SeqStudio™ Flex Series Genetic Analyzer
USER BULLETIN

Instrument use and validation for HID applications

Publication Number   MAN0028463

Revision   B.0

When used for purposes other than Human Identification the instruments cited are for Research Use Only. Not for use in diagnostic procedures.

Thermo Fisher
S C I E N T I F I C

Revision history: MAN0028463 B.0 (English)

Revision	Date	Description

B.0	6 October 2023	• Updated with the following changes for SeqStudio™ Flex Series Instrument Software v1.1.1:
		  – The text of the footer that appears in reports was added.
		  – Information about the enhancements that have been made to the auto-spectral calibration algorithm was added.
		• The actions that require manual spectral calibration were updated.
		• The verification of select Applied Biosystems™ PCR amplification kits using the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series Instrument Software v1.1.1 was added.

A.0	31 May 2023	New user bulletin for the SeqStudio™ Flex Series Genetic Analyzer.

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

Contents

■ **CHAPTER 5** HID instrument verification of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series Instrument Software v1.1.1

■ **APPENDIX A** Documentation and support

1 About the instrument and software

For detailed information on the SeqStudio™ 8 Flex Genetic Analyzer and SeqStudio™ 24 Flex Genetic Analyzer, see the *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide*.

About the instrument

Product description

The SeqStudio™ 8 Flex Genetic Analyzer and the SeqStudio™ 24 Flex Genetic Analyzer are fluorescence-based DNA analysis instruments using capillary electrophoresis technology with 8 or 24 capillaries.

The instrument is a stand-alone instrument, but can be used with the Plate Manager software. The Plate Manager software makes it easier to create plate files.

The instrument can also be linked to the Thermo Fisher™ Connect Platform to access these additional functions:

- Save results and other files to the cloud
- Monitor instrument runs remotely from a computer or from a smart device
- Request assistance using **Smart Help**

The SeqStudio™ 8 Flex Genetic Analyzer and the SeqStudio™ 24 Flex Genetic Analyzer for HID validation was performed according to SWGDAM Validation Guidelines for DNA Analysis Methods (SWGDAM, Dec 2016) and the FBI's Quality Assurance Standards (QAS, July 2020).

Theory of operation

Preparing the instrument

The following runs are required to prepare the instrument for routine operation.

- **Spatial calibration**—Associates the signal from each capillary to a specific position on the CCD camera. Spatial calibration is required after a new capillary array is installed.
- **Spectral calibration**—Generates a matrix for each capillary that compensates for dye overlap and is used to convert the 20–bin data into 4-, 5-, or 6-dye data. Spectral calibration is required before a new dye set/polymer combination is used.
- **Install run**—Ensures that the instrument meets specifications for HID applications after installation. An install run can be used as a performance check as part of routine operation.

During a run

During a run, the instrument:

- Prepares the capillaries by pumping fresh polymer under high pressure from the polymer delivery pump to the waste position in the cathode buffer container (CBC).
- Electrokinetically injects the sample into the capillaries by briefly applying a low voltage.
- Washes the capillary tips in the rinse position of the CBC, then returns the capillary to the buffer position of the CBC.
- Ramps the voltage up to a constant level.

  A high electric field is created between the ground end of the anode buffer container (ABC) and the negative voltage applied to the load header of the capillary array. This field pulls the negatively charged DNA through the separation polymer. The smaller fragments migrate faster than the larger fragments and reach the detector first.

  To ensure optimal separation and maintain denaturation of the DNA, the capillaries are thermally controlled in the oven and in the detection cell. The oven has a Peltier heat unit and fan-circulated air.

  In the detection cell, the dyes attached to DNA are excited by a narrow beam of laser light. The laser light is directed into the plane of the capillaries from both the bottom and top. A small amount of laser light is absorbed by the dyes and emitted as longer wavelength light in all directions.

- Captures the fluorescent light on the instrument optics while blocking the laser light. The light passes through a transmission grating, which spreads the light out. The light is imaged onto a cooled CCD array. For each capillary, 20 zones on the CCD are collected to provide 20-bin data for each capillary.
- Converts the 20-bin data into multi-dye data for the entire run. For HID applications, up to 6 dyes can be used to determine fragment sizes in a single run.

Results
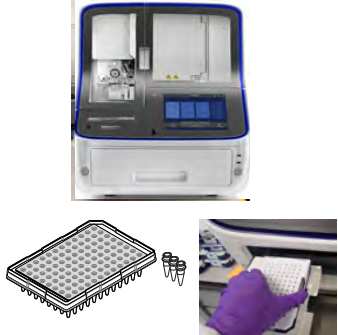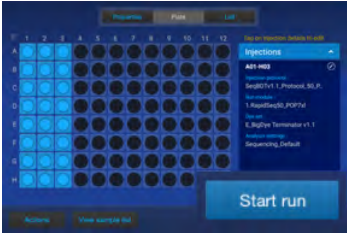
The software generates an electropherogram (intensity plot) for each dye based on the migration of DNA fragments over the run and generates primary analysis results. For HID analysis, the software uses the internal size standard to assign a fragment size to each peak and a sizing quality value to the sample. Results are generated in FSA file format.

For more information on the theory of operation, see *DNA Fragment Analysis by Capillary Electrophoresis User Guide* (Pub. No. 4474504).

Workflow: Create a plate, start and monitor a run, then view results

Use the instrument

Create

Create a plate file on the instrument or in the SeqStudio™ Plate Manager software v2.1.1.

Prepare

Prepare the instrument, prepare the samples and the plate, then load the plate into the instrument.

Link

Link a plate file located in the **Inbox**, on the instrument, on a network or USB drive, or on the Thermo Fisher™ Connect Platform.

Start

Set injection properties, then start the run.

Use the instrument

Monitor

Monitor the run in the home screen by viewing the information for a plate position, or by tapping a plate position to view the plate layout and run progress.

Monitor the run in the **Run Queue** screen by tapping ⊜ **Run queue** in the home screen.

Monitor the run in the SeqStudio™ Flex Remote Monitoring software by opening the InstrumentConnect software, then selecting an instrument.

Monitor the run from a mobile device by launching the InstrumentConnect app, selecting an instrument, then tapping a plate position.

<div style="background:#1a1a5e;color:white;text-align:center">

Use the instrument

</div>

View

View and analyze results in any of the following locations:

- In the home screen, tap the plate position.
- In the **Run Queue** screen, tap an injection.
- In the home screen, select ⊙ **Actions ▸ Run history**.
- In the Remote Monitoring software, tap the plate position.

Instrument consumables handling, usage limits, and expiration

---

IMPORTANT! Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see "Customer and technical support" on page 78.

IMPORTANT! Do not leave the instrument for more than 20 minutes without a pouch (polymer or conditioning reagent), buffers, array, and an array port plug installed. The capillary array and pump can dry out if these consumables are not installed.

Containers and pouches are ready-to-use. Labels are embedded with a radio frequency identification (RFID) tag that the instrument uses to track usage and expiration date.

Configuring the instrument to prevent the use of consumables beyond expiration and usage limits

By default, the instrument is configured to allow the use of consumables beyond expiration and usage limits.

Important notice regarding use of consumables that exceed supported limits

BEFORE DISMISSING THE WARNING THAT THE CONSUMABLES HAVE REACHED SUPPORTED LIMITS AND CONTINUING WITH OPERATION OF THE INSTRUMENT, PLEASE READ AND UNDERSTAND THE FOLLOWING IMPORTANT NOTICE AND INFORMATION:

Thermo Fisher Scientific does not recommend the use of consumables that exceed supported limits. The recommended limits are designed to promote the production of high-quality data and minimize instrument downtime. Reagent and consumable lifetime minimum performance are based on testing and studies that use reagents and consumables that have not exceeded supported limits.

The use of consumables beyond the supported limits may impact data quality or cause damage to the instrument or capillary array. The cost of repairing such damage is *NOT* covered by any Thermo Fisher Scientific product warranty or service plan. Customer use of expired consumables is at customer's

own risk and without recourse to Thermo Fisher Scientific. For example, product warranties do not apply to defects resulting from or repairs required due to misuse, neglect, or accident including, without limitation, operation outside of the environmental or use specifications or not in conformance with Thermo Fisher Scientific instructions for the instrument system, software, or accessories.

Please see your specific service contract or limited product warranty for exact language regarding coverage and ask your Thermo Fisher Scientific representative if you have further questions.

Buffer storage, usage, and limits

Cat. No.	Description	Storage[1]
4393927	Anode Buffer Container 3500/Flex Series (ABC), 4 pack Contains 1X running buffer	2–8°C[2]
4408256	Cathode Buffer Container 3500/Flex Series (CBC), 4 pack Contains 1X running buffer	2–8°C[2]

[1] See the expiration date on the label. Do not use expired product.

[2] Buffers have been qualified to ship at ambient conditions. For a description of the qualification, go to **http://find.thermofisher.com/ce/ambientbuffers/email?CID=fl-we18868**.

Instrument	On-instrument supported limits Lower of:	Guidelines
8-capillary	14 days, 240 injections, or expiration date	The buffer has been verified for use for up to 14 days on the instrument.
24-capillary	14 days, 100 injections, or expiration date	

Polymer storage, usage, and limits

Cat. No.	Description	Storage[1]
4393715	POP-4™ (384) Performance Optimized Polymer	2–8°C
4393710	POP-4™ (960) Performance Optimized Polymer	IMPORTANT! Do not freeze polymer.

[1] See the expiration date on the label. Do not use expired product.

Pouch size	Instrument	On-instrument supported limits Lower of:	Guidelines
384 samples	8-capillary	14 days, 60 injections, or expiration date	The POP-4™polymer has been verified for use for up to 14 days on the instrument at 15–25°C.
	24-capillary	14 days, 20 injections, or expiration date	
960 samples	8-capillary	14 days, 120 injections, or expiration date	At higher temperatures, the limit is 7 days on the instrument.
	24-capillary	14 days, 50 injections, or expiration date	

Note: A polymer pouch includes a small reserve volume that is used for the **Remove bubbles** maintenance wizard, which consumes ~350 µL of polymer. The reserve volume is sufficient to run the wizard ~4 times (including the remove bubbles step during other maintenance wizards). If you manually run the **Remove bubbles** maintenance wizard >4 times, the volume of polymer that is available for samples may be depleted.

Conditioning reagent storage, usage, and limits

Cat. No.	Description	Storage[1]	On-instrument supported limits
4393718	Conditioning Reagent Kit 3500/Flex Series 1 unit (single-use)	2–8°C After removing from storage, use the pouch within 24 hours.	Single use or expiration date

[1] See the expiration date on the label. Do not use expired product.

Capillary array storage and usage

⚠️ **WARNING! SHARP** The electrodes on the load-end of the capillary array have small, blunt ends that can lead to piercing injury.

Cat No.	Description	Storage[1]
A49104	Capillary array 36-cm SeqStudio™ 8 Flex	15–30°C
A49105	Capillary array 36-cm SeqStudio™ 24 Flex	

[1] See the expiration date on the label. Do not use expired product.

Note: Capillary arrays have been verified for 160 injections when used with Thermo Fisher Scientific reagents.

Hi-Di™ Formamide storage and usage

Formamide is an injection solvent that is used to prepare samples. It is not a consumable that is installed on the instrument as are the other consumables listed in this section. It does not include an RFID tag on the label.

Cat. No.	Description	Storage[1]
4401457	5 mL	−25°C to −15°C
4440753	4 × 5 mL	If frequent sampling is required, dispense and freeze small aliquots of Hi-Di™ Formamide. To preserve quality, minimize freeze-thaw cycles and exposure to air and room temperature.
4311320	25 mL	Use of Hi-Di™ Formamide after 8 freeze/thaw cycles has not been validated.

[1] See the expiration date on the label. Do not use expired product.

Chapter 1 About the instrument and software
About the SeqStudio™ Flex Series Instrument Software v1.1.0 and later for HID

1

Maintain the instrument

For HID applications, monthly maintenance tasks should be completed weekly to ensure minimal loss of resolution.

For more information about daily, monthly, annual, and as-needed maintenance tasks, see *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide* (Pub No. 100104689).

About the SeqStudio™ Flex Series Instrument Software v1.1.0 and later for HID

The SeqStudio™ Flex Series Instrument Software is the on-instrument, integrated touchscreen software that runs the SeqStudio™ Flex Series Genetic Analyzer.

The instrument and software include the following features:

- Complete workflow, from plate file setup to review of primary analysis results
- Intuitive display of sample quality, run notifications, and consumables status
- 4-plate capacity, with interactive plate loading during runs
- Ability to prioritize the order of injections and plates
- Automated barcode workflow with the internal barcode reader and optional user-supplied external USB barcode reader
- Enhanced results accuracy with auto-spectral calibration during every run
- Libraries to allow reuse of plate files, injection protocols, and other elements that are required to run HID plates
- Integration of auto-spectral calibration (pull-up reduction) and off-scale recovery.

Note: Auto-spectral calibration and off-scale recovery are enabled in default HID injection protocols. For more information, see the *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide*.

  · Auto-spectral calibration cannot be disabled with SeqStudio™ Flex Series Instrument Software v1.1.0.
  · Auto-spectral calibration can be disabled when you create a custom dye set with SeqStudio™ Flex Series Instrument Software v1.1.1. For information about auto-spectral calibration with software version 1.1.1, see "Auto-spectral calibration in SeqStudio™ Flex Series Instrument Software v1.1.1" on page 14.
  · Off-scale recovery can be disabled when you create a custom dye set with SeqStudio™ Flex Series Instrument Software v1.1.0 and later.

- On-instrument learning aids and assistance:
  – On-instrument tutorial videos with additional videos available on **www.thermofisher.com**
  – Demonstration mode that allows use of most features
  – Step-by-step wizards for instrument maintenance procedures
  – Smart help (requires connection to Thermo Fisher™ Connect Platform) and remote session features to request and obtain assistance from Technical Support

- Optional use with the Security, Audit, and E-Signature (SAE) module for controlled user access
- Application programming interface (API) to allow automation of instrument and software functions

Parts of the software

The home screen is the main screen of the software where you select, run, and monitor plate runs. For more information, see the *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide*.

From the home screen, you access the following screens:

- **Link Plate File** screen where you select the plate file that contains the settings that are used to run and analyze a plate. When you link a plate file, you can modify settings, if needed, and start the plate run.
- **Run Queue** screen where you view, edit, repeat, and cancel injections, and change the order of the injection list.
- **Actions** screen where you access most functions in the software other than running plates and using the run queue. The **Actions** screen includes options for instrument settings, libraries, maintenance and support, and viewing results for completed plates.
- **Dashboard** screens where you view information about instrument status, consumables status, and upcoming maintenance.

Auto-spectral calibration in SeqStudio™ Flex Series Instrument Software v1.1.1

In version 1.1.1 of the instrument software, the auto-spectral calibration algorithm has been updated. With this update, auto-spectral calibration can be optimally applied to most applications.

The auto-spectral calibration function is enabled by default. If desired, you can disable the auto-spectral calibration function when you create a custom dye set. When you perform a run with a custom dye set that does not use the auto-spectral calibration function, the instrument applies the manual spectral calibration to the sample data.

We recommend that you leave auto-spectral calibration enabled by default. If you run samples that do not use the auto-spectral calibration function, perform a manual spectral calibration if any of the following actions are performed or observations are made:

- The capillary array is changed.
- The instrument is moved.
- The optical system is adjusted by Service.
- A decrease in spectral separation in the raw or analyzed data is observed, as seen by an increase in pull-up/pull-down spectral peaks.

Other software

Companion software

The instrument can be used with the following software:

- **Plate Manager software**—An application for creating plate files and sending them to the **Inbox** on the instrument.
- **SeqStudio™ Flex Remote Monitoring software**—An application that allows you to monitor instrument runs from a remote computer or from a mobile device. This application requires connection to Thermo Fisher™ Connect Platform.
- **Security, Auditing, and E-signature software**—Optional module that controls user access, grants permission to specific functions and actions, provides an audit trail, and supports electronic signature.

Desktop secondary analysis software

GeneMapper™ *ID-X* Software v1.7, a secondary analysis software for HID analysis is available for desktop computers.

IMPORTANT! Earlier versions of the GeneMapper™ *ID-X* Software cannot analyze data files generated by the SeqStudio™ Flex Series Genetic Analyzer. Contact Support for information on obtaining the latest versions of software.

Size standard normalization feature

For HID analysis applications, the software includes an optional normalization feature for use with the GeneScan™ 600 LIZ™ Size Standard v2.0. This feature attenuates signal variations associated with instrument, capillary array, sample salt load, and injection variability between capillaries and injections. Normalization can be applied during data collection.

Based on the settings specified in the run module and the data collected for GeneScan™ 600 LIZ™ Size Standard v2.0, the software calculates a normalization factor for each sample based on a threshold setting. The normalization factor is used as a multiplier to adjust the peak height of the sample peaks relative to the GeneScan™ 600 LIZ™ Size Standard v2.0 peaks.

IMPORTANT! Normalization is not applied to samples with failing sizing quality. Select a size standard definition file appropriate for your application that accurately sizes samples. For example, if your application includes small fragments that may be obscured by primer peaks, or large fragments that may not be present due to slower migration rates, specify a size standard definition file that eliminates these fragments from sizing.

To enable the normalization feature, available in the SeqStudio™ Flex Series Instrument Software v1.1.0 and later, and the GeneMapper™ *ID-X* Software v1.7:

- Prepare each sample with the GeneScan™ 600 LIZ™ Size Standard v2.0.
- Use a run module with laboratory-determined optimized normalization settings.
- Select the appropriate normalization size standard in the plate file.

