PrepFiler® Automated Forensic DNA Extraction Kit

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

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Revision Date October 2011

Sample Preparation
DNA Quantification
PCR Amplification
Genetic Analysis
Data Interpretation
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CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the “Safety” appendix in this document.

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

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

Revision history

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4393917 Rev01</td>
<td>10/2008</td>
<td>New user guide.</td>
</tr>
<tr>
<td>4393917 RevB</td>
<td>12/2008</td>
<td>Updated sample input amounts and safety information.</td>
</tr>
<tr>
<td>4393917 RevC</td>
<td>12/2010</td>
<td>Updated user guide to include:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• PrepFiler® Wash Buffer B – An additional wash buffer used for the third (final) wash during extraction to minimize the potential for detergent carryover from PrepFiler® Wash Buffer A. Detergent carryover can inhibit downstream PCR applications.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Modified worktable layout – Added a trough for Wash Buffer B and rearranged the remaining reagent and waste troughs to accommodate the new trough.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• New HID EVOlution™ scripts – New scripts replace existing automated extraction scripts for use with the PrepFiler® Automated Forensic DNA Extraction Kit. The new scripts incorporate the worktable changes, further minimize bubble formation on disposable tips during dispensing steps, and further optimize liquid handling performance. Core liquid handling for operations such as binding, washing, and elution are identical for all scripts. See “Select a script” on page 57 for a list of scripts.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All new HID EVOlution™ systems are delivered with the new scripts. If you are an existing customer, please contact your local Tecan customer support organization to obtain the new scripts. For details on script changes, refer to the service pack revision history file included in the Documents folder on the CD containing the new scripts.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• New procedure for documenting Te-Shake plate adapter temperature – To ensure that the heating plate adapter reaches the recommended temperatures during the air-drying and elution steps of the automated extraction run, we recommend that you use a micro thermocouple and the test procedure in Appendix D on page 83 to verify the empirical temperatures between the PrepFiler® Processing Plate and the Te-Shake plate adapter.</td>
</tr>
</tbody>
</table>
About This Guide

Purpose

This guide provides:

- Procedures for extracting and isolating genomic DNA from forensic samples using the PrepFiler® Automated Forensic DNA Extraction Kit (Chapter 2 on page 19)
- An overview of setting up, operating, and maintaining your own Tecan HID EVolution™ – Extraction System or HID EVolution™ – Combination System (HID EVolution™ system), with references to detailed procedures (Chapter 3 on page 29 and Chapter 4 on page 51)
- Troubleshooting information (Appendix A on page 67)
- A step-by-step description of the automated DNA binding, washing, and elution steps (Appendix E on page 87)

Prerequisites

This guide assumes that:

- You know how to handle forensic samples and prepare them for quantitation and STR analysis.
- The HID EVolution™ – Extraction System or HID EVolution™ – Combination System (a Tecan Freedom EVO® 150 or 200 with the appropriate hardware, software, and scripts) has been installed, configured, tested, and calibrated by Tecan personnel.
- You are trained on the proper operation, maintenance, and troubleshooting of the Tecan HID EVolution™ – Extraction System or HID EVolution™ – Combination System.
- You have access to the Tecan HID EVolution™ – Extraction Application Manual or Tecan HID EVolution™ – Combination Application Manual, the Tecan Freedom EVO® Operating Manual, and other applicable Tecan documentation, and to the PrepFiler® Forensic DNA Extraction Kits User Guide.
• You have referred to the manufacturer’s instrument documentation for important safety information related to the use of the Tecan Freedom EVO® instrument.
• You have a working knowledge of the Microsoft® Windows® operating system, the Internet, and Internet-based browsers.

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

**Note:** Provides information that may be of interest or help but is not critical to the use of the product.

**IMPORTANT!** Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

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

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

**DANGER!** Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the “Safety” appendix for descriptions of the symbols.
Kit description

The PrepFiler® Automated Forensic DNA Extraction Kit contains reagents needed for:

- Manual lysate preparation
- Automated DNA extraction and purification with the HID EVOlution™ – Extraction System and HID EVOlution™ – Combination System.

The kit is designed for extracting and purifying DNA from a variety of common forensic sample types, including body fluid stains and swabs of body fluids. The kit is appropriate for use with samples containing potential inhibitors of the polymerase chain reaction (PCR). The extracted DNA is compatible for use in quantitation using the Quantifiler® Human, Quantifiler® Y Human Male, and Quantifiler® Duo DNA Quantification Kits, and STR amplification using the AmpF/STR® PCR Amplification kits.

After sample lysis, the remaining DNA extraction procedures are performed on a Tecan Freedom EVO® robotic workstation, which automates liquid and magnetic particle handling (see Figure 2 on page 17). The extracted DNA is collected in either a 96-well plate or 1.5-mL tubes, depending on the Freedom EVOware® software script that you select.

Run time is dependent on the number of samples and on the type of HID EVOlution™ system you are using. The HID EVOlution™ – Extraction System can run 80 extractions in about 2 hours. Table 1 on page 11 shows examples of uninterrupted run times for the HID EVOlution™ – Combination System for various numbers of samples.

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Extraction run time</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>1 hour 40 minutes</td>
</tr>
<tr>
<td>32</td>
<td>2 hours 8 minutes</td>
</tr>
<tr>
<td>64</td>
<td>3 hours 25 minutes</td>
</tr>
<tr>
<td>80</td>
<td>3 hours 51 minutes</td>
</tr>
</tbody>
</table>
Supported HID EVOlution™ system configurations

The PrepFiler® Automated Forensic DNA Extraction Kit is supported for use with the HID EVOlution™ – Extraction System and HID EVOlution™ – Combination System, which consist of:

- A Tecan Freedom EVO® 150 or 200 robotic workstation
- The Freedom EVOware® software version 2.1 SP1 or later, configured with the HID EVOlution™ – Extraction application
  
  **Note:** Contact Life Technologies Technical Support for more information on verified configurations. See “Obtaining support” on page 139.

- The necessary hardware, including an 8-channel liquid-handling arm (LiHa), Robotic Manipulator arm (RoMa), and Te-Shake™ adapter with heating block and adapter

  **Note:** The Freedom EVO 150 and 200 instruments can be configured identically and both instruments are supported for use with the HID EVOlution™ – Extraction System. Validation studies were performed on the Freedom EVO 150.

The HID EVOlution™ systems support the following configurations. You can select one configuration per extraction run:

- Perform cell lysis in plates and collect eluate in plates
- Perform cell lysis in plates and collect eluate in tubes
- Perform cell lysis in tubes and collect eluate in tubes
- Perform cell lysis in tubes and collect eluate in plates

  **Note:** Consult the manufacturer’s documentation for the information needed for the safe use of this product.
Kit contents and storage conditions

Kit contents
Each PrepFiler® Automated Forensic DNA Extraction kit contains materials sufficient for up to 960 extractions when used with the applicable standard lysis protocol (see Chapter 2 on page 19). Plastics are sold separately.

Table 2 Materials in PrepFiler® Automated Forensic DNA Extraction Kit (Part No. 4463353)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepFiler® Lysis Buffer</td>
<td>One bottle, 500 mL</td>
</tr>
<tr>
<td>PrepFiler® Magnetic Particles</td>
<td>13 tubes, 1.5 mL each</td>
</tr>
<tr>
<td>PrepFiler® Wash Buffer A Concentrate</td>
<td>One bottle, 500 mL</td>
</tr>
<tr>
<td>PrepFiler® Wash Buffer B Concentrate</td>
<td>One bottle, 250 mL</td>
</tr>
<tr>
<td>PrepFiler® Elution Buffer</td>
<td>One bottle, 200 mL</td>
</tr>
</tbody>
</table>

Table 3 Plastics (sold separately)

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Reagent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4397901</td>
<td>PrepFiler® 96-Well Spin Plate and Filter Plate</td>
<td>10 sets</td>
</tr>
<tr>
<td>4392904</td>
<td>PrepFiler® 96-Well Processing Plate</td>
<td>10 plates</td>
</tr>
<tr>
<td>4392342</td>
<td>PrepFiler® Spin Tubes and Filter Columns</td>
<td>300 Spin Tubes and 100 Filter Columns</td>
</tr>
<tr>
<td>AM12450</td>
<td>Non-stick RNase-free Microfuge Tubes (1.5-mL), certified DNase- and RNase-free</td>
<td>250 tubes</td>
</tr>
</tbody>
</table>

Kit usage
- Do not reuse PrepFiler® Spin, Filter, or Processing Plates.
- To perform lysis or collect eluate in tubes, purchase tubes separately. See “Required materials and instruments” on page 14 for appropriate tubes.

Storage conditions
Store all kit components at room temperature (18 to 25°C).
## Required materials and instruments

Table 4 lists materials and instruments that are required in addition to the reagents and materials supplied with the PrepFiler® Automated Forensic DNA Extraction Kit.

<table>
<thead>
<tr>
<th>Workflow step</th>
<th>Material or instrument</th>
<th>Source‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Common laboratory equipment such as pipettors, aerosol-resistant micropipette tips, and a microcentrifuge</td>
<td>Major laboratory supplier</td>
</tr>
<tr>
<td>Vortexer (a variable speed vortexer is recommended)</td>
<td>Vortexer (a variable speed vortexer is recommended)</td>
<td>Major laboratory supplier</td>
</tr>
<tr>
<td>Lysis [if performing lysis in tubes]</td>
<td>DL-dithiothreitol [Molecular biology grade; ≥98% (TLC), ≥99% (titration)]</td>
<td>Sigma-Aldrich [<a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a> (Part Number D9779)]</td>
</tr>
<tr>
<td></td>
<td>General purpose shaking incubator capable of temperatures up to 70°C</td>
<td>VWR Signature™ Benchtop Shaking Incubator or equivalent [<a href="http://www.vwr.com">www.vwr.com</a> (Model 1575)]</td>
</tr>
<tr>
<td></td>
<td>Non-stick RNase-free Microfuge Tubes (1.5-mL), certified DNase- and RNase-free</td>
<td>Applied Biosystems [Part No. AM12450] or equivalent</td>
</tr>
<tr>
<td></td>
<td><strong>Note</strong>: If you use an equivalent tube, select tubes that allow you to observe the tube contents.</td>
<td></td>
</tr>
<tr>
<td>(If substrate removal is required) PrepFiler® Spin Tubes and Filter Columns</td>
<td>PrepFiler® Spin Tubes and Filter Columns</td>
<td>Applied Biosystems [Part No. 4392342]</td>
</tr>
<tr>
<td>Laboratory microcentrifuge capable of 16,110 × g</td>
<td>Laboratory microcentrifuge capable of 16,110 × g</td>
<td>Major laboratory supplier</td>
</tr>
<tr>
<td>Lysis (if performing lysis in PrepFiler® 96-Well Spin Plate and Filter Plates)</td>
<td>DL-dithiothreitol [Molecular biology grade; ≥98% (TLC), ≥99% (titration)]</td>
<td>Sigma-Aldrich [<a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a> (Part Number D9779)]</td>
</tr>
<tr>
<td></td>
<td>General purpose shaking incubator capable of temperatures up to 70°C</td>
<td>VWR Signature™ Benchtop Shaking Incubator or equivalent [<a href="http://www.vwr.com">www.vwr.com</a> (Model 1575)]</td>
</tr>
<tr>
<td></td>
<td>Benchtop centrifuge with rotor for 96-deep-well plates</td>
<td>Eppendorf Centrifuge 5804 with A-2-DWP swing bucket rotor, or equivalent [<a href="http://www.eppendorf.com">www.eppendorf.com</a>]</td>
</tr>
<tr>
<td></td>
<td>MicroAmp® Multi-Removal Tool</td>
<td>Applied Biosystems [Part No. 4313950]</td>
</tr>
<tr>
<td></td>
<td>MicroAmp® Clear Adhesive Film</td>
<td>Applied Biosystems [Part No. 4306311]</td>
</tr>
<tr>
<td></td>
<td>MicroAmp® Adhesive Film Applicator</td>
<td>Applied Biosystems [Part No. 4333183]</td>
</tr>
<tr>
<td></td>
<td>Deep well plate (for balancing the centrifuge rotor during centrifugation)</td>
<td>VWR Part Number 82006-448 or equivalent [<a href="http://www.vwr.com">www.vwr.com</a>]</td>
</tr>
</tbody>
</table>
# Chapter 1 The PrepFiler® Automated Forensic DNA Extraction Kit

## Required materials and instruments

<table>
<thead>
<tr>
<th>Workflow step</th>
<th>Material or instrument</th>
<th>Source‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automated extraction</td>
<td>DNA Suspension Buffer (low-TE buffer)</td>
<td>Teknova (Cat. No. T0223) <a href="http://www.teknova.com">www.teknova.com</a></td>
</tr>
<tr>
<td></td>
<td>Isopropanol (2-Propanol, ACS reagent grade, ≥99.5%)</td>
<td>Sigma-Aldrich <a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a> (Part Number 190764)</td>
</tr>
<tr>
<td></td>
<td>Ethanol (Molecular biology grade; 95% or 190 proof)</td>
<td>Sigma-Aldrich <a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a> (Part Number E7148)</td>
</tr>
<tr>
<td></td>
<td>Note: Open a new bottle when preparing the PrepFiler® Wash Buffer A and B solutions.</td>
<td></td>
</tr>
<tr>
<td>96-Well Magnetic Ring Stand</td>
<td></td>
<td>Applied Biosystems (Part No. AM 10050)</td>
</tr>
<tr>
<td>Disposable Tips (DiTi), Tecan Pure, Filtered, 1000-µL (30 000 631)†</td>
<td>Tecan (Part No. 30000631) <a href="http://www.tecan.com">www.tecan.com</a></td>
<td></td>
</tr>
<tr>
<td>Disposable Tips (DiTi), Tecan Pure, Filtered, 200-µL (30 000 629)†</td>
<td>Tecan (Part No. 30000629) <a href="http://www.tecan.com">www.tecan.com</a></td>
<td></td>
</tr>
<tr>
<td>100-mL disposable troughs for reagents</td>
<td></td>
<td>Tecan (Part No. 10613048) <a href="http://www.tecan.com">www.tecan.com</a></td>
</tr>
<tr>
<td>Barcodes (optional)</td>
<td>Refer to the Tecan Freedom EVO® Operating Manual, Section 3.5.6 “Positive Identification (PosID)”, for barcode requirements</td>
<td></td>
</tr>
<tr>
<td>If collecting eluate in plates:</td>
<td>MicroAmp® Optical 96-Well Reaction Plate (with or without barcode)</td>
<td>Applied Biosystems (Part No. N8010560 or 4306737)</td>
</tr>
<tr>
<td>If collecting eluate in tubes:</td>
<td>Non-stick RNase-free Microfuge Tubes (1.5-ml), certified DNase- and RNase-free</td>
<td>Applied Biosystems (Part No. AM12450) or equivalent</td>
</tr>
</tbody>
</table>

† Disposable tips that have not been certified by Tecan may not yield the same liquid-handling performance.
‡ Recommended sources. Equivalent materials from other suppliers can be used after appropriate validation studies by the user laboratory.
Workflow for manual lysis and automated extraction

The full workflow is shown below. Details of the manual lysis step and automated DNA extraction step are shown in Figure 1 and Figure 2 on page 17.

1. **Prepare the Reagents and Samples** *(see Chapter 2 on page 19)*
   - Determine sample size or input amount
   - Select a lysis protocol
   - Prepare reagents for lysis, binding, washing, and elution
   - Perform lysis and substrate removal

2. **Set Up the Automation Instrument** *(see Chapter 3 on page 29)*
   - (HID EVOlution™ – Combination System only) Set up the carriers and labware for extraction
   - Perform routine maintenance
   - Set up the disposable pipette tips
   - Set up reagents on the workstation
   - Set up lysate, processing, and elution plates and/or tubes

3. **Run Automated DNA Extraction** *(see Chapter 4 on page 51)*
   - Set up sample information
   - Set up and run a script
   - Complete the run
   - View the output file and the report
Figure 1  Lysis steps are performed manually, in tubes (shown), or in plates (not shown)

Incubate sample with PrepFiler® Lysis Buffer-DTT solution

If sample substrate is present, centrifuge in PrepFiler® Spin Tube/Filter Column to separate substrate and sample lysate.

Figure 2  Automated DNA extraction steps are performed by the HID EVOlution™ - Extraction System or HID EVOlution™ - Combination System

Lysate is processed from the PrepFiler® Spin Plate or 1.5-mL tubes into the PrepFiler® Processing Plate (shown) for automated DNA extraction.
Chapter 1 The PrepFiler® Automated Forensic DNA Extraction Kit

Workflow for manual lysis and automated extraction
Prepare the Reagents and Samples

This chapter covers the PrepFiler® Automated Forensic DNA Extraction Kit standard protocols:

- Workflow ................................................................. 20
- Determine sample size or input amount ....................... 20
- Select a lysis protocol ................................................. 21
- Prepare reagents for lysis, binding, washing, and elution .... 22
- Perform lysis and substrate removal: 1.5-mL tubes standard protocol ........ 23
- Perform lysis and substrate removal: 96-well plate standard protocol ....... 25
Chapter 2 Prepare the Reagents and Samples

Workflow

Determine sample size or input amount (see page 20)

Select a lysis protocol (see page 21)

Prepare reagents for lysis, binding, washing, and elution (see page 22)

Perform lysis and substrate removal:
1.5-mL tubes standard protocol (see page 23)

or

96-well plate standard protocol (see page 25)

Set Up the Automation Instrument (see Chapter 3 on page 29)

Run Automated DNA Extraction (see Chapter 4 on page 51)

Determine sample size or input amount

The PrepFiler® Automated Forensic DNA Extraction Kit is appropriate for most forensic sample types, including body fluids and stains and swabs of body fluids. Determine the appropriate sample size or input amount for use with your sample types. Examples of appropriate sample types and inputs for the standard protocols are shown in Table 5. Optimal input amounts may be affected by factors such as sample age and substrate properties. Each lab should perform studies to independently validate input amounts.

Life Technologies validation studies for the PrepFiler® Automated Forensic DNA Extraction Kit were performed using the standard PrepFiler® protocols and the sample inputs shown in Table 5. The samples that were used in the validation studies were prepared from body fluids.

Table 5 Example sample types and inputs for use with the standard protocols

<table>
<thead>
<tr>
<th>Example sample</th>
<th>Example sample input†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid samples (blood, saliva)</td>
<td>Up to 40 µL</td>
</tr>
<tr>
<td>Blood (on FTA paper or fabric)</td>
<td>Up to 25-mm$^2$ (cutting or punch)</td>
</tr>
<tr>
<td>Body fluids [saliva, semen] on fabric</td>
<td>Up to 25-mm$^2$ (cutting or punch)</td>
</tr>
<tr>
<td>Body fluids on swabs (buccal and other body fluids)</td>
<td>Up to one swab</td>
</tr>
</tbody>
</table>

† It is not necessary to use an entire sample punch or swab.
Select a lysis protocol

When you perform an extraction run, select one lysis protocol from Table 6 based on the sample type and size and on the type of plasticware (plate or tubes) that you want to use for lysis.

