The AutoMate *Express™* Forensic DNA Extraction System

Brad Dixon
Forensic Science Applications Group
Presentation Overview

• Chemistry and Instrument Overview
  • PrepFiler Express™ & PrepFiler Express BTA™ Forensic DNA Extraction Kits
    • Protocol Overviews
    • PrepFiler LySep™ Column and Cartridge Configuration

• AutoMate Express™ Instrument DNA Isolation

• Developmental Validation Data
AutoMate Express™ Forensic DNA Extraction System

- Developed specifically to enable maximum yield of high quality genomic DNA from forensic samples using the PrepFiler Express™ Forensic DNA Extraction Kits
  - Processes 13 samples in ~30 minutes
  - Two protocols on a single script card
    - PrepFiler™ Lysis Buffer and Bone Tooth Adhesive (BTA)
AutoMate Express™ DNA Extraction Workflow

**PrepFiler™ Lysis Buffer (PFLB)**
(majority of samples including trace evidence samples) – high extraction efficiency

**PrepFiler BTA™ Lysis Buffer**
(Bone, tooth, sticky tape, cig. butt) performing delicate lysis

**PFLB or BTA Protocol**

Lysate separation from substrate

DNA for downstream applications

**DNA Binding**

**Wash**

**DNA Elution**
What is the PrepFiler Express™ Kit?

- Contains all reagents needed to perform on-line DNA isolation in an AutoMate Express™ System specific cartridge
- A combination of uniquely structured magnetic particles and a multi-component surface chemistry optimized to provide extremely efficient DNA binding capacity and maximum DNA recovery

Advantages of the Magnetized DNA Complex

- Efficient DNA binding capacity and maximum DNA recovery
- Stable binding during wash steps while removing PCR inhibitors
- Consistent yields of purified DNA separated from the magnetic particles at the elution step

- No organic extraction
- No silica-based purification
- No ion exchangers
- No direct precipitation
PrepFiler Express™ and Express BTA™ Forensic DNA Extraction Kits
Kit Configurations

• PrepFiler Express™ Forensic DNA Extraction Kit*
  • PrepFiler™ Lysis Buffer
  • PrepFiler Express™ Cartridges**
  • Sample Tubes
  • Elution Tubes
  • PrepFiler LySep™ Columns
  • Tips and tip sheaths

• PrepFiler Express BTA™ Forensic DNA Extraction Kit*
  • PrepFiler BTA™ Lysis Buffer
  • Proteinase K
  • PrepFiler Express™ Cartridges**
  • Sample Tubes
  • Elution Tubes
  • PrepFiler LySep™ Columns
  • PrepFiler™ Lysis Tubes (for bone)
  • Tips and tip sheaths

*1M DTT also required but not included in the kit
** Isopropanol and prepared PrepFiler™ Kit wash buffers are included in cartridge format
*** Available as a plastics only kit
PrepFiler Express™/Express BTA™ Cartridge Configuration

- Cartridges are foil sealed and pierced after protocol start.
PrepFiler Express™/Express BTA™ Kit Off-Line Lysis
Add 500 μL Complete PrepFiler Express Lysis Solution to Sample in PrepFiler LySep™ Column Assembly

Incubate 70°C for 40 minutes with shaking (750 rpm)

Centrifuge (10000 g for 2 minutes) PrepFiler LySep™ Column Assembly

Transfer lysate and Sample Tube to AutoMate Express™ Instrument

Perform PrepFiler Express™ Standard Instrument Purification Script
The PrepFiler LySep™ Column
Simple off-line Lysis & Substrate Removal

Current Spin Basket Method

Manual transfer → Spin basket → Spin → Sample tube → DNA purification
The PrepFiler LySep™ Column
Simple off-line Lysis & Substrate Removal

Add Sample, Lysis Buffer & DTT* to PrepFiler LySep™ Column

Remove PrepFiler LySep™ Column and discard

Load on Instrument

Incubate/Shake

High-speed centrifugation enables simple & efficient separation of lysate from substrate
PrepFiler LySep™ Filter Column

• Allows incubation of substrate with lysis buffer in assembled PrepFiler LySep™ Column+Sample Tube configuration
  • Filter unit plus membrane seal prevents any lysis buffer drainage during incubation
• High-speed centrifugation enables separation of lysate from substrate
  • Centrifugation force bursts membrane seal enabling lysate flow-through
• Discard PrepFiler LySep™ Column containing substrate and load hingeless sample tube directly onto the AutoMate Express™ Instrument
• Eliminates the need for a “Tip Dance” protocol
PrepFiler Express BTA™ Kit Protocol: Adhesives

Add 230 µL Complete PrepFiler BTA Lysis Solution to Sample in PrepFiler LySep™ Filter Column Assembly

Incubate 56°C for 40 minutes with shaking (750 rpm)

Centrifuge (10000 g for 2 minutes) PrepFiler LySep™ Column Assembly

Transfer lysate and Sample Tube to AutoMate Express™ Instrument

Perform PrepFiler Express BTA™ Purification Instrument Script
PrepFiler Express BTA™ Kit Protocol: Bones and Teeth

Add 230 µL Complete PrepFiler BTA Lysis Solution to 50mg bone/tooth powder in 2mL screw cap tube

Incubate 56°C for at least 2 hours with shaking (1100 rpm)

Centrifuge (10000 g for 2 minutes) screw cap tube

Transfer supernatant into Sample Tube and load onto AutoMate Express™ Instrument

Perform PrepFiler Express BTA™ Purification Instrument Script
PrepFiler Express BTA™ Kit Protocol: Bone Samples

• Screw Cap:
  • Needed during lysis due to bone effervescence which can pop open snap cap tubes. Also, prevents loss of volume during incubation

• 50mg Bone Powder:
  • Optimal input to recover the highest yield of DNA per mg of starting material.
  • Higher starting weight of bone may increase yield but may decrease available lysate volume

• 2 hour Incubation:
  • Minimum time for incubation but can do up to 18 hours without adverse results.

