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PrepFiler™ Automated Forensic DNA Extraction Kit

Automated Extraction on the HID EVOlution™ - Extraction System

User Guide

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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, with references to detailed procedures (Chapter 3 on page 33 and Chapter 4 on page 53)
- Troubleshooting information (Appendix A on page 69)
- A step-by-step description of the automated DNA binding, washing, and elution steps (Appendix E on page 89)

What's new in this guide

This guide has been updated to include instructions for using a new wash buffer, PrepFiler™ Wash Buffer B, with the PrepFiler™ Automated Forensic DNA Extraction Kit and the related modifications to the HID EVOlution™ – Extraction 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 the first wash buffer. Detergent carryover can inhibit downstream PCR applications. For instructions on preparing Wash Buffer B, see "Prepare reagents for lysis, binding, washing, and elution" on page 21. PrepFiler™ Wash Buffer B will be available in the PrepFiler™ Automated Forensic DNA Extraction Kits in 2011.

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.

New software scripts

New HID EVOlution™ 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:

- PrepFiler_plate_plate_V1_SP2
- PrepFiler_plate_tubes_V1_SP2
- PrepFiler_tubes_plate_V1_SP2
- PrepFiler_tubes_tubes_V1_SP2
- PrepFiler_plate_plate500_V1_SP2
- PrepFiler_plate_tubes500_V1_SP2
- PrepFiler_tubes_plate500_V1_SP2
- PrepFiler_tubes_tubes500_V1_SP2

All new HID EVOlution™ – Extraction 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.

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

New procedure for documenting Te-Shake plate adapter temperatures

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 on page 85 to verify the empirical temperatures between the PrepFiler™ Processing Plate and the Te-Shake plate adapter.

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 (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.
- You have access to the *Tecan HID EVOlution™ – Extraction Application Manual*, the *Tecan Freedom EVO® Operating Manual*, and other applicable Tecan documentation, and to the PrepFiler Forensic DNA Extraction Kit 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.

Safety information

Note: For general safety information, see this section and Appendix F, "Safety" on page 93. When a hazard symbol and hazard type appear by an instrument hazard, see the "Safety" Appendix for the complete alert on the instrument.

Safety alert words

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

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

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. This signal word is to be limited to the most extreme situations.

Instrument safety

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

SDSs

The SDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 95.

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

For more information

Refer to the Applied Biosystems and Tecan documents in the following table for more information. References to the appropriate Tecan documentation are also provided throughout this guide. See "Documentation and Support" on page 99 for a list of related Applied Biosystems and Tecan documents.

For information on...	Refer to the...
Developmental validation experiments that are performed by Applied Biosystems	*PrepFiler™ Forensic DNA Extraction Kit User Guide (PN 4390932)* *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 (PN 4457144)*
Quantitation and amplification setup on the Freedom EVO instrument with the HID EVOlution™ – qPCR/PCR Setup System	*HID EVOlution™ – qPCR/STR Setup System Getting Started Guide (PN 4426903)* *Tecan HID EVOlution™ Application Guide: Automation for Applied Biosystems Human Identification Kits*
Using the PrepFiler kits with other robot systems	See Appendix E on page 89
Extraction-specific operating procedures, maintenance, and troubleshooting information for the Freedom EVO instrument	*Tecan HID EVOlution™ – Extraction Application Manual*
Installing and setting up the Freedom EVO instrument to perform DNA extraction	*Tecan HID EVOlution™ – Extraction Installation Manual* *Tecan Software Manual Instrument Software V6.1 Part 1*
Comprehensive safety information and operating, maintenance, and troubleshooting procedures for the Freedom EVO instrument	*Tecan Freedom EVO® Operating Manual*
Installing, setting up, running, and programming the Freedom EVOware® software	*Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Manual* *Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Getting Started Guide*
Running scripts and troubleshooting script-related error messages	*Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Runtime Controller Manual* *Tecan HID EVOlution™ – Extraction Application Manual*

1 The PrepFiler™ Automated Forensic DNA Extraction Kit

This chapter covers:

- Kit description 12
- Supported extraction system configurations 12
- Kit contents and storage conditions 13
- Required materials and instruments 15
- Workflow for manual lysis and automated extraction 17

Kit description

The PrepFiler™ Automated Forensic DNA Extraction Kit contains Applied Biosystems reagents optimized for use in:

- Performing cell lysis
- Binding genomic DNA to magnetic particles
- Removing PCR inhibitors
- Eluting concentrated purified DNA

The kit can be used with one standard protocol to extract and isolate DNA from most forensic sample types, including body fluids, and 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 for use in STR amplification using the AmpFℓSTR® PCR Amplification kits.

Automated DNA extraction procedures for use with the PrepFiler™ kits were validated on the Tecan Freedom EVO® 150 robotic workstation, which automates liquid and magnetic particle handling. After sample lysis, the remaining steps in the extraction procedure are performed on the Freedom EVO instrument, as shown in Figure 2 on page 18. The extracted DNA is collected in either a plate or tubes, depending on the Freedom EVOware® software script that you select. The Freedom EVO instrument can run 80 extractions in about 2 hours.

Supported extraction system configurations

The PrepFiler Automated kits are supported for use with the HID EVOlution™ – Extraction System, which consists of:

- A TECAN Freedom EVO® 150 or 200 robotic workstation
- The Freedom EVOware® software version 2.1 SP1 configured with the HID EVOlution™ – Extraction application
- 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 – Extraction System supports the following four configurations (select one configuration per extraction run):

- Performing cell lysis in plates and collecting eluate in plates
- Performing cell lysis in plates and collecting eluate in tubes
- Performing cell lysis in tubes and collecting eluate in tubes
- Performing cell lysis in tubes and collecting eluate in plates

Kit contents and storage conditions

Kit contents

Each PrepFiler™ Automated Forensic DNA Extraction Kit contains materials sufficient for up to 960 extractions using the Standard 300-µL protocol. The PrepFiler™ Automated Forensic DNA Extraction Kit with Plastics also includes 96-well plates sufficient for 10 extraction runs. The contents of the kit are described in Table 1.

Table 1 Materials in PrepFiler™ Automated Forensic DNA Extraction Kits (PNs 4397977 and 4393135)

Reagent†	Description	Kit with plastics (PN 4397977)	Kit without plastics (PN 4393135)
PrepFiler Lysis Buffer	One bottle, 500 mL	✔	✔
PrepFiler Magnetic Particles	13 tubes, 1.5 mL each	✔	✔
PrepFiler Wash Buffer Concentrate	One bottle, 500 mL	✔	✔
PrepFiler Elution Buffer	One bottle, 125 mL	✔	✔
PrepFiler 96-Well Spin Plate and Filter Plate	10 sets	✔	no
PrepFiler 96-Well Processing Plate	10 plates	✔	no

† PrepFiler™ Wash Buffer B will be available in the PrepFiler™ Automated Forensic DNA Extraction Kits in 2011. For instructions on preparing Wash Buffer B, see "Prepare reagents for lysis, binding, washing, and elution" on page 21.

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 15 for appropriate tubes.

Storage conditions

The kits are shipped at room temperature.

IMPORTANT! When you receive a kit, immediately store the PrepFiler Magnetic Particles tubes at 4 to 8 °C. *Do not freeze* the PrepFiler Magnetic Particles tubes.

Note: The PrepFiler Magnetic Particles may be shipped in a separate container.

Table 2 PrepFiler™ Automated Forensic DNA Extraction Kit storage conditions

Reagent	Storage conditions
Magnetic Particles	• 4 to 8 °C when you receive the kit • After first use, store at room temperature (18 to 25 °C) for up to 3 months or at 4 °C for longer periods up to the expiration date
Lysis Buffer Wash Buffer Concentrate Prepared Wash Buffer Wash Buffer B Elution Buffer	Room temperature (18 to 25 °C)

Required materials and instruments

Table 3 lists materials and instruments that are required in addition to the reagents and materials supplied with the PrepFiler™ Automated Forensic DNA Extraction Kit.

Table 3 Required materials and instruments

Workflow step	Material or instrument	Source‡
All	Common laboratory equipment such as pipettors, aerosol-resistant micropipette tips, and a microcentrifuge	Major laboratory supplier
	Vortexer (a variable speed vortexer is recommended)	Major laboratory supplier
Lysis (if performing lysis in 96-well plates)	DL-dithiothreitol [Molecular biology grade; ≥98% (TLC), ≥99% (titration)]	Sigma-Aldrich www.sigmaaldrich.com (Part Number D9779)
	General purpose shaking incubator capable of temperatures up to 70 °C	VWR Signature™ Benchtop Shaking Incubator or equivalent www.vwr.com (Model 1575)
	Benchtop centrifuge with rotor for 96-deep-well plates	Eppendorf Centrifuge 5804 with A-2-DWP swing bucket rotor, or equivalent www.eppendorf.com
	MicroAmp® Multi-Removal Tool	Applied Biosystems (PN 4313950)
	MicroAmp® Clear Adhesive Film	Applied Biosystems (PN 4306311)
	MicroAmp® Adhesive Film Applicator	Applied Biosystems (PN 4333183)
	Deep well plate (for balancing the centrifuge rotor during centrifugation)	VWR Part Number 82006-448 or equivalent www.vwr.com
Lysis (if performing lysis in tubes)	DL-dithiothreitol [Molecular biology grade; ≥98% (TLC), ≥99% (titration)]	Sigma-Aldrich www.sigmaaldrich.com (Part Number D9779)
	General purpose shaking incubator capable of temperatures up to 70 °C	VWR Signature™ Benchtop Shaking Incubator or equivalent www.vwr.com (Model 1575)
	RNase-free Microfuge Tubes (1.5-mL), certified DNase- and RNase-free	Applied Biosystems (PN AM12400) or equivalent **Note:** If you use an equivalent tube, select tubes that allow you to observe the tube contents.
	PrepFiler Spin Tubes and Columns	Applied Biosystems (PN 4392342)
	Laboratory microcentrifuge capable of 16,110 × g	Major laboratory supplier

Workflow step	Material or instrument	Source‡
Automated extraction	DNA Suspension Buffer (low-TE buffer) **Note:** DNA suspension buffer is required to prepare PrepFiler™ Wash Buffer B. Prepared PrepFiler™ Wash Buffer B will be available in the PrepFiler™ Automated Forensic DNA Extraction Kits in 2011.	Teknova (Cat. No. T0223) www.teknova.com
	Isopropanol (2-Propanol, ACS reagent grade, ≥99.5%)	Sigma-Aldrich www.sigmaaldrich.com (Part Number 190764)
	Ethanol (Molecular biology grade; 95% or 190 proof) **Note:** Open a new bottle when preparing the PrepFiler Wash Buffer solution.	Sigma-Aldrich www.sigmaaldrich.com (Part Number E7148)
	96-Well Magnetic Ring Stand	Applied Biosystems (PN AM 10050)
	Disposable Tips (DiTi), Tecan Pure, Filtered, 1000-µL (30 000 631)†	Tecan (PN 30000631) www.tecan.com
	Disposable Tips (DiTi), Tecan Pure, Filtered, 200-µL (30 000 629)†	Tecan (PN 30000629) www.tecan.com
	100-mL disposable troughs for reagents	Tecan (PN 10613048) www.tecan.com
	Barcodes (optional)	Refer to the *Tecan Freedom EVO® Operating Manual*, Section 3.5.6 "Positive Identification (PosID)", for barcode requirements
	If collecting eluate in plates: MicroAmp® Optical 96-Well Reaction Plate (with or without barcode)	Applied Biosystems (PN N8010560 or 4306737)
	If collecting eluate in tubes: RNase-free Microfuge Tubes (1.5-mL), certified DNase- and RNase-free	Applied Biosystems (PN AM12450) or equivalent

† 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 18.

