Applied Biosystems

HID EVOlution™ – qPCR/STR Setup System

Getting Started Guide

HID EVOlution ™ – qPCR/STR Setup System

Getting Started Guide

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Contents

About This Guide

Purpose

This guide provides procedures for human identification (HID) customers who want to use the HID EVOlution™ - qPCR/STR Setup System to automate:

- Reaction plate setup for quantitative real-time PCR (qPCR)
- DNA normalization and PCR amplification reaction plate setup for Short Tandem Repeat (STR) analysis

Always refer to this guide for pre-run and post-run handling of samples, kit reagents, and pipetted PCR plates. Refer to Tecan documentation for details on the HID EVOlution™ - qPCR/STR Setup System safety, set up, operating, and maintenance instructions.

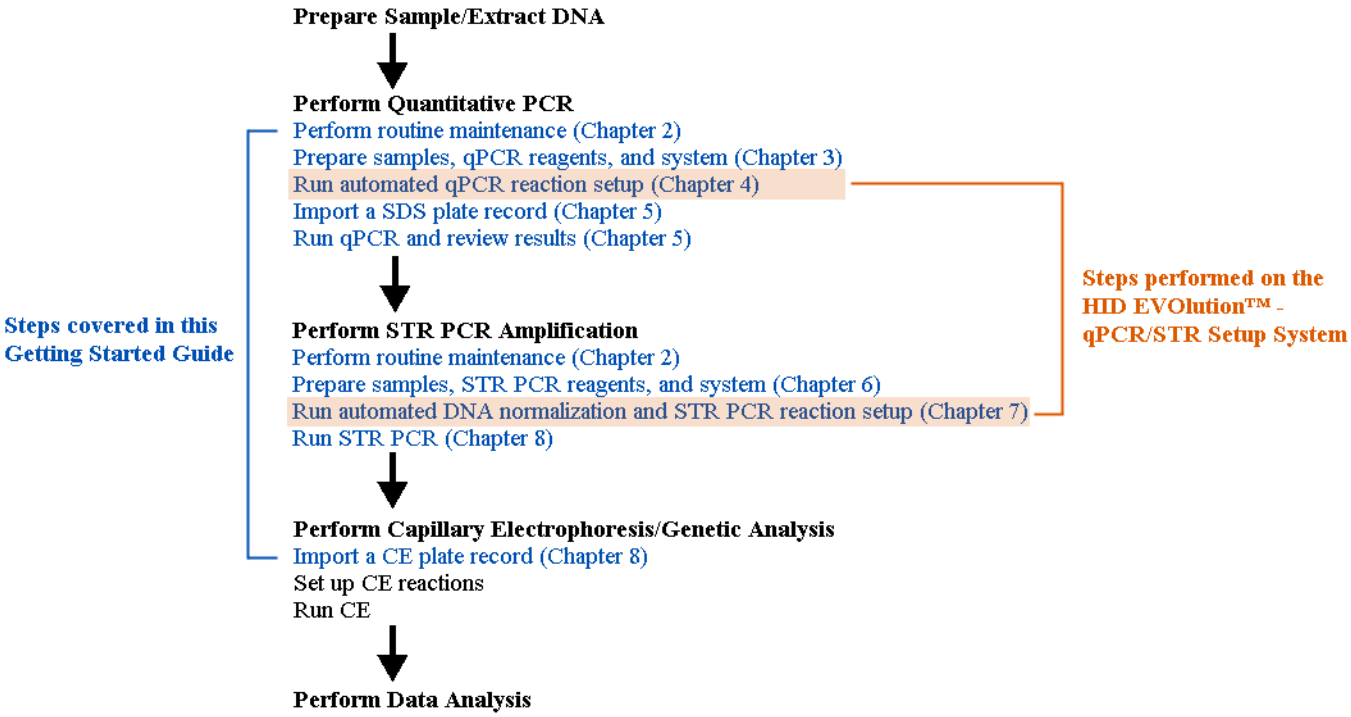


Figure 1 Overview of the automated HID workflow using the HID EVOlution™ - qPCR/STR Setup System. The steps covered in this Getting Started Guide are shown in blue. The steps performed on the HID EVOlution™ system are highlighted in orange.

Assumptions

This guide assumes that:

- You know how to handle forensic samples and prepare them for quantitation and STR analysis.
- The HID EVOlution™ - qPCR/STR Setup System (a Tecan Freedom EVO® 150 or 200 with the appropriate hardware, software, and scripts) has been installed, configured, tested, and calibrated by Tecan personnel.
- You are trained on the proper operation, maintenance, and troubleshooting of the HID EVOlution™ - qPCR/STR Setup System.
- You have access to the Tecan *HID EVOlution™ - qPCR/STR Setup System Application Manual*, the Tecan *Freedom EVO® Operating Manual*, and other applicable Tecan documentation.
- You have referred to the manufacturer's instrument documentation for important safety information related to the use of the Tecan Freedom EVO® instrument.

Safety information

Note: For general safety information, see this Preface and Appendix G, "Safety" on page 141. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

WARNING! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Applied Biosystems instruments*).

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "Obtaining MSDSs" on page 143.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

How to obtain support

For HID support:

- In North America – send an email to **HIDTechSupport@appliedbiosystems.com**, or call **888-821-4443** option **1**.
- Outside North America – contact your local support office.

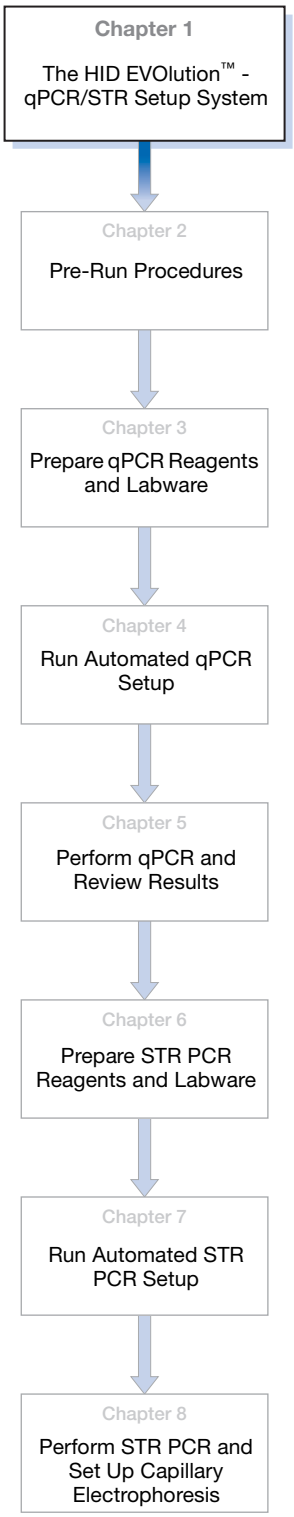
For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

1 The HID EVOlution™ - qPCR/STR Setup System



This chapter covers:

HID EVOlution™ - qPCR/STR Setup System workflow

The HID EVOlution™ - qPCR/STR Setup System automates qPCR and STR PCR reaction setup. Table 1 below describes the steps performed by the user in a human identification (HID) workflow, with the steps that involve the HID EVOlution™ - qPCR/STR Setup System highlighted in blue. A detailed description of the steps is provided in Appendix C on page 89. Figure 1 on page 3 shows the integration of the HID EVOlution™ - qPCR/STR Setup System with the other instruments, software, and data files in the HID workflow.

Table 1 Overview of the automated HID workflow.

HID Workflow	Covered in Chapter...
Prepare Sample/Extract DNA	Extraction procedures are not included in this guide. For automated extraction procedures, see the *PrepFiler™ Automated Forensic DNA Extraction Kit Getting Started Guide.*
Perform Quantitative PCR	
1. Perform routine maintenance	2, "Pre-Run Procedures" on page 9
2. Prepare samples, qPCR reagents, and system	3, "Prepare qPCR Reagents and Labware" on page 23
3. Run automated qPCR reaction setup	4, "Run Automated qPCR Setup" on page 33
4. Import a SDS plate record	5, "Perform qPCR and Review Results" on page 39
5. Run qPCR and review results	5, "Perform qPCR and Review Results" on page 39
Perform STR PCR Amplification	
1. Perform routine maintenance	2, "Pre-Run Procedures" on page 9
2. Prepare samples, STR PCR reagents, and system	6, "Prepare STR PCR Reagents and Labware" on page 45
3. Run automated DNA normalization and STR PCR reaction setup	7, "Run Automated STR PCR Setup" on page 57
4. Run STR PCR amplification	8, "Perform STR PCR and Set Up Capillary Electrophoresis" on page 65
Perform Capillary Electrophoresis/Genetic Analysis	
1. Import a CE plate record	8, "Perform STR PCR and Set Up Capillary Electrophoresis" on page 65
2. Set up CE reactions	
3. Run CE	
Perform Data Analysis	CE and data analysis procedures are not included in this guide. See the AmpFℓSTR Kit User's Guides.

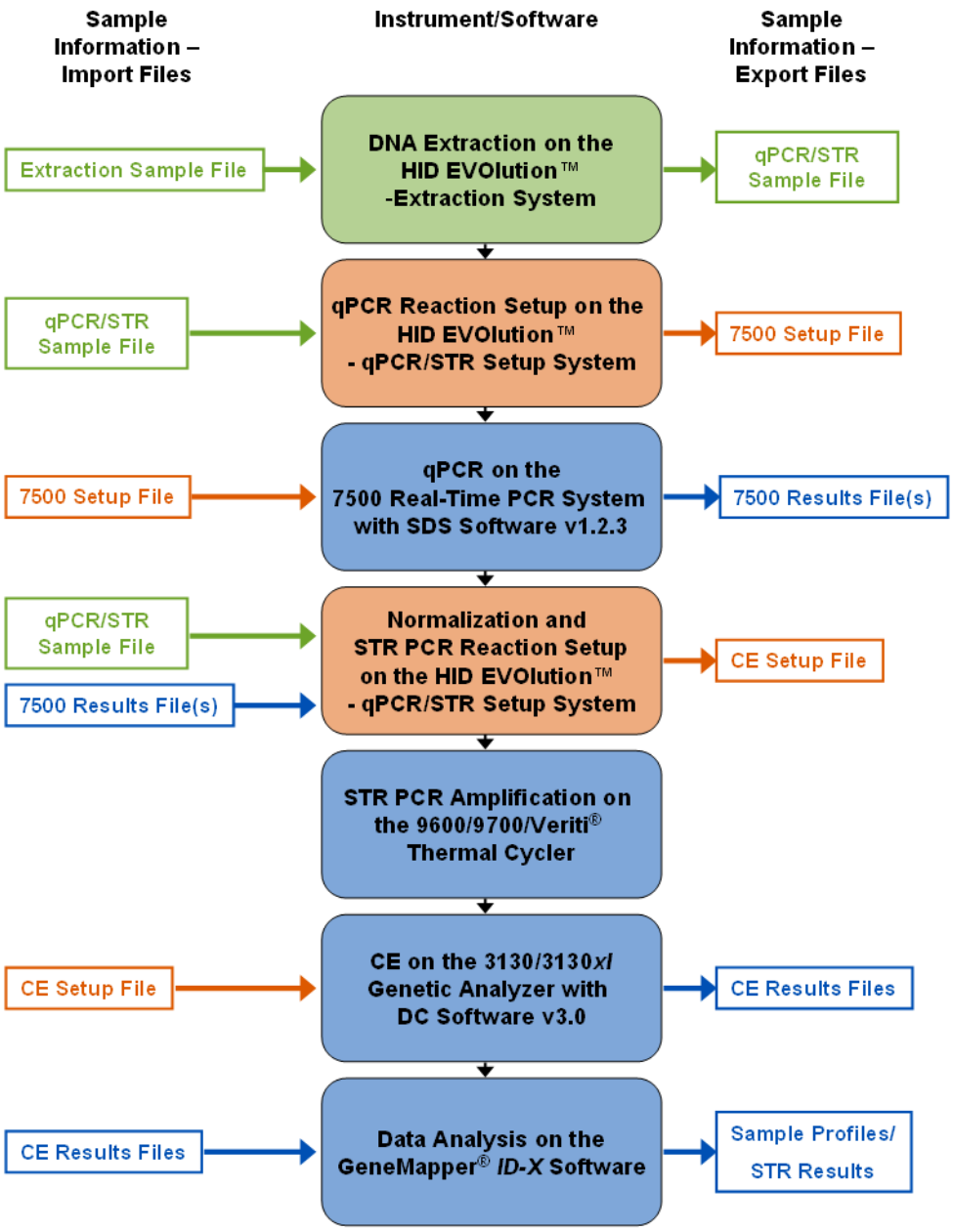


Figure 1 Files used to import sample information to the next HID workflow step. The qPCR/STR Sample file is automatically generated by the HID EVOlution™ – Extraction System, or can be manually created from a template. Use the qPCR/STR Sample file to import the required sample name and information for both qPCR and STR PCR reaction setup (see page 17 for more options). Import the automatically-generated 7500 Setup file as a SDS plate record. Import the automatically-generated CE Setup file as a CE plate record.

Supported system configuration

The HID EVOlution™ - qPCR/STR Setup System consists of:

- A dedicated TECAN Freedom EVO® 150 or 200 robotic workstation.
- The necessary hardware, including a 4-channel liquid handling accessory (LiHa).
- Application software: Freedom EVOware® version 2.1 SP1, SOE version 1.2 SP1, and HID EVOlution qPCR/STR driver 1.1.0.0.25 SP4 *or* Freedom EVOware® version v1.4 SP1, special SOE for HID, and HID EVOlution qPCR/STR driver 1.1.0.0.25 SP4.

  Note: If you are not using the most current driver (1.1.0.0.25 SP4), contact your local Tecan service organization about upgrading. See the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 10, "Customer Support," for contact information.

- Software scripts developed and validated to automate the set up of reaction plates for quantitative real-time PCR and STR PCR amplification for use in HID applications.

Note: The Freedom EVO 150 and 200 instruments can be configured identically and both instruments are supported for use with the HID EVOlution™ - qPCR/STR Setup System. Validation studies were performed on the Freedom EVO 150 and Freedom EVOware® v1.4. Freedom EVOware® v2.1 was validated on the HID EVOlution™ – Combination System with additional verification studies performed on the HID EVOlution™ - qPCR/STR Setup System.

Supported kits

The following Applied Biosystems DNA quantitation and amplification kits have been validated and are supported for use with the HID EVOlution - qPCR/STR Setup System.

Supported Kit	Applied Biosystems Part Number
Quantifiler® Human DNA Quantification Kit	4343895
Quantifiler® Y Human Male DNA Quantification Kit	4343906
AmpFℓSTR® COfiler® PCR Amplification Kit	4304256
AmpFℓSTR® Identifiler® PCR Amplification Kit	4322288
AmpFℓSTR® MiniFiler™ PCR Amplification Kit	4373872
AmpFℓSTR® Profiler Plus® PCR Amplification Kit	4303326
AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit	4382699
AmpFℓSTR® SGM Plus® PCR Amplification Kit	4307133
AmpFℓSTR® Yfiler® PCR Amplification Kit	4359513

Refer to the appropriate Quantifiler® kit and AmpFℓSTR® kit user guides for kit contents and storage conditions.

Required instruments, software, and materials

The additional instruments, software, and materials needed for use but not supplied with the HID EVOlution™ - qPCR/STR Setup System are described in Tables 2 and 3:

Table 2 Required instruments and software

Instrument or Software	Source
Applied Biosystems 7500 Real-Time PCR System and Sequence Detection Software v1.2.3	Applied Biosystems (Contact your local sales representative for computer configurations)
9600/9700 Gold-plated Silver 96-Well GeneAmp® PCR System *or* 9600/9700 Silver 96-Well GeneAmp® PCR System *or* Veriti® 96-well thermal cycler (with silver 96-well sample block or gold-plated silver 96-well sample block)	Applied Biosystems (PN 4314878) Applied Biosystems (PN N8050001) Applied Biosystems (PN 4375786)
Applied Biosystems 3130/3130*xl* Genetic Analyzer and Genetic Analyzer Data Collection Software version 3.0	Applied Biosystems (Contact your local sales representative for computer configurations)

Table 3 Required materials

Material	Source‡
Benchtop centrifuge with 96-well plate adapters	
Disposable pipette tips (DiTis): 50-µL LiHa conductive disposable tips with filter§	Tecan (PN 30032114) www.tecan.com
Disposable pipette tips (DiTis): 200-µL LiHa conductive disposable tips with filter §	Tecan (PN 30000629) www.tecan.com
100 mL disposable troughs for reagents	Tecan (PN 10613048) www.tecan.com
Barcodes (optional)	See the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual* and the Tecan *Freedom EVO® Operating Manual*, Section 3.5.6 “Positive Identification (PosID)” for requirements
MicroAmp® clear adhesive film (to seal PCR product plates for storage)	Applied Biosystems (PN 4306311)
MicroAmp® adhesive film applicator	Applied Biosystems (PN 4333183)
MicroAmp® optical adhesive film (to seal qPCR reaction plates during qPCR)	Applied Biosystems (PN 4360954)
Four 96-well reaction plate adapters (supplied with the system; listed as “adapter plate PCR HID EVOlution” in the *HID EVOlution™ – qPCR/STR Setup System Application Manual*.) **Note:** If you are processing extracted DNA from plates, you will require 2 adapters for qPCR setup and 4 adapters for STR PCR setup. If you are processing extracted DNA from tubes, you need 1 adapter for qPCR setup and 3 for STR PCR setup.	Tecan (PN 30032860)
Six tube racks (optionally supplied with the system; also listed as “16-position tube carrier with vertical cap storage” in the *HID EVOlution™ – qPCR/STR Setup System Application Manual*.)	Tecan (PN 10613035)

Table 3 Required materials *(continued)*

Material	Source‡
Graduated 5-mL self-standing transport tubes (master mix vials) with conical bottom	VWR 89005-596
$T_{10}E_{0.1}$ buffer (10 mM Tris-HCl [pH 8.0] and 0.1 mM Na_2EDTA)	User supplied or Teknova (T0233)
Degassed deionized water	MLS#
MicroAmp® optical 96-well reaction plate (with or without barcode)	Applied Biosystems (PN 4306737 or N8010560)
RNase-free microfuge tubes (1.5 mL), certified DNase- and RNase-free	Applied Biosystems (PN AM12400) or equivalent

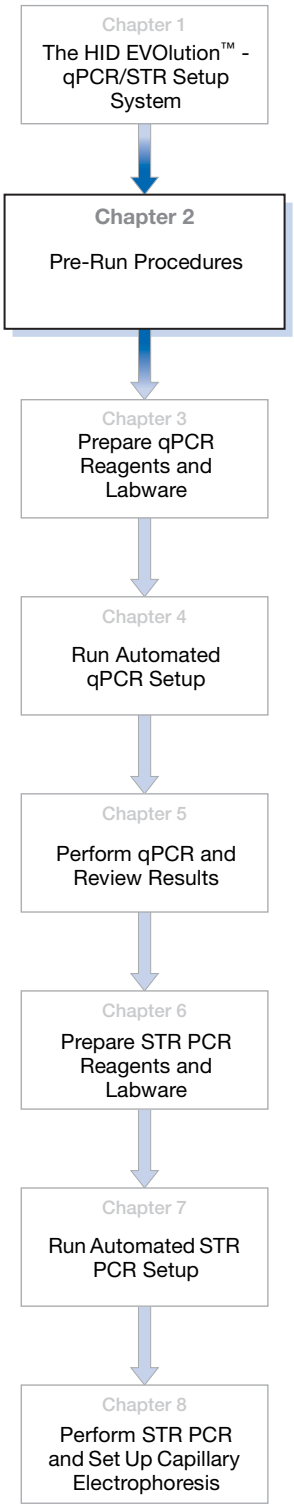
‡ Recommended sources. Equivalent materials from other suppliers can be used after appropriate validation studies by the user laboratory.
§ Disposable tips that have not been certified by Tecan may not yield the same liquid handling performance.
# For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

For more information

For information on:

- DNA standard dilution series preparation and quantitation procedures, see the appropriate *Quantifiler® Kits User's Guide*.
- DNA normalization and amplification procedures, see the appropriate AmpFℓSTR® Kit User Guide as noted in "Applied Biosystems documentation" on page 148.
- Manual DNA extraction procedures, see the *PrepFiler™ Forensic DNA Extraction Kit User Guide*.
- Automated DNA extraction procedures, see the *PrepFiler™ Automated Forensic DNA Extraction Kit Getting Started Guide*.
- Validation experiments performed by Applied Biosystems and the results, see Appendix E, "Validation Experiments and Results" on page 103.
- The HID EVOlution™ - qPCR/STR Setup System, see the "Tecan documentation" on page 149.

2 Pre-Run Procedures



This chapter provides prerequisite procedures for qPCR and STR PCR reaction setup.

One-time tasks

Complete the following tasks on each Real-Time PCR and CE instrument before starting the automated procedures. This will allow you to import the:

- SDS Setup file (generated by the HID EVOlution™ - qPCR/STR Setup System) into the SDS software v1.2.3.
- 7500 Results file(s) into the HID EVOlution™ - qPCR/STR Setup System software.
- CE Setup file (generated by the HID EVOlution™ - qPCR/STR Setup System) into the 3130/3130*xl* instrument Data Collection Software v3.0.

Create detectors for the 7500 Real-Time PCR System

If you have not already done so, create detectors in the SDS Software v1.2.3 for running the Quantifiler® assays. Refer to the Applied Biosystems *Quantifiler® Kits User's Manual* for instructions on creating the detectors.

IMPORTANT! The detectors must have the exact names and capitalization shown below.

Note: These detectors allow you to import the HID EVOlution system-generated 7500 Setup file (ReactionPlate1.txt or <*barcode*>.txt) to the SDS Software. See "About the 7500 Setup file" on page 40 for details.

1. Create the following detectors for the Quantifiler® Human kit:
   - Name = **Quantifiler Human**
     - Reporter Dye = **FAM**
     - Quencher Dye = **None** (make sure **None** is selected)
   - Name = **IPC** (IPC assay)
     - Reporter Dye = **VIC**
     - Quencher Dye = **None** (make sure **None** is selected)
2. Create the following detectors for the Quantifiler® Y Human Male kit:
   - Name = **Quantifiler Y**
     - Reporter Dye = **FAM**
     - Quencher Dye = **None** (make sure **None** is selected)
   - Name = **IPC** (IPC assay)
     - Reporter Dye = **VIC**
     - Quencher Dye = **None** (make sure **None** is selected)

Create an instrument protocol and results group for the CE instrument

If you have not already done so, create an instrument protocol and a results group in the Data Collection Software v3.0 to match the names used in CE Setup file generated by the HID EVOlution™ - qPCR/STR Setup System:

1. After STR PCR reaction setup, use Microsoft® Excel® to open the CE Setup file generated by the HID EVOlution™ - qPCR/STR Setup System.

   Note: The CE Setup file name and location is C:\HIDEVOlution_qPCRSTRfiles\AB3130Input\STRplate_*<rundate>*_*<runtime>*.txt *or* C:\HIDEVOlution_qPCRSTRfiles\AB3130Input*<barcode>*_*<rundate>*_*<runtime>*.txt.

2. In the CE Setup file, note the exact name (including capitalization) of the instrument protocol and the results group.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Container	Description	ContainerT	AppType	Owner	Operator								
2	A9045GIE_20090123		96-Well	Regular	No log in	No log in								
3	AppServer	AppInstance												
4	GeneMapp	GeneMapper_Generic_Instance												
5	Well	Sample Na	Comment	Priority	Sample Ty	Snp Set	Analysis M	Panel	User-Defin	Size Stanc	User-Defin	User-Defin	Results Group 1	Instrument Protocol 1
6	A1	50ng1	50ng1	100	Sample	None	HID_Advar	MiniFiler				50ng1	HIDEvolution	HIDEvolution
7	B1	TE1	TE1	100	Sample	None	HID_Advar	MiniFiler				TE1	HIDEvolution	HIDEvolution
8	C1	50ng2	50ng2	100	Sample	None	HID_Advar	MiniFiler				50ng2	HIDEvolution	HIDEvolution
9	D1	TE2	TE2	100	Sample	None	HID_Advar	MiniFiler				TE2	HIDEvolution	HIDEvolution
10	E1	50ng3	50ng3	100	Sample	None	HID_Advar	MiniFiler				50ng3	HIDEvolution	HIDEvolution
11	F1	TE3	TE3	100	Sample	None	HID_Advar	MiniFiler				TE3	HIDEvolution	HIDEvolution
12	G1	50ng4	50ng4	100	Sample	None	HID_Advar	MiniFiler				50ng4	HIDEvolution	HIDEvolution
13	H1	TE4	TE4	100	Sample	None	HID_Advar	MiniFiler				TE4	HIDEvolution	HIDEvolution
14	A2	TE5	TE5	100	Sample	None	HID_Advar	MiniFiler				TE5	HIDEvolution	HIDEvolution
15	B2	50ng5	50ng5	100	Sample	None	HID_Advar	MiniFiler				50ng5	HIDEvolution	HIDEvolution
16	C2	TE6	TE6	100	Sample	None	HID_Advar	MiniFiler				TE6	HIDEvolution	HIDEvolution
17	D2	10ng1	10ng1	100	Sample	None	HID_Advar	MiniFiler				10ng1	HIDEvolution	HIDEvolution
18	E2	TE7	TE7	100	Sample	None	HID_Advar	MiniFiler				TE7	HIDEvolution	HIDEvolution
19	F2	Positive	Positive	100	Positive C	None	HID_Advar	MiniFiler				Positive	HIDEvolution	HIDEvolution
20	G2	Negative	Negative	100	Negative C	None	HID_Advar	MiniFiler				Negative	HIDEvolution	HIDEvolution
21	H2	ladder	ladder	100	Allelic Lad	None	HID_Advar	MiniFiler				ladder	HIDEvolution	HIDEvolution

   Note: By default, the instrument protocol and the results group are both named "HIDEvolution"; however, individual systems can have different configurations.

3. Confirm that, in the Data Collection software, you have created an instrument protocol and a results group with the same names that you noted in step 2. If not, follow steps 4 and 5 to create them.

4. If necessary, set up the results group in the Data Collection software Results Group Editor:

   a. Select the **General** tab, then enter the results group name exactly as it appears in the CE Setup file (usually **HIDEvolution**).

   b. Select the Analysis tab, then select **GeneMapper_Generic** in the Analysis Type field.

   c. Configure the Destination and Naming tabs according to your laboratory needs.

5. If necessary, set up the instrument protocol in the Data Collection software:
   a. Select **Protocol Manager** from the left navigation pane.
   b. Click **New** at the center left of the Instrument Protocol section, then enter the following:
      - Name: Enter the instrument protocol name exactly as it appears in the CE Setup file (usually **HIDEvolution**).
      - Type: **Regular**
      - Run Module: Select the run module according to the kit and your laboratory needs.
      - Dye Set: Select the dye set according to the kit.
   c. Click **OK**.

Refer to the Applied Biosystems *3130/3130xl Getting Started Guide* for details on creating the instrument protocol and results group.

Before each run: Run maintenance scripts

Run the appropriate maintenance scripts after setting up carriers and racks on the worktable, but before placing samples, reagents, or plasticware on the worktable. See the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual,* Section 7.5, "Maintenance Scripts," and Section 5.2, "Running Maintenance" for details.

Before starting the run, if...	...then run
It is the first run of the day	DailyStartUp_qPCRSTR
It is *not* the first run of the day	Flush_qPCRSTR
When you run DailyStartUp_qPCRSTR or Flush_qPCRSTR, you see: • Air bubbles in the lines *and/or* • Intermittent flow from a DiTi cone	Flush_qPCRSTR one or more times until: • There are no visible air bubbles *and* • Flow from the DiTi cones is constant
There are one or more DiTis on the liquid handling arm (LiHa)	Drop_DiTis_qPCRSTR
You refilled DiTis	Set_200tip_Position_qPCRSTR *and* Set_50tip_Position_qPCRSTR

IMPORTANT! For proper liquid handling, run the Flush_qPCRSTR script before *every* run one or more times until there are no visible air bubbles.

Note: Maintenance scripts are not included with Freedom EVOware® v1.4.

Before each run: Set up extracted DNA samples

You can set up extracted DNA samples in *either*:

- A 96-well plate – Follow the instructions in "Set up extracted DNA samples in a 96-well plate" on page 14
- 1.5-mL tubes – Follow the instructions in "Set up extracted DNA samples in tubes" on page 15

Note: You cannot use *both* a plate and tubes for extracted DNA samples in the same run.

Tables 4 and 5 describe the maximum number of extracted DNA samples that can be processed in a qPCR or STR PCR reaction setup run.

Table 4 Maximum number of samples in a qPCR reaction setup run

Quantifiler® DNA Quantification Kit	Number of extracted samples and controls	Number of reactions in resulting qPCR reaction plate	Other wells in resulting qPCR reaction plate
Quantifiler® Human Kit	up to 80	up to 80	16 wells are used for the DNA standard dilution series (2 replicates of the 8 concentrations in the series)
Quantifiler® Y Human Male Kit	up to 80	up to 80	16 wells are used for the DNA standard dilution series (2 replicates of the 8 concentrations in the series)
Combined set up using both Quantifiler® Human and Y Human Male Kits	up to 32	up to 32 Quantifiler® Human kit reactions up to 32 Quantifiler® Y Human Male kit reactions	32 wells are used for the DNA standard dilution series (4 replicates of the 8 concentrations in the series)

Table 5 Maximum number of samples in a STR PCR reaction setup run

AmpFℓSTR® PCR Amplification Kit	Number of extracted samples and controls	Number of reactions in resulting STR PCR reaction plate	Other wells in resulting STR PCR reaction plate
COfiler®, Identifiler®, MiniFiler™, Profiler Plus®, SEfiler Plus™, or SGM Plus® Kits	up to 88	up to 88	1 well for amplification positive control 1 well for amplification negative control 6 empty wells‡
Yfiler® Kit	up to 87	up to 87	2 wells for amplification positive control 1 well for amplification negative control 6 empty wells‡

‡ Because the STR PCR reaction plate layout is designed for easy transfer of amplified PCR product to a CE plate, six of the wells in the STR PCR reaction plate remain empty as a placeholder for the allelic ladder sample replicates in the CE plate. See "About the STR PCR plate layout" on page 62.

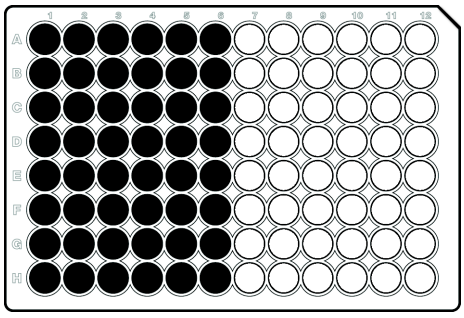
Set up extracted DNA samples in a 96-well plate

1. Confirm that a MicroAmp® Optical 96-Well Reaction Plate (the extracted DNA sample plate) is labeled for identification. If you use barcodes to track samples, move the barcode provided with the plate into the correct position as shown in the Tecan *HID EVOlution™ qPCR/STR Setup System Application Manual*.

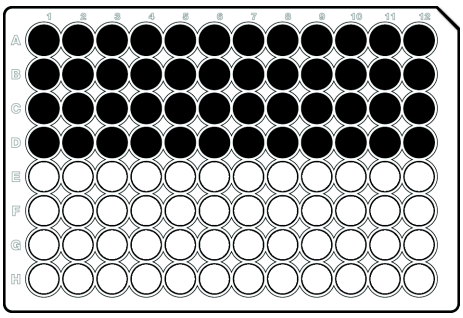
2. Place the first extracted DNA sample in any well position on the plate (for example, you can begin with well number 14).

3. After the first extracted DNA sample, continue placing samples next to one another in *vertical columns* as shown in the "Correct" examples below. Do not leave empty wells between samples. If there is a failed extracted sample in the plate, use blank reagents (water or TE buffer) in that well. See examples of correct extracted DNA sample plate setup below.

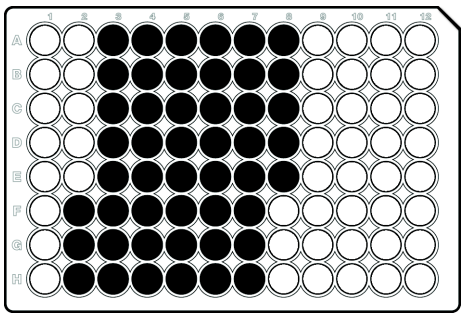
IMPORTANT! Make sure to assign a sample ID to all samples, including failed samples.



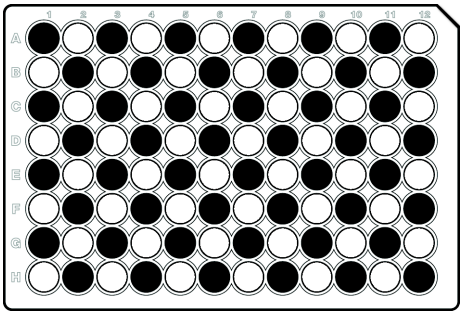
Correct (samples in wells 1 through 48)



Incorrect



Correct (samples in wells 14 through 61)



Incorrect

Set up extracted DNA samples in tubes

1. Confirm the number and labeling of tubes:
   - For qPCR setup, confirm that you have no more than 80 labeled 1.5-mL microcentrifuge tubes containing extracted DNA samples or control samples (or no more than 32 tubes for a combined plate).
   - For STR PCR setup, confirm that you have no more than 88 labeled 1.5-mL microcentrifuge tubes (87 for the Yfiler® kit) containing extracted DNA samples.

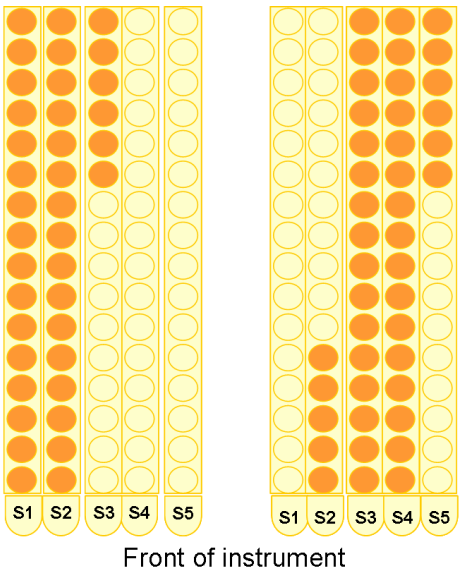
   If you use barcodes to track samples, confirm that barcodes are correctly placed on the tubes (see barcode information in the Tecan *HID EVOlution™ qPCR/STR Setup System Application Manual*).

2. Confirm that the tube racks are correctly positioned:
   - For qPCR setup, confirm that the tube racks S1 through S5 are at grid positions 27-31.
   - For STR PCR setup, confirm that the tube racks S1 through S6 are at grid positions 27-32.

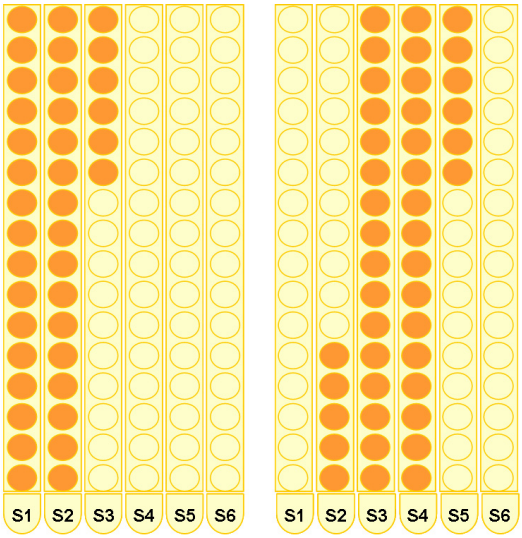
3. Correctly position the sample tubes in the tube racks:
   a. Place the first sample tube in the tube racks (for example, you can begin with rack S1, position 8).
   b. After the first sample tube, continue placing sample tubes from back to front in *vertical columns* as shown in the examples below. Do not leave empty positions between sample tubes.

IMPORTANT! DNA sample tubes must be contiguously loaded. Do not leave empty tube positions between sample tubes.

Examples of correct qPCR setup:



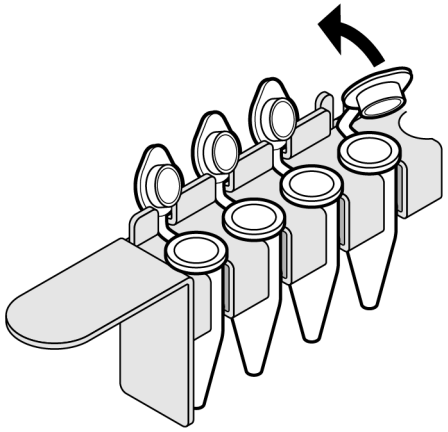
Examples of correct STR PCR setup:



4. Check that the barcodes are in a readable position.

5. Open each tube, securing the tube caps in a fixed upright position as shown below.

IMPORTANT! Open tube caps carefully to prevent contamination and splatter.



Before each run: (Optional) Set up sample information

You have several options for entering sample information to the HID EVOlution™ - qPCR/STR Setup System software. If you chose the sample file option, set up the sample file before the run according to the instructions in "Create a qPCR/STR Sample file" on page 18.

About sample information

During a qPCR or STR PCR reaction setup run, the software prompts you to enter or import the sample name and information for each extracted DNA sample. The sample information is used by the HID EVOlution™ - qPCR/STR Setup System to:

- Set up the reaction plate.
- Generate a Samples Report at the end of the run. The report records each extracted DNA sample starting position in a plate or in tubes and each sample final position in the reaction plate.
- Generate a text file containing the sample information:
  - **qPCR reaction setup run** – Generates a 7500 Setup file that you can import into the SDS software plate document to define the parameters of the 7500 Real-Time PCR System run.
  - **STR PCR reaction setup run** – Generates a CE Setup file that you can import into the Data Collection Software to define the parameters of the 3130/3130*xl* Genetic Analyzer run.

