

## 3500 Series Data Collection Software 3

Pub. No. MAN0010812 Rev. A.0

#### SUBJECT: New Features and HID Validation Summary

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## Purpose of this user bulletin

This user bulletin:

- Provides an overview of the 3500 Series Data Collection Software 3.
- Describes new features of the 3500 Series Data Collection Software 3 that are relevant for performing Human Identification (HID) applications.
- Describes HID validation of Data Collection Software 3 including: methods, analysis, results, and conclusions.



## **Overview of 3500 Series Data Collection Software 3**

Benefits of the software	The Applied Biosystems <sup>®</sup> 3500 Series Data Collection Software 3 supports genotyping applications on the 3500 and 3500xl Genetic Analyzers. It integrates instrument control, data collection, consumable and maintenance quality control, and size calling functions into a single software program. The software streamlines instrument setup, plate setup, data collection, and preliminary data review.			
	With its user-friendly navigation and intuitive dashboard design, the 3500 Data Collection Software 3 enables you to assess the quality of your data as it is analyzed on the instrument. By providing immediate access to size-called data, you can make decisions about the quality of your data as it is generated, without transferring output files into secondary analysis software packages.			
	The system includes pre-configured protocols and plate templates to support rapid and efficient fragment and sequencing run setup. Additionally, it offers Security, Audit, and Electronic-Signature (E-Sig) features to enable you to comply with 21CFR Part 11 requirements.			
Features of the software:	• <b>Dashboard</b> : Provides easy access to common operations. Displays instrument status, an overview of usage of each consumable, and reminders for scheduled tasks.			
	• <b>Spatial and Spectral Calibrations:</b> Enables instrument calibration for different types of consumables and operating conditions. You can review the calibration results and choose to accept or reject results. Reports can be generated and results can be exported. With the History View, you can view recent calibrations.			
	• <b>Install Check for Sequencing:</b> Install Check verifies that the instrument meets the sequencing read length specifications. Easy-to-read reports are provided to review Install Checks. Additionally, with the History View, you can review recent records of performed Install Checks.			
	• Install Check for Fragment/HID Analysis: Install check verifies that the instrument meets the fragment/HID analysis capability and sizing precision needs. Easy-to-read reports are provided to review the Install Check. Additionally, with the History View, you can review recent records of performed Install Checks.			
	• Plate Setup: Feature rich plate and table views are provided to make plate setup easy. You can assign and create resources such as Assays, File Name conventions, and Result Groups, and set well property definitions in one place without having to navigate to different views. You can set convenient defaults for these views in the preferences including default plate type, polymer used, display grid or table view, etc. A variety of features are available to search well contents, to assist in well-to-capillary mapping, to customize how well attributes are displayed, etc. Editing features such as undo, redo, fill, cut, copy, and paste makes plate setup easy.			
	• <b>Data Collection and run monitoring:</b> Various views are provided to enable you to easily link plates, monitor the data collection, and manage injection lists. The Link Plates view allows you link plates to the plate A and B positions on the instrument. The Injection list view displays the list of injections to be performed with progress status, instrument protocol used, and primary analysis status. Real-			

time views (Gel View, Trace View) displays data as it is being collected. The EPT view plots values of various instrument parameters against time. Features such as

zooming and detaching are provided for ease of use.

- **Primary Analysis:** After data collection, sample files are generated for each capillary and primary analysis is performed on these samples. Any error/warning flags regarding sample quality are displayed showing the quality information about the injection, sample name and assay used. The primary analysis algorithms (base-calling and size-calling) remain unchanged from 3500 Series Data Collection Software v1.0.
- Library Management: You can easily manage different library items such as plate records, assays, instrument protocols etc. Features are provided to create, edit, delete, export, and import library items. Search and filter features enable you to find items of interest easily.
- **Instrument Maintenance Wizards:** Wizard-style operations are provided to guide you through procedures to maintain the instrument. Wizards are provided for installing the capillary array, removing bubbles from the polymer pump, washing the pump chamber and channels, filling the array with fresh polymer, replenishing the polymer installed on the instrument, changing the type of polymer, and handling the long term shutdown of the instrument.
- **RFID Consumables Tracking:** RFID (radio frequency identification) consumable tracking evaluates the usage and expiry of consumables at various points before and during the instrument run to inform you about the status of the consumables.
- **Calendar functionality:** Recommended calendar/maintenance tasks (factory default) such as cleaning the anode buffer and periodically restarting the computer are provided. You can also create custom maintenance tasks using calendar like functionality.
- **Review and Quality Check of Primary Analysis Results**: Primary analysis results are automatically displayed in the View Results screens and can be reviewed using the sequencing results view for sequencing applications. Flags indicate the quality of the results, and sample files created during the instrument run are automatically imported into these views for review. During a run, you can re-inject samples after reviewing the results.
- **Reports for Calibrations and Install Checks:** Easy-to-read reports are provided for spatial and spectral calibrations. Summary and detailed reports are available for all applications.
- **Quality Control Reports:** Results can be displayed using various reports available for sequencing analysis such as: Trace Score, Plate, CRL, CRL Distribution, QV20+ and Signal strength.
- **Preferences:** It is possible to setup system or user level preferences for sequencing reports, plot views, tables, date formats, etc.
- **SAE (Security, Audit and E-Signature):** The SAE module is a component of the 3500 Series Data Collection Software 3. System security controls user access to the software. Auditing tracks changes and actions performed by users. E-Signature determines if users are permitted, prompted, or required to provide a user name and password when performing certain actions.
- Runs on Windows<sup>®</sup> 7 Operating System: Life Technologies configured Windows<sup>®</sup> 7 Professional Operating System Service Pack 1. Do not install on any other operating system.

