Rapid DNA Testing Approaches for Reference Samples

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National Institute of Standards and Technology

Mid-Atlantic Association of Forensic Scientists
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Ellicott City, MD

NIST Disclaimer

Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

Points of view are mine and do not necessarily represent the official position of the National Institute of Standards and Technology.

Outline

- Applications of Human Identity Testing
- Current DNA typing process
  - Time and limiting factors
- Benefits of rapid DNA typing
- Current rapid advancements
- How fast am I?
  - Using current laboratory equipment and supplies to type **reference samples** (buccal)

Applications of Human Identity Testing

- Forensic cases -- **matching suspect with evidence**
- Paternity testing -- **identifying father**
- Missing persons investigations
- Military DNA “dog tag”
- Convicted felon DNA databases
- Mass disasters -- **putting pieces back together**
- Historical investigations and genetic genealogy

Involves generation of DNA profiles usually with the same core STR (short tandem repeat) markers and then MATCHING TO A KNOWN SAMPLE

Steps in Forensic DNA Analysis

Steps Involved

- Collection
- Specimen Storage
- **Extraction**
- **Quantitation**
- **Multiplex PCR**
- **STR Typing**
- **Interpretation of Results**
- **Database**
  Storage & Searching
- **Calculation of Match Probability**

Usually 1-2 day process (a minimum of ~8 hours)

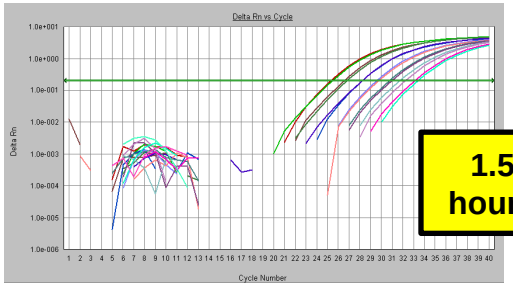
Blood Stain

Buccal swab

Sample Collection & Storage

1.5 hours

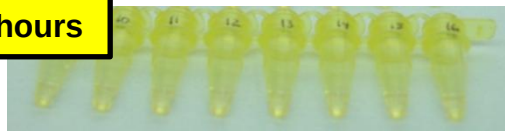
DNA Extraction



1.5 hours

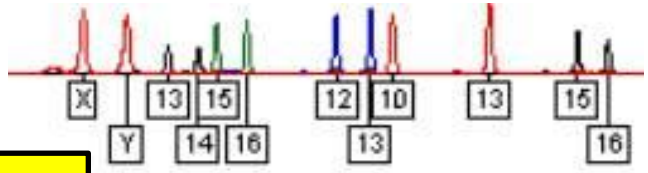
DNA Quantitation

3.5 hours



Multiplex PCR Amplification

DNA separation and sizing



1.5 hours

STR Typing

Interpretation of Results

Statistics Calculated

DNA Database search
Paternity test
Reference sample

Applied Use of Information

Applications of Rapid DNA Typing

- Goal of obtaining a STR profile in less than 2 hours from collection
  - **Single-source reference samples**
- Fully integrated devices in development
- Decrease overall DNA typing times within a laboratory setting
  - Involves non-integrated laboratory equipment and techniques
- Time requirements for forensic DNA typing are being significantly reduced

Interest for Rapid DNA Typing

- Several agencies have an interest in rapid DNA typing technologies
  - **DoJ**: law enforcement, initial information (investigative leads), booking stations
  - **DoD**: field testing, rapid intelligence, mass fatalities
  - **DHS**: kinship determination, border security, immigration
  - **Industry**: security, authentication

Rapid DNA Typing

Fully Integrated Technology

- Portable rapid DNA typing device
- Sample in – Answer out
- Extraction to STR profile within one system
- No user interaction or manual liquid handling
- No need for expertise training
- Several current companies working on this technology

Non-Integrated Technology

- Employs traditional bench top science
- Current equipment and personnel are in place
- Modifications to current protocols and techniques to reduce time
- Several liquid handling steps
- Requires the use of multiple instrument platforms
- Requires expertise training

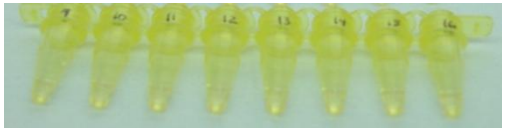
Rapid Advancements in Forensic DNA Typing

