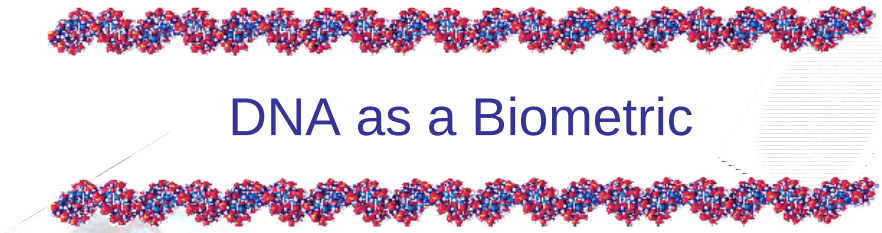

DNA as a Biometric

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

George Washington University
Washington, D.C.
Friday November 12, 2010

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Outline

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

What is a Biometric?

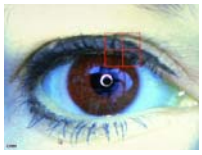
Method for uniquely recognizing humans based upon one or more intrinsic physical or behavioral traits

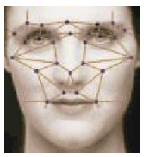
Jain, A. K.; Ross, Arun; Prabhakar, Salil (January 2004), "An introduction to biometric recognition", *IEEE Transactions on Circuits and Systems for Video Technology* **14th** (1): 4–20

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

Enrollment

Verification

Identification

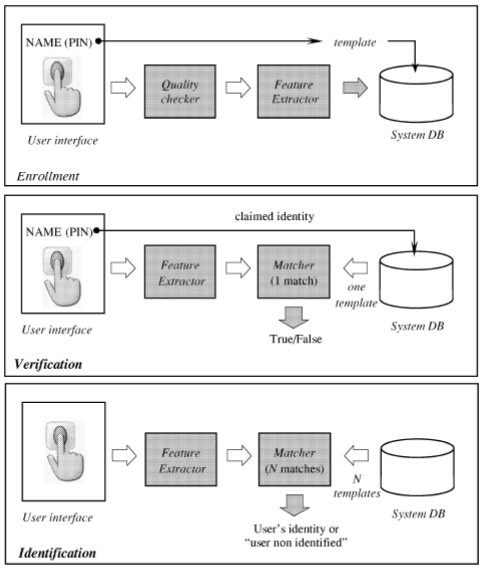


Fig. 1. Block diagrams of enrollment, verification, and identification tasks are shown using the four main modules of a biometric system, i.e., sensor, feature extraction, matcher, and system database.

Biometric ?⟷ Forensic

Person ⟶ Biometric ⟶ Event

Event ⟶ Forensic ⟶ Person

Biometric ?⟷ Forensic

- Pre-event
- Verification
- Identification
- Rapid answer – real time
- Cooperative – non intrusive
- High quality, (not necessarily) stored (quality capture)
- Post Event
- Application to legal issues/problems
- Evidence from an event is analyzed off line afterwards
- Non-cooperative (mask evidence), intrusive
- Data stored

DNA Typing as a Biometric

Advantages

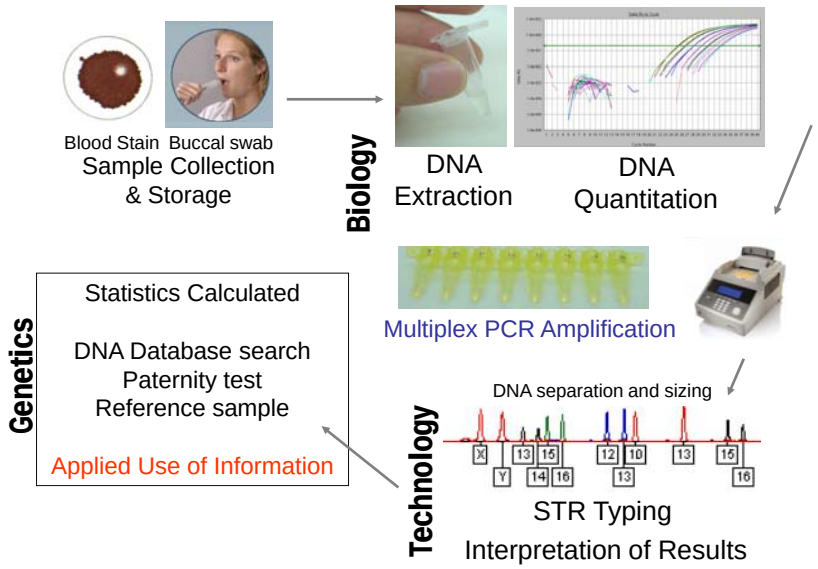
- High level of accuracy (Gold Standard)
- Solid foundation of Forensic DNA Testing (pop stats, molecular biology, court acceptance, protocols, training, education)
- Kinship determination (unique to DNA)
- Potential use for:
  - Phenotype (traits; eye/hair color)
  - Ancestry

Challenges

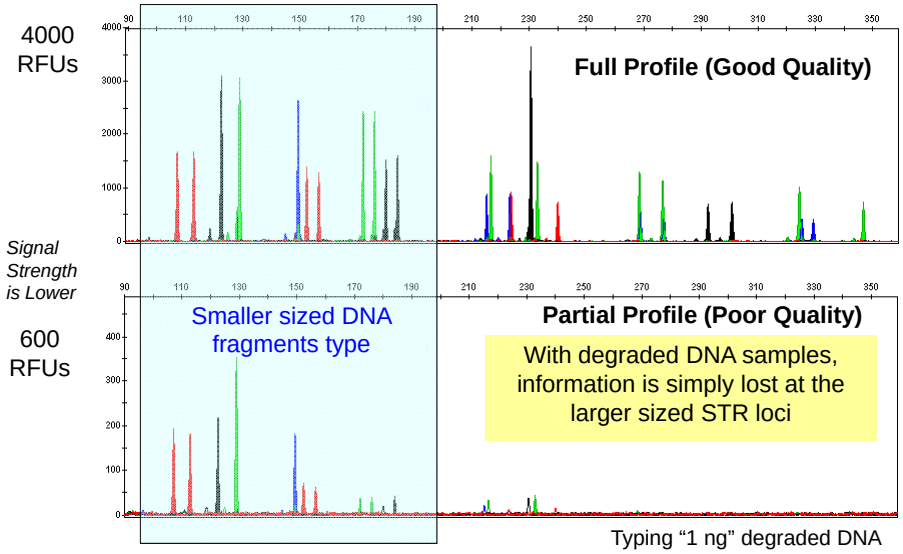
- Expensive
- **Time consuming**
- Sample collection (invasive, stability issues)
- Technical expertise required for analysis
- Low level template, mixtures, PCR inhibition
- Policy/Privacy/Ethical issues

