Automated DNA Quantification, Normalization, and Amplification Setup

USER BULLETIN

for use with:
HID NIMBUS® QNA System
HID NIMBUS® Presto QNA System

Publication Number MAN1000064

Revision B

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

Revision history: MAN1000064 B (English)

Revision	Date	Description
B	1 July 2024	• The characters that are allowed in the worklists were updated (see "Create a worklist" on page 11 and page 28). • Validation information was added (see Chapter 4, "Experiments and results").
A00	20 March 2024	New document for automated DNA quantification, normalization, and amplification setup on the HID NIMBUS® QNA System and the HID NIMBUS® Presto QNA System.

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

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the safety information in the manufacturer's documentation.

IMPORTANT! Before using this product, read and understand the safety information for the KingFisher™ Presto Purification System in the *Thermo Scientific™ KingFisher™ Presto User Manual* (Pub. No. MAN0019872).

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

The Applied Biosystems™ HID NIMBUS® QNA System and HID NIMBUS® Presto QNA System are automated systems that include optimized scripts and use validated software to support high-throughput workflows for streamlined quantification, normalization, and amplification plate preparation. Unauthorized modifications are not supported.

The HID NIMBUS® QNA System and the HID NIMBUS® Presto QNA System can set up 96 reactions for quantification in <45 minutes, and set up 96 reactions for normalization and amplification in <90 minutes.

The system is available in the following configurations:

Configuration	Description
HID NIMBUS® QNA System (Cat. No. A55771)	• Includes the Microlab® NIMBUS® Liquid Handler and the Applied Biosystems™ HID NIMBUS® System Software • Supports automated plate setup for DNA quantification, normalization, and amplification
HID NIMBUS® Presto QNA System (Cat. No. A55769)	• Includes the Microlab® NIMBUS® Liquid Handler, the KingFisher™ Presto Purification System, and the Applied Biosystems™ HID NIMBUS® System Software • Supports automated DNA purification, and automated plate setup for DNA quantification, normalization, and amplification

Note: The system is also available as the HID NIMBUS® Presto System (Cat. No. A55395), configured only for automated DNA purification. For information about DNA purification, see the *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the HID NIMBUS® Presto Systems User Bulletin* (Pub. No. MAN0019368).

System components

The HID NIMBUS® systems for quantification and amplification include the following components:

- Microlab® NIMBUS® Liquid Handler
- HID NIMBUS® System Software
- Laptop computer
- *(HID NIMBUS® Presto QNA System only)* KingFisher™ Presto Purification System
- *(Optional)* Clean Air Protection (CAP) system, with UV lamps

The Microlab® NIMBUS® Liquid Handler is an automated liquid handling platform.

The HID NIMBUS® System Software facilitates automated DNA purification, and automated plate setup for DNA quantification, normalization, and amplification. For information about DNA purification, see the *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the HID NIMBUS® Presto Systems User Bulletin* (Pub. No. MAN0019368).

Compatible kits

To prepare for quantification runs, the HID NIMBUS® QNA System and the HID NIMBUS® Presto QNA System are equipped to work with the Quantifiler™ Trio Automated DNA Quantification Kit (Cat. No. A58787). This kit is designed to quantify the total amount of amplifiable human DNA in a sample and includes all the necessary reagents for detection, amplification, and quantification of three human-specific DNA targets. For more information about the Quantifiler™ Trio Automated DNA Quantification Kit, see the *Quantifiler™ Trio Automated DNA Quantification Kit User Guide* (Pub. No. MAN1000066).

To prepare for amplification runs, the HID NIMBUS® QNA System and the HID NIMBUS® Presto QNA System are equipped to work with various short tandem repeat (STR) multiplex assays. Compatible kits include the following:

- GlobalFiler™ PCR Amplification Kit (Cat. No. 4476135 and Cat. No. 4482815)
- GlobalFiler™ IQC PCR Amplification Kit (Cat. No. A43565)
- VeriFiler™ Plus PCR Amplification Kit (Cat. No. A35495)
- Yfiler™ Plus PCR Amplification Kit (Cat. No. 4484678 and Cat. No. 4482730)
- NGM Detect™ PCR Amplification Kit (Cat. No. A31832)

Deck layouts

To view the deck layout for quantification, see Figure 5 and Figure 6.

To view the deck layout for amplification, see Figure 12 and Figure 13.

To view the deck layout for purification, see the *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the HID NIMBUS® Presto Systems User Bulletin* (Pub. No. MAN0019368).

Note: These layouts are provided for reference. Follow the software prompts for detailed run-specific information about labware locations, reagent positions, and volumes.

IMPORTANT! Use only the input labware that was used at installation for robot axis teaching. If your laboratory wants to change labware, contact Thermo Fisher Scientific Technical Support for more information.

Materials supplied with the HID NIMBUS® systems

Table 1 Deck components

Item	Hamilton Cat. No.
16-Position Sample Tube Carriers	6601693-01
MicroTube Inserts	182238
Carrier with the following configuration: • 4 x NTR Pedestal • 4 x 96-Well Microplate Adapter	6604980-01
200-mL Reagent Trough Carrier	6603240-01
Carrier with the following configuration: • 2 x FTR Pedestal • 6 x 60-mL Reagent Trough Pedestal • NTR Pedestal with Multi-Tube Adapter	6604985-01

Table 1 Deck components *(continued)*

Item	Hamilton Cat. No.
Carrier with the following configuration: • 2 x FTR Pedestal • 2 x Plate Stack Pedestal	6604990-01
CO-RE® Paddles	97021-01
Barcode Reader	97360-01

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Table 2 Materials for automated quantification

Item	Source
Tips, Conductive Non-sterile Filter, 50 µL	A58978
MicroAmp™ Optical 96-Well Reaction Plate (recommended)	N8010560
MicroAmp™ Optical Adhesive Film (recommended)	4311971
RNase-Free Microfuge Tube (1.5 mL) (recommended)	AM12450
Quantifiler™ Trio Automated DNA Quantification Kit	A58787
Waste bags, standard enclosure, 100 count	Hamilton 93894-02

Table 3 Materials for automated amplification

Item	Source
Tips, Conductive Non-sterile Filter, 50 µL	A58978
MicroAmp™ Optical 96-Well Reaction Plate (recommended)	N8010560
RNase-Free Microfuge Tube (1.5 mL) (recommended)	AM12450
60-mL Reagent Reservoir, Self-Standing with Lid	56694-01
TE buffer	12090015
Waste bags, standard enclosure, 100 count	Hamilton 93894-02

Table 3 Materials for automated amplification *(continued)*

Item	Source
PCR amplification kit, one of the following:	
GlobalFiler™ PCR Amplification Kit	4476135 (200 reactions) 4482815 (1,000 reactions)
GlobalFiler™ IQC PCR Amplification Kit	A43565
VeriFiler™ Plus PCR Amplification Kit	A35495
Yfiler™ Plus PCR Amplification Kit	4484678 (100 reactions) 4482730 (500 reactions)
NGM Detect™ PCR Amplification Kit	A31832

Network and password security requirements

Network configuration and security

The network configuration and security settings of your laboratory or facility (such as firewalls, anti-virus software, network passwords) are the sole responsibility of your facility administrator, IT, and security personnel. This product does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the software, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss. It is the responsibility of your facility administrator, IT, and security personnel to prevent the use of any unsecured ports (such as USB, Ethernet) and ensure that the system security is maintained.

Password security

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. All passwords should be reset upon first sign in to the product. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

2 Perform the automated DNA quantification setup run

Workflow

Prepare the automated DNA quantification plate

- **Create a worklist** (page 11)
- **Enter the run settings** (page 14)
- **Load the deck and start the run** (page 19)
- **Remove the quantification plate setup, unused reagents, and labware** (page 25)
- **View the output files** (page 25)

Guidelines for the automated quantification run

- The Quantifiler™ Trio Automated DNA Quantification Kit is sufficient for ≤960 samples, depending on the batch size.
- The HID NIMBUS® QNA System and HID NIMBUS® Presto QNA System are only compatible with 1.5 mL microcentrifuge tubes, 96-well PCR plates, and KingFisher™ 96-well deep-well plates for purified sample input.
- Before loading the instrument deck, remove air bubbles from the surface of reagents and samples. Bubbles can lead to incorrect liquid detection and pipetting errors.
- When loading tubes on the deck, ensure that the tip will not come into contact with any objects, including the tube caps, to prevent false liquid detection. Firmly press down on all labware placed on the instrument deck.

- We do not recommend a reagent or sample volume <35 µL in microcentrifuge tubes. If the eluate volume from purification is <35 µL, use a MicroAmp™ PCR plate as the input labware.
- For information on barcode positioning, see the *NIMBUS HD Operator's Manual* (Pub. No. 98538-01).
- Before the first run of the day, we recommend that you perform daily maintenance. See *NIMBUS HD Operator's Manual* (Pub. No. 98538-01) for instructions.

Create a worklist

For a quantification run, we recommend using a worklist for the samples in XLS, XLSX, or CSV format. Templates are installed on the provided laptop computer.

Alternatively, use the worklist generated from the run-tracking report of the purification workflow. For more information about the purification workflow, see *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the HID NIMBUS® Presto Systems User Bulletin* (Pub. No. MAN0019368).

1. Before each run, navigate to the **ExampleWorklists** folder on the system computer:

   ```
   C:\Program Files (x86)\Hamilton\HID Nimbus
   System\QuantNormAmp\Resources\Example Worklists
   ```

   Note: If you cannot find the worklist templates on the computer, contact your local Thermo Fisher Scientific representative.

2. Copy the required template, then save it with a new file name.

3. In the saved copy, enter information for each sample, as shown in Table 4, Figure 1, and Figure 2.

IMPORTANT! The worklist must include the required information shown in Table 4. Do not change the column headings or leave cells blank. The system only reads XLS, XLSX, and CSV formats.

Table 4 Worklist information for each sample

Required column order	Required column heading	Action
1	**Sample ID**	Enter the sample name or ID associated with the sample. The sample ID must be unique. The following characters are valid in the worklist: • 0–9 • a–z (uppercase and lowercase) **IMPORTANT!** Do not use the following special characters: **$ * ^ + { } [ ] ( ) \ / & % @** These characters interfere with quantification data processing and with Thermo Fisher™ Connect Platform applications. **IMPORTANT!** The following Sample IDs are reserved for the software: **ladder**, **PC**, and **NTC**. Do not use these words as your Sample IDs, whether uppercase or lowercase; for example, the software will filter out **NTC**, **ntc**, **NTc**, and so on. However, your Sample IDs can *contain* these words; for example, the software will accept and process **NTC_sample1**.
2	**Eluate Position**	For tubes, use numeric values. For plates, use alphanumeric values. **Note:** The eluate position on the instrument deck refers to the loading position, regardless of your choice to remove samples from the run during quantification or amplification.
3	**Eluate Barcode**	If you are using barcodes, enter the expected eluate barcode for each sample lysate tube or for the MicroAmp™ PCR plate or KingFisher™ deep-well plate. • **Tubes**—Each tube has a unique barcode. • **Plates**—All sample wells of the plate have the same barcode.

IMPORTANT! To proceed with plate setup, ensure the barcode information on the worklist matches the barcodes loaded on the instrument in the same positions. You must include barcode information in all sample lines. If barcode information is absent in one or more sample lines, you will receive a read error on import. For plates, use the same barcode for each sample. If barcodes are not used, fill the input and eluate barcode cells with ***No Barcode***.

Sample ID	Eluate Position	Eluate Barcode
Sample 1	1	No Barcode
Sample 2	2	No Barcode
Sample 3	3	No Barcode
Sample 4	4	No Barcode
Sample 5	5	No Barcode
Sample 6	6	No Barcode
Sample 7	7	No Barcode
Sample 8	8	No Barcode
Sample 9	9	No Barcode
Sample 10	10	No Barcode
Sample 11	11	No Barcode
Sample 12	12	No Barcode
Sample 13	13	No Barcode
Sample 14	14	No Barcode
Sample 15	15	No Barcode
Sample 16	16	No Barcode
Sample 17	17	No Barcode
Sample 18	18	No Barcode
Sample 19	19	No Barcode
Sample 20	20	No Barcode

Figure 1 Quantification worklist example for tubes

Sample ID	Eluate Position	Eluate Barcode
Sample 1	A1	No Barcode
Sample 2	B1	No Barcode
Sample 3	C1	No Barcode
Sample 4	D1	No Barcode
Sample 5	E1	No Barcode
Sample 6	F1	No Barcode
Sample 7	G1	No Barcode
Sample 8	H1	No Barcode
Sample 9	A2	No Barcode
Sample 10	B2	No Barcode
Sample 11	C2	No Barcode
Sample 12	D2	No Barcode
Sample 13	E2	No Barcode
Sample 14	F2	No Barcode
Sample 15	G2	No Barcode
Sample 16	H2	No Barcode
Sample 17	A3	No Barcode
Sample 18	B3	No Barcode
Sample 19	C3	No Barcode
Sample 20	D3	No Barcode

Figure 2 Quantification worklist example for plates

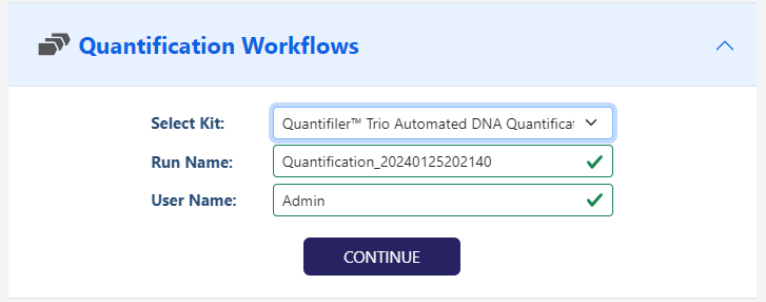
Enter the run settings

1. On the desktop, click **Applied Biosystems HID NIMBUS System Software**.

   Note: If the shortcut is not present on the desktop, navigate to `C:\Program Files (x86)\Hamilton\HID Nimbus System\Application`

2. *(Optional)* Expand the **Input Catalog Number** pane, then scan or enter the Quantifiler™ Trio Automated DNA Quantification Kit catalog number.

3. Expand the **Quantification Workflows** pane, then enter the workflow information.



   a. Use the **Select Kit** dropdown list to select a kit.

   b. *(If needed)* Change the run name, then enter a user name.

   IMPORTANT!
   - The run name and user name fields cannot include special characters other than - (hyphen) and _ (underscore).
   - The run name and user name must be 1–30 characters in length.

   Note: The run name is prepopulated with the workflow type, followed by the date and time (format: Quantification_YYYYMMDDhhmmss). The input run name determines the folder where the run tracking and instrument import files are saved. Use the run name field to organize files across purification, quantification, and amplification workflow runs on the HID NIMBUS® systems.

4. Click **CONTINUE**.

   The settings window is displayed.

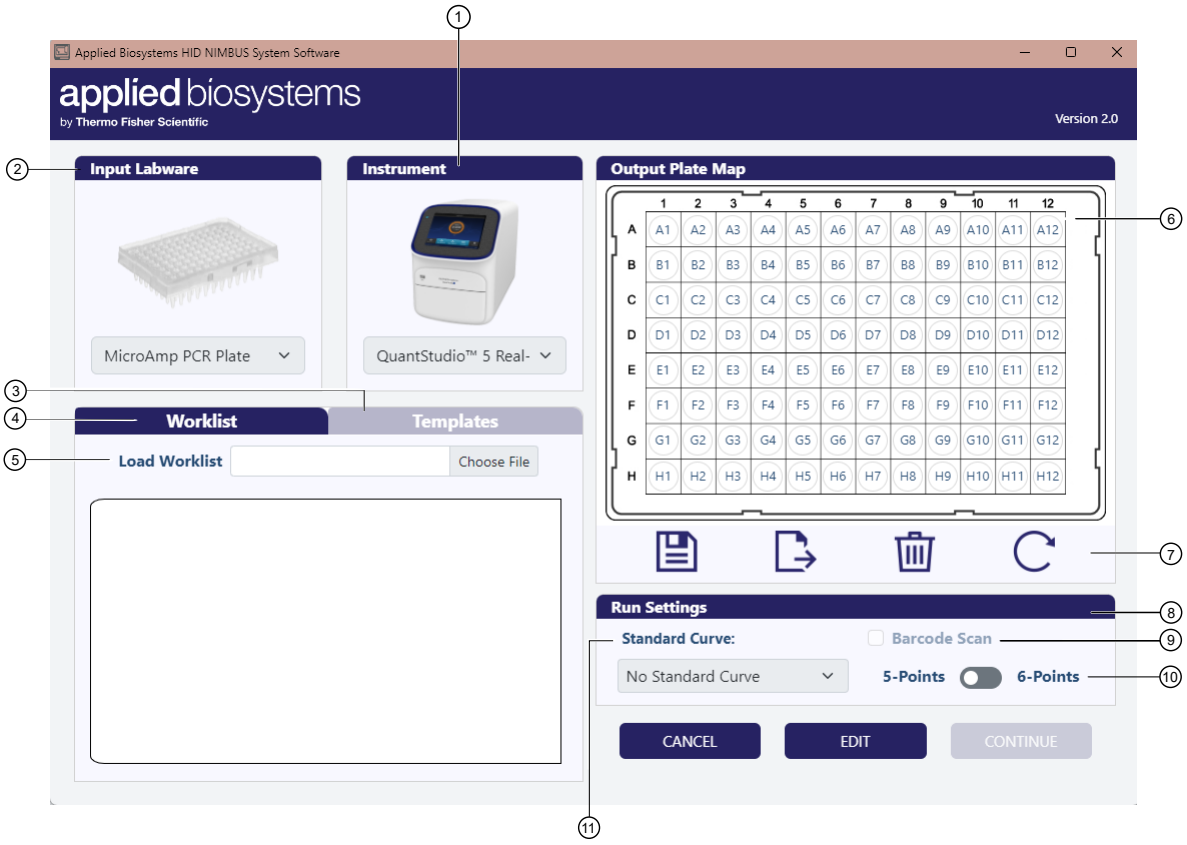


Figure 3 Settings window

1. Select an instrument
2. Select the input labware
3. Select a template
4. **Worklist** tab
5. Import a worklist
6. Plate map
7. Plate map tools
8. **Run Settings** pane
9. Enable or disable barcode scanning
10. Points toggle
11. Select a standard curve option

5. Select the input labware and the instrument for the quantification experiment.
   a. Click **EDIT** to unlock the user input interface.
   b. In the **Parameter Modification** window, click **CONTINUE**.
   c. Use the dropdown list in the **Input Labware** pane to select an option.
      - **MicroAmp PCR plate**
      - **Microcentrifuge Tubes**
      - **KingFisher Deep Well Plate**
   d. Use the dropdown list in the **Instrument** pane to select an option.
      - **QuantStudio™ 5 Real-Time PCR System**
      - **7500 Real-Time PCR System**

e. Click **SAVE** to confirm the selection.

Anytime that you edit the parameters, the edits are tracked in the GUI log file, located at `C:\Program Files (x86)\Hamilton\LogFiles`

6. In the **Worklist** tab, import a worklist.
   a. In the **Worklist** pane, click **Choose File**, or click within the blank file upload field.
   b. Select the prepared worklist or select purification run tracking file.

   Note: Before uploading, ensure that the worklist matches the input labware. For example, if you are using a MicroAmp™ PCR plate, ensure that the worklist is not intended for 1.5-mL microcentrifuge tubes.

   c. *(Optional)* To change a worklist after it has been uploaded, click **Choose File**, or click within the blank file upload field. In the **Changing Worklist** window, click **CONTINUE**.

After the worklist is imported, the samples and controls appear in the **Worklist** pane.

7. In the **Run Settings** pane, use the **Standard Curve** dropdown list to select an option.
   - **Create New Standard Curve**
   - **Use Existing Standard Curve**
   - **No Standard Curve**

Note: If a template was applied, a standard curve option is preselected. To remove the standard curve, in the **Run Settings** pane, use the **Standard Curve** dropdown list to select **No Standard Curve**. Do not drag-and-drop standards to **(Remove Target)**.

8. In the **Run Settings** pane, use the toggle to select **5-Points** or **6-Points**.

IMPORTANT! If a template was applied and you select standard curve points that differ from the template, the standards are removed from the plate map. For example, if you select the **Default - 5 Point Duplicates** template but manually change the number of points to **6-Points**, the standards are removed from the plate map.

9. *(Optional)* Enable barcode scanning.
   a. Click **EDIT** to unlock the user input interface.
   b. In the **Run Settings** pane, select the **Barcode Scan** checkbox.
   c. Click **SAVE** to confirm the selection.

Note: When barcode scanning is on, you are prompted to manually scan the barcodes of the labware and carriers. Your worklist must contain the relevant barcode information to avoid scanning errors.

10. Select an action to set up the plate map.

    - Drag-and-drop each sample from the **Worklist** tab to the desired location of the **Output Plate Map**.

      Note: The positive control and no template control samples dragged onto the **Output Plate Map** are ignored when importing the quantification data file in the amplification workflow. The control sample names are not case-sensitive: The software will filter out these control samples, regardless of upper- or lowercase use.

    - In the **Templates** tab, use the arrows to scroll, select the desired template, then click **APPLY**.

    Note: Samples in the worklist that are not included in the plate map blink red in the worklist tab. Click **(Export Worklist)** to export a worklist for the remaining samples. The file is saved and exported to `C:\Program Files (x86)\Hamilton\Local Outputs\Run Name`, where the run name is the input selected in step 3.

11. *(Optional)* Create and save a template.

    a. Drag-and-drop samples, standards, and controls from the **Worklist** tab to the desired location of the **Output Plate Map**.

    b. Click **(Save Plate Map)**.

    c. In the **Save Current Plate Map** window, enter a template name.

    d. Use the **Template ID** dropdown list to select **Create a new template**.

    e. Click **SUBMIT**.

12. *(Optional)* To reset the plate map, click **(Restart Plate Map)**.

13. *(Optional)* To remove a sample or control from the plate map, drag-and-drop a desired target to **(Remove Target)**.

    The target is immediately removed from a plate map. To add a target back, drag-and-drop the desired target from the **Worklist** tab onto the **Output Plate Map**.

    Note: To remove the standard curve, in the **Run Settings** pane, use the **Standard Curve** dropdown list to select **No Standard Curve**. Do not drag-and-drop standards to **(Remove Target)**.

14. Click **CONTINUE**.

    The method calculates the required number of tips and reagent volumes according to the number of samples to be processed and the information you provided in the settings.

Load the deck and start the run

IMPORTANT! Due to reagent stability at room temperature, load the deck and start the run within 3 hours.

1. Place all reagents according to the diagram in the deck summary screen (see Figure 5 and Figure 6).
   a. Prepare the master mix off-deck, then place the quantification reagents on the deck with the specified volumes.

   Note: If the master mix requirement is >1,500 µL, we recommend that you prepare the master mix in a single tube, then equally split the master mix into two 1.5 mL microcentrifuge tubes (Cat. No. AM12450) before placing the tubes on the deck. Click the **Master Mix** row to view a diagram of the tubes and their contents.

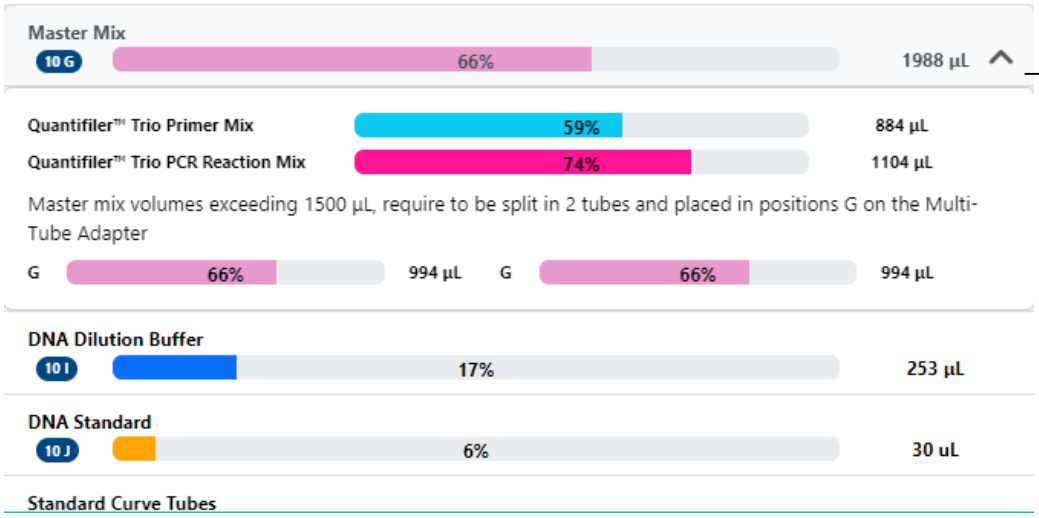


Figure 4 Reagent volumes

① Master Mix row (expanded)

   Note: Reagent percentages are calculated based on the labware on the deck.

   b. Place the pointer over each position number on the deck layout to show tips with instructions.

   c. *(Optional)* Click **Guidance Mode** to follow prompts for how to set up the deck.
   Click **NEXT** to navigate through the videos. Click **FINISH** to close **Guidance Mode**.

   Note: Due to the unavailability of videos for all scenarios, the positions shown in the guidance mode videos are not specific to every use case. To help ensure appropriate guidance for each deck position, read the instructions provided below each video.

   d. Follow the color-coding and letters to load the labware into the correct carriers and positions. When loading the labware, start from the carriers at the back.

IMPORTANT! To avoid a potential collision hazard with the instrument arm and channels, do not leave any objects on the instrument deck that are not specifically called out in the deck loading instructions. This includes de-capping tubes before clicking **CONTINUE**.

