USER GUIDE

applied biosystems® by life technologies™

PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits

for use with: AutoMate *Express*™ Forensic DNA Extraction System

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life technologies™

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About This Guide

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

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

Purpose

This guide is intended for scientists who isolate DNA from forensic samples for applications such as quantitation, Short Tandem Repeat (STR) analysis, and SNP genotyping.

This guide provides:

- Step-by-step instructions for using the PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits for:
  - Manual preparation of lysate from forensic samples
  - Automated extraction and isolation of genomic DNA on the AutoMate *Express*™ Instrument
- A description of the experiments performed by Life Technologies to validate the performance of the PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits with the AutoMate *Express*™ Instrument.

User attention words

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

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

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

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

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

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

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

1 About the Kits

This chapter covers:

PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits description

The PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits contain reagents and plastics needed for:

- Manual lysate preparation
- Automated DNA extraction and purification with the AutoMate *Express*™ Forensic DNA Extraction System

The kits are designed for extracting and purifying DNA from a variety of forensic sample types:

- The **PrepFiler® *Express* Forensic DNA Extraction Kit** is designed for common forensic sample types, including body fluid stains and swabs of body fluids.
- The **PrepFiler® *Express* BTA Forensic DNA Extraction Kit** is designed for challenged forensic sample types such as bone, teeth, and adhesive-containing substrates including cigarette butts, chewing gum, and tape lifts.

The kits are appropriate for use with samples containing potential inhibitors of the polymerase chain reaction (PCR). The extracted DNA is compatible for use in quantitation using the Quantifiler® Human, Quantifiler® Y Human Male, and Quantifiler® Duo DNA Quantification Kits, and STR amplification using the AmpFℓSTR® PCR Amplification kits.

Note: For more information on the PrepFiler® kits extraction chemistry, see the manual lysis and extraction protocols in the *PrepFiler® Forensic DNA Extraction Kit User Guide* (Part no. 4390932).

AutoMate *Express*™ Forensic DNA Extraction System

The AutoMate *Express*™ Forensic DNA Extraction System allows automated, fast, and reliable DNA extraction from up to 13 samples in about 30 minutes. In addition to the PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits, the system consists of:

- **AutoMate *Express*™ Instrument** – The AutoMate *Express*™ Instrument is compatible with the PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits.
- **PrepFiler® *Express* & PrepFiler® *Express* BTA Protocol Card** – This protocol card (provided with the AutoMate *Express*™ Instrument) is pre-programmed with the required extraction protocols for purification of nucleic acids from forensic-type samples. The protocol card directs the movement of the instrument components, the volume of reagents used, and the incubation time.

DNA extraction workflow

Table 1 shows the steps for preparing the sample lysate and running automated DNA extraction. During automated extraction, the AutoMate *Express*™ Instrument:

- Mixes the sample lysate with magnetic particles and other reagents for subsequent DNA binding to magnetic particles in tips
- Separates the DNA-bound magnetic particles from the lysate using magnetic separation
- Thoroughly washes the magnetic particles with wash buffers to remove PCR inhibitors
- Dries the magnetic particles to remove ethanol
- Elutes concentrated purified DNA in elution buffer

Table 1 Lysis and extraction workflow.

Prepare Sample Lysate (see Chapter 2 on page 15)
Prepare samples for lysis (if necessary)
↓
Perform lysis
↓
Remove the substrate from the sample lysate (if necessary)
↓
Set Up and Run Automated DNA Extraction (see Chapter 3 on page 25)
Insert the protocol card and power on the instrument
↓
Load and insert the cartridge rack
↓
Load samples and elution tubes and insert the tip and tube rack
↓
Start the automated extraction run
↓
Store the extracted DNA

Kit contents and storage conditions

Kit contents

Each PrepFiler® *Express* or PrepFiler® *Express* BTA kit contains materials sufficient to perform 52 extractions. The contents of the kits are described in Table 2.

Table 2 PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits Components (volumes sufficient for 52 extractions)

Part	Description	Included with PrepFiler® *Express* Forensic DNA Extraction Kit (Part no. 4441352)	Included with PrepFiler® *Express* BTA Forensic DNA Extraction Kit (Part no. 4441351)
PrepFiler® Lysis Buffer	One bottle, 27 mL	✔	
PrepFiler® BTA Lysis Buffer	One bottle, 13 mL		✔
PrepFiler® *Express* Cartridges	52 cartridges	✔	✔
PrepFiler® Sample Tubes	Minimum 52 pieces	✔	✔
PrepFiler® Elution Tubes	Minimum 52 pieces	✔	✔
PrepFiler® LySep Columns	Minimum 52 pieces	✔	✔
AutoMate *Express*™ Tips and Tip Holders	Minimum 52 sets	✔	✔
PrepFiler® Bone and Tooth Lysate Tubes	Minimum 52 pieces		✔
PrepFiler® Bone and Tooth Lysate Tube Caps	Minimum 52 pieces		✔
Proteinase K	One tube, 400 µL, (concentration 20 mg/mL)		✔

Kit storage conditions

Store all PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits components at ambient temperature (18°C 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® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits.

Table 3 Materials and instruments required but not provided

Item	Source†
AutoMate *Express*™ Instrument (includes the PrepFiler® *Express* & PrepFiler® *Express* BTA Protocol Card)	Life Technologies (contact your local sales representative)
Common laboratory equipment such as pipettors, aerosol-resistant micropipette tips, and a microcentrifuge	Major laboratory supplier (MLS)
Vortexer (a variable-speed vortexer is recommended)	MLS
DL-Dithiothreitol [Molecular biology grade; ≥98% (TLC), ≥99% (titration)]	Sigma-Aldrich‡ www.sigmaaldrich.com (Part No. D9779)
Eppendorf Thermomixer	Eppendorf North America www.eppendorfna.com (Part No. 21516-170) *or* MLS
Laboratory centrifuge capable of 10,000 x g	MLS

† Recommended sources. Equivalent materials from other suppliers can be used after appropriate validation studies by the user laboratory.

‡ For the SDS of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Accessory products

The following products are available for purchase separately from Life Technologies.

Table 4 Accessory products

Item	Part Number
PrepFiler® *Express* & PrepFiler® *Express* BTA Protocol Card	4445165
AutoMate *Express*™ Tip and Tube Rack	4456842
AutoMate *Express*™ Cartridge Rack	4452767
D-ring Exchange Tools	4457423
D-rings, (set of 13)	4448950
AutoMate *Express*™ Install Kit	4441350

2 Prepare Sample Lysate

This chapter covers:

Required materials for lysis and substrate removal

Note: The AutoMate *Express*™ Instrument can run only samples prepared with either the PrepFiler® *Express* kit or the PrepFiler® *Express* BTA kit at one time. Batch the samples accordingly.

- Laboratory equipment and materials listed in "Required materials and instruments" on page 13.
- Sterile tweezers or other tools for transferring samples to PrepFiler® LySep Columns.
- PrepFiler® Sample Tubes
- DTT
- For body fluid samples:
  - PrepFiler® Lysis Buffer
  - PrepFiler® LySep Columns
- For bone or tooth samples:
  - PrepFiler® BTA Lysis Buffer
  - Proteinase K
  - PrepFiler® Bone and Tooth Lysate Tubes and Tube Caps
- For adhesive-containing samples:
  - PrepFiler® BTA Lysis Buffer
  - Proteinase K
  - PrepFiler® LySep Columns

Note: Avoid using an expired kit. Always use the kits before the specified expiration date printed on the package.

Body fluids protocol

For use with kit

PrepFiler® *Express* Forensic DNA Extraction Kit

Sample types and inputs

The PrepFiler® *Express* 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 5. Optimal input amounts may be affected by factors such as sample age and substrate properties. Each lab should perform studies to independently validate input amounts.

Table 5 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
Hair root	Up to 5 mm cutting from root

‡ It is not necessary to use an entire sample punch or swab.

Perform lysis

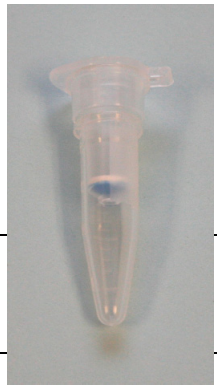
1. If the PrepFiler® Lysis Buffer contains precipitate, heat the solution to 37°C, then vortex the bottle for 5 seconds.
2. Bring the thermal shaker temperature to 70°C.
3. Prepare a fresh 1.0 M DTT solution by dissolving 1.54 g of Dithiothreitol (DTT, MW 154) in 10 mL of molecular-biology grade DNA-free water.

   Note: Alternatively, thaw an aliquot of the desired volume (for example, 100 µL or 500 µL) that you previously prepared fresh, then stored at –20°C for no more than six months.
4. Prepare a fresh PrepFiler® lysis solution. Each sample requires:
   - 500 µL PrepFiler® Lysis Buffer
   - 5 µL freshly-prepared 1 M DTT

   Note: After completing the lysis step, discard unused PrepFiler® lysis solution and DTT.

> ⚠ **WARNING!** Do not add acids, or bases (such as bleach) to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.

5. Insert a PrepFiler® LySep Column into a hingeless PrepFiler® sample tube (together called the "column/tube assembly"), then carefully transfer the sample into the PrepFiler® LySep column.



6. Add 500 µL of freshly-prepared PrepFiler® lysis solution to the column/tube assembly.

IMPORTANT! For effective DNA recovery, make sure that the entire sample is submerged in the lysis solution.

7. Tightly close the lid of the column/tube assembly.
8. Place the column/tube assembly in a thermal shaker, then incubate it at 70°C and 750 rpm for 40 minutes.

   Note: Exceeding the recommended 40 minute incubation time may result in salt precipitation from the lysis buffer before or after centrifugation, potentially leading to instrument crash, tip clogging, or tip filter wetting. If precipitation occurs or the incubation time exceeds 40 minutes, see "Troubleshooting" on page 55 for suggestions for preventing and/or dissolving precipitated salts.

Remove the substrate from the sample lysate

1. Centrifuge the column/tube assembly for 2 minutes at 10,000 × g to transfer the lysate to the sample tube.
2. If the volume of sample lysate collected in the sample tube is less than 300 µL:
   a. Centrifuge the column/tube assembly for an additional 5 minutes.
   b. If the volume is still less than 300 µL, then add PrepFiler® Lysis Buffer to bring the lysate volume to 300 µL.

IMPORTANT! A 300-µL lysate volume is necessary for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated extraction run. Lower lysate volume may cause liquid handling problems.

3. Complete substrate removal as follows:
   a. Carefully remove the PrepFiler® LySep column from the sample tube. If there is clear lysate remaining in the PrepFiler® LySep column, transfer the lysate to the sample tube.

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

      Note: Change gloves frequently when handling tubes. For example, change gloves after removing the PrepFiler® LySep column from the sample tube.

   b. Properly dispose of the PrepFiler® LySep column. Used LySep columns are potentially biohazardous.

c. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler® Sample Tube.

IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated extraction run.

d. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. Do not load any sample tube that contains precipitate on the AutoMate *Express*™ Instrument, or instrument crash, tip clogging, or filter wetting may occur.

4. Proceed directly to the automated extraction run.

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

Bone and tooth protocol

For use with kit

PrepFiler® *Express* BTA Forensic DNA Extraction Kit

Sample types and inputs

The PrepFiler® *Express* BTA Forensic DNA Extraction Kit is appropriate for forensic bone and tooth samples. Examples of appropriate sample types and inputs are shown in Table 6. 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 6 Example sample types and inputs

Sample type	Example sample input
Bone	Up to 50 mg powdered bone
Tooth	Up to 50 mg powdered tooth

Prepare samples for lysis

1. Clean the bone or tooth sample to remove any adhered tissue.
2. Prepare a uniform bone or tooth powder using standard laboratory procedures.
3. Transfer approximately 50 mg of powdered bone or tooth into a new PrepFiler® Bone and Tooth Lysate Tube.

Perform lysis

1. Bring the thermal shaker temperature to 56°C.
2. Prepare a fresh 1.0 M DTT solution by dissolving 1.54 g of Dithiothreitol (DTT, MW 154) in 10 mL of molecular-biology grade DNA-free water.

   Note: Alternatively, thaw an aliquot of the desired volume (for example, 100 µL or 500 µL) that you previously prepared fresh, then stored at –20°C for no more than six months.
3. Prepare a fresh PrepFiler® BTA lysis solution. Each sample requires:
   - 220 µL PrepFiler® BTA Lysis Buffer
   - 3 µL freshly-prepared 1 M DTT
   - 7 µL Proteinase K

   Note: After completing the lysis step, discard unused PrepFiler® BTA lysis solution and DTT.

   ⚠ **WARNING!** Do not add acids, or bases (such as bleach) to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.
4. Add 230 µL of freshly-prepared PrepFiler® BTA lysis solution to the PrepFiler® Bone and Tooth Lysate Tube containing the bone or tooth sample.

5. Screw the cap on the PrepFiler® Bone and Tooth Lysate Tube, vortex it for 5 seconds, then centrifuge it briefly.

   Note: To avoid leaks, make sure that tubes are tightly sealed before vortexing and incubating the tubes.

   To avoid forming a pellet, do not centrifuge longer than 5 seconds.

   After vortexing a tube, check the tube for air bubbles, then re-vortex if necessary to remove bubbles.

6. Place the PrepFiler® Bone and Tooth Lysate Tube in a thermal shaker, then incubate it at 56°C and 1100 rpm for at least 2 hours (sample can be incubated up to 18 hours).

Remove the substrate from the sample lysate

1. Centrifuge the PrepFiler® Bone and Tooth Lysate Tube for 90 seconds at 10,000 × g.
2. Transfer the clear (no sediment) lysate to a new PrepFiler® Sample Tube.

   IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated extraction run.

3. If the volume of sample lysate collected in the sample tube is less than 150 µL, then add PrepFiler® BTA Lysis Buffer to bring the lysate volume to 150 µL.

   IMPORTANT! A 150-µL lysate volume is necessary for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated extraction run. Lower lysate volume may cause liquid handling problems.

4. Proceed directly to the automated extraction run.

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

Adhesive substrates protocol

For use with kit

PrepFiler® *Express* BTA Forensic DNA Extraction Kit

Sample types and inputs

The PrepFiler® *Express* BTA Forensic DNA Extraction Kit is appropriate for most adhesive forensic sample types, including chewing gum, cigarette butts, and tape lift samples. Examples of appropriate sample types and inputs are shown in Table 7. 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 7 Example sample types and inputs

Sample type	Example sample input
Chewing gum	Up to 50 mg (approximately 3×3×5-mm^3 piece)
Cigarette butt	Up to 25-mm^2 cutting of cigarette filter paper **IMPORTANT!** Remove all filter material from the filter paper.
Tape lifts	Up to 2 cm^2 cutting with saliva or blood

Prepare samples for lysis

1. Insert a PrepFiler® LySep Column into a hingeless PrepFiler® sample tube (together called the "column/tube assembly").
2. Follow the appropriate procedure to prepare the sample for lysis.