2 Create a plate, start and monitor a run, then view results

Prepare the samples, plate, and plate assembly

For detailed instructions, see the *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide*.

1. Prepare the samples according to the sample preparation guidelines (see "Sample preparation guidelines").

2. Add samples to the plate according to the plate layout for the plate and your instrument type.

3. Prepare the plate assembly.

   IMPORTANT! Make sure to use the plate base and retainer sets that are designed for the SeqStudio™ Flex instrument. For catalog numbers, see *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide*.

Sample preparation guidelines

Prepare the samples according to the following sample preparation guidelines.

- Plates and tubes:
  - Use MicroAmp™ Optical Reaction Plates or MicroAmp™ Reaction Tubes with a base and retainer set. Use a base and retainer with the same catalog number.
  - Use the appropriate septa for the plates and tubes.
- HID sample preparation:
  - Prepare the HID reactions: 10 µL of the size standard/Hi-Di™ Formamide mixture and 1 µL of the sample or allelic ladder. For more information, see the kit instructions.

    Note: Data quality may be reduced if samples exceed 16–24 hours on the instrument. The software displays an alert if a sample will exceed 24 hours on the instrument by the end of the run. Sample stability is lowered if samples are prepared in an aqueous solution. Using anything other than Hi-Di™ Formamide is not recommended.

  - Ensure that Hi-Di™ Formamide is fresh.
    - Hi-Di™ Formamide should not undergo more than 8 freeze-thaw cycles.
    - Use the same day after thawing.

Create a plate file in the Plate Manager software 2.1.0 and later (desktop)

For information about creating a plate file in the Plate Manager software (desktop), see *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide* (Pub. No. 100104689).

Note: If you do not have the Plate Manager software, proceed to "Create a plate file on the instrument" .

1. In the Windows™ desktop, click ⊞, type **Plate Manager**, then select **Plate Manager** from the start menu.

2. *(First use only)* In the **Select an instrument** screen, select **SeqStudio Flex**.

3. Click **Create a plate file**.

4. Enter the appropriate settings in the **Properties** tab, then click **Next**.

    Note: For the automated barcode workflow, you can scan or manually enter the barcode of the plate.

5. In the **Plate** tab, select one or more injection groups: Click a well to select one injection group; control+click or click-drag to select multiple injection groups; or click ◢ **(Select/deselect)** to select all injection groups.

    Note: *Injection group* refers to each set of 8 or 24 wells on the plate, based on instrument configuration.

Chapter 2 Create a plate, start and monitor a run, then view results
Create a plate file in the Plate Manager software 2.1.0 and later (desktop)

2

6. In the **Injection protocol** field, select a protocol.

An injection protocol specifies the settings that are used to run and analyze the sample.

Instead of selecting an injection protocol, you can select the individual elements of the protocol: **Dye set**, **Run module**, **Analysis settings**, and **Size standard**.
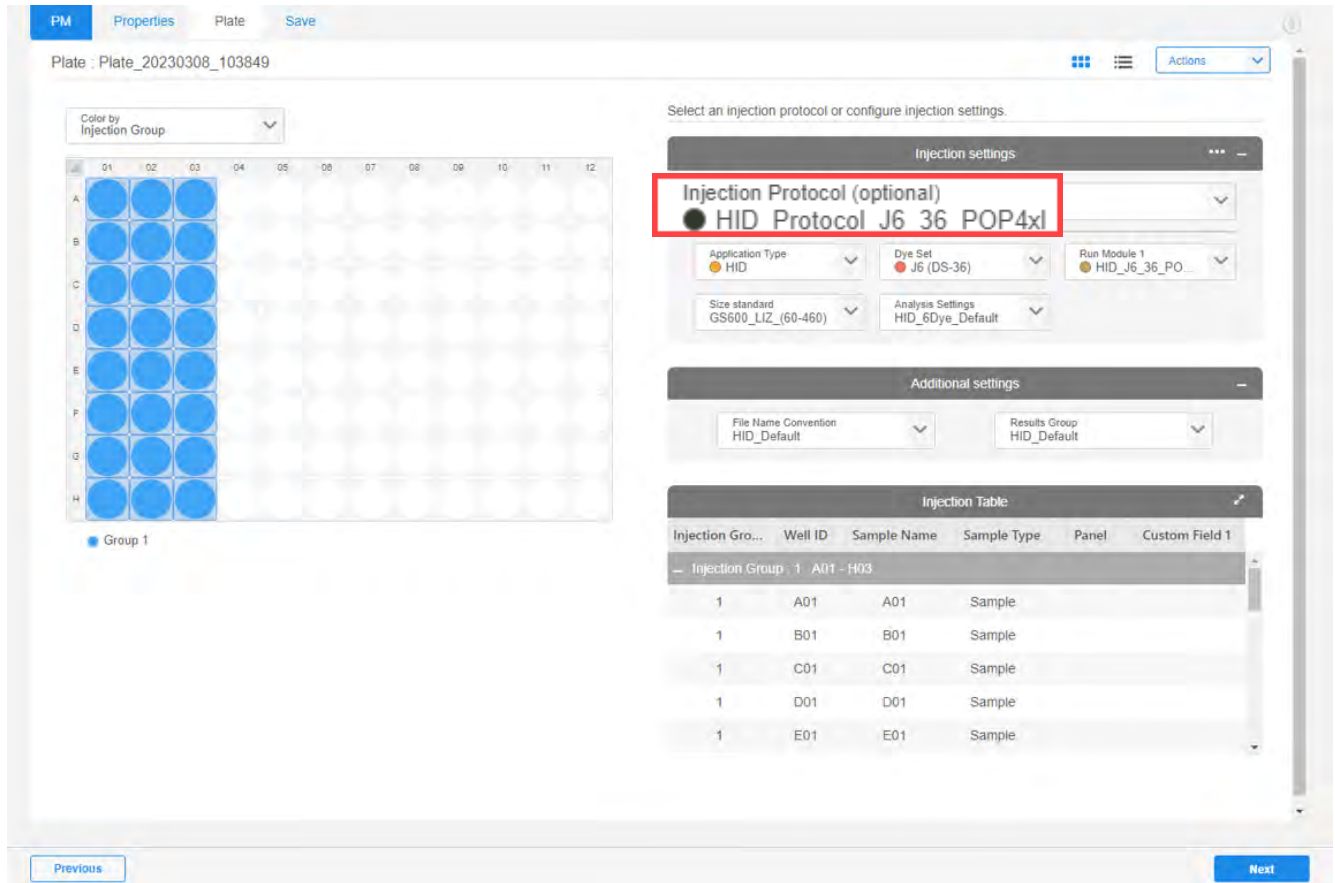
7. Click **Actions ▸ Print plate layout**.
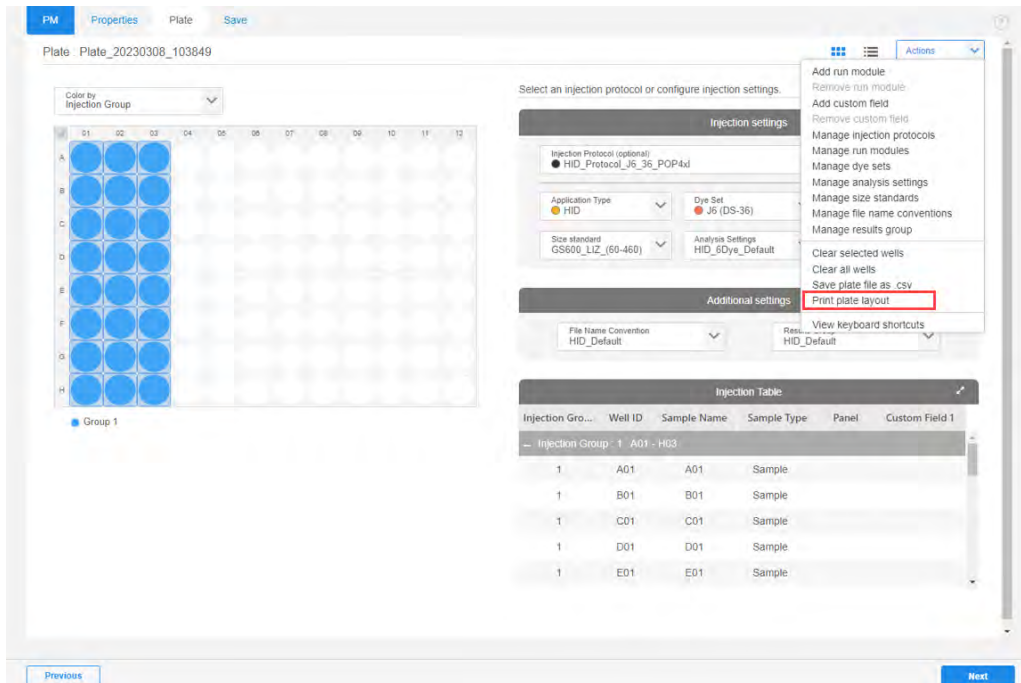
A PDF file of the plate layout is downloaded.

8. Click **Next**, then click **Save**.

    Save the plate file in PSM or CSV format to a USB drive or to a network.

    Proceed to "Load a plate into the instrument" .

    For information on how to set the Plate Manager software (desktop) to send a plate file to the **Inbox** on an instrument, tap ⑦ **(help)** in the top right of the Plate Manager software, or see the *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide*.

Create a plate file on the instrument

1. At the bottom left of the home screen, tap ⊙ **Actions ▸ Create plate file**.

2. Tap a section on the **Create Plate** screen, then enter the appropriate settings in the **Properties** tab.

    Note: For the automated barcode workflow, you can scan or manually enter the barcode of the plate.

3. Tap the **Plate** tab at the top of the screen.

    The plate view is displayed with the first injection group selected.

    Note: *Injection group* refers to each set of 8 or 24 wells on the plate, based on instrument configuration.

4. Tap the injection pane on the right of the screen to display the **Edit Injection Properties** screen.
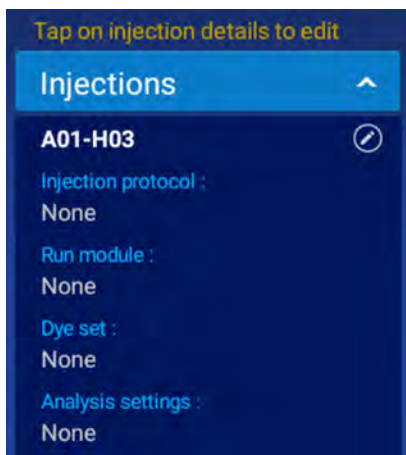
5. In the **Edit Injection Properties** screen, tap the **Injection protocol** field, then select a protocol.

   An injection protocol specifies the settings that are used to run and analyze the sample.

   Instead of selecting an injection protocol, you can select the individual elements of the protocol: **Dye set**, **Run module**, **Analysis settings**, and **Size standard**.

   Note: To use the default settings for sample names (well ID), Sample type (sample), or amplicon or specimen (blank) and do not need to add special fields, you can tap **Done**, tap **Save**, then skip to "Load a plate into the instrument" on page 22.

   ---

6. Tap the **Sample name** field.

   The **Edit Well Properties** screen is displayed.

7. In the **Edit Well Properties** screen, tap **Sample name** in the left column.

8. Tap the entry field for sample name, the enter the sample name, then tap **Enter**.

9. Repeat for all entry fields for the attribute.

10. Repeat for each attribute in the left column, and enter or select the appropriate setting:
    - **Sample type**—**Sample**, **Positive Control**, **Negative Control**, or **Allelic Ladder**.
    - **Panel**—Optional text that can be used by secondary analysis software.

      To successfully import files into GeneMapper™ *ID-X* Software v1.7, use **Custom field 3** instead.
    - **Custom fields 1–10**—Text fields to include additional sample attributes or identifiers that can be used in file name conventions or by secondary analysis applications. Custom fields 1–3 are reserved for the following attributes.

Custom field	Attribute
1	Analysis method
2	Size standard
3	Panel

IMPORTANT! Any entry into custom fields 1–3 overrides the attribute field during an import into GeneMapper™ *ID-X* Software v1.7. To successfully import files, ensure custom fields 1–3 have the same definition as the attributes they override. Custom fields 4–10 do not require a specific definition.

11. Tap **Done** to close the **Edit Well Properties** screen, then tap **Done** again to close the **Edit Injection Properties** screen.

12. Tap **Save**.

Load a plate into the instrument

1. Ensure that the **Drawer unlocked** status is displayed in the home screen.

   Positions in the drawer that are empty are marked as **Available**.

2. Pull the drawer open.

   IMPORTANT! Do not open and close the drawer rapidly. Doing so can cause vibration and disruption of the samples in the plates. Results may be adversely affected.

   IMPORTANT! To avoid injury, do not place your hands into any empty spaces in the drawer.

3. Ensure that the plate retainer is firmly snapped in place on top of the plate, then place the new plate into an empty position. Align the notched corner of the plate with the notched corner of the plate holder.
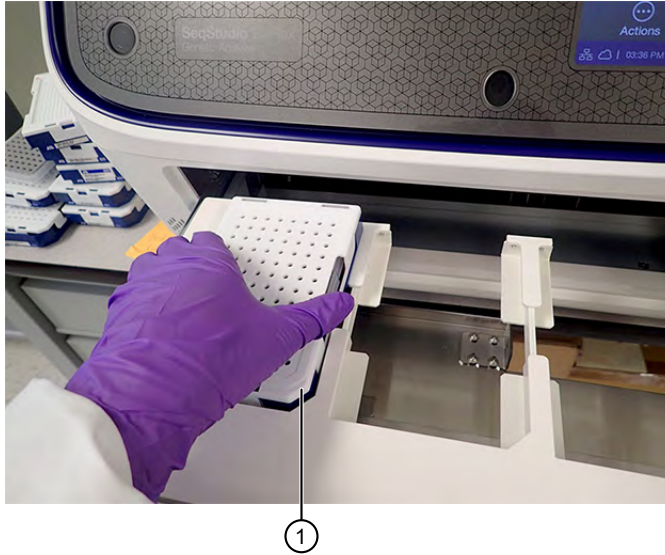
① Notched corner of plate and plate holder in instrument

The position status in the home screen changes to **Link plate file**.

4. Push the drawer closed.

   IMPORTANT! To avoid injury, keep your hands away from any empty spaces in the drawer when you push the drawer closed.

Link a plate file and start a run

1. In the home screen, tap **Link a plate file** in the plate position for the loaded plate.

2. In the **Link Plate File** screen, tap an option, then select the plate file you created earlier.
   If you created the plate file in the Plate Manager software, tap **USB drive** or **Network drive**.
   If you created the plate file on the instrument, tap **My instrument**.
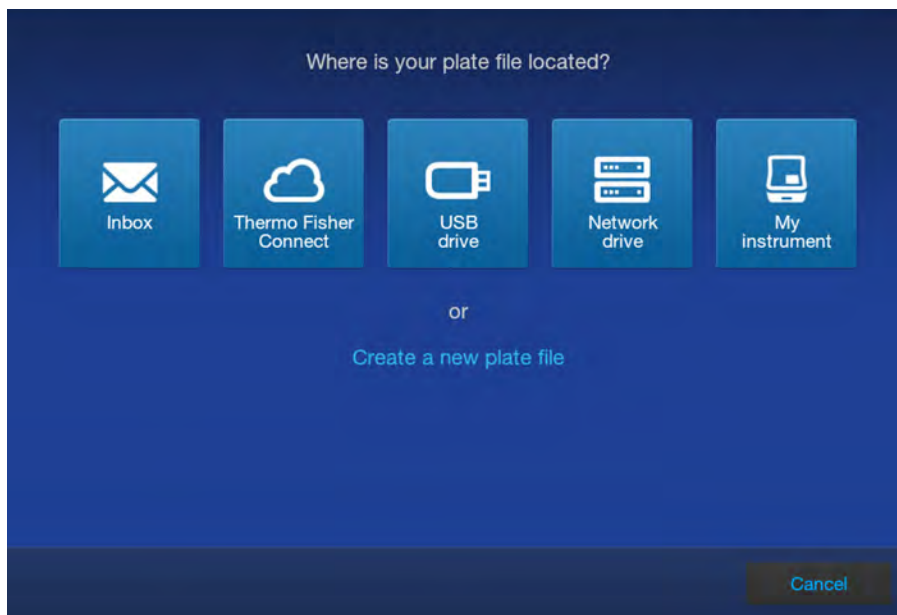
3. Tap **Start run**.