Table 6  Lysis protocol selection

<table>
<thead>
<tr>
<th>If your sample...</th>
<th>And you want to perform lysis in....</th>
<th>Use the lysis protocol...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contains no substrate or Includes a substrate that can be submerged using 300 µL of lysis buffer</td>
<td>1.5-mL tubes</td>
<td>1.5-mL tubes standard protocol [page 23]</td>
</tr>
<tr>
<td></td>
<td>A 96-well plate</td>
<td>96-well plate standard protocol [page 25]</td>
</tr>
<tr>
<td></td>
<td>(For liquid samples only): 96-well plate standard protocol for liquid samples [Appendix C, page 75]</td>
<td></td>
</tr>
<tr>
<td>Includes a substrate that requires more than 300 µL of lysis buffer to submerge your sample</td>
<td>1.5-mL tubes</td>
<td>1.5-mL tubes large-sample (500-µL) protocol [Appendix C, page 81]</td>
</tr>
<tr>
<td></td>
<td>A 96-well plate</td>
<td>96-well plate large-sample (500-µL) protocol [Appendix C, page 78]</td>
</tr>
</tbody>
</table>

**IMPORTANT!** Because each lysis protocol is associated with a separate EVOware software script, you must use the same protocol to prepare all the samples that are included in the same run on the Freedom EVO® instrument.

**IMPORTANT!** The large-sample (500-µL) protocols were not tested as part of the full PrepFiler® Automated Forensic DNA Extraction Kit validation studies that were performed by Life Technologies. The large-sample protocols are intended only for PrepFiler® Automated Forensic DNA Extraction Kit samples that are not submerged by the 300 µL of PrepFiler® Lysis Buffer that is used in the standard protocols. If your laboratory intends to use the large-sample protocols, perform the appropriate validation studies. DNA yields from the large volume protocols may be lower than those that are obtained using the standard protocols with comparable sample input amounts.

If you are unsure whether to use the standard or large-sample protocols, start with a standard protocol, add the recommended amount of lysis buffer, then switch to a large-sample protocol if necessary.

**Note:** With some sample types and inputs, the tube lysis protocol may be the preferred method compared to the plate lysis protocol. See “Experiments and Results” on page 91 for a comparison of the plate and tube lysis protocols.

**Note:** The choice to perform lysis in a plate or tube is independent of the choice to have the DNA eluate collected in plates or tubes.
Prepare reagents for lysis, binding, washing, and elution

⚠️ **WARNING! CHEMICAL HAZARD.** Contact with acids or bases (such as bleach) liberates toxic gases. **DO NOT ADD** acids, or bases (such as bleach) to any liquid wastes containing PrepFiler Lysis Buffer or PrepFiler Magnetic Particles. Wear appropriate protective eyewear, clothing, and gloves.


2. Before each use, incubate the Magnetic Particles at 37°C for 10 minutes, then vortex the Magnetic Particles at medium speed until the particles are completely resuspended.

3. If the PrepFiler® Lysis Buffer contains precipitate, heat the buffer solution to 37°C for 15 minutes, then vortex the bottle for 5 seconds.

4. Prepare Wash Buffers A and B before first use:
   a. 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.
   b. 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.

   **Note:** Prepared wash buffers have a shelf life of 6 months, or the kit expiration date, whichever is sooner, if the containers are kept closed when not in use.

5. Thaw or prepare a fresh 1.0 M solution of DL-dithiothreitol (DTT) in molecular-biology grade DNA-free water.

   **Note:** After completing the lysis step, discard unused DTT.

   **Note:** To prepare fresh DTT, dissolve 1.54 g of dithiothreitol (DTT, MW 154) in 10 mL of molecular-biology grade DNA-free water.

   Aliquots of the desired volume (for example, 100 µL or 500 µL) can be prepared ahead of time, then stored at −20°C for up to 6 months.
Perform lysis and substrate removal: 1.5-mL tubes standard protocol

See “Select a lysis protocol” on page 21 for information on selecting the appropriate protocol.

Required materials
In addition to standard laboratory equipment, you need the following materials. See “Required materials and instruments” on page 14 for details:

- Thermal shaker or shaking incubator
- 1.5-mL microcentrifuge tubes (AB Part No. AM12450 or equivalent)
- PrepFiler® Lysis Buffer
- 1.0 M solution DTT
- If sample substrate is present
  - PrepFiler® Spin Tubes and Columns
  - DNA-free tweezers to move sample substrate

Perform lysis

WARNING! CHEMICAL HAZARD. Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing PrepFiler Lysis Buffer. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate.

2. Bring the shaking incubator to 70°C.
3. Place each sample in a PrepFiler® Spin tube or 1.5-mL microcentrifuge tube.
4. Prepare a fresh lysis buffer solution immediately before each experiment:
   a. Calculate the volumes of components that are needed based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

<table>
<thead>
<tr>
<th>Lysis buffer solution component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One reaction</td>
</tr>
<tr>
<td>PrepFiler® Lysis Buffer</td>
<td>300 µL</td>
</tr>
<tr>
<td>1.0 M DTT</td>
<td>3 µL</td>
</tr>
<tr>
<td></td>
<td>(use 5 µL for samples containing semen)</td>
</tr>
</tbody>
</table>

† Recommended volume; Includes approximately 4% excess volume to compensate for pipetting losses.

b. Combine the required volumes of components, then gently mix.
Chapter 2 Prepare the Reagents and Samples

Perform lysis and substrate removal: 1.5-mL tubes standard protocol

5. Add 300 µL of the lysis buffer solution to each sample.

**IMPORTANT!** If the lysis buffer does not cover the sample substrate (for example, 300 µL may not cover certain types of swabs), bring the lysis buffer and DTT volumes to the volumes specified in step 4 of the large-sample protocol on page 81, then continue following the instructions for the large-sample protocol.

6. Cap the tubes, vortex the tubes for 5 seconds, then centrifuge briefly.

7. Make sure the tubes are well sealed, then place the tubes in a thermal shaker and incubate at 900 rpm and 70°C for 60 minutes.

If sample substrate is present, perform “Remove substrate from sample lysate” on page 24, otherwise continue to Chapter 3, “Set Up the Automation Instrument” on page 29.

**Remove substrate from sample lysate**

1. Label up to 96 new 1.5-mL microcentrifuge tubes.

   **Note:** If you use barcodes to track samples, confirm that barcodes are correctly placed on the tubes (see barcode information in the Tecan HID EVOLUTION™ Extraction System Application Manual).

2. Centrifuge the sample tubes for 2 seconds to collect the condensate from the tube cap.

3. Insert a PrepFiler® Filter Column into a new 1.5-mL PrepFiler® Spin Tube, then carefully transfer the sample tube contents into the filter column:
   - Use a pipette to transfer the liquid contents.
   - Use the pipette tip or sterile tweezers to transfer the substrate.

4. Cap the filter column/spin tube, then centrifuge for 2 minutes at 13,000 to 16,000 × g.

5. Check the volume of sample lysate that is collected in the spin tube. If the volume is less than 180 µL, centrifuge the filter column/spin tube for an additional 5 minutes. If the volume is still less than 180 µL, see “Troubleshooting” on page 67.

6. Remove the filter column from the spin tube, then properly dispose of the filter column.

7. Transfer the lysate into a new, labeled 1.5-microcentrifuge tube.

8. Allow the lysate to come to room temperature before beginning the automated run. Proceed directly to the automated extraction run.

**IMPORTANT!** To avoid precipitation of lysis buffer components, do not chill the sample lysate after performing lysis.

**Note:** If you cannot proceed directly to the automated extraction run, the unprocessed lysate is stable for up to 24 hours at room temperature (20°C) in a sealed tube.
Perform lysis and substrate removal: 96-well plate standard protocol

See “Select a lysis protocol” on page 21 for information on selecting the appropriate protocol.

Figure 3 Overview of the lysis steps (performed manually) when using PrepFiler® Filter and Spin plates

Required materials

In addition to standard laboratory equipment, you need the following materials. See “Required materials and instruments” on page 14 for details:

- PrepFiler® Lysis Buffer
- 1.0 M solution DTT
- PrepFiler® Filter and Spin Plates (shipped assembled)
- Deep-well centrifuge
- Deep-well plate for counterbalancing in centrifuge (VWR Part #82006-448 or equivalent)
- Ice
- MicroAmp® Clear Adhesive Film
- MicroAmp® Adhesive Film Applicator
- Shaking incubator
- MicroAmp® Multi-Removal Tool

WARNING! CHEMICAL HAZARD. Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing PrepFiler Lysis Buffer. Wear appropriate protective eyewear, clothing, and gloves.

1. Review “Plate lysis guidelines and best practices” on page 72.
2. Bring the shaking incubator to 70°C.
   
   Note: Temperatures up to 80 °C are not deleterious to lysis.
3. Make sure that the Filter/Spin Plate unit is tightly assembled by centrifuging the unit as follows:
   a. Confirm that the Filter Plate is placed firmly on top of the Spin Plate as shown below, measure the weight of the assembled plate unit, then place the plate unit in a deep-well centrifuge.

   ![Diagram of Filter/Spin Plate unit]

   b. Create a counterweight to make sure the centrifuge rotor is well-balanced:
      • Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the assembled Filter/Spin Plate unit.
      • Seal the plate.
      • Place the plate in the deep-well centrifuge as a counterweight.
   c. Spin the plates at 650 × g for 2 minutes.

4. Confirm that the PrepFiler® Spin Plate is labeled for identification. If you use barcodes to track samples, move the barcode provided with the plate into the correct position as shown in the Tecan HID EVOlution™ Extraction Setup System Application Manual.

5. Place each sample in a separate well in the top (Filter Plate) of the plate unit. If you are using fewer than 96 samples, see “Plate setup requirements for fewer than 96 samples” on page 28.

6. Prepare a fresh lysis buffer solution immediately before each experiment:
   a. Calculate the volumes of components that are needed based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

       | Lysis buffer solution component | Volume |
       |-------------------------------|--------|
       | PrepFiler® Lysis Buffer        | 300 µL |
       | 1.0 M DTT                      | 3 µL   |

   † Recommended volume; includes approximately 4% excess volume to compensate for pipetting losses.

   b. Combine the required volumes of components, then gently mix.

7. Add 300 µL of the lysis buffer solution to each sample well in the plate unit.

   **IMPORTANT!** If the Lysis Buffer does not cover the sample substrate (for example, 300 µL may not cover certain types of swabs), use the large-sample protocol.

   **Note:** You may observe that some lysis buffer drips through to the Filter Plate during lysis. Lysis is still occurring in the wetted sample.
8. Immediately seal the plate unit with a new sheet of MicroAmp® Clear Adhesive Film.

9. Put the plate unit into a shaking incubator and incubate at 150 rpm and 70°C for 60 minutes.

**IMPORTANT!** Keep the plate unit horizontal during transport to the incubator and throughout incubation to keep lysis solution in contact with sample and to prevent possible cross-contamination.

10. Transfer the sample lysate into the Spin Plate:
   a. Place the plate unit in a deep-well centrifuge.
   b. Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the assembled Filter/Spin Plate unit, seal the plate, then place the plate in the deep-well centrifuge as a counterweight to make sure that the rotor is well balanced.
   c. Centrifuge the plate unit at 650 x g for 1 minute. If all lysate does not pass into the Spin Plate after 1 minute, centrifuge for an additional minute. **Note:** The sample substrate remains in the Filter Plate.

11. While holding the bottom plate, separate the Filter Plate (top) from the Spin Plate (bottom) with the MicroAmp® Multi-Removal Tool.

**IMPORTANT!** If the contents of the Spin Plate are shaken during plate separation, place an adhesive cover on the Spin Plate, place the Spin Plate in the deep-well centrifuge, then briefly centrifuge the Spin Plate to make sure that all contents are collected at the bottom of the wells.

12. Properly dispose of the (top) Filter Plate, which contains the sample substrate. Proceed directly to the automated extraction run.

**IMPORTANT!** To avoid precipitation of lysis buffer components, do not chill the sample lysate after performing lysis.

**Note:** If you cannot proceed directly to the automated extraction run, the unprocessed lysate is stable for up to 24 hours at room temperature (20°C) in a sealed Spin Plate.
Plate setup requirements for fewer than 96 samples

If you prepare fewer than 96 samples in a plate, the Freedom EVOware software scripts require that you place samples in the plate as follows:

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. See examples of correct DNA sample plate setup below.

**IMPORTANT!** When setting up sample information in the HID EVOlution™ Extraction System, make sure to assign a unique sample ID to all wells containing samples or blank reagents. Refer to 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 1 (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 that is generated at the end of the extraction run lists the position of each sample lysate and the position of the corresponding DNA eluate.

**Correct (samples in wells 1 through 48)**

**Incorrect**

**Correct (samples in wells 14 through 61)**

**Incorrect**
This chapter explains how to set up the Tecan Freedom EVO® instrument by providing:

- General procedures for performing an extraction run
- References to Tecan documentation for detailed procedures on setting up the HID EVOlution™ systems

This chapter covers:

- Workflow .......................................................... 30
- (HID EVOlution™ – Combination System only)
  Set up the carriers and labware for extraction ........................................ 31
- Perform routine maintenance ...................................................... 33
- Set up the disposable pipette tips ................................................. 35
- Set up reagents on the workstation ............................................ 36
- Set up lysate, processing, and elution plates and/or tubes ................. 41
- Reference: Worktable layouts ................................................. 47
## Workflow

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
</table>
| 1    | Prepare the Reagents and Samples  
(see Chapter 2 on page 19) |
| 2    | Set Up the Automation Instrument  
(HID EVOlution™ – Combination System only) Set up the carriers and labware for extraction |
| 3    | Perform routine maintenance |
| 4    | Set up the disposable pipette tips |
| 5    | Set up reagents on the workstation |
| 6    | Set up lysate, processing, and elution plates and/or tubes |
| 7    | Run Automated DNA Extraction  
(see Chapter 4 on page 51) |
If the HID EVOlution™ – Combination System was last run for qPCR/STR setup, you must set up the extraction-specific carriers and labware before performing extraction:

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.

2. Set up carriers for the extracted DNA eluate:
   
   - If you want the extracted DNA eluate placed in tubes, make sure that there are six tube racks on grids 1 through 6.
   
   - If you want extracted DNA eluate placed in a 96-well plate, make sure that the metal plate adaptor is 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 1000-µL DiTi boxes as shown below.

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

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

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

6. Set up reagent troughs:
   a. Remove the reagent troughs from previous runs and correctly dispose of the reagents as described in “Complete the run” on page 64.
b. Place new 100-mL reagent troughs on the worktable for PrepFiler® Wash Buffer B and other reagents as shown:

**Note:** The trough layout described below is different from the originally validated layout. The validation of the new trough layout is described in *HID EVOLution™ – Extraction System and HID EVOLution™ – Combination System User Bulletin: Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction* (Part No. 4457144).

![Trough Layout Image]

The worktable should now match the setup shown in “Reference: Worktable layouts” on page 47.

**Perform routine maintenance**

When you perform an extraction run, follow the setup and maintenance procedures in the Tecan documentation and other specific guidelines that Tecan personnel provide.

---

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

Before placing the samples, reagents, and plasticware (DiTis, troughs, plates, and tubes) on the worktable, prepare the Freedom EVO® instrument:
1. Confirm that the carboy next to the instrument contains sufficient system liquid (degassed deionized water) to complete the experiment.

**Note:** Degas deionized water overnight or longer before using it on the system. Run the routine maintenance script each time that you change the system liquid (degassed water) carboy.

**Note:** To avoid introducing air into the system liquid (degassed water) tubing, place the system liquid carboy at the same height as the worktable, and 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 three 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.

2. Use your fingers to gently tighten the DiTi adapter gold cones on the LiHa and the syringe assembly fittings. Refer to the *Tecan Freedom EVO® Operating Manual* for details.

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

3. Run the appropriate maintenance scripts.

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

<table>
<thead>
<tr>
<th>Before starting the run, if...</th>
<th>...then run the script</th>
</tr>
</thead>
<tbody>
<tr>
<td>It is the first run of the day</td>
<td>PrepFiler_DailyStartUp or Combo_DailyStartUp</td>
</tr>
<tr>
<td>It is <em>not</em> the first run of the day</td>
<td>PrepFiler_Flush or Combo_Flush</td>
</tr>
<tr>
<td>When you run DailyStartUp or Flush, you see:</td>
<td>PrepFiler_Flush or Combo_Flush one or more times until:</td>
</tr>
<tr>
<td>• Air bubbles in the lines and/or</td>
<td>• There are no visible air bubbles and</td>
</tr>
<tr>
<td>• Intermittent flow from a DiTi cone</td>
<td>• Flow from the DiTi cones is constant</td>
</tr>
<tr>
<td>There are one or more DiTis on the liquid handling arm (LiHa)</td>
<td>PrepFiler_Drop_DiTis or Combo_Drop_DiTis</td>
</tr>
</tbody>
</table>

4. Confirm that the waste carboy does not need to be emptied.

- For pre-run preparation steps, refer to the *Tecan HID EVOlution - Extraction Application Manual*, Section 4.3.2 “Prepare the Instrument”.
- For maintenance schedules, refer to the *Tecan HID EVOlution - Extraction Application Manual* Section 7.2, "Maintenance Schedule".
- For maintenance procedures, refer to the *Tecan HID EVOlution - Extraction Application Manual* Section 7.3, "Maintenance Tasks".
- For maintenance scripts, refer to the *Tecan HID EVOlution - Extraction Application Manual* Sections 5.2 “Running Maintenance” and 7.5, “Maintenance Scripts”.

For more information
Set up the disposable pipette tips

Terms for pipette tips used on the Freedom EVO® instrument

- **DiTis** – Disposable Tips (DiTi), Tecan Pure, Filtered, 200- and 1000-µL
- **DiTi tray** – Plastic tray containing 96 DiTis
- **DiTi rack** – Aluminum holder for a single tray of 1000-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 4** DiTi terms

**Required materials**

- 1000-µL DiTis: six trays (yellow or white trays; each containing 96 DiTis)
- 200-µL DiTis: three trays (blue or white trays; each containing 96 DiTis)

**Fill DiTi carriers and racks**

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

When you perform an extraction run, set up the pipette tips as described below.

**IMPORTANT!** If nine full DiTi trays are not correctly set up on the Freedom EVO® instrument, the instrument repeatedly searches for the missing DiTi tips, during which time the samples may become unusable.
1. Place three full trays of 1000-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. Confirm 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. Confirm 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!** Make sure that there are no objects placed on shelf positions 1 to 4 or position 8.

2. Place three full trays of 1000-µL DiTis into the DiTi racks on grid 35, positions 1 through 3, as described in step 1a. Confirm 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 through 3. Confirm that the notch in the tray is positioned in the top-left corner. When placing DiTis on the worktable, make sure that the 3-position DiTi carrier on grid 29 contains three 200-µL DiTi trays.

For more information
Refer to the *Tecan HID EVolution - Extraction Application Manual*, Section 4.3.5, “Setup Plasticware and Samples on the Workstation”.

