Rapid DNA Typing & PCR Protocols

Forensics @ NIST
December 3, 2014

Erica Butts
Applied Genetics Group
Biomolecular Measurement Division

National Institute of Standards and Technology
U.S. Department of Commerce

Disclaimer

- Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the U.S. Department of Commerce. 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 endorsement by NIST, nor does it imply that any of the materials, instruments, or equipment identified are necessarily the best available for the purpose.

Outline

- What is Rapid DNA?
- Rapid PCR Protocols
- How Fast am I?
- Rapid DNA Platforms and Testing

Rapid DNA Typing

Modifying Existing Protocols for DNA Typing

- Employs traditional bench top science
- Current equipment and personnel are in place
- Several liquid handling steps
- Requires the use of multiple instrument platforms
- Requires expert training

Fully Integrated Technology "R-DNA Instruments"

- Portable rapid DNA typing device
- Sample in – Answer out < 2 H
- Extraction to STR profile within one system
- No user interaction or manual liquid handling
- **No need for expert training**

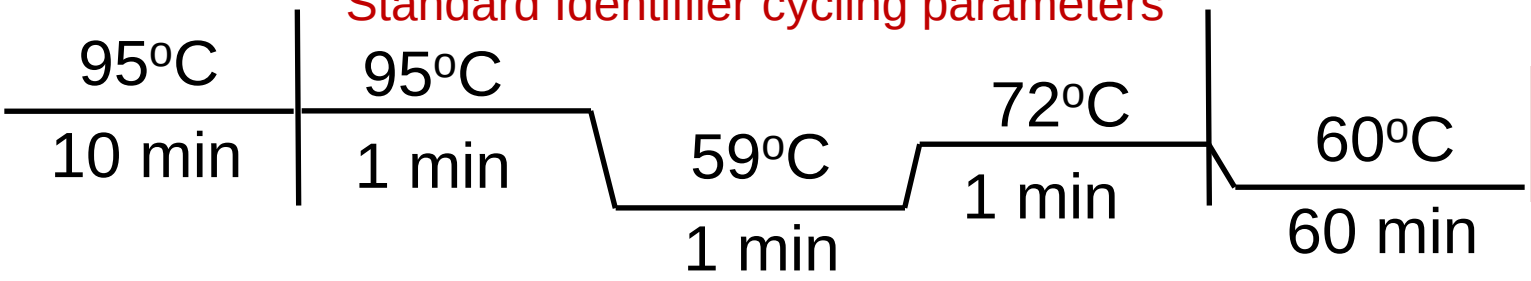
Ways to Decrease DNA Typing Time

Modifying Existing Protocols

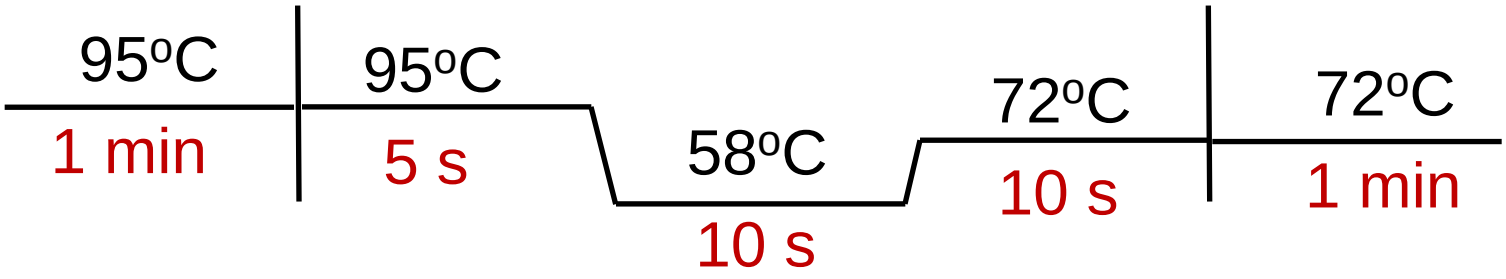
- Eliminate standard extraction protocols
  - Direct PCR for elimination of extraction and quantitation (40 min PCR)
  - Pretreatment techniques for appropriate substrates as a alternative to robotic or manual extraction (20 min incubation)
- Rapid PCR cycling conditions
  - Employ alternate polymerases 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)

