PrepFiler™

Forensic DNA Extraction Kit

User Guide

Applied Biosystems

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Preface

How to use this guide

Purpose of this guide

This guide provides step-by-step instructions for using the PrepFiler™ Forensic DNA Extraction Kit to extract and isolate genomic DNA from forensic samples, and a description of the experiments performed by Applied Biosystems to evaluate the PrepFiler Forensic DNA Extraction Kit.

Audience

This guide is intended for scientists who isolate DNA from forensic samples for the purpose of quantitation and Short Tandem Repeat (STR) analysis.

Text conventions

This guide uses the following conventions:

- **Bold text** indicates user action. For example:
  Add **15 µL** of PrepFiler™ Magnetic Particles to the tube.
- *Italic text* indicates new or important words and is also used for emphasis. For example:
  Before analyzing, *always* prepare fresh matrix.

User attention words

Two user attention words appear in Applied Biosystems user documentation. 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, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: You can also use a heat block to incubate the samples.

IMPORTANT! Do not disturb the magnetic particles pellet.

Safety information

Safety alert words

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

Definitions

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

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

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

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDS" on page vii.)
- 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 MSDS.
- 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 MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDS

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

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

Obtaining MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day.

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

To obtain MSDSs:

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

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

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 Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) 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 MSDS.
- 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 MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

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

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

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

Biological hazard safety

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

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

Additional information about biohazard guidelines is available at:

www.cdc.gov

How to obtain more information

Related documentation

The following related document is available from Applied Biosystems:

- ***PrepFiler™ Forensic DNA Extraction Kit Quick Reference Card*** (PN 4393918) – 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 the User Guide.

For additional documentation, see "How to obtain support" on page xi.

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **http://www.appliedbiosystems.com**, then click the link for **Support**. (See "How to obtain support" on page xi).

How to obtain support

For HID support:

- In North America – send an email to **HIDTechSupport@appliedbiosystems.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 **http://www.appliedbiosystems.com**, then click the link for **Support**. At the Support page, you can:

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

Introduction 1

This chapter covers:

PrepFiler™ Forensic DNA Extraction Kit description 2
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Kit contents and storage conditions. 4
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PrepFiler™ Forensic DNA Extraction Kit description

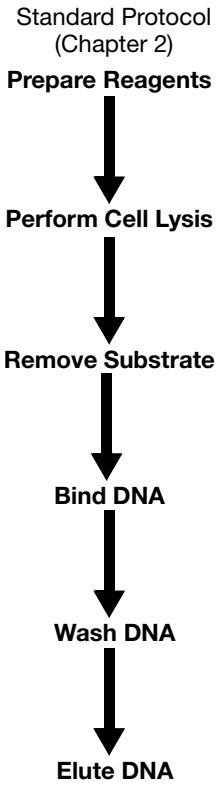
The PrepFiler™ Forensic DNA Extraction Kit contains Applied Biosystems reagents optimized for use in:

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

The kit can be used with one standard protocol to extract and isolate DNA from most forensic sample types, including body fluids, stains and swabs of body fluids, and small tissue samples. The approximate yield from 1 µL of blood that contains 4,000 to 11,000 nucleated blood cells is 25 to 65 ng of DNA. The kit is appropriate for use with samples containing potential inhibitors of the polymerase chain reaction (PCR). The extracted DNA is compatible for use in quantitation using the Quantifiler® Human, Quantifiler® Y Human Male, and Quantifiler® Duo DNA Quantification Kits, and for use in STR amplification using the AmpFℓSTR® PCR Amplification kits.

Extraction workflow overview

This user guide contains a standard extraction protocol that is appropriate for most forensic sample types (see Chapter 2 on page 9), and supplementary protocols that cover additional sample types such as hair, nails, and teeth (see Chapter 3 on page 23). The standard protocol workflow is shown below. The supplementary protocols follow similar workflows, with some differences due to variations in sample preparation and lysis.



Kit contents and storage conditions

Kit contents

Each PrepFiler™ Forensic DNA Extraction Kit contains materials sufficient to perform 100 extractions using the standard protocol (300 µL PrepFiler™ Lysis Buffer per extraction). The contents of the kits are described in Table 1.

Table 1 Materials provided with the PrepFiler™ Forensic DNA Extraction Kits (PNs 4392852 and 4392353)

Reagent	Description	Included with Part Number 4392852‡	Included with Part Number 4392353
PrepFiler™ Lysis Buffer	One bottle, 50 mL	✔	✔
PrepFiler™ Isopropanol	One empty 60 mL bottle (user provides isopropanol)	✔	✔
PrepFiler™ Magnetic Particles	One tube, 1.5 mL	✔	✔
PrepFiler™ Wash Buffer Concentrate	Two 125-mL bottles, each containing 26 mL of concentrate (user adds 74 mL of ethanol to each bottle)	✔	✔
PrepFiler™ Elution Buffer	One bottle, 12.5 mL	✔	✔
PrepFiler™ Filter Columns	100 filter columns	✔	no
PrepFiler™ Spin Tubes	300 spin tubes	✔	no

‡ The kit includes filter columns and spin tubes for sample substrate removal. Extra spin tubes are included for use in the lysis and elution steps.

Storage conditions

The kits are shipped at room temperature.

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

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

Table 2 PrepFiler™ Forensic DNA Extraction Kit storage conditions

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

Required materials and instruments

About thermal shakers

A thermal shaker was used in the validation studies described in Chapter 4 and is recommended for DNA extraction using the PrepFiler™ Forensic DNA Extraction Kit.

You can use a heat block (following the heat block instructions provided in the protocols) instead of a thermal shaker, however the kit performance has not been extensively tested under these conditions, and DNA yield may be lower than would be obtained using a thermal shaker.

Each lab should perform studies to validate the performance of the PrepFiler kit when using methodologies other than a thermal shaker.

Required materials and instruments

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

Table 3 Required materials and instruments

Protocol Step	Material or Instrument	Source‡
All	Common laboratory equipment such as pipettors, aerosol-resistant micropipette tips, and a microcentrifuge	Major laboratory supplier
All	Vortexer (a variable-speed vortexer is recommended)	Major laboratory supplier
Lysis	DL-Dithiothreitol [Molecular biology grade; ≥98% (TLC), ≥99% (titration)]	Sigma-Aldrich www.sigmaaldrich.com (Part Number D9779)
Lysis	RNase-free Microfuge Tubes (1.5 mL), certified DNase- and RNase-free **Note:** 1.5-mL tubes are required for use with the reagent-only version of the PrepFiler™ kit (PN 4392353), which does *not* include PrepFiler™ Filter Columns and Spin Tubes.	Applied Biosystems (PN AM12400) or equivalent **Note:** If you use an equivalent tube, select tubes that allow you to observe the tube contents.
Lysis and elution	(Recommended) Eppendorf Thermomixer R or similar thermal shaker *or* Heat block (see "About thermal shakers" above; a heat block also requires more hands-on processing time than a thermal shaker)	Eppendorf North America www.eppendorfna.com *or* Major laboratory supplier
Substrate removal	Laboratory centrifuge capable of 16,110 x g	Major laboratory supplier
Binding	Isopropanol (2-Propanol, ACS reagent grade, ≥99.5%) **Note:** Open a new bottle when preparing the PrepFiler™ Isopropanol bottle.	Sigma-Aldrich www.sigmaaldrich.com (Part Number 190764)

Table 3 Required materials and instruments *(continued)*

Protocol Step	Material or Instrument	Source‡
Binding	Laboratory shaker for microcentrifuge tubes *or* Vortexer with adaptor for microcentrifuge tubes such as a VWR Microtube Holder (holds forty-eight 0.25-2.0 mL microcentrifuge tubes)	Major laboratory supplier *or* VRW www.vwr.com (Catalog number 12620-876)
Wash	Ethanol (Molecular biology grade; 95% or 190 proof) **Note:** Open a new bottle when preparing the PrepFiler™ Wash Buffer solution.	Sigma-Aldrich www.sigmaaldrich.com (Part Number E7148)
Wash and elution	6-Tube Magnetic Stand	Applied Biosystems (PN AM10055)

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

Chapter 2

Perform Extraction – Standard Protocol

Perform Extraction – Standard Protocol

2

This chapter covers:

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Step 6: Elute DNA. 22

Standard protocol – sample types and inputs

The standard protocol is appropriate for most forensic sample types, including body fluids, stains and swabs of body fluids, and small tissue samples. Examples of appropriate sample types and inputs for the standard protocol are shown in Table 4.

Applied Biosystems validation studies were performed using the standard protocol and the sample inputs shown in Table 4. The samples used in the validation studies were prepared from body fluids. Optimal input amounts may be affected by factors such as sample age and substrate properties. Each lab should perform studies to independently validate input amounts.

Table 4 Example sample types and inputs for use with the standard protocol

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
Tissue fragments (for example, tissue fragments from a razor or other swabbed substrate, or from a fingernail scraping or swabbing)	Up to one swab

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

Required materials

See "Required materials and instruments" on page 6 for details.

For all steps

- Pipettors
- Aerosol-resistant micropipette tips
- Variable-speed vortexer
- Microcentrifuge

For lysis

- PrepFiler™ Lysis Buffer
- 1.0 M solution DL-Dithiothreitol (DTT)
- PrepFiler™ Spin Tube or standard 1.5-mL microcentrifuge tube
- Thermal shaker or heat block

IMPORTANT! Read "About thermal shakers" on page 6 before using a heat block.

For substrate removal

- PrepFiler™ Spin Tube
- PrepFiler™ Filter Column
- Sterile tweezers or other tools for transferring substrate

For binding DNA

- PrepFiler™ Magnetic Particles

Note: Use standard pipette tips for pipetting magnetic particles.

- Isopropanol
- Shaker, or vortexer with adaptor

For washing DNA

- Prepared PrepFiler™ Wash Buffer
- 6-Tube Magnetic Stand

For eluting DNA

- PrepFiler™ Elution Buffer
- Thermal shaker or heat block

IMPORTANT! Read "About thermal shakers" on page 6 before using a heat block.

- 6-Tube Magnetic Stand
- PrepFiler™ Spin Tube or standard 1.5-mL microcentrifuge tube

Tube-handling guidelines

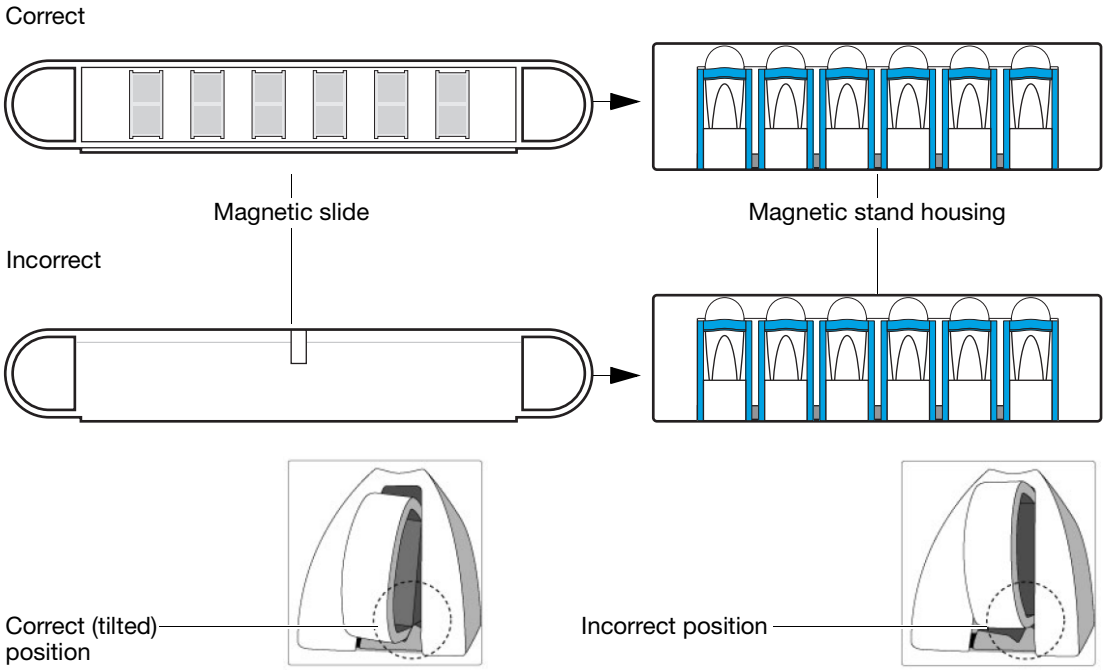
IMPORTANT! Leaking tubes may result in DNA cross-contamination. Avoid cross-contamination by observing the following guidelines.

- Change gloves frequently when handling tubes. For example, change gloves after removing the filter column from the spin tube.
- To avoid leaks, make sure that tubes are tightly sealed before vortexing or incubation.
- After vortexing a tube, check the tube for air bubbles, then re-vortex if necessary to remove bubbles.
- Before opening a tube after vortexing or incubation, centrifuge the tube briefly (approximately two seconds in a microcentrifuge) to collect any residual tube contents from the sides and cap of the tube.

