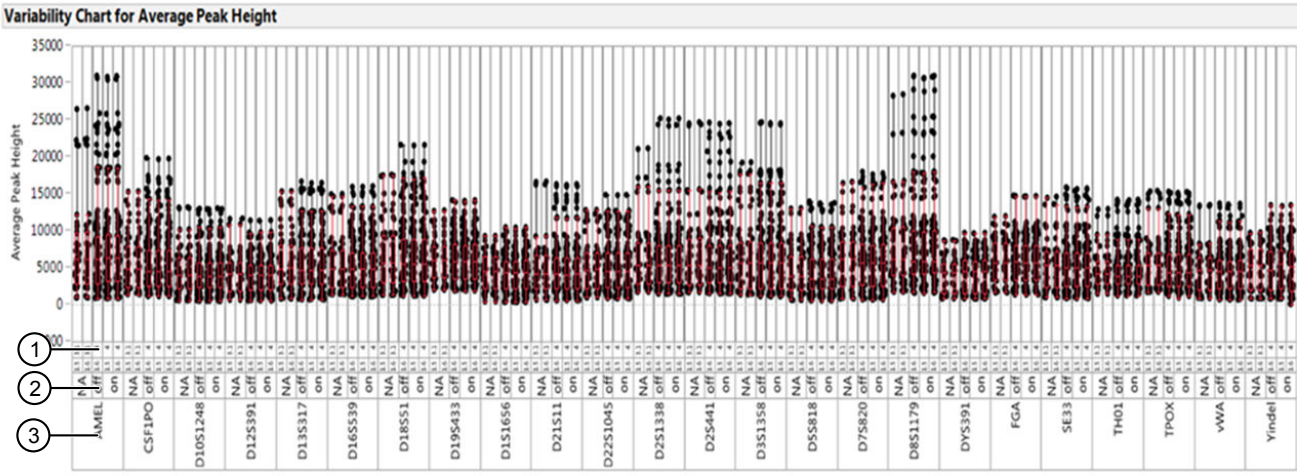


Figure 5 GlobalFiler™ kit sample set: Average peak height is not significantly affected by version of Data Collection or analysis software, or the pull-up reduction feature (some variability between software versions is attributed to different CE plate setups). Data from other kits that were tested showed similar results.

① Software version (Data Collection Software in first row, GeneMapper™ *ID-X* Software in second row)

② Pull-up reduction setting

③ Marker name

Table 8 Software version and pull-up reduction setting for Figure 5

	Software version				
Data Collection Software	**v3.1.2**	**v3.1.2**	**v4**	**v4**	**v4**
GeneMapper™ *ID-X* Software	**v1.5**	**v1.6**	**v1.6**	**v1.5**	**v1.6**
Pull-up reduction	N/A		Off	On	On