Rapid Advancements: Thermal Cycling

Standard Identifiler cycling parameters



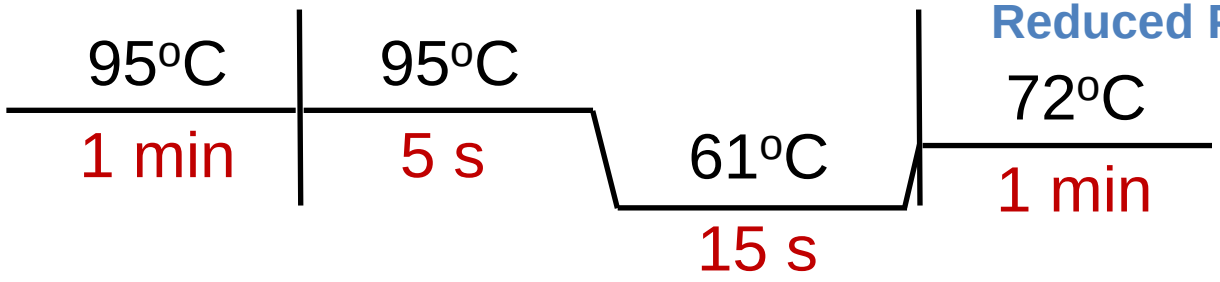
3-step
≈3 hours



3-step
36 min

Demonstration of rapid multiplex PCR amplification involving 16 genetic loci Vallone et al. Forensic Sci Int Genet. 2008 3:42-45

Reduced PCR artifacts



2-step
31 min

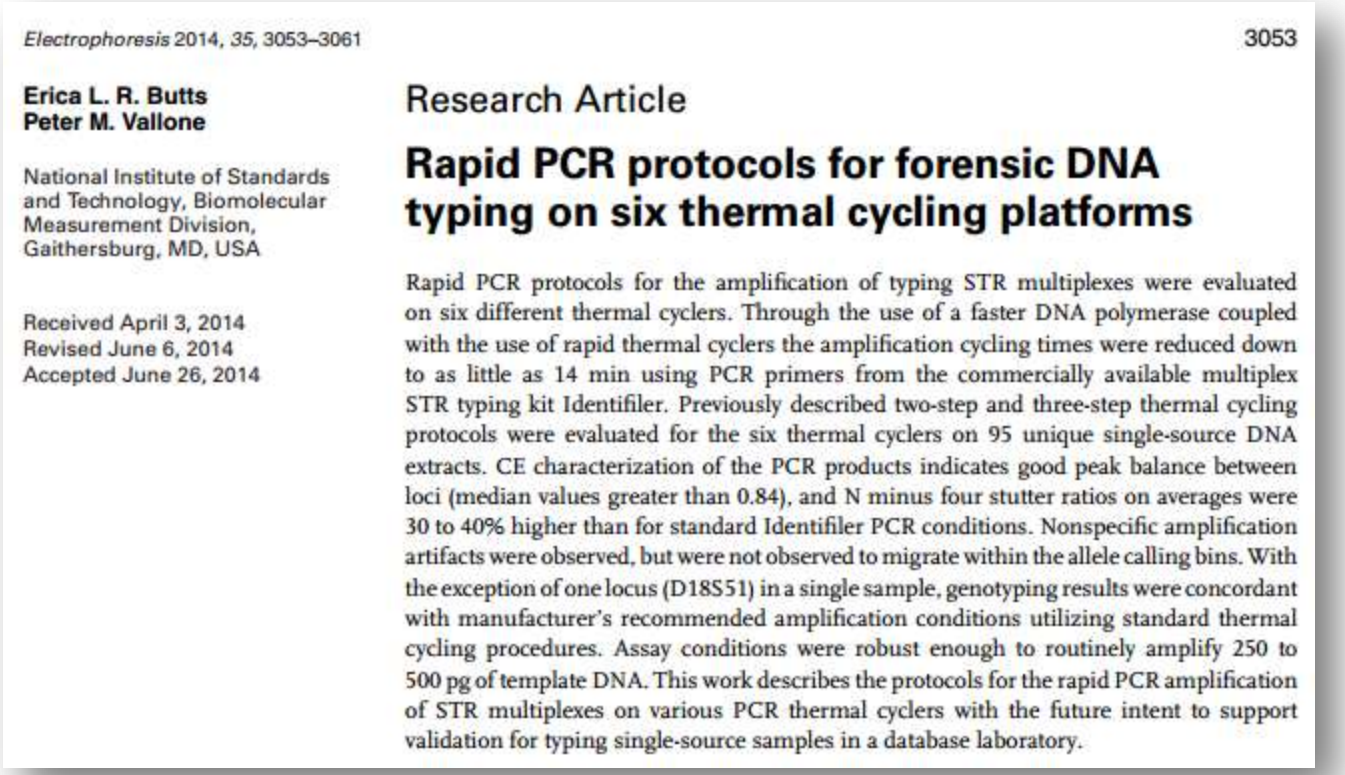
Development of a fast PCR protocol enabling rapid generation of AmpFℓSTR® Identifiler® profiles for genotyping of human DNA Foster and Laurin Investig Genet. (2012) 3:6

