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Automated Extraction of High Quality Genomic DNA from Forensic Evidence Samples Using a Cartridge-Based System

Jason Yingjie Liu¹, Ph.D.^{}, Maxim Brevnov¹, Ph.D., Allison Holt¹, Ph.D., James E. Stray¹, Ph.D., Declan Donovan², Ph.D., Alan B. Dixon¹, MSFS, Jim C. Nurse¹, BS, Manohar R. Furtado¹, Ph.D., and Jaiprakash G. Shewale¹, Ph.D.*

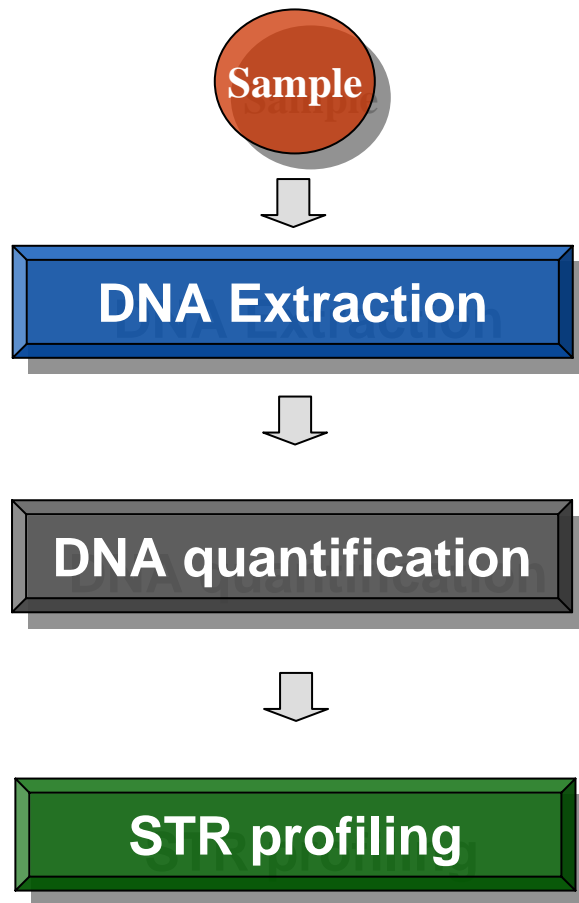
Disclosure

- This presentation includes commercial products from
 - Applied Biosystems (part of Life Technologies)
 - Other Laboratory Suppliers

Presentation Outline

- Introduction of automated benchtop DNA extraction system
- PrepFiler LySep™ column for workflow simplification
- Protocols and examples of DNA extractions using these protocols
- System studies such as cross contamination study, sensitivity study and instrument variation study
- Conclusions

Main Steps in Forensic DNA Analysis



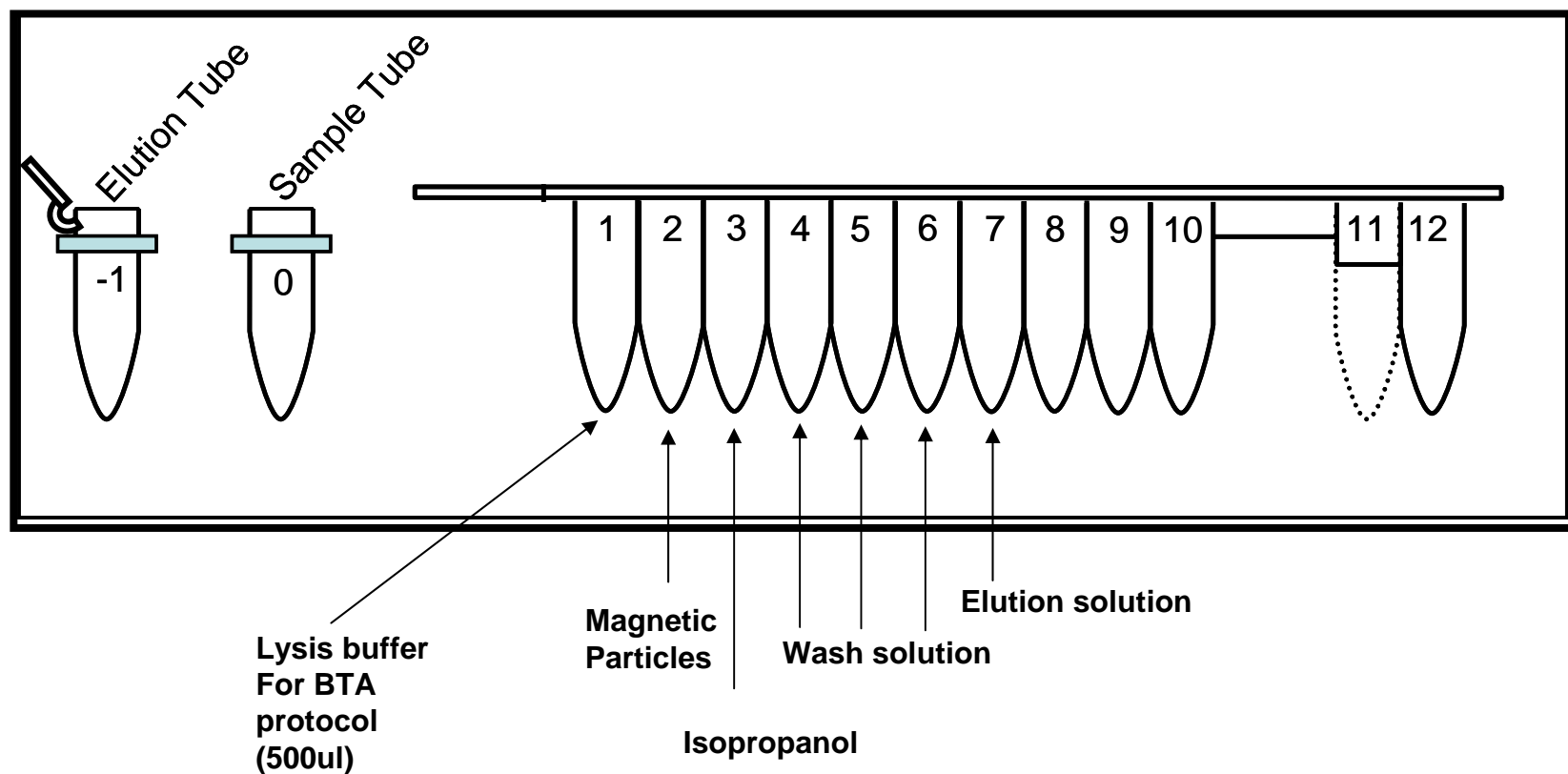
- Suitable for multiple sample types
- Effective removal of inhibitors
- High recovery for minimal input samples
- Simple to use with minimal hands on operations

Automated Benchtop Forensic DNA Extraction System: Highlights

- Core reagents are from the PrepFiler™ Forensic DNA Extraction Kit utilizing magnetic particle based nucleic acid purification technology
- PrepFiler LySep™ column developed to perform lysis and separation of substrate in a single device
- PrepFiler™ lysis buffer for lysis of majority of forensic sample types
- PrepFiler BTA™ Lysis buffer for lysis of bone, tooth or samples containing adhesive
- All required DNA extraction reagents are in a sealed disposable plastic cartridge
- Benchtop instrument for automated purification of DNA

Cartridge Configuration

Universal cartridge suitable for DNA extraction from lysates generated from both PrepFiler™ and PrepFiler BTA™ Lysis Buffers