Options for entering sample information

You have several options for entering sample information. You can:

- **Automatically capture sample information by having the system scan barcodes on the plates and/or tubes** – Select barcodes compatible with the PosID-3, then make sure the barcodes are correctly placed when you set up the worktable. See the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 4.6 "Barcodes" for details. For:
  - **Barcodes on 96-well plates** – After the plate barcode is scanned, you must manually enter or import the sample name and information for each well in the plate. See the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 5.3 "Running a Script" for details.
  - **Barcodes on tubes** – The sample name (barcode) and sample position for each tube are automatically updated in the software when the barcodes are scanned. After the tube barcodes are scanned, you have the option to manually edit the sample information. See the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 5.3 "Running a Script" for details.
- **Import the qPCR/STR Sample file (output) from HID EVOlution™ – Extraction System** – If you used the HID EVOlution™ – Extraction System to perform DNA extraction, use the Extraction System output (qPCR/STR Sample file: HID_<*rundate*> <*runtime*>.csv) from that run. Sample ID and sample position in the sample file must agree with the samples on the worktable.

- **Create a qPCR/STR Sample file from a template** – You can create a qPCR/STR Sample file before running the script and then import the file into the HID EVOlution™ system. Two sample input file templates are provided on the CD with the HID EVOlution software: one for DNA samples in a 96-well plate, and one for DNA samples in 1.5-mL tubes. See "Create a qPCR/STR Sample file" on page 18 and the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 3.4.2 "File Format for Plate Wells/Tubes" for details.
- **Manually enter sample information into the HID EVOlution™ software** – Run the appropriate EVOware software script, then enter the information when you are prompted. Use the **Edit** button in the Sample Information dialog box and manually add sample information. See the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 5.3 "Running a Script" for details.

  Note: If you manually enter sample information for qPCR setup, you will need to re-enter the information for STR PCR setup. To reduce setup time, consider creating a qPCR/STR Sample file for use in qPCR and STR PCR setup as described below.

Note: If you are using barcodes, barcode scanning takes place before you import and/or manually enter information.You can manually enter sample information after barcode scanning and before or after importing a sample file. Generally, the information scanned/imported/entered last overwrites previous sample information. Because initial software configuration can vary depending on customer requirements, you may see different behavior with your system.

Create a qPCR/STR Sample file

1. Open the qPCR/STR Sample file template provided with the software CD:
   a. Select **Start ▸ All Programs ▸ Accessories ▸ Notepad** to open Microsoft Notepad.

      IMPORTANT! Use a text editor such as Microsoft Notepad to edit the sample input file. Do not use Microsoft Excel, which may introduce invalid formatting.

   b. Select **File ▸ Open**, then browse to the template files originally provided in the "Sample Files" folder on the HID EVOlution™ - qPCR/STR Setup System software CD.
   c. Select **SampleFile_96.csv** (if using tubes) or **SampleFile_Plate_96.csv** (if using a plate), then click **Open**.
2. Select **File ▸ Save As**, browse to the directory C:\HIDEVOlution_qPCRSTRfiles (or another location of your choosing), change the file name to ***<UserDefined>*.csv**, then click **Save**.

3. When editing a template, follow the formatting rules that are described in Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 3.4.2 "File Format for Plate Wells/Tubes" and the following guidelines:
   - Do not include empty plate well or tube rack positions between samples.
   - Avoid spaces or other special characters such as commas (,), asterisks (*), or slashes (/).
   - For the sample name field, follow your laboratory naming conventions to assign a unique name to each sample. Make sure that the sample name meets the formatting rules.

4. Save the file with a .csv extension, then close the file.

IMPORTANT! The file extension must be .csv for the file to be imported to the HID EVOlution™ software.

Guidelines: Preventing contamination

PCR assays require special laboratory practices to avoid false positive amplifications. The high sensitivity of these assays may result in the amplification of a single DNA molecule.

To minimize false positives due to the presence of amplified material in your work area, follow these recommended laboratory practices:

- When possible, maintain separate work areas, dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Wear a clean lab coat (not previously worn while handling amplified PCR products or during sample preparation) and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect they are contaminated and before leaving the work area.
- Use positive-displacement pipettes with aerosol-resistant pipette tips.
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes and reaction plates carefully.
- Try not to splash or spray PCR samples.
- When pipetting from a kit component tube, hold the cap of the tube in your gloved hand, or be sure to set it down on a clean, decontaminated surface.
- Keep reactions and components sealed when possible.
- Before opening sealed reagents or reaction tubes or plates, centrifuge the tube or plate briefly (approximately two seconds in a microcentrifuge) to collect any residual tube contents from the sides and cap.
- Clean lab benches and equipment periodically with freshly diluted 10% bleach solution.

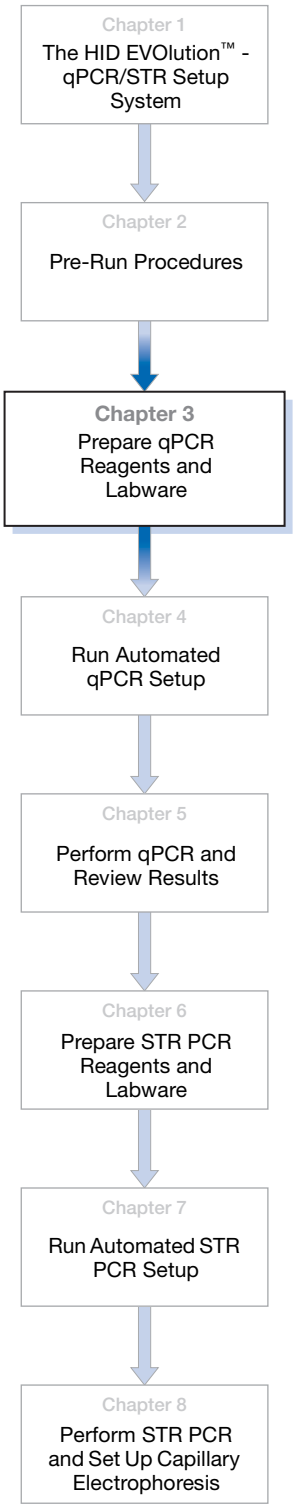
Note: For the Freedom EVO™ workstation, follow with copious amounts of water.

For more information

For information on:

- Tecan's numbering system for plate wells and tube racks, see the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 3.4.5, "Definition of Positions in Multi-Well Racks and Plates."
- Preparing a sample input file, refer to the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 3.4.2 "File Format for Plate Wells/Tubes" and Section 3.4.5, "Definition of Positions in Multi-Well Racks and Plates."
- Manually entering sample information, refer to the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 5.3 "Running a Script."
- Barcode specifications for use on the Freedom EVO instrument, refer to the Tecan *Freedom EVO® Operating Manual*, Section 3.5.6, "Positive Identification (PosID)" and the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 4.6 "Barcodes."

3 Prepare qPCR Reagents and Labware



This chapter provides procedures to prepare reagents, labware, and the Tecan Freedom EVO workstation for quantitative PCR (qPCR) reaction setup.

Review pre-run checklist for qPCR reaction setup

☐	Cool the qPCR reagent block to 4 °C before use to help keep reagents cool on the worktable. It is recommended, when not in use, that you store reagent blocks in a refrigerator at 4 °C.
☐	(Optional) Create a qPCR/STR Sample file that you can use to import sample information during the qPCR and STR PCR setup runs, as described in "Create a qPCR/STR Sample file" on page 18.
☐	If you want the HID EVOlution™ - qPCR/STR Setup System to prepare DNA standards, prepare the $T_{10}E_{0.1}$ buffer (10 mM Tris-HCl [pH 8.0] and 0.1 mM Na_2EDTA) according to the directions in the *Quantifiler® Kits User's Manual*. **IMPORTANT!** Glycogen was *not* used during the Applied Biosystems validation study. If you intend to use glycogen, perform your own validation studies to evaluate the liquid handling performance.
☐	Assemble the materials you will use. See "Required instruments, software, and materials" on page 6 for a complete list of materials and sources. • Extracted DNA samples as described in "Set up extracted DNA samples in a 96-well plate" on page 14 or "Set up extracted DNA samples in tubes" on page 15 • Reagents: – Quantifiler® Human DNA Quantification Kit and/or Quantifiler® Y Human Male DNA Quantification Kit – $T_{10}E_{0.1}$ buffer (10 mM Tris-HCl [pH 8.0] and 0.1 mM Na_2EDTA) – Degassed deionized water (system liquid), 3000 mL per run • Plasticware: – A MicroAmp® Optical 96-Well Reaction Plate (used as the qPCR reaction plate) – If you want the HID EVOlution™ - qPCR/STR Setup System to prepare DNA standards, eight labeled 1.5-mL tubes (PN AM12400 or equivalent) for DNA standards (16 tubes for combined setups) – One 5-mL VWR tube for the Quantifiler® kit master mix (2 tubes for combined setups) – One-half tray of 200-µL DiTis (for 96 reactions) – One and one-half trays of 50-µL DiTis (for 96 reactions) • HID EVOlution™ - qPCR/STR Setup System-specific carriers: – Two 96-well metal microplate/plate adapters – Up to five 16-position tube carriers with vertical cap storage (if extracted DNA samples are in tubes) • (Optional) Barcodes for DNA sample plate or tubes and qPCR reaction plate
☐	Make a list of the lot numbers and expiration dates of the Quantifiler® kit components that will be used in the run for entry into the HID EVOlution™ - qPCR/STR Setup System software.
☐	Set up the carriers and racks according to the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual,* Section 4.3.3, "Set Up Carriers and Racks."
☐	Start up the system and perform routine maintenance, including running the appropriate maintenance scripts, according to the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual,* Section 4.3.2, "Prepare the Instrument," section 5.1 "Starting the System," and "Before each run: Run maintenance scripts" on page 12 in this guide.
☐	Determine the amount of reagents and place the reagents and DNA standard dilution series (if previously prepared) in the reagent block as described in "Set up reagents" on page 25.
☐	Place the reagents, labware, and samples on the worktable as described in "Set up the labware on the worktable" on page 30.

Set up reagents

About minimum required reagent volumes

The tables on page 26 through page 28 list the minimum required reagent volumes for each kit. The volumes in the tables include:

- The volume of reagent that will be added to each reaction in the qPCR reaction plate.
- Excess volume required per sample and per run necessary to compensate for evaporation and pipetting losses during the run.
- Excess volume (dead volume) required per tube (50 µL) or trough (5 mL) on the worktable necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.
- For the Quantifiler® Primer Mix and PCR Reaction Mix, the volume required per tube to ensure that the instrument detects adequate volume to prepare the selected number of reactions. For these reagents, the instrument aspirates 190 µL at a time. In order for the instrument to detect reagent in a tube, the tube must contain a minimum of 240 µL (this is 190 µL plus a 50-µL dead volume). When the reagent volume falls below 240 µL, the instrument is likely to determine that the liquid level is too low for successful aspiration. If the instrument determines that the liquid level is too low, the instrument then looks for a sufficient volume in the next available tube of the same reagent (if available) or pauses the run and displays the error message “Not enough liquid.”

Determine required reagent volumes

Use the following procedure to determine the amount of Quantifiler® kit reagents and $T_{10}E_{0.1}$ buffer that you need to prepare for the run.

Note: Applied Biosystems recommends that you combine reagents from different tubes from the same lot if necessary to meet the minimum volume requirements.

If you want to:

- **Use a DNA standard dilution series that was prepared in the last two weeks** – Start with step 1 below.
- **Let the HID EVOlution™ system prepare the DNA standard dilution series for you** – Start with step 2 on page 27.

1. (Optional) If you want to use a pre-prepared DNA standard dilution series, determine if the series meets the following requirements:
   - The series must have been prepared in the last two weeks
   - The series must have been prepared according to the procedures specified in the appropriate *Quantifiler® Kits User's Manual*
   - You must have the required volumes of the pre-prepared DNA standard dilutions shown in the table:

Required Volumes for Pre-Prepared DNA Standard Dilution Series

Type of qPCR reaction plate	Required Volume
Plate prepared with EITHER Quantifiler® Human or Quantifiler® Y Human Male Quantification Kit	60 µL of each of the eight concentrations of diluted standard. **Note:** This includes the required overfill volume. **Note:** Two replicates of the eight concentrations in the DNA standard dilution series are transferred to the qPCR reaction plate for a total of 16 DNA standard dilution series samples.
Plate prepared with BOTH Quantifiler® Human and Quantifiler® Y Human Male Quantification Kit	You need two pre-prepared DNA standard dilution series, one for each kit. For each of the two DNA standard dilution series, you need 60 µL of each of the eight concentrations of diluted standard. **Note:** This includes the required overfill volume. **Note:** Two replicates of the eight concentrations in the DNA standard dilution series are transferred to the qPCR reaction plate for each of the two DNA standard dilution series for a total of 32 DNA standard dilution series samples.

If the pre-prepared DNA standard dilution series does not meet the requirements, then let the HID EVOlution™ system prepare the DNA standard dilution series for you using the standard and TE buffer volumes shown in step 2 on page 27.

2. If the HID EVOlution™ system is preparing the DNA standard dilution series, use the table below to determine the required volume of undiluted Quantifiler® Human DNA Standard and $T_{10}E_{0.1}$ buffer.

Required Volumes for HID EVOlution™ system-Prepared DNA Standard Dilution Series

Reagent		Available volume in full tube of reagent	Minimum volume‡ required on worktable
Undiluted Quantifiler® Human DNA Standard		120 µL	100 µL
$T_{10}E_{0.1}$ buffer in trough			
	To prepare a reaction plate using EITHER the Quantifiler® Human or Quantifiler® Y Human Male Quantification Kit	NA	6 mL in trough
	To prepare a reaction plate with BOTH the Quantifiler® Human and Quantifiler® Y Human Male Quantification Kits	NA	7 mL in trough

‡ Includes 50 µL dead volume per tube and 5 mL per trough necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.

3. Calculate the required volume of Quantifiler® kit reagents using the Table 6 or Table 7 on page 28.

IMPORTANT! To obtain the required volume of primer mix, you can place up to 3 tubes of primer mix in the reagent block for one run. If you divide the minimum required volume of primer mix across 2 or 3 tubes, each tube must contain a multiple of 190 µL *plus* an additional 50 µL per tube to ensure that the instrument detects adequate volume to prepare the selected number of reactions. See "About minimum required reagent volumes" on page 25.

Table 6 Reagent volumes for qPCR reaction setup with either the Quantifiler® Human or Y Human Male Quantification Kit (25-µL reaction volume).

Reagent	Available Volume in Full Tube of Reagent	Required Volume per Reaction	Extracted DNA Samples per Run	Diluted Standard Samples per Run	Required Excess Reactions per Run	Required Dead Volume‡ per Tube	Minimum Required Volume for 80 extracted DNA samples and 16 diluted standards = [A × (B + C + D)] + E
		A	B	C	D	E	
Quantifiler® Human or Y Human Male Primer Mix	1.4 mL	11.55 µL	up to 80	16	3	50 µL	1194§ µL in one tube#
Quantifiler® PCR Reaction Mix	5 mL	13.75 µL	up to 80	16	3	50 µL	1412‡‡ µL in one tube

‡ A 50-µL dead volume per tube is necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.
§ [11.55 µL/reaction × (80 + 16 + 3 reactions)] + 50 µL/tube = 1193.45 µL in one tube
# If you divide the minimum volume of Primer Mix across multiple tubes, each tube must contain a multiple of 190 µL plus an additional 50 µL per tube to ensure that the instrument detects adequate volume to prepare the selected number of reactions. For example, place at least 1000 µL in the first tube and 240 µL in the second tube.
‡‡ [13.75 µL/reaction × (80 + 16 + 3 reactions)] + 50 µL/tube = 1411.25 µL in one tube

Table 7 Reagent volumes for a combined qPCR reaction setup using both the Quantifiler® Human and Y Human Male Quantification Kits in one qPCR reaction plate (25-µL reaction volume).

Reagent	Available Volume in Full Tube of Reagent	Required Volume per Reaction	Extracted DNA Samples per Run	Diluted Standard Samples per Run	Required Excess Reactions per Run	Required Dead Volume‡ per Tube	Minimum Required Volume for 32 extracted DNA samples and 32 diluted standards = [A × (B + C + D)] + E
		A	B	C	D	E	
Quantifiler® Human Primer Mix	1.4 mL	11.55 µL	up to 32	16	3	50 µL	639 µL in one tube§
Quantifiler® Y Human Male Primer Mix	1.4 mL	11.55 µL	up to 32	16	3	50 µL	639 µL in one tube
Quantifiler® PCR Reaction Mix (for preparing Quantifiler Human reactions)	5 mL	13.75 µL	up to 32	16	3	50 µL	752 µL in one tube
Quantifiler® PCR Reaction Mix (for preparing Quantifiler Y Human Male reactions)	5 mL	13.75 µL	up to 32	16	3	50 µL	752 µL in one tube

‡ A 50-µL dead volume per tube is necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.
§ If you divide the volume of Primer Mix across multiple tubes, each tube must contain a multiple of 190 µL plus an additional 50 µL per tube to ensure that the instrument detects adequate volume to prepare the selected number of reactions.

Place reagents in the qPCR reagent block

Use the following procedure to place the Quantifiler® kit reagents into the correct positions in the chilled qPCR reagent block.

1. Thaw reagents, mix and centrifuge according to the *Quantifiler® Kits User's Manual*.

2. If you are using a previously-prepared DNA standard dilution series, vortex and briefly centrifuge the DNA standard dilutions at low speed before use.

3. Select the appropriate reagent block setup from the following table, then use the appropriate figure to place the reagents and labeled empty tubes in the reagent block.

IMPORTANT! If you are using a pre-prepared DNA standard dilution series, make sure to place each concentration in the correct location in the reagent block.

If you are using...	And the DNA standards...	Set up the reagent block according to...
The Quantifiler® Human kit	Are pre-prepared	Figure 8 on page 76
	Will be prepared by the HID EVOlution™ system	Figure 9 on page 77
The Quantifiler® Y Human Male kit	Are pre-prepared	Figure 10 on page 78
	Will be prepared by the HID EVOlution™ system	Figure 11 on page 79
Both the Quantifiler® Human and Y Human Male kits	Are pre-prepared	Figure 12 on page 80
	Will be prepared by the HID EVOlution™ system	Figure 13 on page 81

Set up the labware on the worktable

After loading the reagent block, use the following procedure to place the reagent block, PCR reaction plate, trough, disposable pipette tips (DiTis), and samples on the worktable. Use Figure 2 on page 32 as a guide.

CAUTION! For important safety information related to the use of the Tecan Freedom EVO instrument, refer to the manufacturer's instrument documentation.

1. Set up the DiTis as described in the Tecan *HID EVOlution™ - qPCR/STR Setup System Application Manual*, Section 4.3.5, "Set Up Plasticware and Samples on the Workstation."

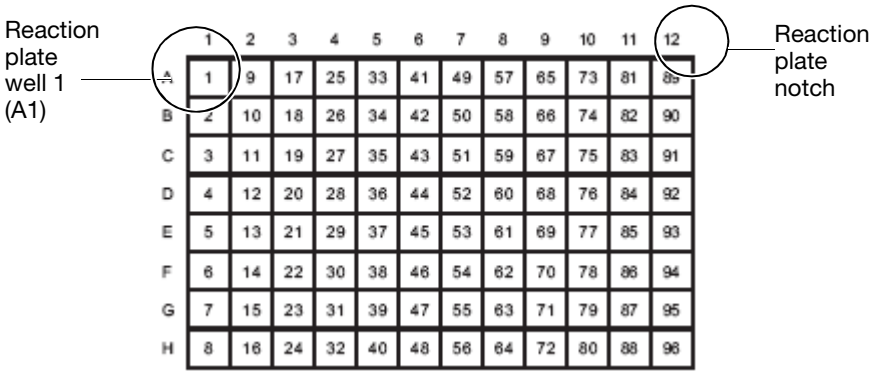
   IMPORTANT! If the DiTi trays are not correctly set up in the two carriers, for example if the 50-µL DiTis are placed in the 200-µL DiTis position, the LiHa may crash or pipetting errors may result.

   If there are no DiTis where the software expects them, the run will pause until you replenish the DiTis. During this pause, the samples may become unstable. To prevent this from occurring, use full DiTi trays, and run the script: **Set_DiTi_Position_200** or **Set_DiTi_Position_50** to set all DiTis to position 1.

2. Confirm that you have correctly loaded the reagent block (see "Place reagents in the qPCR reagent block" on page 29), remove the caps from the prepared reagents, then place the loaded reagent block on grid 15, site position 1.

3. *If the HID EVOlution™ system is preparing the DNA standards*, place 6 mL (Human *or* Y Human Male) or 7 mL (Human *and* Y Human Male) of $T_{10}E_{0.1}$ buffer into a 100-mL trough (see Figure 2, item 7), then place the trough on grid 14, site position 2.

4. Place an empty MicroAmp® Optical 96-Well Reaction Plate (for the qPCR reactions) into the metal plate adapter with well A1 in the top left corner on grid 21, site position 1 (see Figure 2, item 9).

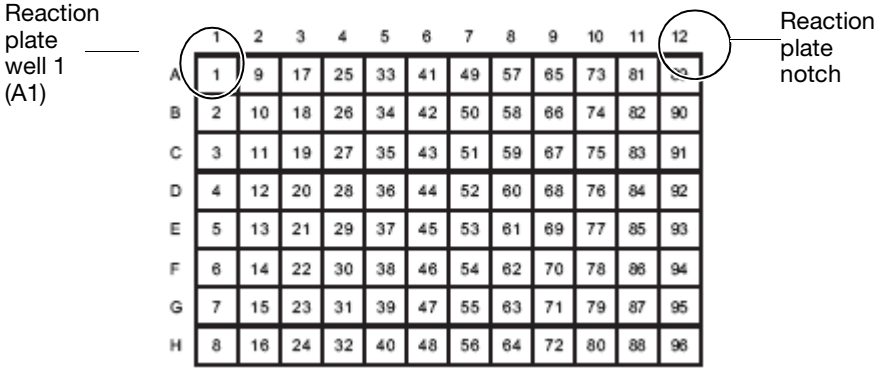


IMPORTANT! To ensure that samples are transferred to the correct wells, confirm that:

- The reaction plate is placed in the metal plate adapter
- The reaction plate wells are aligned with the holes in the metal plate adapter
- Well A1 is positioned in the upper left corner

5. Ensure that the extracted DNA is in either a 96-well plate or in 1.5-mL tubes before setting up the samples on the worktable for qPCR setup. For instructions, see "Set up extracted DNA samples in a 96-well plate" on page 14 or "Set up extracted DNA samples in tubes" on page 15.

6. If the extracted DNA is in a plate, place the extracted DNA sample plate into the metal plate adapter on the worktable in grid 21, site position 3, with well A1 in the top left corner.



IMPORTANT! Confirm that:

- The reaction plate is placed in the metal plate adapter
- The reaction plate wells are aligned with the holes in the metal plate adapter
- Well A1 is positioned in the upper left corner

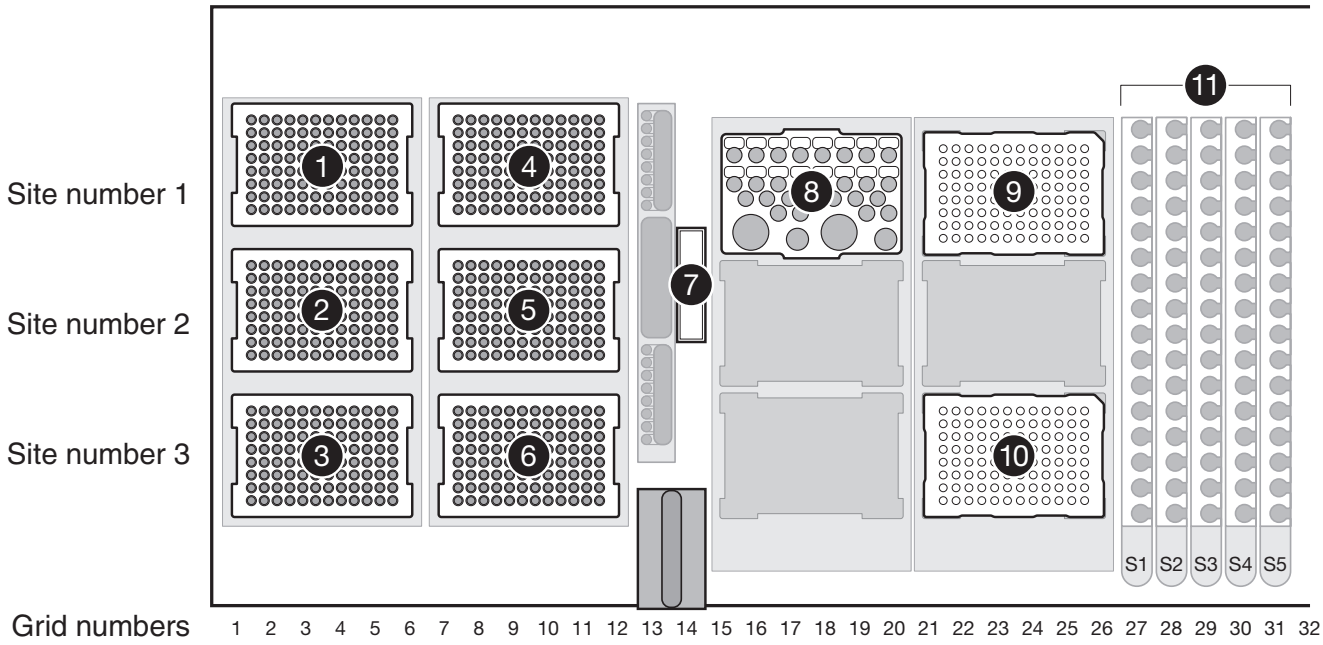


Figure 2 Tecan Freedom EVO® workstation (worktable) layout for a qPCR setup run. Locations for DNA samples in both a plate and tubes are shown.

Note: Locations for DNA samples in both plate and tubes are shown, but only one type of plasticware for extracted DNA can be placed on the workstation for a given run.

1–2. 200-µL disposable pipette tips (DiTis)

3–6. 50-µL DiTis

7. Trough for $T_{10}E_{0.1}$ buffer

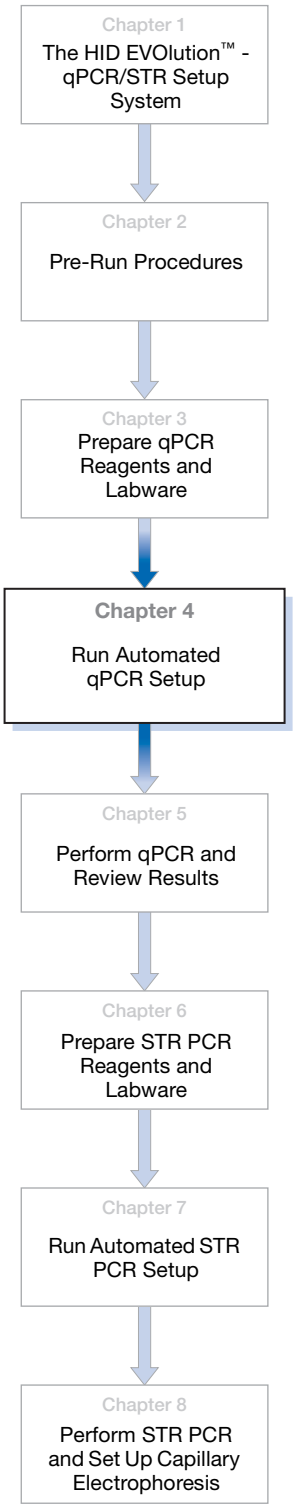
8. Chilled qPCR Reagent Block

9. MicroAmp® Optical 96-Well Reaction Plate (qPCR reaction plate) with 96-well metal plate adapter

10. MicroAmp® Optical 96-Well Reaction Plate (if extracted DNA samples are in a plate) with 96-well metal plate adapter

11. Tube racks S1 through S5 for DNA sample tubes (if extracted DNA samples are in tubes)

4 Run Automated qPCR Setup



This chapter provides procedures for performing a qPCR reaction setup run.

Run a qPCR setup script

About script files

To begin automated qPCR or STR PCR reaction setup on the HID EVOlution™ - qPCR/STR Setup System, you must run a script. Scripts contain the workflow instructions defined for a specific instrument type (the Freedom EVO® 150 and 200 instrument), worktable configuration, and Freedom EVOware® software version to automate a specific procedure. For example, there are six scripts available to set up qPCR reaction plates using the HID EVOlution™ system (see table below) and 14 scripts available to set up a STR PCR reaction plate (see "Run STR PCR setup" on page 58).

Run a script

After performing all tasks, including maintenance, in the "Review pre-run checklist for qPCR reaction setup" on page 24, use the following procedure to begin automated qPCR reaction setup:

1. Select the appropriate EVOware software script for the Quantifiler® kit(s) and plasticware that you are using:

Freedom EVOware® software script selection for qPCR reaction setup

If you are using the kit(s)...	and the sample DNA is in...	...use the script	Maximum number of extracted DNA samples per run
Quantifiler® Human Kit	a 96-well plate	QuantifilerHuman_plate.esc	80
	1.5-mL microcentrifuge tubes	QuantifilerHuman_tubes.esc	80
Quantifiler® Y Human Male Kit	a 96-well plate	QuantifilerY_plate.esc	80
	1.5-mL microcentrifuge tubes	QuantifilerY_tubes.esc	80
Quantifiler® Human AND Y Human Male Kits	a 96-well plate	QuantifilerHumanY_plate.esc	32 total‡
	1.5-mL microcentrifuge tubes	QuantifilerHumanY_tubes.esc	

‡ Two reactions are prepared for each of the 32 extracted DNA samples: one reaction is prepared using the Quantifiler® Human kit reagents, and one reaction is prepared using the Quantifiler® Y Human Male kit reagents.

2. Follow the directions in the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual,* Section 5.3.1, "Running a Quantifiler Script."

IMPORTANT! If you want the HID EVOlution™ - qPCR/STR Setup System to prepare the DNA standard dilution series, make sure to select the **Prepare Standards** checkbox in the Reagent Information window of the script.

Perform post-run tasks

Take care of the qPCR reaction plate

1. Remove the prepared qPCR reaction plate containing the qPCR reactions from the worktable.
2. Seal the qPCR reaction plate with MicroAmp® Optical Adhesive Film (PN 4311971).
3. Place the qPCR reaction plate in a table-top centrifuge with plate holders, then centrifuge the plate at 3000 rpm for approximately 20 seconds to remove any air bubbles.

Clean up the instrument

1. Remove the TE buffer trough, and dispose of any remaining TE buffer. If it is the last run of the day, dispose of the TE buffer trough.

   IMPORTANT! Do not reuse the reagents in the troughs. See "Waste disposal" on page 144.

2. Follow the instructions in the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*, Section 5.3.1, "After Run" on page 87.

About the qPCR reaction plate layout

Regardless of the extracted DNA sample setup, the qPCR reaction plate is always set up in the same way: the sample from the first position in the extracted DNA sample plate or first extracted DNA sample tube is always placed in well A3 of the qPCR reaction plate. The DNA standard dilution series reactions are placed in columns 1 and 2. See Figure 3.

In combined qPCR reaction plates, the first Quantifiler® Human sample reaction is placed in well A3 and the first Quantifiler® Y Human Male sample reaction is placed in well A9. The DNA standard dilution series reactions are placed in columns 1, 2, 7, and 8. See Figure 4 on page 37.

At the end of a qPCR reaction setup run, the HID EVOlution™ - qPCR/STR Setup System generates a report that lists the position of each DNA sample in the extracted DNA sample plate or tubes and in the qPCR reaction plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1_1	STD 2_1	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	STD 1_2	STD 2_2	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	STD 1_3	STD 2_3	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	STD 1_4	STD 2_4	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	STD 1_5	STD 2_5	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	STD 1_6	STD 2_6	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	STD 1_7	STD 2_7	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	STD 1_8	STD 2_8	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Figure 3 Plate layout for Quantifiler® Human or Quantifiler® Y Human Male kits. Eight DNA standards, in duplicate, are placed in columns 1 and 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1_1	STD 2_1	S1	S9	S17	S25	STD 1_1	STD 2_1	S1	S9	S17	S25
B	STD 1_2	STD 2_2	S2	S10	S18	S26	STD 1_2	STD 2_2	S2	S10	S18	S26
C	STD 1_3	STD 2_3	S3	S11	S19	S27	STD 1_3	STD 2_3	S3	S11	S19	S27
D	STD 1_4	STD 2_4	S4	S12	S20	S28	STD 1_4	STD 2_4	S4	S12	S20	S28
E	STD 1_5	STD 2_5	S5	S13	S21	S29	STD 1_5	STD 2_5	S5	S13	S21	S29
F	STD 1_6	STD 2_6	S6	S14	S22	S30	STD 1_6	STD 2_6	S6	S14	S22	S30
G	STD 1_7	STD 2_7	S7	S15	S23	S31	STD 1_7	STD 2_7	S7	S15	S23	S31
H	STD 1_8	STD 2_8	S8	S16	S24	S32	STD 1_8	STD 2_8	S8	S16	S24	S32

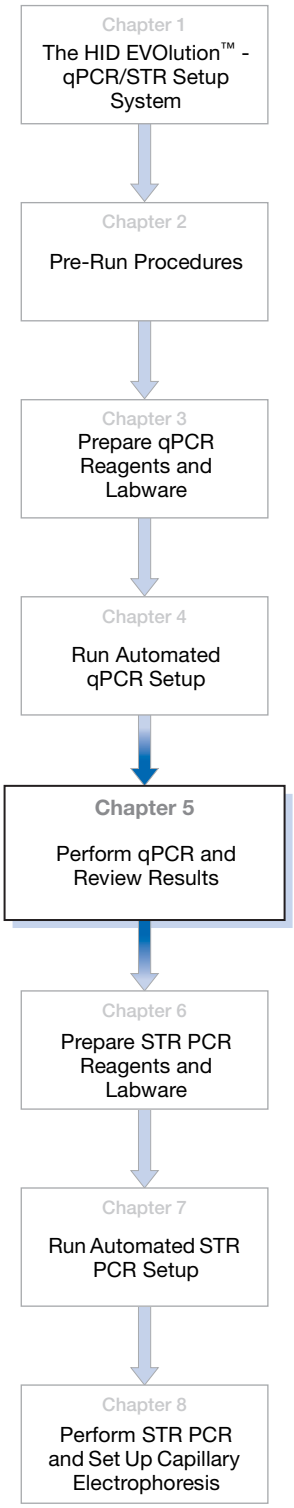
Figure 4 Plate layout for a combined setup using both Quantifiler® Human and Quantifiler® Y Human Male kits. Eight DNA standards, in duplicate, are placed in columns 1 and 2 (Human kit) and in columns 7 and 8 (Y Human Male kit).

For more information

For information on:

- Running quantification scripts, see the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 5.3.1, “Running a Quantifiler Script.”
- How to import sample files, see the Tecan *HID EVOlution™ - qPCR/STR Setup System Application Manual*, Section 5.3.1, “Running a Quantifiler Script.”
- Script error messages, see the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 8.4 “Application Software.”
- The EVOware® software, see the Tecan *EVOware® Standard/EVOware® Plus 2.1 Software Manual* and Tecan *EVOware® Standard/EVOware® Plus 2.1 Software Getting Started Guide*.
- The qPCR Samples Report, see the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 5.3.1, “Running a Quantifiler Script,” and Section 6, “Results.”

5 Perform qPCR and Review Results



This chapter explains where to get relevant information to perform qPCR and review the results.

About the 7500 Setup file

The HID EVOlution™ - qPCR/STR Setup System software generates a 7500 Setup file for each run. The file contains sample information that you can import to an SDS software plate document for the qPCR run on the 7500 Real-Time PCR instrument.

By default, the HID EVOlution™ - qPCR/STR Setup System names the 7500 Setup file "ReactionPlate1.txt" or "<*barcode*>.txt" and saves the file to the C:\HIDEVOlution_qPCRSTRfiles folder. The HID EVOlution™ system automatically archives the 7500 Setup file in the C:\HIDEVOlution_qPCRSTRfiles\Archive folder when you start the next qPCR or STR PCR setup run.

If necessary, create shortcuts on your desktop to the C:\HIDEVOlution_qPCRSTRfiles and C:\HIDEVOlution_qPCRSTRfiles\Archive folders.

ReactionPlate1.txt - Notepad

File Edit Format View Help

```
*** SDS Setup File Version      3
*** Output Plate Size   96
*** Output Plate ID     ReactionPlate1
*** Number of Detectors 2
Detector        Reporter        Quencher        Description     Comments
Quantifiler Human       FAM
IPC     VIC
Well    Sample Name     Detector        Task    Quantity
1       50.000 ng/ul    Quantifiler Human       STND    50.000
1       Standard 50ng/ul        IPC     UNKN
2       50.000 ng/ul    Quantifiler Human       STND    50.000
2       Standard 50ng/ul        IPC     UNKN
3       cfc     Quantifiler Human       UNKN
3       cfc     IPC     UNKN
13      16.700 ng/ul    Quantifiler Human       STND    16.700
13      Standard 16.70ng/ul     IPC     UNKN
14      16.700 ng/ul    Quantifiler Human       STND    16.700
14      Standard 16.70ng/ul     IPC     UNKN
15      vcgg    Quantifiler Human       UNKN
15      vcgg    IPC     UNKN
25      5.560 ng/ul     Quantifiler Human       STND    5.560
25      Standard 5.56ng/ul      IPC     UNKN
26      5.560 ng/ul     Quantifiler Human       STND    5.560
26      Standard 5.56ng/ul      IPC     UNKN
27      vggtfd  Quantifiler Human       UNKN
27      vggtfd  IPC     UNKN
37      1.850 ng/ul     Quantifiler Human       STND    1.850
37      Standard 1.85ng/ul      IPC     UNKN
38      1.850 ng/ul     Quantifiler Human       STND    1.850
38      Standard 1.85ng/ul      IPC     UNKN
39      a       Quantifiler Human       UNKN
39      a       IPC     UNKN
49      0.620 ng/ul     Quantifiler Human       STND    0.620
49      Standard 0.62ng/ul      IPC     UNKN
50      0.620 ng/ul     Quantifiler Human       STND    0.620
50      Standard 0.62ng/ul      IPC     UNKN
61      0.210 ng/ul     Quantifiler Human       STND    0.210
61      Standard 0.21ng/ul      IPC     UNKN
62      0.210 ng/ul     Quantifiler Human       STND    0.210
62      Standard 0.21ng/ul      IPC     UNKN
73      0.068 ng/ul     Quantifiler Human       STND    0.068
73      Standard 0.068ng/ul     IPC     UNKN
74      0.068 ng/ul     Quantifiler Human       STND    0.068
74      Standard 0.068ng/ul     IPC     UNKN
85      0.023 ng/ul     Quantifiler Human       STND    0.023
85      Standard 0.023ng/ul     IPC     UNKN
86      0.023 ng/ul     Quantifiler Human       STND    0.023
86      Standard 0.023ng/ul     IPC     UNKN
```

Transfer and import the 7500 Setup file

IMPORTANT! To successfully import the 7500 Setup file to the SDS software, the detector names created in the SDS software must match those in the 7500 Setup file. See "Create detectors for the 7500 Real-Time PCR System" on page 10.