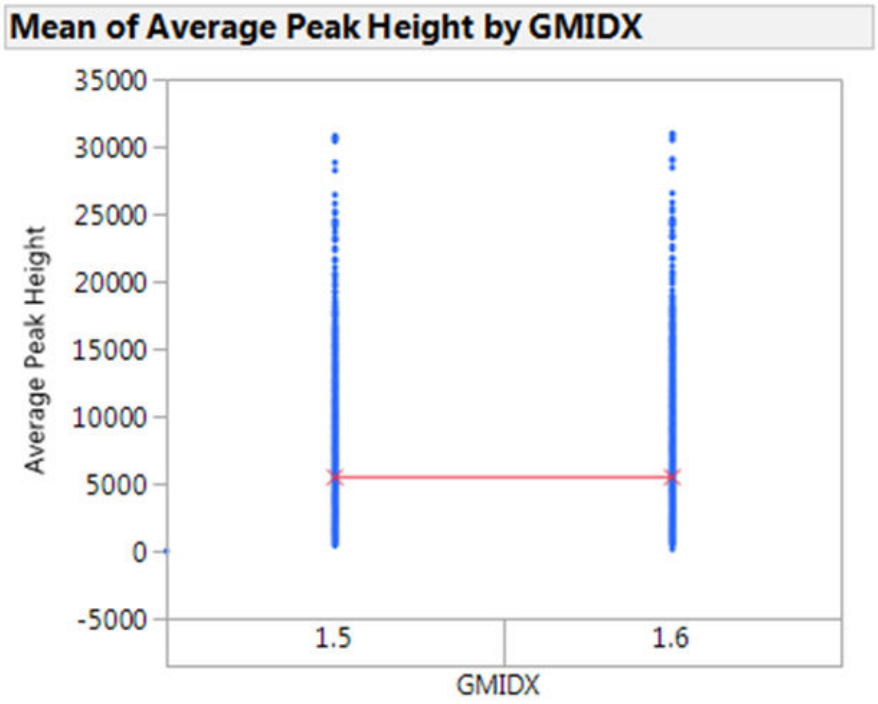


Figure 6 GlobalFiler™ kit sample set: No difference in the mean average peak height (indicated with red "x" and line) in data collected in 3500 Series Data Collection Software 4 and analyzed in GeneMapper™ *ID-X* Software v1.6 and v1.5. Data from other kits that were tested showed similar results.

Pull-up reduction study results

The pull-up reduction study evaluated:

- Pull-up peaks in on-scale data when using Applied Biosystems™ DS-33, DS-36, and DS-37 matrix standards with the G5, J6, J6T, G5-OSR, J6-OSR, or J6T-OSR dye sets.
- Minor contributor peak in GlobalFiler™ positive control mixture of 1:7 (007:9947A) (1 ng)

Kits used: GlobalFiler™, GlobalFiler™ Express, Identifiler™ Plus, NGM Detect™, VeriFiler™ Express, and Yfiler™ Plus kis

Software versions tested: 3500 Series Data Collection Software 4, GeneMapper™ *ID-X* Software v1.6

The data was collected with the pull-up reduction feature enabled and disabled (using an internal Thermo Fisher Scientific software development tool). In the data collected with the pull-up reduction feature enabled, the following results were observed:

- Fewer pull-up peaks above the peak amplitude threshold were observed for all kits tested. The percent reduction in the number of pull-up peaks ranged from approximately 10–55% depending on the sample set. The reduction of pull-up peaks was greater in the samples that contained a higher incidence of pull-up data before the samples were reprocessed with the pull-up reduction feature enabled.
- Overall, more samples with pull-up <1% were observed. Fewer samples with pull-up >5% were observed.
- In the mixture sample, the minor contributor genotype call was not affected by the pull-up reduction feature. The average peak height of the minor contributor was not significantly different before and after pull-up reduction is applied.

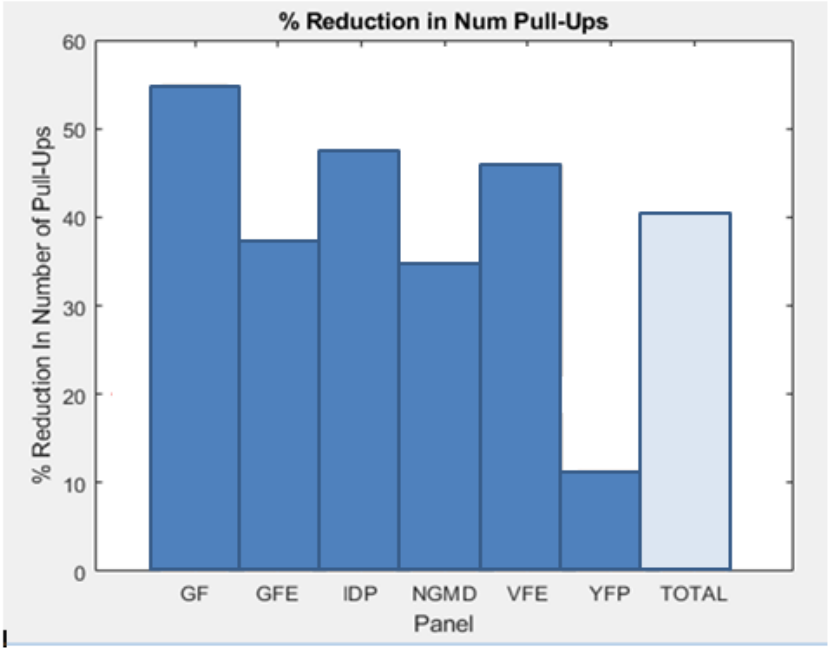


Figure 7 % reduction in pull-up with the pull-up reduction feature enabled (DS-33, DS-36, and DS-37 dye sets)