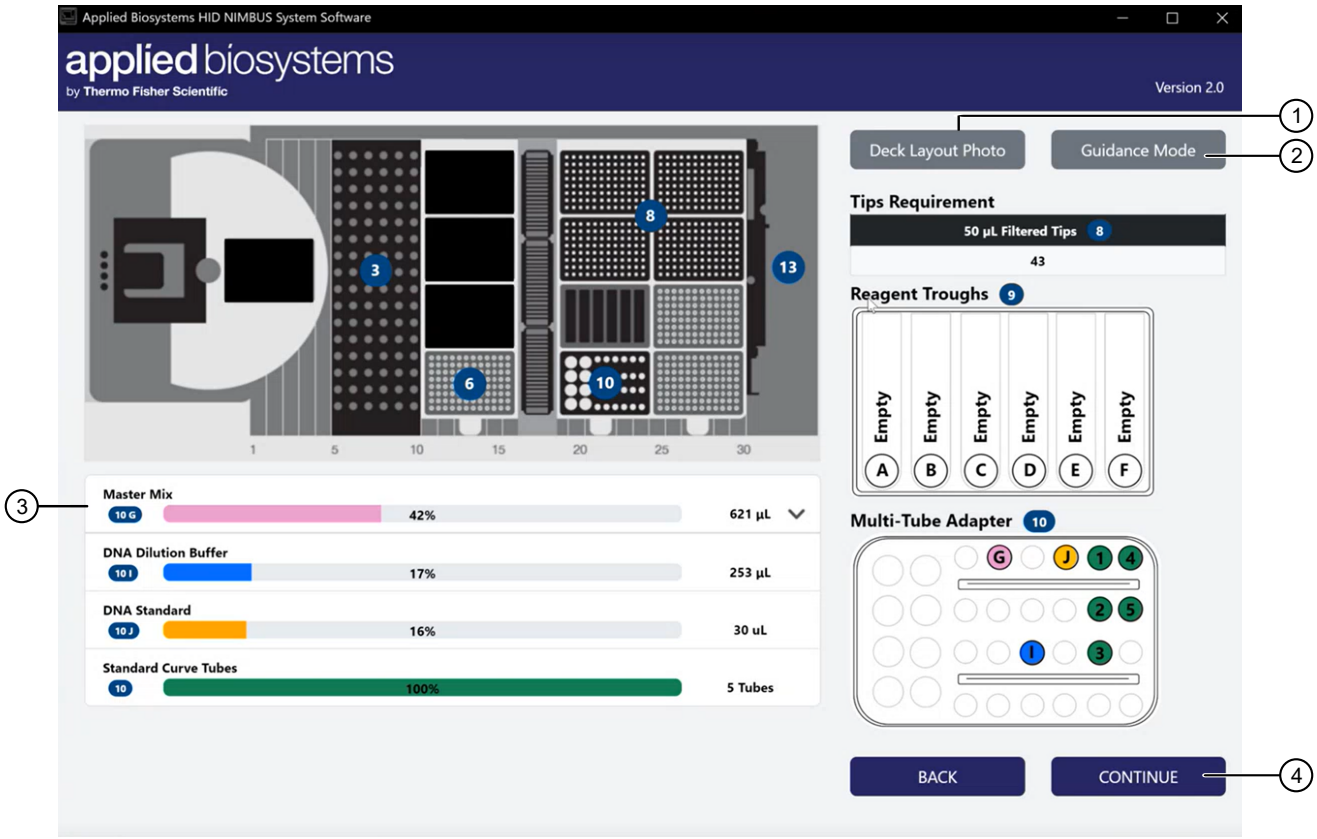


Figure 5 Example quantification deck layout with tubes as the input labware

① Change the deck layout image
② Access instructional videos
③ **Master Mix** row (expandable)
④ Continue to the next screen

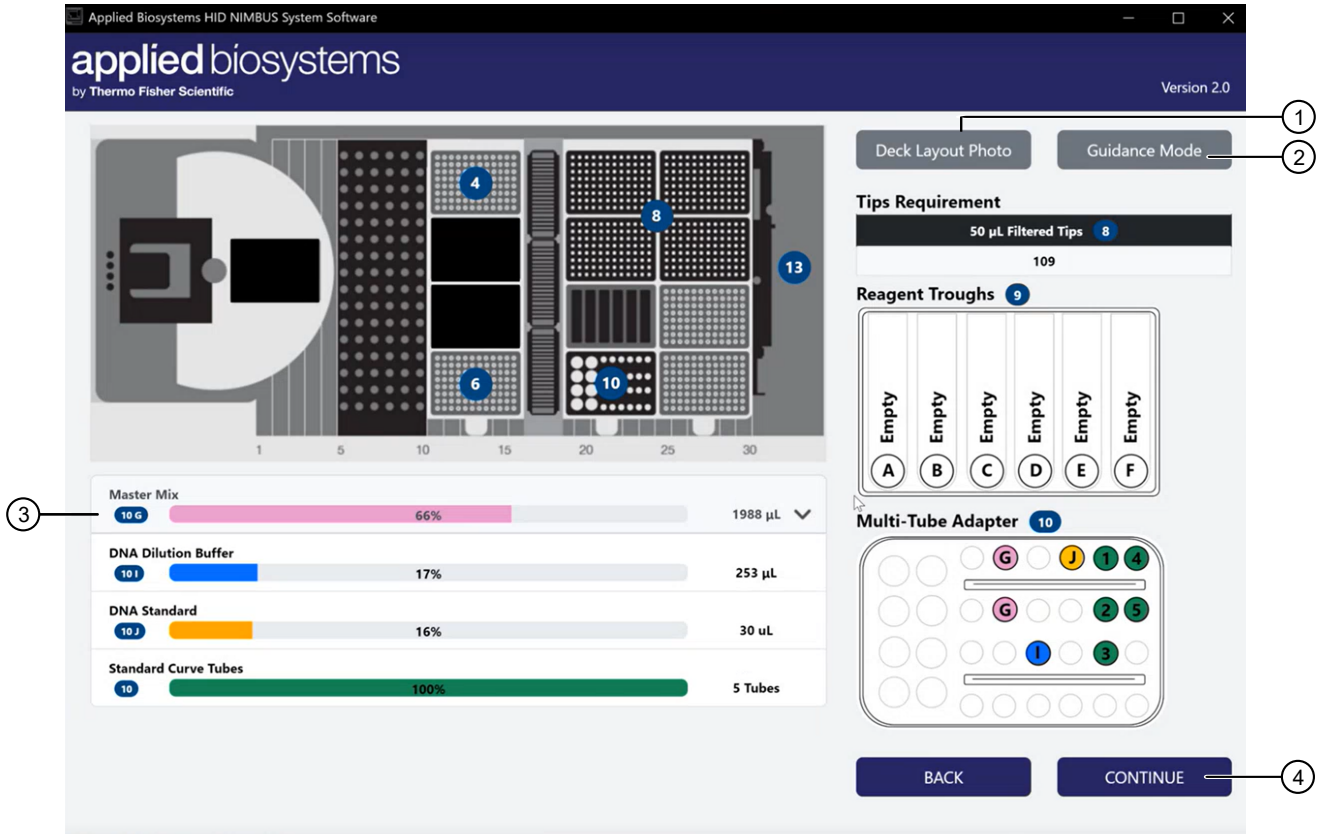


Figure 6 Example quantification deck layout with a plate as the input labware

① Change the deck layout image
② Access instructional videos
③ **Master Mix** row (expandable)
④ Continue to the next screen

2. *(Optional)* If you are using tubes as input labware, load the tubes in the appropriate order: back to front, then left to right, starting with the first carrier at track 5.

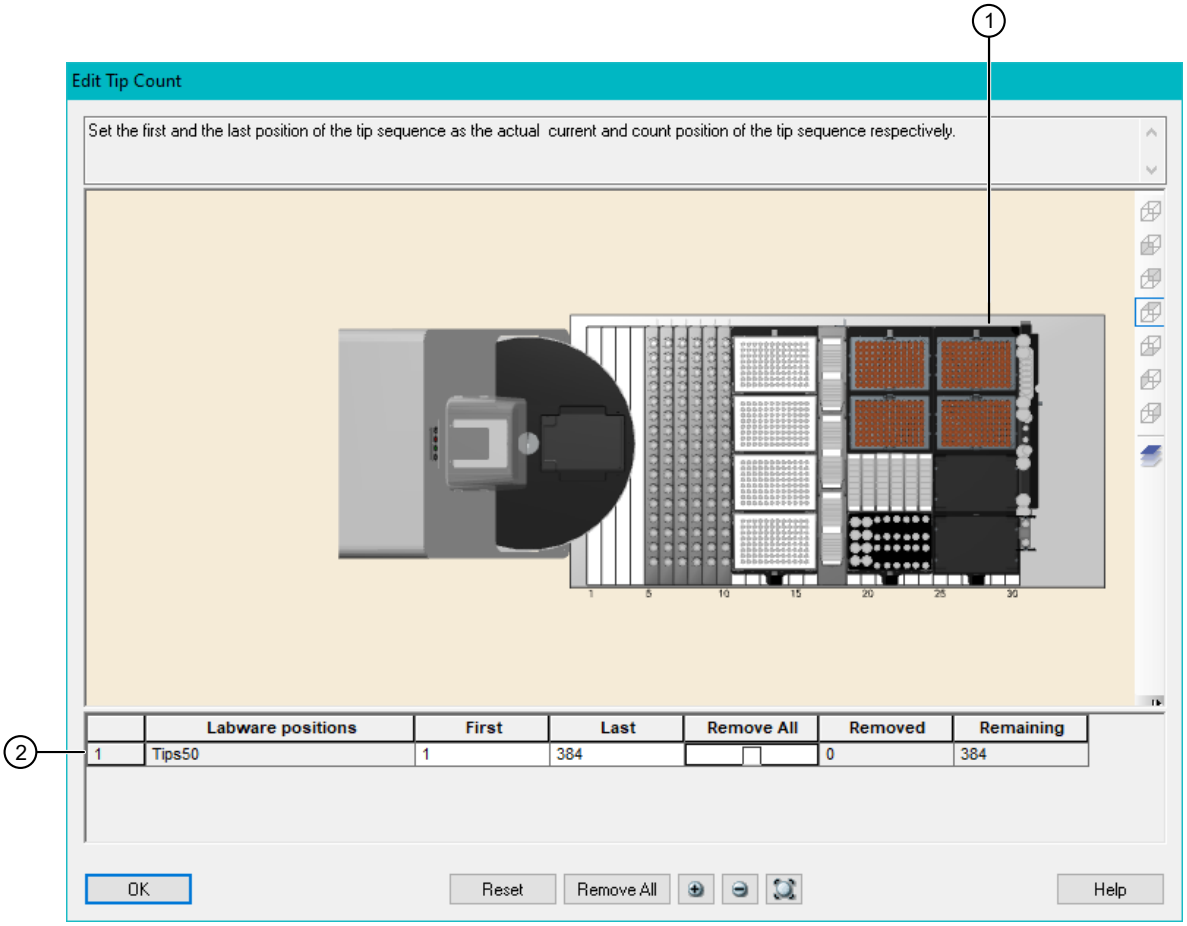
3. *(Optional)* Click **Deck Layout Photo** to change the image format.

   IMPORTANT! Follow the numbered deck layout image when loading the deck. The numbered deck layout image shows deck positions clearly and provides tips with instructions.

4. Click **CONTINUE**.

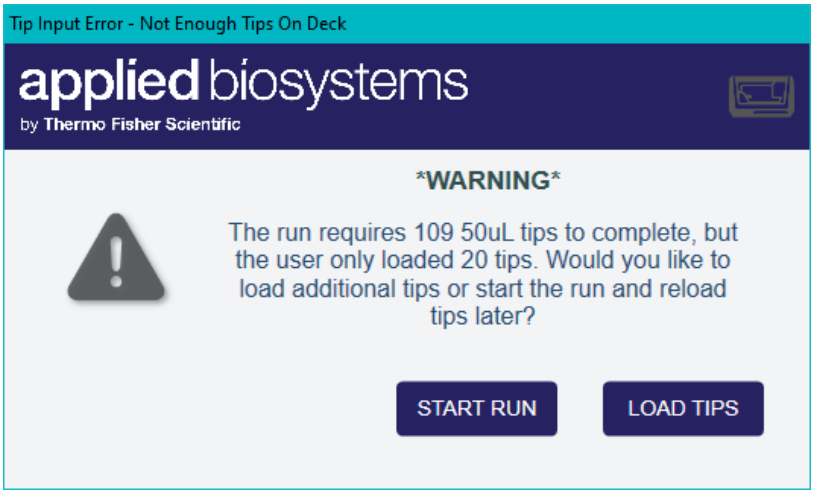
5. *(If needed)* Edit the tip counts for the 50 µL tips (position 8) to reflect the number and placement of tips on each rack. Select an action to edit the tip counts.
   - Click each position to select/deselect the appropriate wells in the diagram. Gray indicates that the well does not have a tip; orange indicates that the well does have a tip.
   - In the **First** and **Last** fields, enter the first and last tip positions.

     IMPORTANT! The total number of 50 µL tips is 384. The top left rack contains tips 1–96, the bottom left rack contains tips 97–192, the top right rack contains tips 193–288, and the bottom right rack contains tips 289–384. See Figure 5 and Figure 6.



① Diagram
② Text fields

Note: If you do not load enough tips to complete the run, you will be warned to load additional tips or start the run at your own risk. We recommend you click **LOAD TIPS** to complete the run. This will avoid pausing midway through the run to load additional tips.



6. *If barcode scanning is on,* scan the carrier and labware barcodes.

Note: The carriers should already be loaded in the correct positions on the deck.

a. At the prompt, slide the specified carrier out of position so that the barcodes can be scanned by the on-deck barcode scanner, then click **Start Scan**.

Note: The scanner reads the carrier and labware barcodes at the same time. You do not need to remove the labware from the carrier.

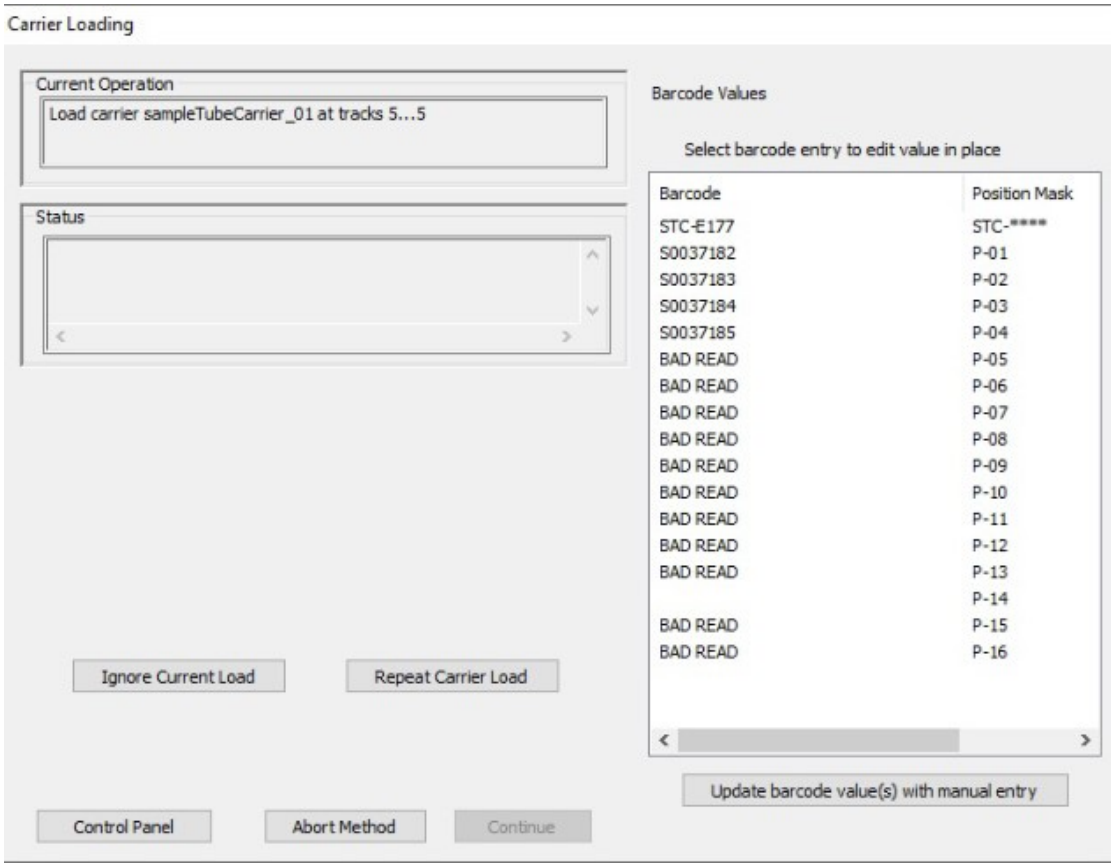


Figure 7 Barcode scanning in progress

b. Push the carrier all the way back into position, then click **Finish Scan**.

c. Repeat substep 6a and substep 6b for each carrier.

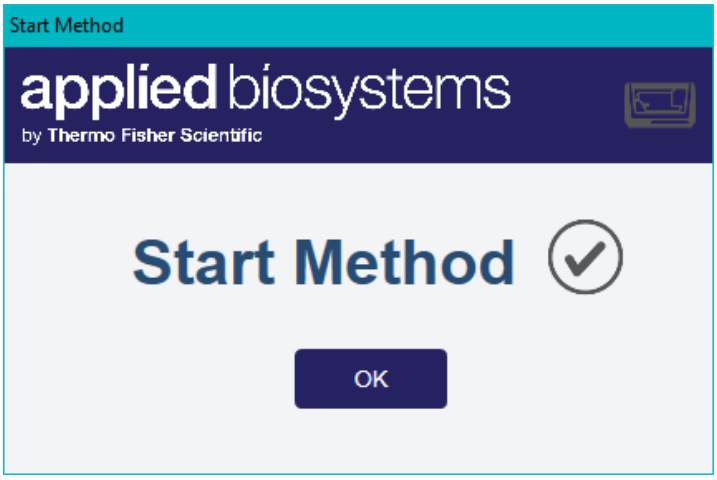
Note: If you are using an input worklist preloaded with barcode information, the system checks that your plates and/or tubes are loaded in the same positions as the worklist. If the loaded positions do not match the worklist, you are prompted to adjust the load positions or abort the run and restart with the correct worklist information.

The HID NIMBUS® System Software performs the following functions:

- **For the microcentrifuge tubes input labware**—Cross-references the scanned barcodes against the expected barcodes supplied in the worklist.
- **For the plate input labware**—Cross-references the scanned barcode against the expected barcode supplied in the worklist.

If the scanned barcode does not match the barcode that was entered into the worklist, you are prompted to correct the load position and rescan or abort the run.

7. Close the instrument door.
   The **Start Method** dialog box is displayed.



8. Click **OK**.
   The door automatically locks and the run starts.

Remove the quantification plate setup, unused reagents, and labware

1. Open the instrument door.
2. Remove the plate that contains the quantification plate setup, seal it with an optical adhesive film, then proceed to setting up the real-time PCR instrument.

   Note: The import file for the real-time instrument is generated by the HID NIMBUS® System Software. To locate the import file, navigate to:

   `C:\Program Files (x86)\Hamilton\Local Outputs\Quantification_YYYYMMDDHHMMSS`
3. Cap and store the remaining Quantifiler™ THP DNA Dilution Buffer.
4. If present on the deck, cap and store the remaining Quantifiler™ THP DNA Standard.
5. Store the low-binding tubes containing the serial dilutions of the standard curve if still within the usable range (14 days stored at 2–8°C).
6. Properly dispose of any unused master mix in the microcentrifuge tubes.

   IMPORTANT! Do not reuse the leftover master mix. Dispose of any unused volumes.
7. Empty the waste container that contains used tips.

IMPORTANT! To properly dispose of all used reagents and labware, see Appendix B, “Safety”.

WARNING! Do not use cleaning or disinfecting solutions which contain hypochlorite, such as bleach, on the instrument.

Clean the HID NIMBUS® QNA systems after each use. For detailed instructions, see the *NIMBUS HD Operator’s Manual* (Pub. No. 98538-01).

View the output files

The output files for each run include a run-tracking report and a log file. Both files are given software-generated unique identifiers.

On the HID NIMBUS® QNA system or HID NIMBUS® Presto QNA system computer, open the output file of interest, based on the run date.

Output file	File name and location	Description
Run-tracking report	QuantificationRunTracking_Quantification_Run Name.xls, located at `C:\Program Files (x86)\Hamilton\Local Outputs\Run Name`	An Excel™ file that includes the following information: • Run Information • Lot Tracking • Run Status List • Worklist • Final Sample List • Pipette Tracking • Pipette Error Report
Real-time PCR import file	Quantification_Run Name_RT.txt, located at `C:\Program Files (x86)\Hamilton\Local Outputs\Run Name`	A TXT file that includes detailed information and is used as an input for the real-time PCR instrument software.
Log file	`<unique identifier>`_Trace.trc, located at `C:\Program Files (x86)\Hamilton\LogFiles`	A TRC file that includes detailed information for each robotic step that was performed during the run.
	Quantification_Run Name_GUILog.txt, located at `C:\Program Files (x86)\Hamilton\LogFiles`	A TXT file that includes information for each instance the edit function is used to modify parameters.

3 Perform the automated DNA normalization and amplification setup run

Workflow

Prepare the automated DNA amplification plate

- **Create a worklist** (page 28)
- **Enter the run settings** (page 31)
- **Load the deck and start the run** (page 36)
- **Remove unused reagents and labware** (page 43)
- **View the output files** (page 44)

Guidelines for the automated amplification run

- The HID NIMBUS® QNA System and HID NIMBUS® Presto QNA System are only compatible with 1.5 mL tubes and 96-well PCR and deep-well plates.
- Before loading the deck, remove air bubbles from the surface of reagents and samples, as bubbles can lead to liquid detection and pipetting errors.
- When loading tubes on the deck, ensure that the tip will not come into contact with any objects, including the tube caps, to prevent false liquid detection.
- The instrument does not dispense an allelic ladder. The positions of the ladders within the plate layout are provided for traceability purposes. These plate positions are empty for CE plate setup.
- To prevent tip collisions, firmly press down all labware when loading the instrument deck.

- For information on barcode positioning, see the *NIMBUS HD Operator's Manual* (Pub. No. 98538-01).
- Before the first run of the day, we recommended that you perform daily maintenance. See *NIMBUS HD Operator's Manual* (Pub. No. 98538-01) for instructions.

Create a worklist

For an amplification run, we recommend using a worklist for the samples in XLS, XLSX, or CSV format. Templates are installed on the provided laptop computer.

Alternatively, use the worklist generated from the run-tracking report of the purification workflow. For more information about the purification workflow, see *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the HID NIMBUS® Presto Systems User Bulletin* (Pub. No. MAN0019368).

1. Before each run, navigate to the **ExampleWorklists** folder on the system computer:

   ```
   C:\Program Files (x86)\Hamilton\HID Nimbus
   System\QuantNormAmp\Resources\Example Worklists
   ```

   Note: If you cannot find the worklist templates on the computer, contact your local Thermo Fisher Scientific representative.

2. Copy the required template, then save it with a new file name.

3. In the saved copy, enter information for each sample, as shown in Figure 8 and Figure 9.

IMPORTANT! The worklist must include the required information shown in Table 5. Do not change the column headings or leave cells blank. The system only reads XLS, XLSX, or CSV formats.

Table 5 Worklist information for each sample

Required column order	Required column heading	Action
1	**Sample ID**	Enter the sample name or ID associated with the sample. The sample ID must be unique. The following characters are valid in the worklist: • 0–9 • a–z (uppercase and lowercase) **IMPORTANT!** Do not use the following special characters: **$ * ^ + { } [ ] ( ) \ / & % @** These characters interfere with quantification data processing and with Thermo Fisher™ Connect Platform applications. **IMPORTANT!** The following Sample IDs are reserved for the software: **ladder**, **PC**, and **NTC**. Do not use these words as your Sample IDs, whether uppercase or lowercase; for example, the software will filter out **NTC**, **ntc**, **NTc**, and so on. However, your Sample IDs can *contain* these words; for example, the software will accept and process **NTC_sample1**.
2	**Eluate Position**	For tubes, use numeric values. For plates, use alphanumeric values.
3	**Eluate Barcode**	If you are using barcodes, enter the expected eluate barcode for each sample lysate tube or for the MicroAmp™ PCR plate or KingFisher™ deep-well plate. • **Tubes**—Each tube has a unique barcode. • **Plates**—All sample wells of the plate have the same barcode.
4	*(Optional)* **Concentration**	Add quantitation data if a separate quantitation data file is not uploaded.

IMPORTANT! To proceed with plate setup, ensure the barcode information on the worklist matches the barcodes loaded on the instrument in the same positions. You must include barcode information in all sample lines. If barcode information is absent in one or more sample lines, you will receive a read error on import. For plates, use the same barcode for each sample. If barcodes are not used, fill the input and eluate barcode cells with ***No Barcode***.

Sample ID	Eluate Position	Eluate Barcode
Sample 1	1	No Barcode
Sample 2	2	No Barcode
Sample 3	3	No Barcode
Sample 4	4	No Barcode
Sample 5	5	No Barcode
Sample 6	6	No Barcode
Sample 7	7	No Barcode
Sample 8	8	No Barcode
Sample 9	9	No Barcode
Sample 10	10	No Barcode
Sample 11	11	No Barcode
Sample 12	12	No Barcode
Sample 13	13	No Barcode
Sample 14	14	No Barcode
Sample 15	15	No Barcode
Sample 16	16	No Barcode
Sample 17	17	No Barcode
Sample 18	18	No Barcode
Sample 19	19	No Barcode
Sample 20	20	No Barcode

Figure 8 Amplification worklist example for tubes

Sample ID	Eluate Position	Eluate Barcode
Sample 1	A1	No Barcode
Sample 2	B1	No Barcode
Sample 3	C1	No Barcode
Sample 4	D1	No Barcode
Sample 5	E1	No Barcode
Sample 6	F1	No Barcode
Sample 7	G1	No Barcode
Sample 8	H1	No Barcode
Sample 9	A2	No Barcode
Sample 10	B2	No Barcode
Sample 11	C2	No Barcode
Sample 12	D2	No Barcode
Sample 13	E2	No Barcode
Sample 14	F2	No Barcode
Sample 15	G2	No Barcode
Sample 16	H2	No Barcode
Sample 17	A3	No Barcode
Sample 18	B3	No Barcode
Sample 19	C3	No Barcode
Sample 20	D3	No Barcode

Figure 9 Amplification worklist example for plates

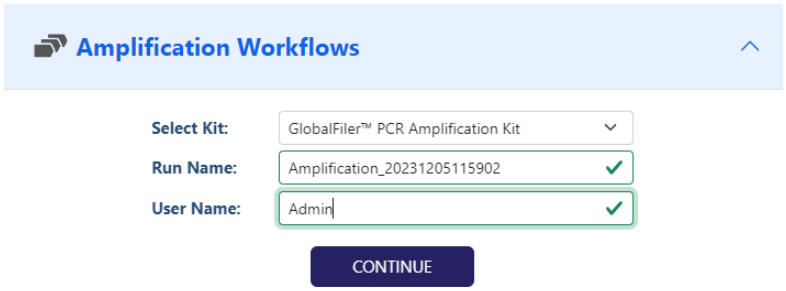
Enter the run settings

1. On the desktop, click **Applied Biosystems HID Nimbus System Software**.

   Note: If the shortcut is not present on the desktop, navigate to `C:\Program Files (x86)\Hamilton\HID Nimbus System\Application`

2. *(Optional)* Expand the **Input Catalog Number** pane, then scan or enter the PCR amplification kit package number.