1. Transfer the 7500 Setup file (generated by the HID EVOlution™ system at the end of the qPCR reaction setup run) to the SDS software computer:
   a. On the HID EVOlution system, navigate to the C:\ HIDEVOlution_qPCRSTRfiles folder, then locate the file (ReactionPlate1.txt or <*barcode*>.txt).
   b. (Optional) Rename the file.
   c. Copy and transfer the ReactionPlate1.txt (or <*barcode*>.txt) file to a location where it can be accessed by the SDS software computer.
2. Start the 7500 Real-Time PCR System and SDS Software:
   a. Start the computer.
   b. Power on the 7500 instrument.
   c. Start the SDS Software.

   Note: For more information on starting the 7500 Real-Time PCR System and software, refer to the Applied Biosystems *7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide*.
3. Import the 7500 Setup file into the Applied Biosystems 7500 SDS Software plate document for use during the quantitation run as follows:
   a. In the SDS software, select **File ▸ New** to create a new Standard Curve (Absolute Quantitation) plate document, then in the new document wizard select **Finish**.
   b. With a blank plate document open, select **File ▸ Import Sample Set Up**.
   c. Browse to locate the 7500 Setup file, select the file (**ReactionPlate1.txt** or **<*barcode*>.txt**), then click **Open**.
   d. Modify the instrument protocol: Delete the **Stage 1 hold step** (50 °C for 2 minutes) and change the Sample Volume to **25**.

4. Save the plate document:
   a. Select **File ▸ Save As**.
   b. In the Save as dialog box:
      - Enter a file name in the File name field. Do not add an extension to the file name.
      - Select **SDS Document (*.sds)** from the Save as type drop-down list.
      - Click **Save**.

Perform qPCR

Perform qPCR as described in the Applied Biosystems *Quantifiler® Kits User's Manual*.

Analyze and export the qPCR results

When the qPCR run is complete, use the following procedures to:

- Analyze and review the qPCR results
- Export the 7500 Results file

1. Analyze and review the 7500 results:
   a. In the SDS software, click **Analyze**.
   b. Omit any blank samples.
   c. Review the standard curve to ensure that results are within the recommended range (see the *Quantifiler® Kits User's Manual*). If necessary, omit standard outliers, then click **Analyze** to recalculate the standard curve.
   d. Review the quantitation data, including the IPC C_T, and consider the condition of the original sample to determine if any samples require additional processing before STR PCR reaction setup.

2. When you are done analyzing the results in the SDS software, export the 7500 Results file and copy to the HID EVOlution™ system computer:
   a. In the SDS software, select **File ▸ Export ▸ Results.**
   b. Navigate to the desired location on your hard drive.
   c. Type a name for the exported file.
   d. Select the export type (.csv).

e. Save the file.

f. Copy the file to the computer where the EVOware™ Software is installed, or to a server that can be accessed from both systems.

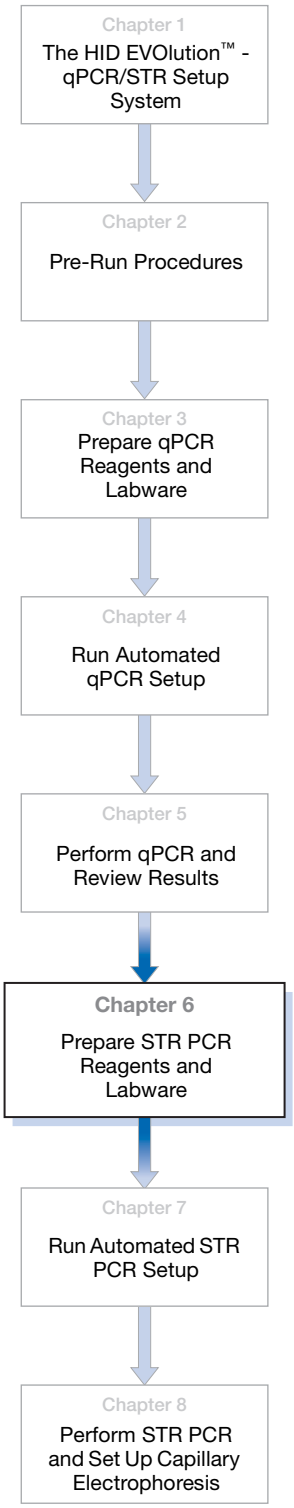
Note: During STR PCR reaction setup, you import the sample information in the 7500 Results file to the HID EVOlution™ - qPCR/STR Setup System. The EVOware uses the information in the 7500 Results file to set up sample information and to help normalize the DNA input amount for STR PCR reaction setup on the HID EVOlution™ - qPCR/STR Setup System.

For more information

For information on:

- The standard curve, refer to the *ABI PRISM® 7000 Sequence Detection System User Guide*, *Quantifiler® Kits User's Manual*, and the *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantification Getting Started Guide*.
- Quantitation, see the Applied Biosystems *Quantifiler® Kits User's Manual*.
- The 7500 Results file, see the *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide*.
- Setting up a plate document and plate document template, see the Applied Biosystems *Quantifiler® Kits User's Manual*.

6 Prepare STR PCR Reagents and Labware



This chapter provides procedures to prepare reagents and labware for STR PCR amplification reaction setup.

Review pre-run checklist for STR PCR reaction setup

☐	Cool the STR PCR reagent block to 4 °C before use to help keep reagents cool on the worktable. It is recommended, when not in use, that you store reagent blocks in a refrigerator at 4 °C.
☐	(Optional) Locate the qPCR/STR Sample file (the .csv sample file that you created and used in qPCR reaction setup), *or* (Optional) Create a qPCR/STR Sample file that you can use to import sample information during the run, as described in "Create a qPCR/STR Sample file" on page 18.
☐	Confirm that the 7500 Results file(s) that you want to use are in .csv format and in a location where the file(s) can be imported to the HID EVOlution™ - qPCR/STR Setup System software. See "Analyze and export the qPCR results" on page 42.
☐	Assemble the materials you will use. See "Required instruments, software, and materials" on page 6 for a complete list of materials and sources. • Extracted DNA samples. Use the plate or tubes containing extracted DNA samples that you prepared and processed in the associated qPCR reaction setup run. See "Set up extracted DNA samples in a 96-well plate" on page 14 or "Set up extracted DNA samples in tubes" on page 15. • Reagents: – Applied Biosystems AmpFℓSTR® kit – $T_{10}E_{0.1}$ buffer (10 mM Tris-HCl [pH 8.0] and 0.1 mM Na_2EDTA). Refer to your specific *AmpFℓSTR® Kit User Guide* for directions on buffer preparation. – Degassed deionized water (system liquid), 3000 mL per run • Plasticware: – A MicroAmp® Optical 96-Well Reaction Plate (to be used as the STR PCR reaction plate) – Two additional MicroAmp® Optical 96-Well Reaction Plates (to be used as pre-dilution plates) – One tray of 200-µL DiTis (for 96 reactions) – Three trays of 50-µL DiTis (for 96 reactions) • HID EVOlution™ - qPCR/STR Setup System carriers: – Four 96-well metal microplate/plate adapters – Up to six 16-position tube carriers with vertical cap storage (if extracted DNA samples are in tubes) • (Optional) Barcodes for DNA sample plate or tubes and STR PCR plate
☐	Make a list of the lot numbers and expiration dates of the AmpFℓSTR® kit components that will be used in the run for entry into the HID EVOlution™ - qPCR/STR Setup System software.
☐	Set up the carriers and racks according to the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual,* Section 4.3.3, "Set Up Carriers and Racks."
☐	Start up the system and perform routine maintenance, including running the appropriate maintenance scripts, according to the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual,* Section 4.3.2, "Prepare the Instrument," section 5.1 "Starting the System," and "Before each run: Run maintenance scripts" on page 12 in this guide.
☐	Determine the required volume of reagents for the run as described in "Determine reagent volumes" on page 47.
☐	Place the reagents in the reagent block as described in "Set up reagents" on page 52.
☐	Place the reagents, labware, and samples on the worktable as described in "Set up the labware on the worktable" on page 54.

Determine reagent volumes

About minimum required reagent volumes

Tables 8 through 11 on pages 48 through 51 list the minimum required reagent volumes for each kit. The volumes in the tables include:

- The volume of reagent that will be added to each reaction in the STR PCR reaction plate.
- Excess volume required per sample and per run necessary to compensate for evaporation and pipetting losses during the run.
- Excess volume (dead volume) required per tube (50 µL) or trough (5 mL) on the worktable necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.
- For the AmpFℓSTR® Primer Set and PCR Reaction Mix or Master Mix, the volume required per tube to ensure that the instrument detects adequate volume to prepare the selected number of reactions. For these reagents, the instrument aspirates 190 µL at a time. In order for the instrument to detect reagent in a tube, the tube must contain a minimum of 240 µL (this is 190 µL plus a 50-µL dead volume). When the reagent volume falls below 240 µL, the instrument is likely to determine that the liquid level is too low for successful aspiration. If the instrument determines that the liquid level is too low, the instrument then looks for a sufficient volume in the next available tube of the same reagent (if available) or pauses the run and displays the error message "Not enough liquid."
- For the $T_{10}E_{0.1}$ buffer in the trough, the volume necessary to ensure adequate buffer for any dilution ratio required for sample normalization.

Reagent volume tables

Use the appropriate table below to determine the required volumes of reagents. For the:

- Identifiler® kit, use Table 8 on page 48
- Yfiler® kit, use Table 9 on page 49
- MiniFiler™ and SEfiler Plus™ kits, use Table 10 on page 50
- COfiler®, Profiler Plus®, and SGM Plus® kits, use Table 11 on page 51

Then prepare the reagents according to "Set up reagents" on page 52.

Table 8 Identifiler® kit reagent volumes (25-µL reaction volume for STR PCR amplification).

Reagent	Available Volume in Full Tube of Reagent	Required Volume per Reaction‡	Extracted DNA Samples per Run	Control Samples per Run	Required Excess Reactions per Run	Required Dead Volume§ per Tube	Minimum Required Volume for 88 Samples and 2 Controls
		A	B	C	D	E	
AmpFℓSTR® Primer Set	1.1 mL	6.05 µL	up to 88	2	4	50 µL	[A × (B + C + D)] + E = 619 µL#
AmpFℓSTR® PCR Reaction Mix	1.1 mL	11.55 µL	up to 88	2	4	50 µL	[A × (B + C + D)] + E = 1136 µL‡‡ in one tube§§
AmpliTaq Gold® DNA Polymerase	50 µL	100 µL combined in one tube## for 1 to 88 samples plus 2 controls					100 µL combined in one tube##
AmpFℓSTR® Control DNA 9947A	300 µL	60 µL for 1 to 88 samples plus 2 controls					60 µL
$T_{10}E_{0.1}$ buffer in reagent block	NA	60 µL for 1 to 88 samples plus 2 controls					60 µL
$T_{10}E_{0.1}$ buffer in trough	NA	25 mL in trough for 1 to 88 samples plus 2 controls					25 mL in trough

‡ Includes excess volume to compensate for evaporation and pipetting losses during the run.
§ A 50-µL dead volume per tube is necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.
# [6.05 µL/reaction × (88 + 2 + 4 reactions)] + 50 µL/tube = 618.7 µL in one tube
‡‡ [11.55 µL/reaction × (88 + 2 + 4 reactions)] + 50 µL/tube = 1135.7 µL in one tube
§§ If you divide the minimum volume of PCR Reaction Mix between two tubes, make sure that each tube contains a multiple of 190 µL plus an additional 50 µL per tube to ensure that the instrument detects adequate volume to prepare the selected number of reactions. For example, place at least 1000 µL in the first tube and 240 µL in the second tube.
You must combine the volumes in two full 50-µL tubes in one tube. The tube containing the combined volume must be one of the original AmpliTaq Gold® DNA Polymerase tubes provided with the kit. Use of another type of tube can cause liquid detection errors.

Table 9 Yfiler® kit reagent volumes (25-µL reaction volume for STR PCR amplification).

Reagent	Available Volume in Full Tube of Reagent	Required Volume per Reaction‡	Extracted DNA Samples per Run	Control Samples per Run	Required Excess Reactions per Run	Required Dead Volume§ per Tube	Minimum Required Volume for 88 Samples and 2 Controls
		A	B	C	D	E	
AmpFℓSTR® Yfiler® Primer Set	0.55 mL	5.5 µL	up to 87	3	3	50 µL	[A × (B + C + D)] + E = 562 µL#
AmpFℓSTR® Yfiler® PCR Reaction Mix	1.1 mL	10.12 µL	up to 87	3	3	50 µL	[A × (B + C + D)] + E = 992 µL‡‡ in one tube§§
AmpliTaq Gold® DNA Polymerase	50 µL	100 µL combined in one tube## for 1 to 87 samples plus 3 controls					100 µL combined in one tube##
AmpFℓSTR® Control DNA 9947A	25 µL	60 µL for 1 to 87 samples plus 3 controls					60 µL
AmpFℓSTR® Control DNA 007	300 µL	60 µL for 1 to 87 samples plus 3 controls					60 µL
$T_{10}E_{0.1}$ buffer in reagent block	NA	60 µL for 1 to 87 samples plus 3 controls					60 µL
$T_{10}E_{0.1}$ buffer in trough	NA	25 mL in trough for 1 to 87 samples plus 3 controls					25 mL in trough

‡ Includes excess volume to compensate for evaporation and pipetting losses during the run.
§ A 50-µL dead volume per tube is necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.
# [5.5 µL/reaction × (87 + 3 + 3 reactions)] + 50 µL/tube = 561.5 µL in one tube
‡‡ [10.12 µL/reaction × (87 + 3 + 3 reactions)] + 50 µL/tube = 991.16 µL in one tube
§§ If you divide the minimum volume of PCR Reaction Mix between two tubes, make sure that each tube contains a multiple of 190 µL plus an additional 50 µL per tube to ensure that the instrument detects adequate volume to prepare the selected number of reactions. For example, place at least 810 µL in the first tube and 240 µL in the second tube.
You must combine the volumes in two full 50-µL tubes in one tube. The tube containing the combined volume must be one of the original AmpliTaq Gold® DNA Polymerase tubes provided with the kit. Use of another type of tube can cause liquid detection errors.

Table 10 MiniFiler™ and SEfiler Plus™ kits reagent volumes (25-µL reaction volume for STR PCR amplification).

Reagent	Available Volume in Full Tube of Reagent	Required Volume per Reaction‡	Extracted DNA Samples per Run	Control Samples per Run	Required Excess Reactions per Run	Required Dead Volume§ per Tube	Minimum Required Volume for 88 Samples and 2 Controls#
		A	B	C	D	E	
AmpFℓSTR® Primer Set	500 µL	5.5 µL	up to 88	2	4	50 µL	[A × (B + C + D)] + E = 567 µL‡‡ in one tube
AmpFℓSTR® Master Mix	500 µL	11 µL	up to 88	2	4	50 µL	[A × (B + C + D)] + E = 1084 µL§§ in one tube##
AmpFℓSTR® Control DNA 007	300 µL	60 µL for 1 to 88 samples plus 2 controls					60 µL
$T_{10}E_{0.1}$ buffer in reagent block	NA	60 µL for 1 to 88 samples plus 2 controls					60 µL
$T_{10}E_{0.1}$ buffer in trough	NA	25 mL in trough for 1 to 88 samples plus 2 controls					25 mL in trough

‡ Includes excess volume to compensate for evaporation and pipetting losses during the run.
§ A 50-µL dead volume per tube is necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.
# If necessary, combine reagents from different tubes from the same lot to meet the minimum volume requirements. When ordering kits, you can request multiple kits from the same lot.
‡‡ [5.5 µL/reaction × (88 + 2 + 4 reactions)] + 50 µL/tube = 567 µL in one tube
§§ [10.12 µL/reaction × (88 + 2 + 4 reactions)] + 50 µL/tube = 1084 µL in one tube
If you divide the minimum volume of PCR Master Mix between two tubes, make sure that each tube contains a multiple of 190 µL plus an additional 50 µL per tube to ensure that the instrument detects adequate volume to prepare the selected number of reactions. For example, place at least 620 µL in the first tube and 515 µL in the second tube.

Table 11 COfiler®/Profiler Plus®/SGM Plus® kits reagent volumes (50-µL reaction volume for STR PCR amplification).

Reagent	Available Volume in Full Tube of Reagent	Required Volume per Reaction‡	Extracted DNA Samples per Run	Control Samples per Run	Required Excess Reactions per Run	Required Dead Volume§ per Tube	Minimum Required Volume for 88 Samples and 2 Controls#
		A	B	C	D	E	
AmpFℓSTR® Primer Set	1.1 mL	12.1 µL	up to 88	2	0.4	50 µL	[A × (B + C + D)] + E = 1144 µL‡‡
AmpFℓSTR® PCR Reaction Mix	1.1 mL	23.1 µL	up to 88	2	0.4	50 µL	[A × (B + C + D)] + E = 2139 µL§§ in one tube##
AmpliTaq Gold® DNA Polymerase	50 µL	100 µL combined in one tube‡‡‡ for 1 to 88 samples plus 2 controls					100 µL combined in one tube‡‡‡
AmpFℓSTR® Control DNA 9947A or 007	300 µL	70 µL for 1 to 88 samples plus 2 controls					70 µL
$T_{10}E_{0.1}$ buffer in reagent block	NA	70 µL for 1 to 88 samples plus 2 controls					70 µL
$T_{10}E_{0.1}$ buffer in trough	NA	30 mL in trough for 1 to 88 samples plus 2 controls					30 mL in trough

‡ Includes excess volume to compensate for evaporation and pipetting losses during the run.
§ A 50-µL dead volume per tube is necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.
# If necessary, combine reagents from different tubes from the same lot to meet the minimum volume requirements. When ordering kits, you can request multiple kits from the same lot.
‡‡ [12.1 µL/reaction × (88 + 2 + 0.4 reactions)] + 50 µL/tube = 1143.84 µL in one tube
§§ [23.1 µL/reaction × (88 + 2 + 0.4 reactions)] + 50 µL/tube = 2138.24 µL in one tube
If you divide the minimum volume of PCR Reaction Mix between two tubes, make sure that each tube contains a multiple of 190 µL plus an additional 50 µL per tube to ensure that the instrument detects adequate volume to prepare the selected number of reactions. For example, place at least 1190 µL in the first tube and 1000 µL in the second tube.
‡‡‡ You must combine the volumes in two full 50-µL tubes in one tube. The tube containing the combined volume must be one of the original AmpliTaq Gold® DNA Polymerase tubes provided with the kit. Use of another type of tube can cause liquid detection errors.

Set up reagents

After using the appropriate table (see "Determine reagent volumes" on page 47) to determine the amount of AmpFℓSTR® kit reagents and $T_{10}E_{0.1}$ buffer that you need to prepare for the run, prepare the reagents and place the reagents in the chilled STR PCR reagent block as follows:

1. Thaw, mix, and centrifuge the reagents according to the procedures in the appropriate AmpFℓSTR® PCR Amplification Kit User Guide.

2. *For kits with tubes of AmpliTaq Gold® DNA Polymerase* – To insure correct pipetting by the HID EVOlution™ system, spin down one full (50-µL) tube of the AmpliTaq Gold® DNA Polymerase, then pipette the entire volume into a second full tube of AmpliTaq Gold® DNA Polymerase from the same lot, for a final volume of 100 µL.

   IMPORTANT! The tube containing the combined volume must be one of the original AmpliTaq Gold® DNA Polymerase tubes provided with the kit. Use of another type of tube can cause liquid detection errors or cause the LiHa to crash.

3. If necessary, combine reagents from different tubes from the same lot to meet the minimum volume requirements.

4. Prepare the DNA control(s) as follows:

   a. If necessary to meet your laboratory target concentrations, dilute a portion of the control DNA with $T_{10}E_{0.1}$ buffer (10 mM Tris-HCl [pH 8.0] and 0.1 mM Na_2EDTA) to meet the minimum required volume shown in the table:

      Note: For example, if you are using the MiniFiler™ kit and your control DNA target concentration is 0.05 ng/µL, combine 30 µL of control DNA (0.1 ng/µL) with 30 µL of low $T_{10}E_{0.1}$ buffer for a total volume of 60 µL.

      Note: The HID EVOlution™ - qPCR/STR Setup System does not dilute the control DNA, instead the system transfers a set volume (10 or 20 µL, depending on the kit) of control DNA to the reaction plate.

AmpFℓSTR® Kit	Recommended Target Concentration for Control DNA (ng/µL)‡	Minimum Required Volume of Diluted Control DNA (µL)§
Identifiler® and Yfiler® Kits	0.1 ng/µL	60
MiniFiler™ and SEfiler Plus™ Kits	0.05 ng/µL	60
Profiler Plus® COfiler®, and SGM Plus® Kits	0.1 ng/µL	70

‡ This is the final recommended target concentration, based on Applied Biosystems developmental validation studies and instrumentation.
§ The minimum required volume is the volume required by the experiment (10 µL or 20 µL) plus a 50 µL dead volume.

b. Place the required volume of diluted control DNA in an empty original control DNA tube (with the narrow chamber and skirt) or another tube with the identical shape.

IMPORTANT! For proper liquid detection the diluted control must be placed into a tube with an identical shape to the original control DNA tube. Using a 1.5 mL tube is not acceptable for proper liquid handling and no control will be transferred to the STR PCR reaction plate.

5. Select the appropriate reagent block setup from the following table, then use the appropriate figure to place the reagents and empty tubes in the correct positions in the chilled reagent block.

Note: There is a 1.5-mL tube of $T_{10}E_{0.1}$ buffer placed in the Reagent block that is separate from the $T_{10}E_{0.1}$ buffer placed in a trough. See "Set up the labware on the worktable" on page 54 for instructions on placing the $T_{10}E_{0.1}$ buffer trough on the worktable.

If you are using the following AmpFℓSTR® PCR Amplification kit...	Set up the reagent block according to...
COfiler® kit	Figure 14 on page 82
Identifiler® kit	Figure 15 on page 83
MiniFiler™ kit	Figure 16 on page 84
Profiler Plus® kit	Figure 17 on page 85
SEfiler Plus™ kit	Figure 18 on page 86
SGM Plus® kit	Figure 19 on page 87
Yfiler® kit	Figure 20 on page 88

Set up the labware on the worktable

After loading the reagent block, use the following procedure to place the reagent block, STR PCR reaction plate, trough, disposable pipette tips (DiTis), and samples on the worktable. Use Figure 5 on page 56 as a guide.

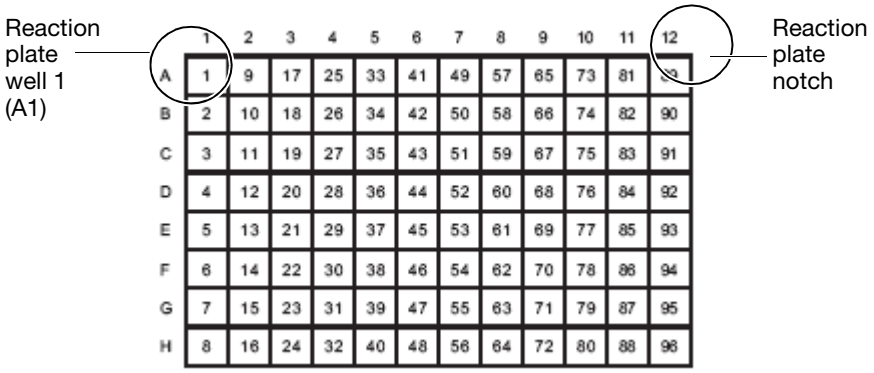
CAUTION! For important safety information related to the use of the Tecan Freedom EVO instrument, refer to the manufacturer's instrument documentation.

1. Set up the DiTis as described in the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 4.3.5., "Set Up Plasticware and Samples on the Workstation."

2. Remove caps from the reagents, then place the loaded reagent block (Figure 5, item 8) on grid 15, site position 1 (See Tables 8 through 11 on pages 48 through 51 for correct volumes).

3. Place $T_{10}E_{0.1}$ buffer (for dilution of DNA samples) into a 100-mL trough (Figure 5, item 7), then place the trough on grid 14, site position 2 (See Tables 8 through 11 on pages 48 through 51 for correct volumes).

4. Place three empty MicroAmp® Optical 96-Well Reaction Plates into the metal plate adapter with well A1 in the top left corner (Figure 5, items 10-12) on:
   - Grid 15, site position 2 (pre-dilution plate)
   - Grid 21, site position 2 (pre-dilution plate)
   - Grid 21, site position 3 (STR PCR reaction plate)



IMPORTANT! To ensure that samples are transferred to the correct wells, confirm for each plate that:
- The plate is placed in the metal plate adapter
- The plate wells are aligned with the holes in the metal plate adapter
- Well A1 is positioned in the upper left corner

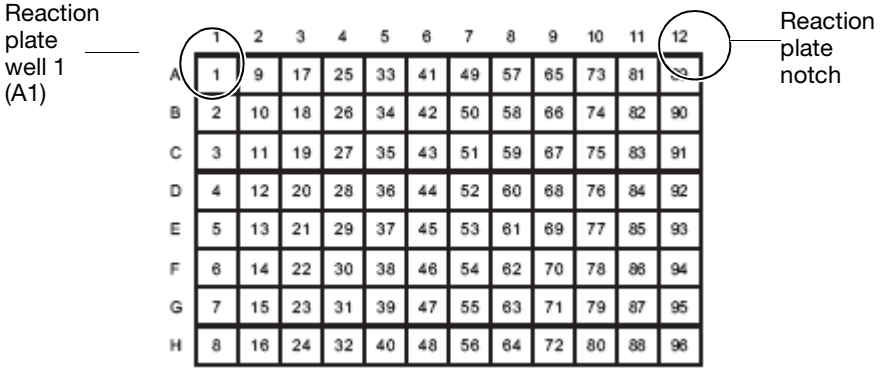
5. Check the extracted DNA samples:

   a. Ensure that the extracted DNA is in either a 96-well plate or in 1.5-mL tubes before setting up the samples on the worktable for STR PCR reaction setup. For instructions, see "Set up extracted DNA samples in a 96-well plate" on page 14 or "Set up extracted DNA samples in tubes" on page 15.

   b. Confirm that there is an adequate volume of each extracted DNA sample.

   IMPORTANT! While the maximum sample volume required for any kit is 20 µL, it is recommended that you check the samples for sufficient volume prior to dilution. A 50 µL total volume is ideal for reliable liquid detection, but you can use lower volumes. If there is not enough sample for the required volume, the entire volume will be aspirated and diluted according to the dilution protocol, regardless of the deficient volume. This deficiency could result in low or no profiles in the downstream analysis.

6. If the extracted DNA is in a plate, place the extracted DNA sample plate into the metal plate adapter on the worktable in grid 21, site position 1, with well A1 in the top left corner.



IMPORTANT! To ensure that samples are transferred to the correct wells, confirm that:

- The extracted DNA plate is placed in the metal plate adapter
- The extracted DNA plate wells are aligned with the holes in the metal plate adapter
- Well A1 is positioned in the upper left corner

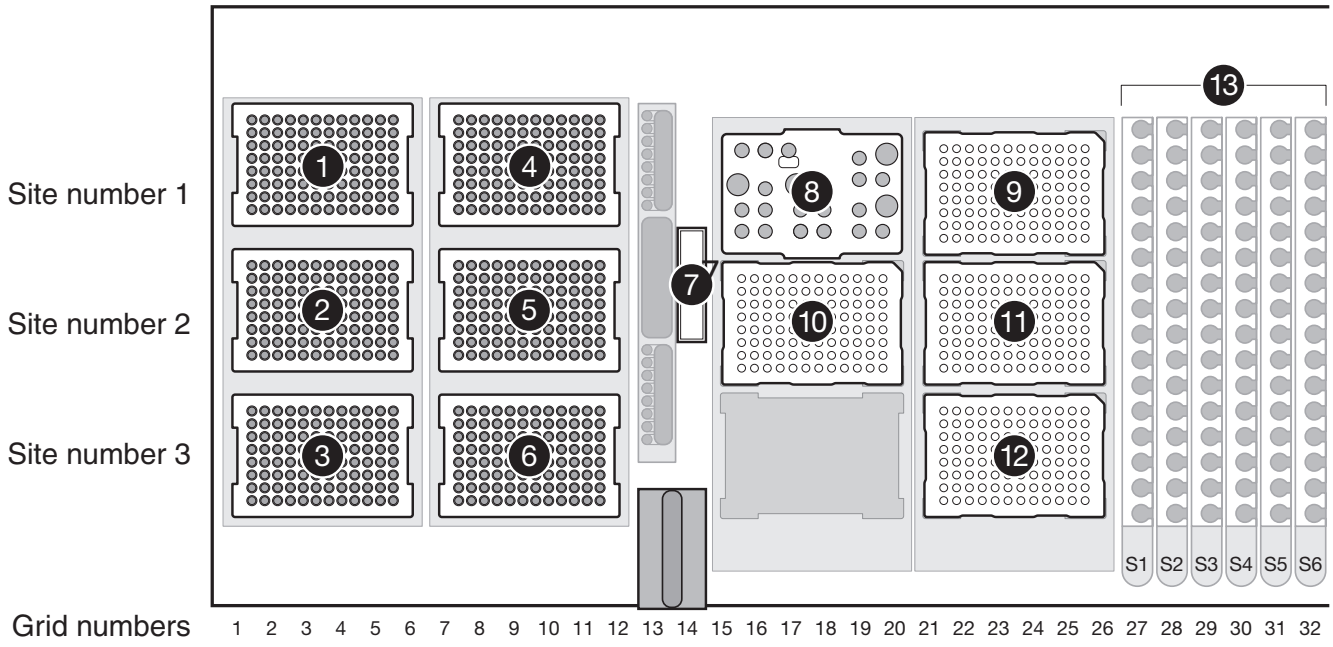


Figure 5 Tecan Freedom EVO® workstation (worktable) layout for a STR PCR setup run. Locations for DNA samples in a plate and tubes are shown.

Note: Only one type of plasticware for extracted DNA can be placed on the workstation for a given run.

1–2. 200-µL disposable pipette tips (DiTis)

3–6. 50-µL DiTis

7. Trough for $T_{10}E_{0.1}$ buffer

8. AB STR PCR Reagent Block

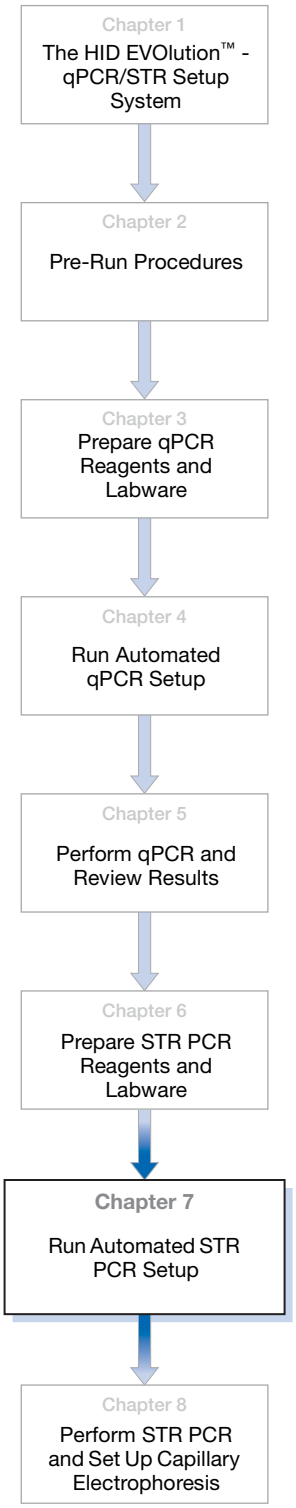
9. MicroAmp® Optical 96-Well Reaction Plate (if samples are in a plate) with 96-well metal plate adapter

10-11. MicroAmp® Optical 96-Well Reaction Plates (for predilution of samples) with 96-well metal plate adapter

12. MicroAmp® Optical 96-Well Reaction Plate (STR PCR reaction plate) with 96-well metal plate adapter

13. Tube racks S1 through S6 for DNA sample tubes (if samples are in tubes)

7 Run Automated STR PCR Setup



This chapter provides procedures for performing a STR PCR reaction setup run.

Run STR PCR setup

After performing all tasks, including maintenance, in the "Review pre-run checklist for STR PCR reaction setup" on page 46, use the following procedure to begin a STR PCR reaction setup run:

1. Select the appropriate EVOware software script for the AmpFℓSTR® kit and plasticware that you are using:

Freedom EVOware® software script selection for STR PCR

If the sample is prepared with...	And the extracted DNA is in...	Samples/run...	Use this script
Identifiler® kit reagents	a 96-well plate	88	Identifiler_plate.esc
	1.5-mL microcentrifuge tubes	88	Identifiler_tubes.esc
Yfiler® kit reagents	a 96-well plate	87	Yfiler_plate.esc
	1.5-mL microcentrifuge tubes	87	Yfiler_tubes.esc
MiniFiler™ kit reagents	a 96-well plate	88	MiniFiler_plate.esc
	1.5-mL microcentrifuge tubes	88	MiniFiler_tubes.esc
COfiler® kit reagents	a 96-well plate	88	COfiler_plate.esc
	1.5-mL microcentrifuge tubes	88	COfiler_tubes.esc
Profiler Plus® kit reagents	a 96-well plate	88	Profiler_plate.esc
	1.5-mL microcentrifuge tubes	88	Profiler_tubes.esc
SEfiler Plus™ kit reagents‡	a 96-well plate	88	SEfilerPlus_plate.esc
	1.5-mL microcentrifuge tubes	88	SEfilerPlus_tubes.esc
SGM Plus® kit reagents	a 96-well plate	88	SGMPlus_plate.esc
	1.5-mL microcentrifuge tubes	88	SGMPlus_tubes.esc

‡ This script is available in Freedom EVOware® version 2.1. To upgrade scripts, contact your local Tecan service organization. See the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 10, "Customer Support," for contact information.

2. Follow the directions in the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual,* Section 5.3.2, "Running a STR-PCR Setup Script" and the additional instructions in steps 3 through 5 below.

3. Before entering or importing sample information, make sure that the sample IDs that you will import or manually enter match the sample IDs in the 7500 Results file(s). If you are using barcodes, make sure that the barcodes on the extracted DNA samples match the barcodes in the 7500 Results file(s).

4. After entering sample information (either manually, by using barcodes, or by importing a qPCR/STR Samples file):

   a. Confirm that the right side of the window displays a message with the number of samples you entered, for example: *60/60 planned samples present*.

   b. If the message says that fewer samples will be processed than entered samples (for example, *15/60 planned samples present*) this indicates that either:

      - Some samples were not given sample information. Click **Edit** to continue entering sample information.
      - Too many samples were indicated at the beginning of the wizard. Click  and start the wizard again, this time entering the correct number of samples to process.

5. After selecting the appropriate 7500 Results file(s), view and edit the information in the Sample Normalization Adjustment window:

   a. Click **View** to open the Sample Normalization Adjustment window.

   Note: The window lists the number of samples automatically selected for processing, and identifies those samples that are outside the acceptable quantity range. Only those samples with data that successfully merged with the 7500 Results file, and which meet the conditions for normalization, are automatically selected for dilution and STR reaction setup. You can manually select or deselect samples for processing, and edit the acceptable quantity range and the target DNA input amount.

   The sample dilution calculations in the Sample Normalization Adjustment window are based on the STR PCR amplification reaction volume, the sample quantification data, and the input amount of DNA that you want to target (Req. Amt (ng)). The software selects the appropriate dilution protocol that brings the DNA input amount after dilution closest to the required DNA target amount. After dilution, 10 µL of diluted DNA (for 25-µL reactions) or 20 µL of diluted DNA (for 50-µL reactions) is added to each PCR reaction.

   b. Review the global settings for all samples in the sample list, and edit the settings if necessary:

   Note: If you change any value(s), the system automatically performs the necessary recalculations.

   - **Process All** – Select this checkbox to process all samples for STR PCR amplification setup. To return to the original settings, select **Cancel**, then select **View** to re-open the window. De-select the Process All checkbox to deselect *all* samples for processing.

- **Global Req. Amount (ng)** – Sets the target DNA amount to be added to each STR PCR reaction in the plate.

  Note: For samples below the target input amount, the sample will not be processed unless you select the process checkbox for the individual sample *or* select **Process All**. If you select this checkbox, then 10 µL of extracted DNA will be added for a 25-µL STR reaction, or 20 µL of extracted DNA will be added for a 50 µL STR reaction.

- **Qty. range (ng/µL)** – Sets the acceptable concentration range for extracted DNA samples. Samples with concentrations above or below the range will not be included in the STR PCR reaction plate.

c. Review the settings for individual samples in the sample list, and edit the settings if necessary:

   Note: If you change any value(s), the system automatically performs the necessary recalculations.

   - **Process** – Select or deselect an individual sample for processing.
   - **Req. Amt (ng)** – Sets the target DNA amount to be added to the STR PCR reaction for a specific extracted DNA sample.

d. (Optional but recommended) When you are done reviewing and editing the information in the Sample Normalization Adjustment window, take a screenshot to capture the information, then name and save the screenshot.

   IMPORTANT! You can use this information later to determine the dilution ratio used for each sample, the amount of extracted DNA removed from the extracted DNA plate or tubes, and the target DNA input amount for each sample.

e. Click **OK** to exit the Sample Normalization Adjustment window.

   Note: The software shows a summary of the total number of samples that will be processed.

Perform post-run tasks

Take care of the STR PCR reaction plate

1. Remove the prepared STR PCR reaction plate containing the STR PCR reactions from the worktable.
2. Seal the STR PCR reaction plate with MicroAmp® Clear Adhesive Film (PN 4306311).
3. Place the STR PCR reaction plate in a table-top centrifuge with plate holders, then centrifuge the plate at 3000 rpm for approximately 20 seconds to remove any air bubbles.

Clean up the instrument

1. Remove the TE buffer trough, and dispose of any remaining TE buffer. If it is the last run of the day, dispose of the TE buffer trough.

