NIST Historical Perspective: Rapid DNA Identification

Erica Romsos

Rapid DNA Technology Forum

Alexandria, VA

August 16, 2017

Forensic Technology
CENTER OF EXCELLENCE
A program of the National Institute of Justice.

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

Disclaimer

We will mention commercial STR kit and instrument names, but we are in no way attempting to endorse any specific products.

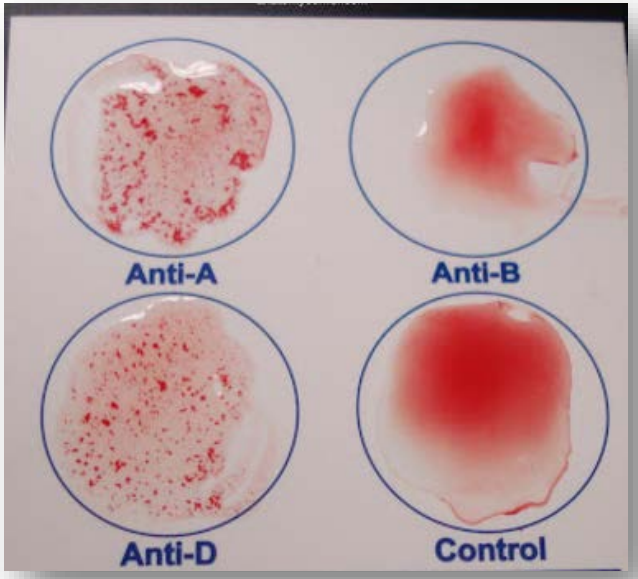
NIST Disclaimer: Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or it imply that any of the materials, instruments or equipment identified are necessarily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

Information presented does not necessarily represent the official position of the National Institute of Standards and Technology or the U.S. Department of Commerce.

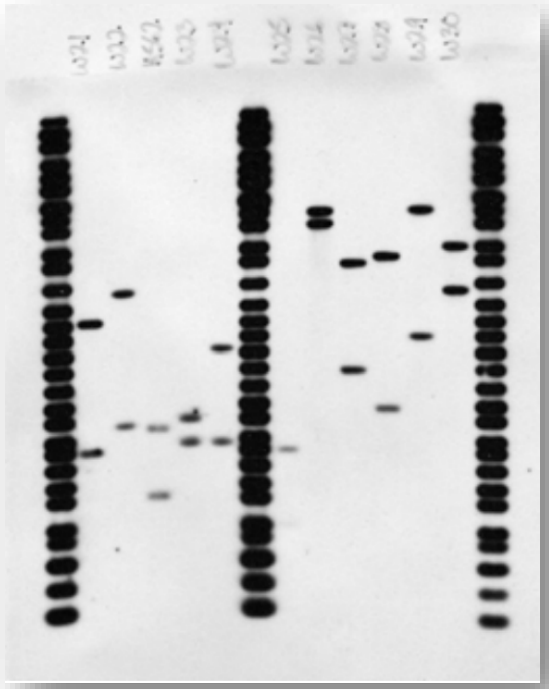
Rapid Overview

- Before there was Rapid DNA
- Rapid DNA Advancements
- NIST’s role in Rapid DNA
- Future of Rapid DNA

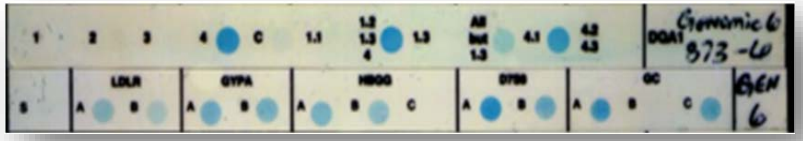
Before there was Rapid DNA



ABO Blood Typing
(~1900-1985)



RFLP
(~1985-mid 1990s)



DQ alpha & Dot Blot Hybridization
(Early-mid 1990s)

42 U.S. Code Part A - DNA Identification

US Code

- § 14131 - Quality assurance and proficiency testing standards
- § 14132 - Index to facilitate law enforcement exchange of DNA identification information

