PrepFiler™ Automated Forensic DNA Extraction Kit

USER BULLETIN

Automated DNA Purification on the HID EVOlution™ Systems

for use with:
HID EVOlution™—Extraction System
HID EVOlution™—Combination System

Publication Number MAN0019298

Revision A.0

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

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

Revision history: Pub. No. MAN0019298

Revision	Date	Description
A.0	22 March 2021	New document.

Contents

1 Product information

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

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

Product description

The PrepFiler™ Automated Forensic DNA Extraction Kit is part of an integrated solution that includes proven PrepFiler™ reagents for use in semi-automated, high-throughput workflows to provide reliable, simplified, and time-saving DNA extraction and purification.

The kit uses magnetic particles with an optimized multi-component surface chemistry to deliver robust and reliable DNA yield from tested routine forensic sample types, including:

- Body fluids (blood, saliva, semen)
- Stains and swabs of body fluids
- Hair roots
- Touch/trace samples

About the HID EVOlution™ systems

The HID EVOlution™—Extraction System and HID EVOlution™—Combination System perform automated DNA purification using a Freedom EVO™ robotic workstation with the PrepFiler™ Automated Forensic DNA Extraction Kit.

The robotic workstation automates liquid and magnetic particle handling, as shown in Figure 1. The purified DNA is collected in a 96-well plate or 1.5-mL tubes, depending on the Freedom EVOware™ software script that you select.

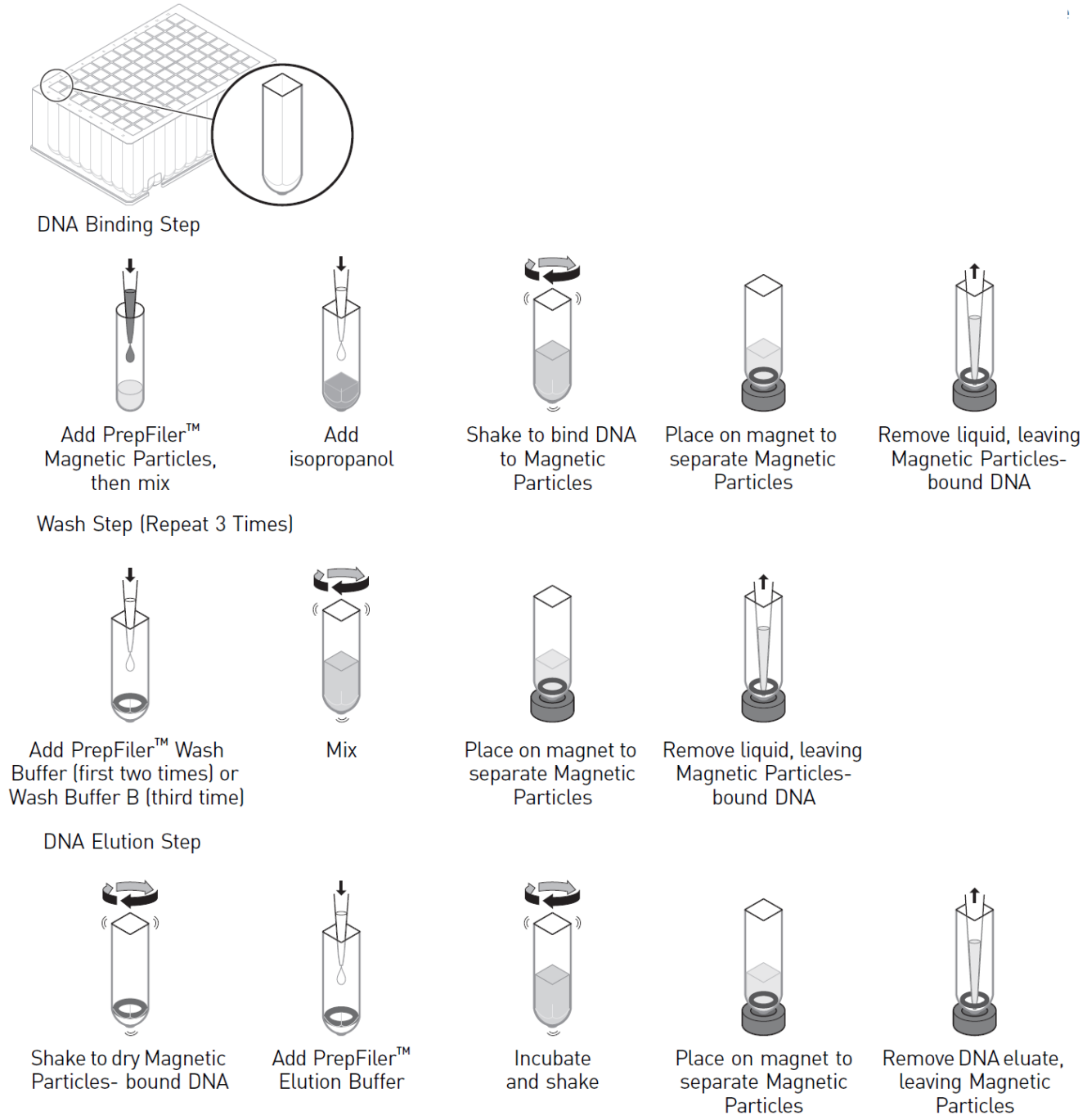


Figure 1 Automated DNA purification steps performed by the HID EVOlution™—Extraction System in plates (shown) or tubes (not shown)

Lysate is transferred from a spin plate or 1.5-mL microfuge tubes into the PrepFiler™ 96-Well Processing Plate (shown) for automated DNA purification.

Purification run times

The automated purification run time is ~3–4 hours for 96 samples, depending on the type of HID EVOlution™ system and configuration that you are using.

System components

The HID EVOlution™—Extraction System and HID EVOlution™—Combination System consist of the following components.

- A Freedom EVO™ 150 robotic workstation or Freedom EVO™ 200 robotic workstation
- Freedom EVOware™ software v2.1 SP1 or later, configured with the HID EVOlution™—Extraction System application

Note: Contact Technical Support for more information on verified configurations. See "Customer and technical support" on page 115.

- 8-channel liquid-handling arm (LiHa)
- Robotic Manipulator arm (RoMa)
- Te-Shake™ plate adapter with heating block and adapter

Plate/tube configurations

The HID EVOlution™ systems support the following plate/tube configurations. You can select one configuration per purification run.

- **Plate-to-plate**—Process lysate from a 96-well plate and collect eluate in a 96-well plate
- **Plate-to-tubes**—Process lysate from a 96-well plate and collect eluate in 1.5-mL tubes
- **Tubes-to-tubes**—Process lysate from 1.5-mL tubes and collect eluate in 1.5-mL tubes
- **Tubes-to-plate**—Process lysate from 1.5-mL tubes and collect eluate in a 96-well plate

Contents and storage

The PrepFiler™ Automated Forensic DNA Extraction Kit is intended for semi-automated workflows, and contains the reagents required for the following procedures:

- Manual sample lysate preparation (DNA extraction)
- Automated DNA purification

The kit is sufficient for ≤960 samples, depending on the batch size.

Table 1 PrepFiler™ Automated Forensic DNA Extraction Kit (Cat. No. 4463353)

Contents	Amount	Storage
PrepFiler™ Lysis Buffer	1 × 500 mL	18–25°C
PrepFiler™ Magnetic Particles	13 × 1.5 mL	
PrepFiler™ Wash Buffer A Concentrate	1 × 500 mL	
PrepFiler™ Wash Buffer B Concentrate	1 × 250 mL	
PrepFiler™ Elution Buffer	1 × 200 mL	

Required materials not supplied

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

Table 2 Reagent preparation

Item	Source
Ethanol (Molecular biology grade; 95% or 190 proof) **Note:** Open a new bottle when preparing the PrepFiler™ Wash Buffer A and Wash Buffer B solutions.	MLS
Clean containers to store the prepared Wash Buffer A and Wash Buffer B solutions; we use: Nalgene™ Square PETG Media Bottles with Closure: Sterile, Shrink-Wrapped Trays	342020-0500 or 342020-1000

Table 3 Automated purification

Item	Source[1]
Isopropanol (2-Propanol, ACS reagent grade, ≥99.5%) **Note:** Purchase isopropanol in small bottles and open fresh bottles frequently to maintain a high-quality grade reagent.	MLS
(If needed) DNA Suspension Buffer (low-TE buffer) **Note:** DNA Suspension Buffer is only needed if you run out of PrepFiler™ Elution Buffer.	MLS
Magnetic-Ring Stand (96 well)	AM10050
Disposable Tips (DiTi), Tecan™ Pure, Filtered, 1,000-µL (30 000 631); six trays (each containing 96 DiTis)	Tecan™ (30000631)[2] **https://www.tecan.com/**
Disposable Tips (DiTi), Tecan™ Pure, Filtered, 200-µL (30 000 629); three trays (each containing 96 DiTis)	Tecan™ (30000629) **https://www.tecan.com/**
100-mL disposable troughs for reagents (5 troughs)	Tecan™ (10613048) **https://www.tecan.com/**
(Optional) Barcodes	See the *Tecan™ Freedom EVO™ Operating Manual*, Section 3.5.6 “Positive Identification (PosID)”, for barcode requirements
PrepFiler™ 96-Well Processing Plate (10 plates)	A47010

Table 3 Automated purification *(continued)*

Item	Source[1]
If collecting eluate in plates: MicroAmp™ Optical 96-Well Reaction Plate (without barcode) or MicroAmp™ Optical 96-Well Reaction Plate with Barcode	N8010560 or 4306737
If collecting eluate in tubes: Nonstick, RNase-free Microfuge Tubes, 1.5 mL; certified DNase- and RNase-free (250 tubes)	AM12450, or equivalent

[1] Recommended sources. Unless otherwise indicated, equivalent materials from other suppliers can be used after appropriate validation studies by the user laboratory.

[2] Disposable tips that have not been certified by Tecan™ may not yield the same liquid-handling performance.

2 Prepare for the automated purification run

Perform lysis

Manually perform lysis according to the *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits User Guide* (Pub. No. 4463349).

Before first use: Prepare the wash buffers

1. Mix 260 mL of PrepFiler™ Wash Buffer A Concentrate with 740 mL of freshly-opened 95% ethanol in a separate, clean container to prepare a 1X solution.
2. Mix 200 mL of PrepFiler™ Wash Buffer B Concentrate with 300 mL of freshly-opened 95% ethanol in a separate, clean container to prepare a 1X solution.

If the containers are kept closed when not in use, the prepared wash buffers have a shelf life of 6 months or the kit expiration date, whichever is earlier.

Before each use: Prepare the magnetic particles

1. Incubate the PrepFiler™ Magnetic Particles tubes at 37°C for 10 minutes.
2. Vortex at medium speed until the particles are completely resuspended and homogenous, then briefly centrifuge.
3. Use one of the following methods to remove any air bubbles:
   - Draw off bubbles with a disposable bulb pipette.
   - Use a clean pipette tip to break up the bubbles.
   - Use a lint-free wipe to absorb the bubbles.

IMPORTANT! Bubbles can interfere with automated liquid detection and aspiration.

3 Set up the robotic workstation

For more information

This chapter provides general procedures for setting up the Freedom EVO™ 150 robotic workstation or Freedom EVO™ 200 robotic workstation for the HID EVOlution™ system.

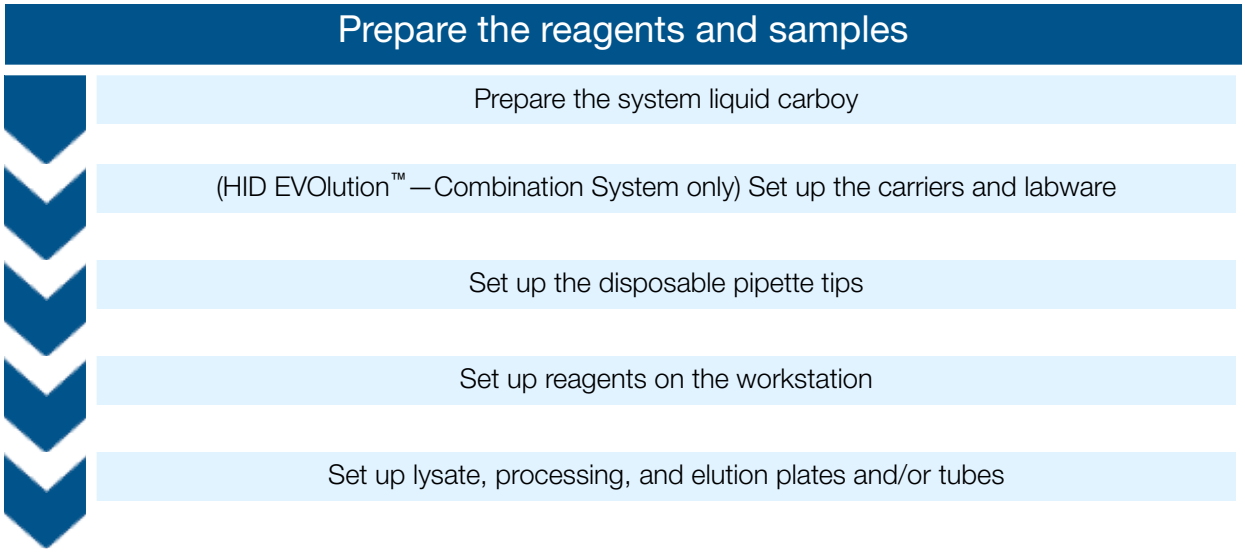
For more information, see the appropriate manufacturer's documentation.

Document	Description	Section
Tecan™ HID EVOlution™—Extraction Application Manual (395372, v2.0, June 2010)	Pre-run preparation step	4.3.2 “Prepare the Instrument”
	Maintenance schedules	7.2, “Maintenance Schedule”
	Maintenance procedures	7.3, “Maintenance Tasks”
	Maintenance scripts	5.2, “Running Maintenance”
		7.5, “Maintenance Scripts”
	Setting up disposable pipette tips	4.3.5, “Setup Plasticware and Samples on the Workstation”
	Setting up reagents	4.3.4, “Setup Reagents on Workstation”
	Place the plates and/or tubes	4.3.5, “Setup Plasticware and Samples on the Workstation”
	Workstation layouts	4.3, "Preparing the Instrument"
		4.4, "Worktable Layouts"

(continued)

Document	Description	Section
Tecan™ HID EVOlution™—Combination Application Manual (395967, v2.0, June 2010)	Pre-run preparation step	5.3.2 “Prepare the Instrument”
	Maintenance schedules	12.2, “Maintenance Schedule”
	Maintenance procedures	12.3, “Maintenance Tasks”
	Maintenance scripts	6.2 and 10.2, “Running Maintenance”
		12.5, “Maintenance Scripts”
	Setting up disposable pipette tips	5.3.5, “Set Up Plasticware and Samples on the Workstation”
	Setting up reagents	5.3.4, “Set Up Reagents on Workstation”
	Place the plates and/or tubes	5.3.5, “Set Up Plasticware and Samples on the Workstation”
	Workstation layouts	5.3, "Preparing the Instrument"
		5.4, "Worktable Layouts"
Tecan™ Freedom EVO™ Operating Manual	Barcode specifications	3.5.6, “Positive Identification (PosID)”

Workflow



Perform routine maintenance

Before placing the samples, reagents, and labware (DiTis, troughs, plates, and tubes), prepare the robotic workstation.

Prepare the system liquid carboy

Ensure that the carboy next to the workstation contains enough system liquid (degassed deionized water) to complete the experiment.

1. Degas the deionized water overnight or longer before using it on the system.

   Note: The time needed for complete degassing varies, depending on the climate in each laboratory and geographical location. In some situations, it may take up to 3 days to fully degas the deionized water. We recommend that each laboratory maintain an additional carboy of fully degassed deionized water to use for replenishment.

2. To avoid introducing air into the system liquid tubing, follow these guidelines:
   - Place the system liquid carboy at the same height as the worktable.
   - Replenish the system liquid as needed before each run to avoid liquid levels dropping below one-quarter carboy during the run.

3. Run the routine maintenance script each time that you change the system liquid carboy.

Empty the waste carboy

Check the waste carboy, and empty if needed.

Tighten the DiTi adapter gold cones

Note: If the cones are loose, the instrument may fail to pick up pipette tips during the run, and liquid delivery will be inconsistent.

Use your fingers to gently tighten the DiTi adapter gold cones on the LiHa and the syringe assembly fittings.

For details, see the *Tecan™ Freedom EVO™ Operating Manual*.

Run maintenance scripts

Before starting the run, run the appropriate maintenance scripts.

IMPORTANT! Watch for air bubbles in the syringes and tubing. Repeat system flushing as needed to remove the air bubbles.

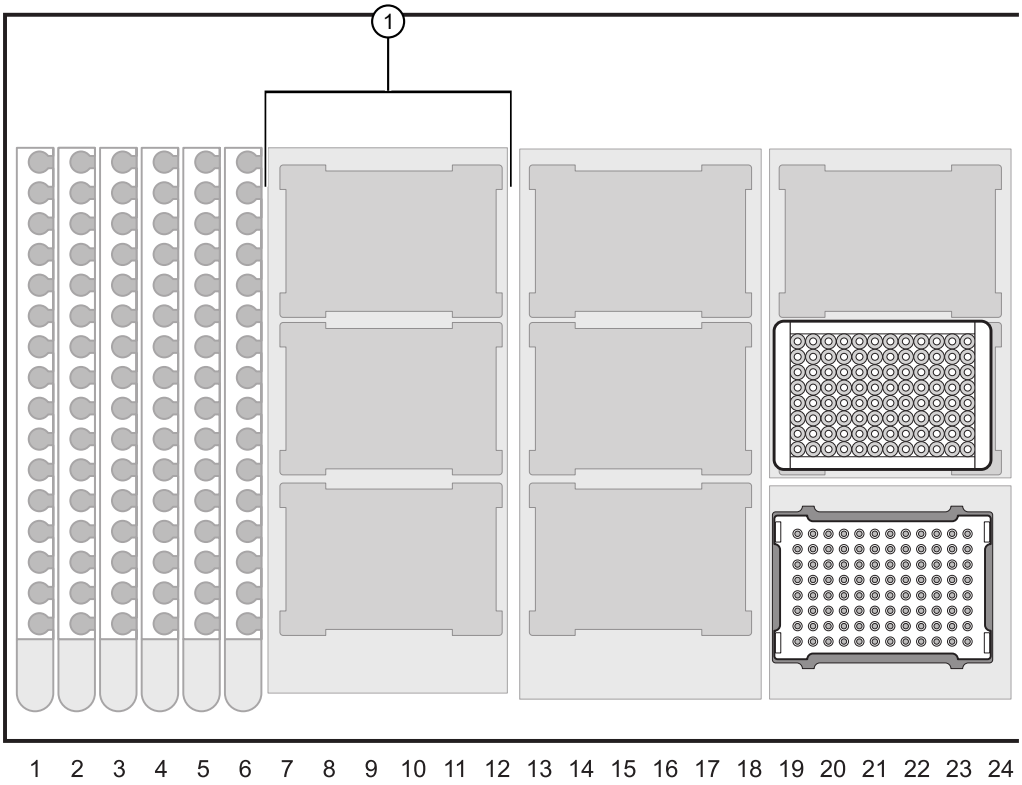
If	Then run
It is the first run of the day	PrepFiler_DailyStartUp or Combo_DailyStartUp
It is *not* the first run of the day	PrepFiler_Flush or Combo_Flush
When you run DailyStartUp or Flush, you see: • Air bubbles in the lines *and/or* • Intermittent flow from a DiTi cone	PrepFiler_Flush or Combo_Flush 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 LiHa	PrepFiler_Drop_DiTis or Combo_Drop_DiTis

(HID EVOlution™—Combination System only) Set up the carriers and labware

If the HID EVOlution™—Combination System was last run for qPCR/STR, you will need to set up the carriers and labware for a purification run.

1. If the DNA lysate is in tubes, remove the 3-position microplate carrier from Grid 7, then place six tube racks on grids 7 through 12.

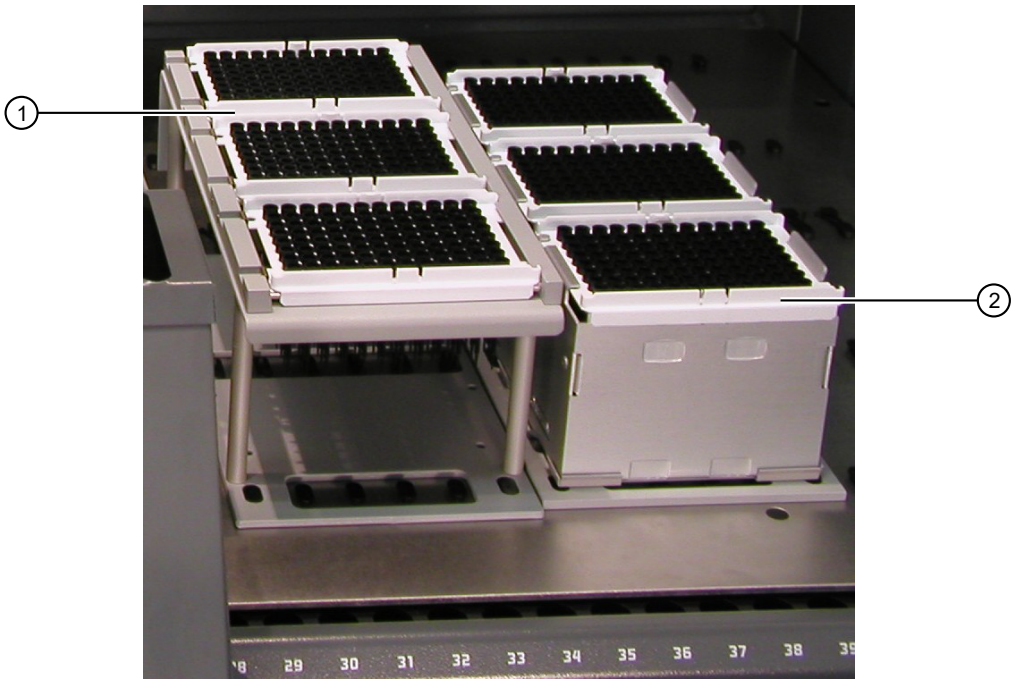
Note: If the DNA lysate is in a plate, you do not need to remove the 3-position microplate carrier.



- 3-position microplate carrier

2. Set up carriers for the DNA eluate.
   - If you want the DNA eluate placed in tubes, position six tube racks on grids 1 through 6.
   - If you want the DNA eluate placed in a 96-well plate, position the metal plate adaptor on grid 13, position 1.

3. Remove the 3-position disposable tips (DiTi) tray carrier from grid 35, then replace it with a flat carrier and three 1,000-µL DiTi boxes as shown.



- 200-µL DiTi trays (blue or white trays)
- 1,000-µL DiTi trays (yellow or white trays)

4. Place the magnetic particle tube block on grid 13, position 2.

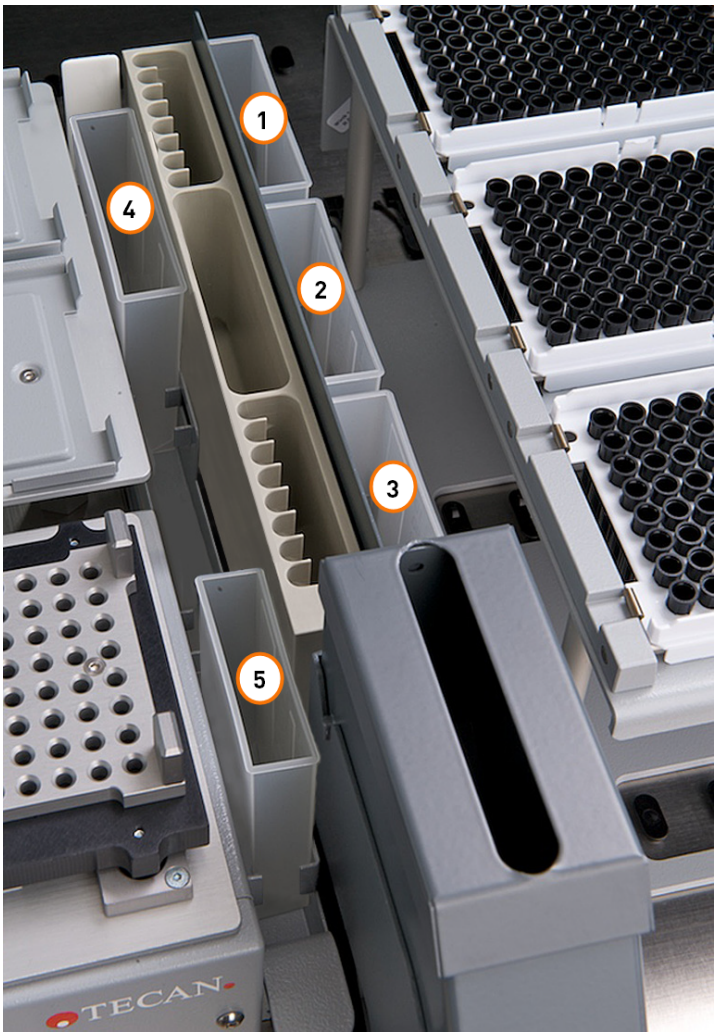
IMPORTANT! Ensure that the tubes and the block containing the tubes are positioned as shown. Incorrect positioning may result in failure to pipet magnetic particles and/or collision of the Liquid Handling (LiHa) arm with the block.

5. Ensure that the 96-well magnetic ring stand is on grid 19, position 2.

6. Set up the reagent troughs.
   a. Remove the reagent troughs from previous runs and correctly dispose of the reagents according to “Complete the run” on page 48.

   b. Place new 100-mL reagent troughs on the worktable for PrepFiler™ Wash Buffer B and other reagents as shown.

Note: The trough layout shown is different from the originally validated layout. The validation of the new trough layout is described in “Validation of PrepFiler™ Wash Buffer B and the related modifications to the workstation layout and scripts” on page 73.



- Elution buffer trough (grid 27, position 1)
- Prepared Wash Buffer B trough (grid 27, position 2)
- Prepared Wash Buffer A trough (grid 27, position 3)
- Isopropanol trough (grid 25, position 1)
- Lysate waste trough (grid 25, position 3)

The workstation should now match the setup shown in“Workstation layouts” on page 30.

Set up the disposable pipette tips

Terms for pipette tips used on the robotic workstation

- **DiTis**—Disposable Tips (DiTi), Tecan™ Pure, Filtered, 200- and 1,000-µL
- **DiTi tray**—Plastic tray containing 96 DiTis
- **DiTi rack**—Aluminum holder for a single tray of 1,000-µL DiTis
- **DiTi carrier**—Aluminum holder for three trays of 200-µL DiTis
- **Orientation nose**—Pin on a DiTi rack to hold the tray in place

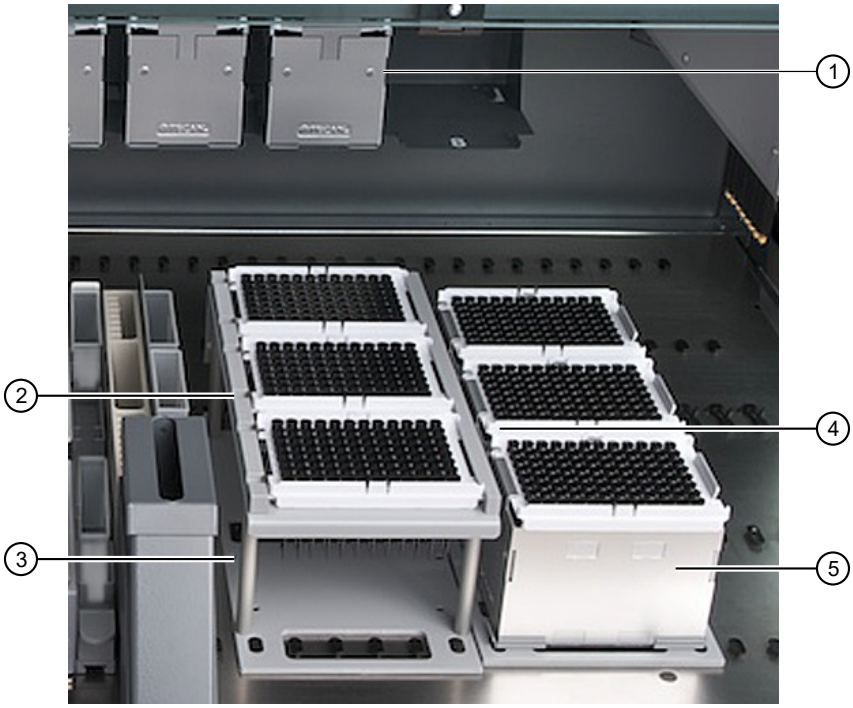


Figure 2 DiTi terms.

1. Racks that contain 1,000-µL DiTis on the rear shelf positions 5, 6, and 7
2. Notch in the DiTi tray
3. Carrier for 200-µL DiTi trays (trays are blue or white)
4. Orientation nose
5. Racks that contain 1,000-µL DiTi trays (trays are yellow or white)

Fill DiTi carriers and racks

Set up the pipette tips for an automated purification run.

IMPORTANT! If nine full DiTi trays are not correctly set up on the Freedom EVO™ robotic workstation, the workstation repeatedly searches for the missing DiTi tips, during which time the samples may become unusable.

1. Place three full trays of 1,000-µL DiTis into the DiTi racks on the rear shelf (shelf positions 5, 6, and 7). For each tray:
   a. Insert the tray into a rack: Ensure that the notch in the tray is aligned with the orientation nose on the rack, snap the tray into the rack, then confirm that the tray fits snugly.
   b. Place the rack on the shelf: Ensure that the orientation pin is positioned toward the back of the shelf, then push the rack *all the way* to the back of the shelf.

      IMPORTANT! Ensure that there are no objects placed on shelf positions 1–4 or position 8.
2. Place three full trays of 1,000-µL DiTis into the DiTi racks on grid 35, positions 1–3, as described in substep 1a. Ensure that the orientation pin is positioned in the top-left corner.
3. Place three full trays of 200-µL DiTis into the carrier on grid 29, positions 1–3. Ensure that the notch in the tray is positioned in the top-left corner.

   IMPORTANT! Ensure that the 3-position DiTi carrier on grid 29 contains *three* 200-µL DiTi trays.

Set up reagents on the workstation

Procedural guidelines

- Calculate the reagent volumes needed based on the number of samples you will process plus the specified overfill and dead volumes.

  Note: The dead volume is independent of the number of samples you run.
- Do not reuse isopropanol, PrepFiler™ Wash Buffer A, PrepFiler™ Wash Buffer B, or PrepFiler™ Elution Buffer from previous runs; always properly dispose of used reagents after each run.
- Do not use water instead of PrepFiler™ Elution Buffer. Instead of PrepFiler™ Elution Buffer, you can prepare low-TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) or purchase DNA Suspension Buffer (low-TE Buffer) from Teknova™.
- Use new reagent troughs each day.

Set up the reagents

1. Place empty troughs on the workstation according to the following table.

Empty trough	Location
PrepFiler™ Elution Buffer	Grid 27, position 1
PrepFiler™ Wash Buffer B	Grid 27, position 2
PrepFiler™ Wash Buffer A	Grid 27, position 3
Isopropanol	Grid 25, position 1
Lysate waste **IMPORTANT!** Do not add acids or bases to any wastes that contain PrepFiler™ Lysis Buffer (guanidine thiocyanate).	Grid 25, position 3

2. Calculate the required PrepFiler™ reagent volumes.

Reagent	Lysis protocol	No. of reactions	Reagent volume per reaction	Overfill volume per run[1]	Dead volume per run[2]	Minimum required volume for 96 samples (A×B)+(A×B×C) +D[3]
		A	B	C	D	
Isopropanol	Standard, 300-µL	Up to 96	180 µL	15%	5 mL	25 mL
	Large-sample, 500-µL[4]	Up to 96	300 µL	15%	5 mL	40 mL
Prepared Wash Buffer A	—	Up to 96	900 µL	15%	5 mL	105 mL
Prepared Wash Buffer B	—	Up to 96	300 µL	15%	5 mL	40 mL
Elution Buffer	—	Up to 96	50 µL	15%	5 mL	11 mL

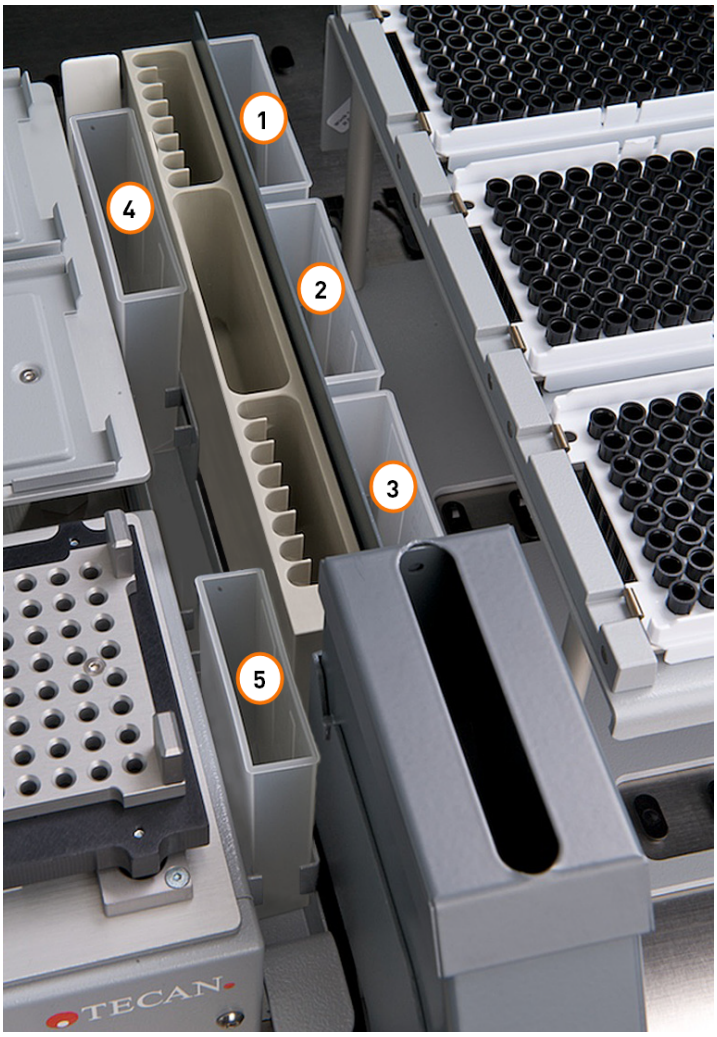
[1] Overfill (excess volume) is needed to compensate for evaporation and pipetting losses during the run.

