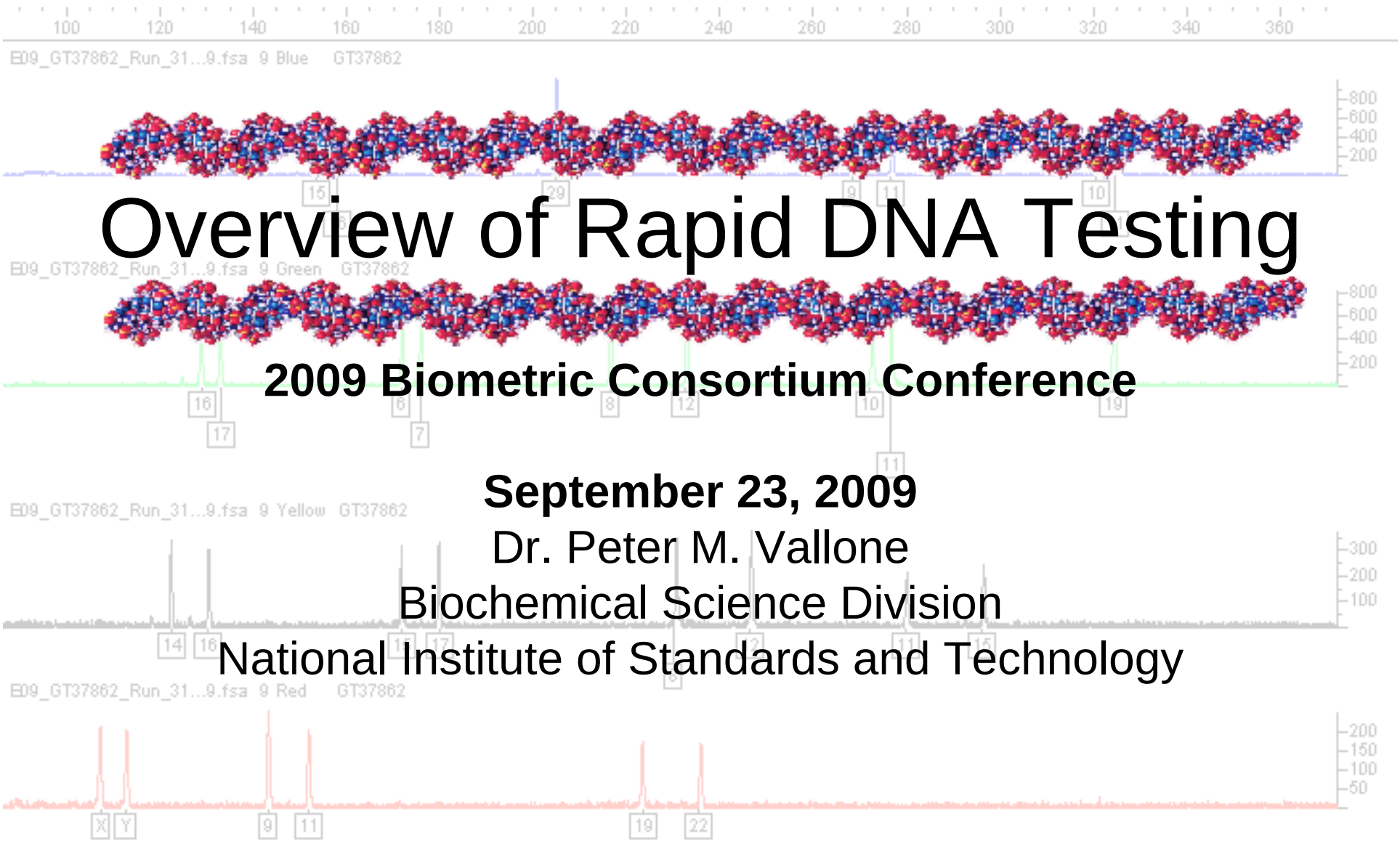


Overview of Rapid DNA Testing

2009 Biometric Consortium Conference

September 23, 2009

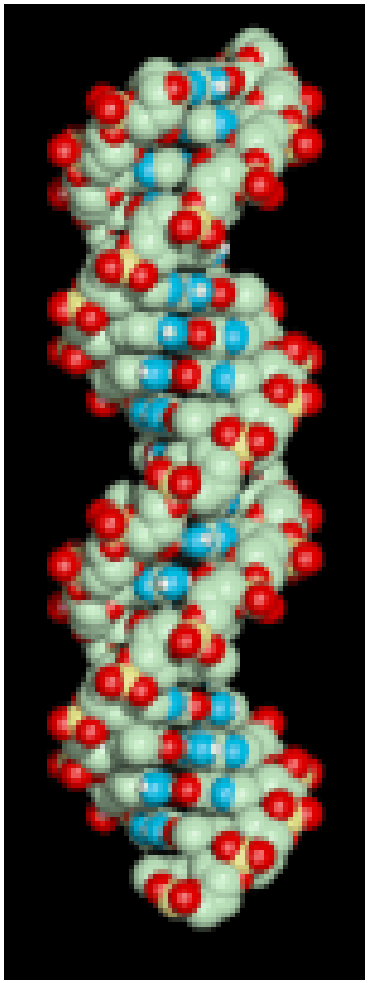
Dr. Peter M. Vallone
Biochemical Science Division
National Institute of Standards and Technology

Outline

- Basics of DNA Typing
- Rapid PCR

Basics of Forensic DNA Testing

General Characteristics of Genomic DNA



- Each person has a unique DNA profile (except identical twins)
- Each person's DNA is the same in every cell (DNA from skin cells will match DNA from blood cells)
- An individual’s DNA profile remains the same throughout life
- Half of your DNA comes from your mother and half from your father

Forensic DNA Testing

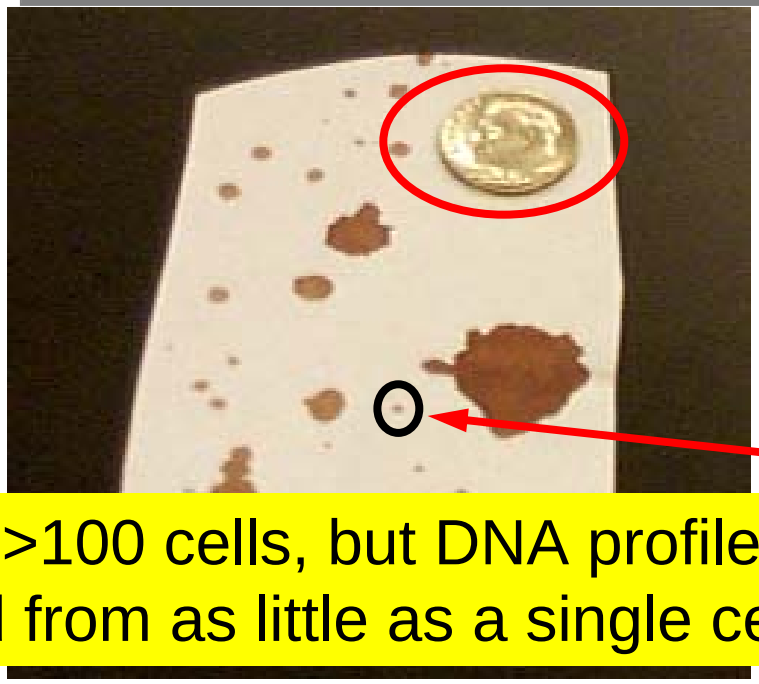
Probe subsets of genetic variation in order to differentiate between individuals

DNA typing must be done efficiently and reproducibly (information must hold up in court)

Typically, we are not looking at genes – little/no information about race, predisposal to disease, or phenotypical information (eye color, height, hair color) is obtained

Sources of Biological Evidence

- **Blood**
- **Semen**
- **Saliva**
- **Urine**
- **Hair**
- **Teeth**
- **Bone**
- **Tissue**



Blood Sample

Only a very small amount of blood is needed to

best results with >100 cells, but DNA profiles can b**ea DNA** recovered from as little as a single cell

Applications

- Forensic cases: matching suspect with evidence
- Paternity testing: identifying father
- Missing persons investigations
- Military DNA “dog tag”
- Convicted felon DNA databases
- Mass fatalities: putting pieces back together
- Historical investigations
- Genetic genealogy
- DNA as a biometric tool