The DNA Identification Act of 1994

Then in 1995…

SRM 2391 PCR-based DNA Profiling Standard Released

National Institute of Standards and Technology

Certificate of Analysis

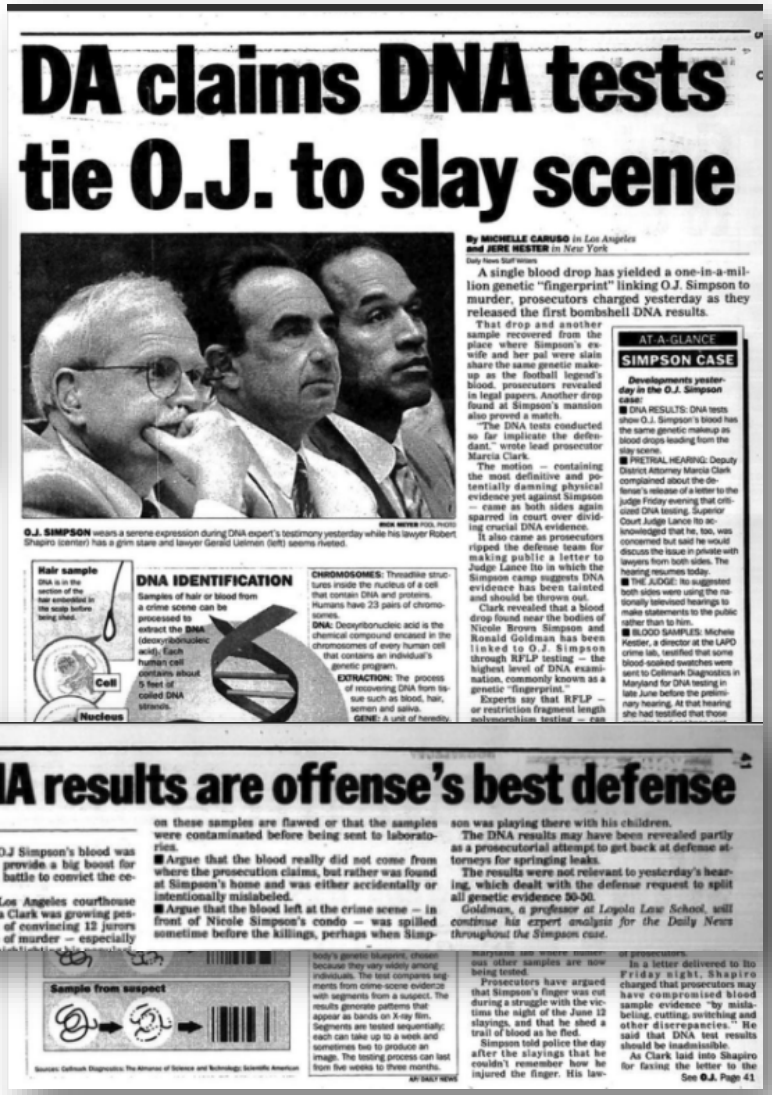
Standard Reference Material® 2391

PCR-based DNA Profiling Standard

DNA typing presented for the first time in a major case

Questions of evidence handling

Validity of DNA typing process not questioned



DA claims DNA tests tie O.J. to slay scene

By MICHELLE CARUSO in Los Angeles and JERE HESTER in New York

A single blood drop has yielded a one-in-a-million genetic "fingerprint" linking O.J. Simpson to murder, prosecutors charged yesterday as they released the first bombshell DNA results.

That drop and another sample recovered from the place where Simpson's ex-wife and her pal were slain share the same genetic makeup as the football legend's blood, prosecutors revealed in legal papers. Another drop found at Simpson's mansion also proved a match.

"The DNA tests conducted so far implicate the defendant," wrote lead prosecutor Marcia Clark.

The motion — containing the most definitive and potentially damning physical evidence yet against Simpson — came as both sides again sparred in court over dividing crucial DNA evidence.

It also came as prosecutors ripped the defense team for making public a letter to Judge Lance Ito in which the Simpson camp suggests DNA evidence has been tainted and should be thrown out.

Clark revealed that a blood drop found near the bodies of Nicole Brown Simpson and Ronald Goldman has been linked to O.J. Simpson through RFLP testing — the highest level of DNA examination, commonly known as a genetic "fingerprint."

Experts say that RFLP — or restriction fragment length polymorphism testing — can

AT-A-GLANCE

SIMPSON CASE

Developments yesterday in the O.J. Simpson case:

■ DNA RESULTS: DNA tests show O.J. Simpson's blood has the same genetic makeup as blood drops leading from the slay scene.

■ PRETRIAL HEARING: Deputy District Attorney Marcia Clark complained about the defense's release of a letter to the judge Friday evening that criticized DNA testing. Superior Court Judge Lance Ito acknowledged that he, too, was concerned but said he would discuss the issue in private with lawyers from both sides. The hearing resumes today.

■ THE JUDGE: Ito suggested both sides were using the nationally televised hearings to make statements to the public rather than to him.

■ BLOOD SAMPLES: Michele Kestler, a director at the LAPD crime lab, testified that some blood-soaked swatches were sent to Cellmark Diagnostics in Maryland for DNA testing in late June before the preliminary hearing. At that hearing she had testified that those

O.J. SIMPSON wears a serene expression during DNA expert's testimony yesterday while his lawyer Robert Shapiro (center) has a grim stare and lawyer Gerald Uelmen (left) seems riveted.

DNA IDENTIFICATION

Samples of hair or blood from a crime scene can be processed to extract the DNA (deoxyribonucleic acid). Each human cell contains about 5 feet of coiled DNA strands.

Hair sample

Cell

Nucleus

CHROMOSOMES: Threadlike structures inside the nucleus of a cell that contain DNA and proteins. Humans have 23 pairs of chromosomes.

DNA: Deoxyribonucleic acid is the chemical compound encased in the chromosomes of every human cell that contains an individual's genetic program.