[2] An extra 5 mL per trough is needed to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.

[3] Includes overfill and dead volume. For example, the required volume of isopropanol for 96 samples when using the standard lysis protocol is (96 × 180 µL) + (96 × 180 µL × 0.15) + 5 mL = 17.28 mL + 2.59 mL + 5 mL = 24.87 mL, rounded up to 25 mL.

[4] The large-sample (500-µL) protocols were not tested as part of our full validation studies. If your laboratory intends to use the large-sample protocols, perform the appropriate validation studies.

3. Add the amounts of PrepFiler™ reagents that you calculated in step 2 to the appropriate trough.



① Elution buffer trough
② Prepared Wash Buffer B trough
③ Prepared Wash Buffer A trough
④ Isopropanol trough
⑤ Lysate waste trough, empty

4. Gently invert two tubes of prepared PrepFiler™ Magnetic Particles to remove large air bubbles, briefly centrifuge the tubes at low speed to collect the contents at the bottom of the tubes, then open the tubes.
   - If a thin film or bubble (caused by surfactants) stretches across the top of the tube, gently break the surface with a clean pipette tip.
   - If there is foam (air bubbles) on the surface of the magnetic particles, remove the foam by pipetting. Surface foam may interfere with liquid level detection during the automated purification run.

5. Place the two tubes of PrepFiler™ Magnetic Particles on the workstation in the first two slots of the metal rack on grid 13, position 2.

IMPORTANT! Ensure that the tubes and the block containing the tubes are positioned as shown. Incorrect positioning may result in failure to pipette magnetic particles and/or collision of the Liquid Handling (LiHa) arm with the block.

Set up lysate, processing, and elution plates and/or tubes

Select a plate/tube configuration

The HID EVOlution™ systems support four plate/tube configurations for the Freedom EVO™ 150 robotic workstation or Freedom EVO™ 200 robotic workstation.

Select a configuration for each automated purification run.

Configuration	Description	Starting labware: Plate or tube that contains the lysate from the sample lysis step	Ending labware: Plate or tube to collect DNA eluate at the end of the run[1]
Plate-to-plate	Process lysate from a 96-well plate and collect eluate in a 96-well plate	PrepFiler™ Spin Plate	MicroAmp™ Optical 96-Well Reaction Plate
Plate-to-tubes	Process lysate from a 96-well plate and collect eluate in 1.5-mL tubes	PrepFiler™ Spin Plate	Nonstick RNase-free Microfuge Tubes (1.5-mL)
Tubes-to-tubes	Process lysate from 1.5-mL tubes and collect eluate in 1.5-mL tubes	Nonstick RNase-free Microfuge Tubes (1.5-mL)	Nonstick RNase-free Microfuge Tubes (1.5-mL)
Tubes-to-plate	Process lysate from 1.5-mL tubes and collect eluate in a 96-well plate	Nonstick RNase-free Microfuge Tubes (1.5-mL)	MicroAmp™ Optical 96-Well Reaction Plate

[1] Your choice is independent of whether the sample lysate is contained in a plate or in tubes

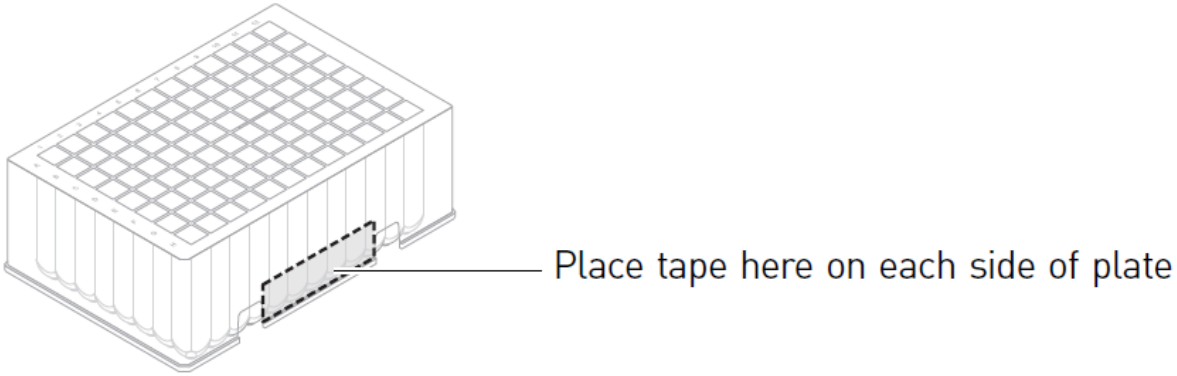
"Workstation layouts" on page 30 shows the placement of plates and tubes for each configuration.

Set up the PrepFiler™ Processing Plate

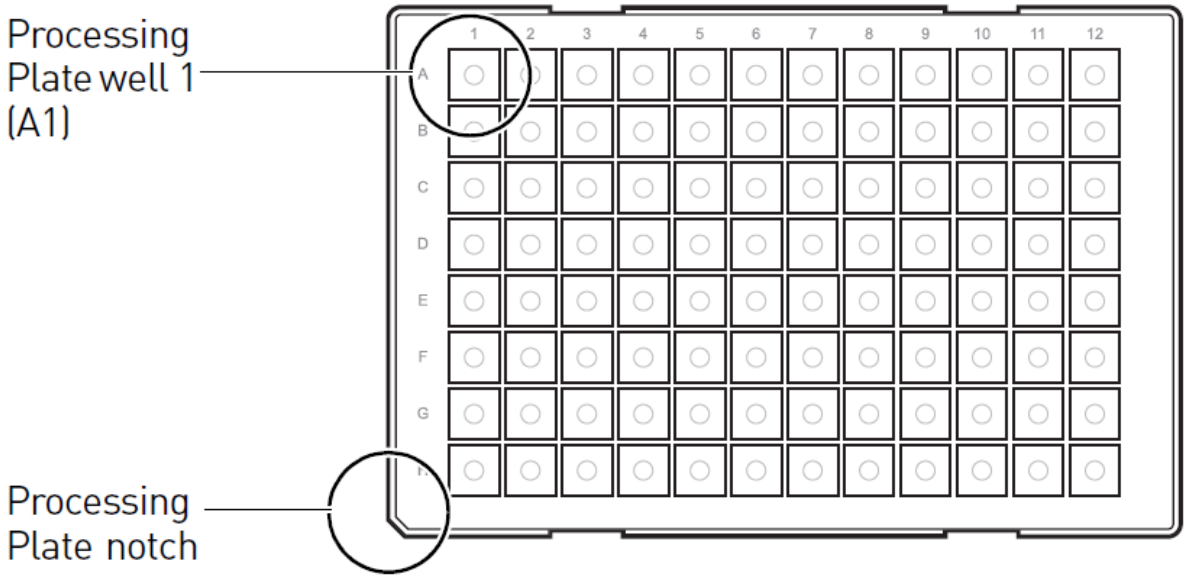
The PrepFiler™ Processing Plate is a square-well plate that is required to process reactions for all four automated purification run configurations.

During the washing and elution steps, the Robotic Manipulator arm (RoMa) moves the processing plate to the 96-Well Magnetic Ring Stand or Te-Shake™ plate adapter.

1. If needed to ensure that the RoMa grips the plate tightly, place a strip of laboratory labeling tape on each side of the PrepFiler™ Processing Plate as shown.



2. Place the PrepFiler™ Processing Plate on the Te-Shake™ plate adapter with well A1 in the top-left position (grid 19, position 3).



3. To ensure that samples are transferred to the correct wells, confirm that:
   - The processing plate is placed on the Te-Shake™ plate adapter with well A1 positioned in the top-left corner
   - The plate wells are aligned with the holes in the Te-Shake™ plate adapter

(If needed) Place barcodes

Perform this procedure if you use barcodes on the plates and/or tubes to track the samples in the HID EVOlution™ software. The system scans the barcodes to automatically capture sample information. For more information, see the *Tecan™ HID EVOlution™ —Extraction Application Manual*, Section 4.5, "Barcodes".

IMPORTANT!

- *If the lysate is in spin/filter plates:* Before the plate barcode is scanned during a run, you must manually enter or import the sample information for each well in the plate. (See "Set up sample and reagent information" on page 40, step 1.)
- *If the lysate is in tubes:* The sample name (barcode) and sample position for each tube are automatically updated in the HID EVOlution™ software when the barcodes are scanned.

1. Select barcodes that are compatible with the PosID-3.
2. Before placing items on the robotic workstation, ensure that the barcodes are correctly placed on the appropriate labware.

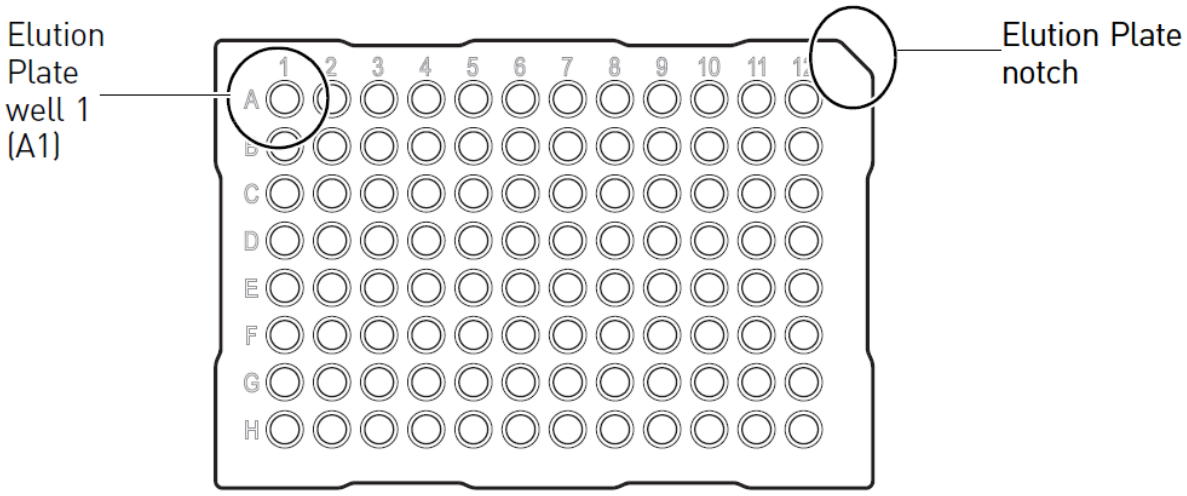
Component	Plate	Tube
Sample lysate	PrepFiler™ Spin Plates	Nonstick RNase-free Microfuge Tubes (1.5-mL)
DNA eluate	MicroAmp™ Optical 96-Well Reaction Plate	Nonstick RNase-free Microfuge Tubes (1.5-mL)

Set up lysate and/or eluate plates

IMPORTANT! To ensure that samples are transferred to the correct wells, confirm the following for each lysate or eluate plate:

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

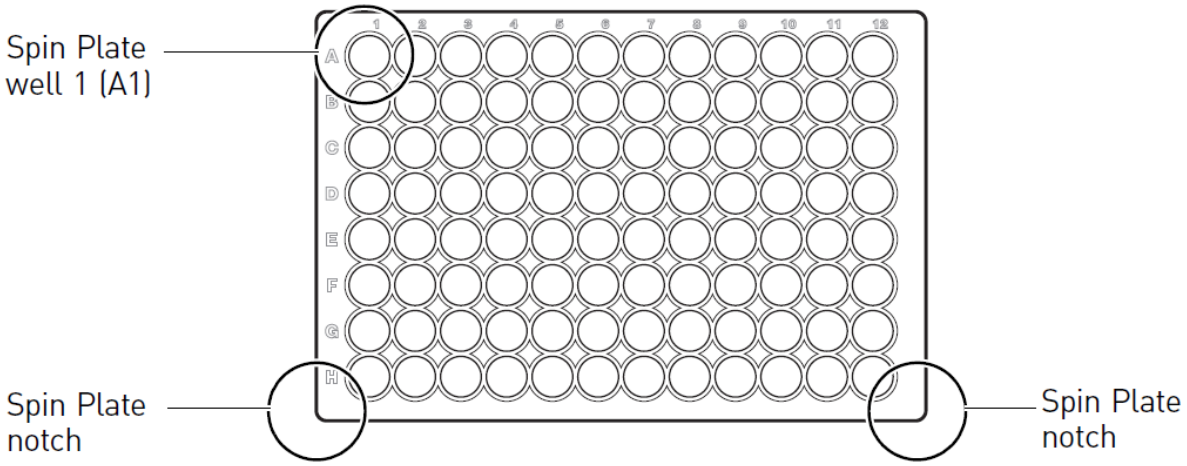
1. If you want DNA eluate to be collected in a plate, place a MicroAmp™ Optical 96-Well Reaction Plate with well A1 in the top-left position (grid 13, position 1).



Note: The DNA eluate corresponding to the first sample is always placed in the first well (A1) of the elution plate. The Report file (PDF) that is generated at the end of the purification run lists the starting position of each sample lysate and the final position of the corresponding DNA eluate.

Note: Using 96-well plates from other manufacturers may result in liquid handling errors if the instrument is not recalibrated for use with the alternate plates.

2. If the lysate is in a PrepFiler™ Spin Plate, place the spin plate with well A1 in the top-left position (grid 13, position 3).



Set up lysate and/or eluate tubes

Set up eluate tubes in tube racks

1. Ensure that:
   - You have new, labeled 1.5-mL microfuge tubes equal to the number of DNA samples to be processed.
   - The tube racks S1–S6 are correctly positioned at grid positions 1–6.

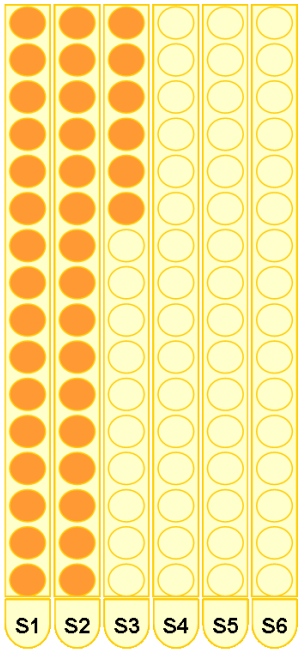
2. Place the first empty 1.5-mL microfuge tube in the tube racks in rack S1, position 1.

   Note: The DNA eluate corresponding to the first sample is always placed in the first tube (1) in the first tube rack (S1). The Report file (PDF) that is generated at the end of the purification run lists the starting position of each sample lysate and the final position of the corresponding DNA eluate.

3. Continue placing empty tubes from back to front in *vertical columns* as shown. Place one empty tube for each sample to be processed. Do not leave empty positions between sample tubes.

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

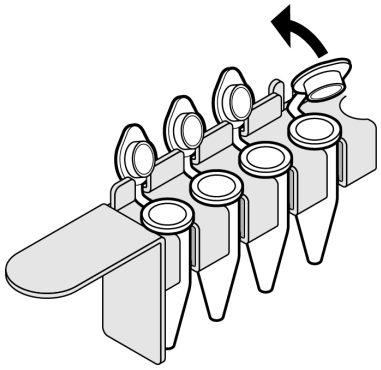
   Example of correct setup



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

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

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



Set up lysate tubes in tube racks

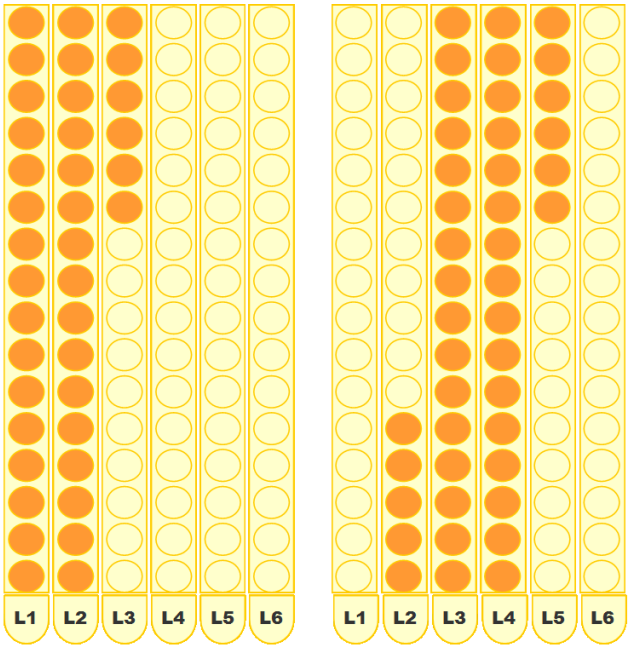
1. Ensure that:
   - You have ≤96 labeled 1.5-mL microfuge tubes that contain DNA sample lysate.
   - The tube racks L1–L6 are correctly positioned at grid positions 7–12.

2. Place the first sample tube in the tube racks. (Unlike the first eluate tube, which must be placed in rack S1, position 1, the first lysate tube may be placed in any position; for example, you can begin with rack L1, position 8.)

3. Continue placing sample tubes from back to front in *vertical columns* as shown. Do not leave empty positions between sample tubes.

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

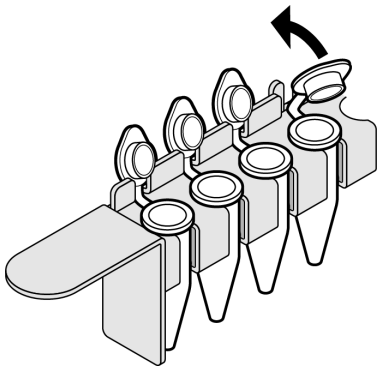
   Examples of correct setup



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

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

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



Workstation layouts

The following figures show the available workstation layouts for the automated purification run configurations.

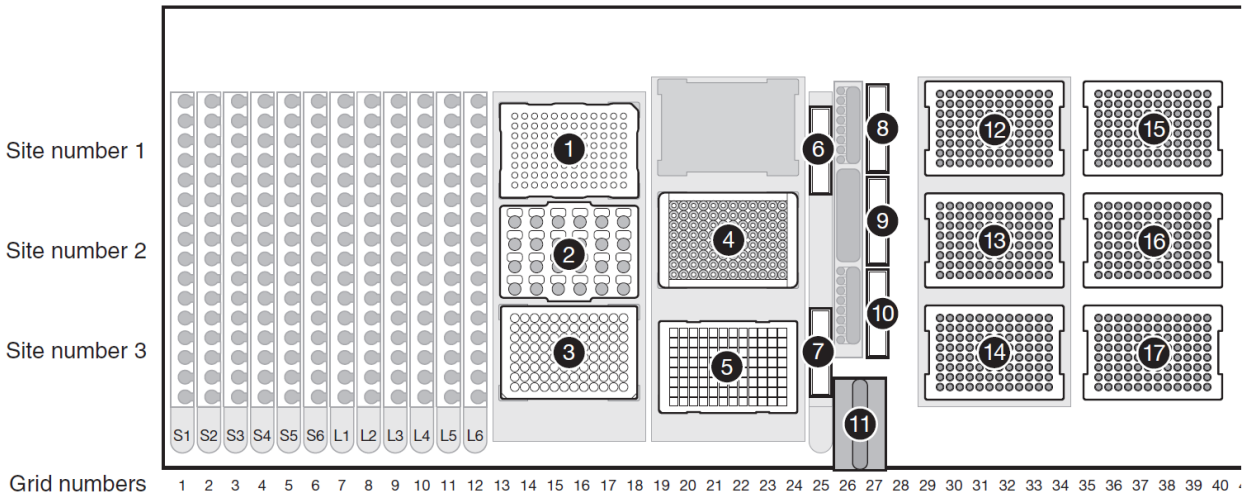


Figure 3 Plate-to-plate workstation layout

① 96-Well Elution Plate
② Block for PrepFiler™ Magnetic Particles
③ PrepFiler™ Spin Plate
④ Magnetic Ring Stand
⑤ PrepFiler™ Processing Plate on Te-Shake™ plate adapter
⑥ Isopropanol trough
⑦ Lysate waste trough
⑧ Elution Buffer trough
⑨ Wash Buffer B trough
⑩ Wash Buffer A trough
⑪ DiTi waste unit
⑫ 200-µL disposable pipette tips (DiTis)
⑬ 200-µL disposable pipette tips (DiTis)
⑭ 200-µL disposable pipette tips (DiTis)
⑮ 1,000-µL DiTis
⑯ 1,000-µL DiTis
⑰ 1,000-µL DiTis

Not shown: Rear shelf with 1,000-µL DiTis in shelf positions 5, 6, and 7

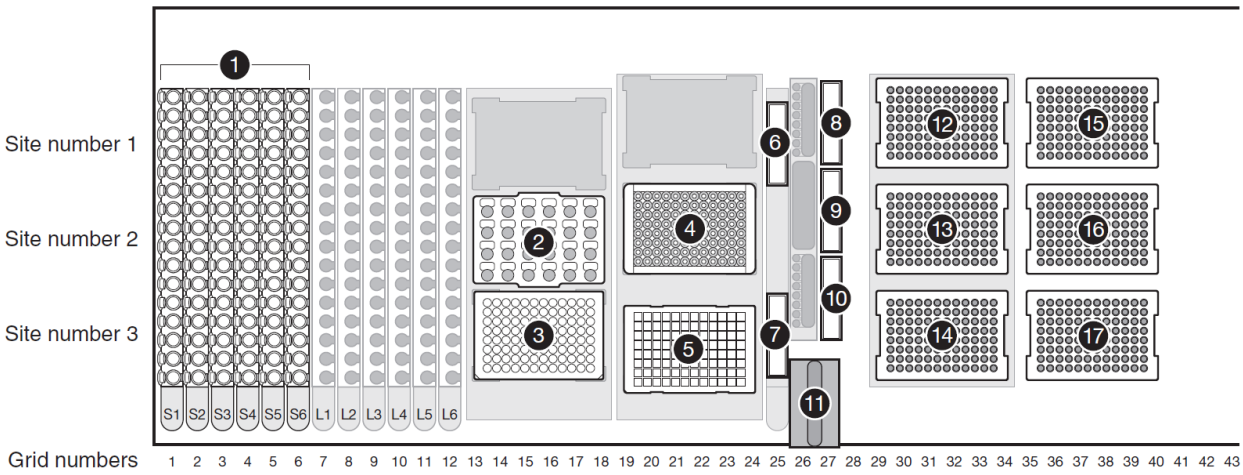


Figure 4 Plate-to-tubes workstation layout

1. Elution tube racks S1 to S6 with microcentrifuge tubes
2. Block for PrepFiler™ Magnetic Particles
3. PrepFiler™ Spin Plate
4. Magnetic Ring Stand
5. PrepFiler™ Processing Plate on Te-Shake™ plate adapter
6. Isopropanol trough
7. Lysate waste trough
8. Elution Buffer trough
9. Wash Buffer B trough
10. Wash Buffer A trough
11. DiTi waste unit
12. 200-µL disposable pipette tips (DiTis)
13. 200-µL disposable pipette tips (DiTis)
14. 200-µL disposable pipette tips (DiTis)
15. 1,000-µL DiTis
16. 1,000-µL DiTis
17. 1,000-µL DiTis

Not shown: Rear shelf with 1,000-µL DiTis in shelf positions 5, 6, and 7

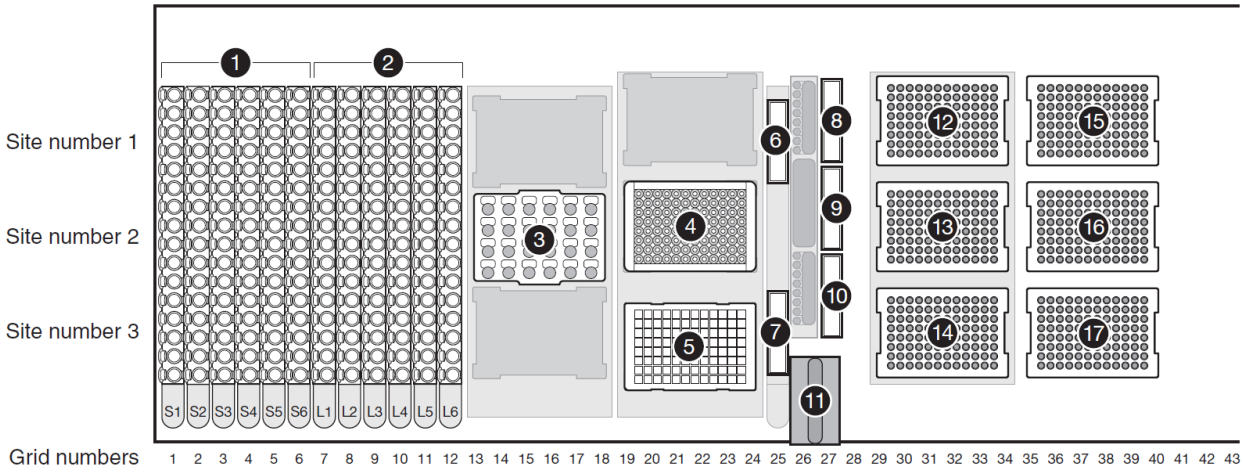


Figure 5 Tubes-to-tubes workstation layout

① Elution tube racks S1 to S6 with microcentrifuge tubes
② Lysate tube racks L1 to L6 with microcentrifuge tubes
③ Block for PrepFiler™ Magnetic Particles
④ Magnetic Ring Stand
⑤ PrepFiler™ Processing Plate on Te-Shake™ plate adapter
⑥ Isopropanol trough
⑦ Lysate waste trough
⑧ Elution Buffer trough
⑨ Wash Buffer B trough
⑩ Wash Buffer A trough
⑪ DiTi waste unit
⑫ 200-µL disposable pipette tips (DiTis)
⑬ 200-µL disposable pipette tips (DiTis)
⑭ 200-µL disposable pipette tips (DiTis)
⑮ 1,000-µL DiTis
⑯ 1,000-µL DiTis
⑰ 1,000-µL DiTis

Not shown: Rear shelf with 1,000-µL DiTis in shelf positions 5, 6, and 7

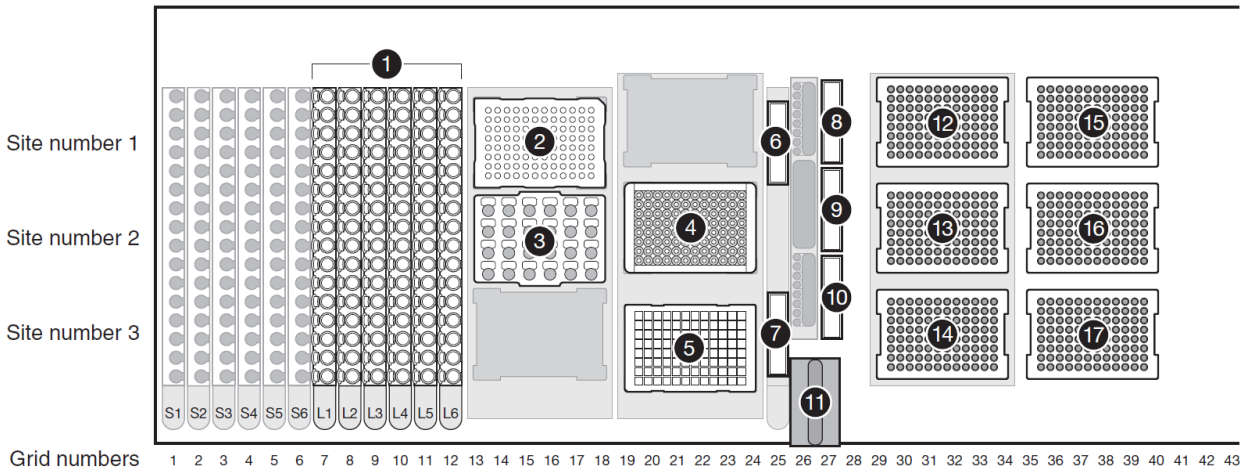


Figure 6 Tubes-to-plate workstation layout

1. Lysate tube racks L1 to L6 with microcentrifuge tubes
2. 96-Well Elution Plate
3. Block for PrepFiler™ Magnetic Particles
4. Magnetic Ring Stand
5. PrepFiler™ Processing Plate on Te-Shake™ plate adapter
6. Isopropanol trough
7. Lysate waste trough
8. Elution Buffer trough
9. Wash Buffer B trough
10. Wash Buffer A trough
11. DiTi waste unit
12. 200-µL disposable pipette tips (DiTis)
13. 200-µL disposable pipette tips (DiTis)
14. 200-µL disposable pipette tips (DiTis)
15. 1,000-µL DiTis
16. 1,000-µL DiTis
17. 1,000-µL DiTis

Not shown: Rear shelf with 1,000-µL DiTis in shelf positions 5, 6, and 7

4 Perform the automated DNA purification run

For more information

This chapter provides general procedures for performing an automated purification run on the HID EVOlution™ systems.

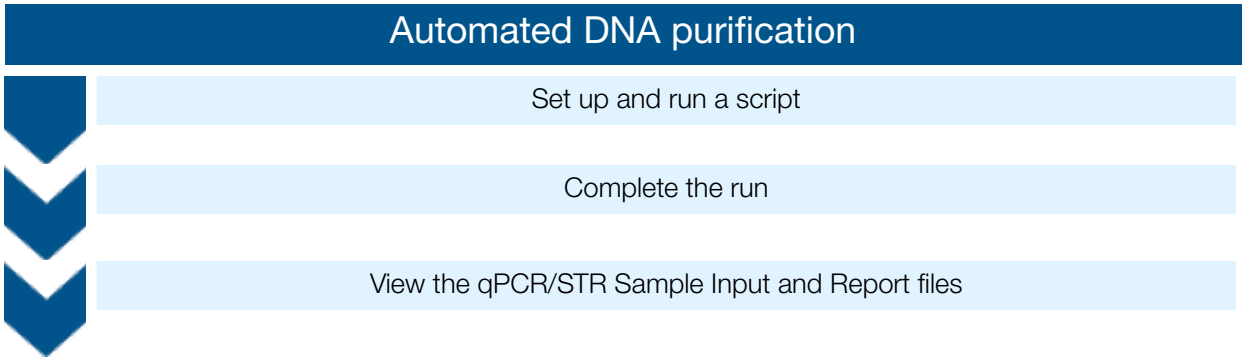
For more information, see the appropriate manufacturer's documentation.

Document	Description	Section
Tecan™ HID EVOlution™—Extraction Application Manual (395372, v2.0, June 2010)	Preparing a sample setup file, including sample naming requirements	3.4, “Sample File”
	Manually entering sample information	5.3, “Running a HID EVOlution—Extraction Script”
	Barcode positioning	4.6, “Barcodes”
	Running extraction scripts	5.3, “Running a HID EVOlution—Extraction Script”
	Script error messages	8.4, “Application Software”
	qPCR/STR Sample Input and Report files	6, “Results”
	Routine cleanup	5.4.2, “Clean Up the Worktable”
	Routine maintenance	7, “Maintenance”
Tecan™ HID EVOlution™—Combination Application Manual (395967, v2.0, June 2010)	Preparing a sample setup file, including sample naming requirements	4.4, “Extraction Sample File”

(continued)

Document	Description	Section
Tecan™ HID EVOlution™—Combination Application Manual (395967, v2.0, June 2010)	Manually entering sample information	6.3, "Running a HID EVOlution—Extraction Script"
	Barcode positioning	5.6, "Barcodes"
	Running extraction scripts	6.3, "Running a HID EVOlution—Extraction Script"
	Script error messages	13.4, "Application Software"
	qPCR/STR Sample Input and Report files	7, "Results from HID EVOlution—Extraction"
	Routine cleanup	10.4, "Removing the Reagents and Cleaning Up the Worktable"
	Routine maintenance	12, "Maintenance"
Tecan™ Freedom EVO™ Operating Manual	Barcode specifications	3.5.6, "Positive Identification (PosID)"
Tecan EVOware™ Standard/EVOware™ Plus 2.1 Software Manual	EVOware™ software	—
Tecan EVOware™ Standard/EVOware™ Plus 2.1 Software Getting Started Guide		—
Tecan HID EVOlution™ Application Guide—Automation for Applied Biosystems Human Identification Kits	HID EVOlution™—qPCR/PCR Setup System	—

Workflow



Before you begin

- If you want to enter reagent information, have the reagent lot numbers and expiration dates available before running the script.
- Ensure that the instrument shield is closed.
- **If a collision occurred during the previous run,** a trained user or Tecan™ Service™ Representative should check the x, y, and z positions before you start a new run. Alternatively, use water in place of reagents and perform a mock run to confirm proper positioning of the robotic movements.
- **If you observe bubbles in the system liquid (degassed water) tubing,** place the system liquid carboy at the same height as the workstation, then replenish the system liquid as needed before each run to avoid liquid levels dropping below one-quarter carboy during the run. The time needed for complete degassing varies depending on the climate in each laboratory and geographical location. In some situations, it may take up to 3 days to fully degas the system liquid. We recommend that each laboratory maintain an additional carboy of fully degassed system liquid to use for replenishment.

Set up and run a script

About script files

Script files contain the workflow instructions for a specific robot, and they can be read only by the software of that robot. For example, Thermo Fisher Scientific provides scripts specifically for use with the PrepFiler™ kits (see page 36 for the list of scripts). ThePrepFiler™ kit scripts are for use with theFreedom EVO™ 150 robotic workstation and Freedom EVO™ 200 robotic workstation, and they can be read only by Freedom EVOware™ software v2.1 with the HID EVOlution™—Extraction application.

You select a script based on three criteria:

- The protocol that you used to prepare sample lysate
- The HID EVOlution™ system that you are using
- The labware that you want to use on the workstation for sample lysate and eluate

Select a script

1. On your desktop, click  to start the EVOware™ Standard software, then enter your user name and password.
2. Select **Edit an existing script**, then click  .

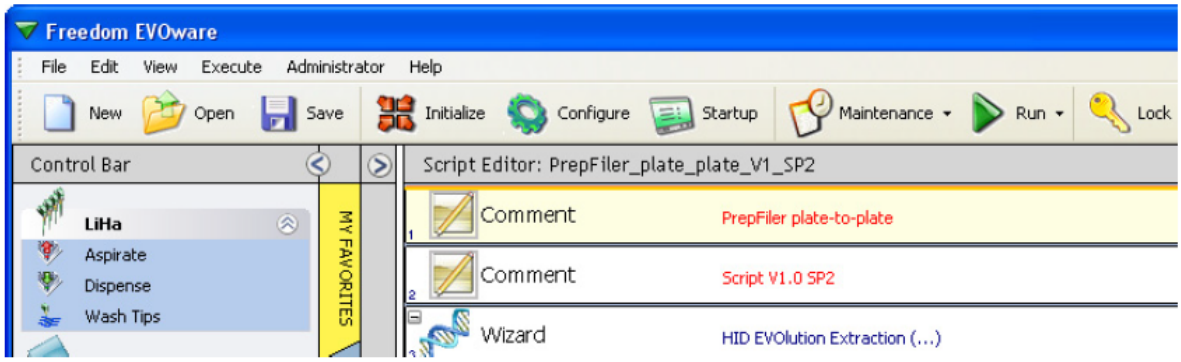
3. In the **Selection** dialog box, select the appropriate script for your HID EVOlution™ system, plate/tube configuration, and lysis protocol, then click ▶.