### Set up reagents on the workstation

When you perform an extraction run, follow these guidelines:

- Before placing reagents on the worktable, prepare the reagents according to the procedures in “Prepare reagents for lysis, binding, washing, and elution” on page 22.
- 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, Wash Buffer A, Wash Buffer B, or Elution Buffer from previous runs; always properly dispose of used reagents after each run.
- Use new reagent troughs each day.
- 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.
Required materials for setup on the Freedom EVO® instrument

- Two tubes of PrepFiler® Magnetic Particles (one full tube and one containing at least 850 µL of magnetic particles)
- Isopropanol
- Prepared PrepFiler® Wash Buffer A
- Prepared PrepFiler® Wash Buffer B
- PrepFiler® Elution Buffer
- Five 100-mL disposable troughs for reagents

Set up reagents

**WARNING! CHEMICAL HAZARD.** Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing PrepFiler Magnetic Particles. Wear appropriate protective eyewear, clothing, and gloves.

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

1. Make sure that you have prepared the PrepFiler® Magnetic Particles, PrepFiler® Wash Buffer A, and PrepFiler® Wash Buffer B according to “Prepare reagents for lysis, binding, washing, and elution” on page 22.

2. Place empty troughs on the worktable:

<table>
<thead>
<tr>
<th>Empty Trough for...</th>
<th>Place in following location...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution buffer</td>
<td>Grid 27, position 1</td>
</tr>
<tr>
<td>Wash buffer B</td>
<td>Grid 27, position 2</td>
</tr>
<tr>
<td>Wash buffer A</td>
<td>Grid 27, position 3</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>Grid 25, position 1</td>
</tr>
<tr>
<td>Lysate waste</td>
<td>Grid 25, position 3</td>
</tr>
</tbody>
</table>

**IMPORTANT!** Do not add acids or bases to any wastes containing lysis buffer (guanidine thiocyanate). See “Chemical safety” on page 134.
3. Using the volumes shown in the table below, calculate the PrepFiler® reagent volumes you need:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Protocol</th>
<th>Reactions (DNA samples) in run</th>
<th>Required reagent volume per reaction</th>
<th>Required overfill volume per run†</th>
<th>Required dead volume per run‡</th>
<th>Minimum required volume for 96 samples (including overfill and dead volume)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropanol</td>
<td>PrepFiler® Automated Forensic DNA Extraction Kit standard (300-µL) lysis protocols</td>
<td>Up to 96</td>
<td>180 µL</td>
<td>15%</td>
<td>5 mL</td>
<td>25 mL</td>
</tr>
<tr>
<td></td>
<td>PrepFiler® Automated Forensic DNA Extraction Kit large-sample (500-µL) lysis protocols††</td>
<td>Up to 96</td>
<td>300 µL</td>
<td>15%</td>
<td>5 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>Prepared Wash Buffer A</td>
<td>-</td>
<td>Up to 96</td>
<td>900 µL</td>
<td>15%</td>
<td>5 mL</td>
<td>105 mL</td>
</tr>
<tr>
<td>Prepared Wash Buffer B</td>
<td>-</td>
<td>Up to 96</td>
<td>300 µL</td>
<td>15%</td>
<td>5 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>-</td>
<td>Up to 96</td>
<td>50 µL</td>
<td>15%</td>
<td>5 mL</td>
<td>11 mL</td>
</tr>
</tbody>
</table>

† Overfill (excess volume) is necessary to compensate for evaporation and pipetting losses during the run.
‡ An extra 5 mL per trough is necessary to ensure that the pipette tips remain submerged during aspiration so that liquid, not air, enters the tips.
§ 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.
†† The large-sample (500-µL) protocols were not tested as part of the validation studies that were performed by Life Technologies. If you intend to use the large-sample protocols, perform the appropriate validation studies.
4. Add the amounts of PrepFiler® kit reagents that you calculated in step 3 to the appropriate trough.

5. After preparing the PrepFiler® Magnetic Particles according to the procedures in “Prepare reagents for lysis, binding, washing, and elution” on page 22, gently invert two tubes of PrepFiler® Magnetic Particles to remove large air bubbles, briefly centrifuge the tubes at low speed to collect any residual tube contents from the sides and cap of the tubes, then open the tubes.
   - If a thin film or bubble (caused by surfactants) stretches across the top of the Magnetic Particles 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 extraction run.
6. Place the two tubes of Magnetic Particles on the worktable in the first two slots of the metal rack on grid 13, position 2.

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

For more information

- Refer to the *Tecan HID EVolution - Extraction Application Manual*, Section 4.3.4, “Setup Reagents on Workstation”.
Set up lysate, processing, and elution plates and/or tubes

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

About automation options

For each automated extraction run:

- Decide which option you want to use. The HID EVOlution™ systems give you the following options:
  - Process lysate from 1.5-mL tubes and collect eluate in 1.5-mL tubes (tubes-to-tubes layout)
  - Process lysate from 1.5-mL tubes and collect eluate in a 96-well plate (tubes-to-plate layout)
  - Process lysate from a 96-well plate and collect eluate in 1.5-mL tubes (plate-to-tubes layout)
  - Process lysate from a 96-well plate and collect eluate in a 96-well plate (plate-to-plate layout)

“Reference: Worktable layouts” on page 47 shows the placement of plates and tubes for each of the four automated extraction run options.

- Set up the worktable with the appropriate plates and/or tubes corresponding to the option you selected. See directions on page 42 through page 46. Depending on the option you selected, use:
  - Either a lysate plate or lysate tubes: The PrepFiler® Spin Plate or the 1.5-mL tubes that contain the lysate from the sample lysis step.
  - Either an elution plate or elution tubes: A MicroAmp® Optical 96-Well Reaction Plate or 1.5-mL tubes to collect eluate at the end of the extraction and isolation process. Your choice is independent of whether the sample lysate is contained in a plate or in tubes.

- Select and run the EVOware software script that corresponds to the lysis protocol that you use and to the worktable setup (see “Set up and run a script” on page 56).

Required materials

- Sample lysate – Contained in a PrepFiler® Spin Plate or 1.5-mL tubes.
- PrepFiler® Processing Plate – A square-well plate that is used on the instrument to process reactions. Before the run, place the plate on the Te-Shake™ adapter. During the washing and elution steps, the Robotic Manipulator arm (RoMa) moves the plate to the 96-Well Magnetic Ring Stand or Te-Shake™ adapter as needed.
- MicroAmp® Optical 96-Well Reaction Plate or 1.5-mL tubes for collecting eluate.
Chapter 3  Set Up the Automation Instrument

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

Set up the PrepFiler® Processing Plate

The PrepFiler® Processing Plate is required for all four automated extraction run options.

1. 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™ 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 plate is placed on the Te-Shake™ adapter with well A1 positioned in the top-left corner
   - The plate wells are aligned with the holes in the Te-Shake™ adapter

Set up lysate and/or eluate plates

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

1. If you use barcodes to track samples, confirm that barcodes are correctly placed on plates before placing the items on the worktable (refer to the Tecan HID EVolution - Extraction Application Manual, Section 4.5, “Barcodes”, for details).
2. 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).

   ![Elution Plate](image)

   **Note:** The DNA eluate corresponding to the first sample is always placed in the first well (A1) of the Elution Plate. The report that is generated at the end of the extraction run lists the plate or tube position of each sample lysate and the 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 alternative plates.

3. 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).

   ![Spin Plate](image)

**Set up lysate and/or eluate tubes**

If you use barcodes to track samples, confirm that barcodes are correctly placed on the tubes before placing the items on the worktable (refer to the Tecan HID EVOLUTION - Extraction Application Manual, Section 4.5, “Barcodes”, for details).

**Set up eluate tubes in tube racks**

If you want to collect eluate in tubes:

1. Confirm that:
   - You have new, labeled 1.5-mL microcentrifuge tubes equal to the number of DNA samples to be processed.
   - The tube racks S1 through S6 are correctly positioned at grid positions 1 through 6.
2. Place the first empty 1.5-mL 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 elution rack (S1). The report that is generated at the end of the extraction run lists the plate or tube position of each sample lysate and the position of the corresponding DNA eluate.

3. After the first sample tube, continue placing empty tubes from back to front in **vertical columns** as shown in the examples below. Place one empty tube for each sample to be processed. Do not leave empty positions between sample tubes.

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

   **Example of correct setup:**

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

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

   **IMPORTANT!** Open tube caps carefully to prevent contamination and splatter.
Set up lysate tubes in tube racks

If the lysate is in 1.5-mL tubes:

1. Confirm that:
   - You have no more than 96 labeled 1.5-mL microcentrifuge tubes containing DNA sample lysate.
   - The tube racks L1 through L6 are correctly positioned at grid positions 7 through 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. After the first sample tube, continue placing sample tubes from back to front in vertical columns as shown in the examples below. Do not leave empty positions between sample tubes.

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

   **Examples of correct setup:**

   ![Examples of correct setup](image)

4. Check that the barcodes are in a readable position.
5. Open each tube, securing the tube caps in a fixed upright position as shown below.

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

- For information on barcode specifications for use on the Freedom EVO® instrument, refer to the *Tecan Freedom EVO® Operating Manual*, Section 3.5.6, “Positive Identification (PosID)”, for details.
- Place the plasticware (plates and/or tubes) according to the *Tecan HID EVolution - Extraction Application Manual*, Section 4.3.5, “Setup Plasticware and Samples on the Workstation”.

For more information
Reference: Worktable layouts

Figures 5 through 8 on page 47 through page 50 show the worktable layouts for each of the four automated extraction run options.

For more information on worktable layouts, refer to the Tecan HIDEVolution™ Extraction Application Manual, 395372, V2.0 (June 2010), (Sections 4.3 and 4.4).

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™ 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
12–14. 200-µL disposable pipette tips (DiTis)
15–17. 1000-µL DiTis
Rear shelf with 1000-µL DiTis in shelf positions 5, 6, and 7 (not shown)
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™ 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
12–14. 200-µL disposable pipette tips (DiTis)
15–17. 1000-µL DiTis
Rear shelf with 1000-µL DiTis in shelf positions 5, 6, and 7 (not shown)
Figure 7  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™ 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
12–14. 200-µL disposable pipette tips (DiTis)
15–17. 1000-µL DiTis
Rear shelf with 1000-µL DiTis in shelf positions 5, 6, and 7 (not shown)
Figure 8 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™ 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
12–14. 200-µL disposable pipette tips (DiTis)
15–17. 1000-µL DiTis
Rear shelf with 1000-µL DiTis in shelf positions 5, 6, and 7 (not shown)
Run Automated DNA Extraction

This chapter explains how to run automated extraction on the HID EVOlution™ systems by providing:

- General procedures for performing an extraction run
- References to Tecan documentation for detailed procedures on running the HID EVOlution™ systems

This chapter covers:

- Workflow ................................................................. 52
- Set up sample information ....................................... 53
- Set up and run a script .............................................. 56
- Complete the run ..................................................... 64
- View the output file and the report ............................. 65
Workflow

Prepare the Reagents and Samples (see Chapter 2 on page 19)

Set Up the Automation Instrument (see Chapter 3 on page 29)

Run Automated DNA Extraction
  Set up sample information
  Set up and run a script
  Complete the run
  View the output file and the report
Set up sample information

You have several options for entering sample information to the HID EVOLution™ - Extraction System software. If you chose the sample file option, set up the sample file before the run according to the instructions in “(Optional) Create a sample input file” on page 54.

About sample information

During an extraction run, the software prompts you to enter or import the sample name and information for each DNA sample. The sample information is used by the HID EVOLution™ - Extraction System to:

- Set up the elution plate or tubes.
- Generate a report at the end of the run. The report records each DNA sample lysate starting position in a plate or in tubes and each extracted sample final position in a plate or in tubes.
- Generate a text file containing the sample information that you can import to the HID EVOLution™ – qPCR/PCR Setup System.

Options for entering sample information

IMPORTANT! When setting up sample information in the HID EVOLution™ - Extraction System, make sure to assign a unique sample ID to each sample. For samples in plates, assign a unique sample ID to all wells containing samples or blank reagents. Refer to the Tecan HID EVOLution - Extraction Application Manual, Section 3.4, “Sample File”, for sample naming requirements.

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

- Automatically capture sample information by having the system scan barcodes on the plates and/or tubes – Select barcodes compatible with the PosID-3, then make sure the barcodes are correctly placed when you set up the worktable. For:
  - Barcodes on Spin (lysate) Plates – Before the plate barcode is scanned, you must manually enter or import the sample name and information for each well in the plate.
  - Barcodes on lysate tubes – The sample name (barcode) and sample position for each tube are automatically updated in the software when the barcodes are scanned.

- Create a sample input file from a template – You can create a sample input file before running the script and then import the file into the HID EVOLution™ – Extraction System. Two sample input file templates are provided on the CD with the HID EVOLution software: one for sample lysate in a 96-well plate, and one for sample lysate in 1.5-mL tubes. Sample ID and sample position in the sample file must agree with the samples on the worktable. See “(Optional) Create a sample input file” on page 54.

- Manually enter sample information into the HID EVOLution™ software – Run the appropriate EVOware software script, then enter the information when you are prompted. Use the Edit button in the Sample Information dialog box to manually add sample information.
To use a sample input file to set up sample information in the HID EVOlution™ system, edit the sample input file template that is provided with the software CD to create a sample input file before starting the extraction run:

Follow these guidelines when creating a sample input file:

- Use a text editor such as Microsoft Notepad to edit the sample input file.

**IMPORTANT!** Do not use Microsoft Excel, which may introduce invalid formatting.

- Follow the formatting rules that are described in the *Tecan HID EVOlution - Extraction Application Manual*, Section 3.4, “Sample File”.
- For the sample name field, follow your laboratory naming conventions to assign a unique name to each sample.
- Save the file with a .csv extension.

### To create a sample input file:

1. Set up the template files on your system:
   a. Create folders for the original and edited template files, for example:
      - C:\PrepFilerTemplateFiles for the template files
      - C:\PrepFilerInputFiles for your edited template files
   b. Copy the following template files from the HID EVOlution™ – Extraction System or – Combination System software CD to the folder that you created for template files:
      - Sample File_Plate_96.csv
      - Sample File_Tubes_96.csv

2. Open the appropriate sample input template:
   a. Select Start ➤ All Programs ➤ Accessories ➤ Notepad to open Microsoft Notepad.
   b. Select File ➤ Open, then browse to C:\PrepFilerTemplateFiles.
   c. Select the appropriate file for sample lysate in a plate or tubes, then click Open.
3. Select File > Save As, browse to the directory C:\PrepFilerInputFiles, change the file name to <UserDefined>.csv, where <UserDefined> is a unique file name of your choosing, then click Save.

4. Edit the plate template following the formatting rules that are described in the Tecan HID EVolution - Extraction Application Manual, Section 3.4.1, “Entering Sample Information” and the following information:
   • Do not include empty plate well or tube rack positions between samples.
   • Avoid spaces or other special characters such as commas (,), asterisks (*), or slashes (/).
   • For the sample name field, follow your laboratory naming conventions to assign a unique name to each sample. Make sure that the sample name meets the formatting rules.

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.

For more information

For details on:
- Preparing a sample setup file, refer to the Tecan HID EVolution - Extraction Application Manual, Section 3.4, “Sample File”.
- Manually entering sample information, refer to the Tecan HID EVolution - Extraction Application Manual, Section 5.3, “Running a HID EVolution - Extraction Script”.
- Barcode specifications for use on the Freedom EVO® instrument, refer to the Tecan Freedom EVO® Operating Manual, Section 3.5.6, “Positive Identification (PosID)”.
- Barcode positioning, refer to the Tecan HID EVolution - Extraction Application Manual, Section 4.6, “Barcodes”.
Set up and run a script

About script files

Script files contain the instructions for a workflow for a specific robot, and they can be read only by the software of that robot. For example, Life Technologies provides scripts specifically for use with the PrepFiler® kit (see page 57 for the list of scripts). These scripts are for use with the Tecan Freedom EVO® 150 and 200 instrument models, and they can be read only by Freedom EVOware® software version 2.1 with the HID EVOlution™ – Extraction application. You select a script based on three criteria:

- Which protocol you used to prepare sample lysate
- Which HID EVOlution™ system you are using
- Which plastics you want to use on the worktable for sample lysate and eluate

Before you begin

- If you want to enter reagent information, have the reagent lot numbers and expiration dates available before running the script.
- Confirm that the instrument shield is closed.

IMPORTANT! 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 correct positioning of the robotic movements.

Note: If you observe bubbles in the system liquid (degassed water) tubing, place the system liquid carboy at same height as worktable, and 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 three 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.
### Select a script

1. On your desktop, click ![EVOware Standard software](image) to start the EVOware Standard software, then enter your user name and password.

2. Select **Run an existing script**, then click ![Run an existing script](image).

3. In the Selection dialog box, select the appropriate script for your system, plate/tube selections, and lysis protocol, then click ![Run](image).

<table>
<thead>
<tr>
<th>If you used this lysis protocol...</th>
<th>And you want the eluted DNA in...</th>
<th>And you are using the HID EVOlution™ system...</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5-mL tubes standard protocol [see page 23]</td>
<td>1.5-mL microcentrifuge tubes</td>
<td>PrepFiler Tubes Tubs V1 SP2</td>
</tr>
<tr>
<td></td>
<td>a 96-well plate</td>
<td>PrepFiler Tubes Plate V1 SP2</td>
</tr>
<tr>
<td>96-well plate standard protocol [see page 25 or page 75]</td>
<td>1.5-mL microcentrifuge tubes</td>
<td>PrepFiler Plate Tubes V1 SP2</td>
</tr>
<tr>
<td></td>
<td>a 96-well plate</td>
<td>PrepFiler Plate Plate V1 SP2</td>
</tr>
<tr>
<td>1.5-mL tubes large-sample protocol‡ [see page 81]</td>
<td>1.5-mL microcentrifuge tubes</td>
<td>PrepFiler Tubes Tubes 500 V1 SP2</td>
</tr>
<tr>
<td></td>
<td>a 96-well plate</td>
<td>PrepFiler Tubes Plate 500 V1 SP2</td>
</tr>
<tr>
<td>96-well plate large-sample protocol‡ [see page 78]</td>
<td>1.5-mL microcentrifuge tubes</td>
<td>PrepFiler Plate Tubes 500 V1 SP2</td>
</tr>
<tr>
<td></td>
<td>a 96-well plate</td>
<td>PrepFiler Plate Plate 500 V1 SP2</td>
</tr>
</tbody>
</table>

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

‡ The large-sample (500-µL) protocols were not tested as part of the validation studies that were performed by Life Technologies. If you intend to use the large-sample protocols, perform the appropriate validation studies.
Chapter 4  Run Automated DNA Extraction

Set up and run a script

4. In the Freedom EVOware script dialog, click \( \text{ } \) to run the script, then click \( \text{ } \) 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 \( \text{ } \) to run a script, you can:
- Cancel the run at any time by clicking \( \text{ } \).
- Pause the run by bringing the EVOware Runtime Controller dialog box to the front of your desktop, then clicking \( \text{ } \).

See “(Optional) Re-cap Magnetic Particles tubes” on page 62 for details on cancelling or pausing a run.

Set up sample and reagent information

1. In the Sample information page, set up sample information in one of the following ways:

   **IMPORTANT!** If you manually enter or import sample information, deselect the Scan Labware checkbox. Otherwise, the system will attempt to scan barcodes and will overwrite previously entered or imported sample information.