Steps Involved

- Collection
- Specimen Storage
- **Extraction**
- Quantitation
- **Multiplex PCR**
- **STR Typing**
- **Interpretation of Results**

DNA Extraction

Robotics
Liquid Extraction
Bypass Extraction



Multiplex PCR Amplification

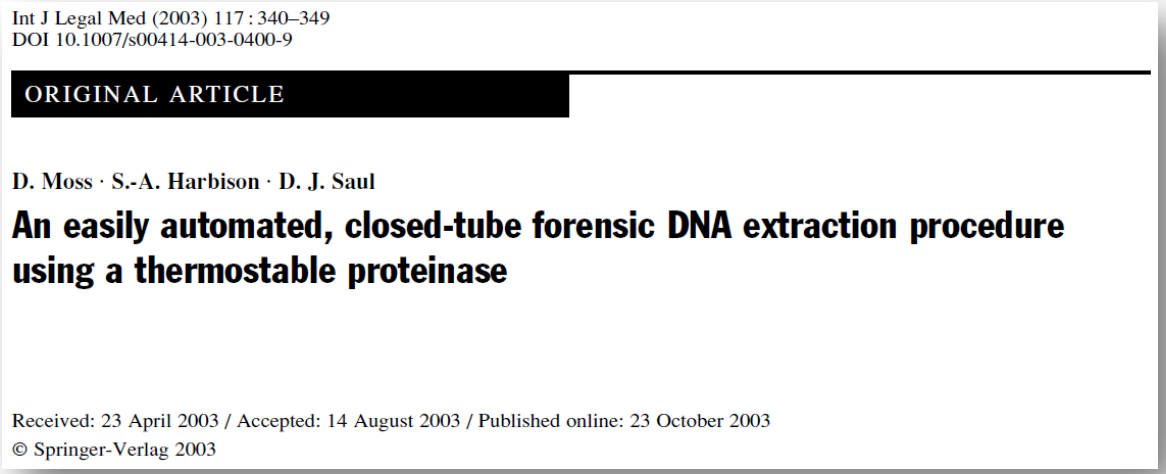
Direct PCR
Alternative Enzymes
Rapid PCR Protocols
Thermal Cyclers

3500 Series CE

Expert Allele Calling Systems

Rapid Advancement: Extraction

- Liquid-Based Extraction
- Thermostable proteinase controlled by a temperature shift regime
  - DNA is released while contaminating nucleases are inactive
- Typically yields between 0.5-2.0 ng/µL



Int J Legal Med (2003) 117 : 340–349
DOI 10.1007/s00414-003-0400-9

ORIGINAL ARTICLE

D. Moss · S.-A. Harbison · D. J. Saul

An easily automated, closed-tube forensic DNA extraction procedure using a thermostable proteinase

Received: 23 April 2003 / Accepted: 14 August 2003 / Published online: 23 October 2003

Rapid Advancement: Amplification

- Alternate DNA Polymerases
  - Faster and more robust
  - Ability to overcome PCR inhibitions
  - 2-5x faster processivity than Taq Gold
  - 1-5 minute hot start
  - Limited post cycling soak
- Direct PCR
  - Bypasses extraction and quantitation
  - Sample (blood or buccal punch) directly into PCR master mix for amplification
    - 1.5 hours (PowerPlex 18D) to 2.5 hours (Identifiler Direct) for amplification

Rapid PCR Protocols

Rapid PCR with alternate polymerases

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Short communication

Demonstration of rapid multiplex PCR amplification involving 16 genetic loci☆

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Optimization and validation of a fast amplification protocol for AmpFlSTR® Profiler Plus® for rapid forensic human identification

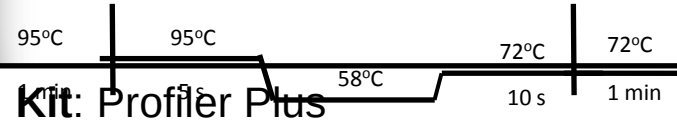
J Forensic Sci, November 2009, Vol. 54, No. 6
doi: 10.1111/j.1556-4029.2009.01200.x
Available online at: interscience.wiley.com

TECHNICAL NOTE

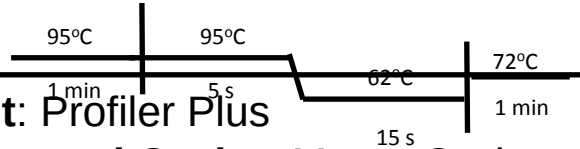
Heidi Giese,[1] Ph.D.; Roger Lam,[1] M.Sc.; Richard Selden,[1] M.D., Ph.D.; and Eugene Tan,[1] Ph.D.