If you used this lysis protocol...	And you want the eluted DNA in...	Then use this script...	
		HID EVOlution™—Extraction System[1]	HID EVOlution™—Combination System[1]
1.5-mL tubes—standard protocol, 300-µL	1.5-mL microfuge tubes	PrepFiler_tubes_tubes_V1_SP2	PrepFiler_tubes_tubesCombo_V1_SP1
	A 96-well plate	PrepFiler_tubes_plate_V1_SP2	PrepFiler_tubes_plateCombo_V1_SP1
96-well plate—standard protocol, 300-µL	1.5-mL microfuge tubes	PrepFiler_plate_tubes_V1_SP2	PrepFiler_plate_tubesCombo_V1_SP1
	A 96-well plate	PrepFiler_plate_plate_V1_SP2	PrepFiler_plate_plateCombo_V1_SP1
1.5-mL tubes—large sample protocol, 500-µL[2]	1.5-mL microfuge tubes	PrepFiler_tubes_tubes500_V1_SP2	PrepFiler_tubes_tubes500Combo_V1_SP1
	A 96-well plate	PrepFiler_tubes_plate500_V1_SP2	PrepFiler_tubes_plate500Combo_V1_SP1
96-well plate—large sample protocol, 500-µL[2]	1.5-mL microfuge tubes	PrepFiler_plate_tubes500_V1_SP2	PrepFiler_plate_tubes500Combo_V1_SP1
	A 96-well plate	PrepFiler_plate_plate500_V1_SP2	PrepFiler_plate_plate500Combo_V1_SP1

[1] Version 1 ("V1") scripts or later. Contact Technical Support for more information on validated and verified scripts.

[2] The large-sample (500-µL) scripts were not tested as part of our validation studies. If you intend to use the large-sample scripts, perform the appropriate validation studies.

4. In the **Freedom EVOware™** script dialog box, click ▶ to run the script, then click ▶ in the **EVOware™ Runtime Controller**.



The Freedom EVOware™ Runtime Controller opens, the system initializes, and the liquid-handling arm (LiHa) and Robotic Manipulator arm (RoMa) move.

Note: After clicking to run a script, you can:

- Cancel the run at any time by clicking .
- Pause the run by bringing the **EVOware™ Runtime Controller** dialog box to the front of your desktop, then clicking .

For details on cancelling or pausing a run, see "(If needed) Re-cap the magnetic particles tubes" on page 46.

Set up sample and reagent information

About sample information

Sample information is used by the HID EVOlution™—Extraction System or HID EVOlution™—Combination System to:

- Set up the elution plate or tubes.
- Generate a Report file (PDF) at the end of the purification run.
- *(HID EVOlution™—Combination System only)* Generate a qPCR/STR Sample Input file (CSV) at the end of the purification run.

For more information on the CSV and PDF files, see "Record file information and exit the script" on page 47 and "View the qPCR/STR Sample Input and Report files" on page 49.

(If needed) Create a sample input file from a template

Perform this procedure if you import sample input files to set up sample information in the HID EVOlution™ software. (See "Set up sample and reagent information" on page 40, step 1.)

IMPORTANT!

- Create the sample input file before starting the purification run.
- Use a text editor such as Microsoft™ Notepad to edit the sample input file. Do not use Microsoft™ Excel™, which may introduce invalid formatting.

1. Set up the template files on your system:
   a. Create the following folders for the original and edited template files:
      - `<installation drive>:\PrepFilerTemplateFiles` for the original template files that are provided with the HID EVOlution™ system software CD
      - `<installation drive>:\PrepFilerInputFiles` for your edited template files
   b. Copy the following template files from the software CD to the folder that you created for the template files:
      - **Sample File_Plate_96.csv**—for sample lysate in a 96-well plate
      - **Sample File_Tubes_96.csv**—for sample lysate in 1.5-mL tubes
2. Open the appropriate template file:
   a. Select **Start** ▸ **All Programs** ▸ **Accessories** ▸ **Notepad** to open Microsoft™ Notepad.

b. Select **File ▸ Open**, then navigate to `<installation drive>:\PrepFilerTemplateFiles`.

c. Select the appropriate template file for sample lysate in a plate or in tubes, then click **Open**.

3. Select **File ▸ Save As**, navigate to `<installation drive>:\PrepFilerInputFiles`, change the file name to `<User Defined>`.csv (where `<User Defined>` is a unique file name of your choosing), then click **Save**.

4. Enter the sample information in the duplicate CSV file. Follow the formatting rules that are described in the *Tecan™ HID EVOlution™—Extraction Application Manual*, Section 3.4, "Sample File".
   - Do not include empty plate well or tube rack positions between samples.
   - Avoid spaces or other special characters such as commas (,), asterisks (*), or slashes (/).
   - Follow your laboratory naming conventions to assign a unique sample name to each sample. For samples in plates, assign a unique sample name to all wells that contain samples or blank reagents.
   - The sample name and sample position in the sample input file must match the samples on the workstation.

5. 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.

Set up sample and reagent information

1. In the **Sample information** page, use one of the options in Table 4 to enter sample information.

Table 4 Options for entering sample information

Option	Action
Manually enter sample information	1. Click **Edit** next to the plate or next to each tube rack. See #1 in Figure 7. 2. Enter sample information as described in the *Tecan™ HID EVOlution™—Extraction Application Manual*, Section 5.3, "Running a HID EVOlution™—Extraction Script". **IMPORTANT!** When entering sample information, assign a unique sample name to all samples. For samples in plates, assign a unique sample name to all wells that contain samples or reagent blanks. (For sample naming requirements, see the *Tecan™ HID EVOlution™—Extraction Application Manual*, Section 3.4, "Sample File".) 3. Click **OK**. 4. Deselect the **Scan labware** checkbox. Otherwise, the system will attempt to scan barcodes and will overwrite the manually entered sample information.
Import a sample input file	1. Create a sample input file according to "(If needed) Create a sample input file from a template" on page 38. 2. Click ..., then navigate to `<installation drive>:\PrepFilerInputFiles`. See #2 in Figure 7. 3. Select the sample input file that you created, then click **OK**. 4. Ensure that the actual sample names and workstation positions match those in the imported sample input file. 5. Deselect the **Scan labware** checkbox. Otherwise, the system will attempt to scan barcodes and will overwrite the imported sample information.
Scan barcodes	1. Place barcodes on the plates and/or tubes according to page 25. 2. Select the **Scan labware** checkbox. See #3 in Figure 7. During a run, the system will scan the barcodes to capture sample information. **IMPORTANT!** • *If the lysate is in spin/filter plates:* Before the plate barcode is scanned during a run, you must manually enter or import the sample information for each well in the plate. • *If the lysate is in tubes:* The sample name (barcode) and sample position for each tube are automatically updated in the HID EVOlution™ software when the barcodes are scanned.

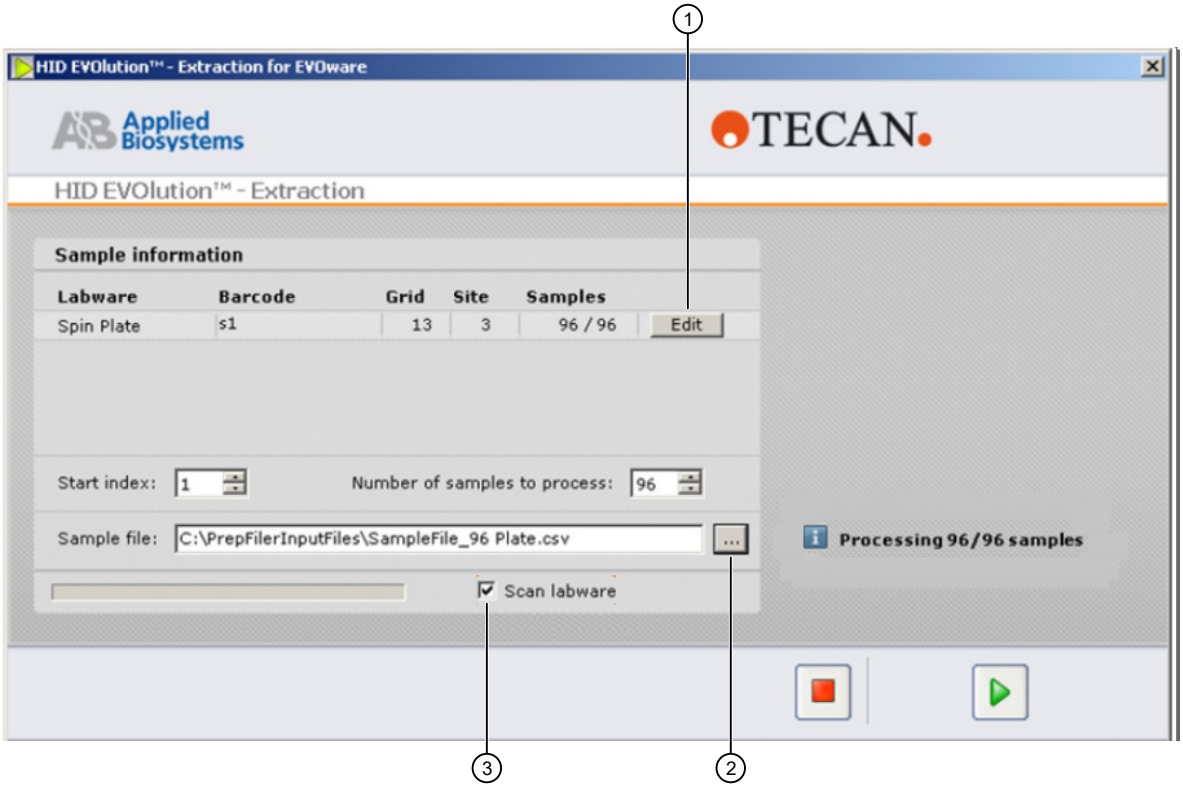


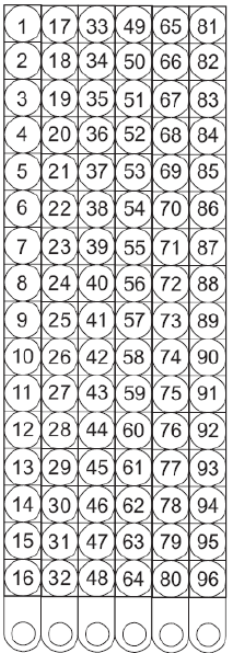
Figure 7 Sample information page (ways to enter sample information)

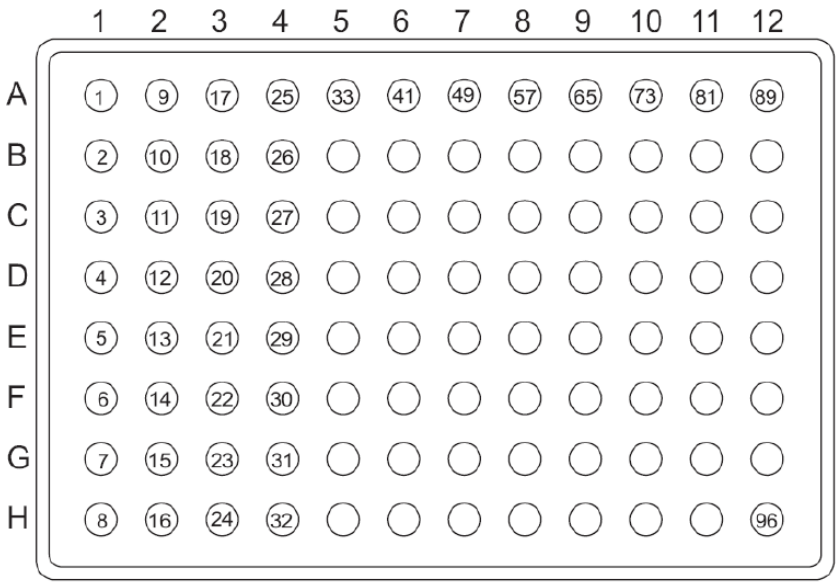
① Manually enter sample information
② Import a sample input file
③ Scan barcodes

IMPORTANT! To avoid situations where the system overwrites previously entered or imported sample information, be aware of the following:

- If you manually enter sample information, then import a sample input file, the information that you manually entered is overwritten by the imported file.
- If your system is set up to use barcodes, any information that you manually entered or imported is overwritten when the sample lysate barcodes are scanned.

2. Enter the position and number of samples.
   a. In the **Start index** field, select a number between 1–96 that corresponds to one of the following:
      - The tube position of the first tube in the sample racks
      - The well position of the first sample in the plate





   b. In the **Number of samples to process** field, select a number between 1–96 that corresponds to the total number of samples that you are running.

   c. Ensure that the number of samples to process (maximum of 96) is correctly shown (for example, if you are processing 16 samples, the message should read "Processing 16/96 samples").

d. Click ▶ to continue.

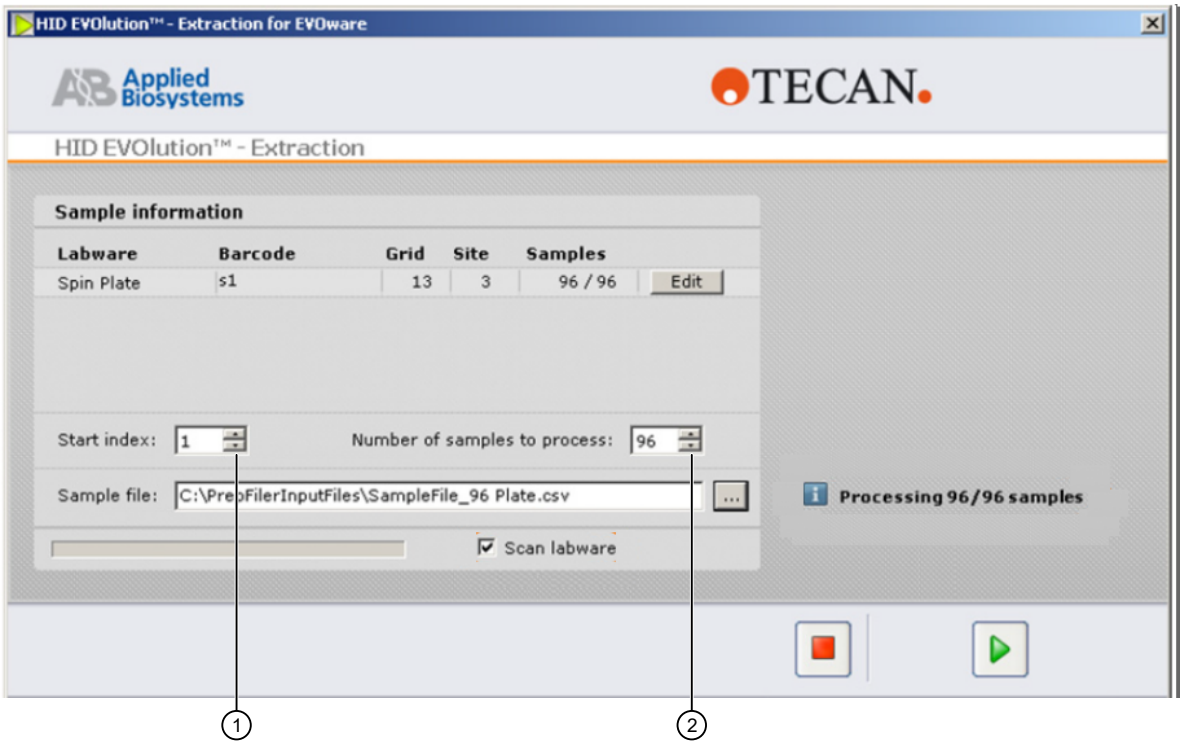


Figure 8 Sample information page (position and number of samples)

① First sample start position
② Number of samples

3. *(Optional)* Record information about the PrepFiler™ kit components that are used for this purification run:

Note: If needed, you can use the kit information for your records and for help with troubleshooting.

a. Click **Record Reagent Information**.

b. In the **Record Reagent Information** page, enter the appropriate lot numbers and expiration dates. Scroll down to see all the fields.

c. Click **OK**, then click ▶ to continue.

Confirm workstation setup and start the run

1. In the **Load worktable** page, compare the listed items to the items on the actual workstation.
   a. Confirm that you correctly loaded the following items:

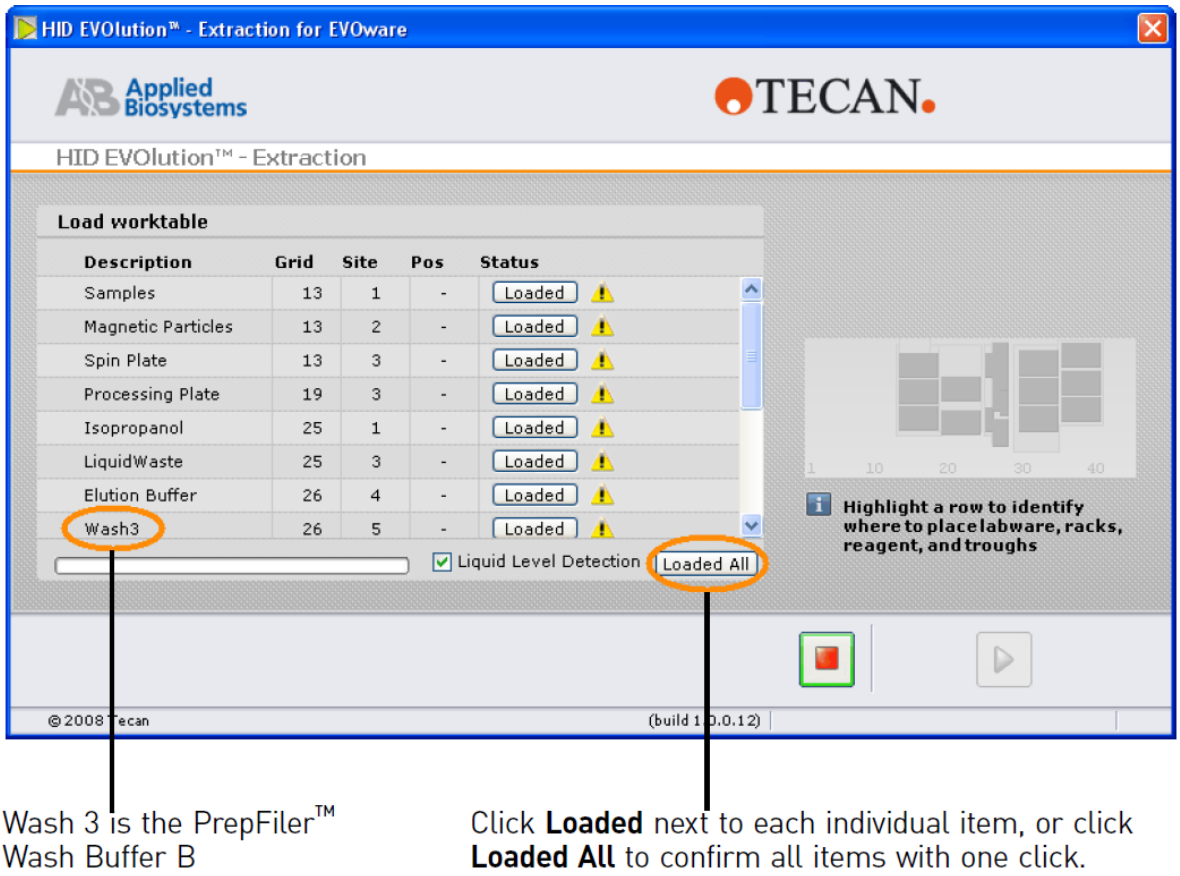
Item	Displayed as
PrepFiler™ 96-Well Processing Plate	Processing Plate
MicroAmp™ Optical 96-Well Reaction Plate or 1.5-mL microfuge tubes to collect the DNA eluate	Samples or tube racks S1–S6
PrepFiler™ Spin Plate or 1.5-mL microfuge tubes that contain the sample lysate	Spin Plate or tube racks L1–L6
Two tubes of PrepFiler™ Magnetic Particles Particles	Magnetic Particles
Isopropanol	Isopropanol
Prepared PrepFiler™ Wash Buffer A	Wash Buffer
Prepared PrepFiler™ Wash Buffer B	Wash3
PrepFiler™ Elution Buffer	Elution Buffer

Note: You can place the cursor on an item in the list to highlight the item in the workstation diagram on the right side of the page.

   b. Ensure that **Liquid Level Detection** is selected.

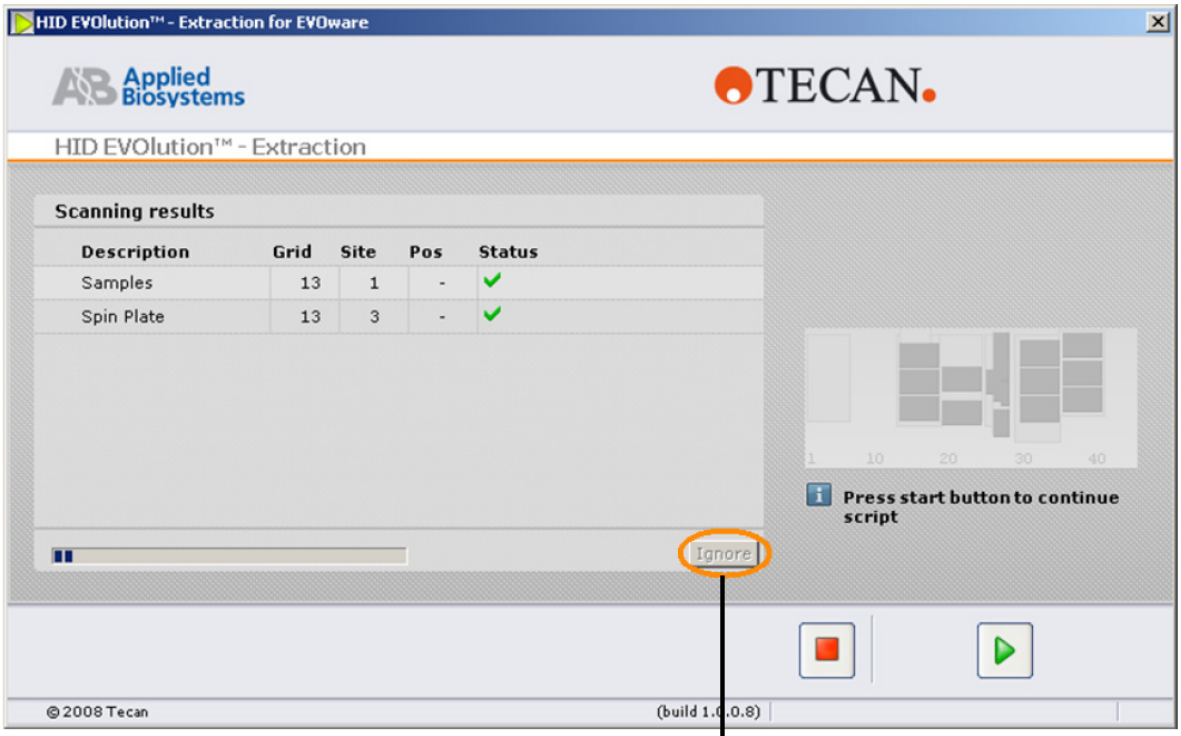
IMPORTANT! When **Liquid Level Detection** is selected, the system checks the isopropanol, wash buffer, and elution buffer liquid levels before starting the run. The system alerts you if the reagent volumes are insufficient for the number of samples that you entered.

2. Click **Loaded All**, then click ▶ to start the inventory scan.



3. In the **Scanning results** page, wait for scanning to finish, then do one of the following:
   - **If you are not using barcodes**—Click **Ignore**, then click ▶ to start the run.
   - **If you are using barcoded plates and/or tubes to track your samples, and the Status column displays:**
     - Only green, click ▶ to start the run.
     - One or more red warnings, confirm that all barcodes are present and in the correct position, then click ▶ to rescan the barcodes.

Note: During the run, the run status is shown next to .



Click **Ignore** to ignore the scanning results unless you use barcoded plates and/or tubes to track your samples.

(If needed) Re-cap the magnetic particles tubes

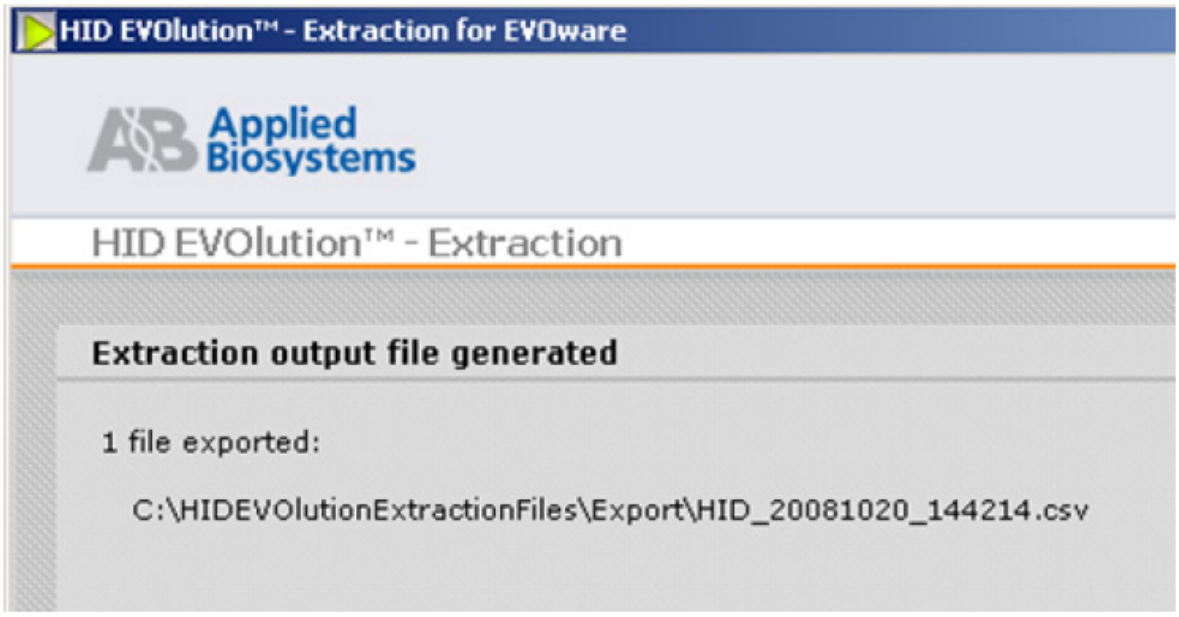
Perform this step during the run if you observe crystal formation on the PrepFiler™ Magnetic Particles tube.

1. After the magnetic particles have been dispensed into all samples, click in the **EVOware™ Runtime Controller** to pause the run.
2. Re-cap the magnetic particles tubes to avoid forming a crust around the rim of the tubes.
3. Click in the **EVOware™ Runtime Controller** to continue the run.

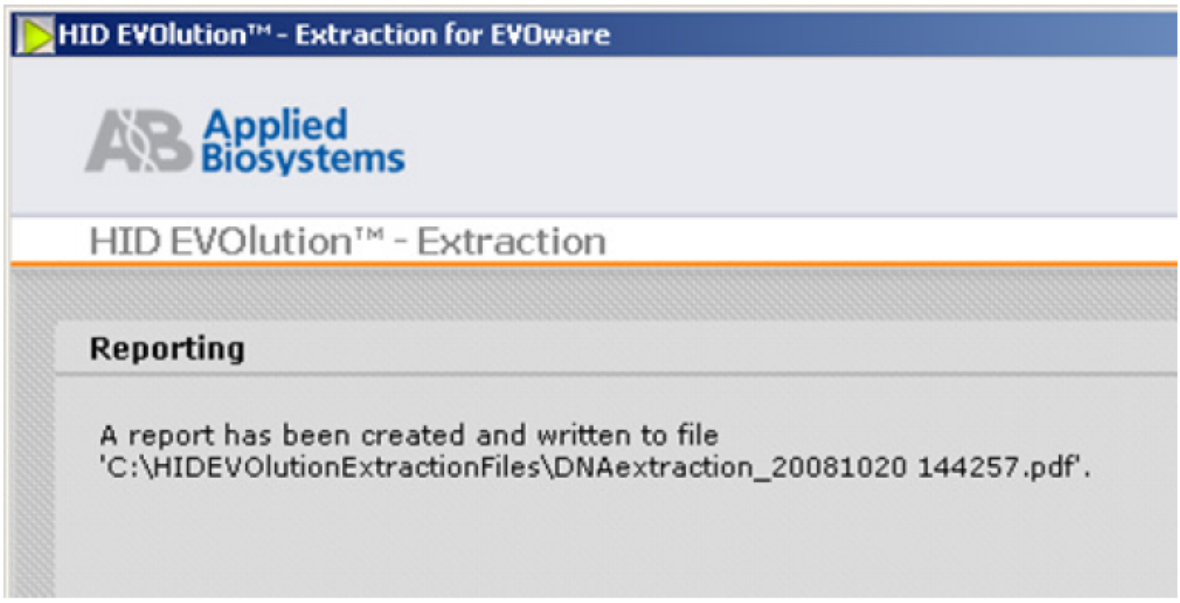
IMPORTANT! Do not click in the **EVOware™ Runtime Controller** or **Freedom EVOware™ script** dialog box. Clicking causes the run to stop and it cannot be restarted.

Record file information and exit the script

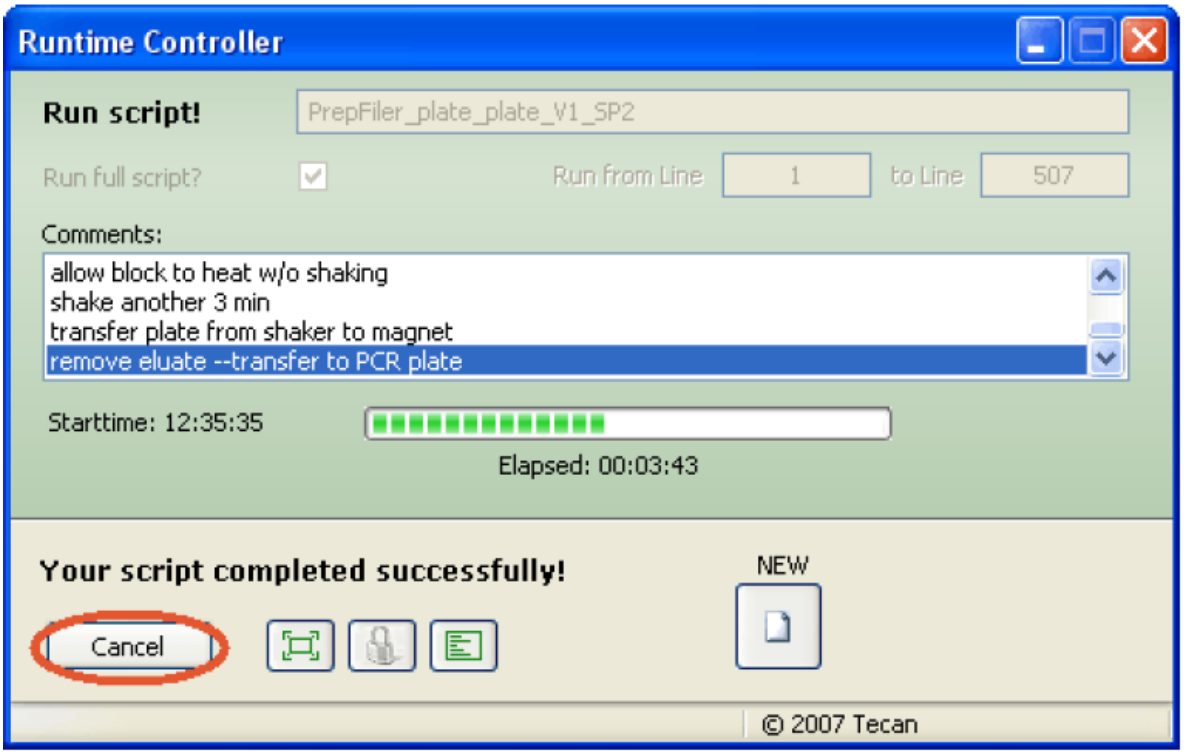
1. *(HID EVOlution™—Combination System only)* In the **Extraction output file generated** page, the software displays the file path and name for the qPCR/STR Sample Input file. Record the file path and name, then click ▶.



2. In the **Reporting** page, the software displays the file path and name for the Report file. Record the file path and name, then click ▶.



3. In the **Runtime Controller** dialog box, click **Cancel** to exit the script.



For more information on the files, see “View the qPCR/STR Sample Input and Report files” on page 49.

Complete the run

1. Open the front panel of the Freedom EVO™ workstation.
2. Remove the MicroAmp™ Optical 96-Well Reaction Plate or 1.5-mL microfuge tubes that contain the DNA eluate from the worktable, seal the plate or tubes, then store them ≤2 weeks at 4°C. For longer storage, store at –20°C.
3. If not capped previously, cap and store the PrepFiler™ Magnetic Particles tubes.
4. Properly dispose of the PrepFiler™ Spin Plate (if used) and PrepFiler™ Processing Plate.
5. Properly dispose of any unused isopropanol, wash buffer, and elution buffer in the reagent troughs.

   IMPORTANT! Do not reuse the reagents in the troughs.
6. *(Last run of day)* Dispose of the reagent troughs.
7. If needed, empty the waste carboy and refill the system liquid carboy.
8. Dispose of the used pipette tips.

9. Perform routine cleanup and maintenance on the Freedom EVO™ workstation. See *Tecan™ HID EVOlution™—Extraction Application Manual*, Section 5.4.2, "Clean Up the Worktable", and Chapter 7, "Maintenance".

IMPORTANT! To clean all workstation surfaces, use deionized water, then wipe with a lint-free lab wipe dampened with laboratory-grade 70% ethanol. Do not use acids, or bases (such as bleach) to clean the workstation. Consult Safety Data Sheets (SDS) and product labeling of cleaning agents, reagents, or chemicals for compatibility with the workstation before cleaning or decontaminating the workstation.