Sample Type	Sample Preparation
Chewing gum	1. In a clean Petri disk, flatten the piece of gum into a pancake shape of approximately 5-mm thickness. 2. Cover the Petri dish, tape it closed, then place the dish in a -80°C freezer for at least 2 hours. 3. Cut and transfer up to 50 mg of gum (approximately 3 mm^2) into the PrepFiler® LySep column prepared in step 1.
Cigarette butt	1. Remove the first 5-mm of filter paper from the end of the cigarette butt, making sure to remove all the filter fibers. 2. Cut the filter paper into 2 to 3 pieces. 3. Transfer the all of the pieces into the PrepFiler® LySep column prepared in step 1.
Tape lift	1. Cut the tape with a razor blade as needed to fit into the PrepFiler® LySep column. 2. Transfer the tape into the PrepFiler® LySep column prepared in step 1. **IMPORTANT!** Make sure that the side of the tape containing the sample does not adhere to the side of the column.

Perform lysis

1. Bring the thermal shaker temperature to 56°C.
2. Prepare a fresh 1.0 M DTT solution by dissolving 1.54 g of Dithiothreitol (DTT, MW 154) in 10 mL of molecular-biology grade DNA-free water.

   Note: Alternatively, thaw an aliquot of the desired volume (for example, 100 µL or 500 µL) that you previously prepared fresh, then stored at –20°C for no more than six months.
3. Prepare a fresh PrepFiler® BTA lysis solution. Each sample requires:
   - 220 µL PrepFiler® BTA Lysis Buffer
   - 3 µL freshly-prepared 1 M DTT
   - 7 µL Proteinase K

   Note: After completing the lysis step, discard unused PrepFiler® BTA lysis solution and DTT.

   WARNING! Do not add acids, or bases (such as bleach) to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.
4. Add 230 µL of freshly-prepared PrepFiler® BTA lysis solution to the column/tube assembly containing the sample.

   IMPORTANT! For effective DNA recovery, make sure that the entire sample is submerged in the lysis solution.
5. Tightly close the lid of the column/tube assembly.
6. Place the column/tube assembly in a thermal shaker, then incubate it at 56°C and 750 rpm for 40 minutes.

Remove the substrate from the sample lysate

1. Centrifuge the column/tube assembly for 2 minutes at 10,000 × g to transfer the lysate to the sample tube.
2. If the volume of sample lysate collected in the sample tube is less than 150 µL:
   a. Centrifuge the column/tube assembly for an additional 5 minutes.
   b. If the volume is still less than 150 µL, then add PrepFiler® BTA Lysis Buffer to bring the lysate volume to 150 µL.

   IMPORTANT! A 150-µL lysate volume is necessary for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated extraction run. Lower lysate volume may cause liquid handling problems.
3. Complete substrate removal as follows:

a. Carefully remove the PrepFiler® LySep column from the sample tube. If there is clear lysate remaining in the PrepFiler® LySep column, transfer the lysate to the sample tube.

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

   Note: Change gloves frequently when handling tubes. For example, change gloves after removing the PrepFiler® LySep column from the sample tube.

b. Properly dispose of the PrepFiler® LySep column. Used LySep columns are potentially biohazardous.

c. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler® Sample Tube.

   IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated extraction run.

4. Proceed directly to the automated extraction run.

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

3 Set Up and Run Automated DNA Extraction

This chapter covers procedures for setting up and running extraction on the AutoMate *Express*™ Instrument:

Note: For detailed instrument operation and troubleshooting information, see the *AutoMate Express*™ *Instrument User Guide*.

Required materials for extraction

- AutoMate *Express*™ Instrument
- PrepFiler® *Express* & PrepFiler® *Express* BTA Protocol Card (provided with instrument)
- From the appropriate kit (PrepFiler® *Express* or PrepFiler® *Express* BTA kit):
  - PrepFiler® *Express* Cartridges

    The cartridges are designed to fit onto the cartridge rack in only one orientation. Cartridges have 10 sealed wells and 2 open wells used for heating tubes. Wells 1 through 7 are pre-filled with the PrepFiler® *Express* reagents required for the protocol (see Figure 1 on page 26); the remaining wells are empty.
  - PrepFiler® Sample Tubes containing the sample lysate prepared according to Chapter 2 on page 15
  - PrepFiler® Elution Tubes
  - AutoMate *Express*™ Tips and Tip Holders

Note: See "Required materials and instruments" on page 13 for details.

Set up the AutoMate *Express*™ Instrument for DNA extraction

Before a run, follow these procedures to set up the instrument:

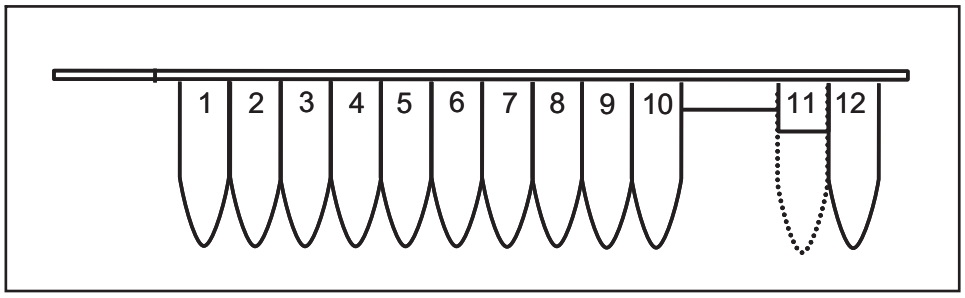
1. "Inspect the PrepFiler® Express cartridges" on page 26.
2. "Insert the protocol card and power on the instrument" on page 27.
3. "Load and insert the cartridge rack" on page 28.
4. "Load samples and elution tubes and insert the tip and tube rack" on page 30.

Note: The AutoMate *Express*™ Instrument can run only one DNA extraction option, either the PF Express option or the PF Express BTA option, at one time. Batch the samples accordingly.

Inspect the PrepFiler® *Express* cartridges

If precipitate forms in compartments 1 or 2 (lysis buffer and magnetic particle suspension), heat the cartridge in an incubator at 37°C for 30 minutes or until the precipitate is no longer visible.

Figure 1 PrepFiler® *Express* cartridge compartments



Compartment	Contents
1	Lysis buffer
2	Magnetic particle suspension
3	Binding solution
4 through 6	Wash buffer
7	Elution buffer
12	Heated chamber for elution

Insert the protocol card and power on the instrument

IMPORTANT! Do not remove or insert the protocol card while the instrument is powered on. Removing the card stops the run, and it may cause instrument data file loss.

If you accidentally remove the protocol card during a run, power off the instrument immediately to minimize potential for instrument data loss.

For guidelines on removing and handling protocol cards, see the *AutoMate Express™ Instrument User Guide*.

1. Confirm that the power switch is in the OFF position.

   Note: If you insert the card while the instrument is on, the instrument does not recognize the card.

2. Open the card slot.

3. Insert the PrepFiler® *Express*and PrepFiler® *Express* BTA Protocol Card in the slot with the arrow pointing toward the instrument and the card label facing left.

   IMPORTANT! Do not remove or insert the AutoMate *Express*™ protocol card while the instrument is powered on.

4. Push the card completely into the card slot, then close the card slot.
5. Power on the instrument.

   When the card is fully inserted in the correct orientation, the display briefly shows information including the instrument version, then displays the Main menu.

6. Press **Start.**

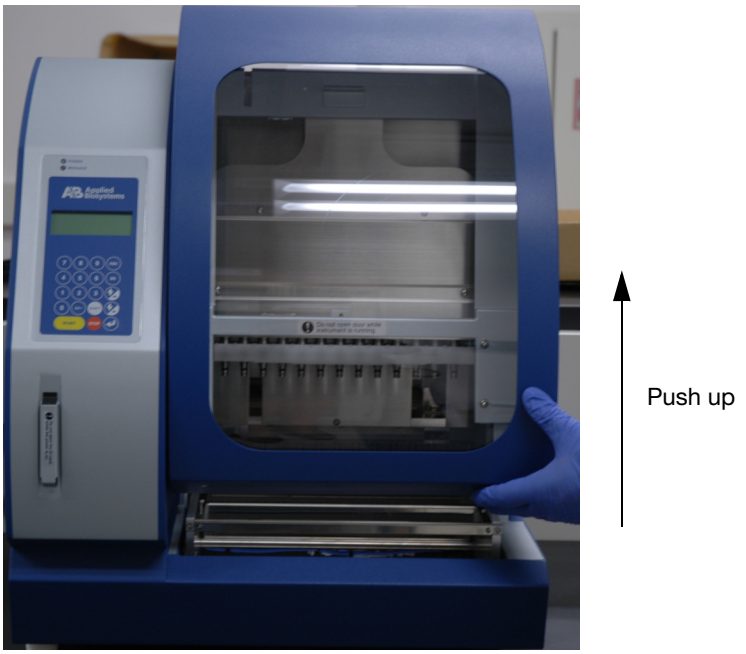
   Note: Press  after following each on-screen prompt.

Load and insert the cartridge rack

Wear gloves when you handle samples or load the cartridges, tips, and tubes in the racks.

Note: To ensure the best pipetting performance, use the cartridge rack and tip and tube rack shipped with the instrument; these racks are calibrated with the instrument at the factory. Before using other racks on a specific instrument, run the installation test to qualify the racks for use on that instrument. Refer to the *AutoMate Express™ Instrument User Guide* for details.

1. Open the instrument door (push up the door), then remove the cartridge rack and tip and tube rack from the instrument.



2. Remove up to 13 cartridges from the kit box.

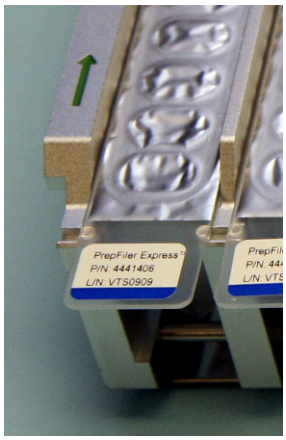
   Note: One cartridge is required per sample. Use only Applied Biosystems® PrepFiler® *Express* cartridges.

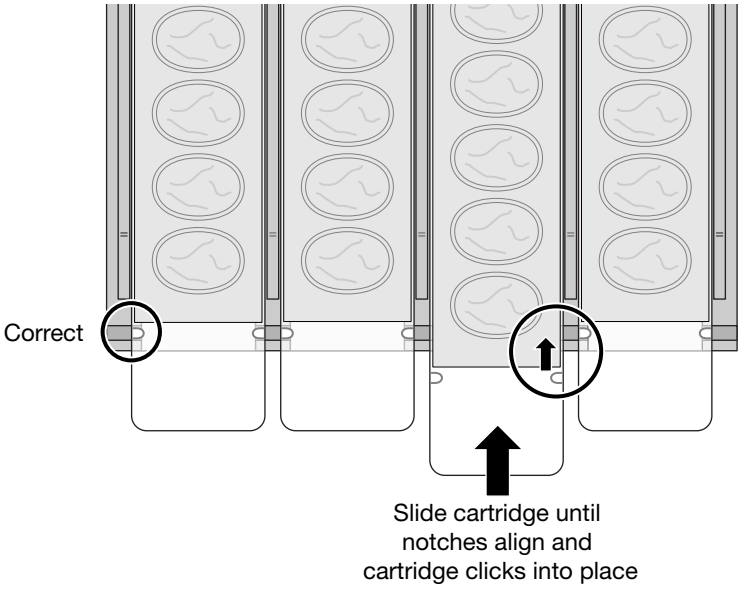
   Note: Do not switch the supplied pre-filled reagents with any other buffers, because the protocols are specifically optimized with the reagents supplied with the kit.

3. Shake and tap the reagent cartridges to resuspend the magnetic particles and to deposit any particles or liquid droplets underneath the foil seal into the compartments. See Figure 1 on page 26.

4. Load the pre-filled reagent cartridges into the cartridge rack by sliding each reagent cartridge along the groove in the direction of the arrow until the reagent cartridge clicks into place. Make sure that the notches in the cartridge align with the notches in the cartridge rack (see the following diagram).

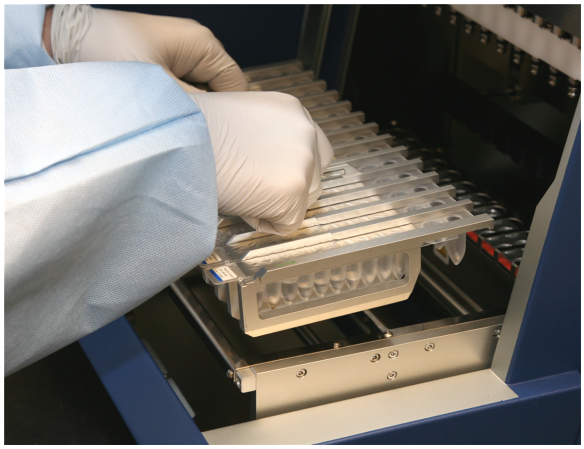
   Note: An incorrectly loaded cartridge rack may cause the instrument to stop during a protocol run.

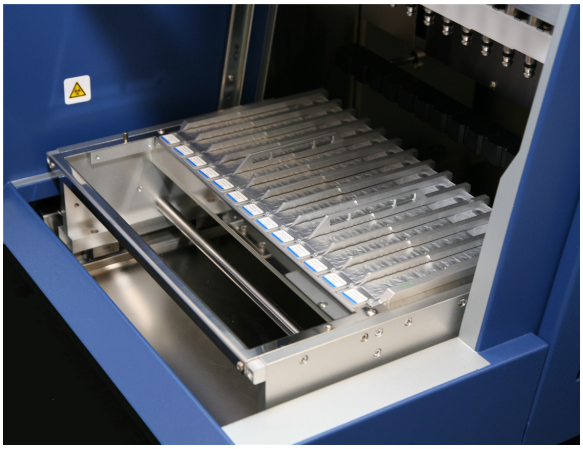




5. Insert the loaded cartridge rack into the instrument.

WARNING! Do not touch the surface of the heat block. The temperature of the heat block can reach 95°C. Touching the block can cause burns.