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

Prepare reagents for lysis, binding, washing, and elution

↓

Determine sample size or input amount

↓

Select a lysis protocol

↓

Perform lysis (see Figure 1 on page 18)

↓

Set Up the Automation Instrument
(see Chapter 3 on page 33)

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

↓

Run Automated DNA Extraction
(see Chapter 4 on page 53)

Set up sample information

↓

Set up and run a script (see Figure 2 on page 18)

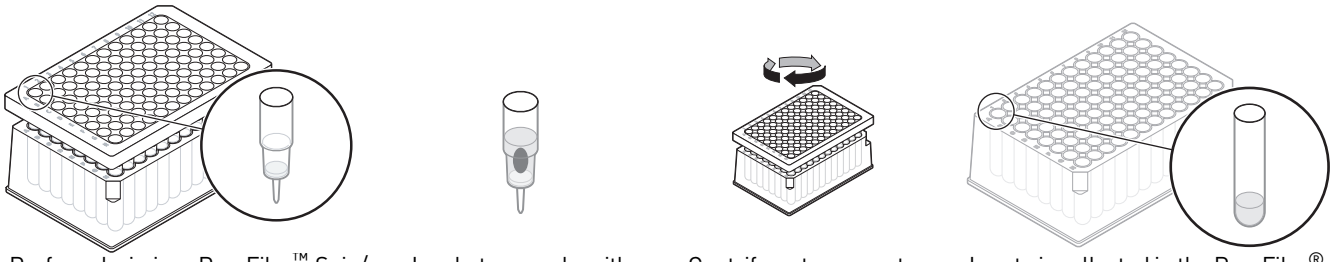
↓

Complete the run

↓

View the output file and the report

Figure 1 Lysis steps (performed manually), in plates (shown) or tubes (not shown).



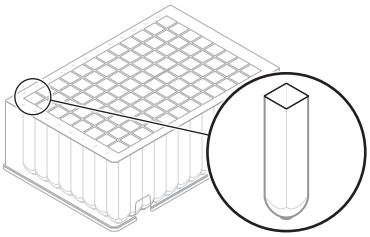
Perform lysis in a PrepFiler™ Spin/ Filter Plate unit (shown) or in 1.5-mL tubes

Incubate sample with PrepFiler™ Lysis Buffer-DTT solution

Centrifuge to separate substrate and sample lysate

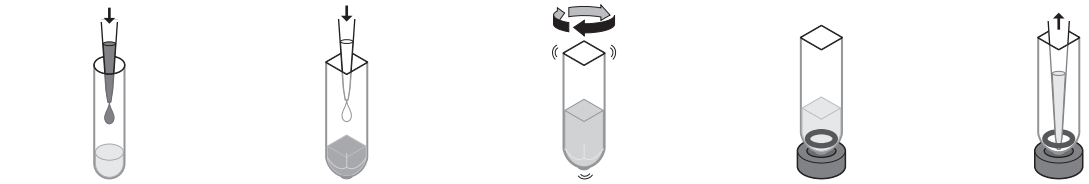
Lysate is collected in the PrepFiler® Spin Plate (shown) or in PrepFiler® Spin Tubes

Figure 2 Automated DNA extraction steps performed by the HID EVOlution™ - Extraction System in plates (shown) or tubes (not shown).



Lysate is processed from the PrepFiler™ Spin Plate or 1.5-mL tubes into the PrepFiler™ Processing Plate (shown) for automated DNA extraction

DNA Binding Step



Add PrepFiler™ Magnetic Particles, then mix

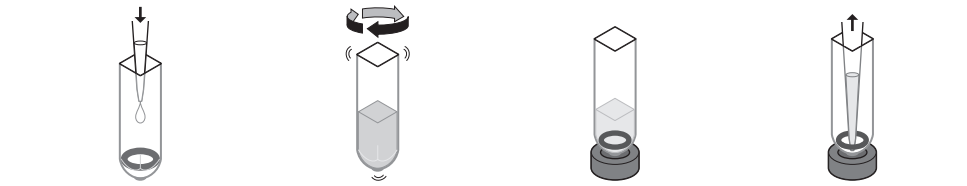
Add isopropanol

Shake to bind DNA to Magnetic Particles

Place on magnet to separate Magnetic Particles

Remove liquid, leaving Magnetic Particles-bound DNA

Wash Step (Repeat 3 Times)



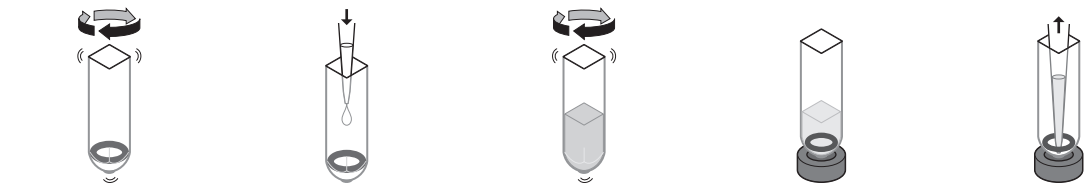
Add PrepFiler™ Wash Buffer (first two times) or Wash Buffer B (third time)

Mix

Place on magnet to separate Magnetic Particles

Remove liquid, leaving Magnetic Particles-bound DNA

DNA Elution Step



Shake to dry Magnetic Particles- bound DNA

Add PrepFiler™ Elution Buffer

Incubate and shake

Place on magnet to separate Magnetic Particles

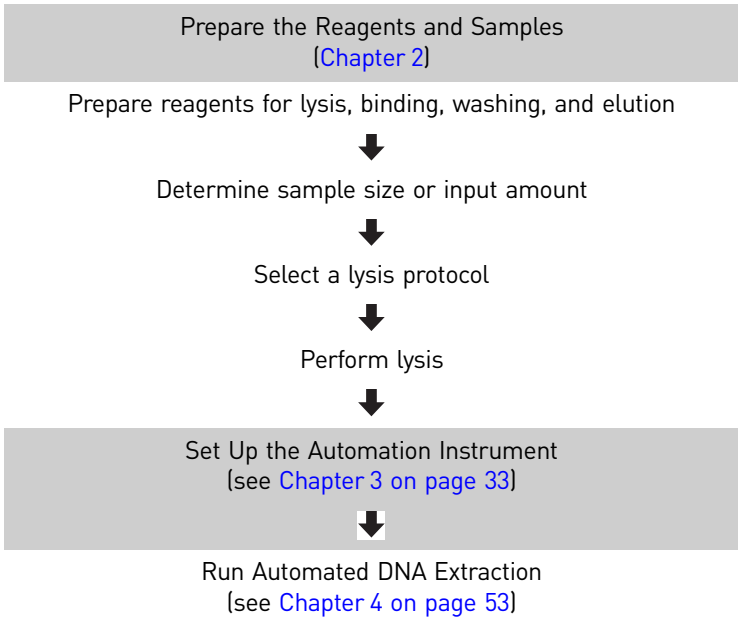
Remove DNA eluate, leaving Magnetic Particles

2 Prepare the Reagents and Samples

This chapter covers:

- Workflow 20
- Prepare reagents for lysis, binding, washing, and elution 21
- Determine sample size or input amount 23
- Select a lysis protocol 24
- Perform lysis in a 96-well plate: standard (300-µL) protocol 25
- Perform lysis in 1.5-mL tubes: standard (300-µL) protocol 30

Workflow


Prepare the Reagents and Samples
(Chapter 2)
Prepare reagents for lysis, binding, washing, and elution
Determine sample size or input amount
Select a lysis protocol
Perform lysis
Set Up the Automation Instrument
(see Chapter 3 on page 33)
Run Automated DNA Extraction
(see Chapter 4 on page 53)

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.

When you perform an extraction run:

- Using a kit that has not been used before, follow the instructions in "Before using each PrepFiler™ kit for the first time" on page 21.
- Before each run, follow the instructions in "Before each assay" on page 22.

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.
- Thaw all reagents completely.
- 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.

Before using each PrepFiler™ kit for the first time

1. Incubate the PrepFiler Magnetic Particles tubes at 37 °C for 30 minutes.

   Note: After incubation, store the tubes at room temperature for up to three months, or at 4 °C for longer periods up to the expiration date.

   Note: Precipitate has been observed to occasionally form in the PrepFiler™ Magnetic Particles tube. Extraction experiments were conducted using PrepFiler™ Magnetic Particles that had formed precipitate. No differences in extraction results were observed between magnetic particles that had and had not previously formed precipitate. PrepFiler Magnetic Particles were stored at 4 °C, then heated to 37 °C ten times with no observed decrease in extraction efficiency. Based on these experiments, it is recommended to incubate the magnetic particles tube for 30 minutes at 37 °C before first use, and again whenever the magnetic particles are stored at 4 °C.

2. Mix 260 mL of PrepFiler Wash Buffer Concentrate with 740 mL of freshly-opened ethanol in a separate, clean container.

   Note: Excess Wash Buffer Concentrate is provided with the kit.

   Note: Prepared Wash Buffer has a shelf life of 6 months if the container is kept closed when it is not in use. Do not transfer Wash Buffer from the trough back to the storage container. Use new Wash Buffer for each run.

3. Prepare Wash Buffer B by adding 95% ethanol to 200 mL DNA Suspension Buffer (low-TE buffer) to bring the final volume to 500 mL.

   Note: PrepFiler™ Wash Buffer B has a shelf life of 6 months if the container is kept closed when it is not in use. Do not transfer Wash Buffer B from the trough back to the storage container. Use new Wash Buffer B for each run.

4. (Optional) Prepare and freeze aliquots of a fresh 1.0 M solution of DL-dithiothreitol (DTT) in DNA-free water:

   a. Dissolve 1.54 g of dithiothreitol (DTT, MW 154) in 10 mL of molecular-biology grade DNA-free water.

   b. Prepare aliquots of the desired volume (for example, 100 µL or 500 µL), then store the aliquots at –20 °C for up to 6 months.

Before each assay

1. If the PrepFiler Magnetic Particles tubes are stored at 4 °C, or if the tubes contain precipitate, incubate the tubes at 37 °C for 30 minutes, vortex the tubes for 5 seconds, then centrifuge briefly.

2. If the lysis buffer contains precipitate, heat the buffer solution to 37 °C, then vortex the bottle for 5 seconds.

3. 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.

4. If you do not have an adequate volume of PrepFiler Elution Buffer to complete the run, substitute prepared low-TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) or purchase DNA Suspension Buffer from Teknova.

   IMPORTANT! Do not use water in place of Elution Buffer.

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. Examples of appropriate sample types and inputs are shown in Table 4.

Applied Biosystems validation studies were performed using the standard protocol and the sample inputs that are shown in Table 5 on page 24. The samples that are used in the validation studies were prepared from body fluids. 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.

Table 4 Example sample types and inputs

Sample type	Example sample input†
Liquid samples (blood, saliva)	Up to 40 µL
Blood (on FTA paper or fabric)	Up to 25-mm^2 (cutting or punch)
Body fluids (saliva, semen) on fabric	Up to 25-mm^2 (cutting or punch)
Body fluids on swabs (buccal and other body fluids)	Up to one swab

† 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 5 based on the sample type and size and on the type of plasticware (plate or tubes) that you want to use for lysis.

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.

Note: With some sample types and inputs, the tube lysis protocol may be the preferred method compared to the plate lysis protocol. Refer to the *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 (PN 4457144)* for a comparison of the plate and tube lysis protocols.

Table 5 Lysis protocol selection

If your sample...	And you want to perform lysis in....	Use the lysis protocol...
Contains no substrate *or* Includes a substrate that can be submerged using 300 µL of lysis buffer	A 96-well plate	Standard (300-µL) plate protocol (page 25)
		(For liquid samples only): Standard (300-µL) protocol for liquid samples (page 76)
	1.5-mL tubes	Standard (300-µL) tube protocol (page 30)
Includes a substrate that requires more than 300 µL of lysis buffer to submerge your sample **IMPORTANT!** The large-sample (500-µL) protocols were not tested as part of the full validation studies that were performed by Applied Biosystems. The large-sample protocol was 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.	A 96-well plate	Large-sample (500-µL) plate protocol (Appendix B, page 80)
	1.5-mL tubes	Large-sample (500-µL) tube protocol (Appendix B, page 83)

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

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.