28 cycles on GeneAmp 9700

Rapid PCR Protocols

Evaluation of both 2-step and 3-step thermal cycling protocols

Six thermal cyclers evaluated for typing single-source reference samples



Electrophoresis 2014, *35*, 3053–3061

Erica L. R. Butts
Peter M. Vallone

National Institute of Standards and Technology, Biomolecular Measurement Division, Gaithersburg, MD, USA

Received April 3, 2014
Revised June 6, 2014
Accepted June 26, 2014

Research Article

Rapid PCR protocols for forensic DNA typing on six thermal cycling platforms

Rapid PCR protocols for the amplification of typing STR multiplexes were evaluated on six different thermal cyclers. Through the use of a faster DNA polymerase coupled with the use of rapid thermal cyclers the amplification cycling times were reduced down to as little as 14 min using PCR primers from the commercially available multiplex STR typing kit Identifiler. Previously described two-step and three-step thermal cycling protocols were evaluated for the six thermal cyclers on 95 unique single-source DNA extracts. CE characterization of the PCR products indicates good peak balance between loci (median values greater than 0.84), and N minus four stutter ratios on averages were 30 to 40% higher than for standard Identifiler PCR conditions. Nonspecific amplification artifacts were observed, but were not observed to migrate within the allele calling bins. With the exception of one locus (D18S51) in a single sample, genotyping results were concordant with manufacturer's recommended amplification conditions utilizing standard thermal cycling procedures. Assay conditions were robust enough to routinely amplify 250 to 500 pg of template DNA. This work describes the protocols for the rapid PCR amplification of STR multiplexes on various PCR thermal cyclers with the future intent to support validation for typing single-source samples in a database laboratory.

"Rapid PCR Protocols for Forensic DNA Typing on Six Thermal Cycling Platforms"

Erica L.R. Butts and Peter M. Vallone

Protocols for 6 Thermal Cyclers

Gene Amp 9700
31 min
96 samples

Life Technologies

Piko
26 min
96 samples

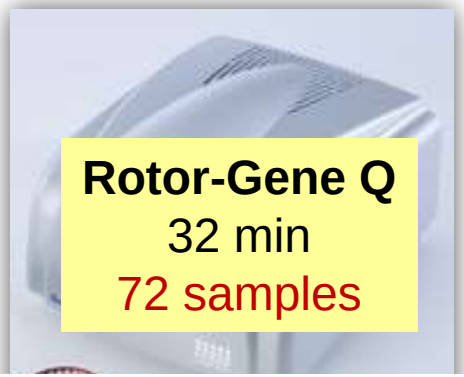
Thermo Scientific

SmartCycler
18 min
16 samples

Cycling times given for a 2-step 28 cycle protocol

Eppendorf

Mastercycler Pro
17 min
96 samples



Rotor-Gene Q
32 min
72 samples

Philisa
14 min
8 samples

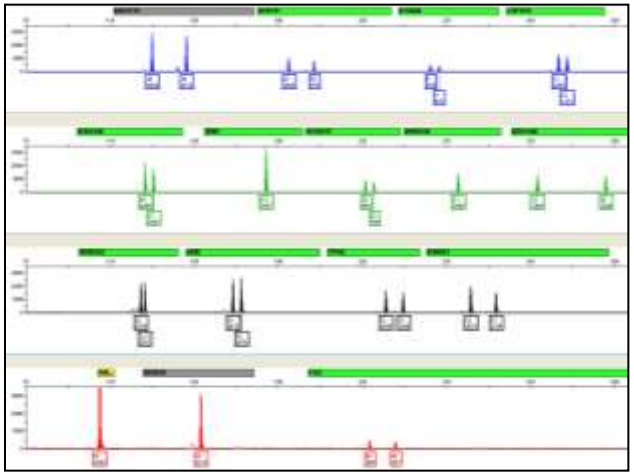
Streck

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

Decreasing DNA Typing Time:

How Fast Am I?