View the qPCR/STR Sample Input and Report files

About the files

As described page 47, two files are automatically generated at the end of each purification run:

- **qPCR/STR Sample Input file** *(HID EVOlution™—Combination System only)*—A CSV file that contains sample information from the purification run. You can use the file as a template to import sample information into the HID EVOlution™—qPCR/PCR Setup System. At the end of each run, the file is automatically saved as follows:

  `<installation drive>:\HIDEVOlutionExtractionFiles\Export\HID_<run date_run time>.csv`

  where: `<installation drive>` and `<run date_run time>` are variable

- **Report file**—A PDF file that contains a record of the reagents used and the samples processed during the purification run, including the starting plate or tube position of each sample lysate and the final position of the corresponding DNA eluate. At the end of each run, the file is automatically saved as follows:

  `<installation drive>:\HIDEVOlutionExtractionFiles\DNAextraction_<run date_run time>.pdf`

  where: `<installation drive>` and `<run date_run time>` are variable

When you start your next run, the files from the previous run are automatically moved to the following folder:

`<installation drive>:\HIDEVOlutionExtractionFiles\Archive\<date_time>`

where: `<installation drive>` and `<date_time>` are variable

For example, if you start your next run on August 15, 2020 at 3:08 PM (15:08 hours), files from the previous run would be archived to the `<installation drive>:\HIDEVOlutionExtractionFiles\Archive\20200815_150800` folder, regardless of the date of the previous run.

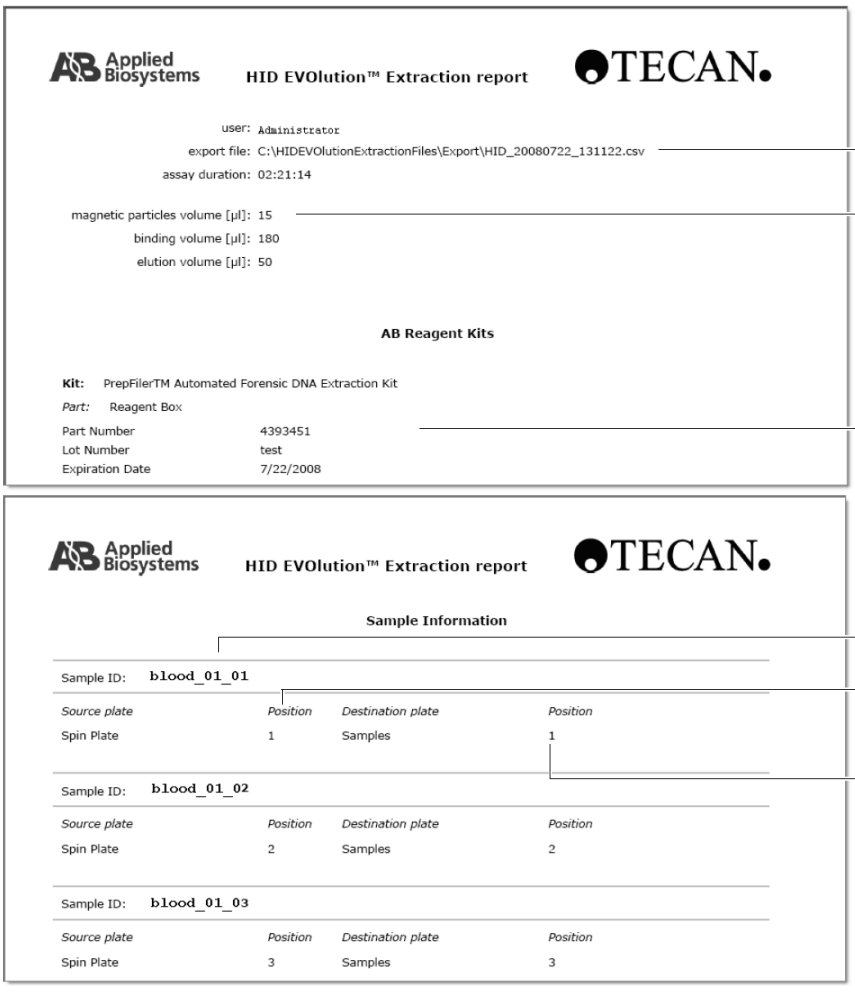
View the files

1. *(Optional)* For quick access to the files, create shortcuts on your desktop to the following folders:
   - **qPCR/STR Sample Input file folder**—`<installation drive>:\HIDEVOlutionExtractionFiles\Export\`
   - **Report file folder**—`<installation drive>:\HIDEVOlutionExtractionFiles\`
   - **Archive folder**—`<installation drive>:\HIDEVOlutionExtractionFiles\Archive\`

2. Navigate to the appropriate folder, then open the files of interest.

   Note: To quickly locate the files, record the file names that are displayed in the software after each run. See “Record file information and exit the script” on page 47.

3. *(Optional)* Print the Report file (PDF), then sign the printout and keep it for your records.



5 Experiments and results

This chapter provides the results of the developmental validation experiments performed by Thermo Fisher Scientific using the PrepFiler™ Automated Forensic DNA Extraction Kit on the HID EVOlution™—Extraction System. These experiments supplement the developmental validation studies, described in the *PrepFiler™ Forensic DNA Extraction Kit User Guide* (Pub. No. 4463348), that were performed to validate the PrepFiler™ Forensic DNA Extraction Kit chemistry.

The PrepFiler™ Automated Forensic DNA Extraction Kit was designed specifically for the lysis and automated purification of DNA from forensic samples. The kit contains reagents needed for cell lysis, binding DNA to magnetic particles, removing PCR inhibitors, and eluting bound DNA. Downstream applications include using the purified DNA in quantitative real-time PCR and in PCR amplification for Short Tandem Repeat (STR) analysis.

The PrepFiler™ Automated Forensic DNA Extraction Kit is not a DNA genotyping assay; the kit is intended to improve the overall yield and quality of DNA isolated from a variety of sample types. By testing the procedure with samples commonly encountered in forensic and parentage laboratories, the validation process establishes attributes and limitations that are critical for sound data interpretation.

Validation of the PrepFiler™ Automated Forensic DNA Extraction Kit on the HID EVOlution™—Extraction System

Overview of experiments and results

We performed developmental validation experiments to evaluate the performance of the PrepFiler™ Automated Forensic DNA Extraction Kit using the HID EVOlution™—Extraction System.

We performed the experiments according to the Revised Validation Guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM) published in Forensic Science Communications Vol. 6, No. 3, July 2004 (**http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/standards/2004_03_standards02.htm/**). These guidelines describe the quality assurance requirements that a laboratory should follow to ensure high quality and integrity of data and to demonstrate the competency of the laboratory. The SWGDAM-based experiments focus on kit performance parameters relevant to the intended use of the kits, that is, the extraction and purification of genomic DNA as a part of the forensic DNA genotyping procedure.

Each laboratory using the PrepFiler™ Automated Forensic DNA Extraction Kit should perform appropriate internal validation studies.

Materials and methods

The following materials and methods were used in all experiments performed as part of the developmental validation. (A detailed list is provided in Table 5.)

- Biological samples from 8 donors obtained from the Serological Research Institute were used to prepare the samples for each experiment.
- Samples were prepared and lysed using the PrepFiler™ Automated Forensic DNA Extraction Kit following the standard 300-µL lysis protocol.
- Genomic DNA was extracted and purified from the lysed samples using the PrepFiler™ Automated Forensic DNA Extraction Kit and the HID EVOlution™—Extraction System. DNA was eluted with 50 µL of elution buffer. Extraction blanks were processed for each study.
- The HID EVOlution™—Extraction System supports four configurations (see “Plate/tube configurations” on page 8) with corresponding software scripts that contain the instructions for the robotic workstation. The core liquid handling script for the binding, washing, and elution operations is identical in all validated scripts. The software script(s) used are described in the "results" section for each study.
- The purified DNA was quantified using the Quantifiler™ Human DNA Quantification Kit on a 7500 Real-Time PCR Instrument. An elution volume of 50 µL was used for all experiments. The quantitation results were analyzed using SDS v1.2.3.
- Quantified DNA was normalized using the HID EVOlution™—qPCR/PCR Setup System and amplified using the AmpFℓSTR™ Identifiler™ PCR Amplification Kit.
- Samples with a target DNA input amount of 1 ng were used for STR PCR amplification. Samples were amplified on a GeneAmp™ PCR System 9700. Electrophoresis was performed on 3130*xl* Genetic Analyzers.
- The STR profiles were analyzed using GeneMapper™ *ID-X* Software v1.0.

Table 5 Summary of materials used in the validation studies

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

Component	Item	Source
Chemistry[1]	Isopropyl alcohol	Sigma-Aldrich™
	TE buffer	Teknova™
	General reagents and materials	MLS
	PrepFiler™ Automated Forensic DNA Extraction Kit	4463353
	Quantifiler™ Human DNA Quantification Kit	4343895
	AmpFℓSTR™ Identifiler™ PCR Amplification Kit	4322288
	AmpFℓSTR™ MiniFiler™ PCR Amplification Kit	4373872
Labware	RNase-free Microfuge Tubes (1.5 mL); certified DNase- and RNase-free	AM12400
	PrepFiler™ Spin Tubes and Filter Columns **Note:** Since these validation experiments were performed, this product has been discontinued. It has been replaced with PrepFiler™ Spin Tubes and Filter Columns, ethylene oxide-treated (Cat. No. A36853).	4392342
	PrepFiler™ 96-Well Processing Plates **Note:** Since these validation experiments were performed, this product has been discontinued. It has been replaced with PrepFiler™ 96-Well Processing Plates (Cat. No. A47010).	4392904
	1,000-µL LiHa disposable tips with filter	Tecan™ (30000631)
	200-µL LiHa disposable tips with filter	Tecan™ (30000629)
	100-mL disposable troughs for reagents	Tecan™ (10613048)
	MicroAmp™ Optical 96-Well Reaction Plate (without barcode) or MicroAmp™ Optical 96-Well Reaction Plate with Barcode	N8010560 or 4306737
	MicroAmp™ Clear Adhesive Film	4306311
Instruments and software	Signature™ Benchtop Shaking Incubators	VWR International, LLC Model 1575 (ZZMFG)
	Magnetic-Ring Stand (96 well)	AM10050
	HID EVOlution™—qPCR/PCR Setup System	Tecan™
	HID EVOlution™—Extraction System	Tecan™
	7500 Real-Time PCR Instrument with SDS v1.2.3	—

Table 5 Summary of materials used in the validation studies *(continued)*

Component	Item	Source
Instruments and software	GeneAmp™ PCR System 9700	—
	3130*xl* Genetic Analyzers	—
	GeneMapper™ *ID-X* Software v1.0	—

[1] Identical lot numbers were used within each validation study.

Precision studies (SWGDAM standard 2.9)

Precision studies experiments

Precision studies were performed to test the precision of DNA recovery within a sample set. Eight replicates of twelve different samples were assayed for DNA concentration and the standard deviation within a replicate set.

Precision experiment A

DNA was extracted and purified from twelve sample types (see Table 6) in eight replicates using thePrepFiler™ Automated Forensic DNA Extraction Kit. A PrepFiler™ Spin Plate (96 wells) was used for lysis, and a MicroAmp™ Optical 96-Well Reaction Plate was used for elution. Each replicate set was arranged in a separate column in the spin plate. All blood samples were prepared from the same donor (Donor 85).

DNA concentration and quality were evaluated with the Quantifiler™ Human DNA Quantification Kit. The DNA concentration and Internal PCR Control (IPC) C_t values were also evaluated for variation among replicates.

Precision experiment B

The experiment described in precision experiment A was also performed using 96 tubes for both the lysis and elution containers.

Table 6 Name, description, and liquid volumes of the experimental samples used in this study

Sample name	Sample description	Body fluid volume
LB-40µL	Liquid human blood	40 µL
LB-30µL	Liquid human blood	30 µL
LB-10µL	Liquid human blood	10 µL
LB-5µL	Liquid human blood	5 µL
LB-2µL	Liquid human blood	2 µL
LB-1µL	Liquid human blood	1 µL
BSC	Human blood stain on non-colored cotton	5 µL
SALSw	Human saliva on cotton swab	50 µL

Table 6 Name, description, and liquid volumes of the experimental samples used in this study *(continued)*

Sample name	Sample description	Body fluid volume
SSC	Human semen stain on non-colored cotton	1 µL
BSCI	Human blood stain on non-colored cotton plus inhibitor mix[1]	5 µL of blood + 1 µL of inhibitor mix
BSD	Human blood stain on denim	5 µL
XB	Extraction blank	NA

[1] The inhibitor mix contains 12.5 mM of indigo, 0.5 mM of hematin, 2.5 mg/mL of humic acid, and 8.75 mg/mL of urban dust extract.

Precision studies results

DNA concentrations obtained in precision experiments A and B are summarized in Figure 9. Average IPC C_t values for the different samples are shown in Figure 9 on the secondary y-axis. Linear regression trend lines of the average DNA concentrations for the liquid blood samples examined in precision experiments A and B are shown in Figure 10.

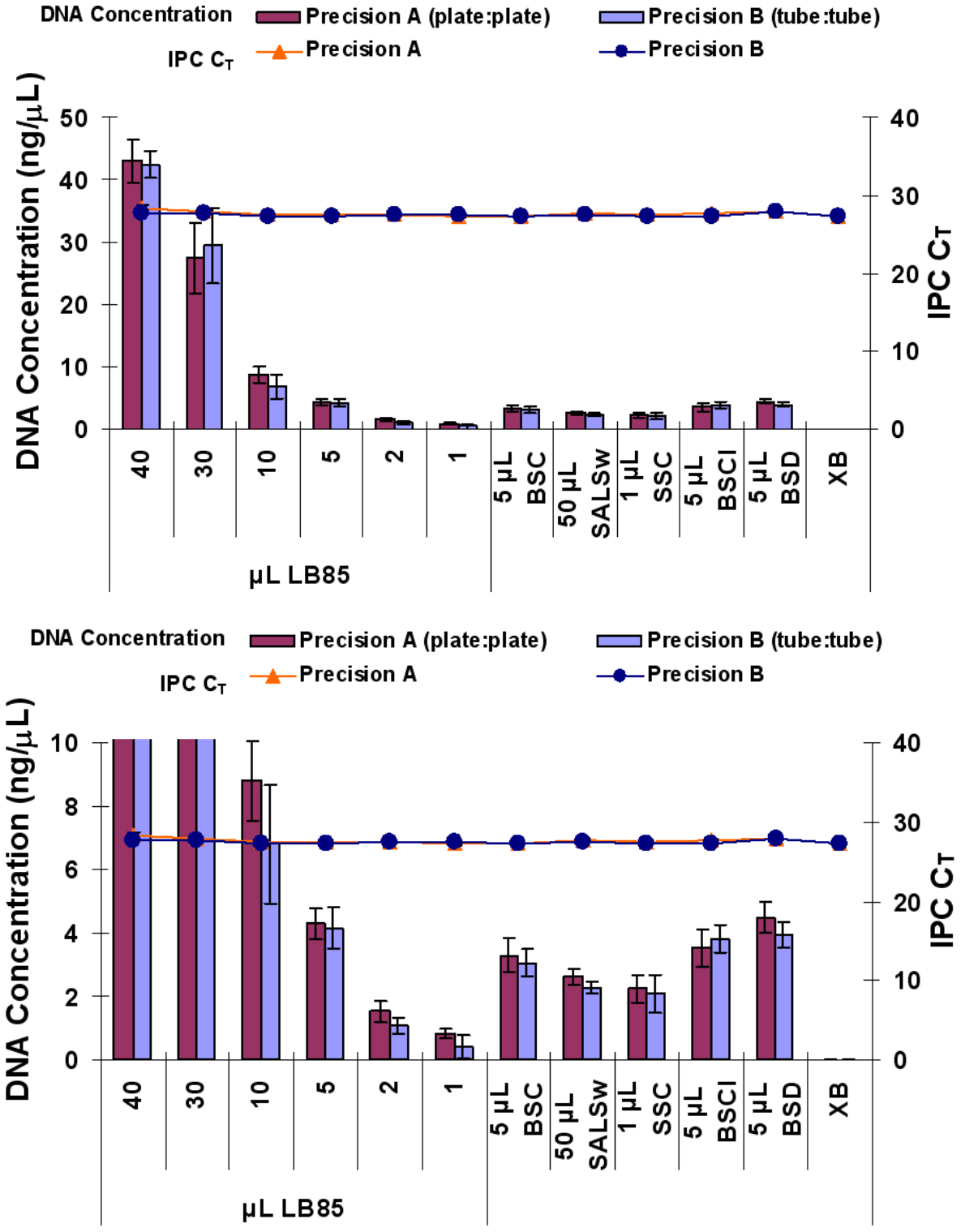


Figure 9 Precision studies A and B—The average DNA concentration and average IPC C_t values for extracted and purified DNA samples

The same data set is shown on two different scales: Concentration ranges 0–50 ng/µL (top) and 0–10 ng/µL (bottom).

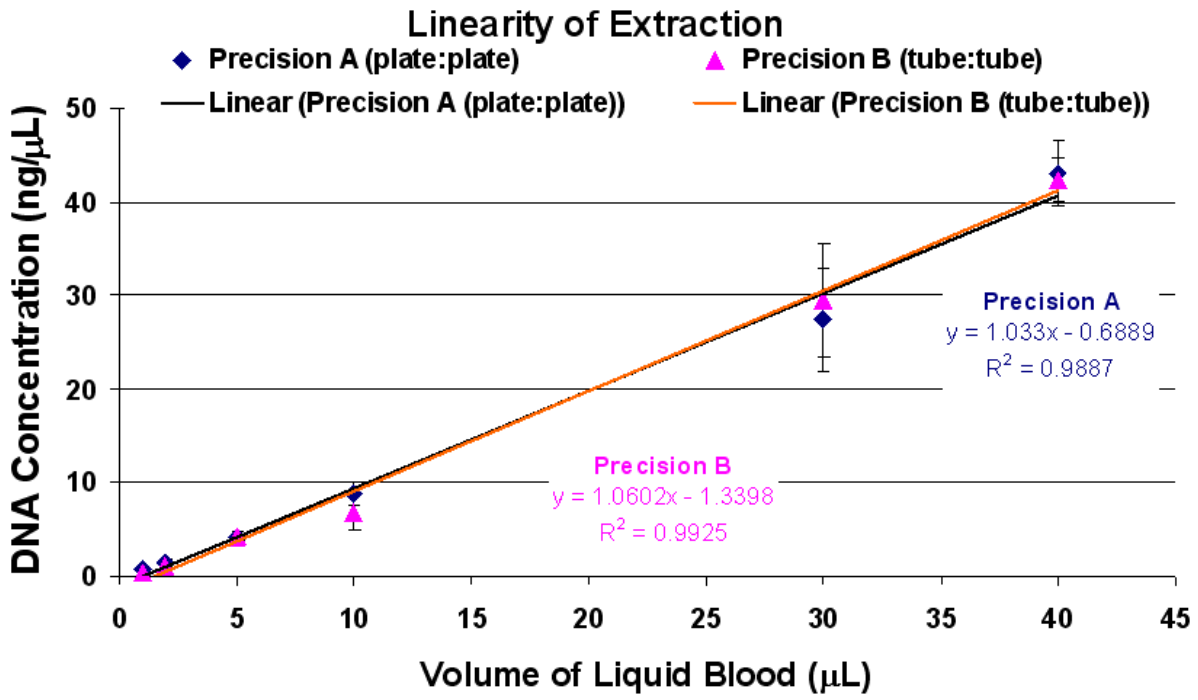


Figure 10 Precision studies A and B—DNA concentration is plotted against liquid blood volume and the linear regression trend is calculated

Table 7 and Table 8 summarize the statistics obtained from precision experiments A (plate-to-plate) and B (tubes-to-tubes).

Table 7 Precision study A—Summarized statistics for the eight replicates

Sample name	n=	DNA concentration			± Standard deviation
		Minimum	Maximum	Average	
Liquid samples					
40 µL LB85	8	38.94 ng/µL	49.74 ng/µL	43.02 ng/µL	3.45 ng/µL
30 µL LB85	8	22.06 ng/µL	36.35 ng/µL	27.42 ng/µL	5.54 ng/µL
10 µL LB85	8	6.87 ng/µL	10.59 ng/µL	8.80 ng/µL	1.25 ng/µL
5 µL LB85	8	3.64 ng/µL	4.83 ng/µL	4.29 ng/µL	0.49 ng/µL
2 µL LB85	8	1.08 ng/µL	2.18 ng/µL	1.52 ng/µL	0.33 ng/µL
1 µL LB85	8	0.62 ng/µL	0.97 ng/µL	0.80 ng/µL	0.15 ng/µL
Solid substrates					
5 µL BSC	8	2.35 ng/µL	4.11 ng/µL	3.30 ng/µL	0.53 ng/µL
50 µL SALSw	8	2.14 ng/µL	2.90 ng/µL	2.63 ng/µL	0.27 ng/µL
1 µL SSC	8	1.79 ng/µL	3.21 ng/µL	2.23 ng/µL	0.45 ng/µL

Table 7 Precision study A—Summarized statistics for the eight replicates *(continued)*

Sample name	n=	DNA concentration			± Standard deviation
		Minimum	Maximum	Average	
5 µL BSCI	8	2.76 ng/µL	4.42 ng/µL	3.51 ng/µL	0.58 ng/µL
5 µL BSD	8	3.63 ng/µL	5.01 ng/µL	4.47 ng/µL	0.49 ng/µL
Extraction blank					
XB	8	0.00 ng/µL	0.00 ng/µL	0.00 ng/µL	0.00 ng/µL

Table 8 Precision study B—Summarized statistics for the eight replicates

Sample name	n=	DNA concentration			± Standard deviation
		Minimum	Maximum	Average	
Liquid samples					
40 µL LB85	8	38.26 ng/µL	45.58 ng/µL	42.39 ng/µL	2.25 ng/µL
30 µL LB85	8	22.59 ng/µL	39.20 ng/µL	29.46 ng/µL	6.01 ng/µL
10 µL LB85	8	4.14 ng/µL	8.81 ng/µL	6.80 ng/µL	1.86 ng/µL
5 µL LB85	8	2.97 ng/µL	4.75 ng/µL	4.17 ng/µL	0.67 ng/µL
2 µL LB85	8	0.80 ng/µL	1.42 ng/µL	1.09 ng/µL	0.25 ng/µL
1 µL LB85	8	0.15 ng/µL	1.04 ng/µL	0.39 ng/µL	0.36 ng/µL
Solid substrates					
5 µL BSC	8	2.29 ng/µL	3.53 ng/µL	3.04 ng/µL	0.44 ng/µL
50 µL SALSw	8	2.04 ng/µL	2.62 ng/µL	2.28 ng/µL	0.16 ng/µL
1 µL SSC	8	0.95 ng/µL	2.79 ng/µL	2.09 ng/µL	0.60 ng/µL
5 µL BSCI	8	3.26 ng/µL	4.76 ng/µL	3.82 ng/µL	0.45 ng/µL
5 µL BSD	8	3.36 ng/µL	4.55 ng/µL	3.94 ng/µL	0.40 ng/µL
Extraction blank					
XB	8	0.00 ng/µL	0.00 ng/µL	0.00 ng/µL	0.00 ng/µL

Reproducibility studies (SWGDAM standard 2.5)

Reproducibility studies experiments

Reproducibility studies were performed to assess the reproducibility of the quantity and quality (as judged by the presence of PCR inhibitors) of DNA obtained from replicate extractions of biological samples.

Using the sample set shown in Table 6, an experiment was repeated on 3 separate days. In each experiment, DNA was extracted and purified from eight replicates. A PrepFiler™ Spin Plate (96 wells) was used for lysis, and a MicroAmp™ Optical 96-Well Reaction Plate was used for elution. The DNA concentration and IPC C_t values were evaluated for reproducibility using the Quantifiler™ Human DNA Quantification Kit.

Reproducibility studies results

Figure 11 shows the average DNA concentration and IPC C_t values for each sample by experiment.

The data from each of the eight replicates from the twelve samples from the three separate experiments were combined. The average and standard deviation were calculated and the summary statistics for all 24 combined replicates are shown in Table 9.

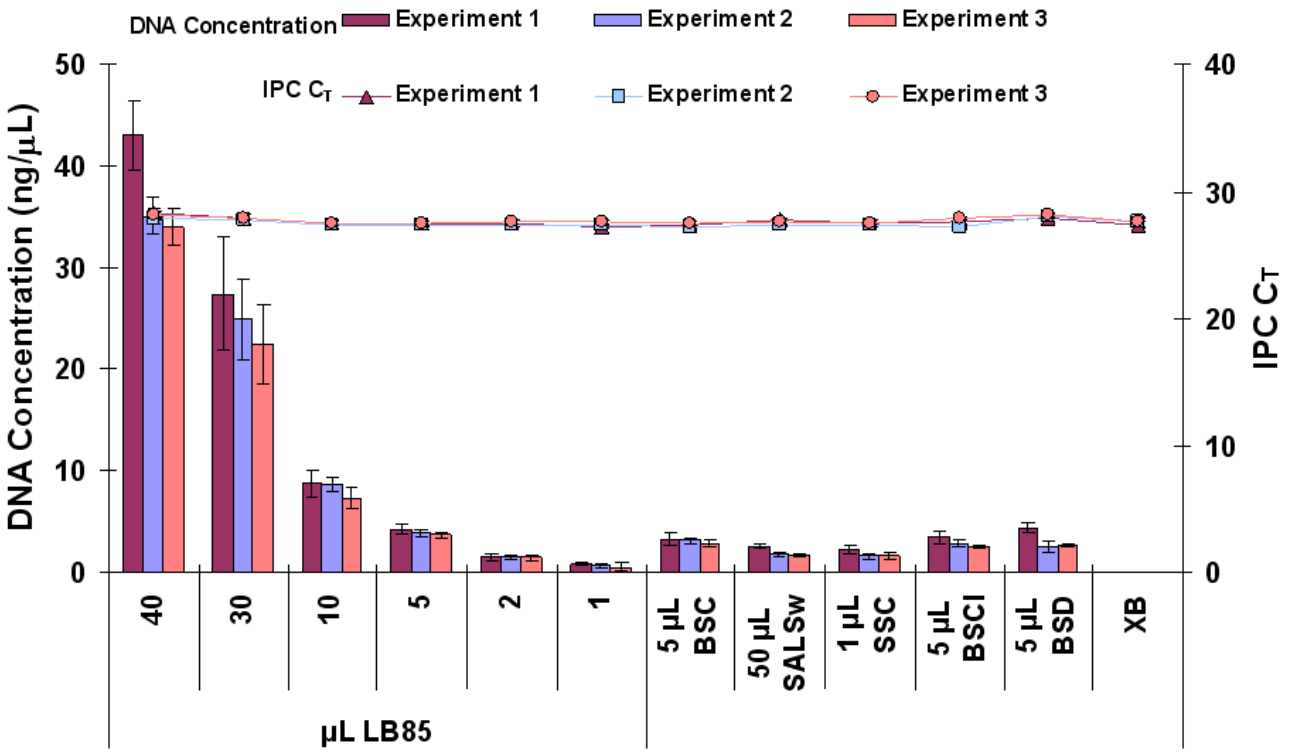


Figure 11 Reproducibility studies—The average DNA concentration and average IPC C_t for the three different experiments

The same data set is shown on two different scales: Concentration ranges 0–50 ng/µL (top) and 0–10 ng/µL (bottom).

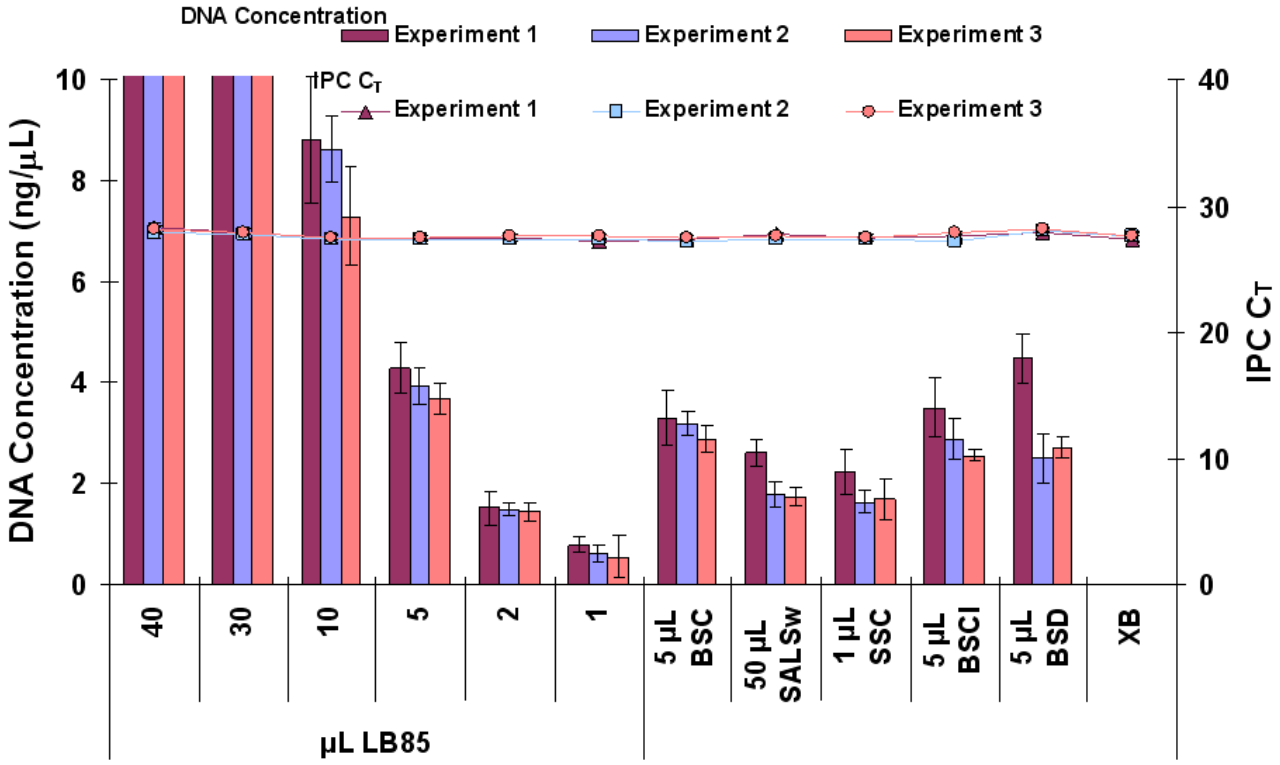


Table 9 Reproducibility studies—The averaged values for all three reproducibility experiments

Sample name	n=	DNA concentration			± Standard deviation
		Minimum	Maximum	Average	
Liquid samples					
40 µL LB85	24	31.37 ng/µL	49.74 ng/µL	37.37 ng/µL	4.74 ng/µL
30 µL LB85	24	18.04 ng/µL	36.35 ng/µL	24.95 ng/µL	4.80 ng/µL
10 µL LB85	24	5.73 ng/µL	10.59 ng/µL	8.24 ng/µL	1.17 ng/µL
5 µL LB85	24	3.30 ng/µL	4.83 ng/µL	3.97 ng/µL	0.45 ng/µL
2 µL LB85	24	1.08 ng/µL	2.18 ng/µL	1.49 ng/µL	0.22 ng/µL
1 µL LB85	24	0.31 ng/µL	1.59 ng/µL	0.62 ng/µL	0.30 ng/µL
Solid substrates					
5 µL BSC	24	2.35 ng/µL	4.11 ng/µL	3.13 ng/µL	0.40 ng/µL
50 µL SALSw	24	1.27 ng/µL	2.90 ng/µL	2.06 ng/µL	0.47 ng/µL
1 µL SSC	24	1.17 ng/µL	3.21 ng/µL	1.86 ng/µL	0.45 ng/µL
5 µL BSCI	24	2.30 ng/µL	4.42 ng/µL	2.99 ng/µL	0.57 ng/µL

Table 9 Reproducibility studies—The averaged values for all three reproducibility experiments *(continued)*

Sample name	n=	DNA concentration			± Standard deviation
		Minimum	Maximum	Average	
5 µL BSD	24	0.17 ng/µL	5.01 ng/µL	2.78 ng/µL	1.53 ng/µL
Extraction blank					
XB	24	0.00 ng/µL	0.00 ng/µL	0.00 ng/µL	0.00 ng/µL

Correlation studies

Correlation studies experiments

Correlation studies were performed to evaluate the performance of the automated protocol relative to the manual protocol.

The sample set shown in Table 6 was extracted and purified in triplicate using the manual extraction and purification protocol (see Chapter 2 of the *PrepFiler™ Forensic DNA Extraction Kit User Guide*). The purified DNA samples were quantified using the Quantifiler™ Human DNA Quantification Kit. To evaluate the performance of the automated protocol relative to the manual protocol, the DNA concentration and IPC C_t data for the manually-purified samples were compared to data generated from the identical samples for the "Reproducibility studies (SWGDAM standard 2.5)" on page 59.

Correlation studies results

Figure 12 shows the data generated from the manually-purified samples compared to the data generated from the same samples purified using the automated protocol. The DNA concentration and the IPC C_t values resulting from both extraction and purification methods are in accordance.