EXTRACTION: The process of recovering DNA from tissue such as blood, hair, semen and saliva.

GENE: A unit of heredity.

O.J. DNA results are offense's best defense

By STAN GOLDMAN

Special to The News

DNA results indicating O.J Simpson's blood was found at the crime scene provide a big boost for prosecutors in their tough battle to convict the celebrity defendant.

The word around the Los Angeles courthouse was that prosecutor Marcia Clark was growing pessimistic over her chances of convincing 12 jurors that Simpson was capable of murder — especially

on these samples are flawed or that the samples were contaminated before being sent to laboratories.

■ Argue that the blood really did not come from where the prosecution claims, but rather was found at Simpson's home and was either accidentally or intentionally mislabeled.

■ Argue that the blood left at the crime scene — in front of Nicole Simpson's condo — was spilled sometime before the killings, perhaps when Simpson was playing there with his children.

The DNA results may have been revealed partly as a prosecutorial attempt to get back at defense attorneys for springing leaks.

The results were not relevant to yesterday's hearing, which dealt with the defense request to split all genetic evidence 50-50.

Goldman, a professor at Loyola Law School, will continue his expert analysis for the Daily News throughout the Simpson case.

Sample from suspect

body's genetic blueprint, chosen because they vary widely among individuals. The test compares segments from crime-scene evidence with segments from a suspect. The results generate patterns that appear as bands on X-ray film. Segments are tested sequentially; each can take up to a week and sometimes two to produce an image. The testing process can last from five weeks to three months.

Sources: Cellmark Diagnostics, The Almanac of Science and Technology, Scientific American

AP/DAILY NEWS

marijuana on which other samples are now being tested.

Prosecutors have argued that Simpson's finger was cut during a struggle with the victims the night of the June 12 slayings, and that he shed a trail of blood as he fled.

Simpson told police the day after the slayings that he couldn't remember how he injured the finger. His lawyers

of prosecutors.

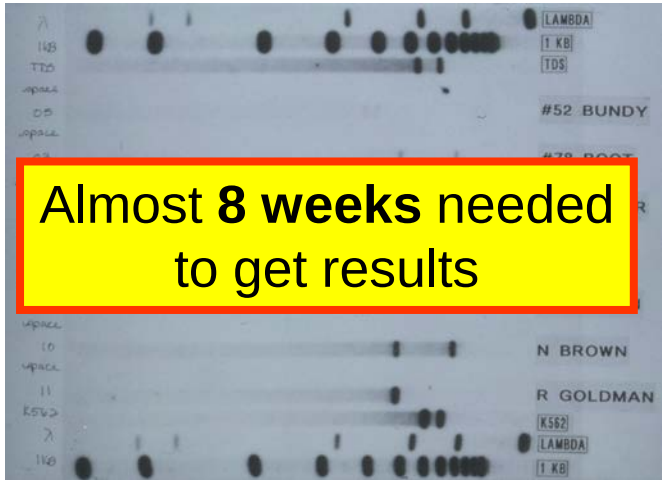
In a letter delivered to Ito Friday night, Shapiro charged that prosecutors may have compromised blood sample evidence "by mislabeling, cutting, switching and other discrepancies." He said that DNA test results should be inadmissible.

As Clark laid into Shapiro for faxing the letter to the

See O.J. Page 41

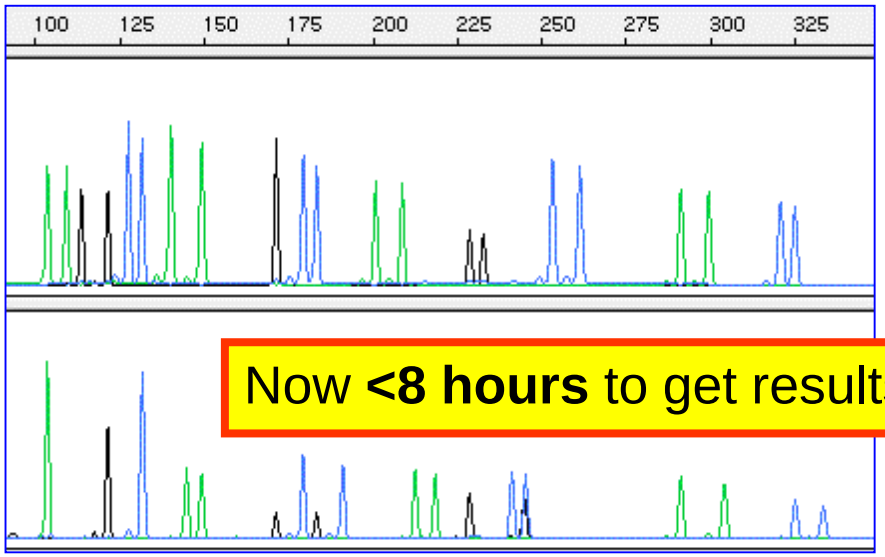
Since 1995…

Forensic DNA typing moved from RFLP to commercial multiplex STR kits



Almost **8 weeks** needed to get results

RFLP discontinued US-wide 2000



Now **<8 hours** to get results

- 1996 first multiplex STR Kits available
- 2000-2001 single coamplification kit of CODIS STRs