Modifying Existing Protocols



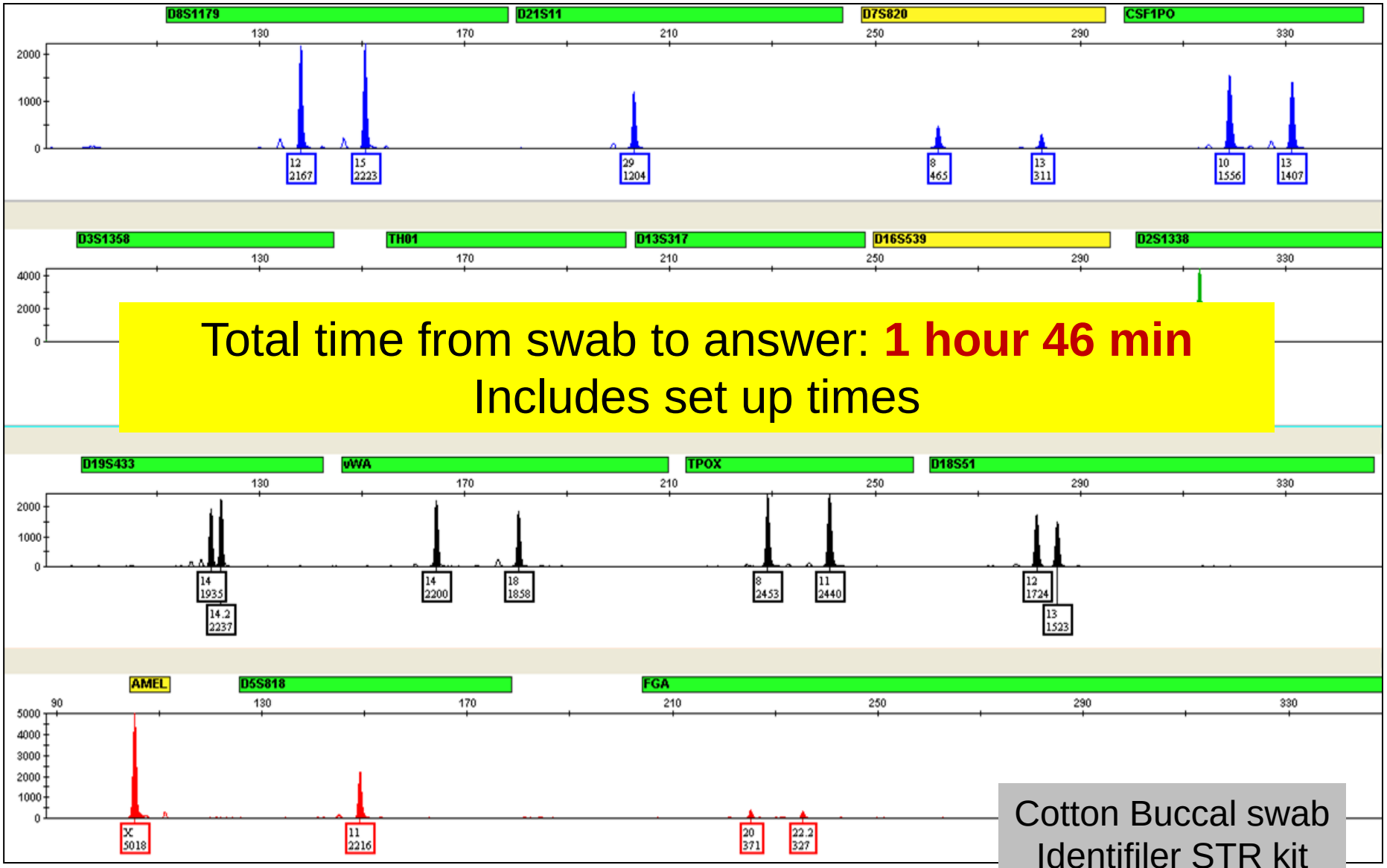
Experimental Design

Fast PCR Single Source Reference Samples	Steps Involved	DirectPCR Single Source Reference Samples
Prep-N-Go Pretreatment (Cotton Buccal)	**Collection**	**None** (1.2 mm Blood Punch)
	Extraction	
Rapid Identifiler Protocol (9700 and Philisa Cyclers)	**Quantitation**	**Globalfiler Experss** (9700)