Monitor a run in the home screen

1. In the home screen, tap a running plate position.
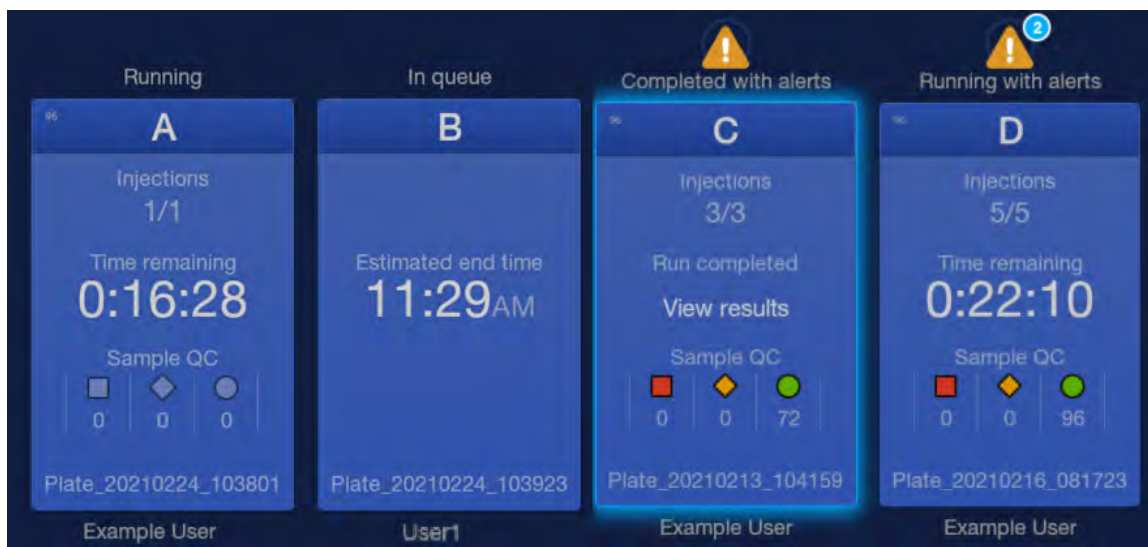
The plate view is displayed.
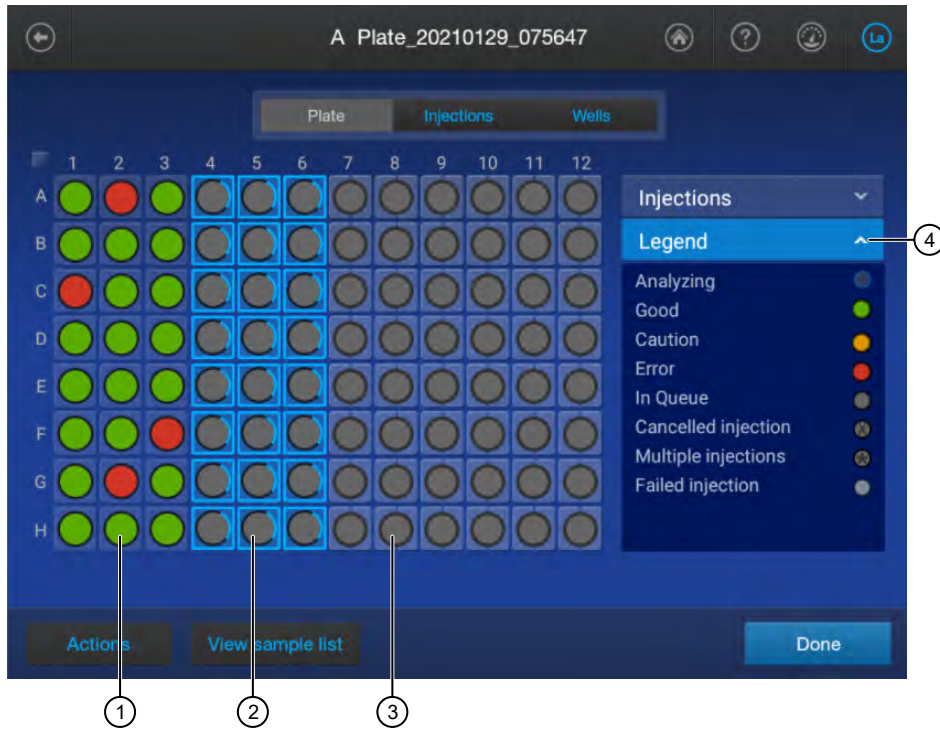
① **Completed** injection
② **Running** injection
③ **In queue** injection
④ Tap to display the plate legend

2. *(Optional)* Tap the **Injections** tab.

3. Tap an injection to display the electropherogram screen.

4. Tap **Done** in the electropherogram screen, then tap the **Wells** tab.

   If needed, swipe up to view all wells.

5. Tap 🛈 **(Information)** to display the details for the well.

   If needed, swipe up to view all wells.

Top of the well information screen.

Bottom of the well information screen.

Monitor the run in the Run queue screen

1. In the home screen, tap ☰ **Run queue**.

2. In the **Run Queue** screen, select an injection or a completed plate with one injection only, then tap **View Plate**.

   The **Run Details** electropherogram view or the plate view is displayed.

View results for a completed run (Run history)

1. In the home screen, tap ⊙ **Actions** ‣ **Run history**.

2. Tap a plate, then tap **View**.

   The results for the plate are displayed.

3. Do any of the following as needed.

   • Tap a well, then tap **View** to view the raw, EPT, or analyzed electropherogram for a well.

   • Tap 🛈 **(Information)** to display the details for the well.

   • Tap 🝖 **(Filter)**, then enter a keyword such as the well number to filter the results (by **Well ID** and **Sample name** attributes) that are listed.

   • Tap the **Result group** tab to list the wells by results group.

   • Tap a well, then tap **Actions**, then select an option to export the results or the run log.

Analysis settings overview

Analysis settings define the settings for peak detection and sizing.

Create a new analysis settings

1. In the home screen, tap ⊙ **Actions ▸ Library ▸ Analysis settings**.
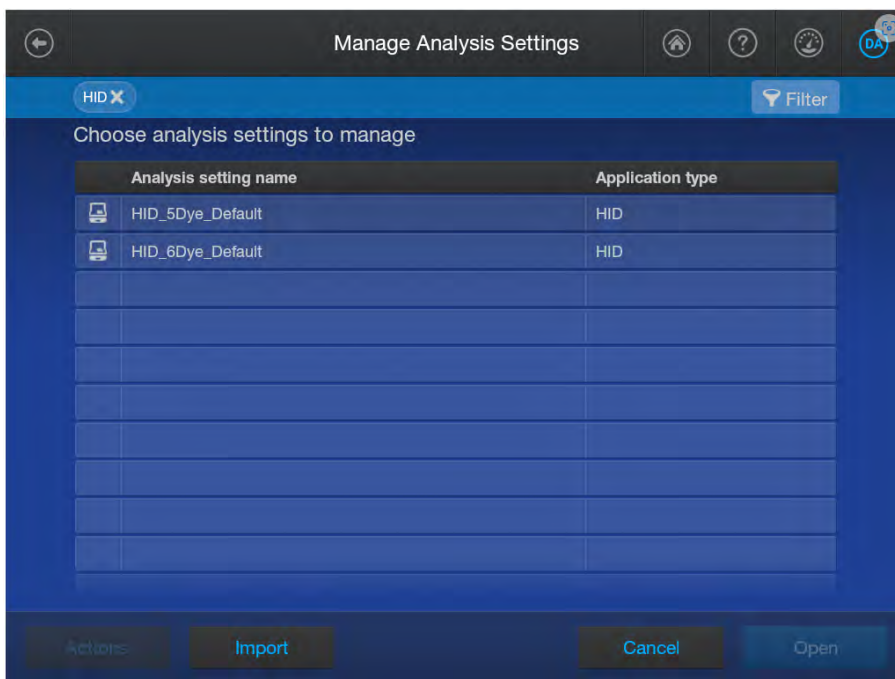
2. Tap a default analysis setting for your application, tap **Open**, then tap **Copy**.

3. Enter a name for the analysis settings, then tap **Enter**.

4. Tap sections of the screen and modify settings as needed.
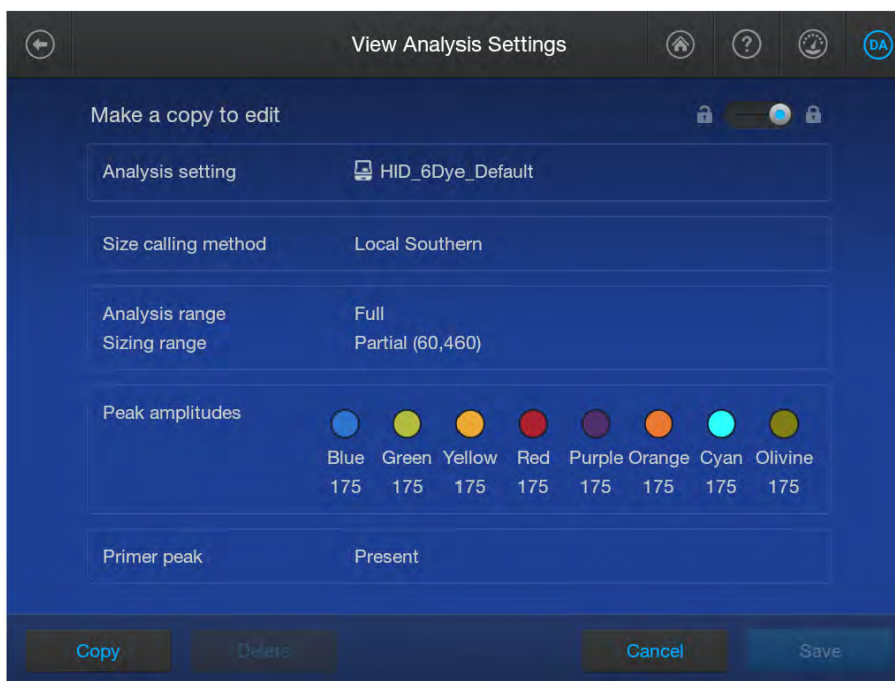
IMPORTANT! Swipe up to see additional settings.

---
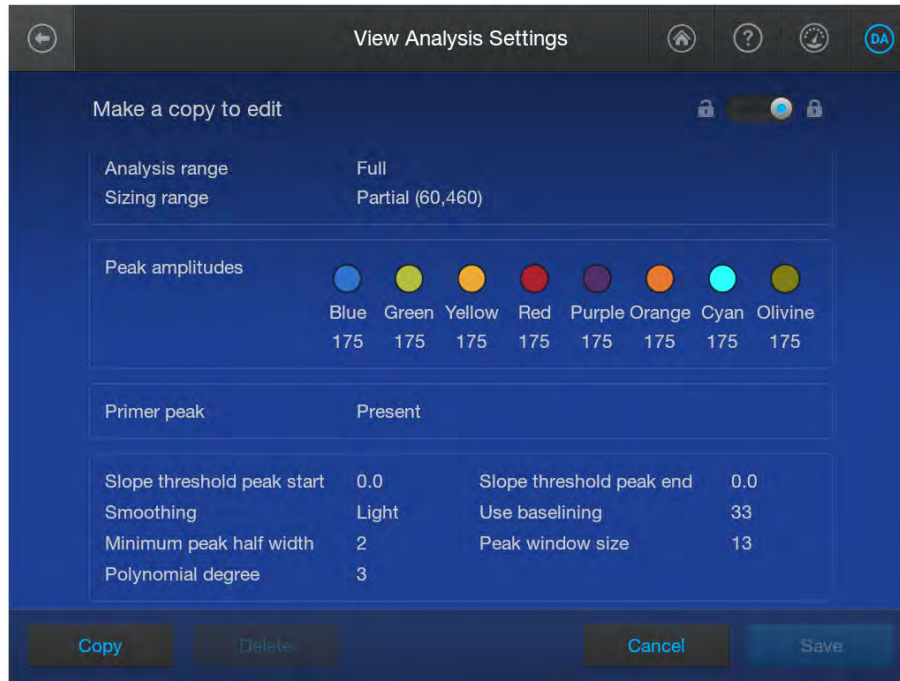
5. Tap **Save**.

3 Run calibrations and install checks

Run a spatial calibration

Spatial calibration overview

A spatial calibration associates the signal from each capillary to a specific position on the CCD camera.

When to perform a spatial calibration

Perform a spatial calibration if you perform any of the following actions:

- Re-install a capillary array
- Replace a capillary array when it expires
- Move the instrument
- Open the detection cell heater door or move the detection cell

Note: If you perform any of the first two actions, the software requires you to run a spatial calibration before you perform a regular run. If you open the detection cell heater door and move the detection cell, the software does not detect that the capillary array is no longer calibrated. It is critical to run a spatial calibration before performing a regular run if you have opened the detection cell heater door or moved the detection cell.

Note: If you use a maintenance wizard to change the capillary array, a spatial calibration is automatically performed during the procedure.

Perform a spatial calibration

A spatial calibration is automatically performed when you use maintenance wizards to install a capillary array.

This section describes how to manually perform a spatial calibration if you interact with the capillary array without using a wizard (for example, if you move the instrument or open the detection cell heater door). You can alternatively run the spatial calibration maintenance wizard.

1. From the home screen, tap ⊙ **Actions ▸ Maintenance ▸ Calibration ▸ Spatial calibration**.

2. Use the default **Fill** setting to fill the capillary array with polymer before starting the calibration.

   Filling the capillary array ensures that no residual fragments are present in the capillary array that can interfere with the spatial calibration and signal optimization.

   Note: If you have previously run a spatial calibration and know that the capillary array is filled with fresh polymer, you can select **No Fill**.

   ---

3. Tap **Recalibrate**.

   Note: If a spatial calibration has not previously been performed for the installed capillary array, the button displays **Start calibration**.

When the spatial calibration is complete, the **Spatial Calibration** screen is displayed.
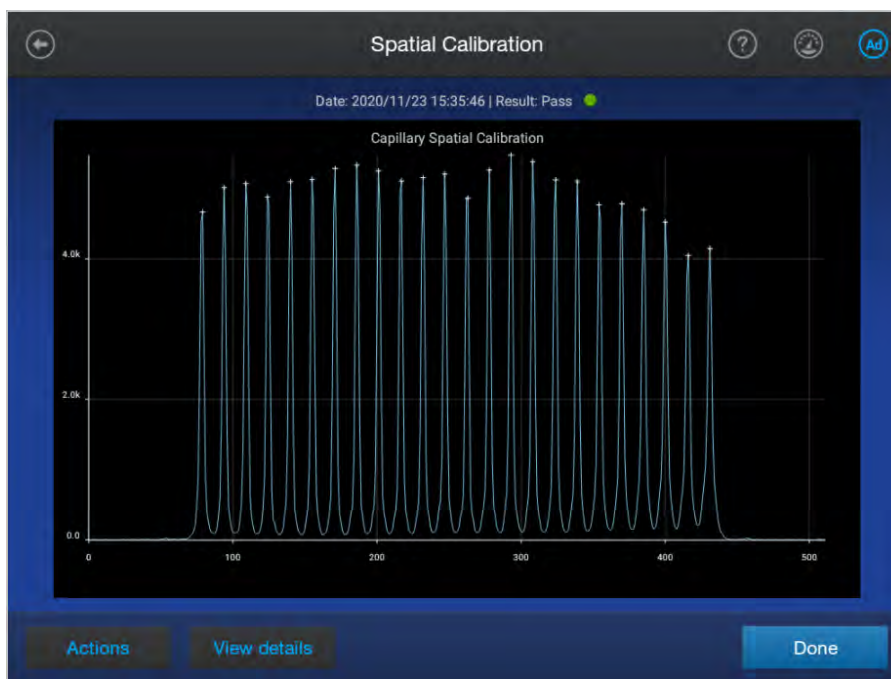
Evaluate the spatial calibration results

1. Note the pass/fail status of the calibration at the top of the screen.

   A **Pass** 🟢 status is required before you can run a spectral calibration.

① Pass/fail result
② Capillary CCD pixel marker
③ Tap for more results

2. Evaluate the spatial calibration profile. Ensure that you see the following:

   • One sharp peak for each capillary. Small shoulders are acceptable.

   • One marker (+) at the apex of every peak. No off-apex markers.

   • An even peak profile (all peaks about the same height).

3. Tap **View details** to view the results for each capillary.

Attribute	Calculation	Threshold
Average peak height	(sum of all peak heights) divided by (number of peaks)	• 8-cap: ≥6,400 RFU • 24-cap: ≥2,500 RFU
Uniformity (peak height similarity)	(standard deviation) divided by (average peak height)	≤0.2
Spacing (capillary spacing)	Maximum spacing – minimum spacing	≤2 pixels
Intensity (individual peak height)	Peak height	1,000 RFU
Signal optimization factor	Described below	—

Analysis on 24-capillary instruments only: A signal optimization factor is calculated for each capillary during spatial calibration. The factor for each capillary is applied during data collection to minimize optical variation effects and to increase signal uniformity between capillaries.

Note: The signal optimization factor is calculated using a fitted curve method. The fitted curve method minimizes background and reduces noise. The adjusted spatial intensity, not the spatial intensity displayed for the capillary, is used to calculate the signal optimization factor.

4. Tap **Done**.

Export spatial calibration results or report

This statement appears in the footer of spatial calibration reports with SeqStudio™ Flex Series Instrument Software v1.1.1: *When used for purposes other than Human Identification the instruments cited are for Research Use Only. Not for use in diagnostic procedures.*

Note: Spatial and spectral calibration reports show the date of first installation of the capillary array. Install run reports show the date of the most recent installation of a capillary array.

1. In the **Spatial calibration** screen, tap **Actions**.

2. Tap the item to export:

   • **Results** (ZIP)—Contains the spatial calibration run file (CID), log files, and information that can be used for troubleshooting.

   Results file name format: `<InstrumentName>_<ArraySerialNumber>_<RunDate>_<Timestamp>.zip`, for example: Instrument1_M323A0C03_20201021_121952.zip

   • **Report** (PDF)—Contains the results of the spatial calibration for your records.

   Report file name format: `<InstrumentName>_<ArraySerialNumber>_<RunDate>_<Timestamp>.pdf`, for example: Instrument1_M323A0C03_20201021_121952.pdf

3. Select the export location, then tap **Next**.

4. When the export is complete, click **Close** to close the message box.

Run a spectral calibration

Overview of spectral calibration

During data collection, the instrument detects the signal of fluorescent dyes that are attached to DNA amplicons. Although each dye emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes. A spectral calibration creates a reference deconvolution matrix (also referred to as a dye matrix) that corrects for the overlapping fluorescence emission spectra of the dyes. A deconvolution matrix is created for each capillary in a capillary array. A dye matrix is created when you run the manual spectral calibration function. The dye matrix is then optimized and updated by the auto-spectral calibration function, which is enabled by default and performed automatically for each capillary during each injection.