AutoMate Express™ Benchtop Instrument

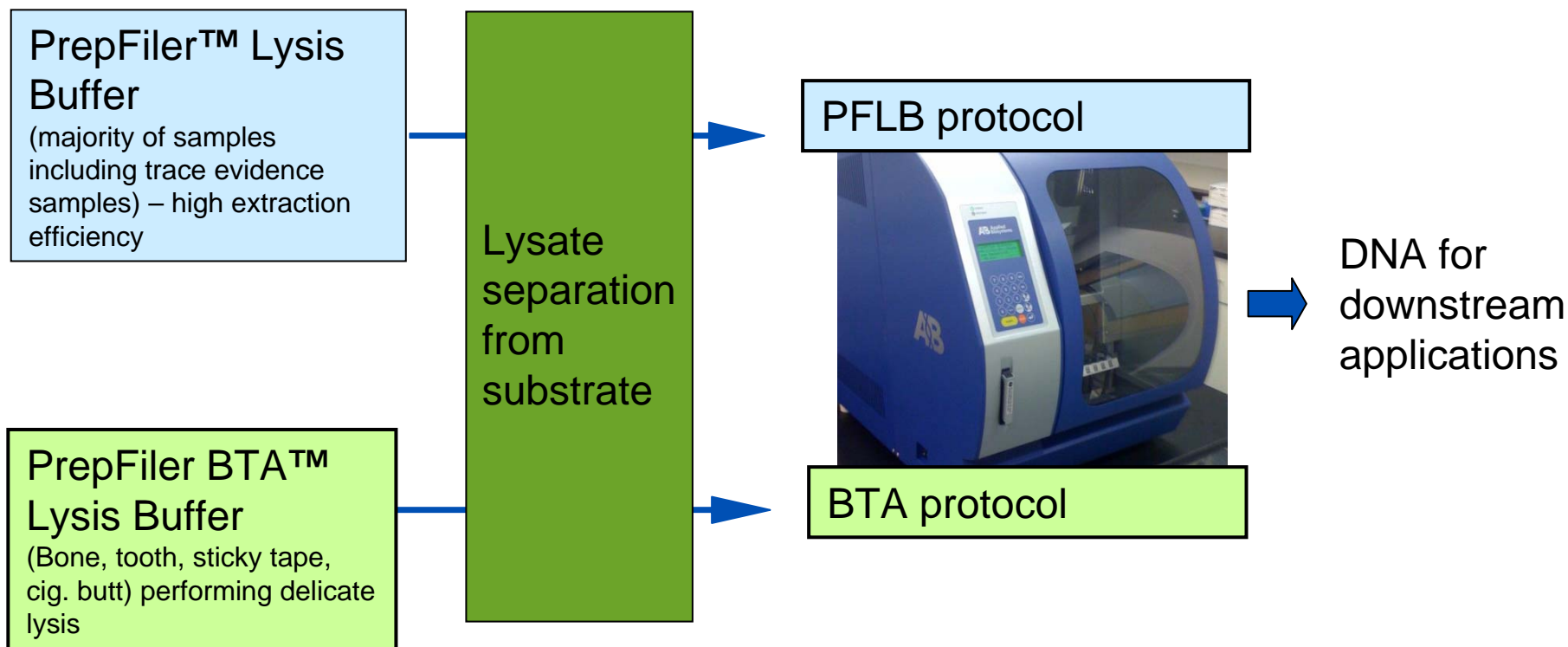
- Processes 13 samples in about 30 minutes
- Elution Vol: 50 µL
- Two protocols (PFLB protocol and BTA protocol) on a single script card



