

# 3500 Series Data Collection Software 4 for HID

## New Features

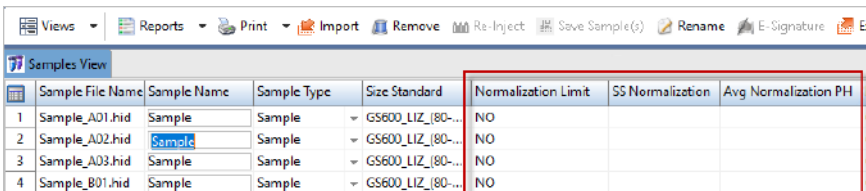
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This user bulletin describes new features. For more information on using the software, see *3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3.1 User Guide* (Pub. No. 100031809).

## Overview of new features in 3500 Series Data Collection Software 4

New feature	Description
General support	<ul style="list-style-type: none"> <li>Windows™ 10, 64-bit operating system (IOT Enterprise) is supported.</li> <li>No license activation or yearly license renewal is required.</li> </ul> <p>The 3500 Series Data Collection Software 4 has been tested with these antivirus software applications:</p> <ul style="list-style-type: none"> <li>Symantec Endpoint Protection 12</li> <li>McAfee Endpoint Security version 10.5</li> </ul> <p><b>IMPORTANT!</b> 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.</p>
Data optimization	<p>See:</p> <ul style="list-style-type: none"> <li>"Signal optimization feature" on page 3</li> <li>"Off-scale recovery feature" on page 5</li> <li>"Pull-up reduction feature" on page 6</li> </ul>

New feature	Description
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 9.
DS-36 install standard (6-dye)	<p>The DS-36 GeneScan™ Installation Standards with GeneScan™ 600 LIZ™ Size Standard v2.0 6-dye install standard is supported. The DS-36 kit includes the GlobalFiler™ Allelic Ladder.</p> <p>A J6 selection is available in the Chemistry list in the <b>Install Check</b> screen. The Calibration history lists the dye set used for the calibration.</p> <p>Pass/fail criteria for HID J6 install check:</p> <ul style="list-style-type: none"> <li>• 26 size standard peaks</li> <li>• 343 ladder peaks</li> <li>• All markers except TH01: <math>\pm 0.7</math> bp of nominal size for the allele</li> <li>• TH01: <ul style="list-style-type: none"> <li>– Seven markers are <math>\pm 0.7</math> bp</li> <li>– Three markers are <math>\pm 0.5</math> bp of nominal size for the allele</li> </ul> </li> <li>• Minimum peak height &gt;400 RFU</li> </ul>
New assays, instrument protocols, QC protocols, and dye sets	Library items have been updated for signal optimization and off-scale data recovery. See "New library items available with 3500 Series Data Collection Software 4" on page 7.
Preferences for reagent use	<p>You can set <b>Instrument Settings</b> preferences to:</p> <ul style="list-style-type: none"> <li>• Prevent an instrument run if reagents exceed on-instrument limits or expiration date.</li> <li>• Control the display and timing of warnings that are related to reagent usage limits and expiration.</li> </ul> <p>See "Set preferences for reagent use" on page 11.</p>
Size standard normalization information	<p>By default, the Samples view displays the following columns.</p> <ul style="list-style-type: none"> <li>• Normalization Limit set in the instrument protocol.</li> <li>• 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.</li> <li>• Avg Normalization PH (peak height) of the size standard peaks used to calculate the factor.</li> </ul> <p>You can change table settings to hide these columns.</p> 

New feature	Description
Consumables log export	<p>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.</p> <ol style="list-style-type: none"> <li>1. In any screen, select <b>Tools &gt; Export Consumables Log</b>.</li> <li>2. Select a location and enter a name for the export file.</li> </ol> <p><b>IMPORTANT!</b> 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.</p>
Injection list export	<p>You can export the injection list from two locations:</p> <ul style="list-style-type: none"> <li>• <b>Preview Run</b> screen at any time before a run or during a run.</li> <li>• <b>Samples view</b> screen when a run is complete.</li> </ul> <p>See "Export the injection list from Preview Run or Samples view" on page 9.</p>
Calendar	Tasks have new <b>Every Two Weeks</b> repeat setting.
EPT plot for completed or terminated runs	<p>You can view the ElectroPhoresis Telemetry (EPT) plot in the <b>Monitor Run</b> screen. The EPT plot shows instrument data conditions for a completed or terminated run until the plate for the run is unlinked.</p> <p><b>Note:</b> <b>EP Voltage</b>, <b>Laser Power</b>, <b>EP Current</b>, and <b>Run Temp</b> are stored in the sample file and is available to view in secondary analysis software.</p>

## Signal optimization feature

The signal optimization feature reduces capillary-to-capillary variation within 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)
- Run module-dependent signal optimization (8-capillary and 24-capillary instruments, optional)

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.