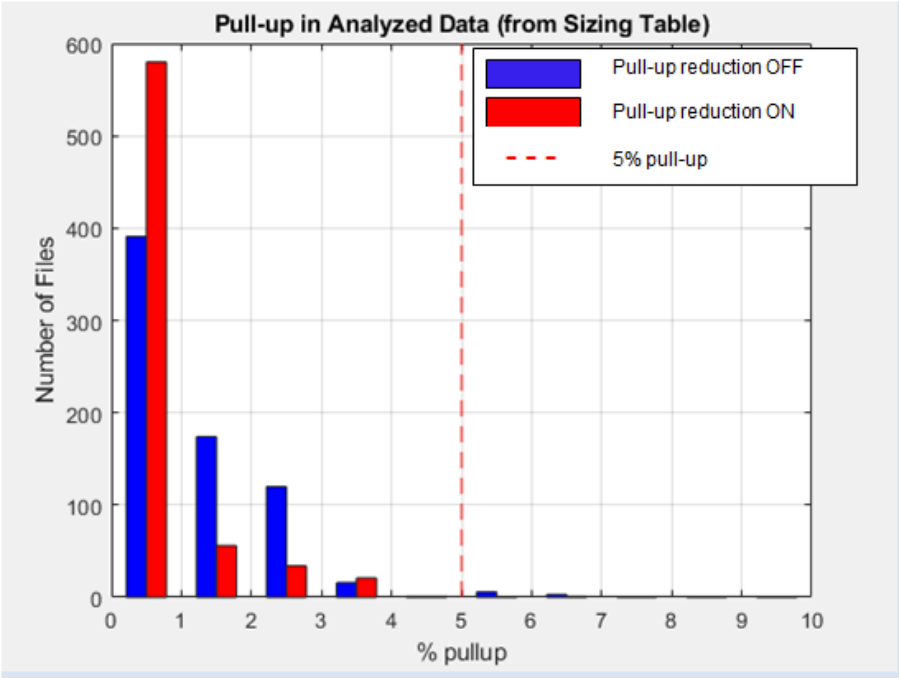


Figure 8 With the pull-up reduction feature disabled, percent pull-up >5% was observed in approximately 1.5% of samples. With the pull-up reduction feature enabled, percent pull-up of >5% was observed in only 0.25% of samples.

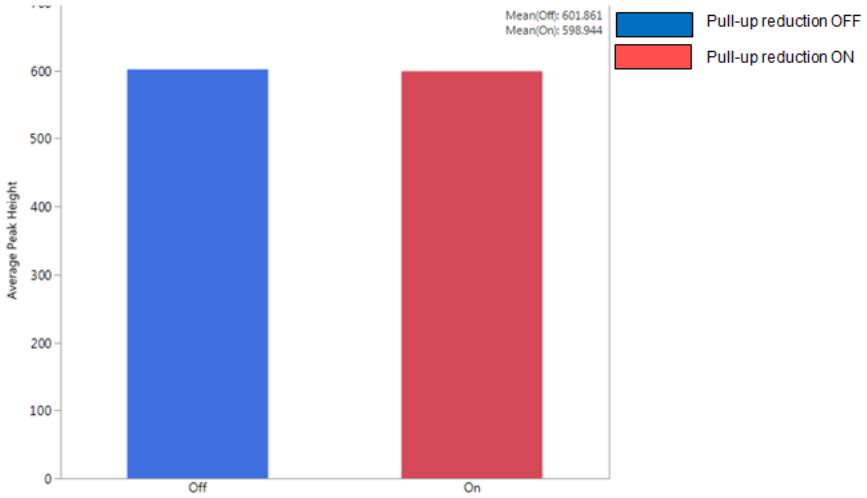


Figure 9 Average peak height of the unshared minor alleles with the pull-up reduction feature disabled (~602 RFU) and enabled (~599 RFU).

Off-scale recovery study results

Off-scale recovery study used Applied Biosystems™ DS-33, DS-36, and DS-37 matrix standards and the G5-OSR, J6-OSR, or J6T-OSR dye sets.