3. Expand the **Amplification Workflows** pane, then enter the workflow information.



   a. Use the **Select Kit** dropdown list to select a kit.

b. Change the run name, if needed, then enter a user name.

IMPORTANT!

- The run name and user name fields cannot include special characters other than - (hyphen) and _ (underscore).
- The run name and user name must be 1–30 characters in length.

Note: The run name is prepopulated with a string in the format of the kit name, followed by the date and time (format: Amplification_YYYYMMDDhhmmss). The input run name determines the folder where the run tracking and instrument import files are saved. Use the run name field to organize files across purification, quantification, and amplification workflow runs on the HID NIMBUS® systems.

4. Click **CONTINUE**.

The settings window is displayed.

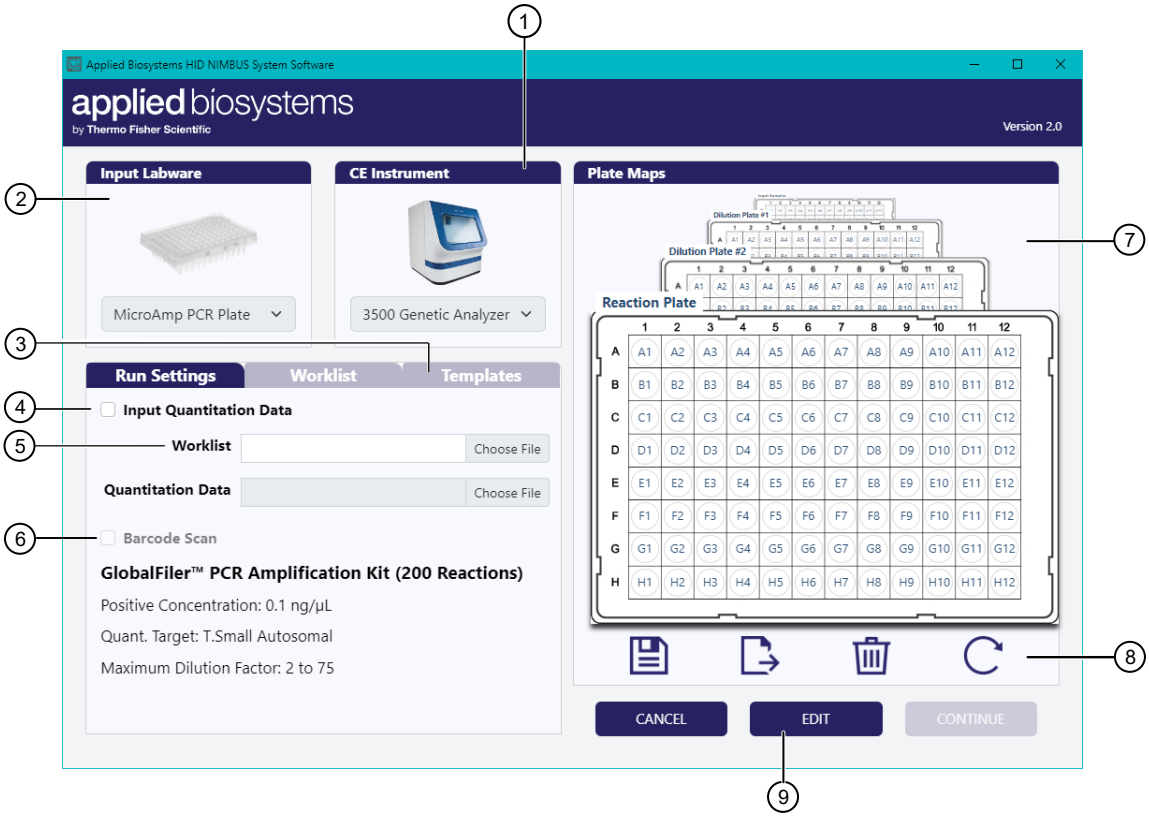


Figure 10 Settings window

1. Select an instrument
2. Select the input labware
3. Select a template
4. **Input Quantitation Data** checkbox
5. Import a worklist
6. Enable or disable barcode scanning
7. Plate maps
8. Plate map tools
9. Enable selection of input labware, instrument, and target concentration

5. Select the input labware and the instrument for the capillary electrophoresis run.

a. Click **EDIT** to unlock the user input interface.

b. In the **Parameter Modification** window, click **CONTINUE**.

c. Use the dropdown list in the **Input Labware** pane to select an option.
   - **MicroAmp PCR plate**
   - **Microcentrifuge Tubes**
   - **KingFisher Deep Well Plate**

d. Use the dropdown list in the **Instrument** pane to select an option.
   - **3500 Genetic Analyzer**
   - **3500xL Genetic Analyzer**
   - **SeqStudio Genetic Analyzer**
   - **SeqStudio 8-Flex Genetic Analyzer**
   - **SeqStudio 24-Flex Genetic Analyzer**

e. Click **SAVE** to confirm the selections.

6. In the **Run Settings** tab, select an action to enter sample information.
   - **Worklist**—Click **Choose File** or click within the **Worklist** file upload field. Select either the prepared worklist or select purification run tracking file.
   - *(Optional)* **Quantitation Data**—Select the **Input Quantitation Data** checkbox, then click **Choose File** or click within the blank file upload field, located to the right of **Quantitation Data**.

     IMPORTANT! If a quantitation data file is not uploaded, a column for quantitation must be present in the worklist. For details, see Table 5.

     Note: Quantitation data are exported from the HID Real-Time PCR Analysis Software. For information about exporting quantitation data, see the *Quantifiler™ Trio Automated DNA Quantification Kit User Guide* (Pub. No. MAN1000066).

7. *(Optional)* Enable barcode scanning.
   a. Click **EDIT** to unlock the user input interface.

   b. In the **Run Settings** pane, select the **Barcode Scan** checkbox.

   c. Click **SAVE** to confirm the selection.

   Note: When barcode scanning is on, you will be prompted to manually scan the barcodes of the labware and carriers.

8. *(Optional)* In the **Worklist** tab, adjust the overall target concentration.
   a. Click **EDIT** to unlock the user input interface.

b. Select an action to adjust the concentration.

- To adjust the target concentration for all samples, use the **Overall Target Concentration** slider scale to adjust the target concentration.
- To adjust the target concentration for individual samples, click the concentration value in the **Conc. Target** column for your sample. In the **Modify Target Concentration** window, enter a new concentration between 0.1 ng and 4.0 ng, and then click submit. Do not include units.

Note: The concentration only displays one decimal. For instance, 0.1 ng and 0.125 ng appear the same, but the calculations for the sample and diluent are different.

Note: If the sample concentration is adjusted after the samples are displayed on the plate map, you must drag-and-drop the sample from the **Reaction Plate** plate map to **(Remove)**, then drag-and-drop the sample from the **Worklist** tab to the **Reaction Plate** plate map. If the overall target concentration is adjusted, you must reapply the template to adjust all the samples. Any individual sample concentrations adjusted previously are reset.

c. Click **SAVE** to confirm the selection.

9. In the **Plate Maps** pane, select an action to set up the reaction plate.

- From the **Worklist** tab, drag-and-drop each sample, control, and ladder to the desired location in the reaction plate.
- In the **Templates** tab, use the arrows to scroll, select the desired template, then click **APPLY**.

Note:

- Templates can be created and saved. For more information, see step 11.
- If a sample concentration is less than 0.001 ng, it is displayed as zero in the software.
- The instrument is not capable of dispensing an allelic ladder. The positions of the ladders within the plate layout are provided for traceability purposes and to include allelic ladder information in the CE import file.
- When a low sample concentration is detected, ⚠ **(Low sample concentration)** is displayed to the left of the sample number in the **Worklist** tab. The sample position also appears orange in the **Worklist** tab and in the plate maps.

10. *(Optional)* To remove a sample, ladder, or control from the plate map, drag-and-drop a desired target to **(Remove)**.

The sample is immediately removed from a plate map. To add a sample back, drag-and-drop the desired sample from the **Worklist** tab to the reaction plate.

11. *(Optional)* Create and save a template.

a. Drag-and-drop samples and controls from the **Worklist** tab to the **Output Plate Map**.

Note: In the **Worklist** tab, samples that are not included in the reaction plate blink red. Click **(Export Worklist)** to export a worklist for the samples that are not included. The file is saved and exported to `C:\Program Files (x86)\Hamilton\Local Outputs\Run Name`.

b. Click **(Save Plate Map)**.

c. In the **Save Current Plate Map** window, enter a template name.

d. Use the **Template ID** dropdown list to select **Create a new template**.

e. Click **SUBMIT**.

12. *(Optional)* To reset the plate map, click **(Restart Plate Map)**.

13. In the **Plate Maps** pane, view or adjust the plate maps.
    - **Input Samples**
    - **Dilution Plate #1**
    - **Dilution Plate #2**
    - **Reaction Plate**

    Note: Click a plate to bring it to the front of the stacked plate maps.

    Note: Place the pointer over each sample number in **Dilution Plate #1** and **Dilution Plate #2** to view the sample and diluent volumes. Place the pointer over each sample number and control in the **Reaction Plate** to view the sample volume, diluent volume, and target concentration.

14. Click **CONTINUE**.
    The method calculates the required number of tips and reagent volumes according to the number of samples to be processed and the information you provided in the **Run Settings** window.

Load the deck and start the run

IMPORTANT! Due to reagent stability at room temperature, load the deck and start the run within 4 hours.

1. Place all the reagents on the deck according to the diagram in the **Deck Summary** screen (see Figure 12 and Figure 13).
   a. Prepare the master mix off-deck, then place the amplification reagents on the deck with the specified volumes.

   Note: If prompted to load two master mix tubes, we recommend that you prepare the master mix in a single tube, then split into two 1.5 mL microcentrifuge tubes (Cat. No. AM12450) before placing the tubes on the deck. Click the **Master Mix** row to view a diagram of the tubes and their contents.

   IMPORTANT! Do not overfill the reagent reservoirs.

   IMPORTANT! Keep the primer mix and the PCR reaction mix protected from direct exposure to light. Excessive exposure to light can affect the fluorescent probes and/or the passive reference dye.

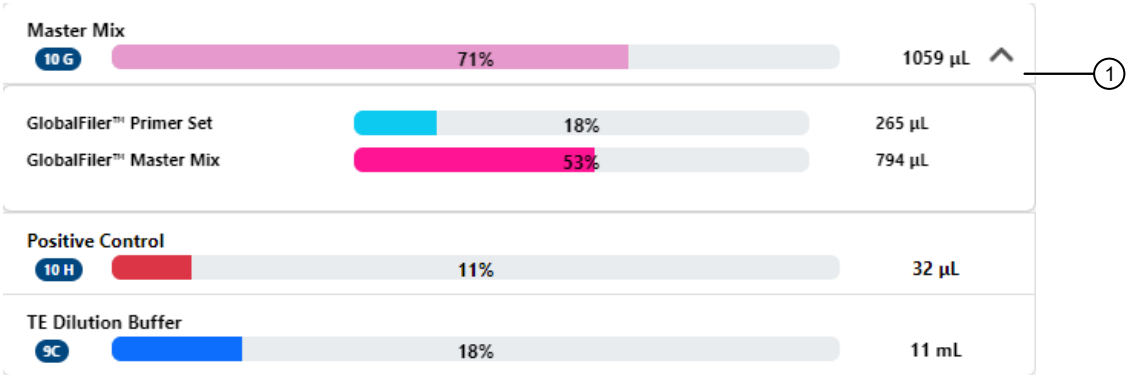


Figure 11 Reagent volumes

① Master Mix row (expanded)

Note: Reagent percentages are calculated based on the labware on the deck.

   b. Fill the 60-mL trough with the specified volume of low TE dilution buffer and place it on deck.

   c. Place the pointer over each position number on the deck layout to show tips with instructions.

   d. *(Optional)* Click **Guidance Mode** to follow prompts for how to set up the deck.
   Click **NEXT** to navigate through the videos. Click **FINISH** to close **Guidance Mode**.

   Note: Due to the unavailability of videos for all scenarios, the positions shown in the guidance mode videos are not specific to every use case. To help ensure appropriate guidance for each deck position, read the instructions provided below each video.

e. Follow the color-coding and letters to load the labware into the correct carriers and positions. When loading the labware, start from the carriers at the back.

f. After all reagents and troughs are filled and placed, click **CONTINUE**.

IMPORTANT! To avoid a potential collision hazard with the instrument arm and channels, do not leave any objects on the instrument deck that are not specifically called out in the deck loading instructions. This includes removing trough lids and de-capping tubes prior to clicking **CONTINUE**.

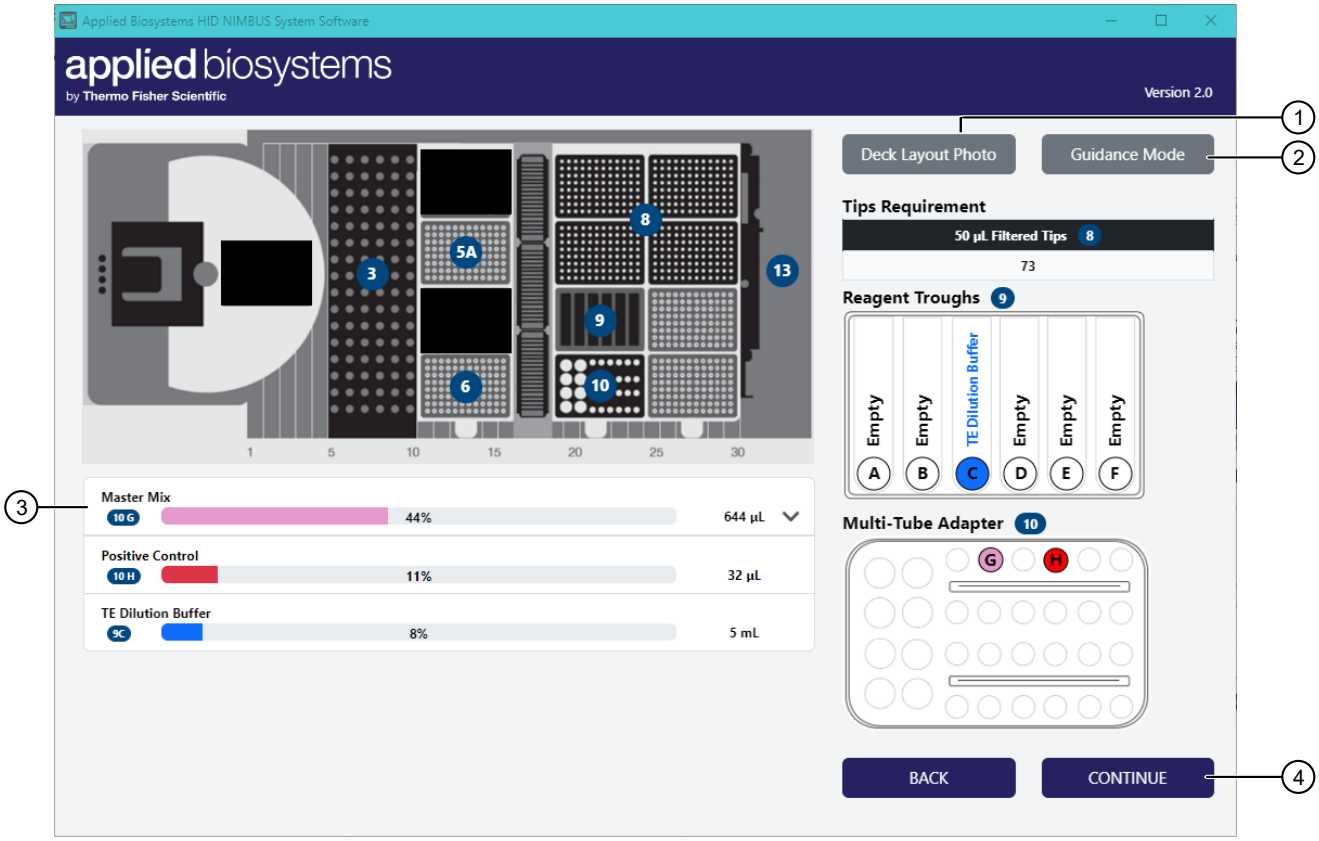


Figure 12 Example amplification deck layout with tubes as the input labware

① Change the deck layout image
② Access instructional videos
③ **Master Mix** row (expandable)
④ Continue to the next screen

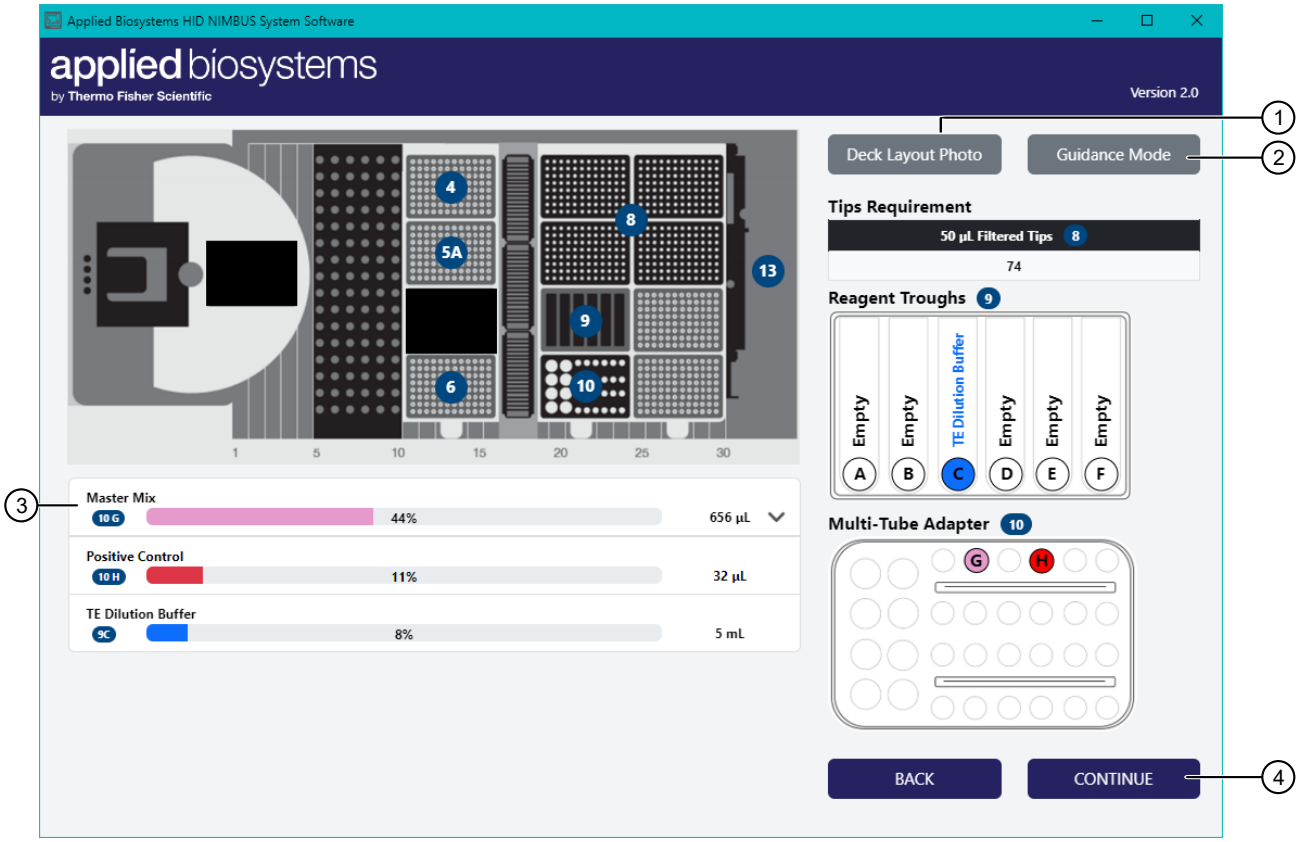


Figure 13 Example amplification deck layout with a plate as the input labware

① Change the deck layout image
② Access instructional videos
③ **Master Mix** row (expandable)
④ Continue to the next screen

2. *(Optional)* If you are using tubes as the input labware, load the tubes in the appropriate order: back to front, then left to right, starting with the first carrier at track 5.

3. *(Optional)* Click **Deck Layout Photo** to change the image format.

   IMPORTANT! Follow the numbered deck layout image when loading the deck. The numbered deck layout image shows deck positions clearly and provides tips with instructions.

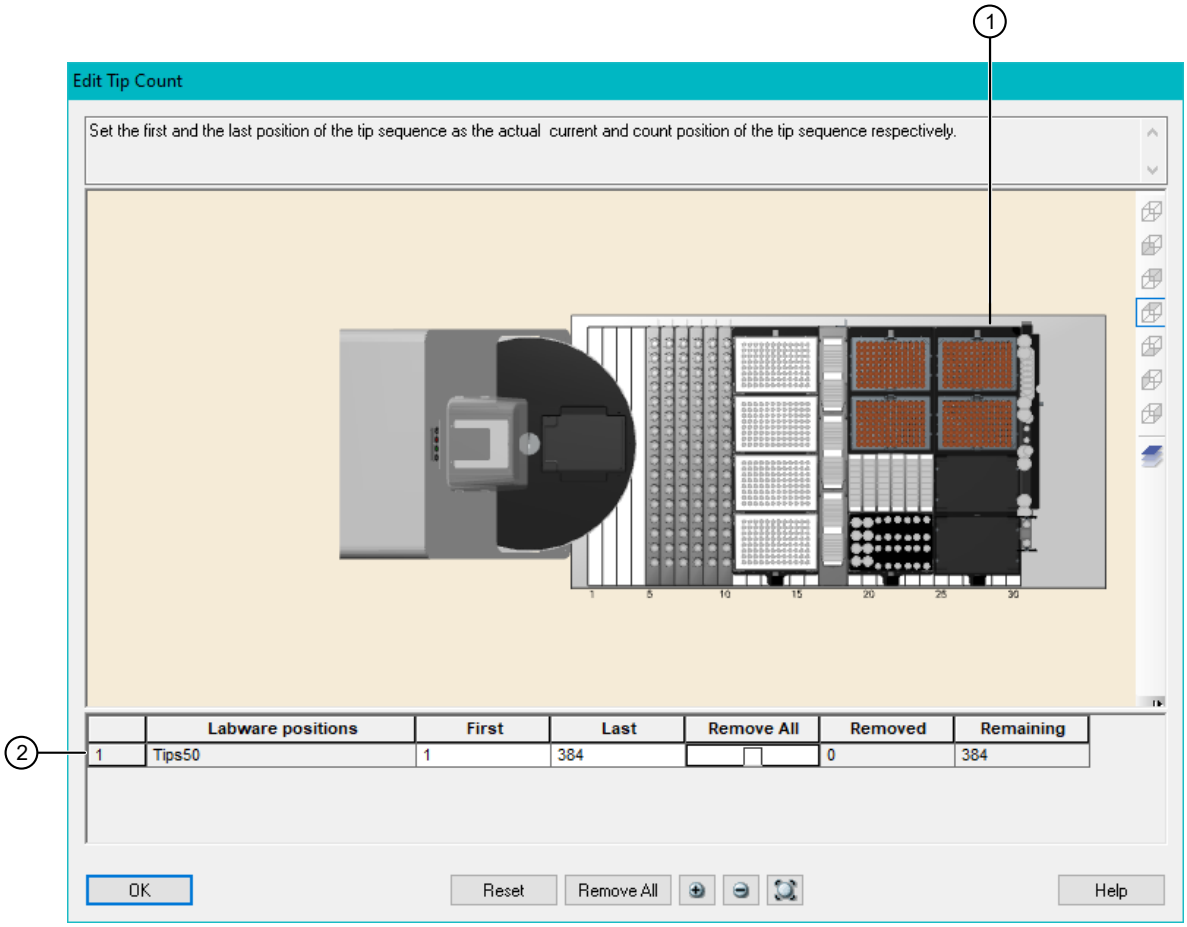
4. Click **CONTINUE**.

   IMPORTANT! If the quantitation data contain samples with **UNDETERMINED** as the C_T value, do not click the **BACK** button on this screen. Clicking **BACK** causes the quantity to be read as **NULL**, and the robot will not transfer the sample to the PCR plate.

   If you do click **BACK**, you will need to click **CANCEL**, then reload the worklist and quantitation data files.

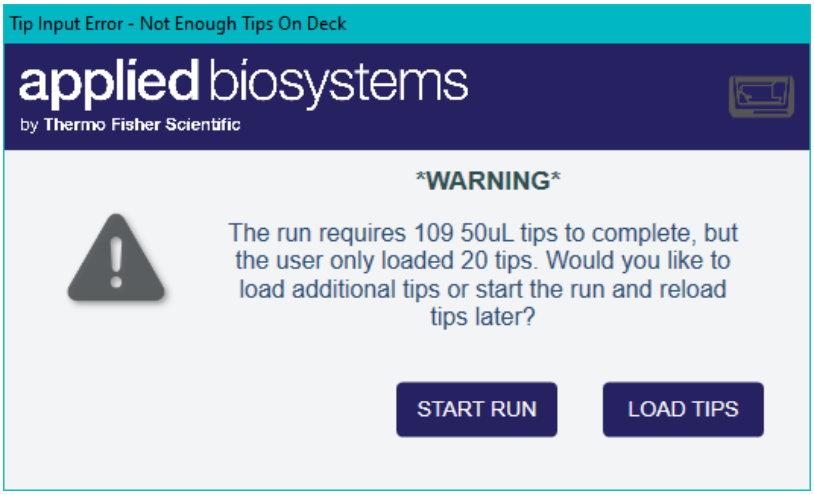
5. *(If needed)* Edit the tip counts for the 50 µL tips (position 8) to reflect the number and placement of tips on each rack. Select an action to edit the tip counts.
   - Click each position to select/deselect the appropriate wells in the diagram. Gray indicates that the well does not have a tip; orange indicates that the well does have a tip.
   - In the **First** and **Last** fields, enter the first and last tip positions.

   IMPORTANT! The total number of 50 µL tips is 384. The top left rack contains tips 1–96, the bottom left rack contains tips 97–192, the top right rack contains tips 193–288, and the bottom right rack contains tips 289–384. See Figure 12 and Figure 13.