## Run module-dependent signal optimization

**To enable this feature:** Use an assay with \_SO suffix. 8-capillary and 24-capillary instruments, optional.

**To disable this feature:** Use an assay without \_SO suffix

A new HID36\_POP4(x1)\_SO run module (SO=signal optimization) has been optimized to minimize injection variability between capillaries. Enhancements to the run module include the introduction of a minimal amount of polymer into the well before the injection and a higher capillary position in the well during the injection. These changes have been shown to improve peak uniformity across capillaries within an injection.

You can use new assays with the \_SO suffix to implement the second component of signal optimization. The \_SO assays use the new HID36\_POP4(x1)\_SO run module (contained in the instrument protocol) (see “New assays” on page 7).

## 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

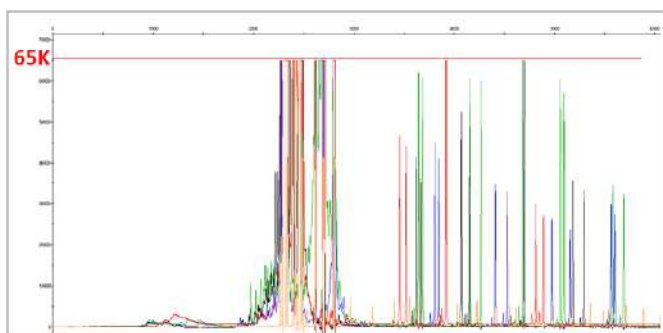
### To disable this feature:

- 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**, **J6**, 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.

## New library items available with 3500 Series Data Collection Software 4

### New assays

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:

- **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)
- **SO**—Contains an SO (Signal Optimization) run module
- **xl**—For use on 24-capillary instruments

	Assay Name
10	AB_AB_J6OSR_LS_POP4_xl_SO
11	AB_AB_J6TOSR_LS_POP4_xl
12	AB_AB_J6TOSR_LS_POP4_xl_SO
13	AB_AB_J6T_LS_POP4_xl
14	AB_AB_J6T_LS_POP4_xl_SO
15	AB_AB_J6_LS_POP4_xl
16	AB_AB_J6_LS_POP4_xl_SO

Assay name first characters <sup>[1]</sup>	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™, Yfiler™ Plus, VeriFiler™ Plus, Huaxia™
AB_J6OSR...	GlobalFiler™ Express, VeriFiler™ Express
AB_J6T...	NGM Detect™, VeriFiler™ Plus

<sup>[1]</sup> 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.

### New instrument protocols

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
- **SO**—Contains an SO (Signal Optimization) run module
- **xl**—For use on 24-capillary instruments

	Instrument Protocol Name
1	AB_AB_HID36_POP4xl_G5OSR_NT3200
2	AB_AB_HID36_POP4xl_G5OSR_NT3200_SO
3	AB_AB_HID36_POP4xl_G5OSR_NT3800
4	AB_AB_HID36_POP4xl_G5OSR_NT3800_SO
5	AB_AB_HID36_POP4xl_G5_NT3200
6	AB_AB_HID36_POP4xl_G5_NT3200_SO
7	AB_AB_HID36_POP4xl_G5_NT3800

Assay name first characters <sup>[1]</sup>	Suggested for use with AmpF(STR™ 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™, SEfiler
AB_HID36_POP4_J6_NT3200...	GlobalFiler™, Yfiler™ Plus, VeriFiler™ Plus, Huaxia
AB_HID36_POP4_J6OSR_NT3200...	GlobalFiler™ Express, VeriFiler™ Express
AB_HID36_POP4xL_J6T_NT3200...	NGM Detect™, VeriFiler™ Plus

<sup>[1]</sup> 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.

## New QC protocols

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

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

<sup>[1]</sup> 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.

## New dye sets

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)



Dye set names	Application
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

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

You can export the injection list from two screens.