   IMPORTANT! Do not reuse the reagents in the troughs. See "Waste disposal" on page 144.

2. Follow the instructions in the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*, Section 5.3.1, "After Run" on page 108.

About the STR PCR plate layout

Regardless of the extracted DNA sample setup, the STR PCR reaction plate is always set up in the same way: the sample from the first position in the extracted DNA sample plate or the first extracted DNA sample tube is always placed in well A1 of the STR PCR reaction plate. See Figure 6 below and Figure 7 on page 63 for STR PCR reaction plate layouts. At the end of the STR PCR setup run, the HID EVOlution™ system generates a report that lists the position of each DNA sample in the extracted DNA sample plate or tubes and in the STR PCR reaction plate.

After STR PCR amplification, you transfer the PCR products from the STR PCR reaction plate to a CE plate for processing on the 3130/3130*xl* Genetic Analyzer. To make the plate-to-plate transfer easier, the STR PCR reaction plate layout mirrors that of the CE plate layout. For example, the HID EVOlution™ - qPCR/STR Setup System leaves six empty wells (designated as LDR) in the STR PCR plate as placeholders for the allelic ladder sample replicates in the CE plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S14	S22	S29	S37	S44	S52	S59	S67	S74	S82
B	S2	S10	S15	S23	S30	S38	S45	S53	S60	S68	S75	S83
C	S3	S11	S16	S24	S31	S39	S46	S54	S61	S69	S76	S84
D	S4	S12	S17	S25	S32	S40	S47	S55	S62	S70	S77	S85
E	S5	S13	S18	S26	S33	S41	S48	S56	S63	S71	S78	S86
F	S6	PTC	S19	S27	S34	S42	S49	S57	S64	S72	S79	S87
G	S7	NTC	S20	S28	S35	S43	S50	S58	S65	S73	S80	S88
H	S8	LDR	S21	LDR	S36	LDR	S51	LDR	S66	LDR	S81	LDR

Figure 6 STR PCR plate layout for all AmpFℓSTR® kits except for the Yfiler® kit. PTC = Amplification positive control (control DNA); NTC = Amplification negative control (TE buffer); LDR = empty wells indicating where allelic ladder sample replicates will be placed in the CE plate. The positive and negative control volumes are 10 µl for 25-µL reactions and 20 µL for 50-µL reactions.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S13	S21	S28	S36	S43	S51	S58	S66	S73	S81
B	S2	S10	S14	S22	S29	S37	S44	S52	S59	S67	S74	S82
C	S3	S11	S15	S23	S30	S38	S45	S53	S60	S68	S75	S83
D	S4	S12	S16	S24	S31	S39	S46	S54	S61	S69	S76	S84
E	S5	D007	S17	S25	S32	S40	S47	S55	S62	S70	S77	S85
F	S6	D9974A	S18	S26	S33	S41	S48	S56	S63	S71	S78	S86
G	S7	NTC	S19	S27	S34	S42	S49	S57	S64	S72	S79	S87
H	S8	LDR	S20	LDR	S35	LDR	S50	LDR	S65	LDR	S80	LDR

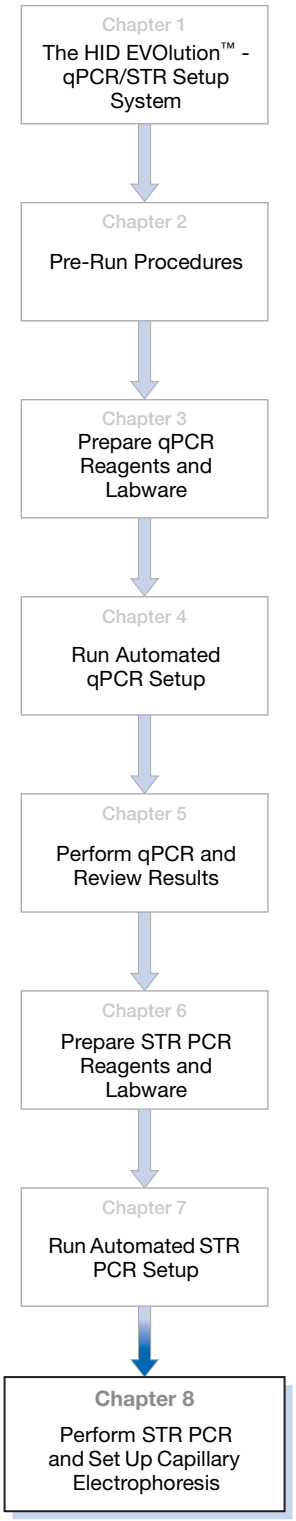
Figure 7 STR PCR plate layout for the AmpFℓSTR® Yfiler® kit. D007 = Amplification positive control (control DNA 007); D9974A = Amplification positive control (control DNA 9947A); NTC = Amplification negative control (TE buffer); LDR = empty wells indicating where allelic ladder sample replicates will be placed in the CE plate. The positive and negative control volumes are 10 µl for 25-µL reactions and 20 µL for 50-µL reactions.

For more information

For information on:

- Reports, see the Tecan *HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 6, "Results."
- Running scripts and on script error messages, see the *Tecan HID EVOlution™ – qPCR/STR Setup System Application Manual*, Section 5.3.2, "Running a STR-PCR Setup Script," and Section 8.4, "Application Software."
- Freedom EVOware® software, see the *Tecan EVOware® Standard/EVOware® Plus 2.1 Software Manual* and *Tecan EVOware® Standard/EVOware® Plus 2.1 Software Getting Started Guide*.

8 Perform STR PCR and Set Up Capillary Electrophoresis



This chapter explains where to get relevant information to perform STR PCR and how to set up for CE.

Run STR PCR amplification

Refer to the appropriate AmpFℓSTR® kit documentation for amplification procedures.

Document	Part number
AmpFℓSTR® COfiler® PCR Amplification Kit User's Manual	4306116
AmpFℓSTR® Identifiler® PCR Amplification Kit User's Manual	4323291
AmpFℓSTR® MiniFiler™ PCR Amplification Kit User's Manual	4374618
AmpFℓSTR® Profiler Plus® PCR Amplification Kit User's Manual	4303501
AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit User's Manual	4385739
AmpFℓSTR® SGM Plus® PCR Amplification Kit User's Manual	4309589
AmpFℓSTR® Yfiler® PCR Amplification Kit User's Manual	4358101

When you are done with amplification (thermal cycling), prepare for CE.

About the CE Setup file

The HID EVOlution™ system software generates a plate file (CE Setup file) for each run. The file contains sample information that you can import to the Data Collection Software plate document for the STR run on the CE instrument (3130/3130*xl* Genetic Analyzer computer).

By default, the HID EVOlution™ - qPCR/STR Setup System names the CE Setup file:

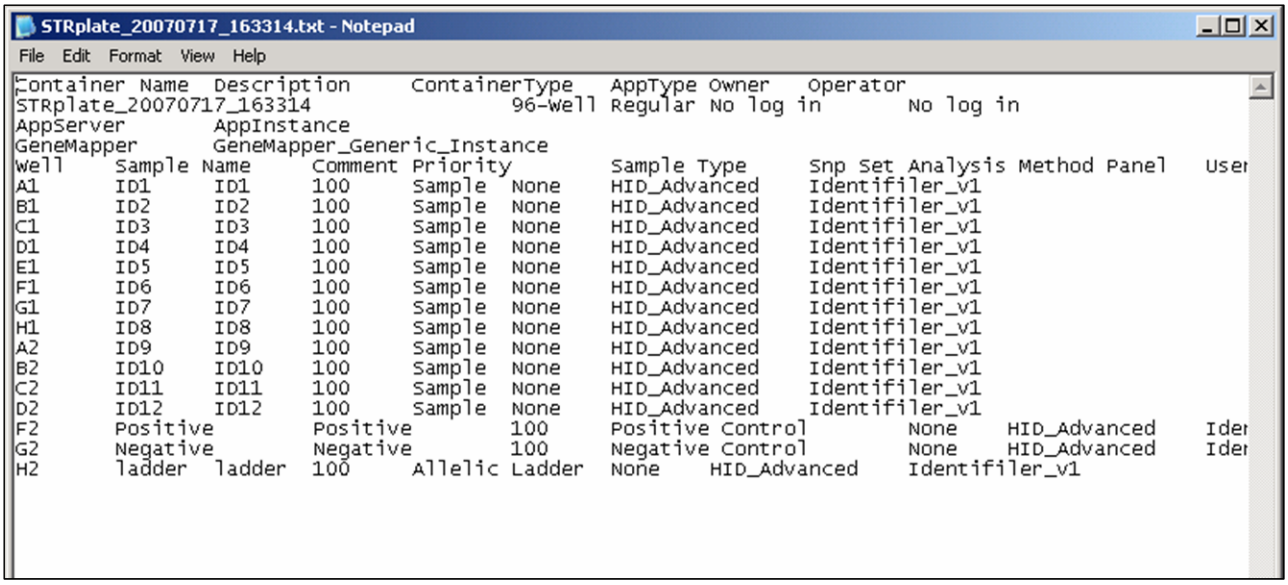
- C:\HIDEVOlution_qPCRSTRfiles\AB3130Input\STRplate_<*rundate*>_<*runtime*>.txt

  or

- C:\HIDEVOlution_qPCRSTRfiles\AB3130Input\<*barcode*>_<*rundate*>_<*runtime*>.txt

The HID EVOlution™ system automatically archives the CE Setup file in the C:\HIDEVOlution_qPCRSTRfiles\AB3130Input\Archive folder when you start the next qPCR or STR PCR setup run.

If necessary, create shortcuts on your desktop to the C:\HIDEVOlution_qPCRSTRfiles\AB3130Input and C:\HIDEVOlution_qPCRSTRfiles\AB3130Input\Archive folders.



STRplate_20070717_163314.txt - Notepad

File Edit Format View Help

```
Container Name  Description     ContainerType   AppType Owner   Operator
STRplate_20070717_163314                96-well Regular No log in       No log in
AppServer       AppInstance
GeneMapper      GeneMapper_Generic_Instance
Well    Sample Name     Comment Priority        Sample Type     Snp Set Analysis Method Panel   User
A1      ID1     ID1     100     Sample  None    HID_Advanced    Identifiler_v1
B1      ID2     ID2     100     Sample  None    HID_Advanced    Identifiler_v1
C1      ID3     ID3     100     Sample  None    HID_Advanced    Identifiler_v1
D1      ID4     ID4     100     Sample  None    HID_Advanced    Identifiler_v1
E1      ID5     ID5     100     Sample  None    HID_Advanced    Identifiler_v1
F1      ID6     ID6     100     Sample  None    HID_Advanced    Identifiler_v1
G1      ID7     ID7     100     Sample  None    HID_Advanced    Identifiler_v1
H1      ID8     ID8     100     Sample  None    HID_Advanced    Identifiler_v1
A2      ID9     ID9     100     Sample  None    HID_Advanced    Identifiler_v1
B2      ID10    ID10    100     Sample  None    HID_Advanced    Identifiler_v1
C2      ID11    ID11    100     Sample  None    HID_Advanced    Identifiler_v1
D2      ID12    ID12    100     Sample  None    HID_Advanced    Identifiler_v1
F2      Positive        Positive        100     Positive Control        None    HID_Advanced    Ider
G2      Negative        Negative        100     Negative Control        None    HID_Advanced    Ider
H2      ladder  ladder  100     Allelic Ladder  None    HID_Advanced    Identifiler_v1
```

Transfer and import the CE Setup file

IMPORTANT! To successfully import the CE Setup file to the 3130/3130*xl* Genetic Analyzer Data Collection software v3.0, the instrument protocol and results group names created in the Data Collection software must match those in the CE Setup file. See "Create an instrument protocol and results group for the CE instrument" on page 11.

1. On the HID EVOlution system, navigate to the C:\HIDEVOlution_qPCRSTRfiles\AB3130Input folder, then locate the CE Setup file with the name that you noted at the end of the run:
   - STRplate_<*rundate*>_<*runtime*>.txt

     or
   - <*barcode*>_<*rundate*>_<*runtime*>.txt
2. Copy and transfer the CE Setup file to a location where it can be accessed by the Data Collection Software computer.
3. In the Data Collection Software:
   a. Select **Plate Manager**, then click **Import**.
   b. Navigate to the CE Setup file that you copied from the HID EVOlution™ - qPCR/STR Setup System software to the CE instrument (3130/3130*xl* Genetic Analyzer computer).
   c. Select the file, click **Open**, then click **OK**.

Prepare the CE Plate

Refer to the applicable *AmpFℓSTR® Kit User Guide* and the Applied Biosystems 3130/3130*xl* Genetic Analyzers User Bulletin: *Using Data Collection Software v3.0 - Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products* (PN 4363787).

For more information

For information on:

- Running STR PCR amplification, refer to the appropriate *AmpFℓSTR® Kit User's Manual*.
- Running CE, see the *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide* or the *AmpFℓSTR® Kit User's Manual* specific to your system.
- For details on the HID EVOlution™ - qPCR/STR Setup System, see the *Tecan HID EVOlution™ – qPCR/STR Setup System Application Manual*.
- Creating an analysis method, instrument protocol, and results group in the Data Collection software, refer to the Applied Biosystems 3130/3130*xl* Genetic Analyzers User Bulletin: *Using Data Collection Software v3.0 - Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products* (PN 4363787).

A Troubleshooting

For troubleshooting problems with:

- DNA yield, see the *Quantifiler® Kits User's Manual* and Table 12 below.
- Setting up and running the automation instrument, see the Tecan *HID EVOlution™ - qPCR/STR Setup System Application Manual*.

Table 12 Troubleshooting qPCR/STR PCR results

Observed problem	Possible reason	Suggested solution
The DNA yield is low or DNA is absent	• The biological sample contains no or a low amount of DNA. • Reagents are missing or improperly positioned on the worktable. • Incorrect automated pipetting occurred because of: – Incorrect or improperly placed DiTis, plates, tubes, or hardware. – Air bubbles or leaks in system. – Dirty or loose DiTi cones. • DiTis were not picked up properly. • Reagent, equipment or instrumentation failure.	Confirm the correct reagent and instrument setup, then re-run the samples: • Confirm reagent and instrument performance - **Manually** set up control DNA samples using the same reagents and instrumentation to confirm that the reagents, protocol, equipment and instrumentation are functioning as expected. • Confirm all necessary reagents are present and correctly positioned on the workstation. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual.* • Confirm that you use the specified DiTis, plates, tubes, and metal racks and carriers in the correct positions. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*. • Clean and finger-tighten the DiTi cones, diluter valves and syringe fittings. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*. • Confirm that you have sufficient system liquid, then flush the system and check for air bubbles and leaks. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*. • Review the LiHa coordinates (x, y, and z positions) of the DiTis. For details, contact Applied Biosystems or refer to the T*ecan Freedom EVOware® Standard 2.1 Freedom EVOware® Plus 2.1 Extended Device Support Software Manual*, Section 9.4.4, "Teaching the Labware Coordinates." • Amplify the maximum volume for STR analysis. • Extract DNA from a different sample that is prepared from the same source. • Concentrate the sample, then repeat the test with the respective Quantifiler® kit before performing STR analysis.

Table 12 Troubleshooting qPCR/STR PCR results *(continued)*

Observed problem	Possible reason	Suggested solution
The sample IPC C_T is higher than the IPC C_T of the no template quantitation control (NTC) or of the quantitation standards (for example, if the sample IPC C_T is approximately two C_T greater than the NTC IPC C_T or the C_T of the standards). *or* Weak amplification (high C_T value and low ΔR_n value) of the human and/or male-specific targets and no or weak amplification of the IPC.	• The DNA concentration is above 25 ng/µL. • Potential presence of PCR inhibitors.	• If the DNA concentration is over 25 ng/µL, dilute the DNA extract, then requantify the sample. • If the DNA concentrate is below 25 ng/µL, or if the diluted DNA eluate still produces high IPC C_T compared to the NTC or quantitation standards, repurify the sample. **IMPORTANT!** Repurification may result in the loss of additional DNA. Consider proceeding to amplification with a kit such as the AmpFℓSTR® MiniFiler™ PCR Amplification kit, which is designed to obtain STR profiles from compromised samples such as those which may be inhibited and/or degraded. • Before the next run: – To ensure correct pipetting, clean and finger tighten the DiTi cones, diluter valves, and syringe fittings. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*. – Confirm that you have sufficient system liquid, then flush the system and check for air bubbles and leaks. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*.
CE signal is too high	Sample concentration is greater than the reported quantification result.	• Requantify (see IMPORTANT above). • Before the next run: – To ensure correct pipetting, clean and finger tighten the DiTi cones, diluter valves, and syringe fittings. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*. – Confirm that you have sufficient system liquid, then flush the system and check for air bubbles and leaks. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*.

Table 12 Troubleshooting qPCR/STR PCR results *(continued)*

Observed problem	Possible reason	Suggested solution
CE signal is too low	• Potential presence of PCR inhibitors. • Incorrect target amount entered into the Sample Normalization Adjustment window. • Not enough DNA. • Presence of inhibitors. • Degraded formamide. • Dilution of DNA sample in H_2O or wrong buffer (e.g., wrong EDTA concentration).	• Confirm reagent and instrument performance - **Manually** set up control DNA samples using the same reagents and instrumentation to confirm that the reagents, protocol, equipment and instrumentation are functioning as expected. • Use the maximum volume permitted for the DNA sample. • If possible, repurify DNA and requantify. Otherwise use the minimal volume possible. • Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide. • Verify that correct TE buffer was used (with 0.1-mM EDTA). • Before the next run: – To ensure correct pipetting, clean and finger tighten the DiTi cones, diluter valves, and syringe fittings. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*. – Confirm that you have sufficient system liquid, then flush the system and check for air bubbles and leaks. Refer to the *Tecan HID EVOlution™ - qPCR/STR Setup System Application Manual*.

B Reagent Block Configurations

This appendix provides the reagent block configurations referred to in Chapter 3, "Prepare qPCR Reagents and Labware" on page 23 and Chapter 6, "Prepare STR PCR Reagents and Labware" on page 45 for the following kits:

IMPORTANT! Prepare the reagents as directed in "Set up reagents" on page 25 and "Set up reagents" on page 52 before placing the reagents in the reagent block.

Quantifiler® DNA Quantification Kits

Quantifiler® Human Kit with pre-prepared standards

50.0	5.556	0.617	0.069
16.667	1.852	0.206	0.023

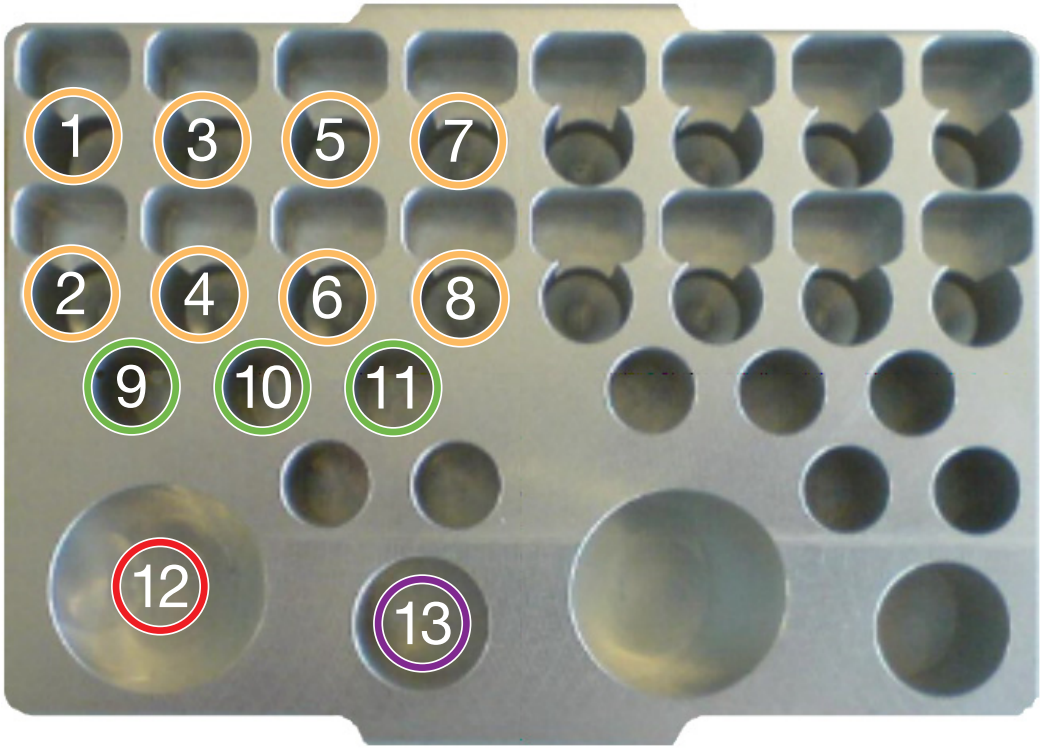


Figure 8 Reagent block configuration for Quantifiler® Human kit with pre-prepared DNA standards.

Legend:

1-8. 1.5-mL tubes of pre-prepared DNA standard dilution series (arrange concentrations as shown in the corresponding orange table).

9-11. Up to three 1.5-mL tube(s) of Quantifiler® Human Primer Mix. Place the first tube in position 9. If you use more than one tube, continue to position 10, then position 11.‡

12. Quantifiler® PCR Reaction mix.

13. Empty 5-mL VWR tube for the master mix.

‡ The required number of tubes with the Quantifiler® Human Primer Mix depends on the number of reactions and the fill level of the tubes. The instrument aspirates 190 µL at a time. In order for the instrument to detect reagent in a tube, the tube must contain a minimum of 240 µL (190 µL plus a 50-µL dead volume). If the volume of reagent in the first tube (position 9) falls below 240 µL, the instrument looks for a sufficient volume in the position 10 tube, then in the position 11 tube. If the tubes are not available or the instrument does not detect sufficient volume, the instrument pauses the run and displays the error message “Not enough liquid.”

Quantifiler® Human Kit with system-prepared standards

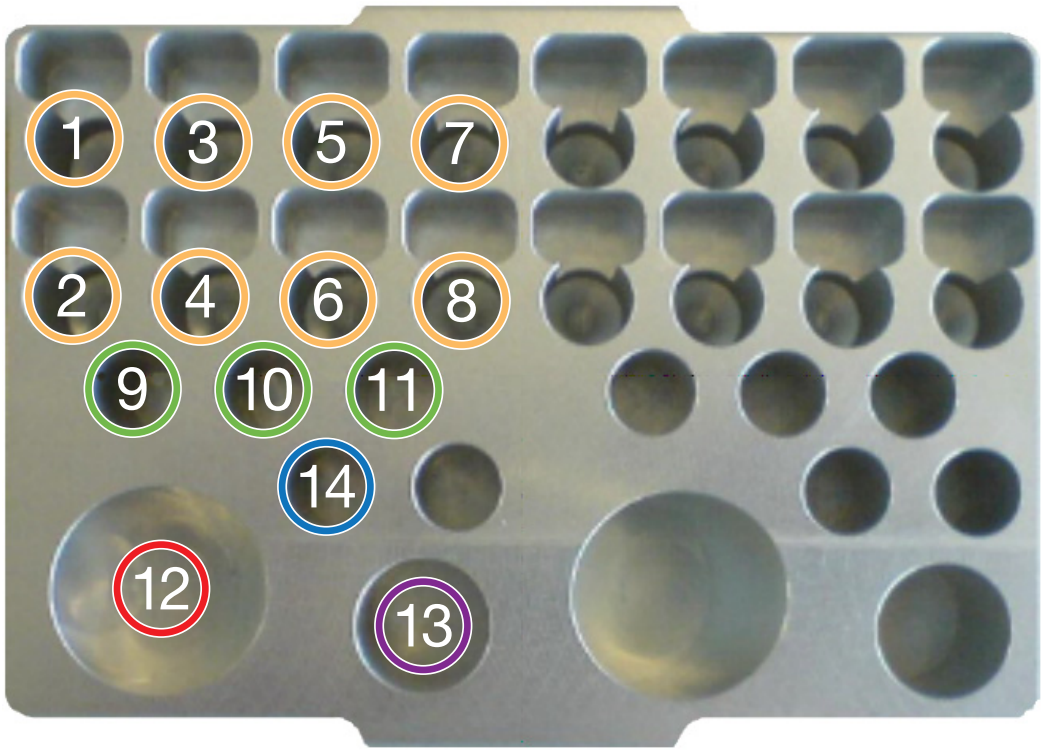


Figure 9 Reagent block configuration for Quantifiler® Human kit with DNA standards prepared by the HID EVOlution™ system.

Legend:

1-8. 1.5-mL empty tubes for DNA standard dilution series.

9-11. Up to three 1.5-mL tube(s) of Quantifiler® Human Primer Mix. Place the first tube in position 9. If you use more than one tube, continue to position 10, then position 11.‡

12. Quantifiler® PCR Reaction mix.

13. Empty 5-mL VWR tube for the master mix.

14. Quantifiler® Human DNA Standard tube.

‡ The required number of tubes with the Quantifiler® Human Primer Mix depends on the number of reactions and the fill level of the tubes. The instrument aspirates 190 µL at a time. In order for the instrument to detect reagent in a tube, the tube must contain a minimum of 240 µL (190 µL plus a 50-µL dead volume). If the volume of reagent in the first tube (position 9) falls below 240 µL, the instrument looks for a sufficient volume in the position 10 tube, then in the position 11 tube. If the tubes are not available or the instrument does not detect sufficient volume, the instrument pauses the run and displays the error message “Not enough liquid.”

Quantifiler® Y Human Male Kit with pre-prepared standards

50.0	5.556	0.617	0.069
16.667	1.852	0.206	0.023

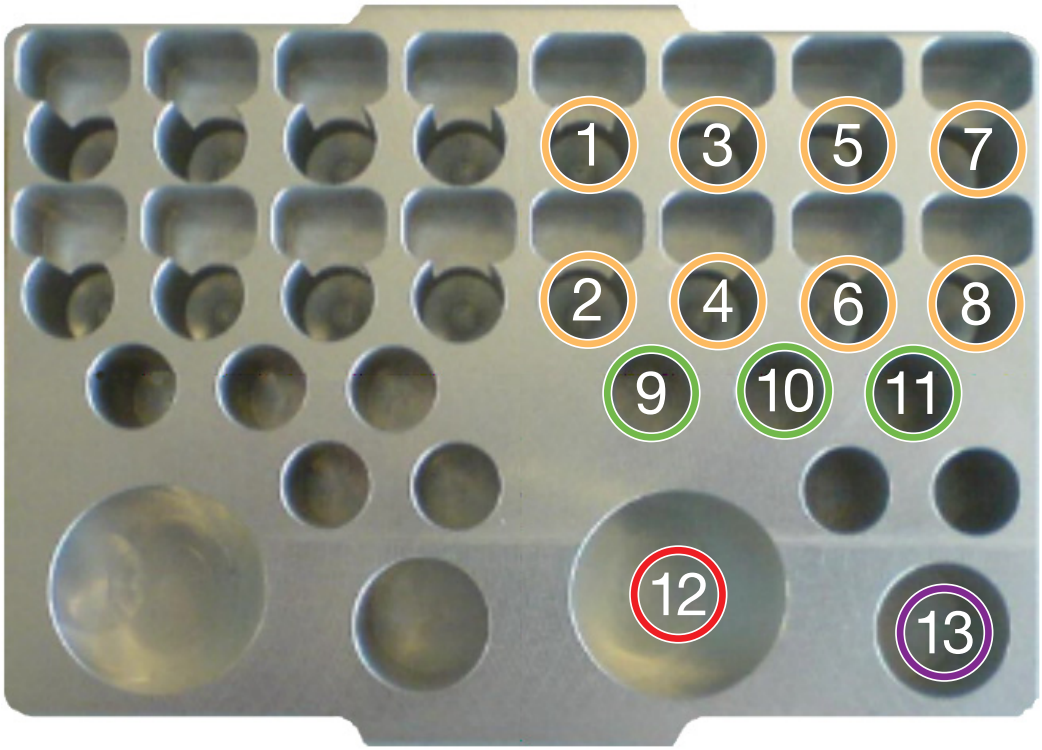


Figure 10 Reagent block configuration for Quantifiler® Y Human Male kit with pre-prepared DNA standards.

Legend:

1-8. 1.5-mL tubes of pre-prepared DNA standards (arrange concentrations as shown in the corresponding orange table).

9-11. Up to three 1.5-mL tube(s) of Quantifiler® Y Human Male Primer Mix. Place the first tube in position 9. If you use more than one tube, continue to position 10, then position 11.‡

12. Quantifiler® PCR Reaction mix.

13. Empty 5-mL VWR tube for the master mix.

‡ The required number of tubes with the Quantifiler® Y Human Male Primer Mix depends on the number of reactions and the fill level of the tubes. The instrument aspirates 190 µL at a time. In order for the instrument to detect reagent in a tube, the tube must contain a minimum of 240 µL (190 µL plus a 50-µL dead volume). If the volume of reagent in the first tube (position 9) falls below 240 µL, the instrument looks for a sufficient volume in the position 10 tube, then in the position 11 tube. If the tubes are not available or the instrument does not detect sufficient volume, the instrument pauses the run and displays the error message “Not enough liquid.”

Quantifiler® Y Human Male Kit with system-prepared standards

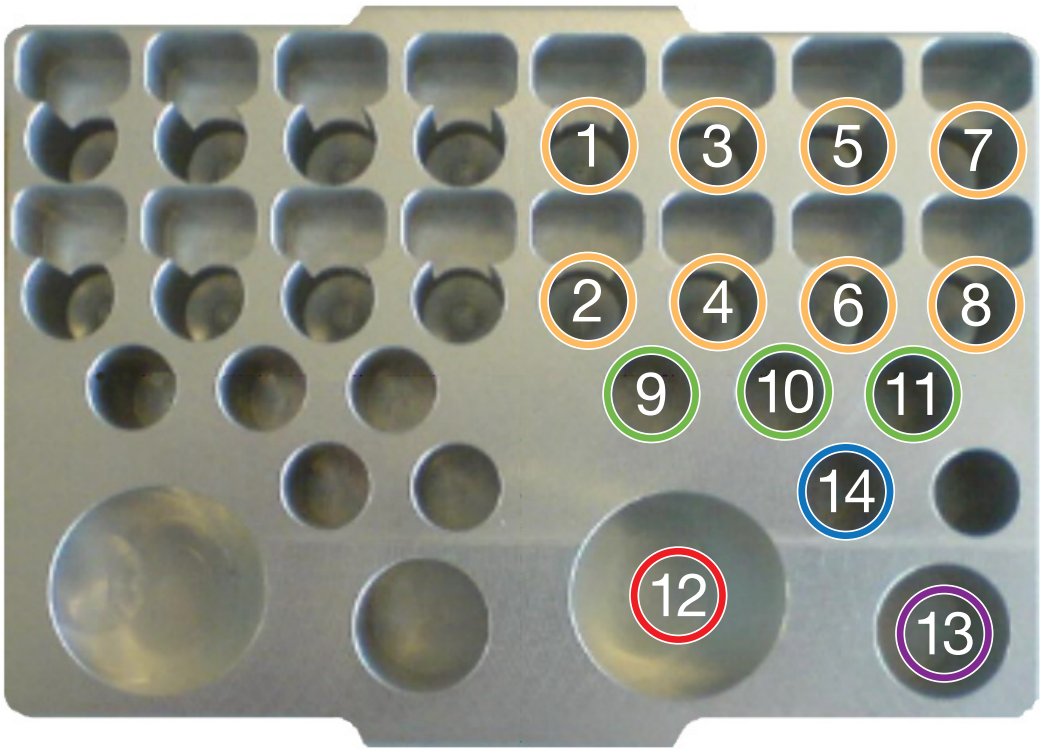


Figure 11 Reagent block configuration for Quantifiler® Y Human Male kit with DNA standards prepared by the HID EVOlution™ system.

Legend:

1-8. 1.5-mL empty tubes for DNA standards dilution series.

9-11. Up to three 1.5-mL tube(s) of Quantifiler® Y Human Male Primer Mix. Place the first tube in position 9. If you use more than one tube, continue to position 10, then position 11.‡

12. Quantifiler® PCR Reaction mix.

13. Empty 5-mL VWR tube for the master mix.

14. Quantifiler® Human DNA Standard tube.

‡ The required number of tubes with the Quantifiler® Y Human Male Primer Mix depends on the number of reactions and the fill level of the tubes. The instrument aspirates 190 µL at a time. In order for the instrument to detect reagent in a tube, the tube must contain a minimum of 240 µL (190 µL plus a 50-µL dead volume). If the volume of reagent in the first tube (position 9) falls below 240 µL, the instrument looks for a sufficient volume in the position 10 tube, then in the position 11 tube. If the tubes are not available or the instrument does not detect sufficient volume, the instrument pauses the run and displays the error message “Not enough liquid.”

Quantifiler® Human and Y Human Male Kits with pre-prepared standards

Quantifiler® Human kit reagents			
50.0	5.556	0.617	0.069
16.667	1.852	0.206	0.023

Quantifiler® Y Human Male kit reagents			
50.0	5.556	0.617	0.069
16.667	1.852	0.206	0.023

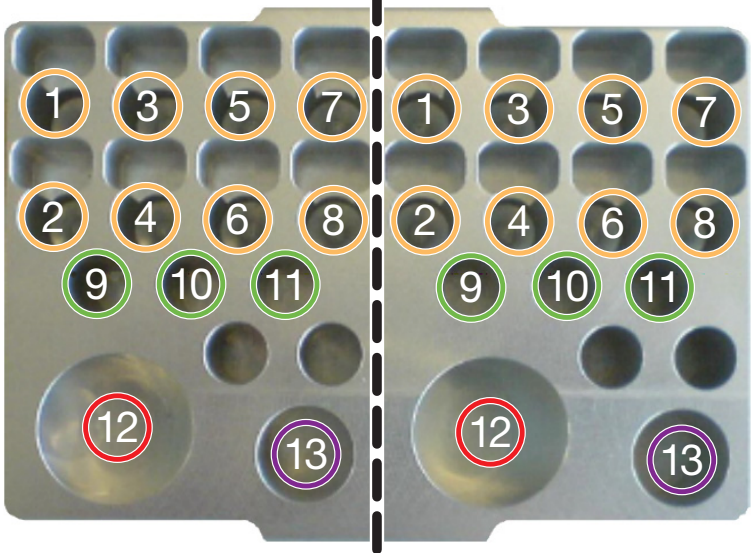


Figure 12 Reagent block configuration for Quantifiler® Human and Y Human Male kits with pre-prepared DNA standards.

Legend:

1-8. 1.5-mL tubes of pre-prepared DNA standards (arrange concentrations as shown in the corresponding orange tables).

9-11. Up to three 1.5-mL tube(s) of Quantifiler® Primer Mix. Place the first tube in position 9. If you use more than one tube, continue to position 10, then position 11.‡

12. Quantifiler® PCR Reaction mix.

13. Empty 5-mL VWR tube for the master mix.

‡ The required number of tubes with the Quantifiler® Primer Mix depends on the number of reactions and the fill level of the tubes. The instrument aspirates 190 µL at a time. In order for the instrument to detect reagent in a tube, the tube must contain a minimum of 240 µL (190 µL plus a 50-µL dead volume). If the volume of reagent in the first tube (position 9) falls below 240 µL, the instrument looks for a sufficient volume in the position 10 tube, then in the position 11 tube. If the tubes are not available or the instrument does not detect sufficient volume, the instrument pauses the run and displays the error message “Not enough liquid.”

Quantifiler® Human and Y Human Male Kits with system-prepared standards

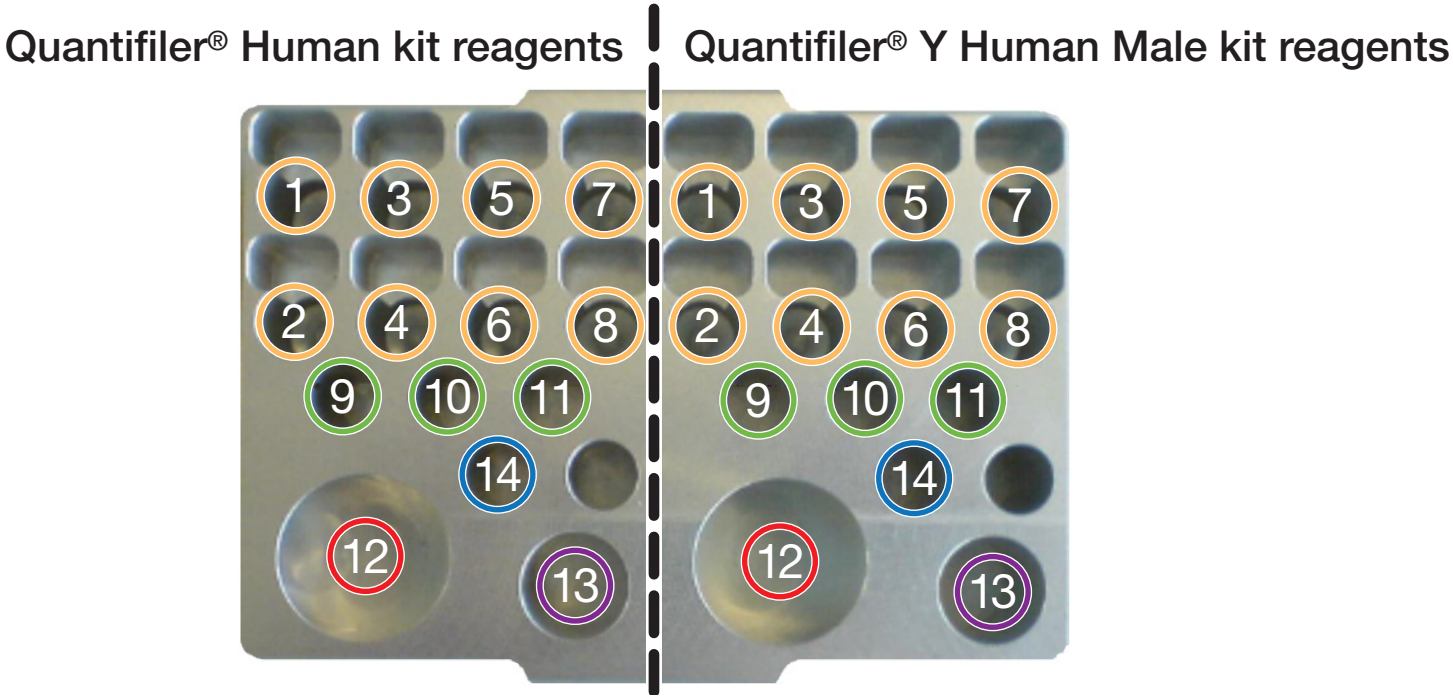


Figure 13 Quantifiler® Human and Y Human Male kits with DNA standards prepared by the HID EVOlution™ system.