For more information, refer to the *Applied Biosystems*<sup>®</sup> 3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3 User Guide (Pub. no. 100025036).

### New features in 3500 Series Data Collection Software 3

#### Overview of new features

The table below lists the software updates and enhancements most relevant for users performing Human Identification (HID) applications. For more information including other software updates, refer to the *3500 Series Data Collection Software 3 Release Notes* (Pub. no. 100026263; located on the 3500 Data Collection Software 3 CD).

**IMPORTANT!** The primary analysis algorithms (base-calling and size-calling) remain unchanged from 3500 Series Data Collection Software v1.0. There were no changes in v2.0 or v3.0 in the core algorithms for instrument control, data collection, spectral multi-componenting, peak detection, sizing, and quality check.

Feature category	Enhancements		
Install check and calibration	Calibration/Install check status displays a warning when a required step is missed.		
	Option to enter notes when accepting or rejecting a calibration run.		
	Option to perform only one spectral calibration run. The system now only requires sufficient consumables for a single run (rather than 3 runs) to start the spectral calibration run.		
Library user	Ability to permanently lock datastore objects to prevent editing.		
interface	Improved ability to filter library objects to hide unnecessary or unused objects:		
	Filter by application type (Sequencing, Fragment, HID, All)		
	<ul> <li>Filter by number of capillaries (8, 24)</li> </ul>		
	Filter by excluding objects whose name matches that of the filter		
	Ability to specify the number of runs retained in the run log.		
User interface operation	Performance improvements to streamline startup sequence, reduce response time and eliminate instances of overlapping "ghost box" windows.		
	Warning appears at software launch if instrument door is open.		
	Option to enter notes when stopping a run prematurely.		
	Option to terminate the injection list when aborting a run.		
	Ability to use the plate grid to select individual samples for re-injection and see the corresponding injection number.		
	Improved ability to set up plates using imported text files that have been modified in Microsoft Excel® 2007.		
	Added user confirmation step after reverse plate-link button is pressed (to help prevent inadvertent plate switching).		
	Progress bar displays elapsed time (rather than percentage) with improved accuracy.		
RFID consumable tracking	Reduced occurrence of RFID hard stops in RUO mode; only remaining hard stop is for the Conditioning Reagent capacity limit.		
	Improved accuracy of the consumable expiration dashboard display to include days and hours. Expiration is now set to occur at 11:59 p.m. local time (rather than noon GMT).		

Feature category	Enhancements		
Wizards	Improved dashboard and UI layout to make wizards more easily accessible.		
	Changed the wizard event name to "Wash Pump and Channels" to be consistent with wizard name.		
	Updated the Wash Pump and Channels and Install Capillary Array wizards with additional instructions to install a fresh Anode Buffer Container when finishing the wizards.		
	New Instrument Reactivation wizard assists in preparing the instrument for operation when no capillary array is already installed on the instrument.		
Reports	Ability to customize report logos using the Preferences menu.		
	Improved consumables reporting to highlight expired consumables in the annotation view.		
	New sample file annotation view for fragment analysis sample files.		
	New utility gathers multiple log files to assist the field service engineer in rapidly gathering troubleshooting information.		
Security, Audit, and e-Signature (SAE)	Optimized amount of information stored in the AuditSummary.aud file to improve performance and reduce need to create new audit files.		
	Disabled the ability to delete pre-defined roles.		
	New "run ended" event added to action log.		
	Option to prohibit pasting into the password field.		
	Option to disable a non-administrative user from editing preferences.		
	New audit event added to document object deletion.		

## Upgrades from previous versions of the software

System requirements	The computer provided with the 3500 instrument contains validated software and settings. Do not update the Windows <sup>®</sup> operating system or firewall settings. Do not rename the computer after the 3500 Series Data Collection Software is installed. For more information, please refer to the "Instrument and computer requirements" section of the <i>Applied Biosystems</i> <sup>®</sup> 3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3 User Guide (Pub. no. 100025036).		
Upgrade from	An upgrade from Data Collection Software v1.0 to v3.0 requires:		
DC v1.0 to v3.0	• A certified system provided by Life Technologies for Data Collection Software 3, with Windows <sup>®</sup> 7 Professional Operating System (OS), Service Pack (SP) 1		
	Installation by your Field Service Engineer		
	<b>Note:</b> This ensures successful migration of your "datastore," including existing spatial calibration, spectral calibration, HID install performance check, plate records, assays, instrument protocols, size standards, and QC protocols.		
Upgrade from DC v2.0 to v3.0	An upgrade from Data Collection Software v2.0 to v3.0 that can be installed by the user.		
	<b>IMPORTANT!</b> If you are upgrading from v2.0 to v3.0, follow the detailed instructions in the <i>3500 Series Data Collection Software 3 Release Notes</i> (Pub. no. 100026263, located on the Software 3 CD). This ensures successful migration of your "datastore," including existing spatial calibration, spectral calibration, HID install performance check, plate records, assays, instrument protocols, size standards, and QC protocols.		