Load samples and elution tubes and insert the tip and tube rack

IMPORTANT! Follow these guidelines to avoid potential problems during the automated extraction run:

- Load the cartridge rack into the instrument first followed by the tip and tube rack. Changing the order of loading the racks may cause the instrument to stop during a run.
- Use only Applied Biosystems® PrepFiler® Sample Tubes and Elution Tubes. Other tubes may be picked up by the nozzle tips due to difference in tube height and shape, stopping the run.
- Before loading each sample tube in the rack, make sure that no salt precipitation is visible in the sample tube. Precipitate in sample tubes may cause instrument crash, tip clogging, or filter wetting. See "Troubleshooting" on page 55 for suggestions on preventing and/or dissolving precipitated salts.
- If you are processing fewer than 13 samples, make sure to load the tips and tubes in the same positions as the reagent cartridges that are loaded in the cartridge rack.

Note: Press  after following each on-screen prompt.

1. Load the tip and tube rack:
   a. **Row S** (fourth row): Load with the PrepFiler® sample tubes containing the lysate.

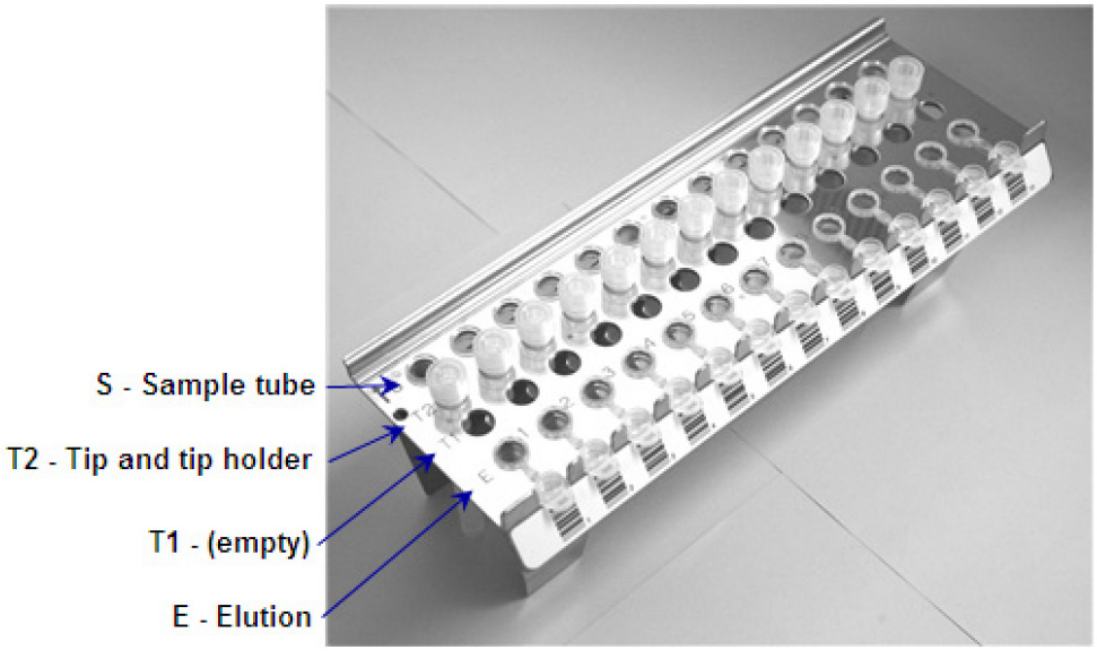
      Note: Make sure that the PrepFiler® LySep columns have been removed from the sample tubes.

   b. **Row T2** (third row): Load with AutoMate *Express*™ Tips inserted into tip holders.

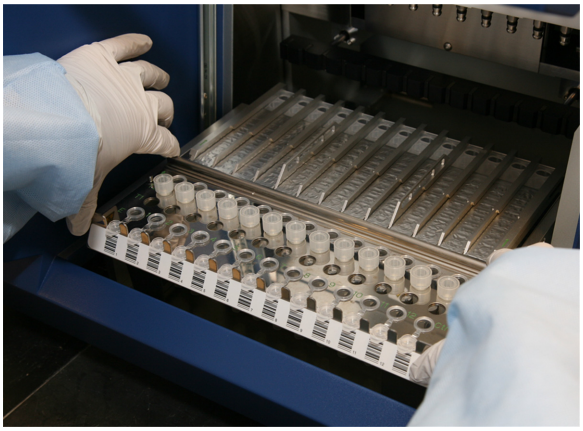
      Note: One tip and tip holder set is required per sample.

   c. **Row T1** (second row): Leave empty.

d. **Row E** (first row): Load with labeled PrepFiler® elution tubes, with the caps open and secured as shown in the following photo.



2. Insert the loaded tip and tube rack into the instrument with row E in the front as shown in the following photos.



Start the automated extraction run

1. Confirm that you have inserted the cartridge rack and tip and tube rack correctly, then close the instrument door.
2. Press , then, if you are using the:
   - **PrepFiler® *Express* kit –** Press **1** to select the PF Express option.

     or
   - **PrepFiler® *Express* BTA kit –** Press **2** to select the PF Express BTA option.

IMPORTANT! For correct operation, make sure that the option matches the kit you are using.

3. Press **Start**.

   The screen shows the steps and the approximate run time remaining.

IMPORTANT! Do not open the door during a protocol run. To pause or cancel the run, see the *AutoMate Express™ Instrument User Guide*.

Note: If you lose power or the power cord is unplugged, the run stops. When the power resumes, the screen displays the Main menu. You cannot resume the run. If the tips are still on the syringe unit when the power resumes, return the tips to the original position as described in the *AutoMate Express™ Instrument User Guide*.

See the *AutoMate Express™ Instrument User Guide* if necessary to troubleshoot issues during the run.

Store the extracted DNA

At the end of the run (the instrument beeps briefly and the digital display shows "Finished Protocol"):

1. Press  to return to the Main menu, then open the instrument door.
2. Remove the cartridge rack and tip and tube rack.
3. Remove and cap the elution tubes containing the purified DNA.

   Note: The isolated DNA can be stored at 4°C for up to two weeks, or at –20°C for longer storage.
4. Properly dispose of the used reagent cartridges, tips, and tubes.

WARNING! The used reagent cartridges may contain the following: guanidine thiocyanate, isopropanol, and ethanol. Refer to Safety Data Sheets and local, state, and national regulations for proper labeling, handling, and disposal.

WARNING! Do not add acids, or bases (such as bleach) to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.

5. Close the instrument door. After each run, clean the tip and tube rack as needed.

   Note: No cooling period is required between runs.

To perform a new run using a different AutoMate *Express™* Instrument protocol card, power off the instrument, then change the protocol card.

4 Experiments and Results

This chapter covers:

Overview

This chapter provides the results of the developmental validation experiments performed to evaluate the performance of the AutoMate *Express*™ Forensic DNA Extraction System (AutoMate *Express*™ System), which consists of the:

- PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits
- AutoMate *Express*™ Instrument
- PrepFiler® *Express* & PrepFiler® *Express* BTA Protocol Card

Life Technologies performed the experiments according to the Revised Validation Guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM) published in Forensic Science Communications Vol. 6, No. 3, July 2004 (**http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/standards/2004_03_standards02.htm/**). These guidelines describe the quality assurance requirements that a laboratory should follow to ensure the quality and integrity of the data and competency of the laboratory.

The experiments focused on kit performance parameters relevant to the intended use of the AutoMate *Express*™ System, that is, for extraction of genomic DNA from forensic samples as a part of a forensic DNA genotyping procedure. Each laboratory using the AutoMate *Express*™ System should perform appropriate internal validation studies.

Materials and methods

The following materials (details in Table 8) and methods were used in all experiments performed as part of the developmental validation:

- Liquid blood samples obtained from the Serological Research Institute (Richmond, California) were used to prepare all blood samples used in the studies. See each study for detailed sample descriptions.
- Lysis was performed using the appropriate kit for the sample type (PrepFiler® *Express* or PrepFiler® *Express* BTA Forensic DNA Extraction Kit) and following the appropriate procedures described in Chapter 2 of this guide.
- Genomic DNA was extracted from the lysed samples using the AutoMate *Express*™ Instrument following the procedures described in Chapter 3 of this guide. DNA was eluted with 50 µL of elution buffer. Extraction blanks were processed for each study.
- Extracted DNA was quantified using the Quantifiler® Duo DNA Quantification Kit on an Applied Biosystems® 7500 Real-Time PCR System. Samples of the same type were grouped together in the same qPCR plate to avoid introducing run-to-run variation. The quantitation results were analyzed using SDS v1.2.3.
- Quantified DNA was processed for STR profiling using the AmpF*ℓ*STR® Identifiler® PCR Amplification Kit. See each study for additional kits used.
- A total of 1 ng of human DNA or up to a maximum of 10 µL of extracted DNA was used for STR PCR amplification. Samples were amplified on a GeneAmp® 9700 thermal cycler. Electrophoresis was performed on Applied Biosystems® 3130*xl* Genetic Analyzers. The STR profiles were analyzed using GeneMapper® *ID-X* Software v1.0.

Table 8 Summary of materials used in the validation studies

Component	Description
Chemistry kits†	PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits (Part no.s 4441352 and 4441351)
	PrepFiler® Forensic DNA Extraction Kit (Part no. 4392852)
	Quantifiler® Duo DNA Quantification Kit (Part no. 4387746)
	AmpFℓSTR® Identifiler® PCR Amplification Kit (Part no. 4322288)
	AmpFℓSTR® MiniFiler™ PCR Amplification Kit (Part no. 4373872)
	AmpFℓSTR® Identifiler® Plus PCR Amplification Kit (Part no. 4427368)
Instruments and software	• AutoMate *Express*™ Instrument with the PrepFiler® *Express* & PrepFiler® *Express* BTA Protocol Card • HID EVOlution™ – qPCR/STR Setup System with Freedom EVOware™ v2.1 • 7500 Real-Time PCR System with SDS v 1.2.3 • GeneAmp® PCR System 9700 Thermal Cycler gold-plated silver 96-well block • 3130*xl* Genetic Analyzer with Data Collection Software v3.0 • GeneMapper® *ID*-X Software version 1.0 • Eppendorf Thermomixer® (Eppendorf North America)

† Identical lot numbers were used within each validation study.

Experiments and results

Sensitivity studies (SWGDAM standard 2.3)

Experiments

Sensitivity studies were performed to determine the range of biological sample amounts that can be reliably processed for extraction of genomic DNA using the AutoMate *Express*™ System.

DNA extractions were performed on four replicates of five volumes of liquid blood samples. One extraction blank was included in each extraction run (see sample layout in Table 9). All samples were processed using the PrepFiler® *Express* Forensic DNA Extraction Kit. Each sample set was extracted once, then quantified in duplicate.

Table 9 Sensitivity study sample sets shown by sample position in the tip and tube rack during extraction

Sample Position	Sample Set 1	Sample Set 2
E1	Blood, 5 µL	Blood, 0.1 µL (5 µL of a 1:50 dilution)
E2	Blood, 5 µL	Blood, 0.1 µL (5 µL of a 1:50 dilution)
E3	Blood, 5 µL	Blood, 0.1 µL (5 µL of a 1:50 dilution)
E4	Blood, 5 µL	Blood, 0.1 µL (5 µL of a 1:50 dilution)
E5	Blood, 1 µL	Blood, 0.025 µL (5 µL of a 1:200 dilution)
E6	Blood, 1 µL	Blood, 0.025 µL (5 µL of a 1:200 dilution)
E7	Blood, 1 µL	Blood, 0.025 µL (5 µL of a 1:200 dilution)
E8	Blood, 1 µL	Blood, 0.025 µL (5 µL of a 1:200 dilution)
E9	Blood, 0.25 µL (5 µL of a 1:20 dilution)	Extraction blank
E10	Blood, 0.25 µL (5 µL of a 1:20 dilution)	—
E11	Blood, 0.25 µL (5 µL of a 1:20 dilution)	—
E12	Blood, 0.25 µL (5 µL of a 1:20 dilution)	—
E13	Extraction blank	—

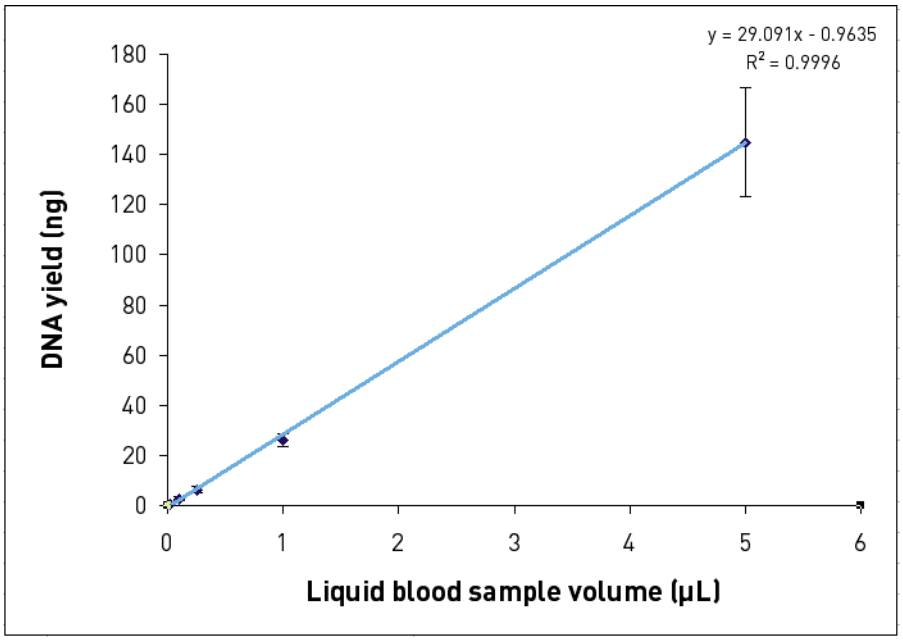
Results

Table 10 shows the average DNA yield for each replicate set. The DNA yield increased proportionately with increasing sample volumes, as shown in Figure 2. DNA was effectively recovered from the smallest sample amount tested (0.025 µL liquid blood). The efficiency of genomic DNA extraction remained linear up to the maximum volume of blood tested (5 µL).