DNA Testing Requires a Reference Sample

A DNA profile by itself is fairly useless because it has no context…

DNA analysis for identity only works by comparison – **you need a reference sample**

Crime Scene Evidence compared to **Suspect(s)** (Forensic Case)
Child compared to **Alleged Father** (Paternity Case)
Victim's Remains compared to **Biological Relative** (Mass Disaster ID)
Soldier's Remains compared to **Direct Reference Sample** (Armed Forces ID)

Steps in Forensic DNA Analysis

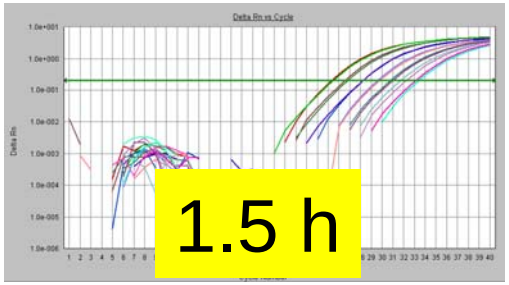
Usually 1-2 day process (a minimum of ~8 hours)

Blood Stain Buccal swab

Sample Collection & Storage

Biology

DNA Extraction



DNA Quantitation

Multiplex PCR Amplification

Technology

DNA separation and sizing

STR Typing

Interpretation of Results

Genetics

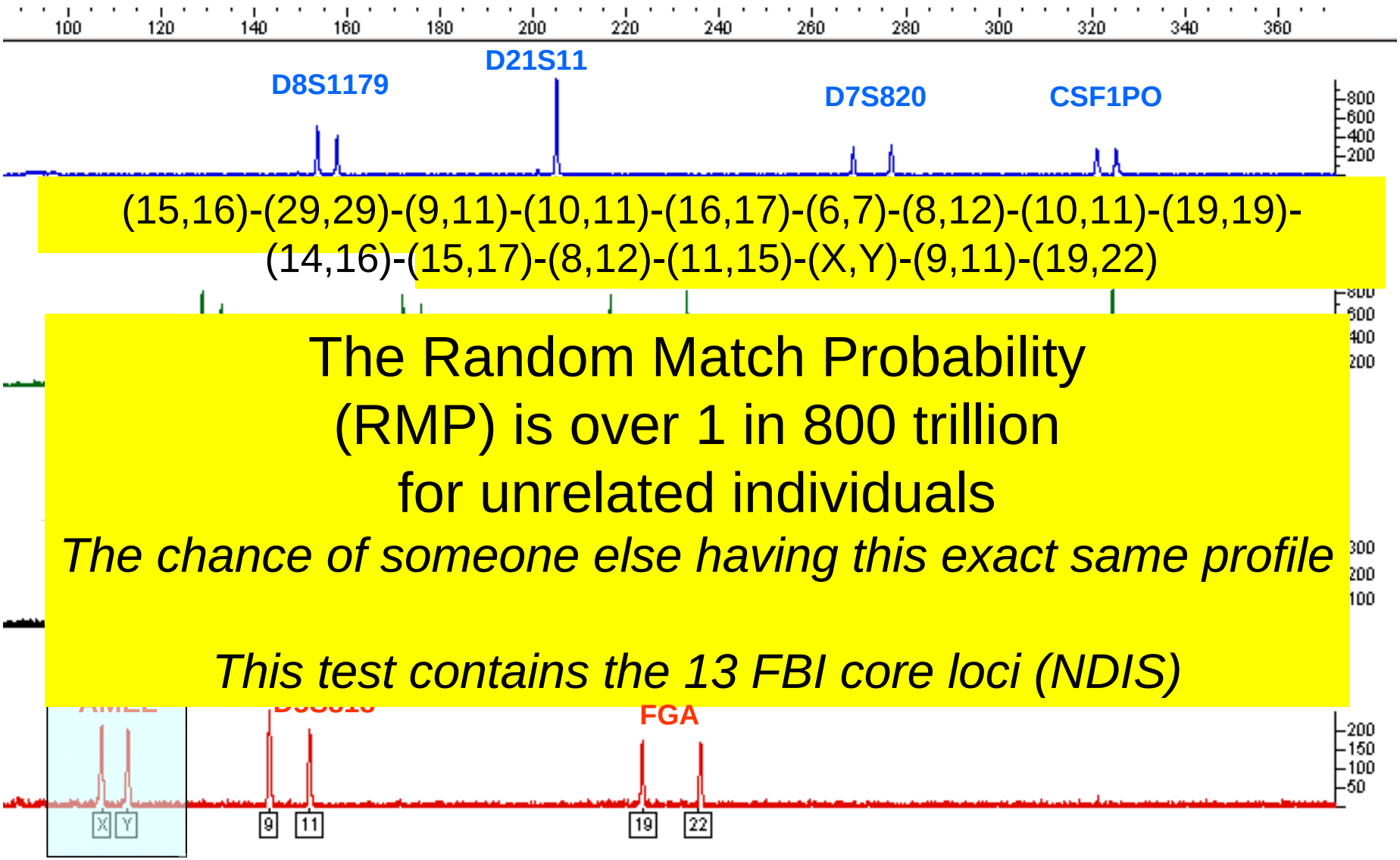
Statistics Calculated

DNA Database search
Paternity test
Reference sample

Applied Use of Information

Identifiler (Applied Biosystems) 15 STR Loci Kit

Information is tied together with multiplex PCR and data analysis



Recent Work with Rapid PCR