① Diagram
② Text fields

Note: If you do not load enough tips to complete the run, you will be warned to load additional tips or start the run at your own risk. To avoid pausing midway through the run to load additional tips, we recommend that you click **LOAD TIPS**.


Tip Input Error - Not Enough Tips On Deck
applied biosystems
by Thermo Fisher Scientific
WARNING
The run requires 109 50uL tips to complete, but the user only loaded 20 tips. Would you like to load additional tips or start the run and reload tips later?
START RUN
LOAD TIPS

6. *If barcode scanning is on,* scan the carrier and labware barcodes.

Note: The carriers should already be loaded in the correct positions on the deck.

a. At the prompt, slide the specified carrier out of position so that the barcodes can be scanned by the on-deck barcode scanner, then click **Start Scan**.

Note: The scanner reads the carrier and labware barcodes at the same time. You do not need to remove the labware from the carrier.

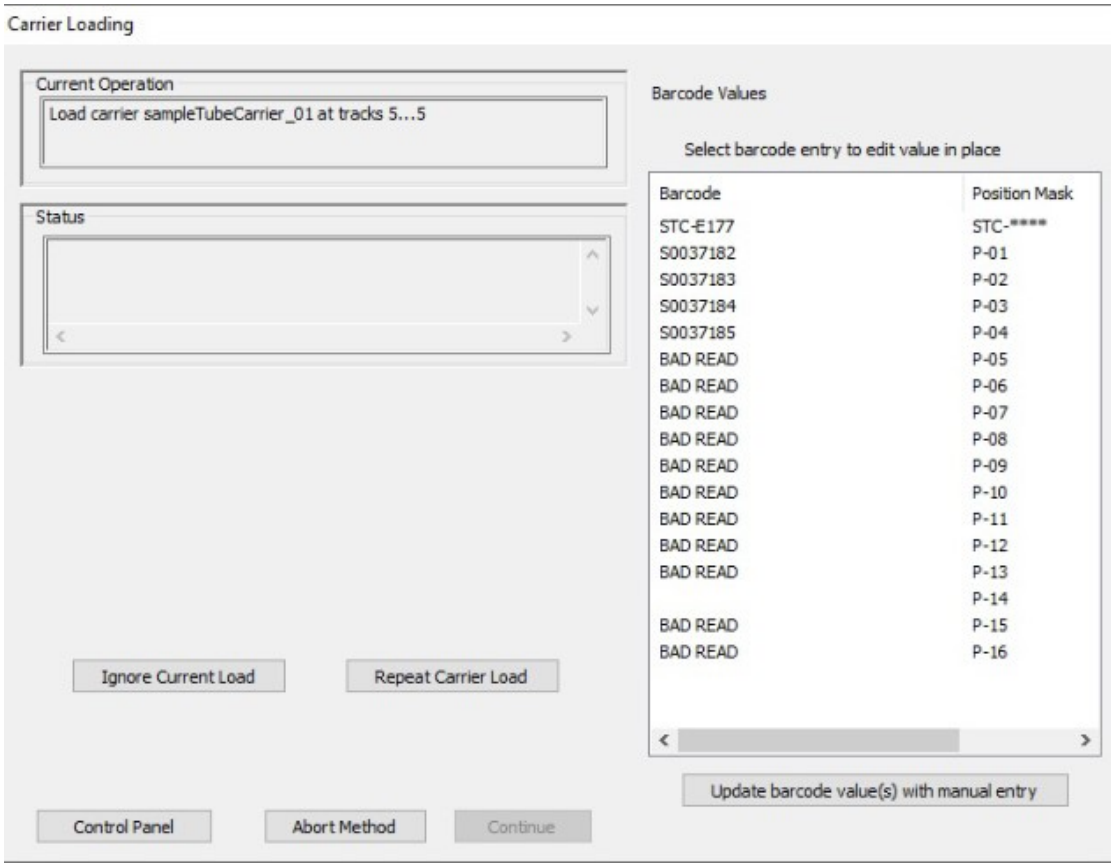


Figure 14 Barcode scanning in progress

b. Push the carrier all the way back into position, then click **Finish Scan**.

c. Repeat substep 6a and substep 6b for each carrier.

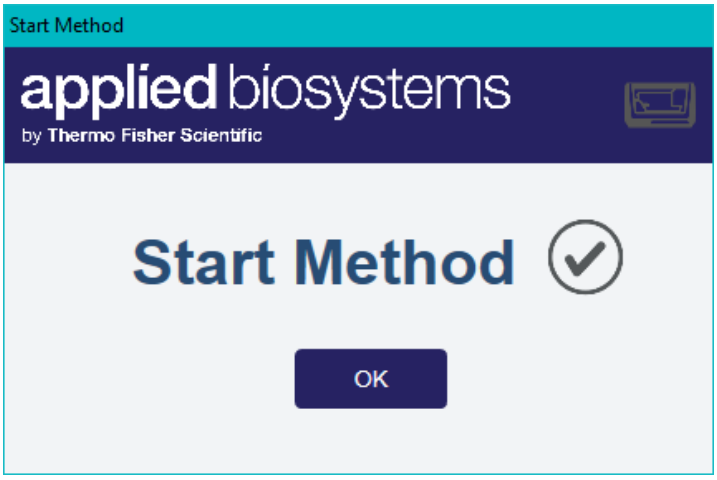
Note: If you are using an input worklist preloaded with barcode information, the system checks that your plates and/or tubes are loaded in the same positions as the worklist. If the loaded positions do not match the worklist, you are prompted to adjust the load positions or abort the run and restart with the correct worklist information.

The HID NIMBUS® System Software performs the following functions:

- **For the microcentrifuge tubes input labware**—Cross-references the scanned barcodes against the expected barcodes supplied in the worklist.
- **For the plate input labware**—Cross-references the scanned barcode against the expected barcode supplied in the worklist.

If the scanned barcode does not match the barcode that was entered into the worklist, you are prompted to correct the load position and rescan or abort the run.

7. Close the instrument door.
   The **Start Method** dialog box is displayed.



8. Click **OK**.
   The door automatically locks and the run starts.

Remove unused reagents and labware

1. Open the instrument door.
2. Remove the plate that contains the amplification plate setup, then proceed to perform the PCR.

   Note: The import file for the capillary electrophoresis instrument is generated by the HID NIMBUS® System Software. To locate the import file, navigate to:

   `C:\Program Files (x86)\Hamilton\Local Outputs\Run Name`
3. Properly dispose of any unused buffer in the reagent troughs.

   IMPORTANT! Do not reuse the reagents in the reagent troughs.
4. *(Recommended)* Discard used reagent reservoirs after each run. Use new reservoirs for each run.
5. Properly dispose of any unused master mix in the microcentrifuge tubes.

   IMPORTANT! Do not reuse the leftover master mix. Dispose of any unused volumes.
6. Empty the waste container that contains used tips.

IMPORTANT! To properly dispose of all used reagents and labware, see Appendix B, “Safety”.

Clean the HID NIMBUS® systems after each use. For detailed instructions, see the *NIMBUS HD Operator’s Manual* (Pub. No. 98538-01).

View the output files

The output files for each run include a run-tracking report and a log file. Both files are given software-generated unique identifiers.

1. On the HID NIMBUS® System Software computer, navigate to `C:\Program Files (x86)\Hamilton\Local Outputs\Amplification_YYYYMMDDHHMMSS`
2. Open the output file of interest, based on the run date.

Output file	File name	Description
Run-tracking report	AmplificationRunTracking_Amplification_YYYYMMDDHHMMSS.xls	An Excel™ file that includes the following information: • Run Information • Lot Tracking • Run Status List • Worklist • Final Sample List • Pipette Tracking • Pipette Error Report
	Amplification_YYYYMMDDHHMMSS_CE.csv	A CSV file that includes detailed information and is used as an input for the CE instrument.
Log file	`<unique identifier>`_Trace.trc, available at `C:\Program Files (x86)\Hamilton\LogFiles`	A TRC file that includes detailed information for each robotic step that was performed during the run.

4 Experiments and results

Objective of the validation

The objective of the validation studies was to demonstrate the capability and reliability of the HID NIMBUS® Presto QNA System (including the v1.2.2 software scripts) to effectively streamline the quantification and amplification workflows. The amplification workflow includes a normalization step to adjust the DNA input to the desired amount.

Note: The validation studies performed on the HID NIMBUS® Presto QNA System are also applicable to the HID NIMBUS® QNA System.

This chapter summarizes the results of the developmental validation studies that we conducted to assess the performance of the HID NIMBUS® Presto QNA System with the Quantifiler™ Trio Automated DNA Quantification Kit and the following PCR amplification kits:

- GlobalFiler™ IQC PCR Amplification Kit
- NGM Detect™ PCR Amplification Kit
- VeriFiler™ Plus PCR Amplification Kit
- Yfiler™ Plus PCR Amplification Kit

Validation studies

We performed the following validation studies according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) *Validation Guidelines for DNA Analysis Methods* (December 2016) and the FBI Quality Assurance Standards (July 2020):

- Cross-contamination
- Precision and accuracy
- Correlation

These studies integrate the developmental validation studies performed for the kits listed in Table 6. The validation studies for each kit are described in the user guides referenced in the table.

Table 6 Integrated developmental validation studies

Kit	User guide
Quantifiler™ HP and Trio DNA Quantification Kits	*Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* (Pub. No. 4485354)
GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits	*GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits User Guide* (Pub. No. 4477604)
NGM Detect™ PCR Amplification Kit	*NGM Detect™ PCR Amplification Kit User Guide*(Pub. No. 100044085)
VeriFiler™ Plus PCR Amplification Kit	*VeriFiler™ Plus PCR Amplification Kit User Guide* (Pub. No. MAN0017493)
Yfiler™ Plus PCR Amplification Kit	*Yfiler™ Plus PCR Amplification Kit User Guide* (Pub. No. 4485610)

Performance comparison

We also conducted a performance comparison of the HID NIMBUS® Presto QNA System automated workflows to manual workflows, as follows:

- Quantification workflow—Yield
- Amplification workflow—Genotype accuracy, intra-color balance, intra-locus balance, and average peak height

Materials used in the validation

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Table 7 Automated qPCR

Item	Source
Quantifiler™ Automation Enhancer	A31287
Quantifiler™ Trio DNA Quantification Kit	4482910
HID Real-Time PCR Analysis Software v1.3 or v1.4	A31150 (v1.3) A78901 (v1.4)
QuantStudio™ 5 Real-Time PCR System for Human Identification, 96-well, 0.2 mL, desktop	A34322
Hamilton® CO-RE® II Conductive Filter Tips, non-sterile, 50 µL	A58978
RNase-Free Microfuge Tube (1.5 mL)	AM12450

Table 7 Automated qPCR *(continued)*

Item	Source
MicroAmp™ Optical Adhesive Film	4360954
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
HID NIMBUS® Presto QNA System	A55769
HID NIMBUS® System Software	A58846

Table 8 Automated PCR

Item	Source
GlobalFiler™ IQC PCR Amplification Kit (200 reactions)	A43565
VeriFiler™ Plus PCR Amplification Kit (200 reactions)	A35495
NGM Detect™ PCR Amplification Kit (200 reactions)	A31832
Yfiler™ Plus PCR Amplification Kit (500 reactions)	4482730
DNA Suspension Buffer (Tris 10 mM, EDTA 0.1 mM) pH 8.0	MLS
Hamilton® CO-RE® II Conductive Filter Tips, non-sterile, 50 µL	A58978
Hamilton® Reagent Reservoirs, 60 mL	56694-01
RNase-Free Microfuge Tube (1.5 mL)	AM12450
MicroAmp™ Optical Adhesive Film	4360954
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
HID NIMBUS® Presto QNA System	A55769
HID NIMBUS® System Software	A58846
ProFlex™ 2 x 96-well PCR System	4484076

Table 9 Capillary electrophoresis

Item	Source
3500xL Genetic Analyzer for Human Identification	4406016
(Software upgrade package, includes the v4.0.1 patch) 3500 Series HID Data Collection Software v4.0.1	A46085
3500 Series Data Collection Software v4.0.2 Patch Installer	N/A
GeneMapper™ *ID-X* Software v1.6	A39975
DS-36 Matrix Standard	4425042
DS-37 Matrix Standard	A31234

Table 9 Capillary electrophoresis *(continued)*

Item	Source
GeneScan™ 600 LIZ™ Size Standard v2.0	4408399
Hi-Di™ Formamide	4311320
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
Plate Septa, 96-well	4315933
Anode Buffer Container 3500/Flex Series	4393927
Cathode Buffer Container 3500/Flex Series	4408256
3500xL Genetic Analyzer 24-Capillary Array, 36 cm	4404687
POP-4™ (960) Performance Optimized Polymer	4393710

Validation protocols

The validation studies described in this chapter were performed on the HID NIMBUS® Presto QNA System with the v1.2.2 software scripts for quantification and amplification.

The HID NIMBUS® QNA System and the HID NIMBUS® Presto QNA System share the same deck carrier components as the HID NIMBUS® Presto System. However, deck loading varies for the two layouts. Reagent tubes and reservoirs are loaded differently in the carrier positions, depending on the run selections made by the user. Similarly, plates loaded in the plate carrier at track 11 vary, depending on user selections and initial sample concentrations requiring normalization.

1. **Sample purification:**
   - **Lysis**—Lysis was performed manually in PrepFiler™ LySep™ Columns with PrepFiler™ Lysis Buffer from the PrepFiler™ Automated Forensic DNA Extraction Kit, according to the *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits User Guide* (Pub. No. 4463349).
   - **DNA purification and elution**—DNA from 1 µL of blood and 2 µL of saliva on swabs were processed with the HID NIMBUS® Presto QNA System, according to the *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the HID NIMBUS® Presto Systems User Bulletin* (Pub. No. MAN0019368).
2. **qPCR**—DNA was quantified using the HID NIMBUS® Presto QNA System quantification workflow with the Quantifiler™ Trio DNA Quantification Kit and a QuantStudio™ 5 Real-Time PCR System for Human Identification.
3. **PCR**—Quantified DNA was normalized and the amplification plate set up using the HID NIMBUS® Presto QNA System amplification workflow, targeting the recommended total DNA input for each PCR amplification kit used according to the corresponding kit user guide. Samples with suboptimal DNA input were amplified at the maximum input obtained using 15 µL of the sample eluate. A ProFlex™ 2 x 96-well PCR System was used to perform the PCR.

4. **Capillary electrophoresis and data analysis**—Capillary electrophoresis was performed on a 3500xL Genetic Analyzer for Human Identification with 3500 Series Data Collection Software v4.0.2 Patch Installer. The data was analyzed with GeneMapper™ *ID-X* Software v1.6. Both steps were performed according to the corresponding kit user guide, with the following parameters:
   - HID36_POP4xl run module
     - 1.2 kV/20 second injection for the NGM Detect™ PCR Amplification Kit
     - 1.2 kV/24 second injection for all other PCR amplification kits
   - Peak Amplitude Threshold (PAT)
     - 50 and 100 RFU for all dye channels for the cross-contamination study
     - 175 RFU for all dye channels for all other studies

Cross-contamination study

Method

To confirm that the HID NIMBUS® Presto QNA System does not introduce cross-contamination during automated quantification and amplification setup, we performed several runs on two different robots using a checkerboard pattern plate layout of the following samples and reagent blanks:

- Quantification workflow (Figure 15):
  - 42 donor DNA wells containing 20 ng/µL of male 007 (M007) DNA
  - 42 TE buffer reagent blank (RB) wells
- Amplification workflow (Figure 16 and Figure 17):
  - 43 or 44 donor DNA wells containing 20 ng/µL of M007 DNA (Yfiler™ Plus PCR Amplification Kit) or 10 ng/µL of M007 DNA (all other PCR amplification kits); normalized to the recommended input target concentration during the amplification setup
  - 46 or 47 TE buffer reagent blank (RB) wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	M007.4	RB8	M007.12	RB16	M007.20	RB24	M007.28	RB32	M007.36	RB40
B	S2	S2	RB4	M007.8	RB12	M007.16	RB20	M007.24	RB28	M007.32	RB36	M007.40
C	S3	S3	M007.5	RB9	M007.13	RB17	M007.21	RB25	M007.29	RB33	M007.37	RB41
D	S4	S4	RB5	M007.9	RB13	M007.17	RB21	M007.25	RB39	M007.33	RB37	M007.41
E	S5	S5	M007.6	RB10	M007.14	RB18	M007.22	RB26	M007.30	RB34	M007.38	RB42
F	RB1	M007.2	RB6	M007.10	RB14	M007.18	RB22	M007.26	RB30	M007.34	RB38	M007.42
G	M007.1	RB3	M007.7	RB11	M007.15	RB19	M007.23	RB27	M007.31	RB35	M007.39	Negative control
H	RB2	M007.3	RB7	M007.11	RB15	M007.19	RB23	M007.27	RB31	M007.35	RB39	Negative control

Figure 15 Quantification workflow—Cross-contamination study checkerboard pattern. Plate layout used for the cross-contamination study: 42 RB wells and 42 high-concentration M007 DNA wells, arranged in a checkerboard pattern in the HID NIMBUS® Presto QNA System processing plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	RB5	M007.8	RB13	M007.15	RB21	M007.23	RB29	M007.30	RB37	M007.38	RB44
B	RB.1	M007.4	RB9	Ladder	RB17	M007.19	RB25	M007.26	RB33	M007.34	RB40	M007.42
C	M007.1	RB6	M007.9	RB14	M007.16	RB22	Ladder	RB30	M007.31	RB38	M007.39	RB45
D	RB2	M007.5	RB10	M007.12	RB18	M007.20	RB26	M007.27	RB34	M007.35	RB41	M007.43
E	M007.2	RB7	M007.10	RB15	M007.17	RB23	M007.24	RB31	M007.32	Ladder	m007.40	RB46
F	RB3	M007.6	RB11	M007.13	RB19	m007.21	RB27	M007.28	RB35	m007.36	RB42	M007.44
G	M007.3	RB8	M007.11	RB16	M007.18	RB24	m007.25	RB32	m007.33	RB39	M007.41	Positive control
H	RB4	M007.7	RB12	M007.14	RB20	M007.22	RB28	m007.29	RB36	M007.37	RB43	Negative control

Figure 16 Amplification workflow—Cross-contamination study checkerboard pattern. Plate layout used for the cross-contamination study: 46 RB wells and 44 high-concentration M007 DNA wells, arranged in a checkerboard pattern in the HID NIMBUS® Presto QNA System processing plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	RB5-8	M007.8	RB13	M007.15	RB21	M007.23	RB29	M007.30	RB37	M007.37	RB45
B	RB.1	M007.4	RB9	Ladder	RB17	M007.19	RB25	M007.26	RB33	M007.34	RB41	M007.41
C	M007.1	RB6	M007.9	RB14	M007.16	RB22	Ladder	RB30	M007.31	RB38	M007.38	RB46
D	RB2	M007.5	RB10	M007.12	RB18	M007.20	RB26	M007.27	RB34	Ladder	RB42	M007.42
E	M007.2	RB7	M007.10	RB15	M007.17	RB23	M007.24	RB31	M007.32	RB39	m007.39	RB47
F	RB3	M007.6	RB11	M007.13	RB19	m007.21	RB27	M007.28	RB35	m007.35	RB43	M007.43
G	M007.3	RB8	M007.11	RB16	M007.18	RB24	m007.25	RB32	m007.33	RB40	M007.40	Positive control
H	RB4	M007.7	RB12	M007.14	RB20	M007.22	RB28	m007.29	RB36	M007.36	RB44	Negative control

Figure 17 Amplification workflow—Cross-contamination study checkerboard pattern. Plate layout used for the cross-contamination study for the VeriFiler™ Plus PCR Amplification Kit: 47 RB wells and 43 high-concentration M007 DNA wells, arranged in a checkerboard pattern in the HID NIMBUS® Presto QNA System processing plate.

Results

Testing for the presence of DNA by qPCR quantification assay

Analysis of the TE buffer reagent blank (RB) eluates for indications of human DNA cross-contamination using the Quantifiler™ Trio DNA Quantification Kit showed the following:

- All RBs and the negative controls returned undetermined C_T values, which indicates no contamination in these wells.
- Internal PCR Control (IPC) values for the RBs, negative controls, and M007 DNA fell within the expected range for samples with no inhibition (data not shown).
- The M007 DNA values were consistent with a concentration approximating 20 ng/µL.

Testing for the presence of DNA by allele activity with the PCR amplification assay

To further assess the potential for human DNA cross-contamination, we used the HID NIMBUS® Presto QNA System to set up the amplification plate (46 RB wells and 44 M007 DNA wells), used PCR amplification kits and a 3500xL Genetic Analyzer for Human Identification to perform amplification, then analyzed the results with GeneMapper™ *ID-X* Software v1.6. To detect allele activity, we used peak amplitude thresholds of 175, 100, and 50 RFU.

GlobalFiler™ IQC PCR Amplification Kit

No allele peaks >50 RFU were detected in any RB wells.

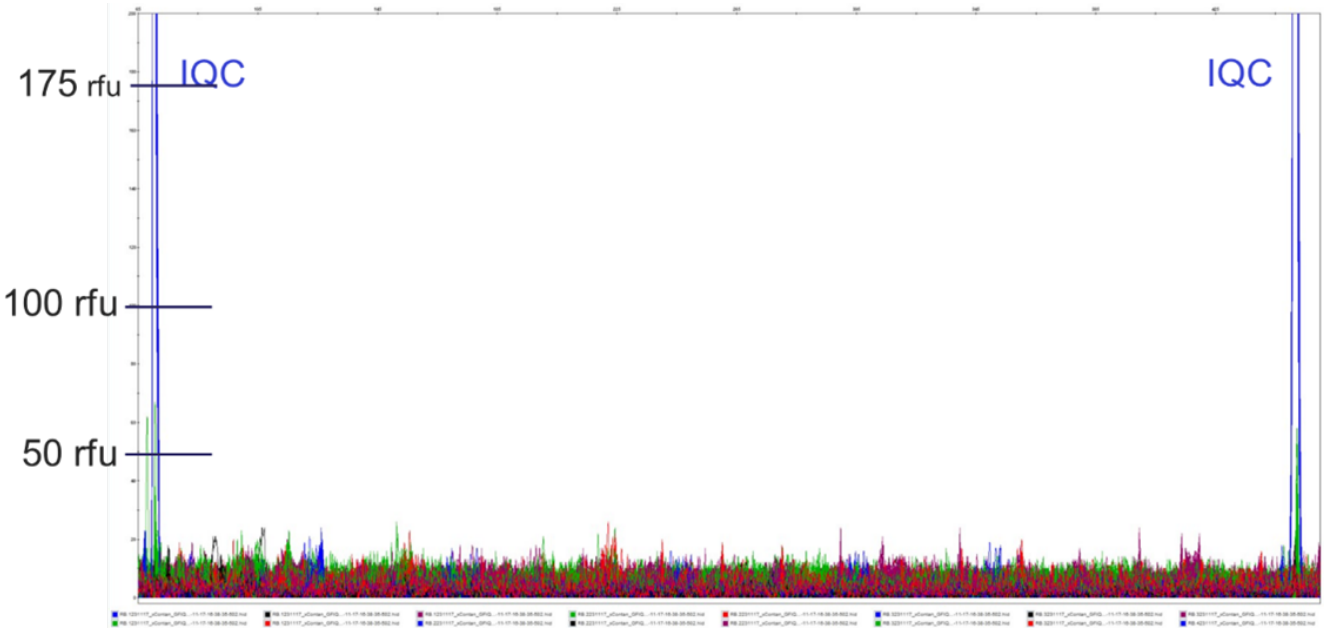


Figure 18 GlobalFiler™ IQC PCR Amplification Kit—Overlay of the electropherograms from all 46 RB wells. The electropherograms from all 46 RB wells were overlaid, displaying all dye channels. The plate was set up using the HID NIMBUS® Presto QNA System and amplified with the GlobalFiler™ IQC PCR Amplification Kit (15 µL of eluate, 29 cycles). The Y-axis is scaled to 200 RFU; no allele peaks >50 RFU were detected.

NGM Detect™ PCR Amplification Kit

No allele peaks >175 RFU were detected in any RB wells. Four peaks >50 RFU were detected, as follows:

- One off-ladder peak
- One spike
- One FAM™ dye artifact
- One in-bin peak (118 RFU) that did not match the donor M007 DNA, allele 19 at locus D19S433

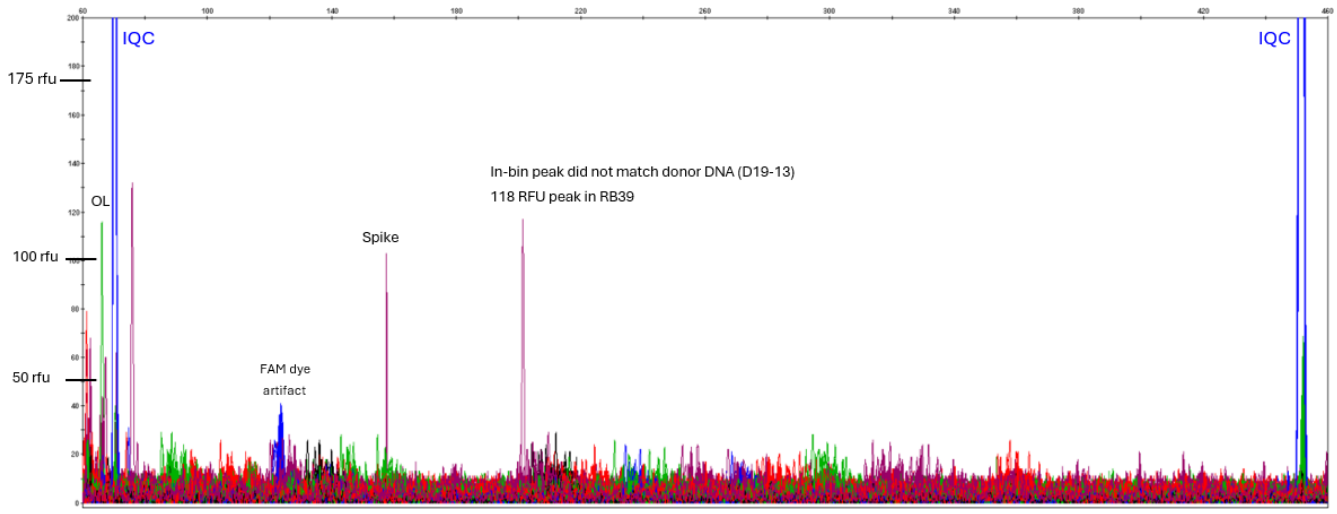


Figure 19 NGM Detect™ PCR Amplification Kit—Overlay of the electropherograms from all 46 RB wells. The electropherograms from all 46 RB wells were overlaid, displaying all dye channels. The plate was set up using the HID NIMBUS® Presto QNA System and amplified with the NGM Detect™ PCR Amplification Kit (15 µL of eluate, 30 cycles). The Y-axis is scaled to 200 RFU; four peaks >50 RFU were detected.