## **Documentation and support**

# Related documentation

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

Document title	Part number
Applied Biosystems <sup>®</sup> 3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3 User Guide	100025036
Applied Biosystems <sup>®</sup> 3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3 Quick Reference Guide	100026299
Applied Biosystems <sup>®</sup> 3500/3500xL Genetic Analyzer User Guide, Data Collection v2.0	4476988
3130, 3730, and 3500 Data Collection Software; Compendium of Software Changes User Bulletin	MAN0010821
3500 and 3500xL Genetic Analyzer Site Preparation Guide	4401689
<i>3500 Series Data Collection Software 3 Release Notes</i> (Located on the 3500 Data Collection Software 3 CD)	100026263
3500/3500xL Genetic Analyzer: Protocols for the analysis of Applied Biosystems® PCR Amplification Kit PCR Products and Validation Summary User Bulletin	4469192
GeneMapper <sup>®</sup> ID-X Software Version 1.4 User Bulletin	4477684
GeneMapper <sup>®</sup> ID-X Software Version 1.3 User Bulletin	4470483
GeneMapper <sup>®</sup> ID-X Software Version 1.2 User Bulletin	4462639

#### **Obtaining support** For HID support:

- In the United States and Canada send an email to HIDTechSupport@lifetech.com, or call 888-821-4443 option 1.
- Outside the United States and Canada contact your local support office.

For the latest services and support information for all locations, go to:

#### www.lifetechnologies.com/support

At the web site, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Search for user documents, Safety Data Sheets (SDSs), vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents.
- Download.pdf documents.
- Obtain information about customer training.
- Download software updates and patches.

## HID validation summary

**Objectives** The objective of the HID validation was to confirm that the performance of the 3500 Data Collection Software 3 meets these requirements:

- 100% operation accuracy for instrument control, calibration, run set up, and data collection with both legacy and current 6-dye Applied Biosystems<sup>®</sup> PCR amplification kits
- 100% data collection accuracy comparing to Data Collection Software v1.0 on Windows<sup>®</sup> Vista OS and Data Collection Software v2.0 on Windows<sup>®</sup> 7 OS, assessing sizing precision and genotyping concordance of genomic DNA samples with representative Applied Biosystems<sup>®</sup> PCR amplification kits
- 100% data concordance of sample files generated on Data Collection Software v3.0 and analyzed by GeneMapper<sup>®</sup> *ID-X* v1.2, v1.3, and v1.4 software
- Free of any critical limitations or defects in the general user interface and workflow.

## Materials and samples

#### Instrumentation and computers

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

Instrumentation	Upgrade path tested	Computer for DC v1.0	Computer for DC v2.0	Computer for DC v3.0
3500	Data Collection v1.0 to v3.0 using the datastore migration utility	Dell <sup>®</sup> OptiPlex <sup>®</sup> 755	N/A	Dell <sup>®</sup> OptiPlex <sup>®</sup> XE2
3500xL	Data Collection v1.0 to v3.0 without data migration (fresh install)	-		Dell <sup>®</sup> OptiPlex <sup>®</sup> XE
	Data Collection v2.0 to v3.0 using the user-installable upgrade pathway	N/A	Dell <sup>®</sup> OptiPlex <sup>®</sup> XE	Dell <sup>®</sup> OptiPlex <sup>®</sup> XE

Make/Model	Processor (CPU)/speed	Memory (RAM)	Operating System (OS)/Service Pack (SP)
Dell <sup>®</sup> OptiPlex <sup>®</sup> 755	Intel <sup>®</sup> Core <sup>™</sup> 2 Duo E8400 3 GHz	2 Gb	$Windows^{\texttt{B}}$ Vista Professional OS (SP1), 32-bit
Dell <sup>®</sup> OptiPlex <sup>®</sup> XE2	Intel <sup>®</sup> Core <sup>™</sup> 2 Duo E8400 3 GHz	4 Gb	Windows® 7 Professional OS (SP1), 32-bit
Dell <sup>®</sup> OptiPlex <sup>®</sup> XE	Intel <sup>®</sup> Core <sup>™</sup> 2 Duo E8400 3 GHz	4 Gb	Windows <sup>®</sup> 7 Professional OS (SP1), 32-bit

Software

Internal software tested:

- 3500 Series Data Collection Software versions 1.0, 2.0, and 3.0
- GeneMapper<sup>®</sup> *ID-X* Software versions 1.2, 1.3, and 1.4

External software used for data analysis:

- Beyond Compare<sup>™</sup> software
- Microsoft Excel<sup>®</sup> software
- Minitab<sup>®</sup> statistical software

Kits

These kits were utilized during this validation study:

- Yfiler<sup>®</sup> PCR Amplification Kit
- Identifiler<sup>®</sup> Plus PCR Amplification Kit
- NGM SElect<sup>™</sup> PCR Amplification Kit
- SGM Plus<sup>®</sup> PCR Amplification Kit
- GlobalFiler<sup>®</sup> PCR Amplification Kit
- GlobalFiler<sup>®</sup> Express PCR Amplification Kit

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

#### Samples

The table below lists studies performed, kits tested, and samples tested.

Study	Samples	PCR amplification kits tested	Dye set	Size standard
Genotype	• 30 male gDNA samples,	SGM Plus <sup>®</sup>	F	GS500 ROX <sup>™</sup>
Concordance and Reproducibility	<ul> <li>2 injections<sup>1</sup></li> <li>6 positive controls from each kit.</li> </ul>	Identifiler <sup>®</sup> Plus	G5	GS600 LIZ <sup>®</sup> v2
		NGM SElect <sup>™</sup>		
	2 injections	Yfiler <sup>®</sup>		
	6 No Template Control     (NTC), 2 injections	GlobalFiler <sup>®</sup>	9F	
	<ul> <li>6 Allelic Ladders from each kit, 2 injections</li> </ul>			
Sizing Precision	GlobalFiler <sup>®</sup> Express Kit Allelic Ladder:	GlobalFiler <sup>®</sup> Express		
	<ul> <li>12 runs on the 3500 instrument with 2 injections of 6 sets of 8 ladders;</li> </ul>			
	<ul> <li>4 runs on the 3500xL instruments with 2 injections of 2 sets of 24 ladders</li> </ul>			

† The gDNA samples were extracted from blood specimens from a variety of donors. They were quantified using the Quantifiler<sup>®</sup> Human DNA Quantification Kit and diluted to approximately 1–2 ng/μL prior to amplification.

## Methods and data analysis

Instrument operation and UI testing	Instrument operation and UI testing was designed to evaluate the Data Collection Series 3 for instrument control, maintenance wizard operation, calibration workflow, and interface ease-of-use.		
Concordance testing	Concordance testing evaluated the Data Collection Series 3 performance in comparison with the pre-upgraded software performance. These aspects were tested in a Windows <sup>®</sup> 7 environment:		
	<ul> <li>Set up of results groups and plate records, control of instrument runs, and collection of sample files</li> </ul>		
	Multi componenting and genotyping		
	<ul> <li>Analysis of sample files generated on DC v3.0 and analyzed by GeneMapper<sup>®</sup> ID-X v1.2, v1.3, and v1.4 software</li> </ul>		
	<ul> <li>Analysis of sizing precision and peak height scaling</li> </ul>		
	Note: The auto-analysis function will not be supported and tested in this version.		

# **Summary table** All samples in this study were amplified and run according to the standard protocols in the applicable User Guide. Analysis was performed on the computers and instruments listed in on page 10 using 36-cm capillary arrays and POP-4 polymer.

**Note:** For step-by-step procedures and data analysis, see "Appendix A. Procedural and data analysis steps" on page 21.

Category Subcategory		Method and analysis		
Instrument	Datastore	Installed Data Collection Software 3 using the upgrade paths described in		
operation and User Interface	Instrument control	Instrumentation and computers on page 10. All computers were connected calibrated 3500 instruments. Ran all wizards, ran spatial and spectral		
testing	Plate and run setup	calibrations, ran HID install performance check and checked the integrity of pre-		
	Data collection	upgrade data. Checked all datastore items, checked SAE menu and functions.		
	SAE	Data analysis included checking for spectral calibration flags, checking all allele peaks per capillary and capillary raw data. In the UI, tested all buttons, icons, pull-down menus for function. Evaluated GeneMapper <sup>®</sup> <i>ID-X</i> sample file accuracy.		
Concordance	Genotyping	Thirty male genomic DNA samples and 6 positive control samples were amplified with the GlobalFiler <sup>®</sup> , Identifiler <sup>®</sup> , NGM SElect <sup>™</sup> , SGM Plus <sup>®</sup> , and Yfiler <sup>®</sup> kits. and analyzed with GeneMapper <sup>®</sup> <i>ID-X</i> software v1.4.		
		Data were analyzed with GeneMapper <sup>®</sup> <i>ID-X</i> software v1.4. The genotypes were compared using Beyond Compare <sup>™</sup> software between DC v1.0, DC v2.0, and DC v3.0, and between the three 3500-series instruments.		
	GeneMapper <sup>®</sup> <i>ID-X</i> Software backward compatibility	All the Yfiler <sup>®</sup> , NGM SElect <sup>™</sup> , Identifiler <sup>®</sup> Plus, and SGM Plus <sup>®</sup> data (.hid files) from DC v3.0 software were analyzed in GeneMapper <sup>®</sup> <i>ID-X</i> software v1.2, v1.3, and v1.4 to confirm that the sample files from the new software were properly analyzed in the older versions of GeneMapper <sup>®</sup> <i>ID-X</i> software.		
		The analysis results, including allele calls, sizing, peak heights, peak areas, and PQV scores, were compared using the Beyond Compare <sup>™</sup> software tool.		
	Sizing precision and accuracy	Sizing precision and size range of alleles were measured based on 12 runs on the 3500 instrument with 2 injections of 6 sets of 8 GlobalFiler <sup>®</sup> Express Allelic Ladders, and 4 runs on the 3500xL instruments with 2 injections of 2 sets of 24 GlobalFiler <sup>®</sup> Express Allelic Ladders.		
		The sizing precision was calculated as the standard deviation of the mean size of each allele in the allelic ladder from all capillaries in each CE run. The size accuracy was calculated as the size range between the minimum and maximum size of each allele in the allelic ladder from all capillaries in each CE run.		
	Peak height scaling	A set of 12 genomic male DNA samples and 1 positive control (007) ranging from minimal to maximal peak height were selected. Samples were amplified with the Yfiler <sup>®</sup> kit and run on the three 3500-series instruments with both pre-upgrade and new data collection software versions.		
		The data were analyzed using GeneMapper <sup>®</sup> <i>ID-X</i> software version 1.4.		