At NIST we are working on new PCR methods to reduce the time for PCR down to 20 minutes

Polymerase Chain Reaction (PCR)

Is a means to create billions of exact copies of the human genome – necessary/essential for DNA typing

~3.5 h → ~20 min?

Multiplex PCR Amplification

Why go Faster?
Applications for Rapid PCR

- Integrated devices (‘Lab on a Chip’)
- Screening at a point of interest (airport, border, crime scene, intelligence community)
- Rapid STR typing ‘in the field’
  - Potential for situations/cases when a quick result is needed
  - Provide initial screening information
- Decrease overall time required for STR typing

DNA as a Biometric tool

Current Efforts Towards Portable/Mobile DNA Devices

- Network Biosystems (Woburn, MA) http://www.netbio.com
- MicroLab Diagnostics and Lockheed Martin (Charlottesville,VA) http://www.microlabdiagnostics.com
- Microchip Biotech (Dublin, CA) http://www.microchipbiotech.com

Goals for Rapid DNA Typing Platforms

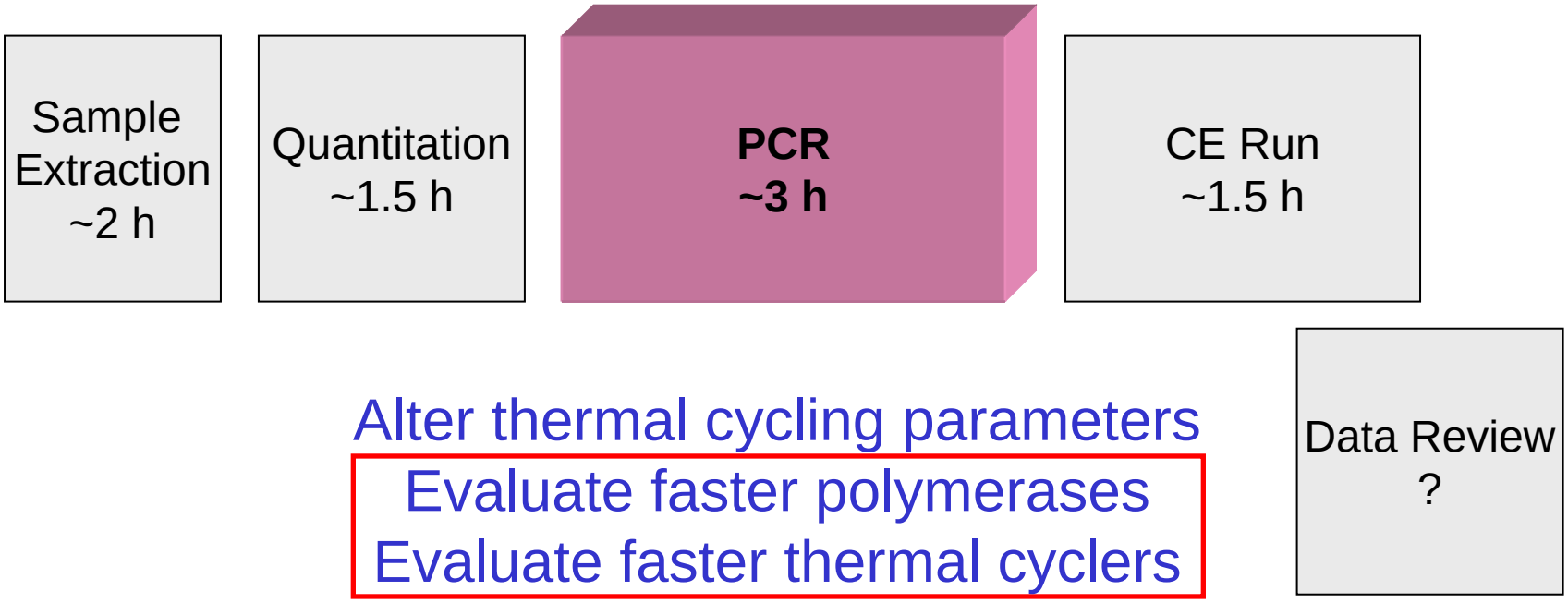
- Create an integrated system capable of taking a swab and perform DNA testing in approximately 1 hour