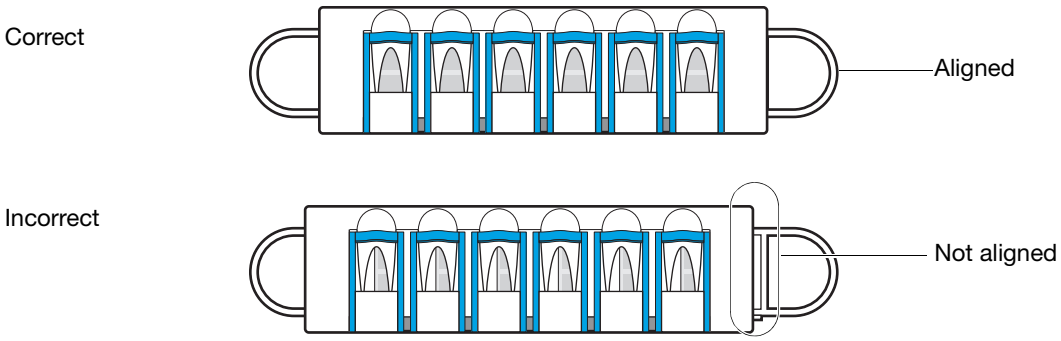
Magnetic stand guidelines

To help optimize magnetic pellet formation, ensure that the magnetic stand is correctly assembled before performing the wash and elution steps:

1. Insert the magnetic slide into the magnetic stand housing with the magnetic slide facing front and in the tilted position:



2. Confirm that the magnetic slide is correctly aligned:



Step 1: Prepare reagents

Prepare reagents before first use and before each extraction assay.

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. PrepFiler™ Magnetic Particles are harmful by inhalation, skin absorption, and if swallowed. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor (or dust). Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. PrepFiler™ Wash Buffer Concentrate causes eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare reagents before first use

IMPORTANT! Perform these steps before using each PrepFiler™ kit for the first time.

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

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

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

2. Measure 60 mL of freshly-opened isopropanol, then add the isopropanol to the empty isopropanol bottle.

   Note: Keep prepared isopropanol closed when it is not in use, and follow the manufacturer's shelf-life recommendations.

3. Measure 74 mL of freshly-opened ethanol, then add the ethanol to *one* of the wash buffer concentrate bottles.

   Note: The PrepFiler™ kit contains two bottles of wash buffer concentrate. For best results, prepare and use one bottle of wash buffer at a time.

   Note: Prepared wash buffer has a shelf life of six months if the container is kept closed when it is not in use.

4. (Optional) Prepare and freeze aliquots of a fresh 1.0 M solution of DL-Dithiothreitol (DTT) in DNA-free water:
   a. Dissolve 1.54 g of Dithiothreitol (DTT, MW 154) in 10 mL of molecular-biology grade DNA-free water.
   b. Prepare aliquots of the desired volume (for example, 100 µL or 500 µL), then store the aliquots at –20 °C for up to six months.

Prepare reagents before each assay

1. If the PrepFiler™ Magnetic Particles tube is stored at 4 °C, or if the tube contains precipitate, incubate the tube at 37 °C for 30 minutes, vortex the tube for 5 seconds, then centrifuge briefly.
2. If the lysis buffer contains precipitate, heat the solution to 37 °C, then vortex the bottle for 5 seconds.
3. If all of the first bottle of wash buffer was used in previous assays, measure 74 mL of freshly-opened ethanol, then add the ethanol to the second bottle of the wash buffer concentrate provided with the kit.
4. Thaw, or prepare a fresh 1.0 M solution of DL-Dithiothreitol (DTT) in molecular-biology grade DNA-free water.

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

Step 2: Perform lysis

About lysis time and temperature

Incubation times *may* be extended for sample types such as fixed stains that may be difficult to lyse.

In our experience, temperature and time for lysis can be varied between 50 °C and 80 °C and 10 and 90 minutes, respectively. It is recommended that the temperature and time for lysis not exceed 80 °C and 90 minutes, respectively.

Overnight incubation is not recommended due to the potential to degrade DNA.

Perform lysis

After preparing the reagents, perform cell lysis.

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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

1. Bring the thermal shaker temperature to 70 °C.
2. Place a sample in a PrepFiler™ Spin Tube or standard 1.5-mL microcentrifuge tube.
3. To the tube that contains the sample, add:
   - **PrepFiler™ Lysis Buffer**: 300 µL
   - **DTT, 1.0 M**: 3 µL (use 5 µL for samples containing semen)

   IMPORTANT! If the lysis buffer does not cover the sample substrate (for example, 300 µL may not cover certain types of swabs), bring the lysis buffer and DTT volumes to the volumes specified in step 4 of the large-sample protocol on page 38, then continue following the instructions for the large-sample protocol.

   Note: To minimize the number of times you pipette, you can pre-mix the lysis buffer and DTT (1.0 M) for all samples, then add 300 µL of the lysis buffer-DTT mixture to each tube. Prepare a fresh lysis buffer-DTT mixture for each experiment.
4. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.

5. Place the tube in a thermal shaker, then incubate it at 70 °C and 900 rpm for the appropriate amount of time from the following table:

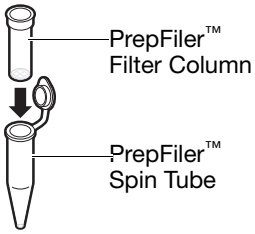
Lysis times at 70 °C incubation temperature	
Sample Type	**Lysis Time (Minutes)**
Liquid body fluids	20
Dried stains or samples on swabs	40
Neat semen samples	90

Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube every 5 minutes.

Step 3: Remove substrate from sample lysate

If a sample substrate is present, perform the following steps to remove it from the lysate before continuing with the extraction procedure.

1. Centrifuge the sample tube for 2 seconds to collect the condensate from the tube cap.
2. Insert a PrepFiler™ Filter Column into a new 1.5 mL PrepFiler™ Spin Tube, then carefully transfer the sample tube contents into the filter column:
   - Use a pipette to transfer the liquid contents.
   - Use the pipette tip or sterile tweezers to transfer the substrate.



3. Cap the filter column/spin tube, then centrifuge it at the maximum g of the centrifuge. For example, centrifuge for 2 minutes at 12,000 to 14,000 rpm or for 5 minutes at 3,000 to 4,000 rpm.
4. Check the volume of sample lysate collected in the spin tube. If the volume is less than 180 µL, then centrifuge the filter column/spin tube for an additional 5 minutes.

   Note: If the volume is still less than 180 µL, see Appendix A on page 61.

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

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

Step 4: Bind genomic DNA to magnetic particles

After performing cell lysis and, if necessary, removing the sample substrate, add magnetic particles to bind the DNA.

WARNING! CHEMICAL HAZARD. PrepFiler™ Magnetic Particles are harmful by inhalation, skin absorption, and if swallowed. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor (or dust). Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Allow the sample lysate to come to room temperature (approximately 5 minutes).

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

2. Vortex the PrepFiler™ Magnetic Particles tube approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge briefly.

   Note: If you are pipetting multiple samples, vortex the magnetic particles tube every 5 minutes until you complete the next step.

3. Pipette 15 µL of magnetic particles into the tube containing the sample lysate.
4. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 10 seconds, then centrifuge it briefly.

   IMPORTANT! This step is required before you add isopropanol in order to promote binding.

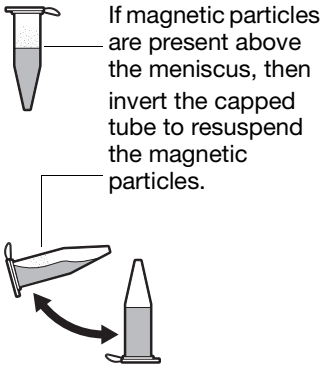
5. Add 180 µL of isopropanol to the sample lysate tube.
6. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 5 seconds, then centrifuge it briefly.
7. Place the sample lysate tube in a shaker or on a vortexer (with adaptor), then mix at room temperature at 1,000 rpm for 10 minutes.

Step 5: Wash bound DNA

After binding the DNA to the magnetic particles, wash the magnetic particles to remove impurities and inhibitors.

WARNING! CHEMICAL HAZARD. PrepFiler™ Wash Buffer Concentrate causes eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Vortex the sample DNA tube:
   a. If magnetic particles are present on the sides of the sample DNA tube above the meniscus, invert the tube to resuspend the particles.
   b. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) for 10 seconds, then centrifuge briefly.

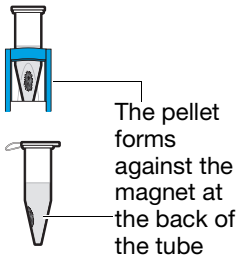


Note: It is acceptable to have magnetic particle aggregates suspended in the solution or on the side of the tube below the meniscus.

2. Confirm that the magnet in the magnetic stand is properly aligned.

Note: See "Magnetic stand guidelines" on page 13.

3. Place the sample DNA tube in the magnetic stand and observe that the magnetic particles form a pellet against the back of the tube. Wait until the size of the pellet stops increasing (approximately 1 to 2 minutes).



Note: Samples containing high levels of proteins or other impurities may require more time.

Note: For some sample types such as blood, the solution may remain colored after the magnetic particles are separated.

4. With the sample DNA tube remaining in the magnetic stand, use a pipette to carefully remove and discard *all* visible liquid phase.

   IMPORTANT! When removing the liquid phase, do not aspirate magnetic particles or disturb the magnetic particle pellet.

   Note: One way to remove the liquid phase is to use a size P200 or P1000 pipettor to remove most of the liquid, then use a size P20 pipettor to remove the remaining liquid.

5. Perform steps a through e *three* times:

   a. Add 300 µL of prepared PrepFiler™ Wash Buffer to the sample DNA tube.

   b. Cap the sample DNA tube and remove the tube from the magnetic stand.

   c. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 5 seconds), then centrifuge briefly.

      Note: It is acceptable to have magnetic particle aggregates suspended in the solution.

   d. Place the sample DNA tube in the magnetic stand for 30 to 60 seconds.

   e. With the sample DNA tube remaining in the magnetic stand, use a pipette to carefully remove and discard *all* visible liquid phase.

      IMPORTANT! When removing the liquid phase, do not aspirate magnetic particles or disturb the magnetic particle pellet.

6. With the sample DNA tube remaining in the magnetic stand, open the tube, then allow the magnetic particles-bound DNA to air-dry for 7 to 10 minutes.

   IMPORTANT! Air-drying for more than 10 minutes may reduce DNA yield.

   IMPORTANT! If the room temperature is >25 °C, reduce the drying time to 5 minutes.

Step 6: Elute DNA

After performing the wash step, resuspend the purified DNA and separate the DNA eluate from the magnetic particles.

1. Bring the thermal shaker temperature to 70 °C.
2. Add 50 µL of PrepFiler™ Elution Buffer to the sample DNA tube.

   Note: Do *not* use water instead of PrepFiler™ Elution Buffer. In place of PrepFiler™ Elution Buffer, you can prepare low TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) or purchase low TE buffer from Teknova (Cat # T0223).

3. Cap the sample DNA tube, vortex it at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 5 seconds), then centrifuge it briefly.
4. Place the sample DNA tube in a thermal shaker, then incubate at 70 °C and 900 rpm for 5 minutes.

   Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube every 2 to 3 minutes.

5. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 2 seconds), then centrifuge briefly.
6. Place the sample DNA tube in the magnetic stand, then wait until the size of the pellet at the side of the tube stops increasing (at least 1 minute).
7. Pipette the liquid in the sample DNA tube (which contains the isolated genomic DNA) to a new spin tube or 1.5-mL microcentrifuge tube for storage.

   IMPORTANT! When removing the liquid phase, do not aspirate magnetic particles or disturb the magnetic particle pellet.

   Note: The isolated DNA can be stored at 4 °C for up to one week, or at –20 °C for longer storage.

8. If the eluted DNA extract is turbid (for example, this may occur in tissue samples with a high fat content), centrifuge the tube for 5 to 7 minutes at maximum speed (approximately 10,000 rpm), then transfer the clear supernatant to a new 1.5-mL microcentrifuge tube.

Chapter 3

Perform Extraction – Supplementary Protocols

Perform Extraction – Supplementary Protocols

3

This chapter covers:

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Supplementary protocols for additional sample types and inputs

This section describes the protocols used to prepare and extract DNA from additional sample types using the PrepFiler™ kit chemistry. These protocols provide experimental guidelines to adapt the PrepFiler™ kit chemistry to extract DNA from a range of sample types that you may encounter in your own laboratory. These protocols have not been thoroughly tested or optimized, and were not used in the validation studies described in Chapter 4. Each lab should perform studies to validate the performance of the PrepFiler kit using these protocols. Table 5 provides example sample types and input amounts for use with the supplementary protocols.