	Multiplex PCR	
ABI 3500 Genetic Analyzer **GeneMapper IDX v1.2**	**STR Typing**	**ABI 3500 Genetic Analyzer** **GeneMapper IDX v1.2**
	Interpretation of Results	

8 unique samples for each method were typed in parallel

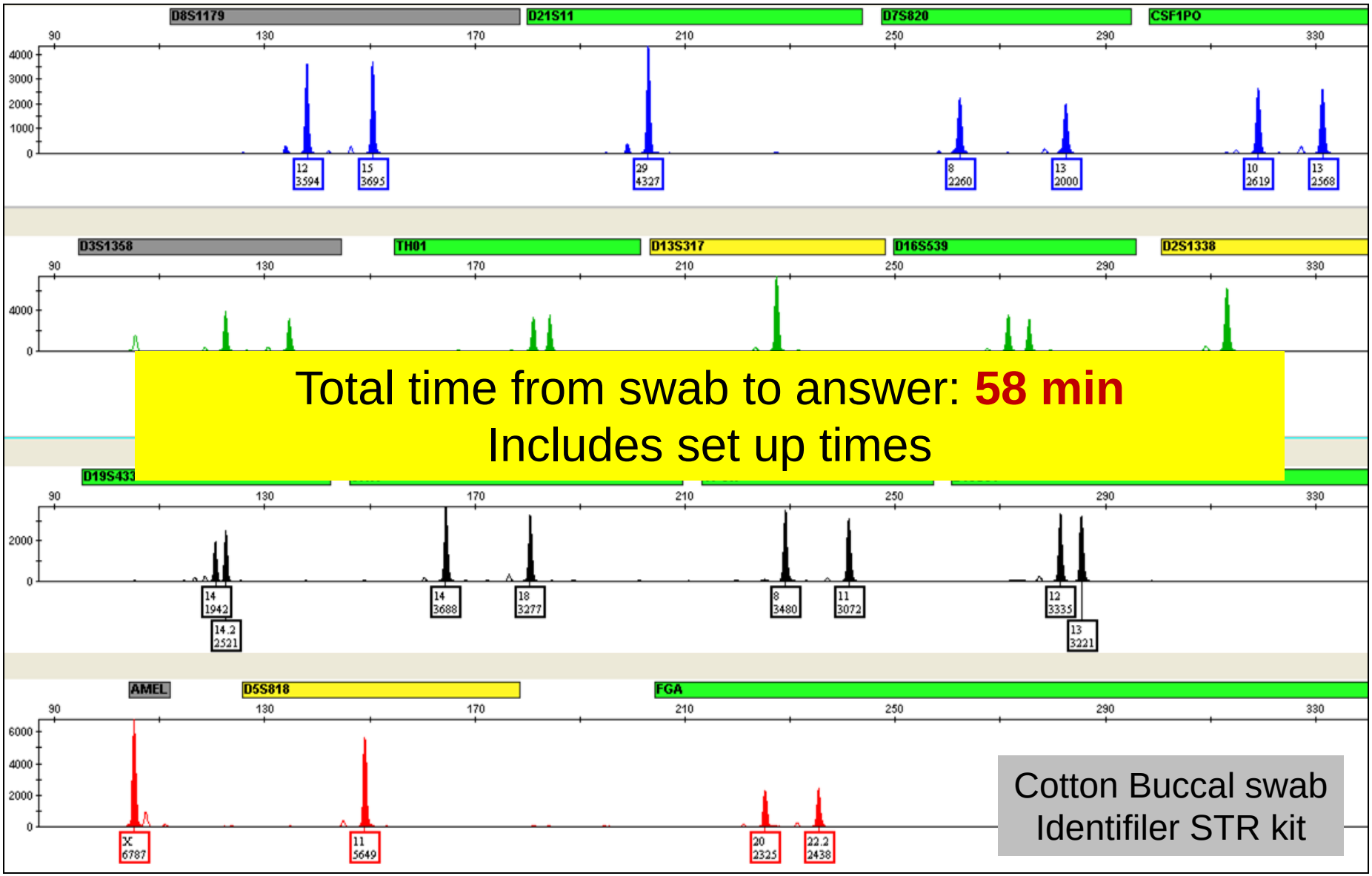
Testing was timed from collection through data interpretation of results, to include all sample transfer steps