Table 10 Sensitivity study average DNA yield

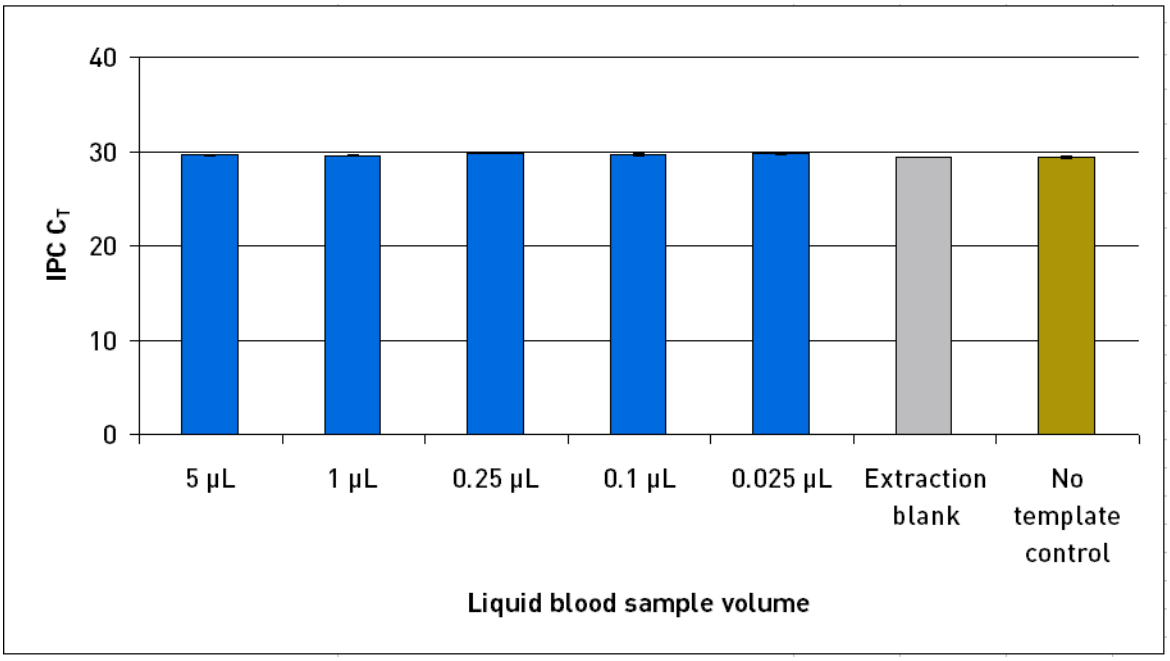
Blood Sample Volume (µL)	Average DNA Yield, ng (n=4)
5 µL	144.89
1 µL	26.03
0.25 µL (5 µL of a 1:20 dilution)	6.39
0.1 µL (5 µL of a 1:50 dilution)	2.68
0.025 µL (5 µL of a 1:200 dilution)	0.65
Extraction blank (XB)	0.00

Figure 2 Sensitivity study average DNA yield



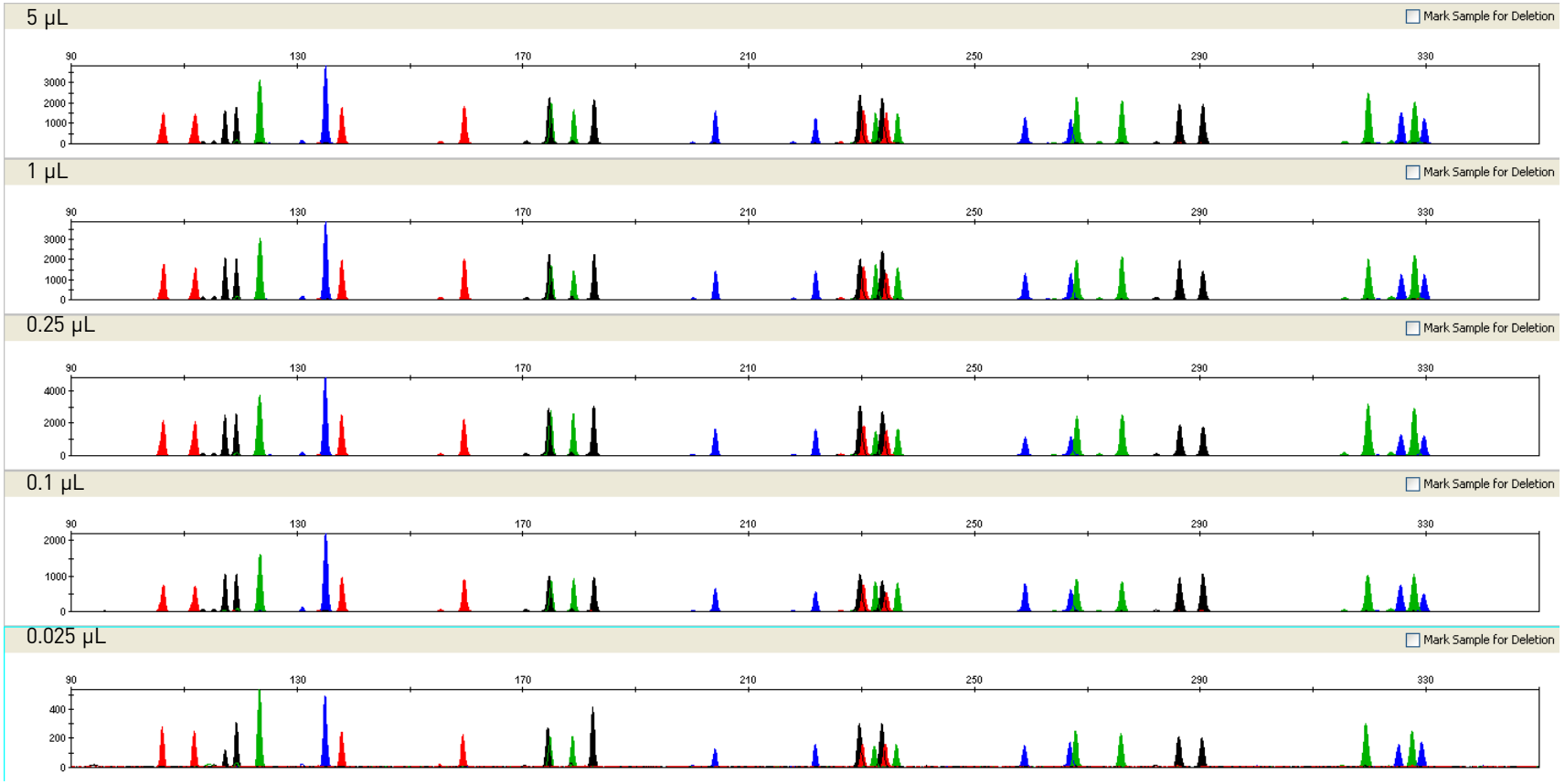
The IPC C_T values for the sensitivity study samples, extraction blanks, and quantitation negative controls (referred to as no template controls or NTCs) were compared to determine the presence or absence of detectable PCR inhibitors in DNA extracted using the AutoMate *Express*™ System. The IPC C_T values for the samples and NTCs were within ±1 C_T unit, indicating that PCR inhibitors were effectively removed during extraction. The IPC C_T values for the extraction blanks and NTCs were also within ±1 C_T unit, indicating that the PrepFiler® *Express* Forensic DNA Extraction Kit reagents did not introduce PCR inhibitors into the sample. A plot of the IPC C_T values for different liquid blood sample volumes is shown in Figure 3.

Figure 3 Sensitivity study IPC C_T



The quality of the DNA extract obtained from the AutoMate *Express*™ System was further evaluated by examining the STR profiles. Full, conclusive STR profiles were obtained from all samples (see Figure 4).

Figure 4 Sensitivity study STR profiles: 5 , 1, 0.25 , 0.1, and 0.025 µL blood samples



Stability studies (SWGDAM standard 2.4)

Experiments

Stability studies were performed to determine the ability of the AutoMate *Express*™ System to extract DNA and remove PCR inhibitors from samples subjected to environmental and chemical insults encountered in forensic samples. The following sample set was used:

- 1 µL blood on blue denim (Canyon River)
- 1 µL blood on cotton with inhibitor mix (12.5 mM indigo, 0.5 mM hematin, 2.5 mg/mL humic acid, and 300 mg/mL urban dust)
- 10 mg pulverized tooth (aged approximately 6 months), exposed to environment/light for 3 days
- 50 mg pulverized, aged bone

Bone and tooth samples were processed with the PrepFiler® *Express* BTA kit. All other sample types were processed with the PrepFiler® *Express* kit. DNA extractions were performed on three replicates of each of the four sample types. One extraction blank was included in each extraction run. Each sample set was extracted once, then quantified in duplicate.

Results

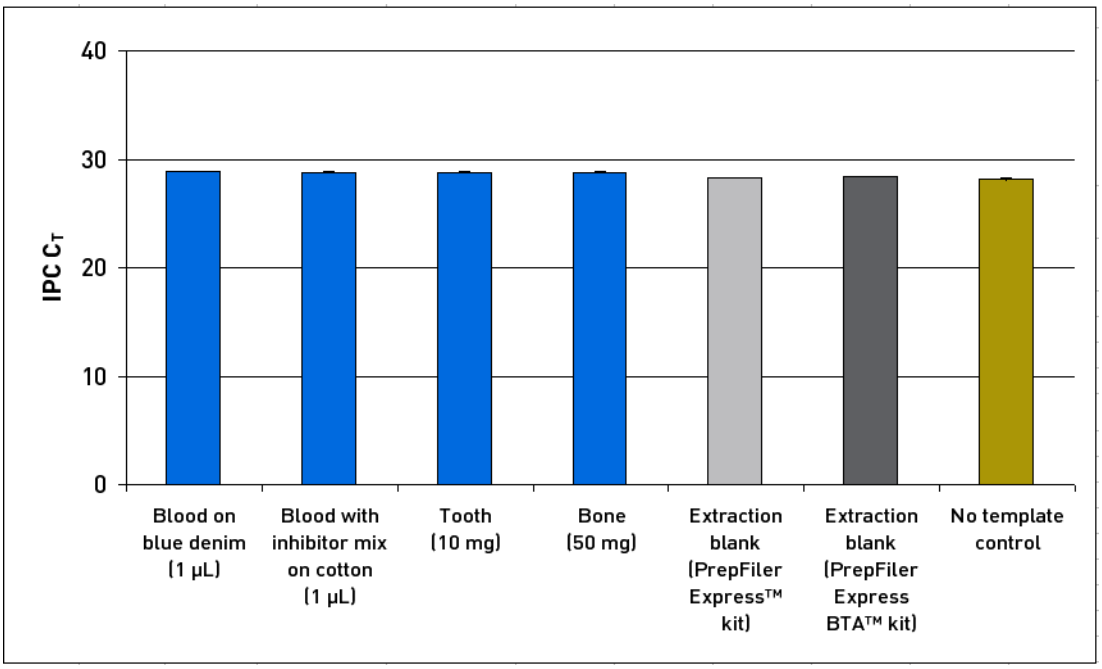
The average DNA concentration and yield for stability study samples are shown in Table 11. The variation in concentrations is within the expected variation introduced through the extraction and quantitation procedures.

Table 11 Stability study average DNA concentration and total yield

Sample Type	Average DNA Concentration (ng/µL)	Average Total Yield (ng)
1 µL blood on blue denim (Canyon river)	0.51	26.85
1 µL blood on cotton with inhibitor mix	0.46	23.76
10 mg pulverized tooth exposed to environment/light	0.16	8.36
50 mg pulverized, aged bone samples	0.01	0.49
Extraction blanks	0	0

Average IPC C_T values for each sample type are shown in Figure 5. The IPC C_T values for the samples and NTCs were within ±1 C_T unit, indicating that PCR inhibitors present in all tested sample types were effectively removed during the extraction of DNA using the AutoMate *Express*™ System.

Figure 5 Stability study IPC C_T



The quality of the DNA extract obtained from the AutoMate *Express*™ System was further evaluated by examining the STR profiles. STR profiles are shown in Figures 6 and 7 on page 42. Full and balanced STR profiles were obtained from blood on blue denim and blood on cotton with inhibitor mix samples (see top two panels of Figures 6).

A ski slope effect was observed for both tooth and bone samples (panels 3 and 4 of Figures 6). Allele drop-out was also observed for the larger loci of the bone samples. The ski slope effect and allele drop-out were still observed after analyzing the bone and tooth samples with AmpFℓSTR® Identifiler® Plus PCR Amplification Kit, which provides a high level of tolerance for known PCR inhibitors introduced by forensic samples (see Figure 7). Therefore, these effects are likely due to sample degradation.

Figure 6 Stability study STR profiles for samples processed with the AmpFℓSTR® Identifiler® PCR Amplification Kit