- Little user interaction (or experience)
- Rugged
- Robust
- Simple data interpretation

Swab in…answer out

Typical STR Typing Workflow

Can the time required for PCR thermal cycling be reduced?



Goal: cycling in less than 40 minutes
Trying simple things first…

Thermal Cyclers

Cepheid SmartCycler
Ramp rate = 10°C/s

Purchased with FBI funding April 2009

Eppendorf Mastercycler pro
Ramp rate = 6°C/sec

Applied Biosystems 9700
Ramp rate = 4°C/s

36 Minute PCR Amplification on AB 9700 Cycler

28 cycles, Identifiler STR kit, 1 ng of DNA

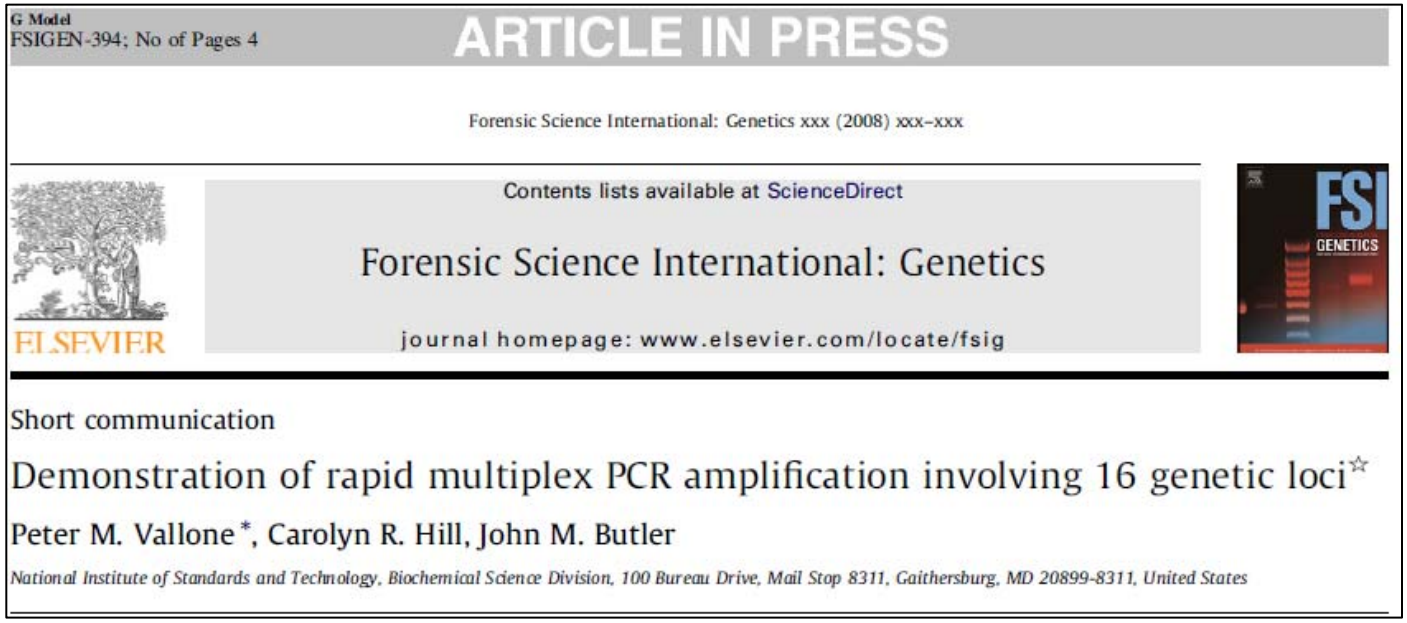
20 Minute PCR Amplification on Cepheid Cycler

