applied biosystems

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

USER GUIDE

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

Catalog Numbers 4441352 and 4441351
Publication Number 4442699
Revision D

Manufacturer's address: Thermo Fisher Scientific | 7 Kingsland Grange | Warrington, Cheshire WA1 4SR | United Kingdom

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

Revision history: Pub. No. 4442699

Revision	Date	Description
D	07 March 2017	Add Quantifiler™ Trio and Quantifiler™ HP DNA Quantification Kits to quantification paragraph. Update Cat. No. for PrepFiler *Express*™ & PrepFiler *Express*™ & PrepFiler *Express* BTA™ Protocol Card v1.1 (variable elution). Change sample lysate minimum volume from 150 µL to 200 µL for PrepFiler *Express* BTA™ kit (bone-tooth and adhesive substrate). Add variable elution studies to Experiments and Results chapter.
C	March 2012	Minor updates to kit contents and legal language.

Contents

1 Product information

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

PrepFiler *Express*™ and PrepFiler *Express* BTA™ kit descriptions

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 with:

- Quantitation using the Quantifiler™ Human, Quantifiler™ Y Human Male, Quantifiler™ Duo, Quantifiler™ Trio, and Quantifiler™ HP DNA Quantification Kits
- STR amplification using Applied Biosystems™ PCR Amplification kits

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* (Pub. No. 4390932).

AutoMate *Express*™ Forensic DNA Extraction System description

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:

- PrepFiler *Express*™ Forensic DNA Extraction Kit
- PrepFiler *Express* BTA™ Forensic DNA Extraction Kit
- AutoMate *Express*™ Instrument
- PrepFiler *Express*™ and 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.

Automated DNA extraction overview

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.

Contents and storage

Table 1 PrepFiler *Express*™ Forensic DNA Extraction Kit

Contents	Amount[1]	Storage conditions
PrepFiler™ Lysis Buffer	27 mL	Store all kit components at room temperature.
PrepFiler™ *Express* Cartridges	52	
PrepFiler™ Sample Tubes	52	
PrepFiler™ Elution Tubes	52	
PrepFiler™ LySep Columns	52	
AutoMate *Express*™ Tips and Tip Holders	52	

[1] Amounts are sufficient for 52 extractions.

Table 2 PrepFiler *Express* BTA™ Forensic DNA Extraction Kit

Contents	Amount[1]	Storage conditions
PrepFiler™ BTA Lysis Buffer	13 mL	Store all kit components at room temperature.
PrepFiler™ *Express* Cartridges	52	
PrepFiler™ Sample Tubes	52	
PrepFiler™ Elution Tubes	52	
PrepFiler™ LySep Columns	52	
AutoMate *Express*™ Tips and Tip Holders	52	
PrepFiler™ Bone and Tooth Lysate Tubes	52	
PrepFiler™ Bone and Tooth Lysate Tube Caps	52	
Proteinase K	400 µL	

[1] Amounts are sufficient for 52 extractions.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source[1]
AutoMate *Express*™ Instrument (includes the PrepFiler *Express*™ and PrepFiler *Express* BTA™ Protocol Card	Contact your local sales representative
Common laboratory equipment such as pipettors, aerosol-resistant micropipette tips, and a microcentrifuge	MLS
Vortexer (a variable-speed vortexer is recommended)	MLS
DL-Dithiothreitol [Molecular biology grade; ≥98% (TLC), ≥99% (titration)]	Sigma-Aldrich[2] **www.sigmaaldrich.com** (Cat. No. D9779)
Eppendorf Thermomixer	Eppendorf North America **www.eppendorfna.com** (Cat. No. 21516-170) or MLS
Laboratory centrifuge capable of 10,000 × *g*	MLS

[1] 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 relevantprecautions.

[2] For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Accessories

The following products are available for purchase separately from Thermo Fisher Scientific.

Item	Cat. No.
PrepFiler *Express*™ & PrepFiler *Express* BTA™ Protocol Card v1.1	A33682
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

Workflow: DNA extraction

Prepare sample lysate

▼

Prepare samples for lysis (if necessary)

▼

Perform lysis

▼

Remove the substrate from the sample lysate (if necessary)

▼

Set up and run automated DNA extraction

▼

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

2 Prepare sample lysate

Required materials for lysis and substrate removal

Note: 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 not supplied" on page 8.
- Sterile tweezers or other tools for transferring samples to PrepFiler™ LySep Columns
- PrepFiler™ Sample Tubes
- Dithiothreitol (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 fluid protocol

For use with kit

PrepFiler *Express*™ Forensic DNA Extraction Kit

Sample types and inputs (body fluid)

The PrepFiler *Express*™ Forensic DNA Extraction Kit is appropriate for most forensic sample types, including stains and swabs of biological fluids. Examples of appropriate sample types and inputs are shown in Table 3. Optimal input amounts may be affected by factors such as sample age and substrate properties. Each lab should perform studies to validate optimum input amounts.

Table 3 Example sample types and inputs for body fluid

Sample type	Example sample input[1]
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

[1] It is not necessary to use an entire sample punch or swab.

Perform lysis (body fluid)

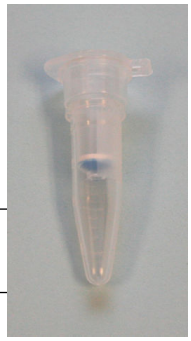
1. If the Lysis Buffer contains precipitate, heat the solution to 37°C, then vortex the bottle for 5 seconds.
2. Bring the thermal shaker temperature to 70°C.
3. Prepare a fresh 1.0-M DTT solution by dissolving 1.54 g of Dithiothreitol (DTT, MW 154) in 10 mL of molecular-biology grade DNA-free water.

   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 6 months.
4. Prepare a fresh lysis solution. Each sample requires:
   - 500 µL Lysis Buffer
   - 5 µL freshly prepared 1 M DTT

   Note: After completing the lysis step, discard unused 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 LySep Column into a hingeless PrepFiler™ sample tube (together called the "column/tube assembly"), then carefully transfer the sample into the LySep column.



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

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

7. Tightly close the lid of the column/tube assembly.

   Do not place labels on tube caps; doing so can cause leakage.

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 can 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 Appendix A, "Troubleshooting" for suggestions for preventing and/or dissolving precipitated salts.

Remove the substrate from the sample lysis (body fluid)

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 that is 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 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 can cause liquid handling problems.

3. Complete substrate removal as follows:

   a. Carefully remove the LySep column from the sample tube. If there is clear lysate remaining in the 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 LySep column from the sample tube.

   b. Properly dispose of the 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. Precipitate can cause the instrument to crash, tips to clog, or filters becoming wet.

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 (bone and tooth)

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 4. Optimal input amounts may be affected by factors such as sample age and substrate properties. Each lab should perform studies to validate optimal input amounts.

Table 4 Example sample types and inputs for bone and tooth

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

Prepare samples for lysis (bone and tooth)

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 (bone and tooth)

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 6 months.

3. Prepare a fresh lysis solution. Each sample requires:
   - 220 µL Lysis Buffer
   - 3 µL freshly prepared 1 M DTT
   - 7 µL Proteinase K

   Note: After completing the lysis step, discard unused 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 lysis solution to the Bone and Tooth Lysate Tube containing the bone or tooth sample.

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

   Note: To avoid leaks, ensure 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 vortex again if needed to remove bubbles.

6. Place the Bone and Tooth Lysate Tube in a thermal shaker, then incubate it at 56°C and 1,100 rpm for at least 2 hours (sample can be incubated up to 18 hours).

Remove the substrate from the sample lysis (bone and tooth)

1. Centrifuge the 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 can cause liquid handling problems during the automated extraction run.

3. If the volume of sample lysate that is collected in the sample tube is less than 200 µL, add Lysis Buffer to bring the lysate volume to 200 µL.

   IMPORTANT! A 200-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 can 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 substrate protocol

For use with kit

PrepFiler *Express* BTA™ Forensic DNA Extraction Kit

Sample types and inputs (adhesive substrate)

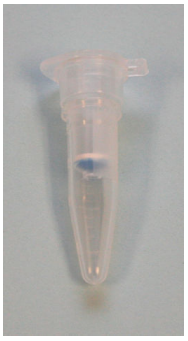
The PrepFiler *Express* BTA™ Forensic DNA Extraction Kit is appropriate for most adhesive forensic sample types, including chewing gum, cigarette butts, and tape lift samples. Examples of appropriate sample types and inputs are shown in Table 5. Optimal input amounts may be affected by factors such as sample age and substrate properties. Each lab should perform studies to validate optimal input amounts.

Table 5 Example sample types and inputs for adhesive substrates

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 (adhesive substrate)

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



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™ 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 (adhesive substrate)

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 6 months.

3. Prepare a fresh lysis solution. Each sample requires:
   - 220 µL Lysis Buffer
   - 3 µL freshly prepared 1 M DTT
   - 7 µL Proteinase K

   Note: After completing the lysis step, discard unused 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 lysis solution to the column/tube assembly.

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

5. Tightly close the lid of the column/tube assembly.
   Do not place labels on tube caps; doing so can cause leakage.

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 lysis (adhesive substrate)

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 that is collected in the sample tube is less than 200 µL:
   a. Centrifuge the column/tube assembly for an additional 5 minutes.

   b. If the volume is still less than 200 µL, add Lysis Buffer to bring the lysate volume to 200 µL.

IMPORTANT! A 200-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 can cause liquid handling problems.

---

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

   b. Properly dispose of the 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

Workflow: Set up the AutoMate *Express*™ Instrument

Set up and run automated DNA extraction

▼

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

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 that are required for the protocol (see Figure 1); the remaining wells are empty.
  - PrepFiler™ Sample Tubes containing the sample lysate prepared according to Chapter 2, "Prepare sample lysate".
  - PrepFiler™ Elution Tubes
  - AutoMate *Express*™ Tips and Tip Holders

Note: See "Required materials not supplied" on page 8 for details.

Protocol card handling

- Store the card in the plastic cover, in its box, protected from light.
- Do not drop or bend the card.
- Do not wipe or clean the card using volatile chemicals such as alcohol or equivalent.
- Do not expose the card to water or any solution.

Inspect cartridges

Inspect the reagent 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. Heat only those cartridges that you plan to use that day.

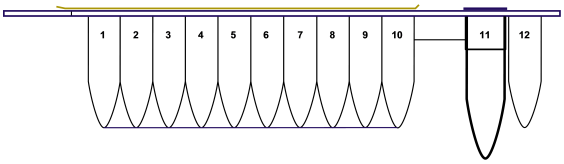


Figure 1 PrepFiler *Express*™ cartridge compartments

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 a protocol card

For guidelines on 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.

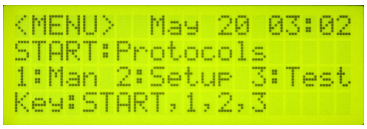
2. Open the card slot.

3. Insert the protocol card in the slot with the arrow pointing toward the instrument and the label facing left.

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 shows the **Main** menu.

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 the card is removed during a run, immediately power off the instrument to minimize the potential for data loss.



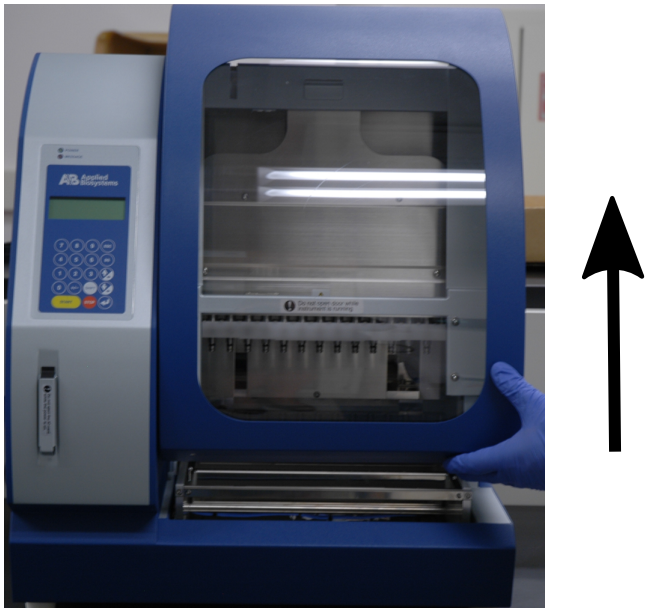
Load and insert the cartridge rack

Note: To ensure the best pipetting performance, use the cartridge rack and tip and tube rack that are shipped with the instrument. These racks are calibrated with the instrument at the factory.