Steps in Forensic DNA Analysis

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

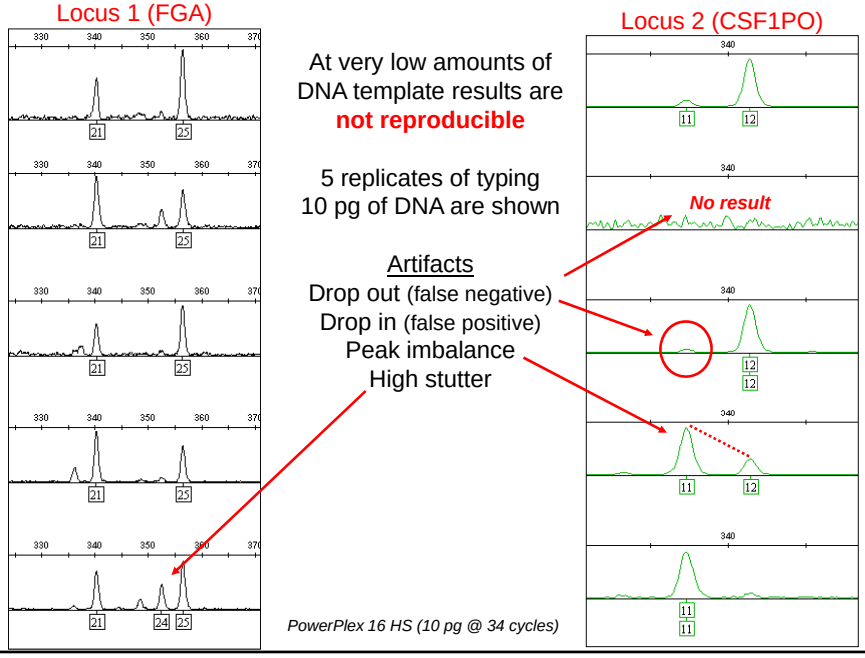


Same DNA with Different Quality



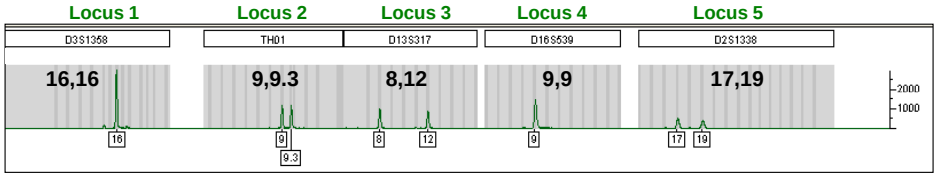
Typing “1 ng” degraded DNA

Low Levels of DNA Template



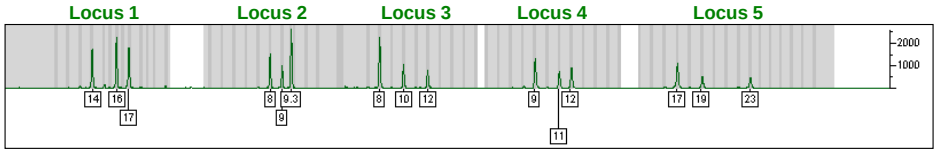
Mixture Interpretation – A Major Challenge…

Single Source Sample



One or two peaks observed at each locus (tested DNA region)

Mixture Sample



More than two peaks observed at more than two loci (tested DNA regions)

Different possible combinations could have given rise to the particular mixture observed

Biometric 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

Interest in Rapid DNA Typing

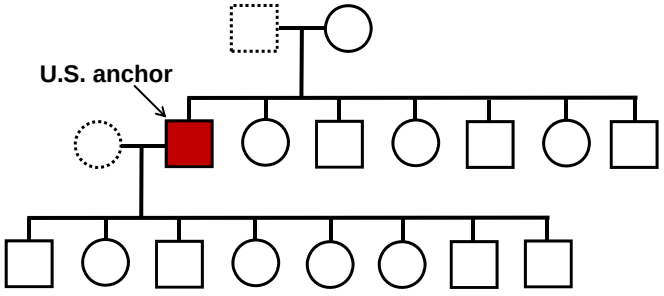
- DoD (field testing, rapid intelligence, mass fatalities)
- DHS (kinship determination, border security, immigration)
- DoJ (law enforcement, initial information)
- Industry (security, authentication)

- 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

Immigration Testing

U.S. Department of Homeland Security



Anchor may sponsor up to 15 relatives (spouse, parents, siblings, children)

79% of refugee claims were fraudulent based on DNA testing or failure to appear for DNA testing (U.S. Dept. of State)

DHS is looking to require DNA to support relationship claims

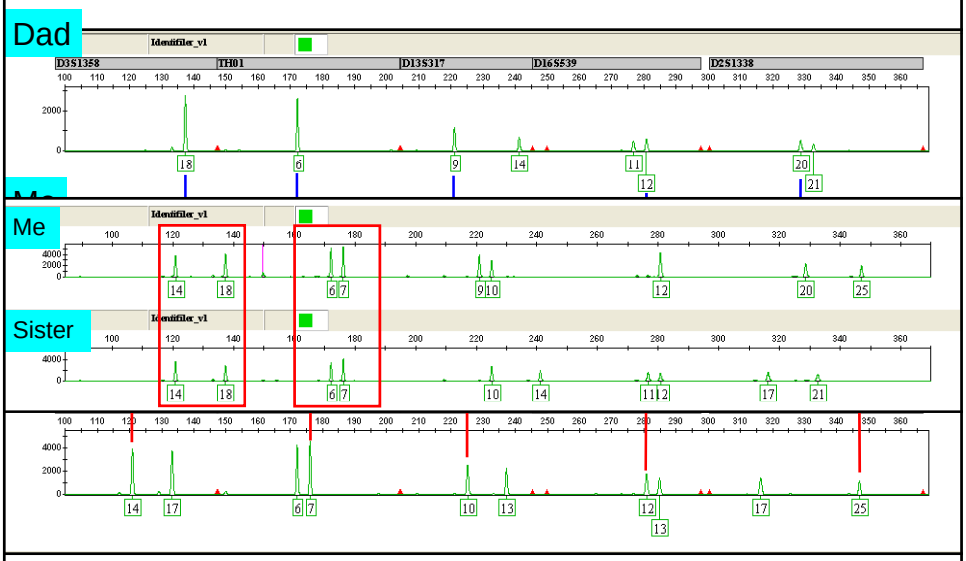
Kinship Testing

- DNA profiles can also be used to evaluate the probability of a specific familial relationship
- As a familial relationship becomes more distant, the ability of DNA to confirm the likelihood of that relationship decreases
  1. Parent-offspring
  2. Siblings
  3. Half siblings = uncle/nephew = grandchild
  4. Cousins

Allele Sharing Probabilities

Relationship	0 alleles	1 alleles	2 alleles
Parent-child	0	1	0
Full siblings	1/4	1/2	1/4
Half siblings	1/2	1/2	0
Cousins	3/4	1/4	0
Uncle-nephew	1/2	1/2	0
Grandparent-grandchild	1/2	1/2	0

Autosomal Paternity Example

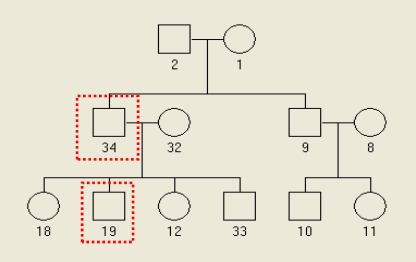


Likelihood Ratio

- Examining or counting shared alleles is qualitative
- Likelihood ratio can be used to help quantify the information

$$LR = \frac{\text{Probability of the evidence (genotypes) given a particular hypothesis}}{\text{Probability of the evidence (genotypes) given an alternative hypothesis}}$$

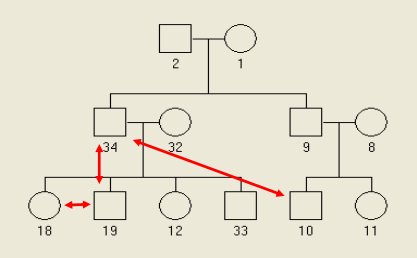
Posing a Hypothesis



- Basic equations for calculating LR are known
- Available in the literature and in commercial software
- Requirements:
- Genotypes of individuals being tested
- Allele frequencies for the loci involved in the testing
- **Two Hypotheses!**

$$LR = \frac{\text{Prob. of observing the genotypes for 19 and 34 given that they are parent–offspring}}{\text{Prob. of observing the genotypes for 19 and 34 given that they are unrelated}}$$

Likelihood Ratios with 15 Loci



Comparison	LR for 34 & 19	LR for 18 & 19	LR for 34 & 10
Parent-Child	1.28E+06		
Full Siblings		2.76E+07	
Half Siblings			
Cousins			
Uncle-Nephew			6.65E-01
Grandparent-Grandchild			
	Parent/ Child	Full Sibs	Uncle/ Nephew