CODIS
COMBINED DNA INDEX SYSTEM

1997-13 Core Loci defined
Launched October 1998

DNA Core Loci Expanding

Thu, Dec 15 2016 by Seth Augenstein

Expanded January 2017

From 2000s to Present Day

The forensic science world is on the brink of a revolution

Human Genome Sequenced

Commercial Multiplex STR Kits >20 Loci

ABI 3100 Genetic Analyzer introduced

ABI 3500 Genetic Analyzer introduced

NDIS exceeds 12 million offender profiles

NEWS

What If All Law Enforcement Agencies Could Do Instant DNA Analysis?

Forensic News
Thu, Oct 4 2012

No human intervention

Minimal operator education

Small liquid volumes

Faster PCR

Expert analysis software

Rugged & Portable

Single instrument

Faster Separation & Detection

No liquid handling steps

Stable reagents

Microfluidics

DNA Typing Process

- **Extraction**
- **Quantitation**
- **Multiplex PCR**
- **Separation & Detection**
- **Interpretation of Results**

Entire DNA typing process in less than 2 hours

Rapid Advancements

- Many efforts made to reduce time within the DNA typing process

- Predominant focus area in reduction: **PCR**

Forensic Science International: Genetics 18 (2015) 90–99

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Review

Rapid PCR of STR markers: Applications to human identification

Erica L. Romsos *, Peter M. Vallone

National Institute of Standards and Technology, 100 Bureau Drive, MS 8314, Gaithersburg, MD 20899-8314, USA

Alternative DNA Polymerases

- Allow for a higher processivity than AmpliTaq Gold
- Higher resistance to inhibitors
- 16-32 fold increase in efficacy with shorter extension times
  - Allows for reduction in PCR thermal cycling time

Nucleic Acids Research, 2004, Vol. 32, No. 3 **1197–1207**
DOI: 10.1093/nar/gkh271

A novel strategy to engineer DNA polymerases for enhanced processivity and improved performance *in vitro*

Yan Wang, Dennis E. Prosen, Li Mei, John C. Sullivan, Michael Finney and Peter B. Vander Horn*

Anatomy of a Polymerase - How Structure Effects Function

frontiers in MICROBIOLOGY

EDITORIAL
published: 01 December 2014
doi: 10.3389/fmicb.2014.00659

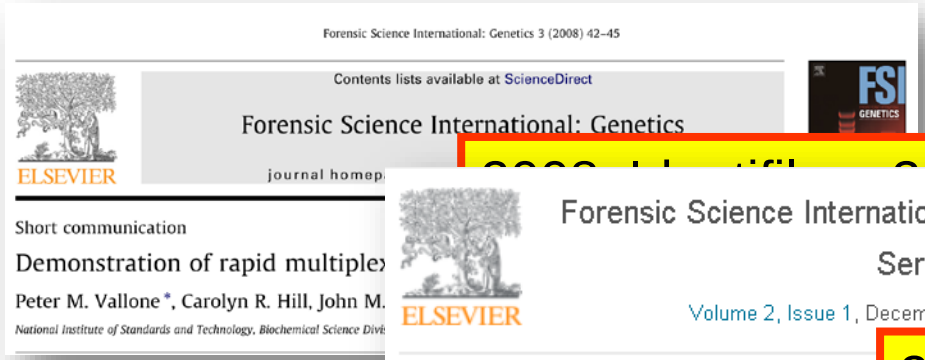
DNA polymerases in biotechnology

Andrew F. Gardner[1] and Zvi Kelman[2,3]*

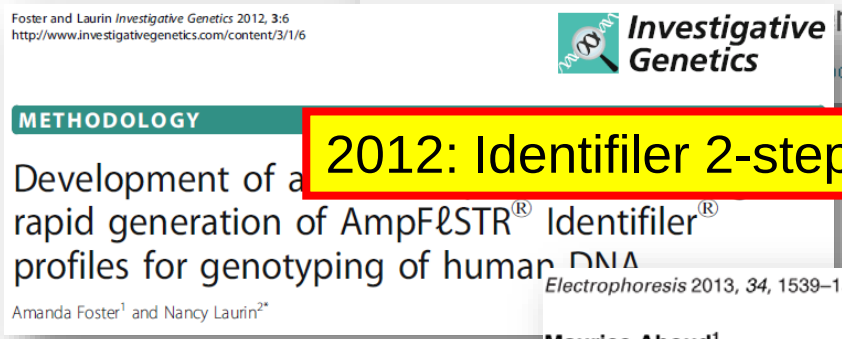
[1] New England Biolabs Inc., Ipswich, MA, USA
[2] National Institute of Standards and Technology, Gaithersburg, MD, USA
[3] Institute for Bioscience and Biotechnology Research, Rockville, MD, USA
*Correspondence: gardner@neb.com