Kits tested: GlobalFiler™ Express, Identifiler™ Direct, and VeriFiler™ Express kits

Software versions tested: 3500 Series Data Collection Software 4, GeneMapper™ *ID-X* Software v1.6, and GeneMapper™ *ID-X* Software v1.5

The following results were observed.

Table 9 Observations for the peak height of the heterozygous allele

Data set/signal (RFU)	GeneMapper™ *ID-X* Software	
	v1.6	v1.5
≥65,000	Peaks are flat-topped and flagged as off-scale (OS).	• Peaks are off-scale. • Signal maximum is 32,000 RFU.
32,000–65,000	Peaks are on-scale with expected peak morphology.	
<32,000	Peaks are on-scale with expected peak morphology.	Peaks are on-scale with expected peak morphology.

Additionally, the following results were evaluated in GeneMapper™ *ID-X* Software v1.6:

- **Genotype concordance and intra-locus peak balance**—When comparing the same sample with peak heights between 32,000–65,000 RFU and <32,000 RFU, there was 100% concordance in genotyping results and intra-locus peak balance ≤10%.
- **Pull-up reduction**—Pull-up reduction was evaluated for the 32,000–65,000 RFU data set in GeneMapper™ *ID-X* Software v1.5 and v1.6. With the off-scale recovery feature disabled, data was saturated in both versions of software. The resulting significant number of pull-up peaks could not be quantified due to the poor quality of the samples. When the same sample set was analyzed with OSR enabled, although data was still saturated in GeneMapper™ *ID-X* Software v1.5, data quality improved and fewer pull-up peaks under the off-scale peaks were observed in both versions of software.

  When comparing the 32,000–65,000 RFU data set in GeneMapper™ *ID-X* Software v1.6 with off-scale recovery enabled with pull-up reduction off versus on, the percent reduction in the number of pull-up peaks above threshold was 12%.

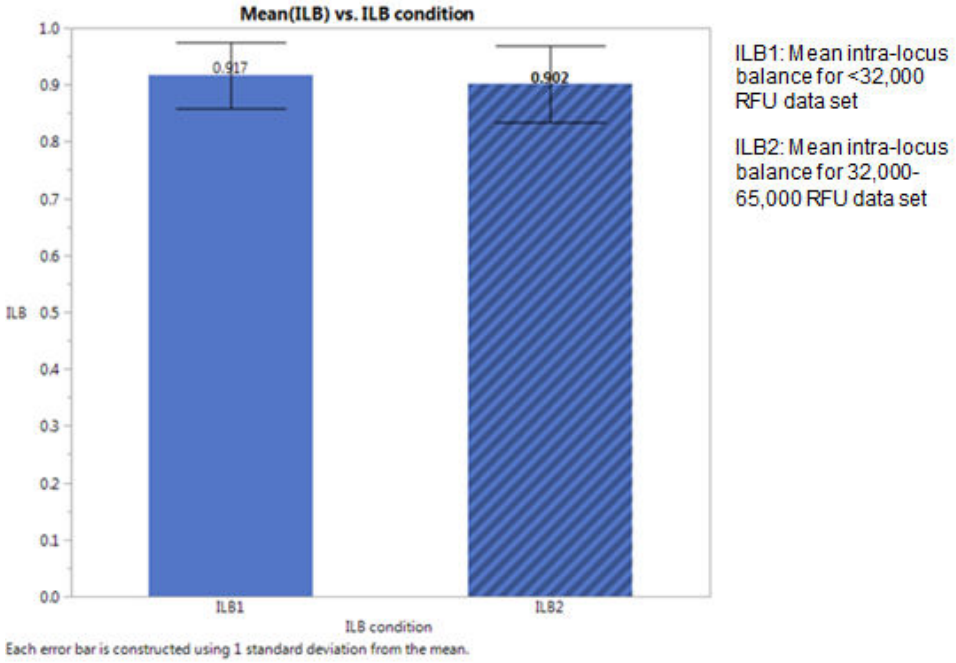


Figure 10 GlobalFiler™ Express sample: Average intra-locus balance for data with all peaks <32,000 RFU (ILB1) was 91.7%. Average intra-locus balance for the same data set with all peaks between 32,000-65,000 RFU (ILB2) was 90.2%. Input DNA and injection parameters were modified to increase sample peak heights. Results for Identifiler™ Direct and VeriFiler™ Express kits were similar.