VeriFiler™ Plus PCR Amplification Kit

Two allele peaks >175 RFU were detected in one RB sample. Nine peaks >50 RFU were detected in different RB samples, as follows:

- Three off-ladder peaks
- Eight in-bin peaks (50–203 RFU) that did not match the donor M007 DNA were observed; for details, see Figure 20. Note that allele 17 at D3S1358 appeared in two different RBs.

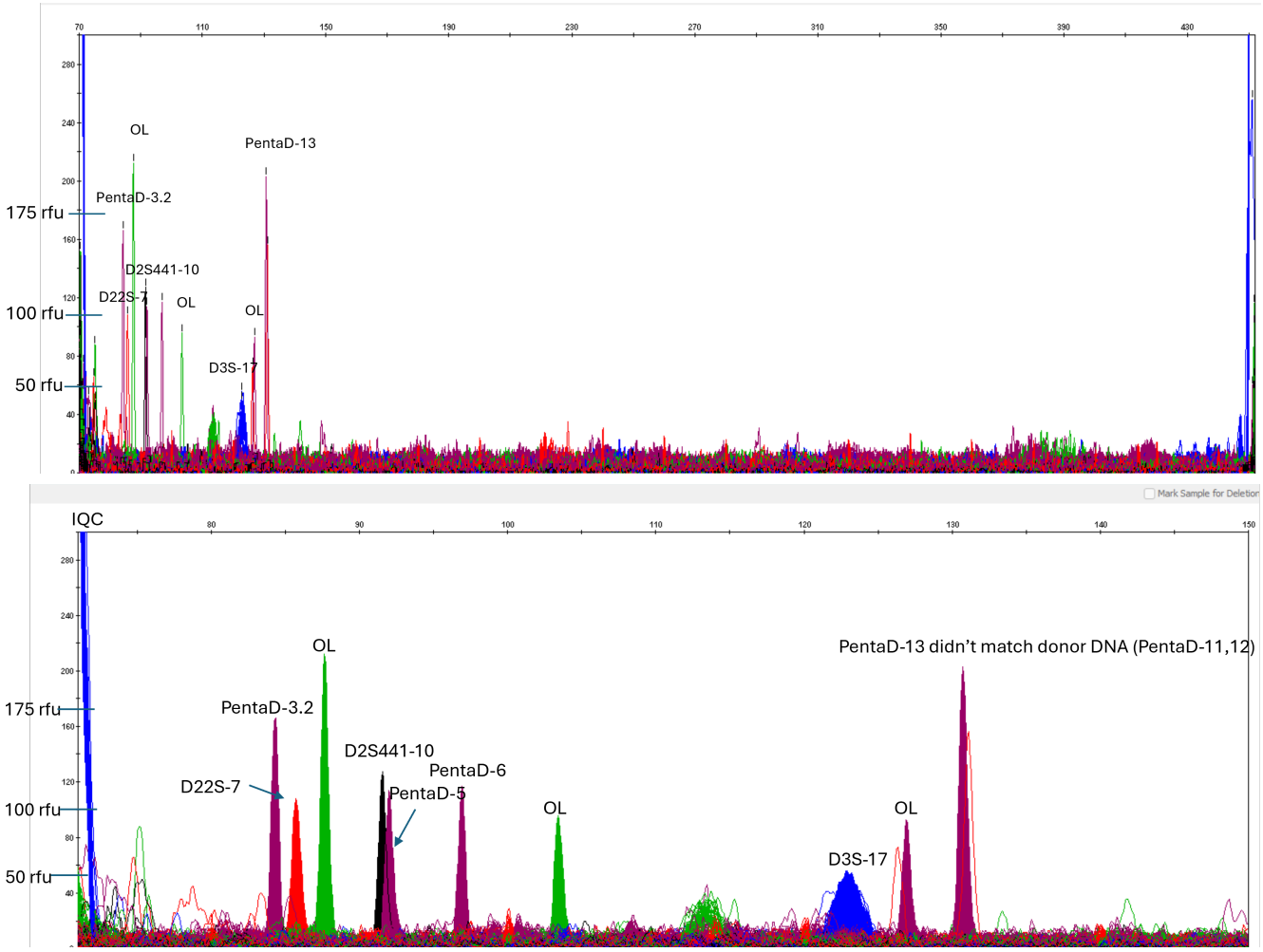


Figure 20 VeriFiler™ Plus PCR Amplification Kit—Overlay of the electropherograms from all 47 RB wells. The electropherograms from all 47 RB wells were overlaid, displaying all dye channels. The plate was set up using the HID NIMBUS® Presto QNA System and amplified with the VeriFiler™ Plus PCR Amplification Kit (15 µL of eluate, 29 cycles). The Y-axis is scaled to 300 RFU; nine peaks >50 RFU were detected.

Yfiler™ Plus PCR Amplification Kit

No allele peaks >175 RFU were detected in any RB wells. Seven peaks >50 RFU were detected, as follows:

- Six off-ladder peaks
- One in-bin peak (66 RFU) that did not match the donor M007 DNA, allele 30 at locus DYS481

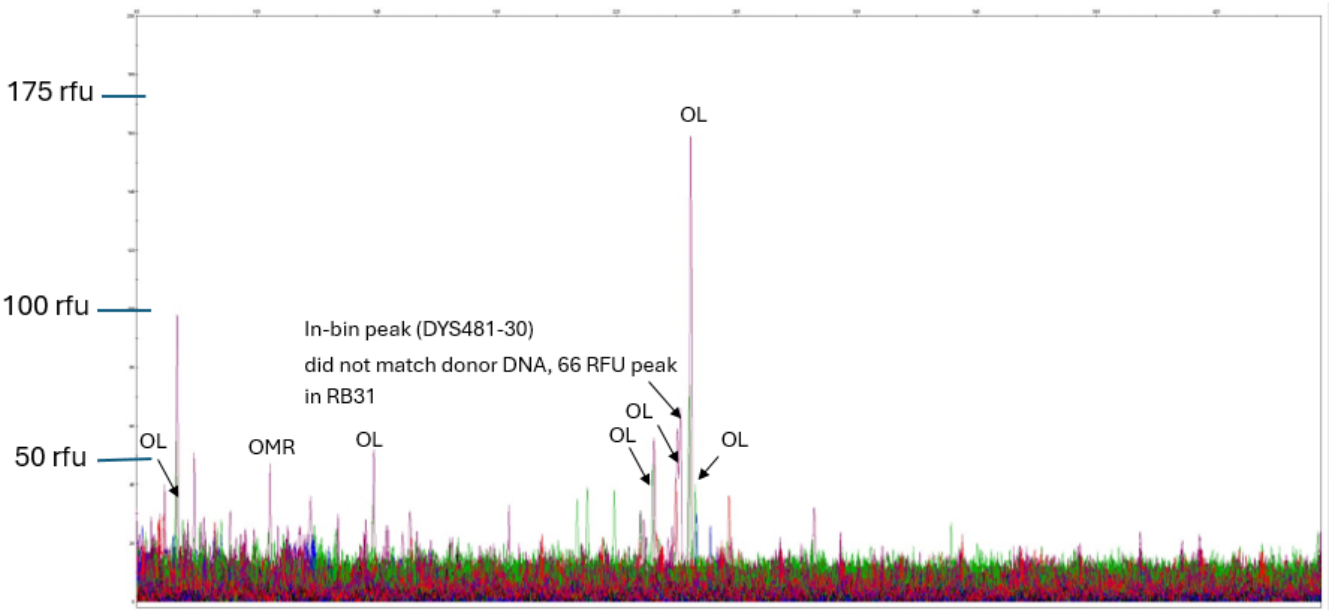


Figure 21 Yfiler™ Plus PCR Amplification Kit—Overlay of the electropherograms from all 46 RB wells. The electropherograms from all 46 RB wells were overlaid, displaying all dye channels. The plate was set up using the HID NIMBUS® Presto QNA System and amplified with the Yfiler™ Plus PCR Amplification Kit (15 µL of eluate, 29 cycles). The Y-axis is scaled to 200 RFU; seven peaks >50 RFU were detected.

Quantification workflow: Precision and accuracy studies

Method

To assess the precision and accuracy of the quantification workflow with the small autosomal and Y targets, we used the Quantifiler™ Trio DNA Quantification Kit to analyze the following samples:

- Two body fluid DNA samples (1 µL of blood and 2 µL of saliva) purified on the HID NIMBUS® Presto QNA System using the new recovery feature starting at the default binding step
- Three input amounts of M007 DNA (10, 2.5, and 0.625 ng/µL)

We ran all samples in duplicate, using the 5-points standard curve created by the HID NIMBUS® Presto QNA System. For the small autosomal target, we set up the sample plate layout twice using Robot 1 (Plate 1 and Plate 2), as shown in Figure 22. We then replicated Plate 1 on two different robots (Robot 1 and Robot 2). For the Y target, we set up the sample plate layout twice using Robot 2 (Plate 1 and Plate 2), as shown in Figure 23. We then replicated Plate 1 on two different robots (Robot 1 and Robot 2).

Additionally, we manually prepared a set of samples that we added to the plates prepared on the robots to assess any differences between the two workflows.

	Robotic						Manual					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	STD1	STD1	S4	S4			STD1	STD1	M007-S1	M007-S1		
B	STD2	STD2	S5	S5			STD2	STD2	M007-S2	M007-S2		
C	STD3	STD3					STD3	STD3	M007-S3	M007-S3		
D	STD4	STD4					STD4	STD4	S4	S4		
E	STD5	STD5					STD5	STD5	S5	S5		
F	M007-S1	M007-S1										
G	M007-S2	M007-S2										
H	M007-S3	M007-S3									NTC	NTC

Figure 22 Quantification workflow: Plate layout for the precision and accuracy study using the small autosomal target. The diagram shows the duplicate samples run for the precision and accuracy study:

- **M007-S1**, **M007-S2**, and **M007-S3** are the M007 DNA input amounts of 10, 2.5, and 0.625 ng/ µL, respectively.
- **S4** is the 1-µL blood sample; **S5** is the 2-µL saliva sample.
- Columns **7**, **8**, **9**, and **10** are the samples analyzed through the manual workflow.
- **NTC** is the no-template control.

	Robotic						Manual					
	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD1	Blood DNA-4	Blood DNA-4			STD1	STD1	Blood DNA-4	Blood DNA-4		
B	STD2	STD2	Saliva DNA-5	Saliva DNA-5			STD2	STD2	Saliva DNA-5	Saliva DNA-5		
C	STD3	STD3	NTc-1#1	NTc-1#1			STD3	STD3	NTc-1#1	NTc-1#1		
D	STD4	STD4	NTc-1#2	NTc-1#2			STD4	STD4	NTc-1#2	NTc-1#2		
E	STD5	STD5	000001	000001			STD5	STD5	000001	000001		
F	M007-S1	M007-S1	10102023	10102023			M007-S1	M007-S1	10102023	10102023		
G	M007-S2	M007-S2					M007-S2	M007-S2				
H	M007-S3	M007-S3					M007-S3	M007-S3			NTC	NTC

Figure 23 Quantification workflow: Plate layout for the precision and accuracy study using the Y target. The diagram shows the duplicate samples run for the precision and accuracy study:

- **M007-S1**, **M007-S2**, and **M007-S3** are the M007 DNA input amounts of 10, 2.5, and 0.625 ng/µL, respectively.
- **000001** and **10102023** are the M007 DNA dilutions at 0.1 and 0.2 ng/µL, respectively.
- **S4** is the 1-µL blood sample; **S5** is the 2-µL saliva sample.
- Columns **7**, **8**, **9**, and **10** are the samples analyzed through the manual workflow.
- **NTC** is the no-template control.

Results

Standard curve metrics

When using the Quantifiler™ Trio DNA Quantification Kit, the automated quantification workflow on the HID NIMBUS® Presto QNA System and the manual quantification workflow returned the expected values for all targets for the following standard curve metrics: slope, R^2, and Y intercept . All slope values are included between –3.2 and –3.5 and R^2 values are >0.990.

Table 10 Standard curve metrics from the automated and manual workflows—small autosomal target.

The table shows the result metrics obtained from the standard curves created using the automated quantification workflow on the HID NIMBUS® Presto QNA System and the manual quantification workflow for the small autosomal experiments. All metrics met the expected results when using the Quantifiler™ Trio DNA Quantification Kit.

Standard curve target	Metric	Robot 1/Plate 1		Robot 1/Plate 2		Robot 2/Plate 1	
		Automated workflow	Manual workflow	Automated workflow	Manual workflow	Automated workflow	Manual workflow
Large autosomal target	Slope	–3.417	–3.271	–3.357	–3.292	–3.493	–3.388
	R^2	1.000	1.000	1.000	1.000	0.997	1.000
	Y intercept	25.078	24.684	25.267	24.666	25.326	24.864
Small autosomal target	Slope	–3.342	–3.321	–3.329	–3.208	–3.322	–3.314
	R^2	0.998	1.000	0.999	0.999	0.995	0.995
	Y intercept	27.356	27.103	27.493	27.007	27.666	27.226
Y chromosome target	Slope	–3.335	–3.304	–3.364	–3.225	–3.411	–3.237
	R^2	0.999	0.999	0.999	0.998	0.999	0.999
	Y intercept	26.289	26.223	26.607	26.144	26.559	26.175
IPC C_T	No. of thresholds met	10	10	10	10	10	10
	No. of thresholds not met	0	0	0	0	0	0

Table 11 Standard curve metrics from the automated and manual workflows—Y target.

The table shows the result metrics obtained from the standard curves created using the automated quantification workflow on the HID NIMBUS® Presto QNA System and the manual quantification workflow for the Y target experiments. All metrics met the expected results when using the Quantifiler™ Trio DNA Quantification Kit.

Standard curve target	Metric	Robot 1/Plate 1		Robot 2/Plate 1		Robot 2/Plate 2	
		Automated workflow	Manual workflow	Automated workflow	Manual workflow	Automated workflow	Manual workflow
Large autosomal target	Slope	-3.545	-3.443	-3.122	-3-370	-3.418	-3.419
	R2	0.999	0.998	0.999	0.999	0.999	0.999
	Y intercept	25.461	24.832	25.255	24 812	25.164	24.756
Small autosomal target	Slope	-3.411	-3.308	-3.39	-3.314	-3.268	-3.303
	R2	0.998	0.999	0.999	1	0.998	1
	Y intercept	27.749	27.360	27.516	27.138	27.386	27.149
Y chromosome target	Slope	-3.398	-3.350	-3.348	-3.329	-3.519	-3.419
	R2	0.999	0.998	0.999	1	0.999	0.999
	Y intercept	26.776	26.320	26.625	26.402	26.591	24.756
IPC CT	No. of thresholds met	10	10	10	10	10	10
	No. of thresholds not met	0	0	0	0	0	0

DNA concentration values—small autosomal target

To assess reproducibility, we compared DNA yields obtained from the small autosomal target of the Quantifiler™ Trio DNA Quantification Kit. We set up Plate 1 replicates on Robot 1 and Robot 2, then set up Plate 2 on Robot 1 using the same purified DNA. The DNA yields for the automated and manual quantification workflows are averaged yields from duplicates in the same plate (see Table 12). The yields from the same sample run on the same robot in a second independent run or on a different robot showed a maximum percentage difference of 23.70 for the automated workflow and 44.96 for the manual workflow. The observed difference in yield is within the expected variation introduced through the quantification process. The HID NIMBUS® Presto QNA System has a precision rate of ≤25%.

Table 12 DNA yields obtained from the automated and manual workflows.

The table provides the average DNA yield results from duplicates analyzed using the Quantifiler™ Trio DNA Quantification Kit and set up using the automated quantification workflow on the HID NIMBUS® Presto QNA System and the manual quantification workflow. The maximum percentage difference observed in the automated quantification workflow is 23.70%; the maximum percentage difference observed in the manual workflow is 44.96%.

Sample	Robot 1/ Plate 1	Robot 1/ Plate 2	Robot 2/ Plate 1	% Difference		
				Robot 1/Plate 1 vs. Robot 1/Plate 2	Robot 1/Plate 1 vs. Robot 2/Plate 1	Robot 1/Plate 2 vs. Robot 2/Plate 1
Automated workflow						
S1	9.58	10.14	10.68	5.62	10.78	5.17
S2	2.44	2.47	2.60	1.15	6.56	5.41
S3	0.61	0.56	0.71	8.85	14.93	**23.70**
S4	0.30	0.33	0.33	8.41	9.57	1.16
S5	1.26	1.35	1.57	7.33	21.94	14.66
Manual workflow						
S1	9.02	8.36	10.14	7.57	11.75	19.28
S2	2.85	2.24	2.80	24.01	1.43	22.60
S3	0.56	0.52	0.82	8.14	37.16	**44.96**
S4	0.23	0.20	0.23	14.22	0.08	14.15
S5	0.83	0.76	0.81	9.51	2.08	7.43

DNA concentration values—Y target

To assess reproducibility, we compared DNA yields obtained from the Y target of the Quantifiler™ Trio DNA Quantification Kit. We set up Plate 1 replicates on Robot 1 and Robot 2, then set up Plate 2 on Robot 2 using the same purified DNA. The DNA yields for the automated and manual quantification workflows are averaged yields from duplicates in the same plate (see Table 13). The yields from the same sample run on the same robot in a second independent run or on a different robot showed a maximum percentage difference of 21.55 for the automated workflow and 38.97 for the manual workflow. The observed difference in yield is within the expected variation introduced through the quantification process. The HID NIMBUS® Presto QNA System has a precision rate of ≤25%.

Table 13 DNA yields obtained from the automated and manual workflows.

The table provides the average DNA yield results from duplicates analyzed using the Quantifiler™ Trio DNA Quantification Kit and set up using the automated quantification workflow on the HID NIMBUS® Presto QNA System and the manual quantification workflow. The maximum percentage difference observed in the automated quantification workflow is 21.55%; the maximum percentage difference observed in the manual workflow is 38.97%.

Sample	Robot 1/ Plate 1	Robot 2/ Plate 1	Robot 2/ Plate 2	% Difference		
				Robot 1/Plate 1 vs. Robot 2/Plate 1	Robot 1/Plate 1 vs. Robot 2/Plate 2	Robot 2/Plate 1 vs. Robot 2/Plate 2
Automated workflow						
M007-S1	11.489	10.967	10.737	4.65	6.77	2.12
M007-S2	2.815	2.636	2.823	6.56	0.27	6.83
M007-S3	0.755	0.691	0.815	8.85	7.65	16.48
Blood DNA-4	0.554	0.483	0.543	13.72	1.97	11.76
Saliva DNA-5	1.157	1.145	1.198	1.01	3.48	4.49
000001	0.114	0.109	0.125	4.34	9.07	13.39
10102023	0.232	0.191	0.237	19.47	2.11	**21.55**
Manual workflow						
M007-S1	11.612	12.046	10.894	3.66	6.39	10.05
M007-S2	2.771	3.160	2.962	13.14	6.68	6.47
M007-S3	0.685	0.725	1.017	5.66	**38.97**	33.49
Blood DNA-4	0.301	0.333	0.385	10.20	24.49	14.38
Saliva DNA-5	0.697	0.723	0 814	3.70	15.48	11.79
000001	0.102	0.114	0.136	10.91	28.33	17.55
10102023	0.166	0.215	0 245	25.49	38.37	13.20

Accuracy—small autosomal quantity values

To evaluate the accuracy of the automated HID NIMBUS® Presto QNA System quantification workflow, we calculated the differences between the expected and observed yields for the small autosomal quantity values. The highest percentage difference observed for Plate 1 on Robot 2 is 44.14. The HID NIMBUS® Presto QNA System met an accuracy value of ≤45%.

Table 14 Expected vs. observed values for the automated quantification workflow

The comparison between the expected and observed yields for the small autosomal target and the automated quantification workflow on the HID NIMBUS® Presto QNA System shows that the maximum percentage difference is 44.14.

Sample	Expected yield	Observed yield			% Difference		
		Robot 1/ Plate 1	Robot 1/ Plate 2	Robot 2/ Plate 1	Robot 1/ Plate 1	Robot 1/ Plate 2	Robot 2/ Plate 1
S1	10	9.58	10.14	10.68	4.25	1.38	6.54
S2	2.5	2.44	2.47	2.60	2.47	1.32	4.09
S3	0.625	0.61	0.56	0.71	1.89	10.74	13.04
S4	0.25	0.30	0.33	0.33	18.06	26.37	27.51
S5	1	1.26	1.35	1.57	22.75	29.96	**44.14**

Accuracy—Y target quantity values

To evaluate the accuracy of the automated HID NIMBUS® Presto QNA System quantification workflow, we calculated the differences between the expected and observed yields for the Y target quantity values. The highest percentage difference observed for Plate 2 on Robot 2 is 30.42. The HID NIMBUS® Presto QNA System met an accuracy value of ≤45%.

Table 15 Table 15 Expected vs. observed values for the automated quantification workflow.

The comparison between the expected and observed yields for the Y target and the automated quantification workflow on the HID NIMBUS® Presto QNA System shows that the maximum percentage difference is 30.42.

Sample	Expected yield	Observed yield			% Difference		
		Robot 1/ Plate 1	Robot 2/ Plate 1	Robot 2/ Plate 2	Robot 1/ Plate 1	Robot 2/ Plate 1	Robot 2/ Plate 2
M007-S1	10	11.489	10.967	10.737	14.89	9.67	7.37
M007-S2	2.5	2.815	2.636	2.823	12.61	5.46	12.91
M007-S3	0.625	0.755	0.691	0.815	20.81	10.57	**30.42**
000001	0.1	0.114	0.109	0.125	14.02	9.18	24.84
10102023	0.2	0.232	0.191	0.237	16.05	4.54	18.53

Amplification workflow: Precision and accuracy studies

Method

Precision and accuracy—GlobalFiler™ IQC, NGM Detect™, and VeriFiler™ Plus kits

To assess the precision and accuracy of the amplification workflow, including the sample normalization step, we used several PCR amplification kits and the small autosomal target quantity values to analyze the following samples:

- The two body fluid DNA samples (1 µL of blood and 2 µL of saliva) previously quantified on Robot 1/ Plate 1
- The three input amounts of M007 DNA (10, 2.5, and 0.625 ng/µL) previously quantified on Robot 1/ Plate 1

We ran all samples in triplicate. We set up the sample plate layout twice using Robot 2 (Plate 1 and Plate 2), as shown in Figure 24 for the PCR amplification kits using the small autosomal target for the normalization. We then replicated Plate 1 on Robot 1, as shown in Figure 25.

Additionally, we manually prepared a set of samples that we added to the plates prepared on the robots to assess any differences between the two workflows.

We analyzed each plate using the following PCR amplification kits:

- GlobalFiler™ IQC PCR Amplification Kit
- NGM Detect™ PCR Amplification Kit
- VeriFiler™ Plus PCR Amplification Kit

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	M007-S3	Blood DNA-S4(Manual)									
B	M007-S1	M007-S3	Blood DNA-S4(Manual)									
C	M007-S1	Blood DNA-S4	Blood DNA-S4(Manual)									
D	M007-S1	Blood DNA-S4	Saliva DNA-S4(Manual)									
E	M007-S2	Blood DNA-S4	Saliva DNA-S4(Manual)									
F	M007-S2	Saliva DNA-S5	Saliva DNA-S4(Manual)									
G	M007-S2	Saliva DNA-S5	Positive control									
H	M007-S3	Saliva DNA-S5	Negative control									

Figure 24 Amplification workflow: Plate layout for the precision and accuracy study—Robot 2. The diagram shows the triplicate samples run for the precision and accuracy study:

- **M007-S1**, **M007-S2**, and **M007-S3** are the M007 DNA input amounts of 10, 2.5, and 0.625 ng/ µL, respectively.
- **S4** is the 1-µL blood sample; **S5** is the 2-µL saliva sample.
- Wells **A3**–**F3** are the blood and saliva samples analyzed through the manual workflow.
- Well **G3** is the positive control; well **H3** is the negative control.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	M007-S3	Blood DNA-S4(Manual)						Positive control(Manual)	Positive control(Manual)		
B	M007-S1	M007-S3	Blood DNA-S4(Manual)						M007-S1(Manual)	Blood DNA-S4(Automate Express)		
C	M007-S1	Blood DNA-S4	Blood DNA-S4(Manual)						M007-S1-(Manual)	Blood DNA-S4(Automate Express)		
D	M007-S1	Blood DNA-S4	Saliva DNA-S4(Manual)						M007-S2(Manual)	Blood DNA-S4(Automate Express)		
E	M007-S2	Blood DNA-S4	Saliva DNA-S4(Manual)						M007-S2-(Manual)	Saliva DNA-S4(Automate Express)		
F	M007-S2	Saliva DNA-S5	Saliva DNA-S4(Manual)						M007-S3(Manual)	Saliva DNA-S4(Automate Express)		
G	M007-S2	Saliva DNA-S5	Positive control						M007-S3-(Manual)	Saliva DNA-S4(Automate Express)		
H	M007-S3	Saliva DNA-S5	Negative control						Ladder	Negative control(Manual)		

Figure 25 Amplification workflow: Plate layout for the precision and accuracy study—Robot 1. The diagram shows the triplicate samples run for the precision and accuracy study:

- **M007-S1**, **M007-S2**, and **M007-S3** are the M007 DNA input amounts of 10, 2.5, and 0.625 ng/ µL, respectively.
- **S4** is the 1-µL blood sample; **S5** is the 2-µL saliva sample.
- Wells **A3**–**F3** and **A9**–**G9** are the samples analyzed through the manual workflow.
- Column **10** are the blood and saliva samples used for the correlation study.

Precision and accuracy—Yfiler™ Plus kit

To assess the precision and accuracy of the Yfiler™ Plus PCR Amplification Kit workflow, including the sample normalization step, we analyzed the following samples:

- The two body fluid DNA samples (1 µL of blood and 2 µL of saliva) previously quantified on Robot 1/ Plate 1
- The three input amounts of M007 DNA (10, 2.5, and 0.625 ng/µL) previously quantified on Robot 1/ Plate 1
- Two additional M007 dilutions at 0.1 and 0.2 ng/ µL: 000001 and 10102023, respectively.