   - Load the sample setup file:
     - Click \( \text{ } \), then browse to the C:\PrepFilerInputFiles directory.
     - Select the file that you prepared in “(Optional) Create a sample input file” on page 54, then click \( \text{ } \).
     - Confirm that the actual sample IDs and worktable positions match those in the imported sample input file.

   - Enter sample information manually: Click the \( \text{ } \) button next to the plate or each tube rack, enter the information as described in the Tecan HID EVOlution - Extraction Application Manual, Section 5.3, “Running a HID EVOlution - Extraction Script”, then click \( \text{ } \).

   **Note:** If you use the PosID to scan in sample information, you may need to enter or import sample information before continuing, depending on how your system is set up. For lysate in barcoded 96-well plates, you must manually enter or import information for each sample in the plate before continuing. Refer to the Tecan HID EVOlution - Extraction Application Manual, Section 5.3, “Running a HID EVOlution - Extraction Script” for more information.
2. In the Sample information page, enter information on the number and positioning of the samples:
   
a. In Start index, select a number between 1 and 96 that corresponds to:
   - The tube position of the first tube in the sample racks
   - The well position of the first sample in the plate

   ![Sample positioning diagram]

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

   c. Confirm 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”), then click .

   ![Software interface screenshot]

   If you are not using barcodes, deselect this checkbox

   Make sure that this message is correct before continuing
3. Record information about the PrepFiler® kit components that are used for this extraction run:

**Note:** Entering kit information is optional. Use the kit information for your records and for help with troubleshooting, if necessary.

   a. Click *Record Reagent Information*.
   
   b. In the Record Reagent Information dialog box, enter the appropriate lot numbers and expiration dates.

   **Note:** Scroll down to see all the fields in the dialog box.

   c. Click *OK*, then click ![Play](image) to continue.

---

**Confirm worktable setup and start the run**

1. In the Load worktable page, compare the listed items to the items on the actual worktable.

   a. Confirm that you correctly loaded:

      - A PrepFiler® Processing Plate (shown as Processing Plate)
      - A MicroAmp® Optical 96-Well Reaction Plate or 1.5-mL tubes to collect the DNA eluate (shown as Samples or tube rack S1 through S6)
      - The PrepFiler® Spin Plate or 1.5-mL tubes containing the sample lysate (shown as Spin Plate or tube racks L1 through L6)
      - Two tubes of PrepFiler® Magnetic Particles (shown as Magnetic Particles)
      - The isopropanol (shown as Isopropanol)
      - The prepared PrepFiler® Wash Buffer A (shown as Wash Buffer)
      - The Wash Buffer B (shown as Wash3)
      - The PrepFiler® Elution Buffer (shown as Elution Buffer)

   **Note:** You can place the pointer on an item in the list to highlight the item in the worktable diagram on the right side of the page.

   b. Make sure **Liquid Level Detection** remains selected.

---

**IMPORTANT!** Select Liquid Level Detection if you want the system to check the isopropanol, Wash Buffer A, Wash Buffer B, 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. After confirming that each item is loaded, click **Loaded All**, then click \[play\] to start the inventory scan.

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

   **Note:** During the run, the run status is shown next to the \[play\]. Click **Ignore** to ignore the scanning results unless you use barcoded plates and/or tubes to track your samples.
**Optional** Re-cap Magnetic Particles tubes

*Note:* Perform this step during the run if you observe crystal formation on the particle tube.

After the Magnetic Particles have been dispensed into all samples:

1. 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 Runtime Controller or Freedom EVOware script dialog, or the run stops and cannot be restarted.

If you intentionally or accidentally stop the run, take the following steps to clear the script recovery status before you start a new run:

- Click **Cancel** in the Runtime Controller.
- In the Freedom EVOware script dialog, note the highlighted orange line; this is the step where the run stopped.
- In the Freedom EVOware script dialog, select **Execute ▶️ Clear Recovery Status** from the menu. You can now start a new run.
Record file information and exit the script

When the run is complete:

1. Record the Extraction output file path and name (C:\HIDEVOlutionExtractionFiles\Export\HID_run date_run time.csv), then click [ ] to continue.

2. Record the report path and file name (C:\HIDEVOlutionExtractionFiles\DNAextraction_run date_run time.pdf), then click [ ] to continue.

3. In the Runtime Controller dialog box, click Cancel to exit the script.
For more information

For details on:

- Running extraction scripts, refer to the *Tecan HID EVolution - Extraction Application Manual*, Section 5.3, “Running a HID EVolution - Extraction Script”.
- Script error messages, refer to the *Tecan HID EVolution - Extraction Application Manual*, Section 8.4, “Application Software”.
- The EVOware® software, refer to the *Tecan EVOware® Standard/EVOware® Plus 2.1 Software Manual* and the *Tecan EVOware® Standard/EVOware® Plus 2.1 Software Getting Started Guide*.

Complete the run

When you perform an extraction run, follow the cleanup and maintenance procedures that are specified in the *Tecan HID EVolution - Extraction Application Manual*, Section 5.4.2, “Clean Up the Worktable”, and Chapter 7, “Maintenance”.

**WARNING! CHEMICAL HAZARD.** Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing PrepFiler Lysis Buffer or PrepFiler Magnetic Particles. Wear appropriate protective eyewear, clothing, and gloves.

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

1. Open the front panel of the Freedom EVO® instrument.
2. Remove the MicroAmp® Optical 96-Well Reaction Plate or 1.5-mL tubes containing the DNA eluate from the worktable, seal the plate or tubes, then store them at 4°C for up to 2 weeks or at –20°C for longer storage.
3. Cap (if not capped previously) and store the PrepFiler® Magnetic Particles tubes.
4. Properly dispose of the PrepFiler® Spin Plate (if used) and Processing Plate.

**IMPORTANT!** Do not reuse the reagents in the troughs. See “Chemical safety” on page 134.

6. (Last run of the day) Dispose of the reagent troughs.
7. If necessary, empty the waste carboy and refill the system liquid carboy.
8. Dispose of the used pipette tips.

**IMPORTANT!** To clean all worktable 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 worktable. Consult Safety Data Sheets (SDS) and product labeling of cleaning agents and reagents or chemicals used on the instrument for compatibility before cleaning or decontaminating the instrument.

---

**View the output file and the report**

When you perform an extraction run, follow these guidelines:

- Save the report file as a record of the reagents that you used and the samples that you processed in the extraction run.

  At the end of a run, the output file and report are automatically saved to the C:\HIDEVOlutionExtractionFiles folder. When you start your next run, the output file and report from the previous run are automatically moved to a C:\HIDEVOlutionExtractionFiles\Archive\date_time folder. For example, if you start your next run on August 15, 2011 at 3:08 pm (15:08 hours), files from the previous run would be archived to the C:\HIDEVOlutionExtractionFiles\Archive\20110815_150800 folder regardless of the date of the previous run.

  For quick access to the generated files, create shortcuts on your desktop to the C:\HIDEVOlutionExtractionFiles and C:\EVOlutionExtractionFiles\Archive folders.

- If you use the HID EVOlution™ – qPCR/PCR Setup System, use the Extraction Output file (HID_run date_run time.csv) to import your sample information to the applications.

To view the report generated at the end of the extraction run:

1. Navigate to the C:\HIDEVOlutionExtractionFiles directory.

2. Open the PDF file with the name that you recorded when the extraction run completed (DNAextraction_run date_run_time.pdf).
3. Review the reagent and sample information in the report:

<table>
<thead>
<tr>
<th>Name of the corresponding export file</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent volumes that were used (per well)</td>
</tr>
<tr>
<td>Kit part number (Lot number and expiration date are shown if you entered this information in the Record Reagent Information dialog box)</td>
</tr>
<tr>
<td>Sample ID</td>
</tr>
<tr>
<td>Well position of the sample lysate in the PrepFiler® Spin Plate</td>
</tr>
<tr>
<td>Well position of the corresponding DNA eluate in the MicroAmp® plate</td>
</tr>
</tbody>
</table>

4. Print and sign the report, then keep the report for your records.

For more information:

- The report and extraction output files, refer to the Tecan HID EVOlution - Extraction Application Manual, Section 6, “Results”.
Use this appendix for troubleshooting problems with lysate, DNA eluate appearance, or DNA yield.

To troubleshoot problems with setting up and running the automation instrument, refer to the Tecan HID EVolution - Extraction Application Manual, Chapter 8, “Troubleshooting”.

<table>
<thead>
<tr>
<th>Observed problem</th>
<th>Possible cause</th>
<th>Suggested solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitate in the PrepFiler® Magnetic Particles tube</td>
<td>Magnetic particles were exposed to low temperatures during the shipping or storage.</td>
<td>Before each use, incubate the Magnetic Particles at 37°C for 10 minutes, then vortex the Magnetic Particles at medium speed until the particles are completely resuspended.</td>
</tr>
</tbody>
</table>
| The volume of collected lysate is low. | • Some lysate remained in the sample substrate after centrifugation.  
• A plate or tube was not properly sealed during incubation or vortexing, resulting in volume loss through leakage or evaporation.  
• A tube was not briefly centrifuged after incubation or vortexing, and droplets on the inside of the tube lid leaked when the tube was opened. | If the lysate volume is >180 µL: Proceed to the next step in the protocol.  
If the lysate volume is <180 µL and the sample required substrate removal:  
1. Centrifuge the plate or tube containing the substrate for an additional 5 minutes.  
2. If the resulting lysate volume is:  
   • >180 µL, continue to the next step in the protocol.  
   • <180 µL, add PrepFiler® Lysis Buffer to bring the lysate volume to 300 µL, then proceed to the next step in the protocol.  
If the lysate volume is <180 µL and the sample did not require substrate removal: Add PrepFiler® Lysis Buffer to bring the lysate volume to 300 µL, then proceed to the next step in the protocol. |
| 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 Life Technologies to determine if the liquid handling (LiHa) arm requires adjustment. Also refer to the Tecan Freedom EVware® Standard 2.1 Freedom EVware® Plus 2.1 Extended Device Support Software Manual, Section 9.4.4, “Teaching the Labware Coordinates”. |
## Troubleshooting

<table>
<thead>
<tr>
<th>Observed problem</th>
<th>Possible cause</th>
<th>Suggested solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>The DNA eluate is colored.</td>
<td>The substrate yielded a colored eluate. For example, some sample substrates contain dyes.</td>
<td><strong>Note:</strong> Color does not necessarily interfere with quantitation or amplification. If you see a shift in IPC C&lt;sub&gt;T&lt;/sub&gt; value in the quantitation run, manually process the DNA eluates using the Repurification Protocol, then requantify the sample. Refer to the <em>PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits User Guide</em>, (PN 4463348), Appendix B, “Repurification Protocol”.</td>
</tr>
</tbody>
</table>
| The DNA eluate volume is low.     | • Incomplete volume transfer occurred because of a loose pipette tip (DiTi cone).                    | • If liquid remains in the Processing Plate, manually pipet the liquid to the correct plate well(s) or tube(s).  
• The z-max of the liquid pipetting arm needs to be adjusted.  
• Before the next run, clean and finger-tighten the DiTi cones and diluter valves. Refer to the *Tecan HID EVolution – Extraction Application Manual*, Section 7.3.2, “Disposable Tip (DiTi) of LiHa” and 7.3.10, “Diluter”.  
• If the problem continues, confirm that the z-max is set correctly for the Processing Plate when the plate is set on the 96-Well Magnetic Ring Stand and used with a 200-µL disposable pipette tip. For details, contact Life Technologies or refer to 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”. |
### Observed problem
The DNA yield is low or DNA is absent.

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Suggested solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>• The biological sample contains no or a low amount of DNA.</td>
<td>1. Confirm that reagent and instrument setup are correct, then re-run the samples:</td>
</tr>
<tr>
<td>• Reagents are missing or improperly positioned on the worktable.</td>
<td>• Confirm all necessary reagents are present and correctly positioned on the workstation. Refer to the Tecan HID EVOlution - Extraction Application Manual, Section 4.3.4 “Setup Reagents on the Workstation”.</td>
</tr>
<tr>
<td>• Incorrect automated pipetting occurred because of:</td>
<td>• Confirm that you use the specified DiTis, plates, tubes, and metal racks and carriers in the correct positions. Refer to the Tecan HID EVOlution - Extraction Application Manual, Section 4.3.5, “Setup Plasticware and Samples on the Workstation”, and Section 4.4, “Worktable Layouts”.</td>
</tr>
<tr>
<td>– Incorrect or improperly placed DiTis, plates, tubes, or hardware.</td>
<td>• Flush the system and check for air bubbles and leaks. Refer to the Tecan HID EVOlution - Extraction Application Manual, Section 7.3.1.2, “Flushing the Liquid System”.</td>
</tr>
<tr>
<td>– Air bubbles or leaks in system.</td>
<td>• Clean and finger-tighten the DiTi cones and diluter valves. Refer to the Tecan HID EVOlution - Extraction Application Manual, Section 7.3.2, “Disposable Tip (DiTi) of LiHa” and 7.3.10, “Diluter”.</td>
</tr>
<tr>
<td>– Dirty or loose DiTi cones.</td>
<td>• Reteach the LiHa the coordinates (x, y, and z positions) of the 200-µL and 1000-µL DiTis. For details, contact Life Technologies or refer to 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”.</td>
</tr>
<tr>
<td>• DiTis were not picked up properly.</td>
<td>2. Amplify the maximum volume for STR analysis.</td>
</tr>
<tr>
<td></td>
<td>3. Extract DNA from a different sample that is prepared from the same source.</td>
</tr>
<tr>
<td>Observed problem</td>
<td>Possible cause</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| The sample IPC CT is higher than the IPC CT of the no template quantitation control (NTC) or of the quantitation standards (for example, if the sample IPC CT is approximately two CT greater than the NTC IPC CT or the CT of the standards). | • Magnetic particles are in the DNA extract.  
• The DNA concentration is above 25 ng/µL.  
• The DNA eluate contains PCR inhibitors due to 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 x 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 over 25 ng/µL, dilute the DNA eluate, then requantify the sample.  
• If the DNA eluate is below 25 ng/µL, or if the diluted DNA eluate still produces high IPC CT compared to the NTC or quantitation standards, follow the Repurification Protocol (refer to the PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits User Guide, (PN 4463348), Appendix B, “Repurification Protocol”) to process the DNA eluate, then requantify the sample.  
**IMPORTANT!** Repurification may result in the loss of additional DNA. Consider proceeding to amplification with a kit such as AmpFSTR® Identifiler Plus™ PCR Amplification Kit, which is designed to obtain STR profiles from inhibited samples, or the AmpFSTR® 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. Refer to the Tecan HID EVOLUTION - Extraction Application Manual, Section 7.3.2, “Disposable Tip (DiTi) of LiHa” and 7.3.10, “Diluter”. |
Preventing Contamination

This appendix covers:
- Reagent preparation guidelines .......................................................... 71
- Tube-handling guidelines ................................................................. 72
- Plate lysis guidelines and best practices ........................................... 72
  Plate-handling guidelines ............................................................. 72
  Best practices for sealing the plate ................................................... 73
  Best practices for using the MicroAmp® Multi-Removal Tool ........... 73
- Laboratory practices to minimize false positives .......................... 74

Reagent preparation guidelines

**IMPORTANT!** When preparing and handling reagents, follow these guidelines to minimize potential for contamination and issues during the extraction run:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or during sample preparation) when performing DNA extractions and preparing samples for PCR amplification.
- Change gloves frequently to prevent contamination of reagents. For example, change gloves after opening tubes containing DNA standards or control DNA to avoid contaminating other kit reagents.
- Label each vial and tube cap to make sure that the caps are replaced on the correct tube or vial.
- Before opening, centrifuge each reagent tube or vial briefly to remove any liquid from the cap and sides of the tube. To avoid forming air bubbles, do not vortex reagent tubes or vials unless directed to do so.
- When uncapping tubes and vials, place the caps in a clean area to minimize the potential for contamination.
- If air bubbles form on the surface of the reagents in the tubes or vials, remove the air bubbles by pipetting. Surface bubbles may interfere with liquid-level detection during the automated extraction run.
Tube-handling guidelines

IMPORTANT! Leaking tubes may result in DNA cross-contamination.

Avoid cross-contamination by observing the following guidelines.

- Change gloves frequently when handling tubes. For example, change gloves after removing the filter column from the spin tube.
- To avoid leaks, make sure that tubes are tightly sealed before vortexing or incubation.
- After vortexing a tube, check the tube for air bubbles, then revortex if necessary to remove bubbles.
- Before opening a tube after vortexing or incubation, centrifuge the tube briefly (approximately two seconds in a microcentrifuge) to collect any residual tube contents from the sides and cap of the tube.
- Open and close all sample tubes carefully and briefly centrifuge tubes before opening them to remove any excess liquid from the lid.
- After substrate removal, always process samples in new 1.5-ml tubes (not PrepFiler® Spin Tubes).

Plate lysis guidelines and best practices

Plate-handling guidelines

IMPORTANT! Leaking plates may result in DNA cross-contamination.

Avoid cross-contamination by observing the following guidelines.

- Change gloves frequently when handling plates. For example, change gloves after separating the filter plate from the spin plate.
- Make sure that the filter/spin-plate unit is tightly assembled before shaking and incubation.
- Use caution when separating the filter plate (top) from the spin plate (bottom) with the MicroAmp® Multi-Removal Tool. If the contents of the spin plate are shaken during plate separation, place an adhesive cover on the spin plate, place the spin plate in the deep-well centrifuge, then briefly centrifuge the spin plate to make sure that all contents are collected at the bottom of the wells.
- Use caution when placing each sample in a separate well in the top (PrepFiler® Filter Plate) of the plate unit. Consider covering unfilled wells to prevent depositing two samples in the same well.
- Cover and uncover all reaction plates carefully.
Best practices for sealing the plate

**IMPORTANT!** Apply a sealing tool with significant downward pressure to make sure that the plate is well sealed:

- Place an adhesive cover on the plate, then rub the flat edge of the applicator back and forth along the long edge of the plate.

- Rub the flat edge of the applicator back and forth along the short edge (width) of the plate.
- Rub the end of the applicator horizontally and vertically between all wells (not shown).

- Rub the end of the applicator around all outside edges of the plate using small back and forth motions to form a complete seal around the outside wells.

Best practices for using the MicroAmp® Multi-Removal Tool

Before using the MicroAmp® Multi-Removal Tool for the first time, use the tool to practice separating an empty Filter Plate from the Spin Plate as shown below. Be aware that when you use the tool on assembled plates that have been processed through lysis, the seal may be tighter than the seal on the empty plates.

Insert the Multi-Tool between the two plates, then gently lift up to partially separate the plates.

Repeat the process on the opposite side. Continue using the Multi-Tool on opposite sides of the plate until the Filter Plate can be easily lifted off the Spin Plate.
Laboratory practices to minimize false positives

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

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

- When possible, maintain separate work areas and dedicated equipment and supplies for:
  - Sample preparation
  - DNA extraction
  - PCR setup
  - PCR amplification
  - Analysis of PCR products

- Prepare PrepFiler® kit reagents in a clean, dedicated area free of amplified PCR products.

- During sample preparation and extraction, separate samples containing high quantities of DNA from those containing low quantities of DNA.