Perform lysis in a 96-well plate: standard (300-µL) protocol

See "Select a lysis protocol" on page 24 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 15 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

Plate lysis guidelines and special procedures

Review this section before performing a protocol for the first time.

Plate-handling guidelines

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

Avoid cross-contamination by observing the following guidelines.

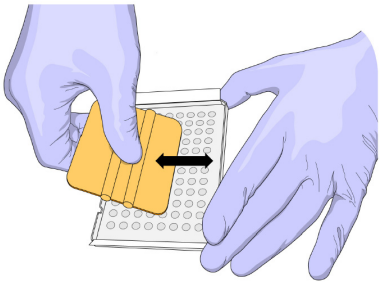
- Change gloves frequently when handling plates. For example, change gloves after separating the filter plate from the spin plate.
- Make sure that the filter/spin-plate unit is tightly assembled before shaking and incubation.
- Use caution when separating the filter plate (top) from the spin plate (bottom) 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.

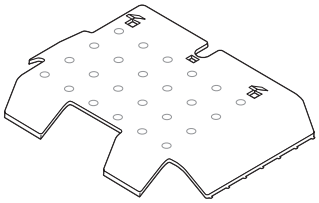
- Rub the flat edge of the applicator back and forth along the short edge (width) of the plate.
- Rub the end of the applicator horizontally and vertically between all wells (not shown).

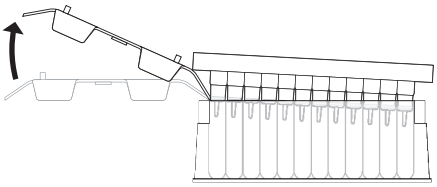


- Rub the end of the applicator around all outside edges of the plate using small back and forth motions to form a complete seal around the outside wells.

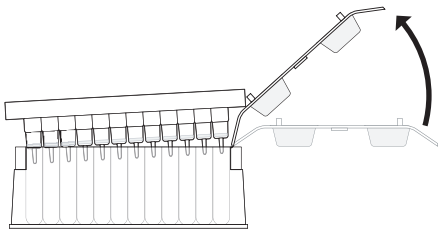
Best practices for using the MicroAmp® Multi-Removal Tool

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.

Plate setup requirements for fewer than 96 samples

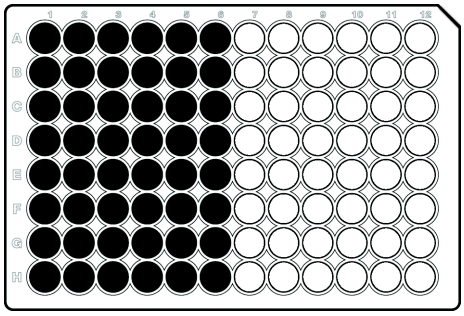
If you prepare fewer than 96 samples in a plate, the Freedom EVOware software scripts require that you place samples in the plate as follows:

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

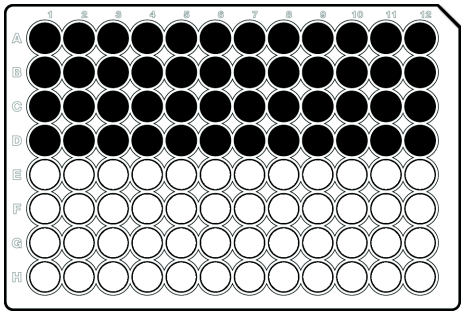
2. After the first DNA sample, continue placing samples next to one another in *vertical columns* as shown in the "Correct" examples below. Do not leave empty wells between samples. If you make a pipetting error, use blank reagents (water or DNA suspension buffer) as needed to avoid leaving empty wells 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.

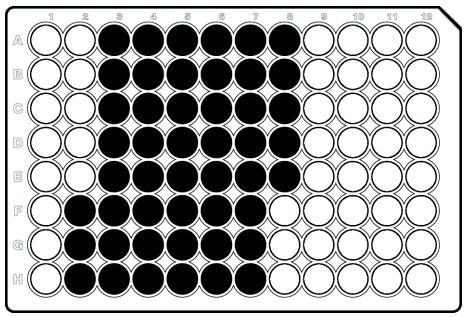
   Note: Regardless of the lysis plate setup, the DNA eluate corresponding to the first sample in the lysis plate is always placed in well position 1 (for eluate that is collected in a 96-well Elution Plate) or rack S1 tube position 1 (for eluate that is collected in tubes). The report that is generated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



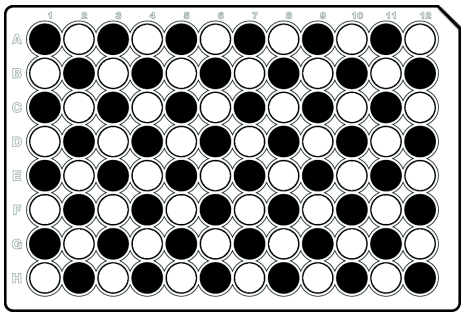
Correct (samples in wells 1 through 48)



Incorrect



Correct (samples in wells 14 through 61)



Incorrect

Perform lysis

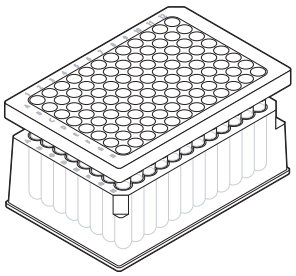
WARNING! CHEMICAL HAZARD. Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing **PrepFiler Lysis Buffer.** Wear appropriate protective eyewear, clothing, and gloves.

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. Place each sample in a separate well in the top (Filter Plate) of the plate unit.

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.

Component	Volume	
	One reaction	Recommended volume for 96 reactions†
PrepFiler Lysis Buffer	300 µL	30 mL
1.0 M DTT	3 µL	300 µL

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

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

5. 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*.

6. Add 300 µL of the PrepFiler Lysis Buffer-DTT mixture to each well of the plate unit that contains a sample.

   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.

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

8. 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.

9. 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.

10. 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.

11. Properly dispose of the (top) Filter Plate that 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.

Perform lysis in 1.5-mL tubes: standard (300-µL) protocol

See "Select a lysis protocol" on page 24 for information on selecting the appropriate protocol.

Required materials

In addition to standard laboratory equipment, you need:

- 1.5-mL microcentrifuge tubes (AB PN AM12450 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

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.

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**: 300 µL
      - **DTT, 1.0 M**: 3 µL (use 5 µL for samples containing semen)

      IMPORTANT! If the lysis buffer does not cover the sample substrate (for example, 300 µL may not cover certain types of swabs), follow the large-sample protocol on page 83.

      Note: To minimize the number of times you pipette, you can pre-mix the lysis buffer and DTT (1.0 M) for all samples, then add 300 µL of the lysis buffer-DTT mixture to each tube. Prepare a fresh lysis buffer-DTT mixture for each experiment.

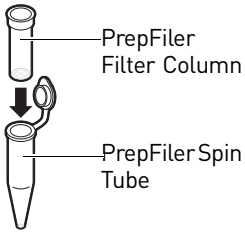
   b. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.
3. Place the tube in a shaking incubator, then incubate the tube at 150 rpm and 70 °C for 60 minutes.

   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. 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 69.

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

      Note: The collected sample lysate remains in the spin tube as you process the lysate in the remaining extraction steps.

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 a sealed Spin Tubes.

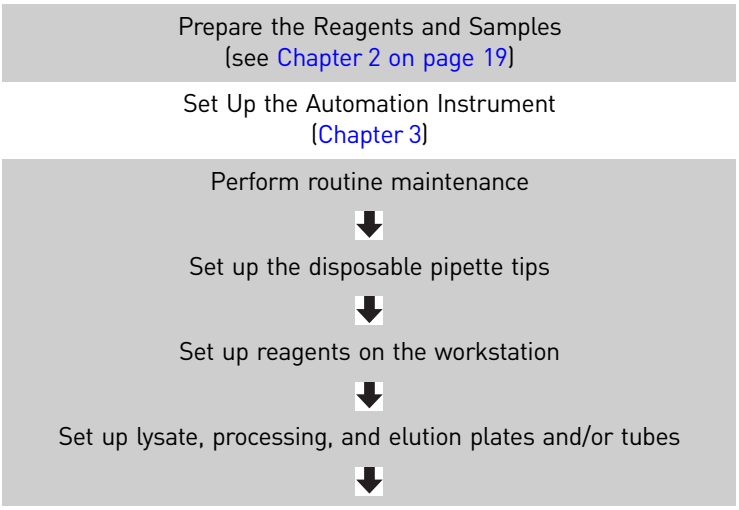
3 Set Up the Automation Instrument

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™ – Extraction system

- Workflow 34
- Perform routine maintenance 34
- Set up the disposable pipette tips. 36
- Set up reagents on the workstation 38
- Set up lysate, processing, and elution plates and/or tubes. 43
- Reference: Worktable layouts 49

Workflow



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.

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

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

For more information

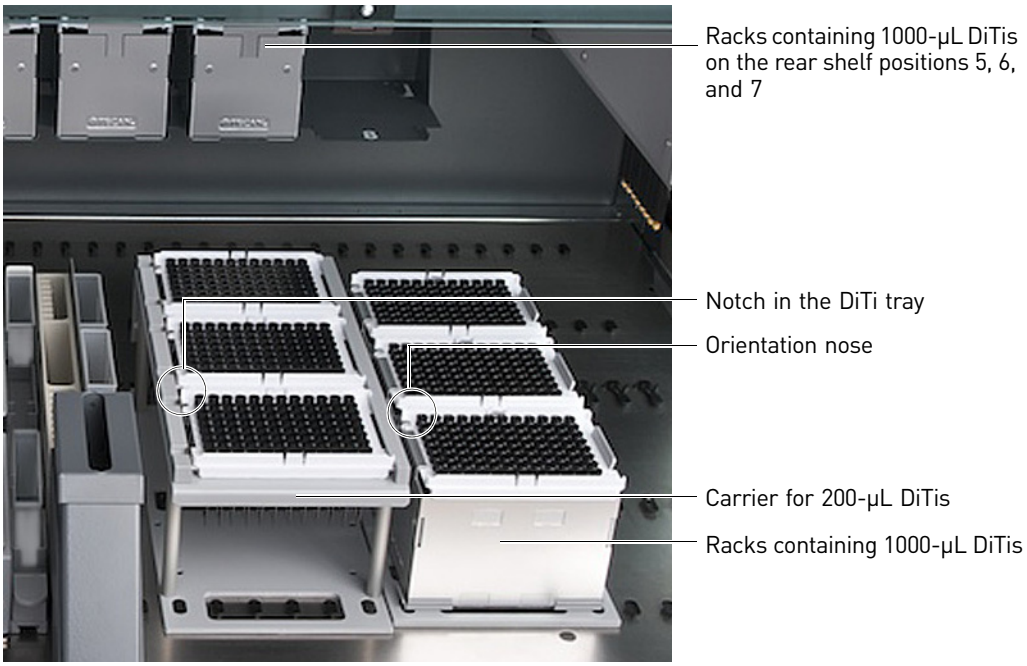
- For pre-run preparation steps, refer to the *Tecan HID EVOlution - Extraction Application Manual*, Section 4.3.2 “Prepare the Instrument”.
- For maintenance schedules, refer to the *Tecan HID EVOlution - Extraction Application Manual* Section 7.2, "Maintenance Schedule".
- For maintenance procedures, 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”.

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 3 DiTi terms.



Required materials

- 1000-µL DiTis: six trays (each containing 96 DiTis)
- 200-µL DiTis: three 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 the other shelf positions.

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 upper 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 upper left corner.

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 21.
- 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, 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
- 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, and PrepFiler™ Wash Buffer B according to "Prepare reagents for lysis, binding, washing, and elution" on page 21.