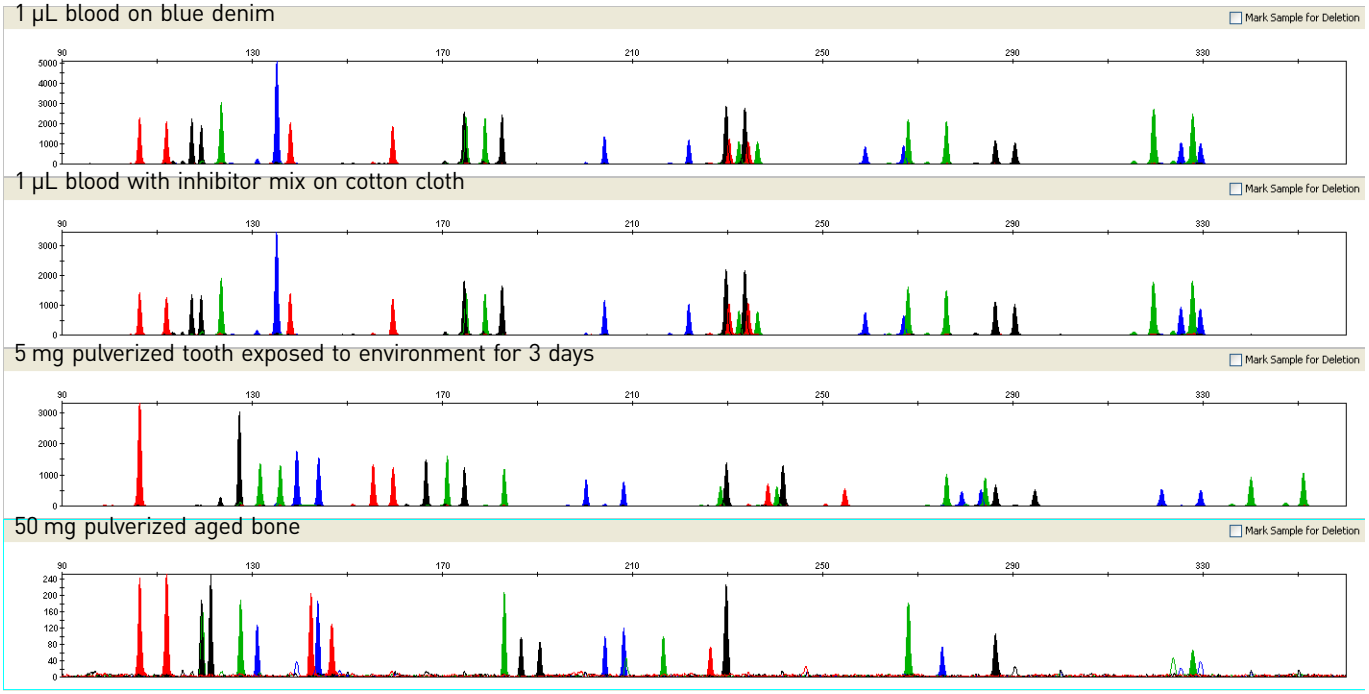
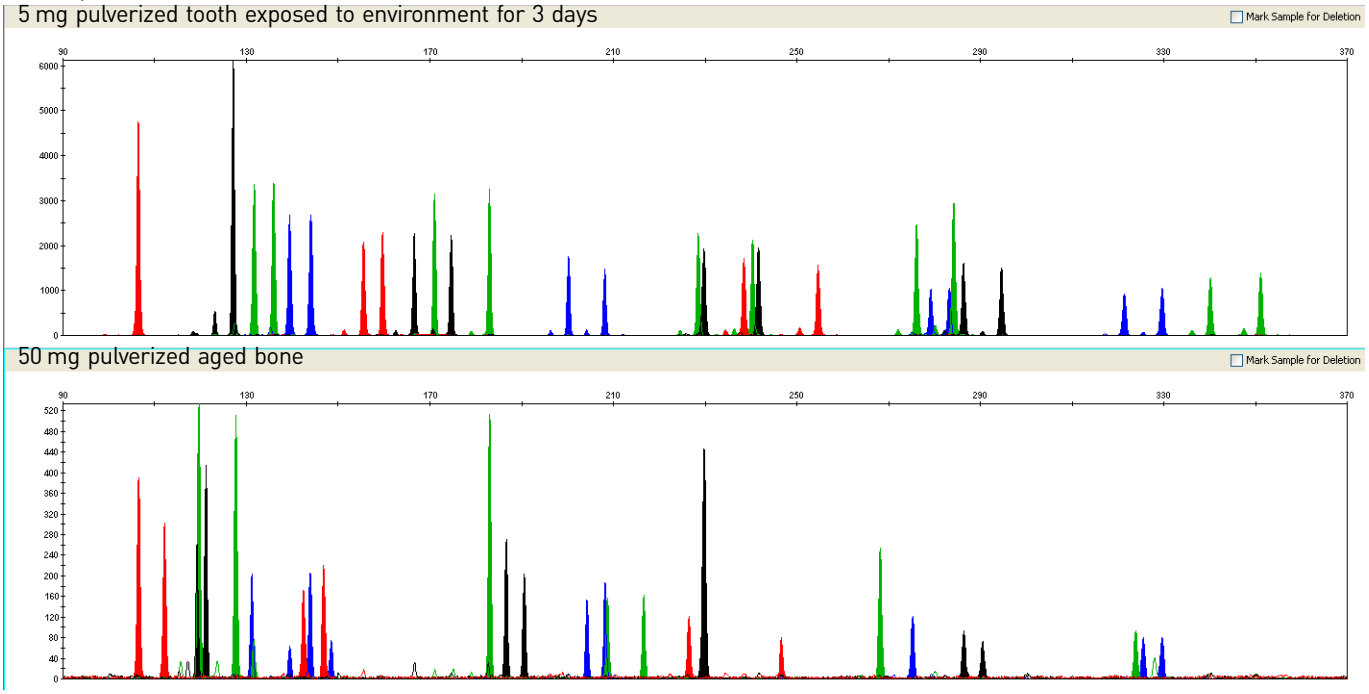


Figure 7 Stability study STR profiles for tooth and bone samples processed with the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit



Reproducibility study (SWGDAM standard 2.5)

Experiments

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

The reproducibility study sample sets are shown in Table 12. Bone and tooth samples were processed with the PrepFiler® *Express* BTA kit; all other sample types were processed with the PrepFiler® *Express* kit. DNA extractions were performed on four replicates of each of the five sample types. One extraction blank was included in each extraction run. Each sample set was extracted three times (once per day on three different days). Extracted samples from all extraction runs were placed in one plate for quantitation to avoid introducing run-to-run variation. Each extracted sample was quantified in duplicate.

Table 12 Reproducibility study sample sets

Sample Set 1 (PrepFiler® *Express* kit)	Sample Set 2 (PrepFiler® *Express* BTA kit)
5 µL epithelial-cell suspension on swab	5 mg pulverized tooth
1 µL semen on cotton cloth	50 mg bone
2 µL bloodstain on cotton	Extraction blank
Extraction blank	

Results

The average DNA yields for reproducibility study samples are shown in Figures 8 and 9 on page 44. Consistent DNA concentrations were obtained for each sample. The variation in concentrations is within the expected variation introduced through the extraction and quantitation procedures.

Average IPC C_T values for each sample type are shown in Figure 10 on page 45. The IPC C_T values for the samples and NTCs were within ±1 C_T unit, indicating that PCR inhibitors present in all tested sample types were effectively removed during the extraction of DNA using the AutoMate *Express*™ System.

The quality of the DNA extracts obtained from the AutoMate *Express*™ System was further evaluated by examining the STR profiles. Full and balanced STR profiles (not shown) were obtained from all samples, with the exception of bone samples, which did not return full profiles due to sample degradation.

Figure 8 Reproducibility study average DNA yield

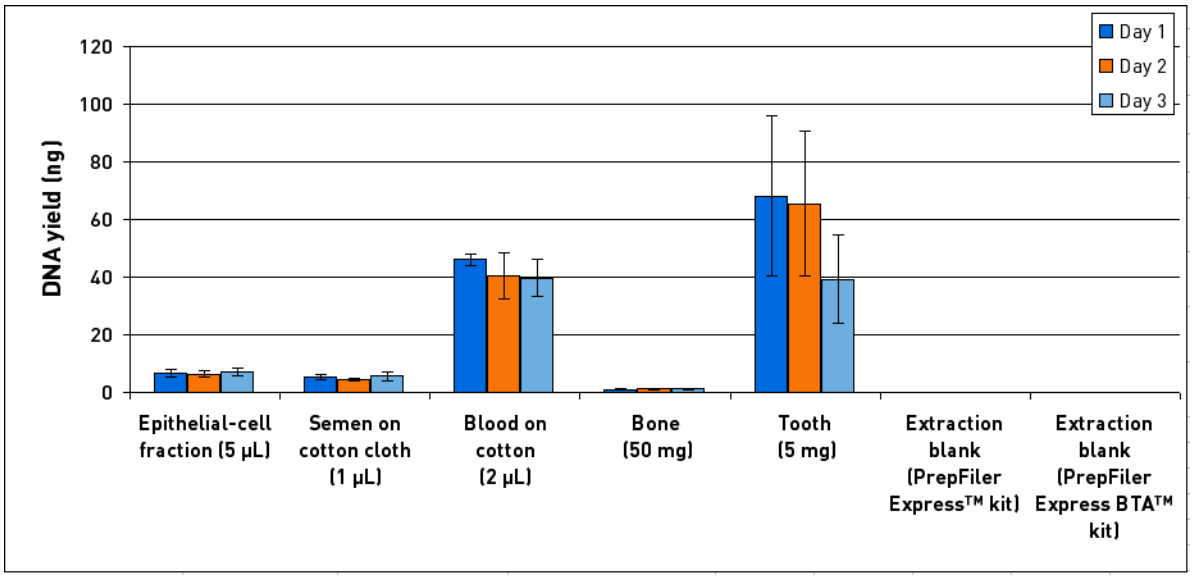


Figure 9 Reproducibility study: Magnified view of Figure 8 focusing on samples with less than 20 ng average DNA yield

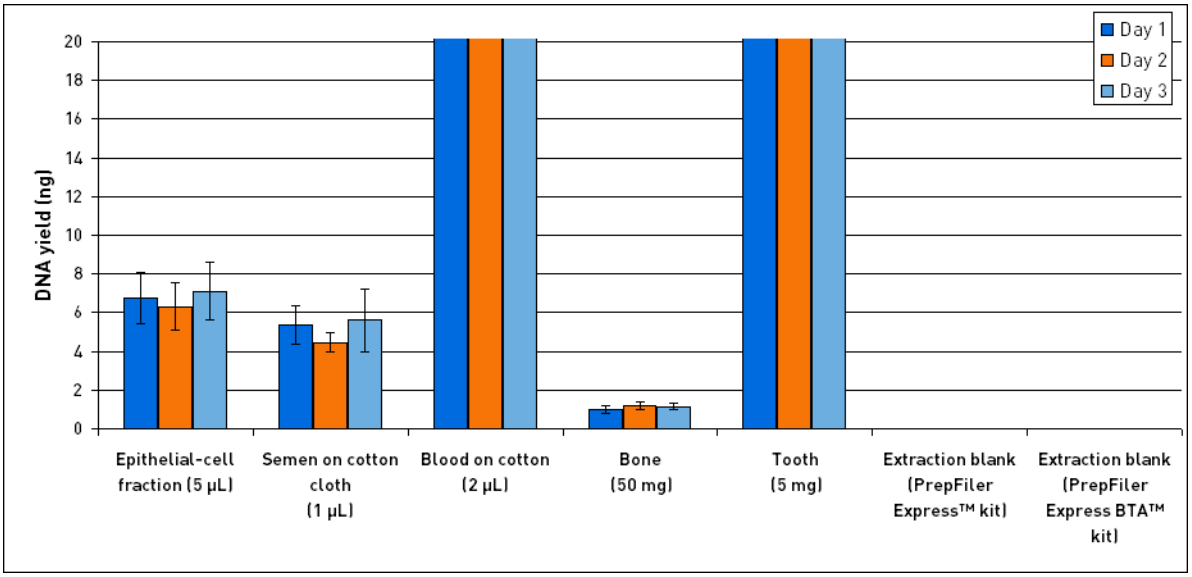
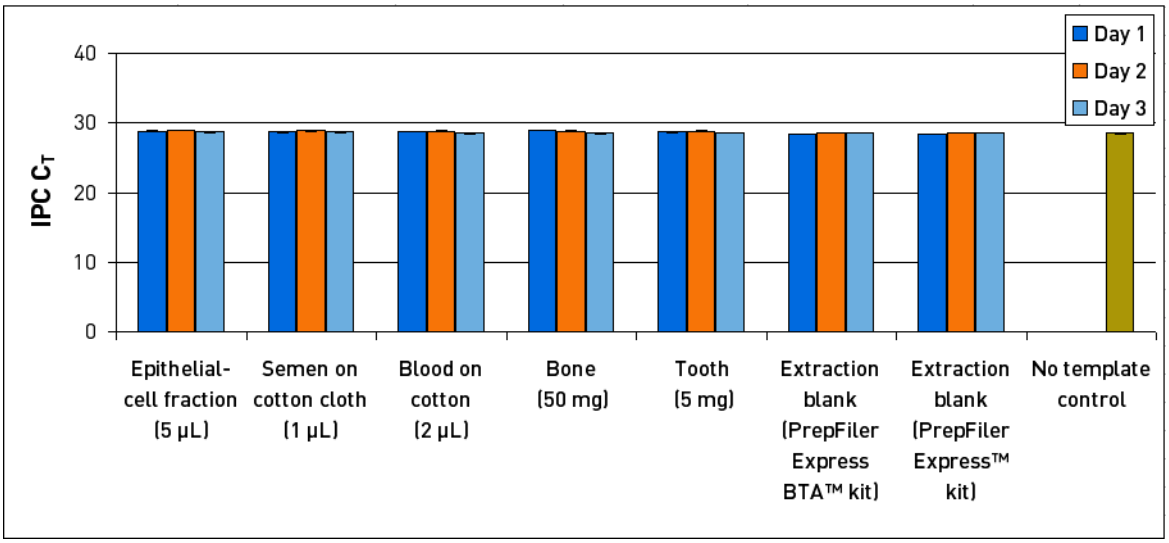


Figure 10 Reproducibility study IPC C_T



Case-type samples study (SWGDAM standard 2.6)

Experiments

Case-type sample studies were performed to evaluate the extraction of genomic DNA by the AutoMate *Express*™ System for different sample types that are commonly processed in a forensic laboratory.

The case-type sample study sample sets are shown in Table 13. Gum and cigarette butt samples were processed with the PrepFiler® *Express* BTA kit; all other sample types were processed with the PrepFiler® *Express* kit. DNA extractions were performed on three replicates of each of the 14 sample types. One extraction blank was included in each extraction run. Each sample set was extracted once, then quantified in duplicate.

Table 13 Case-type samples study samples

Description	Kit/Protocol
2 µL dried blood on acetate fabric	PrepFiler® *Express* kit/ body fluids protocol
1 µL blood on 5-mm black leather punch	
2 µL blood on rayon fabric	
2 µL blood on silk fabric	
2 µL blood on wool fabric	
3 µL diluted blood (1:10) on cotton cloth	
Hair root	
Blood mixture: 1 µL male (donor 239) and 9 µL female (donor 237)	
50 µL saliva stain on cotton	
5 µL saliva on 5-mm FTA punch	
Epithelial-cell fractions†	
Sperm-cell fractions†	
Airwave gum (chewed for 30 minutes, 1/8 of one piece)	PrepFiler® *Express* BTA kit/adhesive substrates protocol
Marlboro Light cigarette butt (approximately 3/4 cm cutting of filter paper)	
Extraction blank	—

† Mock sexual-assault-type samples were prepared by mixing 2 µL sperm positive semen with 50 µL vaginal epithelial cell suspension. Sperm fraction (DE-s fraction) and epithelial cell fraction (DE-e fraction) were generated from the mock sexual-assault-type samples using the procedure described by Gill (Gill, P., Jeffreys, A. J., and Werrett, D. J. 1985. Forensic application of DNA 'fingerprints.' *Nature* 318:577-579). 50 µL of DE-e fraction was added to 450 µL of PrepFiler® Lysis Buffer, then processed for extraction using the PrepFiler® *Express* protocol on the AutoMate *Express*™ Instrument. DE-s fraction was processed according to the PrepFiler® *Express* Forensic DNA Extraction Kit for semen samples.

Results

The average DNA yields for case-type samples are shown in Figure 11 and Figure 12. Variation in DNA yield may occur due to cells that are entrapped and/or bound within the substrate and are inaccessible to the lysis buffer. Variation in DNA concentrations between samples was expected due to the variation in the amount of biological material present in different samples from different donors and different body fluids. All sample types provided DNA in sufficient quantities for downstream applications.

Note: Hair roots were not microscopically examined to determine quality.

Average IPC C_T values for each sample type are shown in Figure 13 on page 48. The IPC C_T values for the samples and NTCs were within ±1 C_T unit, indicating that PCR inhibitors present in all tested sample types were effectively removed during the extraction of DNA using the AutoMate *Express*™ System.