Note: Before using other racks on a specific instrument, run the installation test to qualify the racks for use on that instrument (see the *AutoMate Express™ Instrument User Guide*).

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

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



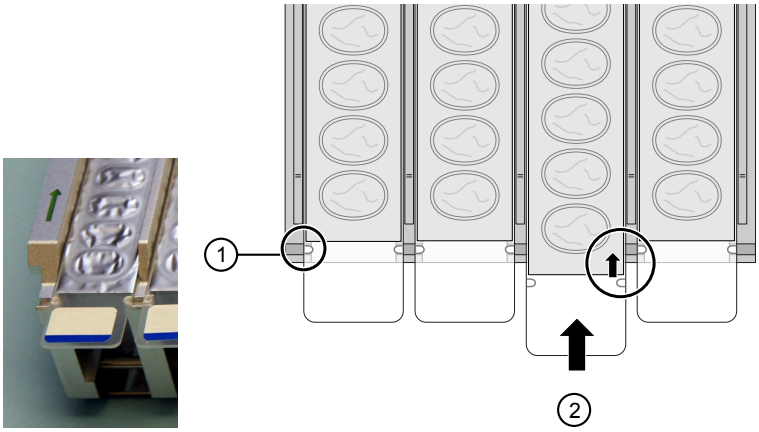
2. Remove up to 13 cartridges from the kit box.

   Note: One cartridge is required per sample. Use only Thermo Fisher Scientific PrepFiler *Express™* reagent cartridges.

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.

4. Load the 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. Ensure that the notches in the cartridge align with the notches in the cartridge rack.

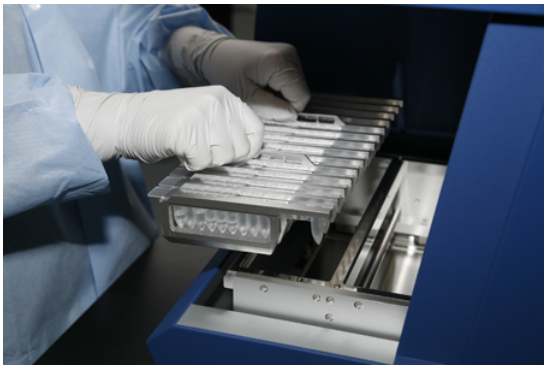
   Note: An incorrectly loaded cartridge rack can cause the instrument to stop during a run.

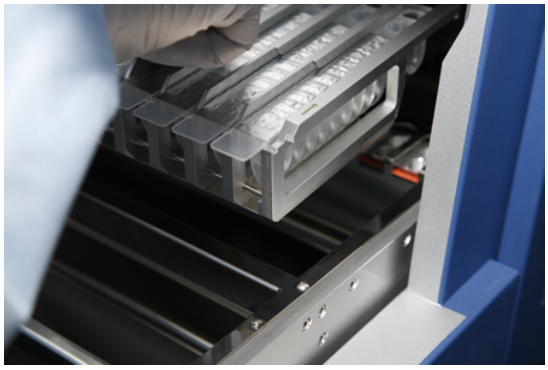


① Correct position
② Slide the cartridge until the notches align and the cartridge clicks into place

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 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. Loading the tip and tube rack first 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 and can stop the run.
- Before loading each sample tube in the rack, make sure that no salt precipitation is observed in the sample tube. Precipitate in sample tubes may cause instrument crash, tip clogging, or filter wetting. See Appendix A, "Troubleshooting" 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 in the following order:
    a. **Row S** (fourth row): Load PrepFiler™ sample tubes containing the lysate.

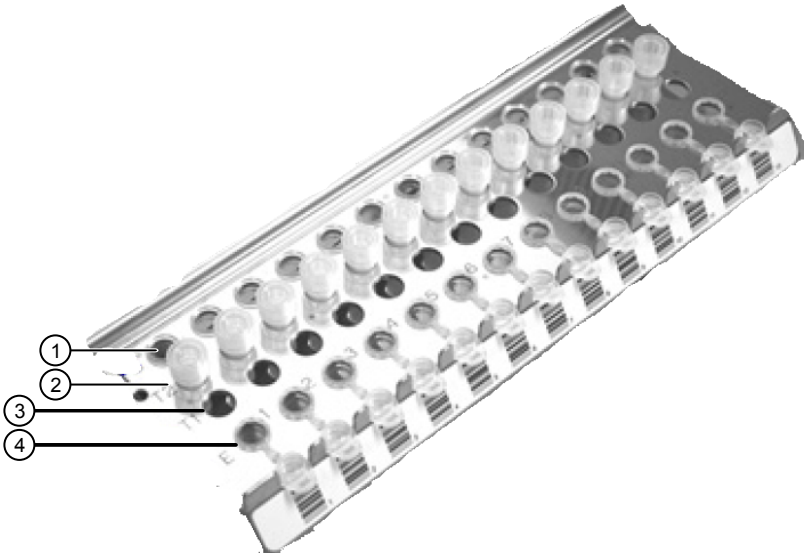
    Note: Make sure that the LySep columns have been removed from the sample tubes.

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.

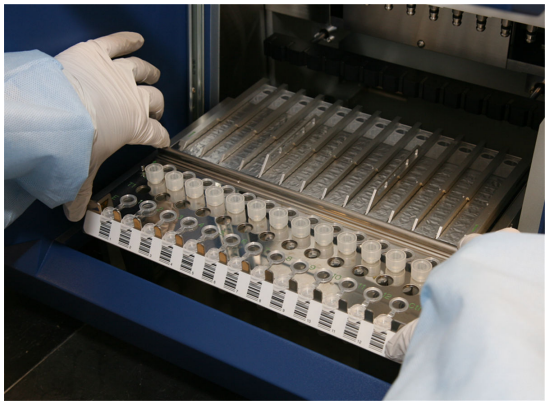
c. **Row T1** (second row): Leave empty.

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



1. Row S
2. Row T2
3. Row T1
4. Row E

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



Start an automated extraction run

1. Ensure that you have loaded and 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.
   - 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 digital display shows 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 positions 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 protocol card, power off the instrument, then change the protocol card.

4 Experiments and results

Overview of experiments and results

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

We performed the experiments according to the Revised Validation Guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM) published in Forensic Science Communications Vol. 6, No. 3, July 2004 (**http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/standards/2004_03_standards02.htm/**). These guidelines describe the quality assurance requirements that a laboratory should follow to ensure 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 6) 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, "Prepare sample lysate".
- Genomic DNA was extracted from the lysed samples using the AutoMate *Express*™ Instrument following the procedures described in Chapter 3, "Set up and run automated DNA extraction". 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 AmpFℓSTR™ 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™ PCR System 9700. Electrophoresis was performed on Applied Biosystems™ 3130xl Genetic Analyzers. The STR profiles were analyzed using GeneMapper™ *ID-X* Software v1.0.

Table 6 Summary of materials used in the validation studies

Component	Description
Chemistry kits[1]	PrepFiler *Express*™ and PrepFiler *Express*™ BTA Forensic DNA Extraction Kits (Cat. No. 4441352 and 4441351)
	PrepFiler™ Forensic DNA Extraction Kit (Cat. No. 4392852)
	Quantifiler™ Duo DNA Quantification Kit (Cat. No. 4387746)
	AmpFℓSTR™ Identifiler™ PCR Amplification Kit (Cat. No. 4322288)
	AmpFℓSTR™ MiniFiler™ PCR Amplification Kit (Cat. No. 4373872)
	AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kits (Cat. 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 gold-plated silver 96-well block
	3130 *xl* Genetic Analyzer with Data Collection Software v3.0

Component	Description
Instruments and software	GeneMapper™ *ID-X* Software version 1.0
	Eppendorf Thermomixer™ (Eppendorf North America)

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

Sensitivity studies (SWGDAM standard 2.3)

Sensitivity studies experiments

Sensitivity studies were performed to determine the range of biological sample amounts that can be reliably processed for extraction of genomic DNA using theAutoMate *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 7). All samples were processed using the PrepFiler *Express*™ Forensic DNA Extraction Kit. Each sample set was extracted once, then quantified in duplicate.

Table 7 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	—

Sensitivity studies results

Table 8 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 8 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

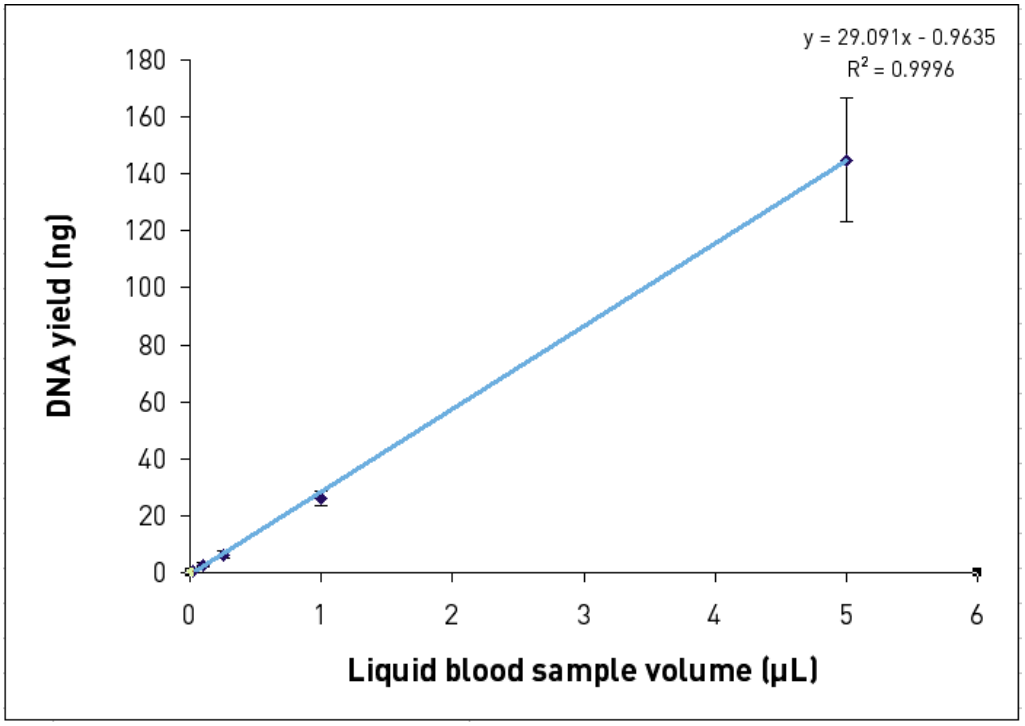


Figure 2 Sensitivity study average DNA yield

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.

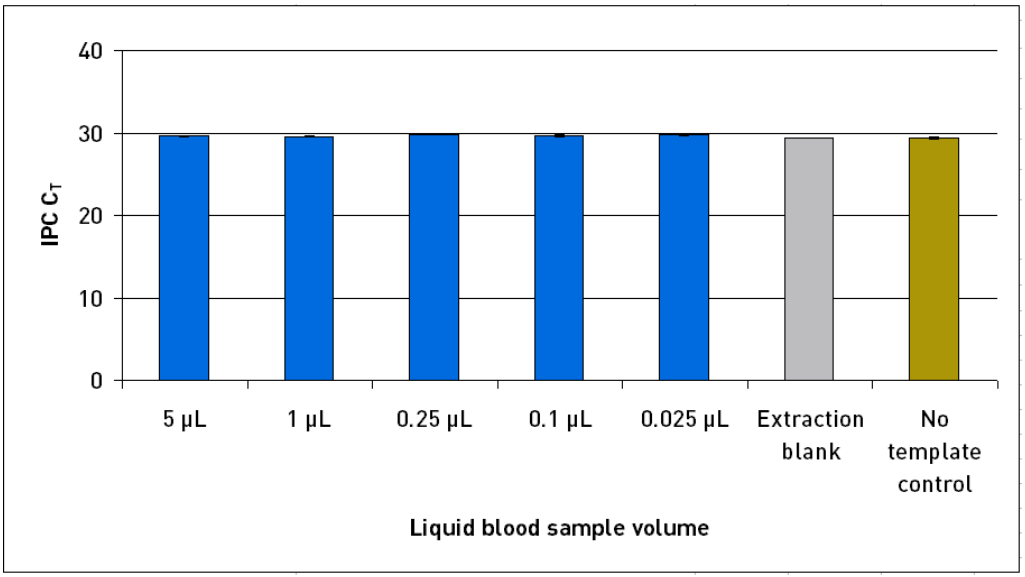


Figure 3 Sensitivity study IPC C_T

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).