## Results

Category	Subcategory	Expected Outcome	Result
Instrument operation and User Interface testing	Datastore	Proper importation of all datastore items, consumable status, and calibration data. Proper implementation of all changes in the Library interface.	Pass. For more information see "SAE module must be disabled prior to upgrade" on page 18, and "Data traces for spectral calibrations and install checks were not migrated" on page 18.
	Instrument control	Proper implementation of all controlled instrument components (thermal, optical, mechanical, and electrical) for consumable exchange, calibration, CE run, and maintenance wizards. Proper implementation of all changes in the user interface.	Pass. For more information see "Reactivation Wizard failure after upgrade from DC 2.0 to 3.0" on page 17, and "Library object filtering fails for assays with multiple instrument protocols" on page 17.
	Plate and run setup	Proper set up of plate records and injection lists with import/export function.	Pass
		Proper implementation of all changes in the user interface.	
	Data collection	Proper collection of sample signals and application of the multi-componenting algorithm. Correct generation of data format and run information.	Pass
	Security, Audit and E-Signature (SAE)	Proper implementation of changes in the SAE functionality.	Pass
Concordance	Genotyping	100% concordance for all allele calls.	Pass
	GeneMapper <sup>®</sup> <i>ID-X</i> Software backward compatibility	100% concordant between GeneMapper <sup>®</sup> <i>ID-X</i> versions 1.2, 1.3, and 1.4.	Pass
	Sizing precision and accuracy	Standard deviation of mean size within a run is ≤0.15 bp for all alleles in the kit ladder.	Pass
		Size range within a run is ≤0.5 bp for the largest allele in the kit ladder.	
	Peak height scaling	Proper evaluation of low-to-high sample signals and accurate calling of off-scale peaks.	Pass

#### All metrics were met as described in this table:

Instrument operation and user interface	All changes in the 3500 Series Data Collection Software 3 passed test criteria and functioned properly, except for the following minor testing observations:
	• The Reactivation Wizard failed in one instance after upgrade from DC 2.0 to 3.0 (see page 17)
	• The Library object filtering failed for assays containing multiple instrument protocols (see page 17)
	• There was an out-of-memory issue when over 800 plate records were in the datastore (see page 19)
	• A "duplicate plate found" error message occurred even after deleting the plate record (see page 19)
	• An incorrect error message occurred when importing plate records that included data objects that do not already exist in the library (see page 20)
Datastore migration	The datastore migration was designed to transfer the entire Datastore folder (except for the Injection Data due to large file size) from the pre-upgrade software system to the new software system. As a result, the original calibration and install check data, and the original assays and plate records continued to function in the new software.
	The upgrade migration pathways from DC v1.0 to v3.0 and DC v2.0 to DC v3.0 were both tested and functioned properly. The spatial calibration, spectral calibration, HID install performance check, plate records, assays, instrument protocols, size standards, and QC protocols were transferred to the new software systems, and successfully generated sample files. However, the following minor issues were observed:
	• The SAE module must be disabled prior to upgrade (see page 18).
	• The data traces for spectral calibrations and install checks were not migrated (see page 18).
Genotype concordance	The genotype calls from all samples were 100% concordant between the data collection software versions and instruments.
GeneMapper <sup>®</sup> <i>ID-X</i> software backward compatibility	The results were 100% concordant between the GeneMapper <sup>®</sup> <i>ID-X</i> software versions 1.2, 1.3, and 1.4.
Sizing precision and accuracy	For all runs in this study, sizing precision and accuracy was well within passing specifications. Minimal variation was observed between pre-upgrade and new versions of data collection software.
	Figures 1 through 3 on pages 15 and 16 display box plots showing sizing precision (standard deviation) and size range across the 3 instruments that were tested with pre- upgrade and new data collection software versions. For all runs, the sizing precision and accuracy was well within the passing specifications (dotted blue lines) of $\leq 0.15$ bp for sizing precision, and $\leq 0.5$ bp for size range.