Legend:

1-8. 1.5-mL empty tubes for DNA standards.

9-11. Up to three 1.5-mL tube(s) of Quantifiler® Primer Mix. Place the first tube in position 9. If you use more than one tube, continue to position 10, then position 11.‡

12. Quantifiler® PCR Reaction mix.

13. Empty 5-mL VWR tube for the master mix.

14. Quantifiler® Human DNA Standard tube.

‡ The required number of tubes with the Quantifiler® Primer Mix depends on the number of reactions and the fill level of the tubes. The instrument aspirates 190 µL at a time. In order for the instrument to detect reagent in a tube, the tube must contain a minimum of 240 µL (190 µL plus a 50-µL dead volume). If the volume of reagent in the first tube (position 9) falls below 240 µL, the instrument looks for a sufficient volume in the position 10 tube, then in the position 11 tube. If the tubes are not available or the instrument does not detect sufficient volume, the instrument pauses the run and displays the error message “Not enough liquid.”

AmpFℓSTR® PCR Amplification Kits

AmpFℓSTR® COfiler® PCR Amplification Kit

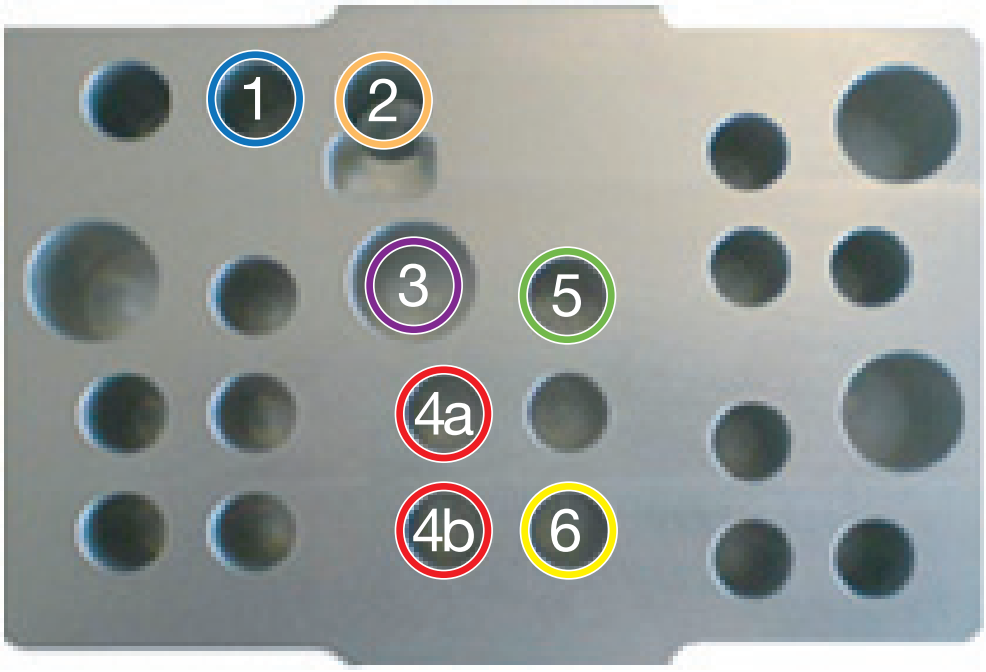


Figure 14 Reagent block configuration for COfiler® kit.

Legend:

1. Control DNA 9947A diluted as necessary. See step 4 on page 52.
2. 1.5-mL tube containing $T_{10}E_{0.1}$ buffer.
3. Empty VWR tube for master mix preparation.

4a-4b. AmpFℓSTR® PCR Reaction Mix.

5. COfiler® Primer Mix.
6. AmpliTaq Gold® DNA polymerase.‡

‡ You must combine the volumes in two full 50-µL tubes in one tube. The tube containing the combined volume must be one of the original AmpliTaq Gold® DNA Polymerase tubes provided with the kit. Use of another type of tube can cause liquid detection errors.

AmpFℓSTR® Identifiler® PCR Amplification Kit

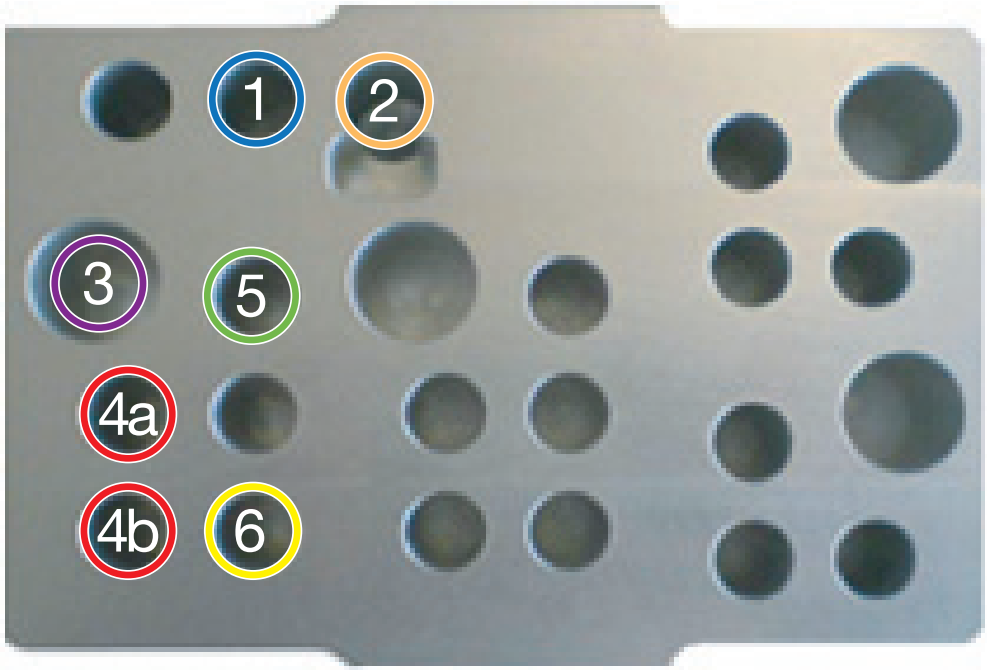


Figure 15 Reagent block configuration for Identifiler® kit.

Legend:

1. Control DNA 9947A diluted as necessary. See step 4 on page 52.
2. 1.5-mL tube containing $T_{10}E_{0.1}$ buffer.
3. Empty VWR tube for master mix preparation.

4a-4b. AmpFℓSTR® PCR Reaction Mix.

5. Identifiler® Primer Mix.
6. AmpliTaq Gold® DNA polymerase.‡

‡ You must combine the volumes in two full 50-µL tubes in one tube. The tube containing the combined volume must be one of the original AmpliTaq Gold® DNA Polymerase tubes provided with the kit. Use of another type of tube can cause liquid detection errors.

AmpFℓSTR® MiniFiler™ PCR Amplification Kit

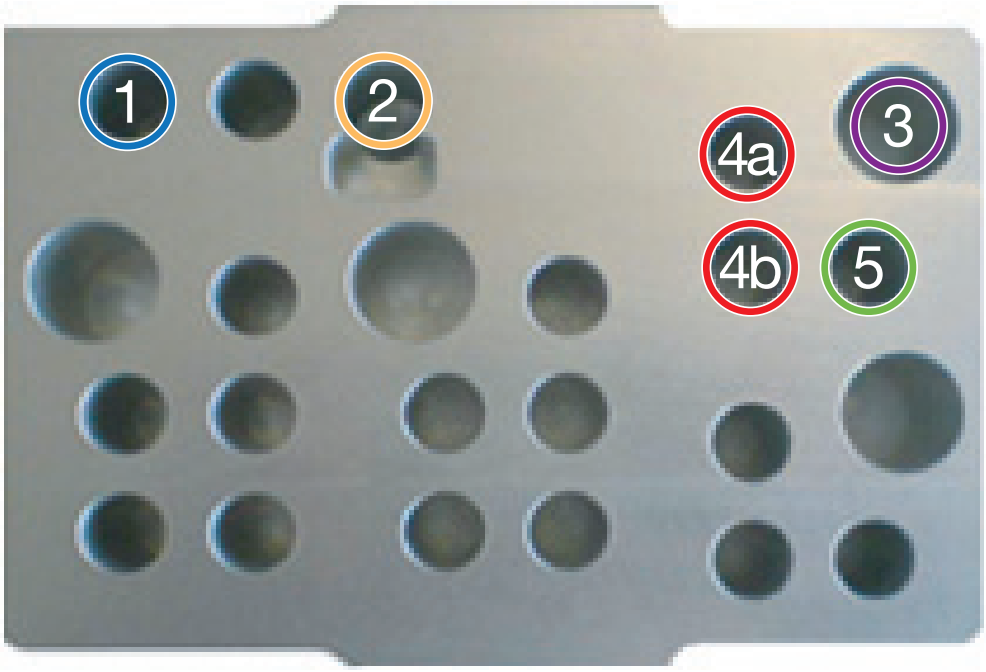


Figure 16 Reagent block configuration for MiniFiler™ kit.

Legend:

1. Control DNA 007 diluted as necessary. See step 4 on page 52.
2. 1.5-mL tube containing $T_{10}E_{0.1}$ buffer.
3. Empty VWR tube for master mix preparation.

4a-4b. MiniFiler™ Master Mix.

5. MiniFiler™ Primer Mix.

AmpFℓSTR® Profiler Plus® PCR Amplification Kit

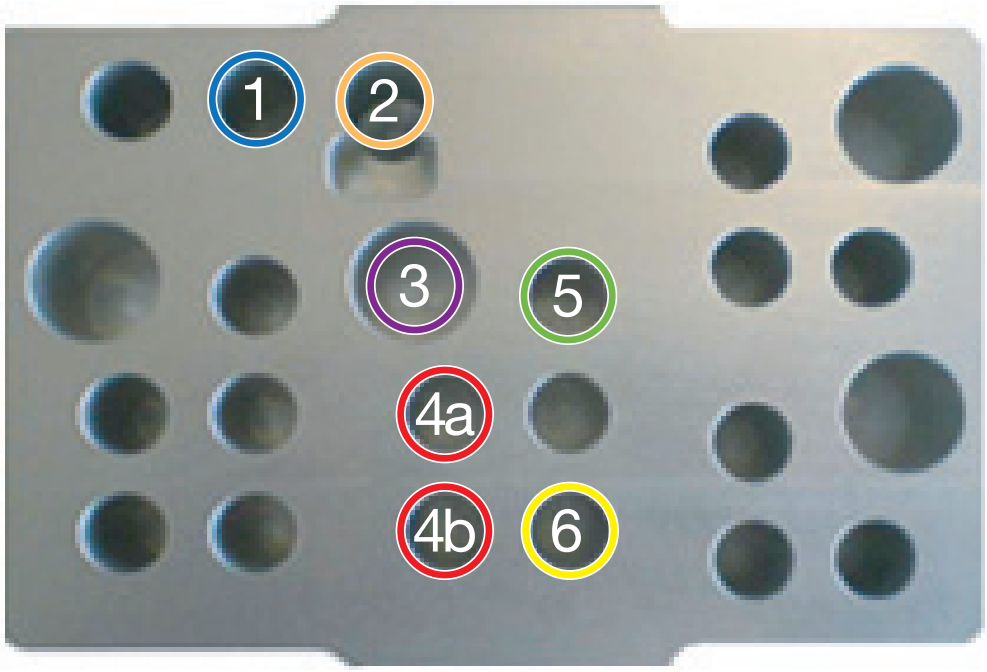


Figure 17 Reagent block configuration for Profiler Plus® kit.

Legend:

1. Control DNA 9947A diluted as necessary. See step 4 on page 52.
2. 1.5-mL tube containing $T_{10}E_{0.1}$ buffer.
3. Empty VWR tube for master mix preparation.

4a-4b. AmpFℓSTR® PCR Reaction Mix.

5. Profiler Plus® Primer Mix.
6. AmpliTaq Gold® DNA polymerase.‡

‡ You must combine the volumes in two full 50-µL tubes in one tube. The tube containing the combined volume must be one of the original AmpliTaq Gold® DNA Polymerase tubes provided with the kit. Use of another type of tube can cause liquid detection errors.

AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit

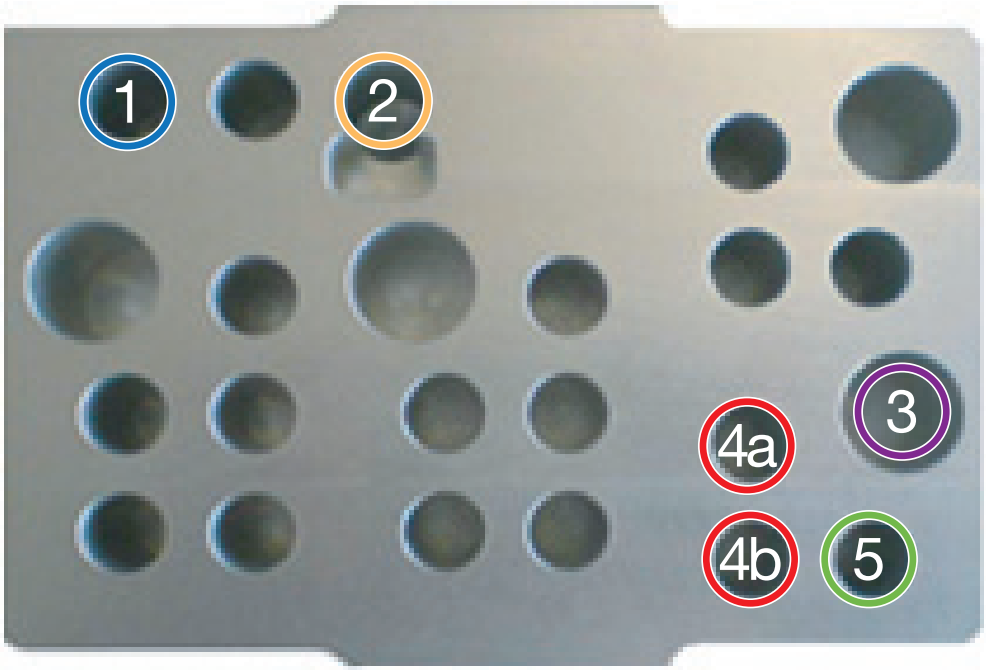


Figure 18 Reagent block configuration for SEfiler Plus™ kit.

Legend:

1. Control DNA 007 diluted as necessary. See step 4 on page 52.
2. 1.5-mL tube containing $T_{10}E_{0.1}$ buffer.
3. Empty VWR tube for master mix preparation.

4a-4b. SEfiler Plus™ Master Mix.

5. SEfiler Plus™ Primer Mix.

AmpFℓSTR® SGM Plus® PCR Amplification Kit

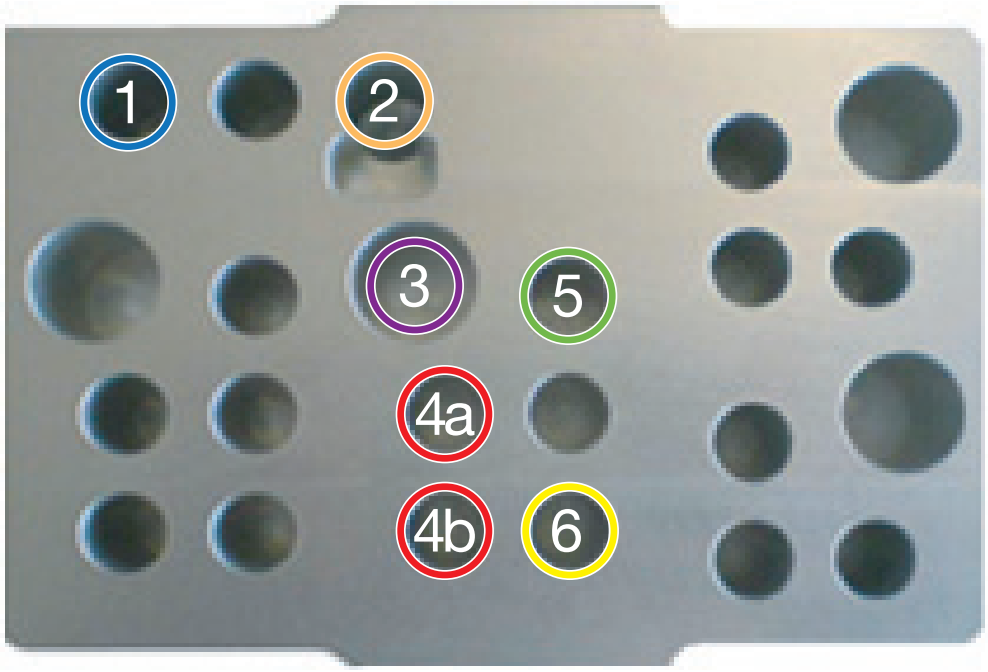


Figure 19 Reagent block configuration for SGM® Plus kit.

Legend:

1. Control DNA 007 diluted as necessary. See step 4 on page 52.
2. 1.5-mL tube containing $T_{10}E_{0.1}$ buffer.
3. Empty VWR tube for master mix preparation.

4a-4b. AmpFℓSTR® PCR Reaction Mix.

5. SGM® Plus Primer Mix.
6. AmpliTaq Gold® DNA polymerase.‡

‡ You must combine the volumes in two full 50-µL tubes in one tube. The tube containing the combined volume must be one of the original AmpliTaq Gold® DNA Polymerase tubes provided with the kit. Use of another type of tube can cause liquid detection errors.

AmpFℓSTR® Yfiler® PCR Amplification Kit

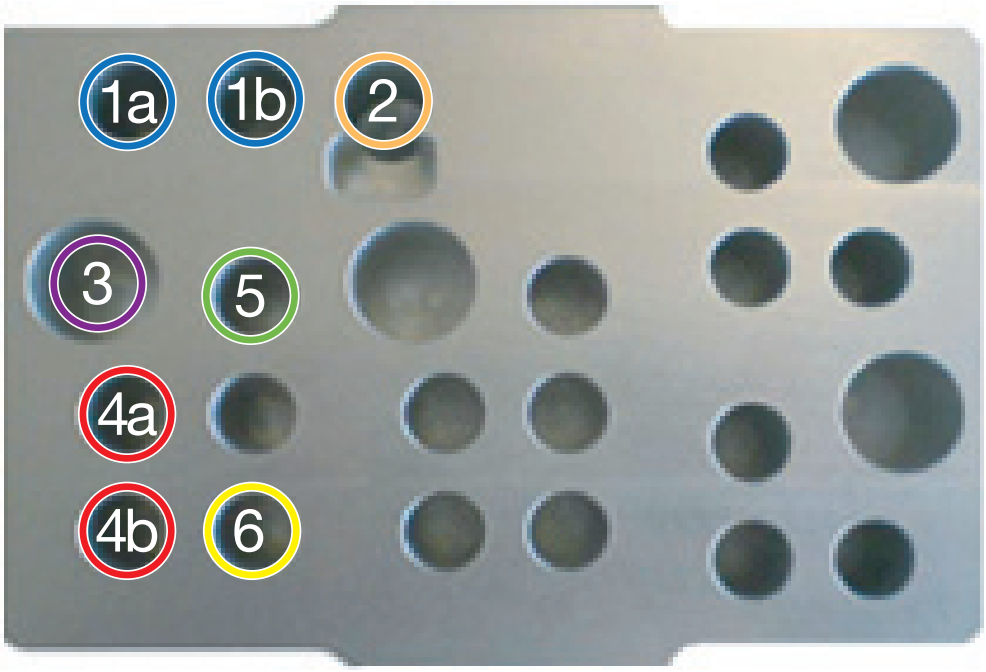


Figure 20 Reagent block configuration for Yfiler® kit.

Legend:

1a. Control DNA 007 diluted as necessary. See step 4 on page 52.

1b. Control DNA 9947A diluted as necessary. See step 4 on page 52.

2. 1.5-mL tube containing $T_{10}E_{0.1}$ buffer.

3. Empty VWR tube for master mix preparation.

4a-4b. Yfiler® PCR Reaction Mix.

5. Yfiler® Primer Mix.

6. AmpliTaq Gold® DNA polymerase.‡

‡ You must combine the volumes in two full 50-µL tubes in one tube. The tube containing the combined volume must be one of the original AmpliTaq Gold® DNA Polymerase tubes provided with the kit. Use of another type of tube can cause liquid detection errors.

C HID EVOlution™ - qPCR/STR Setup System Detailed Workflow Description

The steps performed by the user and by the HID EVOlution™ - qPCR/STR Setup System are described in the table and text below.

Table 13 Overview of the automated HID workflow.

HID Workflow	Covered in Chapter...
Prepare Sample/Extract DNA	Extraction procedures are not included in this guide. For automated extraction procedures, see the *PrepFiler™ Automated Forensic DNA Extraction Kit Getting Started Guide.*
Perform Quantitative PCR	
1. Perform routine maintenance	2, "Pre-Run Procedures" on page 9
2. Prepare samples, qPCR reagents, and system	3, "Prepare qPCR Reagents and Labware" on page 23
3. Run automated qPCR reaction setup	4, "Run Automated qPCR Setup" on page 33
4. Import a SDS plate record	5, "Perform qPCR and Review Results" on page 39
5. Run qPCR and review results	5, "Perform qPCR and Review Results" on page 39
Perform STR PCR Amplification	
1. Perform routine maintenance	2, "Pre-Run Procedures" on page 9
2. Prepare samples, STR PCR reagents, and system	6, "Prepare STR PCR Reagents and Labware" on page 45
3. Run automated DNA normalization and STR PCR reaction setup	7, "Run Automated STR PCR Setup" on page 57
4. Run STR PCR amplification	8, "Perform STR PCR and Set Up Capillary Electrophoresis" on page 65
Perform Capillary Electrophoresis/Genetic Analysis	
1. Import a CE plate record	8, "Perform STR PCR and Set Up Capillary Electrophoresis" on page 65
2. Set up CE reactions	CE and data analysis procedures are not included in this guide. See the AmpFℓSTR Kit User's Guides.
3. Run CE	
Perform Data Analysis	

1. **Prepare Sample/Extract DNA** from the forensic sample. DNA extraction with the PrepFiler™ Forensic DNA Extraction Kits can be performed manually, or automated on the HID EVOlution™ – Extraction System.

   Note: Extraction procedures are not included in this guide. For automated extraction procedures, see the *PrepFiler™ Automated Forensic DNA Extraction Kit Getting Started Guide.*

2. **Perform quantitative PCR** to quantify the total amount of amplifiable DNA.

   a. Prepare for automated qPCR reaction setup:
      - Place extracted DNA samples in 96-well plates or 1.5-mL tubes.
      - (Optional) Create a qPCR/STR Sample file containing sample names and information for import to the HID EVOlution™ - qPCR/STR Setup System software.
      - Prepare Quantifiler® kit reagents for use on the HID EVOlution™ - qPCR/STR Setup System.
      - Prepare the HID EVOlution™ - qPCR/STR Setup System.

   b. Run automated qPCR reaction setup on the HID EVOlution™ - qPCR/STR Setup System. The HID EVOlution™ system:
      - (Optional) Imports sample information from a qPCR/STR Sample file.

        Note: You can prepare a qPCR/STR Sample file before the run, or, if you used the HID EVOlution™ – Extraction System for DNA extraction, the HID EVOlution™ – Extraction System generates a PCR/STR Sample file (*.csv output file) that you can import for the qPCR or STR PCR reaction setup run. See "Options for entering sample information" on page 17.

      - Prepares the qPCR master mix.
      - (Optional) Prepares the DNA standard dilution series from the control DNA standard.
      - Transfers the master mix, DNA standard dilution series, and samples into a qPCR reaction plate and mixes the reaction components.
      - Generates a 7500 Setup file that can be imported into the 7500 Real-Time PCR System SDS software v1.2.3 to set up the plate document for the qPCR run on the 7500 Real-time System.

      Note: The HID EVOlution™ - qPCR/STR Setup System can prepare up to 80 qPCR reactions and 16 DNA standard dilution series reactions per run. See "Before each run: Set up extracted DNA samples" on page 13.

   c. Run qPCR using the 7500 Real-Time PCR System with SDS Software v1.2.3.
      - Transfer the qPCR reaction plate to the 7500 instrument.
      - Import the 7500 Setup file to the SDS software.
      - Run qPCR.
      - Analyze and review the qPCR results.

3. **Perform STR amplification** to amplify specific STR loci in a single PCR amplification sample:

   a. Prepare for automated STR PCR amplification reaction setup:
      - Place extracted DNA samples in 96-well plates or 1.5-mL tubes.
      - Prepare AmpFℓSTR® kit reagents for use on the HID EVOlution™ - qPCR/STR Setup System.
      - Prepare the HID EVOlution™ - qPCR/STR Setup System.

   b. Run automated STR PCR reaction setup on the HID EVOlution™ - qPCR/STR Setup System.

      The HID EVOlution™ system:
      - Reads the sample information from either the imported qPCR/STR Sample input file (*.csv) or the manually-entered sample list.
      - Reads the 7500 Results file(s) that you import from the 7500 Real-Time System SDS Software v1.2.3.
      - Confirms that the sample ID names from the Sample input file or sample list match the 7500 Results (output) file(s).
      - Determines the dilution ratio for each sample, if required, based on the measured DNA concentration, the required DNA input volume, or the user-defined target DNA input amount for the STR PCR reactions.
      - Dilutes the extracted DNA samples as needed to normalize the sample concentrations.
      - Prepares the master mix.
      - Transfers the master mix and normalized DNA samples into a STR PCR reaction plate and mixes the reaction components.
      - Generates a CE Setup file that can be imported into to the CE instrument (3130/3130*xl* Genetic Analyzer) Data Collection Software v3.0 to automatically populate the plate record.

      Note: The HID EVOlution™ - qPCR/STR Setup System can prepare up to 88 STR PCR reactions (87 when using the AmpFℓSTR® Yfiler® PCR Amplification Kit) plus 2 or 3 controls per run. See "Before each run: Set up extracted DNA samples" on page 13.

   c. Transfer the STR PCR reaction plate to the 9600/9700 or Veriti® thermal cycler and perform PCR amplification.

4. **Perform capillary electrophoresis/genetic analysis** to separate and detect amplified STR products.
   - Prepare the CE plate.
   - Import the CE Setup file to the Data Collection Software v3.0.
   - Run CE on a 3130/3130*xl* Genetic Analyzer.

   Note: Any AB HID validated CE platform can be used, but the electronic CE Setup file was formatted and validated for Data Collection Software v3.0 for the 3130/3130*xl* Genetic Analyzers.

5. **Perform data analysis** to analyze the CE results with GeneMapper® *ID-X* Software or GeneMapper® *ID* Software v3.2.

D Dilution Protocols

This appendix covers:

DNA input amount must be normalized before STR PCR amplification

If the concentration (ng/µL) of an extracted DNA sample is higher than the target DNA input (ng) for the AmpFℓSTR® PCR Amplification kit, then the extracted DNA sample must be diluted to normalize the DNA input in the STR PCR reaction plate. The HID EVOlution™ system automates DNA input normalization using a one- or two-step dilution process. The workflow and examples of the dilution ratios used to achieve this are in the sections that follow.

Terms used in the Freedom EVOware® software

Required amount of DNA The target amount of DNA (ng) you want in each reaction in the STR PCR reaction plate.

Quantity The concentration of the extracted DNA (ng/µL) as shown in the SDS software. Also shown as "Qty" in the EVOware software Sample Normalization window.

Configured volume The volume of extracted DNA sample that is placed in the STR PCR reaction plate. The volume (10 or 20 µL) is pre-defined in the EVOware software based on the AmpFℓSTR® kit that you use. This volume is not user-editable.

Guidelines for successful normalization

- **Provide an adequate volume of extracted DNA** – While the maximum sample volume required for any kit is 20 µL, it is recommended that you check the samples for sufficient volume prior to dilution. A 50 µL total volume is ideal for reliable liquid detection, but you can use lower volumes.

  IMPORTANT! If there is insufficient sample for the required volume, the entire volume will be aspirated and diluted according to the dilution protocol, regardless of the deficient volume. This deficiency could result in low or no profiles in the downstream analysis.

- **Set up the system** – When you load plasticware for an STR PCR setup run, *always* load two pre-dilution plates on the worktable to ensure there are adequate wells for dilution.
- **Select samples for processing** – During the STR PCR setup run, review and adjust (if necessary) the information in the Sample Normalization Adjustment window (see step 5 on page 59). When selecting samples for processing, be aware of the limits to the HID EVOlution™ system sample normalization (see page 95).

Limits to the HID EVOlution™ system sample normalization

The HID EVOlution™ system software uses the target amount of DNA to select the required dilution protocol. It is not possible for the HID EVOlution™ system to dilute exceptionally high concentrations of DNA to the smallest target amount. Given a two-step dilution process and a maximum volume of 200 µL per dilution step, the maximum achievable dilution for a 1 ng target is 1:4000 for a 25-µL total reaction volume and 1:8000 for a 50-µL total reaction volume.

In the Sample Normalization Adjustment window, if you select a sample that requires more than a 1:4000 dilution (or 1:8000 dilution for a 50-µL reaction volume), the sample will only be diluted by 1:4000 or 1:8000, respectively. After dilution, HID EVOlution™ system will place the maximum transferable volume (either 10 or 20 µL) into the STR PCR reaction plate. Consequently, the HID EVOlution™ system may add too much DNA sample to the STR PCR plate. This could result in an off-scale DNA data profile.

Table 14 lists the maximum and minimum concentrations that can be diluted to the specified target amount for either a 10-µL or 20-µL transfer. Use Table 14 to determine the maximum concentration that can be diluted for the target DNA input amount (ng). For example, the HID EVOlution™ system cannot dilute a 1000 ng/µL sample to achieve a 0.1 ng target input amount within these limitations.

The default limits in the HID EVOlution™ system software are set to the maximum and minimum concentrations of the standard curve for the Quantifiler® kits (0.023 to 50 ng/µL). Samples with concentrations outside these limits will not be processed, unless you manually edit the concentration range or select the individual sample(s) for processing.

Table 14 Concentrations within the HID EVOlution™ system dilution range.

Target Amount (ng)	10 µL transfer into STR PCR plate		20 µL transfer into STR PCR plate	
	Min (ng/µL) transfer	Max (ng/µL) transfer	Min (ng/µL) transfer	Max (ng/µL) transfer
0.1	0.01	40	0.005	40
0.125	0.013	50	0.063	50
0.5	0.05	200	0.025	200
1.0	0.1	400	0.05	400
1.5	0.15	600	0.15	600
2.0	0.2	800	0.2	800
2.5	0.25	1000	0.125	1000
5.0	0.5	2000	0.25	2000
10.0	1.0	4000	0.5	4000

About DNA normalization on the HID EVOlution™ system

How the HID EVOlution™ system determines when to dilute an extracted DNA sample

1. For each extracted DNA sample, the system uses the following values to determine if the sample needs to be diluted before it is pipetted to the STR PCR reaction plate.
   - **CONC** – Concentration (ng/µL) of the extracted DNA sample (shown as QTY in the 7500 Results file)
   - **VOL** – Volume (µL) of extracted DNA sample added to a STR PCR reaction (either 10 µL or 20 µL, depending on the kit)
   - **AMOUNT** – Target amount of DNA (ng) that you want in the STR PCR reaction. AMOUNT is the "Required Amount of DNA" defined by the user in the Sample Normalization Adjustment window. The usual range is 0.1 to 2.5 ng; the default is 1 ng.

If CONC x VOL is...	Then...
≤ AMOUNT	No dilution is necessary; if you select this sample for processing, the HID EVOlution™ system transfers the maximum allowable volume of sample (either 10 or 20 µL) to the STR PCR plate.
> AMOUNT	The HID EVOlution™ system determines which dilution protocol to use to achieve an amount of DNA in the STR PCR reaction no more than 15% above or below the target amount that you specified. See "Dilution protocols with examples" on page 98.

2. Where necessary, the system calculates the required dilution ratio:
   DIL = CONC x VOL / AMOUNT, where DIL is the Dilution Ratio, for example a DIL of 1.548 represents a dilution ratio of 1:1.548.

3. Based on the required dilution ratio, the system selects one of the dilution protocols shown in Table 15 on page 100 (for kits with a 10-µL addition) or Table 16 on page 101 (for kits with a 20-µL addition).

How the HID EVOlution™ system dilutes a sample

The maximum dilution in a one-step dilution is 1:20 for kits with a 25-µL total reaction volume, and 1:40 for kits with a 50-µL total reaction volume. Any higher dilutions are performed as two-step dilutions.

The HID EVOlution™ system will dilute a sample with a greater concentration than the target quantity/maximum transferable volume at least once.

1. The system performs the first dilution (D1).

   Note: The maximum dilution ratio for D1 is 1:20 for a 25-µL total reaction volume and 1:40 for 50-µL total reaction volumes.

2. If the first dilution (D1) is sufficient to reach the target amount of DNA, the HID EVOlution™ system transfers the maximum allowable volume of sample (either 10 or 20 µL) from D1 to the STR PCR reaction plate.

3. If the first dilution (D1) is still too concentrated to reach the target amount of DNA, then the HID EVOlution™ system performs a second dilution (D2).

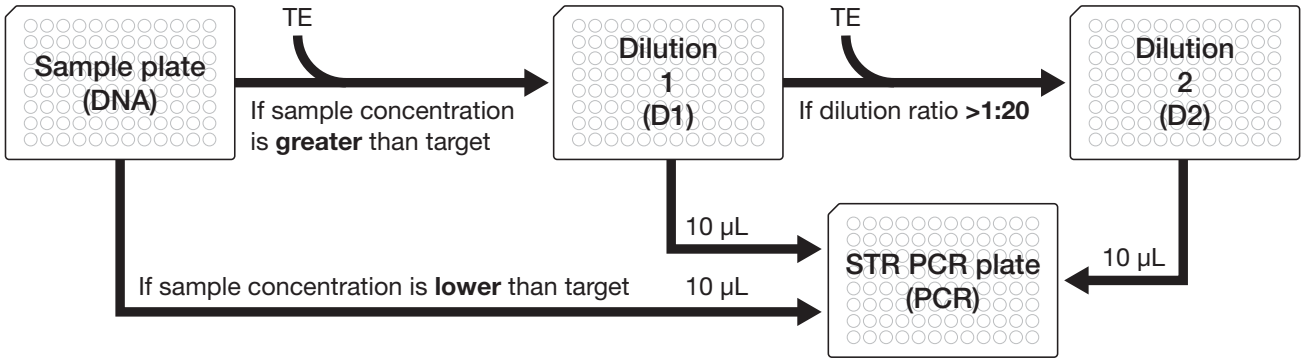
   Note: The maximum dilution ratio for D2 is 1:20 for a 25-µL total reaction volume and 1:40 for 50-µL total reaction volumes.

4. The HID EVOlution™ system transfers the maximum allowable volume of sample (either 10 or 20 µL) from D2 to the STR PCR plate.

In a two-step dilution, the final dilution ratio equals the first dilution times the second dilutions. For example, two dilutions each of 1:20 will result in a 1:400 final dilution ratio.

The workflows in Figure 21 are for demonstration purposes only.

Workflow for normalization using a 10-µL addition



Workflow for normalization using a 20-µL addition

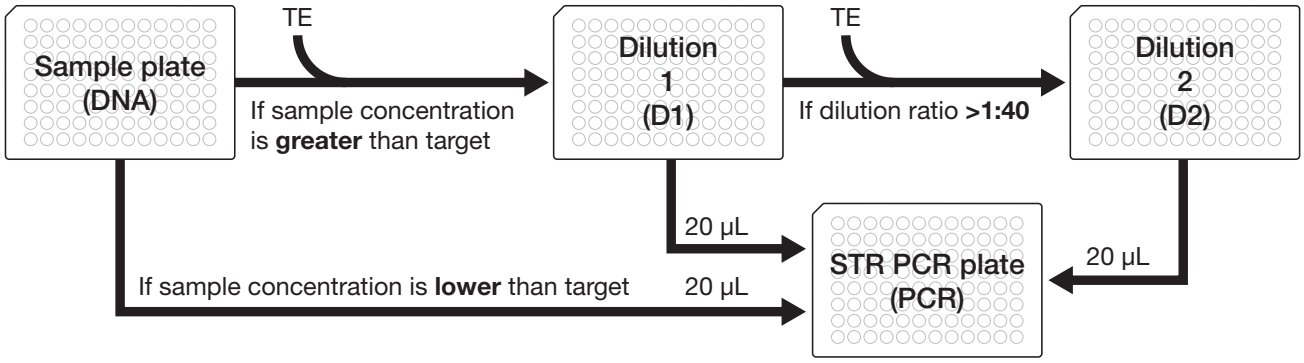


Figure 21 Normalization workflows (10 µL and 20 µL). Samples with DNA concentrations lower than the target will not be processed unless you manually select these samples for processing in the Sample Normalization Adjustment window.

Order of processing and placement of D1 and D2 in the pre-dilution plates

- The HID EVOlution™ - qPCR/STR Setup System processes all of the samples that require a one-step dilution first, then processes all of the samples that require a two-step dilution.
- For two-step dilutions, the first (D1) and second (D2) dilutions occur in the same predilution plate in adjacent wells (for example: wells 41 and 49 or wells A6 and A7).
- The two predilution plates loaded onto the worktable *do not* correspond to the number of dilutions a sample requires; rather, the two plates provide enough wells in the event that all samples require a two-step dilution.
- The D2 dilutions do not necessarily occur in a second plate.They can occur in the first plate in wells that are adjacent to the D1 dilutions.
- Additionally, when the samples that require a two-step dilution are being normalized, the HID EVOlution™ system processes them starting in a new column of the plate.This could leave some wells empty following the normalization of one-step diluted samples.

Dilution protocols with examples

About the HID EVOlution™ - qPCR/STR Setup System dilution protocols

In order to cover a dynamic range for dilutions from 1:1 to 1:4000 (or 1:8000 for the kits that use 20 µL of sample), different volumes are used in the dilution steps. The dilution protocols used by the HID EVOlution - qPCR/STR Setup System were developed based on the following considerations: Minimized DNA extract consumption, pipetting accuracy, and mechanical characteristics of the pipetting system. The dilution volumes are based on the physical properties of the system (increments of volumes in 83.3-nL steps) while minimizing sample consumption and maximizing pipetting accuracy. The maximal deviation from calculated-to-executed dilution is less than +/- 15% at the end of each dilution range.