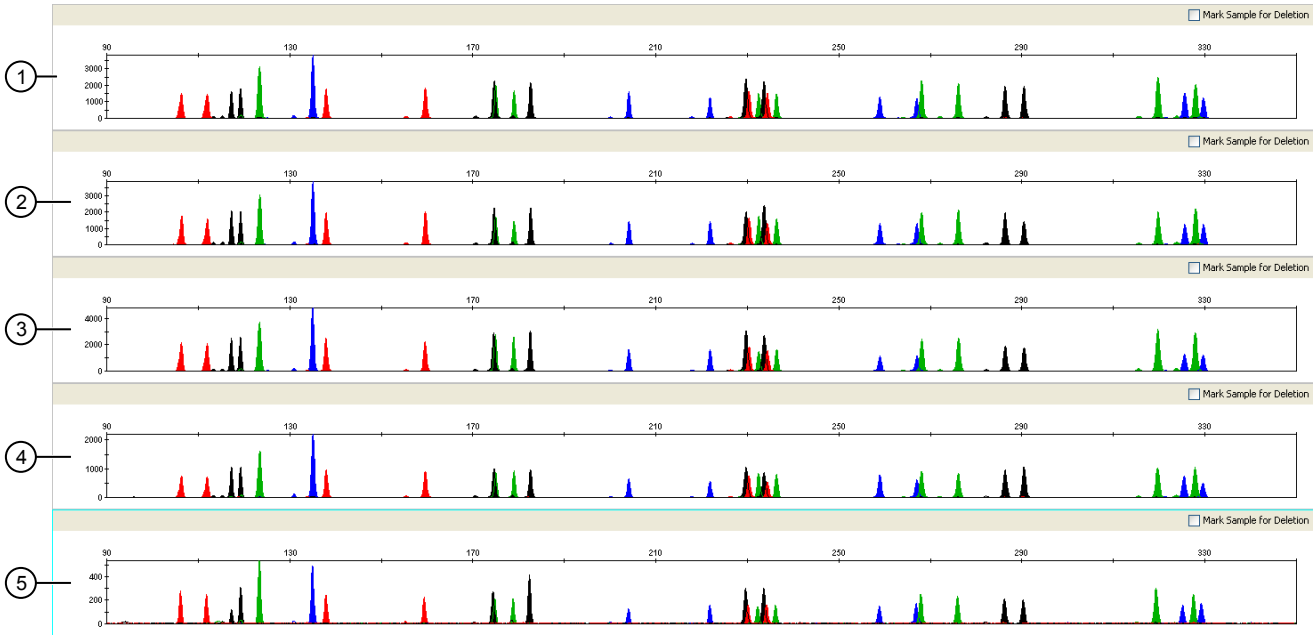


Figure 4 Sensitivity study STR profiles: 5, 1, 0.25, 0.1, and 0.025 µL blood samples

① 5 µL
② 1 µL
③ 0.25 µL
④ 0.11 µL
⑤ 0.25 µL

Stability studies (SWGDAM standard 2.4)

Stability studies 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.

Stability studies results

The average DNA concentration and yield for stability study samples are shown in Table 9. The variation in concentrations is within the expected variation introduced through the extraction and quantitation procedures.

Table 9 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 CT unit, indicating that PCR inhibitors present in all tested sample types were effectively removed during the extraction of DNA using the AutoMate *Express*™ System.

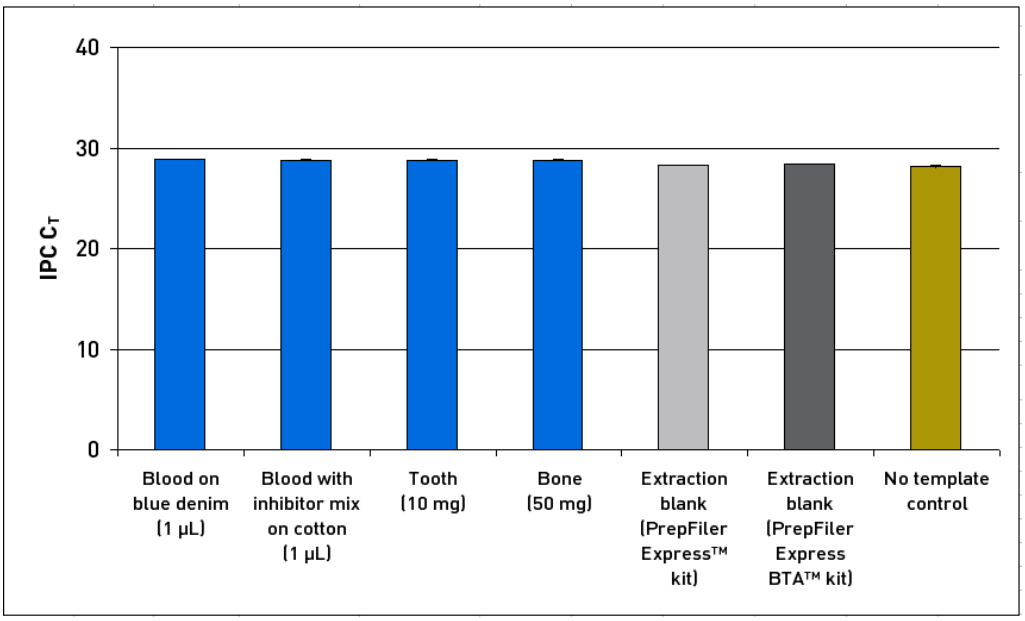


Figure 5 Stability study IPC C_T

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 Figure 6 and Figure 7. 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 Figure 6).

A ski slope effect was observed for both tooth and bone samples (panels 3 and 4 of Figure 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 Kits, 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.

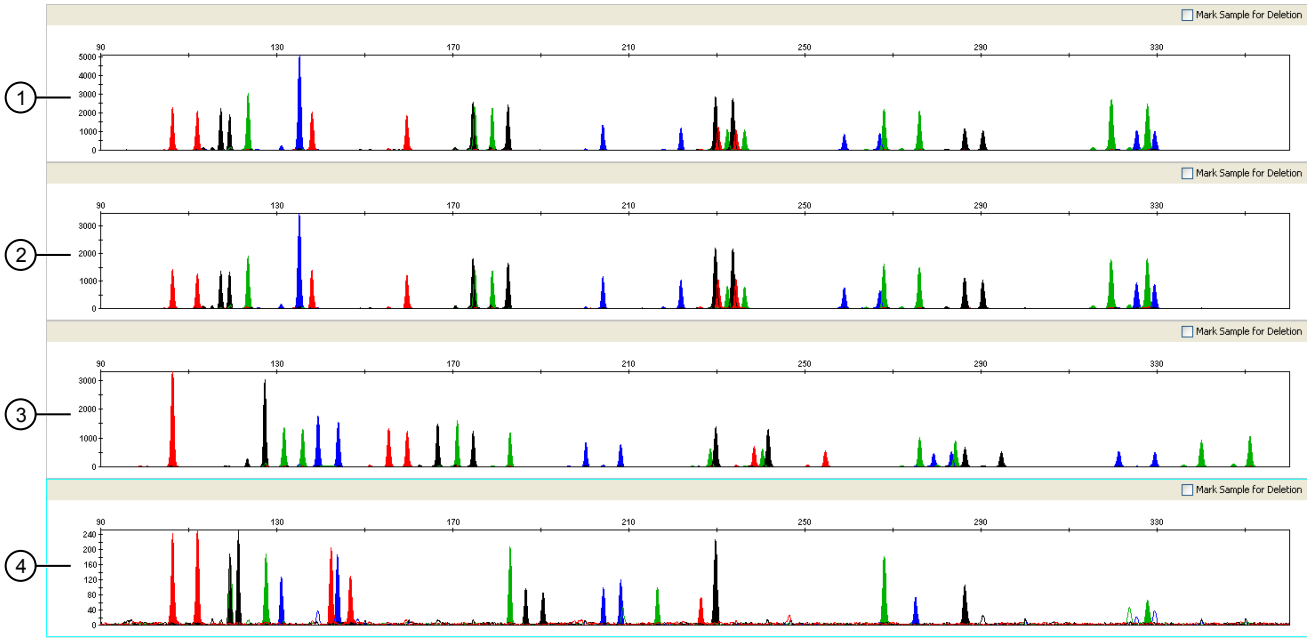


Figure 6 Stability study STR profiles for samples processed with the AmpFℓSTR™ Identifiler™ PCR Amplification Kit

① 1 µL blood on blue denim
② 1 µL blood with inhibitor mix on cotton cloth
③ 5 mg pulverized tooth exposed to environment for 3 days
④ 50 mg pulverized aged bone

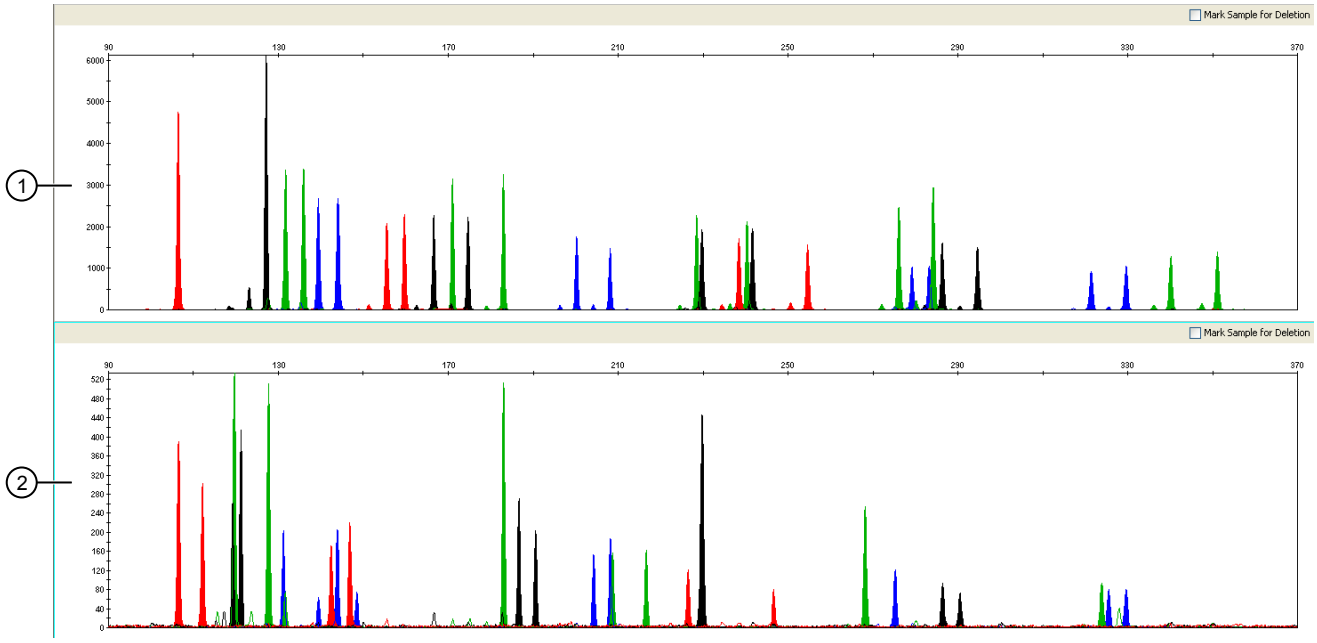


Figure 7 Stability study STR profiles for tooth and bone samples processed with the AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kits

① 5 mg pulverized tooth exposed to environment for 3 days
② 50 mg pulverized aged bone

Reproducibility studies (SWGDAM standard 2.5)

Reproducibility studies experiments

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

The reproducibility study sample sets are shown in Table 10. 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 10 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	

Reproducibility studies results

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

Average IPC C_T values for each sample type are shown in Figure 10. 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.