28 cycles, Identifiler STR kit, 1 ng of DNA

19 Minute PCR Amplification on Eppendorf Cycler

28 cycles, Identifiler STR kit, 1 ng of DNA

Rapid PCR Article



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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

Vallone, P.M., Hill, C.R., Butler, J.M. (2008) Demonstration of rapid multiplex PCR amplification involving 16 genetic loci. *FSI Genetics* 3(1): 42-45.

Rapid Amplification of Commercial STR Typing Kits
Presented at the International Society of Forensic Genetics (ISFG) meeting in Buenos Aires Argentina (September 16, 2009)
(Voted Best Poster Presentation)

Rapid PCR Summary

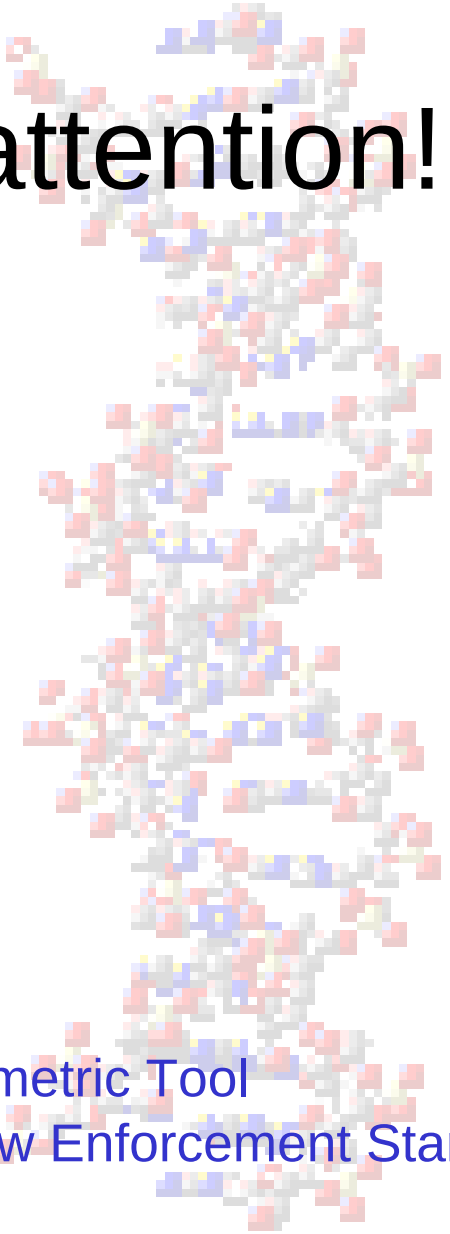
- Rapid multiplex PCR amplification is possible
  - Compatible with commercial STR typing kits
  - Provides same genotypes as standard cycling
- Fast (optimized) polymerases are needed
- Further work
  - Applying techniques to integrated platforms
  - Formal validation of technique
  - Sharing results with PCR community
  - Understanding the kinetics of PCR

Thank you for your attention!

Questions?

Peter.Vallone@nist.gov

301-975-4872



Outside funding agencies:
FBI - Evaluation of Forensic DNA Typing as a Biometric Tool
NIJ – Interagency Agreement with the Office of Law Enforcement Standards