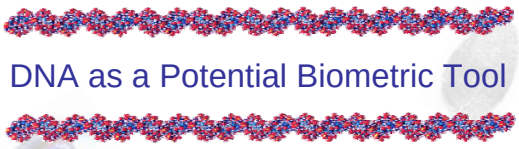


DNA as a Potential Biometric Tool

Dr. Peter M. Vallone
National Institute of Standards and Technology

Advances in Forensic DNA Analysis
AAFS 62nd Annual Scientific Meeting
Seattle, WA
Monday February 22, 2010

peter.vallone@nist.gov (301)-975-4872

Outline

- What is a Biometric?
- Rapid PCR Protocols
- DNA Typing on Integrated Systems

What is a Biometric?

Method for uniquely recognizing humans based upon one or more intrinsic physical or behavioral traits

Jain, A. K.; Ross, Arun; Prabhakar, Salil (January 2004), "An introduction to biometric recognition", *IEEE Transactions on Circuits and Systems for Video Technology* **14th** (1): 4–20

Current Biometrics

Some commonly measured features

- Physical
  - Fingerprints (Palm/hand geometry)
  - Iris, retinal
  - Face
  - Odor/scent
  - DNA?
- Behavioral
  - Gait
  - Voice
  - Vein (IR thermogram)
  - Hand geometry
  - Handwriting





Characteristics of a Biometric

- Universality
  - each person should have the characteristic
- Uniqueness
  - is how well the biometric separates individuals from another
- Permanence
  - measures how well a biometric resists aging and variance over time
- Collectability
  - ease of acquisition for measurement

Jain, A. K.; Ross, Arun; Prabhakar, Salil (January 2004), "An introduction to biometric recognition", *IEEE Transactions on Circuits and Systems for Video Technology* **14th** (1): 4–20

Characteristics of a Biometric

(practical considerations)

- Performance
  - accuracy, **speed**, and robustness of technology used
- Acceptability
  - degree of approval of a technology
- Circumvention
  - ease of use of a substitute

Jain, A. K.; Ross, Arun; Prabhakar, Salil (January 2004), "An introduction to biometric recognition", *IEEE Transactions on Circuits and Systems for Video Technology* **14th** (1): 4–20

Modes of Use

- Enrollment – Capturing and storing the biometric into a database
- Verification – A one to one comparison of a captured biometric with a stored template to verify/confirm identity
- Identification – A one to many comparison of the captured biometric against a biometric database in attempt to identify an unknown individual

DNA Typing as a Biometric

Advantages

- High level of accuracy (Gold Standard)
- Solid foundation of Forensic DNA Testing (pop stats, molecular biology, court acceptance, protocols, training, education)
- Kinship determination (unique to DNA)
- Potential use for:
  - Phenotype (traits; eye/hair color)
  - Ancestry

Challenges

- Expensive
- Time consuming
- Sample collection (invasive, stability issues)
- Technical expertise required for analysis
- Low level template, mixtures, PCR inhibition
- Policy/Privacy/Ethical issues

Interest in Rapid DNA Typing

- DoD (field testing, rapid intelligence, mass fatalities)
- DHS (kinship determination, border security, immigration)
- DoJ (law enforcement, initial information)
- Industry (security, authentication)
- Each customer will have specific requirements
  - sample input
  - information output
  - degrees of 'accuracy'

The time required for generating a STR profile will have to be significantly reduced

Sample Collection

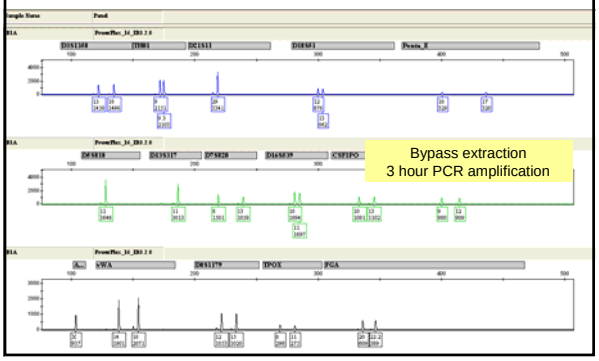
- Similar to collecting a reference sample
  - Single source, human specific
  - No quantitation
- Collected on site (buccal swab)
  - Not sample limited
    - > 100 ng of template DNA
  - No mixtures, no LCN, no inhibitors