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or during sample preparation) when performing DNA extractions and preparing samples for PCR amplification.

- Change gloves whenever you suspect they are contaminated and before leaving the work area.

- Use positive-displacement pipettes or aerosol-resistant pipette tips.

- Never bring amplified PCR products into the DNA extraction or PCR setup areas.

- Try not to splash or spray PCR samples.

- When pipetting from a kit component tube, hold the cap of the tube in your gloved hand, or be sure to set it down on a clean, decontaminated surface.

- Keep reactions and components sealed when possible.

- Clean lab benches and equipment periodically with 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 worktable.

Consult safety data sheets (SDS) and product labeling of cleaning agents and reagents or chemicals used on the instrument for compatibility before cleaning or decontaminating the instrument.
Supplementary Lysis Protocols for the PrepFiler® Automated Forensic DNA Extraction Kit

This appendix covers:

- 96-well plate standard (300-µL) protocol for liquid samples .................... 75
- 96-well plate large-sample (500-µL) protocol ............................................. 78
- 1.5-mL tubes large sample (500-µL) protocol ............................................. 81

96-well plate standard (300-µL) protocol for liquid samples

Required materials

In addition to standard laboratory equipment, you need the following materials. See “Required materials and instruments” on page 14 for details:

- 1.5-mL microcentrifuge tubes (use RNase-free Microfuge Tubes (1.5-mL), certified DNase- and RNase-free (Applied Biosystems Part No. AM12450) or equivalent tubes that allow you to observe the tube contents and that fit on the automated system)
- Laboratory microcentrifuge capable of 16,110 × g from a major laboratory supplier
- PrepFiler® Lysis Buffer
- 1.0 M solution DTT
- PrepFiler® Filter and Spin Plates (shipped assembled)
- Deep-well centrifuge
- Deep-well plate for counterbalancing in centrifuge (VWR Part #82006-448 or equivalent)
- Ice
- MicroAmp® Clear Adhesive Film
- MicroAmp® Adhesive Film Applicator
- Shaking incubator
- MicroAmp® Multi-Removal Tool
Appendix C  Supplementary Lysis Protocols for the PrepFiler® Automated Forensic DNA Extraction Kit
96-well plate standard (300-µL) protocol for liquid samples

Perform lysis

**WARNING! CHEMICAL HAZARD.** Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing PrepFiler Lysis Buffer. Wear appropriate protective eyewear, clothing, and gloves.

1. Bring the shaking incubator temperature to 70°C.

   **Note:** Temperatures up to 80°C are not deleterious to lysis.

2. Make sure that the Filter/Spin Plate unit is tightly assembled by centrifuging the unit as follows:
   a. Confirm that the Filter Plate is placed firmly on top of the Spin Plate as shown below, measure the weight of the assembled plate unit, then place the plate unit in a deep-well centrifuge.
   b. Create a counterweight to make sure the centrifuge rotor is well-balanced:
      • Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the assembled Filter/Spin Plate unit.
      • Seal the plate.
      • Place the plate in the deep-well centrifuge as a counterweight.
   c. Spin the plates at 650 × g for 2 minutes.

3. Confirm that the PrepFiler® Spin Plate is labeled for identification. If you use barcodes to track samples, move the barcode provided with the plate into the correct position as shown in the Tecan HID EVOLUTION™ Extraction Setup System Application Manual.

4. Prepare the PrepFiler® Lysis Buffer-DTT mixture:
   a. Calculate the volumes of components that are needed based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

   **IMPORTANT!** Prepare fresh Lysis Buffer-DTT mixture for each experiment.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One reaction</td>
</tr>
<tr>
<td>PrepFiler® Lysis Buffer</td>
<td>300 µL</td>
</tr>
<tr>
<td>1.0 M DTT</td>
<td>3 µL</td>
</tr>
</tbody>
</table>

   † Includes approximately 4% excess volume to compensate for pipetting losses.

   b. Combine the required volumes of components, then gently mix.
5. Prepare each sample:
   a. Add 300 µL of the Lysis Buffer-DTT mixture to a separate 1.5-mL tube that contains no more than 30 µL of liquid sample (such as blood or saliva). If adding liquid blood, invert the tube containing blood several times to mix before adding to the 1.5-mL tube.
   b. Cap the tube, vortex the tube at high speed for approximately 15 seconds, then centrifuge the tube at maximum speed for 20 seconds.
   c. Aspirate the entire liquid sample with a pipette, then dispense the sample into a new well in the Filter/Spin Plate unit.

6. Immediately seal the plate unit with a new sheet of MicroAmp® Clear Adhesive Film.

7. Place the plate unit into the shaking incubator, then incubate the plate unit at 150 rpm and 70°C for 60 minutes.

   **IMPORTANT!** Keep the plate unit horizontal during transport to the incubator and throughout incubation to keep lysis solution in contact with sample and to prevent possible cross-contamination.

8. Transfer the sample lysate into the Spin Plate:
   a. Place the plate unit in a deep-well centrifuge.
   b. Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the assembled Filter/Spin Plate unit, seal the plate, then place the plate in the deep-well centrifuge as a counterweight to make sure that the rotor is well balanced.
   c. Centrifuge the plate unit at 650 × g for 1 minute. If all lysate does not pass into the Spin Plate after 1 minute, centrifuge for an additional minute.

   **Note:** The sample substrate remains in the Filter Plate.

9. While holding the bottom plate, separate the Filter Plate (top) from the Spin Plate (bottom) with the MicroAmp® Multi-Removal Tool.

   **IMPORTANT!** If the contents of the Spin Plate are shaken during plate separation, place an adhesive cover on the Spin Plate, place the Spin Plate in the deep-well centrifuge, then briefly centrifuge the Spin Plate to make sure that all contents are collected at the bottom of the wells.

10. Properly dispose of the (top) Filter Plate. Proceed directly to automated extraction run.

   **IMPORTANT!** To avoid precipitation of lysis buffer components, do not chill the sample lysate after performing lysis.

   **Note:** If you cannot proceed directly to the automated extraction run, the unprocessed lysate is stable for up to 24 hours at room temperature (20°C) in a sealed Spin Plate.
96-well plate large-sample (500-µL) protocol

You may choose to evaluate the large-sample protocol if you require more than 300 µL of Lysis Buffer to submerge your sample. If you are unsure about using the large-sample protocol, start with the standard protocol, add the recommended amount of Lysis Buffer, then consider evaluating the large-sample protocol if necessary. See “Select a lysis protocol” on page 21 for information on selecting the appropriate protocol.

Review “Plate lysis guidelines and best practices” on page 72 before performing a protocol for the first time.

IMPORTANT! The large-sample (500-µL) protocols were not tested as part of the full validation studies that were performed by Life Technologies. The large-sample protocol is intended only for samples that are not submerged by the 300 µL of PrepFiler® Lysis Buffer that is used in the standard protocol. If your laboratory intends to use the large-sample protocols, perform the appropriate validation studies. DNA yields from the large volume protocol may be lower than those that are obtained using the standard protocols with comparable sample input amounts.

Required materials

In addition to standard laboratory equipment, you need the following materials. See “Required materials and instruments” on page 14 for details:

- PrepFiler® Lysis Buffer
- 1.0 M solution DTT
- PrepFiler® Filter and Spin Plates (shipped assembled)
- 1.5-mL microcentrifuge tubes (for liquid samples)
- Deep-well centrifuge
- Deep-well plate for counterbalancing in centrifuge (VWR Part #82006-448 or equivalent)
- Ice
- MicroAmp® Clear Adhesive Film
- MicroAmp® Adhesive Film Applicator
- Shaking incubator
- MicroAmp® Multi-Removal Tool

Perform lysis

⚠️ WARNING! CHEMICAL HAZARD. Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing PrepFiler Lysis Buffer. Wear appropriate protective eyewear, clothing, and gloves.

1. Bring the shaking incubator temperature to 70°C.

   Note: Temperatures up to 80°C are not deleterious to lysis.
2. Make sure that the Filter/Spin Plate unit is tightly assembled by centrifuging the unit as follows:
   a. Confirm that the Filter Plate is placed firmly on top of the Spin Plate as shown below, measure the weight of the assembled plate unit, then place the plate unit in a deep-well centrifuge.

   ![Centrifuge Unit Image]

   b. Create a counterweight to make sure the centrifuge rotor is well-balanced:
      - Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the assembled Filter/Spin Plate unit.
      - Seal the plate.
      - Place the plate in the deep-well centrifuge as a counterweight.
   c. Spin the plates at 650 × g for 2 minutes.

3. Place each sample in a separate well in the top (filter) plate of the plate unit.

4. Calculate the volumes of components that are needed based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepFiler® Lysis Buffer</td>
<td>500 µL</td>
</tr>
<tr>
<td>1.0 M DTT</td>
<td>5 µL</td>
</tr>
</tbody>
</table>

**Component** | **Volume**
--- | ---
PrepFiler® Lysis Buffer | 500 µL | 50 mL
1.0 M DTT | 5 µL | 500 µL

† Includes approximately 4% excess volume to compensate for pipetting losses.

5. Combine the required volumes of components, then gently mix.

**IMPORTANT!** Prepare fresh Lysis Buffer-DTT mixture for each experiment.

6. Confirm that the PrepFiler® Spin Plate is labeled for identification. If you use barcodes to track samples, move the barcode provided with the plate into the correct position as shown in the Tecan HID EVOLUTION™ Extraction Setup System Application Manual.

7. Add 500 µL of the PrepFiler® Lysis Buffer-DTT mixture to each well of the plate unit that contains samples.

**Note:** You may observe that some lysis buffer drips through to the Filter Plate during lysis. Lysis is still occurring in the wetted sample.
8. Immediately seal the plate unit with a new sheet of MicroAmp® Clear Adhesive Film.

9. Place the plate unit into the shaking incubator, then incubate the plate unit at 150 rpm and 70°C for 60 minutes.

**IMPORTANT!** Keep the plate unit horizontal during transport to the incubator and throughout incubation to keep lysis solution in contact with sample and to prevent possible cross-contamination.

10. Transfer the sample lysate into the Spin Plate:
   a. Place the plate unit in a deep-well centrifuge.
   b. Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the assembled Filter/Spin Plate unit, seal the plate, then place the plate in the deep-well centrifuge as a counterweight to make sure that the rotor is well balanced.
   c. Centrifuge the plate unit at 650 × g for 1 minute. If all lysate does not pass into the Spin Plate after 1 minute, centrifuge for an additional minute.  
   **Note:** The sample substrate remains in the Filter Plate.

11. While holding the bottom plate, separate the Filter Plate (top) from the Spin Plate (bottom) with the MicroAmp® Multi-Removal Tool.

**IMPORTANT!** If the contents of the Spin Plate are shaken during plate separation, place an adhesive cover on the Spin Plate, place the Spin Plate in the deep-well centrifuge, then briefly centrifuge the Spin Plate to make sure that all contents are collected at the bottom of the wells.

12. Properly dispose of the (top) Filter Plate, which contains the sample substrate. Proceed directly to automated extraction run.

**IMPORTANT!** To avoid precipitation of lysis buffer components, do not chill the sample lysate after performing lysis.

**Note:** If you cannot proceed directly to the automated extraction run, the unprocessed lysate is stable for up to 24 hours at room temperature (20°C) in a sealed Spin Plate.
1.5-mL tubes large sample (500-µL) protocol

You may choose to evaluate the large-sample protocol if you require more than 300 µL of Lysis Buffer to submerge your sample. If you are unsure about using the large-sample protocol, start with the standard protocol, add the recommended amount of Lysis Buffer, then consider evaluating the large-sample protocol if necessary. See “Select a lysis protocol” on page 21 for information on selecting the appropriate protocol.

Review “Tube-handling guidelines” on page 72 before performing a protocol for the first time.

IMPORTANT! The large-sample (500-µL) protocols were not tested as part of the full validation studies that were performed by Life Technologies. The large-sample protocol is intended only for samples that are not submerged by the 300 µL of PrepFiler® Lysis Buffer that is used in the standard protocol. If your laboratory intends to use the large-sample protocols, perform the appropriate validation studies. DNA yields from the large volume protocol may be lower than those that are obtained using the standard protocols with comparable sample input amounts.

Required materials

In addition to standard laboratory equipment, you need:

- 1.5-mL microcentrifuge tubes (AB Part No. AM12400 or equivalent)
- PrepFiler® Lysis Buffer
- 1.0 M solution DTT
- PrepFiler® Spin Tubes and Columns
- If sample substrate is present, DNA-free tweezers to move sample substrate
- Shaking incubator

Perform lysis

WARNING! CHEMICAL HAZARD. Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing PrepFiler Lysis Buffer. Wear appropriate protective eyewear, clothing, and gloves.

1. Bring the shaking incubator temperature to 70°C.

2. Prepare each sample tube:
   a. Place a sample in a standard 1.5-mL microcentrifuge tube, then add:
      - PrepFiler® Lysis Buffer: 500 µL
      - DTT, 1.0 M: 5 µL

      IMPORTANT! The use of more than 500 µL of lysis buffer is not recommended.

      Note: To minimize the number of times you pipet, you can pre-mix the lysis buffer and DTT (1.0 M) for all samples, then add 500 µL of the lysis buffer-DTT mixture to each tube. Prepare a fresh lysis buffer-DTT mixture for each experiment.

   b. Cap the tube, vortex it for 5 seconds, then centrifugate it briefly.
3. Place the tube in a shaking incubator, then incubate the tube at 900 rpm and 70°C for 60 minutes.

**IMPORTANT!** Before incubation, make sure that the tubes are well sealed.

4. Label up to 96 PrepFiler® Spin Tubes.

**Note:** If you use barcodes to track samples, confirm that barcodes are correctly placed on the tubes (see barcode information in the *Tecan HID EVOLUTION™ Extraction System Application Manual*).

5. Centrifuge the sample tube for 2 seconds to collect the condensate from the tube cap.

6. Remove the substrate from the sample lysate:
   a. Insert a PrepFiler® Filter Column into a new, labeled 1.5-mL PrepFiler® Spin Tube, then carefully transfer the sample tube contents into the filter column:
      - Use a pipette to transfer the liquid contents.
      - Use the pipette tip or sterile tweezers to transfer the substrate.
   b. Cap the filter column/spin tube, then centrifuge it for 2 minutes at 13,000 to 16,000 × g.
   c. Remove the filter column from the spin tube, then properly dispose of the filter column.
   d. Transfer the lysate into a new, labeled 1.5-microcentrifuge tube.

7. Allow the lysate to come to room temperature before beginning the automated run. Proceed directly to automated extraction run.

**IMPORTANT!** To avoid precipitation of lysis buffer components, do not chill the sample lysate after performing lysis.

**Note:** If you cannot proceed directly to the automated extraction run, the unprocessed lysate is stable for up to 24 hours at room temperature (20°C) in sealed Spin Tubes.
The temperatures set in the extraction scripts are the temperatures for the heating plate (C in Figure 9), not the heating plate adapter (D in Figure 9). To ensure that the heating plate adapter reaches the recommended temperatures during the automated extraction run, we recommend that you use a micro thermocouple and the test procedure on page 84 to verify the empirical temperatures between the PrepFiler® Processing Plate and the Te-Shake™ plate adapter.

- The temperature readings at the end of timer 11 should be no less than 30°C or more than 35°C.
- The temperature readings at the end of timer 14 should not be less than 65°C or more than 75°C.

If the temperatures are not within these ranges,

- Contact your Tecan or Life Technologies representative for assistance.

or

- Verify the extraction 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.

---

**Figure 9** Te-Shake™ components (from the Tecan HID EVOlution™ – Extraction Application Manual)

<table>
<thead>
<tr>
<th>No.</th>
<th>Plain Text Designation</th>
<th>Tecan p/n</th>
<th>Label Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Te-Shake mounting plate with two additional positions</td>
<td>300155605</td>
<td>PLATE MOUNTING 2 TE-SHAKE CMR</td>
</tr>
<tr>
<td>B</td>
<td>Shaker plate for heating block</td>
<td>10760726</td>
<td>PLATE SHAKER HEATING PLATE TE-SHAKE</td>
</tr>
<tr>
<td>C</td>
<td>Heating plate for microplate/tube adapters</td>
<td>30015374</td>
<td>PLATE HEATING TE-MAGS/ TE-SHAKE IP</td>
</tr>
<tr>
<td>D</td>
<td>Heating plate adapter for VWR plate</td>
<td>90055318</td>
<td>BLOCK HEATING VWR PLATE CPL</td>
</tr>
</tbody>
</table>
For this procedure, you need a digital thermometer and a thermocouple probe such as the following:

- Sper Scientific Type-K, Type-J Thermometer 800005 (VWR catalog number 14003-070)
- Sper Scientific Type-K Beaded Wire 800077 (VWR catalog number 14003-136)

**To measure the actual temperature between the plate adapter and the processing plate:**

1. Attach the micro-thermocouple probe:
   a. Tape the micro-thermocouple probe near the center of the Te-Shake™ plate adapter. Make sure 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™ so that the probe is secure and does not interfere with pick up or set down of the processing plate by the RoMa. Make sure that there is sufficient slack between the heating plate and Te-Shake™ to allow the Te-Shake™ to move without pulling on the probe.

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

3. Set up the worktable for a water run for a small number of samples.

   Note: To perform a water run for a small number of samples, fill 2 to 3 wells in a PrepFiler® Spin Plate with 50 µL of water or TE buffer. Fill each reagent trough with 30 mL water, and place two PrepFiler® Magnetic Particles tubes containing water on the worktable. Otherwise, set up the worktable according to Chapter 3, “Set Up the Automation Instrument” on page 29.

4. Begin a run according to the instructions in Chapter 4, “Run Automated DNA Extraction” on page 51. Select any extraction script (for example, PrepFiler_plate_plate_V1_SP1).

5. During the run, view the timers in the Waiting dialog box, and 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, you can click Continue in the Waiting dialog box to skip timers 1 through 9. The first heating step begins at timer 10 (at approximately script line 416).
Automation Guidelines

The following are general guidelines for automating the binding, wash, and elution steps of the PrepFiler® kit extraction procedure.

Before the automated steps

Prepare reagents and perform lysis manually as described in Chapter 2 on page 19.

Binding step

1. Place the PrepFiler® Spin Plate (shown) or 1.5-µL tubes containing the lysate on the robot.

2. To each well or tube, add 15 µL of PrepFiler® Magnetic Particles, then, with the robot at the default mixing speed, pipet the well contents up and down until the particles are completely resuspended.

   Note: Mixing speed does not affect Magnetic Particle performance.

3. Transfer the contents of each well or tube to a separate well in the PrepFiler® Processing Plate.

4. Add isopropanol to each well:

<table>
<thead>
<tr>
<th>If you used this lysis protocol...</th>
<th>Add this volume of isopropanol to each well...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard lysis protocols for the PrepFiler® Automated Forensic DNA Extraction Kit</td>
<td>180 µL</td>
</tr>
<tr>
<td>Large-volume lysis protocols for the PrepFiler® Automated Forensic DNA Extraction Kit</td>
<td>300 µL</td>
</tr>
</tbody>
</table>
5. Shake the plate at 500–1000 rpm for 10 minutes to bind the DNA to the magnetic particles.