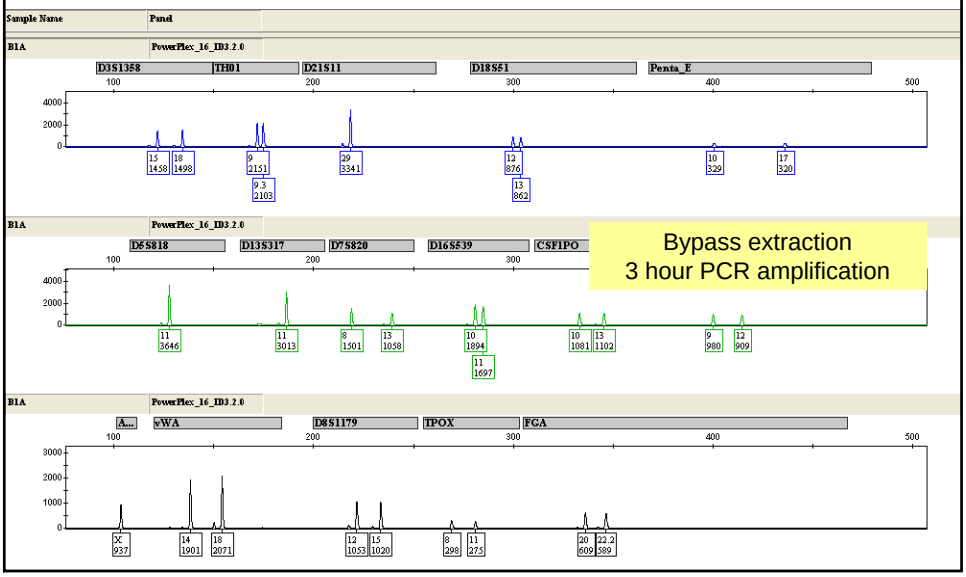
LR calculations were performed with GeneMarker HIDv1.90

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

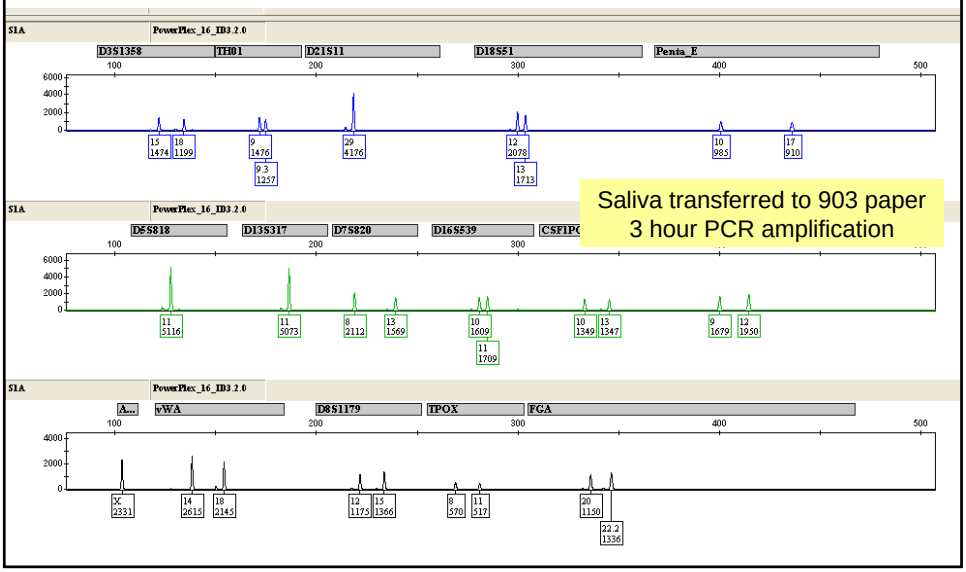
Direct PCR

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 = 10°C/s

Eppendorf
Mastercycler pro
Ramp rate = 6°C/sec

Qiagen Rotor-Gene
Ramp rate = 15°C/s

PCR Thermal Cycling Profile

28 cycles of PCR

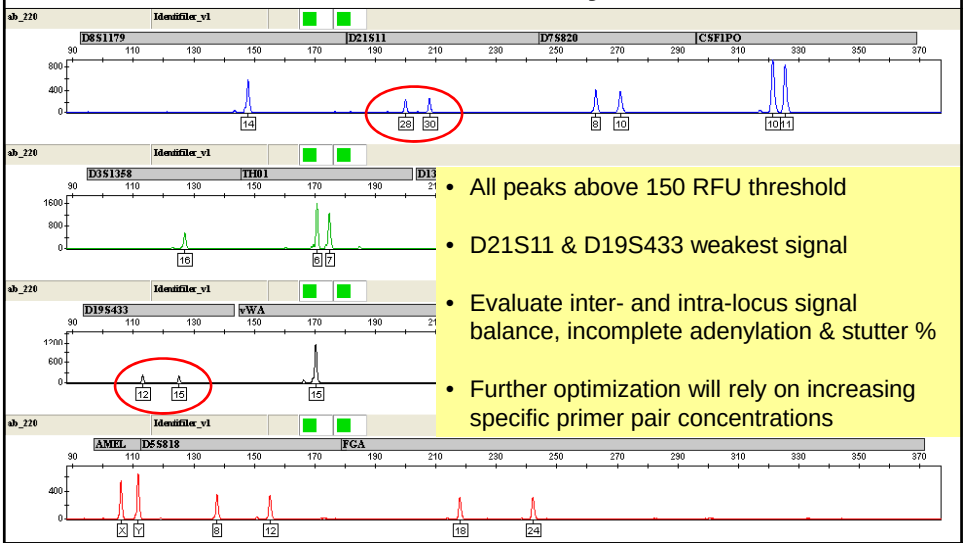
95°C	95°C	58°C	72°C	60°C
10 min	1 min	1 min	1 min	60 min

95°C	95°C	58°C	72°C	72°C
1 min	5 s	10 s	10 s	1 min

Sub 36 min run time

Maximum heating rate of ~4 to 10°C/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

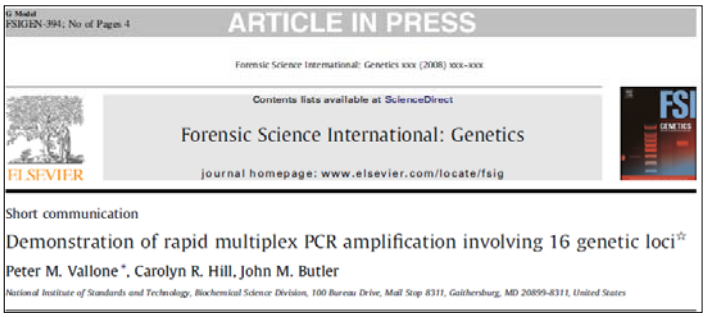
20 Minute PCR Amplification on Cepheid Cycler