Rapid PCR Protocols

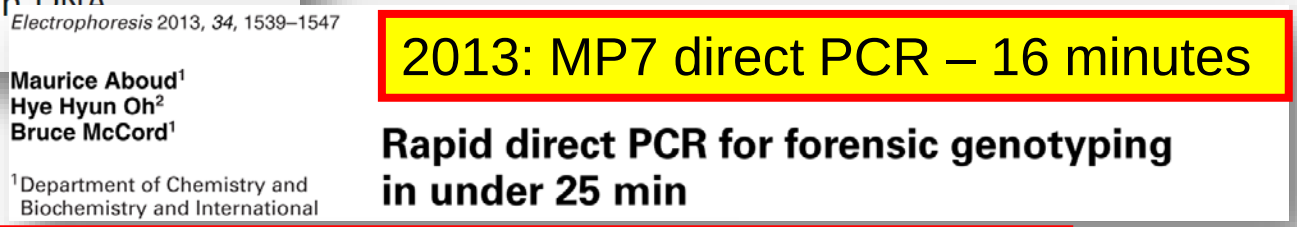
Standard PCR Cycling
Time: ~3 Hours



2009: Identifiler Optimized - 36 minutes

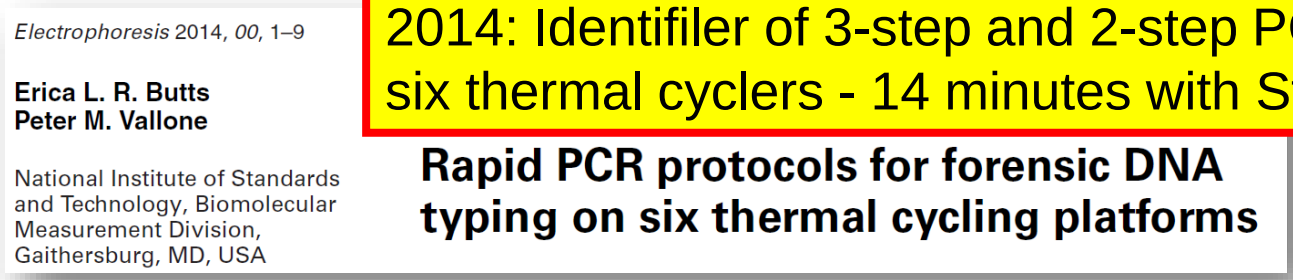


2012: Identifiler 2-step PCR – 26 minutes



2013: MP7 direct PCR – 16 minutes

2014: Identifiler of 3-step and 2-step PCR across six thermal cyclers - 14 minutes with Streck Philisa



Rapid PCR on a Chip

- Reduction of PCR volume
- Miniaturization and integration with microfluidic

Table 3
Summary of studies performing PCR of STR markers on a chip.

E.L. Romsos, P.M. Vallone / Forensic Science International: Genetics 18 (2015) 90–99

Assay/Primers	Polymerase	Thermal cycling	Time (min)	Reference number
AmpFlSTR Blue	AmpliTaq Gold	Miniature analytical thermal cycler instrument	60	[68]
Profiler Plus	SpeedSTAR	Custom thermal cycler	17.3	[70]
PowerPlex ESI 17	Not Reported	Custom hydrogel μSPEAM beads	110	[71]
MiniFiler	Taq Gold	Non-contact infrared	90	[76]
IdentiFiler	SpeedSTAR	Non-contact infrared	45	[76]
IdentiFiler				[77]
IdentiFiler				[79]

JOURNAL OF FORENSIC SCIENCES

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

Katie M. Hors
Ph.D.

Forensic
Devices: A Review

ic devices

Forensic Integration - Microfluidics

Integrated Microfluidic Systems for DNA Analysis

Samuel K. Njoroge, Hui-Wen Chen, Małgorzata A. Witek, and Steven A. Soper

Review of microfluidic systems which were composed of 2+ microdevices

An integrated sample-in-answer-out microfluidic chip for rapid human identification by STR analysis†

Delphine Le Roux,‡[a] Brian E. Root,‡[b] Jeffrey A. Hickey,[b] Orion N. Scott,[b] Anchi Tsuei,[b] Jingyi Li,[b] David J. Saul,[c] Luc Chassagne,[a] James P. Landers§[d] and Philippe de Mazancourt§*[a]

Integration of liquid extraction with a pre-existing microfluidic PCR platform and µCE

Anal. Chem. **2010,** *82,* 6991–6999

Integrated Microfluidic System for Rapid Forensic DNA Analysis: Sample Collection to DNA Profile

Andrew J. Hopwood,*,† Cedric Hurth,‡ Jianing Yang,‡ Zhi Cai,‡ Nina Moran,† John G. Lee-Edghill,† Alan Nordquist,‡ Ralf Lenigk,‡ Matthew D. Estes,‡ John P. Haley,† Colin R. McAlister,† Xiaojia Chen,‡ Carla Brooks,‡ Stan Smith,‡ Keith Elliott,† Pieris Koumi,† Frederic Zenhausern,*,‡ and Gillian Tully†