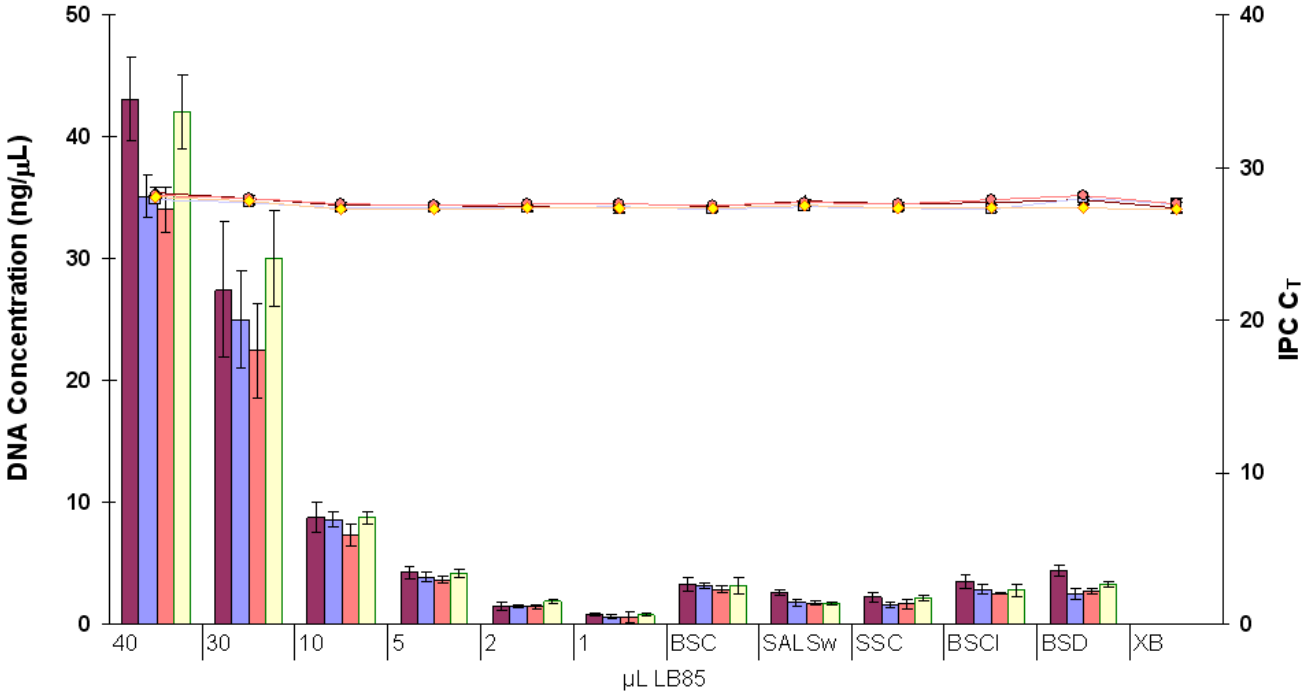


Figure 12 Correlation study—The graph shows the average DNA concentration (barchart) and IPC C_t values (line graph) obtained for the three replicates of each manually-purified sample compared to the data generated from the identical samples purified using the automated protocol (reproducibility experiments 1–3)

Table 10 Correlation study—Legend

Experiment		Sample name											
		40	30	10	5	2	1	BSC	SALSw	SSC	BSCI	BSD	XB
Concentration													
	Reproducibility experiment 1	43.02	27.42	8.80	4.29	1.52	0.80	3.30	2.63	2.23	3.51	4.47	0.00
	Reproducibility experiment 2	35.1	24.99	8.62	3.94	1.50	0.60	3.20	1.79	1.64	2.89	2.51	0.00
	Reproducibility experiment 3	34.00	22.44	7.29	3.68	1.46	0.56	2.89	1.75	1.70	2.57	2.72	0.00
	Manual purification	41.99	29.97	8.74	4.21	1.90	0.80	3.19	1.75	2.20	2.81	3.33	0.00
IPC C_t													
	Reproducibility experiment 1	28.31	27.91	27.51	27.46	27..44	27.27	27.36	27.75	27.54	27.66	27.89	27.30
	Reproducibility experiment 2	27.89	27.70	27.28	27.29	27.32	27.36	27.24	27.41	27.30	27.17	27.98	27.58
	Reproducibility experiment 3	28.15	27.91	27.54	27.52	27.57	27.58	27.47	27.68	27.55	27.87	27.13	27.58
	Manual purification	28.03	27.76	27.25	27.24	27.28	27.34	27.27	27.48	27.31	27.27	27.31	27.21

Cross-contamination studies (SWGDAM standard 3.6)

Cross-contamination studies experiments

These studies were performed to evaluate the potential for cross-contamination.

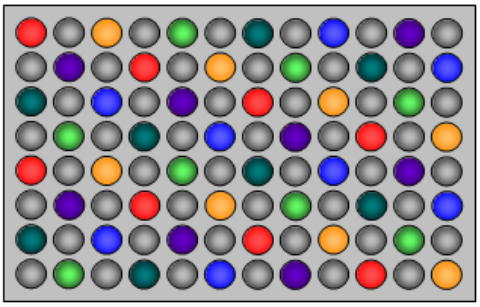
Checkerboard plate:plate experiment

For lysis, 10-µL samples of blood from six different donors were arranged in combination with extraction blanks in a PrepFiler™ Spin Plate (96 wells). The samples were arranged in a checkerboard format, such that samples from the same donor were not in adjacent sample wells (see Figure 13a). Samples were eluted into a MicroAmp™ Optical 96-Well Reaction Plate. The DNA was quantified using the Quantifiler™ Human DNA Quantification Kit. All extraction blanks were amplified with the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit using 10 µL of eluate.

Checkerboard tubes:plate experiment

An experiment similar to the plate:plate experiment was performed to test the use of microcentrifuge tubes and a 96-well reaction plate. For lysis, 10-µL samples of blood from eight different donors were arranged in combination with extraction blanks in a checkerboard format using microcentrifuge tubes (see Figure 13c).The samples were eluted into a 96-well reaction plate.

a



b

Donor 1	76-female	♀
Donor 2	77-male	♂
Donor 3	83-male	♂
Donor 4	90-male	♂
Donor 5	91-female	♀
Donor 6	92-female	♀
Donor 7	93-female	♀
Donor 8	94-male	♂
	lysis sol'n	

c

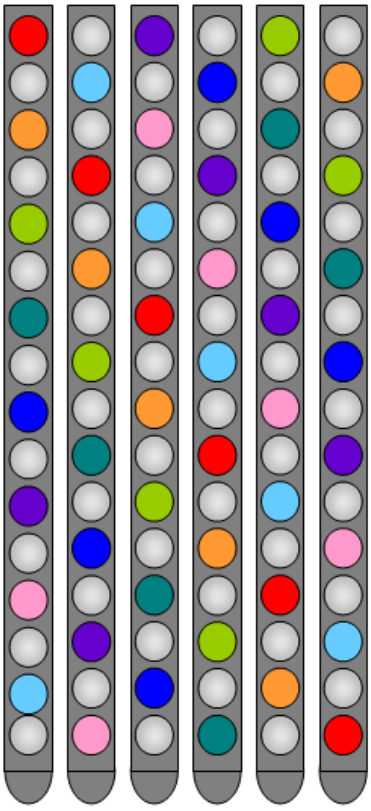


Figure 13 Cross-contamination study setup

a. Checkerboard format with 6 donors on a plate; b. Checkerboard format using 8 donors in tubes; c. Liquid blood donors

Cross-contamination studies results

Checkerboard plate:plate experiment

Of the 48 extraction blanks, six wells produced a C_t value <40. Of the wells with a C_t value <40, only one well yielded a detectable profile with the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit analysis and this profile was not attributable to any of the blood donors.

Checkerboard tubes:plate experiment

Of the 48 extraction blanks, one well had a C_t value <40. No detectable AmpFℓSTR™ MiniFiler™ PCR Amplification Kit profile was observed in any of the analyzed wells.

STR studies

STR studies experiments

The goal of the DNA extraction and purification step in the STR analysis workflow is to extract and purify DNA of sufficient quality and quantity to produce conclusive STR profiles. The quality of the purified DNA extract obtained from the PrepFiler™ Automated Forensic DNA Extraction Kit was further evaluated by examining the STR profiles.

The extracted and purified DNA samples described in precision experiment A (eight replicates of 12 samples; see Table 6) were amplified using the AmpFℓSTR™ Identifiler™ PCR Amplification Kit. 1 ng of human DNA, as determined by the Quantifiler™ Human DNA Quantification Kit, was used as the template DNA.

STR studies results

Full STR profiles were obtained from all extracted and purified DNA samples (see Figure 14). No cross-contamination was observed.

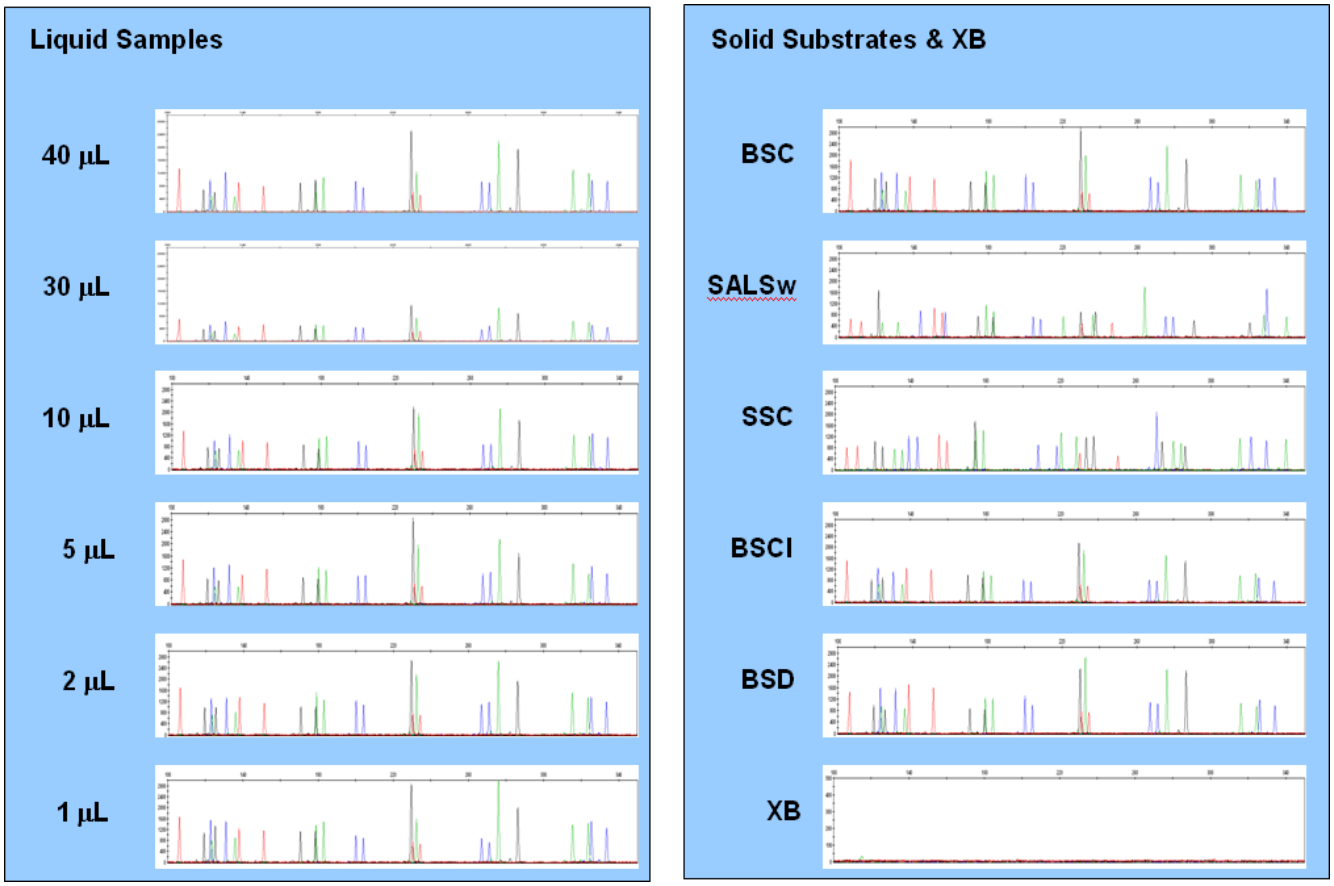


Figure 14 STR study—AmpFℓSTR™ Identifiler™ PCR Amplification Kit STR profiles for the various sample types tested

On the left, liquid blood samples (from a single donor) show complete profiles (RFU=3,000). On the right, solid substrate samples (from different donors) each show a different profile (RFU=3,000). The extraction blank (XB) is also shown on the right (RFU=500).

The interlocus balance was calculated for each of the 96 individual profiles. The eight replicate measurements were averaged across each dye for each sample type and the standard deviation was calculated. The average interlocus balance for each of the eleven sample types and the positive amplification control 9947a is shown in Figure 15.

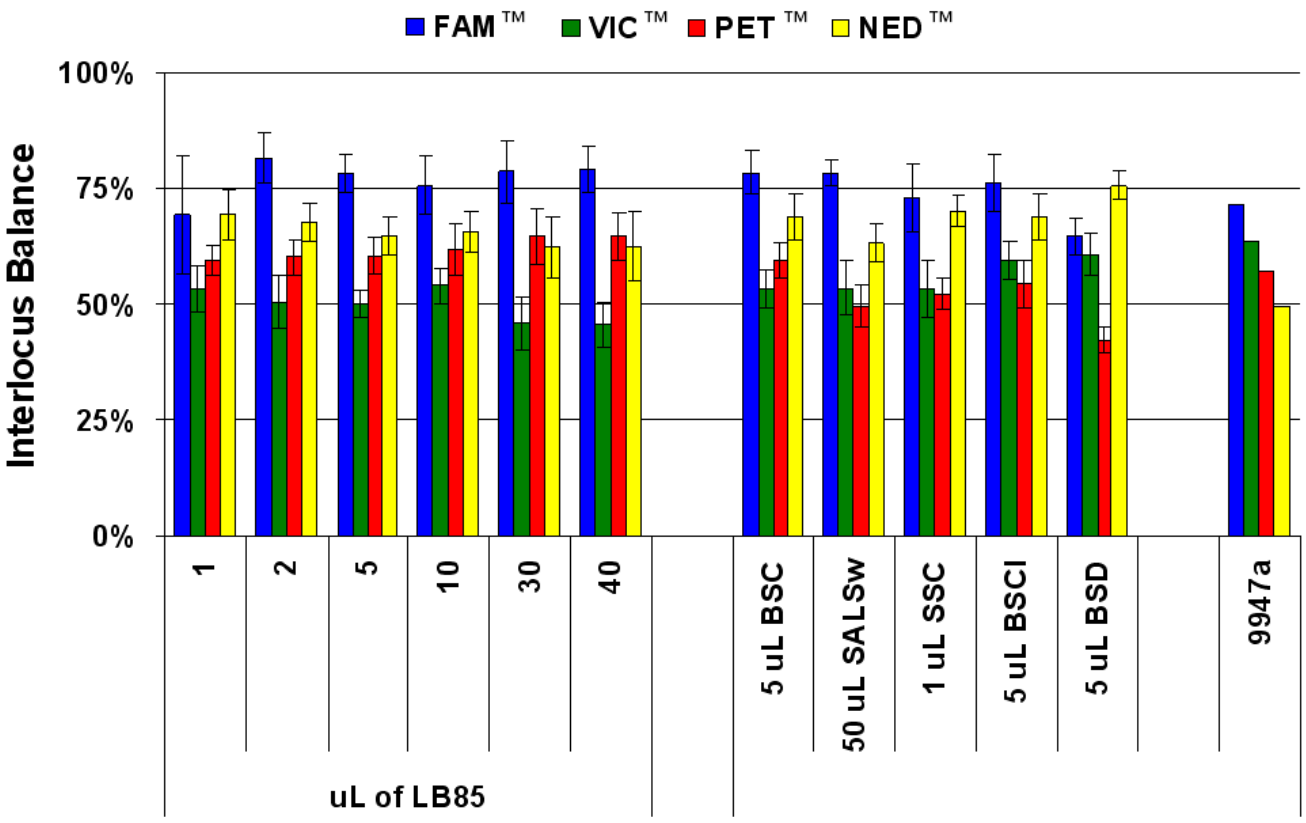


Figure 15 STR study—The average interlocus balance for each sample type (eight replicates each)

Liquid blood samples are shown on the left, and samples spotted on solid substrates are shown on the right. A single replicate of 9947a was used as a positive control.

Heterozygote peak height ratios were calculated for each profile. The eight replicate measurements were averaged for each sample type and the standard deviation was calculated. The average heterozygote peak height ratio for each of the eleven sample types, as well as a positive control, is displayed in Figure 16. The liquid blood graph (left side) does not include homozygous loci for these samples.

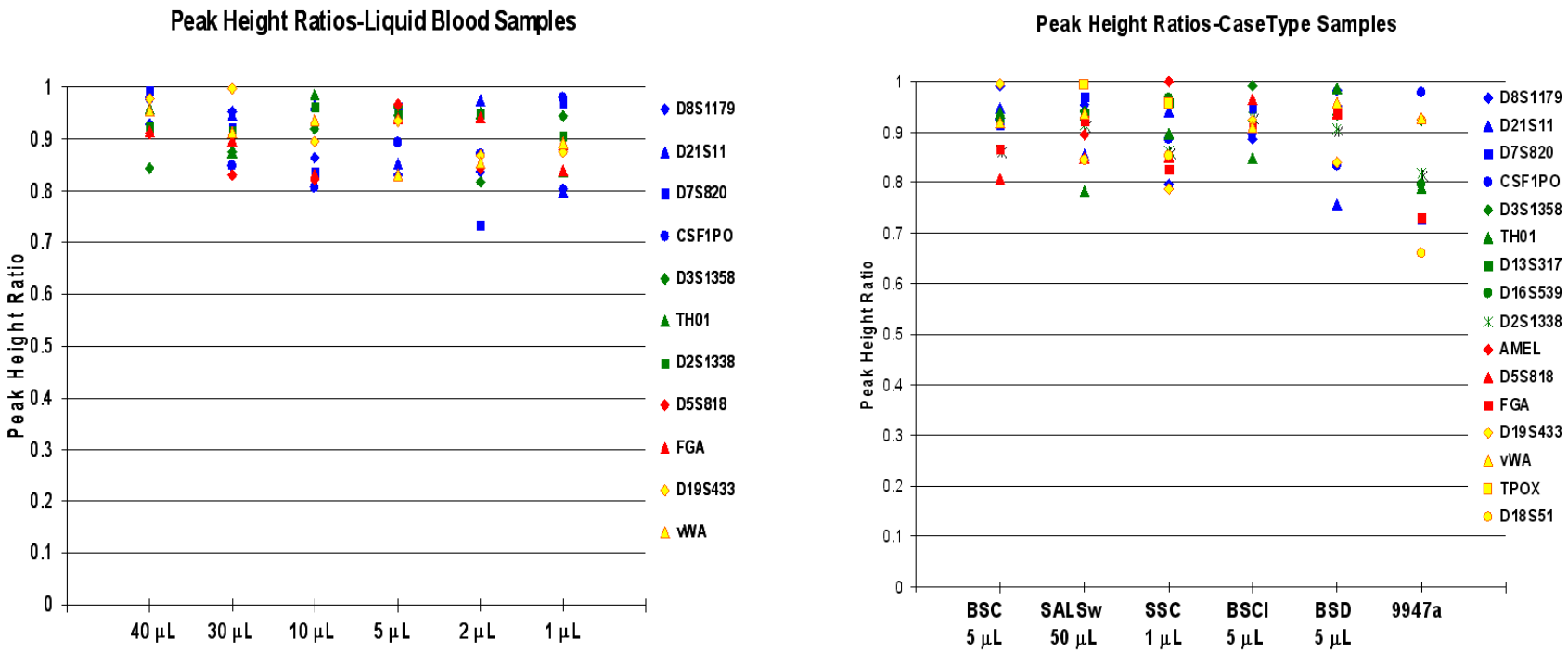


Figure 16 STR study—The average peak height ratio is shown by locus for the eight replicates of each sample type

The left panel represents data from a range of starting volumes of liquid blood from a single donor and includes heterozygote loci. The right panel includes heterozygote loci for each of the samples spotted on solid substrates as well as the positive control 9947a.

Verification studies for remaining scripts

Verification studies experiments

Four software scripts containing the DNA purification instructions for the robotic workstation were developed:

- **Plate:plate**—Beginning with lysate in a 96-well plate and collecting the eluate in a 96-well plate
- **Plate:tubes**—Beginning with lysate in a 96-well plate and collecting the eluate in tubes
- **Tubes:tubes**—Beginning with lysate in tubes and collecting the eluate in tubes
- **Tubes:plate**—Beginning with lysate in tubes and collecting the eluate in a 96-well plate

The core liquid handling script for operations such as binding, washing, and elution is identical in all four scripts. The plate:plate script was the primary script used during developmental validation, including in the cross-contamination study. Verification studies were performed to test the other three scripts.

Plate:tubes experiment

To test the performance of the PrepFiler™ Spin Plate (96 wells) as a source vessel and microfuge tubes as elution vessels, the lysate from 10-µL blood samples from six different donors was arranged in a checkerboard pattern in combination with extraction blanks in such a way that samples from the same donor were not in adjacent sample wells (see Figure 13a).

Tubes:plate experiment

The tubes:plate experiment performed for the cross-contamination study also served as the tubes:plate experiment for the verification studies (see “Cross-contamination studies experiments” on page 64): To test the performance of microfuge tubes as source vessels and the MicroAmp™ Optical 96-Well Reaction Plate as an elution vessel, the lysate from 10-µL blood samples from eight different donors was arranged in a checkerboard format in combination with extraction blanks in such a way that samples from the same donor were not in adjacent sample wells (see Figure 13c). Microfuge tubes that contained the lysate were placed in tube racks L1–L6 and the DNA eluate was collected in a 96-well reaction plate.

Tubes:tubes experiment

To test the performance of microfuge tubes as source vessels and elution vessels, the lysate from 10-µL blood samples from eight donors was arranged in a checkerboard format in combination with extraction blanks in such a way that samples from the same donor were not in adjacent sample wells (see Figure 13c). Microfuge tubes that contained the lysate were placed in tube racks L1–L6 and the DNA eluate was collected in microfuge tubes in tube racks S1–S6.

The DNA from all three verification experiments was quantified using the Quantifiler™ Human DNA Quantification Kit.

Verification studies results

Data from each experiment were reviewed for well-to-well cross-contamination and overall consistency in DNA yield (see Figure 17 and Table 11).

- **Plate:tubes verification experiment**—Of the 48 extraction blanks tested, a C_t value <40 was observed in three wells.
- **Tubes:tubes verification experiment**—See the results of the cross-contamination study tubes:plate experiment ("Checkerboard tubes:plate experiment" on page 65).
- **Tubes:plate verification experiment**—All of the 48 extraction blanks resulted in C_t values >40.

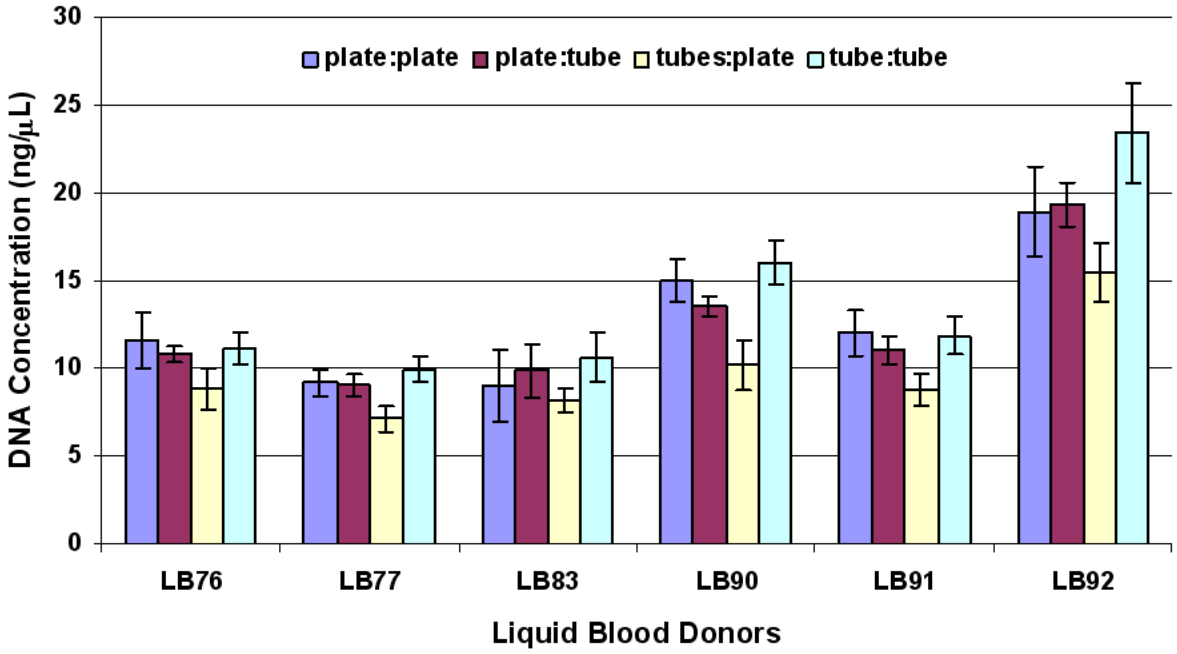


Figure 17 Verification studies—The average DNA concentration for each of the six or eight replicates for each liquid blood donor

Only six of the eight donors are shown for simplicity, with the remaining donors showing similar results.

Table 11 Verification studies—Average total DNA yield (ng)

The average total DNA yield (ng) was calculated for each liquid blood donor for all four automated purification methods and compared to the expected yield from 4,000 or 11,000 nucleated blood cells per 1 µL.

Sample	Total DNA yield	± Standard deviation
LB76	529.90 ng	56.38 ng
LB77	440.71 ng	35.89 ng
LB83	469.50 ng	74.40 ng
LB90	684.27 ng	59.06 ng

Table 11 Verification studies—Average total DNA yield (ng) *(continued)*

Sample	Total DNA yield	± Standard deviation
LB91	545.45 ng	51.12 ng
LB92	963.90 ng	109.31 ng
LB93	589.44 ng	54.92 ng
LB94	683.53 ng	61.66 ng
Expected yield (ng)		
4,000 cells/µL	250 ng	NA
11,000 cells/µL	650 ng	NA

Additional cross-contamination studies

Additional cross-contamination studies experiments

Additional studies were performed to monitor cross-contamination during lysis using the filter plate and DNA isolation on the HID EVOlution™—Extraction System. The extracted and purified samples (including extraction reagent blanks) were processed to:

- Quantitate human DNA using the Quantifiler™ Human DNA Quantification Kit
- Perform STR typing using the AmpFℓSTR™ Identifiler™ PCR Amplification Kit and AmpFℓSTR™ MiniFiler™ PCR Amplification Kit

Two PrepFiler™ Spin Plates (96 wells) were prepared for lysis. Each plate contained 10-µL samples of blood from six different donors arranged in combination with extraction blanks in a checkerboard format, such that samples from the same donor were not in adjacent sample wells in the lysis or elution plates (same format as the "Checkerboard plate:plate experiment" on page 64; see also Figure 13a). The open wells were covered with MicroAmp™ Clear Adhesive Film while the liquid blood samples were dispensed to avoid any aerosol transfer, and the movement of the pipette was controlled to reduce aerosol formation. The samples were processed using the plate:plate script and eluted into two MicroAmp™ Optical 96-Well Reaction Plates. All samples were quantified using the Quantifiler™ Human DNA Quantification Kit and amplified with the AmpFℓSTR™ Identifiler™ PCR Amplification Kit and AmpFℓSTR™ MiniFiler™ PCR Amplification Kit following the standard kit protocols.

Additional cross-contamination studies results

DNA quantitation using the Quantifiler™ Human DNA Quantification Kit

None of the extraction blanks in plate 1 exhibited the presence of human DNA as determined by the Quantifiler™ Human DNA Quantification Kit; the C_t values for the human target were Undetermined. In plate 2, only one well (well B11) exhibited a C_t value of 39.94, which is attributed to higher background and not necessarily due to cross-contamination (see the STR results below).

STR profiling using the AmpFℓSTR™ Identifiler™ and MiniFiler™ kits

STR profiling results were generated from samples in plates 1 and 2 using the Identifiler™ and MiniFiler™ kits. The results were analyzed using a 50-RFU detection threshold. All samples in both extraction/purification plates exhibited single source, conclusive, and complete STR profiles. Further, none of the samples exhibited detectable mixed profiles. None of the extraction blank wells exhibited partial or complete STR profiles. Extraction blank well B11 from plate 2, which exhibited a C_t value of 39.94, did not exhibit an STR profile using either the Identifiler™ or MiniFiler™ kit.

The results from the two plates of extracts processed for quantitation and STR profiling are summarized in Table 12.

Table 12 Summary of additional cross-contamination studies

A total of 192 samples were extracted and purified, of which 96 were extraction blanks and 96 were samples originating from six human donors.

Sample type	Number of samples		
	Plate 1	Plate 2	Total
Samples analyzed (including extraction blanks)	96	96	192
Extraction blanks	48	48	96
Extraction blanks with C_t <40	0	1	1
Extraction blanks exhibiting peaks called as alleles in the Identifiler™ kit run[1]	0	0	0
Extraction blanks exhibiting peaks called as alleles in the MiniFiler™ kit run[1]	0	0	0

[1] Using the standard cutoff (50 RFU)

Conclusions

The PrepFiler™ Automated Forensic DNA Extraction Kit was developed for the isolation of genomic DNA from a variety of biological samples. Validation studies confirmed that the kit provides robust and reliable results in obtaining genomic DNA from forensic biological samples for downstream applications such as real-time quantitative PCR and PCR for STR profiling.

- The PrepFiler™ Automated Forensic DNA Extraction Kit was validated following the SWGDAM standards.
- The utility of the extraction and purification methods in forensic DNA analysis was demonstrated using forensic-type samples.
- The kit is effective in maximizing the amount of DNA obtained from samples that contain small and large quantities of biological material.
- The DNA that was extracted and purified was free of PCR inhibitors as determined by the IPC C_t values using the Quantifiler™ Human DNA Quantification Kit.
- The PrepFiler™ Automated Forensic DNA Extraction Kit exhibited clean operations and did not introduce any detectable cross-contamination of human DNA.

Validation of PrepFiler™ Wash Buffer B and the related modifications to the workstation layout and scripts

Overview of experiments and results

We performed developmental validation experiments to evaluate the following:

- A new wash buffer, PrepFiler™ Wash Buffer B, for use with the PrepFiler™ Automated Forensic DNA Extraction Kit
- Related modifications to the HID EVOlution™—Extraction System and HID EVOlution™—Combination System

The new buffer and system modifications provide a more robust automated protocol and improve overall performance.

PrepFiler™ Wash Buffer B

An additional wash buffer, PrepFiler™ Wash Buffer B, is used for the third (final) wash during DNA purification to minimize the potential for detergent carryover from PrepFiler™ Wash Buffer A. Detergent carryover can inhibit downstream PCR applications.

Modified workstation layout

The worktable layout has been modified to add a trough for PrepFiler™ Wash Buffer B and to rearrange the remaining reagent and waste troughs to accommodate the new trough. For information on setting up the workstation, see the appropriate document for your instrument:

- *TecanHID EVOlution™—Extraction System Application Manual*, 395372, v2.0 (June 2010), Sections 4.3 and 4.4
- *Tecan HID EVOlution™—Combination System Application Manual*, 395967, v2.0 (June 2010), Sections 4.3 and 4.4

New software scripts

New HID EVOlution™ scripts (see Table 13) replace existing automated purification scripts for use with the PrepFiler™ Automated Forensic DNA Extraction Kit. The new scripts incorporate the workstation changes, improvements to eliminate bubble formation on disposable tips during dispensing steps, and additional changes to optimize liquid handling performance and pathways.

All new HID EVOlution™—Extraction Systems and HID EVOlution™—Combination Systems are delivered with the new scripts. If you are an existing customer, contact your local Tecan customer support organization to obtain the new scripts.

For details on script changes, see the service pack revision history file included in the **Documents** folder on the CD that contains the new scripts.

Table 13 New Freedom EVOware™ software purification scripts

System	Script names
HID EVOlution™—Extraction System	• PrepFiler_plate_plate_V1_SP2 • PrepFiler_plate_tubes_V1_SP2 • PrepFiler_tubes_plate_V1_SP2 • PrepFiler_tubes_tubes_V1_SP2 • PrepFiler_plate_plate500_V1_SP2[1] • PrepFiler_plate_tubes500_V1_SP2 • PrepFiler_tubes_plate500_V1_SP2 • PrepFiler_tubes_tubes500_V1_SP2
HID EVOlution™—Combination System	• PrepFiler_plate_plateCombo_V1_SP1 • PrepFiler_plate_tubesCombo_V1_SP1 • PrepFiler_tubes_plateCombo_V1_SP1 • PrepFiler_tubes_tubesCombo_V1_SP1 • PrepFiler_plate_plateCombo500_V1_SP1[1] • PrepFiler_plate_tubesCombo500_V1_SP1 • PrepFiler_tubes_plateCombo500_V1_SP1 • PrepFiler_tubes_tubesCombo500_V1_SP1

[1] The 500-mL scripts were not validated as part of the studies described in this chapter.

Note: Core liquid handling for operations such as binding, washing, and elution are identical for all scripts.

Materials and methods

The following materials and methods were used in all experiments performed as part of the developmental validation of the PrepFiler™ Wash Buffer B and the new scripts. (Detailed lists are provided in Table 14 and Table 15.)

- PrepFiler™ Wash Buffer B was prepared by adding 95% ethanol to 200 mL of low-TE buffer to bring the final volume to 500 mL.
- Biological fluids and tissues used in the studies are listed in Table 15. See each study for a description of the samples used.
- Lysis was performed as described in "Sample lysis method" on page 77.
- Extracted genomic DNA was purified from the lysed samples on the HID EVOlution™—Extraction System and/or HID EVOlution™—Combination System. The standard 300-µL scripts were used in all studies. Except where noted, lysate was processed from a 96-well plate and eluate collected in a 96-well plate. Final elution volumes varied depending on humidity and room temperature, and ranged between 40–46 µL. Extraction blanks were included in each study.

- Purified DNA extract was set up for qPCR on the HID EVOlution™—qPCR/PCR Setup System using the Quantifiler™ Human DNA Quantification Kit. If study samples filled more than one qPCR plate, samples of the same type were grouped together in the same qPCR plate to avoid introducing run-to-run variation.
- Purified DNA extract from each sample was quantified using the Quantifiler™ Human DNA Quantification Kit on a 7500 Real-Time PCR Instrument. The quantitation results were analyzed using SDS v1.2.3.
- Quantified DNA from each sample was normalized and set up for amplification using the HID EVOlution™—Combination System. The AmpFℓSTR™ Identifiler™ PCR Amplification Kit was used for all studies except the cross-contamination study, which used the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit.
- A target input of 1 ng of DNA was used for STR PCR amplification. For samples with concentrations <0.10 ng/µL, a maximum of 10 µL of purified DNA extract was added to each STR reaction. Samples were amplified on a GeneAmp™ PCR System 9700. Electrophoresis was performed on a 3130*xl* Genetic Analyzer.
- The STR profiles were analyzed using GeneMapper™ *ID-X* Software v1.0.