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 xxx (2008) xxx–xxx

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

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

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

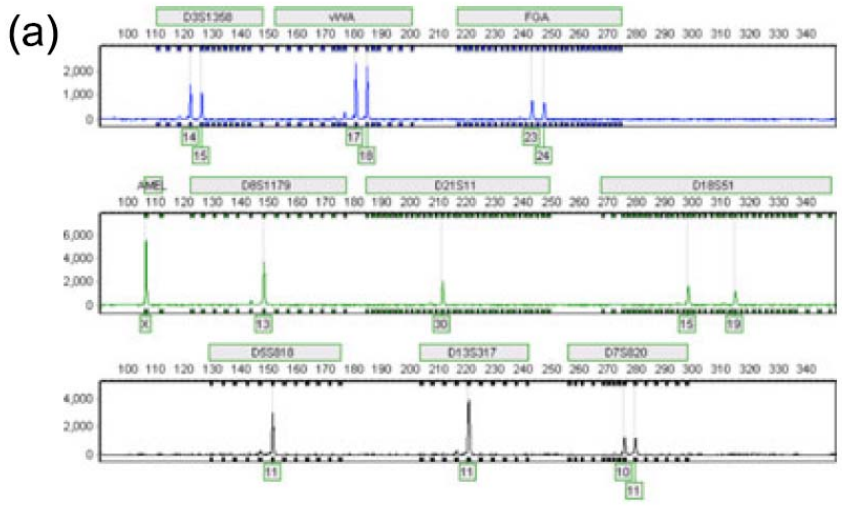
TECHNICAL NOTE

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

- 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

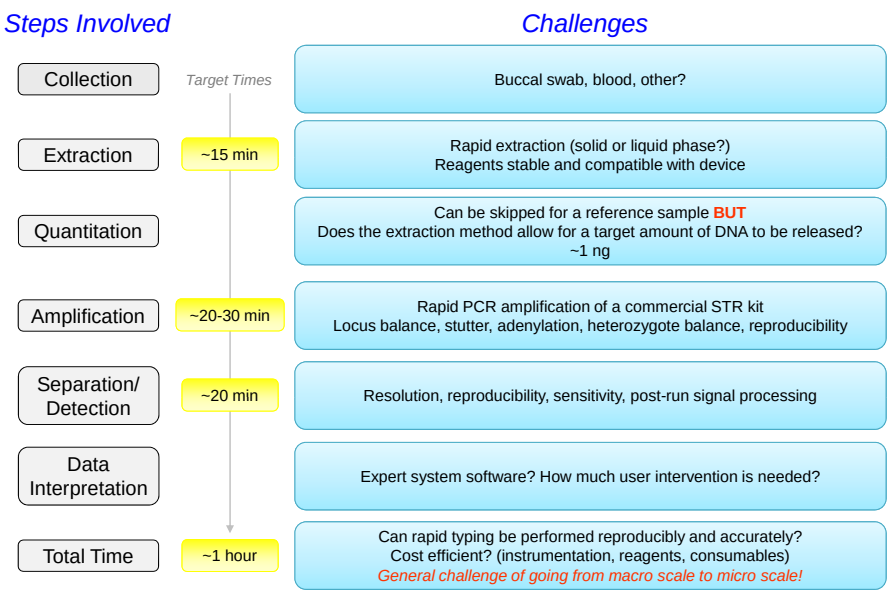
Goals for Rapid DNA Typing Systems

- 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)



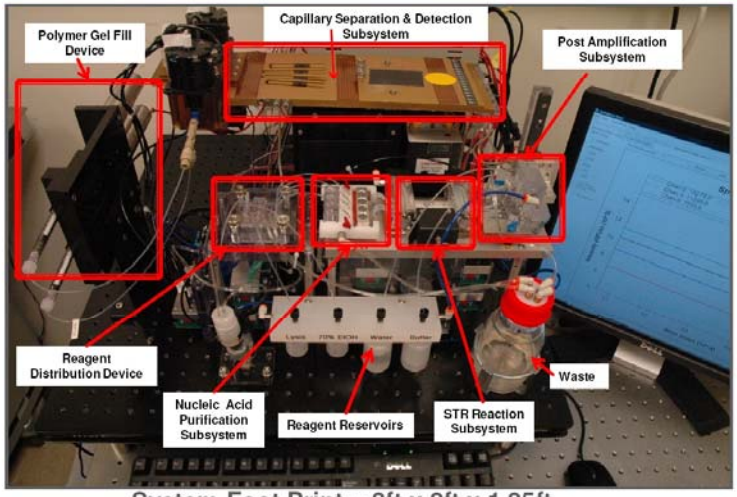
Rapid DNA Typing Systems Under Development

- Systems are currently under development and are not yet commercially available
- Network Biosystems (Woburn, MA) http://www.netbio.com
- ZyGEM and Lockheed Martin (Charlottesville,VA) http://www.zygem.com
- IntegenX (Pleasanton, CA) http://www.integenx.com
- Forensic Science Service (UK) http://www.forensic.gov.uk/

Use of DNA as a Biometric Tool American Academy of Forensic Science, Feb 22, 2010, Seattle, WA
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Biometrics Consortium Conference September, 2010 Tampa, FL
http://www.biometrics.org/bc2010/program.pdf

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

Apollo 200: 4-Channel Integrated Breadboard



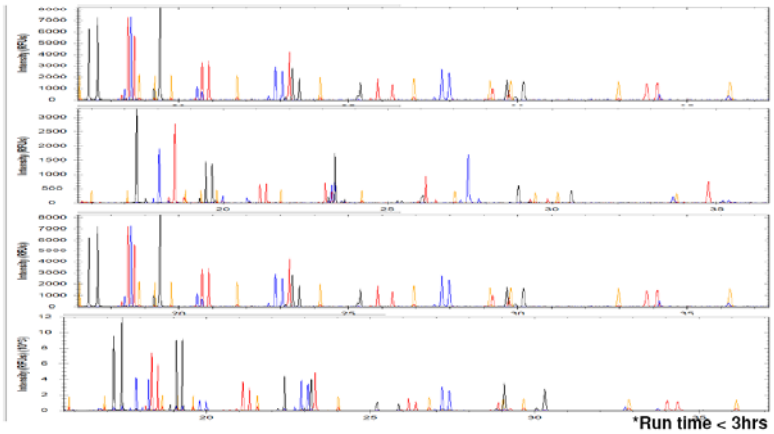
System Foot Print = 2ft x 2ft x 1.25ft

 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)

Apollo 200: Fully Integrated and Automated Runs

Representative full profile: Buccal Swab to STR Profile fully integrated and automated run using ABI® IdentiFiler Reagents.

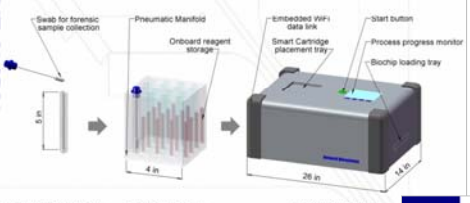
*Run time < 3hrs (system #14)

8

Integrate. Automate. Simplify.

Network Biosystems (slide courtesy of Dr. Eugene Tan)

NetBio Rapid Sample-to-ID: Forensic Process Flow, Instrument, and Consumables



Evidence Collection Device | Smart Cartridge (16 Samples) | Fully Integrated Instrument

Network Biosystems

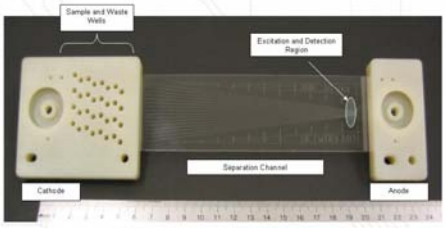
16-Sample Single Use Disposable Microfluidic Biochip for Rapid PCR

Dimensions: 25 mm x 75 mm

Network Biosystems 121109

Network Biosystems (slide courtesy of Dr. Eugene Tan)

16-Sample Microfluidic Biochip for Separation and Detection



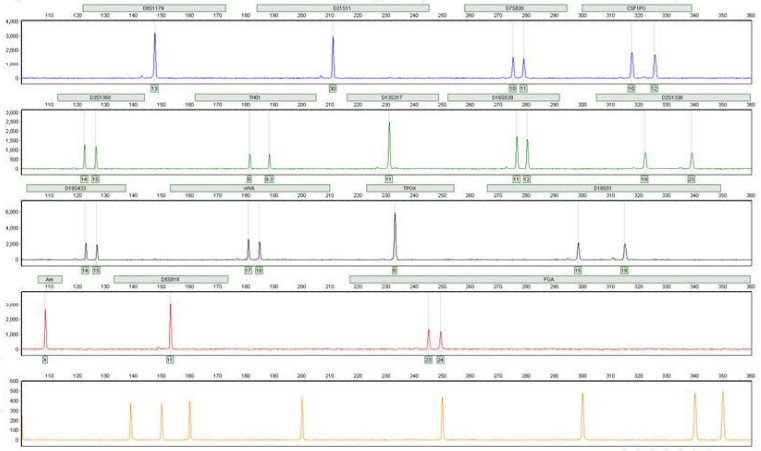
Network Biosystems 121109

NetBio Genebench-FX™ Separation and Detection Platform

Network Biosystems 121109

Network Biosystems (slide courtesy of Dr. Eugene Tan)