Prep-N-Go → 9700 (2-step) → 3500

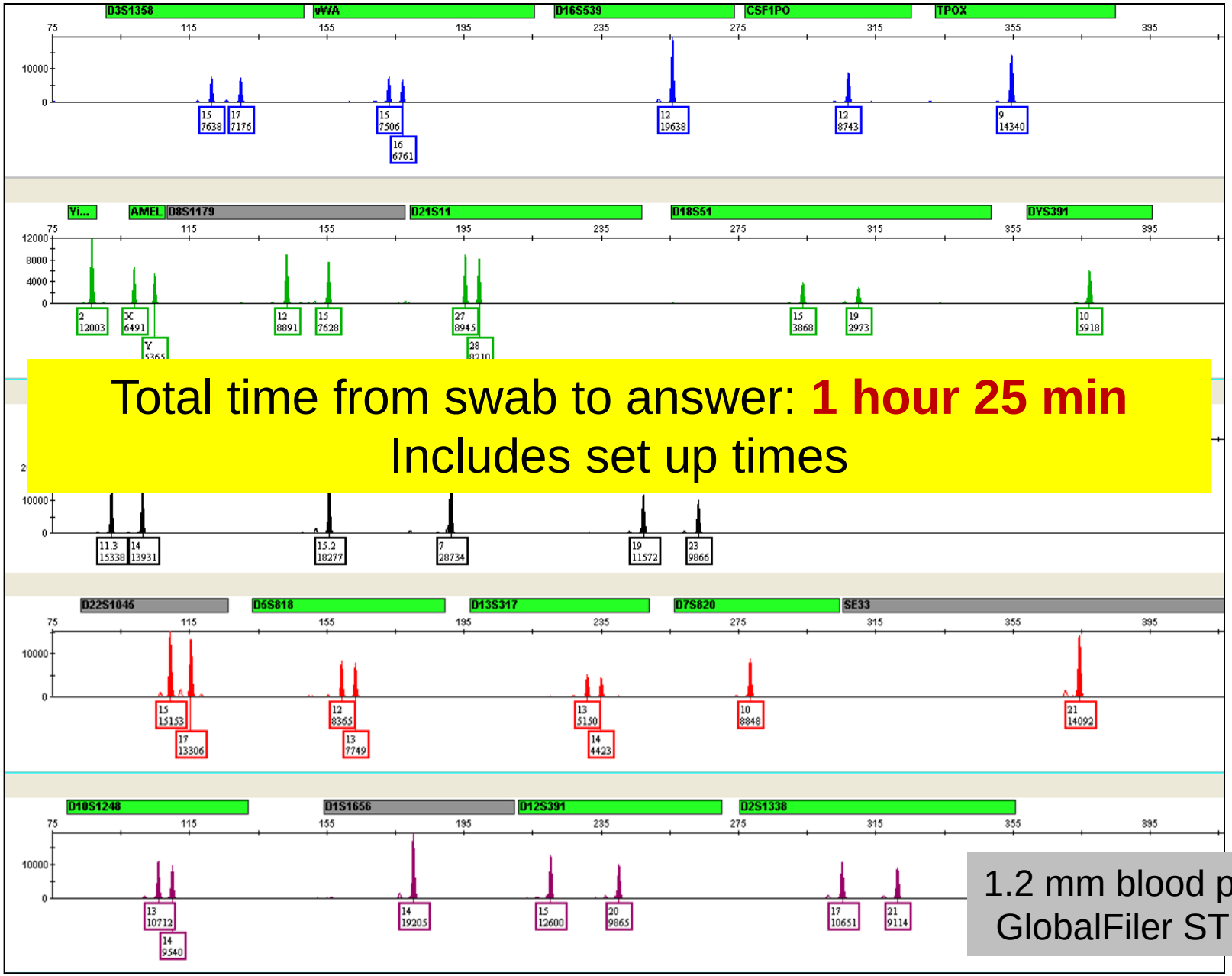


Cotton Buccal swab
Identifiler STR kit

Prep-N-Go → Philisa (2-step) → 3500



FTA → 9700 → 3500



Total time from swab to answer: **1 hour 25 min**
Includes set up times

1.2 mm blood punch
GlobalFiler STR kit

Summary

- Several areas exist to decrease the time for DNA typing
- A common technique is to modify the PCR amplification protocol
- STR genotype results can be generated in less than 2 hours
  - With **standard laboratory equipment** and protocols
  - Overall time includes: collection, sample handling, and liquid transfer steps

Rapid DNA Instrumentation

Swab in → Profile Out Platforms

Testing Performed at NIST

Rapid DNA Instruments

Testing on behalf of Chris Miles DHS S&T

ANDE (NetBio)

- Electrophoresis takes place on chip
- One biochipset
  - Stored at RT
  - Shelf life ≈ 6 months
- RFID tagged swabs

PowerPlex 16 loci
≈86 min runtime
(5 samples)

RapidHIT 200 (IntegenX)

- Electrophoresis takes place on an 8 capillary array
- Kit = 4 components
  - Stored between RT-4°C
  - Shelf life ≈ 5 months @ 4°C
- Cotton swabs

PowerPlex 16 loci
≈90 min runtime
(5 samples)
GlobalFiler Express loci
≈116 min runtime
(1-7 samples)

Sample Analysis Methods

- **Rapid DNA Analysis**: describes the fully automated (hands free) process of developing a CODIS Core STR profile from a reference sample buccal swab. The "swab in – profile out" process consists of automated extraction, amplification, separation, detection and allele calling without human intervention.

- **Modified Rapid DNA Analysis**: describes the automated (hands free) process of developing a CODIS Core STR profile from a known reference sample. This process consists of integrated extraction, amplification, separation, and detection without human intervention, but requires human interpretation and technical review.

http://www.fbi.gov/about-us/lab/biometric-analysis/codis/rapid-dna-analysis

NIST R-DNA Interlaboratory Study **Fall 2013**

- Presented last September at BCC
- Two R-DNA developers
- Three testing sites
- A total of 350 reference buccal swabs run