Figure 1 Box plot of sizing precision and size range following a clean installation of DC v3.0 without data migration

Figure 2 Box plot of sizing precision and size range following migration of data from DC v2.0 to DC v3.0





Figure 3 Box plot of sizing precision and size range following migration of data from DC v1.0 to DC v3.0

# **Peak height scaling** Testing confirmed that peak height scaling was consistent across all instruments and data collection software versions. The new Data Collection Software 3 properly labelled and flagged offscale peaks.

## Testing observations

Reactivation Wizard failure after upgrade from DC 2.0 to 3.0	In one instance, the Reactivation Wizard failed on the 3500xL upgrade from DC 2.0 to 3.0. Following the wash step, the wizard failed to complete with no option to proceed. The problem was not reproducible on the instrument or any other instrument, and was not observed again. The exact cause of this failure is unknown.
	In rare situations, it appears that migrating DC 2.0 spectral calibration pre-upgrade data may cause wizards to fail. If this occurs during the Reactivation Wizard, then restart the software and the instrument (according to the 3500 system start-up procedure), and run a bubble-removal routine to complete the reactivation.
	If the problem persists, it may be necessary to delete all files that start with "Run" in the folder D:\Applied Biosystems\3500\datastore\SpectralCalibrations and delete all files in D:\AppliedBiosystems\3500\datastore\mdcsystemdata\InjectionDataSpectralCal and its subfolders. It will be necessary to re-run the spectral calibrations for the dye sets in use. If the issue remains unresolved, then Data Collection Software 3 should be reinstalled.
Library object filtering fails for assays with multiple instrument protocols	When filtering by number of capillaries, the library filter incorrectly filtered out an assay edited to have multiple instrument protocols (run modules). The modified assay was not seen in the assay list when the filter was enabled. To view an assay containing multiple instrument protocols, you must disable the filtering. This applies to the library assay view and to the assay selection boxes.

#### SAE module must be disabled prior to upgrade

The DC v3.0 SAE (Security, Audit and E-Signature) functions are disabled by default, unless the user provides the new SAE license during installation. If the SAE module is enabled in DC v1.0 or DC v2.0, and the SAE license is not provided during DC v3.0 installation, a potential conflict of status may cause software failures. These include inability to finish a run and collect sample files, or inability to save a plate record. This is demonstrated in the following screenshot:



To correct this, specific instructions describing how to disable the SAE module before the upgrade are included in the *3500 Series Data Collection Software 3 Release Notes* (Pub. no. 100026263). It is essential to follow these instructions for a successful user installable upgrade from v2.0 to v3.0. For upgrades from v1.0 to v3.0, the Field Service Engineer will ensure successful migration of data and reactivation of the SAE module.

**IMPORTANT!** If you are upgrading from v2.0 to v3.0, follow the detailed instructions in the *3500 Series Data Collection Software 3 Release Notes* (Pub. no. 100026263). This prevents the error described above, and enables successful migration of your "datastore."

Data traces for spectral calibrations and install checks were not migrated

When the spectral calibrations (from v1.0 to v3.0) and install checks (all instances) are migrated to the new software, the data traces are not migrated. Therefore, when you view the calibration or install check in the History tab, the Intensity-vs.-Scan Number plot is blank. This is a cosmetic display issue only. The calibration information is correct, and is used in subsequent runs. Future calibrations or install checks display the trace data.

# Out-of-memory issue

An out-of-memory issue occurred when the upper limit (> 800) was reached on the number of plates in the Plate Record folder. The software displayed an error message with the following description in the Load Plates for Run Workflow: "Could not create the view: An unexpected exception was thrown". If this occurs, you will be able to create the plate record, but unable to link the plate, or create an injection list. The plate records in the Datastore must be deleted or transferred out of the Plate Record folder in order for the software to function properly. This issue does not appear to be specific to DC v3.0, and is expected to occur in previous versions upon reaching the upper limit of plate records.



#### Duplicate-platesfound error message

If you delete an imported plate record, and re-import the same plate record, an error message may appear for "Duplicate Plate Found." This occurs only when auditing is enabled. The created plate will permanently exist in the audit records, even when the deleted plate (.xml file) was not found in the software UI or in the Plate Record.

It's preferable to change the plate name in the plate record and re-import the plate upon duplication.



#### Incorrect error when importing plate records

You may observe an incorrect error message when importing plate records that include data objects that do not exist in the library. The software warning message states "Plate contain(s) resources "exist" in Library by name". The error message should say "not exist." This will not affect the plate import functionality. The software will proceed with the plate import, but the plate view will not have the non-existing data objects. You will need to create new data objects. This issue does not appear to be specific to DC v3.0, and is expected to occur in previous as well.