2. Place empty troughs on the worktable:

Empty Trough for...	Place in following location...
Elution buffer	Grid 27, position 1
Wash buffer B	Grid 27, position 2
Wash buffer	Grid 27, position 3
Isopropanol	Grid 25, position 1
Lysate waste **IMPORTANT!** Do not add acids or bases to any wastes containing lysis buffer (guanidine thiocyanate). See "Chemical waste safety guidelines" on page 96.	Grid 25, position 3

3. Using the volumes shown in the table below, calculate the PrepFiler™ reagent volumes you need:

Reagent		Reactions (DNA samples) in run	Required reagent volume per reaction	Required overfill volume per run‡	Required dead volume per run§	Minimum required volume for 96 samples (including overfill and dead volume)††
		A	B	C	D	(A×B)+(A×B×C)+D
Isopropanol	**If you used the standard (300-µL) lysis protocol**	Up to 96	180 µL	15%	5 mL	25 mL
	If you used the large-sample (500-µL) lysis protocol†	Up to 96	300 µL	15%	5 mL	40 mL
Prepared Wash Buffer		Up to 96	900 µL	15%	5 mL	105 mL
Wash Buffer B		Up to 96	300 µL	15%	5 mL	40 mL
Elution Buffer		Up to 96	50 µL	15%	5 mL	11 mL

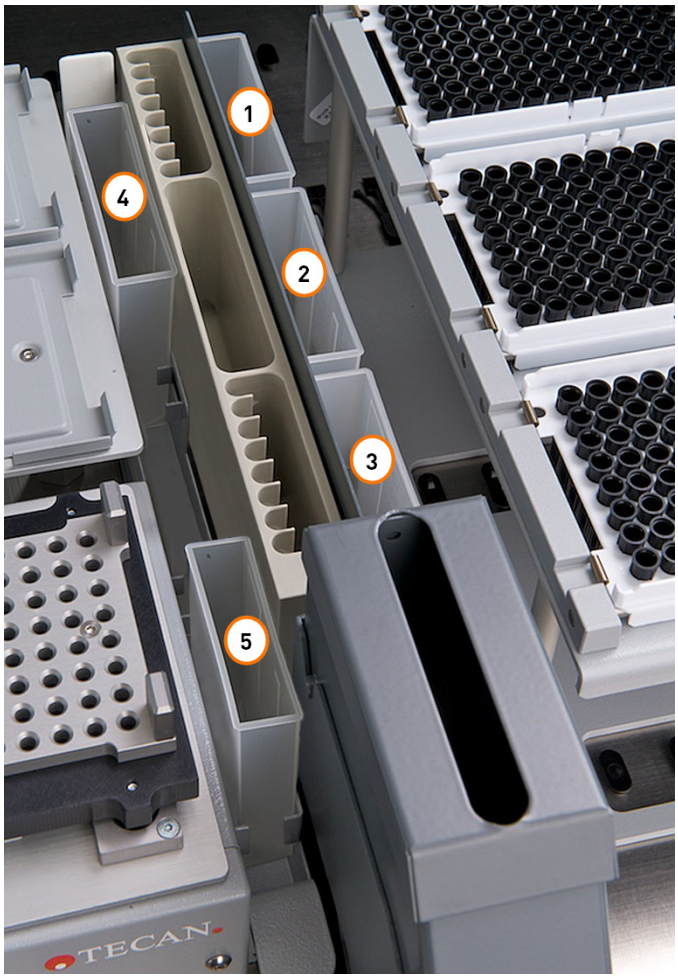
† The large-sample (500-µL) protocols were not tested as part of the validation studies that were performed by Applied Biosystems. If you intend to use the large-sample protocols, perform the appropriate validation studies.

‡ 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.

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

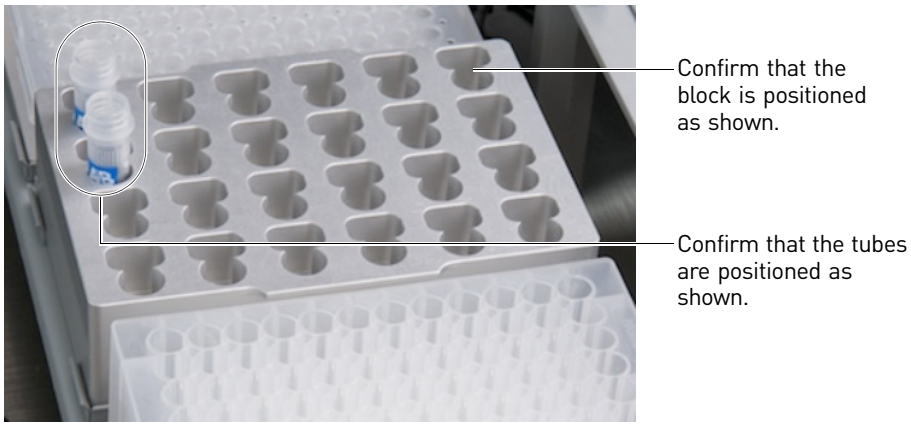


1. Elution Buffer trough
2. Wash Buffer B trough
3. Prepared Wash Buffer trough
4. Isopropanol trough
5. Lysate waste trough (empty)

5. 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 pipette 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™ – Extraction System gives you the following options:
  - Process lysate from a 96-well plate and collect eluate in a 96-well plate (plate-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 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)

  "Reference: Worktable layouts" on page 49 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 45 through page 48. Depending on the option you selected, you will 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 58).

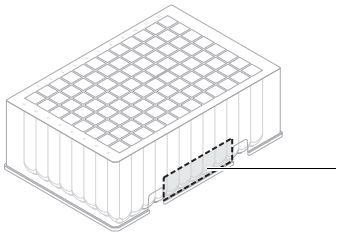
Required materials

- 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

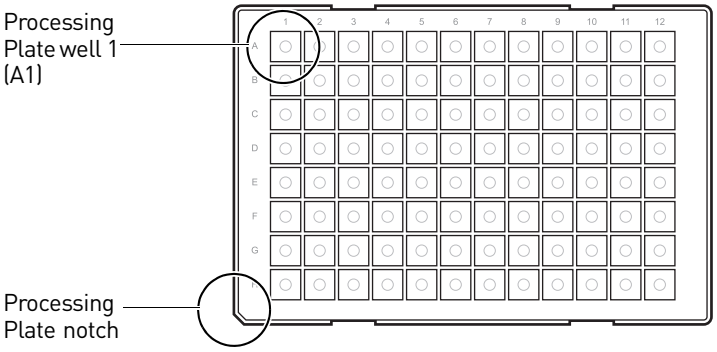
Set up the PrepFiler™ Processing Plate

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 upper left corner
   - The plate wells are aligned with the holes in the Te-Shake™ adapter

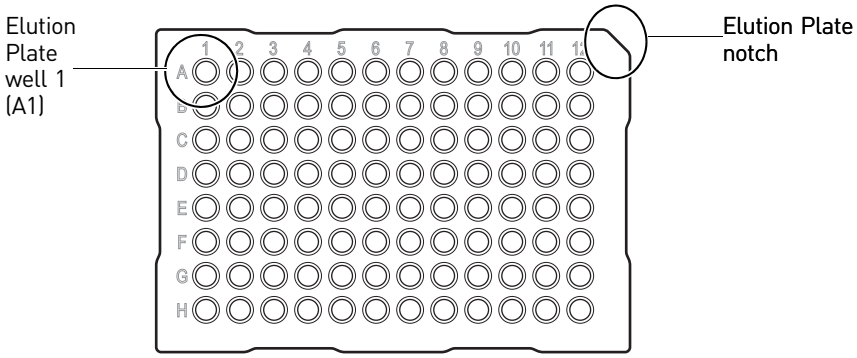
Set up lysate and/or eluate plates

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 upper 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).

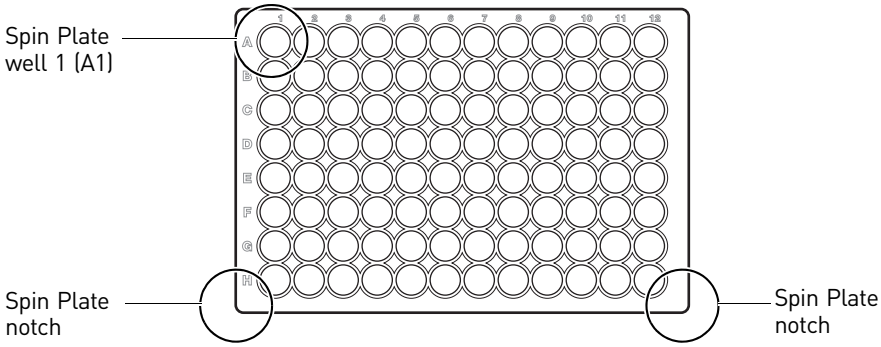
2. If you want DNA eluate to be collected in a plate, place a MicroAmp® Optical 96-Well Reaction Plate with well A1 in the top left position (grid 13, position 1).



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 alternate 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).



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 elute 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.

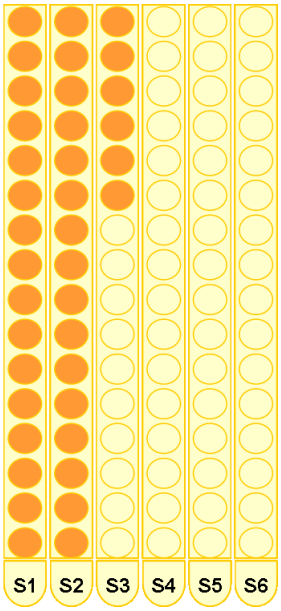
2. Place the first empty 1.5-mL tube in the tube racks in rack S1, position 1.

   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.

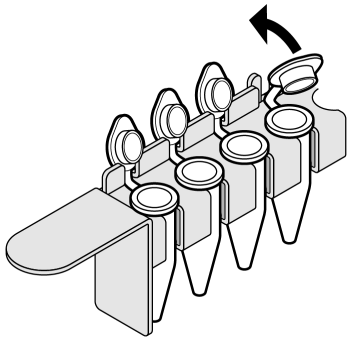
   Example of correct setup:



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 below.

   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:

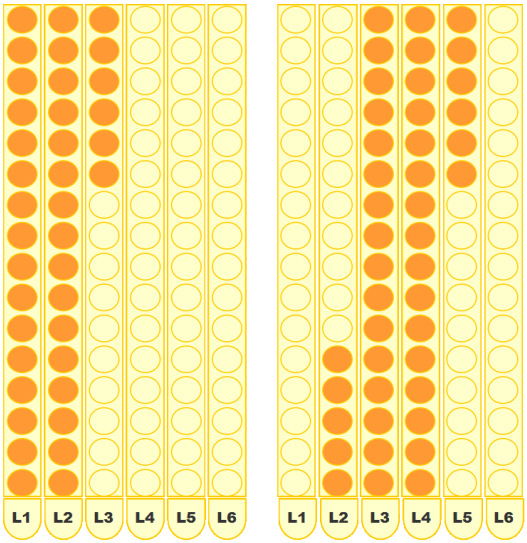
1. Confirm that:
   - You have no more than 96 labeled 1.5-mL microcentrifuge tubes containing DNA sample lysate.
   - The tube racks L1 through L6 are correctly positioned at grid positions 7 through 12.

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.