DNA Analysis Approaches (non-integrated)

Steps Involved	*Traditional Protocols*	*Rapid Improvements (Buccal)*	*Rapid Improvements (Direct PCR)*
Collection			
Extraction	1.5 hours (Manual)	30 min. (Automated Extraction Qiagen EZ1 Advanced)	 Blood Stain
Quantitation	1.5 hours (qPCR)	1.5 hours	
Amplification	3.5 hours	<36 min. (Rapid PCR conditions)	3 hour (Using PowerPlex 16 HS or Identifiler Direct for direct amplification from a 1.2mm blood punch)
Separation/ Detection	1 hour (Capillary Electrophoresis ABI 3130xl)	1 hour	1 hour
Data Interpretation	Time may vary depending on software, sample quality and analyst expertise		
Total Time	Minimum ~7.5 hours	~4 hours	~4 hours

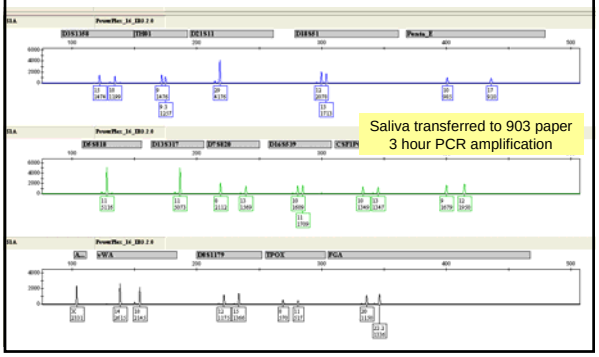
Direct PCR

PowerPlex16 HS (25 µL reaction, 32 cycles)
1.2 mm **blood** punch (903 paper)



Direct PCR

PowerPlex16 HS (25 µL reaction, 32 cycles)
1.2 mm **saliva** punch (903 paper)



Saliva transferred to 903 paper
3 hour PCR amplification

Recent Work with Rapid PCR

Developing rapid PCR protocols
Multiplex amplification of STR kits in 20 - 30 min

- Evaluating faster polymerases
- Faster thermal cyclers
- Deviating from standard STR typing kit protocols

~3.5 h → ~20 min?

Multiplex PCR Amplification

DNA Polymerases

- Takara
  - SpeedStar
- Fermentas
  - PyroStart Master Mix
- Qiagen
  - QIAGEN Fast Cycling PCR Kit
- New England Biolabs/Finnzymes
  - Phusion DNA Polymerases

General characteristics

2 - 5x faster processivity than TaqGold

1-5 min hot start at 95°C

Minimal post-cycling 'soak'

Thermal Cyclers

Cepheid SmartCycler
Ramp rate = 10ºC/s

Eppendorf
Mastercycler pro
Ramp rate = 6ºC/sec

Qiagen Rotor-Gene
Ramp rate = 15ºC/s

PCR Thermal Cycling Profile

28 cycles of PCR

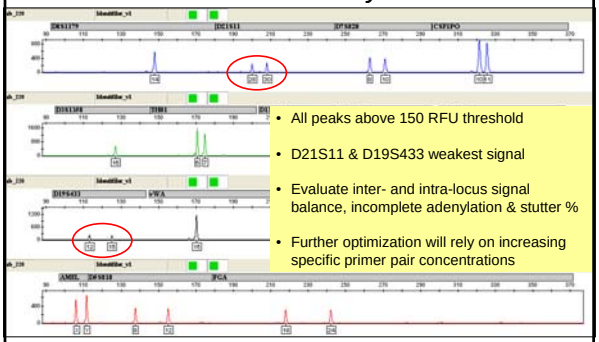
95ºC	95ºC	58ºC	72ºC	60ºC
10 min	1 min	1 min	1 min	60 min