• Supernatant Processing:
  • After incubation, centrifugation required to pellet bone powder
  • Process supernatant only
AutoMate Express™ Instrument
DNA Isolation
Setup AutoMate *Express™* Instrument

- Ensure Protocol Card is properly inserted
- Do Not Turn on Instrument without card inserted!
- Do Not Remove IC Card if the instrument is on!
- Power on AutoMate *Express™* Instrument
AutoMate Express™ Instrument Run

1. Load cartridge rack with appropriate number of reagent cartridges

2. Load tip and tube rack
   - Sample lysate
   - Tips and tip holders
   - Elution tube

3. Select appropriate PrepFiler Express™ Protocol

4. Start run
AutoMate Express™ DNA Isolation

Wash 3X

*Not to Scale
Instrument Run and Completion

- Both the PrepFiler Express™ Standard and BTA Kits take approximately 30 minutes to complete.
- When the instrument has completed the run, the instrument will beep briefly and display Finished Protocol on the display screen.
- Open the door, remove samples and store short term at 4°C or long term at -20°C.
- Discard the sample tubes, cartridges, tips, and tip holders into biohazardous waste containers.
Developmental Validation Data
The blood series demonstrates a proportional increase in DNA yield as a function of blood input.

<table>
<thead>
<tr>
<th>Blood Sample Volume (µL)</th>
<th>Average DNA Yield, ng (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>144.89</td>
</tr>
<tr>
<td>1</td>
<td>26.03</td>
</tr>
<tr>
<td>0.25</td>
<td>6.39</td>
</tr>
<tr>
<td>0.1</td>
<td>2.68</td>
</tr>
<tr>
<td>0.025</td>
<td>0.65</td>
</tr>
<tr>
<td>XB</td>
<td>0.00</td>
</tr>
</tbody>
</table>

N=4 for each blood sample
Sensitivity STR Data: Representative Profiles

5 uL

1 uL

0.25 uL

0.1 uL

0.025 uL
Correlation Study-Low Input Samples

![Graph showing DNA Yield (ng) for different samples and methods.](image)

**DNA Yield (ng)**

- **Blood on cotton cloth (0.3 µL)**
- **Semen on cotton cloth (1 µL)**
- **Bone (50 mg)**
- **Extraction blank**

**Methods**

- AutoMate Express™ System
- Method A
- Method B
Correlation Study-High Input Samples

DNA Yield (ng)

- Blood on cotton cloth (2 µL)
- Blood on FTA paper (2 µL)
- E-cells on swab (50 µL)
- Blood on blue denim (1 µL)
- Extraction blank

Methods:
- AutoMate Express™ System
- Method A
- Method B
Correlation Study-Total Allele Count

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>% Alleles Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood on cotton cloth (0.3 µL)</td>
<td>100</td>
</tr>
<tr>
<td>Blood on cotton cloth (2 µL)</td>
<td>100</td>
</tr>
<tr>
<td>Blood on FTA paper (2 µL)</td>
<td>100</td>
</tr>
<tr>
<td>E-cells on swab (50 µL)</td>
<td>100</td>
</tr>
<tr>
<td>Semen on cotton cloth (1 µL)</td>
<td>100</td>
</tr>
<tr>
<td>Blood on blue denim (1 µL)</td>
<td>100</td>
</tr>
<tr>
<td>Bone (50 mg)</td>
<td>55</td>
</tr>
</tbody>
</table>

Method A and Method B comparison:
- AutoMate Express™
- Method A
- Method B

Note: The comparison shows the percentage of alleles recovered for different sample types using two different methods, with Method A generally showing higher recovery rates.
Reproducibility-Low Input Samples

- Epithelial-cell fraction (5 µL)
- Semen on cotton cloth (1 µL)
- Bone (50 mg)
- Extraction blank (PrepFiler Express™ kit)
- Extraction blank (PrepFiler Express BTA™ kit)

Total DNA Yield (ng)
Reproducibility-High Input Samples

![Graph showing total DNA yield for different samples and extraction methods over three days.](image-url)
Case Type Samples

![Graph showing DNA yield for various case type samples.](image)
Summary

• Robust, easy-to-use system optimized for use with PrepFiler™ Chemistry

• Effectively isolates high quality genomic DNA from a broad range of forensic samples

• Successfully removes inhibitors to PCR commonly found in forensic samples maximizing the performance of downstream applications.

• Extracted DNA validated for use with the Quantifiler® and AmpFSTR® kits
For Research, Forensic, or Paternity Use Only. Not intended for any animal or human therapeutic or diagnostic use.

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

NOTICE TO PURCHASER: Limited Use Label License
The products shown in this presentation may be covered by one or more Limited Use Label License(s). Please refer to the respective product documentation or the Applied Biosystems website under www.appliedbiosystems.com for the comprehensive license information. By use of these products, the purchaser accepts the terms and conditions of all applicable Limited Use Label Licenses. These products are sold for research use only, and are not intended for human or animal diagnostic or therapeutic uses unless otherwise specifically indicated in the applicable product documentation or the respective Limited Use Label License(s).