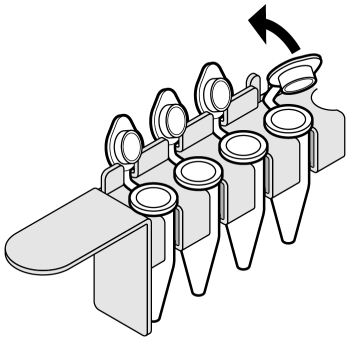
Examples of correct setup:



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 more information

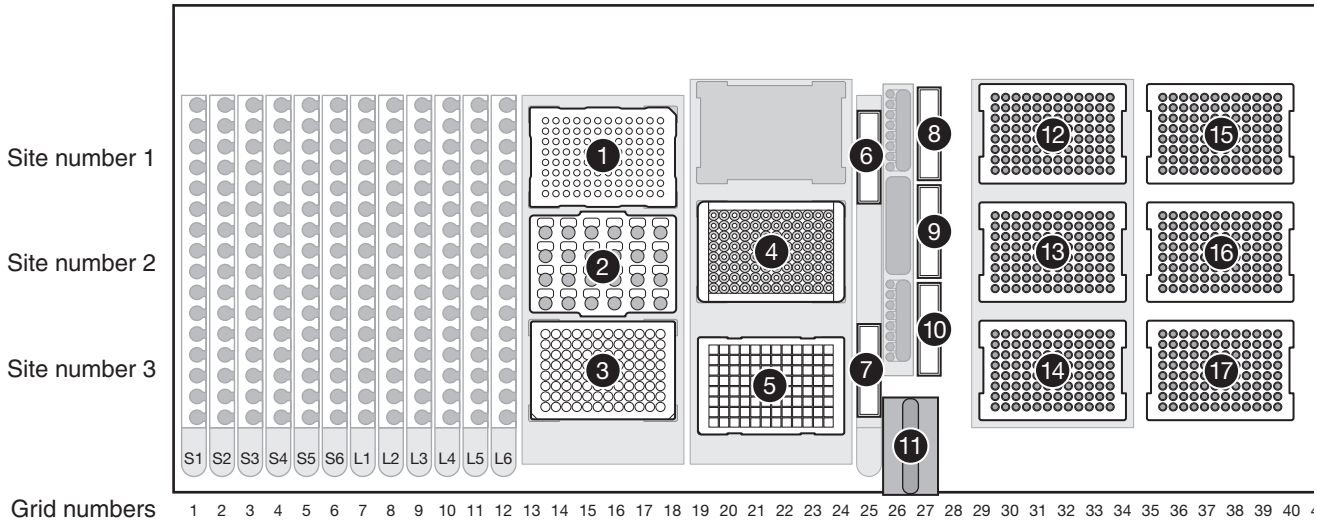
- For information on barcode specifications for use on the Freedom EVO® instrument, refer to the *Tecan Freedom EVO® Operating Manual*, Section 3.5.6, "Positive Identification (PosID)", for details.
- Place the plasticware (plates and/or tubes) according to the *Tecan HID EVOlution - Extraction Application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

Reference: Worktable layouts

Figures 4 through 7 on page 49 through page 52 show the worktable layouts for each of the four automated extraction run options.

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

Figure 4 Plate-to-plate workstation layout.



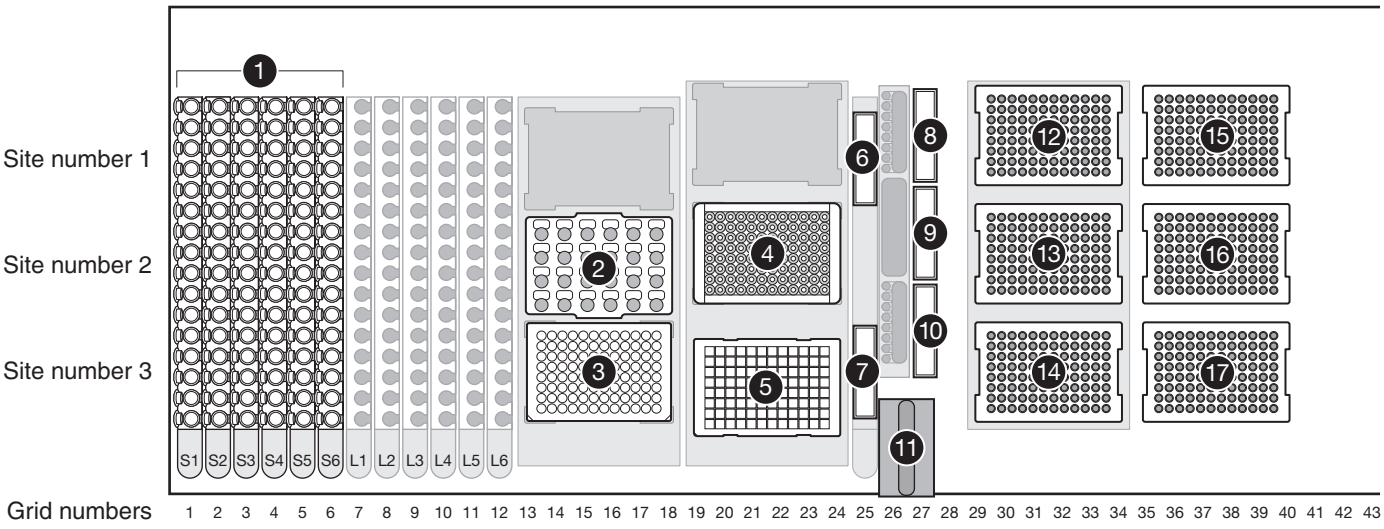
1. 96-Well Elution Plate
2. Block for PrepFiler™ Magnetic Particles
3. PrepFiler™ Spin Plate
4. Magnetic Ring Stand
5. PrepFiler™ Processing Plate on Te-Shake™ adapter
6. Isopropanol trough
7. Lysate waste trough
8. Elution Buffer trough
9. Wash Buffer B trough
10. Wash Buffer trough
11. DiTi waste unit

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 5 Plate-to-tubes workstation layout.



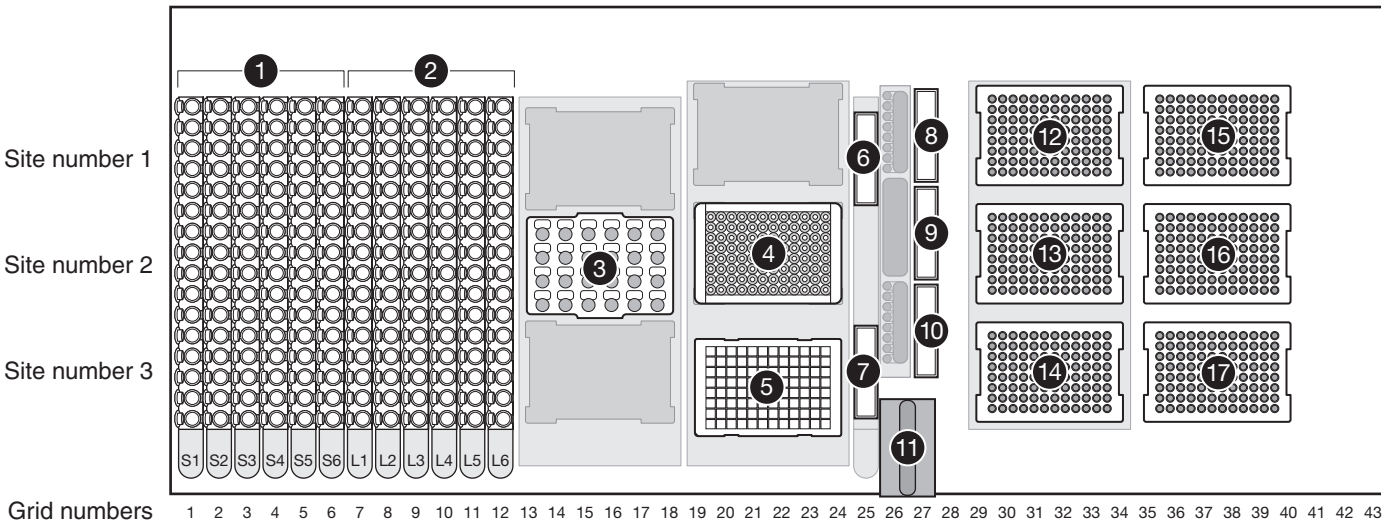
1. Elution tube racks S1 to S6 with microcentrifuge tubes
2. Block for PrepFiler™ Magnetic Particles
3. PrepFiler™ Spin Plate
4. Magnetic Ring Stand
5. PrepFiler™ Processing Plate on Te-Shake™ adapter
6. Isopropanol trough
7. Lysate waste trough
8. Elution Buffer trough
9. Wash Buffer B trough
10. Wash Buffer 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-tubes workstation layout.



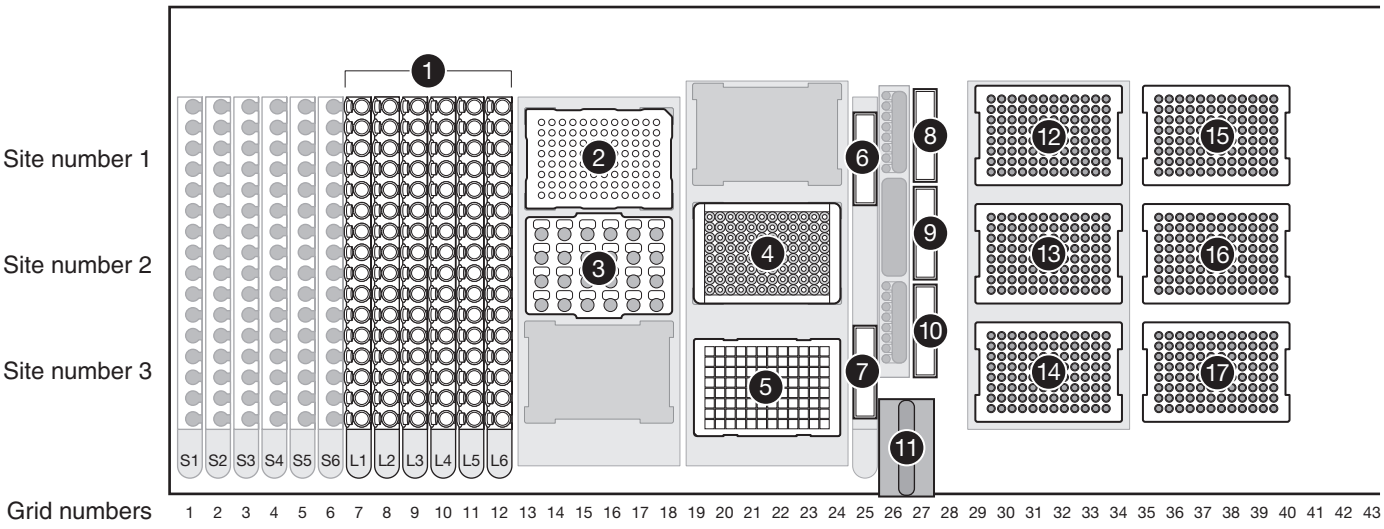
1. Elution tube racks S1 to S6 with microcentrifuge tubes
2. Lysate tube racks L1 to L6 with microcentrifuge tubes
3. Block for PrepFiler™ Magnetic Particles
4. Magnetic Ring Stand
5. PrepFiler™ Processing Plate on Te-Shake™ adapter
6. Isopropanol trough
7. Lysate waste trough
8. Elution Buffer trough
9. Wash Buffer B trough
10. Wash Buffer 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 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 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)

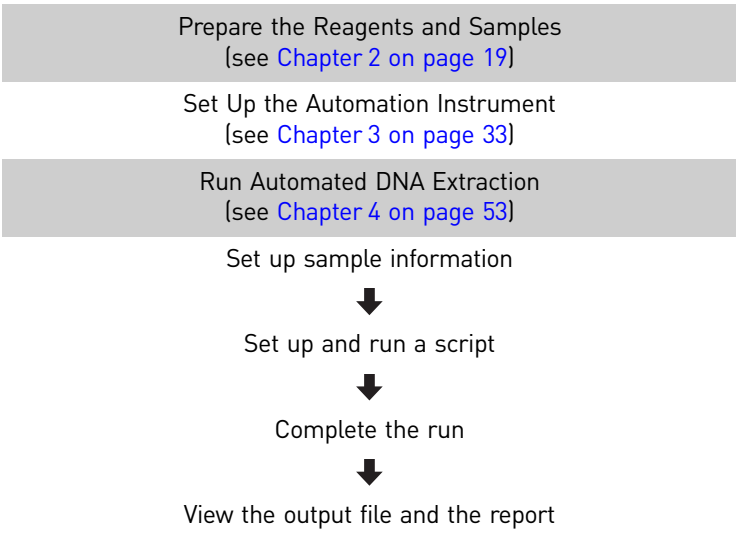
4 Run Automated DNA Extraction

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

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

- Workflow 54
- Set up sample information 54
- Set up and run a script 58
- Complete the run 66
- View the output file and the report 67

Workflow



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 56.