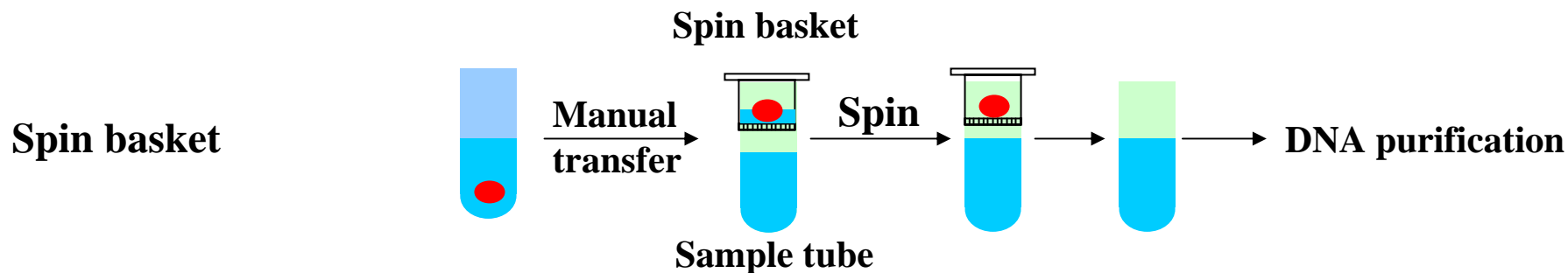
Cartridge rack

Tip and tube rack

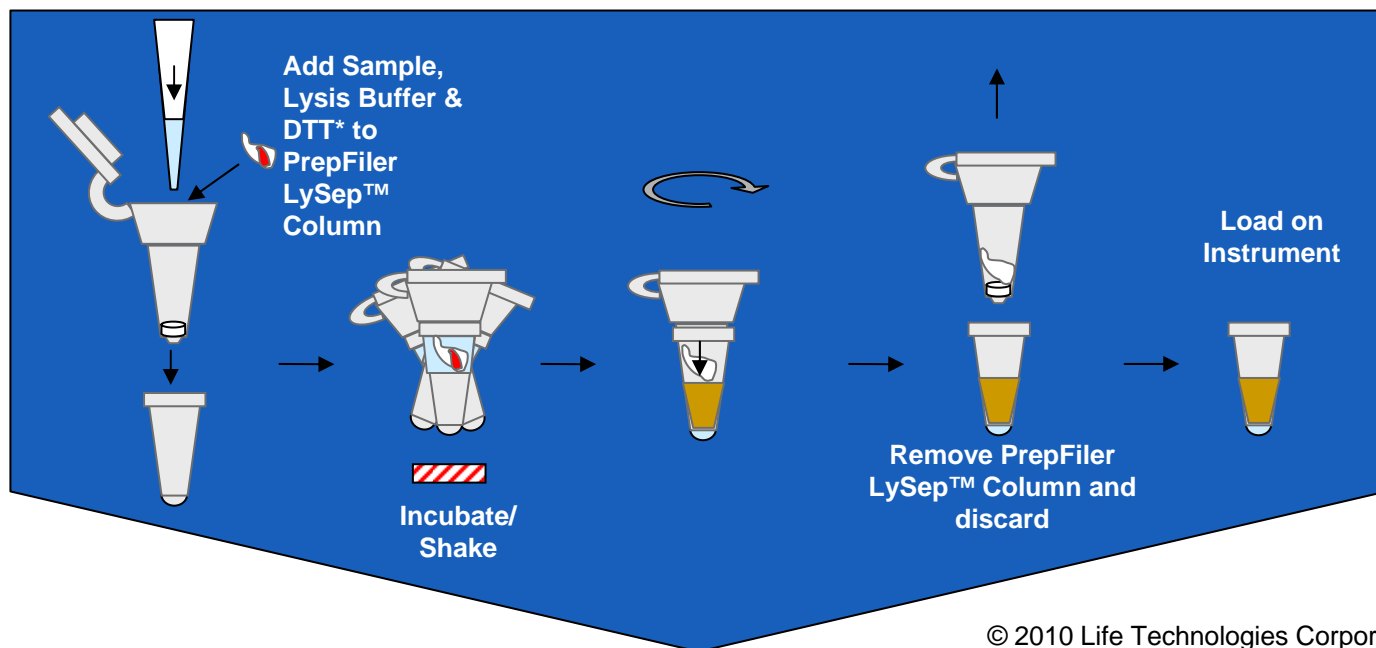
Overview of the Workflows

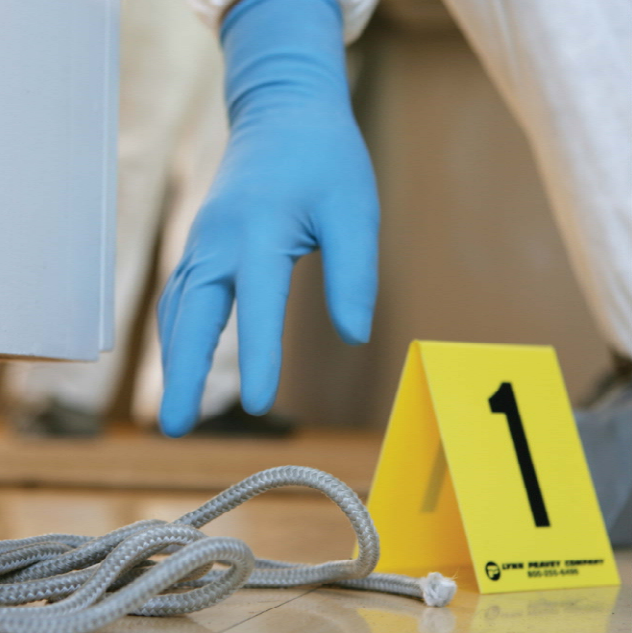


PrepFiler LySep™ column: Lysis and substrate separation in a single device



PrepFiler LySep™ column





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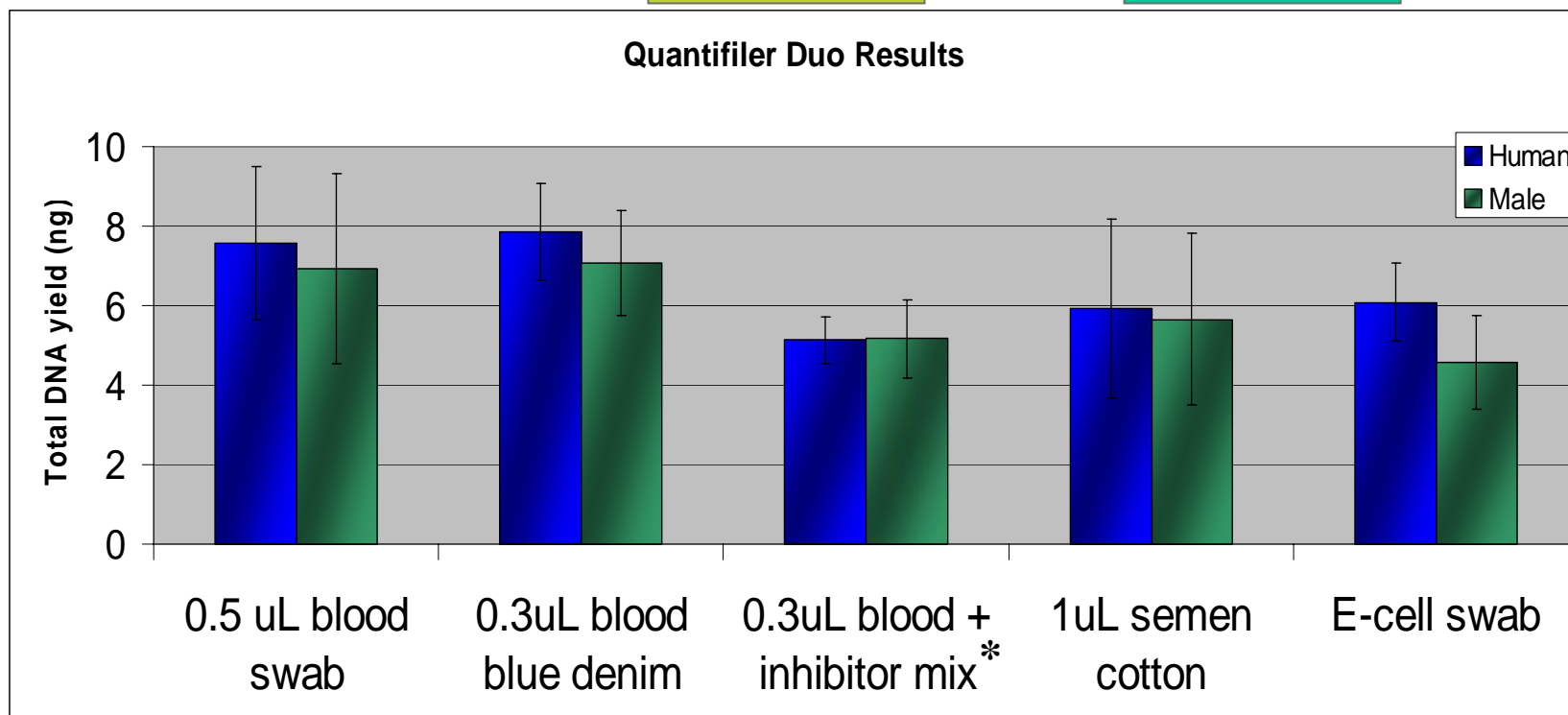
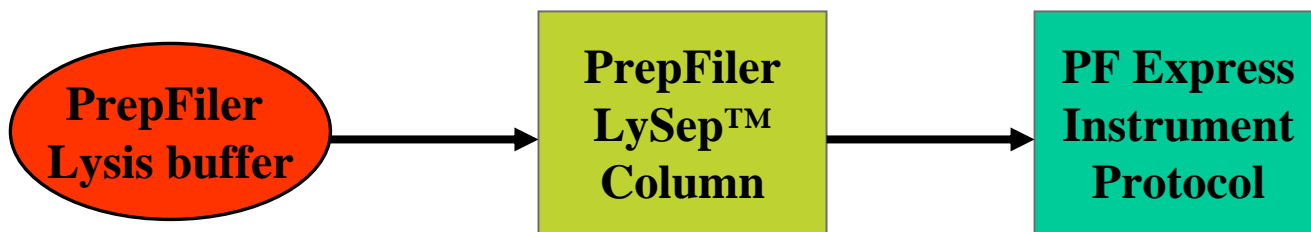
AAFS

2010

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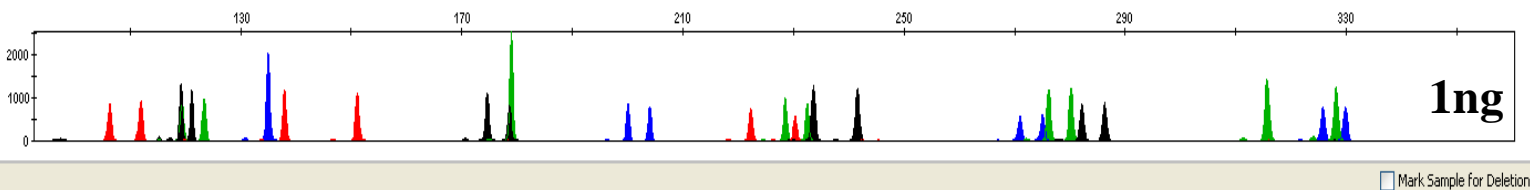
Protocol Options

Protocol 1: PrepFiler Express™ Extraction

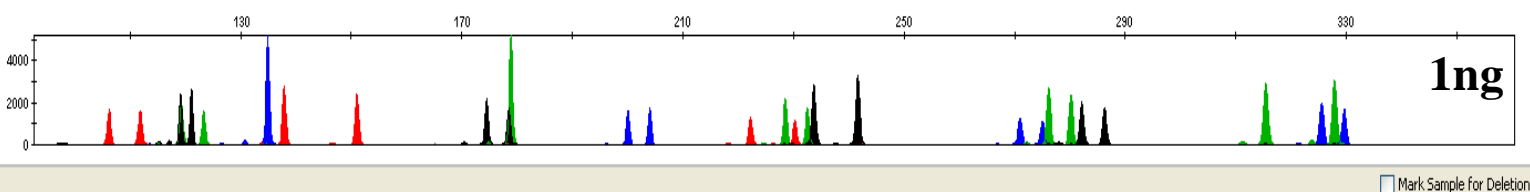


* Inhibitor mix: 12.5mM Indigo, 0.5mM Hematin, 2.5mg/mL Humic acid and 300mg/mL urban dust