Figure 11 Case-type sample study average DNA yield

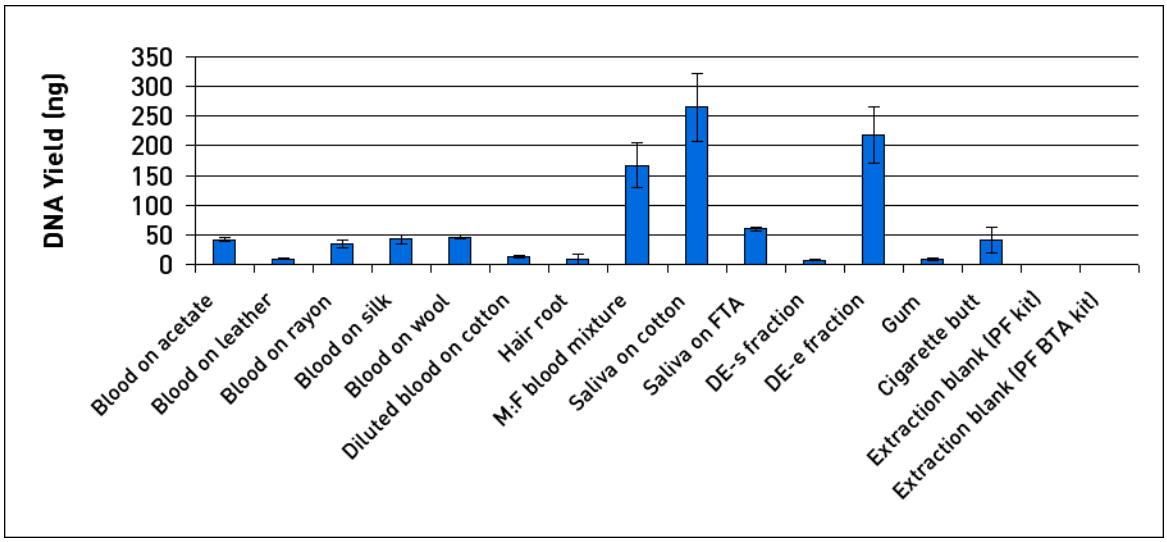


Figure 12 Case-type sample study: Magnified view of Figure 11 focusing on samples with less than 50 ng average DNA yield

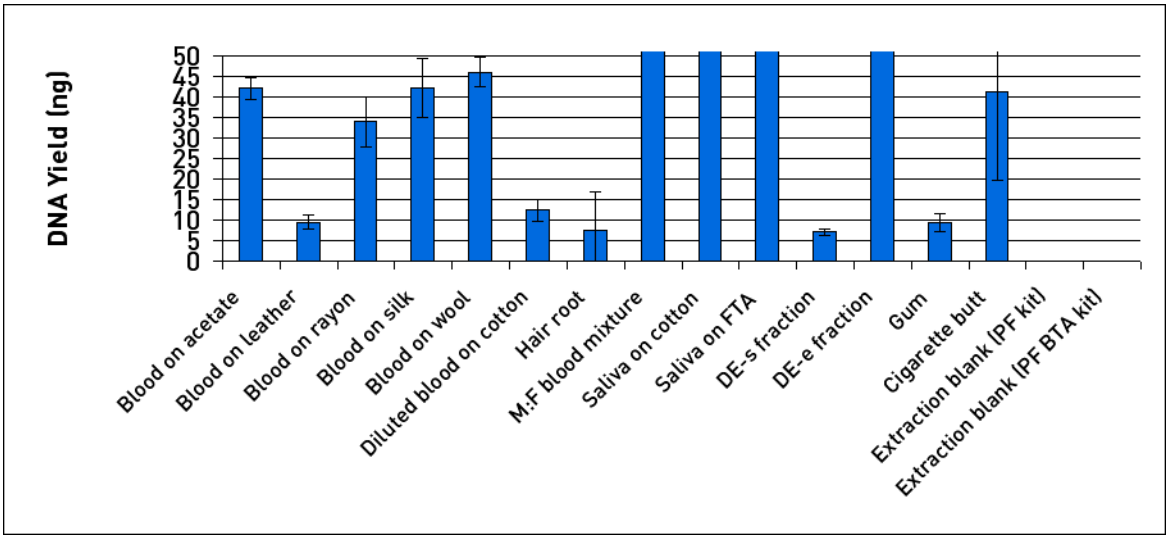
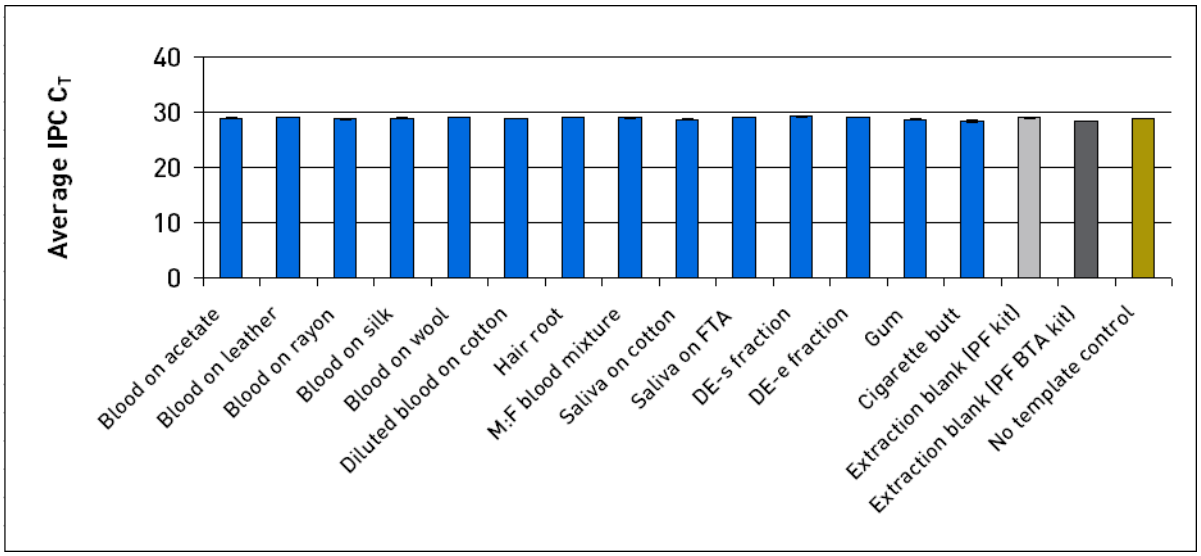


Figure 13 Case-type sample study IPC C_T



The quality of the DNA extract obtained from the AutoMate *Express*™ System was further evaluated by examining the STR profiles. Conclusive STR profiles were obtained from all samples and are shown in Figure 14 below and Figures 15 and 16 on page 49.

Figure 14 Case-type samples study

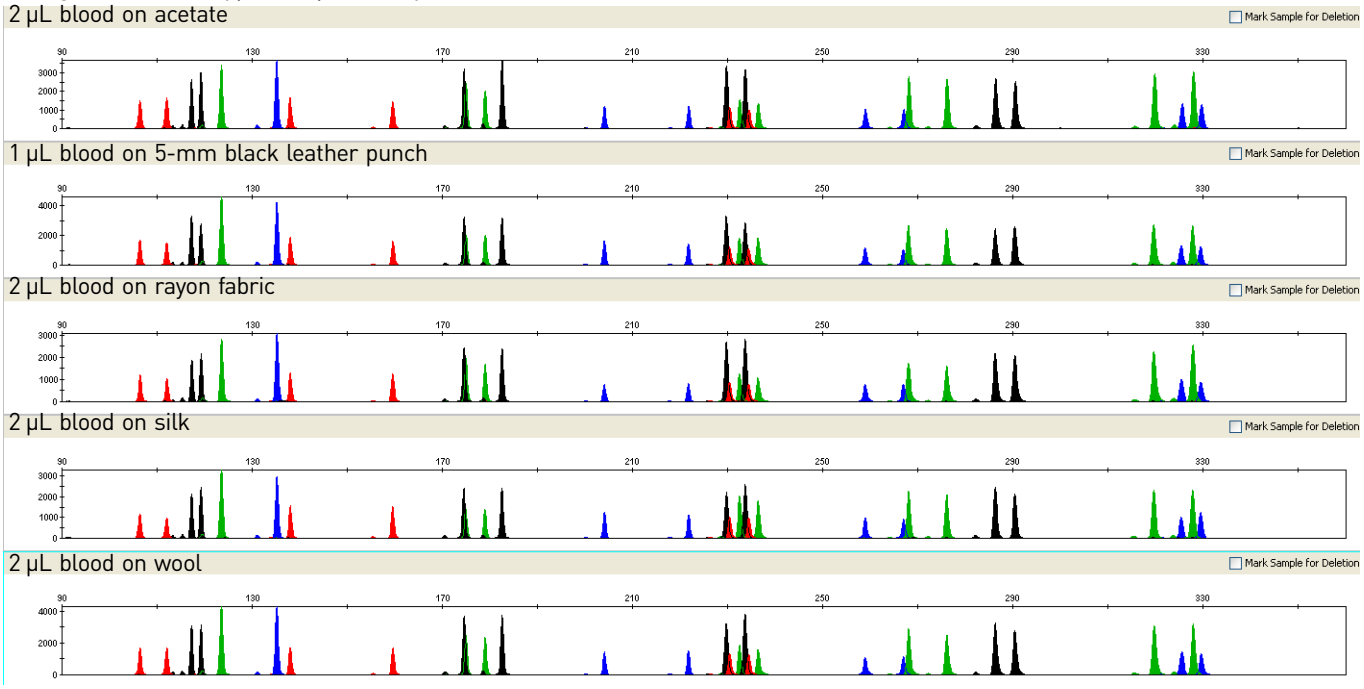


Figure 15 Case-type samples study

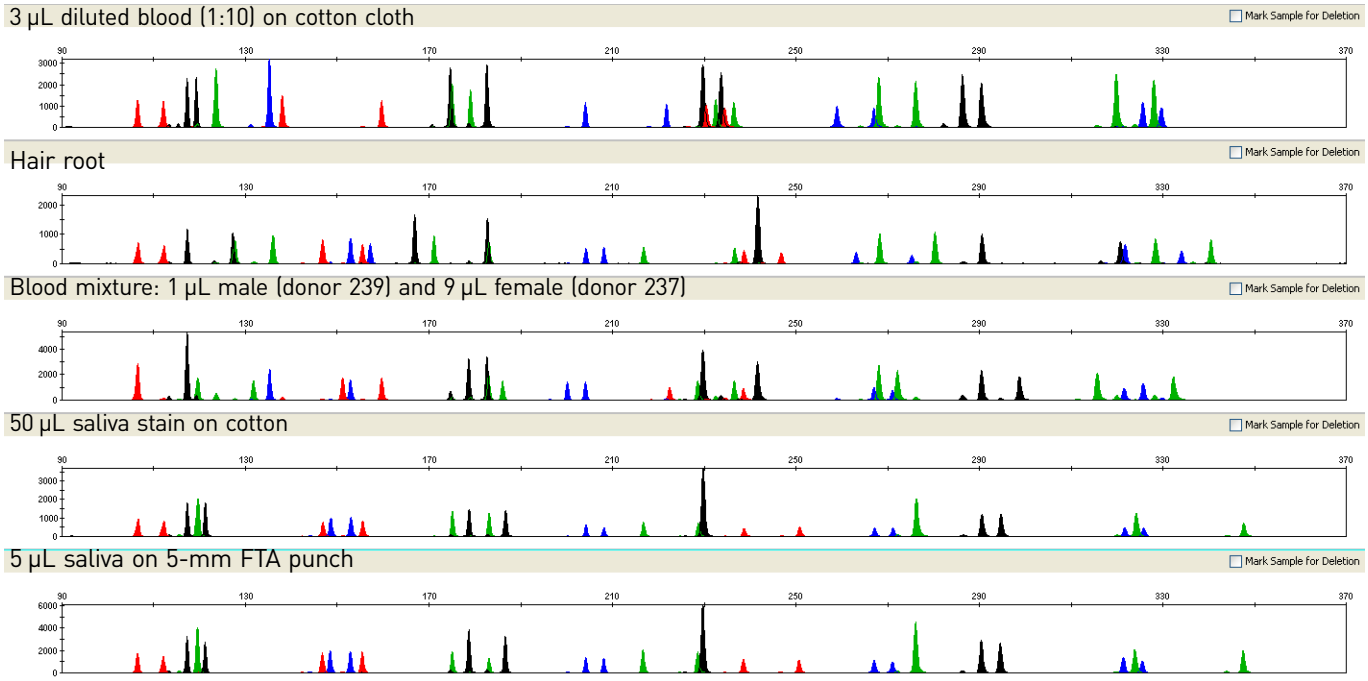
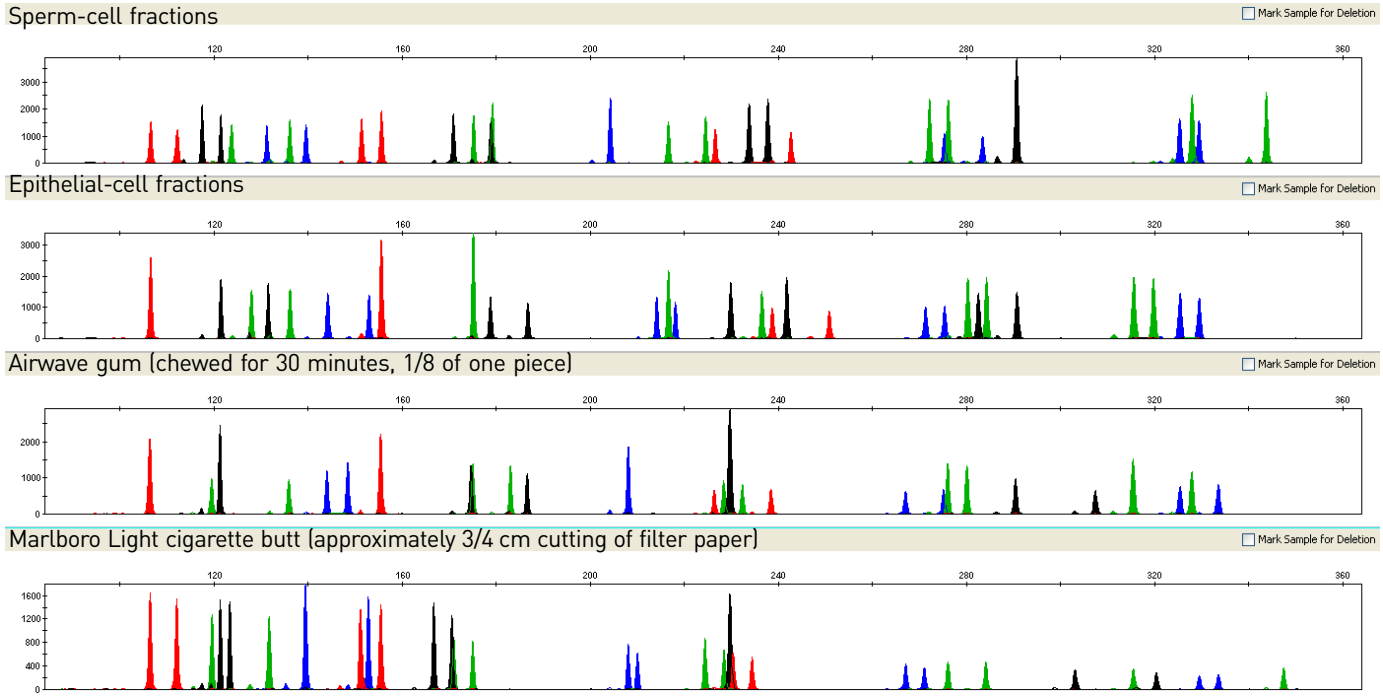


Figure 16 Case-type samples study



Contamination study (SWGDAM standard 3.6)

Experiments

Contamination studies were performed to confirm that the AutoMate *Express*™ instrument liquid handling does not introduce cross-contamination.