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

Sample Type	Example Sample Input
Blood/soil mixture	Up to 50 mg blood/soil mixture
Epithelial- or sperm-cell fraction lysate	Epithelial cell fraction lysate – Up to 150 µL lysate Sperm cell fraction lysate – Up to 200 µL lysate **Note:** Use the procedure described by Gill (Gill *et al*., 1985)‡ to separate and lyse the epithelial- and sperm-cell fractions before using the PrepFiler™ kit.
Hair	Up to 3 mm cutting from root
Nail clipping	Up to 5 mm clipping
Paraffin-embedded tissue	Up to 3 × 3 mm piece of tissue, or up to 5 × 5 mm section from a tissue slide obtained by scraping or swabbing
Tooth	Up to 50 mg powdered tooth
Large sample	The sample types and inputs are the same as those for the standard protocol (see "Standard protocol – sample types and inputs" on page 10), but the large-sample protocol uses a larger volume of PrepFiler™ Lysis Buffer. **IMPORTANT!** Use the large-sample protocol *only* for standard-protocol-type samples that are not submerged by the 300 µL of PrepFiler™ Lysis Buffer used in the standard protocol.

‡ Gill, P., Jeffreys, A. J., and Werrett, D. J. 1985. Forensic application of DNA 'fingerprints.' *Nature* 318:577-579

Blood/soil mixture protocol

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. PrepFiler™ Magnetic Particles are harmful by inhalation, skin absorption, and if swallowed. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor (or dust). Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: In addition to the "Required materials" on page 11, phosphate-buffered saline is required for blood/soil mixtures.

1. Prepare reagents as directed in "Step 1: Prepare reagents" on page 14.
2. Place approximately 50 mg of the blood/soil mixture in a 1.5-mL microcentrifuge tube.
3. Add 100 µL of 1✕ phosphate-buffered saline (PBS) to the tube.
4. Close the tube, vortex it for 10 seconds, then centrifuge it for 30 seconds.

   IMPORTANT! Do not centrifuge for longer than 30 seconds.

5. Transfer approximately 70 µL of clear supernatant (free of residual soil) to a new 1.5-mL microcentrifuge tube.
6. Bring the thermal shaker temperature to 70 °C.
7. To the tube that contains the sample, add 500 µL of PrepFiler™ Lysis Buffer.
8. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.
9. Place the tube in a thermal shaker, then incubate at 70 °C and 900 rpm for 30 minutes.

   Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube every 5 minutes.

10. Centrifuge the tube at maximum speed (≈16,110 x g) for 5 minutes.
11. Transfer the clear (free of residual soil) supernatant to a new 1.5-mL microcentrifuge tube.

12. Allow the sample lysate to come to room temperature (approximately 5 minutes).

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

13. Vortex the PrepFiler™ Magnetic Particles tube approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge briefly.

Note: If you are pipetting multiple samples, vortex the magnetic particles tube every 5 minutes until you complete the next step.

14. Pipette 20 µL of magnetic particles into the tube containing the sample lysate.
15. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 10 seconds, then centrifuge it briefly.

IMPORTANT! This step is required before you add isopropanol in order to promote binding.

16. Add 300 µL of isopropanol to the sample lysate tube.
17. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 5 seconds, then centrifuge it briefly.
18. Place the sample lysate tube in a shaker or on a vortexer (with adaptor), then mix at room temperature at 1,000 rpm for 10 minutes.
19. Follow the standard protocol procedures to wash and elute the DNA as directed in "Step 5: Wash bound DNA" on page 20 and "Step 6: Elute DNA" on page 22.

Epithelial- and sperm-cell fraction protocols

Note: The procedures below describe the use of the PrepFiler™ kit to extract DNA from sperm- and epithelial-cell fraction lysates prepared using a procedure based on the protocol described by Gill, et al. (Gill P., Jeffreys A.J. and Werrett, D.J. 1985. Forensic application of DNA 'fingerprints'. *Nature* 318:577-579). These procedures are similar to the standard protocol, but support higher sample lysate volumes. Although you perform lysis before beginning DNA isolation using the PrepFiler™ kit, the addition of PrepFiler™ Lysis Buffer in the appropriate proportion is still required for optimal use of the extraction chemistry.

Epithelial-cell fraction protocol

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: See "Required materials" on page 11 for a list of required materials.

1. Prepare reagents as directed in "Step 1: Prepare reagents" on page 14.
2. Before using the PrepFiler™ kit, use the procedure described by Gill (Gill *et al.*, 1985) to separate and lyse the epithelial- and sperm-cell fractions.
3. Place up to 150 µL of epithelial-cell fraction lysate into a 1.5-mL microcentrifuge tube.

   Note: The volumes of PrepFiler™ kit reagents used in this procedure do not support the use of more than 150 µL of epithelial-cell fraction lysate.

4. Add PrepFiler™ Lysis Buffer to bring the total volume to 300 µL.
5. Follow the standard protocol procedures to bind, wash and elute the DNA as directed in "Step 4: Bind genomic DNA to magnetic particles" on page 19, "Step 5: Wash bound DNA" on page 20 and "Step 6: Elute DNA" on page 22.

Sperm-cell fraction protocol

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. PrepFiler™ Magnetic Particles are harmful by inhalation, skin absorption, and if swallowed. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor (or dust). Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: See "Required materials" on page 11 for a list of required materials.

1. Prepare reagents as directed in "Step 1: Prepare reagents" on page 14.
2. Before using the PrepFiler™ kit, use the procedure described by Gill (Gill *et al.*, 1985) to separate and lyse the epithelial- and sperm-cell fractions.
3. Place up to 200 µL of sperm-cell fraction lysate into a 1.5-mL microcentrifuge tube.

   Note: The volumes of PrepFiler™ kit reagents used in this procedure do not support the use of more than 200 µL of sperm-cell fraction lysate.

4. Add PrepFiler™ Lysis Buffer to bring the total volume to 500 µL.
5. Allow the sample lysate to come to room temperature (approximately 5 minutes).

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

6. Vortex the PrepFiler™ Magnetic Particles tube approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge briefly.

   Note: If you are pipetting multiple samples, vortex the magnetic particles tube every 5 minutes until you complete the next step.

7. Pipette 15 µL of magnetic particles into the tube containing the sample lysate.
8. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 10 seconds, then centrifuge it briefly.

   IMPORTANT! This step is required before you add isopropanol in order to promote binding.

9. Add 300 µL of isopropanol to the sample lysate tube.
10. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 5 seconds, then centrifuge it briefly.
11. Place the sample lysate tube in a shaker or on a vortexer (with adaptor), then mix at room temperature at 1,000 rpm for 10 minutes.
12. Follow the standard protocol procedures to bind, wash and elute the DNA as directed in "Step 5: Wash bound DNA" on page 20 and "Step 6: Elute DNA" on page 22.

Hair protocol

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. PrepFiler™ Magnetic Particles are harmful by inhalation, skin absorption, and if swallowed. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor (or dust). Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: See "Required materials" on page 11 for a list of required materials.

1. Prepare reagents as directed in "Step 1: Prepare reagents" on page 14.
2. Cut the hair root approximately 3 mm from the tip.
3. Use pointed tweezers to place the cutting in a PrepFiler™ Spin Tube or 1.5-mL microcentrifuge tube.
4. Bring the thermal shaker temperature to 70 °C.
5. To the tube that contains the sample, add:
   - **PrepFiler™ Lysis Buffer**: 100 µL
   - **DTT, 1.0 M**: 3 µL
6. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.
7. Place the tube in a thermal shaker, then incubate it at 70 °C and 900 rpm for 40 minutes.

   Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube every 5 minutes.

8. Remove the sample substrate as directed in "Step 3: Remove substrate from sample lysate" on page 18.
9. Allow the sample lysate to come to room temperature (approximately 5 minutes).

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

10. Vortex the PrepFiler™ Magnetic Particles tube approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge briefly.

    Note: If you are pipetting multiple samples, vortex the magnetic particles tube every 5 minutes until you complete the next step.

11. Pipette 15 µL of magnetic particles into the tube containing the sample lysate.
12. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 10 seconds, then centrifuge it briefly.

    IMPORTANT! This step is required before you add isopropanol in order to promote binding.

13. Add 100 µL of isopropanol to the sample lysate tube.
14. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 5 seconds, then centrifuge it briefly.
15. Place the sample lysate tube in a shaker or on a vortexer (with adaptor), then mix at room temperature at 1,000 rpm for 10 minutes.
16. Follow the standard protocol procedures to wash and elute the DNA as directed in "Step 5: Wash bound DNA" on page 20 and "Step 6: Elute DNA" on page 22.

Nail cuttings protocol

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: See "Required materials" on page 11 for a list of required materials.

1. Prepare reagents as directed in "Step 1: Prepare reagents" on page 14.
2. Place a nail clipping (approximately 5 mm long) in a 1.5-mL microcentrifuge tube.
3. Bring the thermal shaker temperature to 37 °C.
4. To the tube that contains the sample, add:
   - **PrepFiler™ Lysis Buffer**: 300 µL
   - **DTT, 1.0 M**: 3 µL
5. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.
6. Place the tube in a thermal shaker, then incubate it at 37 °C and 900 rpm for 20 minutes.

   Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube every 5 minutes.

7. Centrifuge the tube at maximum speed (≈16,110 x g) for 5 seconds, then pipette the clear solution (free of nail clipping residue) into a new 1.5 mL microcentrifuge tube.
8. Cap the tube, then incubate the tube at 70 °C for 20 minutes at 900 rpm.

   Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube every 5 minutes.

9. Follow the standard protocol procedures to bind, wash and elute the DNA as directed in "Step 4: Bind genomic DNA to magnetic particles" on page 19, "Step 5: Wash bound DNA" on page 20 and "Step 6: Elute DNA" on page 22.

Paraffin-embedded tissue protocol

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. PrepFiler™ Magnetic Particles are harmful by inhalation, skin absorption, and if swallowed. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor (or dust). Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: In addition to the "Required materials" on page 11, low-TE buffer, SDS, and Proteinase K are required for paraffin-embedded tissue samples.

Note: Due to slide preparation procedures, the DNA extracted from paraffin-embedded tissue may be fragmented. For this reason, the AmpFℓSTR® MiniFiler™ PCR Amplification Kit maybe better suited for PE tissue samples scraped from slides.

1. Prepare reagents as directed in "Step 1: Prepare reagents" on page 14.
2. Bring the thermal shaker to 56 °C.
3. Combine the following components to prepare a Proteinase K lysis solution:
   - 980 µL low-TE buffer
   - 5 µL SDS (10%)
   - 15 µL Proteinase K (20 mg/mL)
4. Place the tissue sample (up to a 3 × 3 mm piece of tissue, or up to an approximately 5 × 5 mm section obtained by scraping or swabbing a tissue slide surface) into a 1.5-mL microcentrifuge tube.

   Note: Minimize the amount of paraffin collected with the tissue sample.

5. Add 100 µL of Proteinase K lysis solution to the tube containing the tissue sample.
6. Cap the tube, place the tube in a thermal shaker, then incubate the tube at 56 °C and 900 rpm for 1 hour.

   Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube at least 4 times per hour.

7. Incubate the tube at 95 °C and 900 rpm for 15 minutes.
8. Cool the tube to room temperature, then centrifuge it for 30 seconds at maximum speed (≈16,110 x g).
9. Bring the thermal shaker temperature to 70 °C.
10. To the tube that contains the sample, add 500 µL of PrepFiler™ Lysis Buffer.
11. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.
12. Place the tube in a thermal shaker, then incubate at 70 °C and 900 rpm for 20 minutes.

Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube every 5 minutes.

13. Centrifuge the tube at maximum speed (≈16,110 x g) for 2 minutes.
14. Transfer the clear (no sediment) supernatant to a new 1.5-mL microcentrifuge tube.
15. Allow the sample lysate to come to room temperature (approximately 5 minutes).

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

16. Vortex the PrepFiler™ Magnetic Particles tube approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge briefly.

Note: If you are pipetting multiple samples, vortex the magnetic particles tube every 5 minutes until you complete the next step.

17. Pipette 15 µL of magnetic particles into the tube containing the sample lysate.
18. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 10 seconds, then centrifuge it briefly.

IMPORTANT! This step is required before you add isopropanol in order to promote binding.

19. Add 300 µL of isopropanol to the sample lysate tube.
20. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 5 seconds, then centrifuge it briefly.
21. Place the sample lysate tube in a shaker or on a vortexer (with adaptor), then mix at room temperature at 1,000 rpm for 10 minutes.
22. Follow the standard protocol procedures to wash and elute the DNA as directed in "Step 5: Wash bound DNA" on page 20 and "Step 6: Elute DNA" on page 22.

Tooth protocol

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: In addition to the "Required materials" on page 11, a mild detergent is required for tooth samples.

1. Prepare reagents as directed in "Step 1: Prepare reagents" on page 14.
2. Clean the tooth thoroughly with a mild detergent such as SoftCIDE® hand soap.
3. Dry the tooth for 24 hours at room temperature.
4. Grind the tooth to a fine powder.
5. Transfer approximately 50 mg of powdered tooth to a 1.5-mL microcentrifuge tube.
6. Bring the thermal shaker temperature to 50 °C.
7. To the tube that contains the sample, add:
   - **PrepFiler™ Lysis Buffer**: 300 µL
   - **DTT, 1.0 M**: 3 µL
8. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.
9. Place the tube in a thermal shaker, then incubate at 50 °C and 900 rpm for a minimum of 4 hours, but no more than 18 hours.