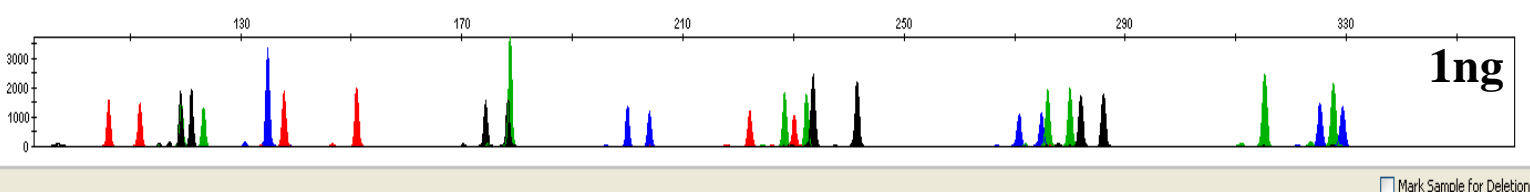
Identifiler® STR Kit Profiles



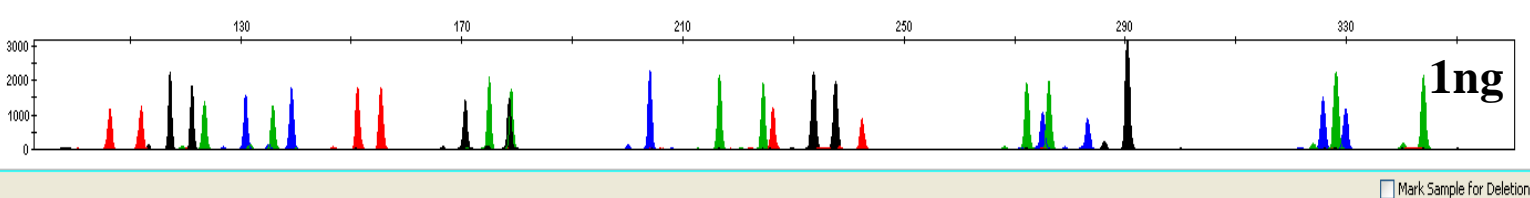
0.5 uL blood swab



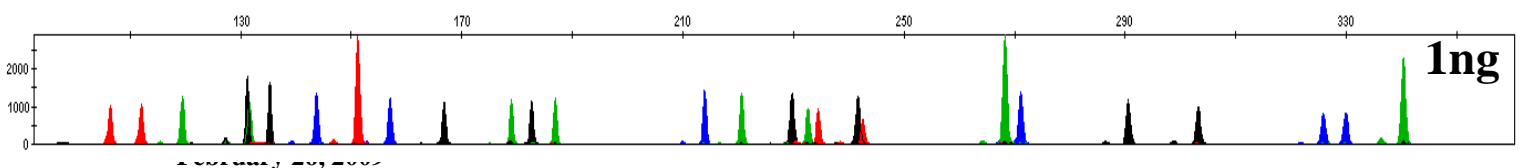
**0.3uL blood
blue denim**



**0.3uL blood
w. inhibitors**

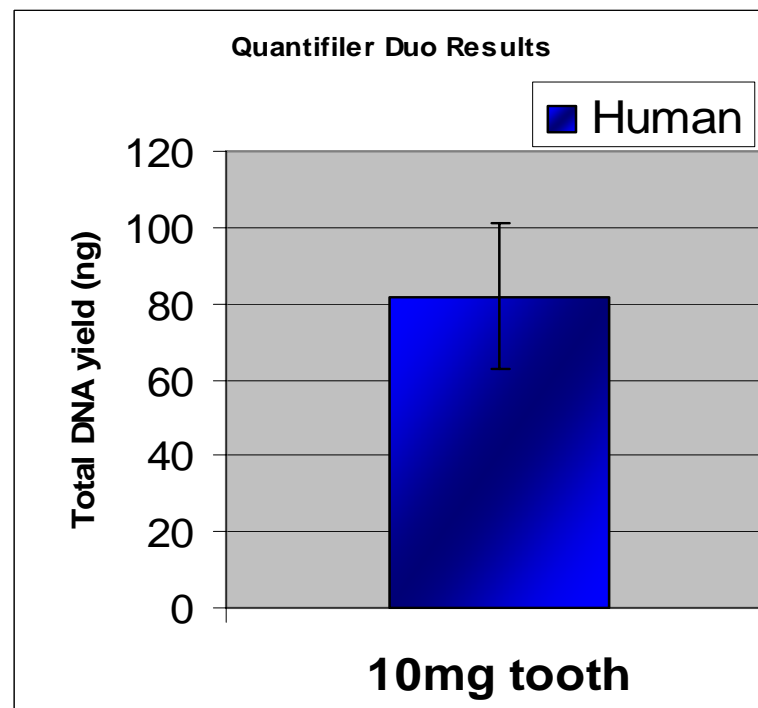
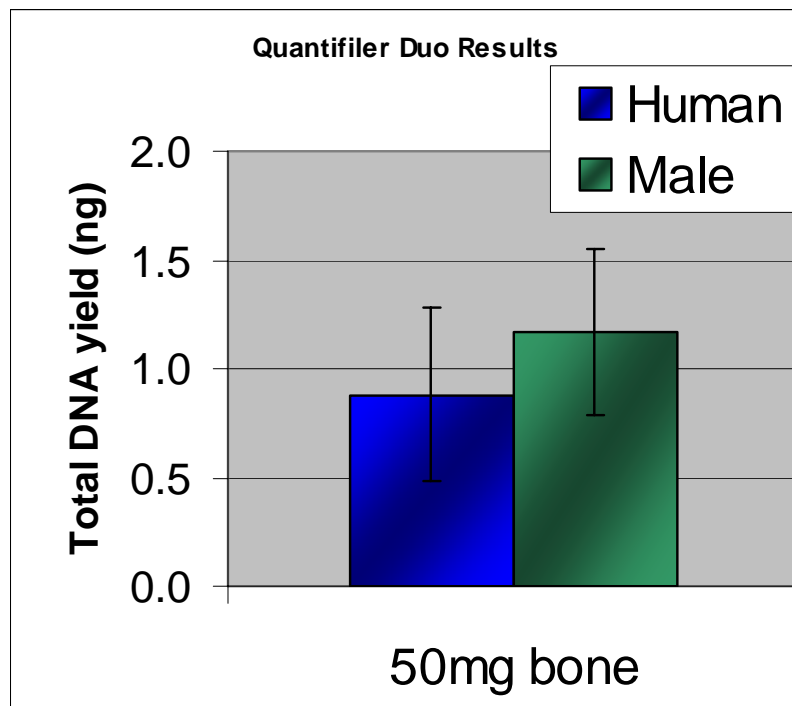
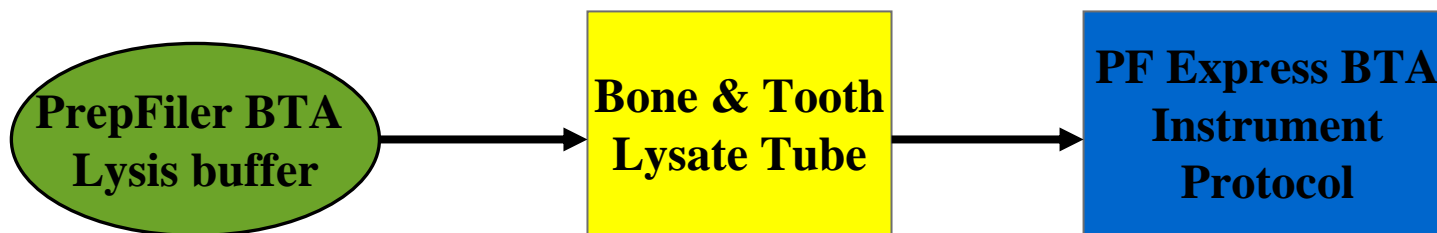