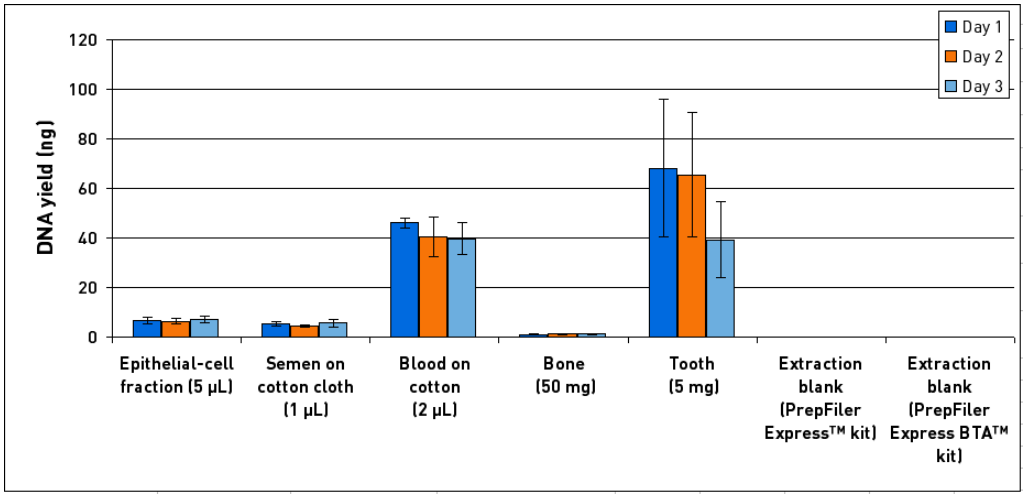


Figure 8 Reproducibility study average DNA yield

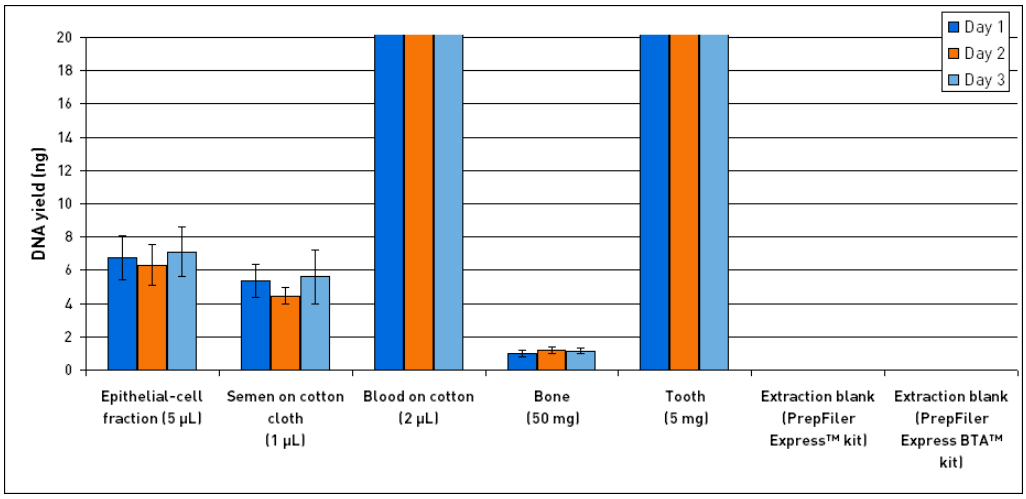


Figure 9 Reproducibility study: Magnified view of Figure 8 focusing on samples with less than 20 ng average DNA yield

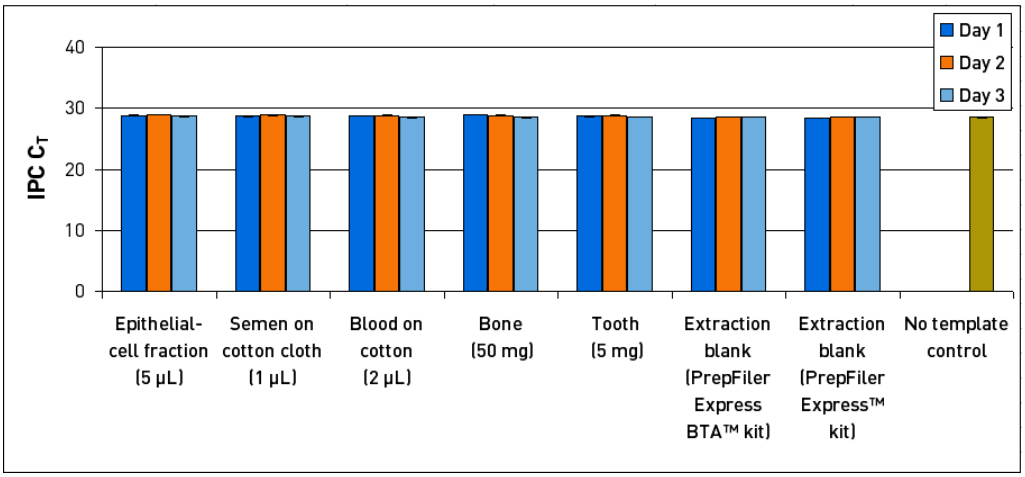


Figure 10 Reproducibility study IPC C_T

Case-type samples study (SWGDAM standard 2.6)

Case-type sample study 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 11. 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 11 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	

Description	Kit/Protocol
Epithelial-cell fractions[1]	PrepFiler *Express*™ kit/body fluids protocol
Sperm-cell fractions[1]	
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	—

[1] 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.

Case-type sample study 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. 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.

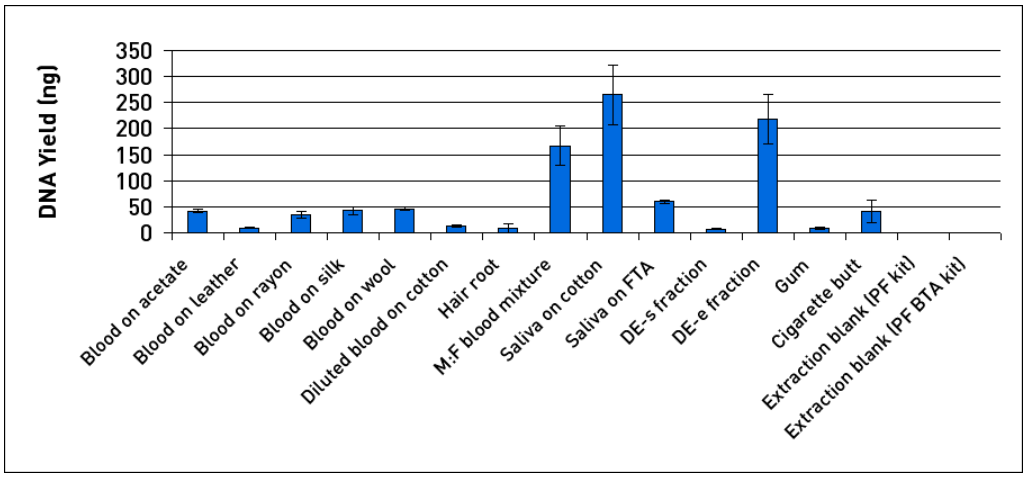


Figure 11 Case-type sample study average DNA yield

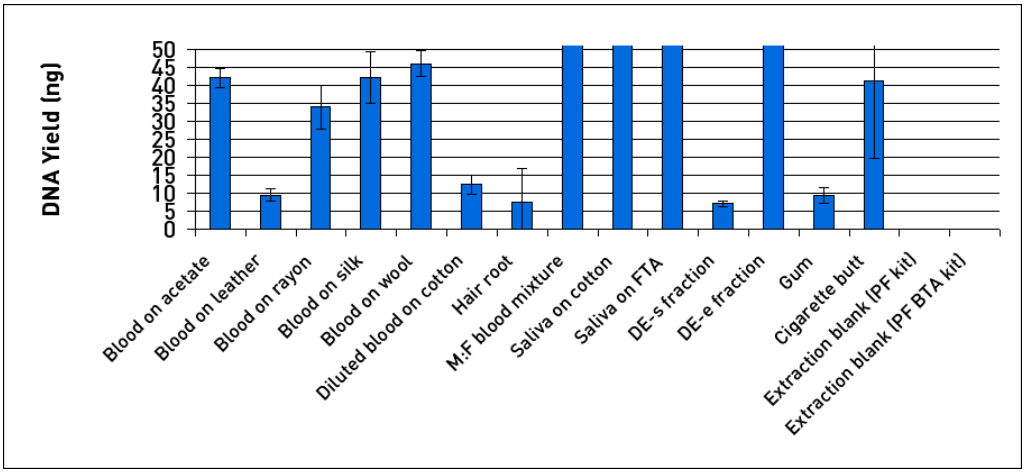


Figure 12 Case-type sample study: Magnified view of Figure 11 focusing on samples with less than 50 ng average DNA yield

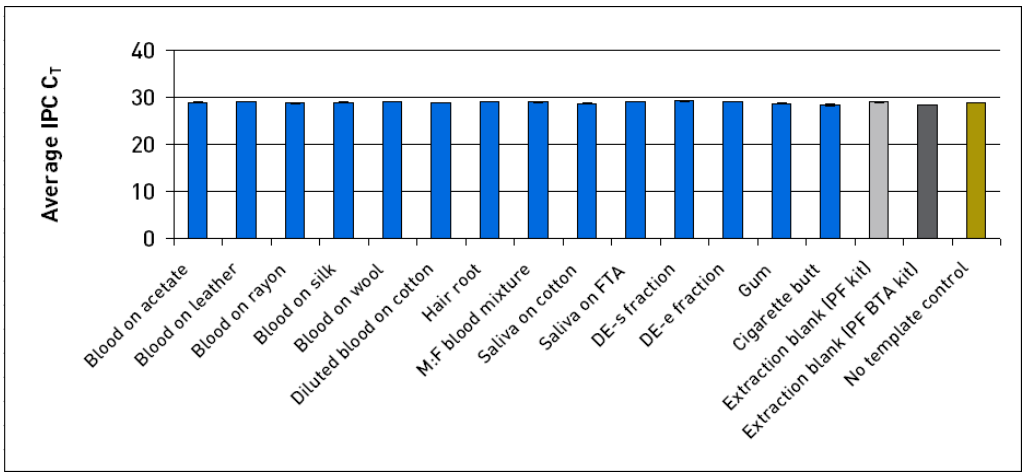


Figure 13 Case-type sample study IPC C_T

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, Figure 15, and Figure 16.

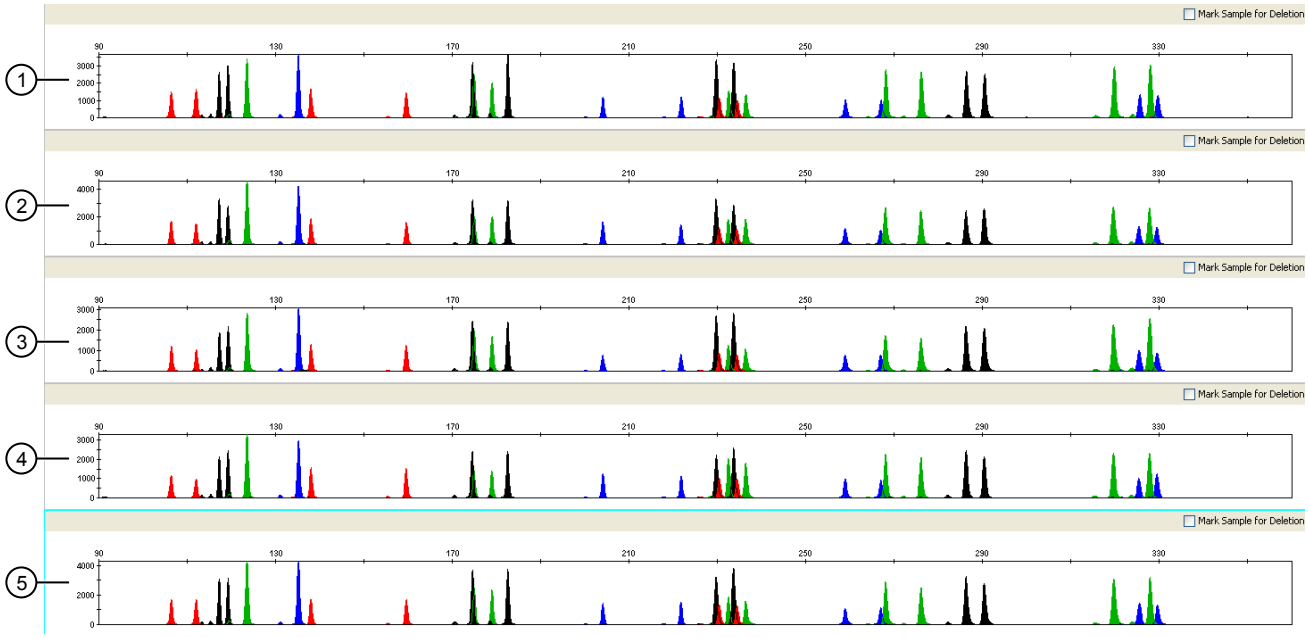


Figure 14 Case-type samples study

① 2 µL blood on acetate
② 1 µL blood on 5-mm black leather punch
③ 2 µL blood on rayon fabric
④ 2 µL blood on silk
⑤ 2 µL blood on wool