- Success defined as the automated calling of the 13 core STR loci
- **Overall success = 87.4%**

http://www.cstl.nist.gov/biotech/strbase/pub_pres/Vallone_BCC_Talk_Sept2013.pdf

Update since last year

September 2013-2014

- Run a total of 452 single source samples between both R-DNA platforms
  - 727 total (Not including negative controls, tests with non-buccal swabs)
- Success measured by concordant CODIS 13 loci called **Overall success = 84.8%**
- Two instrument upgrades for each platform
- Two software upgrades for each platform

Participation in developmental validation studies

- IntegenX RH200 (PowerPlex 16 chemistry)
  - 100 samples (NIST provided buccal swabs)
  - Age range (~1.5 years old)
  - 10 unique individuals
  - Results contributed to concordance and aged swab study
- NetBio ANDE (PowerPlex 16 chemistry)
  - 150 samples (reference swabs) provided by NetBio
  - Samples run over 3 weeks
  - Results provided back to NetBio/GEHC electronically

DV data is in the hands of the developers in the support of peer-reviewed studies

Positive and negative control experiments to support SWGDAM

- Over the past year the SWGDAM R-DNA Committee was involved in drafting recommendations for SWGDAM regarding R-DNA
  - FBI Director has approved the changes proposed via the 2 R-DNA Addendums Effective December 1, 2014
- NIST supported this work by performing Control Data Experiments to provide data to SWGDAM R-DNA Committee
  - To what extent are positive and negative controls needed?
    - Presence or absence of signal from a positive or negative control is not a good indicator of the success of other lanes

This led the recommendation that positive and negative controls are not required for every run

Controls are required for Cartridge/reagents check (lot check) under Standard 9 AND Performance Check under Standard 10

Making materials traceable to NIST SRM 2391c

- QAS 9.5.5 The laboratory shall check its DNA procedures ***annually*** or ***whenever substantial changes*** are made to a procedure against an appropriate and **available NIST standard reference material** *or* ***standard traceable to a NIST standard***.