Table 14 Summary of materials used in the PrepFiler™ Wash Buffer B validation studies

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

Component	Item	Source
Chemistry	Isopropyl alcohol	Sigma-Aldrich™
	TE buffer	Teknova™
	Ethanol (95% molecular-biology grade)	Sigma-Aldrich™
	PrepFiler™ Automated Forensic DNA Extraction Kit with Plastics **Note:** Since these validation experiments were performed, this product has been discontinued. It has been replaced with PrepFiler™ Automated Forensic DNA Extraction Kit (Cat. No. 4463353).	4397977 (Lot Number 0901004)
	PrepFiler™ Wash Buffer B (from the PrepFiler™ Automated Forensic DNA Extraction Kit, prepared as indicated above)	
	Quantifiler™ Human DNA Quantification Kit	4343895 (Lot Number 0906112)
	AmpFℓSTR™ Identifiler™ PCR Amplification Kit	4322288 (Lot Numbers 0901114, 0905120, and 0907123)
	AmpFℓSTR™ MiniFiler™ PCR Amplification Kit	4373872 (Lot Number 0909021)
	Cartridge-based, silica magnetic bead extraction kit	Company A
	Cartridge-based, silica magnetic bead extraction kit	Company B

Table 14 Summary of materials used in the PrepFiler Wash Buffer B validation studies *(continued)*

Component	Item	Source
Labware	MicroAmp™ Optical 96-Well Reaction Plate (without barcode) or MicroAmp™ Optical 96-Well Reaction Plate with Barcode	N8010560 or 4306737
	RNase-free Microfuge Tubes (1.5 mL); certified DNase- and RNase-free	AM12400
	200 µL and 1,000 µL tips	Tecan™
	100-mL troughs for reagents	Tecan™
	Puritan™ Cotton Tipped Wooden Swabs (without glue)	VWR™
	SERI Washed Cotton Cloth	Serological Research Institute (Richmond, CA)
HID EVOlution™—Extraction System	Freedom EVO™ 150 robotic workstation, including the following hardware:	
	• 8-channel Liquid Handling accessory (LiHa; channels 1–4 used for qPCR/STR setup and channels 5–8 used for purification)	Tecan™
	• Robotic Manipulator arm (RoMa)	
	• Te-Shake™ plate adapter with heating block	
	• Magnetic-Ring Stand (96 well)	AM10050
	Freedom EVOware™ software v2.1 SP1, configured with HID EVOlution™—Extraction application v1.0 SP1	Tecan™
	Eight new scripts (see Table 13)	
	Windows™ XP Professional operating system	
HID EVOlution™—Combination System	Freedom EVO™ 150 robotic workstation, including the following hardware:	
	• 8-channel Liquid Handling accessory (LiHa; channels 1–4 used for qPCR/STR setup and channels 5–8 used for purification)	Tecan™
	• Robotic Manipulator arm (RoMa)	
	• Te-Shake™ plate adapter with heating block	
	• Magnetic-Ring Stand (96 well)	AM10050
	Freedom EVOware™ software, v2.1 SP1 configured with the following:	
	• HID EVOlution™—Extraction application v1.0 SP1	Tecan™
	• HID EVOlution™ driver v1.0.0.25 SP4	
	• HID EVOlution™—qPCR/PCR driver v1.0.0.26 SP5	
	• Sample Oriented EVOware™ v1.2 SP1	

Table 14 Summary of materials used in the PrepFiler Wash Buffer B validation studies *(continued)*

Component	Item	Source
HID EVOlution™ —Combination System	Eight new scripts (see Table 13)	Tecan™
	Windows™ XP Professional operating system	
Other instruments and software	3130*xl* Genetic Analyzer with Data Collection Software v3.0	—
	GeneMapper™ *ID-X* Software v1.0	—
	GeneAmp™ PCR System 9700 (gold-plated silver 96-well block)	—
	7500 Real-Time PCR Instrument with SDS v1.2.3	—
	Signature™ Benchtop Shaking Incubators	VWR International, LLC Model 1575 (9120890; serial number 10032308)
	Eppendorf™ 1.5-mL Tubes Thermomixer	Eppendorf™ Model 5350

Table 15 Biological samples used in the PrepFiler™ Wash Buffer B validation studies

Sample type	Donor reference number	Gender	Source
Human peripheral blood	233	Female	Serological Research Institute (Richmond, CA)
Human peripheral blood	238	Female	
Human peripheral blood	240	Female	
Human peripheral blood	236	Male	
Semen	—	Male	
Saliva	Anonymous	Male	In house

Sample lysis method

To test the performance of the automated purification system, we took steps to minimize potential variation because of differences between manual lysis methods. Except where noted in individual studies, sample lysis was performed using the PrepFiler™ Automated Forensic DNA Extraction Kit with Plastics and the following procedure based on the standard (300-µL) tube protocol from the *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits User Guide* (Pub. No. 4463349).

1. Samples were placed in nuclease-free 1.5-mL polypropylene microcentrifuge tubes.
2. 300 µL of PrepFiler™ Lysis Buffer and 3 µL of DTT, 1.0 M, were added to each sample.
3. The tubes were vortexed for 10 seconds, then briefly centrifuged.
4. The tubes were incubated in a thermal mixer at 900 rpm for 40 minutes at 70°C.

5. The substrate and residual lysis buffer were transferred from the tubes to the wells of a PrepFiler™ 96-Well Spin Plate and Filter Plate assembly, then centrifuged at 2,000 rpm for 1 minute to recover clarified lysates.
6. The filter plate was separated from the spin plate. The spin plate that contained the lysed sample was used as the source plate during automated purification.

Script validation

We performed script validation to compare the new scripts to the original scripts.

Note: Because core liquid handling for operations such as binding, washing, and elution are identical for all new scripts, we performed script validation with only one of the new scripts: PrepFiler_plate_plateCombo.

Before performing the PrepFiler™ Wash Buffer B validation studies, we tested the new workstation layout, new buffer, and new PrepFiler_plate_plateCombo script on the HID EVOlution™—Combination System using the following sample types:

- A dilution series of blood dried on cotton-tipped wooden swabs
- A low-input case-type sample set identical to that shown in Table 24

The performance of the new scripts was equivalent to the original scripts with respect to DNA recovery. No indication of PrepFiler™ Wash Buffer A carryover was observed in the STR profiles. Figure 18 and Figure 19 show the results for each sample type.

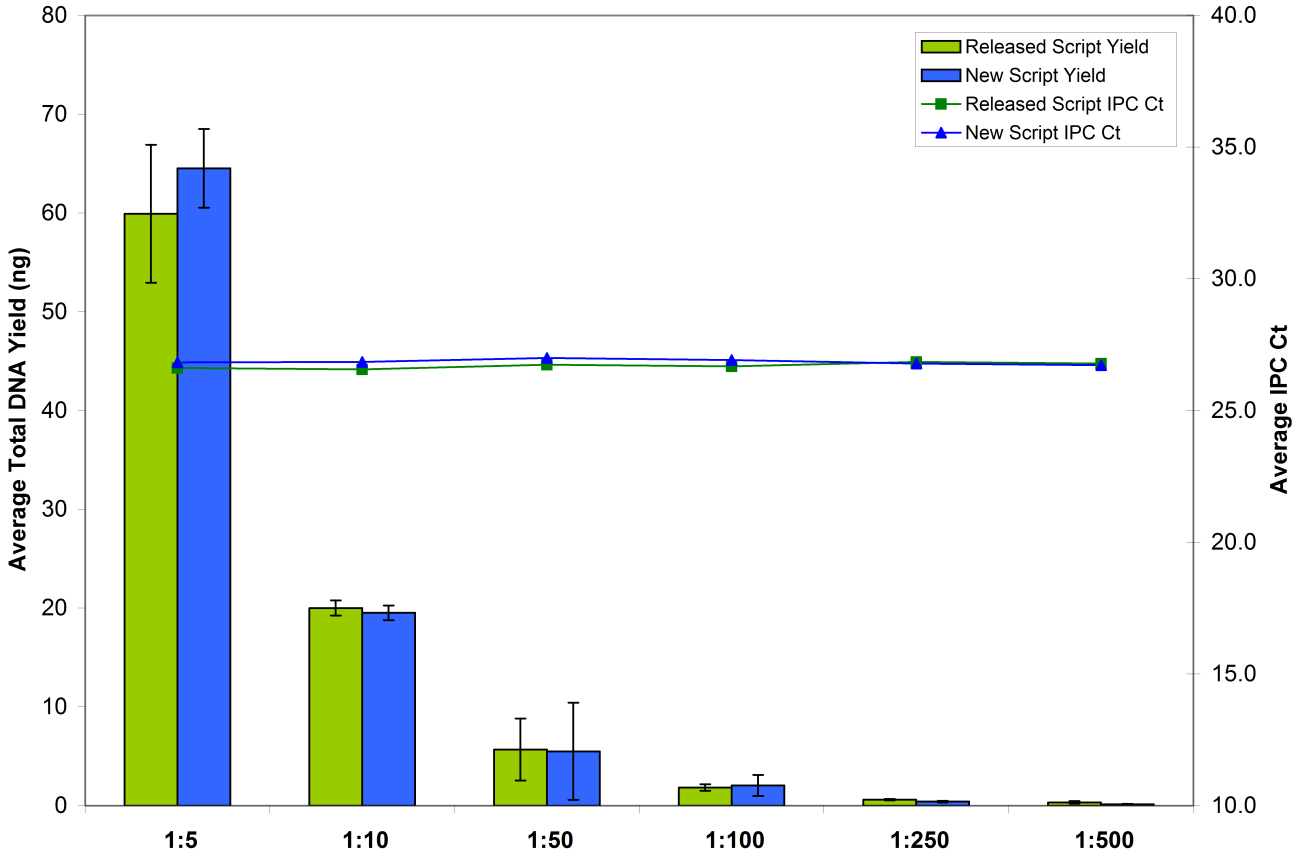


Figure 18 Diluted blood on swabs—DNA yield from original (green) and new (blue) scripts

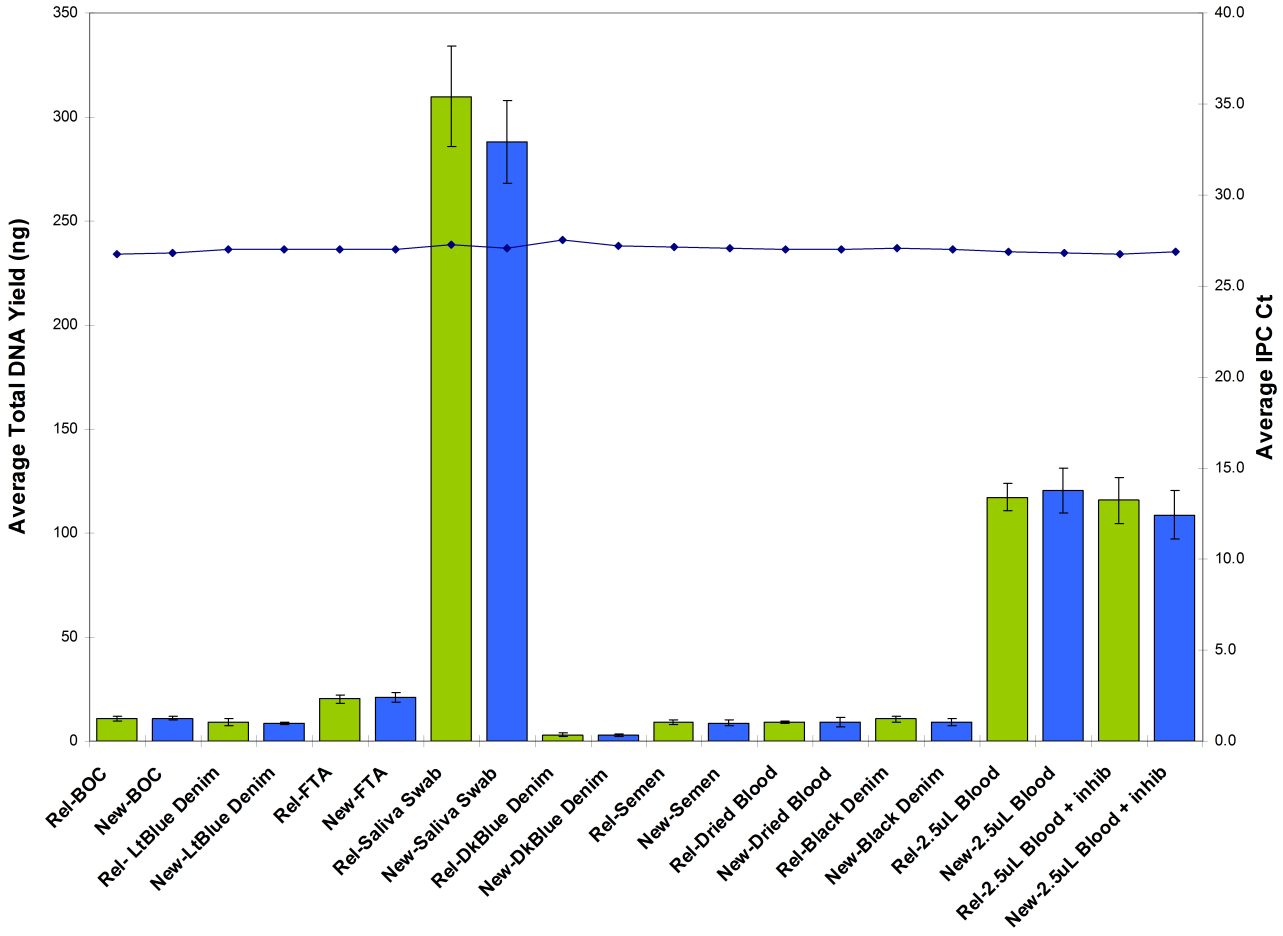


Figure 19 Low-input samples—DNA yield from original (green) and new (blue) scripts

Precision and sensitivity studies

Precision and sensitivity studies experiments

Precision and sensitivity studies were performed to test the consistency of DNA yield and quality from extracted and purified DNA. Seven replicates of a blood sample dilution series were extracted and purified. The average and standard deviation DNA yield and the IPC C_t within each replicate set were examined.

Table 16 Experiment setup for precision and sensitivity studies

Experiment setup	HID EVOlution™—Combination System	HID EVOlution™—Extraction System
Sample set	Dilution series using blood from Donor 238	Dilution series using blood from Donor 233
	Blood was diluted using the following dilution series: 1:5, 1:10, 1:50, 1:100, and 1:250. 5 µL of diluted blood was then spotted on SERI cotton cloth and Puritan swabs.	
Lysis method	See “Sample lysis method” on page 77.	

Table 16 Experiment setup for precision and sensitivity studies *(continued)*

Experiment setup	HID EVOlution™—Combination System	HID EVOlution™—Extraction System
Purification run setup (layout and replicates)	For each automated purification run, seven replicates of each dilution were set up in a plate as shown in Table 17. Each plate contained one substrate blank per dilution concentration (five extraction blanks per plate).	
Extraction script	PrepFiler_plate_plateCombo_V1_SP1	PrepFiler_plate_plate_V1_SP2
Number of purification runs	The experiment (purification through STR PCR) was performed three times (once per day on 3 different days).	
qPCR method	qPCR setup was performed on the HID EVOlution™ – Combination System using the Quantifiler® Human DNA Quantification kit.	
STR PCR method	DNA normalization and STR PCR setup was performed on the HID EVOlution™—Combination System using the AmpFℓSTR™ Identifiler™ PCR Amplification Kit. A target input of 1 ng of DNA was used for STR PCR amplification. For samples with concentrations <0.10 ng/µL, a maximum of 10 µL of purified DNA extract was added to each STR reaction.	

Table 17 DNA lysate plate layout for precision and sensitivity studies

	Dilution 1:5	Dilution 1:10	Dilution 1:50	Dilution 1:100	Dilution 1:250
A	Replicate 1	Replicate 1	Replicate 1	Replicate 1	Replicate 1
B	Replicate 2	Replicate 2	Replicate 2	Replicate 2	Replicate 2
C	Replicate 3	Replicate 3	Replicate 3	Replicate 3	Replicate 3
D	Replicate 4	Replicate 4	Replicate 4	Replicate 4	Replicate 4
E	Replicate 5	Replicate 5	Replicate 5	Replicate 5	Replicate 5
F	Replicate 6	Replicate 6	Replicate 6	Replicate 6	Replicate 6
G	Replicate 7	Replicate 7	Replicate 7	Replicate 7	Replicate 7
H	Extraction Blank 1	Extraction Blank 2	Extraction Blank 3	Extraction Blank 4	Extraction Blank 5

Precision and sensitvity studies results

The new scripts returned DNA in similar yield and with more efficient STR PCR amplification than the original scripts. Background amplification products were not observed from substrate blanks. IPC C_t was within normal range for all samples, and consistent results were obtained across the runs and between the two platforms.

For the HID EVOlution™—Combination System:

- DNA concentration results are shown in Table 19 below and Figure 21 on page 117
- Average peak heights are shown in Figure 22 on page 118

For the HID EVOlution™—Extraction System:

- DNA concentration results are shown in Table 20 and Figure 23 on page 119
- Average peak heights are shown in Figure 24 on page 119

Table 18 HID EVOlution™—Combination System—Precision and sensitivity studies

Average and standard deviation DNA concentration for the seven replicates for each dilution concentration

Sample dilution	Run 1		Run 2		Run 3	
	Average concentration	Conc. standard deviation	Average concentration	Conc. standard deviation	Average concentration	Conc. standard deviation
(1:5)	1.331 ng/µL	0.084	1.171 ng/µL	0.302	1.060 ng/µL	0.032
(1:10)	0.700 ng/µL	0.067	0.733 ng/µL	0.043	0.563 ng/µL	0.068
(1:50)	0.135 ng/µL	0.011	0.131 ng/µL	0.009	0.107 ng/µL	0.017
(1:100)	0.057 ng/µL	0.009	0.068 ng/µL	0.007	0.046 ng/µL	0.004
(1:250)	0.027 ng/µL	0.006	0.022 ng/µL	0.007	0.012 ng/µL	0.002
Average IPC C_t	27.478 ± 0.172 ng/µL	—	—	—	—	—

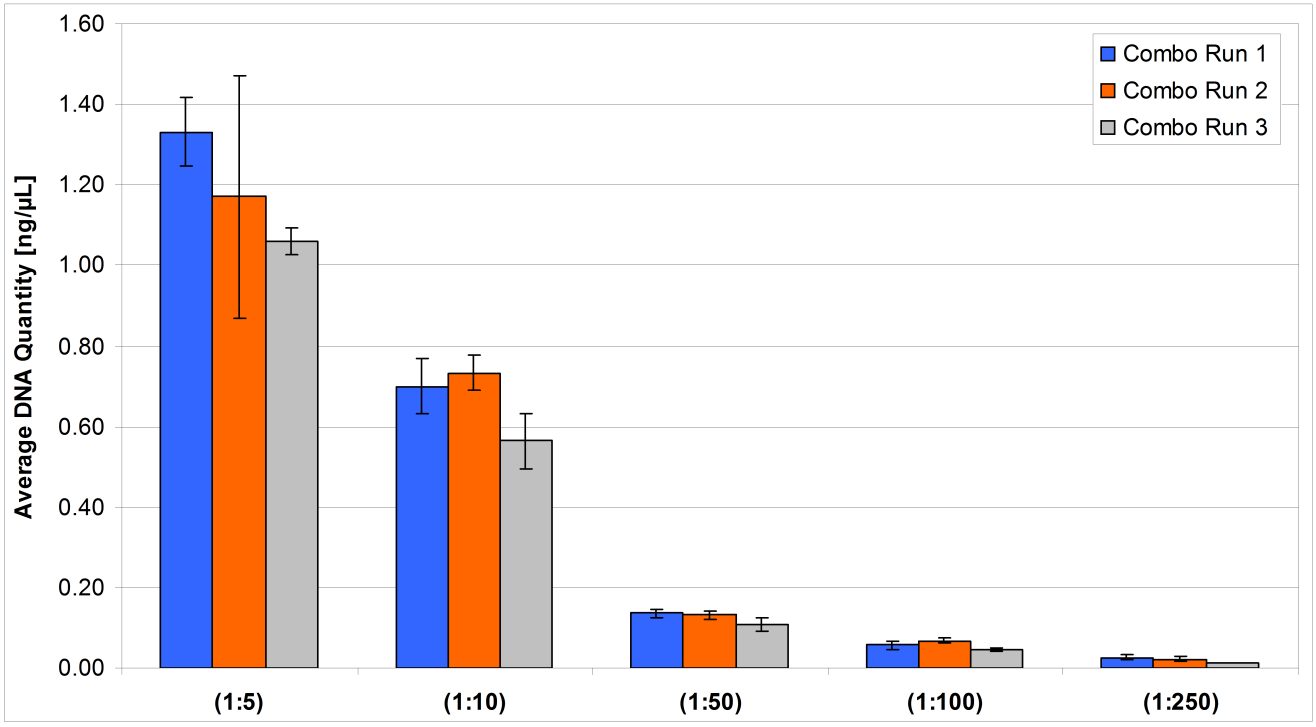


Figure 20 HID EVOlution™—Combination System—Average DNA concentration (ng/µL) for the seven replicates for each dilution concentration

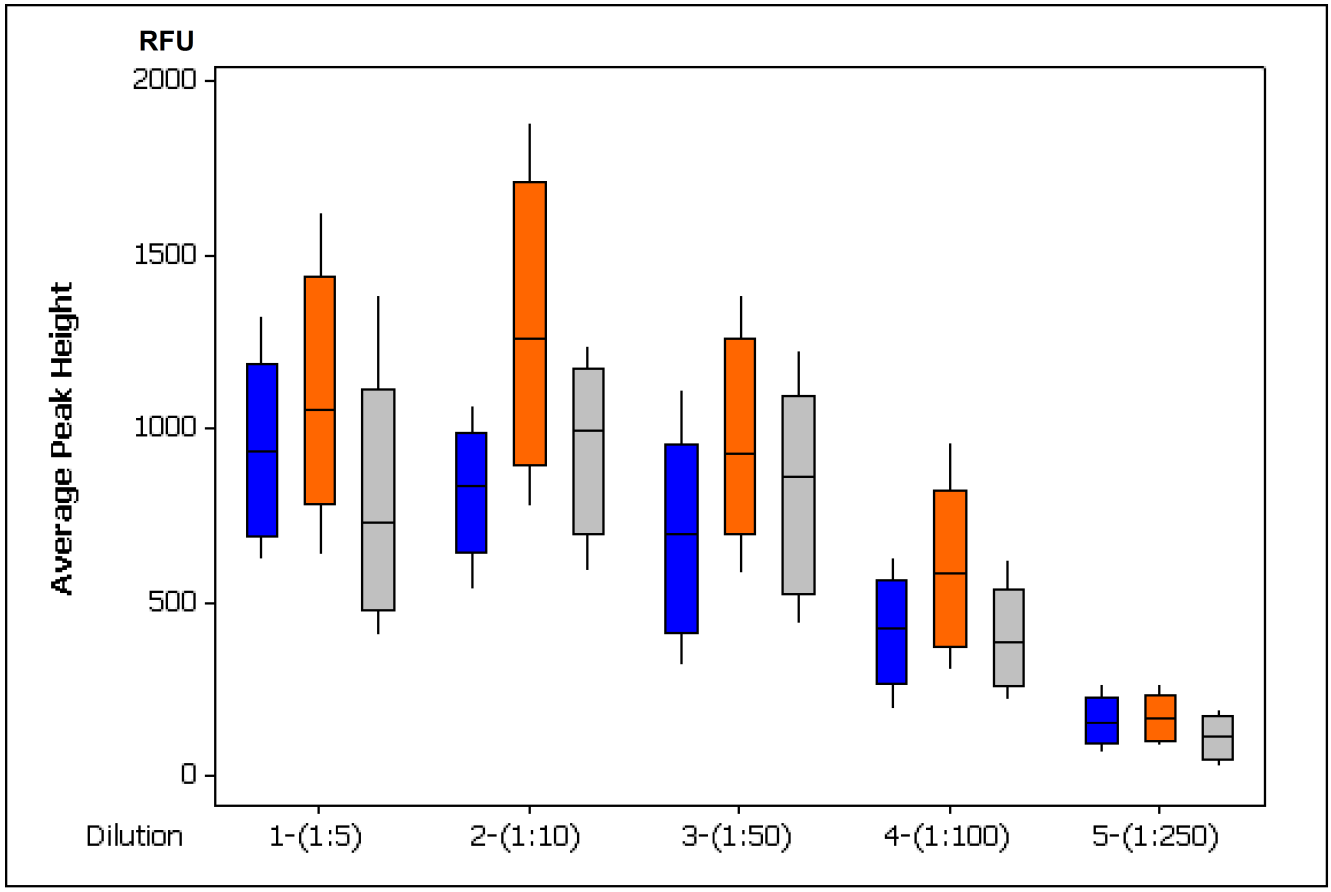


Figure 21 HID EVOlution™—Combination System—Average peak heights from AmpFℓSTR™ Identifiler™ PCR Amplification Kit STR profiles

Table 19 HID EVOlution™—Extraction System—Precision and sensitivity studies

Average and standard deviation DNA concentration for the seven replicates for each dilution concentration

Sample dilution	Run 1		Run 2		Run 3	
	Average concentration	Conc. standard deviation	Average concentration	Conc. standard deviation	Average concentration	Conc. standard deviation
(1:5)	1.359 ng/µL	0.156	1.270 ng/µL	0.208	1.202 ng/µL	0.155
(1:10)	0.547 ng/µL	0.043	0.585 ng/µL	0.115	0.570 ng/µL	0.036
(1:50)	0.108 ng/µL	0.007	0.109 ng/µL	0.008	0.105 ng/µL	0.011
(1:100)	0.045 ng/µL	0.008	0.045 ng/µL	0.006	0.052 ng/µL	0.007
(1:250)	0.020 ng/µL	0.002	0.016 ng/µL	0.007	0.025 ng/µL	0.009
Average IPC C_t	26.46 ± 0.211 ng/µL	—	—	—	—	—

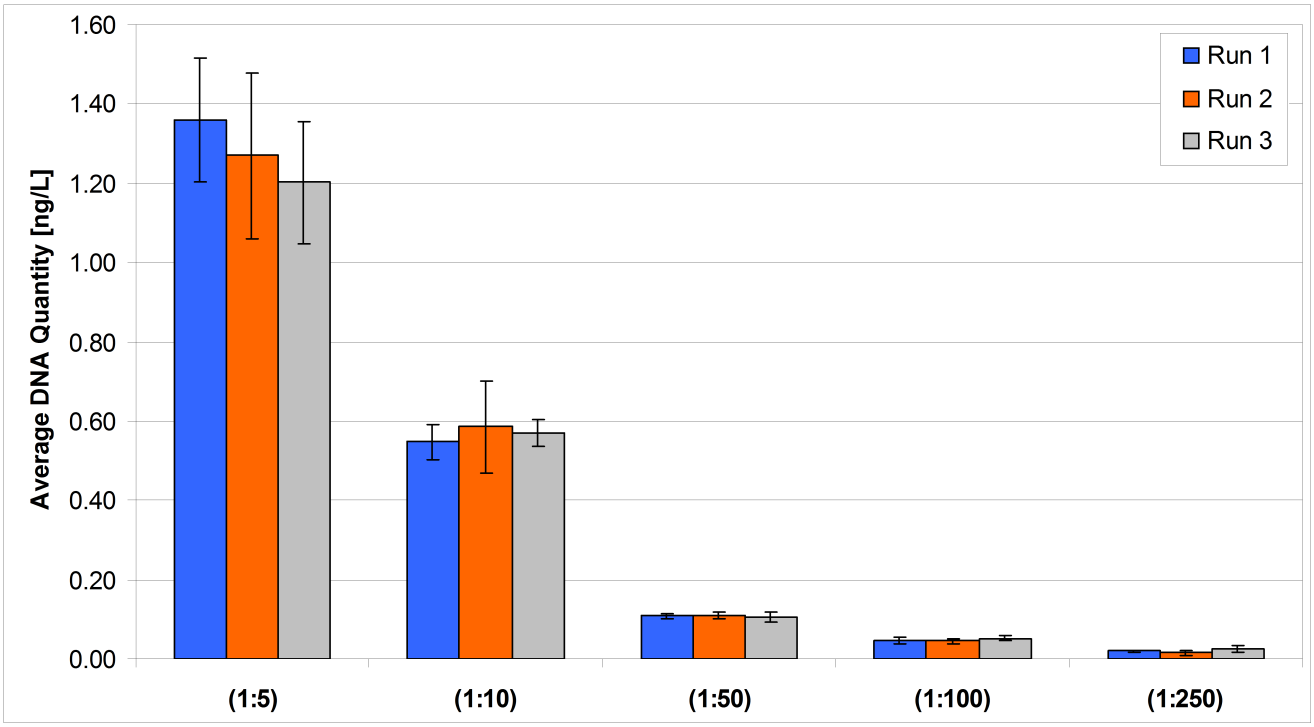


Figure 22 HID EVOlution™—Extraction System—Average DNA concentration (ng/µL)

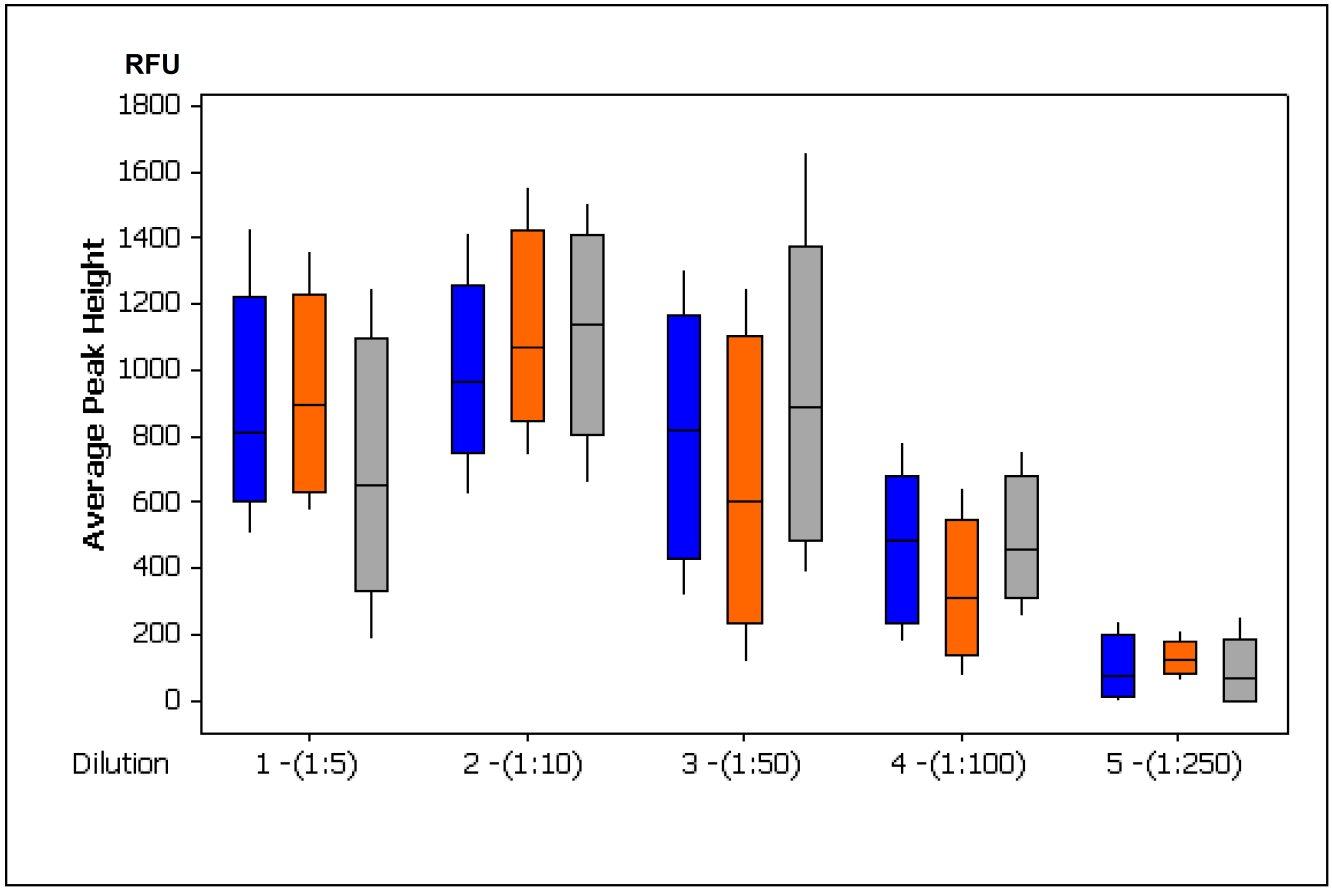


Figure 23 HID EVOlution™—Extraction System—Average peak heights from AmpFℓSTR™ Identifiler™ PCR Amplification Kit STR profile

Cross-contamination studies

Cross-contamination studies experiments

Cross-contamination studies were performed to confirm that the new scripts, wash buffer, and workstation layout do not introduce cross-contamination between samples. Liquid blood samples and extraction blanks were aliquoted in a checkerboard/column layout, then extracted and purified. The quantitation and STR results were examined for potential contamination in the extraction blanks. Consistency in the quality of the extracted and purified DNA, as determined by the presence of PCR inhibitors, was also assessed.