When you create a custom dye set, the auto-spectral calibration setting can be disabled if needed. For runs using a custom dye set without the auto-spectral calibration, the instrument applies the manual spectral calibration to the sample data.

In the auto-spectral calibration process, the instrument uses spectral data from the samples to optimize and update the dye matrix from the manual calibration, which generates an auto-spectral dye matrix. This feature optimally reduces pull-up (false secondary peaks under a true peak) that can be caused by changes in the spectral characteristics from the original manual calibration dye matrix. The instrument performs an auto-spectral calibration for factory-provided and custom dyes.

For later runs, one of the following occurs:

- The run uses its own auto-spectral dye matrix.
- The run borrows from the previous auto-spectral dye matrix or from the manual calibration if no auto-spectral dye matrix has been made.

When to perform a manual spectral calibration

Perform a spectral calibration if you do any of the following actions:

- Use a dye set/polymer combination that you have not previously calibrated
- Use a custom dye set (a dye set that is not available from Thermo Fisher Scientific) or a dye set definition that is not factory-provided
- After you enable or disable off-scale recovery
- After you disable auto-spectral calibration
- Observe a decrease in spectral separation (pull-up/pull-down in peaks) in the raw or analyzed data
- After performance of maintenance by Service that affects the optical systems
- After the instrument is moved
- You frequently run samples for which auto-spectral calibration cannot be performed (these samples generate a "Calibration from manual..." calibration status)

Under most conditions, manual spectral calibrations are not needed after the capillary array is changed because of the auto-spectral calibration feature. If an auto-spectral calibration cannot be performed and no saved auto-spectral calibration is available for the capillary position, the most recent manual spectral calibration is applied to the samples. When routinely running samples for which auto-spectral calibration cannot be performed, it is important to perform a manual spectral calibration after you change the capillary array or move the instrument, to provide a valid manual calibration for samples.

System dye sets versus custom dye sets

System dye sets are available from Thermo Fisher Scientific:

- G5 (DS-33)
- J6 (DS-36)
- J6-T (DS-37)

Custom dye sets are any dyes that are not available from Thermo Fisher Scientific or any user-created dye set definitions. For example, if you open a factory-provided dye set and make a copy of it, the copy is considered a custom dye set.

Estimated spectral calibration run times

The run time for a POP-4™ polymer (36 cm) is approximately 28 minutes. A 50-cm configuration is not supported for HID.

Note: The run time does not include the time for the oven to preheat.

Prepare for spectral calibration

Prepare the spectral calibration standard

For the instructions to prepare the standard, see the product information sheet. To find the product information sheet, go to **www.thermofisher.com**, search for the product, then scroll to the bottom of the product page.

Prepare the matrix standard appropriate for your application as described in the product insert.

Name	Cat. No.
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
DS-37 Matrix Standard Kit (Dye set J6-T)	A31234

Perform a spectral calibration

Note: The first time you perform a spectral calibration (required for each dye set/polymer configuration), you may notice pull-down peaks (or mirror image peaks). While the run is in progress, these pull-down peaks will eventually correct themselves. When the run is complete, the pull-down peaks disappear.

1. Load the calibration plate in the instrument.

2. In the home screen, tap ⊙ **Actions** ▸ **Maintenance** ▸ **Calibration** ▸ **Spectral Calibration**.

3. Tap the screen to select the plate type you are using for calibration.

3

4. Tap the injection group for the dye set in the plate, then tap **Dye set**.
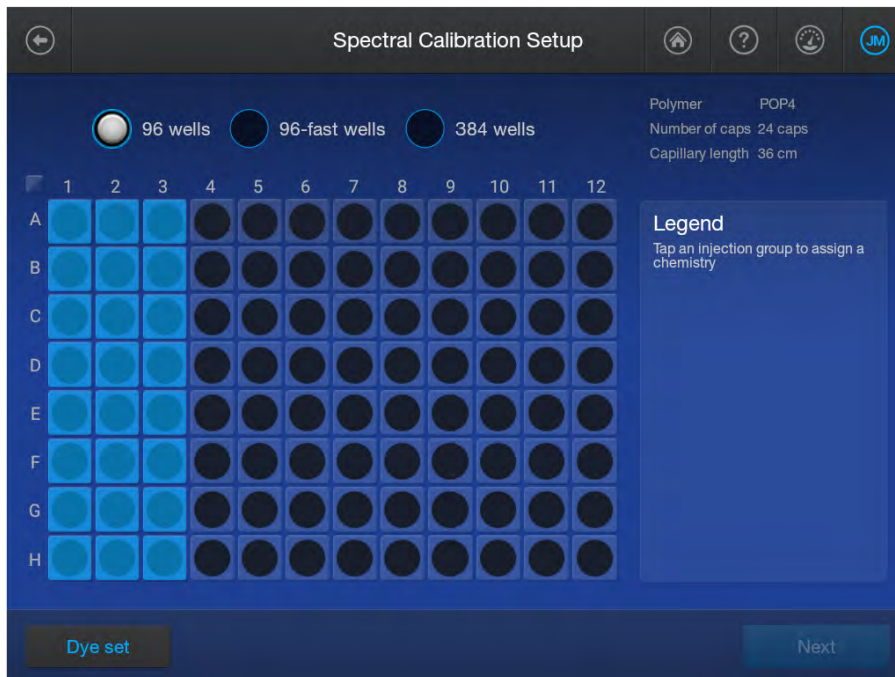
5. Tap **Matrix**, then tap the appropriate calibration standard.

   IMPORTANT! The **Matrix** tab for HID analysis is displayed by default.
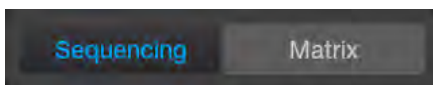
6. Tap **Next**.
   The home screen is displayed with capillary information sharing settings.

7. Select capillary information sharing settings as needed:
   - **Allow borrowing**—Determines whether the software can use spectral calibration data from an adjacent capillary if the spectral calibration from a capillary does not meet the quality thresholds. If Auto Retry is also enabled, spectral calibration information can also be borrowed from the additional runs that can be performed.
   - **Auto Retry**—Determines whether up to 3 calibration runs are performed if the first calibration run fails.

8. Tap the plate position that corresponds to the location of the spectral calibration plate in the instrument.

9. Tap **Start calibration**.
   The home screen is displayed. The status of the spectral calibration is shown in the appropriate plate position. The spectral calibration run is also listed in the **Run queue**.

When the spectral calibration finishes, the status changes to **Completed** in the home screen and the **Run queue**.

View spectral calibration results

1. When the spectral calibration is complete, tap the plate position in the home screen or in the **Run Queue** screen.
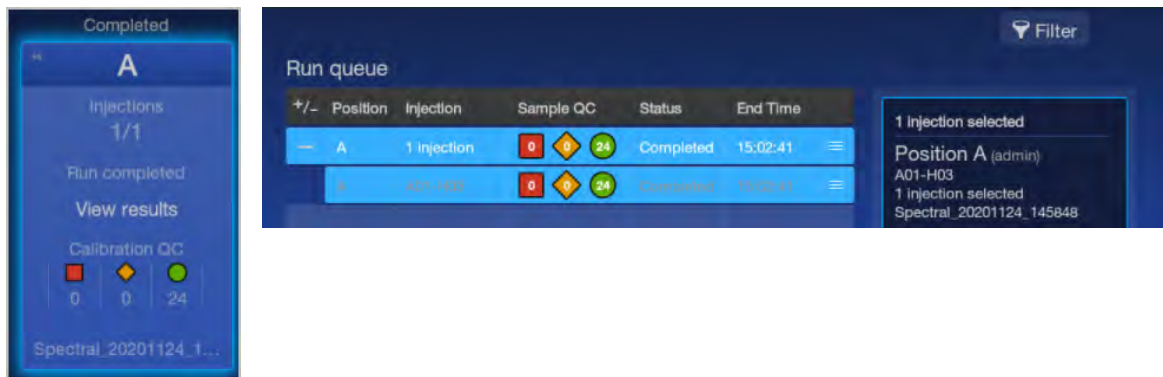
Note: If data for a capillary is borrowed, it is listed in the ◆ **(caution)** category.

Example of **Calibration QC** with a borrowed capillary:

2. In the **Run Details** screen, tap the spectral calibration of interest.

The **Spectral Calibration** electropherogram screen is displayed.

The thumbnail pane is at the far left of the screen. Press-drag the pane to the right until the calibration spectra are displayed.
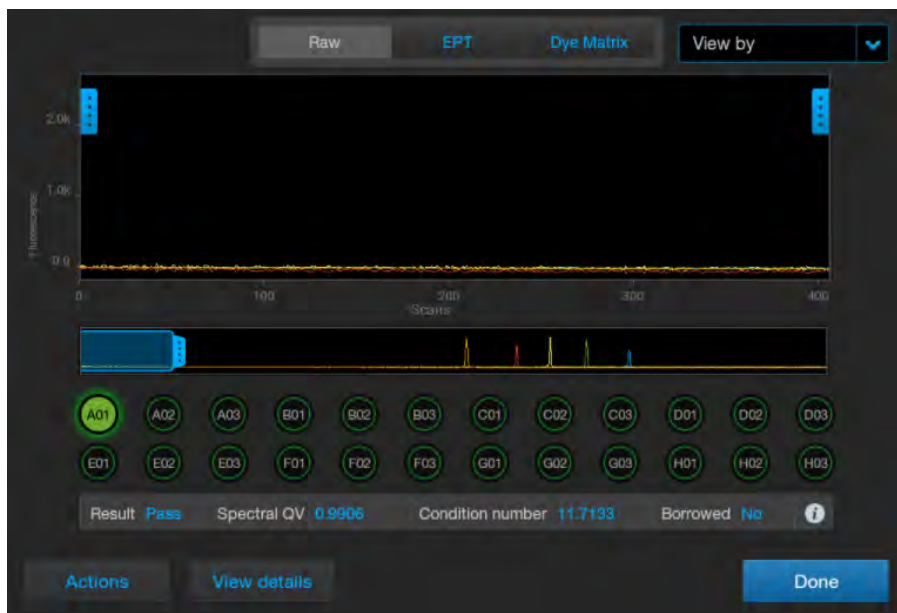
Note: Wells with borrowed spectral calibration are flagged in yellow (caution).
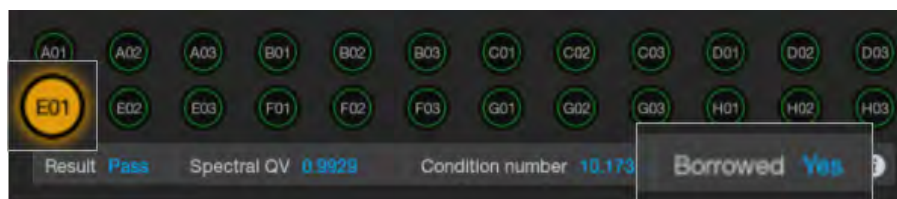
In this screen, you can:

- Tap a well to view the results for the well. The results for the well are displayed below the well positions.
- Tap 🛈 to display the file name for the well.
- Tap the **Raw**, **EPT**, or **Dye Matrix** tab to display different views of the data.
- Tap **Actions**, then tap **Pause instrument**, **Export logs**, or **Export report**.
- Tap **View details** to display the details for each well.

3. Tap **View details** to display the details for each capillary.
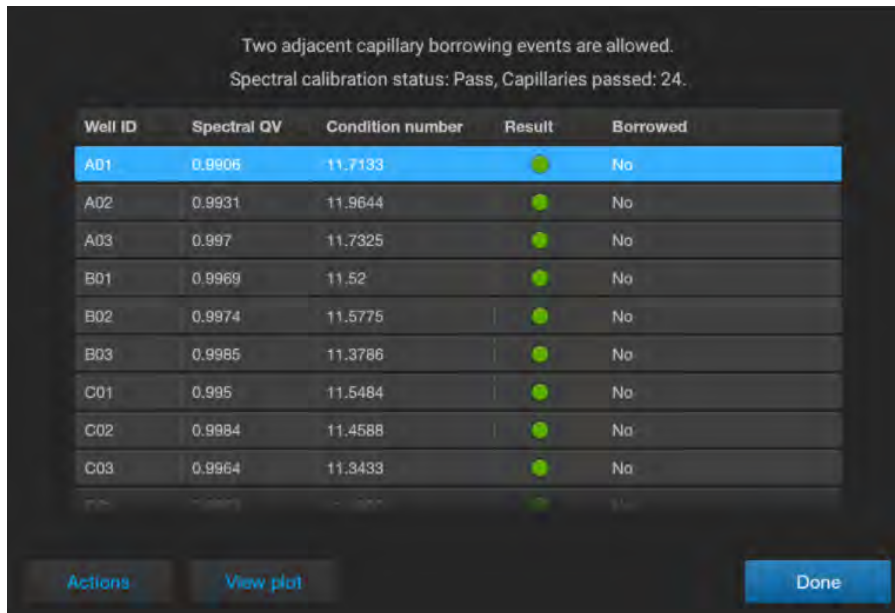
Spectral QV (quality value) and Condition number definitions and limits

Spectral QV

A **Spectral QV** (quality value) reflects the confidence that the individual dye emission signals can be separated from the overall measured fluorescence signal. It is a measure of the consistency between the final matrix and the data from which it was computed. A **Spectral QV** of 1.0 indicates high consistency, providing an ideal matrix with no detected pull-up/pull-down peaks.

In rare cases, a high **Spectral QV** can be computed for a poor matrix. This can happen if the matrix standard contains artifacts, leading to the creation of one or more extra peaks. The extra peaks cause the true dye peak to be missed by the algorithm, and can lead to a higher **Spectral QV** than would be computed with the correct peak.

Condition number

A **Condition number** indicates the amount of overlap between the dye peaks in the fluorescence emission spectra of the dyes in the dye set.

If there is no overlap in a dye set, the **Condition number** is 1.0 (ideal conditions), the lowest possible value. The condition number increases with increasing peak overlap.

Spectral QV and Condition number limits

Dye set	Minimum spectral QV	Matrix condition number upper limit
G5 (DS-33)	0.95	13.5
J6 (DS-36)	0.95	8.0
J6-T (DS-37)	0.95	8.0

Export spectral calibration logs or report

This statement appears in the footer of spectral calibration reports with SeqStudio™ Flex Series Instrument Software v1.1.1: *When used for purposes other than Human Identification the instruments cited are for Research Use Only. Not for use in diagnostic procedures.*

Note: Spatial and spectral calibration reports show the date of first installation of the capillary array. Install run reports show the date of the most recent installation of a capillary array.

Note: You can also export these items from the **Spectral calibration history** option.

1. In the **Spectral Calibration** plot screen or the results per capillary screen, tap **Actions**.

2. Tap the item to export:

   - **Logs** (ZIP)—Contains the spectral calibration report (PDF), data files (FSA or AB1), spectral calibration run file (CID), and log files that can be used for troubleshooting.

     Log file name format: `<InstrumentName>_<DyeSet>_<RunDate>_<Timestamp>.zip`, for example: Instrument1_J6 (DS-36)_20230220_202403.zip

   - **Report** (PDF)—**QC report** Contains the results of the spectral calibration for your records.

     Report file name format: `<InstrumentName>_<DyeSet>_<RunDate>_<Timestamp>.pdf`, for example: Instrument1_J6 (DS-36)_20230220_202403.pdf

3. Select the export location, then tap **Next**.
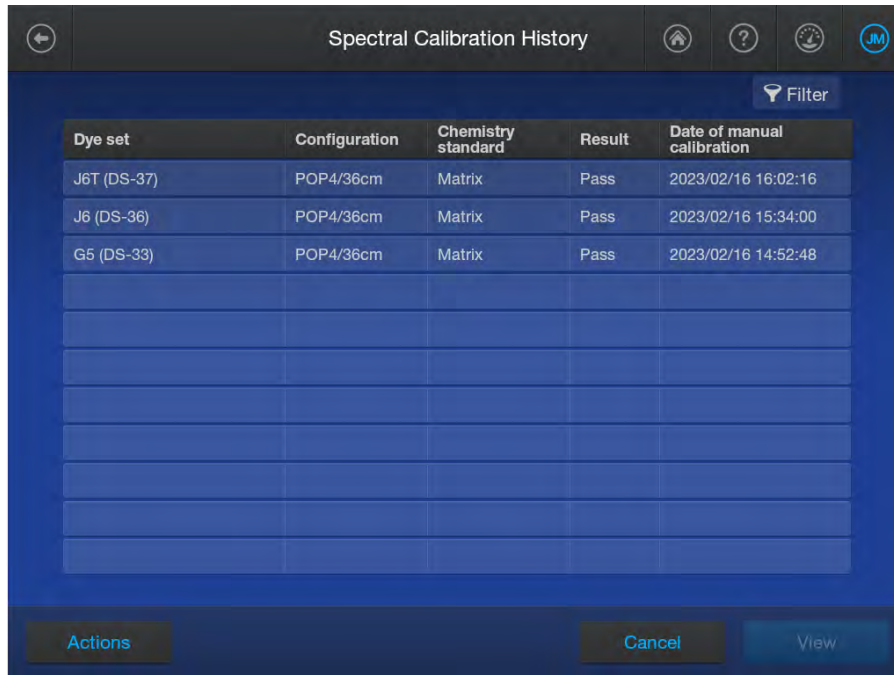
   Note: It can take up to 15 seconds for the instrument to recognize a USB drive after the drive is inserted in a USB port. exFAT-formatted USB drives are recommended.