   Note: Lysis time may vary depending on the sample age and condition. To maximize yield from older samples, a longer incubation time may be necessary.

   Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube every 5 minutes.

10. Vortex the tube for 5 seconds.
11. Centrifuge the tube at maximum speed (≈16,110 x g) for 3 minutes.
12. Transfer the clear (no sediment) supernatant to a new 1.5-mL microcentrifuge tube.

13. If the supernatant volume is less than 300 µL, add PrepFiler™ Lysis Buffer to bring the supernatant volume to 300 µL.
14. Follow the standard protocol procedures to bind, wash and elute the DNA as directed in "Step 4: Bind genomic DNA to magnetic particles" on page 19, "Step 5: Wash bound DNA" on page 20 and "Step 6: Elute DNA" on page 22.

Large-sample protocol

About the large-sample protocol

Use the large-sample protocol if your sample is appropriate for use with the standard protocol (see "Standard protocol – sample types and inputs" on page 10), but the sample substrate is not completely immersed by the 300 µL of PrepFiler™ Lysis Buffer used in the standard protocol. Table 6 summarizes the differences between the standard and large-sample protocol.

Table 6 Differences between standard and large-sample protocols

Protocol Step	Reagent	Volume (µL)	
		Large-Sample Protocol	Standard Protocol
Lysis	PrepFiler™ Lysis Buffer	500 µL **IMPORTANT!** The use of more than 500 µL of lysis buffer is not recommended.	300 µL
Lysis	DTT, 1.0 M	5 µL (use 8 µL for samples containing semen)	3 µL (use 5 µL for samples containing semen)
Binding	Isopropanol	300 µL	180 µL

Note: This protocol provides guidelines for DNA extraction from larger-sized sample substrates. This protocol was evaluated separately from the validation studies described in Chapter 4. DNA yields may be lower than those obtained using the standard protocol. When using the large-sample protocol, you may need to allow additional time for magnetic pellet formation.

Large-sample protocol

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. PrepFiler™ Magnetic Particles are harmful by inhalation, skin absorption, and if swallowed. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor (or dust). Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: See "Required materials" on page 11 for a list of required materials.

1. Prepare reagents as directed in "Step 1: Prepare reagents" on page 14.
2. Bring the thermal shaker temperature to 70 °C.
3. Place a sample in a PrepFiler™ Spin Tube or standard 1.5-mL microcentrifuge tube.
4. To the tube that contains the sample, add:
   - **PrepFiler™ Lysis Buffer**: 500 µL
   - **DTT, 1.0 M**: 5 µL (use 8 µL for samples containing semen)

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

   Note: To minimize the number of times you pipette, you can pre-mix the lysis buffer and DTT (1.0 M) for all samples, then add 500 µL of the lysis buffer-DTT mixture to each tube. Prepare a fresh lysis buffer-DTT mixture for each experiment.
5. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.

6. Place the tube in a thermal shaker, then incubate it at 70 °C and 900 rpm for the appropriate amount of time from the following table:

Lysis times at 70 °C incubation temperature	
Sample Type	**Lysis Time (Minutes)**
Liquid body fluids	20
Dried stains or samples on swabs	40
Neat semen samples	90

Note: You can use a heat block instead of a thermal shaker. Read "About thermal shakers" on page 6 before using a heat block. If you use a heat block, briefly vortex and centrifuge the tube every 5 minutes.

7. Remove the sample substrate as directed in "Step 3: Remove substrate from sample lysate" on page 18.
8. Allow the sample lysate to come to room temperature (approximately 5 minutes).

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

9. Vortex the PrepFiler™ Magnetic Particles tube approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge briefly.

Note: If you are pipetting multiple samples, vortex the magnetic particles tube every 5 minutes until you complete the next step.

10. Pipette 15 µL of magnetic particles into the tube containing the sample lysate.
11. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 10 seconds, then centrifuge it briefly.

IMPORTANT! This step is required before you add isopropanol in order to promote binding.

12. Add 300 µL of isopropanol to the sample lysate tube.
13. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 5 seconds, then centrifuge it briefly.
14. Place the sample lysate tube in a shaker or on a vortexer (with adaptor), then mix at room temperature at 1,000 rpm for 10 minutes.
15. Follow the standard protocol procedures to wash and elute the DNA as directed in "Step 5: Wash bound DNA" on page 20 and "Step 6: Elute DNA" on page 22.

Experiments and Results 4

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Overview

This chapter provides results of the developmental validation experiments performed by Applied Biosystems using the PrepFiler™ Forensic DNA Extraction Kit.

Importance of validation

The performance of the PrepFiler™ Forensic DNA Extraction Kit was evaluated by conducting validation studies with samples commonly encountered in forensic and parentage laboratories. The validation process establishes critical attributes and limitations for sound data interpretation.

Experiments

Experiments to evaluate the performance of the PrepFiler Forensic DNA Extraction Kit were performed at Applied Biosystems according to the Revised Validation Guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM) published in Forensic Science Communications Vol. 6, No. 3, July 2004 (**http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm**). These guidelines describe the quality assurance requirements that a laboratory should follow to ensure 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 kit for extraction of genomic DNA from forensic samples as a part of a forensic DNA genotyping procedure. Each laboratory using the PrepFiler Forensic DNA Extraction Kit should perform appropriate internal validation studies.

The following materials and methods were used in all experiments performed as part of the developmental validation:

- Biological samples obtained from the Serological Research Institute (Richmond, California) were used to prepare the samples for each experiment.
- Genomic DNA was extracted from the samples using the PrepFiler Forensic DNA Extraction Kit following the standard protocol described in Chapter 2. Extraction blanks were processed for each study.
- Extracted DNA from each sample was quantified in duplicate using the Quantifiler® Human DNA Quantification Kit on an Applied Biosystems 7500 Real-Time PCR System. An elution volume of 50 µL was used for all experiments. The quantitation results were analyzed using SDS software v 1.2.3.
- Quantified DNA from each sample was processed using the AmpFℓSTR® Identifiler® PCR Amplification Kit. Samples with a target DNA input amount of 1 ng were amplified on a GeneAmp® 9700 thermal cycler. Electrophoresis was performed on an Applied Biosystems 3100 Genetic Analyzer. The STR profiles were analyzed using GeneMapper® *ID* software v 3.2.1.

Sensitivity study (SWGDAM Guideline 2.3)

Sensitivity studies were performed to determine the range of biological sample amounts that can be reliably processed for extraction of genomic DNA using the PrepFiler™ Forensic DNA Extraction Kit.

Experiment

DNA extractions were performed in triplicate on 40.0, 30.0, 5.0, 2.0, and 0.1 µL of liquid human peripheral blood samples from one male and one female donor. To process the 0.1 µL sample volume, 1.0 µL of blood was mixed with 9 µL of 1× PBS, then 1.0 µL of the diluted blood sample was used in the experiment. Four replicate extraction reagent blanks were included in the experimental set.

DNA concentration results

The DNA concentration ranged from 0.13 to 53.88 ng/µL, and from 0.28 to 57.67 ng/µL, for donors 1 and 2, respectively. The average DNA concentrations for each sample are summarized by sample volume in Table 7.

Table 7 Sensitivity studies: Average DNA concentrations

Blood Sample Volume	Average DNA concentration, ng/µL (n=3)	
	Blood Donor 1	Blood Donor 2
40.0 µL	53.88	57.67
30.0 µL	40.82	42.14
5.0 µL	6.38	8.72
2.0 µL	2.88	3.85
0.1 µL	0.13	0.28
XB (extraction blank)	0.00	0.00

For both samples, the DNA concentration increased proportionately with increasing sample volumes, as shown in Figure 1. The efficiency of genomic DNA extraction remained linear up to the maximum volume of blood tested (40 µL).

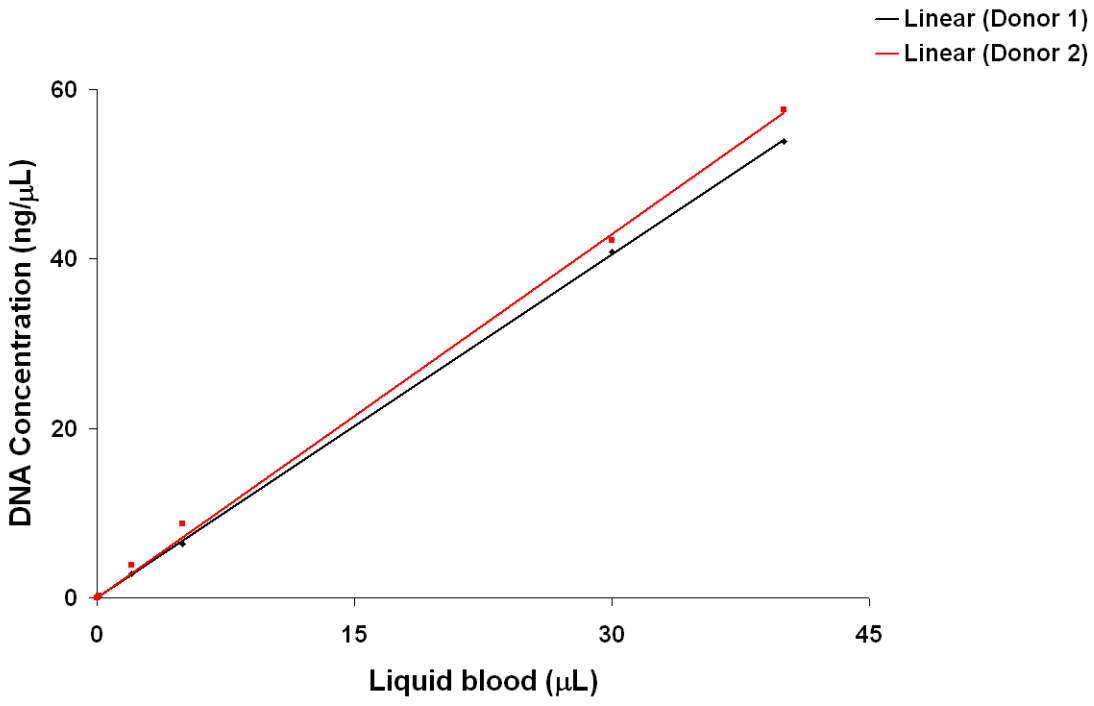

Figure 1 Sensitivity studies: Linearity of the DNA concentrations

For the smallest sample amount tested (0.1 µL of blood), the PrepFiler kit effectively recovered DNA (See Table 7 on page 44). The DNA concentration increased proportionately with increasing volume of blood processed, indicating that PrepFiler™ Magnetic Particles were not saturated at the high end (40 µL) of the tested sample volume. Therefore, the PrepFiler kit can be used to process small and large amounts of biological material. Actual DNA yields may vary because of sample-to-sample differences in nucleated cell count.

IPC C_T results

Forensic samples commonly contain PCR inhibitors (such as hematin in blood). Because extracted DNA is processed in PCR applications, it is desirable that the extraction method remove PCR inhibitors present in the sample. Additionally, it is important that the extraction reagents do not introduce PCR inhibitors to the sample.

The presence of PCR inhibitors in a sample affects the amplification of the internal PCR control (IPC) contained in the Quantifiler® Human DNA Quantification Kit. Typically, an upward shift of the IPC C_T is expected in the presence of PCR inhibitors.

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 PCR inhibitors in DNA extracted using the PrepFiler kit. 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 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 2.

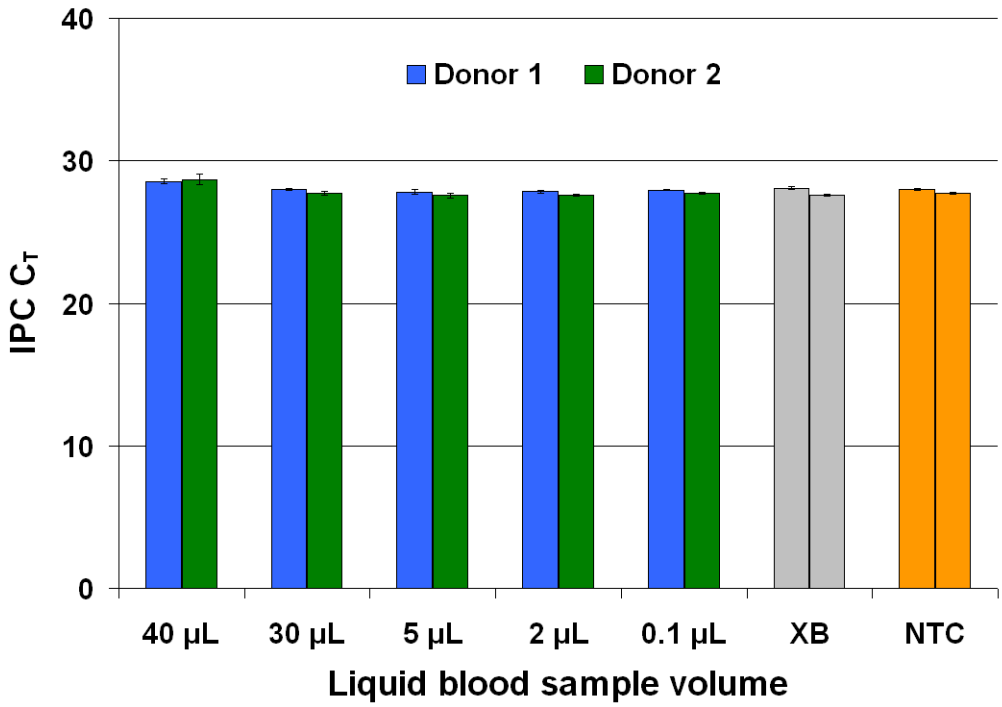

Figure 2 Sensitivity studies: IPC C_T values

Stability study (SWGDAM Guideline 2.4)

Stability studies were performed to determine the ability of the PrepFiler™ Forensic DNA Extraction Kit to extract DNA and remove PCR inhibitors from samples subjected to environmental and chemical insults. Forensic samples commonly contain compounds that inhibit the amplification of nucleic acids during PCR. It is important to remove PCR inhibitors during DNA extraction, because the inhibitors can reduce reaction efficiency, and in some cases completely inhibit PCR.