Research and Development, Forensic Science Service, Trident Court 2960 Solihull Parkway, Birmingham Business Park, Birmingham UK B37 7YN, and Center for Applied NanoBioscience and Medicine, The University of Arizona College of Medicine, 425 N. Fifth Street, Phoenix, Arizona 85004

First demonstrated fully integrated device without any manual intervention

Run time: 4 hours

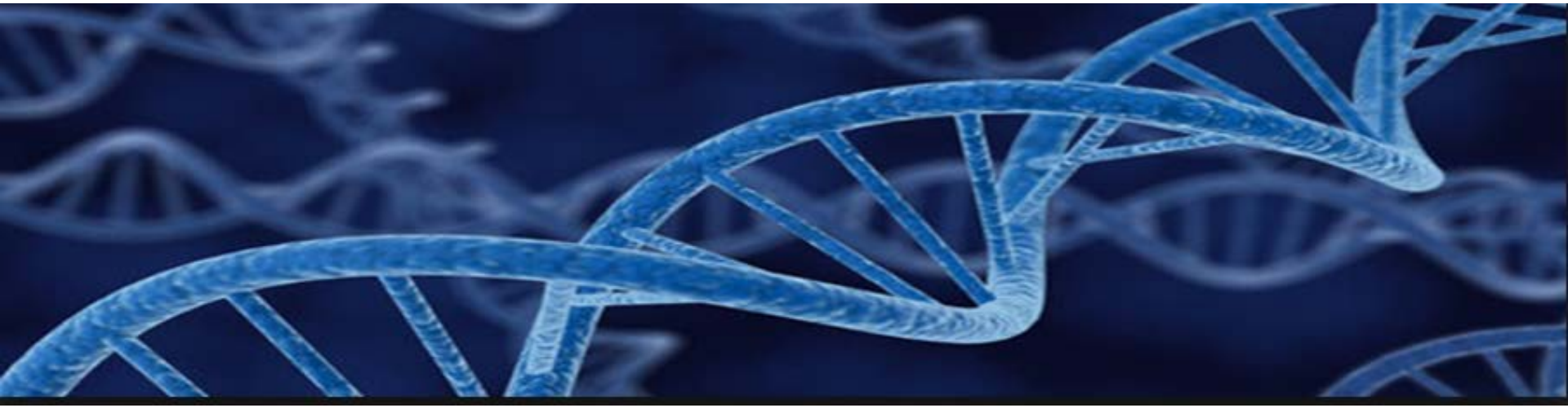
Commercial RDNA Instruments

IntegenX

- RapidHIT 200
  - PowerPlex 16HS
  - Globalfiler
- RapidHIT ID
  - Globalfiler

NetBio/ANDE

- ANDE/DNAScan
  - PowerPlex 16
- ANDE
  - FlexPlex (27)



Rapid Advancements: NIST Edition

How does NIST fit into Rapid DNA Identification?

Who are we?

- NIST falls within the Department of Commerce
  - Mission: to promote innovation and industrial competitiveness by advancing measurement science, standards, and technology
- Our focus is on making measurements
  - Robust examinations of technology
  - Collaboration with other federal/state/local users
  - Collaboration with industry
- Interagency collaboration with the FBI and DHS

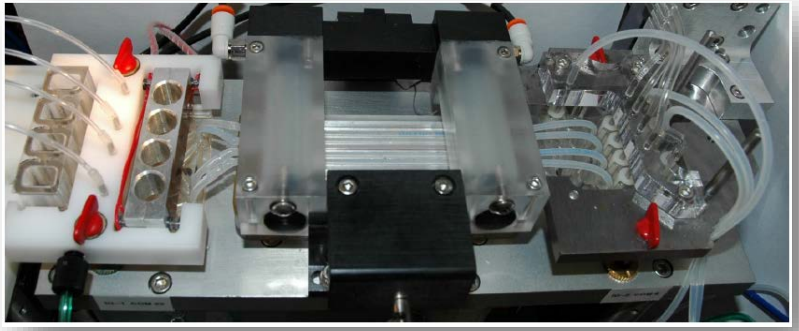
Rapid Advancements: NIST Edition

2008 2010 2012 2014 2016 2018

2008: Demonstration of RPCR – 36 min

2009: Optimization of RPCR – 36 min

2010: Apollo 200 Testing (136 samples)



Forensic Science International: Genetics 3 (2008) 42–45

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☆

Peter M. Vallone *, Carolyn R. Hill, John M. Butler

National Institute of Standards and Technology, Biochemical Science Division, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899-8311, United States

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

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

Research article

Rapid amplification of commercial STR typing kits

Peter M. Vallone [a,*], Carolyn R. Hill [a], Daniele Podini [b], John M. Butler [a]

[a] National Institute of Standards and Technology, Gaithersburg, MD 20899-8312, USA

[b] Department of Forensic Sciences, The George Washington University, 2036 H St NW, Washington, DC 20052, USA

ARTICLE

Inside the Black Box: Testing and Validation of a Rapid DNA Instrument

Wed, Sep 28 2011 by Peter M. Vallone, Ph.D.