Table 20 Experiment setup for cross-contamination studies

Experiment setup	HID EVOlution™—Combination System	HID EVOlution™—Extraction System
Sample set	Blood from Donor 238	Blood from Donor 233
Lysis method and purification run setup (layout and replicates)	For each automated purification run, 40 sample replicates and 40 extraction blanks were arranged as shown in Figure 24 and Figure 25. To test only for cross-contamination introduced during automated liquid handling, steps were taken to minimize potential cross-contamination during sample preparation, lysis, and manual dispensing of samples and extraction blanks: • Before beginning this study, 80 extraction blanks were run on each platform using the cross-contamination study methodology to create a baseline for spurious signals and to confirm that the instruments, labware, and reagents used in the study were not a source of contamination. • Lysates (equivalent to 20 µL of liquid blood in 300 µL PrepFiler™ Lysis Buffer + 3 µL of DTT) and extraction blanks were prepared in batch, then dispensed into plates or tubes as follows: – For plate-to-plate extraction: With the filter plate removed, 323 µL of extraction blank lysate was added to the designated spin plate wells (see the plate map in Figure 24), then the filter plate was placed on top of the spin plate. After lysis, 323 µL of blood lysate was added to the designated wells. The spin/filter plate unit was centrifuged at 2,000 rpm for 1 minute to recover clarified lysates. – For tube-to-tube extraction: 1.5-mL tubes to receive the blood lysate were placed in the tube racks (see the tube positions in Figure 25). 323 µL of blood lysate was distributed to each sample tube. 1.5-mL tubes to receive the extraction blank lysate were placed in the tube racks, 323 µL of extraction blank lysate was added to each tube, then the tube racks were placed on the workstation.	
Extraction scripts	**Validation** • PrepFiler_plate_plateCombo_V1_SP1 • PrepFiler_tube_tubeCombo_V1_SP1	**Validation** • PrepFiler_plate_plate_V1_SP2 • PrepFiler_tube_tube_V1_SP2
	Verification[1] • PrepFiler_tube_tubeCombo_V1_SP1[2] • PrepFiler_plate_tubeCombo_V1_SP1 • PrepFiler_tube_plateCombo_V1_SP1	**Verification**[1] • PrepFiler_tube_tube_V1_SP2‡[2] • PrepFiler_plate_tube_V1_SP2 • PrepFiler_tube_plate_V1_SP2

Table 20 Experiment setup for cross-contamination studies *(continued)*

Experiment setup	HID EVOlution™—Combination System	HID EVOlution™—Extraction System
Number of purification runs	For each of the two extraction scripts, the experiment (purification through STR PCR) was performed two times (once per day on 2 different days).	
STR PCR method[3]	All extraction blanks were processed through STR PCR. STR PCR setup was performed on the HID EVOlution™—Combination System using the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit.	
qPCR method[3]	For this study, qPCR was performed after STR PCR. qPCR setup was performed on the HID EVOlution™—Combination System using the Quantifiler™ Human DNA Quantification Kit.	

[1] Verification studies methodology was identical to that of the validation studies, with the exception that 2-µL blood samples were used instead of 20-µL blood samples.

[2] For continuity, this script was run in both validation and verification studies.

[3] As a precaution, STR PCR with the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit was performed before quantitative PCR to eliminate cross-contamination that could result from accessing the DNA plate during qPCR. A sample sheet and 7500 Results files (CSV) were created to allow 10 µL of each extraction blank eluate to be directly dispensed in 15 µL of AmpFℓSTR™ MiniFiler™ PCR Amplification Kit reaction mix.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	X5	S9	X13	S17	X21	S25	X25	S33	X33		
B	X1	S5	X9	S13	X17	S21	S26	X26	S34	X34		
C	S2	X6	S10	X14	S18	X22	S27	X27	S35	X35		
D	X2	S6	X10	S14	X18	S22	S28	X28	S36	X36		
E	S3	X7	S11	X15	S19	X23	S29	X29	S37	X37		
F	X3	S7	X11	S15	X19	S23	S30	X30	S38	X38		
G	S4	X8	S12	X16	S20	X24	S31	X31	S39	X39		
H	X4	S8	X12	S16	X20	S24	S32	X32	S40	X40		

Figure 24 Cross-contamination studies—Plate layout

Plate layout for processing lysate from an 96-well plate and for collecting eluate in a 96-well plate for cross-contamination studies (S = blood lysate/eluate, X = extraction blank lysate/eluate)

	S1	S2	S3	S4	S5
1	S1	X9	S17	X25	X33
2	X1	S9	X17	X26	X34
3	S2	X10	S18	X27	X35
4	X2	S10	X18	X28	X36
5	S3	X11	S19	X29	X37
6	X3	S11	X19	X30	X38
7	S4	X12	S20	X31	X39
8	X4	S12	X20	X32	X40
9	S5	X13	S21	S25	S33
10	X5	S13	X21	S26	S34
11	S6	X14	S22	S27	S35
12	X6	S14	X22	S28	S36
13	S7	X15	S23	S29	S37
14	X7	S15	X23	S30	S38
15	S8	X16	S24	S31	S39
16	X8	S16	X24	S32	S40

Figure 25 Cross-contamination studies—Tube positions

Tube positions for processing lysate from tubes and for collecting eluate in tubes for cross-contamination studies (S = blood lysate/eluate, X = extraction blank lysate/eluate)

Cross-contamination studies results

Validation studies

For the HID EVOlution™—Extraction System, no evidence of liquid handling cross-contamination was seen in any of the 160 total extraction blanks.

For the HID EVOlution™—Combination System, no evidence of liquid handling cross-contamination was seen in any of the 160 total extraction blanks.

The IPC C_t values of the extracted blood samples and extraction blanks matched, indicating that no PCR inhibitors were present in the extracted DNA.

Verification studies

The remaining new scripts were tested using the cross-contamination study methodology with 2-µL blood samples and extraction blanks. No cross-contamination was observed in the extraction blanks.

Case-type sample studies

Case-type sample studies experiments

Case-type sample studies were performed to test performance when running low-input, case-type samples. Ten different sample types were extracted and purified in replicates of four, then DNA quality was evaluated by examining DNA yield and the STR profiles. To compare lysis methods, lysis was performed in tubes and in plates for the same sample set.

Table 21 Experiment setup for case-type sample studies

Experiment setup	HID EVOlution™—Combination System	HID EVOlution™—Extraction System
Sample set	Ten low-input, case-type samples. See Table 22.	
Lysis method	**Lysis in tubes** Sample lysis was performed in tubes, transferred to a spin/filter plate unit for substrate removal (see "Sample lysis method" on page 77), then processed on the automated system using a plate-to-plate script.	
	Lysis in plates 1. Dried samples were distributed into the wells of the spin/filter plate unit. The unit was placed on ice for 30 minutes to chill the chambers of the spin plate. 2. Lysis was initiated by adding a mixture of 300 µL of PrepFiler™ Lysis Buffer and 3 µL of 1 M DTT per well. 3. The spin/filter plate unit was sealed with adhesive film, then shaken at 300 rpm for 40 minutes at 60°C. 4. Lysates were centrifuged at 2,000 rpm for 1 minute to recover clarified lysates.	
Purification run setup (layout and replicates)	To eliminate potential plate-to plate variation that could occur during purification and quantitation, all lysates (from lysis in tubes and lysis in plates) were consolidated in one plate for purification and quantitation.	
Extraction script	PrepFiler_plate_plateCombo_V1_SP1	NA
Number of purification runs	1	NA
qPCR method	qPCR setup was performed on the HID EVOlution™—Combination System using the Quantifiler™ Human DNA Quantification Kit.	
STR PCR method	DNA normalization and STR PCR setup were performed on the HID EVOlution™—Combination System using the AmpFℓSTR™ Identifiler™ PCR Amplification Kit. A target input of 1 ng of DNA was used for STR PCR amplification. For samples with concentrations <0.10 ng/µL, a maximum of 10 µL of purified DNA extract was added to each STR reaction.	

Table 22 Low-input, case-type sample set

Plate column	Sample source/substrate	Body fluid volume	Number of replicates	Plate well position[1]
1	Blood dried on white cotton cloth (SERI, 4-mm punch)	0.2 µL	4	A1 to H1
2	Blood dried on unwashed light blue denim (4-mm)	0.2 µL	4	A2 to H2
3	Blood dried on FTA paper (4-mm punch)	0.5 µL	4	A3 to H3
4	Saliva dried on cotton swab (Puritan wooden)	5.0 µL	4	A4 to H4
5	Blood dried on unwashed dark blue denim (4-mm)	0.2 µL	4	A5 to H5
6	Semen dried on white cotton cloth (SERI, 4-mm punch)	1.0 µL	4	A6 to H6
7	Dried blood in 1.5-mL tube	0.2 µL	4	A7 to H7
8	Blood dried on unwashed black denim (4-mm punch)	0.2 µL	4	A8 to H8
9	Blood spiked with PBS dried on pre-washed white cotton cloth (SERI; 4 µL of blood + 1 µL of PBS, 3 µL on 4-mm punch)	2.5 µL	4	A9 to H9
10	Blood spiked with inhibitor mix dried on pre-washed white cotton cloth (SERI; 4 µL of blood + 1 µL of PBS, 3 µL on 4-mm punch)	2.5 µL	4	A10 to H10

[1] Four replicates each from lysis in tubes and lysis in plates.

Case-type sample studies results

DNA yield and average IPC C_t for samples lysed in plates and tubes are shown in Figure 26 and Figure 27. With some sample types and inputs, the tube lysis protocol may be the preferred method compared to the plate lysis protocol.

The AmpFℓSTR™ Identifiler™ PCR Amplification Kit STR profiles (not shown) were complete except for those derived from the dark blue and black denim samples that were tested in this study. While not true for all denim sample types, some of the STR profiles generated from the dark blue and black denim samples in the study showed signs of PCR inhibition.

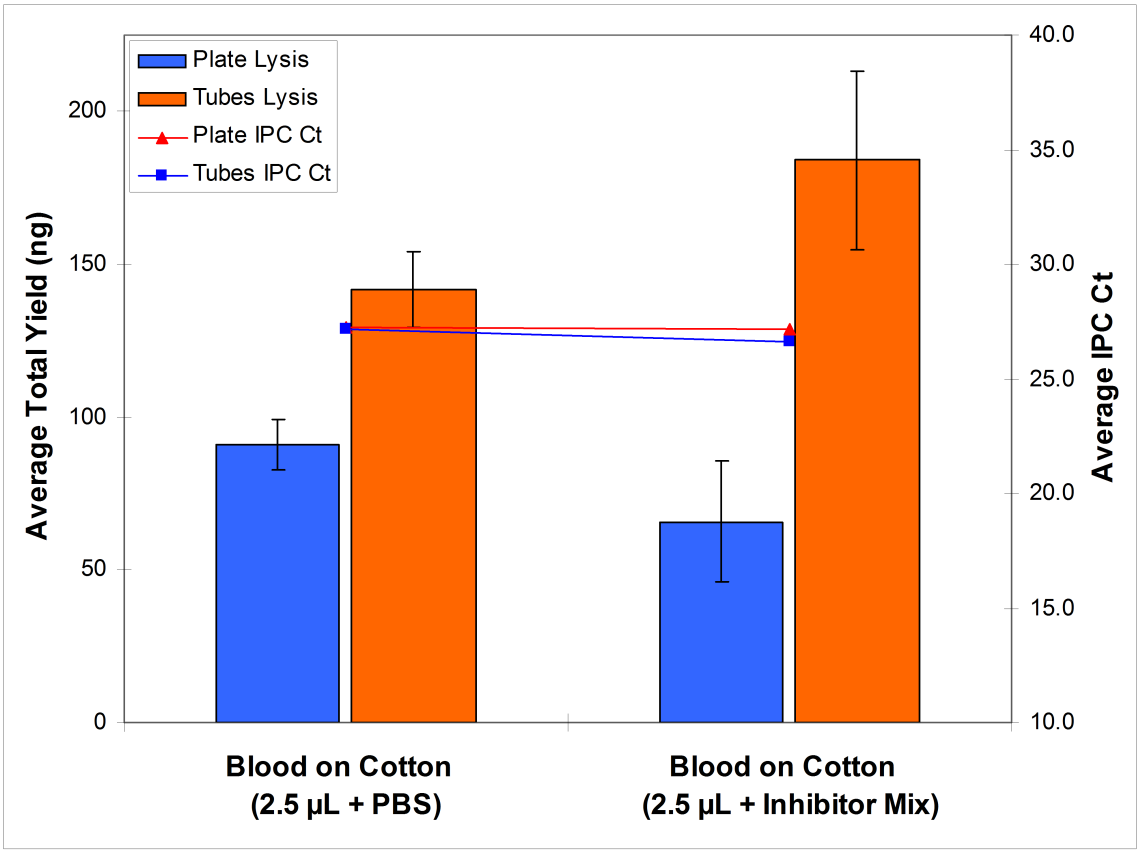


Figure 26 Total DNA yield for sample lysis in plates and in tubes—2.5 µL of blood (with and without inhibitor mix) on cotton

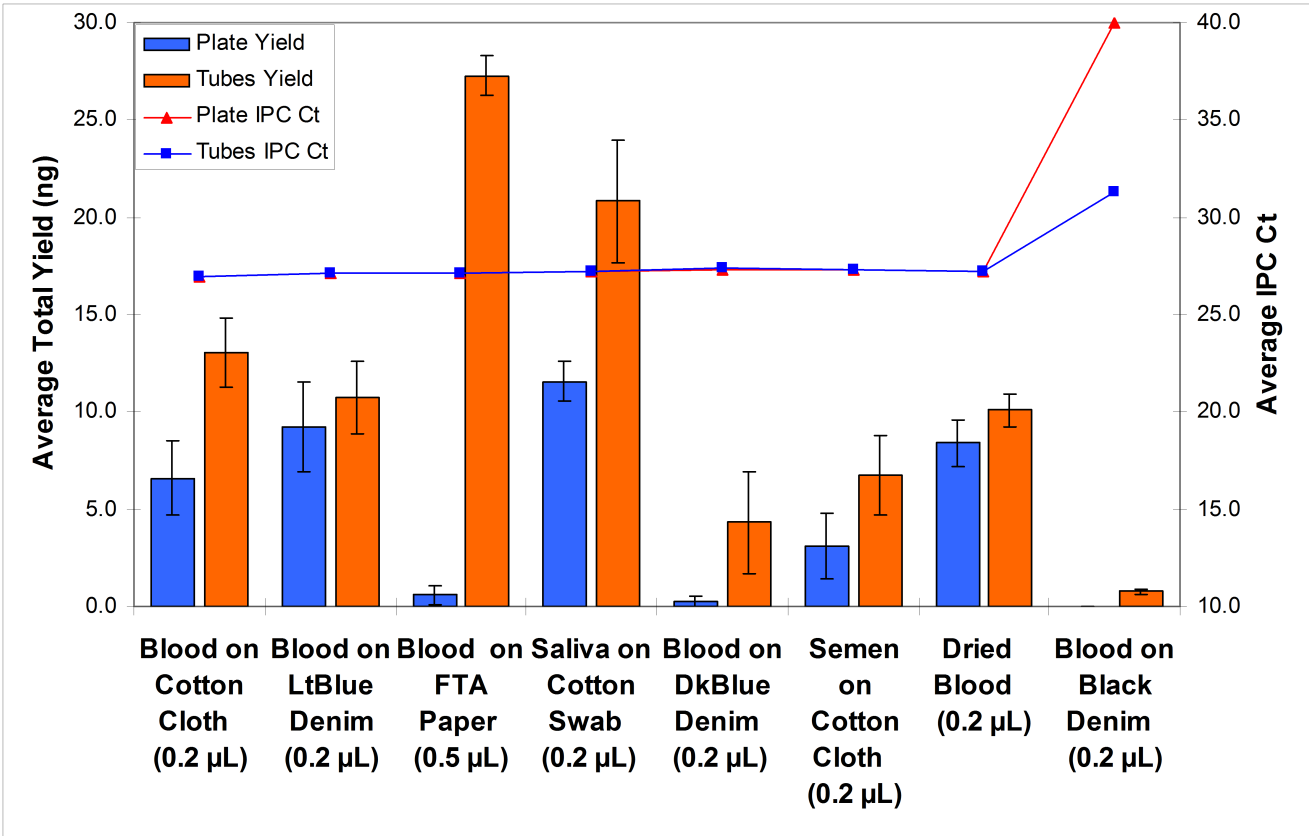


Figure 27 Total DNA yield for 8 case-type samples comparing lysis in plates and in tubes

Comparative analysis studies

Comparative analysis studies experiments

Comparative analysis studies were performed to compare the quantity and quality of DNA purified on the HID EVOlution™—Combination System and HID EVOlution™—Extraction System to other platforms. The DNA yield and STR profiles from the "Case-type sample studies" on page 89 were compared to the data generated on other automated purification systems.

Table 23 Experiment setup for comparative analysis studies

Experiment setup	Cartridge-based, silica magnetic bead extraction kit and platform from Company A	Cartridge-based, silica magnetic bead extraction kit and platform from Company B
Sample set	Ten low-input, case-type samples. See Table 24.	
Sample preparation	Company A kit using the trace protocol with carrier RNA	Company B kit
Lysis method	Samples listed in Table 24 were lysed in 1.5-mL tubes according to the manufacturer's recommended protocol for low-input samples.	Samples listed in Table 24 were lysed in 1.5-mL tubes according to the manufacturer's recommended protocol for small casework samples.
Number of purification runs	1	1
qPCR method	qPCR setup was performed on the HID EVOlution™—Combination System using the Quantifiler™ Human DNA Quantification Kit.	
STR PCR method	DNA normalization and STR PCR setup were performed on the HID EVOlution™—Combination System using the AmpFℓSTR™ Identifiler™ PCR Amplification Kit. A target input of 1 ng of DNA was used for STR PCR amplification. For samples with concentrations <0.10 ng/µL, a maximum of 10 µL of purified DNA extract was added to each STR reaction.	

Table 24 Comparative analysis studies sample set and results for average DNA concentration (ng/µL)

Plate column	Sample source/substrate	Body fluid volume	Average DNA concentration (n=4)		
			PrepFiler™ Automated Forensic DNA Extraction Kit[1]	Company A	Company B
1	Blood dried on white cotton cloth (SERI, 4-mm punch)	0.2 µL	0.33 ng/µL	0.15 ng/µL	0.08 ng/µL
2	Blood dried on unwashed light blue denim (4-mm)	0.2 µL	0.27 ng/µL	0.16 ng/µL	0.11 ng/µL
3	Blood dried on FTA paper (4-mm punch)	0.5 µL	0.68 ng/µL	0.24 ng/µL	0.12 ng/µL

Table 24 Comparative analysis studies sample set and results for average DNA concentration (ng/µL) *(continued)*

Plate column	Sample source/substrate	Body fluid volume	Average DNA concentration (n=4)		
			PrepFiler™ Automated Forensic DNA Extraction Kit[1]	Company A	Company B
4	Saliva dried on cotton swab (Puritan wooden)	5.0 µL	0.52 ng/µL	0.32 ng/µL	0.05 ng/µL
5	Blood dried on unwashed dark blue denim (4-mm)	0.2 µL	0.11 ng/µL	0.014 ng/µL	0.17 ng/µL
6	Semen dried on white cotton cloth (SERI, 4-mm punch)	1.0 µL	0.17 ng/µL	0.07 ng/µL[2]	0.03 ng/µL[2]
7	Dried blood in 1.5-mL tube	0.2 µL	0.25 ng/µL	0.21 ng/µL	0.01 ng/µL
8	Blood dried on unwashed black denim (4-mm punch)	0.2 µL	0.02 ng/µL	0.18 ng/µL	0.09 ng/µL
9	Blood spiked with PBS dried on pre-washed white cotton cloth (SERI; 4 µL of blood + 1 µL of PBS, 3 µL on 4-mm punch)	2.5 µL	3.5 ng/µL	1.81 ng/µL	1.38 ng/µL
10	Blood spiked with inhibitor mix dried on pre-washed white cotton cloth (SERI; 4 µL of blood + 1 µL of PBS, 3 µL on 4-mm punch)	2.5 µL	4.6 ng/µL	3.53 ng/µL	1.29 ng/µL
	Average elution volume		40 µL	45 µL	41 µL

[1] Lysis in 1.5-mL tubes incubated in a thermal shaker for 40 minutes at 70°C

[2] Overnight lysis at 56°C.

Comparative analysis studies results

The quality of the DNA extracted and purified with the PrepFiler™ Automated Forensic DNA Extraction Kit on the HID EVOlution™—Combination System was high and generally yielded overall peak heights and intracolor balance metrics that met or exceeded results obtained from DNA isolated using Company A and Company B methods.

All extraction blank results demonstrated clean liquid handling. Average yields for the various low-input samples are listed in Table 24.

Figure 28 and Figure 29 display average total yields, in nanograms, of DNA recovered using Company A kit and platform, Company B kit and platform, or the PrepFiler™ Automated Forensic DNA Extraction Kit on the HID EVOlution™—Combination System. Yields were normalized to total elution volume (see Table 24, last row). The overall yield is generally higher for the HID EVOlution™ system with lysis performed in 1.5-mL tubes.

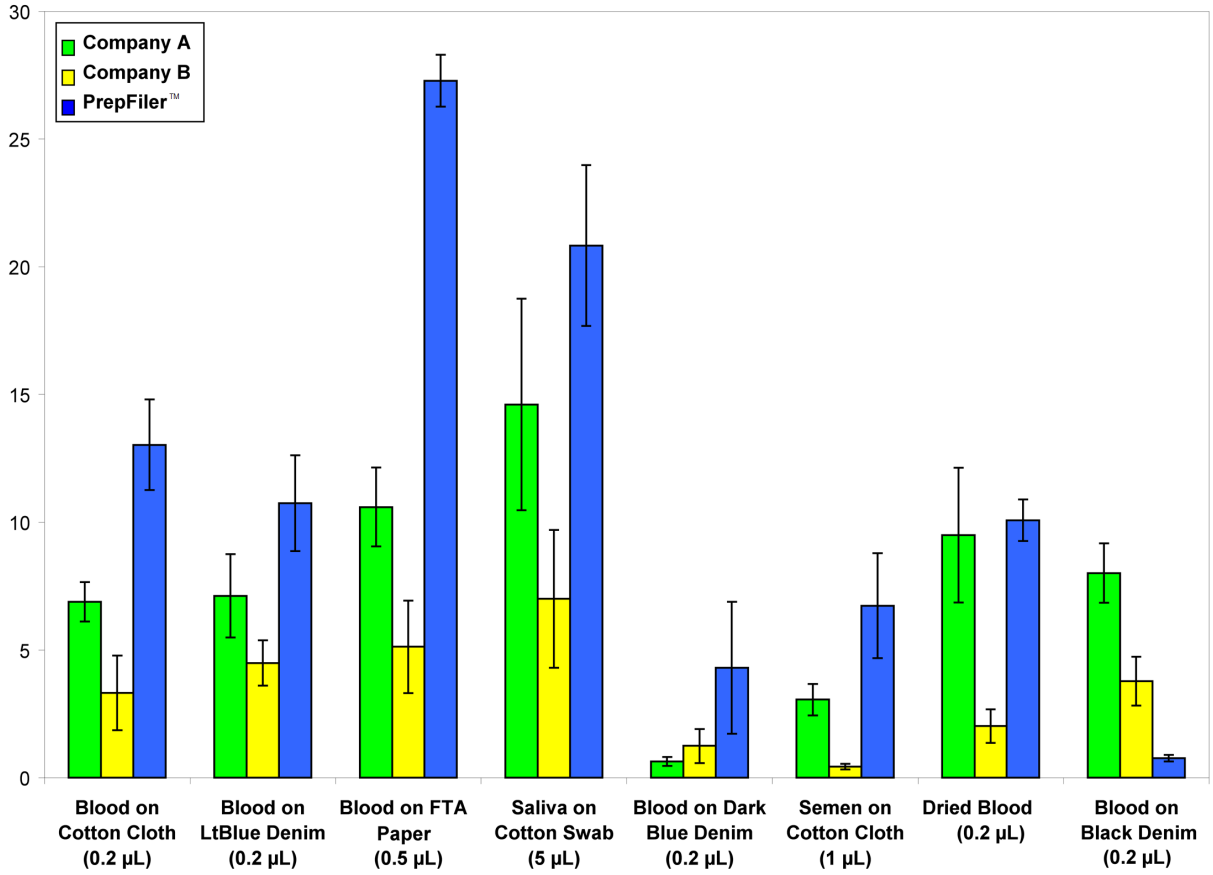


Figure 28 Comparative overall yield (ng)

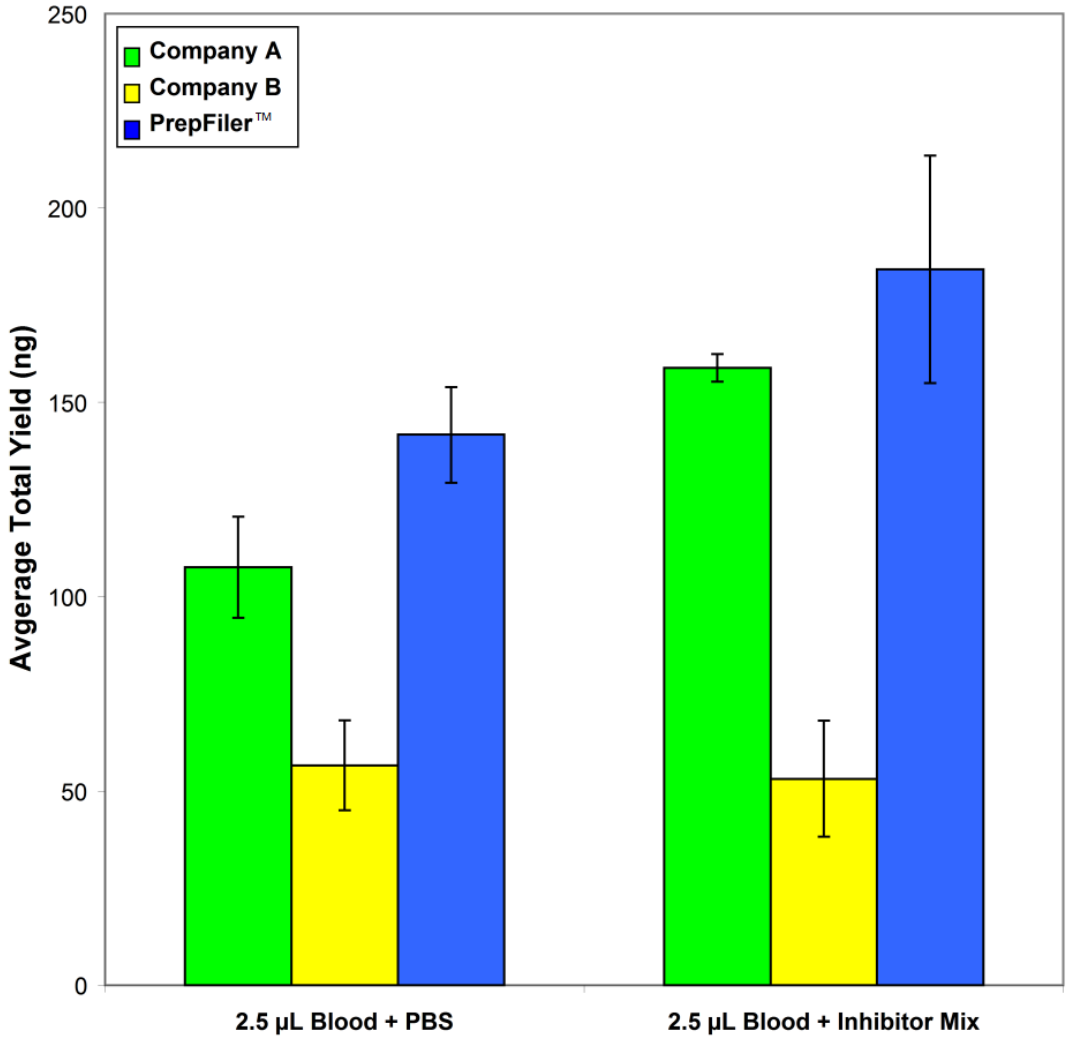


Figure 29 Comparative yield for 2.5 µL of blood and blood spiked with inhibitors

Figure 30 shows intracolor balance results for six samples. Samples 5 and 8 were omitted because of low DNA input and stochastic effects. Figure 31 shows the intracolor balance for samples 9 and 10.

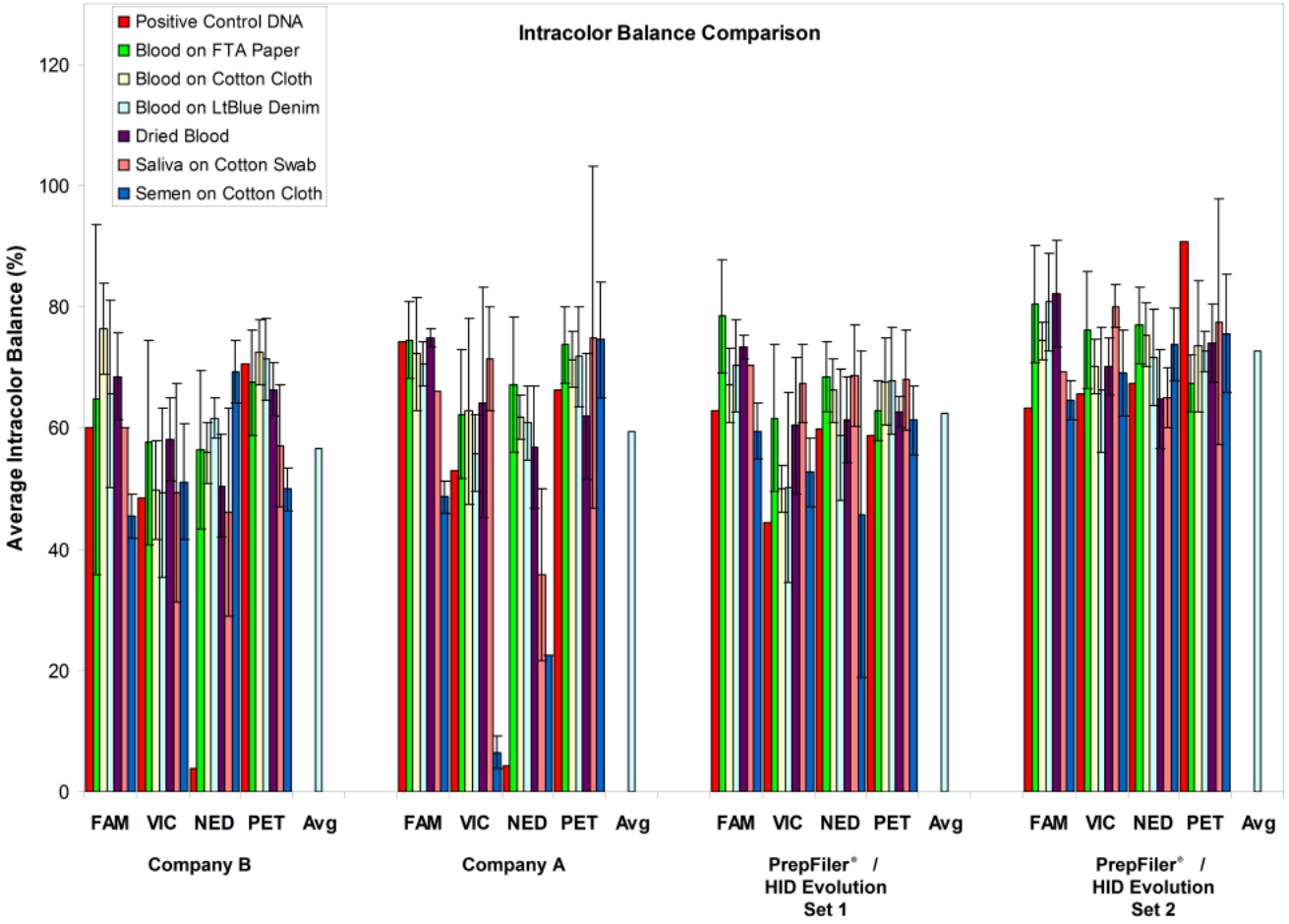


Figure 30 Intracolor balance between purification platforms obtained with the AmpFℓSTR™ Identifiler™ PCR Amplification Kit

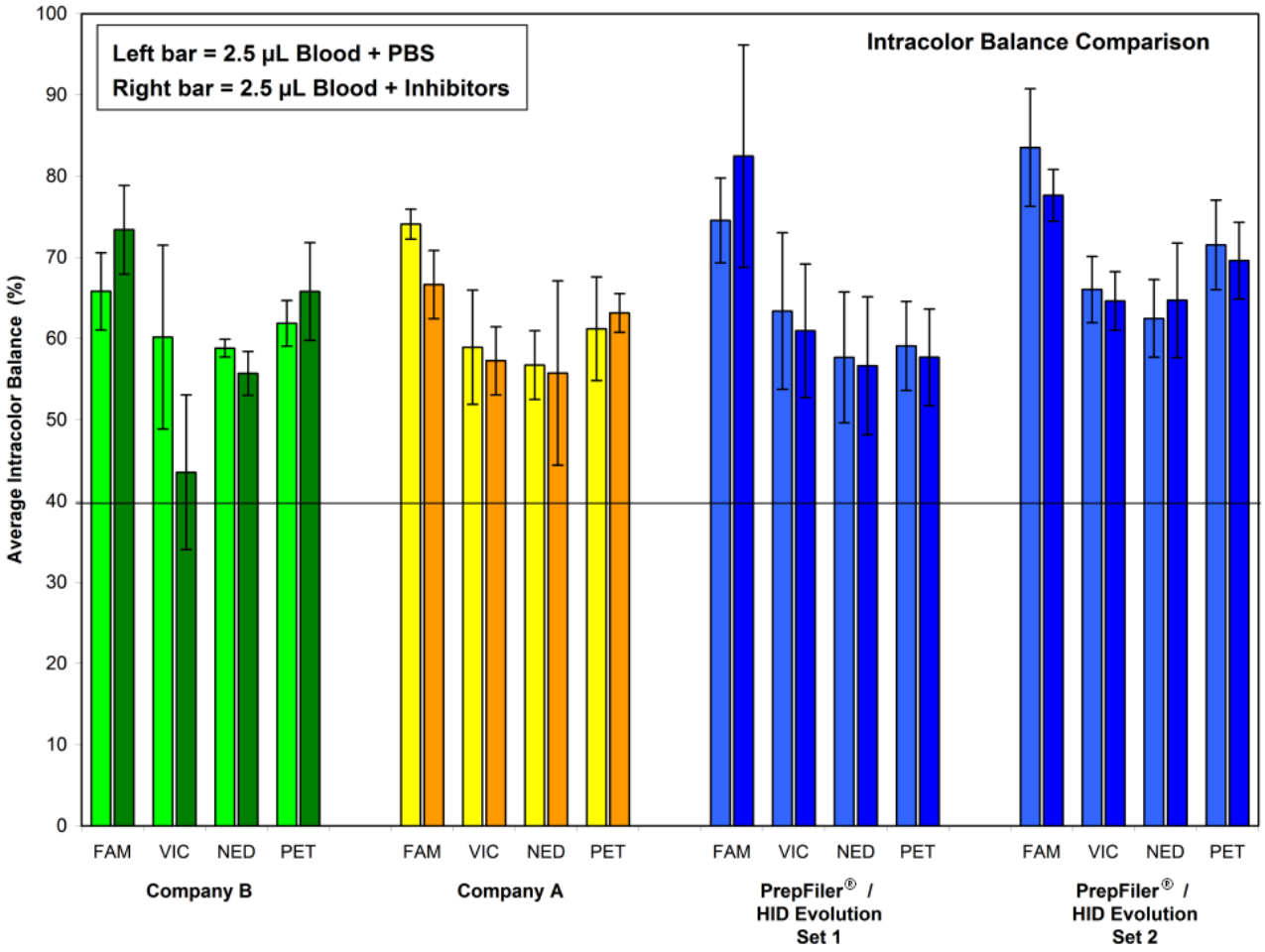


Figure 31 Intracolor balance obtained with the AmpFℓSTR™ Identifiler™ PCR Amplification Kit with DNA recovered from 2.5 µL of blood on cotton cloth spiked with an inhibitor cocktail

Samples were spiked with phosphate buffered saline (first bars) or an inhibitor cocktail (second bars). The intracolor balances for these samples were all >40% for all dye labels.