We ran all samples in triplicate. We set up the sample plate layout twice using Robot 2 (Plate 1 and Plate 2), as shown in Figure 26. We then replicated Plate 1 on Robot 1 using the same plate layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ladder	M007-S3	Blood DNA-4 (Manual)	NTc-1#1	000001							
B	M007-S1	M007-S3	Blood DNA-4 (Manual)	Ladder	000001							
C	M007-S1	Blood DNA-S4	Blood DNA-4 (Manual)	NTc-1#1	10102023							
D	M007-S1	Blood DNA-S4	Saliva DNA-5 (Manual)	NTc-1#1	10102023							
E	M007-S2	Blood DNA-S4	Saliva DNA-5 (Manual)	NTc-1#2	10102023							
F	M007-S2	Saliva DNA-S5	Saliva DNA-5 (Manual)	NTc-1#2								
G	M007-S2	Saliva DNA-S5	Positive control	NTc-1#2								
H	M007-S3	Saliva DNA-S5	Negative control	000001								

Figure 26 Amplification workflow: Plate layout for the precision and accuracy study. The diagram shows the triplicate samples run for the precision and accuracy study:

- **M007-S1**, **M007-S2**, and **M007-S3** are the M007 DNA input amounts of 10, 2.5, and 0.625 ng/ µL, respectively.
- **000001** and **10102023** are the M007 DNA dilutions at 0.1 and 0.2 ng/ µL, respectively.
- **S4** is the 1-µL blood sample; **S5** is the 2-µL saliva sample.
- Wells **A3**–**F3** are the samples analyzed through the manual workflow.

Results

We analyzed the STR profiles from the automated and manual amplification workflows on a 3500xL Genetic Analyzer for Human Identification, then compared the profiles. We evaluated genotype accuracy, intra-color balance (ICB), intra-locus balance (ILB), and average peak height. To evaluate genotype accuracy, we used the profile comparison tool in GeneMapper™ *ID-X* Software v1.6. The evaluation results showed the following:

- All STR profiles from the different workflows were 100% concordant within each other and to the reference profile.
- The ICB and ILB from plate-to-plate, robot-to-robot, and automated-to-manual workflows are comparable for all PCR amplification kits used; no statistically significant differences were observed.
- The average peak heights for the automated and manual workflows are comparable for all PCR amplification kits tested.

The results are summarized in Table 16. The results of the ICB and ILB evaluation for each kit are illustrated in the figures in “ICB, ILB, and average peak height evaluation results for the PCR amplification kits” on page 69.

Table 16 Summary of the results obtained for each PCR amplification kit for genotype accuracy, ICB, and ILB

All profiles from the different workflows were 100% concordant within each other and to the reference profile. The ICB and ILB from plate-to-plate, robot-to-robot, and automated-to-manual workflows are comparable for all PCR amplification kits used; no statistically significant differences were observed.

PCR amplification kit	Genotype accuracy	Intra-color balance (ICB)	Intra-locus balance (ILB)
GlobalFiler™ IQC PCR Amplification Kit	100%	Average ICB ≥40% with 95% confidence	Average ILB ≥65% for all loci
NGM Detect™ PCR Amplification Kit	100%	Average ICB ≥40% with 95% confidence	Average ILB ≥50% for all loci
VeriFiler™ Plus PCR Amplification Kit	100%	Average ICB ≥50% with 95% confidence	Average ILB ≥70% for all loci
Yfiler™ Plus PCR Amplification Kit	100%	Average ICB ≥40% with 95% confidence Low VIC™ dye ICB in the saliva sample mainly driven by an almost doubled peak height at DYS460. The observation could be explained by the donor's DYS385 region duplication, which may extend to include DYS460. DYS385 a/b and DYS460 are adjacent in the Yq11.222 region of the Y chromosome.	N/A

Correlation study

Method

We compared the performance of the HID NIMBUS® Presto QNA System quantification and amplification workflows to a semi-automated workflow that included:

- DNA purification using the AutoMate *Express*™ Forensic DNA Extraction System
- Manual setup for quantification using the Quantifiler™ Trio DNA Quantification Kit
- Manual setup for amplification using the GlobalFiler™ IQC PCR Amplification Kit

Results

DNA concentration

The small autosomal target C_T values for the blood and saliva samples were within ±1.5 C_T units. The DNA concentration was greater than or equal to that of the AutoMate *Express*™ Forensic DNA Extraction System. For results, see Table 17.

Table 17 Correlation study comparison

Sample	Small autosomal target				Average quantity	
	HID NIMBUS® Presto QNA System		AutoMate *Express*™ Forensic DNA Extraction System		HID NIMBUS® Presto QNA System	AutoMate *Express*™ Forensic DNA Extraction System
Blood	29.02 C_T	29.2 C_T	29.43 C_T	29.30 C_T	0.3 ng/µL	0.23 ng/µL
Saliva	27.03 C_T	27.02 C_T	28.16 C_T	27.99 C_T	1.26 ng/µL	0.56 ng/µL

Intra-color, intra-locus balance and peak height

We amplified the blood and saliva samples using the GlobalFiler™ IQC PCR Amplification Kit. We made the following observations:

- The ICB from the different workflows is comparable and ≥40%. No statistically significant differences were observed, as shown in Figure 27.
- The ILB from the different workflows is comparable and ≥60%. No statistically significant differences were observed, as shown in Figure 28.
- The average heterozygous peak heights for the blood and saliva samples is higher for the HID NIMBUS® Presto QNA System for both analyzed samples, as shown in Figure 29.

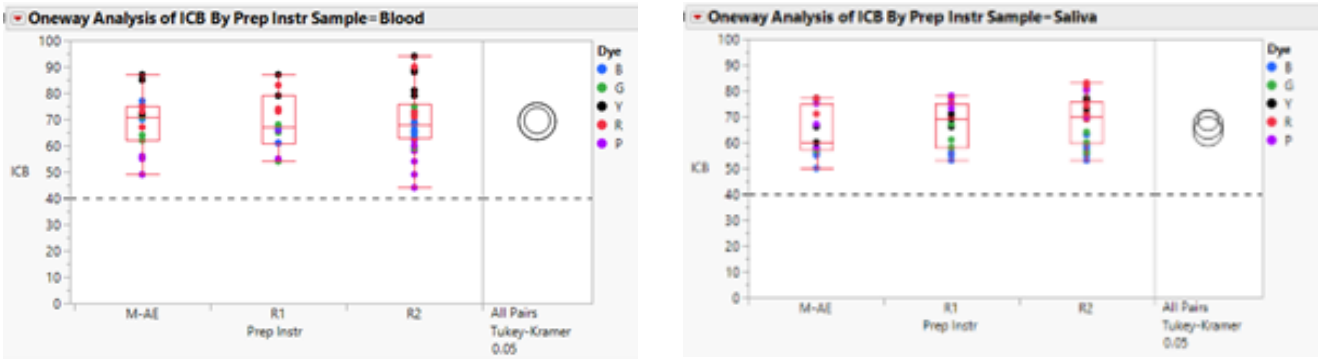


Figure 27 Correlation study: Intra-color balance from the blood and saliva samples using the GlobalFiler™ IQC PCR Amplification Kit. The figure shows the intra-color balance of the blood and saliva samples analyzed with the HID NIMBUS® Presto QNA System automated workflow and the AutoMate *Express*™ Forensic DNA Extraction System semi-automated workflow. The ICB is ≥40%.

- **M-AE** is the AutoMate *Express*™ Forensic DNA Extraction System semi-automated workflow
- **R1** is Robot 1
- **R2** is Robot 2

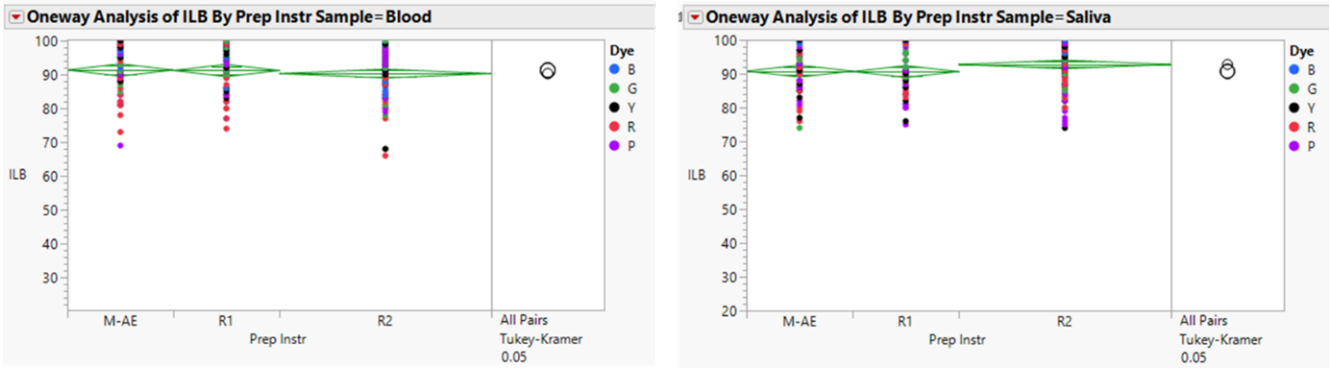


Figure 28 Correlation study: Intra-locus balance from the blood and saliva samples using the GlobalFiler™ IQC PCR Amplification Kit. The figure shows the intra-locus balance of the blood and saliva samples analyzed with the HID NIMBUS® Presto QNA System automated workflow and the AutoMate *Express*™ Forensic DNA Extraction System semi-automated workflow. The ILB is ≥60%.

- **M-AE** is the AutoMate *Express*™ Forensic DNA Extraction System semi-automated workflow
- **R1** is Robot 1
- **R2** is Robot 2

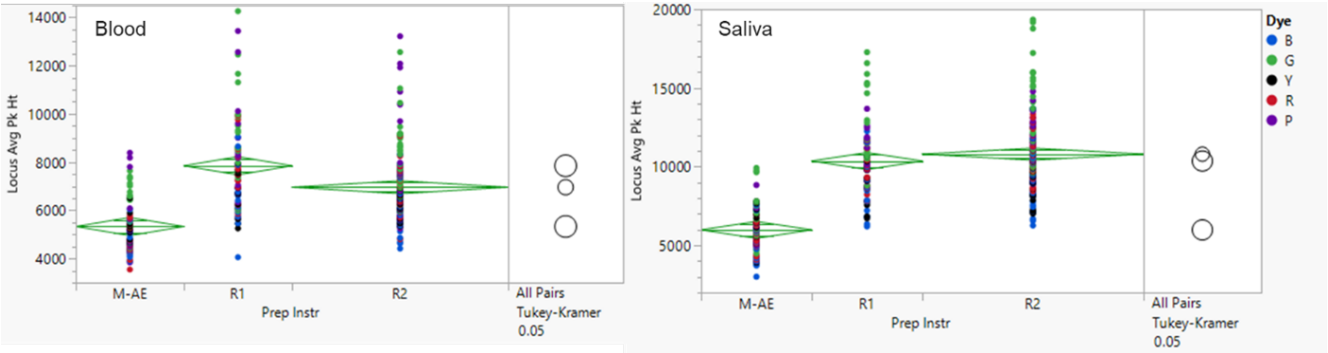


Figure 29 Correlation study: Average heterozygous peak heights from the blood and saliva samples using the GlobalFiler™ IQC PCR Amplification Kit. The figure shows the average heterozygous peak heights of the blood and saliva samples analyzed with the HID NIMBUS® Presto QNA System automated workflow and the AutoMate *Express*™ Forensic DNA Extraction System semi-automated workflow. The average heterozygous peak height is higher for the HID NIMBUS® Presto QNA System for both analyzed samples.

- **M-AE** is the AutoMate *Express*™ Forensic DNA Extraction System semi-automated workflow
- **R1** is Robot 1
- **R2** is Robot 2

Conclusion

The validation studies demonstrated that the automated quantification and amplification workflows implemented on the HID NIMBUS® Presto QNA System, using the Quantifiler™ Trio Automated DNA Quantification Kit and several PCR amplification kits, provides robust and reliable results in obtaining genomic DNA from forensic biological samples for downstream applications such as real-time quantitative PCR and PCR for STR profiling. The validation study results indicated the following:

- No cross-contamination was introduced by the system when used for quantification and amplification setup.
- The system can reliably set up plates using the automated quantification workflow with the validated script. The HID NIMBUS® Presto QNA System has a precision rate of ≤25% and an accuracy rate of ≤45%.
- The system can reliably set up plates using the automated amplification workflow with the validated script. The HID NIMBUS® Presto QNA System ICB and ILB from plate-to-plate, robot-to-robot, and automated-to-manual workflows are comparable for all PCR amplification kits used; no statistically significant differences were observed.
- Correct, complete, and consistent genotypes were obtained with all PCR amplification kits validated on the system with a 100% accuracy rate.
- The performance of the automated quantification and amplification setup workflows using the HID NIMBUS® Presto QNA System is comparable to the established manual quantification and amplification setup workflows using the AutoMate *Express*™ Forensic DNA Extraction System. The workflows yielded equivalent results on both systems.

ICB, ILB, and average peak height evaluation results for the PCR amplification kits

GlobalFiler™ IQC PCR Amplification Kit

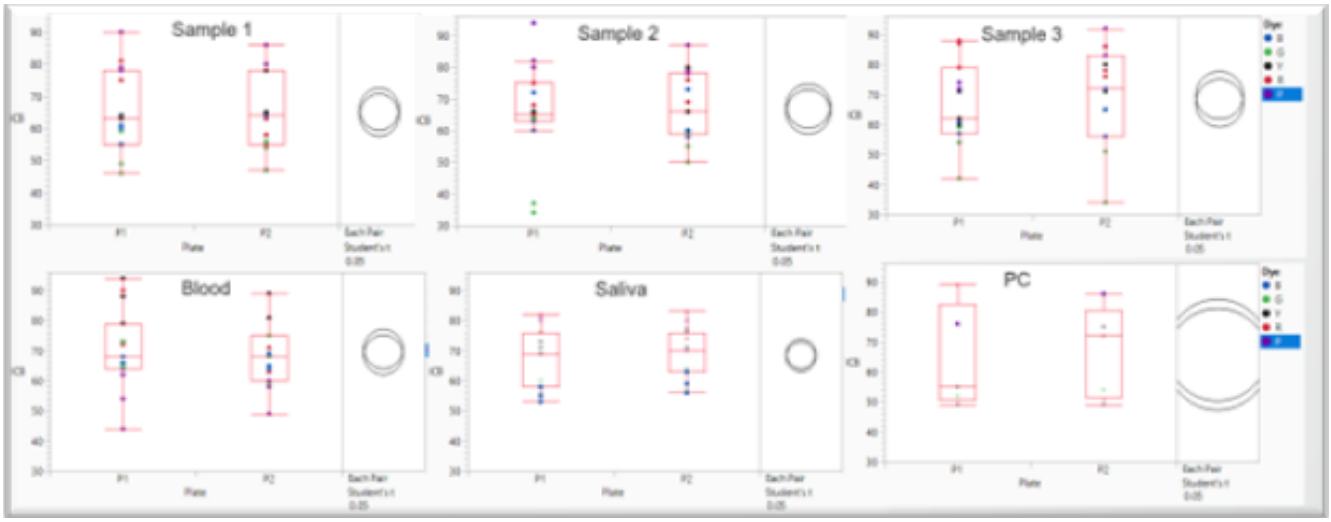


Figure 30 Intra-color balance from the analyzed samples using the GlobalFiler™ IQC PCR Amplification Kit. The figure shows the intra-color balance from Plate 1 and Plate 2 set up on Robot 2. The ICB is ≥40% with 95% confidence.

- **P1** is Plate 1; **P2** is Plate 2
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

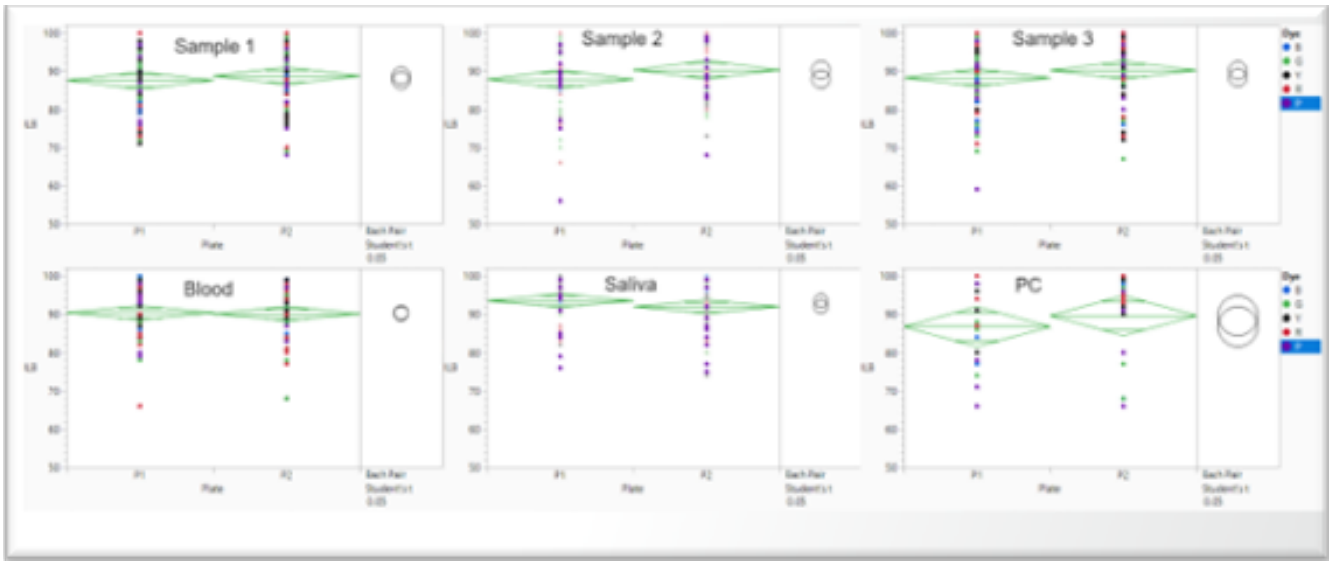
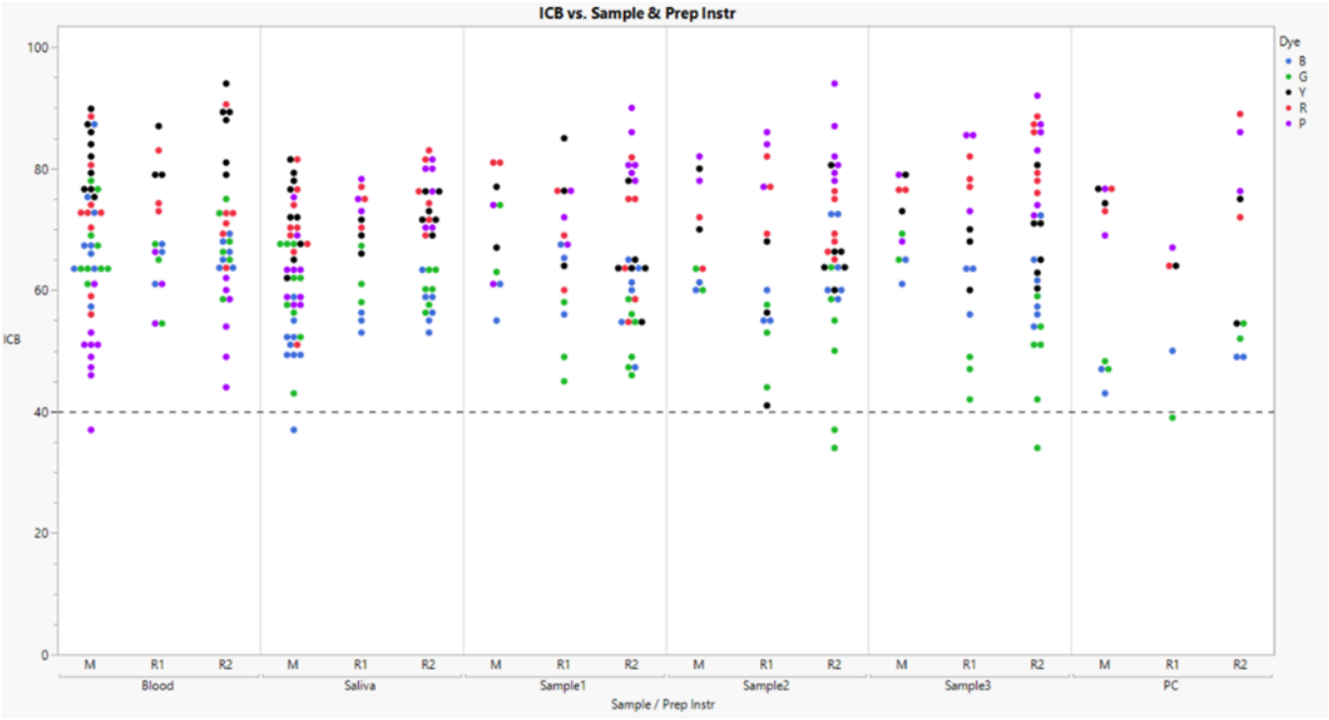


Figure 31 Intra-locus balance from the analyzed samples using the GlobalFiler™ IQC PCR Amplification Kit. The figure shows the intra-locus balance from Plate 1 and Plate 2 set up on Robot 2. The ILB is ≥65% for all loci.

- **P1** is Plate 1; **P2** is Plate 2
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control



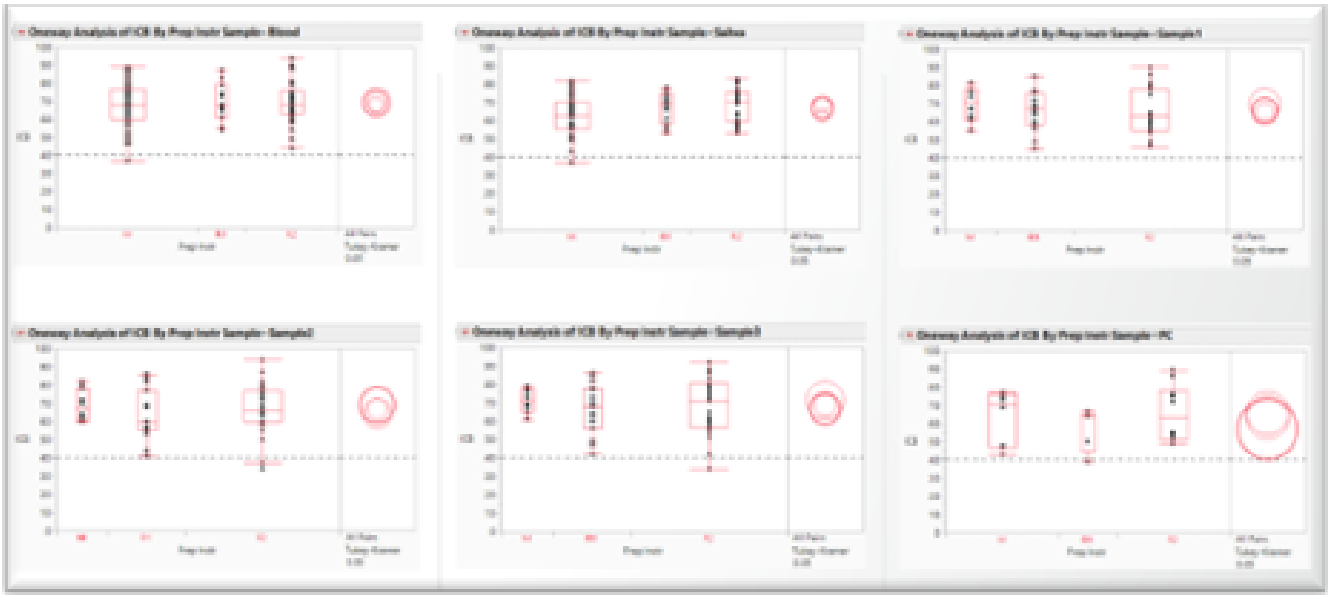


Figure 32 Intra-color balance from the same samples analyzed using a different workflow/instrument with the GlobalFiler™ IQC PCR Amplification Kit. The figures show the ICB comparison from each sample across the different workflows/instruments. The ICB is ≥40% with 95% confidence for all evaluated samples.

- **R1** is Robot 1; **R2** is Robot 2
- **M** is manual
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

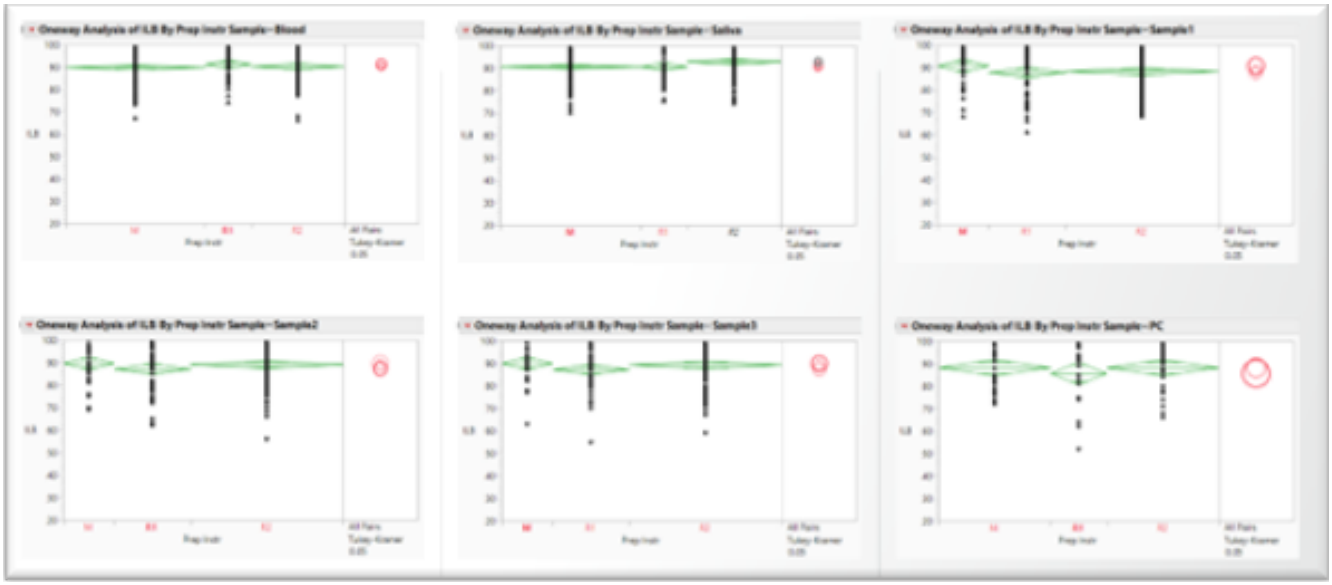


Figure 33 Intra-locus balance from the same samples analyzed using a different workflow/instrument with the GlobalFiler™ IQC PCR Amplification Kit. The figure shows the ILB comparison from each sample across the different workflows/instruments. The ILB is ≥65% for all loci.