4. When the export is complete, click **Close** to close the message box.

View and export the spectral calibration history

This statement appears in the footer of spectral calibration reports with SeqStudio™ Flex Series Instrument Software v1.1.1: *When used for purposes other than Human Identification the instruments cited are for Research Use Only. Not for use in diagnostic procedures.*

1. In the home screen, tap ⊙ **Actions ▸ Maintenance ▸ Calibration ▸ Spectral calibration history**.

Dye set	Configuration	Chemistry standard	Result	Date of manual calibration
J6T (DS-37)	POP4/36cm	Matrix	Pass	2023/02/16 16:02:16
J6 (DS-36)	POP4/36cm	Matrix	Pass	2023/02/16 15:34:00
G5 (DS-33)	POP4/36cm	Matrix	Pass	2023/02/16 14:52:48

Spectral Calibration History — Filter — Actions — Cancel — View

2. *(Optional)* Tap ▽ **Filter** to filter the list by polymer, result, and/or dye set.

3. To export the list of calibrations that have been run on the instrument, tap **Actions ▸ Export report**.

   The spectral calibration history report contains the results of all spectral calibrations performed on the instrument.

   Report file name format: `<InstrumentName>_SpectralHistoryReport_ <ReportGenerationDate>_<Timestamp>.pdf`, for example: Instrument1_SpectralHistoryReport_20201124_162247.pdf

4. To view the details of an individual spectral calibration, tap a dye set, then tap **View**.
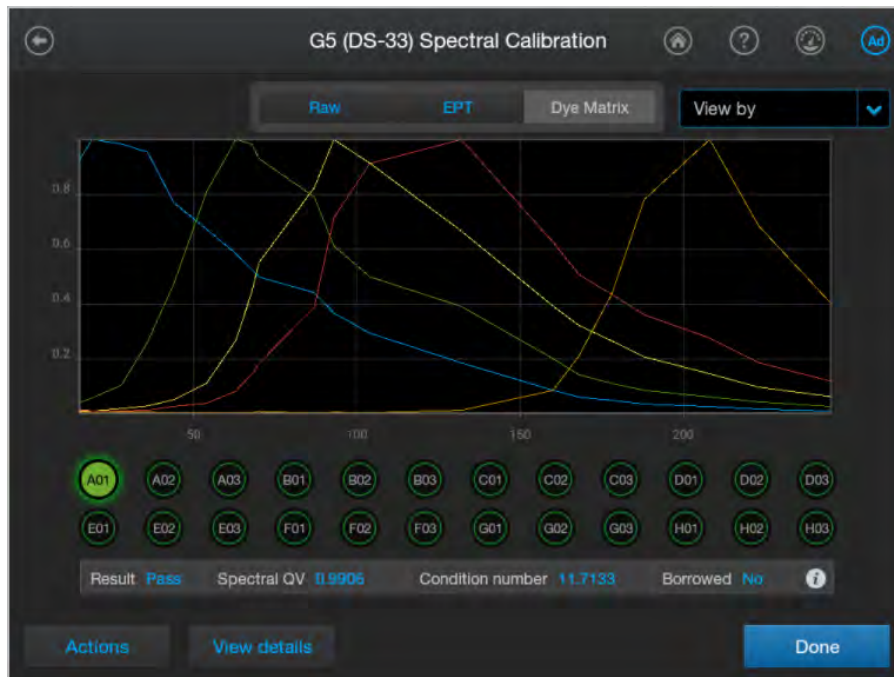
5. To export the log or report for the individual spectral calibration, tap **Actions**, then tap the item to export (these options generate the same files you can generate at the end of a spectral calibration):

- **Logs** (ZIP)—Contains the spectral calibration report (PDF), data files (FSA or AB1), spectral calibration run file (CID), and log files that can be used for troubleshooting.

  Log file name format: `<InstrumentName>_<DyeSet>_<RunDate>_<Timestamp>.zip`, for example: Instrument1_J6 (DS-36)_20230220_202403.zip

- **Report** (PDF)—Contains the results of the spectral calibration for your records.

  Report file name format: `<InstrumentName>_<DyeSet>_<RunDate>_<Timestamp>.pdf`, for example: Instrument1_J6 (DS-36)_20230220_202403.pdf

Perform an install run

Overview of install run

Install runs ensure that the instrument meets specifications for HID analysis. For more information, see "Pass/fail criteria and results for HID install checks" on page 50.
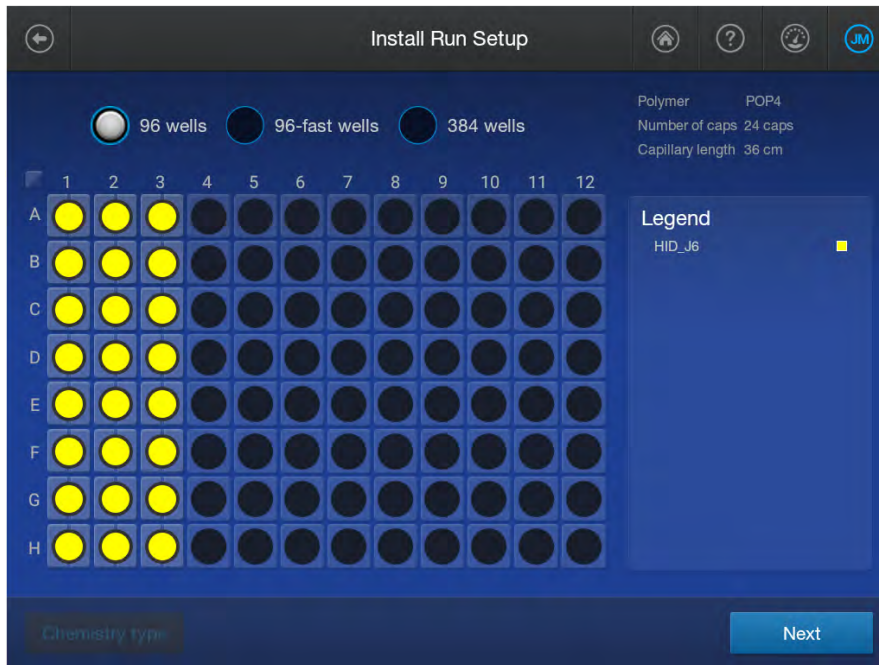
Figure 1   Example install run plate

When to perform an install run

You can perform an install run under the following conditions:

- If at least one install run for HID analysis is not present in the **Install Run History** list. This can occur if the install run for the application type has never been performed, or if all install runs for the application type have been deleted from the instrument.

- At any time to verify that the instrument meets specifications.

Estimated install run times

The run time for a POP-4™ polymer (36 cm) is approximately 37 minutes. A 50-cm configuration is not supported for HID.

The number of capillaries on the instrument does not affect run time.

Prepare for the install run

Prepare the instrument

1. Display the consumables dashboard by tapping ⊙ **(Dashboard)** at the top right of the home screen (if the consumables dashboard is not displayed, tap ❮ or ❯).

2. Check the consumables status.

    • Ensure the consumables are not expired.

    • Ensure that adequate injections remain for consumables.

    IMPORTANT! For install runs, use polymer that has been on the instrument ≤48 hours. Slight peak shifts can occur with polymer that has been on the instrument >48 hours.

    ---

3. Ensure that buffer levels are at the fill lines.

4. Check the oven and detection cell temperature to ensure that they are at the correct temperature.

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

Prepare the HID install run standard

Prepare the appropriate standard according to the procedure in the product information sheet.

The following reagents are required:

• 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)

Note: A manual calibration for the J6 dye is required before the install check.

Prepare the standard plate

IMPORTANT! Do not use warped or damaged plates.

---

1. Load the standards in any injection position in the plate.

   The example below shows injection position 1, but you can specify the starting well for an injection position.

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

2. Briefly centrifuge the plate that contains the standards.

3. Verify that each standard is positioned correctly in the bottom of its well.

   IMPORTANT! If the contents of any well contain bubbles or are not located at the bottom of the well, briefly centrifuge the plate, remove the plate from the centrifuge, and verify that each standard is positioned correctly in the bottom of its well.

   ---
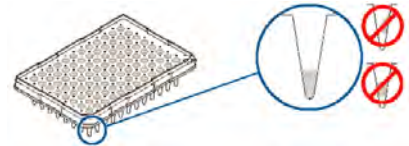
4. Store the plate on ice until you prepare the plate assembly and load the plate in the instrument.

5. Prepare the plate assembly as described in "Prepare the samples, plate, and plate assembly" on page 17.

Perform an install run

IMPORTANT! Ensure that polymer has been installed for <48 hours before you run a HID install check. If you use older polymer, the install run may fail because allele peaks may not align with the expected system-specified bins.

---

1. Load the install run plate in the instrument.

2. In the home screen, tap ⊙ **Actions ▸ Maintenance ▸ Install run ▸ Install run**.

3. Tap the screen to select the plate type you are using for the install run.

4. Tap the injection group for the install standard in the plate, then tap **Chemistry type**.
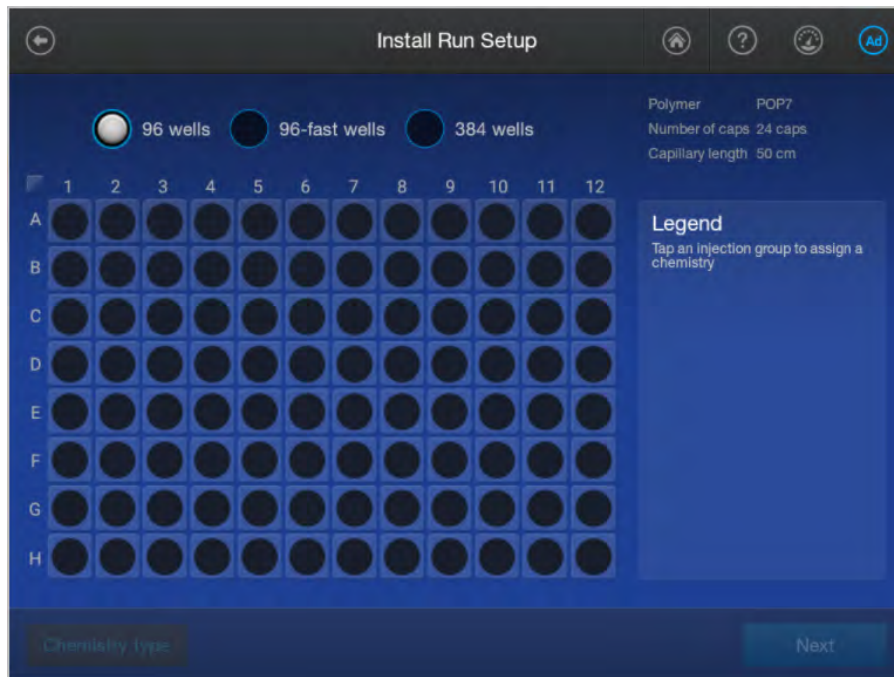
5. Tap the **HID_J6** for install run.

6. Tap **Next**.
   The home screen is displayed with capillary information sharing settings.
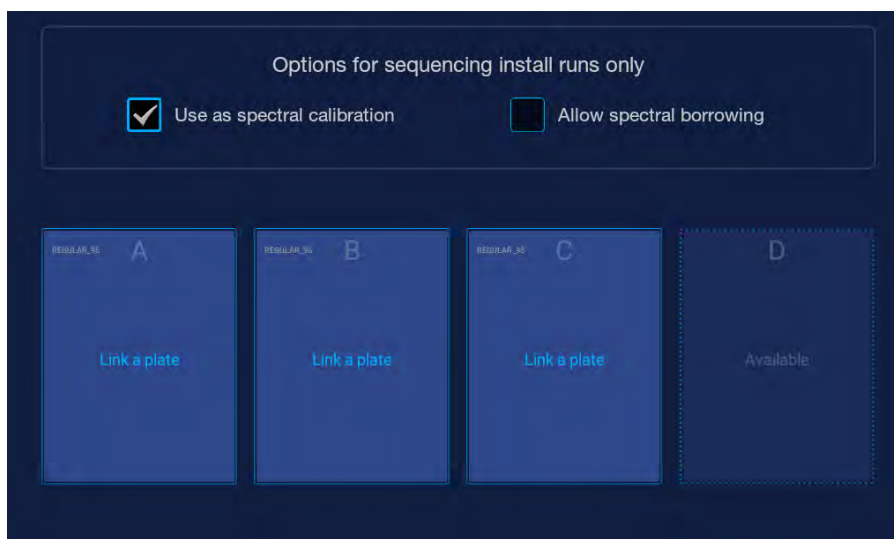
7. Tap the plate position that corresponds to the location of the plate in the instrument.

8. Tap **Start Run**.
   The home screen is displayed and shows the status of the install run in the appropriate plate position. The install run is also listed in the **Run queue**.
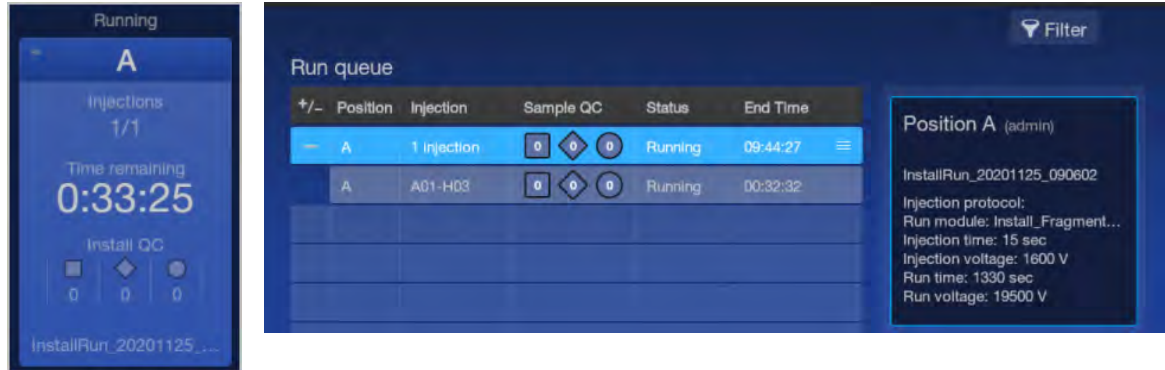
When the install run is complete, the home screen and the **Run queue** status displays **Completed**.

View HID analysis install run results

1. When the install run is complete, tap the plate position in the home screen or in the **Run queue**.
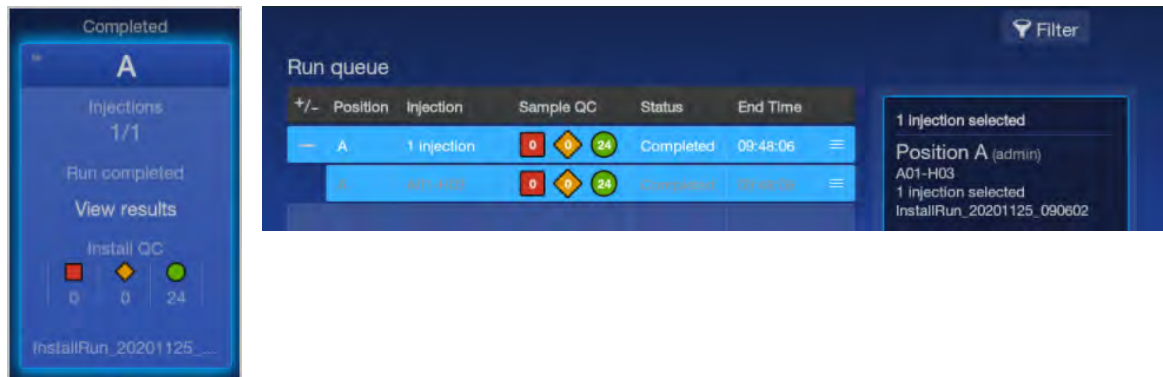
2. In the **Run Details** screen, tap the install run of interest.

   The install run results screen is displayed with summary results.
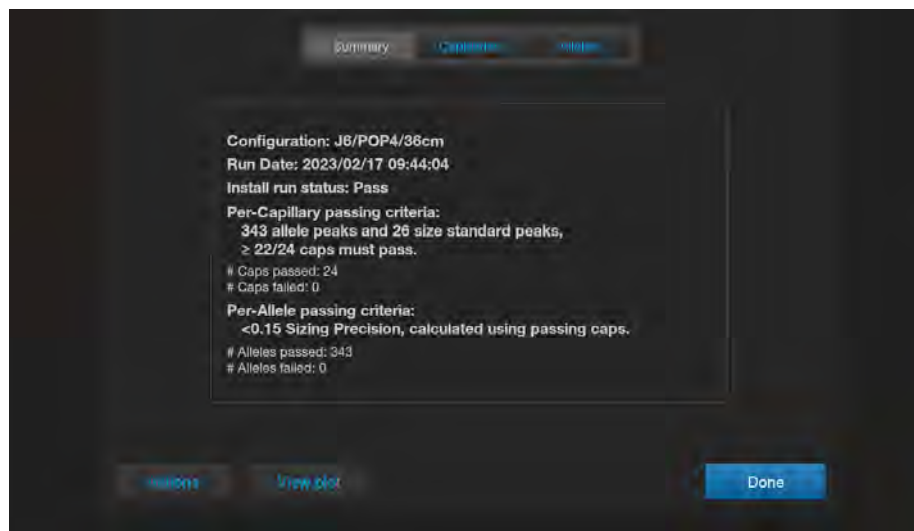
3. Tap **Capillaries** to view results for each capillary. Tap 🛈 **(Information)** to display the **Sizing quality** and other information for a capillary.

4. Tap **Alleles** to display the sizing information for alleles.

For a description of results, see "Pass/fail criteria and results for HID install checks" .

IMPORTANT! If fewer than 343 alleles per capillary are identified, ensure that polymer has been installed on the instrument for <48 hours. Older polymer can cause the fragment install run to fail.