6. Place the plate on the 96-Well Magnetic Ring Stand (Part No. AM10050) for 5 minutes to separate the magnetic particles.

7. Remove all of the liquid phase without disturbing the magnetic particles.

Wash step (repeat three times)

1. Place the plate on the Shaker.

2. Add wash buffer to each well:
   - **First wash step**: Add 600 µL of prepared PrepFiler® Wash Buffer A
   - **Second wash step**: Add 300 µL of prepared PrepFiler® Wash Buffer A
   - **Third wash step**: Add 300 µL of prepared PrepFiler® Wash Buffer B

3. Shake the plate at 500–1000 rpm for 1 minute.

4. Place the plate on the 96-Well Magnetic Ring Stand for 1 minute.

5. Remove all the liquid phase without disturbing the magnetic particles.
Elution step

1. Place the plate on the Shaker.

2. Shake the plate at 500–1000 rpm for 5 minutes to allow the magnetic particles to dry.

3. To each well, add 50 µL of PrepFiler® Elution Buffer.

4. Incubate and shake the plate at 500–1000 rpm at 70°C for 5 minutes.

5. Place the plate on the 96-Well Magnetic Ring Stand for 7 minutes.

6. Remove all of the DNA-containing liquid phase without disturbing the magnetic particles.

7. Pipet the DNA-containing liquid phase into a new 96-well plate or new 1.5-mL tubes.
Experiments and Results

This chapter covers:

- Validation of the PrepFiler® Automated Forensic DNA Extraction Kit on the Tecan HID EVOlution™ – Extraction System
  - Overview ................................................................. 92
  - Materials and methods ........................................... 93
  - Validation results .................................................. 94
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- Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction
  - Purpose of these validation studies .......................... 110
  - Validation studies overview ................................... 111
  - Materials and methods .......................................... 111
  - Script validation .................................................... 114
  - Precision and sensitivity studies ............................. 116
  - Cross-contamination studies .................................. 120
  - Case-type sample studies ...................................... 122
  - Comparative analysis studies ................................. 126
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Validation of the PrepFiler® Automated Forensic DNA Extraction Kit on the Tecan HID EVOLution™ – Extraction System

Overview

This chapter provides the results of the developmental validation experiments performed by Applied Biosystems 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, that were performed to validate the chemistry used by both the PrepFiler® Forensic DNA Extraction and PrepFiler® Automated Forensic DNA Extraction Kits.

The PrepFiler® Automated Forensic DNA Extraction Kit was designed specifically for the automated extraction of DNA from forensic samples. The kit contains reagents necessary for the lysis of cells, binding of DNA to magnetic particles, removal of PCR inhibitors, and elution of bound DNA. Downstream applications include the use of the extracted 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; it 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.

Experiments to evaluate the performance of the PrepFiler® Automated Forensic DNA Extraction Kit using the Tecan HID EVOLution™ – Extraction System were performed at Applied Biosystems 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://www2.fbi.gov/hq/lab/fsc/backissu/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 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:

- Biological samples from 8 donors were obtained from the Serological Research Institute (Richmond, California) and were used to prepare the samples for each experiment.
- Samples were prepared and lysed for DNA extraction using the PrepFiler® Automated Forensic DNA Extraction Kit following the standard 300-µL lysis protocol described in this user guide.
- Genomic DNA was extracted from the lysed samples using the PrepFiler® Automated Forensic DNA Extraction Kit and the HID EVolution™ – Extraction System, which consists of:
  - A TECAN Freedom EVO® 150 or 200 robotic workstation
    - **Note**: The Freedom EVO 150 and 200 instruments can be configured identically and both instruments are supported for use with the HID EVolution™ – Extraction System. Validation studies were performed on the Freedom EVO 150.
  - The Freedom EVOware® software version 2.1 SP1 configured with the HID EVolution™ – Extraction application
  - The necessary hardware, including an 8-channel liquid-handling accessory (LiHa), Robotic Manipulator arm (RoMa), and Te-Shake with heating block and adapter
- DNA was eluted with 50 µL of elution buffer. Extraction blanks were processed for each study.
- The HID EVolution™ – Extraction System supports four configurations with corresponding software scripts which contain the instructions for the robotic workstation. The configurations are:
  - Plate:plate – Performing cell lysis in a 96-well plate and collecting eluate in a 96-well plate
  - Plate:tubes – Performing cell lysis in a 96-well plate and collecting eluate in tubes
  - Tubes:tubes – Performing cell lysis in tubes and collecting eluate in tubes
  - Tubes:plate – Performing cell lysis in tubes and collecting eluate in a 96-well plate
- The core liquid handling script for the binding, washing, and elution operations is identical in all validated scripts. The software script(s) used in each study are described in the Validation Results section.
- Extracted DNA from each sample was quantified using the Quantifiler® Human DNA Quantification Kit on an Applied Biosystems 7500 Real-Time PCR System. An elution volume of 50 µL was used for all experiments. The quantitation results were analyzed using SDS software v 1.2.3.
- Quantified DNA from each sample was normalized using the Tecan HID EVolution™ – qPCR/PCR Setup System and amplified using the AmpFISTR® Identifiler® PCR Amplification Kit. Samples with a target DNA input amount of 1 ng were amplified on a GeneAmp® 9700 thermal cycler. Electrophoresis was performed on an Applied Biosystems 3130xl Genetic Analyzer.
- The STR profiles were analyzed using GeneMapper® ID-X software v1.0.
Additional instruments and materials

In addition to the materials provided with the PrepFiler® Automated Forensic DNA Extraction Kit and the HID EVOlution™ – Extraction System, the following additional instruments and materials were used:

- Isopropyl alcohol, Sigma-Aldrich, St. Louis, MO
- TE buffer, Teknova, Hollister, CA
- All other general reagents and materials were purchased from major laboratory suppliers
- Signature™ Benchtop Shaking Incubators, Model #1575 ZZMFG
- RNase-free Microfuge Tubes (1.5 mL), certified DNase and RNase-free, Applied Biosystems (PN AM12400) or equivalent
- PrepFiler® Spin Tubes and Columns, Applied Biosystems (PN 4392342)
- 96-Well Magnetic Ring Stand, Applied Biosystems (PN AM 10050)
- 1000-µL LiHa disposable tips with filter, Tecan (PN 30000631) www.tecan.com
- 200-µL LiHa disposable tips with filter, Tecan (PN 30000629)
- 100-mL disposable troughs for reagents, Tecan (PN 10613048)
- MicroAmp™ Optical 96-Well Reaction Plate (with or without barcode), Applied Biosystems (PN N8010560 or 4306737)

Validation results

Precision studies (SWGDAM Guideline 2.9)

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.

Experiments

Precision experiment A – DNA was extracted from twelve sample types (see Table 8 on page 95) in eight replicates using the PrepFiler® Automated Forensic DNA Extraction Kit. A PrepFiler® 96-Well Spin Plate (96-well spin plate) was used for lysis, and a MicroAmp™ Optical 96-Well Reaction Plate (96-well PCR plate) was used for elution. Each replicate set was arranged in a separate column in the 96-well 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) Cₜ values were also evaluated for variation among replicates.

Precision experiment B – The experiment described above in precision experiment A was also performed using 96 tubes for both the lysis and elution containers.
Table 8  Name, description, and liquid volumes of the experimental samples used in this report.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample Description</th>
<th>Body Fluid Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB-40µL</td>
<td>Liquid human blood</td>
<td>40</td>
</tr>
<tr>
<td>LB-30µL</td>
<td>Liquid human blood</td>
<td>30</td>
</tr>
<tr>
<td>LB-10µL</td>
<td>Liquid human blood</td>
<td>10</td>
</tr>
<tr>
<td>LB-5µL</td>
<td>Liquid human blood</td>
<td>5</td>
</tr>
<tr>
<td>LB-2µL</td>
<td>Liquid human blood</td>
<td>2</td>
</tr>
<tr>
<td>LB-1µL</td>
<td>Liquid human blood</td>
<td>1</td>
</tr>
<tr>
<td>BSC</td>
<td>Human blood stain on non-colored cotton</td>
<td>5</td>
</tr>
<tr>
<td>SALSw</td>
<td>Human saliva on cotton swab</td>
<td>50</td>
</tr>
<tr>
<td>SSC</td>
<td>Human semen stain on non-colored cotton</td>
<td>1</td>
</tr>
<tr>
<td>BSCI</td>
<td>Human blood stain on non-colored cotton plus inhibitor mix†</td>
<td>5 µL blood + 1 µL inhibitor mix</td>
</tr>
<tr>
<td>BSD</td>
<td>Human blood stain on denim</td>
<td>5</td>
</tr>
<tr>
<td>XB</td>
<td>Extraction blank</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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

Results

DNA concentrations obtained in precision experiments A and B are summarized in **Figure 10 on page 96**. Average IPC $C_T$ values for the different samples are shown in **Figure 10** 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 11 on page 97**.
Figure 10  Precision studies A and B: The average DNA concentration and average IPC C<sub>T</sub> values for extracted DNA samples. The same data set is shown on two different scales: for the concentration ranges 0 to 50 ng/µL (top) and 0 to 10 ng/µL (bottom).
Figure 11  Precision studies A and B: DNA concentration is plotted against liquid blood volume and the linear regression trend is calculated.

Table 9 below and Table 10 on page 98 summarize the statistics obtained from precision experiments A (plate to plate) and B (tubes to tubes).

**Table 9**  Precision study A: Summarized statistics for the eight replicates.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>n=</th>
<th>DNA Concentration (ng/µL)</th>
<th>± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td><strong>Liquid Samples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 µL LB85</td>
<td>8</td>
<td>38.94</td>
<td>49.74</td>
</tr>
<tr>
<td>30 µL LB85</td>
<td>8</td>
<td>22.06</td>
<td>36.35</td>
</tr>
<tr>
<td>10 µL LB85</td>
<td>8</td>
<td>6.87</td>
<td>10.59</td>
</tr>
<tr>
<td>5 µL LB85</td>
<td>8</td>
<td>3.64</td>
<td>4.83</td>
</tr>
<tr>
<td>2 µL LB85</td>
<td>8</td>
<td>1.08</td>
<td>2.18</td>
</tr>
<tr>
<td>1 µL LB85</td>
<td>8</td>
<td>0.62</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Solid Substrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 µL BSC</td>
<td>8</td>
<td>2.35</td>
<td>4.11</td>
</tr>
<tr>
<td>50 µL SALSw</td>
<td>8</td>
<td>2.14</td>
<td>2.90</td>
</tr>
<tr>
<td>1 µL SSC</td>
<td>8</td>
<td>1.79</td>
<td>3.21</td>
</tr>
<tr>
<td>5 µL BSCI</td>
<td>8</td>
<td>2.76</td>
<td>4.42</td>
</tr>
<tr>
<td>5 µL BSD</td>
<td>8</td>
<td>3.63</td>
<td>5.01</td>
</tr>
<tr>
<td><strong>Extraction Blank</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XB</td>
<td>8</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 10  Precision study B: Summarized statistics for the eight replicates.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>n=</th>
<th>DNA Concentration (ng/µL)</th>
<th>± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td><strong>Liquid Samples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 µL LB85</td>
<td>8</td>
<td>38.26</td>
<td>45.58</td>
</tr>
<tr>
<td>30 µL LB85</td>
<td>8</td>
<td>22.59</td>
<td>39.20</td>
</tr>
<tr>
<td>10 µL LB85</td>
<td>8</td>
<td>4.14</td>
<td>8.81</td>
</tr>
<tr>
<td>5 µL LB85</td>
<td>8</td>
<td>2.97</td>
<td>4.75</td>
</tr>
<tr>
<td>2 µL LB85</td>
<td>8</td>
<td>0.80</td>
<td>1.42</td>
</tr>
<tr>
<td>1 µL LB85</td>
<td>8</td>
<td>0.15</td>
<td>1.04</td>
</tr>
<tr>
<td><strong>Solid Substrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 µL BSC</td>
<td>8</td>
<td>2.29</td>
<td>3.53</td>
</tr>
<tr>
<td>50 µL SALSw</td>
<td>8</td>
<td>2.04</td>
<td>2.62</td>
</tr>
<tr>
<td>1 µL SSC</td>
<td>8</td>
<td>0.95</td>
<td>2.79</td>
</tr>
<tr>
<td>5 µL BSCI</td>
<td>8</td>
<td>3.26</td>
<td>4.76</td>
</tr>
<tr>
<td>5 µL BSD</td>
<td>8</td>
<td>3.36</td>
<td>4.55</td>
</tr>
<tr>
<td><strong>Extraction Blank</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XB</td>
<td>8</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Reproducibility studies (SWGDAM Guideline 2.5)

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.

Experiment

Using the sample set shown in Table 8 on page 95, an extraction experiment was repeated on three separate days. In each experiment, DNA was extracted from eight replicates. A 96-well spin plate was used for lysis, and a 96-well PCR plate was used for elution. The DNA concentration and IPC C_{T} values were evaluated for reproducibility using the Quantifiler® Human DNA Quantification Kit.

Results

Figure 12 on page 99 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 11 on page 100.
Figure 12  Reproducibility studies: The average DNA concentration and average IPC $C_T$ for the three different experiments. The same data set is shown at two different scales: for the concentration ranges 0 to 50 ng/µL (top) and 0 to 10 ng/µL (bottom).
Table 11 Reproducibility studies: The averaged values for all three reproducibility experiments.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>n=</th>
<th>DNA Concentration (ng/µL)</th>
<th>± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td><strong>Liquid Samples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 µL LB85</td>
<td>24</td>
<td>31.37</td>
<td>49.74</td>
</tr>
<tr>
<td>30 µL LB85</td>
<td>24</td>
<td>18.04</td>
<td>36.35</td>
</tr>
<tr>
<td>10 µL LB85</td>
<td>24</td>
<td>5.73</td>
<td>10.59</td>
</tr>
<tr>
<td>5 µL LB85</td>
<td>24</td>
<td>3.30</td>
<td>4.83</td>
</tr>
<tr>
<td>2 µL LB85</td>
<td>24</td>
<td>1.08</td>
<td>2.18</td>
</tr>
<tr>
<td>1 µL LB85</td>
<td>24</td>
<td>0.31</td>
<td>1.59</td>
</tr>
<tr>
<td><strong>Solid Substrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 µL BSC</td>
<td>24</td>
<td>2.35</td>
<td>4.11</td>
</tr>
<tr>
<td>50 µL SALSw</td>
<td>24</td>
<td>1.27</td>
<td>2.90</td>
</tr>
<tr>
<td>1 µL SSC</td>
<td>24</td>
<td>1.17</td>
<td>3.21</td>
</tr>
<tr>
<td>5 µL BSCI</td>
<td>24</td>
<td>2.30</td>
<td>4.42</td>
</tr>
<tr>
<td>5 µL BSD</td>
<td>24</td>
<td>0.17</td>
<td>5.01</td>
</tr>
<tr>
<td><strong>Extraction Blank</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XB</td>
<td>24</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Correlation studies**

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

**Experiment**

The sample set shown in Table 8 on page 95 was extracted in triplicate using the manual extraction protocol (refer to Chapter 2 of the PrepFiler® Forensic DNA Extraction Kit User Guide). The extracted 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 Cₜ data for the manually-extracted samples were compared to data generated from the identical samples for the reproducibility studies described on page 98.

**Results**

Figure 13 on page 101 shows the data generated from the manually-extracted samples compared to the data generated from the same samples extracted using the automated protocol. The DNA concentration and the IPC Cₜ values resulting from both extraction methods are in accordance.
Figure 13  Correlation study: The graph shows the average DNA concentration (barchart) and IPC C<sub>T</sub> values (line graph) obtained for the three replicates of each manually-extracted sample compared to the data generated from the identical samples extracted using the automated protocol (reproducibility experiments 1 through 3).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sample Name</th>
<th>40</th>
<th>30</th>
<th>10</th>
<th>5</th>
<th>2</th>
<th>1</th>
<th>BSC</th>
<th>SALSw</th>
<th>SSC</th>
<th>BSCI</th>
<th>BSD</th>
<th>XB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproducibility experiment 1</td>
<td></td>
<td>43.02</td>
<td>27.42</td>
<td>8.80</td>
<td>4.29</td>
<td>1.52</td>
<td>0.80</td>
<td>3.30</td>
<td>2.63</td>
<td>2.23</td>
<td>3.51</td>
<td>4.47</td>
<td>0.00</td>
</tr>
<tr>
<td>Reproducibility experiment 2</td>
<td></td>
<td>35.1</td>
<td>24.99</td>
<td>8.62</td>
<td>3.94</td>
<td>1.50</td>
<td>0.60</td>
<td>3.20</td>
<td>1.79</td>
<td>1.64</td>
<td>2.89</td>
<td>2.51</td>
<td>0.00</td>
</tr>
<tr>
<td>Reproducibility experiment 3</td>
<td></td>
<td>34.00</td>
<td>22.44</td>
<td>7.29</td>
<td>3.68</td>
<td>1.46</td>
<td>0.56</td>
<td>2.89</td>
<td>1.75</td>
<td>1.70</td>
<td>2.57</td>
<td>2.72</td>
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</tr>
<tr>
<td>Manual extraction</td>
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<td>29.97</td>
<td>8.74</td>
<td>4.21</td>
<td>1.90</td>
<td>0.80</td>
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<td>1.75</td>
<td>2.20</td>
<td>2.81</td>
<td>3.33</td>
<td>0.00</td>
</tr>
<tr>
<td>IPC C&lt;sub&gt;T&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproducibility experiment 1</td>
<td></td>
<td>28.31</td>
<td>27.91</td>
<td>27.51</td>
<td>27.46</td>
<td>27.44</td>
<td>27.27</td>
<td>27.36</td>
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<td>27.54</td>
<td>27.66</td>
<td>27.89</td>
<td>27.30</td>
</tr>
<tr>
<td>Reproducibility experiment 2</td>
<td></td>
<td>27.89</td>
<td>27.70</td>
<td>27.28</td>
<td>27.29</td>
<td>27.32</td>
<td>27.36</td>
<td>27.24</td>
<td>27.41</td>
<td>27.30</td>
<td>27.17</td>
<td>27.98</td>
<td>27.58</td>
</tr>
<tr>
<td>Reproducibility experiment 3</td>
<td></td>
<td>28.15</td>
<td>27.91</td>
<td>27.54</td>
<td>27.52</td>
<td>27.57</td>
<td>27.58</td>
<td>27.47</td>
<td>27.68</td>
<td>27.55</td>
<td>27.87</td>
<td>27.13</td>
<td>27.58</td>
</tr>
<tr>
<td>Manual extraction</td>
<td></td>
<td>28.03</td>
<td>27.76</td>
<td>27.25</td>
<td>27.24</td>
<td>27.28</td>
<td>27.34</td>
<td>27.27</td>
<td>27.48</td>
<td>27.31</td>
<td>27.27</td>
<td>27.31</td>
<td>27.21</td>
</tr>
</tbody>
</table>
Contamination studies (SWGDAM Guideline 3.6)

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

Experiments

**Checkerboard plate:plate experiment** – For lysis, 10-µL samples of blood from six different donors were arranged in combination with extraction blanks in a 96-well spin plate. The samples were arranged in a checkerboard format, such that samples from the same donor were not in adjacent sample wells (see Figure 14a). Samples were eluted into a 96-well PCR plate. The DNA was quantified using the Quantifiler® Human DNA Quantification Kit. All extraction blanks were amplified with the AmpFSTR® 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 PCR 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 14c). The samples were eluted into a 96-well PCR plate.