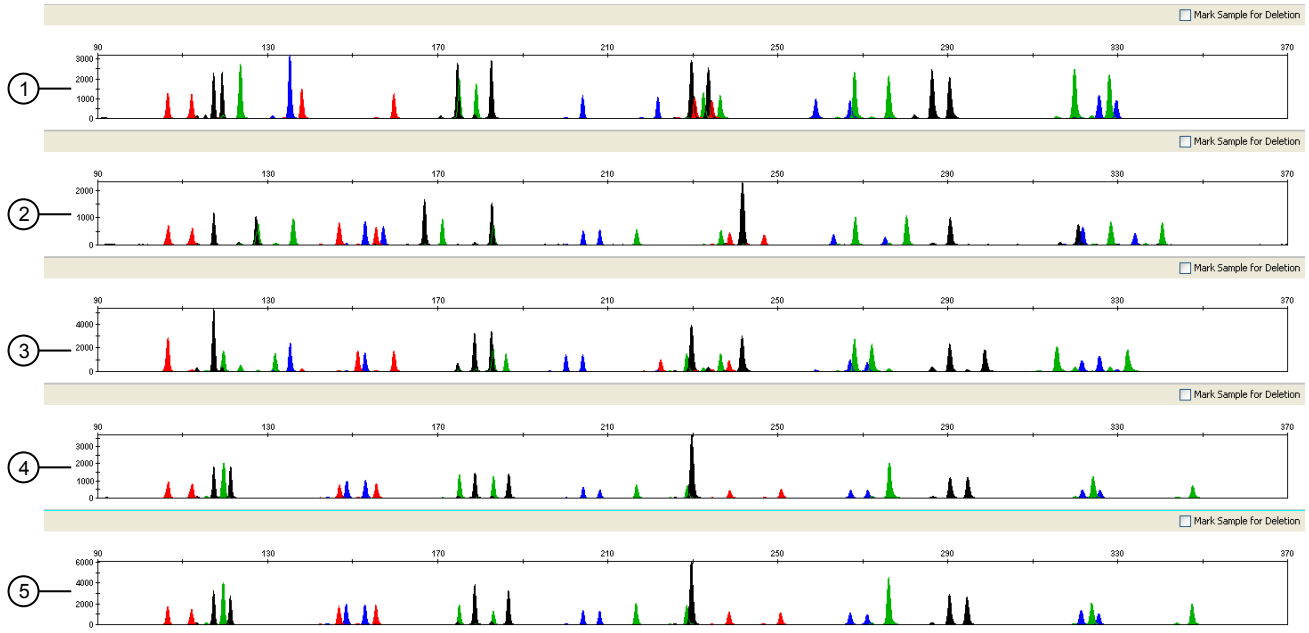


Figure 15 Case-type samples study

① 3 µL diluted blood (1:10) on cotton cloth
② Hair root
③ Blood mixture: 1 µL male (donor 239) and 9 µL female (donor 237)

④ 50 µL saliva stain on cotton

⑤ 5 µL saliva on 5-mm FTA punch

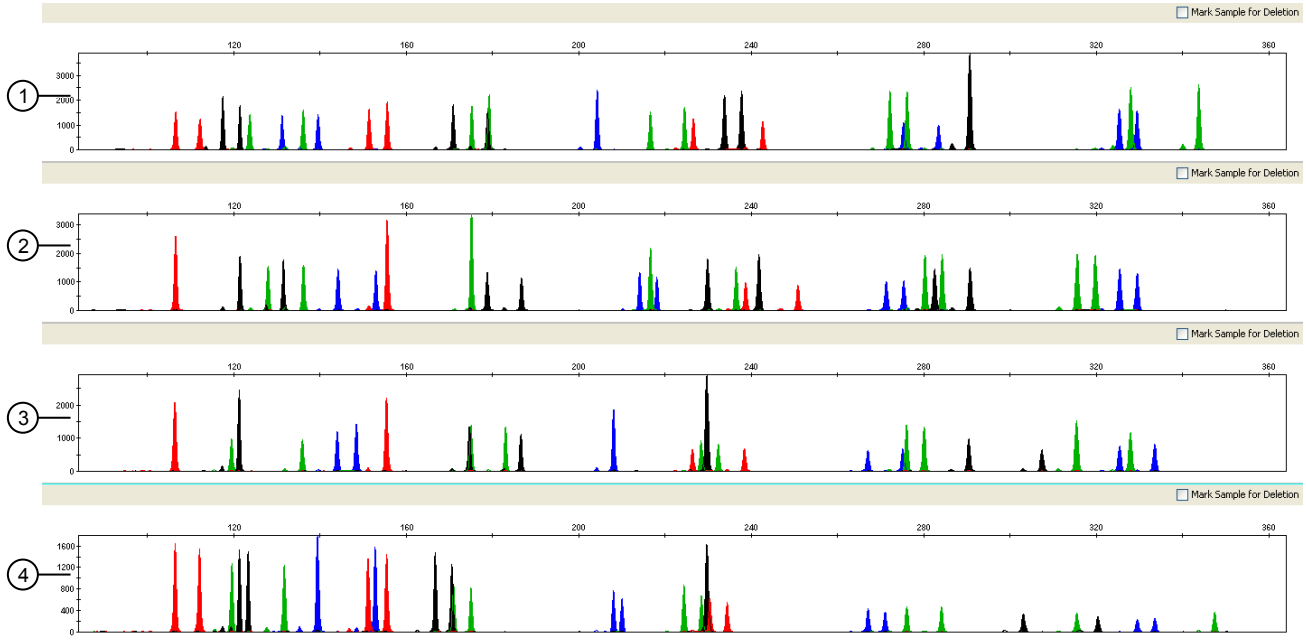


Figure 16 Case-type samples study

① Sperm-cell fractions

② Epithelial-cell fractions

③ Airwave gum (chewed for 30 minutes, 1/8 of one piece

④ Marlboro Light cigarette butt (approximately 3/4 cm cutting of filter paper)

Contamination study (SWGDAM standard 3.6)

Contamination study 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 12).

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 12 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 1X PBS	10 µL blood
E2	10 µL 1X PBS	10 µL blood	10 µL 1X PBS
E3	10 µL blood	10 µL 1X PBS	10 µL blood
E4	10 µL 1X PBS	10 µL blood	10 µL 1X PBS
E5	10 µL blood	10 µL 1X PBS	10 µL blood
E6	10 µL 1X PBS	10 µL blood	10 µL 1X PBS
E7	10 µL blood	10 µL 1X PBS	10 µL blood
E8	10 µL 1X PBS	10 µL blood	10 µL 1X PBS
E9	10 µL blood	10 µL 1X PBS	10 µL blood
E10	10 µL 1X PBS	10 µL blood	10 µL 1X PBS
E11	10 µL blood	10 µL 1X PBS	10 µL blood
E12	10 µL 1X PBS	10 µL blood	10 µL 1X PBS
E13	10 µL blood	10 µL 1X PBS	10 µL blood

Contamination study 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

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.
- Quality and quantity of DNA 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 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 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:
  a. Prepare a Proteinase K digestion solution by mixing 9.44 mL of bone incubation buffer with 560 µL of stock Proteinase K solution.
  b. Add 500 µL of freshly prepared PK digestion solution to the sample.
  c. Incubate the sample tube at 56°C for 1 hour.
  d. Centrifuge the sample tube at 5,000 rpm for 5 minutes to separate the remaining bone substrate.
  e. Transfer the solution to a new 1.5-mL tube.
  f. 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 13). 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 Quantification Kit. Each extracted sample was quantified one time. 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 13 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	50 mg bone
Bloodstain, 2 µL on 5-mm cotton cloth Extraction blank	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	

Correlation study 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 show the average total DNA yields (ng), and Figure 19 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.

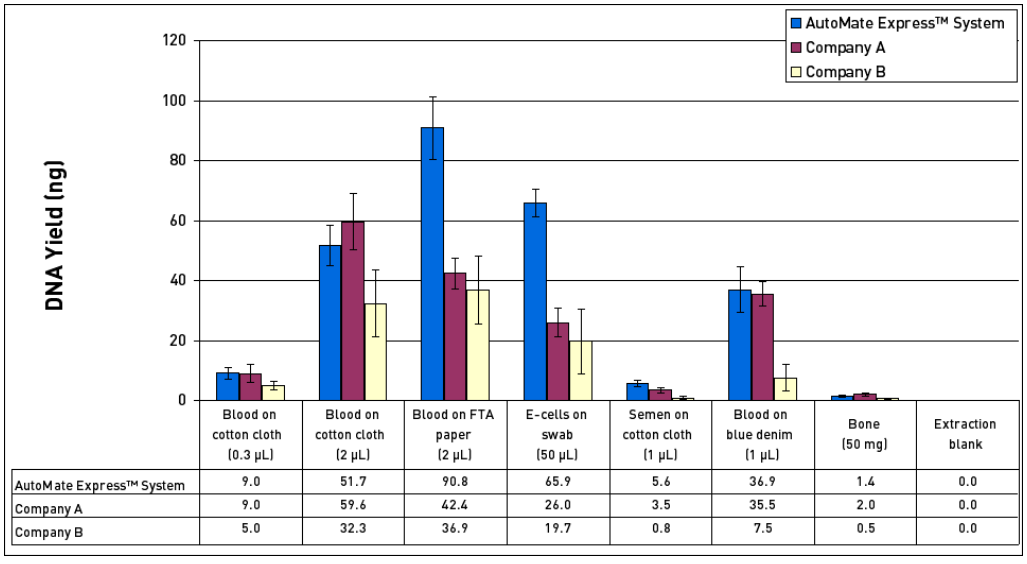


Figure 17 Correlation study average DNA yield

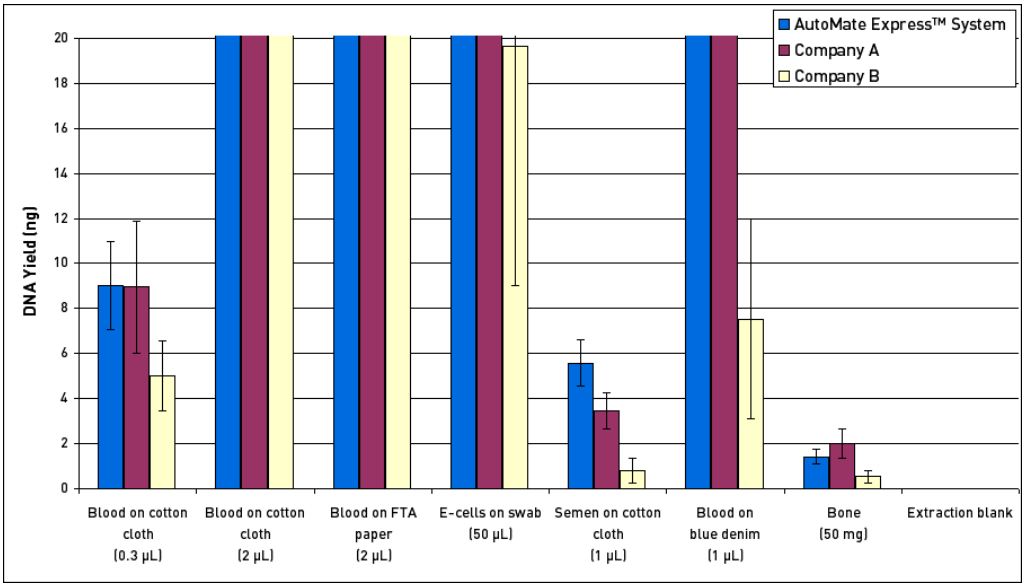


Figure 18 Correlation study: magnified view of Figure 17 focusing on samples with less than 20 ng average DNA yield