[ Impor	t Plates	
<u> </u>	Plate contain(s) resources exist in Library by name. Select Continue to import or Cancel to exit. Click the "Details" button below for more information.	
cpa_to_ - Assay - Result - File N	seq_3500_C9903GZ5 "IDP" can't be found in either current plate or Library. ts Group "IDPRG" can't be found in either current plate or Library. ame Convention "IDFNC" can't be found in either current plate or Libra	ny.

# For more information

For more information including other minor software limitations detected by the software development team, please refer to the *3500 Series Data Collection Software 3 Release Notes* (Pub. no. 100026263; located on the 3500 Data Collection Software 3 CD).

#### Conclusions

Based on the studies performed, we conclude the following:

- All changes in the 3500 Series Data Collection Software 3 passed testing criteria and functioned properly (with minor testing observations noted on page 17).
- The genotype calls from all genomic DNA samples and positive control samples were 100% concordant between the data collection software versions v1.0, v2.0, and v3.0, and between the three 3500 series instruments.
- Sizing precision was comparable between data collection software versions v1.0, v2.0, and v3.0.
- Peak height on data collection software versions v1.0, v2.0, and v3.0 were on the same scale. The new data collection software v3.0 was able to properly label and flag offscale peaks.
- Data from Data Collection Software 3 is compatible with GeneMapper<sup>®</sup> *ID-X* software versions 1.2, 1.3, and 1.4. The allele calls, sizing, peak heights, peak areas, and PQV scores were 100% concordant between the GeneMapper<sup>®</sup> *ID-X* software versions.
- The upgrade migration pathways from DC v1.0 to v3.0 and DC v2.0 to DC v3.0 functioned properly. The spatial calibration, spectral calibration, HID install performance check, plate records, assays, instrument protocols, size standards, and QC protocols were correctly transferred to the new software systems, and were used to successfully generate sample files. For more information on minor testing observations, see "SAE module must be disabled prior to upgrade" on page 18, and "Data traces for spectral calibrations and install checks were not migrated" on page 18.

## Appendix A. Procedural and data analysis steps

Procedure: Instrument operation and UI testing	This portion of testing evaluated the Data Collection Software 3 for proper instrument control, maintenance wizard operation, calibration workflow, and updates to the user interface.
	1. Installed the v3.0 data collection software on all computers, and connected them to the 3500 and 3500xL instruments with proper calibration files.
	2. Using the proper wizards with each data collection software, installed the capillary array, polymer, and anode/cathode buffers. Ran the Instrument Reactivation Wizard, Install Array Wizard, and Wash Pump and Channels Wizard to confirm the implementation of the changes. Also ran the Remove Bubble and Replenish Polymer Wizard to check performance.
	<b>3.</b> Ran the spatial calibration 3 times on the instrument with a fresh DC v3.0 installation (no datastore migration). The two instruments with datastore folder migration (upgrade from DC v2.0 or DC v1.0) were checked for the proper transfer of the pre-upgrade spatial calibration data into the new software.
	<b>4.</b> Ran the DS32, DS33, and DS36 spectral calibration 3 times on the instrument with a fresh DC v3.0 installation. On the two instruments with datastore folder migration (upgrade from DC v2.0 or DC v1.0), checked for proper transfer of the pre-upgrade spectral calibration data into the new software.

	<b>5.</b> Ran the HID Install Standard performance check 3 times on the instrument with a fresh DC v3.0 installation. On the two instruments with datastore folder migration (upgrade from DC v2.0 or DC v1.0), checked for proper transfer of the pre-upgrade HID Install Standard data into the new software.
	<b>6.</b> Opened the software Library, and checked all the changes and new features for the datastore items.
	<b>7.</b> Opened the software SAE menu, and checked all the changes and improvements for security, audit, and e-signature functions
	<b>8.</b> During the instrument operation for genotyping concordance and sizing precision runs, checked all the changes and improvements for plate setup, reinjection, status tracking, run stopping, and consumable tracking.
	<b>9.</b> After collecting run data, checked all the changes and improvements for sample file annotation, customized report logo, and troubleshooting information.
Data analysis: Instrument operation and UI	<ol> <li>Reviewed the spectral calibration results by checking the flags to ensure all capillaries passed the calibration. Reviewed the raw data from each capillary as well as the condition numbers and quality values.</li> </ol>
testing	<b>2.</b> Reviewed the HID Install Standard performance check results by checking the flags to ensure all 205 allele peaks were found in each capillary and the sizing quality and precision from each capillary met specifications. This included review of raw data from each capillary.
	<b>3.</b> For each test, checked the executable buttons, icons, or pull down menus that were necessary to verify correct function and performance, and that appropriate information was provided.
	<ol><li>After selecting any options, typing in information, or completing a step in any test, checked to see if the software status had been updated correctly.</li></ol>
	<b>5.</b> Evaluated the accuracy of information in the sample files in GeneMapper <sup>®</sup> <i>ID-X</i> software.
Procedure: Concordance	This portion of testing evaluated the Data Collection Software 3 performance in a Windows <sup>®</sup> 7 environment with regard to the following aspects:
testing	• Proper set up of the results groups and plate records, as well as control of the instrument runs and collection of sample files without error;
	• The sample files collected from the new software were properly multi- componented and generated concordant genotypes as compared to the sample files from DC v1.0 and v2.0
	<ul> <li>The sample files generated with DC v3.0 could be properly analyzed in GeneMapper<sup>®</sup> ID-X v1.2, v1.3, and v1.4 software</li> </ul>
	• The sizing precision and peak height scaling was comparable in different versions of the Data Collection Software.
	Procedural steps:
	1. After running all the samples and kits described above (see the table on page 11) with double injections on the 3 instruments with the pre-upgrade versions of the data collection software, the Data Collection Software v3.0 was installed on the instruments and computers.