Microfluidic Biochip STR Analysis

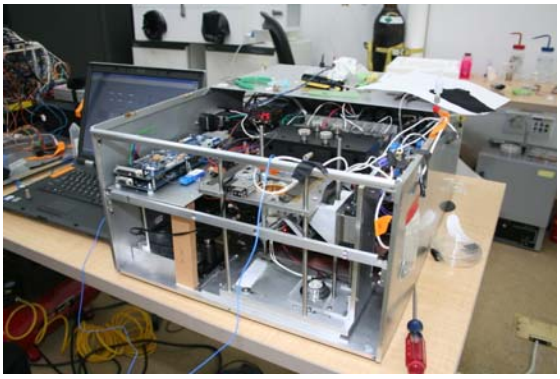


Network Biosystems 121109

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

DNA Analysis for Human ID *RapID.™*

2009 Alpha Design

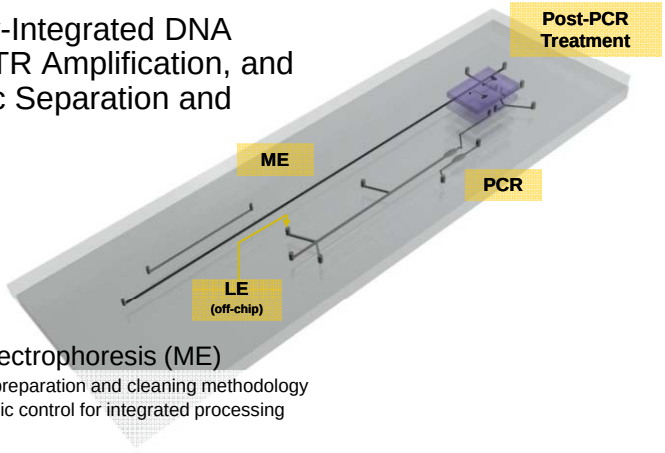


- Wet swab sample to analysis In ~ 75 min; partial profile
  - •Liquid Extraction
  - •PCR
  - •Separation & Detection
- Smaller box 24” by 10” by 18”
- More ruggedized design
- Improved valving
- IR light source improvement
- On-box syringe heater
- Analysis software improvements
- Configured for future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



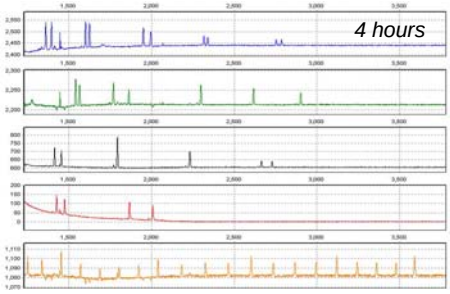
  - PCR-Micro Electrophoresis (ME)
    - Modified chip preparation and cleaning methodology
    - Optimized fluidic control for integrated processing

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

Primary Aim:
Evidential Quality

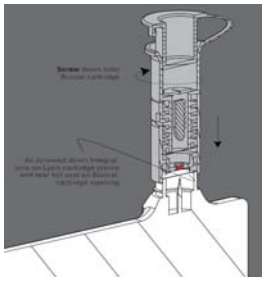
THE UNIVERSITY OF ARIZONA COLLEGE OF MEDICINE PHOENIX
in partnership with Arizona State University

- 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)



THE UNIVERSITY OF ARIZONA COLLEGE OF MEDICINE PHOENIX
in partnership with Arizona State University

- 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/
- Biometrics Task Force
  - http://www.biometrics.dod.mil/

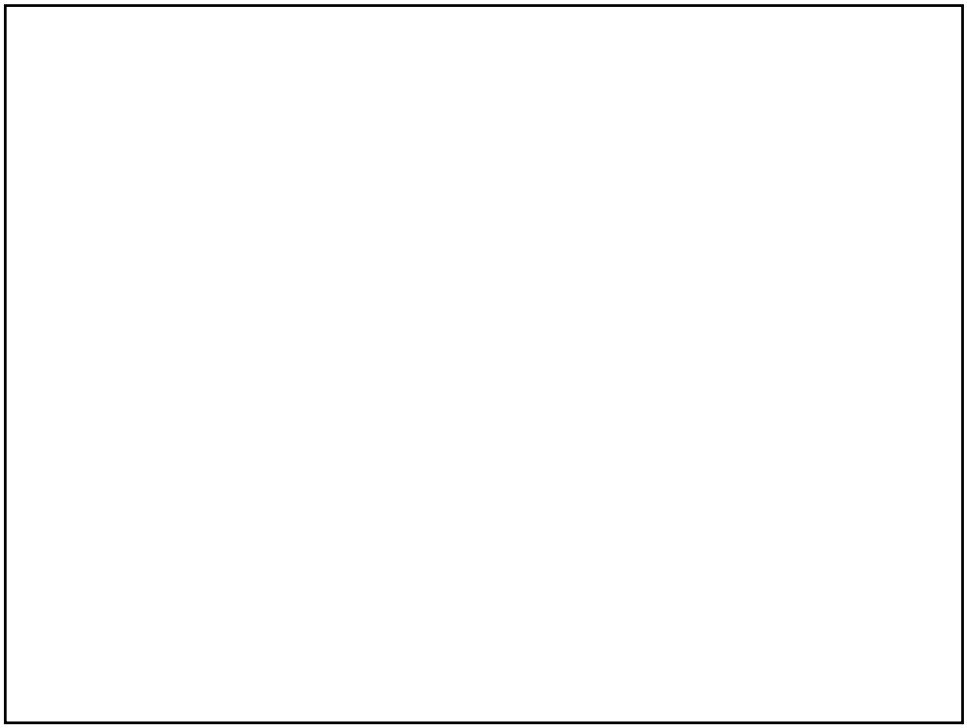
Acknowledgements

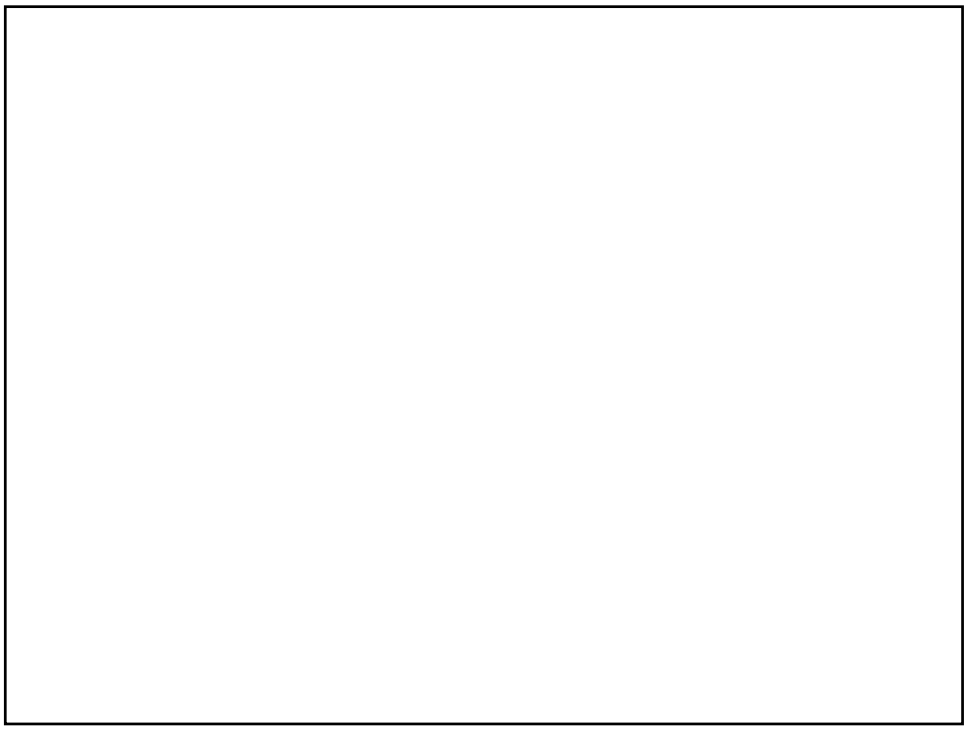
- Erica Butts (NIST)
- Dr. Kristen Lewis (NIST)

- 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





Rapid PCR

Applications

- Faster sample-to-answer
- Increase throughput
- Integrated platforms for forensics and biometrics
- Single source reference samples = 1ng of DNA

Develop a PCR protocol for typing the Applied Biosystems Identifiler STR kit in less than 1 hour

Initial Questions

- Robustness
- Sensitivity
- PCR artifacts
- Locus-to-locus balance

- Validation
- Mixtures
- PCR Inhibitors

Common Thermal Cycling Times

Can we reduce PCR cycling times? What are the effects or limitations?