To assess the overall performance of the DNA purified using the three extraction and purification chemistries tested, the average peak heights for all ten sample types listed in Table 24 were calculated and plotted in a box-plot format in Figure 32.

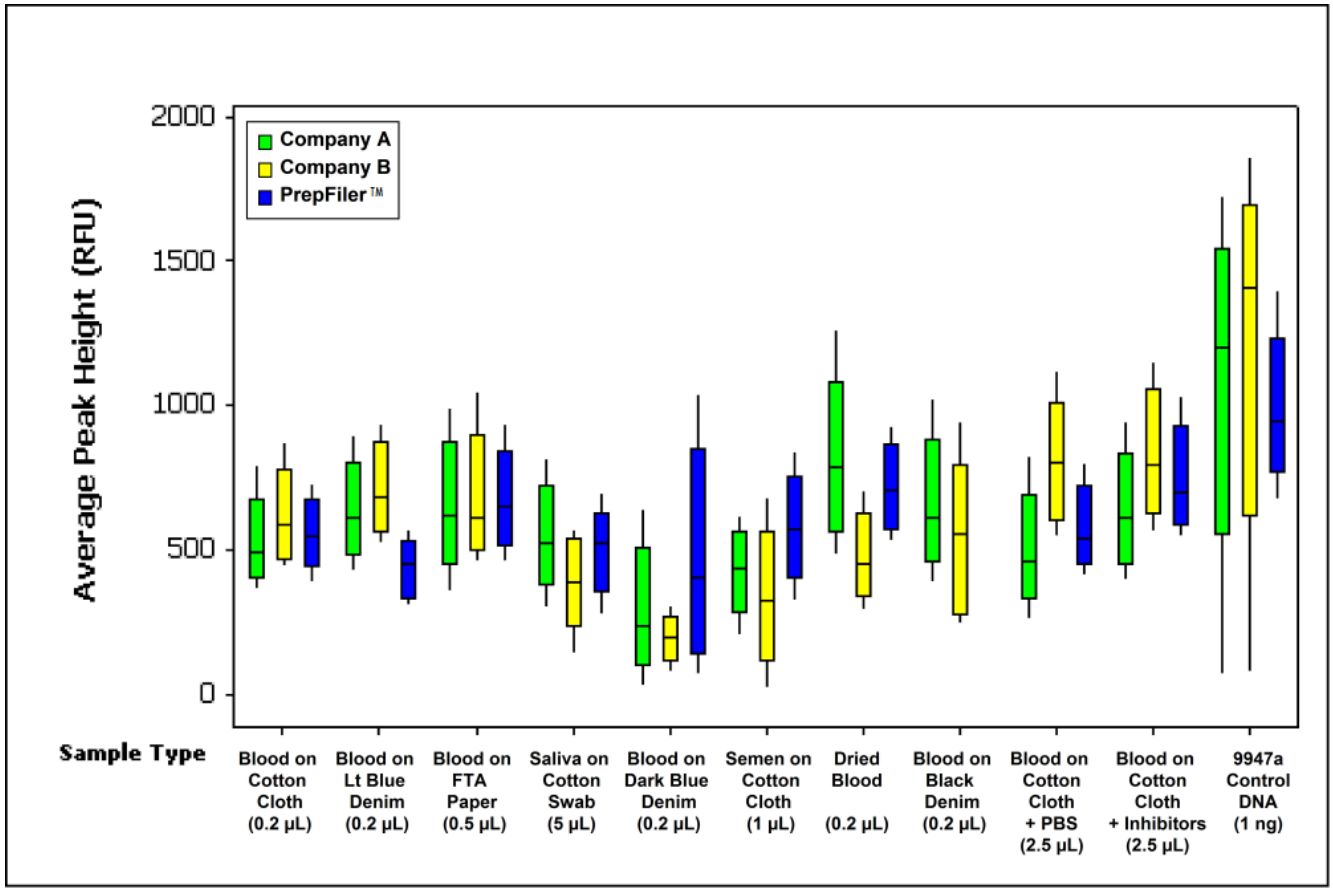


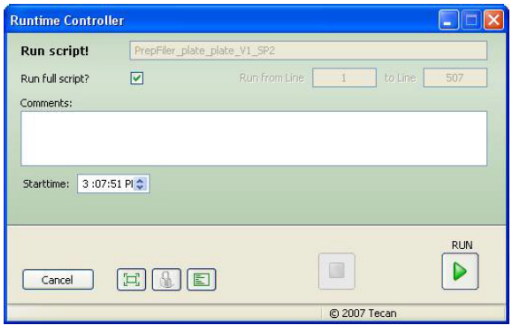
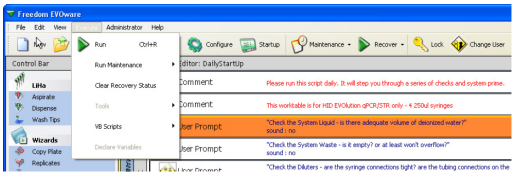
Figure 32 Average AmpFℓSTR™ Identifiler™ PCR Amplification Kit peak height for low-input, case-type samples

Conclusions

PrepFiler™ Wash Buffer B and the scripts listed in Table 13 were validated on the HID EVOlution™—Extraction System and the HID EVOlution™—Combination System. High-quality genomic DNA, obtained from a variety of biological samples, was determined to be suitable for downstream applications.

- Precision and sensitivity studies confirmed that the new scripts perform as well as the original scripts.
- Cross-contamination studies of 240 extraction blanks co-extracted with 20 µL of whole blood samples and amplified with the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit confirmed that the new scripts operate with clean liquid handling.
- Low-input, case-type samples studies demonstrated that high-quality STR profiles were obtained for the majority of samples tested. No PCR inhibition was observed in samples that contained known PCR inhibitors.
- Comparative analysis studies demonstrated that extraction and purification with the PrepFiler™ Automated Forensic DNA Extraction Kit on the HID EVOlution™—Extraction System or HID EVOlution™—Combination System, with the new scripts, yielded more DNA, and in most cases produced better STR profiles, than comparable extraction and purification chemistries. Some denim samples showed signs of PCR inhibition.
- With some sample types and inputs, the tube lysis protocol may be the preferred method compared to the plate lysis protocol.

A Troubleshooting

Observation	Possible cause	Recommended action
A run is stopped	You intentionally or accidentally stopped a run.	Clear the script recovery status before you start a new run: 1. Click **Cancel** in the **Runtime Controller**.  2. In the **Freedom EVOware™** script dialog box, note the highlighted orange line; this is the step where the run stopped. 3. In the **Freedom EVOware™** script dialog box, select **Execute ▸ Clear Recovery Status** from the menu. You can now start a new run. 
The DNA eluate contains magnetic particles	• Small magnetic particles (fines), which migrate more slowly towards the magnet, or particle aggregates, which hinder particle migration, were present. • The liquid handling arm on the Freedom EVO™ instrument needs to be adjusted.	Place the plate or tube containing the DNA eluate in a deep-well centrifuge, spin at 650 × *g* for 5 minutes, then pipet the clear DNA extract into a new plate or tube. If the problem persists over multiple runs, contact Technical Support to determine if the liquid handling (LiHa) arm requires adjustment. Also see the *Tecan™ Freedom EVOware™ Standard 2.1 Freedom EVOware™ PLUS™ 2.1 Extended Device Support Software Manual*, Section 9.4.4, "Teaching the Labware Coordinates".

Observation	Possible cause	Recommended action
The DNA eluate is colored **Note:** Color does not necessarily interfere with quantitation or amplification.	The substrate yielded a colored eluate. For example, some sample substrates contain dyes.	If you see a shift in the IPC C_t value in the quantitation run, manually process the DNA eluates using the Repurification Protocol, then requantify the sample. See the *PrepFiler™ Forensic DNA Extraction Kit User Guide*, Appendix B, "Repurification Protocol".
The DNA eluate volume is low	Incomplete volume transfer occurred because of a loose pipette tip (DiTi cone).	• If liquid remains in the PrepFiler™ 96-Well Processing Plate, manually pipet the liquid to the correct plate wells or tubes. • Before the next run, clean and finger-tighten the DiTi cones and diluter valves. See the *Tecan™ HID EVOlution™—Extraction Application Manual*, Section 7.3.2, "Disposable Tip (DiTi) of LiHa" and Section 7.3.10, "Diluter".
	The z-max of the liquid pipetting arm was not properly adjusted.	Confirm that the z-max is set correctly for the PrepFiler™ 96-Well Processing Plate when the plate is set on the Magnetic-Ring Stand (96 well) and used with a 200-µL disposable pipette tip. For details, contact Technical Support or see the *Tecan™ Freedom EVOware™ Standard 2.1 Freedom EVOware™ PLUS™ 2.1 Extended Device Support Software Manual*, Section 9.4.4, "Teaching the Labware Coordinates".

Observation	Possible cause	Recommended action
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 the system. – Dirty or loose DiTi cones. • DiTis were not picked up properly.	1. Confirm that reagent and instrument setup are correct, then re-run the samples: • Confirm all necessary reagents are present and correctly positioned on the workstation. See the *Tecan™ HID EVOlution™ - Extraction Application Manual*, Section 4.3.4, "Setup Reagents on the Workstation". • Confirm that you used the specified DiTis, plates, tubes, and metal racks and carriers in the correct positions. See the *Tecan™ HID EVOlution™ - Extraction Application Manual*, Section 4.3.5, "Setup Plasticware and Samples on the Workstation", and Section 4.4, "Worktable Layouts". • Flush the system and check for air bubbles and leaks. See the *Tecan™ HID EVOlution™ - Extraction Application Manual*, Section 7.3.1.2, "Flushing the Liquid System". • Clean and finger-tighten the DiTi cones and diluter valves. See the *Tecan™ HID EVOlution™ - Extraction Application Manual*, Section 7.3.2, "Disposable Tip (DiTi) of LiHa" and 7.3.10, "Diluter". • Reteach the LiHa coordinates (x, y, and z positions) of the 200-µL and 1,000-µL DiTis. For details, contact Technical Support or see the *Tecan™ Freedom EVOware™ Standard 2.1 Freedom EVOware™ PLUS™ 2.1 Extended Device Support Software Manual*, Section 9.4.4, "Teaching the Labware Coordinates". 2. Amplify the maximum volume for STR analysis. 3. Extract DNA from a different sample that is prepared from the same source.

Observation	Possible cause	Recommended action
The sample IPC C_t is greater than the IPC C_t of the no template control (NTC) or quantitation standards **Details:** For example, the sample IPC C_t is ~2 C_t is greater than the NTC IPC C_t or the standards C_t.	• Magnetic particles are in the DNA extract. • The DNA concentration is >25 ng/µL. • The DNA eluate contains PCR inhibitors because of excessive amounts of inhibitors in the sample.	• If magnetic particles are in the DNA eluate, place the plate or tube containing the DNA eluate in a deep-well centrifuge, centrifuge at 650 × *g* for 5 minutes, pipet the clear DNA extract solution into a new plate or tube, then process for quantitation. • If the DNA concentration is >25 ng/µL, dilute the DNA eluate, then requantify the sample. • If the DNA eluate is <25 ng/µL, or if the diluted DNA eluate still produces high IPC C_t compared to the NTC or quantitation standards, follow the Repurification Protocol to process the DNA eluate, then requantify the sample. See the *PrepFiler™ Forensic DNA Extraction Kit User Guide*, Appendix B, "Repurification Protocol". **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 inhibited and/or degraded samples. • Before the next run, to ensure correct pipetting, clean and finger-tighten the DiTi cones and diluter valves. See the *Tecan™ HID EVOlution™ - Extraction Application Manual*, Section 7.3.2, "Disposable Tip (DiTi) of LiHa" and 7.3.10, "Diluter".

B Plate setup requirements for <96 samples

If you prepare <96 samples in a plate, the Freedom EVOware™ software scripts require a specific plate layout.

Place samples in the plate (<96 samples)

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

2. After the first 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 you make a pipetting error, use blank reagents (water or DNA suspension buffer) as needed to avoid leaving empty wells between samples.

IMPORTANT! When setting up sample information in the HID EVOlution™ - Extraction System, assign a unique sample ID to all wells that contain samples or blank reagents. See the *Tecan™ HID EVOlution™ - Extraction Application Manual*, Section 3.4, "Sample File", for sample naming requirements.

Note: Regardless of the lysis plate setup, the DNA eluate corresponding to the first sample in the lysis plate is always placed in well position A1 (for eluate that is collected in a 96-well Elution Plate) or rack S1 tube position 1 (for eluate that is collected in tubes). The Report file (PDF) that is generated at the end of the purification run lists the starting position of each sample lysate and the final position of the corresponding DNA eluate.

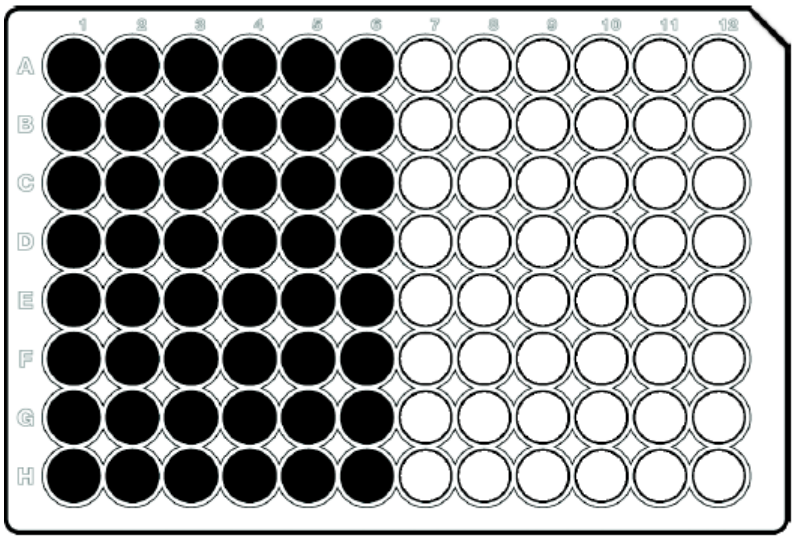


Figure 33 Correct (samples in wells 1–48)

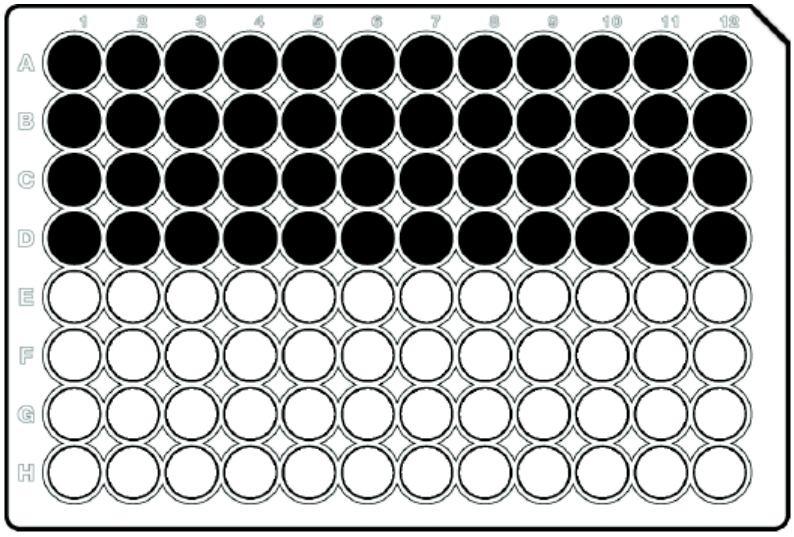


Figure 34 Incorrect

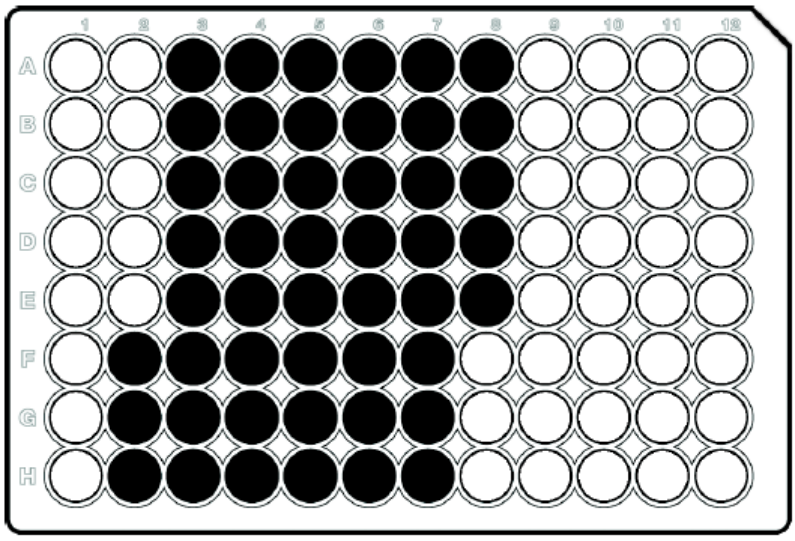


Figure 35 Correct (samples in wells 14–61)

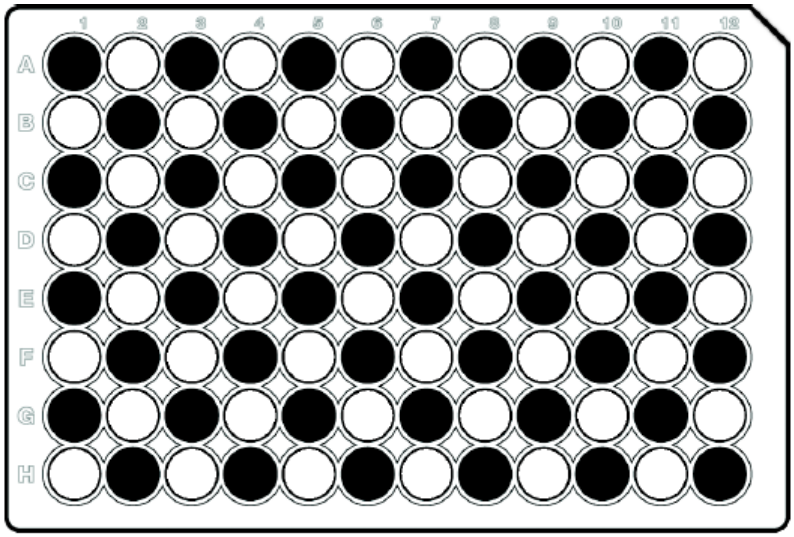


Figure 36 Incorrect

C

(One-time procedure) Document Te-Shake™ plate adapter temperatures

The temperatures set in the extraction scripts are the temperatures for the heating plate (4 in Figure 37), not the heating plate adapter (3 in Figure 37).

To ensure that the heating plate adapter reaches the recommended temperatures during the automated purification run, perform procedure on 108.

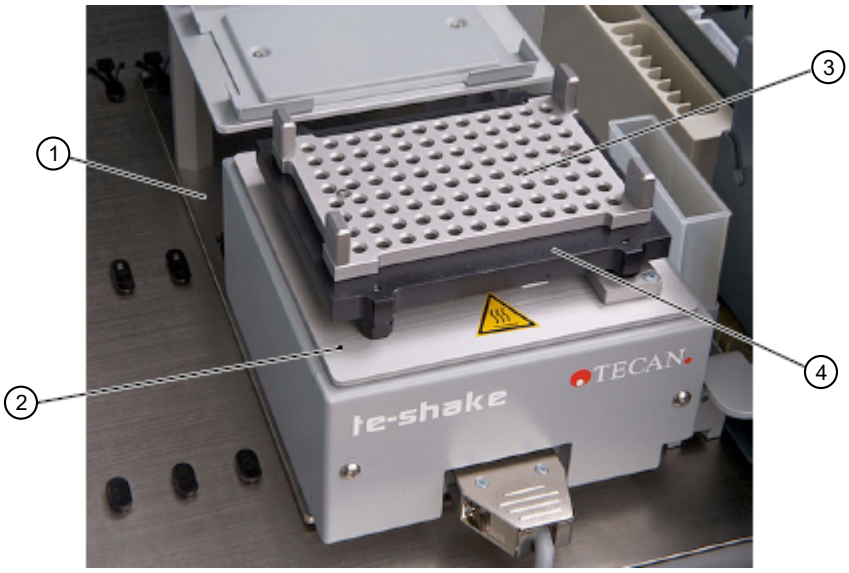


Figure 37 Te-Shake™ plate adapter components

① Mounting plate with two additional positions (Tecan™ Cat. No. 30015506; PLATE MOUNTING 2 TE-SHAKE CMR)
② Shaker plate for the heating block (Tecan™ Cat. No. 10760726; PLATE SHAKER HEATING PLATE TE-SHAKE)
③ Heating plate adapter for the VWR plate (Tecan™ Cat. No. 30035318; BLOCK HEATING VWR PLATE CPL)
④ Heating plate for the plate/tube adapters (Tecan™ Cat. No. 30015374; PLATE HEATING TE-MAGS/TE-SHAKE MP)

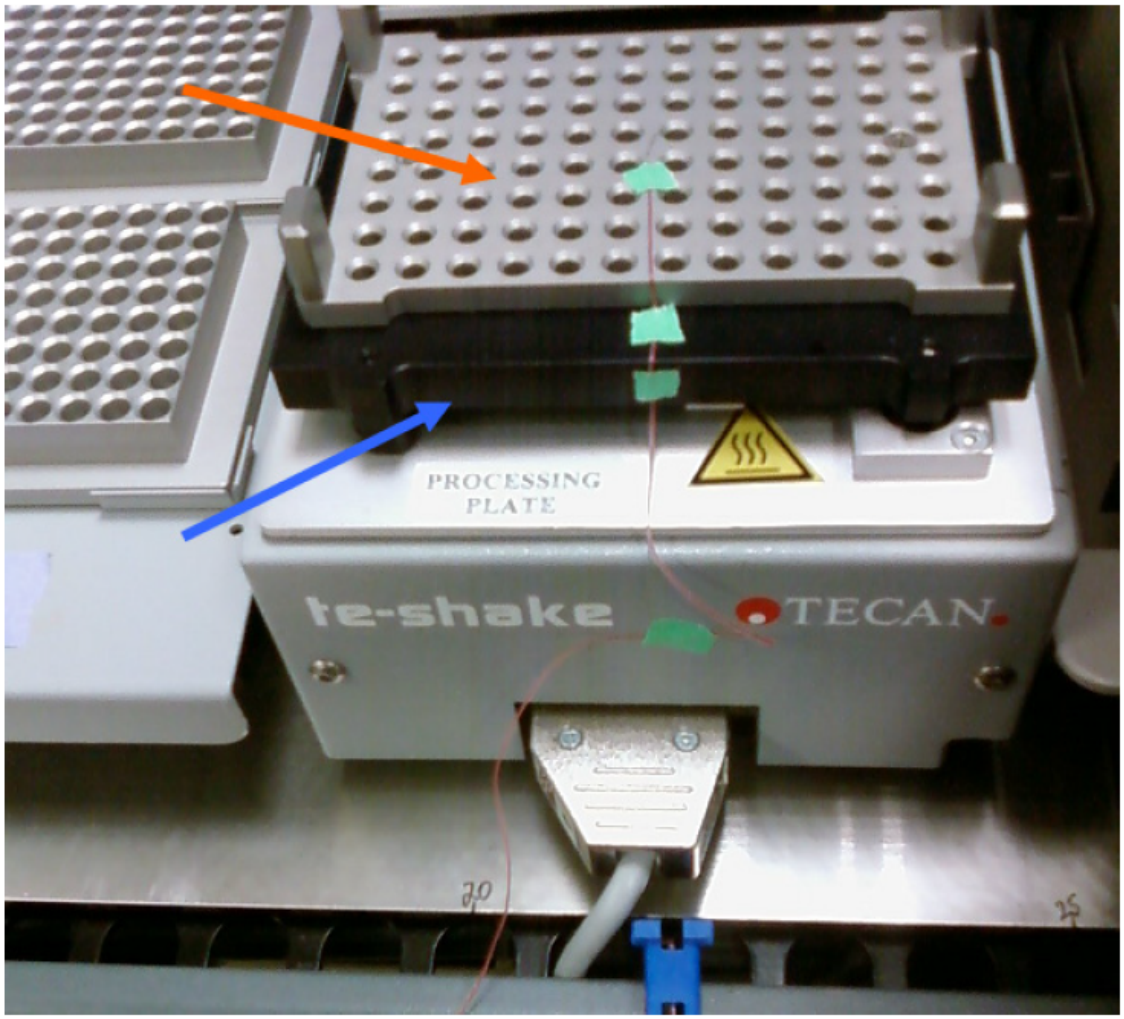
Required materials not supplied

- Digital thermometer, such as Sper Scientific Type-K, Type-J Thermometer 800005 (VWR™ Cat. No. 14003-070)
- Micro-thermocouple probe, such as Sper Scientific Type-K Beaded Wire 800077 (VWR™ Cat. No. 14003-136)

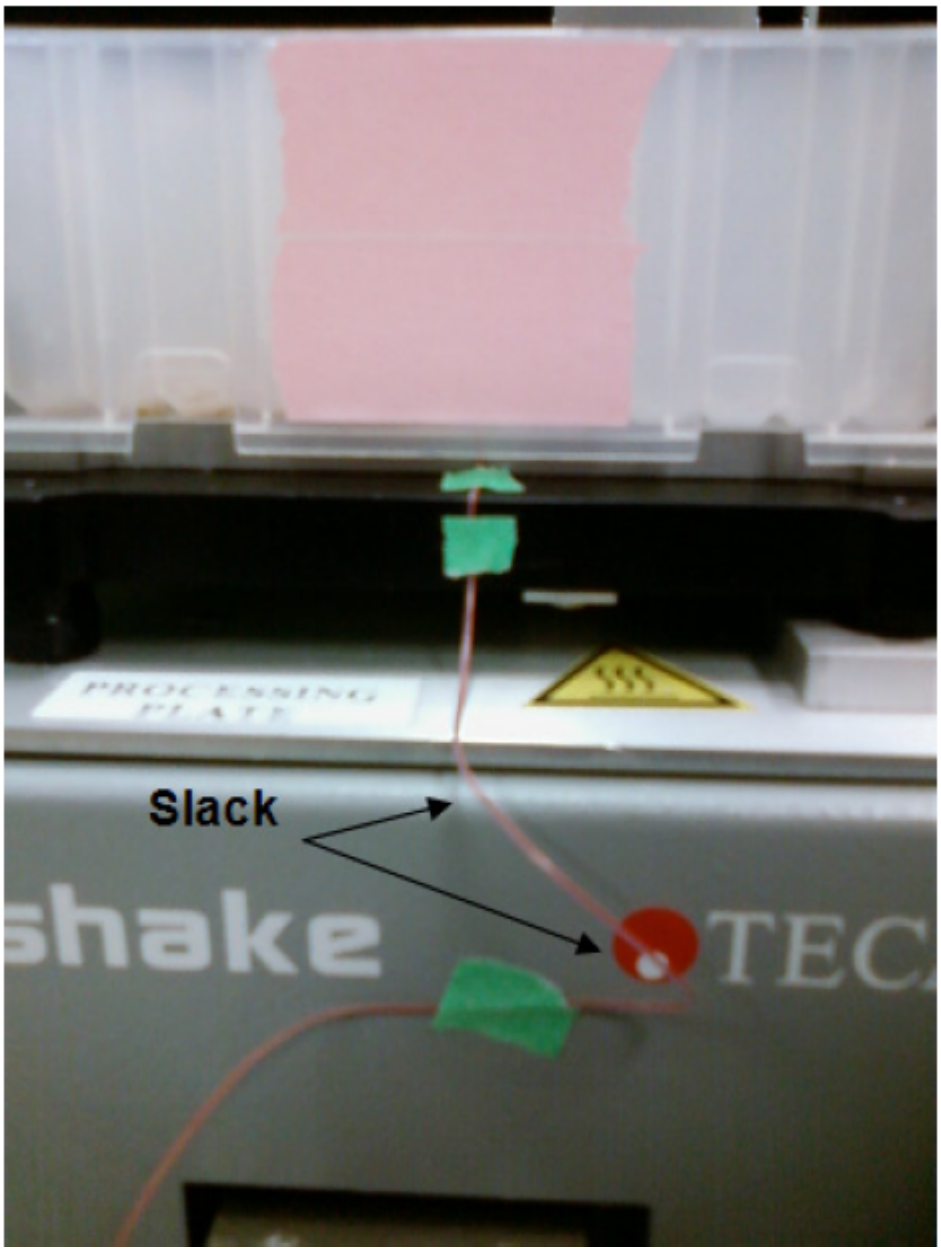
Measure the plate and adapter temperatures

Measure the empirical temperatures between the PrepFiler™ 96-Well Processing Plate and the Te-Shake™ plate adapter.

1. Attach the micro-thermocouple probe:
   a. Tape the micro-thermocouple probe near the center of the Te-Shake™ plate adapter. Ensure that the probe does not cross the holes of the plate adapter.



b. Tape the probe to the heating plate and to the Te-Shake™ plate adapter so that the probe is secure and does not interfere with the RoMa (picking up or setting down the PrepFiler™ 96-Well Processing Plate plate). Ensure that there is sufficient slack between the heating plate and Te-Shake™ plate adapter to allow the adapter to move without pulling on the probe.



2. Before beginning the test run, record the ambient temperature.

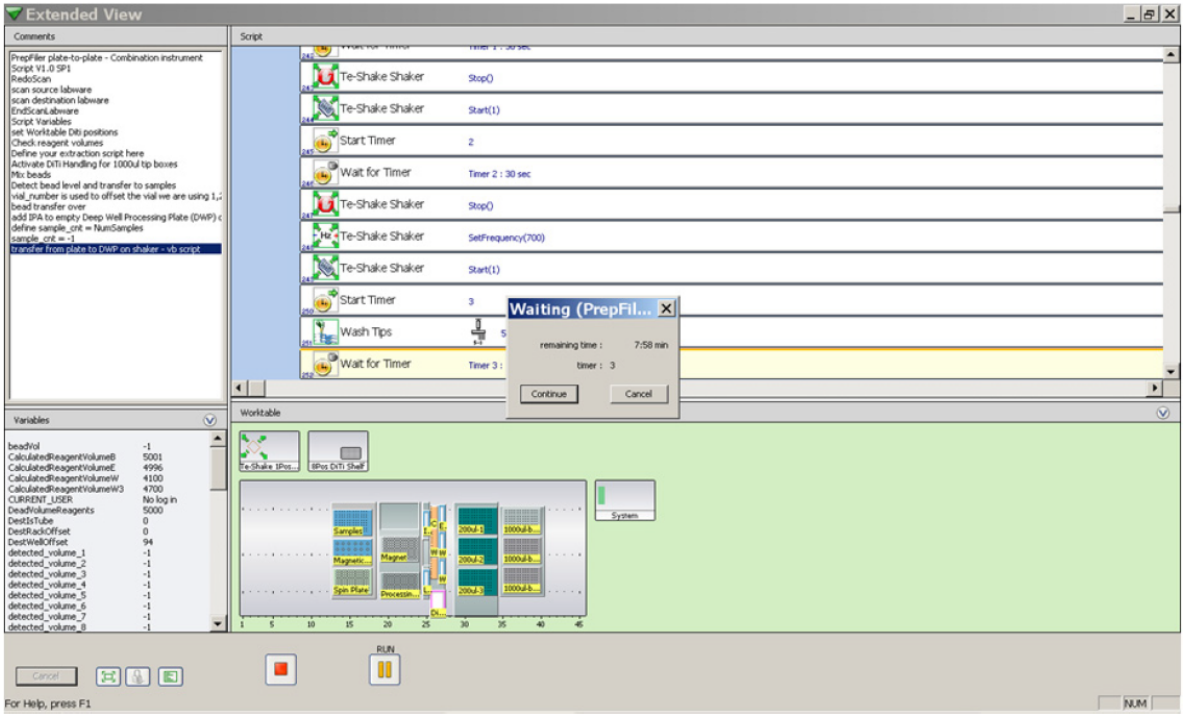
3. Following the procedures in Chapter 3, “Set up the robotic workstation”, set up the workstation for a water run for a small number of samples.
   a. Fill 2–3 wells in a PrepFiler™ Spin Plate with 50 µL of water or TE buffer.
   b. Fill each reagent trough with 30 mL of water.

c. Place two PrepFiler™ Magnetic Particles tubes that contain water on the workstation.

4. Start a run according to the instructions in Chapter 4, “Perform the automated DNA purification run”. Select any extraction and purification script (for example, PrepFilter_plate_plate_V1_SP1).

5. During the run, view the timers in the **Waiting** dialog box, then record the temperatures at the following times:
   - At the beginning of timer 10 and the end of timer 11 (air-drying phase)
   - At the beginning of timer 12 and the end of timer 14 (DNA elution phase).

Note: To save time, click **Continue** in the **Waiting** dialog box to skip timers 1–9. The first heating step begins at timer 10 (approximately script line 416).



- The temperature readings at the end of timer 11 should be 30–35℃.
- The temperature readings at the end of timer 14 should 65–75 ℃.

If the temperatures are not within these ranges,

- Contact your Tecan™ or Thermo Fisher Scientific representative for assistance.

  or

- Verify the extraction and purification performance (DNA yield and STR quality), conduct optimization studies, then adjust the set temperatures and elution volume in the scripts to meet your performance standards. Note that optimal temperatures may vary slightly depending on altitude and relative humidity.

D

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Safety information for instruments not manufactured by Thermo Fisher Scientific

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

Chemical safety

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

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

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.

- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

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

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

Biological hazard safety

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

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number
PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits User Guide	4463349
PrepFiler™ and PrepFiler™ BTA Forensic DNA Extraction Kits User Guide	4463348
PrepFiler™ Automated Forensic DNA Extraction Kit: Automated DNA Purification on the HID EVOlution™ Systems User Bulletin	MAN0019298
PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the ID NIMBUS® Presto Workstation User Bulletin	MAN0019368

Customer and technical support

For support:

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

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

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

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

Limited product warranty

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