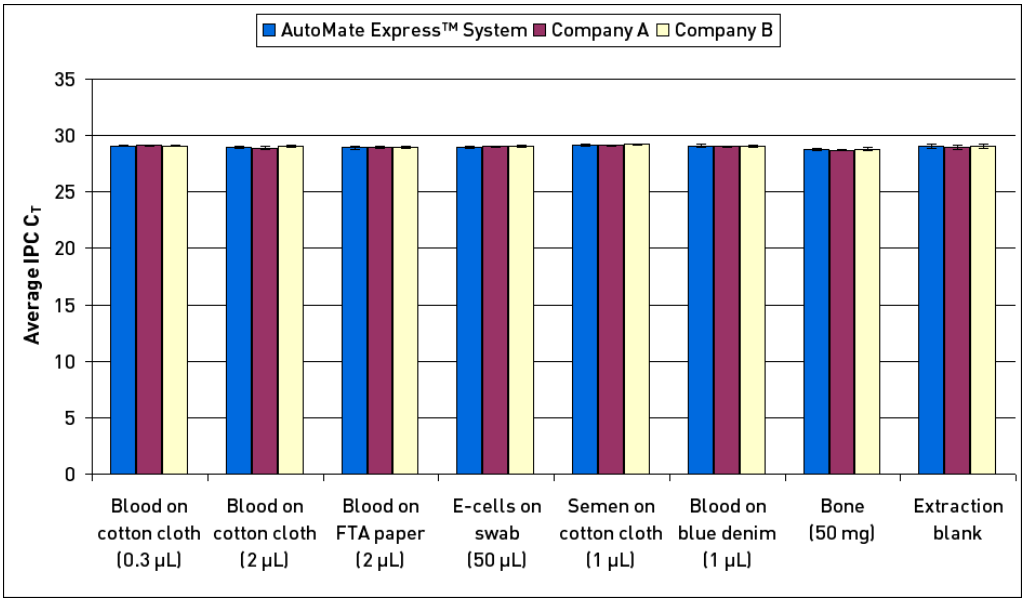


Figure 19 Correlation study IPC C_T

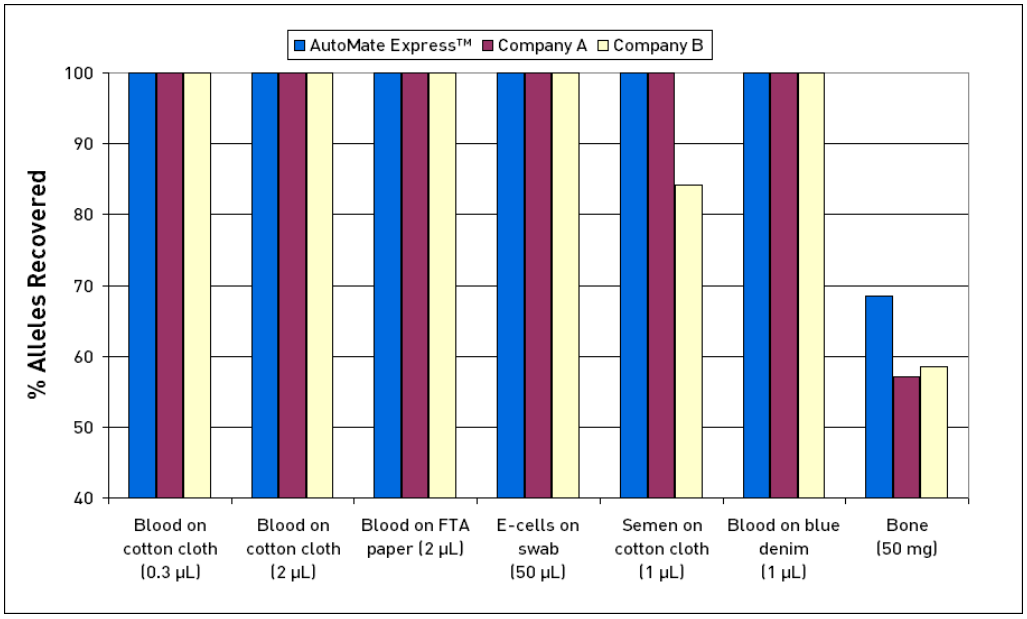


Figure 20 Correlation study percent alleles recovered by sample type

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 CT values using the Quantifiler™ Duo DNA Quantification Kit.
- The reagents and operations of the AutoMate *Express*™ 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 retesting 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.

Variable elution studies

The PrepFiler *Express*™ & PrepFiler *Express* BTA™ Protocol Card v1.1 supports elution volumes of 20–250 µL. We performed experiments to determine the effect of different elution volumes on eluted DNA concentration, DNA recovery, and STR profile quality.

Materials and methods

Component	Description
Samples	1 single-source blood sample, 1 single-source saliva sample
Chemistry kits	• PrepFiler *Express*™ Forensic DNA Extraction Kit • PrepFiler *Express* BTA™ Forensic DNA Extraction Kit • Quantifiler™ Trio DNA Quantification Kit • GlobalFiler™ PCR Amplification Kit
Instruments and software	• AutoMate *Express*™ Forensic DNA Extraction System with PrepFiler *Express*™ & PrepFiler *Express* BTA™ Protocol Card v1.1 • 7500 Real-Time PCR System with HID Software v1.2 • 3500/3500xL Genetic Analyzer with Data Collection Software v3.0 • GeneMapper™ ID-X Software v1.5

1. Lysate from each sample was generated using the PrepFiler *Express*™ kit and PrepFiler *Express* BTA™ kit. After extraction, the lysate from each kit and each elution volume was pooled to minimize sample input variation.
2. The pooled lysate from the PrepFiler *Express*™ kit was aliquotted in to 500 µL volumes.

   The pooled lysate from the PrepFiler *Express* BTA™ kit was aliquotted in to 230 µL volumes.
3. Three replicates of the lysate from each sample was extracted on the AutoMate *Express*™ Forensic DNA Extraction System using the respective protocol for the kit at elution volumes of 20 µL, 30 µL, 40 µL, 50 µL, 100 µL, 200 µL and 250 µL.
4. Samples were quantified in duplicate using the Quantifiler™ Trio DNA Quantification Kit on a 7500 instrument.
5. Samples were prepared using the GlobalFiler™ kit, then run on a 3500/3500xL Genetic Analyzer.
6. Samples were genotyped using the GeneMapper™ *ID-X* Software with a Peak Amplitude Threshold of 175 RFU.

PrepFiler *Express*™ kit results: Blood samples

As shown in Figure 21, DNA concentration increases with decreasing elution volume. Therefore, the use of smaller elution volume will increase the DNA concentration, which is helpful for processing low input DNA samples. However, the use of low elution volume may result in reduced DNA recovery (Figure 22) as shown by the 20 µL elution volume, which recovers about 80% of DNA compared to the 50 µL elution volume.

When 1 ng of total DNA was amplified with the GlobalFiler™ kit, full, well-balanced profiles were produced form all samples tested (Figure 23). Because there was no change to the extraction chemistry or the protocol with the exception of the elution volume, this is as expected.

PrepFiler *Express*™ kit results: Blood samples DNA concentration

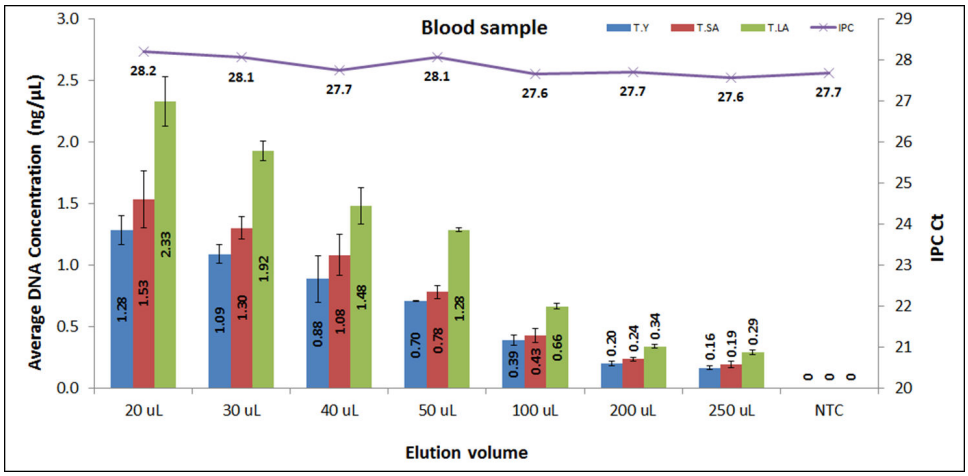


Figure 21 The effect of elution volume on DNA concentration of blood samples processed using PrepFiler *Express*™ kit. Each data point is a replicates of three. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

PrepFiler *Express*™ kit results: Blood samples DNA recovery

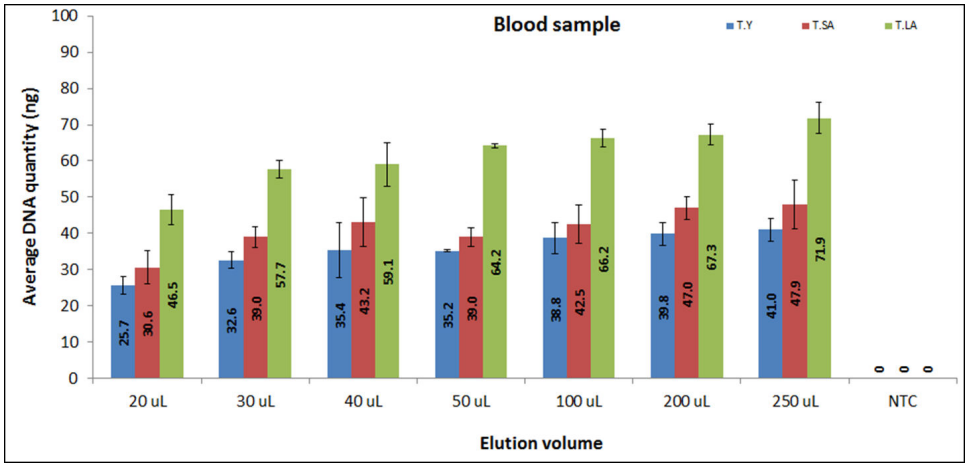


Figure 22 The effect of elution volume on DNA recovery of blood samples processed using the PrepFiler *Express*™ kit. Each data point is a replicates of three. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

PrepFiler *Express*™ kit results: Blood samples STR profiles

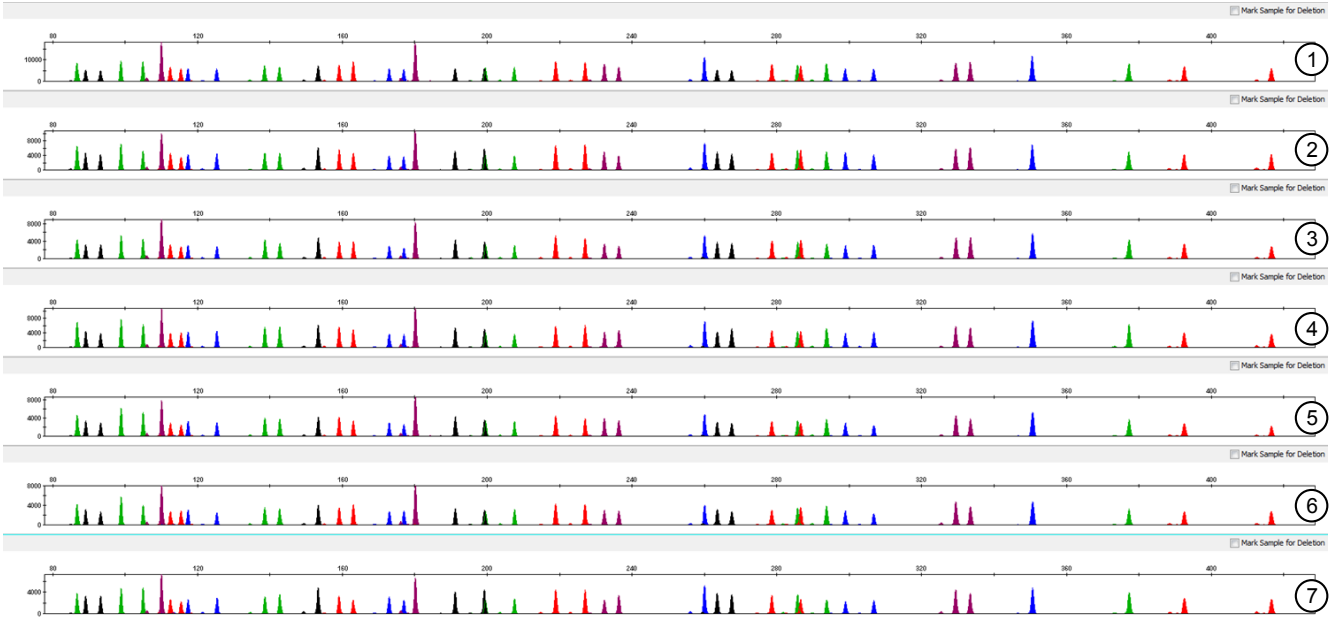


Figure 23 The effect of elution volume on GlobalFiler™ kit STR profile intracolor balance of blood samples processed using PrepFiler *Express*™ kit. STR PCR input amount: 1ng. There are three replicates with each of the seven elution volumes.