Fast Multiplexed Polymerase Chain Reaction for Conventional and Microfluidic Short Tandem Repeat Analysis

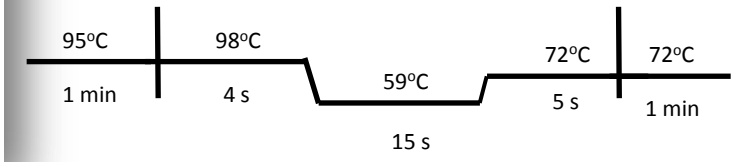
Kit: Identifiler
Thermal Cycler: 9700
Enzyme(s): PyroStart & SpeedSTAR
Time: 36 min



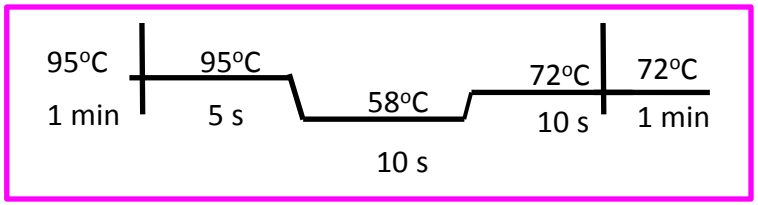
Kit: Profiler Plus
Thermal Cycler: PTC-200
Enzyme(s): SpeedSTAR
Time: 26 min



Kit: Profiler Plus
Thermal Cycler: MasterCycler
Enzyme(s): SpeedSTAR
Time: 19 min



Rapid PCR Protocols: Thermal Cyclers



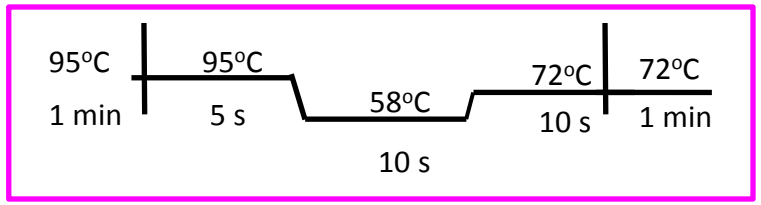
GeneAmp 9700 (Applied Biosystems)

Heating rate: 4°C/s
Heating mechanism: Peltier block (Al)
Tube format: 0.2 mL
96 reactions per instrument
28 cycles = 36 min

Mastercycler Pro (Eppendorf)

Heating rate: 6°C/s
Heating mechanism: Peltier block (Ag)
Tube format: 0.2 mL
96 reactions per instrument
28 cycles = 19 min

Rapid PCR Protocols: Thermal Cyclers



SmartCycler (Cepheid)

Heating rate: 10°C/s
Heating mechanism: heating plates and air circulating fan
Tube format: proprietary 25 μL tubes
16 reactions per instrument
28 cycles = 20 min

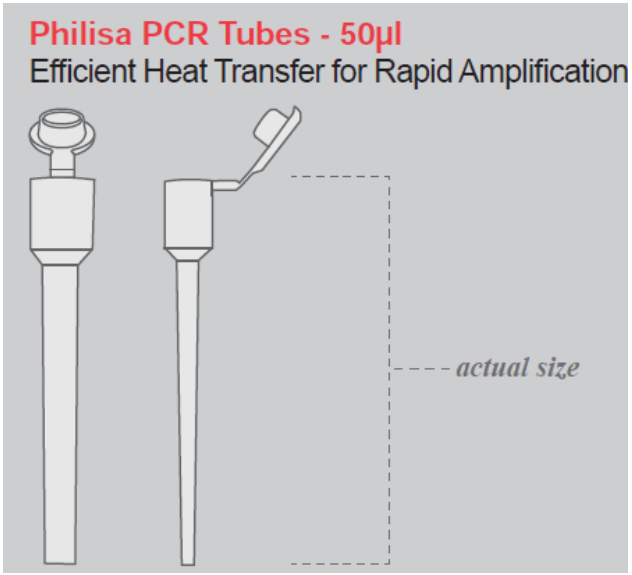
Rotor-Gene Q (Qiagen)