Conclusions

Based on the studies performed, the following conclusions were made:

- General operations in the 3500 Series Data Collection Software 4 functioned properly for instrument control, calibration, run set up, and data collection with both legacy and current 5 and 6-dye Applied Biosystems™ PCR amplification kits.
- Sizing precision, accuracy, and resolution were comparable between data collected in 3500 Series Data Collection Software 4 and 3500 Series Data Collection Software 3 v3.1.2.
- Genotyping of gDNA sample and kit positive controls was 100% concordant between data collected in 3500 Series Data Collection Software 4 and 3500 Series Data Collection Software 3 v3.1.2.
- The pull-up reduction and off-scale recovery features significantly reduced the number of pull-up peaks seen in the data and the number of samples/ markers flagged as off-scale when using Applied Biosystems™ 5 or 6-dye chemistry. Both of these features should reduce the time spent on manual edits and/or review during data analysis.
- Peak height variation and signal uniformity across capillaries in an injection is improved by the signal optimization feature.
- 3500 Series Data Collection Software 4 has no critical limitations or defects in the general user interface and workflow.

3500 Series Data Collection Software 3 v3.1.2 signal optimization validation

For a description of the signal optimization feature, see "Signal optimization feature" on page 4.

Objective of the validation

The signal optimization study had the following objectives:

- Determine the optimized injection parameters (z-offset and introduction of polymer before injection) to minimize peak height variation across capillaries in an injection.
- Evaluate the effect of the spatial-calibration–dependent signal optimization factor and the run-module–dependent signal optimization on peak height variation across capillaries in an injection.

Materials

Instruments tested: Two 3500xL and two 3500 instruments with 3500 Series Data Collection Software 3 v3.1.2 and one 3500 with 3500 Series Data Collection Software 3 v3.1.

Kits tested: GlobalFiler™ PCR Amplification Kit, GlobalFiler™ Express PCR Amplification Kit, and Identifiler™ Plus PCR Amplification Kit

Software tested: 3500 Series Data Collection Software 3 v3.1.2

Studies, samples, and methods (testing matrix)

Study	Samples	Methods and Analysis	Replicates
Peak height variability	Control DNA 007 amplified with GlobalFiler™ and Identifiler™ Plus at 1 ng and 125 pg	• 3500xL—Set up 1 full 96-well plate per instrument with sample. • 3500—Set up 1 quadrant of plate (8-cap) per instrument with sample. • With spatial-calibration–dependent signal optimization factor enabled, inject each well 5 times. Use a different run module for each injection (see "Peak height variability study results" on page 38). • Calculate the %CV and Maximum-to-Minimum ratio of peak heights for each run. • Recalculate the %CV and Maximum-to-Minimum ratio of peak heights for each run with a spatial optimization factor of 1 (disables the spatial-calibration–dependent signal optimization). Compare the results to the data collected with the spatial-calibration–dependent signal optimization factor enabled.	5 injections per sample for each kit and DNA input (20 injections total)