http://www.nist.gov/traceability/
From the QAS
http://www.fbi.gov/about-us/lab/biometric-analysis/codis/qas_testlabs

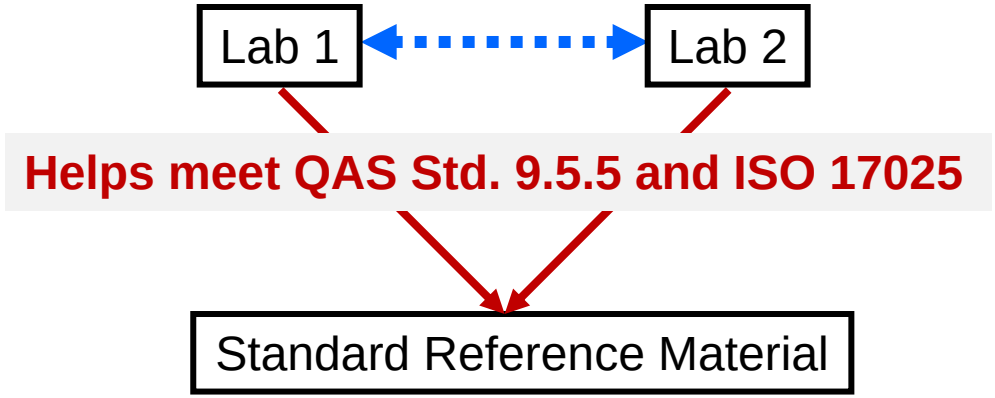
Standard **R**eference **M**aterial 2391c : PCR-Based DNA Profiling Standard

- Components A through D are DNA extracts in liquid form
- Components E and F are cells spotted on 903 paper or FTA paper

No buccal swabs in SRM 2391c

The paper components may not contain enough cells for R-DNA analysis

Genomic DNAs characterized for the expanded CODIS core loci and Y-STRs



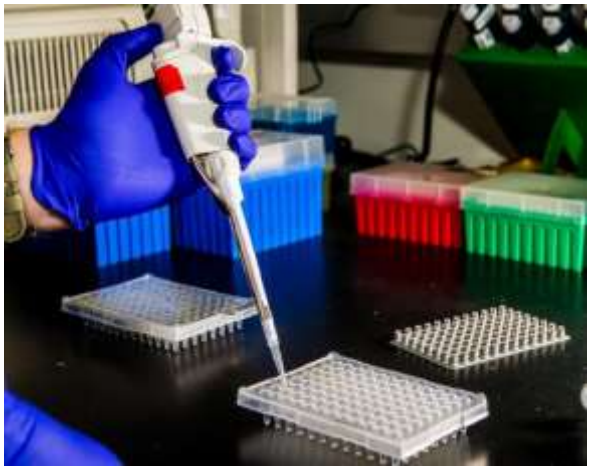
Calibration with SRMs enables confidence in comparisons of results between laboratories

How to make a NIST traceable swabs (SRM 2391c) - example

Collect a lot of 10 Buccal swabs from single individual

You are making this lot of swabs traceable to the SRM

Extract the DNA from two swabs from the lot (traditional lab methods)



Amplify extracted swabs along with components from SRM 2391c

Verify SRM 2391c allele calls are accurate against the certificate and make allele calls for the (now) traceable swab lot

How to make a NIST traceable swabs (SRM 2391c)

- These swabs can be used on R-DNA instruments now as a NIST traceable material
  - Must confirm typing results after running on a R-DNA platform
  - The process must be repeated to make another traceable lot of materials

- Use of traceable swabs:
  - **Annually or when upgrades are made (9.5.5 of QAS)**

  also if desired
  - During a critical reagents and R-DNA cartridge check (Standard 9)
  - R-DNA performance check (Standard 10)

Summary

- Continuing to run R-DNA platforms with newer kits/chemistries
- Continuing to provide data in support of discussion within the SWGDAM R-DNA committee for Rapid DNA Instrumentation
- Example of material traceability to SRM 2391c for R-DNA platforms

Acknowledgements

Peter Vallone, David Duewer

Contact Info:
erica.butts@nist.gov
301-975-5107

Funding

DHS – Rapid DNA Prototype and Kinship Performance Evaluation

FBI - the Evaluation of Forensic DNA Typing as a Biometric Tool