95ºC	95ºC	58ºC	72ºC	72ºC
1 min	5 s	10 s	10 s	1 min

Sub 36 min run time

Maximum heating rate of ~4 to 10ºC/s (cycler dependent)

36 Minute PCR Amplification on AB 9700 Cycler



- All peaks above 150 RFU threshold
- D21S11 & D19S433 weakest signal
- Evaluate inter- and intra-locus signal balance, incomplete adenylation & stutter %
- Further optimization will rely on increasing specific primer pair concentrations

28 cycles, Identifiler STR kit, 1 ng of DNA, 10 µL rxn volume

36 Minute PCR Amplification on Rotor-Gene Cycler

28 cycles, Identifiler STR kit, 1 ng of DNA, 10 µL rxn volume

20 Minute PCR Amplification on Cepheid Cycler

28 cycles, Identifiler STR kit, 1 ng of DNA, 10 µL rxn volume

19 Minute PCR Amplification on Eppendorf Cycler

28 cycles, Identifiler STR kit, 1 ng of DNA, 10 µL rxn volume

Rapid PCR Article


ARTICLE IN PRESS

Forensic Science International: Genetics

Short communication

Demonstration of rapid multiplex PCR amplification involving 16 genetic loci

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

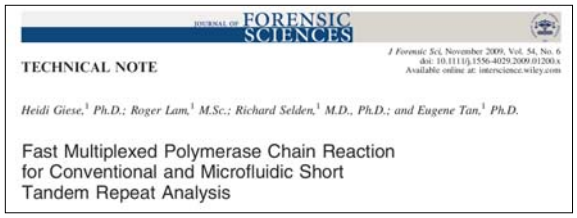
Vallone, P.M., Hill, C.R., Butler, J.M. (2008) Demonstration of rapid multiplex PCR amplification involving 16 genetic loci. *FSI Genetics* 3(1): 42-45.

Rapid PCR Amplification of STR Typing Kits 20th Annual International Symposium on Human Identification (Promega Meeting) October 14, 2009, Las Vegas, NV

Rapid Amplification of Commercial STR Typing Kits, International Society of Forensic Genetics (ISFG), September 16, 2009, Buenos Aires, Argentina

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Rapid PCR of STRs on a chip-based thermal cycler


JOURNAL OF FORENSIC SCIENCES

TECHNICAL NOTE

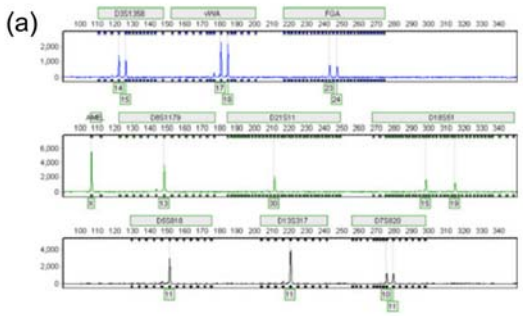
J Forensic Sci, November 2009, Vol. 54, No. 6

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

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

- Multiplex amplifications in microfluidic biochip-based thermal cycler in 17.3 min
- Full CODIS-compatible profiles were generated using the Profiler Plus ID, COfiler and Identifiler primer sets

Profiler Plus® ID Profile



Giese et. al., Fast Multiplexed Polymerase Chain Reaction for Conventional and Microfluidic Short Tandem Repeat Analysis *J Forensic Sci*, Vol. 54, 1287-1296