Study	Samples	Methods and Analysis	Replicates
Sizing precision	GlobalFiler™ and Identifiler™ Plus allelic ladder	• 3500xL—Set up 1 full 96-well plate per instrument with sample. • 3500—Set up 1 quadrant of plate (8-cap) per instrument with sample. • Inject each well 5 times. Use a different run module for each injection (see "Materials" on page 36). • Calculate the standard deviation of the mean size of each allele in the ladder for each run.	5 injections per sample for each kit and DNA input (20 injections total)
Genotype concord-ance	10 gDNA samples amplified with GlobalFiler™ and Identifiler™ Plus at 1 ng	• Run each sample on each instrument with 3500 Series Data Collection Software 3 v3.1.2. • Run each sample on each instrument with 3500 Series Data Collection Software 3 v3.1. • Compare allele calls.	2 injections per sample
Carryover	• Control DNA 9947A amplified with GlobalFiler™ at 1ng, 2ng, and 3ng • No-template controls	• Set up two full CE plates on one 3500xL with the following injection list: – NTC for baseline – Sample DNA and NTC alternated for each injection • Inject each well one time with the HID36_POP4xl run module. • Inject each well one time with the HID36_POP4xl_SO run module (describe page 38). • Evaluate carryover using a peak amplitude threshold of 50 RFU. • Calculate %CV, Maximum-to-Minimum ratio of peak heights, and average peak height for each run.	1 injection per well per run module
Sample stability	• 6 DNA Control 007 samples amplified with GlobalFiler™ • 4 Bode Buccal DNA Collector™ swabs amplified with GlobalFiler™ Express • 6 buccal swabs amplified with GlobalFiler™ Express • 8 Blood FTA samples amplified with GlobalFiler™ Express	• Set up two CE plates with all samples. • Day 0— – Inject both CE plates on one 3500xL instrument with DCv3.1.2 with the HID36_POP4xL run module. – Inject both CE plates on one 3500xL instrument with DCv3.1.2 with the HID36_POP4xl_SO run module (describe page 38). • Store the CE plates at 4°C and room temperature. • Day 3—Repeat the Day 0 steps. • Day 8—Reinject the CE plate stored at 4°C with both run modules. Evaluate resolution, migration time, peak heights, and intra-color balance.	1 injection per run module for each time point for each sample

Peak height variability study results

The study evaluated the effect of the spatial-calibration–dependent signal optimization factor (software algorithm) and the run-module–dependent signal optimization (injection parameter optimization) on peak height variation across capillaries in an injection.

The injection parameters tested were z-offset and introduction of polymer before an injection. The study results were used to create the new HID36_POP4(xl)_SO run module that optimizes signal.

Five run modules with different injection parameters were used for the study.

- HID36_POP4(xl) standard run module: Polymer is not introduced into wells before an injection. Z-offset is between the setting in run module 3 and 4.
- Run module 2 and 3: Polymer is introduced into wells before an injection. Z-offset is higher than the standard run module.

  Data was collected with spatial-calibration–dependent signal optimization factor disabled and enabled. Data was analyzed using the SO factor from the spatial calibration and with an SO factor of 1 (disables the spatial-calibration–dependent signal optimization).
- Run module 4 and 5: Polymer is introduced into wells before an injection. Z-offset is lower than the standard run module.

Data collected with run modules 2 and 3 (with higher z-offsets) showed improvement in signal variation when compared to the standard HID36_POP4(xl) run module. For the new HID36_POP4(xl)_SO run module, a z-offset that was between the standard and the extreme high setting was selected to lessen the risk of missed injection (although no missed injections were seen during this study).

With the spatial-calibration–dependent signal optimization factor enabled and the new HID36_POP4(xl)_SO run module, the following results were observed.

- When data was collected with the new HID36_POP4(xl)_SO run module and with the signal optimization factor enabled, an overall improvement in %CV and Maximum-to-Minimum ratio on the 3500xL and 3500 instruments (see Table 10).

  Note: On one of the 3500xL instruments, no improvement to %CV or Maximum-to-Minimum ratio was observed. This instrument showed less signal variation across the capillaries compared to the other instruments. Data for an outlier capillary in the spatial calibration data caused an overcorrection in the calculation of the signal optimization factor. It was determined that the signal optimization factor calculation was functioning as designed. To ensure that the signal optimization factor is calculated accurately, repeat a spatial calibration if outlier data is observed.
- When the data was recalculated with the SO factor set to 1, there was no significant impact on the 3500 instrument %CV and Maximum-to-Minimum ratio. Based on this observation, the spatial-calibration–dependent signal optimization factor was disabled for 3500 instruments (data not shown).