- **R1** is Robot 1; **R2** is Robot 2
- **M** is manual
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

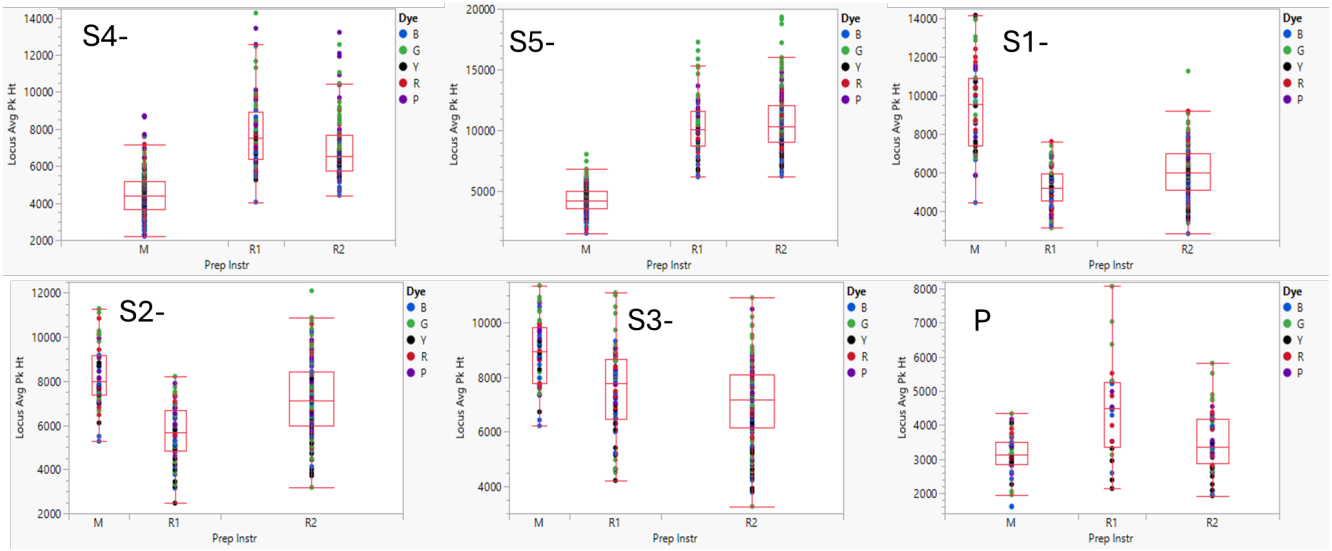


Figure 34 Average heterozygous peak heights from the same samples analyzed using a different workflow/instrument with the GlobalFiler™ IQC PCR Amplification Kit. The figure shows the average peak height of the samples analyzed with the HID NIMBUS® Presto QNA System automated workflow and the manual workflow. The average peak height is comparable between the two workflows.

- **R1** is Robot 1; **R2** is Robot 2
- **M** is manual
- **S1**, **S2**, and **S3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively; **S4** is the 1-µL blood sample; **S5** is the 2-µL saliva sample
- **P** is the positive control

NGM Detect™ PCR Amplification Kit

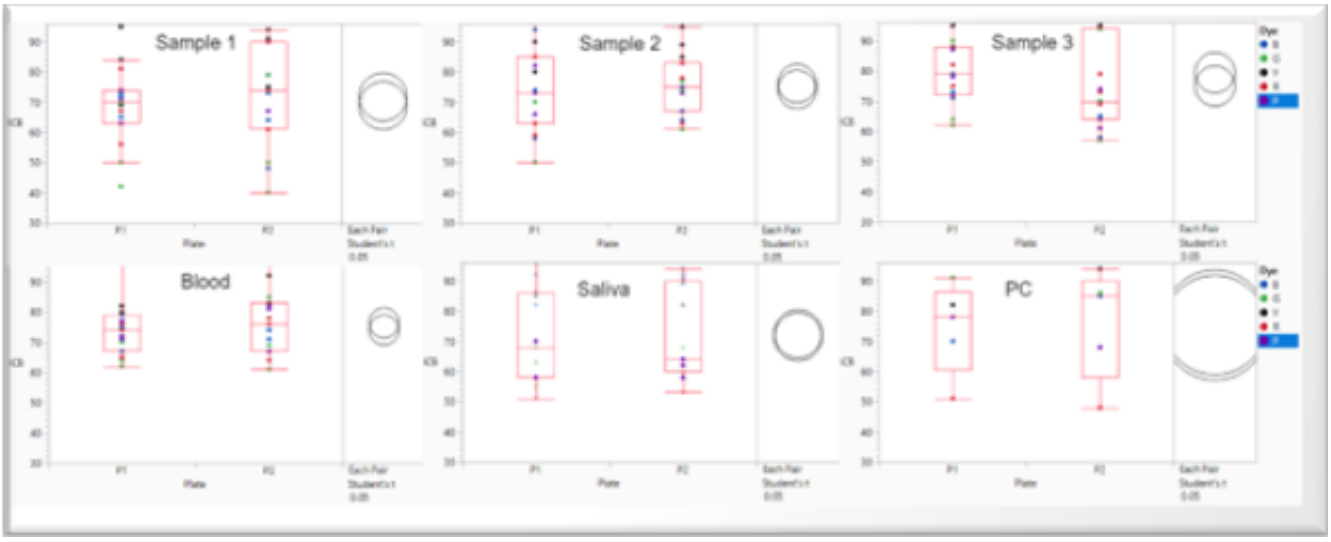


Figure 35 Intra-color balance from the analyzed samples using the NGM Detect™ PCR Amplification Kit. The figure shows the intra-color balance from Plate 1 and Plate 2 set up on Robot 2. The ICB is ≥40% with 95% confidence.

- **P1** is Plate 1; **P2** is Plate 2
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

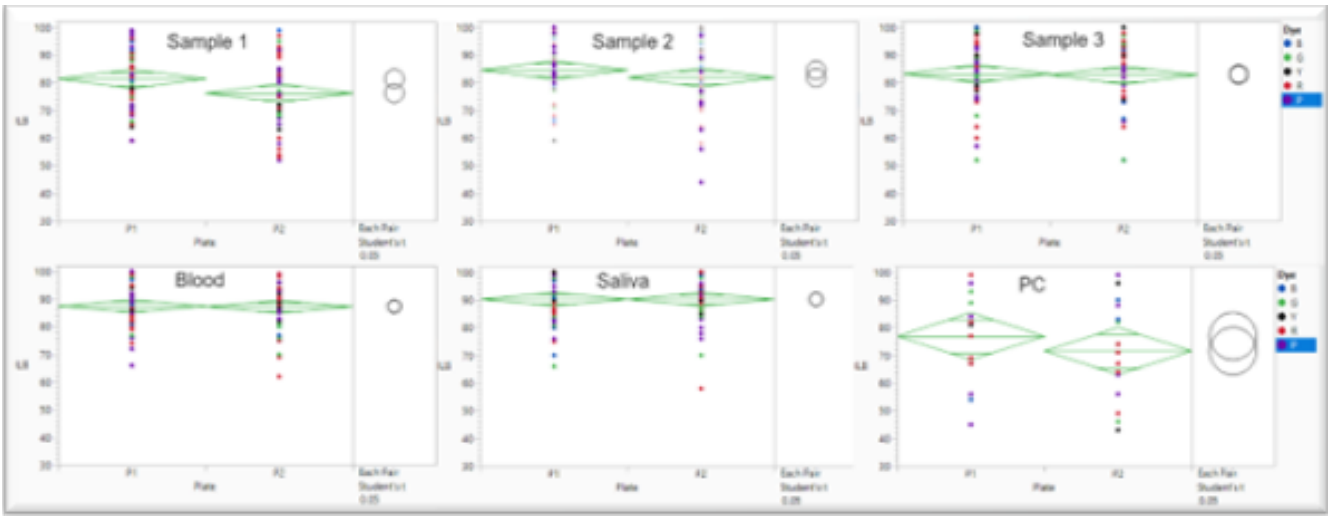


Figure 36 Intra-locus balance from the analyzed samples using the NGM Detect™ PCR Amplification Kit. The figure shows the intra-locus balance from Plate 1 and Plate 2 set up on Robot 2. The ILB is ≥50% for all loci.

- **P1** is Plate 1; **P2** is Plate 2
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

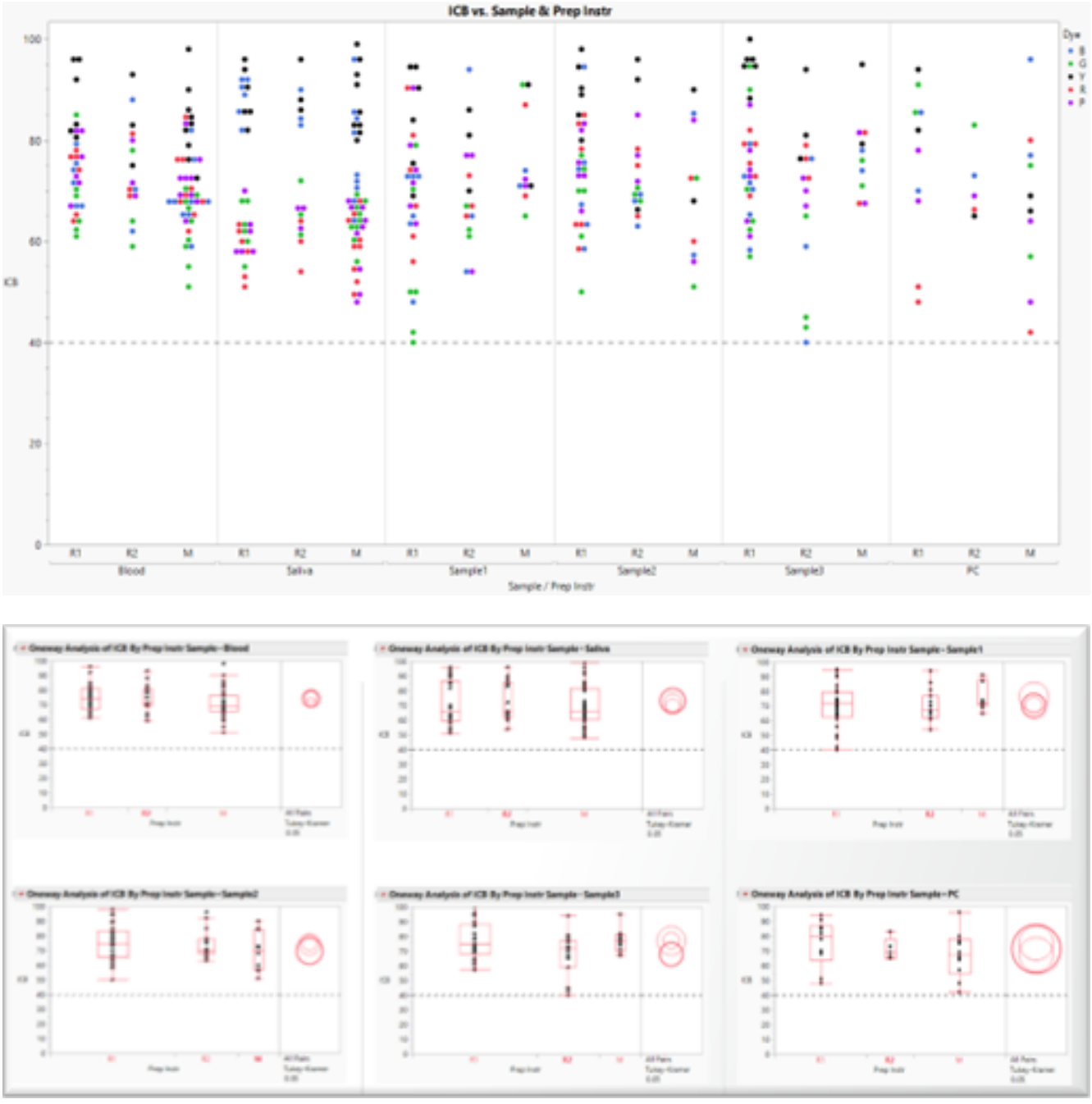


Figure 37 Intra-color balance from the same samples analyzed using a different workflow/instrument with the NGM Detect™ PCR Amplification Kit. The figures show the ICB comparison from each sample across the different workflows/instruments. The ICB is ≥40% with 95% confidence for all evaluated samples.

- **R1** is Robot 1; **R2** is Robot 2
- **M** is manual
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

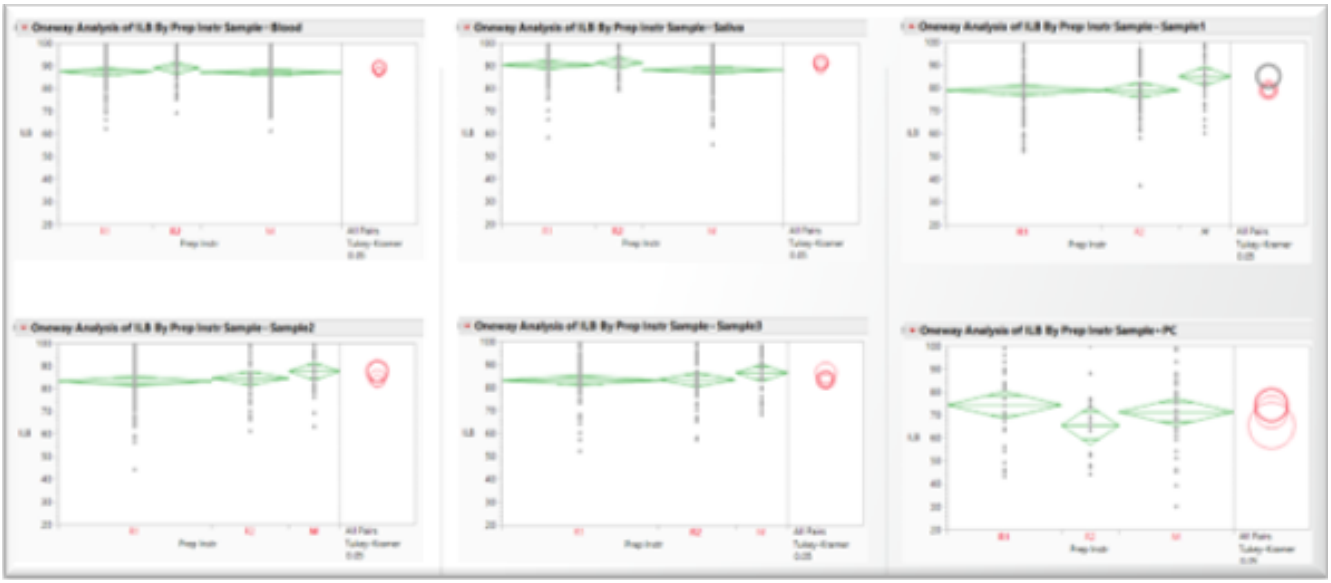


Figure 38 Intra-locus balance from the same samples analyzed using a different workflow/instrument with the NGM Detect™ PCR Amplification Kit. The figure shows the ILB comparison from each sample across the different workflows/instruments. The ILB is ≥50% for all loci.

- **R1** is Robot 1; **R2** is Robot 2
- **M** is manual
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

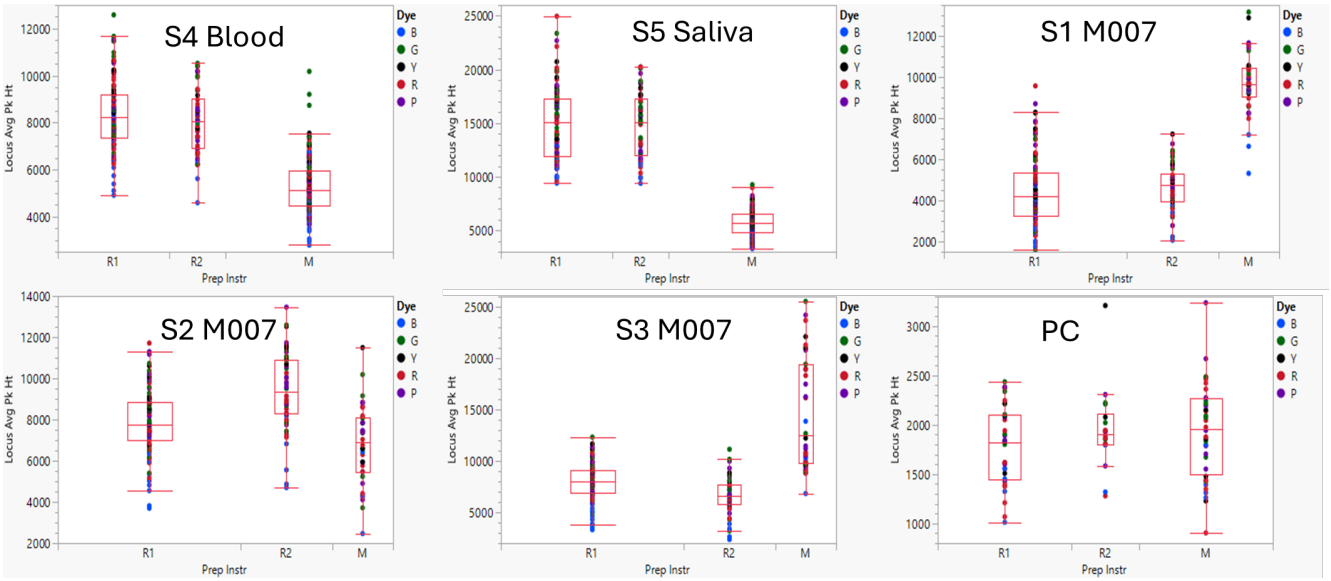


Figure 39 Average heterozygous peak heights from the same samples analyzed using a different workflow/instrument with the NGM Detect™ PCR Amplification Kit. The figure shows the average peak height of the samples analyzed with the HID NIMBUS® Presto QNA System automated workflow and the manual workflow. The average peak height is comparable between the two workflows.

- **R1** is Robot 1; **R2** is Robot 2
- **M** is manual
- **S1**, **S2**, and **S3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively; **S4** is the 1-µL blood sample; **S5** is the 2-µL saliva sample
- **PC** is the positive control

VeriFiler™ Plus PCR Amplification Kit

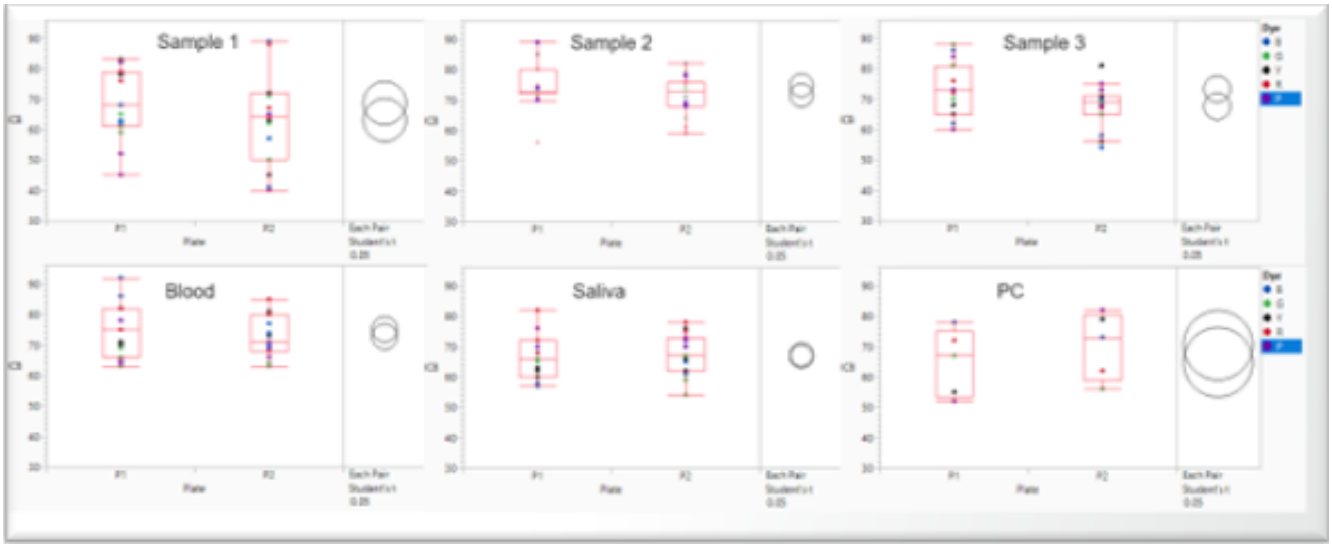


Figure 40 Intra-color balance from the analyzed samples using the VeriFiler™ Plus PCR Amplification Kit. The figure shows the intra-color balance from Plate 1 and Plate 2 set up on Robot 2. The ICB is ≥50% with 95% confidence.

- **P1** is Plate 1; **P2** is Plate 2
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

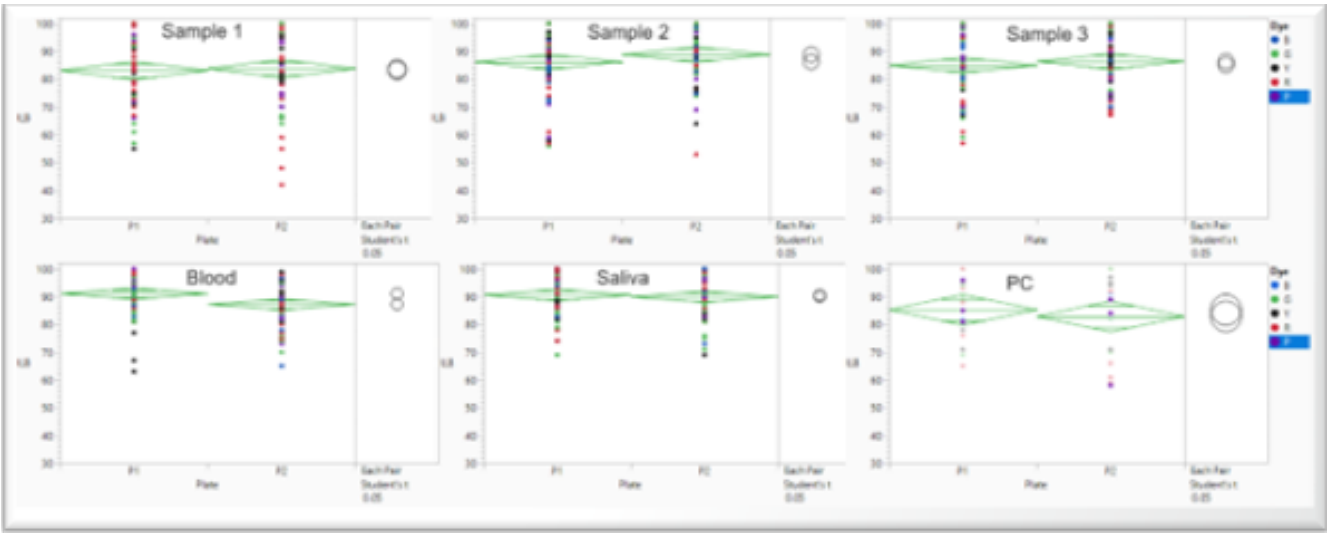
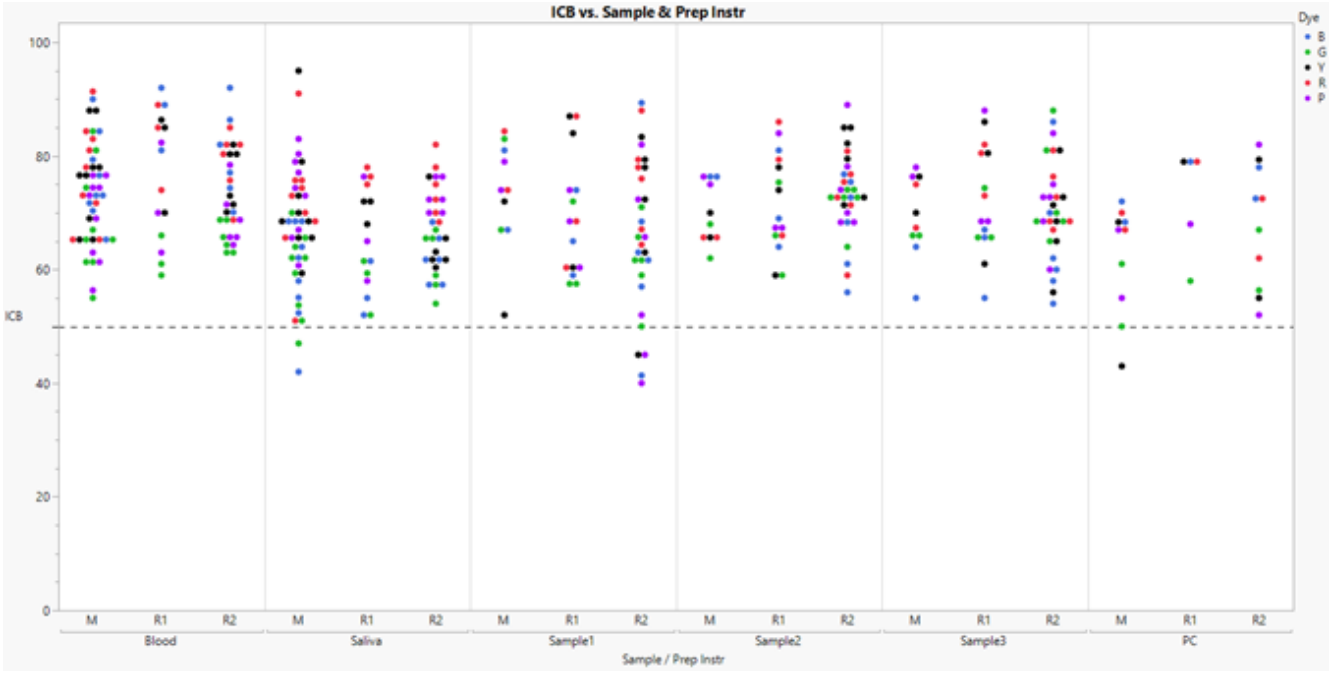


Figure 41 Intra-locus balance from the analyzed samples using the VeriFiler™ Plus PCR Amplification Kit. The figure shows the intra-locus balance from Plate 1 and Plate 2 set up on Robot 2. The ILB is ≥70% for all loci.