Screen	Procedure
<b>Preview Run</b> Provides a CSV, XLS, or TXT export file that lists injections in the order in which they are displayed on the screen.	<ol style="list-style-type: none"> <li>1. Select <b>Preview Run</b> in the left pane.</li> <li>2. Click <b>Export</b>.</li> </ol>
<b>Samples view</b> Provides a CSV, XLS, or TXT export file that lists samples in the order in which they are displayed on the screen.	<ol style="list-style-type: none"> <li>1. Select <b>View Fragment/HID results</b> in the left pane.</li> <li>2. Create a table setting that includes <b>Injection Start Date</b> column.</li> <li>3. Sort the table by sample file name, then by <b>Injection Start Date</b> column (which also includes the time of the injection).</li> <li>4. Click <b>Export</b>.</li> </ol>

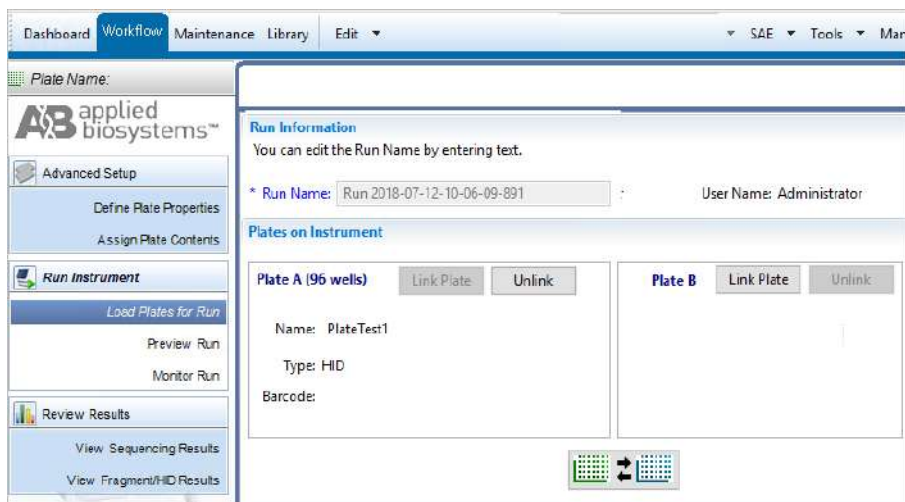
## Pause a run and load a new plate (flexible plate loading)

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

1. Display the **Monitor Run** screen: 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 indicating 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)	<p>By default, the software allows use of reagents that exceed expiry data and on-instrument limits.</p> <p>An Administrator can prevent the use of reagents that exceed on-instrument limits by changing the Instrument Settings system preference.</p> <p>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.</p> <p>Deselect the reagents that will not allow runs unless they are within on-instrument limits (to set a "hard stop").</p> <p>If you do not have Administrator role, these options are not active.</p>
Enable warning messages (Figure 3)	<p>You can control the display and timing of warnings that are related to reagent usage limits and expiration.</p> <ul style="list-style-type: none"> <li>• Expiration warnings are displayed when a reagent is expired or exceeds on-instrument limits.</li> <li>• Pre-expiration warnings are displayed the following number of days before a reagent is expired or exceeds on-instrument limits: <ul style="list-style-type: none"> <li>– ABC and CBC: 7 days</li> <li>– Array and polymer: 14 days</li> </ul> </li> </ul>

Allow runs with reagents that exceed on-instrument limits

☒ Buffer expired / usage limit

☒ Polymer expired / usage limit

☒ Conditioning reagent expired

☒ Array expired

☒ Array usage

Figure 2 Allow runs with reagents that exceed limits

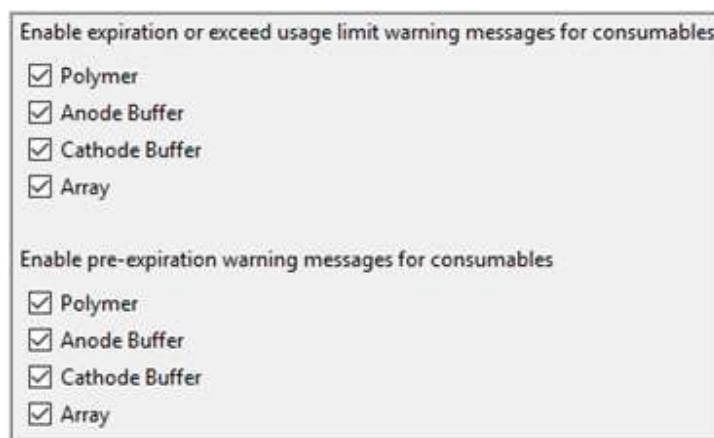


Figure 3 Enable warning messages

3. Click OK.

## Documentation and support

### Related documentation

Document	Publication number
<i>Software Release Notes 3500 Series Data Collection Software 4.0</i>	100078600
<i>3500/3500xL Genetic Analyzer User Guide</i>	100031809

### Customer and technical support

For support:

- **In North America**—Send an email to [HIDTechSupport@thermofisher.com](mailto:HIDTechSupport@thermofisher.com), or call 888-821-4443 **option 1**.
- **Outside North America**—Contact your local support office.
- For latest services and support information for all locations, go to [thermofisher.com/support](http://thermofisher.com/support).



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Revision	Date	Description
A	21 August 2018	New document.

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