5. Tap **View plot** to display the plot for each capillary (**Raw** and **Analyzed** tabs are shown below).
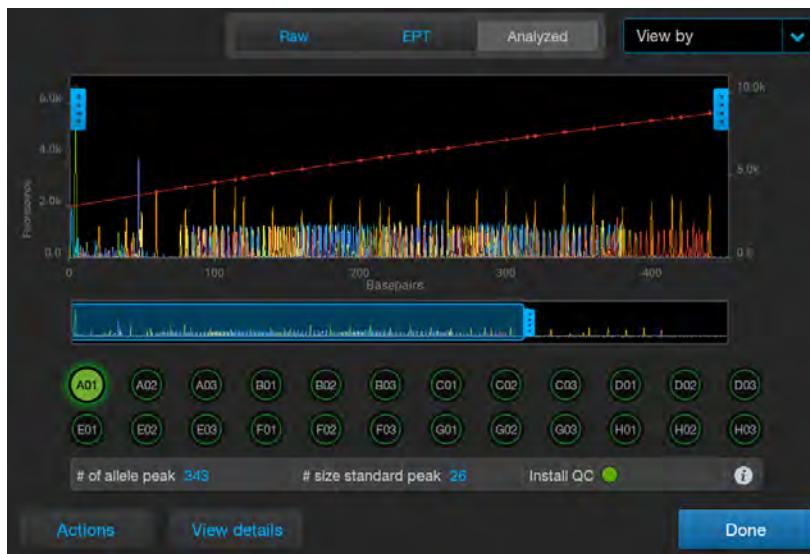
The red line in the plot is the sizing curve. The install run uses the **Local Southern** sizing method. You can tap **View by** to filter results by Pass (🟢) or Flagged (🟡 or 🔴).

Pass/fail criteria and results for HID install checks

The HID install run passes if both the capillary passing criteria and the allele passing criteria are met. The HID install check passing criteria includes the following:

- The required number of capillaries must pass the per-capillary passing criteria (see "Per-capillary passing criteria" on page 50).

- All alleles in the passing capillaries must pass the per-allele passing criteria (see "Per-allele passing criteria" on page 51).

Per-capillary passing criteria

The per-capillary passing criteria includes the following:

- Alleles are within their expected size range.

- The required number of allele peaks and size standard peaks must be present (see Table 1).

- The required number of capillaries must pass per injection (see Table 2).

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

- All markers except D1S1656, D12S391, D2S441, and TH01: ±0.7 bp of nominal size for the allele
- D1S1656
  - Ten markers are ±0.7 bp of the nominal size for the allele
  - Six markers are ±0.5 bp of the nominal size for the allele
- D12S391
  - Thirteen markers are ±0.7 bp of the nominal size for the allele
  - Two markers are ±0.5 bp of the nominal size for the allele
- D2S441
  - Nine markers are ±0.7 bp of the nominal size for the allele
  - Two markers are ±0.5 bp of the nominal size for the allele
- TH01:
  - Seven markers are ±0.7 bp of nominal size for the allele
  - Three markers are ±0.5 bp of nominal size for the allele

Table 1   Required number of allele peaks and size standard peaks

Result	Acceptance criteria
# Allele peaks	343 per capillary
# Size standard peaks	26 per capillary

Table 2   Required number of capillaries that must pass

Instrument type	Number of capillaries required to pass
24-capillary instrument	≥22 out of 24 capillaries
8-capillary instrument	≥7 out of 8 capillaries

Per-allele passing criteria

Only alleles from passing capillaries are used to calculate the **Average Peak Height** and the **Sizing Precision** passing criteria. For the install run to pass, all alleles in the passing capillaries must pass the per-allele passing criteria.

Table 3   Per-allele passing criteria

Result	Threshold passing criteria
Average peak height	>400 RFU
Sizing precision (standard deviation of the observed allele fragment sizes for all capillaries)	<0.15 base pairs (bp)

The following information is included in an exported install report.

Table 4   Install report

Result	Description	Threshold
Min Peak Height	Minimum of peak heights for observed allele peaks of the included capillaries.	>175 RFU
Sizing precision	Standard deviation per allele of the observed allele fragment sizes for all capillaries	<0.15 for expected alleles
Pass/Fail	Alleles with a sizing precision and required peak height that do not meet thresholds fail.	
For information only		
Nominal Size	Expected allele fragment peak size (bp).	
Mean size	Average fragment size for the observed allele peaks.	
Mean peak height	Average peak height for the observed allele peaks.	
Peak Height % >Min	Percentage of observed allele peaks with a peak height above the minimum threshold.	
Sizing Precision	Standard deviation of the observed allele fragment sizes vs the expected fragment sizes.	
Sizing Accuracy	Difference between the expected allele size and the mean allele size.	

View, export, or delete an HID install run history

This statement appears in the footer of HID install run reports with SeqStudio™ Flex Series Instrument Software v1.1.1: *When used for purposes other than Human Identification the instruments cited are for Research Use Only. Not for use in diagnostic procedures.*

Note: Spatial and spectral calibration reports show the date of first installation of the capillary array. Install run reports show the date of the most recent installation of a capillary array.

1. In the home screen, tap ⊙ **Actions ▸ Maintenance ▸ Install run ▸ Install run history**.

2. *(Optional)* Tap 🔽 **Filter** to filter the list by polymer, result, and/or dye set.

3. To export an individual install run that has been run on the instrument, tap a row, then tap **Actions ▸ Export report**.

   The install run history report contains the results of the install run performed on the instrument.

   Report file name format: `<InstrumentName>_InstallHistoryReport_<ReportGenerationDate>_<Timestamp>.pdf`, for example: Instrument1_InstallHistoryReport_20201124_162247.pdf

4. To delete an install run history, tap a row, then tap ⊙ **Actions ▸ Delete**.

   IMPORTANT! At least one history for each chemistry type must be retained. If you delete the only run history for a chemistry type, it cannot be retrieved. You will have to perform a new install run for the chemistry type before you can perform regular runs.

5. To view the details of an individual install run, tap a row, then tap **View**.

6. To export the log or report for the individual install run, tap **Actions**, then tap the item to export (these options generate the same files you can generate at the end of an install run):

   - **Logs** (ZIP)—Contains the install report (PDF), data files (FSA or AB1), install run file (CID), and log files that can be used for troubleshooting.

     Log file name format: `<InstrumentName>_<InstallRunType>_<RunDate>_<Timestamp>.zip`, for example: Instrument1_HID_J6 (DS-36)_20230220_230248.zip

   - **Report** (PDF)—Contains the results of the install run for your records.

     Report file name format: `<InstrumentName>_<InstallRunType>_<RunDate>_<Timestamp>.pdf`, for example: Instrument1_HID_J6 (DS-36)_20230220_230248.pdf

Export HID analysis install run logs or report

This statement appears in the footer of HID install run reports with SeqStudio™ Flex Series Instrument Software v1.1.1: *When used for purposes other than Human Identification the instruments cited are for Research Use Only. Not for use in diagnostic procedures.*

Note: Spatial and spectral calibration reports show the date of first installation of the capillary array. Install run reports show the date of the most recent installation of a capillary array.

1. In the plot screen or the results per capillary screen, tap **Actions**.

2. Tap the item to export:

   • **Logs** (ZIP)—Contains the install run report (PDF), data files (FSA), peak tables (CSV), install run file (CID), and log files.

   Log file name format: `<InstrumentName>_<InstallRunType>_<RunDate>_<Timestamp>.zip`, for example: Instrument1_HID_J6 (DS-36)_20230220_230248.zip

   • **Report** (PDF)—Contains the results of the install run.

   Report file name format: `<InstrumentName>_<InstallRunType>_<RunDate>_<Timestamp>.pdf`, for example: Instrument1_HID_J6 (DS-36)_20230220_230248.pdf

3. Select the export location, then tap **Next**.

   Note: It can take up to 15 seconds for the instrument to recognize a USB drive after the drive is inserted in a USB port. exFAT-formatted USB drives are recommended.

4. When the export is complete, click **Close** to close the message box.

HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series Instrument Software v1.1.0

Objective of the validation

The objective of the validation is to confirm the performance of the SeqStudio™ Flex Series Genetic Analyzer, with Applied Biosystems™ PCR amplification kits for forensic DNA profiling. System performance testing includes general instrument operation, consumables, and SeqStudio™ Flex Series Instrument Software v1.1.0.

The validation was performed according to SWGDAM Validation Guidelines for DNA Analysis Methods (SWGDAM, Dec 2016) and the FBI's Quality Assurance Standards (QAS, July 2020). Each laboratory should perform internal validation studies to establish appropriate guidelines, protocols, and procedures before implementing a new technology.

Materials, equipment, and software used for the validation

PCR amplification kits

PCR amplification kit	Cat. No.
Casework kits	
GlobalFiler™ IQC PCR Amplification Kit	A43565
NGM Detect™ PCR Amplification Kit	A31832
AmpFℓSTR™ NGM SElect™ PCR Amplification Kit	4457889
VeriFiler™ Plus PCR Amplification Kit	A35495

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Materials, equipment, and software used for the validation

4

(continued)

PCR amplification kit	Cat. No.
Yfiler™ Plus PCR Amplification Kit	4484678
Database kits	
GlobalFiler™ Express PCR Amplification Kit	4476609
VeriFiler™ Express PCR Amplification Kit	A32014
AmpFℓSTR™ NGM SElect™ Express PCR Amplification Kit	4472193

Instruments and software

Three SeqStudio™ 8 Flex Genetic Analyzers, and five SeqStudio™ 24 Flex Genetic Analyzers were used throughout the validation. Not all instruments were used in every study, however, each instrument was used in at least one study. Five instruments were used in each study: two SeqStudio™ 8 Flex Genetic Analyzers and three SeqStudio™ 24 Flex Genetic Analyzers. Each instrument was installed and calibrated by field service engineers before the experiments. One 3500xL Genetic Analyzer with 3500 Series HID Data Collection Software v4.0.1 was used for comparative studies. The spatial and spectral calibrations were performed on all instruments with DS-33 (G5), DS-36 (J6), and DS-37 (J6-T) matrix standards. The HID install run was performed on all instruments with the HID install standard (GlobalFiler™ Allelic Ladder) and the GeneScan™ 600 LIZ™ Size Standard v2.0. All instruments met all specifications for spatial and spectral calibrations, and the HID install run.

All short tandem repeat (STR) amplifications were performed on a ProFlex™ 96-well PCR System. All DNA quantification was performed on a 7500 Real-Time PCR System for human identification using the Quantifiler™ Trio DNA Quantification Kit.

All data were collected using the SeqStudio™ Flex Series Instrument Software v1.1.0 with off-scale recovery and internal standard normalization enabled. For details of the on-instrument settings used, see Table 5.

Note: Off-scale recovery is enabled by default in all system dye sets for HID analysis.

Table 5 On-instrument analysis settings

Parameter	Settings					
	G5 dye set (5-dye)		J6 dye set (6-dye)		J6-T dye set (6-dye)	
	SeqStudio™ 8 Flex	SeqStudio™ 24 Flex	SeqStudio™ 8 Flex	SeqStudio™ 24 Flex	SeqStudio™ 8 Flex	SeqStudio™ 24 Flex
Size standards	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)	GS600_LIZ_(60-460)
Run module						
Run module name	HID_G5_36_POP4	HID_G5_36_POP4xl	HID_J6_36_POP4	HID_J6_36_POP4xl	HID_J6T_36_POP4	HID_J6T_36_POP4xl
Injection time	15 sec	24 sec	15 sec	24 sec	15 sec	24 sec
Injection voltage	1,200 V	1,200 V	1,200 V	1,200 V	1,200 V	1,200 V
Run time	1,210 sec	1,210 sec	1,550 sec	1,550 sec	1,550 sec	1,550 sec
Run voltage	15,000 V	15,000 V	13,000 V	13,000 V	13,000 V	13,000 V
Analysis settings[1]						
Analysis settings name	HID_5Dye_Default	HID_5Dye_Default	HID_6Dye_Default	HID_6Dye_Default	HID_6Dye_Default	HID_6Dye_Default
Size calling method	Local Southern	Local Southern	Local Southern	Local Southern	Local Southern	Local Southern
Analysis range	Full	Full	Full	Full	Full	Full
Sizing range	80,400	80,400	60,460	60,460	60,460	60,460
Peak amplitude threshold	175	175	175	175	175	175
Primer peak	Present	Present	Present	Present	Present	Present

[1] Values used for on-instrument settings only. See Table 6 for the analysis settings used in GeneMapper™ ID-X Software v1.7 for the purposes of the validation studies.

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Materials, equipment, and software used for the validation

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series Instrument Software v1.1.0

Materials, equipment, and software used for the validation

4

Data were analyzed using the GeneMapper™ *ID-X* Software v1.7 with minimum thresholds determined for each kit, and with internal standard normalization disabled. For details of the GeneMapper™ *ID-X* Software v1.7 analysis settings used, see Table 6.

Table 6   GeneMapper™ *ID-X* Software v1.7 analysis settings

Parameter	Settings		
	G5 dye set (5-dye)	**J6 dye set (6-dye)**	**J6-T dye set (6-dye)**
Size Standard	GS600 LIZ (60-460)	GS600 LIZ (60–460)	GS600 LIZ (60–460)
Smoothing	Light	Light	Light
Size Calling Method	Local Southern	Local Southern	Local Southern
Baseline Window	51	33	33
Peak Amplitude Threshold	Kit-specific thresholds are provided in "Minimum detection threshold and contamination study" on page 60.		
Minimum Peak Half Width	2	2	2
Polynomial Degree	3	3	3
Peak Window Size	15	13	11
Normalization	Unchecked		

4 Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Materials, equipment, and software used for the validation

Samples

All DNA or FTA blood punch samples (except ladders) were amplified according to the standard protocols in the applicable kit user guide, including the recommended DNA input and reaction volume. Any deviations from the default conditions are noted.

Table 7 Studies performed and kits tested

Study	Samples	PCR amplification kit[1]
Minimum detection threshold and contamination	No-template controls (NTC)	NGM SElect™, NGM Detect™, GlobalFiler™ IQC, Yfiler™ Plus, VeriFiler™ Plus, NGM SElect™ Express, GlobalFiler™ Express, VeriFiler™ Express kits
Genotyping	Kit positive control (0.5 ng or 1 ng) 23 gDNA samples (0.5 ng or 1 ng) 23 blood FTA 1.2-mm punches	NGM SElect™, NGM Detect™, GlobalFiler™ IQC, Yfiler™ Plus, VeriFiler™ Plus, NGM SElect™ Express, GlobalFiler™ Express, VeriFiler™ Express, kits
Sizing	Allelic ladder	NGM SElect™, NGM Detect™, GlobalFiler™ IQC, Yfiler™ Plus, VeriFiler™ Plus, NGM SElect™ Express, GlobalFiler™ Express, VeriFiler™ Express, kits
Sensitivity	Kit positive control samples: 31 pg and 125 pg	NGM SElect™, NGM Detect™, GlobalFiler™ IQC, Yfiler™ Plus, and VeriFiler™ Plus kits
Mixture analysis	DNA Control 007:9947A 1:2, 2:1, 1:7 and 7:1 • 0.5 ng for NGM Detect™ and VeriFiler™ Plus kits • 1 ng for NGM SElect™ and GlobalFiler™ IQC kits DNA Control 007:IB-0996 1:2, 2:1,1:7, 7:1 for the Yfiler™ Plus kit (1 ng)	NGM SElect™, NGM Detect™, GlobalFiler™ IQC, Yfiler™ Plus, and VeriFiler™ Plus kits
Resolution	Data from the Genotyping Study and the Sensitivity study	NGM SElect™, NGM Detect™, GlobalFiler™ IQC, Yfiler™ Plus, VeriFiler™ Plus, NGM SElect™ Express, GlobalFiler™ Express, VeriFiler™ Express, kits

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series Instrument Software v1.1.0

Validation methods and results

4

Table 7 Studies performed and kits tested *(continued)*

Study	Samples	PCR amplification kit[1]
Crosstalk and carryover	DNA Control 007 • 2 ng for NGM SElect™NGM Detect™ and GlobalFiler™ IQC kits • 4 ng for Yfiler™ Plus, and VeriFiler™ Plus kits Hi-Di™ Formamide (crosstalk and carryover) NTC (carryover)	NGM SElect™, NGM Detect™, GlobalFiler™ IQC, Yfiler™ Plus, and VeriFiler™ Plus kits

[1] Casework kit-recommended DNA inputs: 0.5 ng (NGM Detect™ and VeriFiler™ Plus kits) and 1 ng (NGM SElect™, GlobalFiler™ IQC, and Yfiler™ Plus kits).