Current State of Rapid PCR Protocols

- Rapid amplification of at least 16 loci is possible
  - 17.3 minutes
- Faster DNA polymerases and thermal cyclers are required
- Optimized rapid STR typing kits could be produced for
  - chip based thermal cyclers
  - standard bench top cyclers
- Success with ~1 ng of DNA template (single source)
- Sub 45 minute PCR will be essential for rapid typing in a integrated/ portable system

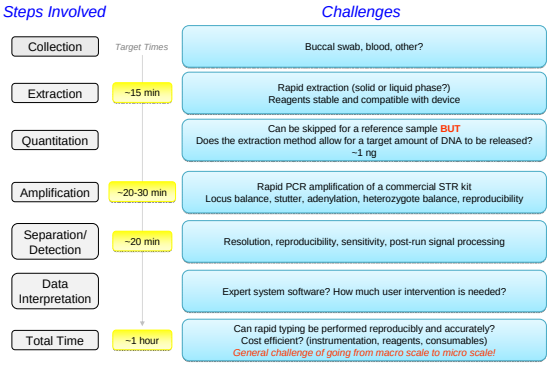
Goals for Rapid DNA Typing Systems

- Develop an integrated system capable of performing DNA testing in less than 1 hour

- Little user interaction (or experience)
- Rugged
- Robust
- Simple data interpretation
- 4-16 samples per run
- Disposable chips (with reagents on board)

Swab in…answer out

DNA Analysis Approach (integrated)



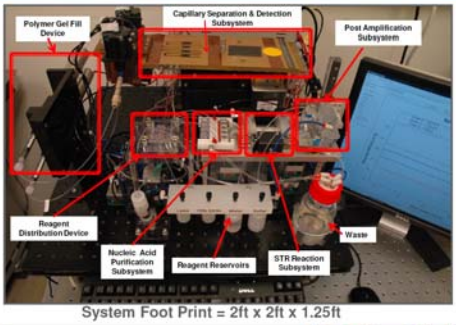
Rapid DNA Typing Systems Under Development

- The following slides were generously shared by the developers of integrated DNA typing instrumentation
- The systems are currently under development and are not yet commercially available
- Network Biosystems (Woburn, MA) http://www.netbio.com
- MicroLab Diagnostics and Lockheed Martin (Charlottesville,VA) http://www.microlabdiagnostics.com
- Microchip Biotechnologies, Inc. (Pleasanton, CA) http://www.microchipbiotech.com
- Forensic Science Service (UK) http://www.forensic.gov.uk/

Friday Session I - Robotics and New Technology 8 AM – 10 AM

Microchip Biotechnologies, Inc. (slide courtesy of Helen Franklin)

Apollo 200: 4-Channel Integrated Breadboard



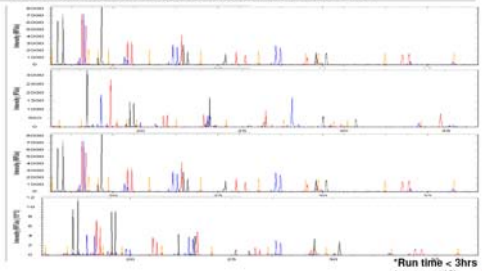
System Foot Print = 2ft x 2ft x 1.25ft

 2 Integrate. Automate. Simplify.

Microchip Biotechnologies, Inc. (slide courtesy of Helen Franklin)

Apollo 200: Fully Integrated and Automated Runs

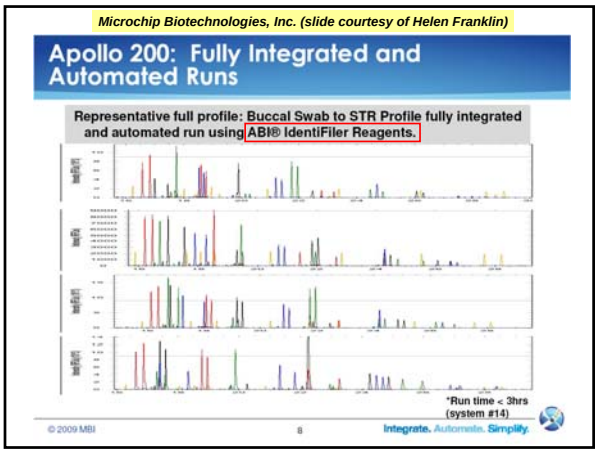
Representative full profile: Buccal Swab to STR Profile fully integrated and automated run using Promega® PowerPlex 16 HS.