Heating rate: 15°C/s
Heating mechanism: Air chamber (spinning rotor)
Tube format: 0.1 mL
72 tube reactions per instrument
28 cycles = 36 min

Rapid Advancement: Thermal Cyclers

Streck Philisa

Heating rate: 15°C/s
Heating mechanism: Peltier block (Al)
Tube format: proprietary 50 µL tubes
8 reactions per instrument
28 cycles = 14 min



Require the use of gel loading tips to load PCR product into CE setup plate due to tube design

Comparative Throughput (Cycling)

Cycler	# Samples	Fastest Cycling Time (min)	Runs to Complete 96 Samples	Cycling time for 96 Samples (min)
9700	96	36	1	36
Smart Cycler	16	20	6	120
MasterCycler	96	16	1	16
Rotor-Gene	72	36	2	72
Phisila	8	14	12	168

While cycling times may be rapid, the throughput in some cases is reduced from the standard 96-well format

Separation and Detection

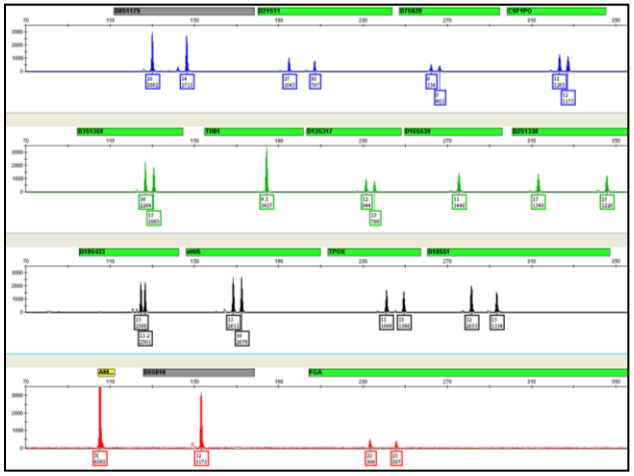
31xx Series

- 4 capillaries
- 16 capillaries
- 48 minutes per run
- GeneMapper ID 3.2

3500 Series

- 8 capillaries
- 24 capillaries
- 36 minutes per run
- Requires GeneMapper IDX v1.2

How Fast Am I?



Experimental Design

- Timed testing from sample collection to finalizing data processing
- 8 Buccal swabs evaluated for all testing
  - Rapid sampling and non-invasive
  - 3500 is an 8-capillary
  - Phisila amplifies 8 samples at a time
- Liquid Extraction protocol tested
  - Zygem Saliva Kit
- Direct PCR tested
  - PowerPlex 18D
- Two thermal cyclers evaluated
  - Applied Biosystems 9700
  - Streck Phisila

ZyGEM Liquid-Based Extraction

Saliva Kit

- Buccal swab washed with 500 µL DNA-free H_20
- 20 µL solution added to reaction
- 75 C Incubation
- 95 C Incubation
- 2 µL solution added to PCR reaction

PCR Setup

Rapid Identifiler

- 2 µL ZyGEM extraction solution
- 2 µL Identifiler Primers
- 5 µL Takara Perfect Real Time Mix
- 0.25 µL Takara Polymerase
- 0.75 µL Water

Amplification on the ABI 9700 and Streck Phisila

PowerPlex 18D

- One 1.2 mm punch from a Whatman Easicollect
- 5 µL PowerPlex 18D Primers
- 5 µL PowerPlex 18D Master Mix
- 15 µL Water

Amplification on the ABI 9700

Separation and Detection Setup

- 1 µL of each amplified product was diluted in 8.5 µL HiDi, 0.5 µL GeneScan LIZ 600 v2.0 (Identifiler)
- 1 µL of each amplified product was diluted in 10 µL HiDi, 0.5 µL CC5 ILS 500 (PowerPlex 18D)

- Separated on ABI 3500 Genetic Analyzer
- 8 capillary 36 cm array with POP-4
- Injected at 1.2 kV for 7 seconds
- GeneMapper ID-X v1.2

How Fast Am I: ZyGEM 9700?