The stability studies examined compromised samples that represent various challenges encountered in forensic samples. The PCR inhibitors contained in the samples included hematin and denim dyes, both of which are thought to be co-extracted with DNA, as well as soil and other contaminants introduced through environmental exposure.

Experiment

The following samples were prepared using blood from two donors:

Sample Name	Sample Description
BSCI (Blood Stain, Cotton, Inhibitors)	Two samples consisting of 5 µL of blood and 1.0 µL of inhibitor mix on cotton cloth. The inhibitor mix contained 12.5 mM indigo, 0.5 mM hematin, 2.5 mg/mL humic acid, and 8.75 mg/mL urban dust extract‡.
BSD (Blood Stain, Denim)	Two samples consisting of 5 µL of blood spotted on denim.
BSC7 (Blood Stain, Cotton, 7-day exposure)	Two samples consisting of 10 µL of blood spotted on cotton cloth, then exposed to the outdoor environment for 7 days.

‡ Urban dust extract is the decanted supernatant resulting from suspending 87.5 mg of urban dust (NIST Standard Reference Material® Number 1649a) in 1 L of TE buffer (10 mM Tris, 0.1 mM EDTA, 8.0 pH), then shaking the suspension for 18 hours at room temperature. 3 µL of the urban dust extract was used to prepare 100 µL of inhibitor mix.

DNA was extracted in triplicate from 5-mm punches of each sample using the PrepFiler kit. Four replicate extraction blanks (XB) were also processed.

Results

The DNA concentration for both blood samples ranged from 4.53 to 5.50 ng/µL for BSCI, from 2.88 to 4.24 ng/µL for BSD, and from 6.78 to 9.91 ng/µL for BSC7. The variation in concentrations is within the expected variation introduced through the extraction and quantitation procedures. The average DNA concentrations from the samples investigated for the stability study are summarized in Table 8 on page 47.

Table 8 Stability studies: Average DNA concentrations

Sample Type	Blood Sample 1		Blood Sample 2	
	ng/µL	Total (ng)	ng/µL	Total (ng)
BSCI (Blood Stain, Cotton, Inhibitors)	5.0	250	4.85	242.5
BSD (Blood Stain, Denim)	3.86	193	3.3	165
BSC7 (Blood Stain, Cotton, 7-day exposure)	8.68	434	7.33	366.5
XB (Extraction Blank)	0	0	0	0

Thus, the PrepFiler kit efficiently extracted genomic DNA from samples that were exposed to environmental and chemical insults. DNA concentrations may vary for different blood samples, substrates, and environmental conditions.

Figure 3 shows the average IPC C_T values obtained during quantitation of the stability study samples.

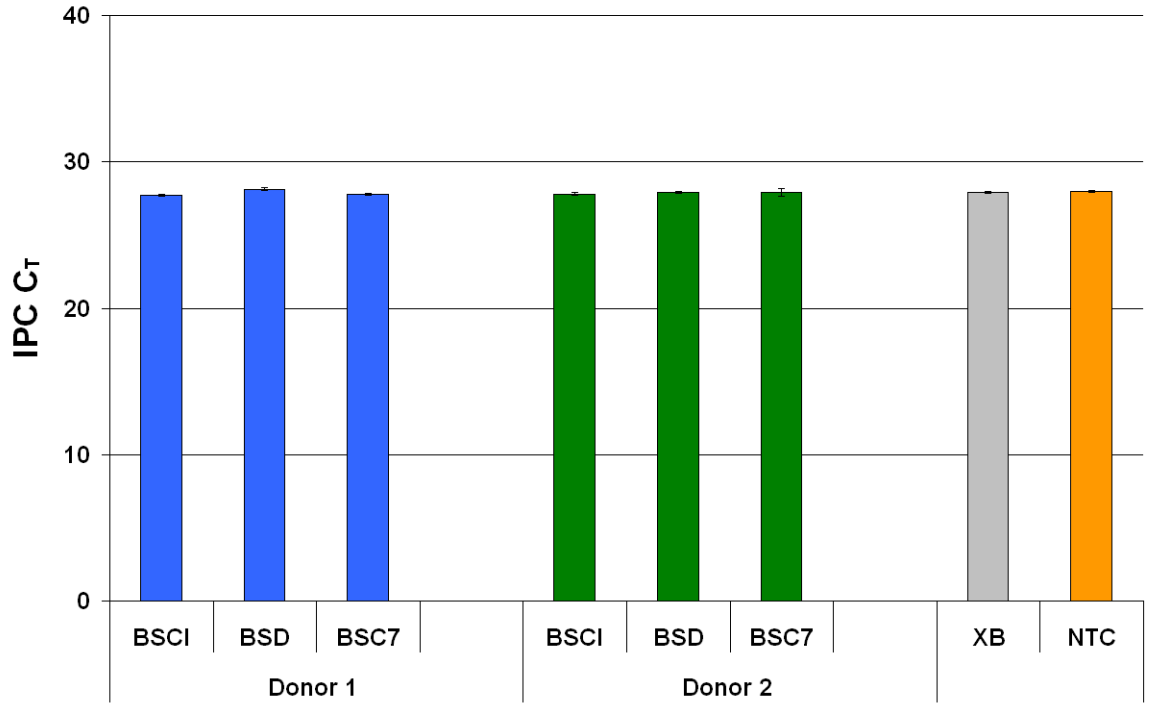

Figure 3 Stability studies: IPC C_T values

The IPC C_T values for the samples and NTCs were within ±1 C_T unit, indicating that PCR inhibitors present in all three tested sample types (blood with inhibitor mix, blood with denim dyes, and blood subject to environmental exposure) were effectively removed during the extraction of DNA using the PrepFiler Forensic DNA Extraction Kit.

Reproducibility study (SWGDAM Guideline 2.5)

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

Experiment

Using one sample set, an extraction experiment was repeated on three separate days. In each experiment, DNA was extracted in duplicate from the following samples (5-mm punch or whole swab) using the PrepFiler™ Forensic DNA Extraction Kit:

Sample Names	Sample Description
BSC-1 and BSC-2 (Blood Stain, Cotton-1 and Blood Stain, Cotton-2)	Two samples, each consisting of 5 µL blood stains on cotton fabric.
SAL-1 and SAL-2 (Saliva-1 and Saliva-2)	Two swab samples, each prepared using 50 µL of human saliva.
SSC (Semen Stain, Cotton)	One sample consisting of 1 µL semen stain spotted on cotton fabric.
XB (Extraction Blank)	Extraction blank.

Results

Table 9 summarizes the DNA concentrations and standard deviation values from the three extraction experiments. Average concentrations are presented graphically in Figure 4 on page 49. Consistent DNA concentrations were obtained for each sample. Variations within results for each sample are due to variation in both extraction and quantitation procedures. The standard deviation values for the DNA concentrations from different samples studied ranged from 0.36 to 0.74. Variation in DNA concentrations between different samples can occur due to the variation in the amount of biological material present in samples from different donors and different body fluids.

Table 9 Reproducibility studies: DNA concentration summary

Sample	Concentration (ng/µL)			Standard Deviation
	Minimum	Maximum	Average	
BSC–1 (Blood Stain, Cotton-1)	5.43	7.06	6.25	0.66
BSC–2 (Blood Stain, Cotton-2)	3.69	6.00	4.82	0.74
SAL–1 (Saliva-1)	4.27	5.94	5.20	0.63
SAL–2 (Saliva-2)	3.02	4.48	3.70	0.54
SSC (Semen Stain, Cotton)	2.74	3.68	3.43	0.36
XB (Extraction Blank)	N/A	N/A	N/A	N/A

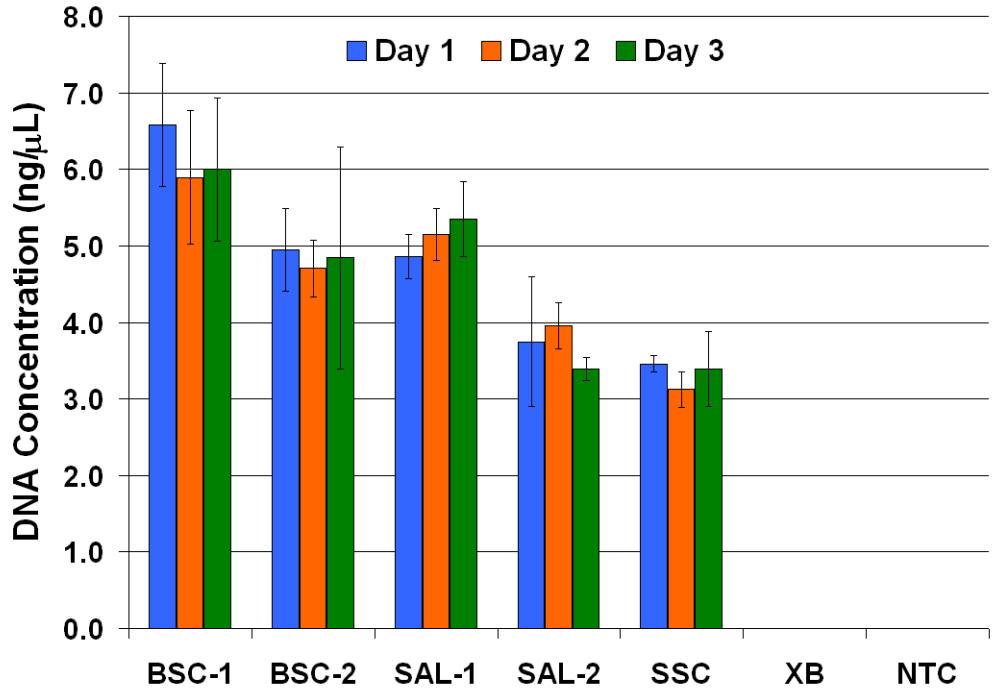

Figure 4 Reproducibility studies: Average DNA concentration

Figure 5 shows the IPC C_T values from the quantitation experiments in the reproducibility studies. The sample IPC C_T values were within ±1 C_T unit of that for the NTC IPC C_T, confirming that the PCR inhibitors were effectively removed during the extraction.

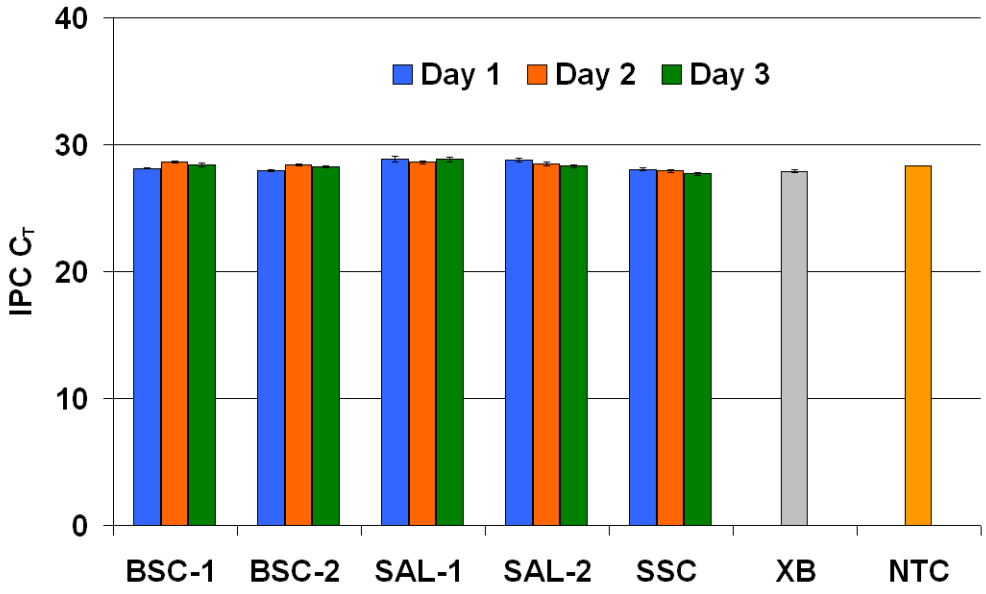

Figure 5 Reproducibility study: IPC C_T values

Case-type sample study (SWGDAM Guideline 2.6)

Case-type sample studies were performed to evaluate the extraction of genomic DNA by the PrepFiler™ Forensic DNA Extraction Kit for different sample types that are commonly processed in a forensic laboratory.