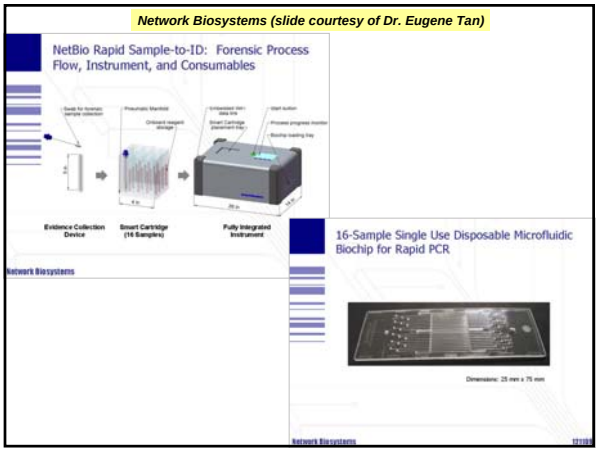
*Run time < 3hrs (system #13)

 7 Integrate. Automate. Simplify.

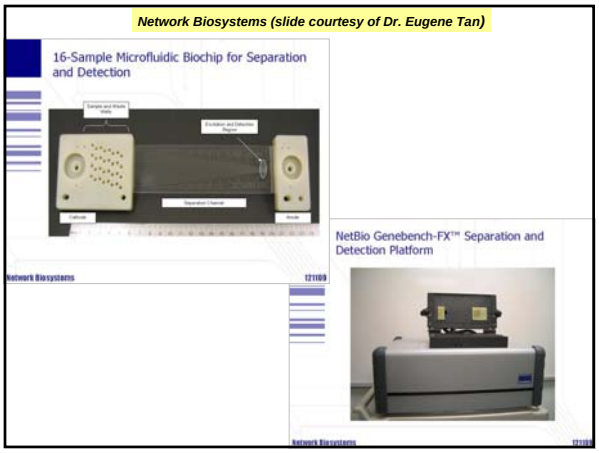
Microchip Biotechnologies, Inc. (slide courtesy of Helen Franklin)



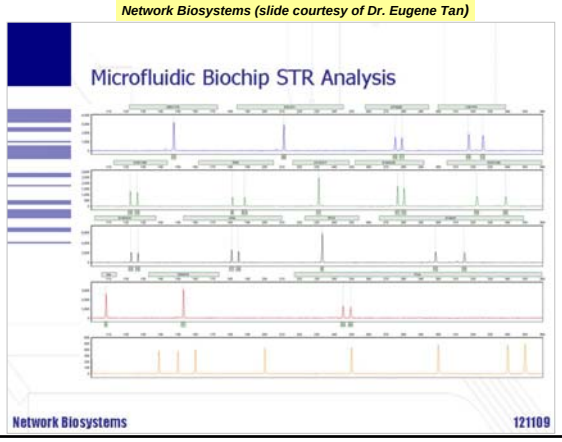
Network Biosystems (slide courtesy of Dr. Eugene Tan)



Network Biosystems (slide courtesy of Dr. Eugene Tan)



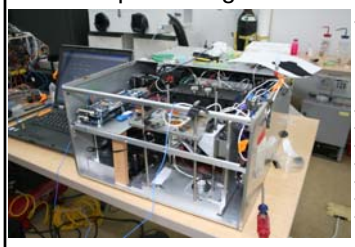
Network Biosystems (slide courtesy of Dr. Eugene Tan)



Lockheed Martin & MicroLab Diagnostics (slide courtesy of Dr. Joan Bienvenue)