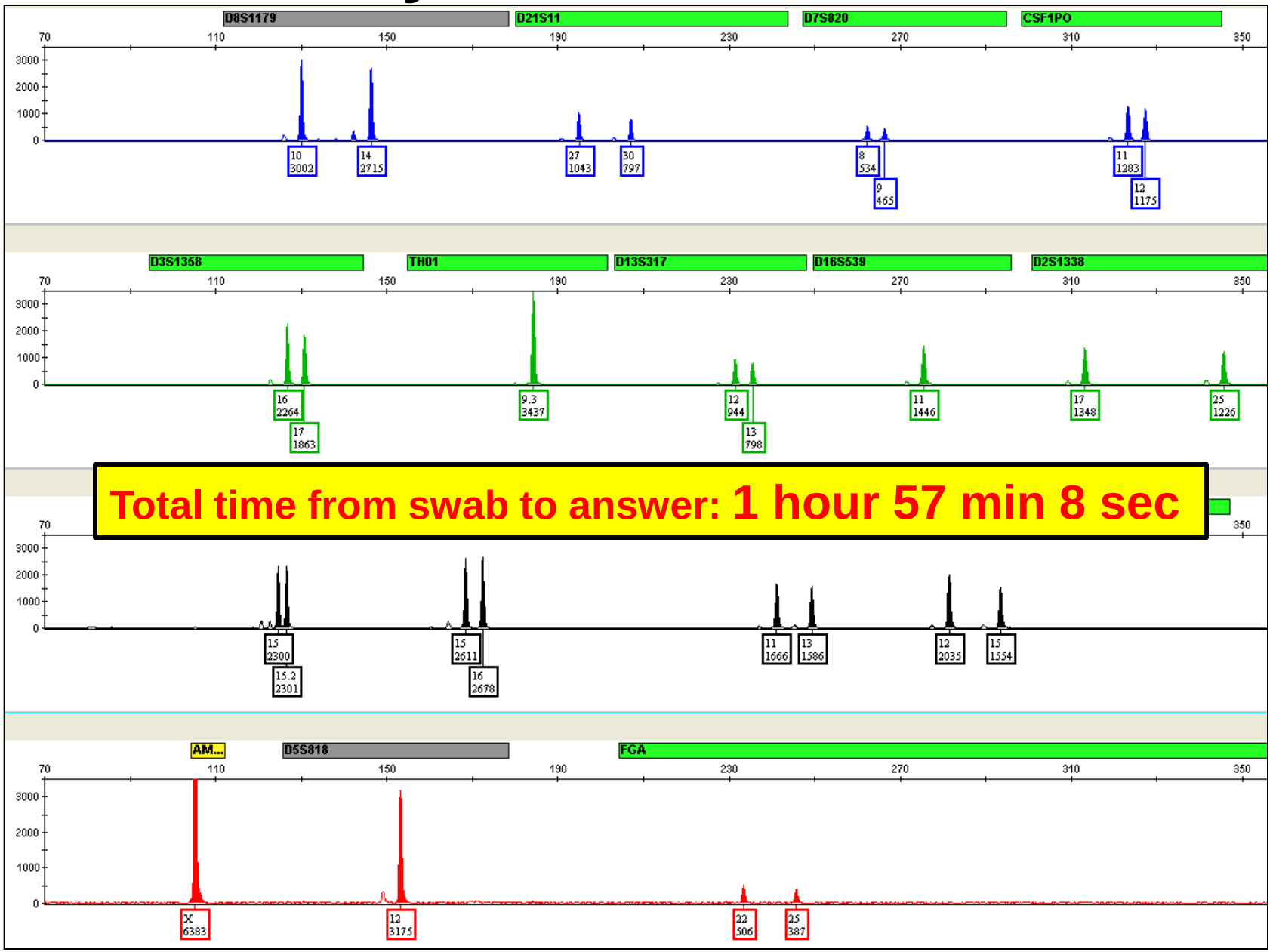
Extraction Setup	6 min
Extraction	20 min
PCR Setup	7 min 18 sec
PCR	36 min
3500 Setup	4 min 50 sec
3500 Run	38 min
Data Analysis	5 min