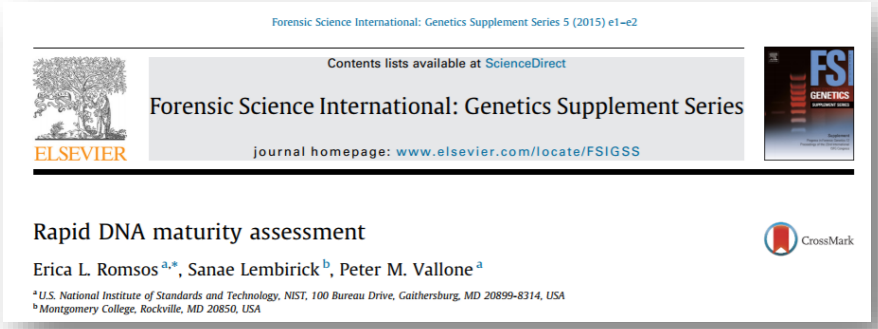
Rapid Advancements: NIST Edition

2012-2014: Ongoing Communication with Developers

2008 2010 2012 2014 2016 2018

09/2012: First prototypes delivered to NIST (DHS Instruments)

2013: NIST Interlab Study (350 samples)



Forensic Science International: Genetics Supplement Series 5 (2015) e1–e2

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

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

Rapid DNA maturity assessment

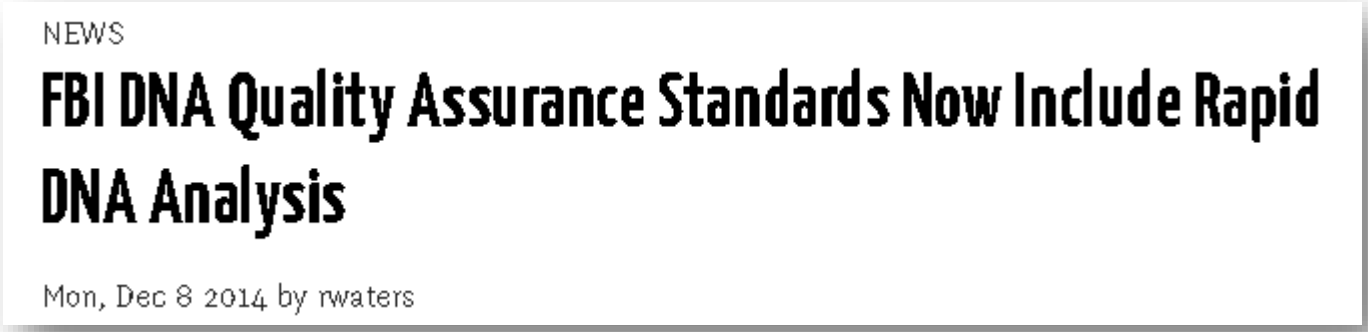
Erica L. Romsos[a,*], Sanae Lembirick[b], Peter M. Vallone[a]

[a] U.S. National Institute of Standards and Technology, NIST, 100 Bureau Drive, Gaithersburg, MD 20899-8314, USA
[b] Montgomery College, Rockville, MD 20850, USA

2014: NIST Maturity Assessment (280 samples)

NIST: 2012 to 2014

- Many developmental changes and upgrades during 2012-2013 timeframe
  - Software, hardware, reagents, consumables, etc
- Over 700 samples run between both platforms
- NIST participation in the Rapid-DNA committee within the Scientific Working Group on DNA Analysis Methods (SWGDAM)


NEWS

FBI DNA Quality Assurance Standards Now Include Rapid DNA Analysis

Mon, Dec 8 2014 by rwaters

NIST: Interlabs and Maturity Assessments

- Collection and distribution of samples to all participating laboratories
  - IL: 3 laboratories, 350 samples total
  - MA: 7 laboratories, 280 samples total
- Coordination of all testing sites to include return of all data to NIST for analysis and review
- Analysis and compilation of all data
- Summary of results presented across multiple meetings within the forensic and biometric communities

NIST
National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

Email:
Erica.Romsos@nist.gov

Rapid DNA Maturity Assessment

Erica L. Romsos[1], Sanae Lembirick[2], and Peter M. Vallone[1]
[1] U.S. National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-8314, USA
[2] Montgomery College, Rockville, MD 20850, USA

iSFG

P-148

Rapid Advancements: NIST Edition

2008 — 2010 — 2012 — 2014 — 2016 — 2018

2015: Rapid DNA Review Article

2015: NIST Participates in IXI PP16 DV (100 samples)

2016: NIST Participates in ANDE PP16 DV (150 samples)