10-µL blood samples and extraction blanks were arranged in the AutoMate *Express*™ tip and tube rack in an alternating pattern. A total of 20 blood samples (from one donor) and 19 extraction blanks were processed with the PrepFiler® *Express* kit (see sample layout in Table 14).

All extraction blanks were processed for STR profiling with the AmpFℓSTR® Identifiler® PCR Amplification Kit. Any samples that exhibited peaks above 50 RFU were additionally processed for STR profiling in triplicate with the AmpFℓSTR® MiniFiler™ PCR Amplification Kit.

Table 14 Contamination study sample set shown by sample position in the tip and tube rack during extraction

Sample Position	Sample Set 1 (PrepFiler® *Express* kit)	Sample Set 2 (PrepFiler® *Express* kit)	Sample Set 3 (PrepFiler® *Express* kit)
E1	10 µL blood	10 µL 1XPBS	10 µL blood
E2	10 µL 1XPBS	10 µL blood	10 µL 1XPBS
E3	10 µL blood	10 µL 1XPBS	10 µL blood
E4	10 µL 1XPBS	10 µL blood	10 µL 1XPBS
E5	10 µL blood	10 µL 1XPBS	10 µL blood
E6	10 µL 1XPBS	10 µL blood	10 µL 1XPBS
E7	10 µL blood	10 µL 1XPBS	10 µL blood
E8	10 µL 1XPBS	10 µL blood	10 µL 1XPBS
E9	10 µL blood	10 µL 1XPBS	10 µL blood
E10	10 µL 1XPBS	10 µL blood	10 µL 1XPBS
E11	10 µL blood	10 µL 1XPBS	10 µL blood
E12	10 µL 1XPBS	10 µL blood	10 µL 1XPBS
E13	10 µL blood	10 µL 1XPBS	10 µL blood

Results

One extraction blank exhibited a single peak with a height of 70 RFU at the vWA locus. The sample was then processed for STR profiling in triplicate with the highly-sensitive AmpFℓSTR® MiniFiler™ PCR Amplification Kit. The vWA peak could not be verified, due to the absence of this locus in the MiniFiler kit. However, the STR profiles generated with the MiniFiler kit did not exhibit any alleles, so the peak was determined to be spurious.

Correlation study

Experiments

Correlation studies were performed to evaluate the quality (as judged by the presence of PCR inhibitors) and quantity of DNA obtained using the AutoMate *Express*™ System as compared to the DNA quantity and quality obtained using other commercially available cartridge-based, silica magnetic bead extraction kits and instruments.

Experiments were run on the AutoMate *Express*™ System and with two other extraction methods:

- **AutoMate *Express*™ Forensic DNA Extraction System** – Bone samples were processed with the PrepFiler® *Express* BTA kit, all other sample types were processed with the PrepFiler® *Express* kit.
- **Company A** – Samples were prepared and extracted using reagents according to the manufacturer's recommendations.
  - Bone samples (50 mg bone powder) were incubated with 400 µL of 0.5 M EDTA at 37°C for 40 hours, then for 3 hours with proteinase K. 1 µL of carrier RNA solution (1 µg) was added to each lysate before DNA extraction on the instrument.
  - Hair samples were incubated for 3 hours with proteinase K. 1 µL of carrier RNA solution (1 µg) was added to each lysate before DNA extraction on the instrument.
- **Company B** – Samples were prepared and extracted using reagents according to the manufacturer's recommendations, with the exception that the bone protocol was modified to accommodate a 50 mg input of bone powder sample. The following protocol was used to prepare bone samples for DNA extraction on the instrument:
  - Prepare a proteinase K digestion solution by mixing 9.44 mL of bone incubation buffer with 560 µL of stock proteinase K solution.
  - Add 500 µL of freshly-prepared PK digestion solution to the sample.
  - Incubate the sample tube at 56°C for 1 hour.
  - Centrifuge the sample tube at 5000 rpm for 5 minutes to separate the remaining bone substrate.
  - Transfer the solution to a new 1.5-mL tube.
  - Add 400 µL of lysis buffer to the solution, then vortex briefly.

For each method, DNA extractions were performed on 6 replicates of the bone sample and 12 replicates of each of the other 6 sample types (see Table 15 on page 52). One extraction blank was included in each extraction run. Extracted DNA was set up for qPCR prepared on the HID EVOlution™ – qPCR/STR Setup System using the Quantifiler® Duo DNA Quantitation Kit. Each extracted sample was quantified once. After quantitation, samples were normalized and set up for amplification using the HID EVOlution™ – qPCR/PCR Setup System with the AmpFℓSTR® Identifiler® PCR Amplification Kit.

Table 15 Correlation study sample sets

Sample Set 1 (PrepFiler® *Express* kit)	Sample Set 2 (PrepFiler® *Express* BTA kit)
Blood, 3 µL of a 1:10 dilution	50 mg bone
Bloodstain, 2 µL on 5-mm cotton cloth	Extraction blank
Blood, 2 µL on 5-mm FTA punch	
Epithelial cell suspension, 50 µL on cotton swab	
Semen, 1 µL on 5-mm cotton cloth	
Bloodstain, 1 µL on 5-mm blue denim	
Extraction blank	

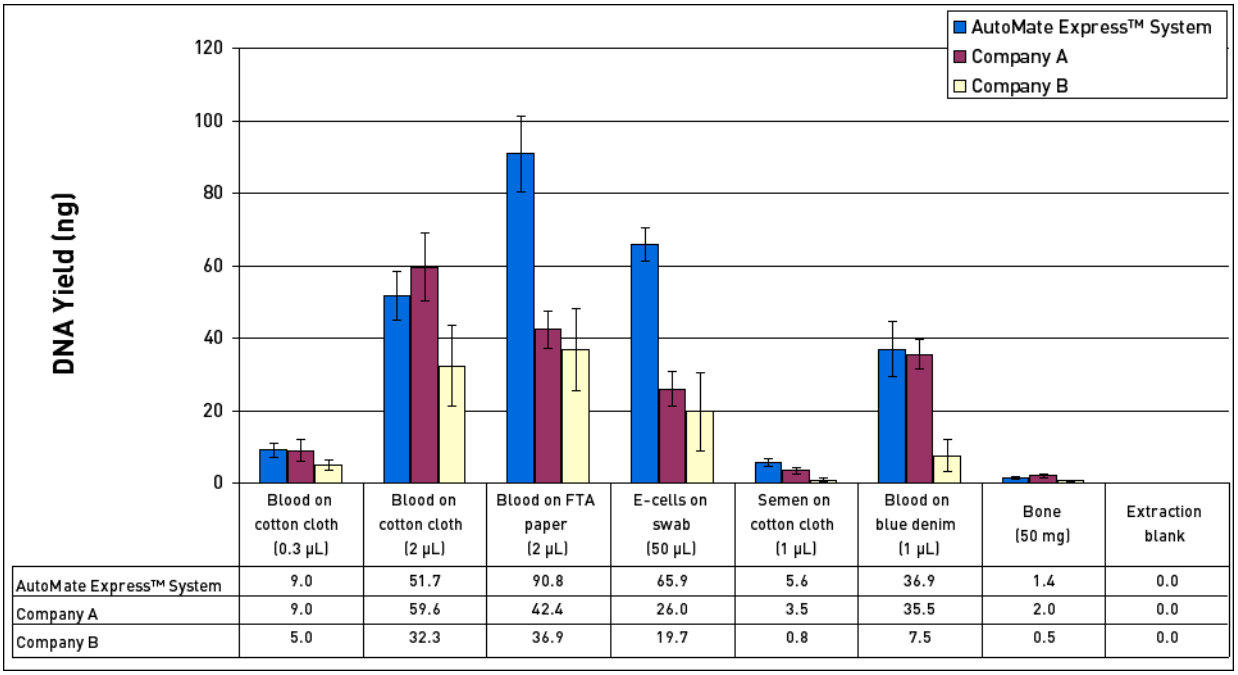
Results

The quantitation results for each extraction method were compared. For all sample types investigated, the AutoMate *Express*™ System DNA yield and concentration was comparable to or higher than that of the other extraction methodologies.

Figure 17 and Figure 18 on page 53 show the average total DNA yields (ng), and Figure 19 on page 53 shows the IPC C_T obtained using the AutoMate *Express*™ System and two other methods. In all figures, the data for extraction blanks is the combined data for PrepFiler® *Express* and PrepFiler® *Express* BTA Kits extraction runs.

The AutoMate *Express*™ System obtained STR profiles comparable to or better than the profiles obtained with Company A and B methods and recovered more alleles for aged bone samples. See Figure 20 on page 54.

Figure 17 Correlation study average DNA yield



	Blood on cotton cloth (0.3 µL)	Blood on cotton cloth (2 µL)	Blood on FTA paper (2 µL)	E-cells on swab (50 µL)	Semen on cotton cloth (1 µL)	Blood on blue denim (1 µL)	Bone (50 mg)	Extraction blank
AutoMate Express™ System	9.0	51.7	90.8	65.9	5.6	36.9	1.4	0.0
Company A	9.0	59.6	42.4	26.0	3.5	35.5	2.0	0.0
Company B	5.0	32.3	36.9	19.7	0.8	7.5	0.5	0.0

Figure 18 Correlation study: magnified view of Figure 17 on page 52 focusing on samples with less than 20 ng average DNA yield