How to determine the volume of extracted DNA sample used in each reaction

The amount of the original (extracted DNA) sample used in a 1- or 2-step dilution is not included in the STR Samples Report provided at the end of the run. To determine the amount:

1. Determine the dilution ratio used for the sample. The final dilution ratio for each sample is shown in the Sample Normalization Adjustment window. The STR Samples report also shows the dilution ratio(s). If you are using the information from the Samples report to determine the dilution ratio for a 2-step dilution, you will need to multiply the ratio for each of the steps together to determine the final dilution ratio.

2. Locate the dilution ratio in Table 15 on page 100 (for a 10-µL addition) or in Table 16 on page 101 (20-µL addition). The volume of extracted DNA sample used is shown in the same row in the next column. This is true for any target DNA input amount, but note that the first 2 columns of these tables apply only for 1-ng target DNA input amounts.

Dilution protocol examples

Example of no dilution/direct transfer – If you select a sample for processing that has a T Amt (target amount) less than the Global Req. Amount (as defined in the Sample Normalization Adjustment window), the HID EVOlution™ system transfers the maximum allowable volume of the sample (either 10 or 20 µL) to the STR PCR plate. For example, if the Global Req. Amount is 1 ng, and you select a 0.025 ng/µL sample prepared with the Identifiler® kit for processing, the HID EVOlution™ system transfers 10 µL of the sample (containing 0.25 ng of DNA) to the STR PCR plate.

Example 1-step dilution – Using Table 15 on page 100, if you have a sample concentration of 1.2 ng/µL and you are targeting a total input amount of 1 ng, the sample will need to be diluted 12 times. The closest dilution protocol to this is 1:13.3, and so the sample will be diluted 1:13.3 times resulting in a final concentration of 0.902 ng/µL. To perform this dilution, the HID EVOlution™ system transfers 3 µL of sample from the original DNA sample plate and adds it to 37 µL of TE in the predilution plate (D1). The system will then thoroughly mix the TE and sample DNA before transferring 10 µL from D1 to the STR PCR plate (D1 to PCR). A single dilution step is sufficient for up to a 20-times dilution for a 10-µL addition (Table 15 on page 100) or up to a 40-times dilution for a 20-µL addition (Table 16 on page 101).

Example 2-step dilution – A more concentrated sample needs stepwise dilutions to economize sample usage but still get a thoroughly mixed dilution. For example, if you have a sample containing 3.9 ng/µL, it will need to be diluted 36 times to be normalized for a 10-µL addition to the STR PCR plate. To perform this stepwise dilution, the HID EVOlution™ system starts by transferring 2 µL of the original DNA sample and mixes it with 38 µL of TE in the first dilution (D1). This first dilution is only a 1:20 dilution. To further dilute the sample, the HID EVOlution™ system aspirates and mixes 22 µL of the D1 dilution with 18 µL of TE in the second dilution (D2). The two dilution steps take place in adjacent wells of the same predilution plate. The second predilution plate is required when a large number of samples are processed and/or a large number of dilutions are required. Finally, the HID EVOlution™ system will aspirate and add 10 µL from D2 directly to the STR PCR Plate (D2 to PCR).

Dilution protocol tables

The maximum total dilution is 1:4000 for a 25-µL total reaction volume (or 1:8000 for a 50-µL total reaction volume). Below are two example dilution protocol tables that target 1 ng of DNA; Table 15 is for the 5-dye kits that require 10 µL of diluted DNA (25 µL total reaction volume) and Table 16 on page 101 is for the 4-dye kits that require 20 µL of diluted DNA (50 µL total reaction volume) in the STR PCR plate.

Note: The 10- and 20-µL dilution tables are example dilution protocols for a 1-ng target DNA input amount. The dilution protocols used by the HID EVOlution™ system software will change based on the modifiable parameters such as the target DNA input amount (see step 5 on page 59). See either the Sample Normalization Adjustment window or the STR Samples report for the specific dilution used for an individual sample.

Table 15 Dilution protocols for 10-µL transfer. Columns 1 and 2 apply only for a DNA target input amount of 1 ng.

If your sample concentration is between...		Then the dilution ratio is...	First Dilution Mixture (D1)		Second Dilution Mixture (D2)		Volume D1 or D2 added to STR PCR reaction plate (µL)
Min (ng/µL)	Max (ng/µL)		Volume of extracted DNA added to D1 (µL)	TE added to D1 (µL)	Volume from D1 added to D2 (µL)	TE added to D2 (µL)	
0.000	0.115	1:1	Sample is transferred directly from the extracted DNA plate or tube to the STR PCR plate				10
0.115	0.155	1:1.3	18.55	6.45	0	0	10
0.155	0.209	1:1.8	11	9	0	0	10
0.209	0.279	1:2.5	8.16	11.84	0	0	10
0.279	0.369	1:3.2	6.16	13.84	0	0	10
0.369	0.490	1:4.3	4.66	15.34	0	0	10
0.490	0.649	1:5.7	3.5	16.5	0	0	10
0.649	0.857	1:7.5	2.66	17.34	0	0	10
0.857	1.143	1:10	3	27	0	0	10
1.143	1.500	1:13.3	3	37	0	0	10
1.500	1.846	1:17.2	2.32	37.68	0	0	10
1.846	2.297	1:20	2	38	0	0	10
2.297	3.097	1:27	2	38	29.68	10.32	10
3.097	4.174	1:36	2	38	22.00	18.00	10
4.174	5.581	1:50	2	38	16.32	23.68	10
5.581	7.385	1:64	2	38	12.32	27.68	10
7.385	9.796	1:86	2	38	9.32	30.68	10
9.796	12.97	1:114	2	38	7.00	33.00	10
12.97	17.14	1:150	2	38	5.32	34.68	10
17.14	22.98	1:200	2	38	4.00	36.00	10
22.98	30.86	1:270	2	88	6.66	33.34	10
30.86	40.76	1:360	2	88	5.00	35.00	10
40.76	54.00	1:468	2	88	3.83	36.17	10
54.00	72.00	1:630	2	88	2.85	37.15	10
72.00	93.91	1:837	2	88	2.15	37.85	10
93.91	120.00	1:1080	2	88	2.50	57.50	10
120.00	154.29	1:1337	2	88	2.02	57.98	10
154.29	205.71	1:1800	2	88	2.00	78.00	10
205.71	266.67	1:2480	1.5	148.5	2.42	57.58	10
266.67	342.86	1:2990	1.5	148.5	2.01	57.99	10
342.86	400.00	1:4000	1.5	148.5	2.00	78	10

Table 16 Dilution protocols for 20-µL transfer. Columns 1 and 2 apply only for a DNA target input amount of 1 ng.

If your sample concentration is between:		Then the dilution ratio is:	First Dilution Mixture (D1)		Second Dilution Mixture (D2)		Volume D1 or D2 added to STR PCR reaction plate (µL)
Min (ng/µL)	Max (ng/µL)		Volume of extracted DNA added to D1 (µL)	TE added to D1 (µL)	Volume from D1 added to D2 (µL)	TE added to D2 (µL)	
0.000	0.115	1:1	Sample is transferred directly from the extracted DNA plate or tube to the STR PCR plate				20
0.115	0.155	1:1.3	19.77	6.89	0	0	20
0.155	0.209	1:1.8	18.34	15	0	0	20
0.209	0.281	1:2.4	16.34	23.66	0	0	20
0.281	0.378	1:3.3	12.16	27.84	0	0	20
0.378	0.511	1:4.4	9	31	0	0	20
0.511	0.686	1:6	6.66	33.34	0	0	20
0.686	0.906	1:8	5	35	0	0	20
0.906	1.171	1:10.4	3.84	36.16	0	0	20
1.171	1.500	1:13.3	3	37	0	0	20
1.500	1.920	1:17.1	2.34	37.66	0	0	20
1.920	2.400	1:21.7	2.76	57.24	0	0	20
2.400	3.000	1:26.7	2.25	57.75	0	0	20
3.000	3.692	1:34.5	2.32	77.68	0	0	20
3.692	4.593	1:40	2	78	0	0	20
4.593	6.194	1:52	2	78	10.34	29.66	20
6.194	8.348	1:72.6	2	78	18.00	22.00	20
8.348	11.23	1:96	2	78	23.66	16.34	20
11.23	15.12	1:132	2	78	27.84	12.16	20
15.12	20.43	1:176	2	78	31.00	9.00	20
20.43	27.43	1:240	2	78	33.34	6.66	20
27.43	36.23	1:320	2	78	35.00	5.00	20
36.23	48.00	1:416	2	78	36.16	3.84	20
48.00	64.00	1:564	2	78	37.16	2.84	20
64.00	83.48	1:740	2	78	37.84	2.16	20
83.48	109.71	1:964	2	78	57.51	2.49	20
109.71	142.22	1:1280	2	158	37.50	2.50	20
142.22	182.86	1:1600	2	158	38.00	2.00	20
182.86	240.00	1:2136	2	158	57.75	2.25	20
240.00	295.38	1:2760	2	158	77.68	2.32	20
295.38	355.56	1:3200	2	158	78	2	20
355.56	457.14	1:4000	2	98	158	2	20

Table 16 Dilution protocols for 20-µL transfer. Columns 1 and 2 apply only for a DNA target input amount of 1 ng. ***(continued)***

If your sample concentration is between:		Then the dilution ratio is:	First Dilution Mixture (D1)		Second Dilution Mixture (D2)		Volume D1 or D2 added to STR PCR reaction plate (µL)
Min (ng/µL)	Max (ng/µL)		Volume of extracted DNA added to D1 (µL)	TE added to D1 (µL)	Volume from D1 added to D2 (µL)	TE added to D2 (µL)	
457.14	600.00	1:5360	1.5	148.5	117.76	2.24	20
600.00	738.46	1:6900	1.5	148.5	118.26	1.74	20
738.46	800.00	1:8000	1.5	148.5	118.5	1.50	20

E Validation Experiments and Results

This appendix covers:

Overview

This appendix describes the experiments performed by the Applied Biosystems Human Identification (HID) team to validate automated qPCR/STR reaction setup on the Tecan HID EVOlution™ - qPCR/STR Setup System using Applied Biosystems chemistries, instruments, and software.

The experimental design followed the validation guidelines published by the Scientific Working Group on DNA Analysis Methods (SWGDAM). Validation experiments were carried out to assess the functionality of the HID EVOlution™ scripts, and to assess the overall assay precision, accuracy, reproducibility, and potential for cross-contamination in qPCR/STR setup experiments automated by the instrument. The experimental results were compared to data generated from manually prepared samples.

IMPORTANT! Laboratories should conduct their own validation studies with their own specific workflow and instruments to set their own standards for internal use.

Importance of validation

The HID EVOlution™ system was tested using the HID EVOlution™ Software v1.0; the Freedom EVOware® Software v1.4; and the Tecan Freedom EVO 150 Liquid Handling Workstation with a 4-channel LiHa, a PosID-3 barcode reader, and carriers and reagent blocks that were arranged according to the specific kit used and shown in this guide and in the *HID EVOlution™ – qPCR/STR Setup System Application Manual*. Freedom EVOware® v2.1 was validated on the HID EVOlution™ – Combination System with additional verification studies performed on the HID EVOlution™ - qPCR/STR Setup System.

The Applied Biosystems kits that were validated include the Quantifiler® Human DNA Quantification kit, Quantifiler® Y Human Male DNA Quantification kit, AmpFℓSTR® Identifiler® PCR Amplification kit, AmpFℓSTR® MiniFiler™ PCR Amplification kit, AmpFℓSTR® Yfiler® PCR Amplification kit, AmpFℓSTR® SGM Plus® PCR Amplification kit, AmpFℓSTR® Profiler Plus® Amplification kit, AmpFℓSTR® COfiler® PCR Amplification kit, and AmpFℓSTR® SEfiler™ PCR Amplification kit. The SEfiler Plus™ kit was validated separately; the results are not presented in this chapter, but are in line with the other kit validation results presented here. For kit part numbers, see "Supported kits" on page 5.

The experimental data that were generated demonstrated that the HID EVOlution™ system v1.0 was functional and consistent in its ability to:

- Produce accurate and reproducible results when used with the Quantifiler® Human and Quantifiler® Y Human Male kits
- Import 7500 Results files generated with SDS software v1.2.3
- Normalize samples based on imported real-time PCR results
- Provide accurate and reliable STR results when used with all supported Applied Biosystems 4- and 5- dye STR amplification kits
- Generate Samples Reports and importable 7500 Setup and CE Setup files appropriate to each kit, instrument, and software configuration

Materials and methods

Samples

Genomic DNA samples obtained from Biochain (Hayward, CA), Sigma Chemical Company (St. Louis, MO) and Serological Research Institute (Richmond, CA) were used in all studies. DNA standard dilution series were made using the Quantifiler® Human DNA Standard provided in the kit being tested.

Reagents

A single lot of each kit was used during the validation studies. TE buffer containing 0.1% EDTA was purchased from Teknova (Hollister, CA). Applied Biosystems POP-4™ (PN 4316355), GeneScan™ 500 LIZ® Size Standard (PN4322682), GeneScan™ 500 ROX™ Size Standard (PN 403039, for 50-µL reactions), Hi-Di™ Formamide (PN 4311320), and Running Buffer 1✕ (prepared from 310 Running Buffer, 10✕ (PN 402824)) were used for the CE run.

Instrumentation, consumables, and software

The Tecan Freedom EVO® 150 Liquid Handling Workstation, which is equipped with a 4-channel liquid handling arm, was used to set up the qPCR reactions, normalize DNA sample concentration, and to set up STR PCR reactions for STR analysis using the HID EVOlution™ Software v1.0, the Freedom EVOware® Software v1.4 SP1, the Tecan HID EVOlution™ driver v1.0.0.12, and the Tecan Sample Oriented EVOware Limited Edition SP1 (See "Tecan documentation" on page 149 for a list of Tecan manuals detailing required parts and configuration). The 100-mL troughs were purchased from Tecan (Durham, NC) and the 1.5-mL tubes were purchased from Ambion (Austin, TX). Prior to starting the validation, the Freedom EVO® 150 Liquid Handling workstation was installed and calibrated by Tecan. The recommended maintenance schedule (see the *HID EVOlution™ qPCR/STR Setup System Application Manual*) was followed.

- **Quantification:** The Tecan Freedom EVO® 150 Liquid Handling Workstation was used to set up the real-time PCR reaction plates according to the protocol outlined in the *Quantifiler Kits User Guide*. For PCR amplification, up to three 7500 instruments were used, all running the SDS Software v.1.2.3.

  A 7500 Results file was generated after the analysis using the 7500 SDS Real-Time Software export tool, then imported into the HID EVOlution™ Software for use during STR PCR amplification setup.

- **Amplification:** The Tecan Freedom EVO® 150 Liquid Handling Workstation was used to set up the STR PCR reaction plates according to the kit-specific protocols described in the individual AmpFℓSTR® PCR Amplification kit user guides. Positive amplification controls were manually diluted prior to amplification setup and the aliquot placed in the appropriate well of the reagent block.

  After importing the 7500 Results file(s) into the HID EVOlution™ Software, extracted DNA samples with concentrations < 0.1 ng/µL and negative controls were selected for direct transfer of the maximum allowable volume (10 or 20 µL) into the STR PCR reaction plate. Extracted DNA samples with concentrations between 0.1 ng/µL and 50 ng/µL were diluted according to the software parameters. See Appendix D, "Dilution Protocols" on page 93 for additional details about dilution protocols.

  PCR amplification was carried out in calibrated 9700 thermal cyclers.

- **Capillary electrophoresis:** Capillary electrophoresis plates were prepared manually. Master mixes of Hi-Di™ Formamide and GeneScan™ 500 LIZ® Size Standard (GeneScan™ 500 ROX™ Size Standard for 50-µL reactions), PCR product, and kit-specific allelic ladder were prepared and added manually to the samples, according to the procedure described in each kit-specific user guide.

  The CE Setup file generated by the HID EVOlution™ Software during the STR PCR setup run was imported to the Data Collection Software v3.0 on a 3130*xl* Genetic Analyzer to create a plate record.

  Capillary Electrophoresis was performed on an ABI PRISM 3130*xl* Genetic Analyzer with Data Collection Software v3.0 using recommended protocols.

Data analysis

For analysis of the Quantifiler® kit results, the 7500 SDS Real-Time Software v.1.2.3 was used. All runs were analyzed using the *Manual* analysis mode, with the baseline set from **3 to 15** and the threshold set at **0.2**.

The R^2 and slope values of the standard curve were calculated by the software and compared with previously observed ranges from the Quantifiler® kit validations. A slope value of −3.3 represents 100% efficiency of PCR. A slope range of −3.0 to −3.6 is typically observed for the different AB TaqMan® assays. Average tolerances for acceptable efficiency are from 90% to 110% with a variation ≤ 2%.

After capillary electrophoresis, samples were analyzed with the 3130*xl* Genetic Analyzer using the Data Collection Software v. 3.0. All samples were genotyped using the GeneMapper® *ID* Software v.3.2 using standard analysis methods (see the *GeneMapper ID Software v 3.1 User Guide,* PN 4338775).

For statistical analysis, box plot charts were created with the MiniTab Statistical Software, version 15, which graphically summarized the distribution of the average replicate peak height for each sample concentration. For other analysis, Microsoft® Excel® was used to calculate averages and standard deviations, and to produce bar charts.

Precision and reproducibility studies: DNA standard dilution series preparation and reaction setup

Experiment

To evaluate the ability of the HID EVOlution™ system to precisely prepare and transfer a DNA standard dilution series, 40 DNA standard dilution series were prepared on the system. The plates were prepared using Quantifiler® Human and Quantifiler® Y Human Male DNA Quantification kits. The DNA standard dilution series consisted of eight concentrations: 50 ng/µL, 16.7 ng/µL, 5.56 ng/µL, 1.85 ng/µL, 0.62 ng/µL, 0.21 ng/µL, 0.068 ng/µL, and 0.023 ng/µL. Two replicates of each concentration from each of the 40 DNA standard dilution series were transferred by the system to a plate, creating a total of eight plates. Each plate also contained a manually-prepared DNA standard dilution series.

The eight plates were then run on a single 7500 Real-Time PCR System. For qPCR, the standard thermal cycling protocol described in the *Quantifiler® Kits User's Manual*, Chapter 3 (PN 4344790) was used for all 7500 instrument runs.

To evaluate the reproducibility and precision of the automated DNA standard dilution series setup across multiple 7500 instruments, an additional 35 DNA standard dilution series were prepared by the HID EVOlution™ system and run on three different 7500 instruments, for a total of 75 DNA standard dilution series tested. After qPCR, the R^2 and slope values calculated by the 7500 System SDS Software v1.2.3 were compared to the original Quantifiler® kit validation results for each kit type, as detailed in the *Quantifiler® Kits User's Manual*, Chapter 6.4 (PN 4344790).

Results

Table 17 shows the observed slope and R^2 ranges obtained from both the automated and the manual qPCR reaction setups. A slope value of –3.3 represents 100% efficiency of PCR and a slope range of –3.0 to –3.6 is typically accepted to account for stochastic variation in samples. The data indicate that the efficiency for Quantifiler® Y Human Male ranged from 97.4% to 104.5% with a variance from the standard curve ≤ 1.6%. The efficiency of the Quantifiler® Human samples ranged from 92.6% to 112.6% with a variance from the standard curve of ≤ 1.3%.

Table 17 Slope and R^2 values for Quantifiler Human and Y Human Male kits (ND = not determined).

		HID EVOlution™ system prepared standards: range observed for one 7500 instrument (n = 40)	HID EVOlution™ system prepared standards: range observed for three different 7500 instruments (n = 75)	Observed range published in the Quantifiler® Kits User's Manual
Quantifiler® Human kit	**Slope**	-3.052 to -3.370	-3.052 to -3.512	-2.9 to -3.3
	R^2	0.991 to 0.999	0.987 to 0.999	> 98%
Quantifiler® Y Human Male kit	**Slope**	-3.217 to -3.386	ND	3.0 to -3.6
	R^2	0.984 to 0.991	ND	> 98%

Precision and reproducibility studies: qPCR reaction setup scripts

Quantifiler® Human reaction setup precision and reproducibility study

Experiment

qPCR reaction setup precision and reproducibility studies were performed with a single female genomic DNA sample. The genomic DNA was quantified using the Quantifiler® Human DNA Quantification kit and based on the results, the original female genomic DNA stock was manually diluted to 0.025, 0.05, 0.1, 1.0, 5.0, and 25.0 ng/µL of DNA. Each concentration was then transferred either into microcentrifuge tubes or into a 96-well plate that served as a source DNA vessel.

The Quantifiler® kit reagents, twelve replicates of each sample DNA concentration, and the DNA standard dilution series were dispensed by the HID EVOlution™ system into a qPCR reaction plate. qPCR was run on a single 7500 Real-Time PCR System. Each study (plates or tubes) was repeated three times.

Automated setup of Quantifiler® Human kit reactions from a plate

Samples containing 0.025, 0.05, 0.1, 1.0, 5.0, and 25.0 ng/µL of DNA in a 96-well source plate were quantified for 12 replicates in three runs. In Figure 22, the red boxes represent plate study 1 (P1), the green boxes represent plate study 2 (P2), and the blue boxes represent plate study 3 (P3).

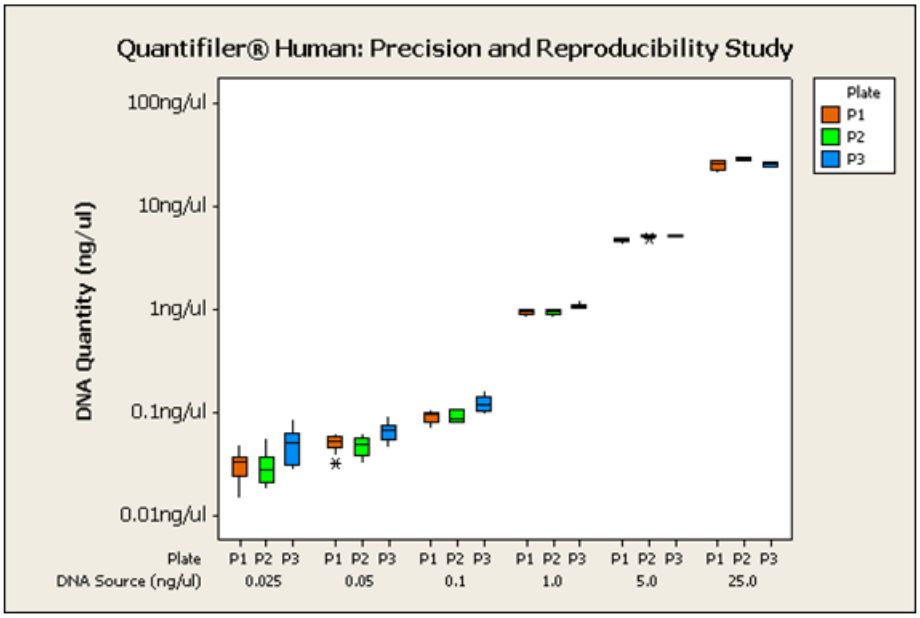


Figure 22 Precision and reproducibility study for the automated reaction setup of Quantifiler® Human kit reactions using extracted DNA from a 96-well plate.

Automated setup of Quantifiler® Human kit reactions from tubes

Samples containing 0.025, 0.05, 0.1, 1.0, 5.0, and 25.0 ng/µL of DNA in 1.5-mL microcentrifuge tubes were quantified for 12 replicates in three runs. In Figure 23, the red boxes represent tube study 1 (Tube1), the green boxes represent tube study 2 (Tube2), and the blue boxes represent tube study 3 (Tube3).

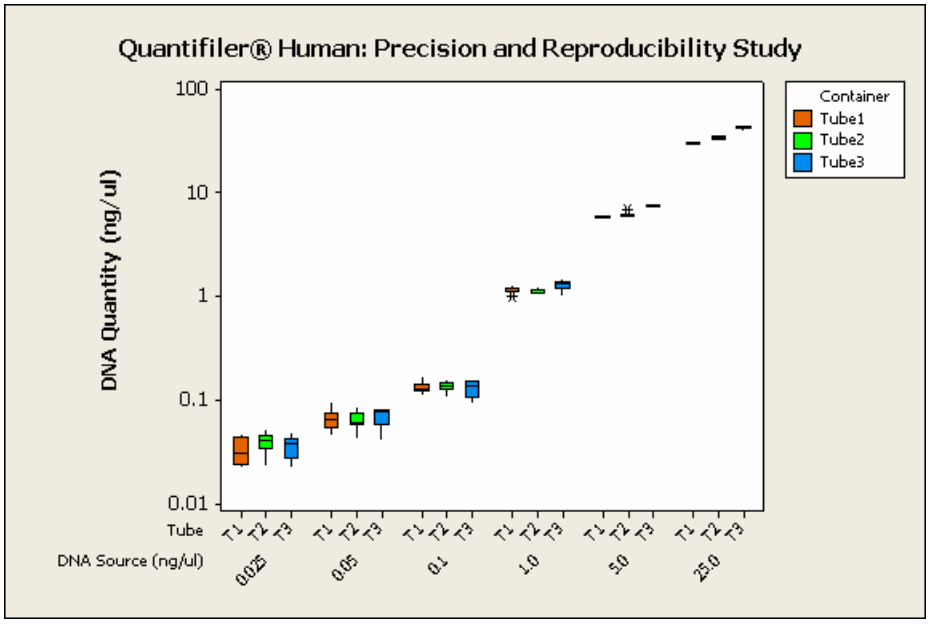


Figure 23 Precision and reproducibility study for the automated setup of Quantifiler® Human kit reactions using extracted DNA from microcentrifuge tubes.

Quantifiler® Human kit results

Figure 24 below and Table 18 on page 110 show the average DNA concentration across a range of DNA concentrations and compare the results to those for samples set up manually.

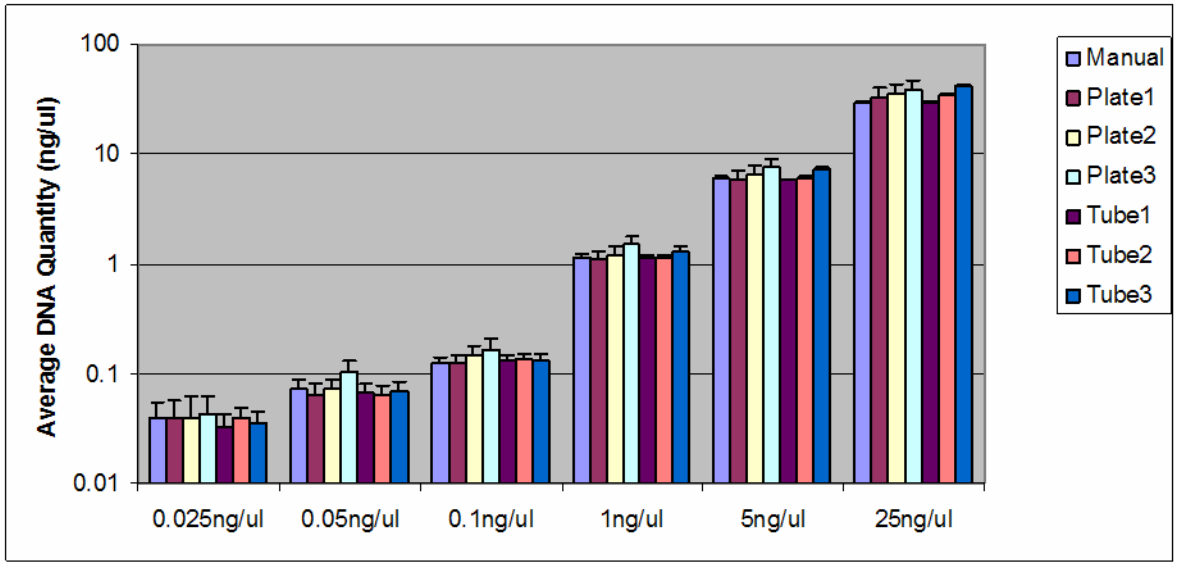


Figure 24 Quantification results comparing Quantifiler® Human kit reactions set up manually and with the HID EVOlution™ system (n =12).

Table 18 Average quantity and standard deviation for quantification of human DNA using the Quantifiler® Human DNA Quantification kit.

Sample	No. of Replicates	Tube 1	Tube 2	Tube 3	Plate 1	Plate 2	Plate 3	Manual
Average quantity (ng/µL)								
25	12	29.458	33.984	30.809	25.043	28.388	25.512	29.654
5	12	5.743	6.116	5.435	4.646	5.037	5.052	5.930
1	12	1.140	1.129	1.107	0.927	0.932	1.072	1.161
0.1	12	0.132	0.136	0.114	0.090	0.091	0.120	0.127
0.05	12	0.067	0.065	0.070	0.050	0.047	0.066	0.073
0.025	12	0.033	0.040	0.032	0.031	0.030	0.050	0.039
Standard deviation								
25	12	0.793	1.342	0.701	2.538	1.336	1.238	1.488
5	12	0.177	0.232	0.198	0.201	0.132	0.146	0.330
1	12	0.075	0.057	0.037	0.050	0.054	0.062	0.063
0.1	12	0.016	0.015	0.022	0.011	0.012	0.021	0.014
0.05	12	0.015	0.013	0.010	0.009	0.010	0.011	0.018
0.025	12	0.010	0.009	0.006	0.010	0.011	0.018	0.016

Quantifiler® Y Human Male kit precision study

Experiment

The experiment described in "Quantifiler® Human reaction setup precision and reproducibility study" on page 108 was repeated with male genomic DNA using the Quantifiler Y Human Male Kit.

Automated setup of Quantifiler® Y Human Male kit reactions from a plate and tubes

The precision of the automated Quantifiler Y Human Male reaction setup was also tested across a range of DNA concentrations using both plate and tube configurations as source vessels.

Quantifiler® Y Human Male kit results

Figure 25 on page 111 (top) shows a box and whisker plot of the average DNA concentrations for extracted DNA samples transferred from a 96-well plate. Figure 25 (bottom) shows the same for samples transferred from microcentrifuge tubes. Table 19 on page 113 shows average concentration and standard deviation results.

In determining the accuracy of automated Quantifiler® Y Human Male reaction setup using the HID EVOlution™ system, the greatest standard deviation in the samples at probable range ≥ 1 ng/µL varied ≤ 5% from the average for all three experiments, regardless of the source vessel.

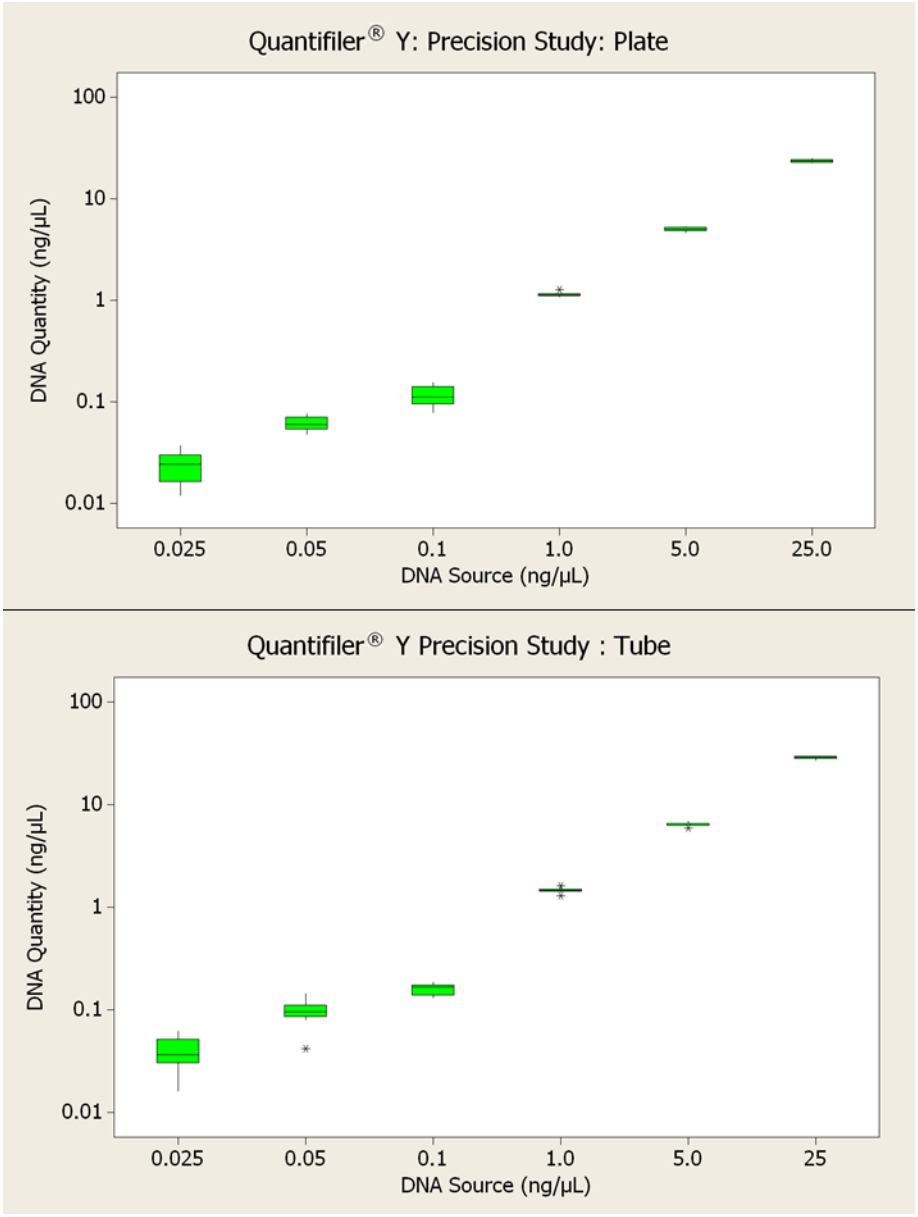


Figure 25 Precision study to verify Quantifiler® Y Human Male kit script; plate (top) and tube (bottom) (n=12).

Combined Quantifiler® Human and Quantifiler® Y Human Male reaction setup precision study

Experiment The HID EVOlution™ qPCR scripts (either Quantifiler_Human Y_plate.esc or Quantifiler_Human Y_tubes.esc), which run both the Quantifiler® Human and Y Human Male assays on the same plate, were tested for assay setup precision using different quantities of human DNA on the same 96-well optical plate. Samples containing about 0.025, 0.05, 0.1, 1.0, 5.0, and 25.0 ng/µL of DNA were quantified for 12 replicates in three runs.

Figure 26 shows a box and whisker plot of the average DNA quantity for each input amount and quantification kit. The red boxes represent samples run with Quantifiler® Human Quantification kit reagents, and the green boxes represent DNA samples run with the Quantifiler® Y Human Male Quantification kit reagents.

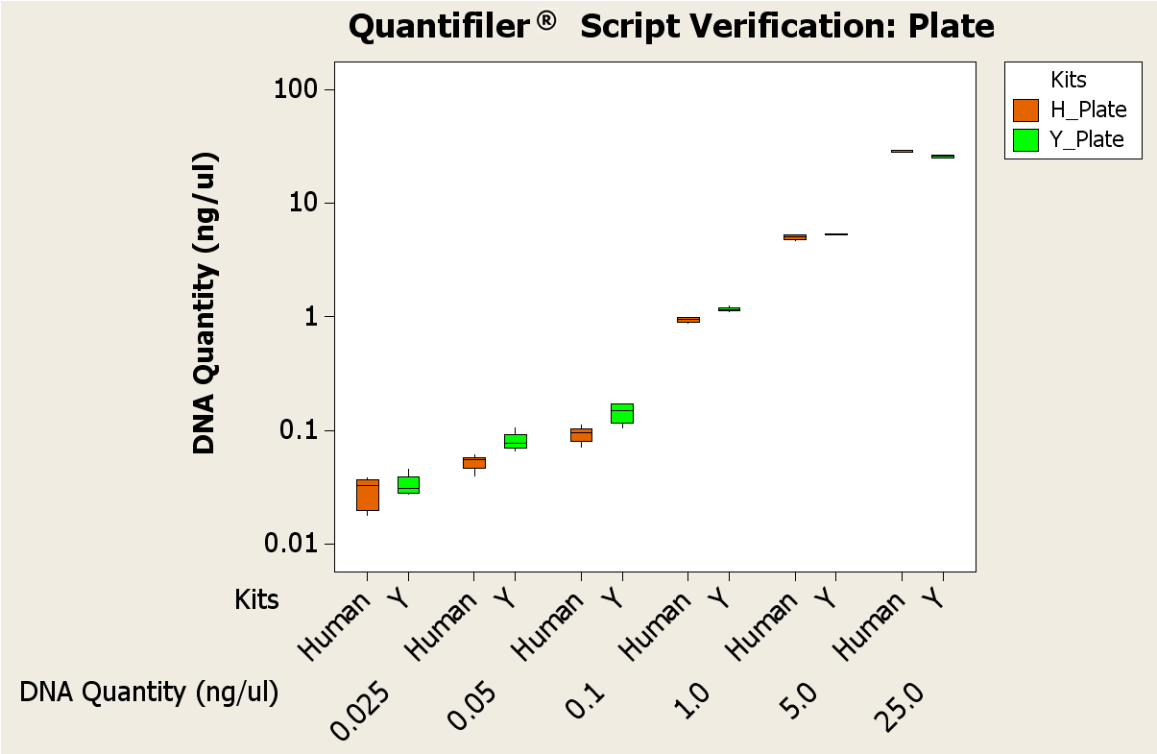


Figure 26 Precision study for automated Quantifiler® Human and Y Human Male kit reaction setup using extracted DNA from a 96-well plate.

Results In determining the accuracy of the Quantifiler® Human and Y Human Male Quantification assay and reaction setup using the combined qPCR setup script on the HID EVOlution™ system, the average concentrations and standard deviations were calculated (see Table 19).

Table 19 Average concentration and standard deviation for automated reaction setup using the Quantifiler® Human and Y Human Male DNA Quantification Kits and each of the following scripts: QuantifilerHuman_plate.esc, QuantifilerHuman_tubes.esc, QuantifilerY_plate.esc, QuantifilerY_tubes.esc, QuantifilerHumanY_plate.esc, and QuantifilerHumanY_tubes.esc.