Final: 1 hour 57 min 8 sec

FOR 8 SAMPLES

This protocol allowed for the use of multi-channel and automatic pipettes for setup of extraction and PCR

ZyGEM & 9700


D8S1179
D21S11
D7S820
CSF1PO
D3S1358
TH01
D13S317
D16S539
D2S1338
AM...
D5S818
FGA
Total time from swab to answer: 1 hour 57 min 8 sec

How Fast Am I: ZyGEM & Phisila?

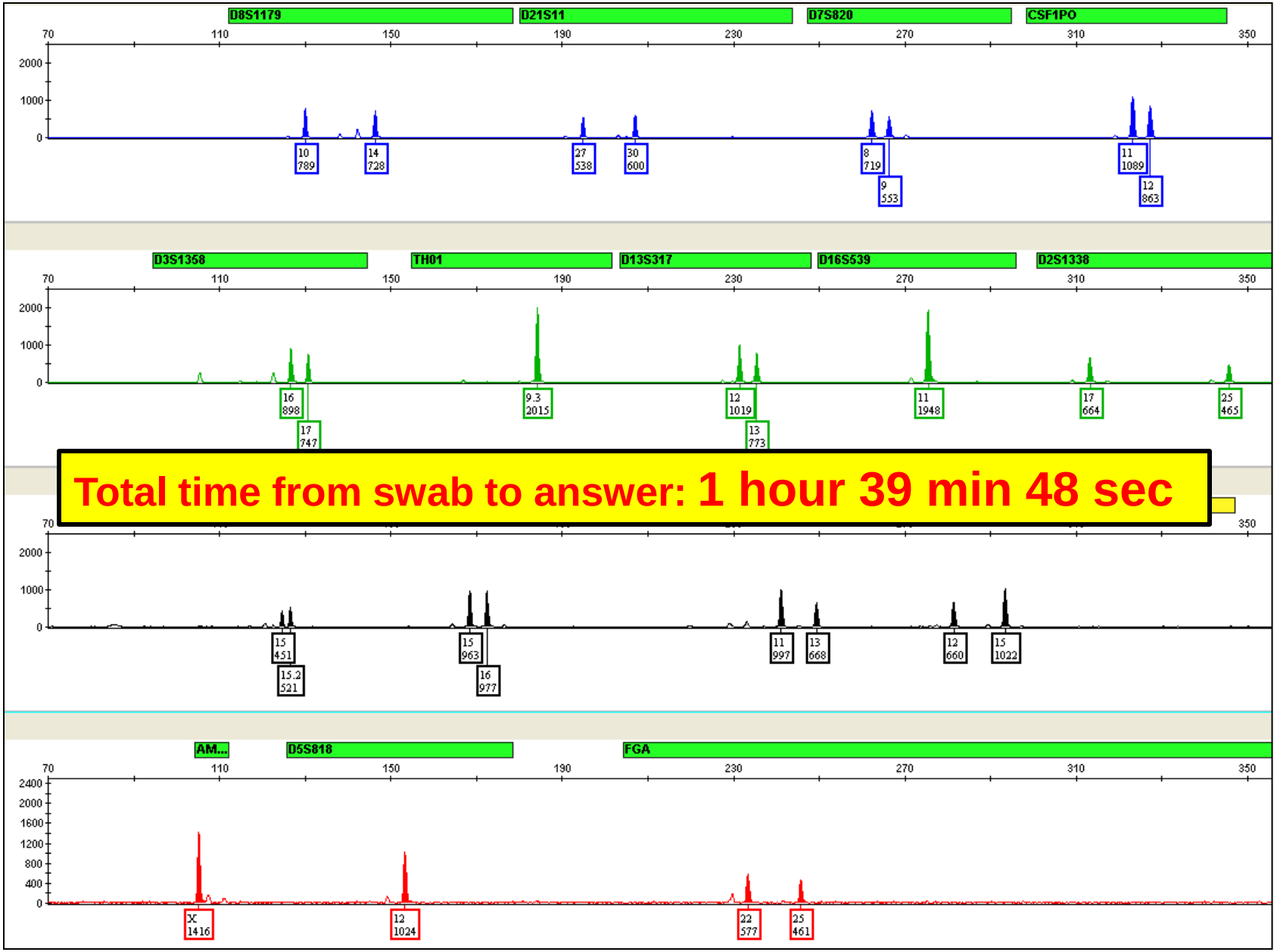
Extraction Setup	6 min
Extraction	20 min
PCR Setup	6 min 48 sec
PCR	14 min
3500 Setup	10 min
3500 Run	38 min
Data Analysis	5 min