- **P1** is Plate 1; **P2** is Plate 2
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control



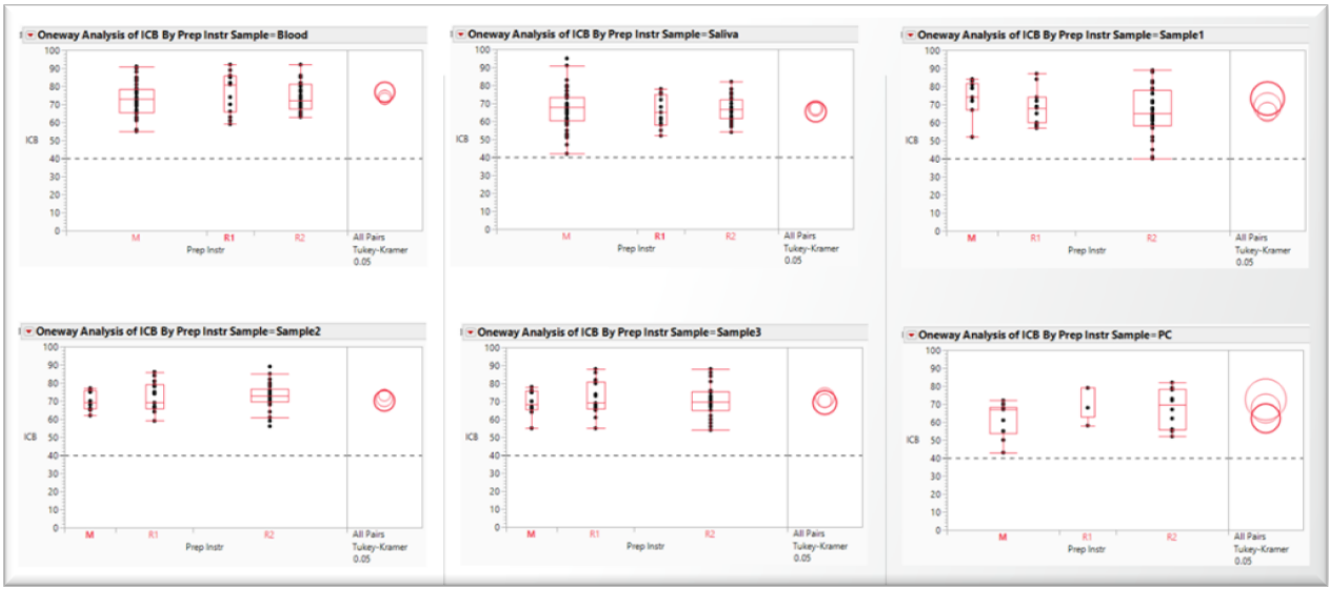


Figure 42 Intra-color balance from the same samples analyzed using a different workflow/instrument with the VeriFiler™ Plus PCR Amplification Kit. The figures show the ICB comparison from each sample across the different workflows/instruments. The ICB is ≥50% with 95% confidence for all evaluated samples.

- **R1** is Robot 1; **R2** is Robot 2
- **M** is manual
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

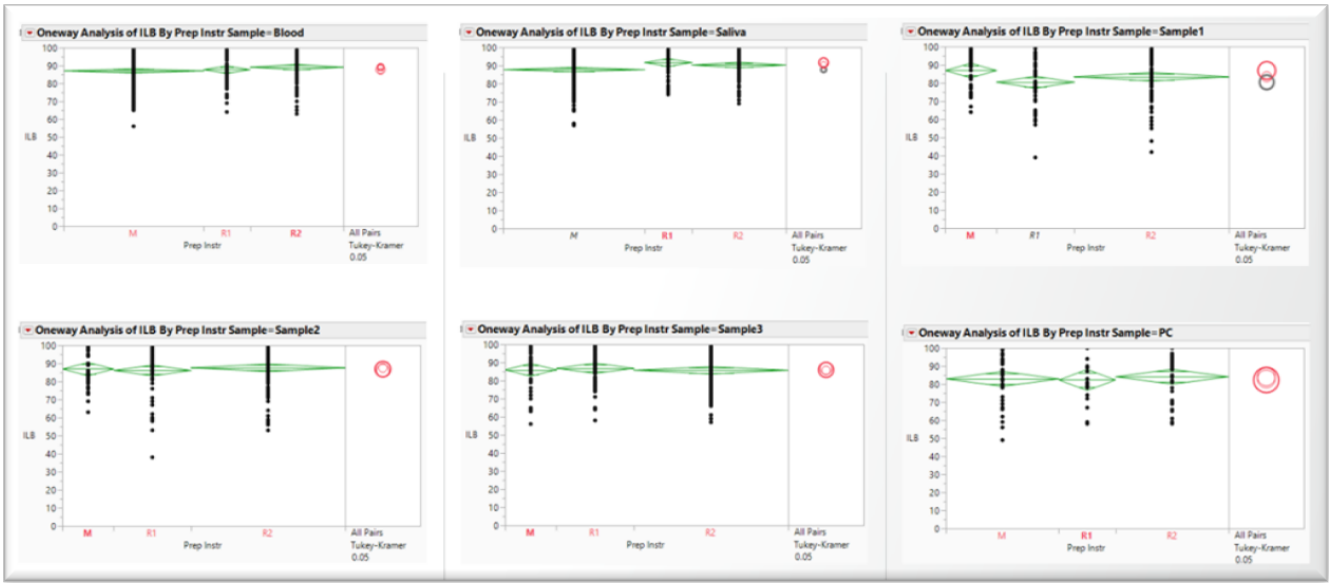


Figure 43 Intra-locus balance from the same samples analyzed using a different workflow/instrument with the VeriFiler™ Plus PCR Amplification Kit. The figure shows the ILB comparison from each sample across the different workflows/instruments. The ILB is ≥70% for all loci.

- **R1** is Robot 1; **R2** is Robot 2
- **M** is manual
- **Sample 1**, **Sample 2**, and **Sample 3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **PC** is the positive control

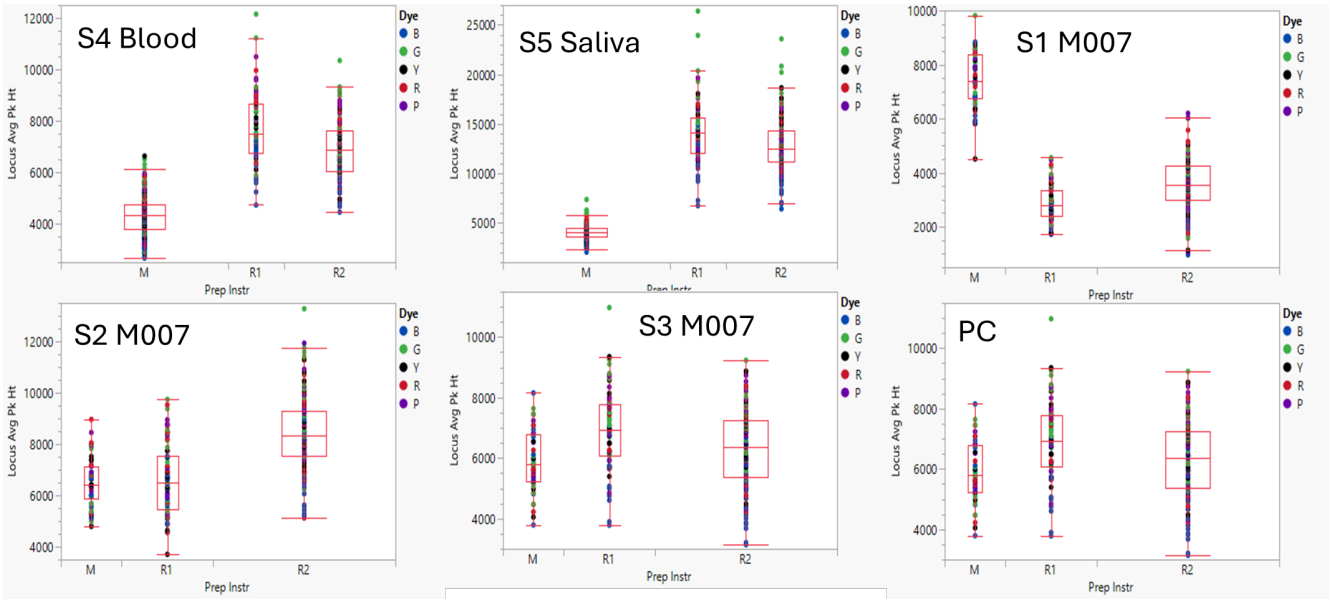


Figure 44 Average heterozygous peak heights from the same samples analyzed using a different workflow/instrument with the VeriFiler™ Plus PCR Amplification Kit. The figure shows the average peak height of the samples analyzed with the HID NIMBUS® Presto QNA System automated workflow and the manual workflow. The average peak height is comparable between the two workflows.

- **R1** is Robot 1; **R2** is Robot 2
- **M** is manual
- **S1**, **S2**, and **S3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively; **S4** is the 1-µL blood sample; **S5** is the 2-µL saliva sample
- **PC** is the positive control

Yfiler™ Plus PCR Amplification Kit

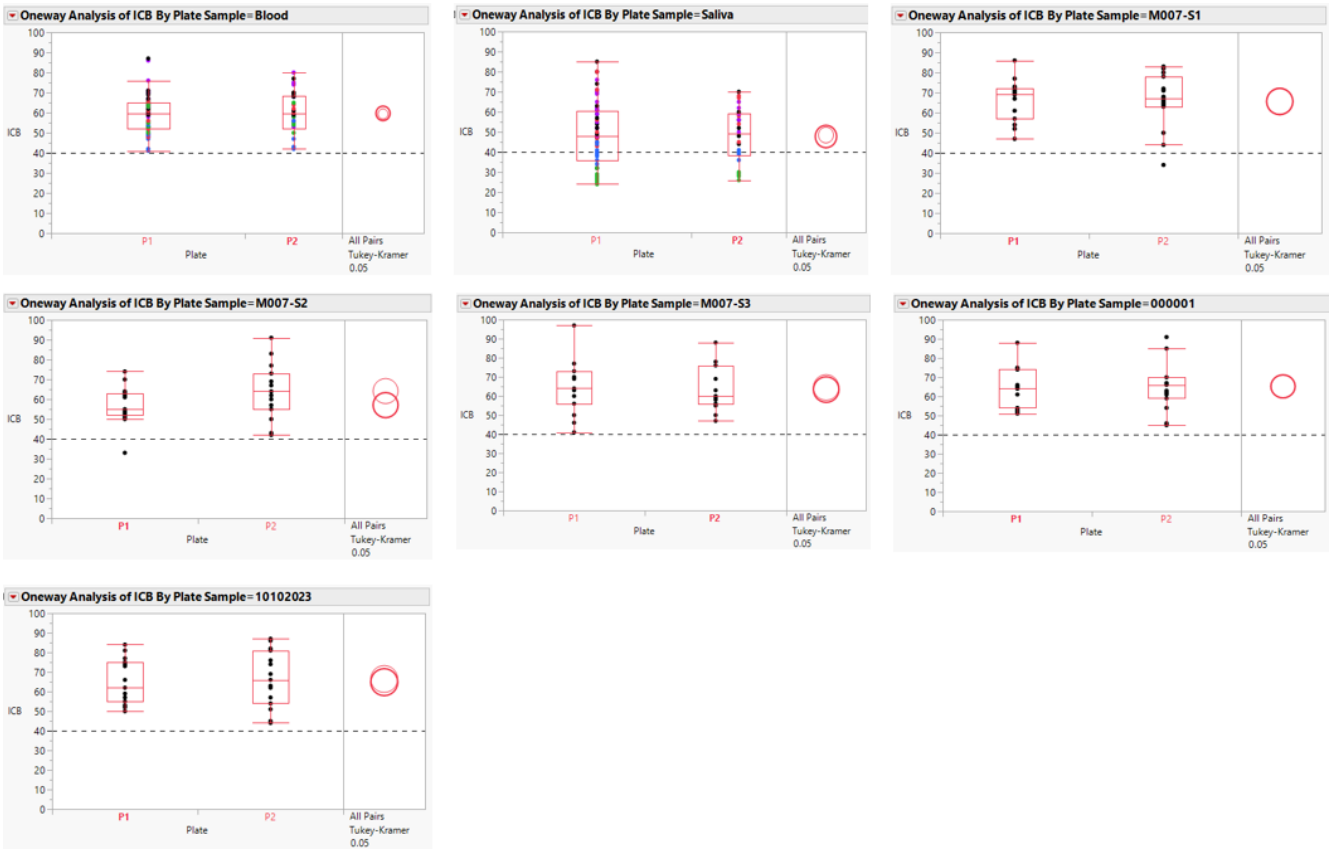


Figure 45 Intra-color balance from the analyzed samples using the Yfiler™ Plus PCR Amplification Kit. The figure shows the intra-color balance from Plate 1 and Plate 2 set up on Robot 2. The ICB is ≥40% with 95% confidence; the saliva sample showed lower peak height at DYS460.

- **P1** is Plate 1; **P2** is Plate 2
- **M007-S1**, **M007-S2**, and **M007-S3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **000001** and **10102023** are M007 dilutions at 0.1 and 0.2 ng/µL, respectively

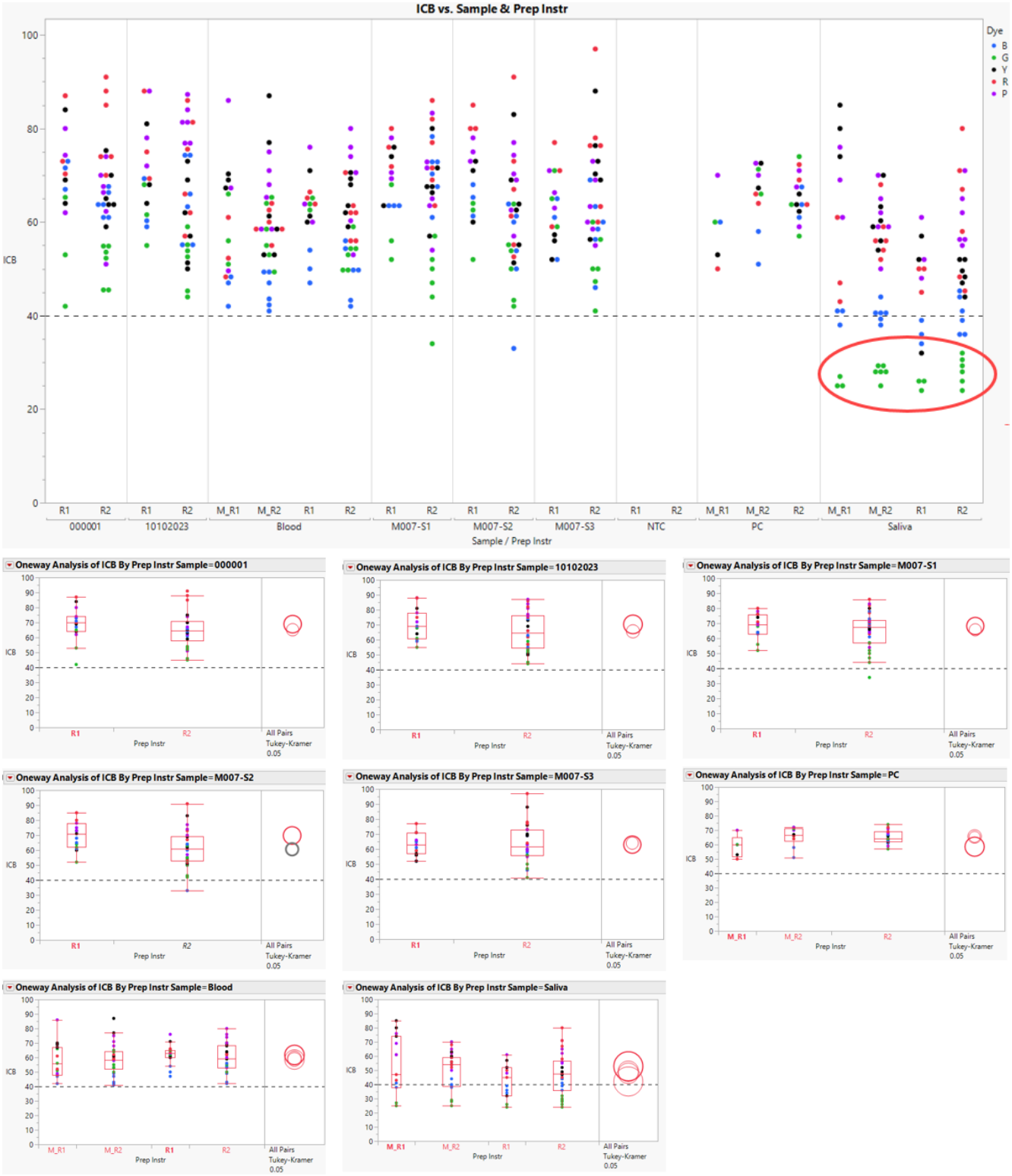


Figure 46 Intra-color balance from the same samples analyzed using a different workflow/instrument with the Yfiler™ Plus PCR Amplification Kit. The figures show the ICB comparison from each sample across the different workflows/instruments. The ICB is ≥40% with 95% confidence for all evaluated samples; the saliva sample showed lower peak height at DYS460 (red circle in the top figure).

- **R1** is Robot 1; **R2** is Robot 2

- **M_R1** and **M_R2** are manual samples added to Robot 1 and Robot 2 prepared plates.
- **M007-S1**, **M007-S2**, and **M007-S3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **000001** and **10102023** are M007 dilutions at 0.1 and 0.2 ng/µL, respectively
- **PC** is the positive control

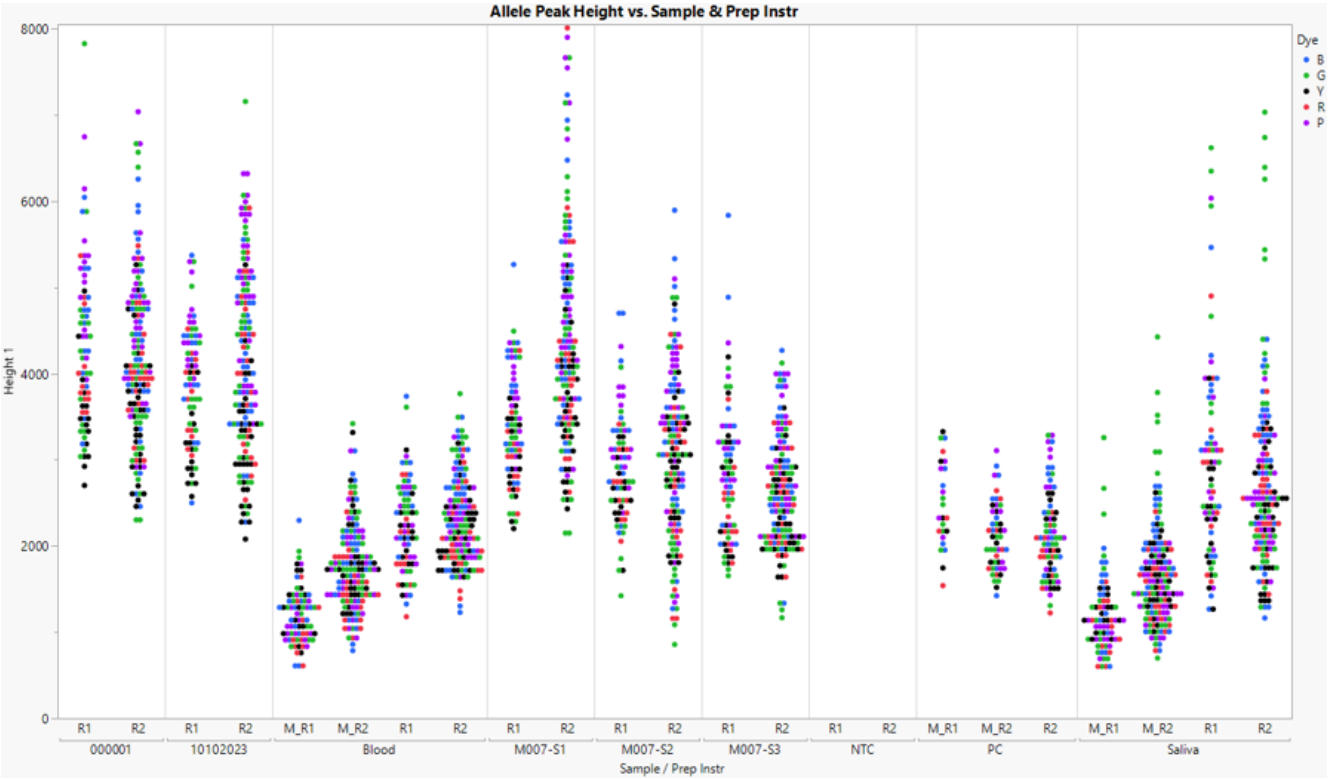
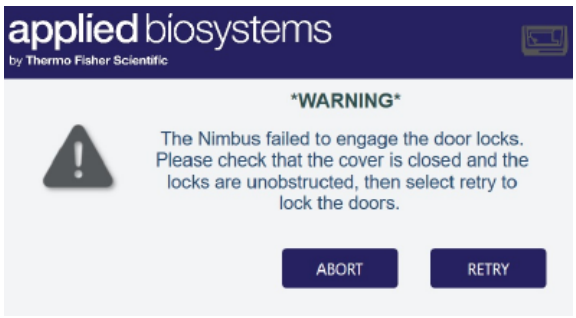


Figure 47 Average heterozygous peak heights from the same samples analyzed using a different workflow/instrument with the Yfiler™ Plus PCR Amplification Kit. The figure shows the average peak height of the samples analyzed with the HID NIMBUS® Presto QNA System automated workflow and the manual workflow. The average peak height is comparable between the two workflows.

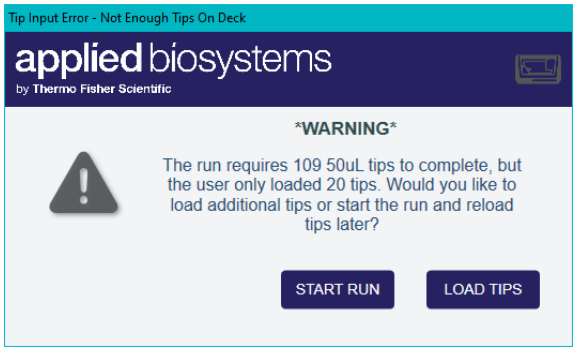
- **R1** is Robot 1; **R2** is Robot 2
- **M_R1** and **M_R2** are manual samples added to Robot 1 and Robot 2 prepared plates.
- **M007-S1**, **M007-S2**, and **M007-S3** are M007 at 10, 2.5, and 0.625 ng/µL, respectively
- **000001** and **10102023** are M007 dilutions at 0.1 and 0.2 ng/µL, respectively
- **PC** is the positive control

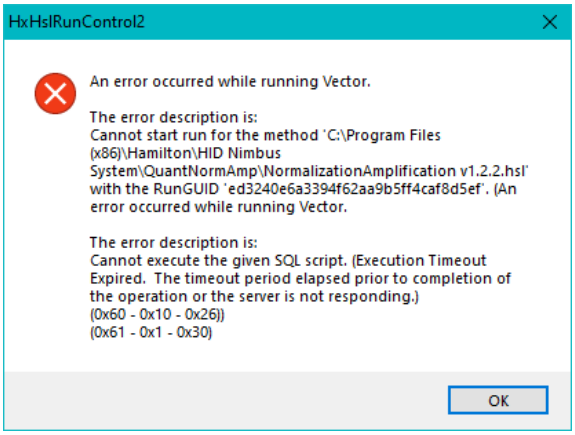
A

Troubleshooting

Observation	Possible cause	Recommended action
Aspiration is incomplete	The wrong labware was used. Using labware that is different from what is specified in the method and used at installation for axis position teaching may cause the tips to hit the tube bottom or aspirate at an insufficient depth.	Use the labware specified by the method. At installation, the robot axis positions are taught relative to specific plastics. Ensure that the same plastics that were used at installation are used for each step of the process.
A door lock error message is displayed 	The instrument was started with the door unlocked.	1. Close the instrument door. 2. Click **RETRY**. The system will lock the door.
	The instrument run was paused, then resumed with the door unlocked.	1. Close the instrument door. 2. In the error message, click **Launch Control Panel**. 3. In the **Control Panel** dialog box, click **Lock Door**, then close the dialog box. 4. In the error message, click **RETRY** to continue the run.

Observation	Possible cause	Recommended action
A clot error message is displayed	A clot was detected during aspiration.	Click the appropriate option in the error message: • **Repeat**—Retries the step that generated the error. • **Cancel**—Aborts the run, unless an automatic error recovery has been added to the method. • **Abort**—Aborts the run and ignores any automatic error recovery options. • **Air**—Raises the tips to clearance height and aspirates air before continuing the run. • **Bottom**—Retries aspiration from the bottom of the reagent reservoir, but with the liquid-level detection turned off. • **Exclude**—Continues the run without using any channel that generated the error in the remaining pipetting steps.

Observation	Possible cause	Recommended action
A liquid-level error message is displayed	The reagent volumes in the reagent reservoirs are too low.	Click the appropriate option in the error message: • **Repeat**—Retries the step that generated the error. • **Air**—Raises the tips to clearance height and aspirates air before continuing the run. • **Bottom**—Retries aspiration from the bottom of the reagent reservoir, but with the liquid-level detection turned off. • **Exclude**—Continues the run without using any channel that generated the error in the remaining pipetting steps. • **Unlock**—Unlocks the door so that the operator can correct the problem before continuing.
An insufficient tips error message is displayed 	There are not enough tips to continue the run.	Load additional tips, then fill out the tip counter appropriately to continue. In the error message, click **START RUN** to continue the run.

Observation	Possible cause	Recommended action
A run control error is displayed 	An attempt to launch a MED file from Venus Run Control.	Use the HID NIMBUS® System Software.

B Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition, and associated monographs)
  www.who.int/publications/i/item/9789240011311

C Documentation and support

Related documentation

Document	Publication number
PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the HID NIMBUS® Presto Systems User Bulletin	MAN0019368
HID NIMBUS® Systems Site Preparation Guide	MAN0026468
Quantifiler™ Trio Automated DNA Quantification Kit Product Information Sheet	MAN1000067
Quantifiler™ Trio Automated DNA Quantification Kit User Guide	MAN1000066

Customer and technical support

For support:

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

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

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Limited product warranty

Life Technologies Corporation and its affiliates 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 questions, contact Life Technologies at **www.thermofisher.com/support**.