Experiment

DNA was extracted in duplicate from the following samples (5-mm punch or whole swab) using the PrepFiler kit. The first five samples were prepared using blood from the same donor.

Sample Name	Sample Description
BFTA (Blood, FTA)	Blood stain on FTA paper, 2 µL spot.
BSC (Blood Stain, Cotton)	Blood stain on non-colored cotton fabric, 2 µL spot.
BSCI (Blood Stain, Cotton, Inhibitor)	Inhibitor-treated blood stain on non-colored cotton fabric, 2 µL spot.
BSD (Blood Stain, Denim)	Blood stain on blue denim, 2 µL spot.
LB (Liquid Blood)	Liquid blood, 2 µL.
DE, e–fraction and DE, s–fraction (Differential Extraction, Epithelial Cell Fraction and Differential Extraction, Sperm Cell Fraction)	Sperm- and epithelial-cell fractions from sexual-assault-type sample. Samples were prepared by placing 50 µL of saliva from a female donor and 5 µL of sperm-positive semen on a swab. The samples were processed using the differential extraction method described by Gill (Gill, *et al*., 1985)‡. 150 µL of the epithelial cell fraction lysate and 200 µL of the sperm fraction lysate were processed for isolation of DNA using the PrepFiler kit chemistry.
MBSC (Mixed Blood Stain, Cotton)	Mixed-blood stain on non-colored cotton fabric prepared using 1 µL blood from human male donor and 4 µL of blood from female donor.
SSC (Semen Stain, Cotton)	Semen (sperm positive) stain on non-colored cotton fabric, 1 µL spot.
SalSw (Liquid Saliva, Swab)	Saliva on cotton swab, 50 µL.
XB	Extraction blank.

‡ Gill, P., Jeffreys, A. J., and Werrett, D. J. 1985. Forensic application of DNA 'fingerprints.' *Nature* 318:577-579.

Results

Figure 6 on page 51 summarizes the results for different sample types commonly encountered in the forensic laboratory. The concentration of DNA from 2 µL of liquid blood was as high as 3.85 ng/µL. The concentration of DNA for different blood stain samples prepared using 2 µL of blood ranged between 1.72 and 2.35 ng/µL. Variation in DNA concentration 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. The results demonstrate that the PrepFiler Forensic DNA Extraction Kit is efficient and useful for genomic DNA extractions from forensic case-type samples.

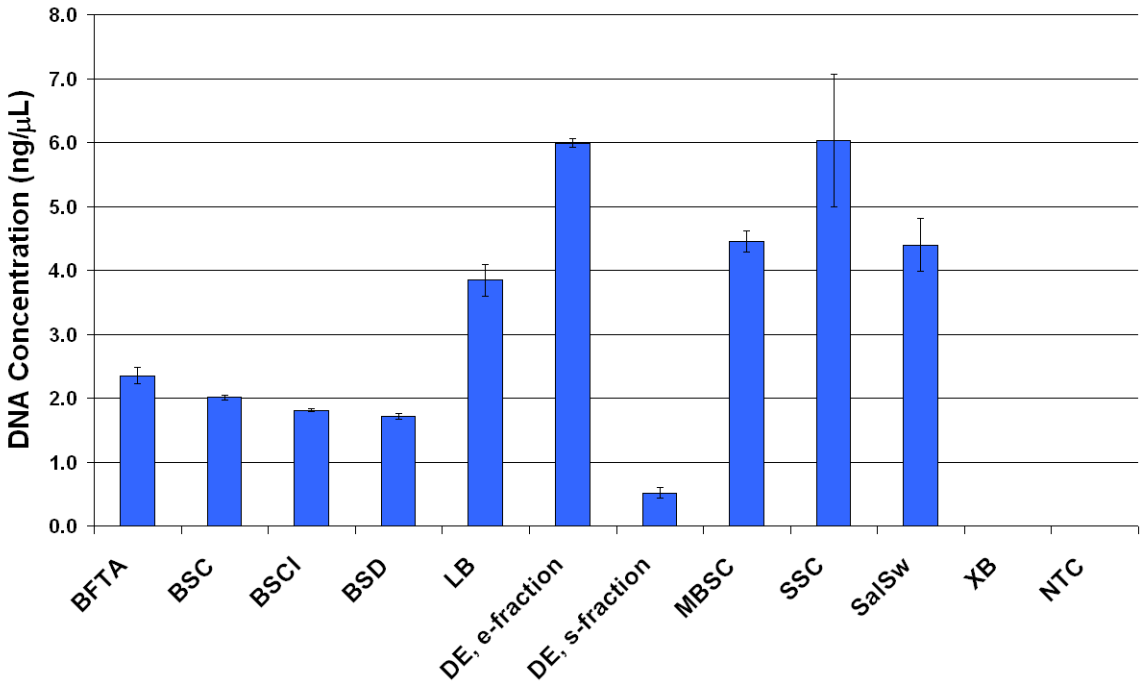

Figure 6 Case type samples: Average DNA concentrations

The IPC C_T values for all case-type samples investigated were within ±1 of that obtained for the NTC, indicating effective removal of PCR inhibitors from the biological samples and substrates. The results are shown in Figure 7.

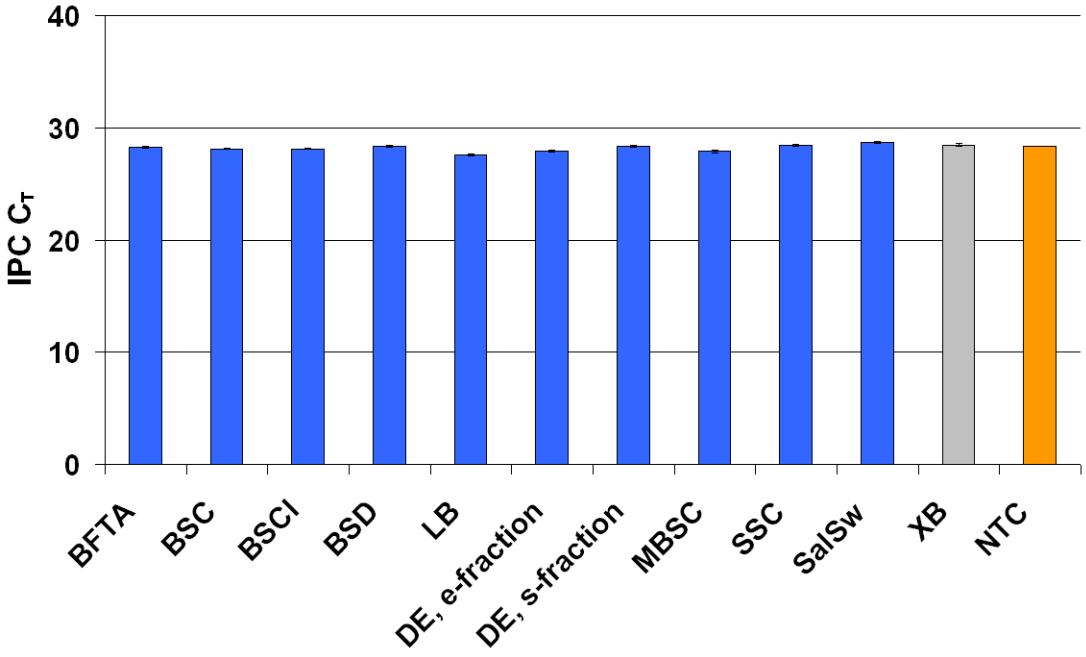

Figure 7 Case-type samples: IPC C_T values

Contamination study (SWGDAM Guideline 3.6)

During the sensitivity, stability, reproducibility, and case-type sample studies, a total of twenty extraction blanks were processed using the Quantifiler® Human DNA Quantification kit and the AmpFℓSTR® Identifiler® PCR Amplification Kit. None of the extraction blanks exhibited detectable quantities of genomic DNA. Thus, the reagents and operations of the PrepFiler™ Forensic DNA Extraction Kit did not introduce any detectable human DNA.

Correlation study

The quantity and quality of extracted DNA can have an effect on the success of sample analysis and the overall quality of the final result. Low recovery of DNA following extraction may result in the amplification of a suboptimal input amount leading to imbalance in heterozygous peak heights and allele drop out. The failure to remove inhibitors during the extraction process may result in poor amplification of certain loci or the inability to generate a complete profile. Correlation studies were performed to evaluate the quality (as judged by the presence of PCR inhibitors) and quantity of DNA obtained using the PrepFiler™ Forensic DNA Extraction Kit as compared to the DNA quantity and quality obtained using other standard and/or commercially available methods.

Experiment

Experiments were run using the PrepFiler kit and four other extraction methods:

- Phenol:Chloroform
- Promega DNA IQ™ (casework protocol)
- QIAGEN EZ1™ Investigator Kit using the BioRobot EZ1 for sample processing
- QIAGEN QIAamp® DNA Micro Kit

For each extraction method, a 5-mm punch or whole swab of each sample described in the table below was extracted in duplicate. An elution volume of 50 µL was used for all methods.

Sample Name	Sample Description
LB (Liquid Blood)	Liquid blood, 2 µL.
BFTA (Blood, FTA)	Blood stain on FTA paper, 2 µL spot.
BSC (Blood Stain, Cotton)	Blood stain on non-colored cotton fabric, 2 µL spot.
BSCI (Blood Stain, Cotton, Inhibitor)	Inhibitor-treated blood, stained on non-colored cotton fabric, 2 µL spot.
BSD (Blood Stain, Denim)	Blood stain on blue denim, 2 µL spot.
SSC (Semen Stain, Cotton)	Semen (sperm positive) stain on non-colored cotton fabric, 1 µL spot.
SalSw (Liquid Saliva, Swab)	Saliva on cotton swab, 50 µL.
XB (Extraction Blank)	Extraction blank.

Results

The quantitation results for each extraction method were compared. For all sample types investigated, the PrepFiler kit produced the highest concentration and total yield of DNA, with the exception that the PrepFiler kit and phenol:chloroform method produced similar results for the blood stain on denim (BSD) sample. Table 10 shows the total DNA yields (ng), and Figure 8 and Table 11 on page 55 show the DNA concentrations (ng/µL) obtained using the PrepFiler kit and the other four methods.

Table 10 Correlation study: Total DNA yield (ng) for commonly-used DNA extraction methods (R_1 = Extraction Replicate 1, R_2 = Extraction Replicate 2)

Sample	PrepFiler™ Kit		Promega DNA IQ™ Kit		Phenol: Chloroform		QIAGEN EZ1™ Investigator Kit		QIAGEN QIAamp® DNA Micro Kit	
	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
BFTA (Blood, FTA)	76.5	78	21	21.5	19.5	36	20	22.5	10	19
BSC (Blood Stain, Cotton)	60	107	46.5	38	67.5	38.5	29.5	34.5	17.5	23.5
BSCI (Blood Stain, Cotton, Inhibitor)	74	82.5	29	33	37.5	33.5	33	22	19.5	18
BSD (Blood Stain, Denim)	66	57	26.5	25	57.5	69.5	20.5	21.5	19	25
SSC (Semen Stain, Cotton)	161	196.5	70.5	66.5	84	74	87	89	49.5	55
SalSw (Liquid Saliva, Swab)	149.5	160	11	19	53.5	72	36	38.5	15.5	14.5
LB (Liquid Blood)	94.5	89.5	29.5	38.5	30	30	51.5	42	26.5	30.5
XB (Extraction Blank)	0	0	0	0	0	0	0	0	0	0