1uL semen cotton

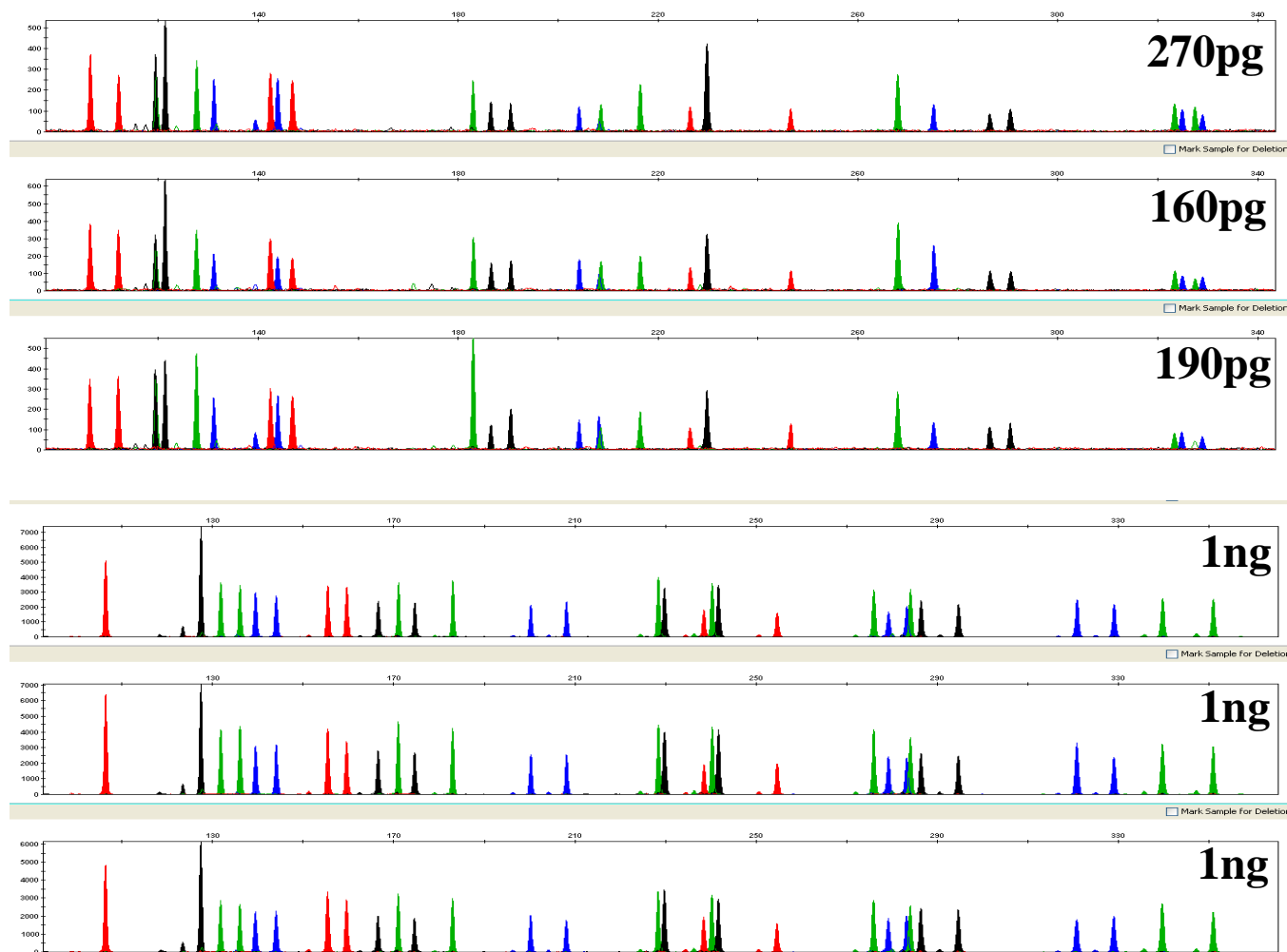


E-cell swab

Protocol 2: PrepFiler Express BTA™ Bones/Teeth Sample Extraction



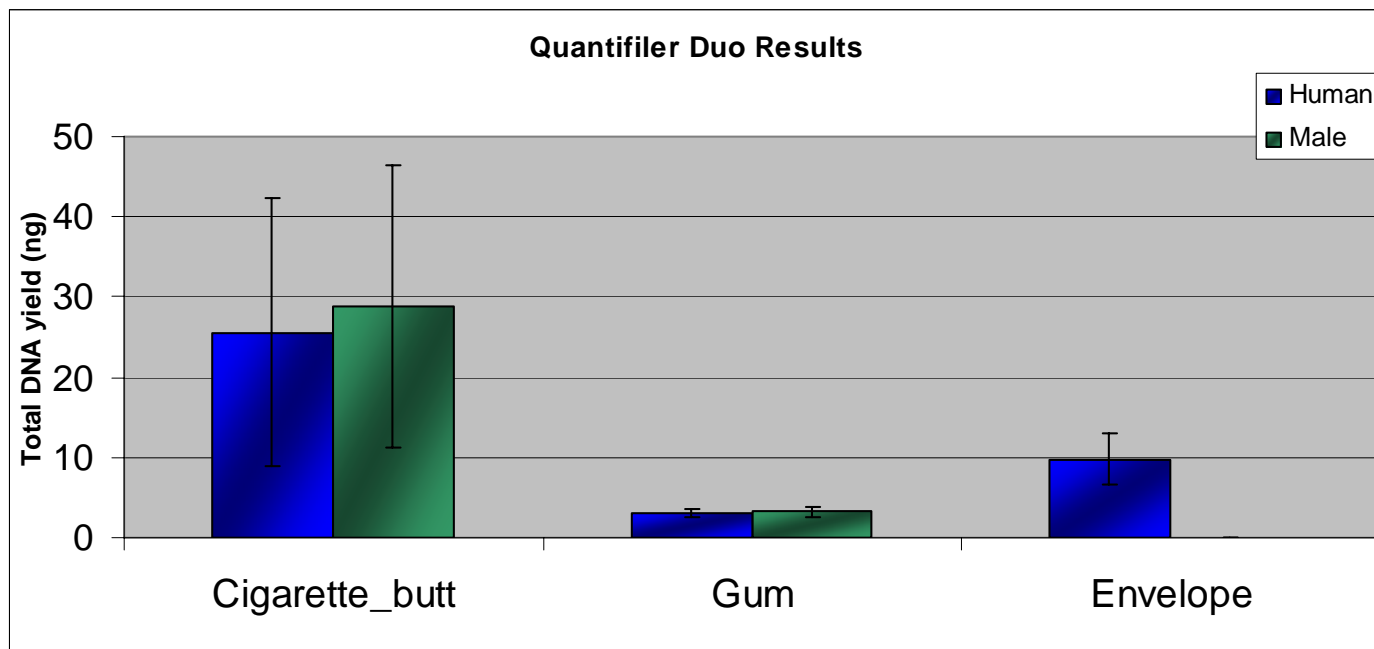
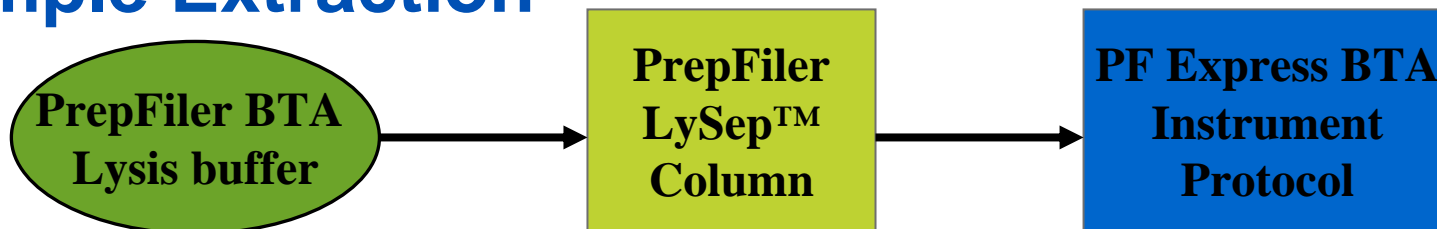
Identifiler® STR Kit Profiles



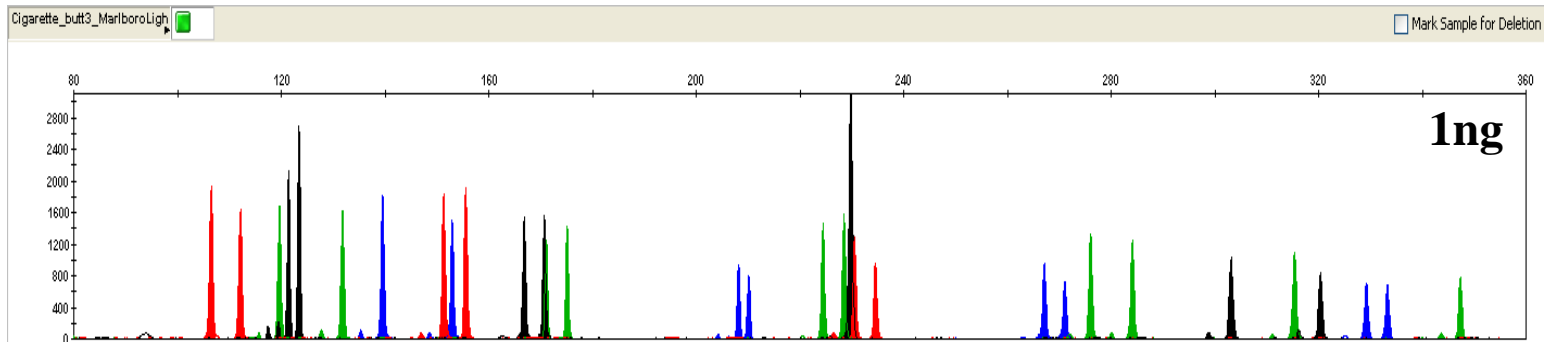
50mg bone

10mg tooth

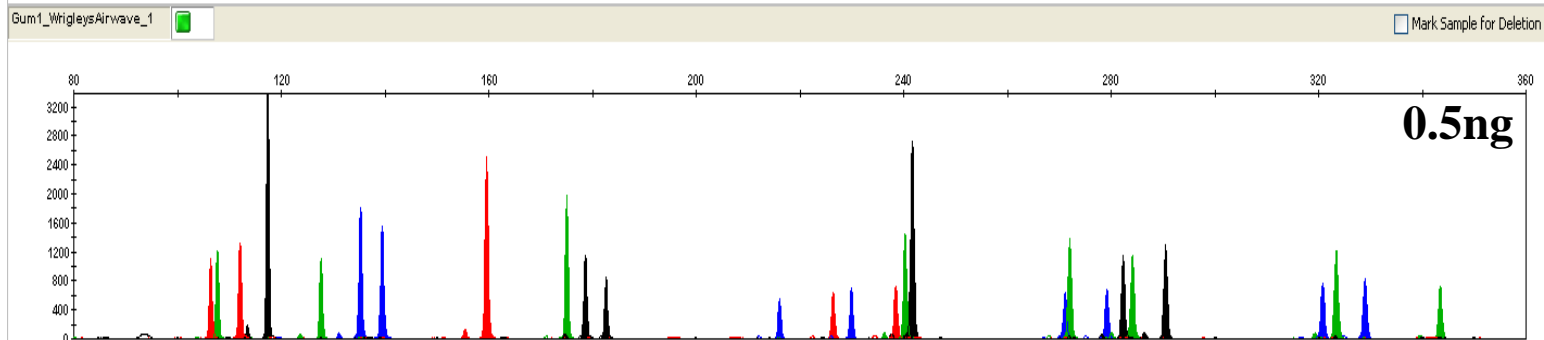
Protocol 3: PrepFiler Express BTA™ Adhesive Sample Extraction