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 all samples. 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 56.
- **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.

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

- If you manually enter sample information and then import a sample setup file, the information that you entered is overwritten by the imported file.
- If your system is set up to use barcodes, any information that you enter manually or by importing a sample setup file is overwritten when the sample lysate barcodes are scanned.
- If your system is set up to use barcodes, but you are not using barcodes for a particular run, you must deselect the Scan Labware checkbox in the Sample Information screen of the HID EVOlution™ – Extraction wizard. Otherwise any sample information that you manually entered or imported is overwritten.

(Optional) Create a sample input file

To use a sample input file to set up sample information in the HID EVOlution™ - Extraction 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 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, Applied Biosystems provides eight scripts for use with the PrepFiler™ kits; 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:

- The scripts for use with lysate that is prepared using a standard (300-µL) protocol are:
  - Sample lysate in plates and eluate in plates
  - Sample lysate in plates and eluate in tubes
  - Sample lysate in tubes and eluate in tubes
  - Sample lysate in tubes and eluate in plates
- The scripts for use with lysate that is prepared using a large-volume sample (500-µL) protocol are:
  - Sample lysate in plates and eluate in plates
  - Sample lysate in plates and eluate in tubes
  - Sample lysate in tubes and eluate in tubes
  - Sample lysate in tubes and eluate in plates

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 proper 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.

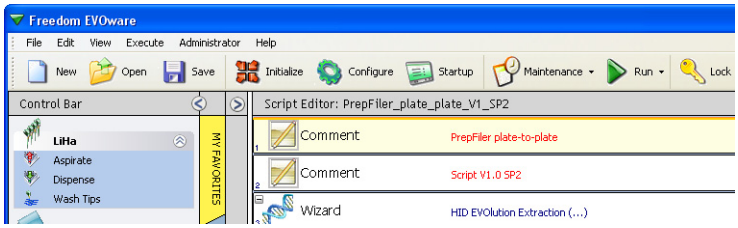
Select a script

1. On your desktop, click  to start the EVOware Standard software, then enter your user name and password.
2. Select **Edit an existing script**, then click  .
3. In the Selection dialog box, select the appropriate script for your lysis protocol and plate/tube selections, then click  .

If the sample lysate is in...	And you want the eluted DNA in...	If the lysis volume is 300 µL, use the script...	If the lysis volume is 500 µL, use the script†...
a 96-well Spin Plate	a 96-well plate	PrepFiler_plate_plate_V1_SP2	PrepFiler_plate_plate500_V1_SP2
	1.5-mL microcentrifuge tubes	PrepFiler_plate_tubes_V1_SP2	PrepFiler_plate_tubes500_V1_SP2
1.5-mL microcentrifuge tubes	a 96-well plate	PrepFiler_tubes_plate_V1_SP2	PrepFiler_tubes_plate500_V1_SP2
	1.5-mL microcentrifuge tubes	PrepFiler_tubes_tubes_V1_SP2	PrepFiler_tubes_tubes500_V1_SP2

† The large-sample (500-µL) scripts were not tested as part of the validation studies that were performed by Applied Biosystems. If you intend to use the large-sample scripts, perform the appropriate validation studies.

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



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

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

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

See "(Optional) Re-cap Magnetic Particles tubes" on page 64 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:

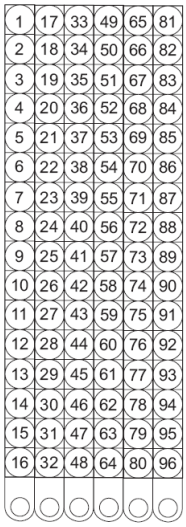
   IMPORTANT! If you manually enter or import sample information, deselect the Scan Labware checkbox. Otherwise, the system will attempt to scan barcodes and will overwrite previously entered or imported sample information.

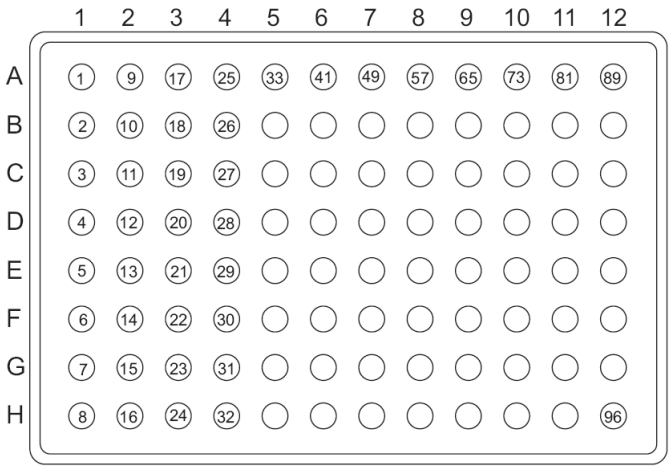
   - Load the sample setup file:
     - Click ..., then browse to the **C:\PrepFilerInputFiles** directory.
     - Select the file that you prepared in "(Optional) Create a sample input file" on page 56, then click **OK**.
     - Confirm that the actual sample IDs and worktable positions match those in the imported sample input file.
   - Enter sample information manually: Click the **Edit** button next to the plate or each tube rack, enter then information as described in the *Tecan HID EVOlution - Extraction Application Manual*, Section 5.3, "Running a HID EVOlution - Extraction Script", then click **OK**.

   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:

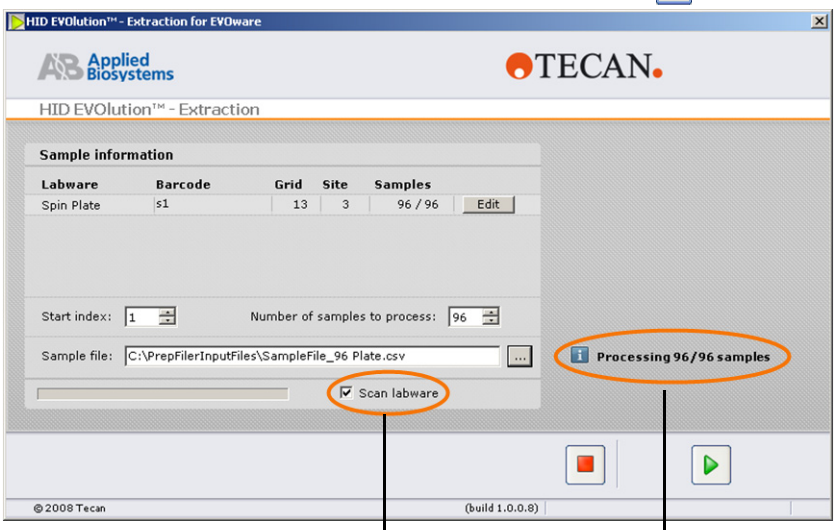
   a. In Start index, select a number between 1 and 96 that corresponds to:
      - The tube position of the first tube in the sample racks, or
      - The well position of the first sample in the plate





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 (out of a maximum of 96) is correctly shown (for example, if you are processing 16 samples, the message should read" Processing 16/96 samples"), then click .



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 to continue.

Confirm worktable setup and start the run

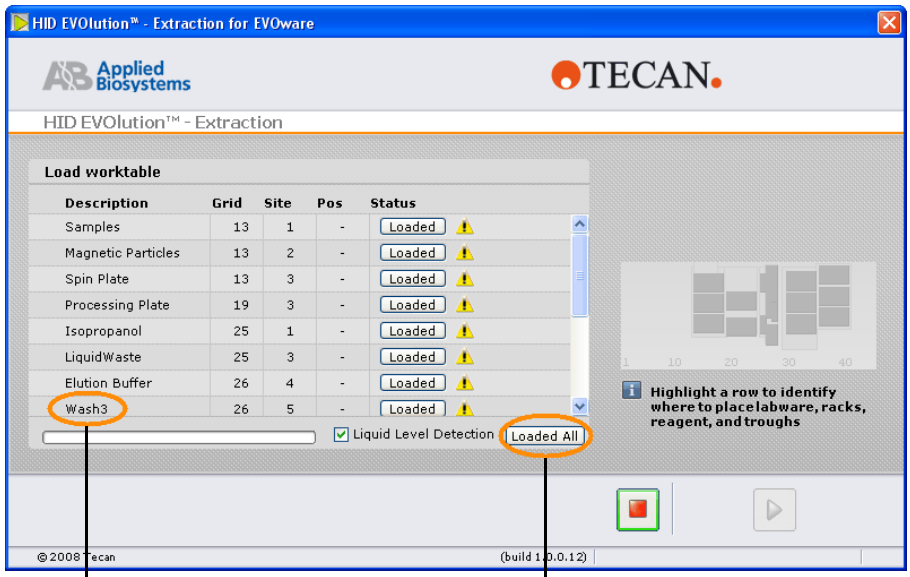
1. In the Load worktable page, compare the listed items to the items on the actual worktable.
   a. Confirm that you correctly loaded:
      - A PrepFiler Processing Plate (shown as Processing Plate)
      - A MicroAmp® Optical 96-Well Reaction Plate or 1.5-mL tubes to collect the DNA eluate (shown as 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 (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, 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 ▶ to start the inventory scan.

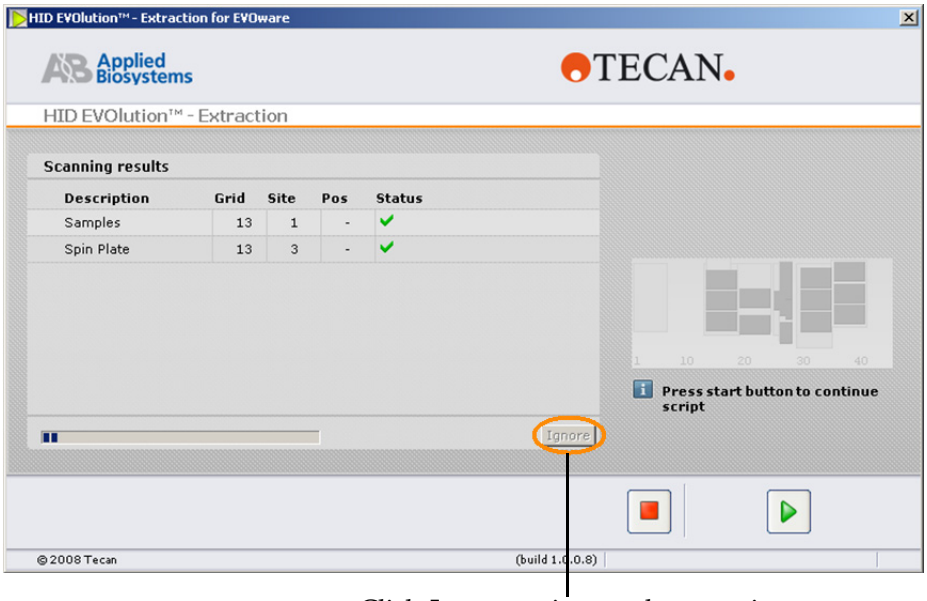


Wash 3 is the PrepFiler™ Wash Buffer B

Click **Loaded** next to each individual item, or click **Loaded All** to confirm all items with one click.

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

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



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:

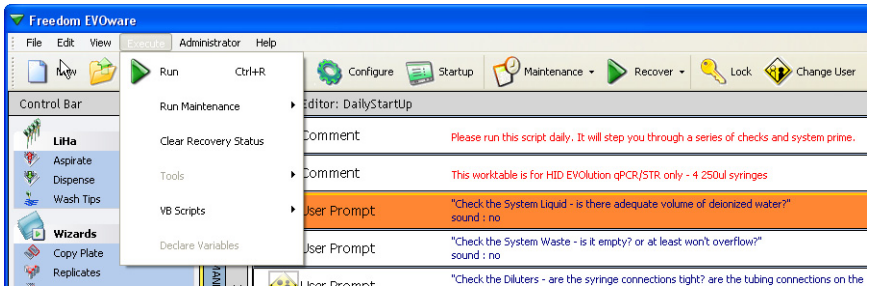
1. Click in the EVOware Runtime Controller to pause the run.
2. Re-cap the Magnetic Particles tubes to avoid forming a crust around the rim of the tubes.
3. Click in the EVOware Runtime Controller to continue the run.