Final: 1 hour 39 min 48 sec

FOR 8 SAMPLES

Time consuming part:
transfer of PCR product into 96-well plate for CE due to the use of gel loading tips and individual transfer

ZyGEM & Phisila

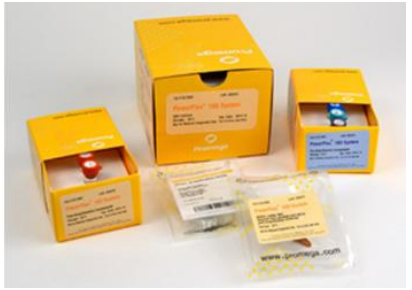


How Fast Am I: PP18D & 9700?

Extraction Setup	NONE
Extraction	NONE
PCR Setup	3 min 5 sec
PCR	1 hour 25 min
3500 Setup	4 min 50 sec
3500 Run	38 min
Data Analysis	5 min

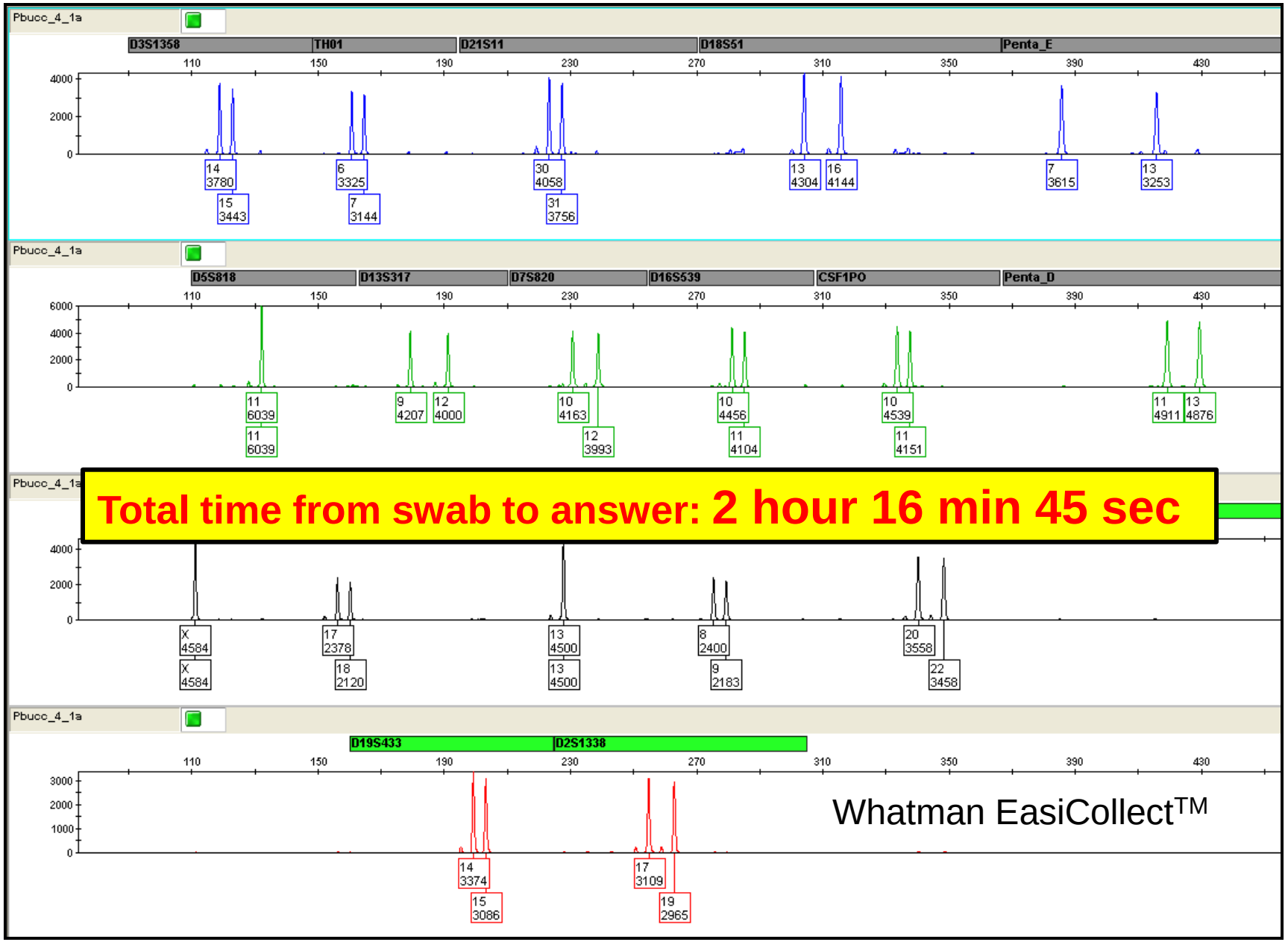
Final: 2 hour 16 min 45 sec

FOR 8 SAMPLES



This protocol allowed for the use of multi-channel and automatic pipettes for setup of extraction and PCR

PP18D & 9700


Pbucc_4_1a
D3S1358
TH01
D21S11
D18S51
Penta_E
14 3780
15 3443
6 3325
7 3144
30 4058
31 3756
13 4304
16 4144
7 3615
13 3253
Pbucc_4_1a
D5S818
D13S317
D7S820
D16S539
CSF1PO
Penta_D
11 6039
11 6039
9 4207
12 4000
10 4163
12 3993
10 4456
11 4104
10 4539
11 4151
11 4911
13 4876
Pbucc_4_1a
Total time from swab to answer: 2 hour 16 min 45 sec
X 4584
X 4584
17 2378
18 2120
13 4500
13 4500
8 2400
9 2183
20 3558
22 3458
Pbucc_4_1a
D19S433
D2S1338
14 3374
15 3086
17 3109
19 2965
Whatman EasiCollect™

Decrease DNA Typing Time

- Eliminate standard extraction protocols
  - Direct PCR for elimination of extraction and quantitation (90 min PCR)
  - Liquid extraction as a alternative to robotic or manual extraction of buccal swabs (20 min extraction)
- Rapid PCR cycling conditions
  - Employ alternate polymerases and thermal cyclers (14-36 min PCR)