Thermal Cycling Times for Current STR Typing Kits

Year	Run on a 9700 thermal cycler	Hot start	Time per cycle	Cycles	Post soak	Total time
1997/98	Profiler Plus/Cofiler	11 min	3 min	28	60 min	2:52
1999	SGM Plus	11 min	3 min	28	45 min	2:53
2000	PowerPlex 16	12 min	1 min 45 s	32	30 min	3:00
2001	Identifiler	11 min	3 min	28	60 min	2:58
2003	PowerPlex Y	12 min	1 min 45 s	32	30 min	3:18
2004	Yfiler	11 min	3 min	30	80 min	2:45
2007	PowerPlex S5	2 min	4 min	30	45 min	3:21
2007	minifiler	11 min	3 min 20 s	30	45 min	3:16
2009	ESI 16, 17 ESX 16,17	2 min	4 min	30	45 min	3:22
2009	PowerPlex 16 HS	2 min	1 min 45 s	32	30 min	2:42
2009	NGM	11 min	3 min 20 s	29	10 min	2:33
2009	Identifler Direct	11 min	3 min	26	25 min	2:34
2010	Idenfiler Plus	11 min	3 min 20 s	28	10 min	2:18

DNA Polymerases

- AmpliTaq Gold® is typically used
  - Heat activated (avoid non-specific PCR products)
- SpeedS
  - Extens
  - (comp ses)
  - Still us
  - Hot-start formulation is antibody mediated

Fermentas
~~PyroStart Master Mix~~

Qiagen
QIAGEN Fast Cycling PCR Kit

New England Biolabs/Finnzymes
Phusion DNA Polymerases

Thermal Cyclers

1. GeneAmp 9700 (Applied Biosystems)
2. Mastercycler Pro S (Eppendorf)
   - Peltier based
3. Rotor-Gene Q (Qiagen)
   - Air heated and cooled
4. SmartCycler (Cepheid)
   - Hot plates for heating, fans for cooling

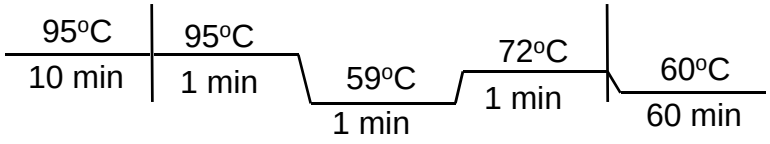
Intended for real-time PCR

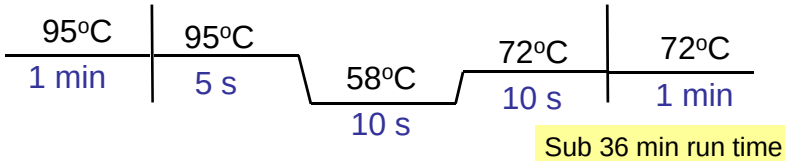
- Cycling for most STR kits is run in '9600 emulation mode' (1°C/s)

PCR Thermal Cycling Profile

Identifiler STR kit

28 cycles of PCR





Sub 36 min run time

Maximum heating/cooling rate of ~2 to 6°C/s (cycler dependent)

Rapid PCR Conditions

- 1 X Takara PCR mastermix, 1 U SpeedStar polymerase
  - *Premix Ex Taq*™ (Perfect Real Time)
- 10 μL total reaction in a thin walled tube (8-strip)
- 2 μL of Identifiler PCR primer mix
- ~1 ng of template DNA

- Utilize maximum ramp rate on thermal cyclers
  - GeneAmp 9700 = 1.6°C/s (36 min)
  - Rotor-Gene Q = 1.6°C/s (36 min)
  - SmartCycler = 5.8°C/s (20 min)
  - Mastercycler Pro S = 6.8°C/s (19 min)

Effective heating/cooling rates

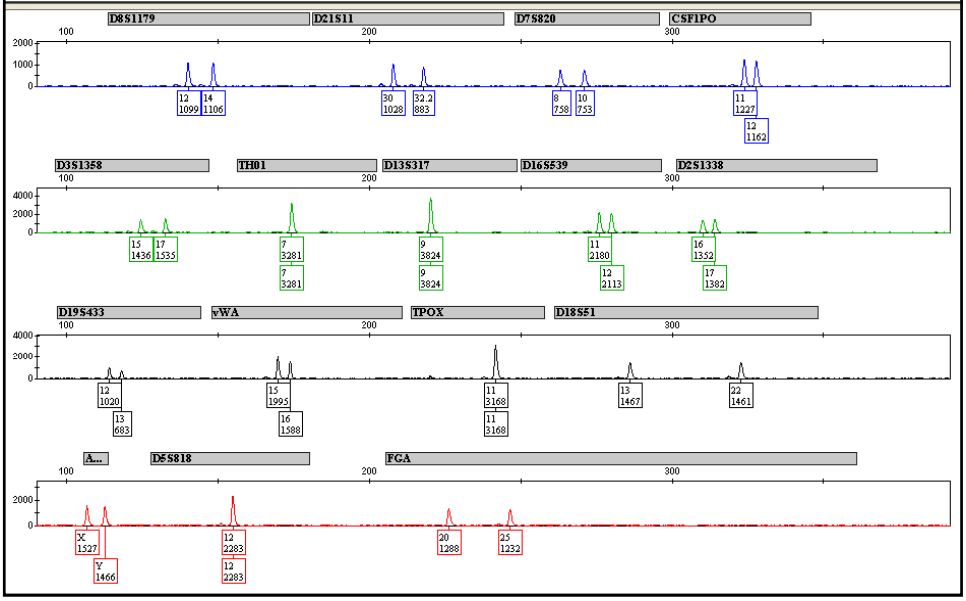
Experimental Design

- Type a set of NIST population samples (n=95)
- Evaluate
  - Full profiles (% success and concordance)
  - Identify artifacts of rapid PCR (adenylation, other)
  - Heterozygote peak height balance
  - Stutter %
  - Signal balance (locus-to-locus) and intensity
  - Sensitivity
- Not inhibitors or mixtures