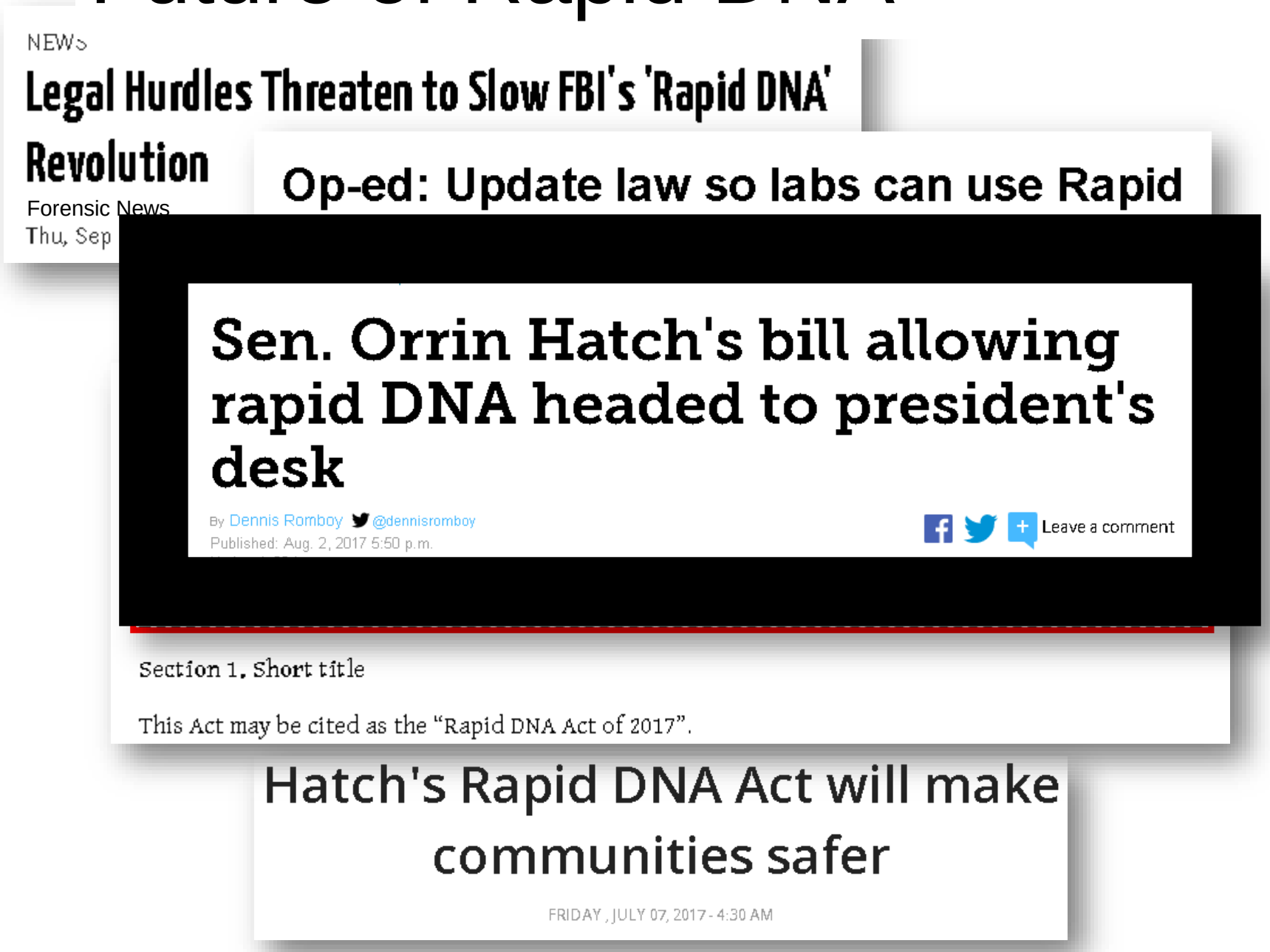
2016: NIST Collaboration with LGC ParaDNA

2017: NIST Participates in ANDE FlexPlex DV (250 samples)

NIST: 2017 and Beyond

- Continuing to provide data in support of discussion within the SWGDAM R-DNA committee
- Subject matter experts for R-DNA for DHS
- Continued support to the R-DNA community and developers

Future of Rapid DNA



NEWS

Legal Hurdles Threaten to Slow FBI's 'Rapid DNA' Revolution

Forensic News
Thu, Sep

Op-ed: Update law so labs can use Rapid

Sen. Orrin Hatch's bill allowing rapid DNA headed to president's desk

By Dennis Romboy @dennisromboy
Published: Aug. 2, 2017 5:50 p.m.

Leave a comment

Section 1. Short title

This Act may be cited as the "Rapid DNA Act of 2017".

Hatch's Rapid DNA Act will make communities safer

FRIDAY , JULY 07, 2017 - 4:30 AM

Future of Rapid DNA

- FBI integration of commercial Rapid DNA profiles into CODIS with search against NDIS
  - Rapid DNA Instrumentation within Police Booking Stations
- DHS continual efforts to employ Rapid DNA typing for immigration, refugee status, and mass fatality response operations for kinship testing

Acknowledgements

NIST – Applied Genetics Group
Peter Vallone
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Sanae Lembirik

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DHS S&T (Chris Miles)

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