Validation methods and results

Table 8 Validation results summary

Study	Test cases	Passing criteria	Result
Minimum detection threshold and contamination For more information, see "Minimum detection threshold and contamination study" on page 60	Contamination	No allelic data should be observed using minimum peak amplitude thresholds in the NTCs.	Pass
Genotyping For more information, see "Genotyping study" on page 62	Concordance	All allele calls are 100% concordant with the 3500xL genotype results.	Pass
	Reproducibility	STR profiles from the same samples or controls run on different instruments, and across injections, are 100% concordant.	Pass
	Pull-up	Mean pull-up is <3%.	Pass
Sizing For more information, see "Sizing study" on page 63	Precision	The sizing precision does not exceed a standard deviation of 0.15 bp within an injection (8- or 24-capillary) for all alleles.	Pass
	Accuracy	The size difference does not exceed 0.5 bp per injection (8- or 24-capillary) for all alleles.	Pass
	Allelic ladder passing rate	Allelic ladders should pass more than 95% of the time.	Pass
Sensitivity For more information, see "Sensitivity study" on page 66	Lower end limits	Full, on-scale profiles are observed at 125 pg of DNA input.	Pass
		Partial profiles are observed at the lowest amplified input of 31 pg.	Pass

Table 8 Validation results summary *(continued)*

Study	Test cases	Passing criteria	Result
Mixture analysis For more information, see "Mixture analysis study" on page 67	Male:Female	Minor contributor alleles are 100% detectable and concordant in a 1:2, 2:1, 1:7, and a 7:1 mixture.	Pass
	Male:Male		
Resolution For more information, see "Resolution study" on page 67	Resolution	The instrument shall reliably detect and resolve alleles that differ in length by a single base pair from 60–470 bp.	Pass
Crosstalk and carryover For more information, see "Crosstalk and carryover study" on page 68	Crosstalk	Crosstalk observed should be ≤0.2%.	Pass
	Carryover	Carryover observed should be ≤0.1% in NTC blanks and ≤1% in Hi-Di™ Formamide blanks.	Pass

Minimum detection threshold and contamination study

Minimum threshold values are established based on the baseline noise of the instrument and the kit. Each HID laboratory should evaluate background noise for each instrument based on internal validation studies and determine whether variable thresholds should be considered based on factors including instrument and/or dye set.

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

Background capillary electrophoresis instrument signal may be attributed to electrical noise from the instrument electronics or background Raman signal from the water in the polymer. Because of these factors, it is impossible to eliminate all the baseline noise in a detection system.

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Validation methods and results

4

Experimental design

To calculate minimum threshold values, no-template control (NTC) data for each kit on five instruments was analyzed at 1 RFU. Calculations were performed for limit of detection (LOD, average RFU plus 3 standard deviations), limit of quantification (LOQ, average RFU plus 10 standard deviations), and minimum threshold (LOQ rounded up to the multiple of five). Unless otherwise noted, an average minimum threshold was calculated across five instruments per dye for each kit and used as the peak amplitude threshold (PAT) settings in the GeneMapper™ *ID-X* Software for the purpose of developmental validation.

Method and analysis	Replicates
1. Run NTCs for each kit in 8 or 24 wells. 2. Analyze the data with a peak amplitude threshold of 1 RFU. Calculate the baseline noise, limit of detection, and limit of quantitation. 3. Determine an appropriate peak amplitude threshold per kit to be used for this validation. 4. Evaluate the NTCs for contamination.	8 wells x 2 injections per kit (8-capillary) and 24 wells x 1 injection per kit (24-capillary) on 5 instruments.

Results

Using calculated minimum thresholds as the peak amplitude threshold settings in the no-template controls, no allelic data was observed for any of the kits tested.

Table 9   Minimum thresholds calculated and used for the peak amplitude thresholds for each kit in GeneMapper™ *ID-X* Software v1.7

Kit	Dye channel				
	Blue	Green	Yellow	Red	Purple
GlobalFiler™ IQC	26	37	23	29	32
NGM Detect™	30	38	21	34	42
NGM SElect™	31	43	68	100	N/A
VeriFiler™ Plus	42	60	30	42	47
Yfiler™ Plus	27	43	22	32	42
GlobalFiler™ Express	32	51	26	43	44
NGM SElect™ Express	24	72	116	99	N/A
VeriFiler™ Express	35	59	27	41	50

Genotyping study

The genotype concordance and reproducibility test cases were conducted to demonstrate that the STR profiles of the kits are complete and accurate, by comparing known STR profiles run on the 3500xL genetic analyzer, to kit data generated by runs on the SeqStudio™ Flex Series Genetic Analyzers using the SeqStudio™ Flex Series Instrument Software v1.1.0.

To demonstrate the performance of the auto-spectral calibration features optimized in this system, pull-up was analyzed for the genotyping samples for each of the 5 casework kits.

Experimental design

Method and analysis	Replicates
1. Casework kits—Amplify the positive control (007) and 23 gDNA samples at the kit recommended input (0.5 ng or 1 ng). 2. Database kits—Amplify 4 ng of the positive control (007) and 23 direct (FTA blood punch) samples. 3. Run the amplified samples on the SeqStudio™ Flex Series Genetic Analyzers and a 3500xL Genetic Analyzer. 4. Compare the genotypes calls across the SeqStudio™ Flex Series Genetic Analyzers and the 3500xL Genetic Analyzer. 5. Evaluate the direct amplification samples for full, on-scale results. 6. Analyze pull-up (peak height of pull-up peak divided by peak height of the parent peak) for the control and gDNA samples for the casework kits (SeqStudio™ Flex data only).	8 wells x 2 injections per kit (8-capillary) and 24 wells x 1 injection per kit (24-capillary) on 5 instruments. gDNA sample: 1 well per sample x 2 injections. Inject the same plate setup for each kit on a 3500xL instrument.

Results

Concordance and reproducibility

For all the kits tested, the results demonstrated that the genotype profiles from the SeqStudio™ 8 Flex Genetic Analyzers, SeqStudio™ 24 Flex Genetic Analyzers, and the 3500xL Genetic Analyzer were 100% concordant with each other. All FTA blood samples generated full, on-scale profiles.

Table 10   Concordance results (control DNA and 23 gDNA or direct samples per instrument)

Kit	Concordance
GlobalFiler™ IQC	100%
NGM Detect™	100%
NGM SElect™	100%
VeriFiler™ Plus	100%
Yfiler™ Plus	100%
VeriFiler™ Express	100%

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Validation methods and results

4

**Table 10 Concordance results (control DNA and 23 gDNA or direct samples per
instrument)** *(continued)*

Kit	Concordance
NGM SElect™ Express	100%
GlobalFiler™ Express	100%

Pull-up

The average pull-up percentage for all pull-up peaks observed across the kits was ≤1%. In all kits, the
majority of the pull-up peaks were <3%, with only two kits showing any pull-up >5% (VeriFiler™ Plus
and Yfiler™ Plus kits).

Table 11 Pull-up results for five kits tested across five instruments

Kit	Mean pull-up	Percent of pull-up <3%	Percent of pull-up <5%	Average parent peak heights
GlobalFiler™ IQC	0.60%	100%	100%	10,679 RFU
NGM Detect™	0.70%	98.2%	100%	13,448 RFU
NGM SElect™	1%	100%	100%	11,015 RFU
VeriFiler™ Plus	0.74%	99.94%	99.94%	Not calculated
Yfiler™ Plus	1%	85.04%	95.89%	Not calculated

Sizing study

To assess the ability of the SeqStudio™ Flex Series Genetic Analyzers to reproducibly measure the size
of an amplified DNA fragment, an allelic ladder was injected multiple times on the SeqStudio™ 8 Flex
Genetic Analyzers and the SeqStudio™ 24 Flex Genetic Analyzers for each kit.

Experimental design

Method and analysis	Replicates
1. Run allelic ladder (1 µL/well) for each kit in 8 wells. 2. Calculate the sizing precision (standard deviation) of each allele per injection (8 capillaries) and per 3 injections. 3. For sizing accuracy, calculate the size difference of each allele in an allelic ladder, and the mean size of the allele of all ladders of all three consecutive injections on the same plate. 4. Calculate the allelic ladder passing rate for the initial injections for all kits. For GlobalFiler™ IQC, NGM Detect™, and NGM SElect™ kits, calculate the allelic ladder passing rate for reinjections at 16 and 18 hours after the initial injections.	8 wells x 3 consecutive injections per kit on 5 instruments. For GlobalFiler™ IQC kits, NGM Detect™, and NGM SElect™ allelic ladders, reinject the allelic ladders at 16 and 18 hours after the initial injections.

4   Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Validation methods and results

Results

Allelic ladders for all kits passed CGQ and SQ specifications in GeneMapper™ *ID-X* Software v1.7 ≥99% of the time on initial injections (see Table 12). The three allelic ladders that did not meet CGQ and SQ specifications (one each for VeriFiler™ Plus, NGM Detect™, and GlobalFiler™ Express) failed due to poor resolution.

All GlobalFiler™ IQC, NGM Detect™, and NGM SElect™ allelic ladders passed CGQ and SQ specifications at the 16 hour and 18 hour time points.

The standard deviation in sizing within an injection and across multiple injections was <0.15 bp across five SeqStudio™ Flex Series Genetic Analyzers tested for each kit. The mean size difference of each allele in the ladder was <0.5 bp. For the data distribution of sizing precision and accuracy of all alleles from wells containing allelic ladders from all kits, see Figure 2 and Figure 3. The data are displayed in box and whisker plots to show mean distribution of results and outliers.

Table 12   Ladder Passing Rate

Kit	Ladder pass rate
GlobalFiler™ IQC	100% (184/184)
VeriFiler™ Plus	99% (127/128)
Yfiler™ Plus	100% (128/128)
NGM SElect™	100% (184/184)
NGM Detect™	99% (183/184)
GlobalFiler™ Express	99% (127/128)
NGM SElect™ Express	100% (128/128)
VeriFiler™ Express	100% (128/128)

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Validation methods and results

4
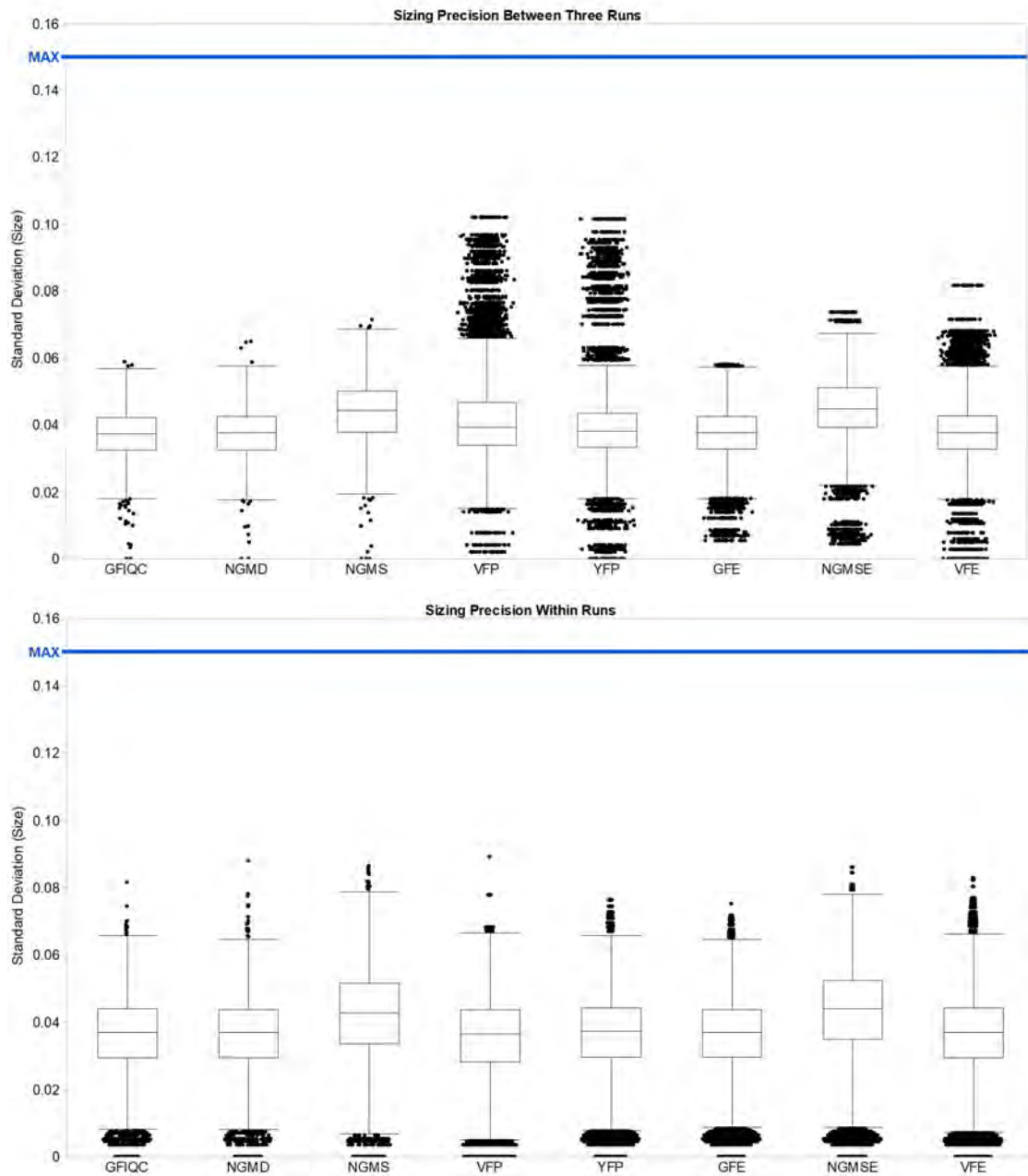
Figure 2 Sizing precision of an allelic ladder from all kits.

The allelic ladder is in eight wells, each injected three times for a total of 24 data points per instrument across five instruments. The top image shows precision per all three injections, and the bottom image shows precision per injection for each marker. The blue line in each pane is at 0.15 bp.

4

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Validation methods and results
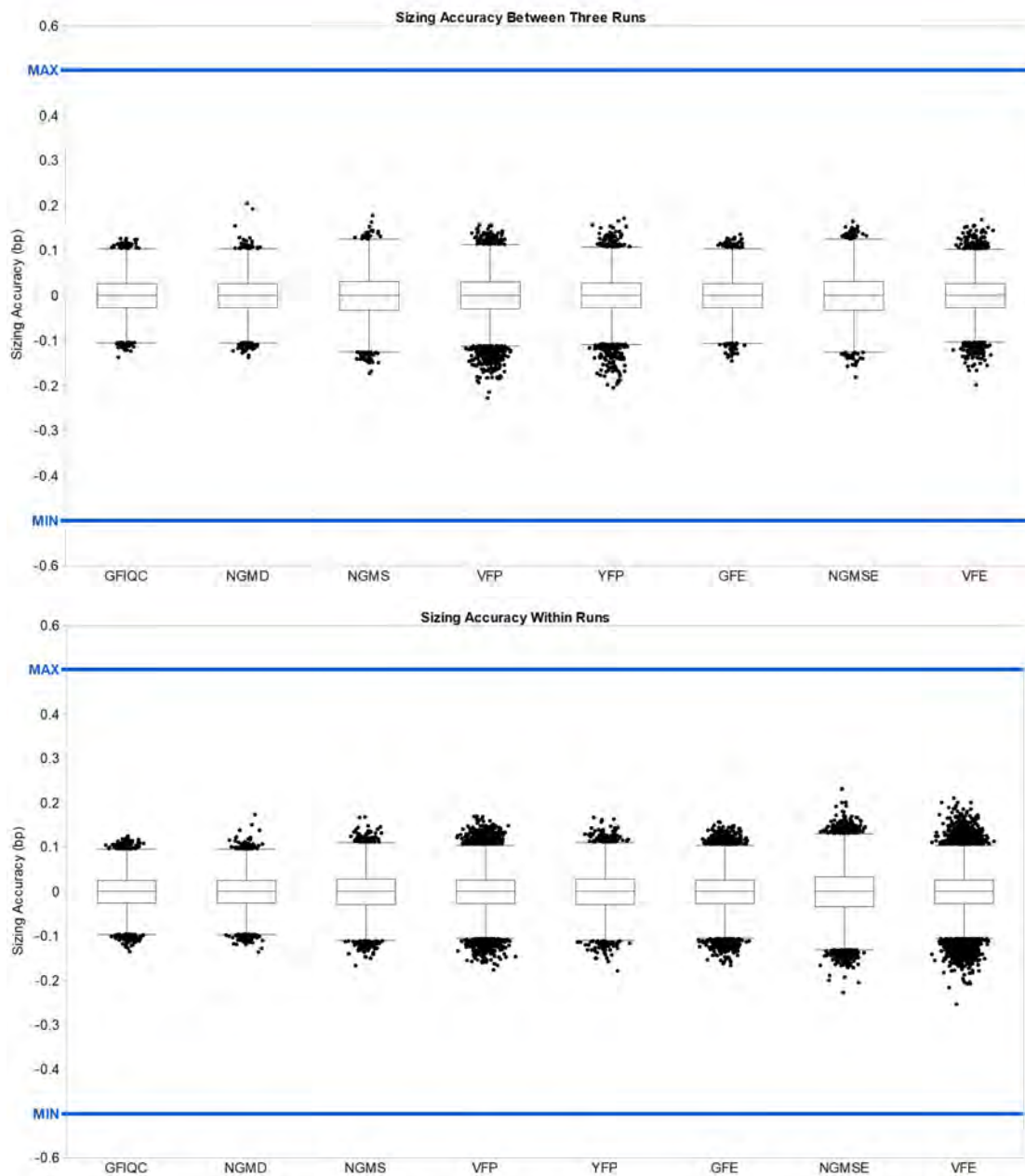
Figure 3   Sizing accuracy of an allelic ladder from all kits.

The allelic ladder is in eight wells, each injected three times for a total of 24 data points across three total injections. The
top image shows precision per all three injections, and the bottom image shows precision per injection for each marker.
The blue line in each pane is at 0.5 bp.

Sensitivity study

The sensitivity series tested the systems ability to generate full or partial profiles with lower DNA input
amounts (125 pg and 31 pg).

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Validation methods and results

4

Experimental design

Method and analysis	Replicates
1. Amplify 31 pg and 125 pg of the kit positive control (007) for the 5 casework kits. 2. Run the control samples on the SeqStudio™ Flex Series Genetic Analyzers. 3. Evaluate dropout at both DNA inputs.	8 wells/dilution for up to 5 injections per kit on 5 instruments.