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.

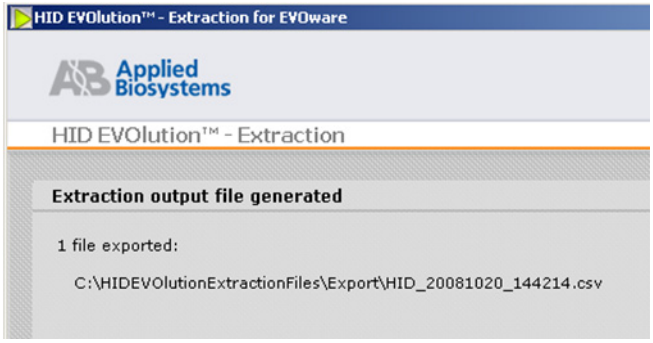
- In the Freedom EVOware script dialog, note the highlighted orange line; this is the step where the run stopped.
- In the Freedom EVOware script dialog, select **Execute ▸ Clear Recovery Status** from the menu. You can now start a new run.



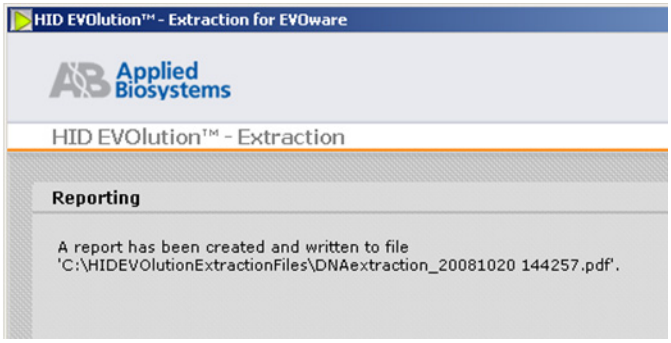
Record file information and exit the script

When the run is complete:

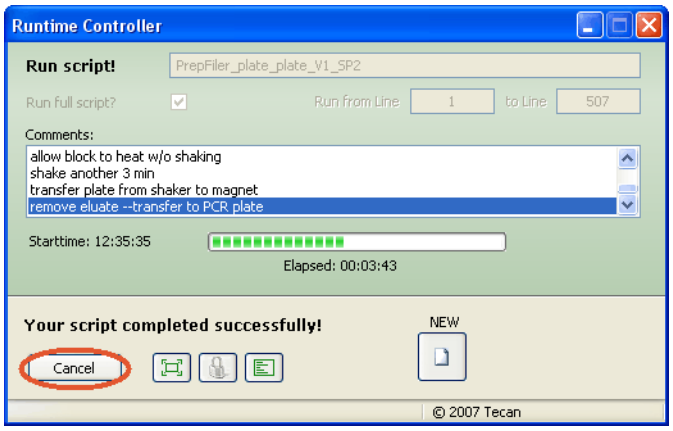
1. Record the Extraction output file path and name (**C:\HIDEVOlutionExtractionFiles\Export\HID_*run date_run time*.csv**), then click ▶ to continue.



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 clean up 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.
5. Properly dispose of any unused isopropanol, Wash Buffer, Wash Buffer B, and Elution Buffer in the reagent troughs.

   IMPORTANT! Do not reuse the reagents in the troughs. See "Waste disposal" on page 96.
6. (Last run of 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.

9. Perform routine cleanup and maintenance on the Freedom EVO instrument.

   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.

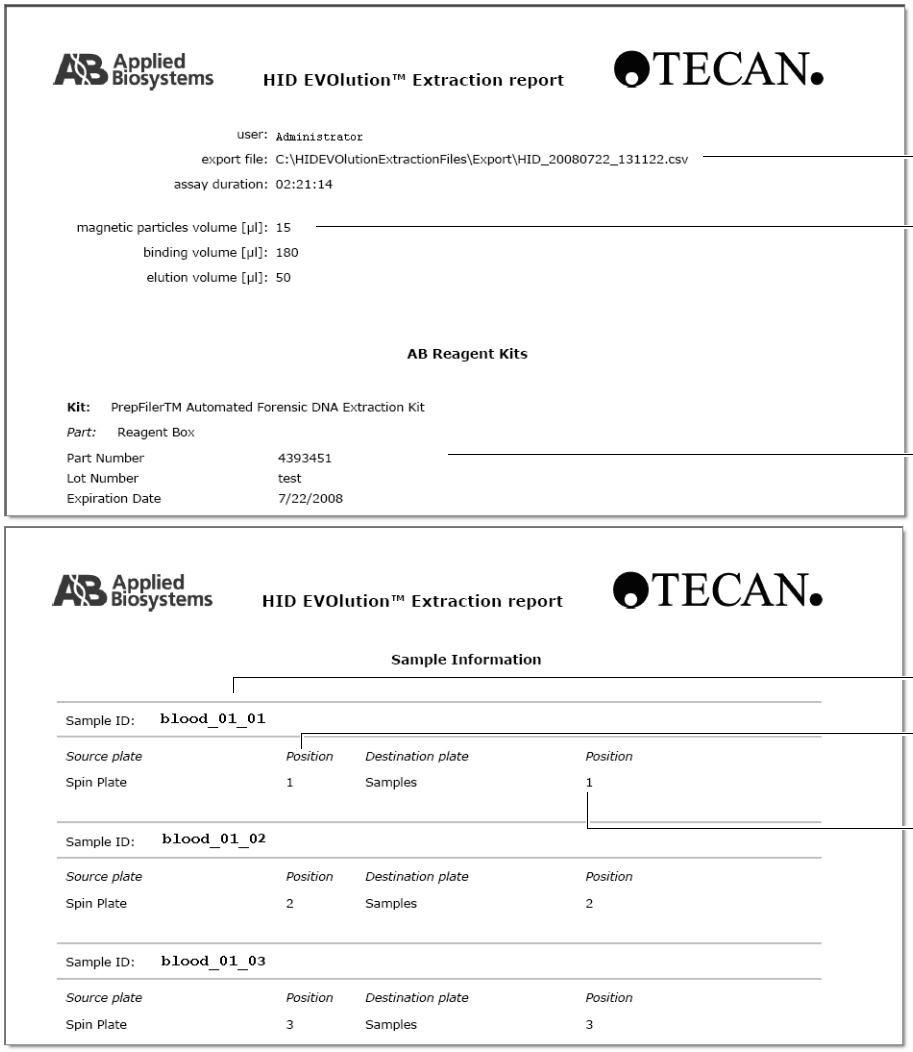
  At the end of a run, the output file and report are automatically saved to the C:\HIDEVOlutionExtractionFiles folder. When you start your next run, the output file and report from the previous run are automatically moved to a C:\HIDEVOlutionExtractionFiles\Archive*date_time* folder. For example, if you start your next run on August 15, 2010 at 3:08 pm, files from the previous run would be archived to the C:\HIDEVOlutionExtractionFiles\Archive\20100815_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 at the end of the extraction run (**DNAextraction_*run date_run_time*.pdf**).

3. Review the reagent and sample information in the report:



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

For more information

For details on:

- The report and extraction output files, refer to the *Tecan HID EVOlution - Extraction Application Manual*, Section 6, "Results".
- The HID EVOlution™ – qPCR/PCR Setup System, refer to the *Tecan HID EVOlution™ Application Guide – Automation for Applied Biosystems Human Identification Kits*.

A Troubleshooting

For troubleshooting problems with:

- Setting up and running the automation instrument, refer to the *Tecan HID EVOlution - Extraction Application Manual*, Chapter 8, "Troubleshooting".
- Lysate, DNA eluate appearance, or DNA yield, see Table 6.

Table 6 Troubleshooting extraction results

Observed problem	Possible cause	Suggested solution
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 pipette the clear DNA extract into a new plate or tube. If the problem persists over multiple runs, contact Applied Biosystems to determine if the liquid handling (LiHa) arm requires adjustment. Also 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	Possible cause	Suggested solution
The DNA eluate is colored.	The substrate yielded a colored eluate. For example, some sample substrates contain dyes.	**Note:** Color does not necessarily interfere with quantitation or amplification. If you see a shift in IPC C_T value in the quantitation run, manually process the DNA eluates using the Repurification Protocol, then requantify the sample. Refer to the *PrepFiler Forensic DNA Extraction Kit User Guide*, Appendix B, "Repurification Protocol".
The DNA eluate volume is low.	• Incomplete volume transfer occurred because of a loose pipette tip (DiTi cone). • The z-max of the liquid pipetting arm needs to be adjusted.	• If liquid remains in the Processing Plate, manually pipette the liquid to the correct plate well(s) or tube(s). • 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 Applied Biosystems 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	Possible cause	Suggested solution
The DNA yield is low or DNA is absent.	• The biological sample contains no or a low amount of DNA. • Reagents are missing or improperly positioned on the worktable. • Incorrect automated pipetting occurred because of: – Incorrect or improperly placed DiTis, plates, tubes, or hardware. – Air bubbles or leaks in system. – Dirty or loose DiTi cones. • DiTis were not picked up properly.	1. Confirm that correct reagent and instrument setup, then re-run the samples: • 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". • 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". • 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". • 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". • Reteach the LiHa the coordinates (x, y, and z positions) of the 200-µL and 1000-µL DiTis. For details, contact Applied Biosystems 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". 2. Amplify the maximum volume for STR analysis. 3. Extract DNA from a different sample that is prepared from the same source.

Observed problem	Possible cause	Suggested solution
The sample IPC C_T is higher than the IPC C_T of the no template quantitation control (NTC) or of the quantitation standards (for example, if the sample IPC C_T is approximately two C_T greater than the NTC IPC C_T or the C_T of the standards).	• 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 × g for 5 minutes, pipette 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 C_T compared to the NTC or quantitation standards, follow the Repurification Protocol (refer to the *PrepFiler Forensic DNA Extraction Kit User Guide*, 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 the AmpFℓSTR® MiniFiler™ PCR Amplification Kit, which is designed to obtain STR profiles from compromised samples such as those which may be inhibited and/or degraded. • Before the next run, to ensure correct pipetting, clean and finger tighten the DiTi cones 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".

B Preventing Contamination

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.

C Supplementary Lysis Protocols

This appendix covers:

- Perform lysis in a 96-well plate: standard (300-µL) protocol for liquid samples 76
- Perform lysis in a 96-well plate: large sample (500-µL) protocol 80
- Perform lysis in 1.5-mL tubes: large sample (500-µL) protocol 83

Perform lysis in a 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 15 for details:

- 1.5-mL microcentrifuge tubes (use RNase-free Microfuge Tubes (1.5-mL), certified DNase- and RNase-free Applied Biosystems (PN AM12450) or equivalent tubes that allow you to observe the tube contents and 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

Perform lysis

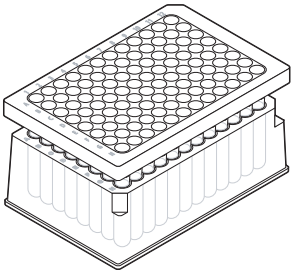
WARNING! CHEMICAL HAZARD. Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing **PrepFiler Lysis Buffer.** Wear appropriate protective eyewear, clothing, and gloves.

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.

Component	Volume	
	One reaction	Recommended volume for 96 reactions†
PrepFiler Lysis Buffer	300 µL	30 mL
1.0 M DTT	3 µL	300 µL

† 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 that 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.

Perform lysis in a 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 24 for information on selecting the appropriate protocol.

Review "Plate lysis guidelines and special procedures" on page 25 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 Applied Biosystems. The large-sample protocol was 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 15 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

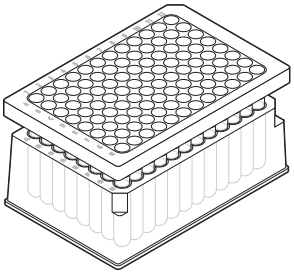
WARNING! CHEMICAL HAZARD. Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing **PrepFiler Lysis Buffer.** Wear appropriate protective eyewear, clothing, and gloves.