	2. The 3500 instrument was upgraded from DC 1.0 to 3.0 using the datastore migration utility, which transferred the entire datastore folder (except for the Injection Data) from the old computer to the new one. The system was then checked to confirm all the datastore items, consumable status, and calibration data were properly imported and remained unchanged in the new software version.
	<b>3.</b> One 3500xL instrument had a fresh install of DC 3.0 (no data migration). The installation wizards and calibrations were re-run and the performance compared between the software versions.
	<b>4.</b> One 3500xL instrument was upgraded from DC 2.0 to 3.0 using the user installable upgrade/migration pathway on the same computer. The system was then checked to confirm all the expected datastore items, consumable status, and calibration data were properly imported and remained unchanged in the new software version.
	<b>5.</b> In the DC v3.0 software, all the default run modules, instrument protocols, size standard files, QC protocols, and assays were reviewed to ensure all the 3500 HID assays were correctly included.
	<b>6.</b> The software instructions were followed to create results group and file naming conventions that contained the sample name, instrument name, capillary, well, and run number (injection number). The same settings were used in all software versions on all computers.
	<b>7.</b> The samples/kits described on page 11 were analyzed again (with 2 injections per plate) in the new Data Collection Software version for comparison purposes.
Data analysis: Concordance testing	<ol> <li>For each test, checked the executable buttons, icons, or pull-down menus that were necessary to verify correct function and performance, and checked that appropriate information was provided.</li> </ol>
5	<b>2.</b> After selecting any options, typing in information, or completing a step in any test, checked to see if the software status had been updated correctly.
	<b>3.</b> Reviewed the raw data display in the data collection software.
	<b>4.</b> Reviewed the analyzed data display in the DC v3.0, and confirmed that clicking the data review button during data collection would not corrupt the results.
	<b>5.</b> In the DC v3.0 HID result review, checked to confirm the peak heights of all peaks were properly scaled, including offscale and low height peaks. Confirmed in GeneMapper <sup>®</sup> <i>ID</i> - <i>X</i> v1.4 software that the offscale marks were properly assigned to the saturated peaks.
	<b>6.</b> In the DC v3.0 HID result review, checked to confirm that the sizing quality and broad peak flags were properly applied to the applicable samples.
	<b>7.</b> Imported all sample files into GeneMapper <sup>®</sup> <i>ID-X</i> v1.4 software and reviewed the raw data information for accuracy and to identify any samples requiring reanalysis.

- **8.** Analyzed all the DC v1.0, v2.0, and v3.0 sample files in GeneMapper<sup>®</sup> *ID-X* v1.4 using the same analysis method, panel, and size standard in all software versions with a 175 RFU peak amplitude detection threshold. Analysis was conducted with and without normalization. Visually reviewed the plots and tables and made any necessary edits to remove off-ladder peaks and artifact peaks.
- **9.** Exported the cleaned genotype tables (the Combined Tables) in .txt format, and used Beyond Compare<sup>™</sup> software to compare the allele calls between different data collection versions for genotype concordance.
- **10.** Analyzed all the DC v3.0 sample files in GeneMapper<sup>®</sup> *ID-X* software versions 1.4, 1.3, and 1.2 (except for samples amplified with the GlobalFiler<sup>®</sup> and GlobalFiler<sup>®</sup> Express kits, which are not supported on versions 1.3 and 1.4) using the same analysis method, panel, and size standard in all software versions with a 175 RFU peak amplitude detection threshold.
- 11. Exported the cleaned genotype tables (the Combined Tables) in .txt format. Used Beyond Compare<sup>™</sup> software to compare the allele calls, sizes, peak heights, and PQV values between different GeneMapper<sup>®</sup> *ID-X* versions for data backward compatibility.
- **12.** For the sizing precision genotype tables, we used the Microsoft Excel<sup>®</sup> and Minitab software to calculate the sizing precision and size range for each allelic ladder allele, in each run, and over the entire 96-well plate. Plotted and compared the results between different DC software versions.

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#### Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288 For support visit lifetechnologies.com/support



lifetechnologies.com 15 August 2014