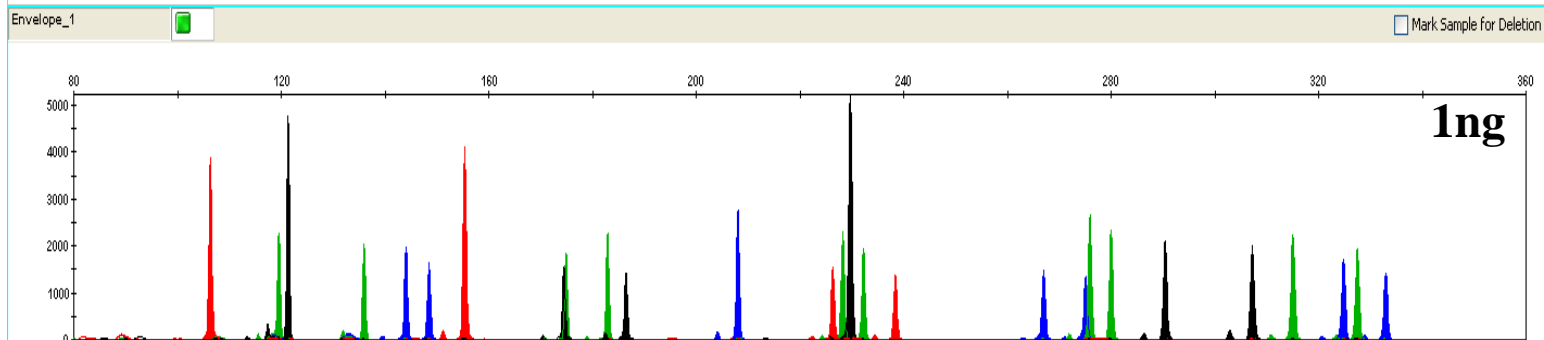
Identifiler® STR Kit Profiles



Cigarette butt

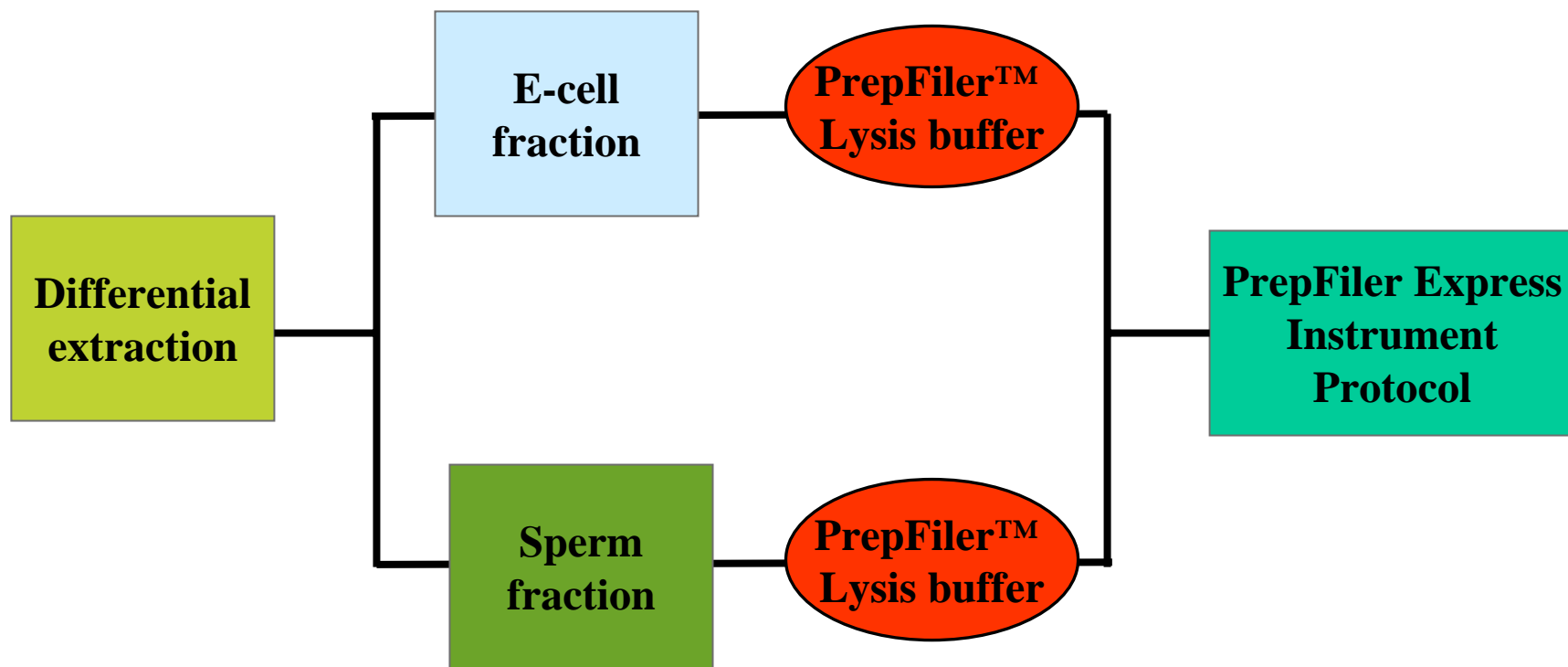


Chewed Gum



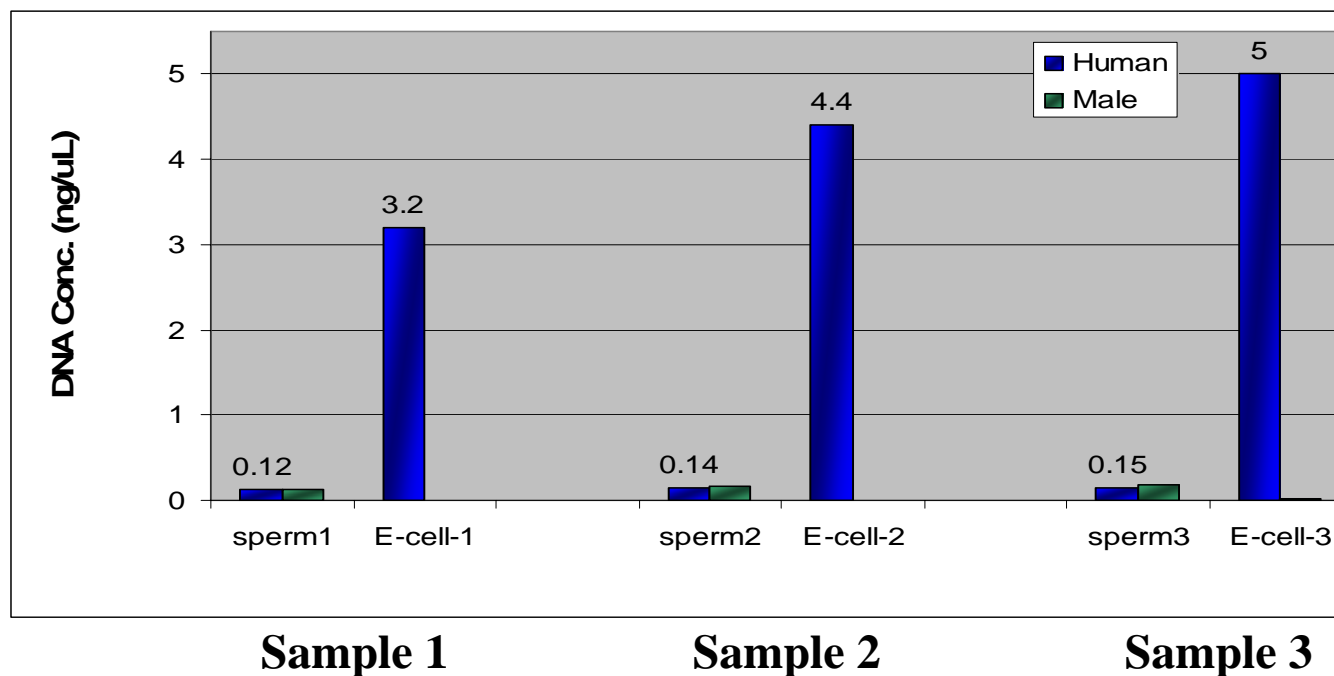
Envelope

PrepFiler Express™ Extraction Protocol for Processing of Sexual Assault Samples



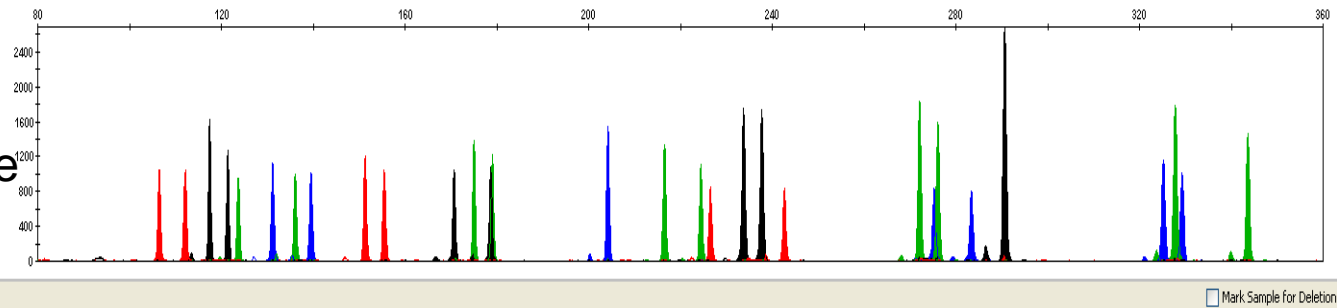
Mock sexual assault samples: E-cell/sperm ratio, 20:1