DNA Analysis for Human ID ***RapI.D.™***

2009 Alpha Design

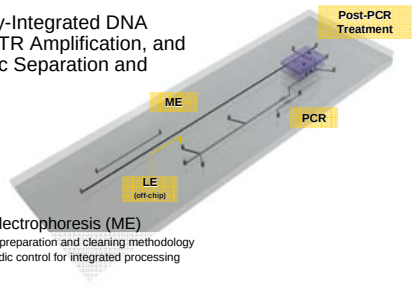


- Wet swab sample to analysis In ~ 75 min; partial profile
  - •Liquid Extraction
  - •PCR
  - •Separation & Detection
- Smaller box 24" by 10" by 18"
- More ruggedized design
- Improved valving
- IR light source improvement
- On-box syringe heater
- Analysis software improvements
- Configured for future growth

Lockheed Martin & MicroLab Diagnostics (slide courtesy of Dr. Joan Bienvenue)

Integrated DNA Analysis

- Microfluidically-Integrated DNA Purification, STR Amplification, and Electrophoretic Separation and Detection

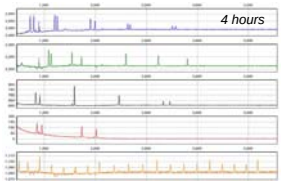


  - PCR-Micro Electrophoresis (ME)
    - Modified chip preparation and cleaning methodology
    - Optimized fluidic control for integrated processing

Forensic Science Service (slide courtesy of Keith Elliott & Dr. Gillian Tully)

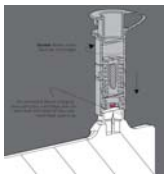
Primary Aim:
Evidential Quality

- Current status
  - Single sample: reduce error
  - Off chip lysis - flexibility
  - Lysate to profile with no manual intervention
  - Plastic integrated cartridge
  - Integrated transfer to glass CE chip (1.2bp resolution)



FORENSIC SCIENCE SERVICE

Forensic Science Service (slide courtesy of Keith Elliott & Dr. Gillian Tully)



- In development
  - Integration of sample collection cartridge
  - Single pre-loaded plastic cartridge for whole process
  - Fully automated “sample to name” <2h
  - Multiple cartridge loading capability for multiple sample analysis with maximum flexibility

FORENSIC SCIENCE SERVICE

Benefits of Developing Integrated Devices

Potential

- Pushing technology and reagent development forward
  - Faster methods of DNA extraction
  - Faster PCR cycling protocols, optimized STR kits
  - Alternative chip electrophoresis, faster separations
- Advances can be applied to benefit DNA typing performed in a lab setting
  - after proper validation studies

Benefits of Developing Integrated Devices

- Functional prototypes should be available for testing in the next 12-18 months
- 3-4 year horizon until concordance testing and validation
- The use of rapid DNA testing as a biometric would have an impact in various areas:
  - field testing, reference samples, rapid intelligence, mass fatalities, kinship determination, airport and border security, immigration, booking stations
  - other identification needs e.g., bioagent/pathogen detection, clinical diagnostics

Resources & Websites

- FBI Biometric Center of Excellence
  - http://www.biometriccoe.gov
- Biometric Consortium
  - http://www.biometrics.org/
- Biometrics.gov
  - http://www.biometrics.gov/default.aspx
- IEEE Biometrics Council
  - http://ieee-biometrics.org/
- Biometric Task Force
  - http://www.biometrics.dod.mil/

Acknowledgements

- Erica Butts (NIST)
- Dr. Kristen Lewis (NIST)

- Helen Franklin (Microchip Biotechnologies)
- Dr. Eugene Tan (Network Biosystems)
- Dr. Joan Bienvenue (Lockheed Martin)
- Dr. Gillian Tully, Keith Elliott and Dr. Andrew Hopwood (FSS)

- FBI for funding (Evaluation of DNA as a Biometric)

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
peter.vallone@nist.gov