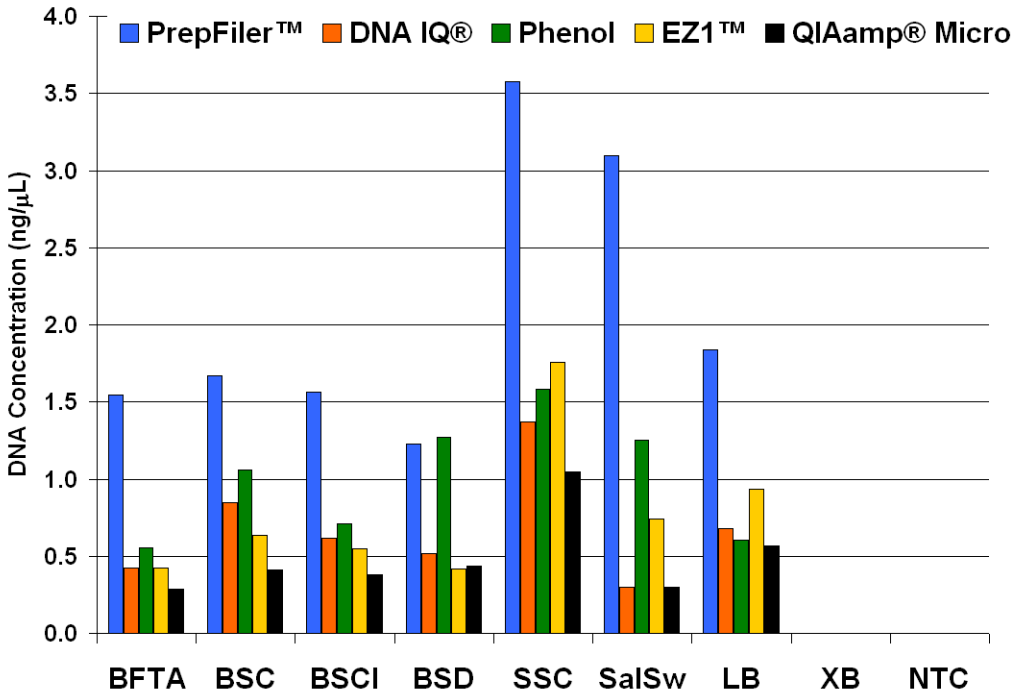

Figure 8 Correlation studies: DNA concentration results for commonly-used DNA extraction methods

Table 11 Correlation study: DNA concentration (ng/µL) for commonly-used DNA extraction methods (R_1 = Extraction Replicate 1, R_2 = Extraction Replicate 2)

Sample	PrepFiler™ Kit		Promega DNA IQ™ Kit		Phenol: Chloroform		QIAGEN EZ1™ Investigator Kit		QIAGEN QIAamp® DNA Micro Kit	
	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
BFTA (Blood, FTA)	1.53	1.56	0.42	0.43	0.39	.072	0.4	0.45	0.2	0.38
BSC (Blood Stain, Cotton)	1.2	2.14	0.93	0.76	1.35	0.77	0.59	0.69	0.35	0.47
BSCI (Blood Stain, Cotton, Inhibitor)	1.48	1.65	0.58	0.66	0.75	0.67	0.66	0.44	0.39	0.36
BSD (Blood Stain, Denim)	1.32	1.14	0.53	0.5	1.15	1.39	0.41	0.43	0.38	0.5
SSC (Semen Stain, Cotton)	3.22	3.93	1.41	1.33	1.68	1.48	1.74	1.78	0.99	1.1
SalSw (Liquid Saliva, Swab)	2.99	3.2	0.22	0.38	1.07	1.44	0.72	0.77	0.31	0.29
LB (Liquid Blood)	1.89	1.79	0.59	0.77	0.6	0.6	1.03	0.84	0.53	0.61
XB (Extraction Blank)	0	0	0	0	0	0	0	0	0	0

The IPC C_T results for each method were also compared. As shown in Figure 9, the IPC C_T values for all sample types and methods of extraction investigated were within ±1 of that obtained for the NTC. Thus, the ability of the PrepFiler kit to remove the PCR inhibitors is comparable to that of other commonly used extraction methods.

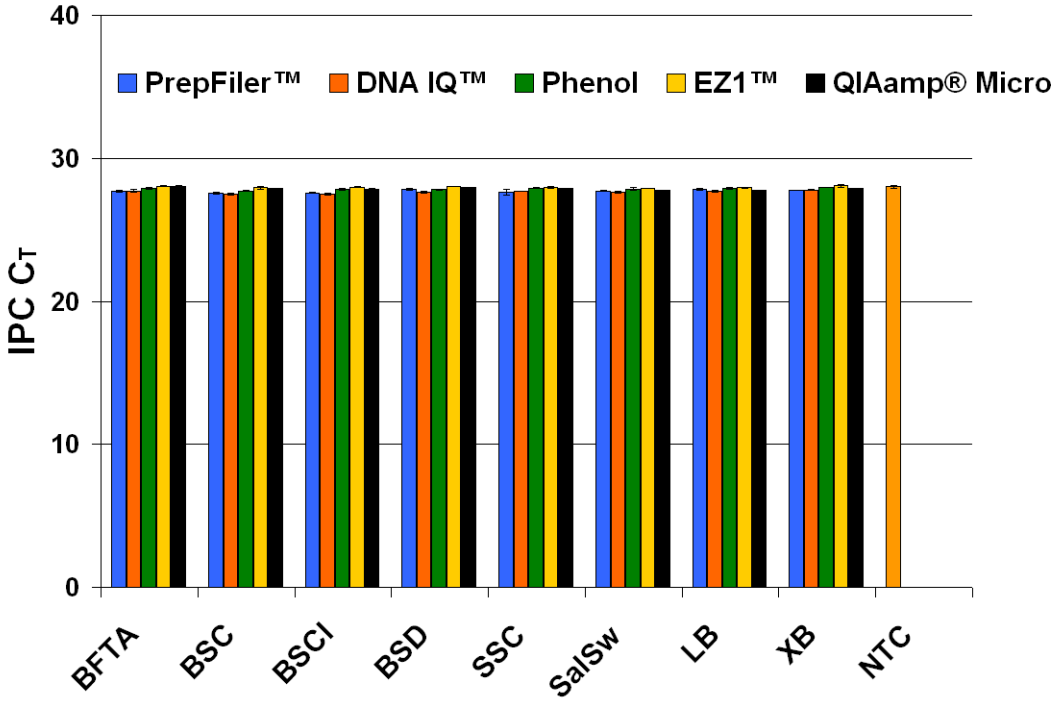

Figure 9 Correlation studies: IPC C_T values for commonly-used DNA extraction methods

Correlation with STR analysis

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

Experiment

The DNA extracts from each sample in the sensitivity, stability, reproducibility, case-type samples, and correlation studies were processed for STR analysis. Total human DNA quantitation values from the Quantifiler® Human DNA Quantification Kit were used to determine the volume of DNA extract for amplification. A target DNA input amount of 1 ng was used with the AmpFℓSTR® Identifiler® PCR Amplification Kit (if the DNA concentration was less than 1 ng/10 µL, 10 µL of DNA extract was used).

Results

STR profiles were analyzed for all of the samples from all of the studies. All samples produced full, balanced STR profiles that were devoid of PCR artifacts and that had peak heights equivalent to input amounts, demonstrating that the PrepFiler kit is effective in isolating high-quality genomic DNA. Representative profiles are shown in Figure 10 through Figure 13.

The profiles shown in Figure 10 represent DNA extracted from high (40 µL) and low (0.1 µL) volumes of blood from the same donor. Both samples are potentially challenging, as high blood volumes also contain high amounts of the PCR inhibitor hematin, while low blood volumes contain proportionally low amounts of DNA. Similar results were obtained for other samples in the sensitivity study.

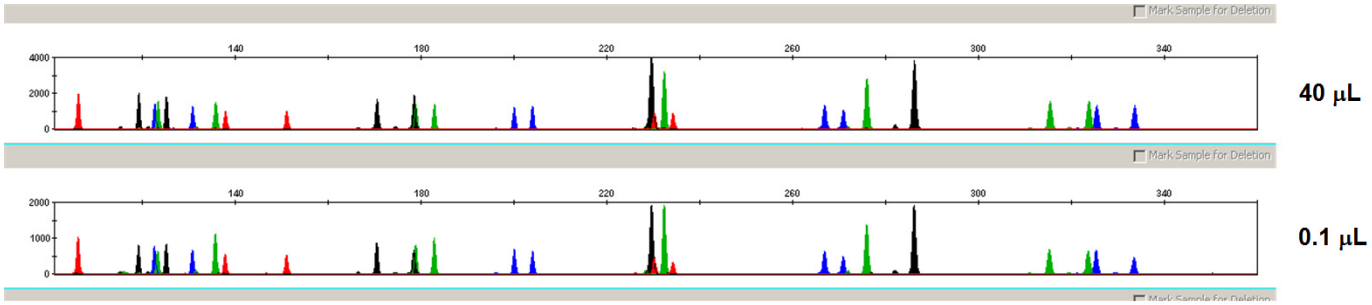

Figure 10 Identifiler® kit profiles for the liquid blood samples from blood donor 2

Figure 11 shows STR profiles for the compromised samples used in the stability studies:

- **BSCI** – 5µL blood with 1 µL inhibitor mix on cotton cloth
- **BSC-7** – 10 µL blood on cotton fabric exposed to the environment for 7 days
- **BSD** – 5 µL blood on denim

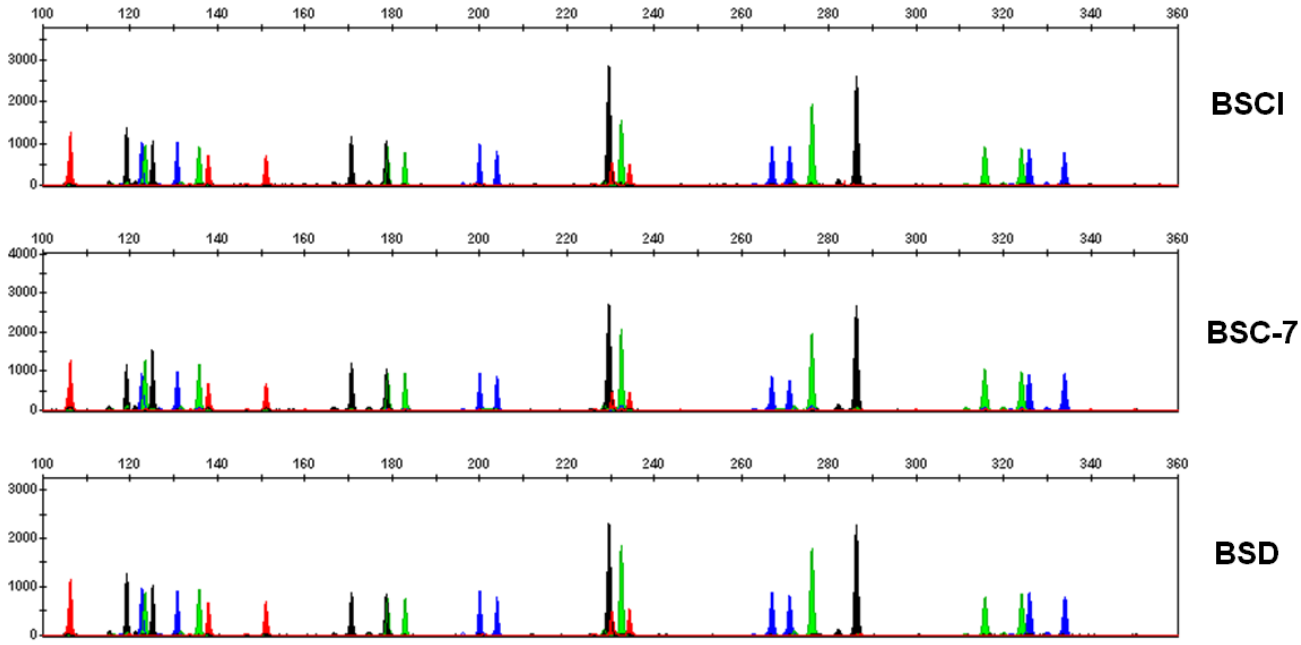

Figure 11 Identifiler® kit profiles for the compromised samples

Figure 12 on page 59 and Figure 13 on page 60 show STR profiles for the forensic sample types used in the case-type studies:

- **LB** – Liquid blood, 2 µL
- **BFTA** – Blood stain on FTA paper, 2 µL spot
- **BSC** – Blood stain on non-colored cotton fabric, 2 µL spot
- **BSCI** – Inhibitor-treated blood stain on non-colored cotton fabric, 2 µL spot
- **BSD** – Blood stain on blue denim, 2 µL spot
- **SSC** – Semen (sperm positive) stain on non-colored cotton fabric, 1 µL spot
- **SAL** – Saliva on cotton swab, 50 µL
- **DE, e–fraction** and **DE, s–fraction** – Epithelial- and sperm-cell fractions from sexual-assault-type sample
- **MBSC** – Mixed-blood stain on non-colored cotton fabric prepared using 1 µL blood from human male donor and 4 µL of blood from female donor