  - Throughput may vary for cyclers resulting in increased overall cycling times for standard 96-well CE setup (8-96 samples)

Conclusions

- Several areas exist to decrease the time for DNA typing
- Most common approach is to reduce thermal cycling parameters
- STR genotype results were generated in less than 2 hours
  - With **standard laboratory equipment** and protocols
  - Overall time includes: collection, sample handling, and liquid transfer steps

Future work

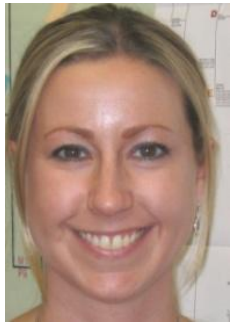
- Test additional forms of rapid extraction
  - Additional liquid extraction kits/enzymes
- Test additional thermal cyclers
  - Optimization of rapid PCR protocols
  - Development of additional rapid PCR protocols with additional STR typing kits
- Optimization of rapid direct protocols

Acknowledgments

NIST Team for This Work

Funding from the **FBI Biometrics Center of Excellence** 'Forensic DNA Typing as a Biometric Tool'

Pete Vallone



Becky Hill

Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards

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301-975-5107

SAVE THE DATE

Forensics@NIST 2012

Three day symposium on cutting edge forensic science research at NIST

Date: November 28-30th, 2012
Time: 9:00 am to 5:00 pm
Location: NIST (Gaithersburg, Maryland)

For more information:

www.nist.gov/oles/forensics-2012.cfm

Note: registration is required

FORENSIC SCIENCES