Results

All samples tested for all kits produced full profiles at 125 pg of DNA input, and produced profiles with at least 1 allele for 31 pg of DNA input.

Mixture analysis study

Experimental design

Method and analysis	Replicates
1. Set up and amplify the mixture samples: 007:9947A or 007:IB-0996 at 1:2, 2:1, 1:7, and 7:1. 2. Run each mixture pair at all ratios on the SeqStudio™ Flex Series Genetic Analyzers. 3. Evaluate the minor genotype calls.	3 or 4 wells for up to 5 injections (8-capillary) and 5 or 6 wells for up to 5 injections (24-capillary) on 5 instruments.

Results

Two test cases were used to evaluate the performance of the SeqStudio™ Flex Series Genetic Analyzer running DNA samples with multiple contributors: Male:Female and Male:Male (Yfiler™ Plus kit only). The kit-recommended input for each kit was amplified for each reaction. The contribution from the minor contributor in the 1:7 and 7:1 mixtures was either 125 pg or 62.5 pg. In the 1:2 and 2:1 mixtures, the minor contributor contribution was either 0.333 pg or 0.176 pg.

For all injections across five instruments, full profiles were generated for the minor contributor.

Resolution study

The study tested the ability of the system to reliably detect and resolve alleles that differ in length by a single base pair (bp).

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series Instrument Software v1.1.0

Validation methods and results

Experimental design

Method and analysis	Replicates
1. Evaluate single base pair resolution up to 470 bp using a Thermo Fisher Scientific software development tool. 2. For GlobalFiler™ IQC, NGM Detect™, and NGM SElect™ kits, calculate the resolution for reinjections at 16 and 18 hours after the initial injections.	Genotyping and sensitivity samples. For GlobalFiler™ IQC, NGM Detect™, and NGM SElect™ kits, reinject the sampels at 16 and 18 hours after the initial injection.

Results

The resolution of the size standard peaks co-injected with samples amplified with all kits up to 470 bp was reviewed. This study also included testing with GlobalFiler™ IQC, NGM Detect™, and NGM SElect™ kit data when the samples were reinjected at 16- and 18-hour time points.

Single-base resolution was achieved between 60–470 bp more for >90% of samples for each kit. Similar results were obtained for GlobalFiler™ IQC, NGM Detect™, and NGM SElect™ samples at the 16- and 18-hour time points.

Crosstalk and carryover study

Crosstalk is signal detection from adjacent wells in an injection. Carryover is a physical transfer of DNA from well to well between injections.

Experimental design

Test case	Method and analysis	Replicates
Crosstalk	1. Set up a checkerboard pattern of DNA Control 007 (2 ng or 4 ng) and Hi-Di™ Formamide. 2. After each injection containing positive control, inject a compete Hi-Di™ Formamide blank injection. 3. Evaluate the Hi-Di™ Formamide blanks in the injections containing positive control for evidence of crosstalk.	8 wells for 2 injections (8-capillary) and 24 wells for 2 injections (24-capillary) on 5 instruments.
Carryover	1. Set up a zebra pattern of DNA Control 007 (2 ng), followed by wither NTCs or Hi-Di™ Formamide. 2. Evaluate the NTC blanks and Hi-Di™ Formamide blanks for evidence of carryover.	8 wells (8-capillary) and 24 wells (24-capillary) on 5 instruments. 1X control DNA injection followed by NTC. 1X control DNA injection followed by Hi-Di™ Formamide.

Chapter 4 HID instrument validation of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.0
Conclusion

4

Results

Crosstalk

No crosstalk peaks were observed in Hi-Di™ Formamide blanks ≥0.2% in wells adjacent to wells containing DNA Control 007 samples.

Carryover

No carryover peaks were observed in Hi-Di™ Formamide blanks >1% or in NTC blanks >0.1% following injections of DNA Control 007 samples.

Conclusion

The SeqStudio™ Flex Series Genetic Analyzers generate high-quality data with Applied Biosystems™ PCR amplification kits analyzed with GeneMapper™ *ID-X* Software v1.7. The studies demonstrate that this instrument meets the same specifications for concordance, reproducibility, precision, accuracy, sensitivity, resolution, crosstalk, and carryover as the 3500xL Genetic Analyzer.

Controls, samples, and allelic ladders injected 16 and 18 hours after the initial injection continued to pass specifications for resolution, allelic ladder passing rate, and other product requirements of the system.

General operations of the SeqStudio™ 8 Flex Genetic Analyzer and the SeqStudio™ 24 Flex Genetic Analyzer for HID functioned properly for instrument control, calibration, run setup, and data collection.

5 HID instrument verification of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series Instrument Software v1.1.1

Objective of the verification

The objective of the verification is to test the performance of an Applied Biosystems™ PCR amplification kit from each Applied Biosystems™ dye set on the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series Instrument Software v1.1.1. Performance testing includes minimum detection threshold, genotyping, and mixture analysis.

Materials, equipment, and software used for the verification

PCR amplification kits

PCR amplification kit	Cat. No.
GlobalFiler™ IQC PCR Amplification Kit	A43565
AmpFℓSTR™ NGM SElect™ PCR Amplification Kit	4457889
VeriFiler™ Plus PCR Amplification Kit	A35495

Chapter 5 HID instrument verification of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.1
Materials, equipment, and software used for the verification

5

Instruments and software

One SeqStudio™ 8 Flex Genetic Analyzer and one SeqStudio™ 24 Flex Genetic Analyzer were used throughout the verification. The spatial and spectral calibrations were performed on both instruments with DS-33 (G5), DS-36 (J6), and DS-37 (J6-T) matrix standards. The HID install run was performed on all instruments with the HID install standard (GlobalFiler™ Allelic Ladder) and the GeneScan™ 600 LIZ™ Size Standard v2.0. All instruments met all specifications for spatial and spectral calibrations, and the HID install run.

All STR amplifications were performed on a ProFlex™ 96-well PCR System. All DNA quantification was performed on a 7500 Real-Time PCR System for human identification using the Quantifiler™ Trio DNA Quantification Kit.

All data were collected using the SeqStudio™ Flex Series Instrument Software v1.1.1 with off-scale recovery and internal standard normalization enabled. HID run modules were used with auto-spectral calibration enabled and disabled. For details of the on-instrument settings used, see Table 5 on page 56.

Note: To perform verification with auto-spectral calibration disabled, a custom dye-set was used, and no other settings were changed.

Data were analyzed using GeneMapper™ *ID-X* Software v1.7 with minimum thresholds determined for each kit, and with internal standard normalization disabled. For details of the GeneMapper™ *ID-X* Software v1.7 analysis settings used, see Table 6 on page 57.

Note: Auto-spectral calibration and off-scale recovery are enabled by default in all system dye sets for HID analysis.

Samples

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

Table 13   Studies performed and kits tested

Study	Samples	PCR amplification kit[1]
Minimum detection threshold	No-template controls (NTC)	GlobalFiler™ IQC, NGM SElect™, VeriFiler™ Plus kits
Genotyping	Kit positive control (0.5 ng or 1 ng) 23 gDNA samples (0.5 ng or 1 ng)	GlobalFiler™ IQC, NGM SElect™, VeriFiler™ Plus kits
Mixture analysis	DNA Control 007:9947A 1:2, 2:1, 1:7 and 7:1 • 0.5 ng for the VeriFiler™ Plus kit • 1 ng for the NGM SElect™ and GlobalFiler™ IQC kits NIST SRM 2391d Component D	GlobalFiler™ IQC, NGM SElect™, VeriFiler™ Plus kits

[1]   Casework kit-recommended DNA inputs: 0.5 ng (VeriFiler™ Plus kit) and 1 ng (NGM SElect™ and GlobalFiler™ IQC kits).

Verification methods and results

Table 14   **Verification results summary**

Study	Test cases	Passing criteria	Result
Minimum detection threshold For more information, see "Minimum detection threshold and contamination study" on page 60	Thresholds	No allelic data should be observed using minimum peak amplitude thresholds in the NTCs.	Pass
Genotyping For more information, see "Genotyping study" on page 62	Concordance	All allele calls are 100% concordant.	Pass
	Reproducibility	STR profiles from the same samples or controls run on different instruments, and across injections, are 100% concordant.	Pass
	Pull-up	Mean pull-up is <3%.	Pass
Mixture analysis For more information, see "Mixture analysis study" on page 67	Male:Female	Minor contributor alleles are 100% detectable and concordant in a 1:2, 2:1, 1:7, and a 7:1 mixture.	Pass
	Male:Male		

Minimum detection threshold

Minimum threshold values are established based on the baseline noise of the instrument and the kit. Each HID laboratory should evaluate background noise for each instrument based on internal validation studies and determine whether variable thresholds should be considered based on factors including instrument and/or dye set.

Experimental design

To calculate minimum threshold values, no-template control (NTC) data for each kit on two instruments were analyzed at 1 RFU. Calculations were performed for limit of detection (LOD, average RFU plus 3 standard deviations), limit of quantification (LOQ, average RFU plus 10 standard deviations), and minimum threshold (LOQ rounded up to the multiple of five). Unless otherwise noted, an average minimum threshold was calculated across two instruments per dye for each kit and used as the peak amplitude threshold (PAT) settings in the GeneMapper™ *ID-X* Software for the purpose of verification.

Method and analysis	Replicates
1. Run NTCs for each kit in 7 or 23 wells. 2. Analyze the data with a peak amplitude threshold of 1 RFU. Calculate the baseline noise, limit of detection, and limit of quantitation. 3. Determine an appropriate peak amplitude threshold per kit to be used for this verification. 4. Evaluate the NTCs for contamination.	8 wells x 2 injections per kit (8-capillary) and 24 wells x 1 injection per kit (24-capillary) on 2 instruments. 7 or 23 wells per kit x 1 injection each with auto-spectral calibration enabled and disabled.

Chapter 5 HID instrument verification of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.1
Verification methods and results

5

Results

Using calculated minimum thresholds as the peak amplitude threshold settings in the no-template controls, no allelic data were observed for any of the kits tested.

Table 15   Minimum thresholds calculated and used for the peak amplitude thresholds for each kit in GeneMapper™ *ID-X* Software v1.7

Dye channel	SeqStudio™ 8 Flex Genetic Analyzer			SeqStudio™ 24 Flex Genetic Analyzer		
	GlobalFiler™ IQC	VeriFiler™ Plus	NGM SElect™	GlobalFiler™ IQC	VeriFiler™ Plus	NGM SElect™
Auto-spectral calibration enabled						
Blue	20	30	30	20	30	35
Green	75	60	50	35	70	65
Yellow	15	25	85	20	30	105
Red	30	40	130	30	40	155
Purple	35	40	N/A	35	45	N/A
Orange	25	30	30	30	40	35
Auto-spectral calibration disabled						
Blue	20	30	35	20	35	35
Green	70	50	55	35	65	65
Yellow	15	25	85	20	30	110
Red	30	45	125	30	55	155
Purple	35	40	N/A	35	45	N/A
Orange	25	30	30	30	35	35

Genotyping study

The genotype concordance and reproducibility test cases were conducted to demonstrate that the STR profiles of the kits are complete and accurate, by comparing known STR profiles.

To demonstrate the performance of the auto-spectral calibration features optimized in this system, pull-up was analyzed for the genotyping samples for each of the three kits. The results were compared to the pull-up observed when the auto-spectral calibration feature was disabled.

5 Chapter 5 HID instrument verification of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.1
Verification methods and results

Experimental design

Method and analysis	Replicates
1. Amplify the positive control (007) and 23 gDNA samples at the kit recommended input (0.5 ng or 1 ng). 2. Compare the genotype calls across the SeqStudio™ Flex Series Genetic Analyzers. 3. Analyze pull-up (peak height of pull-up peak divided by peak height of the parent peak).	gDNA sample: 1 well per sample x 2 injections. 23 wells per kit x 2 injections each with auto-spectral calibration enabled and disabled.

Results

Concordance and reproducibility

For all the kits tested, the results demonstrated that the genotype profiles from the SeqStudio™ 8 Flex Genetic Analyzer and the SeqStudio™ 24 Flex Genetic Analyzer with auto-spectral calibration enabled or disabled were 100% concordant with each other.

Table 16   Concordance results (control DNA and 23 gDNA samples per instrument)

Kit	Concordance
GlobalFiler™ IQC	100%
NGM SElect™	100%
VeriFiler™ Plus	100%

Pull-up

The average pull-up percentage for all pull-up peaks observed across the kits was ≤1%. In all kits, the majority of the pull-up peaks were <3%.

Table 17   Pull-up results for the GlobalFiler™ IQC kit tested across two instruments

Analysis	8-capillary instrument		24-capillary instrument	
	Auto-spectral on	Auto-spectral off	Auto-spectral on	Auto-spectral off
Total number of pull-up calls	1,402	857	5,123	1,883
Mean pull-up	0.70%	0.60%	0.70%	0.60%
Percent of pull-up <3%	99.6%	99.8%	99.4%	99.8%
Percent of pull-up <5%	99.8%	99.8%	99.7%	99.9%
DNA Control 007				
Number of runs	24	24	48	48
Average parent peak height	4,613 RFU	4,108 RFU	6,143 RFU	6,106 RFU

Chapter 5 HID instrument verification of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.1
Verification methods and results

5

Table 17   Pull-up results for the GlobalFiler IQC kit tested across two instruments *(continued)*

Analysis	8-capillary instrument		24-capillary instrument	
	Auto-spectral on	Auto-spectral off	Auto-spectral on	Auto-spectral off
Average pull-up peak height	36 RFU	32 RFU	47 RFU	47 RFU
gDNA samples				
Number of runs	46	46	46	46
Average parent peak height	7,993 RFU	7,571 RFU	10,421 RFU	9,964 RFU
Average pull-up peak height	56 RFU	47 RFU	68 RFU	59 RFU

Table 18   Pull-up results for the NGM SElect™ kit tested across two instruments

Analysis	8-capillary instrument		24-capillary instrument	
	Auto-spectral on	Auto-spectral off	Auto-spectral on	Auto-spectral off
Total number of pull-up calls	265	113	1,746	501
Mean pull-up	1.1%	0.9%	1.9%	1.3%
Percent of pull-up <3%	100%	100%	90.4%	99.6%
Percent of pull-up <5%	100%	100%	99.8%	100%
DNA Control 007				
Number of runs	24	24	48	48
Average parent peak height	5,236 RFU	5,655 RFU	6,180 RFU	7,034 RFU
Average pull-up peak height	51 RFU	42 RFU	128 RFU	101 RFU
gDNA samples				
Number of runs	46	46	46	46
Average parent peak height	10,610 RFU	10,016 RFU	12,904 RFU	13,348 RFU
Average pull-up peak height	108 RFU	81 RFU	215 RFU	149 RFU

Table 19   Pull-up results for the VeriFiler™ Plus kit tested across two instruments

Analysis	8-capillary instrument		24-capillary instrument	
	Auto-spectral on	Auto-spectral off	Auto-spectral on	Auto-spectral off
Total number of pull-up calls	1,441	967	5,038	2,084
Mean pull-up	0.8%	0.9%	0.8%	0.8%
Percent of pull-up <3%	99.6%	99.9%	99.9%	100%
Percent of pull-up <5%	99.7%	99.9%	100%	100%

Table 19 Pull-up results for the VeriFiler Plus kit tested across two instruments *(continued)*

Analysis	8-capillary instrument		24-capillary instrument	
	Auto-spectral on	Auto-spectral off	Auto-spectral on	Auto-spectral off
DNA Control 007				
Number of runs	24	24	48	48
Average parent peak height	6,289 RFU	5,498 RFU	7,815 RFU	7,603 RFU
Average pull-up peak height	52 RFU	55 RFU	61 RFU	68 RFU
gDNA samples				
Number of runs	46	46	46	46
Average parent peak height	9,239 RFU	8,618 RFU	11,805 RFU	11,488 RFU
Average pull-up peak height	66 RFU	68 RFU	84 RFU	82 RFU

Mixture analysis study

Experimental design

Method and analysis	Replicates
1. Set up and amplify the mixture samples: 007:9947A at 1:2, 2:1, 1:7, and 7:1. 2. Run mixture at all ratios on the SeqStudio™ Flex Series Genetic Analyzers. 3. Run the NIST SRM 2391d Component D. 4. Evaluate the minor genotype calls.	5 or 6 wells for 9947A:007 mixture and 7 wells of NIST SRM 2391d, injected twice each with auto-spectral calibration enabled and disabled.

Results

Two test cases were used to evaluate the performance of the SeqStudio™ Flex Series Genetic Analyzer running DNA samples with multiple contributors. The kit-recommended input for each kit was amplified for each reaction. The contribution from the minor contributor in the 1:7 and 7:1 mixtures was either 125 pg or 62.5 pg. In the 1:2 and 2:1 mixtures, the contribution from the minor contributor was either 0.333 pg or 0.176 pg.

For all injections across two instruments, full profiles were generated for the minor contributor.

Chapter 5 HID instrument verification of the SeqStudio™ Flex Series Genetic Analyzer with SeqStudio™ Flex Series
Instrument Software v1.1.1
Conclusion

5

Conclusion

The SeqStudio™ Flex Series Genetic Analyzers generate high-quality data with Applied Biosystems™ PCR amplification kits analyzed with GeneMapper™ *ID-X* Software v1.7.

General operations of the SeqStudio™ 8 Flex Genetic Analyzer and the SeqStudio™ 24 Flex Genetic Analyzer for HID functioned properly for instrument control, calibration, run setup, and data collection.

A Documentation and support

Related documentation

Document	Publication number
SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide	100104689
GeneMapper™ ID-X Software v1.7 Administration Guide	MAN0029245
GeneMapper™ ID-X Software v1.7 Installation Guide	MAN0029246

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.