![Figure 14 Contamination study setup: a. Checkerboard format with 6 donors on a plate; b. Checkerboard format using 8 donors in tubes; c. Liquid blood donors](image)

Results

**Checkerboard plate:plate experiment** – Of the 48 extraction blanks, six wells produced a CT value below 40. Of the wells with a CT value below 40, only one well yielded a detectable profile with the MiniFiler™ 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 below 40. No detectable MiniFiler™ kit profile was observed in any of the analyzed wells.

STR study

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

Experiment

The extracted DNA samples described in precision experiment A (eight replicates of 12 samples; see Table 8 on page 95) were amplified using the AmpFSTR® Identifiler® PCR Amplification Kit. 1 ng of human DNA, as determined by the Quantifiler® Human DNA Quantification Kit, was used as the template DNA.

Results

Full STR profiles were obtained from all extracted DNA samples (see Figure 15). No cross-contamination was observed.

Figure 15  STR study: Identifiler® kit STR profiles for the various sample types tested. On the left, liquid blood samples (from a single donor) show complete profiles (RFU=3000). On the right, solid substrate samples (from different donors) each show a different profile (RFU=3000). 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 16.

**Figure 16** STR study: The average interlocus balance for each sample type (eight replicates each) is shown. 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 17 on page 105. The liquid blood graph (Figure 17, on left) does not include homozygous loci for these samples.
Figure 17 STR study: The average peak height ratio is shown by locus for the eight replicates of each sample type. The left panel represents data obtained 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

Four software scripts containing the DNA extraction 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 contamination study. Verification studies were performed to test the other three scripts.
Experiment

**Plate:tubes experiment** – To test the performance of the 96-well spin plate as a source vessel and microcentrifuge 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 14a on page 102).

**Tubes:plate experiment** – The tubes:plate experiment performed for the contamination study also served as the tubes:plate experiment for the verification studies (see “Experiments” on page 102): To test the performance of microcentrifuge tubes as source vessels and the 96-well PCR 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 14c on page 102). Microcentrifuge tubes containing the lysate were placed in tube racks L1-L6 and the DNA eluate was collected in a 96-well PCR plate.

**Tubes:tubes experiment** – To test the performance of microcentrifuge 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 14c on page 102). Microcentrifuge tubes containing the lysate were placed in tube racks L1-L6 and the DNA eluate was collected in microcentrifuge tubes in tube racks S1-S6.

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

Results

Data from each experiment were reviewed for well-to-well contamination and overall consistency in DNA yield (see Figure 18 on page 107 below and Table 12 on page 107). For the:

- **Plate:tubes verification experiment** – Of the 48 extraction blanks tested, a $C_T$ value less than 40 was observed in 3 wells.

- **Tubes:plate verification experiment** – See the results for the contamination study tubes:plate experiment (“Results” on page 103).

- **Tubes:tubes verification experiment** – All of the 48 extraction blanks resulted in $C_T$ values greater than 40.
Figure 18  Verification studies: The average DNA concentration for each of the 6 or 8 replicates for each liquid blood donor is shown. Only six of the eight donors are shown for simplicity, with the remaining donors showing similar results.

Table 12  Verification studies: The average total DNA yield (ng) was calculated for each liquid blood donor for all four automated extraction methods and compared to the expected yield from 4000 or 11,000 nucleated blood cells per one microliter.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Yield (ng)</th>
<th>±Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB76</td>
<td>529.90</td>
<td>56.38</td>
</tr>
<tr>
<td>LB77</td>
<td>440.71</td>
<td>35.89</td>
</tr>
<tr>
<td>LB83</td>
<td>469.50</td>
<td>74.40</td>
</tr>
<tr>
<td>LB90</td>
<td>684.27</td>
<td>59.06</td>
</tr>
<tr>
<td>LB91</td>
<td>545.45</td>
<td>51.12</td>
</tr>
<tr>
<td>LB92</td>
<td>963.90</td>
<td>109.31</td>
</tr>
<tr>
<td>LB93</td>
<td>589.44</td>
<td>54.92</td>
</tr>
<tr>
<td>LB94</td>
<td>683.53</td>
<td>61.66</td>
</tr>
</tbody>
</table>

Expected Yield (ng)

- 4000 cells/µL 250 n/a
- 11,000 cells/µL 650 n/a

Additional contamination studies

Additional contamination studies were performed in order to monitor for cross-contamination during the operations of lysis using the filter plate and isolation of DNA on the Tecan HID EVOlution™ – Extraction System. The extracted samples (including extraction reagent blanks) were processed for quantitation of human DNA using the Quantifiler® Human DNA Quantification Kit and STR typing using the Identifiler® and MiniFiler™ kits.
Experiment

Two 96-well spin plates 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 102; see also Figure 14a on page 102). 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 96-well PCR plates. All samples were quantified using the Quantifiler® Human DNA Quantification Kit and amplified with the AmpFSTR® Identifiler® and MiniFiler™ PCR Amplification Kits following the standard kit protocols.

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 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 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 13.

Table 13 Summary of additional contamination studies. A total of 192 extractions were processed, of which 96 were extraction blanks and 96 were samples originating from six human donors.

<table>
<thead>
<tr>
<th></th>
<th>Plate 1</th>
<th>Plate 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples analyzed (including extraction blanks)</td>
<td>96</td>
<td>96</td>
<td>192</td>
</tr>
<tr>
<td>Number of extraction blanks</td>
<td>48</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>Number of extraction blanks with C_T &lt; 40</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Number of extraction blanks exhibiting peaks called as alleles in Identifiler® kit run†</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of extraction blanks exhibiting peaks called as alleles in MiniFiler™ kit run‡</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

† Using 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. The PrepFiler® Automated Forensic DNA Extraction Kit was validated following the SWGDAM guidelines and the utility of the extraction method in forensic DNA analysis was
demonstrated using forensic-type samples. The PrepFiler® kit is effective in maximizing the amount of DNA obtained from samples that contain both small and large quantities of biological material. The DNA that was extracted was free of PCR inhibitors as determined by the IPC C<sub>T</sub> values using the Quantifiler® Human DNA Quantification Kit. The reagents and operations of the PrepFiler® Automated Forensic DNA Extraction Kit exhibited clean operations and did not introduce any detectable cross contamination of human DNA. Validation studies confirmed that the PrepFiler® Automated Forensic DNA Extraction 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.
Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

Purpose of these validation studies

Developmental validation experiments were performed to evaluate:

- 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 extraction to minimize the potential for detergent carryover from PrepFiler® Wash Buffer A. Detergent carryover can inhibit downstream PCR applications.

Modified worktable layout

The worktable layout has been modified to add a trough for Wash Buffer B and to rearrange the remaining reagent and waste troughs to accommodate the new trough.

For information on setting up the worktable, refer to the appropriate application manual:

- Tecan HID EVOlution™ – Extraction 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 14 on page 111) replace existing automated extraction scripts for use with the PrepFiler® Automated Forensic DNA Extraction Kit. The new scripts incorporate the worktable 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 and Combination Systems are delivered with the new scripts. If you are an existing customer, please contact your local Tecan customer support organization to obtain the new scripts.

For details on script changes, refer to the service pack revision history file included in the Documents folder on the CD containing the new scripts.
Validation studies overview

The following validation studies were conducted:

- **Script validation** – Compares the performance of the new scripts to the previously released scripts.
- **Precision and sensitivity** – Tests the consistency of extracted DNA yield and quality.
- **Cross-contamination** – Determines if instrument liquid handling is the source of any cross-contamination among samples.
- **Case-type samples** – Tests performance with low-input, case-type samples.
- **Comparative chemistry** – Compares the performance (DNA quantity and quality) of the PrepFiler® Automated Forensic DNA Extraction Kit on the HID EVOlution™ systems against other commercially available extraction platforms.

Materials and methods

The following materials (details in Table 15 on page 112) and methods were used in all experiments performed as part of the validation of the new wash buffer and scripts:

- PrepFiler® Wash Buffer B was prepared by adding 95% ethanol to 200 mL low-TE buffer (from Teknova) to bring the final volume to 500 mL.
- Biological fluids and tissues used in the studies are listed in Table 16 on page 114. See each study for a description of the samples used.
- Lysis was performed as described in “Sample lysis” on page 114.
Appendix F  Experiments and Results  
Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

• Genomic DNA was extracted 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 and 46 µL. Extraction blanks were included in each study.

• Extracted DNA was set up for qPCR on the HID EVOlution™ – qPCR/STR 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.

• Extracted DNA from each sample was quantified using the Quantifiler® Human DNA Quantification Kit on an Applied Biosystems 7500 Real-Time PCR System. The quantitation results were analyzed using SDS software v1.2.3.

• Quantified DNA from each sample was normalized and set up for amplification using the Tecan HID EVOlution™ – Combination System. The AmpFiSTR® Identifiler® PCR Amplification Kit was used for all studies except contamination, which used the AmpFiSTR® MiniFiler™ PCR Amplification Kit.

• A target input of 1 ng of DNA was used for STR PCR amplification. For samples with concentrations less than 0.10 ng/µL, a maximum of 10 µL of extracted DNA was added to each STR reaction. Samples were amplified on a GeneAmp® 9700 thermal cycler. Electrophoresis was performed on an Applied Biosystems 3130xl Genetic Analyzer. The STR profiles were analyzed using GeneMapper® ID-X software v1.0.

Table 15  Materials used in validation studies

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HID EVOlution™ – Extraction System</td>
<td>• Tecan Freedom EVO® 150 robotic workstation&lt;br&gt;• The necessary hardware, including:&lt;br&gt;− 8-channel Liquid Handling accessory [LiHa]&lt;br&gt;− Robotic Manipulator arm [RoMa]&lt;br&gt;− Te-Shake with heating block and adaptor&lt;br&gt;− Applied Biosystems 96-Well Magnetic Ring Stand&lt;br&gt;• The Freedom EVOware® software v2.1 SP1 configured with the HID EVOlution™ – Extraction application v1.0 SP1&lt;br&gt;• Windows XP Professional operating system&lt;br&gt;• Eight new extraction scripts (see Table 14 on page 111)</td>
</tr>
</tbody>
</table>
Appendix F  Experiments and Results

Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
</table>
| HID EVOlution™ – Combination System | · Tecan Freedom EVO® 150 robotic workstation  
· The necessary hardware, including:  
  – 8-channel Liquid Handling accessory (LiHa; channels 1 through 4 used for qPCR/STR setup and channels 5 through 8 used for extraction)  
  – Robotic Manipulator arm (RoMa)  
  – Te-Shake with heating block and adaptor  
  – Applied Biosystems 96-Well Magnetic Ring Stand  
· The Freedom EVOware® software version 2.1 SP1 configured with:  
  – HID EVOlution™ – Extraction application v1.0 SP1  
  – Tecan HID EVOlution driver 1.0.0.25 SP4  
  – Tecan HID EVOlution qPCR/STR driver 1.0.0.26 SP5  
  – Tecan Sample Oriented EVOware 1.2 SP1  
· Windows XP Professional operating system  
· Eight new extraction scripts (see Table 14 on page 111) |
| Life Technologies instruments and software | · 3130xl Genetic Analyzer Data Collection Software v3.0  
· GeneMapper® ID-X Software version 1.0  
· GeneAmp® PCR System 9700 Thermal Cycler gold-plated silver 96-well block  
· 7500 Real-Time PCR System with SDS Software v1.2.3 |
| Other instruments | · VWR Benchtop Shaking Incubators (Model No. 1575, S/N 10032308, P/N 9120890)  
· Eppendorf 1.5-mL Tubes Thermomixer (Number 5350 28629; Hamburg, Germany) |
| Consumables | · PrepFiler® Wash Buffer B (prepared by Life Technologies; see “Materials and methods” on page 111)  
· MicroAmp® Optical 96-Well Reaction Plate (with or without barcode) Applied Biosystems (PN N8010560 or 4306737)  
· RNase-free Microfuge Tubes (1.5-mL), certified DNase and RNase-free Applied Biosystems (PN AM12400)  
· 200 µL and 1000 µL tips (obtained from Tecan Group Ltd.)  
· 100 mL reagent troughs (Tecan Group Ltd.)  
· TE buffer (Teknova, Hollister, CA)  
· Isopropyl alcohol (Sigma-Aldrich, St. Louis, MO)  
· Ethanol (95% molecular-biology grade; Sigma-Aldrich, St. Louis, MO)  
· Puritan Cotton Tipped Wooden Swabs (without glue) (VWR)  
· SERI Washed Cotton Cloth (Serological Research Institute, Richmond, CA) |
| Chemistry Kits | PrepFiler® Automated Forensic DNA Extraction Kit with Plastics (PN 4397977/Lot Number 0901004)  
Quantifiler® Human DNA Quantification Kit (PN 4343895/Lot Number 0906112)  
AmpFSTR® Identifier® PCR Amplification Kit (PN 4322288/Lot Number 0901114, 0905120, and 0907123)  
AmpFSTR® MiniFiler™ PCR Amplification Kit (PN 4373872/Lot Number 0909021)  
Cartridge-based, silica magnetic bead extraction kit from Company A  
Cartridge-based, silica magnetic bead extraction kit from Company B |
Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

### Sample lysis

In order to test the performance of the automated extraction system, steps were taken to minimize potential variation due to 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 this user guide:

1. Samples were placed in nuclease-free 1.5-mL polypropylene microcentrifuge tubes.
2. 300 µL of PrepFiler® Lysis Buffer and 3 µL DTT, 1.0 M was added to each sample.
3. Tubes were vortexed for 10 seconds, then centrifuged briefly.
4. Tubes were incubated in a thermal mixer at 900 RPM and 70°C for 40 minutes.
5. The substrate and residual lysis buffer were transferred from the tubes to the wells of PrepFiler® Spin/Filter Plate assembly and centrifuged at 2000 RPM for 1 minute to recover clarified lysates.
6. The Filter Plate was separated from the Spin Plate. The Spin Plate containing the lysed sample was used as the source plate during automated extraction.

### Script validation

Script validation was performed to compare the performance of the new scripts to the originally-released scripts. Core liquid handling for operations such as binding, washing, and elution are identical for all new scripts. Before performing the validation studies described in this chapter, the performance of the new worktable layout, new buffer, and the new PrepFiler_plate_plateCombo script was tested 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 25 on page 126

The performance of the new scripts was equivalent to the originally released scripts with respect to DNA recovery. No indication of Wash Buffer A carryover was observed in the STR profiles. Figure 19 and Figure 20 on page 115 show results from the blood dried on cotton swabs dilution series and from the low-input case-type samples set, respectively.

### Table 16  Biological samples used in validation studies

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Donor reference number</th>
<th>Gender</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human peripheral blood</td>
<td>233</td>
<td>Female</td>
<td>Serological Research Institute, Richmond, CA</td>
</tr>
<tr>
<td>Human peripheral blood</td>
<td>238</td>
<td>Female</td>
<td>Serological Research Institute, Richmond, CA</td>
</tr>
<tr>
<td>Human peripheral blood</td>
<td>240</td>
<td>Female</td>
<td>Serological Research Institute, Richmond, CA</td>
</tr>
<tr>
<td>Human peripheral blood</td>
<td>236</td>
<td>Male</td>
<td>Serological Research Institute, Richmond, CA</td>
</tr>
<tr>
<td>Saliva</td>
<td>Anonymous</td>
<td>Male</td>
<td>In house</td>
</tr>
<tr>
<td>Semen</td>
<td>Anonymous</td>
<td></td>
<td>Serological Research Institute, Richmond, CA</td>
</tr>
</tbody>
</table>

Sample type Donor reference number Gender Source

- Human peripheral blood 233 Female Serological Research Institute, Richmond, CA
- Human peripheral blood 238 Female Serological Research Institute, Richmond, CA
- Human peripheral blood 240 Female Serological Research Institute, Richmond, CA
- Human peripheral blood 236 Male Serological Research Institute, Richmond, CA
- Saliva Anonymous Male In house
- Semen Anonymous Male Serological Research Institute, Richmond, CA
Figure 19  Comparison of released and new scripts for DNA yield from diluted blood on swabs

Figure 20  DNA yield from released (green) and new (blue) scripts on low-input samples
**Experiments**

To test the consistency of extracted DNA yield and quality, seven replicates of a blood sample dilution series were extracted. The average and standard deviation DNA yield and the IPC $C_T$ within each replicate set were examined.