① 20 µL
② 30 µL
③ 40 µL
④ 50 µL
⑤ 100 µL
⑥ 200 µL
⑦ 250 µL

PrepFiler *Express*™ kit results: Saliva samples

As shown in Figure 24, DNA concentration increases with decreasing elution volume. Therefore, the use of smaller elution volume will increase the DNA concentration, which is helpful for processing low input DNA samples. The DNA recovery is also fairly constant among all seven elution volumes (Figure 25).

When 1 ng of total DNA was amplified with the GlobalFiler™ kit, full, well-balanced profiles were produced from all samples tested (Figure 26) (the total DNA input for the 200 µL and 250 µL elution volumes was 0.75 ng and 0.63 ng, respectively). Because there was no change to the extraction chemistry or the protocol with the exception of the elution volume, this is as expected.

PrepFiler *Express*™ kit results: Saliva samples DNA concentration

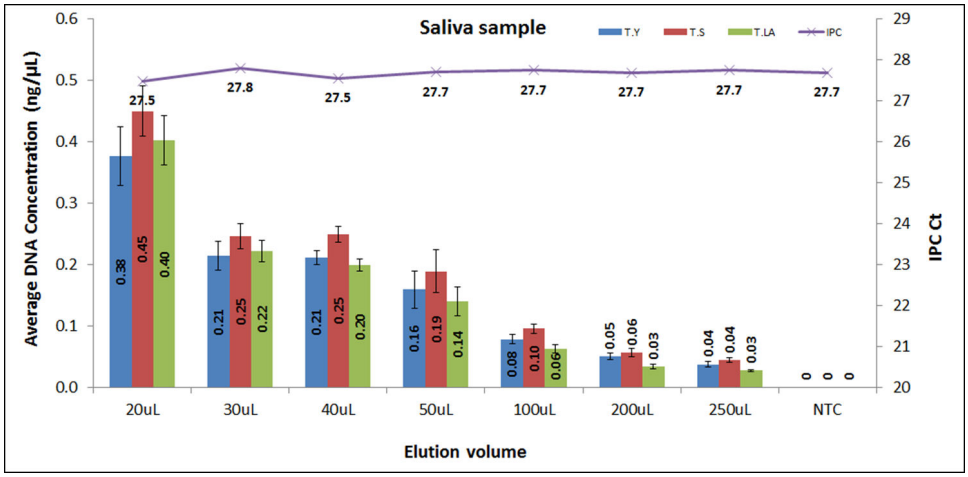


Figure 24 The effect of elution volume on DNA concentration of saliva samples processed using PrepFiler *Express*™ kit. Each data point is a replicates of three. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

PrepFiler *Express*™ kit results: Saliva samples DNA recovery

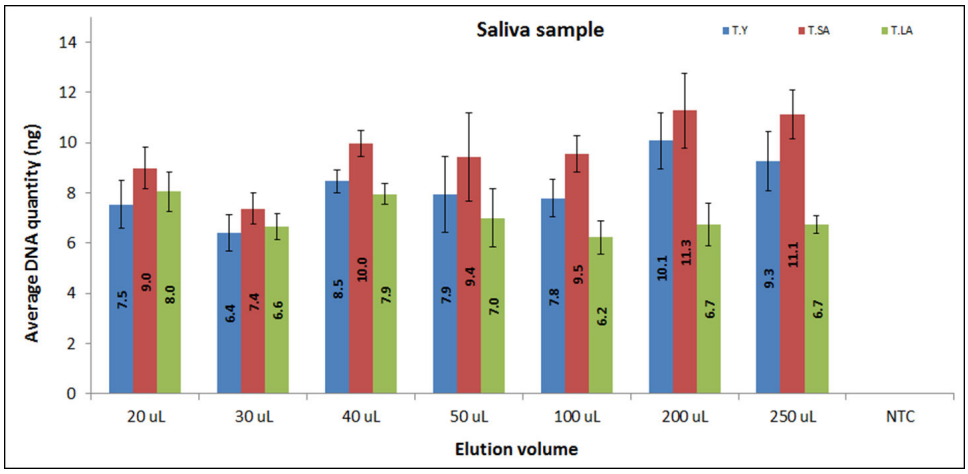


Figure 25 The effect of elution volume on DNA recovery of saliva samples processed using PrepFiler *Express*™ kit. Each data point is a replicates of three. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

PrepFiler *Express*™ kit results: Saliva samples intracolor balance

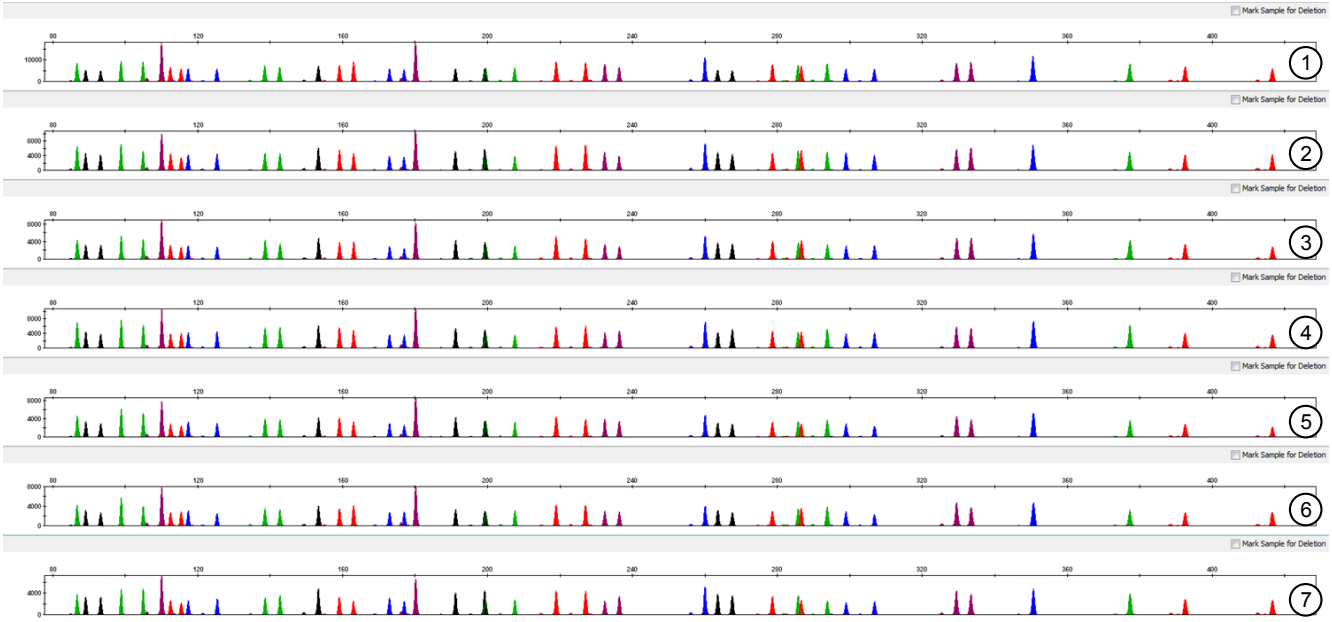


Figure 26 Saliva manual: The effect of elution volume on GlobalFiler™ kit STR profile intracolor balance of saliva samples processed using PrepFiler *Express*™ kit. STR PCR input amount: 1ng. There are three replicates with each of the seven elution volumes.

① 20 µL
② 30 µL
③ 40 µL
④ 50 µL
⑤ 100 µL
⑥ 200 µL
⑦ 250 µL

PrepFiler *Express* BTA™ kit results: Blood samples

As shown in Figure 27, DNA concentration increases with decreasing elution volume. Therefore, the use of smaller elution volume will increase the DNA concentration, which is helpful for processing low input DNA samples. However, the use of low elution volume may result in reduced DNA recovery (Figure 28) as shown by the 20 µL elution volume, which recovers about 94% of DNA recovered by the 50 µL elution volume.

When 1 ng of total DNA was amplified with the GlobalFiler™ kit, full, well-balanced profiles were produced from all samples tested (Figure 29). Because there was no change to the extraction chemistry or the protocol with the exception of the elution volume, this is as expected.

PrepFiler *Express* BTA™ kit results: Blood samples DNA concentration

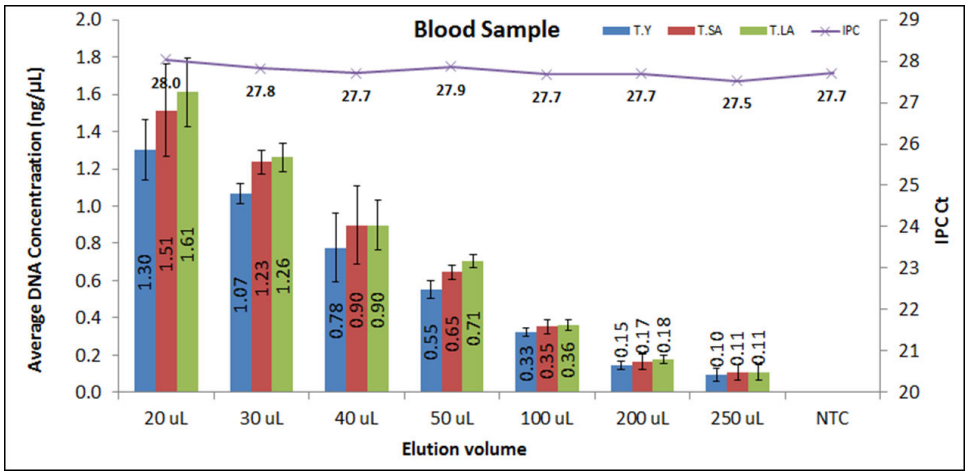


Figure 27 The effect of elution volume on DNA concentration of blood samples processed using the PrepFiler *Express* BTA™ kit. Each data point is a replicates of three. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

PrepFiler *Express* BTA™ kit results: Blood samples DNA recovery

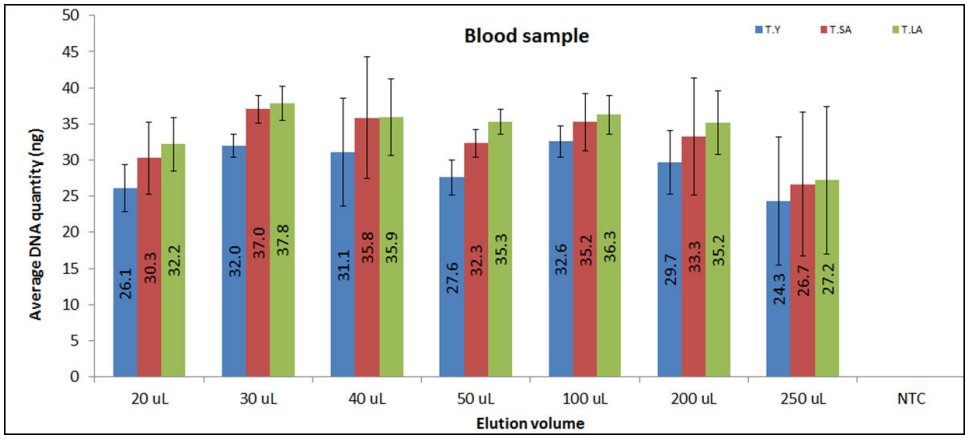


Figure 28 . The effect of elution volume on DNA recovery of blood samples processed using PrepFiler *Express* BTA™ kit. Each data point is a replicates of three. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