Separated on a 3130xl
Injection = 3kV for 5 s
Allele calling threshold = 50 RFUs

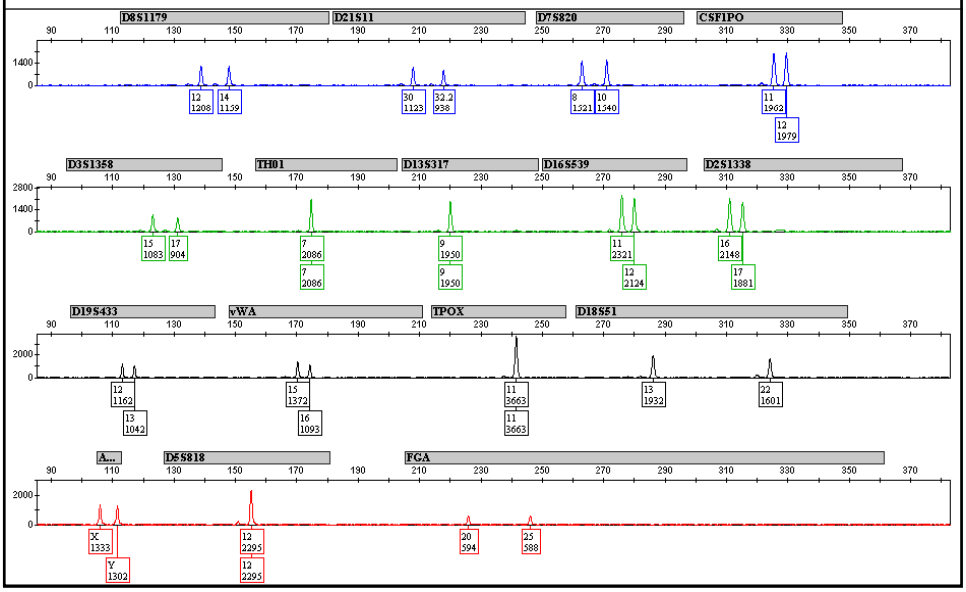
Identifiler STR Profile

GeneAmp 9700 – 36 min PCR



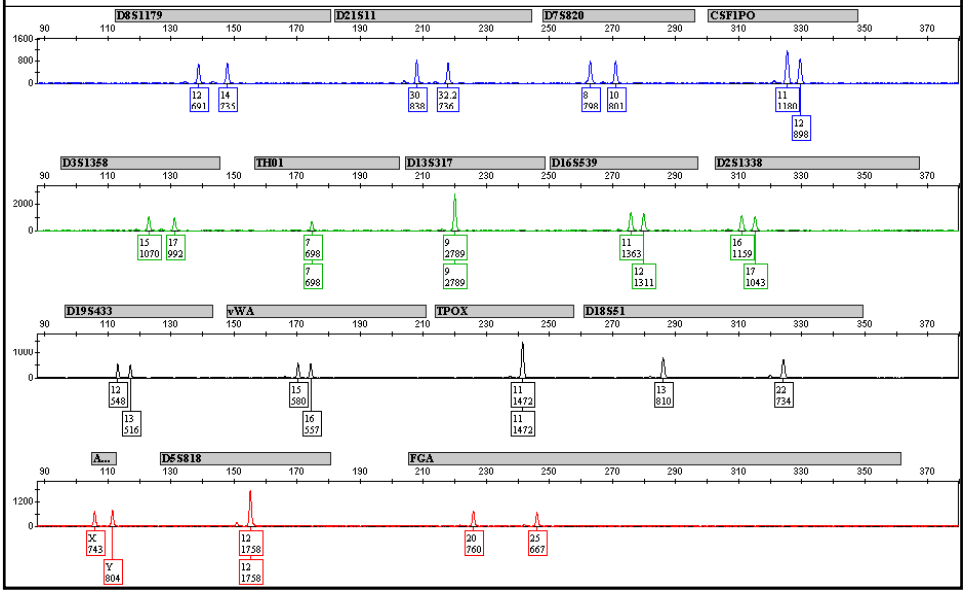
Identifiler STR Profile

Mastercycler Pro S - 19 min PCR



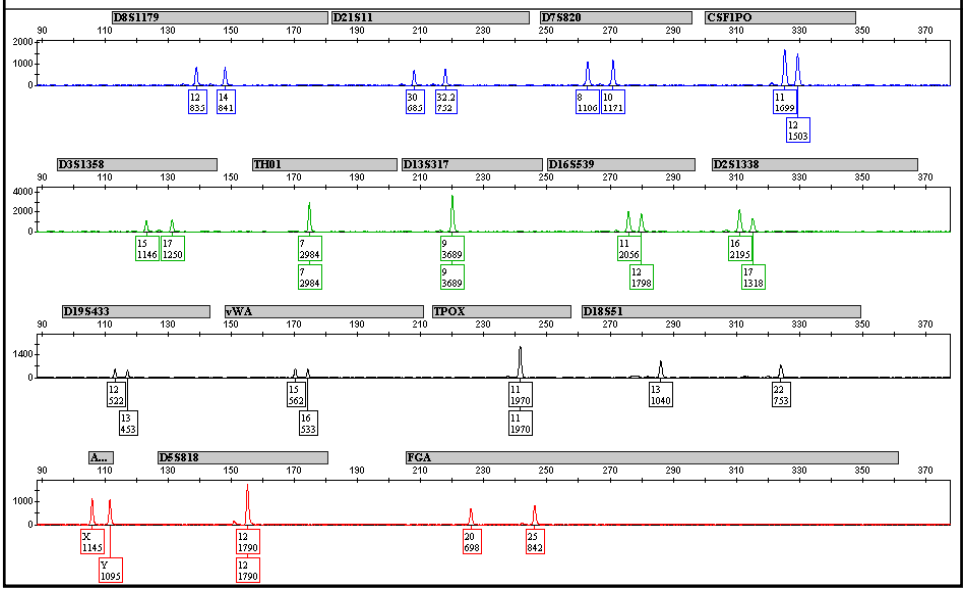
Identifiler STR Profile

Rotor-Gene Q – 36 min PCR



Identifiler STR Profile

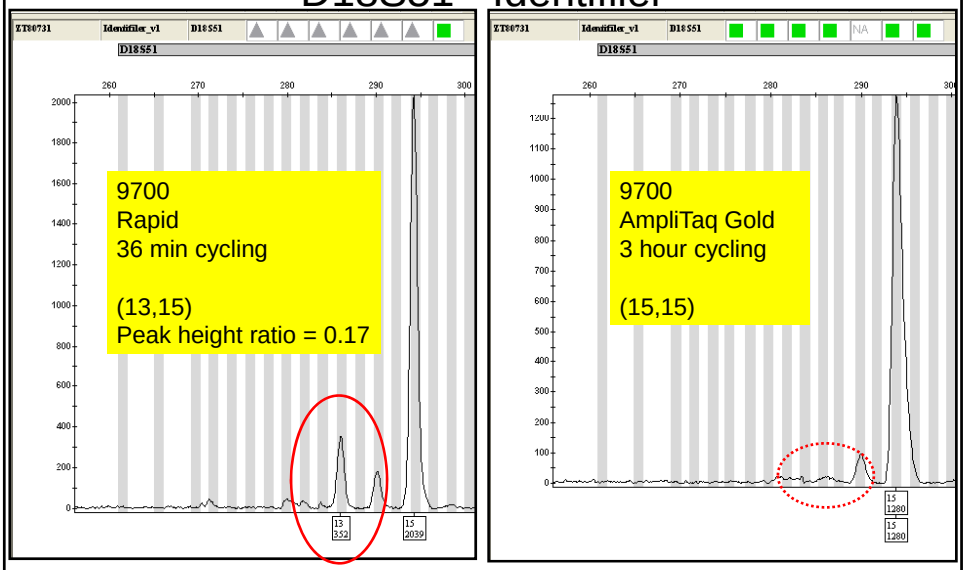
SmartCycler – 20 min PCR



Summary of Initial Results

- All 95 samples were successfully typed on each thermal cycler using the rapid PCR protocol
- One sample gave a discordant genotype

Discordant sample
D18S51 - Identifiler



Discordant sample

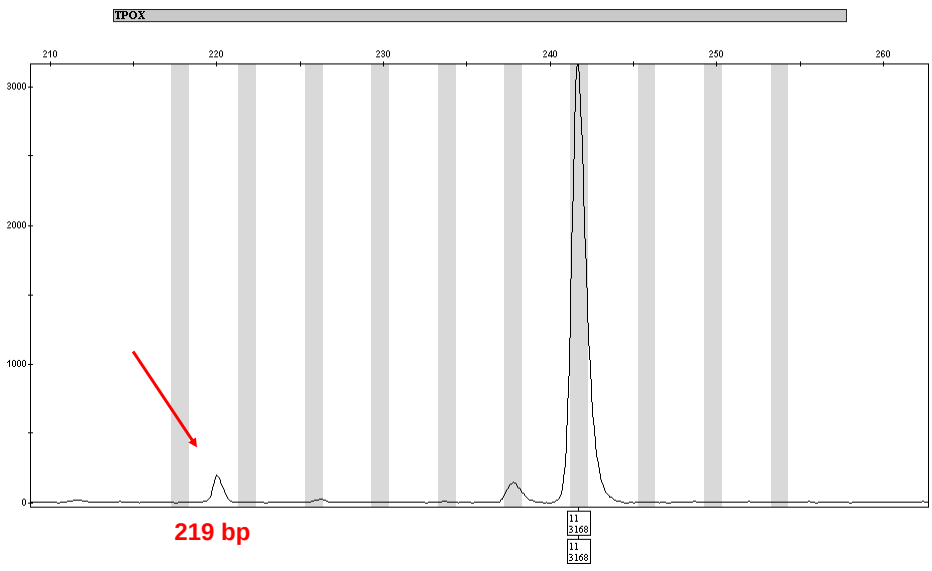
- Amplification with PowerPlex 16 indicated a (13,15) genotype
- A SNP under the binding site an Identifiler PCR primer results in the null allele*