Quantification results

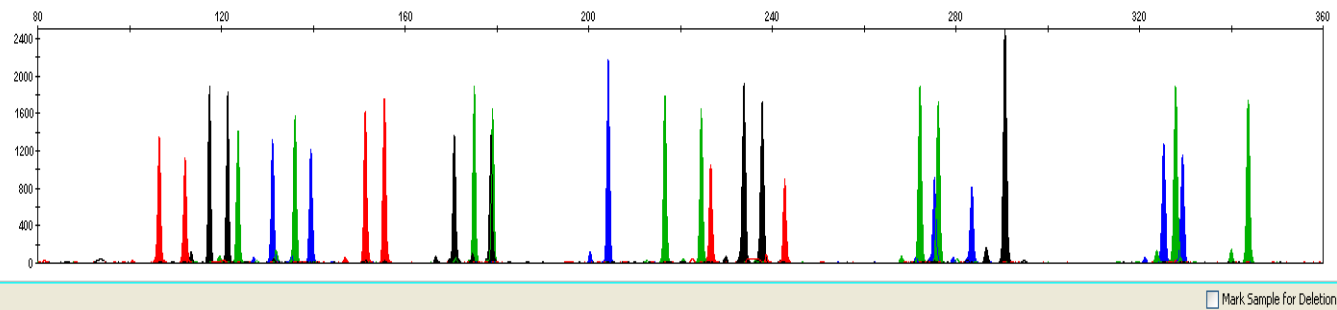


Identifiler® STR Kit Profiles

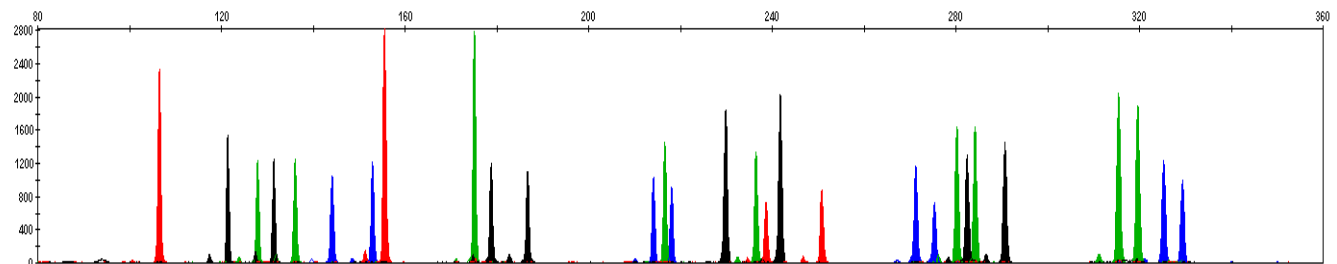
Sperm reference

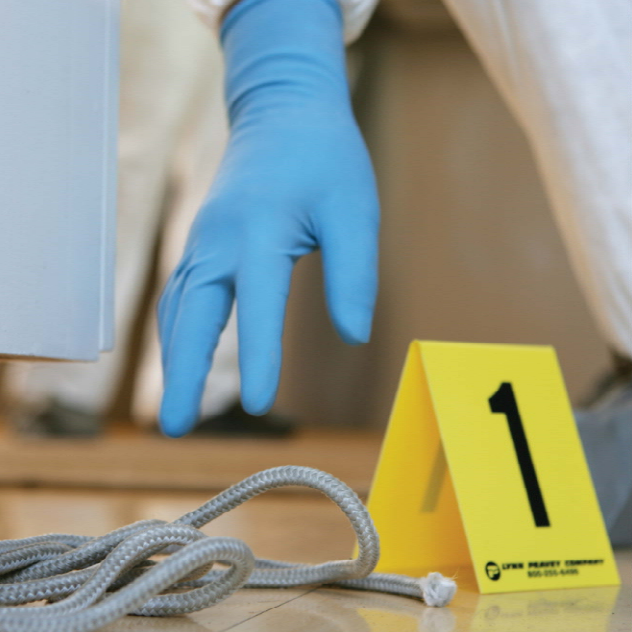


Sperm fraction



E-cell fraction





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Cross-Contamination, Sensitivity and Instrument Variation Studies

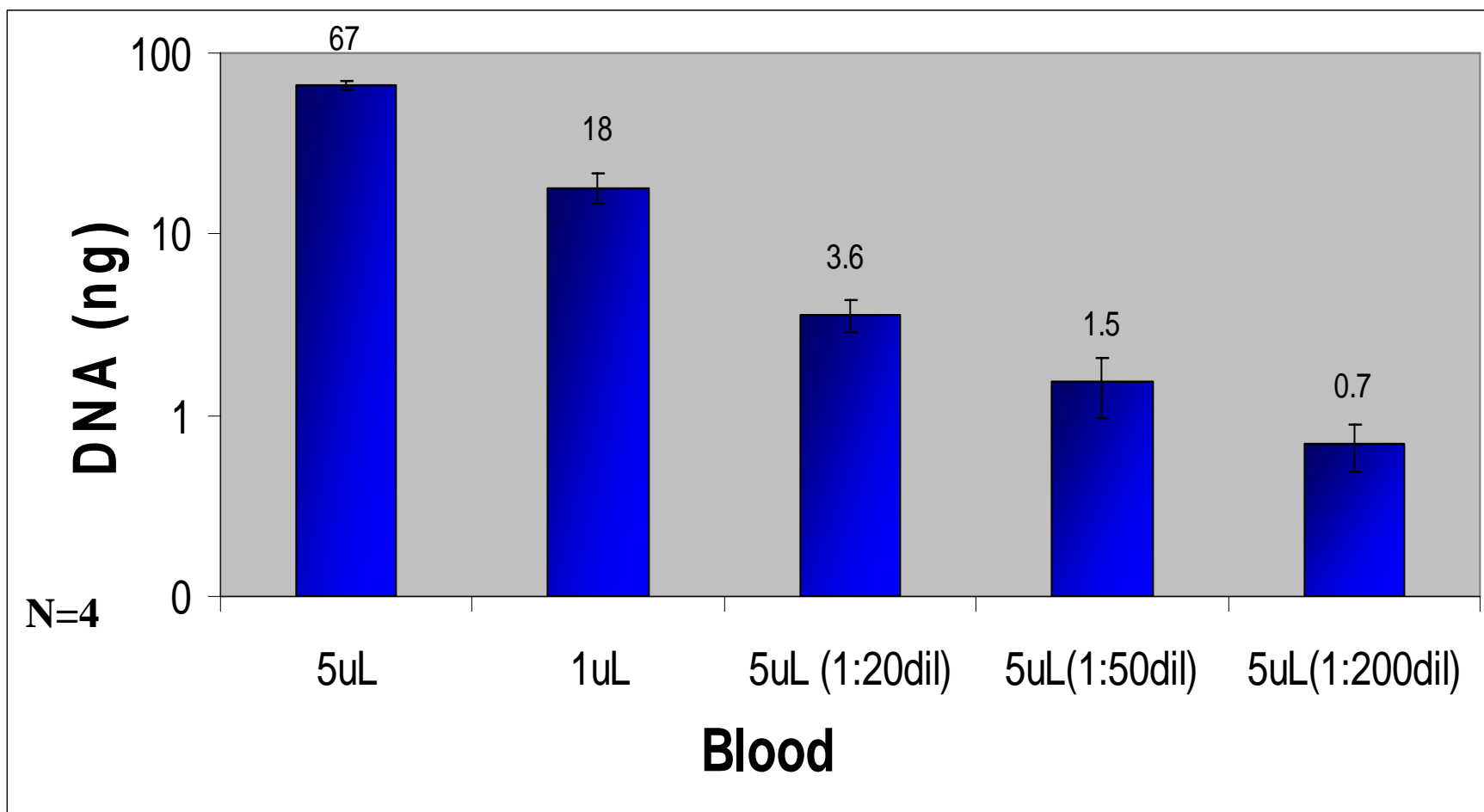
Cross-Contamination Study

10uL Blood
10uL 1xPBS

Cartridge rack 1		Cartridge rack 2		Cartridge rack 3	
1		1		1	
2		2		2	
3		3		3	
4		4		4	
5		5		5	
6		6		6	
7		7		7	
8		8		8	
9		9		9	
10		10		10	
11		11		11	
12		12		12	
13		13		13	