PrepFiler *Express* BTA™ kit results: Blood samples STR profiles

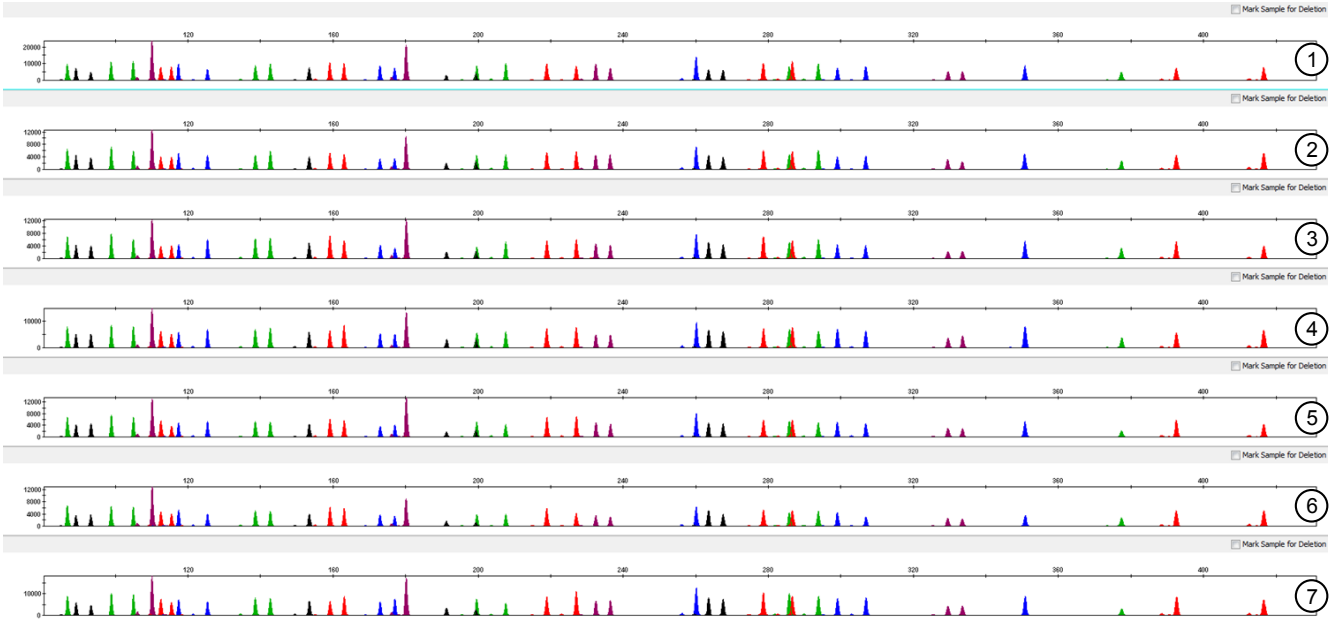


Figure 29 The effect of elution volume on GlobalFiler™ kit STR profiles of blood samples processed using PrepFiler *Express* BTA™ kit. STR PCR input amount: 1ng. There are three replicates with each of the seven elution volumes.

① 20 µL
② 30 µL
③ 40 µL
④ 50 µL
⑤ 100 µL
⑥ 200 µL
⑦ 250 µL

PrepFiler *Express* BTA™ kit results: Saliva samples

As shown in Figure 30, DNA concentration increases with decreasing elution volume. Therefore, the use of smaller elution volume will increase the DNA concentration, which is helpful for processing low input DNA samples. The DNA recovery is also fairly constant among all seven elution volumes (Figure 31).

When 1 ng of total DNA was amplified with the GlobalFiler™ kit, full, well-balanced profiles were produced from all samples tested (Figure 32) (the total DNA input for the 200 µL and 250 µL elution volumes was 0.75 ng and 0.63 ng, respectively). Because there was no change to the extraction chemistry or the protocol with the exception of the elution volume, this is as expected.

PrepFiler *Express* BTA™ kit results: Saliva samples DNA concentration

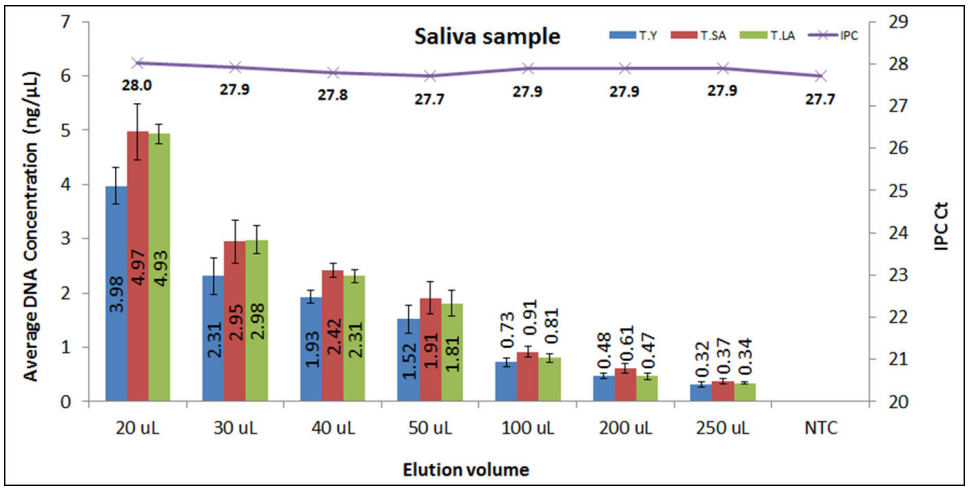


Figure 30 The effect of elution volume on DNA concentration of saliva samples processed using PrepFiler *Express* BTA™ kit. Each data point is a replicates of three. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

PrepFiler *Express* BTA™ kit results: Saliva samples DNA recovery

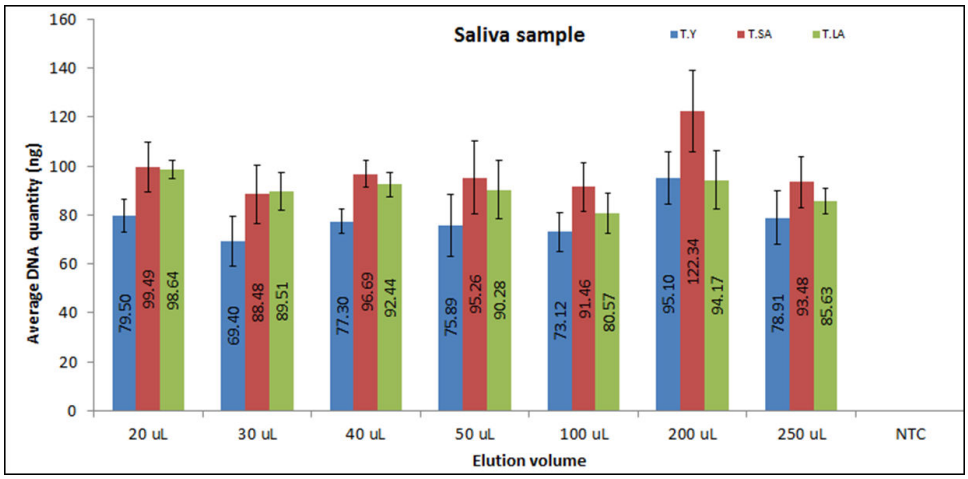


Figure 31 The effect of elution volume on DNA recovery of saliva samples processed using PrepFiler *Express* BTA™ kit. Each data point is a replicates of three. T.Y: male target; T.SA: small autosomal target; T.LA: large autosomal target; IPC: internal positive control.

PrepFiler *Express* BTA™ kit results: Saliva samples intracolor balance

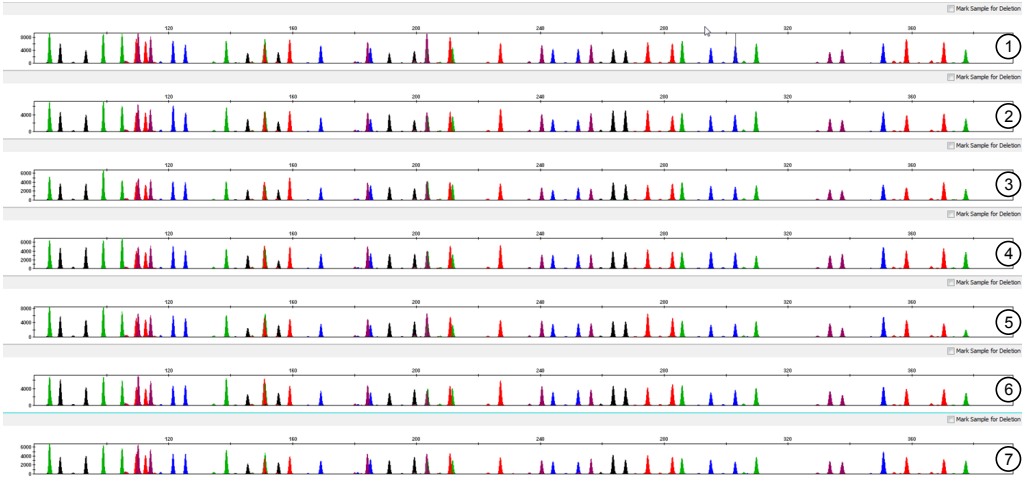


Figure 32 The effect of elution volume on GlobalFiler™ kit STR profile intracolor balance of saliva samples processed using PrepFiler *Express* BTA™ kit. STR PCR input amount: 1ng. There are three replicates with each of the seven elution volumes.

① 20 µL
② 30 µL
③ 40 µL
④ 50 µL
⑤ 100 µL
⑥ 200 µL
⑦ 250 µL

Conclusions

DNA concentration increased with decreased elution volume in the blood and saliva samples tested.

DNA recovery was fairly consistent at 20 µL, 30 µL, 40 µL, 50 µL, 100 µL, 200 µL and 250 µL elution volumes with saliva samples. A slight reduction in DNA recovery was observed with 20 µL elution volume with blood samples.

STR profile quality was not affected by different elution volumes.

The 50 µL standard elution volume can continue to be used for diverse sample and substrate types. Elution volumes >50 µL can be considered for high-quality–high-quantity samples such as reference samples. Elution volumes <50 µL can be considered for low-quantity samples such as touch samples. Perform the necessary internal validation studies to ensure that the different elution volumes perform as expected in the laboratory system before implementation.

A

Troubleshooting

Troubleshooting after lysis incubation

Observation	Possible cause	Recommended action
Precipitate is observed in a LySep Column before centrifugation	• Tube was chilled for more than 40 minutes. • Lysate was chilled. • Low temperature in the laboratory.	• Do either of the following: – Use a pipette to mix the sample lysate and dissolve the salt precipitate. – 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 is observed in a sample tube after centrifugation	• Tube was chilled for more than 40 minutes. • Lysate was chilled. • Low temperature in the 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.

Troubleshooting before loading the cartridges in the cartridge rack

Observation	Possible cause	Recommended action
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 Cartridges at 37°C for 30 minutes or until precipitate is no longer visible.

Troubleshooting during an automated extraction run

Observation	Possible cause	Recommended action
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 instrument.
During the run: No liquid is present in the tip or liquid in the tip does not move After the run: No elution volume.	No sample was added to the tube, causing a wet filter barrier on the tip and blockage of nozzles.	Add samples to tubes, load new reagent cartridges, replace wetted tips as needed, then perform the run again.
	The sample volume is lower than the recommended volume, causing a wet filter barrier on the tip and blockage of nozzles.	In future runs, use the recommended sample volume in the user guide for the kit you are using. Long-term operation with lower-than-recommended sample volumes can lead to problems with liquid handling performance.

Troubleshooting after eluting the DNA

Observation	Possible cause	Recommended action
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.

Troubleshooting after quantifying the extracted DNA

Observation	Possible cause	Recommended action
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.
	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 before automated extraction.

Observation	Possible cause	Recommended action
Sample IPC C_T is higher than quantitation NTC or quantitation standards IPC C_T For example, during quantitation using a Quantifiler™ kit,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.	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).

Troubleshooting after performing STR analysis

Observation	Possible cause	Recommended action
Unbalanced STR profile **Note:** These causes and solutions are related to sample preparation. For other possible causes and solutions, see the applicable STR 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 (shortterm) 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

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

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

Chemical safety

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

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

Biological hazard safety

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number	Description
AutoMate Express™ Instrument User Guide	4441982	Describes the AutoMate *Express*™ Instrument hardware and software and provides information on preparing, maintaining, and troubleshooting the system.
PrepFiler Express™ and PrepFiler Express™ BTA Forensic DNA Extraction Kits Quick Reference	4443104	Provides abbreviated procedures for preparing and extracting DNA.
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 during the development of the PrepFiler™ Forensic DNA Extraction Kit .

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.