	Human plate	Human tube	Y plate	Y tube	Combo plate Human	Combo plate Y	Combo tube Human	Combo tube Y
N =	**N = 36**	**N = 36**	**N = 12**	**N = 12**	**N = 5**	**N = 5**	**N = 5**	**N = 5**
Average concentration (ng/µL)								
0.025 ng/µL	0.037	0.035	0.024	0.038	0.029	0.033	0.025	0.028
0.125 ng/µL	0.054	0.067	0.062	0.097	0.056	0.080	0.057	0.074
0.1 ng/µL	0.100	0.127	0.115	0.161	0.092	0.144	0.113	0.167
1 ng/µL	0.977	1.125	1.143	1.467	0.933	1.154	1.156	1.554
5 ng/µL	4.911	5.765	5.003	6.433	4.990	5.314	6.116	7.038
25 ng/µL	26.314	31.417	23.637	28.493	28.608	25.590	36.960	35.120
Standard deviation								
0.025 ng/µL	0.02	0.01	0.009	0.014	0.008	0.007	0.010	0.018
0.125 ng/µL	0.01	0.01	0.009	0.024	0.003	0.013	0.005	0.025
0.1 ng/µL	0.02	0.02	0.025	0.019	0.013	0.025	0.015	0.027
1 ng/µL	0.09	0.06	0.050	0.080	0.040	0.042	0.042	0.141
5 ng/µL	0.25	0.34	0.198	0.242	0.217	0.068	0.269	0.208
25 ng/µL	2.31	2.15	0.751	0.800	0.521	0.621	1.113	1.423

Precision and reproducibility studies: STR PCR amplification reaction setup scripts

Identifiler® and SGM Plus® STR PCR amplification reaction setup precision and reproducibility studies

Experiment

Precision and reproducibility studies were conducted to evaluate the sample dilution and liquid transfer steps of the automated STR PCR amplification setup protocols. Each AmpFℓSTR® kit script was verified for functionality, precision, and reproducibility. This study was performed using 0.025, 0.1, 0.5, 1, 2, 5, 10, and 50 ng/µL DNA samples that were prepared on the HID EVOlution™ system and quantified using the Quantifiler® Human kit on the 7500 Real-Time PCR System. Eleven replicates of each DNA concentration were prepared, and placed in either a plate or in tubes. The HID EVOlution™ system was then used to normalize the DNA concentration and prepare the STR PCR amplification reactions. The experiment was repeated three times for extracted DNA in tubes and three additional times for extracted DNA in a 96-well plate. Kit-specific positive-control and negative-control reactions were prepared by the HID EVOlution™ system.

Peak height averages and standard deviations were calculated to evaluate the precision and reproducibility of the automated STR PCR setup. Box and whisker plots were generated from the Identifiler® data (Figure 27 on page 115) and the SGM Plus® data (Figure 28 on page 116) using peak height averages from all kit-specific loci amplified. Representative STR profiles and peak height average data from the remaining validated kits are detailed in "Other AmpFℓSTR® kits: Supplemental precision and reproducibility studies" on page 117.

Results

Figure 27 on page 115 is data from the Identifiler® STR PCR setup. The Identifiler® kit assay setup is a 25-µL PCR reaction volume made up of 10 µL of normalized DNA and 15 µL of reaction mix. The overall peak height average for all Identifiler® PCR assay setup experiments, regardless of the source vessel (plate or tubes), was 1239 RFU with a standard deviation of 509, (not including the 0.025 ng samples). For tubes only, the peak height average was 1332 RFU ± 523, while for plates only, the peak height average was 1144 RFU ± 474. Genotype concordance was 100%. All samples with input amounts of 1.0 ng of DNA (DNA starting concentrations of ≥ 0.1 ng/µL) resulted in a peak height ratio of ≥ 70%. The variance in peak height averages was < 16% between source vessels. In conclusion, sample dilution and normalization was successful, regardless of the source vessel and starting DNA concentration (for DNA concentrations ≥ 0.1 ng/µL).

The extracted DNA samples with a starting concentration of 0.025 ng/µL were not diluted; 10 µL of DNA was directly transferred to the PCR reaction plate. Samples with approximately 0.25 ng of DNA added to each PCR reaction displayed expected peak height averages that were smaller than the other samples due to the reduced mass of DNA. The average peak height for reactions prepared from 0.025 ng/µL DNA samples was 332.69 for samples in plates and 391.86 for samples in tubes. The combined peak

height average for 0.25 ng input DNA samples in plates and tubes was 362.27, which is 29% of the average peak height for reactions with approximately 1.00 ng of input DNA (DNA starting concentrations of ≥ 0.1 ng/µL). This result is consistent with the reduced amount of input DNA.

In conclusion, the direct transfer of extracted DNA samples during automated Identifiler® assay setup, using a concentration less than the desired target input amount, was successful regardless of whether the samples started in a plate or in a tube.

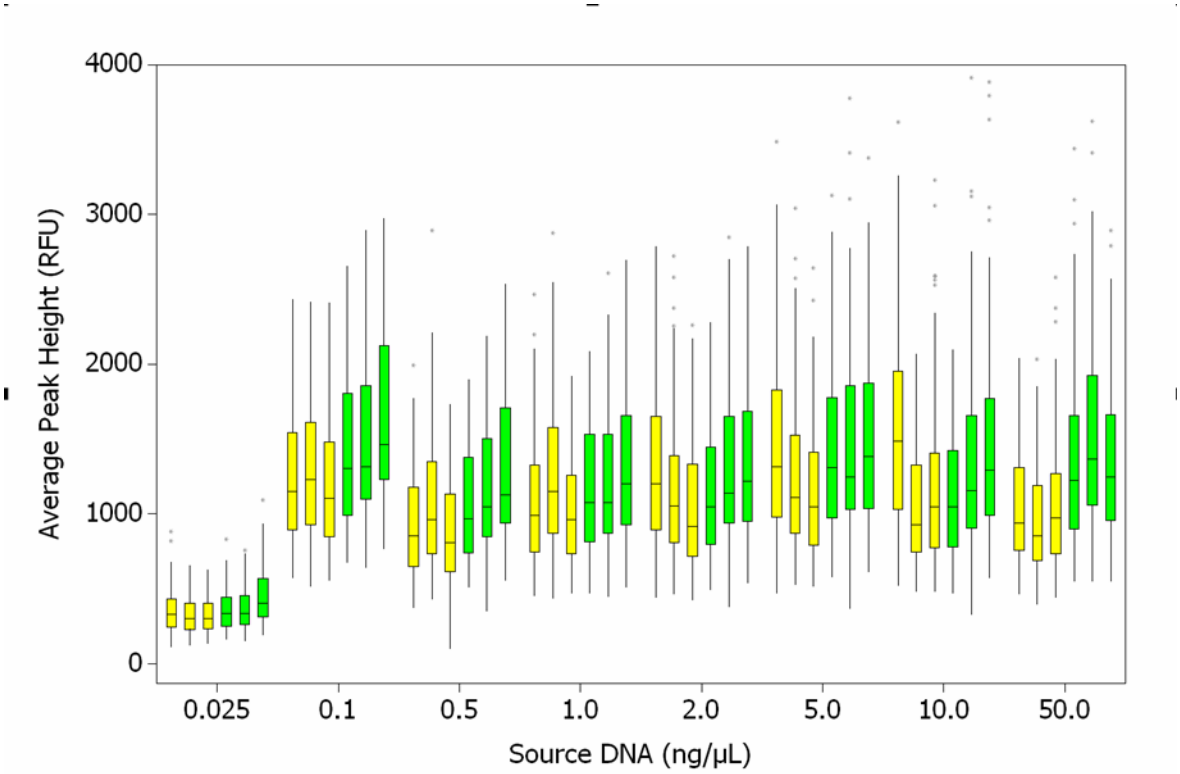


Figure 27 Identifiler® kit precision and reproducibility studies depicting the average peak height distribution of each study. Samples were present either in plates (green) or in tubes (yellow). Studies were carried out on separate days, each represented by a separate box plot in the appropriate color.

Figure 28 on page 116 is data from the SGM Plus® STR PCR setup. The SGM Plus® kit used a 50-µL PCR reaction volume, with a 20-µL volume for normalized DNA (diluted as necessary). The overall peak height average for all experiments using the SGM Plus® kit, regardless of the source vessel, was 938 RFU with a standard deviation of 354 (not including the 0.025 ng samples). For tubes, the peak height average was 958 RFU ± 344. For plates, the peak height average was 919 RFU ± 362. Genotype concordance was 100% and all samples with input amounts ≥ 0.1 ng/µL resulted in a peak height ratio of ≥ 70%.

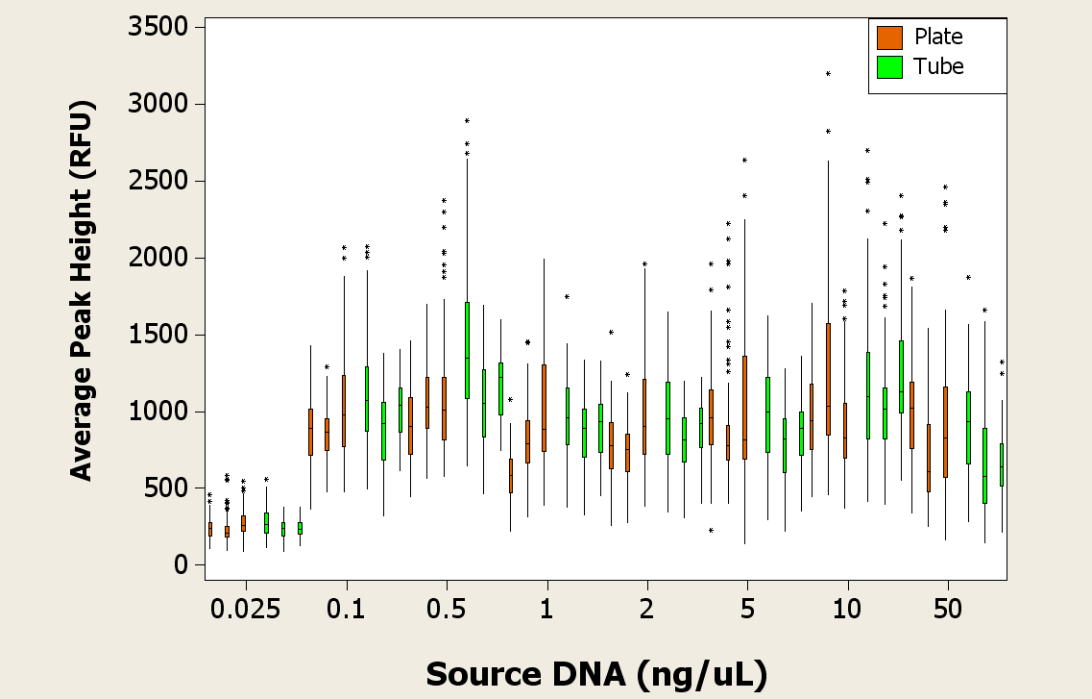


Figure 28 SGM Plus® kit reproducibility studies depicting the average peak height distribution of each study. Samples were either in a plate (brown) or in a tube (green). Studies were carried out on three separate days.

In conclusion, sample dilution and normalization, including the direct transfer for automated SGM Plus® kit samples, was successful regardless of the DNA starting concentration and source vessel.

Other AmpFℓSTR® kits: Supplemental precision and reproducibility studies

The remaining data from the precision and reproducibility study of the STR PCR amplification setup script validation are shown in the following figures (sample data from the Identifiler® and SGM Plus® kits were detailed in the preceding sections). Representative samples of STR profiles are also included.

- Precision studies for Profiler Plus® kit peak height averages
- Precision studies for COfiler® kit peak height averages
- Precision studies for SEfiler™ kit peak height averages
- Precision studies for Yfiler® kit peak height averages
- Precision studies for MiniFiler™ kit peak height averages
- SGM Plus® kit STR profiles
- Profiler Plus® kit STR profiles
- COfiler® kit STR profiles

Figure 29 below and Figures 30 and 31 on page 118 show average peak height across different starting concentrations. *Positive* refers to the positive control used in the experiment. Red bars represent sample data generated when processing DNA extracts from a 96-well plate (source vessel) and green bars represent sample data generated when processing DNA extracts from tubes (source vessels).

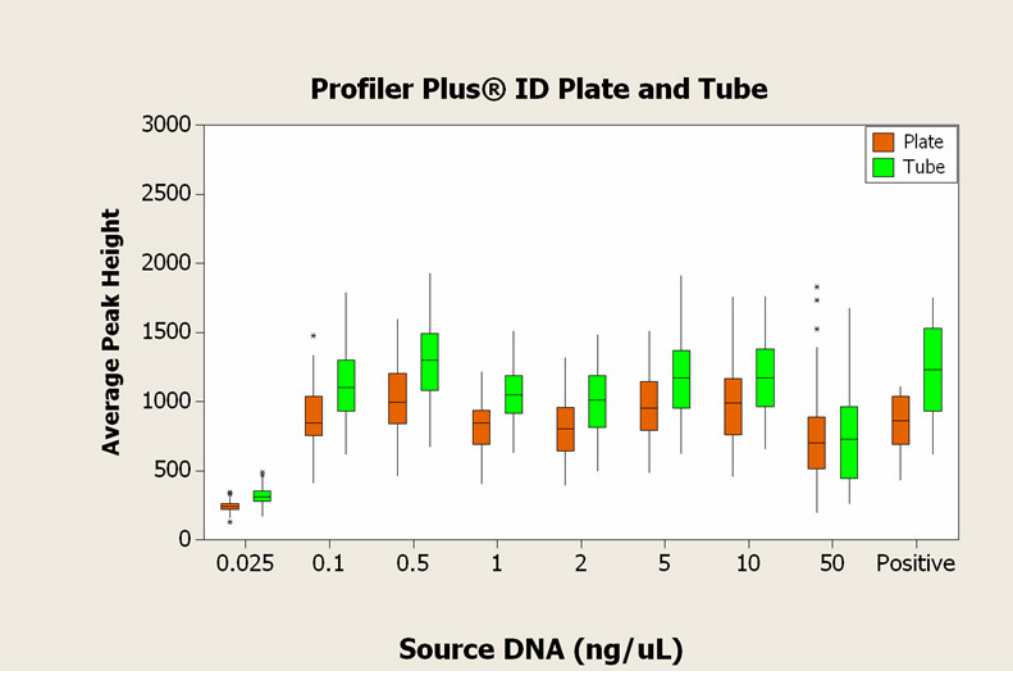


Figure 29 Precision studies for the Profiler Plus® kit.

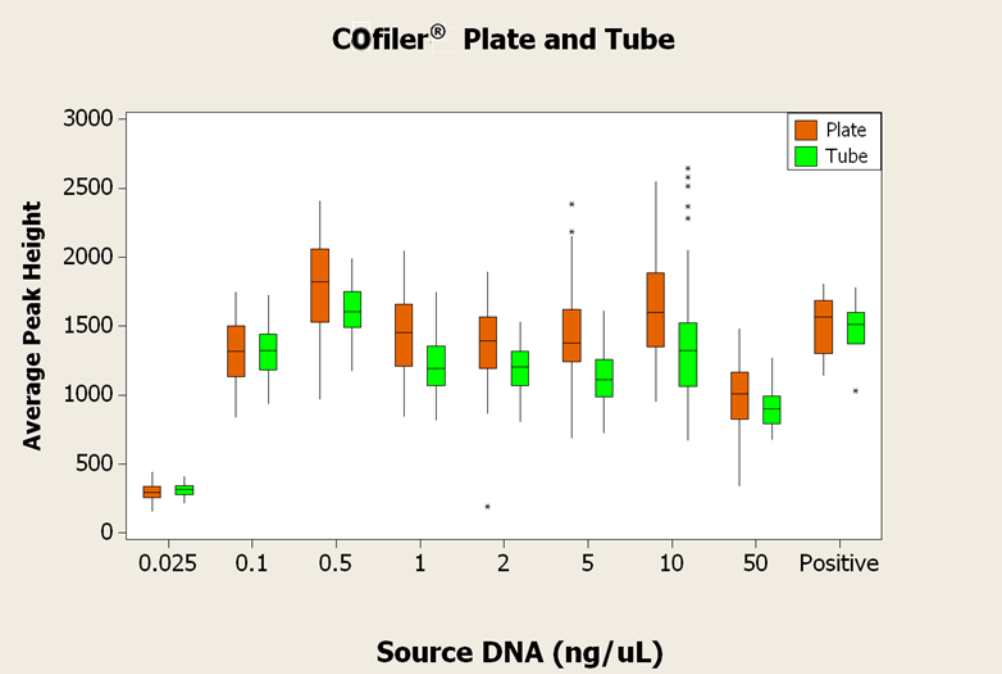


Figure 30 Precision studies for the COfiler® kit.

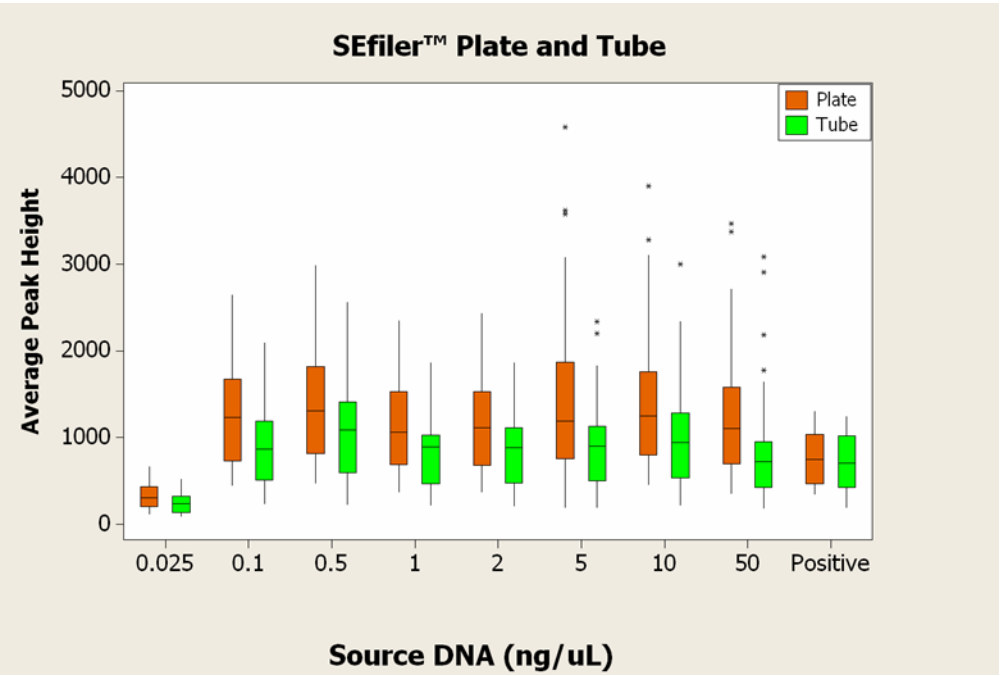


Figure 31 Precision studies for the SEfiler™ kit.

Figures 32 and Figure 33 on page 119 show peak height averages across different starting concentrations. *(Male) Control* refers to the positive control used in the experiment. Green bars represent sample data processed from a plate (-P) and yellow bars represent sample data processed from tubes (-T) (the source vessels).

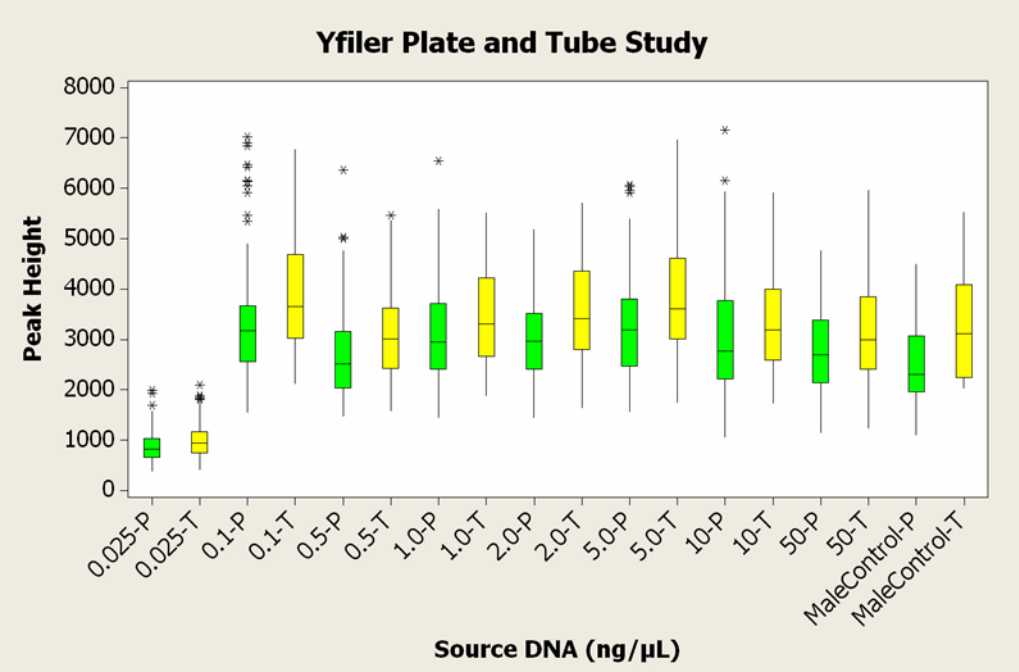


Figure 32 Precision studies for the Yfiler® kit.

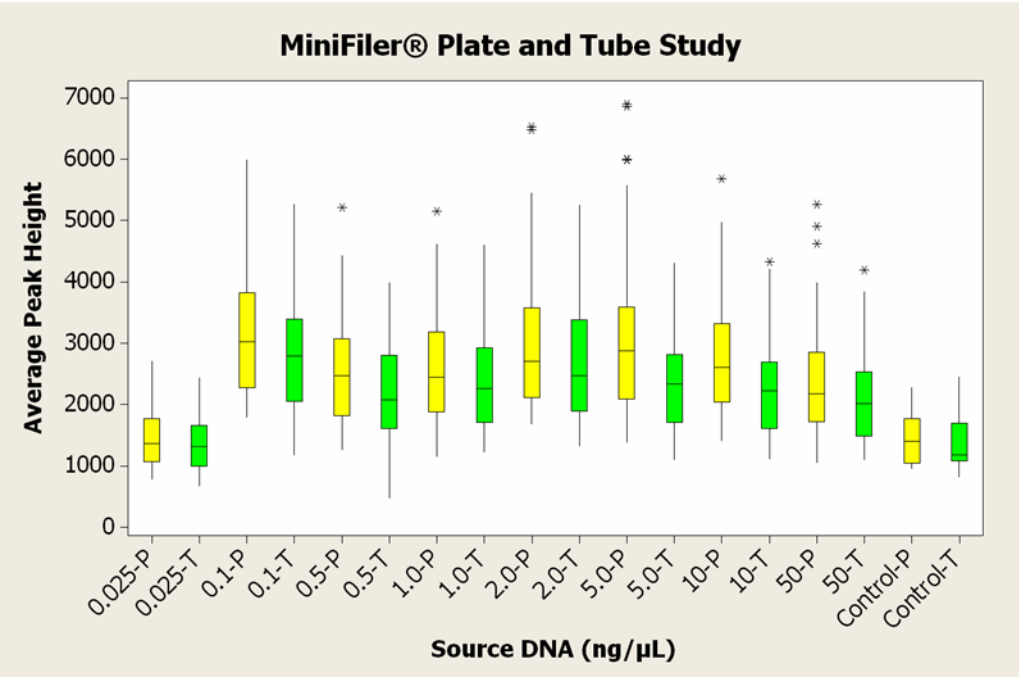


Figure 33 Precision Studies for the MiniFiler™ kit.

Figure 34 on page 120 shows representative STR profiles from a range of extracted DNA concentrations. 10 µL of the 0.025 ng/µL extracted DNA samples was transferred by the HID EVOlution™ system directly to the PCR reaction plate. For extracted DNA samples with concentration > 0.025 ng/µL, the concentration of each DNA sample was normalized to 0.1 ng/µL, and 10 µL of normalized sample was transferred by the HID

EVOlution™ system to the PCR reaction plate. The x-axis of plot indicates base pair size and the y-axis indicates the RFU. The source DNA concentration is as follows: Panel A = 50 ng/µL, Panel B = 10 ng/µL, Panel C= 5 ng/µL, Panel D = 2 ng/µL, Panel E = 1 ng/µL, Panel F = 0.5 ng/µL, Panel G = 0.1 ng/µL, and Panel H = 0.025 ng/µL.

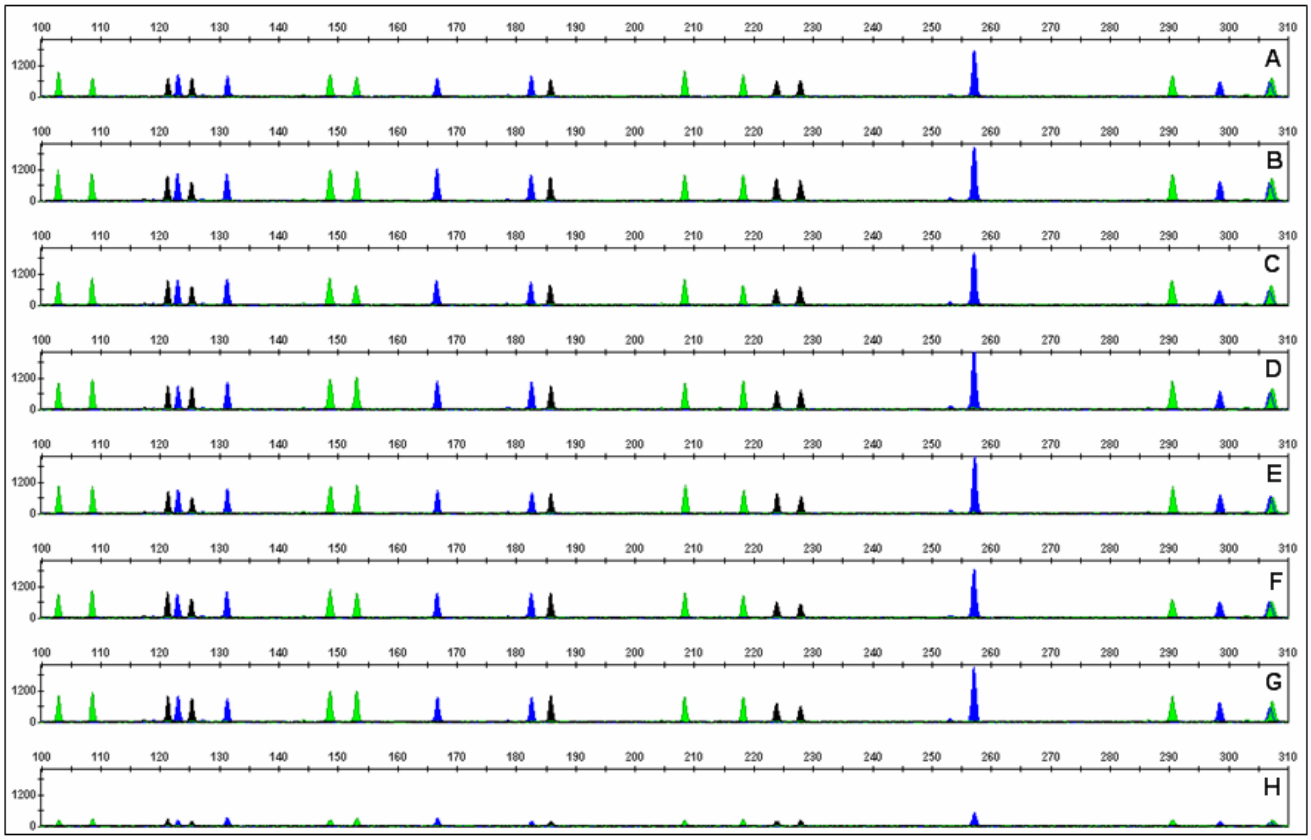


Figure 34 SGM Plus® kit electropherograms.

Figure 35 on page 121 and Figure 36 on page 121 show representative STR profiles from a range of extracted DNA concentrations. 10 µL of the 0.025 ng/µL extracted DNA samples was transferred by the HID EVOlution™ system directly to the PCR reaction plate. For extracted DNA samples with concentration > 0.025 ng/µL, the sample concentrations were normalized to 0.1 ng/µL by the HID EVOlution™ system, and 10 µL of normalized sample was transferred to the PCR reaction plate.

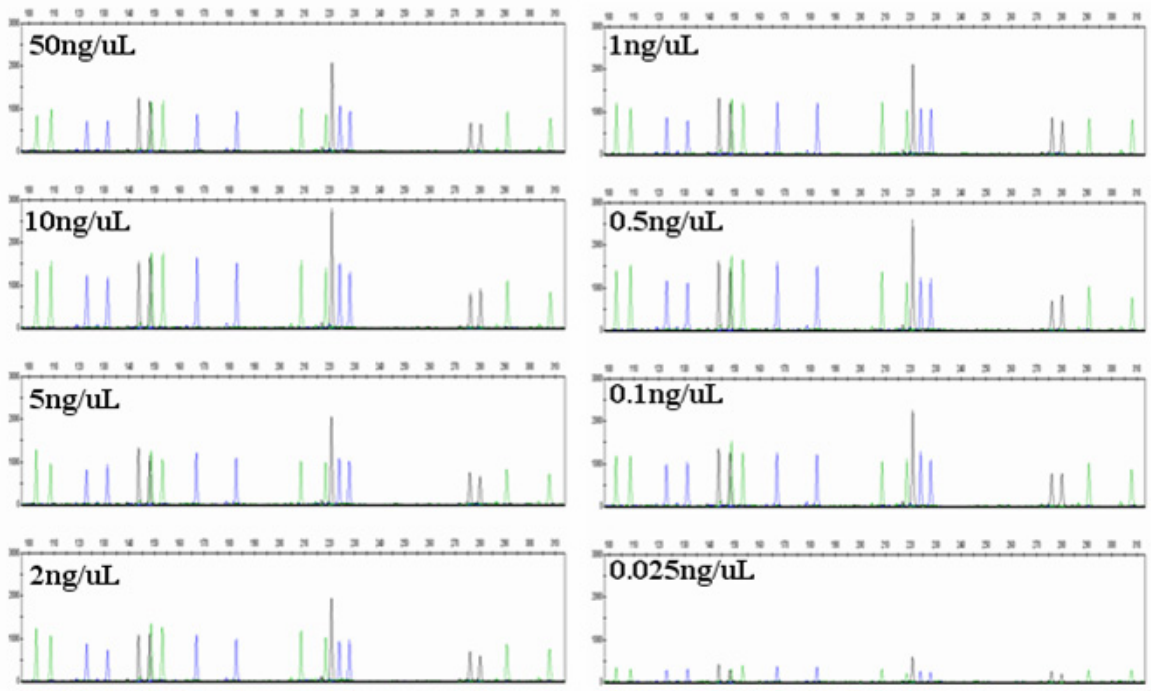


Figure 35 Profiler Plus® kit STR profiles.

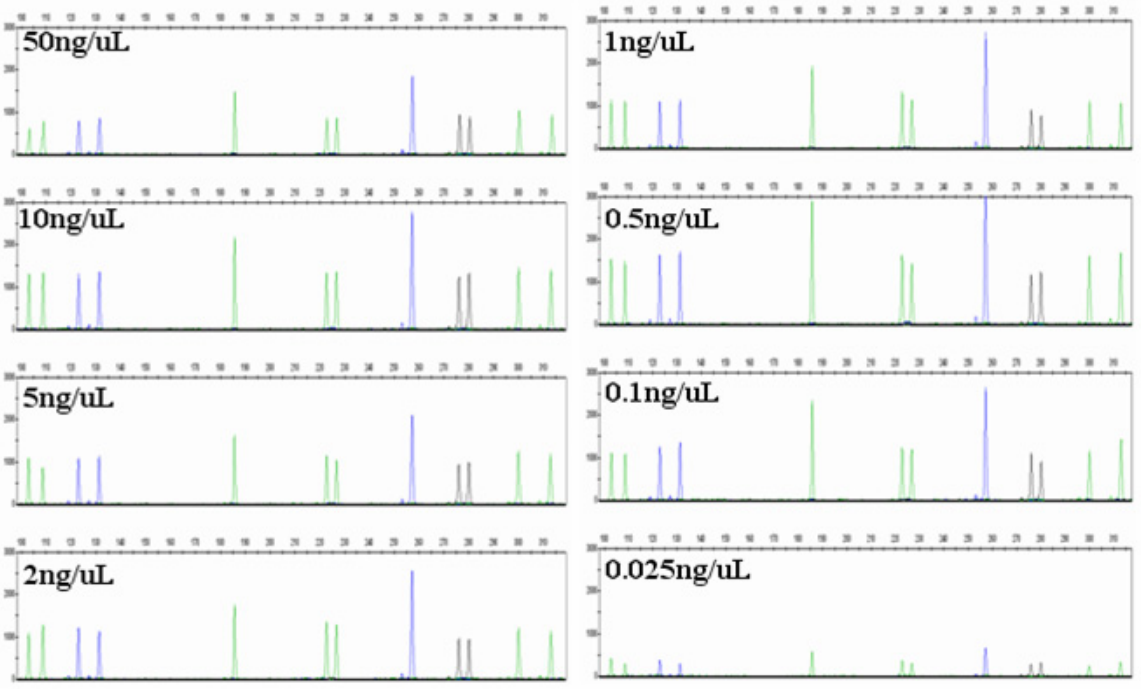


Figure 36 COfiler® kit STR profiles.

Identifiler® and SGM Plus® STR PCR amplification reaction setup accuracy study

Experiment

The AmpFℓSTR® kits were grouped based on the PCR reaction volume:

- 25 µL for Identifiler®, Yfiler®, and MiniFiler™ kits
- 50 µL for SGM Plus®, Profiler Plus®, COfiler®, and SEfiler™ kits

Each script was verified for functionality. One kit-specific script from each group was selected to test for further validation: Identifiler® and SGM Plus® kits.

The accuracy studies were performed using 0.025, 0.1, 0.5, 1, 2, 5, 10, and 50 ng/µL DNA samples that were quantified using the Quantifiler® Human kit on the HID EVOlution™ system. Eleven replicates of each DNA concentration were prepared, and placed in either a plate or in tubes. The HID EVOlution™ system was then used to normalize the DNA concentration and prepare the STR PCR amplification reactions. The experiment was repeated three times for extracted DNA in tubes and three additional times for extracted DNA in a 96-well plate. Peak height averages were calculated and compared for samples prepared on the HID EVOlution™ system and samples prepared manually.

Results

For Identifiler®, the peak height variation was comparable to data generated from manually prepared samples (see Figure 37 and Table 20 on page 123). The peak heights observed for samples with less than 0.1 ng/µL had lower peak heights, as expected, and the reduction correlated with the DNA input amount.

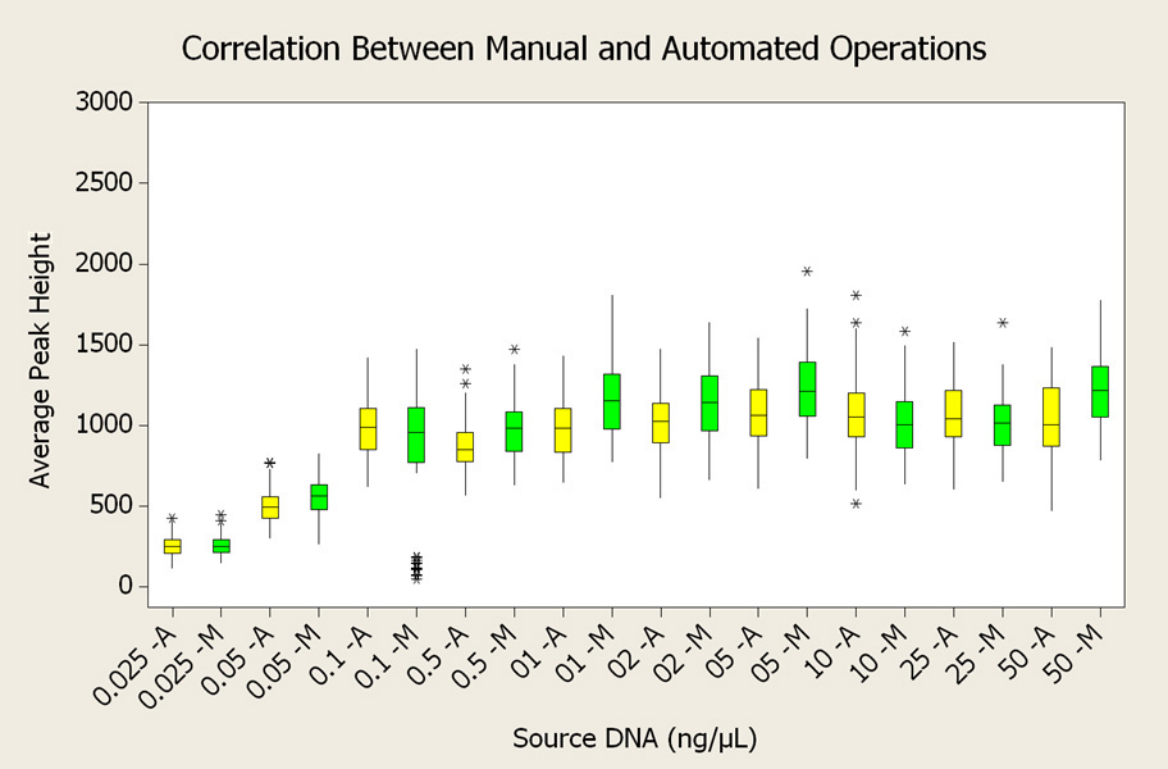


Figure 37 Average peak heights at varying starting DNA concentrations. Results from Identifiler® PCR amplification reactions prepared by the HID EVOlution™ system are shown in yellow and those prepared manually are shown in green.

Table 20 Summary of Peak Height Averages for Identifiler® from samples (n=11) with different starting DNA concentrations before normalization, diluted and prepared either manually or with the HID EVOlution™ system (automated).