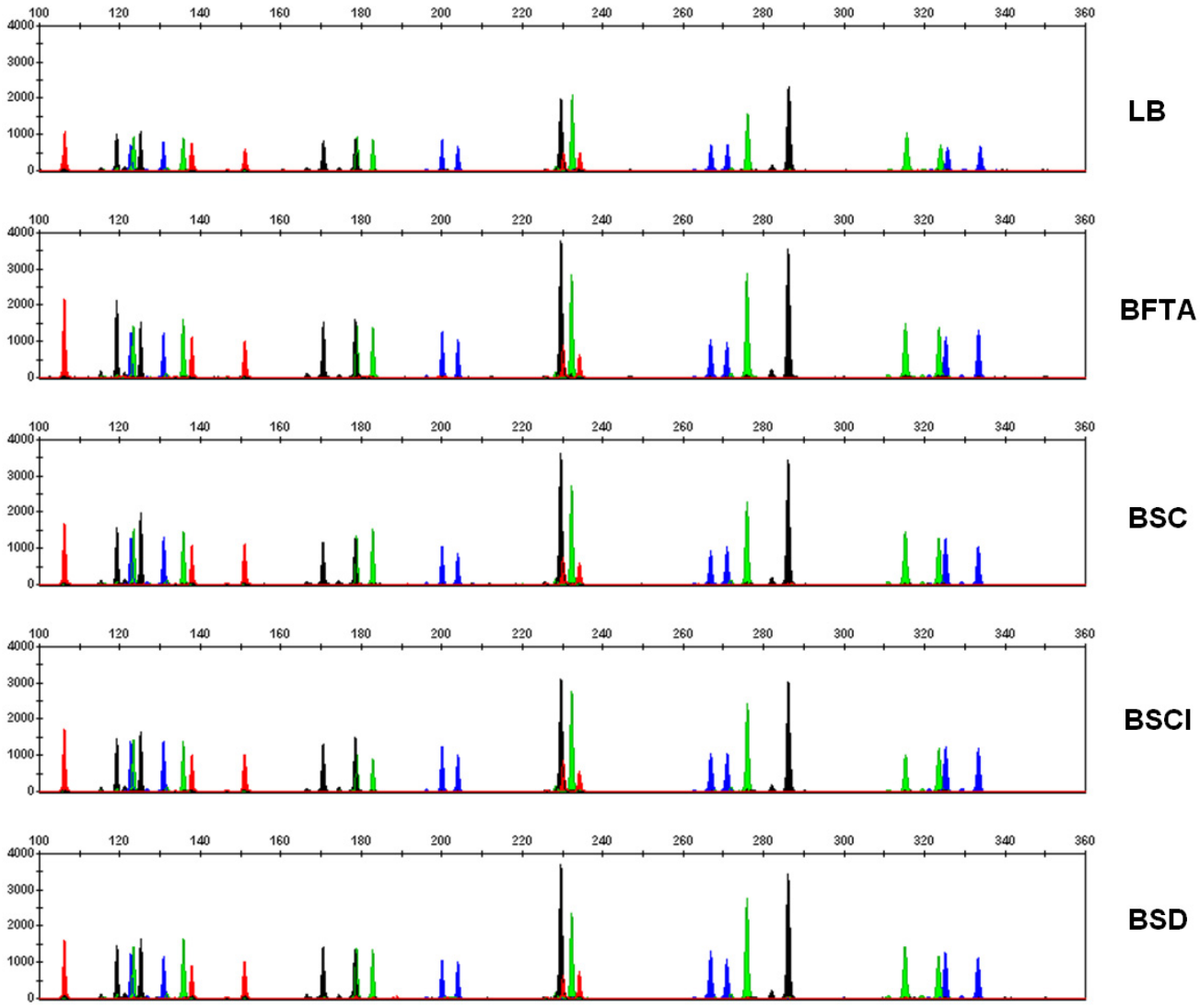

Figure 12 Identifiler® kit profiles for the case-type samples

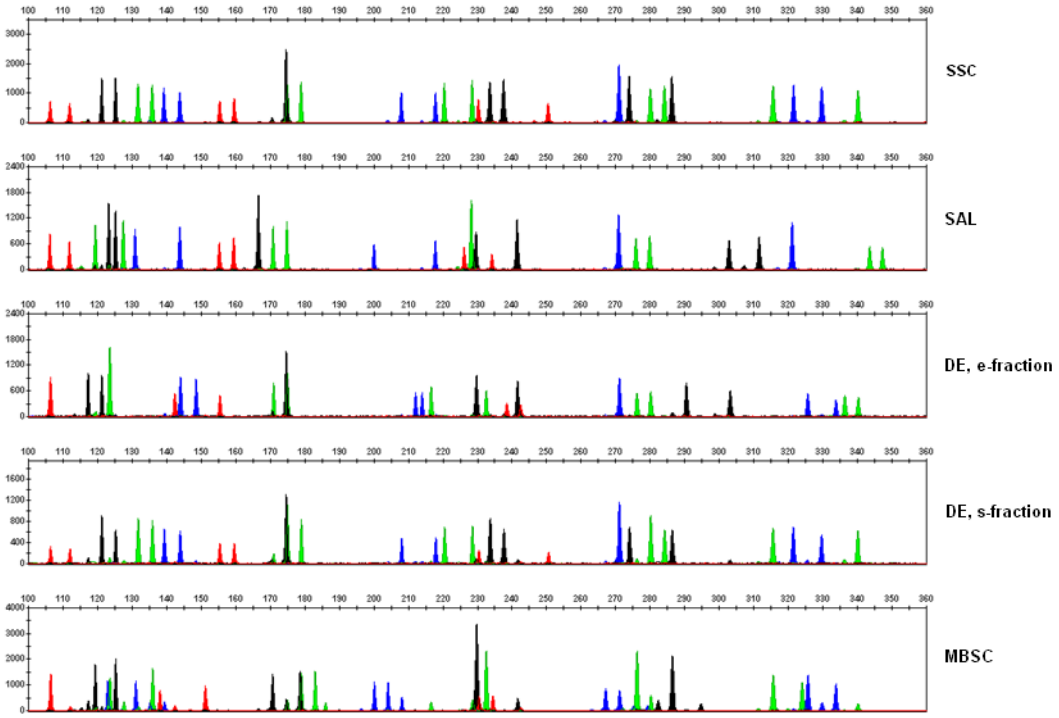

Figure 13 Identifiler® kit profiles for the case-type samples

Troubleshooting A

The information provided in this section applies to the extraction protocols described in Chapter 2, "Perform Extraction – Standard Protocol" on page 9 and Chapter 3, "Perform Extraction – Supplementary Protocols" on page 23.

Observation	Possible Cause	Recommended Action
After performing lysis (and, if applicable, substrate removal)...		
Volume of collected lysate is low	• Some lysate remained in the sample substrate after centrifugation. • A tube lid was not properly sealed during incubation or vortexing, resulting in volume loss through leakage or evaporation. • A tube was not briefly centrifuged after incubation or vortexing, and droplets on the inside of the tube lid leaked when the tube was opened.	**If the lysate volume is >180 µL:** Proceed to the next step in the protocol.
		If the lysate volume is <180 µL and the sample required substrate removal: 1. Centrifuge the PrepFiler™ Filter Column with PrepFiler™ Spin Tube containing the substrate for an additional 5 minutes. 2. If the resulting lysate volume is: • >180 µL, continue to the next step in the protocol. • <180 µL, add PrepFiler™ Lysis Buffer to bring the lysate volume to 300 µL, then proceed to the next step in the protocol.
		If the lysate volume is <180 µL and the sample *did not* require substrate removal: Add PrepFiler™ Lysis Buffer to bring the lysate volume to 300 µL, then proceed to the next step in the protocol.
Before pipetting magnetic particles...		
PrepFiler™ Magnetic Particles tube contains precipitate	Magnetic particles were exposed to low temperatures during the shipping or storage.	To dissolve precipitate that may have formed during shipping or storage, incubate the PrepFiler™ Magnetic Particles tube(s) at 37 °C for 30 minutes at 900 rpm before first use. After incubation, you can store the magnetic particles tube at room temperature for up to 3 months. If longer-term storage is necessary, store the magnetic particle tubes at 4 °C, then incubate the tube at 37 °C for 30 minutes at 900 rpm before use.

Observation	Possible Cause	Recommended Action
After adding magnetic particles...		
When using the magnetic stand, all of the magnetic particles do not migrate to the magnet within one to two minutes	• The magnet was improperly aligned in the magnetic stand. • The presence of lipids, polysaccharides, or other such macromolecules slowed particle migration.	1. Remove the tubes from the stand, check the magnet alignment, then place the tubes back in the magnetic stand. See "Magnetic stand guidelines" on page 13. 2. Keep the DNA sample tube in the magnetic stand for 5 minutes or until all particles have visibly migrated to the magnet. **Note:** The particle migration speed may increase after the first wash step.
During the elution step...		
The magnetic particles are not completely suspended in solution after adding the PrepFiler™ Elution Buffer and vortexing the solution	At the end of the wash step, the magnetic particle pellet was overdried due to: • Drying for more than 10 minutes at room temperature. *or* • Drying at a temperature >25 °C.	1. Vortex the elution buffer-magnetic particles solution at maximum speed for an additional 10 to 15 seconds. 2. If the magnetic particles are still not completely suspended, tap the side of the tube with your finger, or pipette the suspension up and down several times. **IMPORTANT!** Make sure that the magnetic particles are not lost due to adhering to the pipette tip. 3. If the magnetic particles are still not completely suspended, then perform the incubation step (step 4 on page 22) with the following modification: 2 to 3 times during incubation, remove the DNA tube from the thermal shaker and vortex the tube at maximum speed for 5 seconds.

Observation	Possible Cause	Recommended Action
After eluting the DNA...		
The DNA eluate contains magnetic particles	• The magnet was improperly aligned in the magnetic stand. • The magnetic pellet formed during the elution step did not contain all magnetic particles. • Small magnetic particles (fines), which migrate more slowly towards the magnet, or particle aggregates which hinder particle migration were present. • Magnetic particles were captured during removal of the eluate due to overly aggressive pipetting.	1. Remove the tubes from the stand, check the magnet alignment, then place the tubes back in the magnetic stand. See "Magnetic stand guidelines" on page 13. 2. Keep the DNA sample tube in the magnetic stand for 5 minutes or until all particles have visibly migrated to the magnet, then pipette out the clear DNA eluate into a new tube and process for quantitation.
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, follow the Repurification Protocol (see Appendix B, "Repurification Protocol" on page 65) to process the DNA eluate, then requantify the sample. **Note:** If you identify a sample type that typically produces colored eluate, you can modify your protocol for that sample type to wash the DNA an additional one to two times during the wash step before the final elution step.

Observation	Possible Cause	Recommended Action
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. For example, insufficient addition of magnetic particles may result in little or no formation of the magnetic particle pellet when the sample tube is set into the magnetic stand. • Magnetic particles and isopropanol were added to the sample lysate while the 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. • Sample was overdried at end of wash step. • The DNA eluate contains PCR inhibitors due to excessive amount of inhibitors in the sample.	1. Review protocol steps and reagent additions. 2. Amplify the maximum volume for STR analysis. 3. Extract DNA from a different sample prepared from the same source.
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)	• Magnetic particles are present in the DNA extract.	• If magnetic particles are present in the DNA eluate, place the tube containing the DNA eluate in the magnetic stand for 5 minutes, vortex the tube, pipette the clear DNA extract solution into a new tube, then process for quantitation.
	• 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.	• If the DNA eluate is below 25 ng/µL, or if the diluted DNA eluate still produces high IPC C_T compared to the NTC or quantitation standards, follow the Repurification Protocol (see Appendix B, "Repurification Protocol" on page 65) to process the DNA eluate, then requantify the sample. **Note:** Repurification may result in the loss of additional DNA. Consider proceeding to amplification with a kit such as the AmpFℓSTR® MiniFiler™ PCR Amplification Kit, which is designed to obtain STR profiles from compromised samples such as those which may be inhibited and/or degraded.

Repurification Protocol B

Use the repurification protocol if, after extracting DNA from a sample using the standard or supplementary protocols, it is necessary to repurify the DNA eluate. Repurification of DNA eluate is one of possible actions you can take to troubleshoot the observed problems "The DNA eluate is colored" (see page 63) and "Sample IPC C_T is higher than IPC C_T of NTC or quantitation standards" (see page 64).

Note: The following steps are performed after completing "Step 6: Elute DNA" on page 22.

WARNING! CHEMICAL HAZARD. PrepFiler™ Lysis Buffer in contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, and swallowed. Causes eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapor. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. PrepFiler™ Magnetic Particles are harmful by inhalation, skin absorption, and if swallowed. Causes eye, skin, and respiratory tract irritation. Do not taste or swallow. Avoid breathing vapor (or dust). Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Allow the DNA eluate to come to room temperature.
2. Bring the thermal shaker temperature to 70 °C.
3. Add 250 µL of PrepFiler™ Lysis Buffer to the tube containing 50 µL of DNA eluate.
4. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.
5. Vortex the PrepFiler™ Magnetic Particles tube approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge briefly.

   Note: If you are pipetting multiple samples, vortex the magnetic particles tube every 5 minutes until you complete the next step.

6. Pipette 15 µL of PrepFiler™ Magnetic Particles into the tube containing the DNA eluate.

7. Cap the tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 10 seconds, then centrifuge it briefly.
8. Add 180 µL of isopropanol to the tube.
9. Cap the tube, vortex it at *low* speed (approximately 500 to 1,200 rpm) for 5 seconds, then centrifuge it briefly.
10. Place the tube in a shaker or on a vortexer (with adaptor), then mix at 1,000 rpm at room temperature for 10 minutes.
11. Follow the wash and elution steps in sections "Step 5: Wash bound DNA" on page 20 and "Step 6: Elute DNA" on page 22.

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Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

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