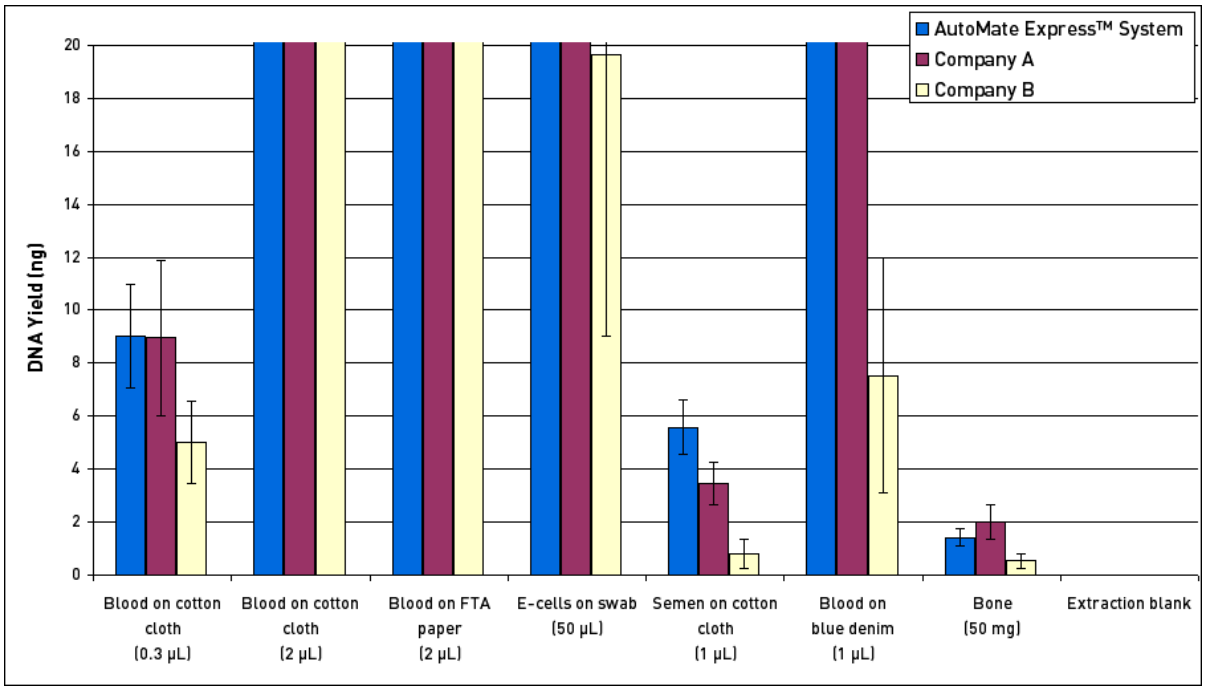


Figure 19 Correlation study IPC C_T

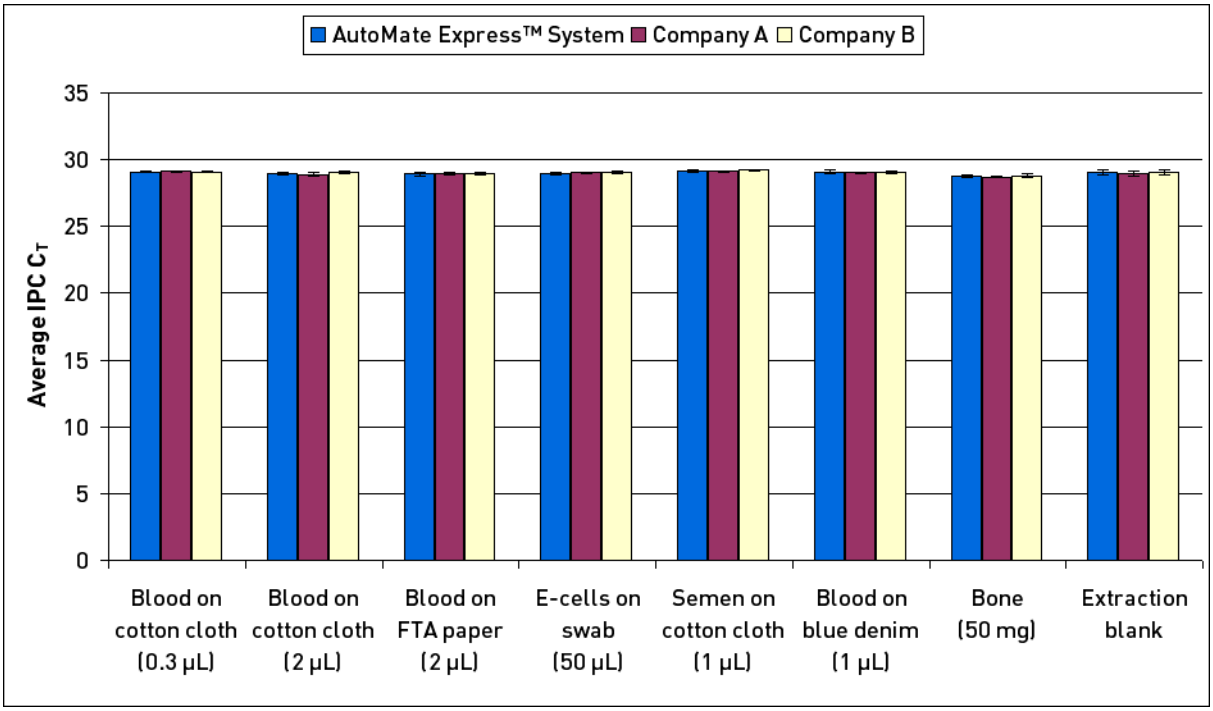
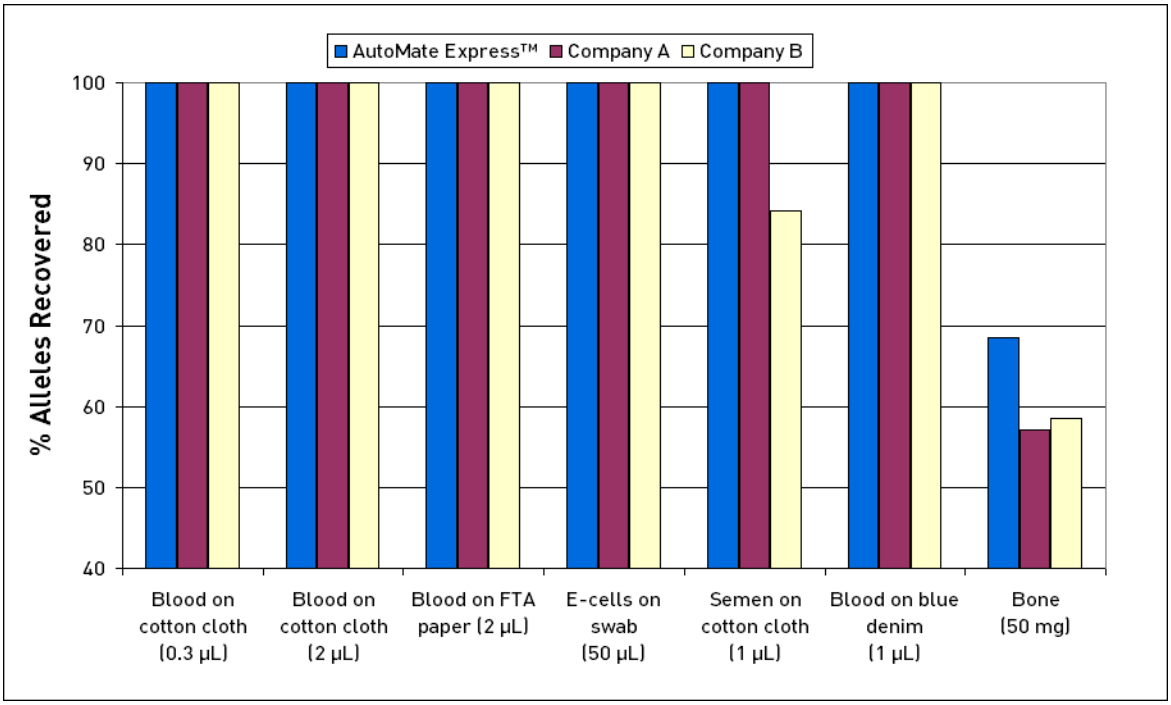


Figure 20 Correlation study percent alleles recovered by sample type



Conclusions

Validation studies confirmed that the AutoMate *Express*™ System provides robust and reliable results in obtaining genomic DNA from forensic biological samples for downstream applications such as real-time quantitative PCR and PCR for STR profiling:

- The AutoMate *Express*™ System provides reliable results at different DNA input amounts and is effective in maximizing the amount of DNA obtained from samples that contain both small and large quantities of biological material.
- The utility of the extraction method in forensic DNA analysis was demonstrated using forensic-type samples.
- The DNA that was extracted was free of PCR inhibitors as determined by the IPC C_T values using the Quantifiler® Duo DNA Quantification Kit.
- The reagents and operations of the AutoMate *Express*™ System exhibited clean operations and did not introduce any detectable cross-contamination of human DNA. Of the 19 extraction blanks co-extracted with 10-µL whole blood samples and processed for STR profiling with the AmpFℓSTR® Identifiler® PCR Amplification Kit, only 1 showed a possible allele; subsequent re-testing with the AmpFℓSTR® MiniFiler™ PCR Amplification Kit indicated that the peak was spurious and was not the result of liquid handling by the AutoMate *Express*™ System.

A

Troubleshooting

Review the information in the following table to troubleshoot your experiments using the PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits.

To troubleshoot operation of the AutoMate *Express*™ Instrument, see the *AutoMate Express*™ *Instrument User Guide*.

Observation	Possible Cause	Suggested Solution
After lysis incubation...		
Precipitate observed in a PrepFiler® LySep Column before centrifugation.	• Tube incubated for more than 40 minutes • Lysate chilled • Low temperature in laboratory	• Use a pipette to mix the sample lysate and dissolve the salt precipitate *or* • Vortex the LySep Column/sample tube assembly at high speed before centrifuging In future runs: • Do no incubate tubes for more than 40 minutes • Do not chill the sample lysate in a refrigerator or centrifuge
Precipitate observed in a sample tube after centrifugation.	• Tube incubated for more than 40 minutes • Lysate chilled • Low temperature in laboratory	Heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. In future runs: • Do no incubate tubes for more than 40 minutes • Do not chill the sample lysate in a refrigerator or centrifuge
Before loading the cartridges in the cartridge rack...		
PrepFiler® *Express* Cartridges contain precipitate in some compartments.	Cartridges were exposed to low temperatures during the shipping or storage.	To dissolve precipitate that may have formed during shipping or storage, incubate the PrepFiler® *Express* Cartridges at 37°C for 30 minutes or until precipitate is no longer visible.

Observation	Possible Cause	Suggested Solution
During the automated extraction run...		
AutoMate *Express*™ Instrument tip filters become wet	Precipitate in sample tube, resulting in partial or full clogging of the pipette tip.	Heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. In future runs, do not load sample tubes containing any precipitate onto the AutoMate *Express*™ Instrument.
During run: No liquid in tip, or liquid in tip not moving	No sample added to tube, leading to wet filter barrier on the tip and blockage of nozzles.	Add samples to tubes, load new reagent cartridges, then perform the run again.
After run: No elution volume	Sample volume is lower than the recommended volume, leading to wet filter barrier on the tip and blockage of nozzles.	In future runs, use the recommended sample volume for the protocol you are using. Long-term operation with lower-than-recommended sample volumes can lead to issues with liquid handling performance.
After eluting the DNA...		
The DNA eluate is colored	Substrate yielded a colored eluate. For example, some sample substrates contain dyes.	**Note:** Color does not necessarily interfere with quantitation or amplification. If a shift in IPC C_T value is observed in the quantitation run, dilute and re-quantify the sample.
	DNA contaminated with heme.	Minimize the amount of blood or blood-stained sample used.
After quantifying extracted DNA...		
No or low yield of DNA	• Biological sample contains no or low amount of DNA. • Missed protocol steps or reagent additions during the lysis step. • Automated extraction was performed while the sample lysate temperature was still above room temperature, preventing the binding of DNA to the magnetic particles. **Note:** This problem may occur when processing liquid samples. For samples that require substrate removal, the substrate removal step provides time for the sample lysate to come to room temperature.	1. Review lysis protocol steps and reagent additions. 2. Amplify the maximum volume for STR analysis. 3. Extract DNA from a different cutting from the sample.

Observation	Possible Cause	Suggested Solution
No or low yield of DNA *(continued)*	The DNA eluate contains PCR inhibitors due to excessive amount of inhibitors in the sample.	Evaluate the IPC C_T value and see suggested solution for "Sample IPC C_T is higher than IPC C_T of NTC" below.
	Incomplete lysis.	Decrease the amount of starting material used. Make sure to add Proteinase K during lysis, if it is specified in the protocol. Make sure that the sample is completely immersed in the lysis solution.
	Poor quality of starting material.	Make sure to process the sample immediately after collection or store the sample at the appropriate temperature. The yield and quality of DNA isolated depends on the starting material.
	Insufficient amount of magnetic particles added.	During shipping, some magnetic particles solution may adhere to the sealing foil of the cartridge. To collect the magnetic particles solution from the foil, tap the cartridge to deposit the magnetic particles solution at the bottom of the tube before loading the cartridge in the cartridge rack.
	Clogged tips resulting in DNA loss.	Ensure that the lysate does not contain any particulate material that can clog the tip. If needed, centrifuge the sample prior to automated extraction.
During quantitation using a Quantifiler® kit, if the sample IPC C_T is higher than the IPC C_T of the no template quantitation control (NTC) or quantitation standards (for example, if the sample IPC C_T is approximately two C_T greater than the standards or NTC IPC C_T)	The DNA concentration is above 25 ng/µL.	If DNA concentration is over 25 ng/µL, dilute the DNA eluate, then requantify the sample.
	The DNA eluate contains PCR inhibitors due to excessive amount of inhibitors in the sample.	Dilute and requantify the eluate. If the eluate contains inhibitors: 1. If there is limited amount of sample and low amount of inhibitors, dilute and requantify the eluate. 2. Consider proceeding to amplification with a kit such as the AmpFℓSTR® MiniFiler™ PCR Amplification Kit. This kit is designed to obtain STR profiles from compromised samples (for example, samples that may be inhibited and/or degraded).

Observation	Possible Cause	Suggested Solution
After performing STR analysis...		
Unbalanced STR profile	**Note:** The following causes and solutions are related to sample preparation. For other possible causes and solutions, see the applicable AmpFℓSTR® kit user guide.	
	Inhibition.	Dilute and requantify the eluate. If the eluate contains inhibitors: 1. If there is limited amount of sample and low amount of inhibitors, dilute and requantify the eluate. 2. Consider proceeding to amplification with a kit such as the AmpFℓSTR® MiniFiler™ PCR Amplification Kit. This kit is designed to obtain STR profiles from compromised samples (for example, samples that may be inhibited and/or degraded).
	Inappropriate storage.	Aliquot purified DNA and store at 4°C (short-term) or -20°C (long-term). Avoid repeated freezing and thawing.
	Biodegradation of DNA or poor quality of sample DNA.	Maintain a sterile environment while working (wear gloves and use DNase-free reagents).

B

Safety

This appendix covers:

Instrumentation safety

Symbols on instruments

Safety symbols on instruments

The following symbols may be displayed on Life Technologies instruments.

Hazard symbol	English	Français
	**CAUTION!** Risk of danger. Consult the user guide for further safety information associated with this symbol.	**ATTENTION!** Risque de danger. Pour plus d'information au sujet des risques associes, consulter le manual d'utilisation.
	**CAUTION!** Hot surface.	**ATTENTION!** Surface brûlante.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	CAUTION! Risk of electric shock.	**ATTENTION!** Risque de choc electrique.
	**CAUTION!** Risk of injury due to moving parts.	**ATTENTION!** Piece mobile. Risque de blessure.
	**CAUTION!** Sharp points.	**ATTENTION!** Angle tranchant.
	Biohazard.	**DANGER!** Biologique.

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Life Technologies instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.

Environmental symbols on instruments

The following symbol applies to all Life Technologies electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Life Technologies Customer Service office for equipment pick-up and recycling. See **www.lifetechnologies.com** for a list of customer service offices in the European Union.

General instrument safety

WARNING! PHYSICAL INJURY HAZARD. Using the instrument in a manner not specified by Life Technologies may result in personal injury or damage to the instrument.

Moving and lifting the instrument

CAUTION! PHYSICAL INJURY HAZARD. Do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See "Obtaining SDSs" on page 69.

Cleaning or decontaminating the instrument

CAUTION! Using a cleaning or decontamination method other than that specified by the manufacturer may result in damage to the instrument.

Physical hazard safety

Moving parts

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Solvents and pressurized fluids

WARNING! PHYSICAL INJURY HAZARD. Always wear eye protection when working with solvents or any pressurized fluids.

Electrical safety

WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

WARNING! ELECTRICAL HAZARD. Grounding circuit continuity is required for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

WARNING! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

WARNING! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The instrument system has an installation (overvoltage) category of II, and is classified as portable equipment.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- Canadian EMC standard
- European safety and EMC standards
- Australian EMC Standards

U.S. and Canadian safety standards

The AutoMate *Express*™ Instrument has been tested to and complies with standard:

UL 61010-1/CSA C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

The bar code scanner provided with the AutoMate *Express*™ Instrument is a class 1 laser device.

Canadian EMC standard

This instrument has been tested to and complies with ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."

European safety and EMC standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

EN 61010-2-081, "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

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 "Obtaining SDSs" on page 69.)
- 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.

Chemical waste safety

Chemical waste hazards

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

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Life Technologies 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

WARNING! Do not add acids, or bases (such as bleach) to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.

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

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

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

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **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**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Safety alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see "User attention words" on page 7.

Specific alerts for chemicals

WARNING! Do not add acids, or bases (such as bleach) to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.

Specific alerts for instrumentation

WARNING! Do not touch the surface of the heat block. The temperature of the heat block may be very high (up to 95°C) and can cause burns.

Documentation and Support

Related documentation

The following related documents are available at **www.lifetechnologies.com**:

Document	Part number	Description
AutoMate Express™ Instrument User Guide	4441982	Describes procedures for installing, operating, maintaining, and troubleshooting the AutoMate *Express*™ Instrument.
PrepFiler® Express and PrepFiler® Express BTA Kits Quick Reference Card	4443104	Provides brief, step-by-step 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 this User Guide.
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 Life Technologies during the development of the PrepFiler® Forensic DNA Extraction Kit.

Note: To open the user documentation, use the Adobe® Reader® software available from **www.adobe.com**.

Note: For additional documentation, see "Obtaining support" on page 70.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

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

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.lifetechnologies.com/support

At the website, you can:

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

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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