Table 10 Peak height %CV Maximum-to-Minimum peak ratio data for the standard and _SO HID36_POP4(xl) run module and with spatial-calibration–dependent signal optimization factor

Instrument and kit	%CV				Maximum-to-Minimum ratio			
	1ng		125 pg		1ng		125 pg	
	Run module							
	Standard	SO	Standard	SO	Standard	SO	Standard	SO
3500xL–GlobalFiler™	15.56	7.17	14.96	7.26	1.95	1.45	1.85	1.35
3500xL–Identifiler™ Plus	10.07	10.38	10.40	12.12	1.43	1.48	1.47	1.58
3500–GlobalFiler™	15.63	13.96	17.14	12.70	1.57	1.61	1.83	1.50
3500–Identifiler™ Plus	11.77	9.16	13.52	6.86	1.37	1.30	1.43	1.21

Sizing precision, genotype concordance, sample stability, and carryover study results

Study	Results
Sizing precision	No significant difference in sizing precision results was observed with any of the 5 run modules described in "Peak height variability study results" on page 38. The standard deviation of the mean size of each allele met the specification of ≤0.15bp.
Genotype concordance	Geontypes for each gDNA sample were 100% concordant between 3500 Series Data Collection Software 3 v3.1.2 and 3500 Series Data Collection Software 3 v3.1.
Sample stability	After 3 days storage at 4°C and room temperature and 8 days storage at 4°C: • No significant difference in sample stability was observed between the SO run module and the standard run module. • No effect on resolution, sample peak heights, or intra-color balance was observed at any time points with either run module. • Migration was slower after 8 days storage at 4°C when samples were injected with the SO run module. • Size standard peak heights were lower after 3 days storage at room temperatures and 8 days storage at 4°C when samples were injected with the SO run module.
Carryover	For all samples run with the SO run module: • No additional carryover was observed (see the table below). • %CV, Max:Min, and average peak height data was also evaluated. • For all DNA inputs %CV and Maximum-to-Minimum ratio were reduced. • No significant difference was observed in average peak heights.

Table 11 Carryover incidence and magnitude of carryover with the HID36_POP4xl_SO (SO) run module and the HID36_POP4xl run module (standard).

DNA input	Carryover incidences by Regular Module	Magnitude	Carryover incidences by SO module	Magnitude
1ng	0	—	1	4%
2ng	4	9%	1	2%
3ng	1	2%	1	4%

Conclusions

The 3500 Series Data Collection Software 3 v3.1.2 signal optimization feature reduces the peak height variation across capillaries in an injection.

On the 3500xL instrument, a greater reduction in variation was observed when using the SO run module in addition to the automatic spatial calibration-based signal optimization.

On the 3500 instrument, the automatic spatial calibration-based signal optimization did not have a significant effect on peak height variation across capillaries in an injection.

Sizing precision, genotype concordance, sample stability, and carryover were not impacted by the signal optimization feature.

Note: A subsequent study concluded that one of the elements of the new SO run module, specifically the mechanism of introducing polymer, was leading to artifacts in the data. Based on this investigation, we are updating our recommendations to reduce signal variation across capillaries. For more information, see *Technical Bulletin: Updated Recommendations for use of the Signal Optimization Features in 3500 Series Data Collection Software 4 for HID*.

Documentation and support

Related documentation

Document	Publication number
Software Release Notes 3500 Series Data Collection Software 4.0	100078600
3500/3500xL Genetic Analyzer User Guide	100031809
GeneMapper™ ID-X Software v1.6 New Features and Software Verification User Bulletin	100073905
Technical Bulletin: Updated Recommendations for use of the Signal Optimization Features in 3500 Series Data Collection Software 4 for HID	—

Customer and technical support

For support:

- **In North America**—Send an email to **HIDTechSupport@thermofisher.com**, or call **888-821-4443 option 1**.
- **Outside North America**—Contact your local support office.

For the latest services and support information for all locations, go to **thermofisher.com/support** to obtain the following information.

- Worldwide contact telephone numbers
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Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision history: Pub. No. 100075298

Revision	Date	Description
D	28 August 2019	Update for v4.0.1 release: Add section on v4.0.1 changes. Add GlobalFiler™ IQC PCR Amplification Kit information. Remove references to assays with _SO suffix (signal optimization). Add v4.0.1 verification section. Add note to v3.2.1 validation conclusion.
C	01 April 2019	Remove references to size standard normalization Table 5 GeneMapper™ ID-X Software Analysis Settings; Results summary: Sizing precision and accuracy; Signal optimization study results. Replace Figure 4.
B	28 February 2019	Add validation sections for 3500 Series Data Collection Software 4 and 3500 Series Data Collection Software v3.3 v3.2.1. Add details on library changes.
A	21 August 2018	New document.

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