<table>
<thead>
<tr>
<th>Experimental setup</th>
<th>HID EVOlution™ – Combination System</th>
<th>HID EVOlution™ – Extraction System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample set</td>
<td>Dilution series using blood from Donor 238</td>
<td>Dilution series using blood from Donor 233</td>
</tr>
<tr>
<td></td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Lysis method</td>
<td>See “Sample lysis” on page 114.</td>
<td></td>
</tr>
<tr>
<td>Extraction setup (layout and replicates)</td>
<td>For each extraction run, seven replicates of each dilution were set up in a plate as shown in Table 18. Each plate contained one substrate blank per dilution concentration (5 extraction blanks per plate).</td>
<td></td>
</tr>
<tr>
<td>Extraction script(s)</td>
<td>PrepFiler_plate_plateCombo_V1_SP1</td>
<td>PrepFiler_plate_plate_V1_SP2</td>
</tr>
<tr>
<td>Number of extraction runs</td>
<td>The experiment (extraction through STR PCR) was performed three times (once per day on 3 different days).</td>
<td></td>
</tr>
<tr>
<td>qPCR method</td>
<td>qPCR setup was performed on the HID EVOlution™ – Combination System using the Quantifiler® Human DNA Quantification kit.</td>
<td></td>
</tr>
<tr>
<td>STR PCR method</td>
<td>DNA normalization and STR PCR setup was performed on the HID EVOlution™ – Combination System using the AmpFSTR® Identifiler® kit. A target input of 1 ng of DNA was used for STR PCR amplification. For samples with concentrations less than 0.10 ng/µL, a maximum of 10 µL of extracted DNA was added to each STR reaction.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 18 DNA lysate plate layout for precision and sensitivity studies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dilution 1:5</strong></td>
</tr>
<tr>
<td>A Replicate 1</td>
</tr>
<tr>
<td>B Replicate 2</td>
</tr>
<tr>
<td>C Replicate 3</td>
</tr>
<tr>
<td>D Replicate 4</td>
</tr>
<tr>
<td>E Replicate 5</td>
</tr>
<tr>
<td>F Replicate 6</td>
</tr>
<tr>
<td>G Replicate 7</td>
</tr>
<tr>
<td>H Extraction Blank 1</td>
</tr>
</tbody>
</table>

**Results**

The new scripts returned DNA in similar yield and with more efficient STR PCR amplification than the original released versions of the automated 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 19  HID EVOlution™ – Combination System: Precision and sensitivity studies; average and standard deviation DNA concentration for the seven replicates for each dilution concentration

<table>
<thead>
<tr>
<th>Sample dilution</th>
<th>Run 1: Average concentration (ng/µL)</th>
<th>Concentration standard deviation</th>
<th>Run 2: Average concentration (ng/µL)</th>
<th>Concentration standard deviation</th>
<th>Run 3: Average concentration (ng/µL)</th>
<th>Concentration standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1:5)</td>
<td>1.331</td>
<td>0.084</td>
<td>1.171</td>
<td>0.302</td>
<td>1.060</td>
<td>0.032</td>
</tr>
<tr>
<td>(1:10)</td>
<td>0.700</td>
<td>0.067</td>
<td>0.733</td>
<td>0.043</td>
<td>0.563</td>
<td>0.068</td>
</tr>
<tr>
<td>(1:50)</td>
<td>0.135</td>
<td>0.011</td>
<td>0.131</td>
<td>0.009</td>
<td>0.107</td>
<td>0.017</td>
</tr>
<tr>
<td>(1:100)</td>
<td>0.057</td>
<td>0.009</td>
<td>0.068</td>
<td>0.007</td>
<td>0.046</td>
<td>0.004</td>
</tr>
<tr>
<td>(1:250)</td>
<td>0.027</td>
<td>0.006</td>
<td>0.022</td>
<td>0.007</td>
<td>0.012</td>
<td>0.002</td>
</tr>
<tr>
<td>Average IPC C_7</td>
<td>27.478 ± 0.172</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 21  HID EVOlution™ – Combination System: Average DNA concentration (ng/µL) for the seven replicates for each dilution concentration
Figure 22  HID EVOlution™ – Combination System: Average peak heights from AmpF/STR® Identifiler® kit STR profiles

Table 20  HID EVOlution™ – Extraction System: Precision and sensitivity studies; average and standard deviation DNA concentration for the seven replicates for each dilution concentration

<table>
<thead>
<tr>
<th>Sample dilution</th>
<th>Run 1 Average concentration (ng/µL)</th>
<th>Concentration standard deviation</th>
<th>Run 2 Average concentration (ng/µL)</th>
<th>Concentration standard deviation</th>
<th>Run 3 Average concentration (ng/µL)</th>
<th>Concentration standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1:5]</td>
<td>1.359</td>
<td>0.156</td>
<td>1.270</td>
<td>0.208</td>
<td>1.202</td>
<td>0.155</td>
</tr>
<tr>
<td>[1:10]</td>
<td>0.547</td>
<td>0.043</td>
<td>0.585</td>
<td>0.115</td>
<td>0.570</td>
<td>0.036</td>
</tr>
<tr>
<td>[1:50]</td>
<td>0.108</td>
<td>0.007</td>
<td>0.109</td>
<td>0.008</td>
<td>0.105</td>
<td>0.011</td>
</tr>
<tr>
<td>[1:100]</td>
<td>0.045</td>
<td>0.008</td>
<td>0.045</td>
<td>0.006</td>
<td>0.052</td>
<td>0.007</td>
</tr>
<tr>
<td>[1:250]</td>
<td>0.020</td>
<td>0.002</td>
<td>0.016</td>
<td>0.007</td>
<td>0.025</td>
<td>0.009</td>
</tr>
<tr>
<td>Average IPC C_T</td>
<td>26.46 ± 0.211</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix F  Experiments and Results

Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

Figure 23  HID EVOlution™ – Extraction System: Average DNA concentration (ng/µL)

Figure 24  HID EVOlution™ – Extraction System: Average peak heights from AmpFSTR® Identifiler® kit STR profile
## Cross-contamination studies

### Experiments

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

### Table 21 Experimental setup for cross-contamination studies

<table>
<thead>
<tr>
<th>Experimental setup</th>
<th>HID EVOLution™ – Combination System</th>
<th>HID EVOLution™ – Extraction System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample set</td>
<td>Blood from Donor 238</td>
<td>Blood from Donor 233</td>
</tr>
</tbody>
</table>
| Lysis method and extraction setup (layout and replicates) | For each extraction run, 40 sample replicates and 40 extraction blanks were arranged as shown in Figures 25 and 26 on page 122. In order 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, plasticware, 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 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 plate map in Figure 25 on page 121], 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 plate unit was centrifuged at 2000 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 tube positions in Figure 26 on page 122]. 323 µL of blood lysate was distributed to each sample tube. Next, 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 work table. |
| Extraction script(s) | Validation:  
  - PrepFiler_plate_plateCombo_V1_SP1  
  - PrepFiler_tube_tubeCombo_V1_SP1  
  Verification†:  
  - PrepFiler_tube_tubeCombo_V1_SP1†  
  - PrepFiler_plate_tubeCombo_V1_SP1  
  - PrepFiler_tube_plateCombo_V1_SP1 | Validation:  
  - PrepFiler_plate_plate_V1_SP2  
  - PrepFiler_tube_tube_V1_SP2  
  Verification†:  
  - PrepFiler_tube_tube_V1_SP2†  
  - PrepFiler_plate_tube_V1_SP2  
  - PrepFiler_tube_plate_V1_SP2 |
| Number of extraction runs | For each of the two extraction scripts, the experiment [extraction through STR PCR] was performed two times [once per day on 2 different days]. |
Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

**Experimental setup**

<table>
<thead>
<tr>
<th>STR PCR method§</th>
<th>HID EVolution™ – Combination System</th>
<th>HID EVolution™ – Extraction System</th>
</tr>
</thead>
<tbody>
<tr>
<td>All extraction blanks were processed through STR PCR. STR PCR setup was performed on the HID EVolution™ – Combination System using the AmpFiSTR® MiniFiler™ kit.</td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>

† 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.

‡ For continuity, this script was run in both validation and verification studies.

§ As a precaution, STR PCR with the AmpFiSTR® MiniFiler™ 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 format) were created to allow 10 µL of each extraction blank eluate to be directly dispensed in 15 µL of AmpFiSTR® MiniFiler™ kit reaction mix.

**Figure 25** 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)
Figure 26  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)

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

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.

Experiments

To test performance when running low-input, case-type samples, 10 different sample types were extracted 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 22 Experimental setup for case-type sample studies

<table>
<thead>
<tr>
<th>Experimental setup</th>
<th>HID EVOLution™ – Combination System</th>
<th>HID EVOLution™ – Extraction System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample set</td>
<td>Ten low-input, case-type samples. See Table 23 on page 124.</td>
<td></td>
</tr>
</tbody>
</table>
| Lysis method           | Lysis in tubes: Sample lysis was performed in tubes, transferred to a Spin/Filter Plate assembly for substrate removal (see "Sample lysis" on page 114), 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 assembly and the assembly placed on ice for 30 minutes to chill the chambers of the lower spin plate.  
2. Lysis was initiated by adding a mixture of 300 µL of PrepFiler® lysis buffer and 3 µL 1M DTT per well.  
3. The Spin/Filer Plate assembly was sealed with adhesive film and placed into a 60°C oven and shaken for 40 minutes at 300 RPM.  
4. Lysates were centrifuged at 2000 RPM for 1 minute to recover clarified lysates. |                                    |
| Extraction setup       | To eliminate potential plate-to-plate variation that could occur during extraction and quantitation, all lysates (from lysis in tubes and lysis in plates) were consolidated in one plate for extraction and quantitation. |                                    |
| Extraction script(s)   | PrepFiler_plate_plateCombo_V1_SP1     | N/A                               |
| Number of extraction runs | 1                                   | N/A                               |
| 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 AmpFSTR® Identifiler® kit.  
A target input of 1 ng of DNA was used for STR PCR amplification. For samples with concentrations less than 0.10 ng/µL, a maximum of 10 µL of extracted DNA was added to each STR reaction. |                                    |
Appendix F  Experiments and Results
Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

Table 23  Low-input, case-type sample set

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

† 4 replicates each from lysis in tubes and lysis in plates.

Results

DNA yield and average IPC $C_T$ for samples lysed in plates and tubes are shown in Figure 27 and Figure 28 on page 125. With some sample types and inputs, the tube lysis protocol may be the preferred method compared to the plate lysis protocol. The Identifiler® 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. Additional studies are being conducted to further optimize the protocols for plate lysis, and for dark blue and black denim and other challenging sample types.
Figure 27  Total DNA yield for sample lysis in plates and in tubes - 2.5 µL blood (with and without inhibitor mix) on cotton

Figure 28  Total DNA yield for 8 case-type samples comparing lysis in plates and in tubes
Comparative analysis studies

Experiments

The ultimate goal of any forensic DNA extraction is to maximize DNA yield and quality to facilitate improved downstream performance of quantitative real-time PCR and PCR for short tandem repeat analysis. To compare the quantity and quality of DNA extracted on the HID EVOlution™ – Extraction and Combination System platforms to other platforms, the DNA yield and STR profiles from the “Case-type sample studies” on page 122 were compared to the data generated on other automated extraction platforms:

Table 24 Experimental setup comparative analysis studies

<table>
<thead>
<tr>
<th>Experimental setup</th>
<th>Cartridge-based, silica magnetic bead extraction kit and platform from Company A</th>
<th>Cartridge-based, silica magnetic bead extraction kit and platform from Company B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample set</td>
<td>Ten low-input, case-type samples. See Table 25 on page 126.</td>
<td></td>
</tr>
<tr>
<td>Sample preparation</td>
<td>Company A kit using the trace protocol with carrier RNA</td>
<td>Company B kit</td>
</tr>
<tr>
<td>Lysis and extraction method</td>
<td>Samples listed in Table 25 on page 126 were lysed in 1.5-mL tubes according to the manufacturer’s recommended protocol for low-input samples.</td>
<td>Samples listed in Table 25 on page 126 were lysed in 1.5-mL tubes according to the manufacturer’s recommended protocol for small casework samples.</td>
</tr>
<tr>
<td>Number of extraction runs</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>qPCR method</td>
<td>qPCR setup was performed on the HID EVOlution™ – Combination System using the Quantifiler® Human DNA Quantification kit.</td>
<td></td>
</tr>
<tr>
<td>STR PCR method</td>
<td>DNA normalization and STR PCR setup was performed on the HID EVOlution™ – Combination System using the AmpF®STR® Identifiler® kit. A target input of 1 ng of DNA was used for STR PCR amplification. For samples with concentrations less than 0.10 ng/µL, a maximum of 10 µL of extracted DNA was added to each STR reaction.</td>
<td></td>
</tr>
</tbody>
</table>

Table 25 Comparative analysis studies sample set and results for average DNA concentration (ng/µL)

<table>
<thead>
<tr>
<th>Plate column</th>
<th>Sample source/substrate</th>
<th>Body fluid volume (µL)</th>
<th>Average DNA concentration (ng/µL); (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PrepFiler® Automated Forensic DNA Extraction Kit†</td>
</tr>
<tr>
<td>1</td>
<td>Blood dried on white cotton cloth (SERI, 4 mm punch)</td>
<td>0.2</td>
<td>0.33</td>
</tr>
<tr>
<td>2</td>
<td>Blood dried on unwashed light blue denim (4 mm)</td>
<td>0.2</td>
<td>0.27</td>
</tr>
<tr>
<td>3</td>
<td>Blood dried on FTA paper (4 mm punch)</td>
<td>0.5</td>
<td>0.68</td>
</tr>
<tr>
<td>4</td>
<td>Saliva dried on cotton swab (Puritan wooden)</td>
<td>5.0</td>
<td>0.52</td>
</tr>
<tr>
<td>5</td>
<td>Blood dried on unwashed dark blue denim (4 mm)</td>
<td>0.2</td>
<td>0.11</td>
</tr>
<tr>
<td>6</td>
<td>Semen dried on white cotton cloth (SERI, 4 mm punch)</td>
<td>1</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Appendix F  Experiments and Results

Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

Results

The quality of the DNA extracted 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 25.

Figure 29 and Figure 30 on page 128 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 25 on page 126, last row). The overall yield is generally higher for the HID EVOlution™ system with lysis performed in 1.5-mL tubes.

<table>
<thead>
<tr>
<th>Plate column</th>
<th>Sample source/substrate</th>
<th>Body fluid volume (µL)</th>
<th>PrepFiler® Automated Forensic DNA Extraction Kit†</th>
<th>Company A</th>
<th>Company B</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Dried blood in 1.5-mL tube</td>
<td>0.2</td>
<td>0.25</td>
<td>0.21</td>
<td>0.01</td>
</tr>
<tr>
<td>8</td>
<td>Blood dried on unwashed black denim (4 mm punch)</td>
<td>0.2</td>
<td>0.02</td>
<td>0.18</td>
<td>0.09</td>
</tr>
<tr>
<td>9</td>
<td>Blood spiked with PBS dried on pre-washed white cotton cloth (SERI; 4 µL blood plus 1 µL PBS, 3 µL on 4 mm punch)</td>
<td>2.5</td>
<td>3.5</td>
<td>1.81</td>
<td>1.38</td>
</tr>
<tr>
<td>10</td>
<td>Blood spiked with inhibitor mix dried on pre-washed white cotton cloth (SERI; 4 µL blood plus 1 µL PBS, 3 µL on 4 mm punch)</td>
<td>2.5</td>
<td>4.6</td>
<td>3.53</td>
<td>1.29</td>
</tr>
</tbody>
</table>

† Lysis in 1.5 mL tubes incubated at 70°C for 40 minutes in a thermal shaker.
‡ Overnight lysis at 56°C.

Average elution volume (µL)

<table>
<thead>
<tr>
<th></th>
<th>PrepFiler® Automated Forensic DNA Extraction Kit†</th>
<th>Company A</th>
<th>Company B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average elution volume</td>
<td>40 µL</td>
<td>45 µL</td>
<td>41 µL</td>
</tr>
</tbody>
</table>
Appendix F  Experiments and Results
Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

Figure 29  Comparative overall yield (ng)

![Comparative overall yield (ng)](image)

Figure 30  Comparative yield for 2.5 µL blood and blood spiked with inhibitors

![Comparative yield for 2.5 µL blood and blood spiked with inhibitors](image)
Figure 31 shows intracolor balance results for six samples. Samples 5 and 8 were omitted due to low DNA input and stochastic effects. Figure 32 on page 130 shows the intracolor balance for samples 9 and 10.
Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction

Figure 32  Intracolor balance obtained with the Identifiler® kit with DNA recovered from 2.5 µL 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 above 40% for all dye labels.

To assess the overall performance of the DNA purified using the three extraction chemistries tested, the average peak heights for all 10 sample types listed in Table 25 on page 126 were calculated and plotted in a box-plot format in Figure 33 on page 131.
Conclusions

The PrepFiler® Wash Buffer B and the scripts listed in Table 14 on page 111 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.

- Sensitivity and precision studies confirmed that the new scripts perform as well as the previously released scripts.
- Cross-contamination studies of 240 extraction blanks co-extracted with 20 µL 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 containing known PCR inhibitors.
- Comparative chemistries studies demonstrated that extraction 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 other comparable extraction chemistries. Some denim samples tested showed signs of PCR inhibition. Further studies are being conducted to improve extraction performance for these challenging sample types.
- With some sample types and inputs, the tube lysis protocol may be the preferred method compared to the plate lysis protocol. Additional studies are being conducted to further optimize the protocols for plate lysis.
Appendix F  Experiments and Results

Validation of PrepFiler® Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction
**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, etc). To obtain SDSs, see the “Documentation and Support” section in this document.

**Safety information for instruments not manufactured by Life Technologies**

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

The HID EVOlution™ – Extraction System and HID EVOlution™ – Combination System are not designed or built by Life Technologies. 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, and 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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's 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 necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

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

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

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

**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:
- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: [www.cdc.gov/biosafety](http://www.cdc.gov/biosafety)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: [www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)
- Your company’s/institution’s Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: [www.cdc.gov](http://www.cdc.gov)

In the EU:
Documentation and Support

Related documentation

Portable document format (PDF) versions of this guide and the following related guide are available at www.appliedbiosystems.com:

<table>
<thead>
<tr>
<th>Document</th>
<th>Part number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits User Guide</td>
<td>4463348</td>
<td>Provides an overview of manual procedures for extraction of genomic DNA, and results of the developmental validation experiments performed by Life Technologies during the development of the PrepFiler® Automated Forensic DNA Extraction Kit.</td>
</tr>
<tr>
<td>HID EVOlution™ – qPCR/STR Setup System Getting Started Guide</td>
<td>4426903</td>
<td>Describes automated quantitation setup and amplification setup on the Freedom EVO® instrument with the HID EVOlution™ – qPCR/STR Setup System</td>
</tr>
</tbody>
</table>

To open the user documentation available at www.appliedbiosystems.com, use the Adobe® Acrobat® Reader® software available from www.adobe.com

Note: For additional documentation, see “Obtaining support” on page 139.

The following related documents are available from Tecan:

<table>
<thead>
<tr>
<th>Document</th>
<th>Document ID</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tecan HID EVolution® – Extraction Application Manual</td>
<td>395372</td>
<td>Extraction-specific operating procedures, safety, maintenance, and troubleshooting information for the Freedom EVO® instrument</td>
</tr>
<tr>
<td>Tecan HID EVolution® – Combination Application Manual</td>
<td>395967</td>
<td>Extraction-specific operating procedures, safety, maintenance, and troubleshooting information for the Freedom EVO® instrument</td>
</tr>
<tr>
<td>Tecan HID EVolution® – Extraction Installation Manual</td>
<td>395392</td>
<td>Includes PrepFiler® kit-specific information for installing and setting up the instrument. Installing and setting up the Freedom EVO® instrument to perform DNA extraction</td>
</tr>
<tr>
<td>Tecan Software Manual Instrument Software V6.1 Part 1</td>
<td>392888</td>
<td>Installing and setting up the Freedom EVO® instrument to perform DNA extraction</td>
</tr>
<tr>
<td>Tecan Freedom EVO® Operating Manual</td>
<td>392886</td>
<td>Comprehensive safety information and operating, maintenance, and troubleshooting procedures for the Freedom EVO® instrument</td>
</tr>
</tbody>
</table>
Documentation and Support

Obtaining information from the Help system

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

- Press F1.
- Select Start ➤ All Programs ➤ Tecan ➤ EVOware ➤ Help.

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

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

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.appliedbiosystems.com/sds

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

<table>
<thead>
<tr>
<th>Document</th>
<th>Document ID</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Manual Limited Device Support (General Purpose)</td>
<td>393804</td>
<td>Includes comprehensive information on installing, setting up, running, and programming the EVOware® software.</td>
</tr>
<tr>
<td>Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Manual Extended Device Support (Research Use Only)</td>
<td>393172</td>
<td></td>
</tr>
<tr>
<td>Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Getting Started Guide</td>
<td>393318</td>
<td>Includes procedures for installing and running the EVOware® software</td>
</tr>
<tr>
<td>Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Runtime Controller Manual</td>
<td>394329</td>
<td>Includes procedures for running scripts and troubleshooting script-related error messages.</td>
</tr>
</tbody>
</table>

Portable document format (PDF) versions of the Freedom EVOware manuals listed above are also available on the Freedom EVOware CD. PDF versions of the HID EVOlution – Extraction manuals are also available on the HID EVOlution – Extraction CD.

**Note:** To open the user documentation included on the CD, use the Adobe® Acrobat® Reader® software available from www.adobe.com
Obtaining support

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

For the latest services and support information for all locations, go to:
www.appliedbiosystems.com

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