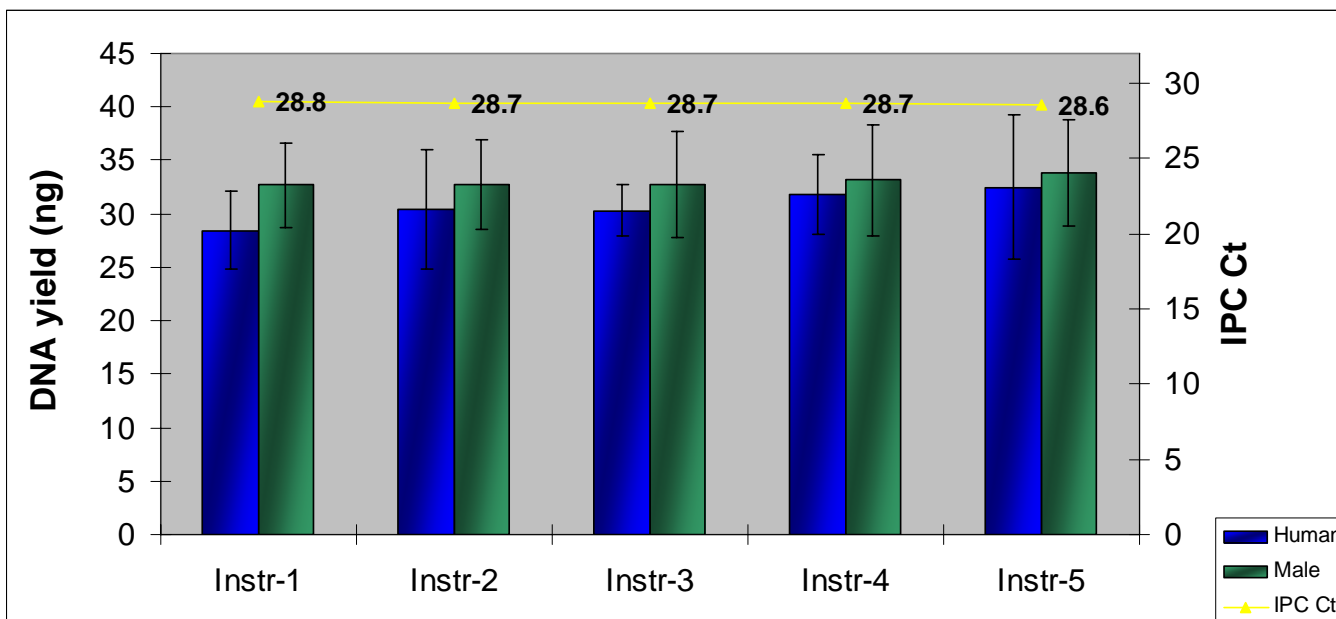
-AutoMate Express™ positions alternate with 10uL blood and 10uL 1xPBS
-MiniFiler profiles for all extraction blanks did not show cross-contamination

Sensitivity Study

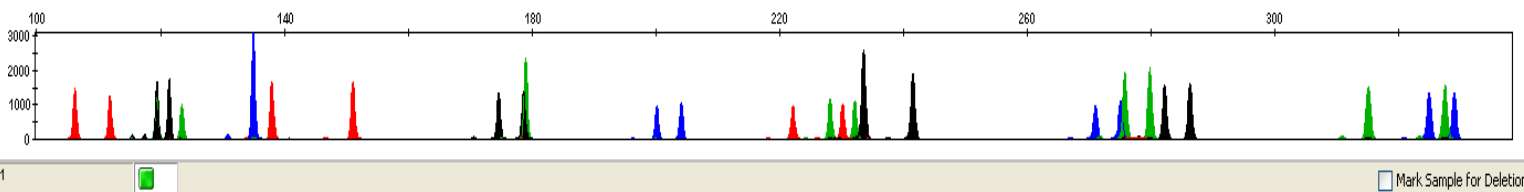


Instrument to Instrument Variation Study

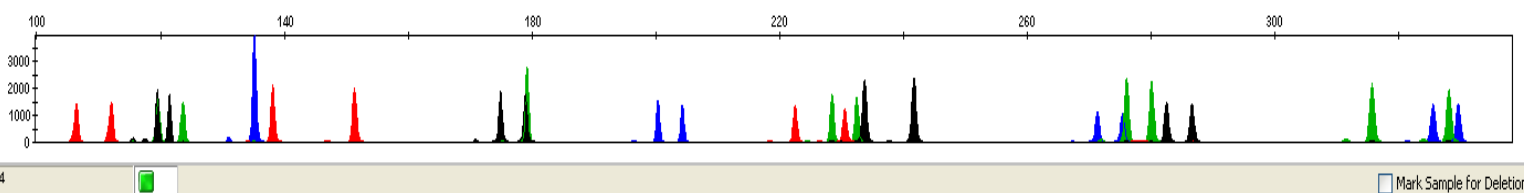
- 5 AutoMate Express™ instruments tested
- 2uL liquid blood processed in 12 replicates on each instrument using PrepFiler Express™ protocol
- Quantifiler® Duo and Identifiler® kits used for downstream analysis
- Consistent DNA yield obtained from each instrument



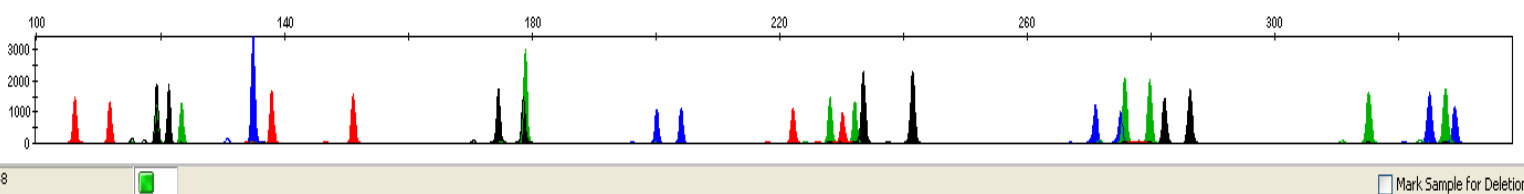
Representative STR Profiles



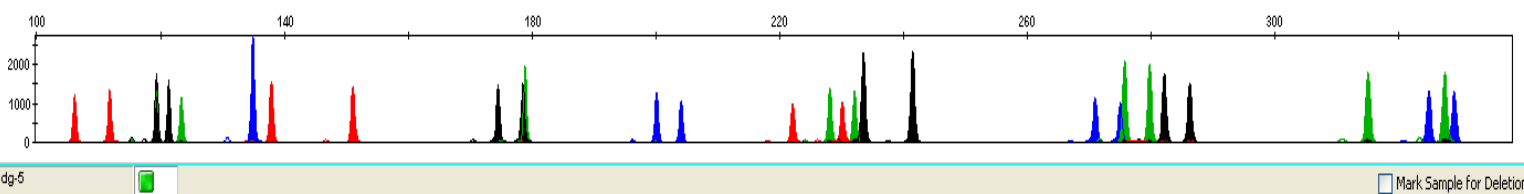
Instr-1



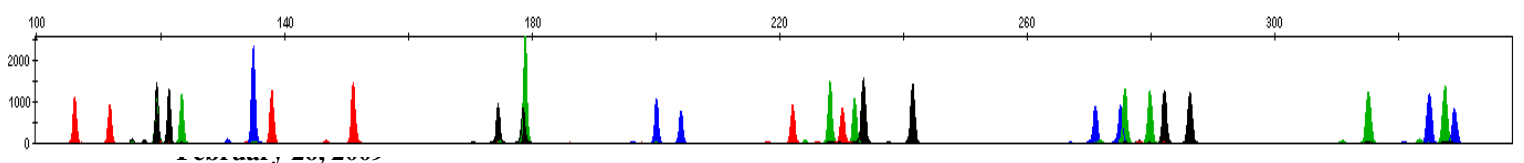
Instr-2



Instr-3



Instr-4



Instr-5

Conclusions:

- AutoMate Express™ benchtop system developed for extraction of DNA from forensic samples
 - 13 samples can be processed simultaneously in about 30 minutes
 - PrepFiler LySep™ column greatly simplifies lysis workflow and reduces risk for contamination
 - Minimal instrument to instrument variability
- PrepFiler Express™ chemistry capable of extracting DNA free of PCR inhibitors from samples containing low quantities of biological material
- Dedicated PrepFiler Express BTA™ lysis buffer for extracting DNA from challenging samples like bones, teeth, and adhesives.
- High quality STR profiles are generated from a broad range of forensic samples

Acknowledgements

Lori Hennessy

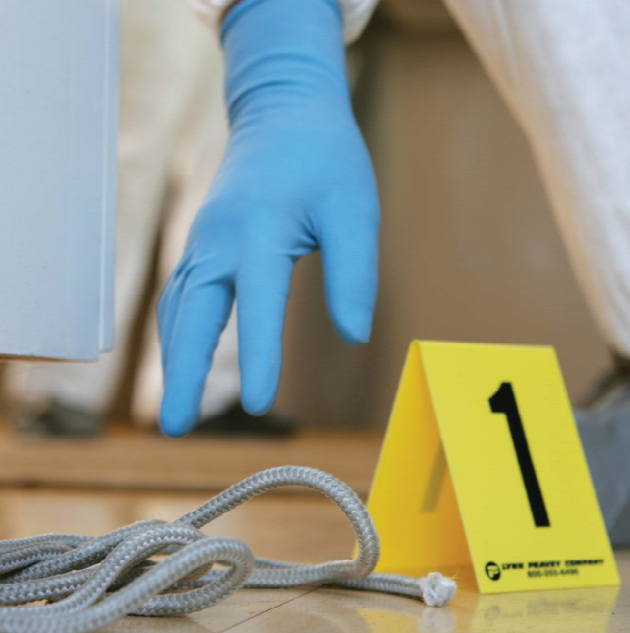
Lisa Calandro

Chang Zhong

Robert Lagacé

Clarissa Kao

Thomas McElroy



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