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.

   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.

Component	Volume	
	One reaction	Recommended volume for 96 reactions†
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 that 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.

Perform lysis in 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 24 for information on selecting the appropriate protocol.

Review "Plate lysis guidelines and special procedures" on page 25 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 Applied Biosystems. The large-sample protocol was 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 PN 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 pipette, 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 centrifuge it briefly.

3. Place the tube in a shaking incubator, then incubate the tube at 150 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.

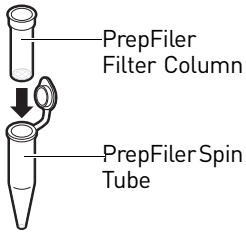
Note: If you use barcodes to track samples, confirm that barcodes are correctly placed on the tubes (see barcode information in the Tecan *HID EVOlution™ Extraction System Application Manual*).

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. Check the volume of sample lysate that is collected in the spin tube. If the volume is less than 180 µL, then centrifuge the filter column/spin tube for an additional 5 minutes. If the volume is still less than 180 µL, see "Troubleshooting" on page 69.

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

Note: The collected sample lysate remains in the spin tube as you process the lysate in the remaining extraction steps.

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.

D One-time procedure: Document Te-Shake™ plate adapter temperatures

The temperatures set in the extraction scripts are the temperatures for the heating plate (C in Figure 8), not the heating plate adapter (D in Figure 8). 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 86 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 Applied Biosystems 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 8 Te-Shake™ components (from the Tecan *HID EVOlution™ – Extraction Application Manual*)

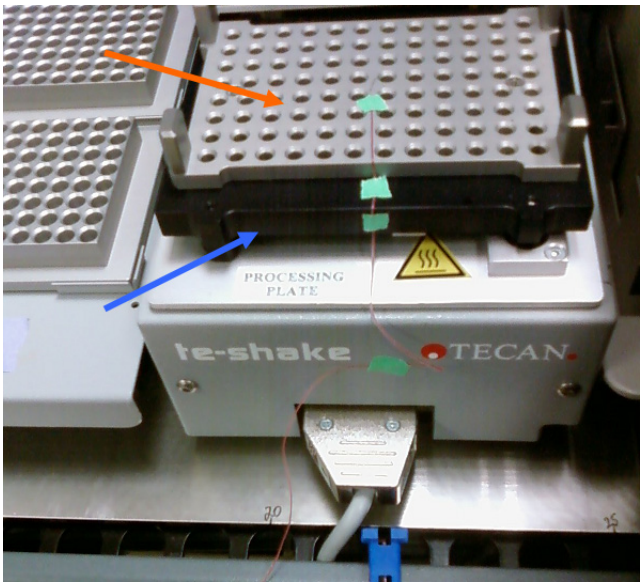
No.	Plain Text Designation	Tecan p/n	Label Designation
A	Te-Shake mounting plate with two additional positions	30015506	PLATE MOUNTING 2 TE-SHAKE CMR
B	Shaker plate for heating block	10760726	PLATE SHAKER HEATING PLATE TE-SHAKE
C	Heating plate for microplate/tube adapters	30015374	PLATE HEATING TE-MAGS/ TE-SHAKE MP
D	Heating plate adapter for VWR plate	30035318	BLOCK HEATING VWR PLATE CPL.

For this procedure, you need a digital thermometer and a thermocouple probe such as the following:

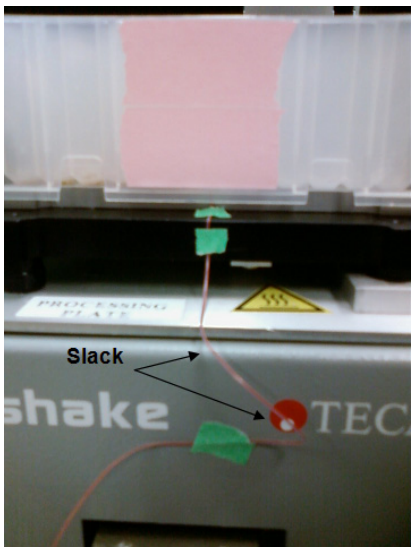
- Sper Scientific Type-K, Type-J Thermometer 800005 (VWR catalog number 14003-070)
- Sper Scientific Type-K Beaded Wire 800077 (VWR catalog number 14003-136)

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.

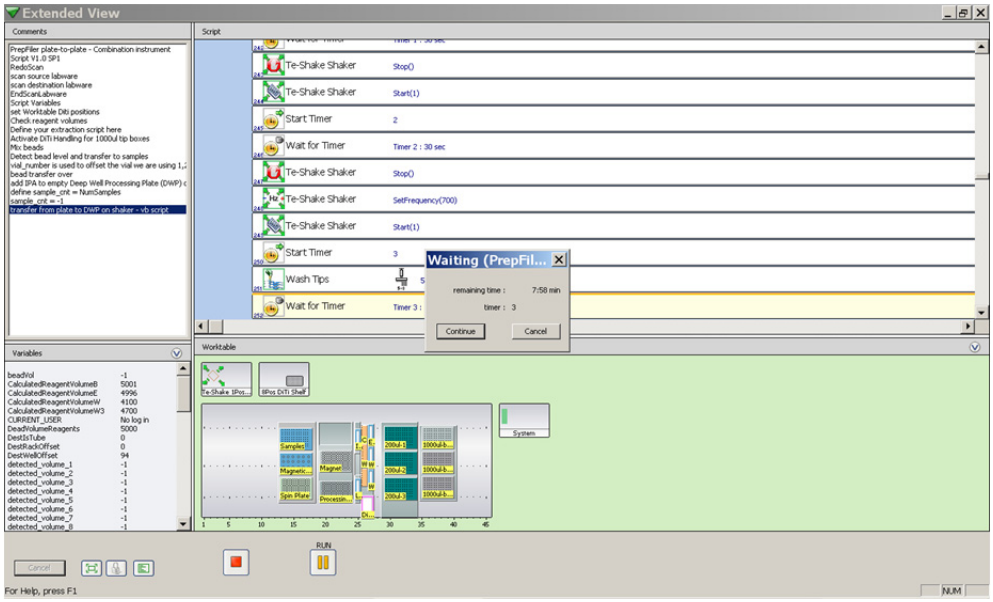
3. Set up the worktable for a water run for a small number of samples.

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 33.

4. Begin a run according to the instructions in Chapter 4, "Run Automated DNA Extraction" on page 53. 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).



E Automation Guidelines

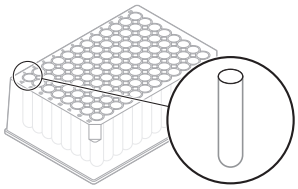
The following are general guidelines for automating the binding, wash, and elution steps of the PrepFiler™ kit extraction procedure.

Before the automated steps

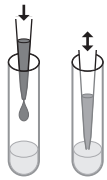
Prepare reagents and perform lysis manually as described in Chapter 2 on page 21.

Binding step

1. Place the PrepFiler Spin Plate containing the lysate on the robot.

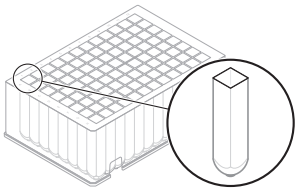


2. To each well, add 15 µL of PrepFiler Magnetic Particles, then, with the robot at the default mixing speed, pipette the well contents up and down until the particles are completely resuspended.

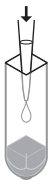


Note: Mixing speed does not affect Magnetic Particle performance.

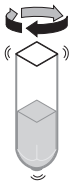
3. Transfer the contents of each well to a separate well in the PrepFiler Processing Plate.



4. To each well, add 180 µL of isopropanol.

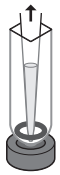


5. Shake the plate at 500–1000 rpm for 10 minutes to bind the DNA to the magnetic particles.



6. Place the plate on the 96-Well Magnetic Ring Stand (PN AM10050) for 5 minutes to separate the magnetic particles.

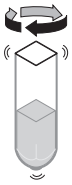
7. Remove all the liquid phase without disturbing the magnetic particles.



Wash step (repeat three times)

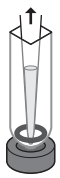
1. Place the plate on the Shaker.
2. To each well, add 300 µL of prepared PrepFiler Wash Buffer (first two times) or 300 µL PrepFiler® Wash Buffer B (third time).

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.

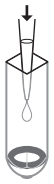
5. Remove all the liquid phase without disturbing the magnetic particles.



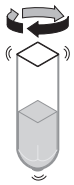
Elution step

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.



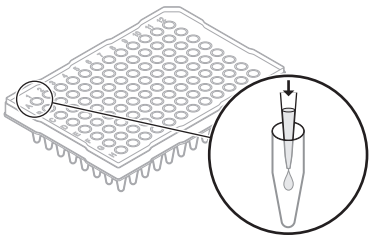
4. Incubate and shake the plate at 500–1000 rpm at 70 °C for 5 minutes.



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

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

7. Pipette the DNA-containing liquid phase into a new 96-well plate or new 1.5-mL tubes.



F

Safety

This appendix covers:

- Instrumentation safety 94
- Chemical safety 94
  - General chemical safety 94
  - SDSs 95
  - Chemical waste safety 95
  - Biological hazard safety 97
- Safety alerts 97
  - Specific alerts for chemicals 97
  - General alert for instrumentation. 97

Instrumentation safety

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

Chemical safety

General chemical safety

Chemical hazard warning

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

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

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

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

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 95.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

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

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

Obtaining SDSs

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

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

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

Chemical waste safety

Chemical waste hazards

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

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

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

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

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

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

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://www.cdc.gov/biosafety/publications/bmbl5/index.htm**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Safety alerts

Specific alerts for chemicals

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.

General alert for instrumentation

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

Documentation and Support

Related documentation

The following related documents are shipped with the system:

Document	Part number	Description
PrepFiler Forensic DNA Extraction Kit User Guide	4390932	Provides an overview of manual procedures for extraction of genomic DNA, and results of the experiments performed by Applied Biosystems during the development of the PrepFiler kit.
PrepFiler Forensic DNA Extraction Kit Quick Reference Card	4393918	Provides brief, step-by-step manual procedures for isolating genomic DNA. It is designed to be used as a reference in the laboratory after you become familiar with the content in the User Guide.
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	4457144	Describes the validation studies performed by Applied Biosystems to validate PrepFiler™ Wash Buffer B and the related worktable and script modifications.
HID EVOlution™ – qPCR/STR Setup System Getting Started Guide	4426903	Describes automated quantitation setup and amplification setup on the Freedom EVO instrument with the HID EVOlution™ – qPCR/STR Setup System

The following related documents are available from Tecan:

Document	Document ID	Description
Tecan HID EVOlution® – Extraction Application Manual	395372	Includes PrepFiler kit-specific safety information, operating procedures, and maintenance and troubleshooting information.
Tecan HID EVOlution® – Extraction Installation Manual	395392	Includes PrepFiler kit-specific information for installing and setting up the instrument.
Tecan Freedom EVO® Operating Manual	392886	Includes comprehensive safety information, operating procedures, and maintenance and troubleshooting information for the Freedom EVO® instrument.
Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Manual Limited Device Support (General Purpose)	393804	Includes comprehensive information on installing, setting up, running, and programming the EVOware® software.
Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Manual Extended Device Support (Research Use Only)	393172	

Document	Document ID	Description
Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Getting Started Guide	393318	Includes procedures for installing and running the EVOware® software
Tecan Freedom EVOware® Standard 2.1/Plus 2.1 Software Runtime Controller Manual	394329	Includes procedures for running scripts and information on error messages.

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**

Note: For additional documentation, see "Obtaining support" on page 101.

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 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 Applied Biosystems web site, you can:

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

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applied biosystems™
by life technologies™

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone 760.603.7200
www.lifetechnologies.com

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