Peak Height Averages (RFU)					
Starting DNA Concentration (ng/uL)	**Manual (RFU)**	**Manual Average**	**Automated (RFU)**	**Automated Average**	**Percent Difference (Manual vs. Automated)**
0.025	250.66	1074 ± 128	250.99	1005 ± 63	100%
0.05	561.20		493.50		114%
0.1	852.30		985.80		86%
0.5	986.70		873.00		113%
1	1160.60		977.40		119%
2	1125.70		1000.50		113%
5	1224.80		1061.80		115%
10	1025.00		1072.50		96%
25	1008.30		1041.90		97%
50	1211.30		1033.40		117%

Figure 38 below and Table 21 on page 125 show data from the SGM Plus® kit STR PCR setup. The STR PCR setup was run using a 50-µL PCR reaction script. The peak height variation is analogous to the data generated from the manually prepared samples. The combined average peak height for all samples ≥ 0.1 ng/µL was 983 RFU ± 80 for the manual setup, and 1066 RFU ± 174 for the automated setup.

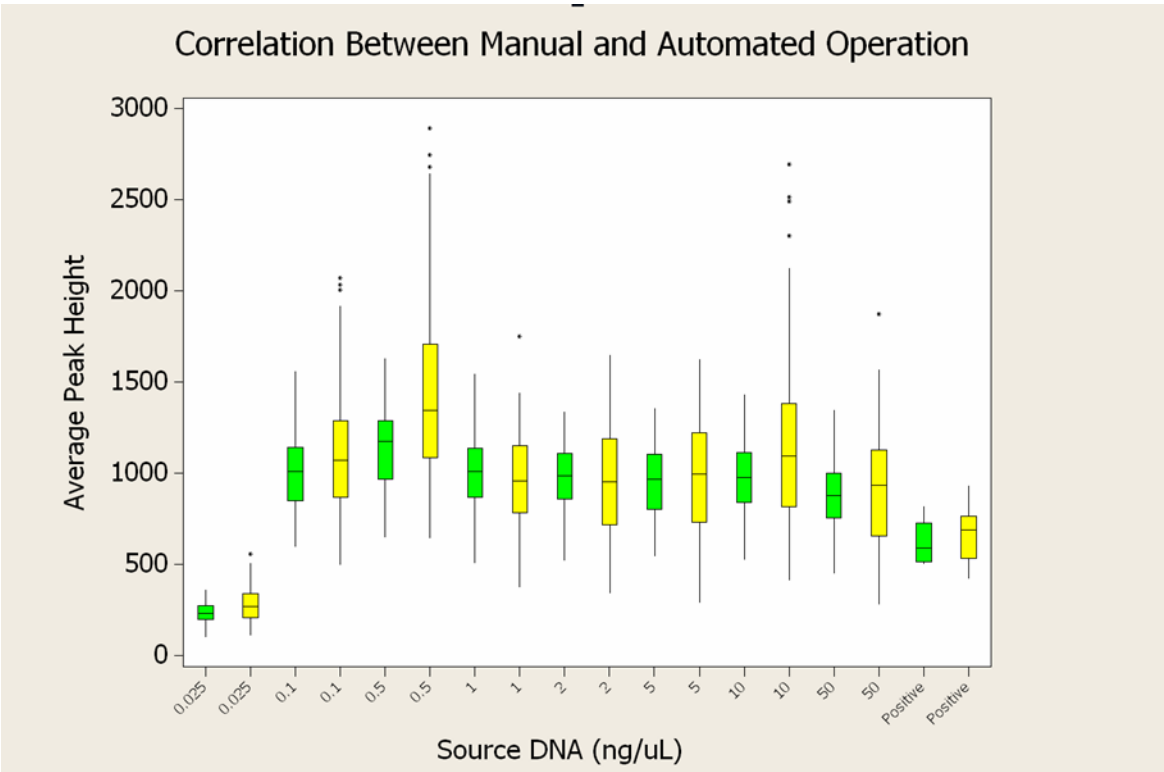


Figure 38 Average peak heights at varying starting DNA concentrations. Results from SGM Plus® PCR amplification reactions prepared by the HID EVOlution™ system are shown in yellow; those prepared manually are shown in green (Positive = Positive Amplification Control).

Table 21 Summary of peak height averages for SGM Plus® from samples (n=11) with different starting DNA concentrations before normalization, diluted and prepared either manually or with the HID EVOlution™ system (automated).

Peak Height Averages (RFU)					
Starting DNA Concentration (ng/uL)	**Manual (RFU)**	**Manual Average**	**Automated (RFU)**	**Automated Average**	**Percent Difference (Manual vs. Automated)**
0.025	234.13		278.13		84%
0.1	1000.70	983 ± 80	1093.50	1066 ± 174	92%
0.5	1139.10		1412.90		81%
1	992.80		952.30		104%
2	956.40		963.10		99%
5	947.00		989.20		96%
10	977.40		1146.80		85%
25	874.30		904.40		97%
50	234.13		278.13		84%

Complete system check and precision study

Experiment To evaluate the precision of the entire HID EVOlution™ system workflow, genomic DNA was diluted with $T_{10}E_{0.1}$ buffer to obtain concentrations of 50.0, 10.0, 5.0, 2.0, 1.0, 0.5, and 0.025 ng/µL. Ten replicates of each concentration were quantified (80 samples in total) using the Quantifiler® Human kit. The quantification standards were prepared by the HID EVOlution™ system.

1. A qPCR/STR Setup file containing sample names and information was created, then imported to the HID EVOlution™ - qPCR/STR Setup System software for use in qPCR reaction setup.

2. The 7500 Setup file generated by the HID EVOlution™ software (output in .txt format) was used as the 7500 Setup file (input) for the 7500 instrument to perform qPCR.

3. The SDS software performed data analysis and produced a 7500 Results file (output in .csv format).

4. The qPCR/STR Setup file containing sample names and information that was created in step 1 was imported to the HID EVOlution™ - qPCR/STR Setup System software for use in STR PCR amplification reaction setup.

5. The 7500 Results file (output) was used as the 7500 Results file (input) to dilute and normalize the samples on the HID EVOlution™ system. All samples with concentrations < 0.1 ng/µL (0.025 ng/µL) were identified by the HID EVOlution™ system to receive 10 µL of DNA extract. The HID EVOlution™ system generated a CE Setup file (output in .txt format) and a STR Samples report.

6. The samples were then prepared for AmpFℓSTR® Identifiler® kit amplification on the 9700 thermal cycler.

7. The CE Setup file (output) generated by the HID EVOlution software (step 5) was imported to create a plate record into the 3130/3130*xl* Genetic Analyzer Data Collection software for capillary electrophoresis/fragment analysis.

Figure 39 on page 127 shows the steps used during HID EVOlution™ system check and precision validation.

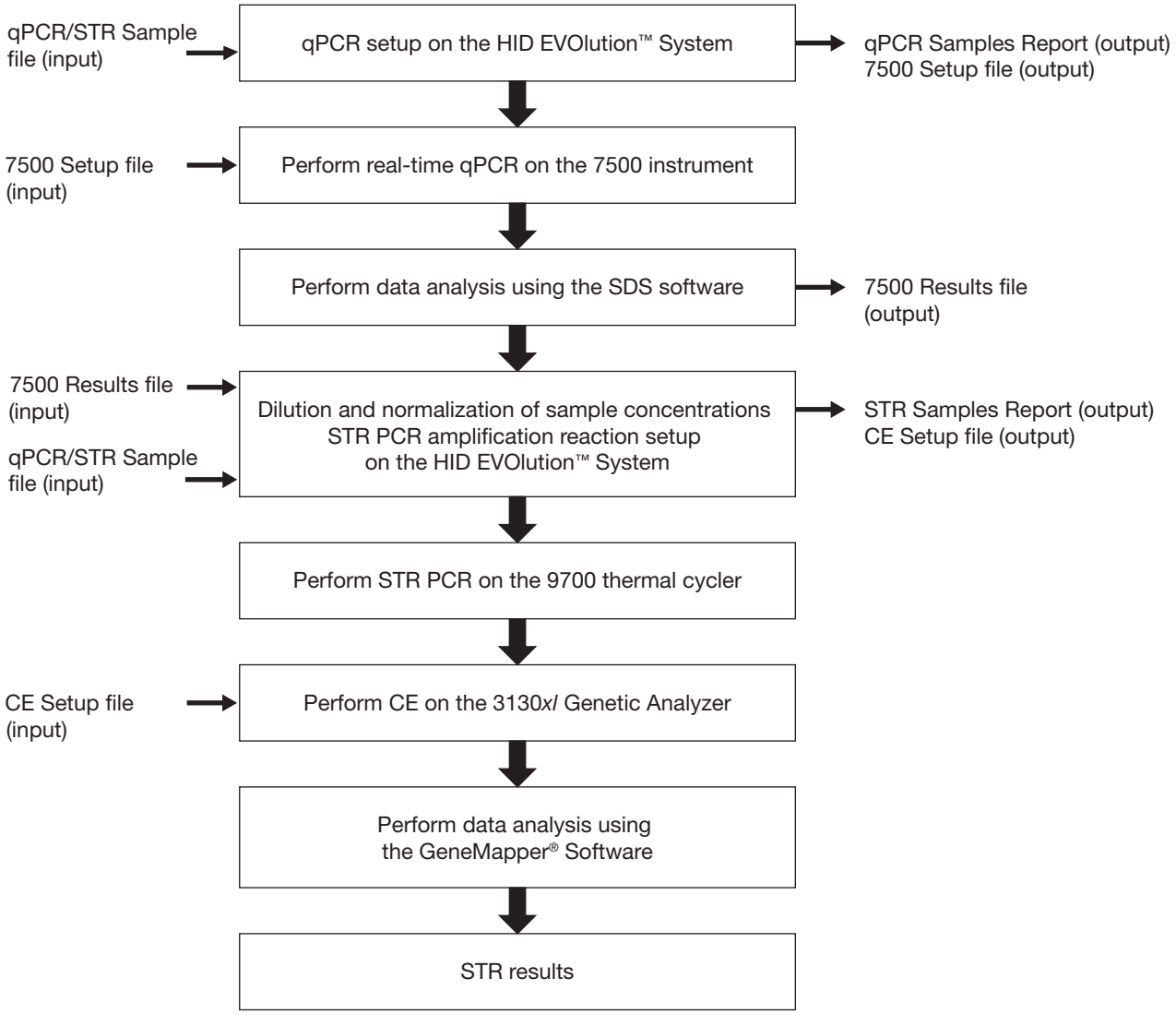


Figure 39 HID EVOlution™ system process.

Results The quantification results obtained (Figure 40) were similar to the expected results across a range of concentrations from 50 ng/µL to 25 pg/µL. Quantification results were as expected for the sample DNA concentrations. Sample-to-sample reproducibility was observed among all ten replicates in each of the eight sample concentrations. A maximum standard deviation was observed at the 50 ng/µL concentration of 1.104. Other standard deviations were < 0.2 indicating minimal sample-to-sample variation.

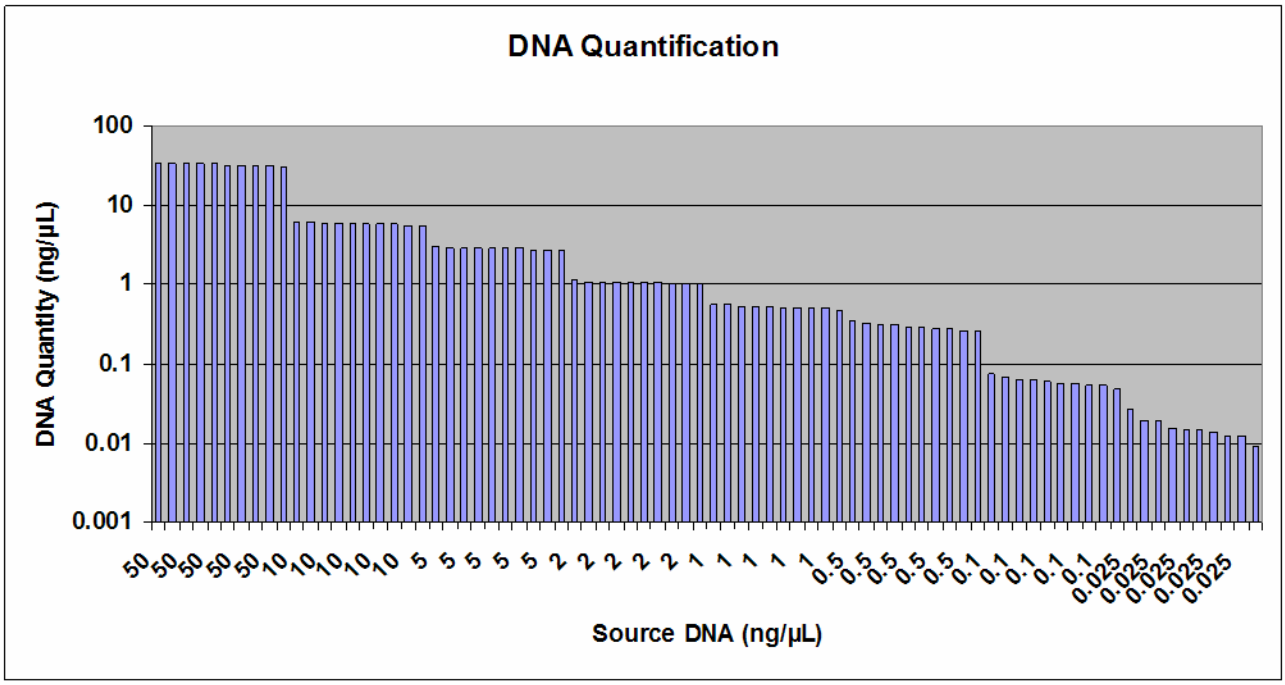


Figure 40 Quantitative results from complete system check study.

Figure 41 on page 129 shows the results of the STR PCR amplification analysis. Extracted DNA samples were first quantified using the Quantifiler® Human script, then normalized for the Identifiler® PCR reaction setup (n=176). The figure shows the average allele peak height for all the replicates normalized across a range of DNA concentrations. The peak height averages were within the acceptable range of 1000-3000 RFU.

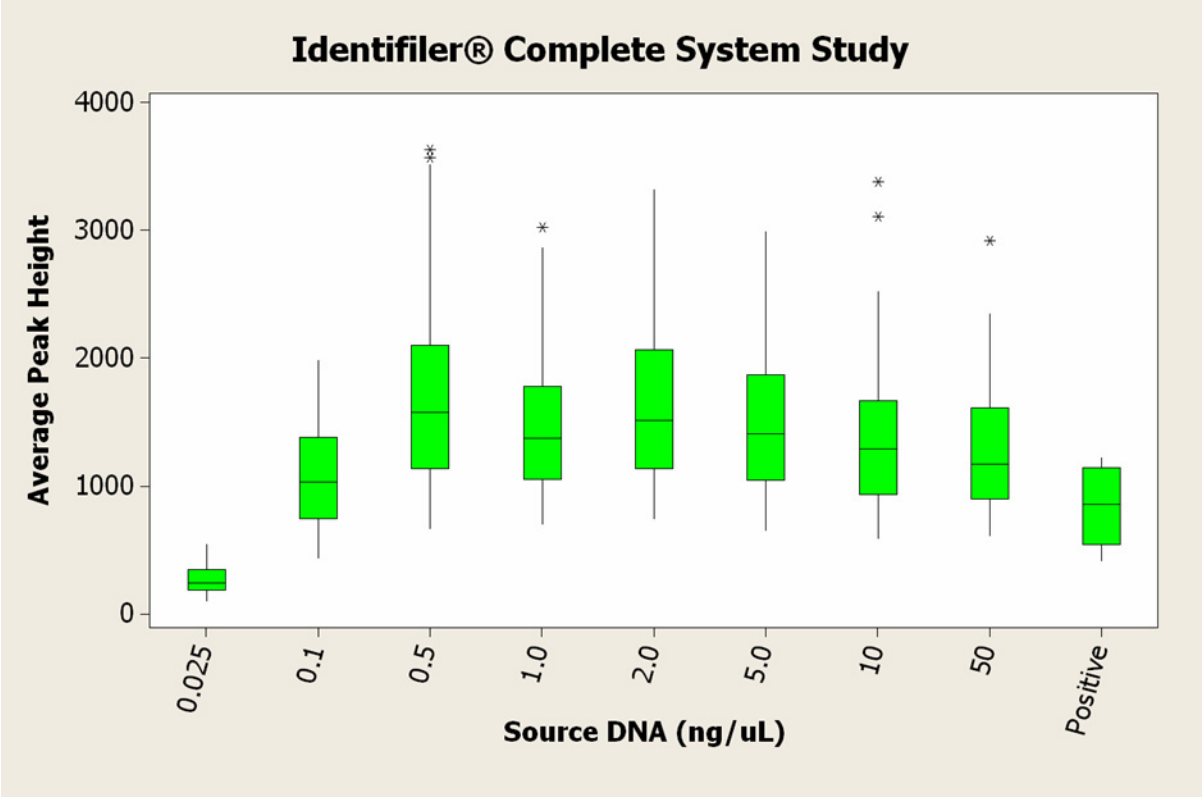


Figure 41 Identifiler® kit complete system study.

The following figures show the Identifiler® kit intralocus balance and intracolor balance profiles. The profiles were evaluated as a measure of the relative changes across the loci and the dye sets. The intralocus balance for almost all samples containing ≥ 0.1 ng/µL DNA concentrations was > 70% (Figure 42 on page 130) and within the acceptable threshold. The intracolor balance was > 40% (Figure 43 on page 130).

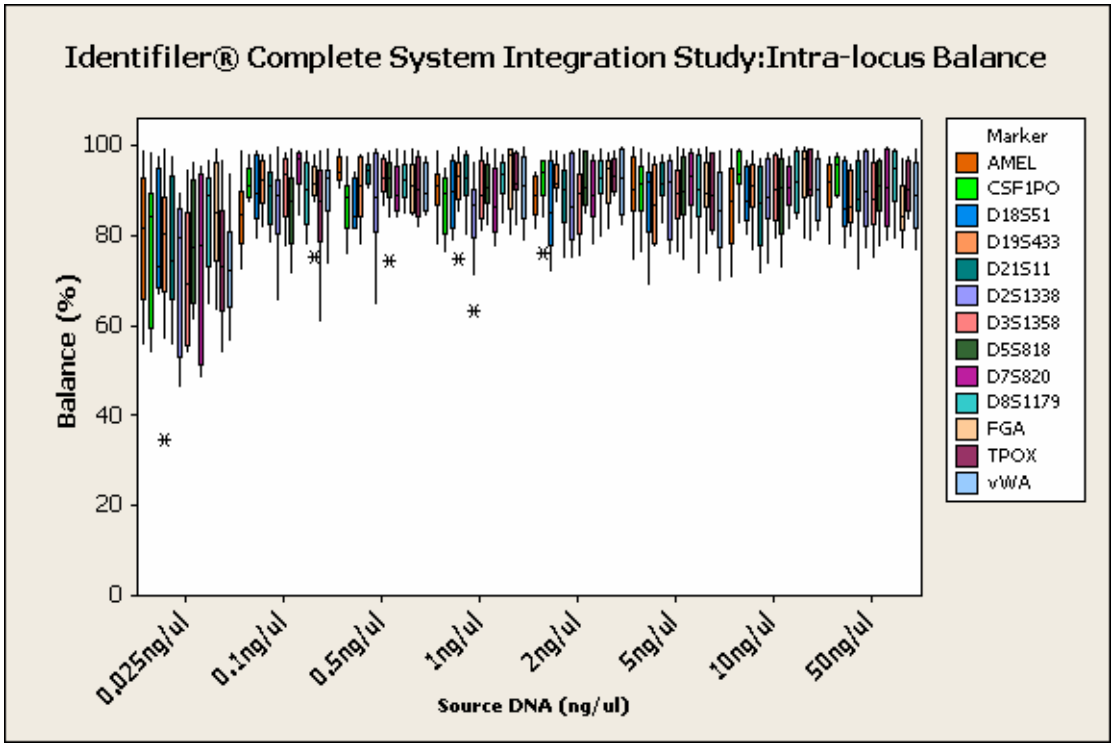


Figure 42 Intralocus balance for Identifiler® kit complete system study.

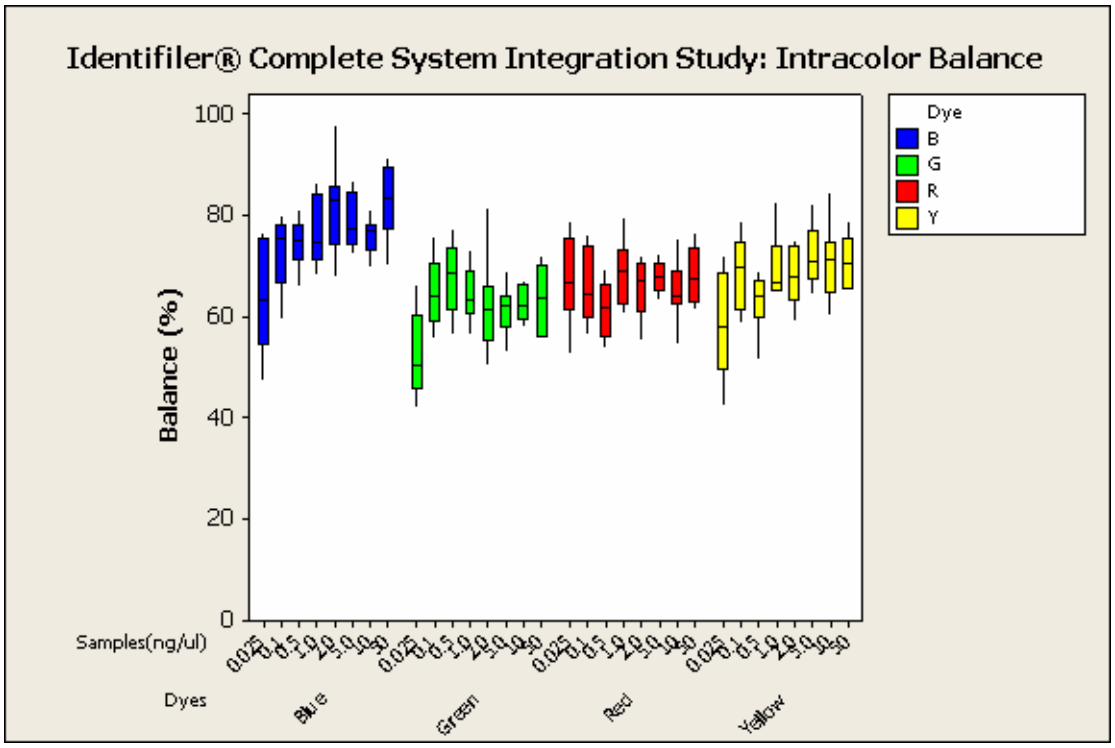


Figure 43 Intracolor balance for Identifiler® kit complete system study.

Concordance and position ID confirmation study

Experiment

For simplicity and consistency during qPCR and STR analyses, the controls and allelic ladder positions on a PCR plate are predetermined. As a result, sample positions in the source and PCR plates may be inconsistent. Sample positions during qPCR and STR PCR amplification may also be inconsistent because of the different control types being used. This inconsistency is compounded by the sample dilution transfers that are required for normalization. As a result, tracking the location of the samples as they are transferred into the PCR reaction plates is imperative.

The HID EVOlution™ Software tracks sample names and positions during processing and generates a final report summarizing this information. This precise information is required to properly process the samples.

Positional integrity of the samples was demonstrated by processing 80 previously typed human samples using the HID EVOlution™ system for quantification, normalization, and STR PCR set up. For the quantification workflow, the Quantifiler® Human kit was used. For STR analysis, the Identifiler® kit samples were in tubes and the SGM Plus® kit samples were in a 96-well plate. Both the sample tubes and the plate were labeled with barcodes.

For additional information about various plate layouts, see "About the qPCR reaction plate layout" on page 36 for qPCR layouts and "About the STR PCR plate layout" on page 62 for STR PCR layouts.

Results

The resulting STR profile for each sample was compared with the known profile. A 100% concordance was observed. The position of each profile was compared with its expected location in the qPCR and STR PCR plates and with the sample report generated by the software. A 100% comparison was observed. In conclusion, the HID EVOlution™ Software tracked the sample name and position correctly during processing.

Contamination study

Experiment

The potential for contamination in the automated quantification and STR protocols (including normalization) was evaluated. Samples that contained 100 µL of 100 ng/µL of DNA were alternated with samples that contained TE buffer only. On each 96-well plate, 40 replicates of the DNA and TE buffer were set up in a checkerboard or vertical stripe pattern. These plates served as source plates for a qPCR reaction that used the Quantifiler® Human kit and script. The negative samples were evaluated for a C_T value < 40. If a negative sample had a C_T value < 40, it was evaluated manually for an STR profile using the MiniFiler™ kit.

A separate set of similarly patterned source plates were used to test the STR PCR setup operations. Samples were processed for quantification using the Quantifiler® Human kit, diluted to normalize DNA concentrations, and then prepared for STR PCR amplification using the Identifiler® kit.

Results

All TE wells in the striped-pattern layout exhibited C_T values > 40, indicating that there was no cross-contamination during the qPCR reaction setup. Of the 40 TE wells in the checkerboard layout, one well exhibited a C_T value of 38. This sample was amplified using MiniFiler™ and standard HID analysis methods. It showed no STR profile. The absence of a profile confirmed that the C_T value of 38 was a result of stochastic variation in the qPCR reaction and not due to a cross-contamination of the sample during automated setup.

A separate set of source plates, with the same source DNA as above and the same layout patterns, was used to test the STR PCR setup operations for cross-contamination. The 80 wells containing TE (40 from each pattern) were evaluated for profiles after fragment analysis. This set of TE studies also showed no STR profile.

Conclusion

Automated protocols were developed for and validated on the HID EVOlution™ System for the following: Quantifiler® Human, Quantifiler® Y Human Male, Identifiler®, Yfiler®, MiniFiler™, SGM Plus®, Profiler Plus®, SEfiler™, and COfiler® kits. Validation studies demonstrated that the liquid handling protocols provided robust and reliable results, using a range of DNA quantities. In conclusion, the following observations were made.

- Quantification scripts:
  - Quantification scripts produced standard-curve results that were within acceptable limits as defined by PCR efficiency and extensive Applied Biosystems experience manually preparing Quantifiler® kits.
  - Variation between scripts employing either tubes or plates was minimal, as was the variation between automation and manually prepared samples.
- STR PCR amplification scripts:
  - STR PCR amplification scripts produced average peak-height results that were within acceptable limits, as defined by instrument-specific parameters, with results within the desired peak height range of 1000-3000 RFU.
  - Variation between scripts employing either tubes or plates was minimal, as was the variation between automation and manually prepared samples.
- Entire workflow
  - Sample names and information were accurately tracked and reported throughout the workflow.
  - Quantification data was properly integrated, and sample normalization was accurately defined.
  - STR electropherograms exhibited desired peak-height averages, intralocus balance, and intracolor balance, resulting in acceptable STR profiles.

References

- Scientific Working Group on DNA Analysis Methods. 2004. Revised Validation Guidelines (approved July 2003). *Forensic Science Communications* Volume 6, Number 3. Available at: **www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm**
- DNA Advisory Board. 2000. Quality Assurance Standards for Forensic DNA Testing Laboratories (approved October 1998). *Forensic Science Communications* Volume 2, Number 3. Available at: **www.fbi.gov/hq/lab/fsc/backissu/july2000/codispre.htm**

F Automation Guidelines

This appendix contains general guidelines to assist you with automating qPCR reaction setup, DNA normalization, and STR PCR amplification reaction setup on other liquid-handling platforms:

qPCR reaction setup automation guidelines

The following are general guidelines for automating the preparation of DNA standards and PCR reaction Master Mix, and the aliquoting and mixing the Reaction Mix standards and samples for the Quantifiler® reaction setup assays.

Before the automated process

Prepare reagents and standards as described in the Quantifiler® and AmpFℓSTR® kits.

1. If using plates, be sure to arrange samples in a way that is compatible with the system.
2. If preparing standards, prepare $T_{10}E_{0.1}$ buffer:
   - 10 mM Tris-HCl (pH 8.0)
   - 0.1 mM Na_2 EDTA
   - 20 µg/mL glycogen (optional)

Prepare a DNA standard dilution series (optional)

Prepare a DNA standard dilution series according to recommendations in the *Quantifiler® Kit User's Manual*.

Prepare the reactions

Calculate the volume of each component needed to prepare the reactions based on the following volumes:

- Quantifiler® Human Primer Mix or Y Human Male Primer Mix - 10.5 µL
- Quantifiler® PCR Reaction Mix - 12.5 µL

Note: Include excess reactions in the calculations to compensate for the loss that occurs during reagent transfers.

To prepare the reactions:

1. Dispense the required volume of primer mix into a separate, empty tube for Master Mix preparation.
2. Dispense the required volume of PCR reaction mix into the Master Mix tube, then mix well.
3. Dispense 23 µL of Master Mix into each reaction well.
4. Add 2 µL of DNA standard or sample to each well.

IMPORTANT! As you add standards or samples, be sure to mix the components of the reaction thoroughly. Applied Biosystems recommends running duplicates of the eight DNA quantification standards for each assay and on each reaction plate.

For plate setup examples, see "About the qPCR reaction plate layout" on page 36.

Upon completing the automated process

When you are done with the automated process:

- Seal the reaction plate with the clear adhesive film.
- Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove any bubbles.
- Run the PCR reaction as described in the *Quantifiler Kits User's Manual*.
- Remove all reagents from worktable.
- Close the sample tubes to prevent contamination and evaporation.
- Flush the liquid system.
- Clean the worktable and carriers to avoid cross contamination.

STR PCR reaction setup automation guidelines

The following are general guidelines for automating the dilutions for normalization, mixing the STR PCR reaction mix for amplification, and dispersing diluted samples and reaction mix into a reaction plate for the STR kits.

Before the automated process

Extract DNA, then:

1. Quantify the concentration of each sample.
2. Identify the target concentration and sample input volume for the STR kit to be used.

Target concentrations and input volumes

STR kit	Target mass (ng)	Sample input volume (µL)	Reaction volume (µL)
COfiler®	0.5 to 1.25	20	50
Identifiler®	0.5 to 1.25	10	25
MiniFiler™	0.5 to 0.75	10	25
Profiler Plus®	0.5 to 1.25	20	50
SEfiler Plus™	0.5 to 0.75	10	25
SGM Plus®	0.5 to 1.25	20	50
Yfiler®	0.5 to 1.0	10	25

Prepare the worktable

After extracting DNA:

1. Place the DNA samples on the worktable.
2. Place empty tube or vial for preparation of Master Mix on worktable. Place two new 96-well plates on the worktable for use in dilutions.
3. Place thawed STR PCR reagents on the worktable. Centrifuge reagents before placing on worktable for maximum volume accessibility.
4. Place $T_{10}E_{0.1}$ buffer on the worktable.
5. Place a new 96-well plate for the PCR reaction on the worktable.

Normalize the DNA samples

Dilute samples according to the exact concentration determined for each individual sample.

For the Tecan Freedom EVO series DNA dilution protocols and a description of dilution ratios, volumes of sample used, and volumes of TE used for dilution, see Appendix D, “Dilution Protocols” on page 93 or the Tecan *HID EVOlution™ Application Guide – Automation for Applied Biosystems Human Identification Kits* (Section 10, Appendix C).

For instruments other than the Tecan Freedom EVO series, use the description below to construct a dilution protocol within the parameters of your specific instrument.

- Add TE buffer to the predilution plate, then dilute the first dilution step samples in well X. For a two-step dilution, dilute with a second step in well X+8.
- Mix thoroughly in all steps to assure a homogeneous solution.
- Vary the volumes for the TE buffer and the volume of sample transferred from the tube for dilutions of 1:23 to 1:4000. Single-step dilutions require a dilution ratio between 1:1 to 1:22.9, while two-step dilutions require a higher dilution ratio.
- Use the minimum recommended final volume recommended by the instrument manufacturer for an individual dilution, to ensure liquid handling precision.

IMPORTANT! If the concentration of the original control DNA needs to be diluted, dilute a portion of the control DNA with low TE buffer and place the diluted control tube into the reagent rack for amplification setup. Ensure that the final volume of the diluted control DNA is at least 50 µL.

Prepare the reactions

Calculate the volume of each component needed to prepare the reactions, using the table below.

Note: Include at least 10% excess reactions in the calculations to compensate for the loss that occurs during reagent transfers. For MiniFiler™ and SEfiler Plus™ kits, add the required volume of Master Mix and Primer Mix only. No additional polymerase is needed.

Target concentrations and input volumes

Component	Volume Per Reaction (µL)						
	COfiler®	Identifiler®	MiniFiler™	Profiler Plus®	SEfiler Plus™	SGM Plus®	Yfiler®
Primer Mix	11.0	5.5	5.0	11.0	5.0	11.0	5.0
PCR Reaction Mix	21,0	10.5	NA	21.0	NA	21.0	9.2
AmpliTaq Gold® Polymerase	1.0	0.5	NA	1.0	NA	1.0	0.8
MiniFiler™ or SEfiler Plus™ Master Mix	NA	NA	10.0	NA	10.0	NA	NA

To prepare the reactions:

1. Dispense the required volume of primer mix into a separate, empty tube for Master Mix preparation.
2. Dispense the required volume of PCR reaction mix into the Master Mix tube, then mix well.
3. Dispense the required volume of AmpliTaq Gold® Polymerase into the Master Mix tube, then mix well.

4. Dispense the required volume of Master Mix into each reaction well. See the table below.

Master Mix for reaction wells

	COfiler®	Identifiler®	MiniFiler™	Profiler Plus®	SEfiler Plus™	SGM Plus®	Yfiler®
Master Mix	30.0	15.0	15.0	30.0	15.0	30.0	15.0

5. Dispense the required volume of DNA sample into each reaction mix, then mix well. See the table below.

DNA sample volume for reaction wells

	COfiler®	Identifiler®	MiniFiler™	Profiler Plus®	SEfiler Plus™	SGM Plus®	Yfiler®
DNA sample volume (minimum)	20.0	10.0	10.0	20.0	10.0	20.0	10.0

6. Add DNA controls (PTC and NTC) as specified in the particular kit in use.

Note: Remember that the Yfiler® kit uses an extra PTC.

Upon completing the automated process

When you are done with the automated process:

- Seal the reaction plate with clear adhesive film.
- Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove any bubbles.
- Run the PCR reaction as described in the kit-specific User's Manual.
- Remove all reagents from worktable.
- Close the sample tubes to prevent contamination and evaporation.
- Flush the liquid system.
- Clean the worktable and carriers to avoid cross contamination.

G Safety

This appendix covers:

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. 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. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 143.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.

- Ensure that the instrument 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.

Biological hazard safety

General biohazard

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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Safety alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see "Safety alert words" on page ix.

Documentation

This appendix covers:

Related documentation

Applied Biosystems documentation

The following related documents are available for use with the system:

Document	Part number	Description
HID EVOlution™ - qPCR/STR Setup System Getting Started Guide	4426903	Provides brief, step-by-step procedures for setting up qPCR and STR PCR. It is designed to help you quickly learn to use the HID EVOlution™ - qPCR/STR Setup System.
Quantifiler® Kits (Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit) User's Manual	4344790	Includes information about manual quantitation specific to your kit.
AmpFℓSTR® COfiler® PCR Amplification Kit User's Manual	4306116	Includes information about normalization and amplification specific to your kit.
AmpFℓSTR® Identifiler® PCR Amplification Kit User's Manual	4323291	Includes information about normalization and amplification specific to your kit.
AmpFℓSTR® MiniFiler™ PCR Amplification Kit User's Manual	4374618	Includes information about normalization and amplification specific to your kit.
AmpFℓSTR® Profiler Plus® PCR Amplification Kit User's Manual	4303501	Includes information about normalization and amplification specific to your kit.
AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit User's Manual	4385739	Includes information about normalization and amplification specific to your kit.
AmpFℓSTR® SGM Plus® PCR Amplification Kit User's Manual	4309589	Includes information about normalization and amplification specific to your kit.
AmpFℓSTR® Yfiler® PCR Amplification Kit User's Manual	4358101	Includes information about normalization and amplification specific to your kit.
3130*xl* Getting Started Guide	4352715	Includes information about using the 3130*xl* genetic analyzer.
GeneMapper® *ID* v 3.1 User Guide	4357520	Includes information specific to AmpFℓSTR® data analysis.
GeneMapper *ID* Software Version 3.2 User Bulletin	4352543	Includes detailed information on the features and capabilities of GeneMapper *ID* Software version 3.2, including support of the AmpFℓSTR® Yfiler® PCR Amplification Kit.
7300/7500 Real-Time PCR System Installation and Maintenance Guide	4347828	Includes information on the 7500 System and SDS Software v1.2.3.

Tecan documentation

Refer to the Tecan documents in the following table for details relating to the Tecan Freedom EVO® instrument and software, and the HID EVOlution™ system. References to the appropriate Tecan documentation are also provided throughout this guide.

For information on...	Refer to...
The HID EVOlution™ system application	*Tecan HID EVOlution™ qPCR/STR Setup System Application Manual (PN 394918)*
Installing and setting up the Freedom EVO instrument	*Tecan HID EVOlution™ Installation Manual* *Tecan Software Manual Instrument Software V6.1 Part 1* *Freedom EVOware® Getting Started Guide*
Comprehensive safety information, operating, maintenance, and troubleshooting procedures for the Freedom EVO instrument	*Tecan Freedom EVO® Operating Manual (PN 392886)* *Tecan Freedom EVO® Maintenance and Service Logbook (PN 392185)* *Tecan Freedom EVO® Daily/Weekly Maintenance Checklist (PN 392818)*
Installing, setting up, running, and programming the Freedom EVOware® software	*Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Manual* *Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Getting Started Guide* *Freedom EVOware® Software Manual Extended Device Support* (Research Use Only) (PN 393172) *Freedom EVOware® Software Manual Limited Dev. Support* (General Purpose) (PN 393804)
Running scripts and troubleshooting script-related error messages	*Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Runtime Controller Manual (PN 394329)*

Note: For additional documentation, see "How to obtain support" on page x.

Obtaining information from the Help system

The Tecan Freedom EVOware® v2.1 has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click in the toolbar of the Tecan Freedom EVOware® 2.1 window.
- Select **Help ▸ Contents and Index**.
- Press **F1**.

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic
- Searching an alphabetized index

Note: The Help system is specifically for the Tecan Freedom EVOware® 2.1 system, not the HID EVOlution™ - qPCR/STR Setup System. Changing the Freedom EVOware® 2.1 system parameters could lead to problems running qPCR/STR PCR scripts. Consult with a system administrator before making any system changes.

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating *only* to documentation. To order documents, download PDF files, or for help with a technical question, see "How to obtain support" on page x.

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