*Hill et al., (2007) J. Forensic Sci. 52: 870-873

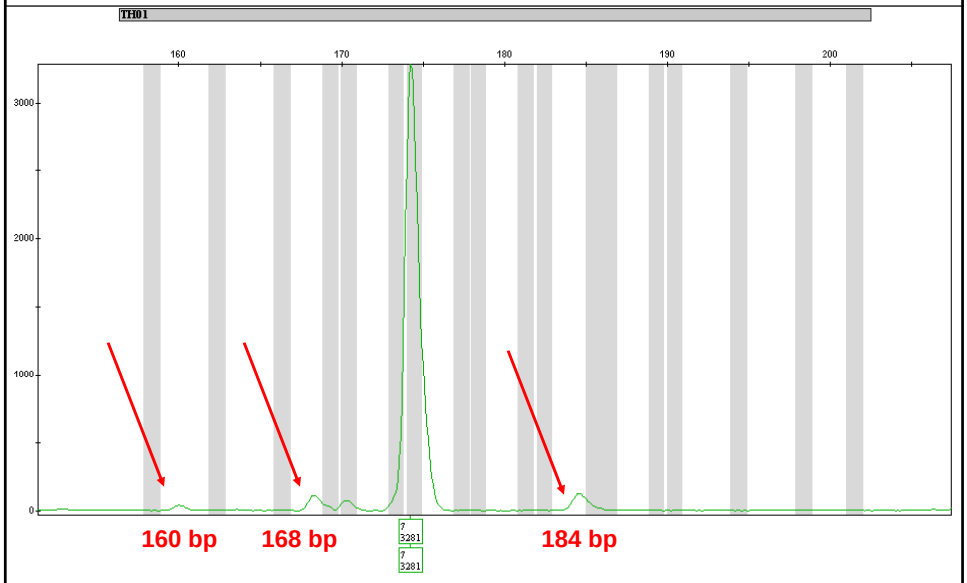
- Still present with a 59ºC annealing temperature
  - But with lower signal intensity

Rapid PCR Artifacts

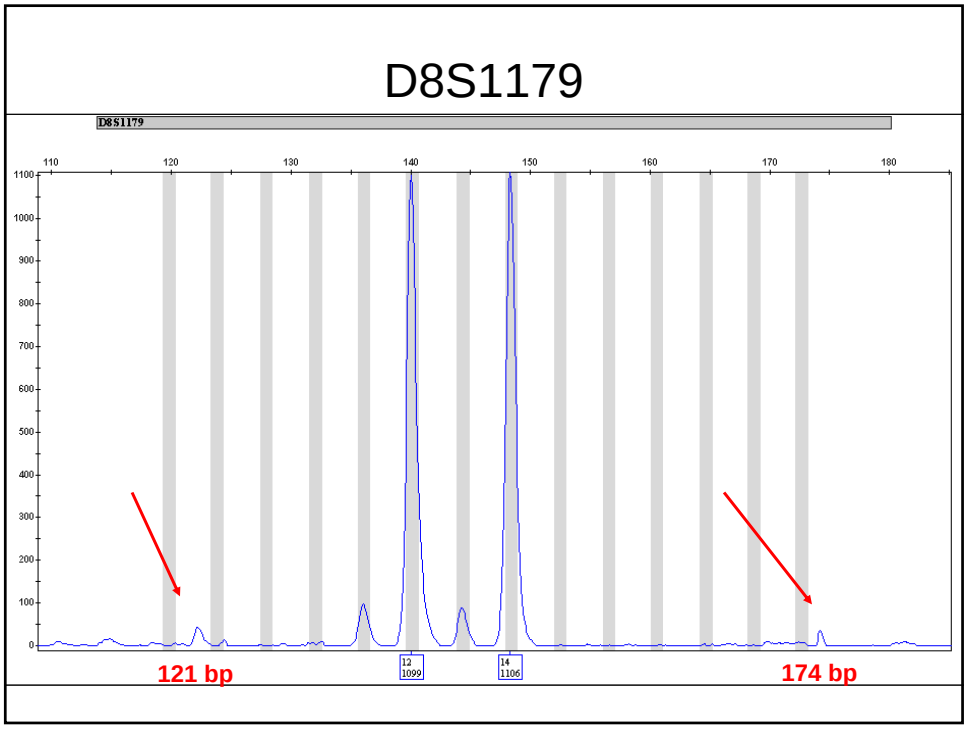
TPOX



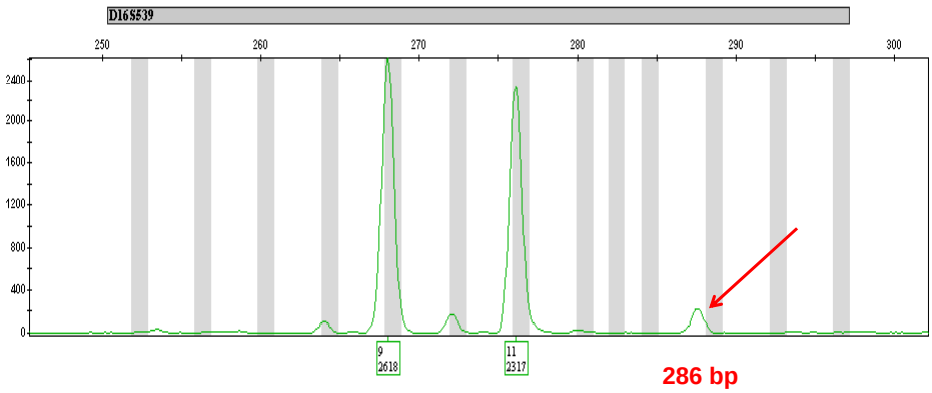
TH01



D8S1179



D16S539



Summary of PCR Artifacts

N = 95

Number of times an artifact was observed

PCR Artifacts Observed	9700	Smart Cycler	Master Cycler Pro	Rotor-Gene
D16S539 (287 bp)	35	4	1	6
D8S1179 (121 bp)	6	0	1	3
D8S1179 (174 bp)	14	10	1	7
TH01 (160 bp)	28	2	1	11
TH01 (168 bp)	83	32	1	40
TH01 (184 bp)	59	19	0	25
TPOX (219 bp)	77	13	2	22

Artifacts only called above 50 RFUs

PCR artifacts did not affect allele calls (not in bins) and exhibited signal intensities similar to stutter peaks

Artifact signal intensities varied based on cycler

Heterozygote peak height ratios

n = 95	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
9700	0.89	0.91	0.90	0.89	0.90	0.90	0.89	0.91	0.88	0.88	0.89	0.90	0.91	0.92	0.90	0.89
SmartCycler	0.90	0.88	0.89	0.87	0.88	0.89	0.88	0.89	0.88	0.89	0.87	0.90	0.86	0.90	0.88	0.89
Mastercycler pro	0.89	0.89	0.89	0.89	0.90	0.90	0.87	0.89	0.88	0.90	0.89	0.89	0.88	0.93	0.90	0.89
Rotor-Gene Q	0.88	0.85	0.86	0.88	0.88	0.90	0.86	0.88	0.88	0.88	0.87	0.89	0.86	0.90	0.88	0.89
9700 Taq Gold	0.87	0.89	0.88	0.84	0.84	0.88	0.83	0.88	0.84	0.87	0.86	0.88	0.84	0.88	0.88	0.86
SD <0.1																

- Average PHR for rapid PCR conditions > 0.85
- Standard deviation per locus < 0.1 (n=95)
- 1 ng of DNA amplified with the rapid PCR protocols exhibited heterozygote peak height balance comparable to traditional kit cycling conditions

Stutter Intensity

N > 80 for all points

	9700 Taq		9700		SmartCy		MasterC		Rotor-G	
Locus	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CSF1PO	3.9	1.8	7.4	4.3	6.8	2.6	7.5	3.3	6.7	2.5
D13S317	4.1	2.7	7.7	5.7	6.7	4.3	7.5	4.2	6.9	5.0
D16S539	4.2	2.2	7.7	3.3	7.7	2.6	8.0	2.3	7.5	3.7
D18S51	7.8	2.7	11.8	5.9	10.6	4.2	12.6	5.5	10.9	4.4
D19S433	7.2	2.4	10.4	3.9	9.6	2.4	10.0	2.5	11.3	4.1
D21S11	5.3	1.5	9.2	1.9	9.6	2.4	10.1	1.9	9.7	2.6
D2S1338	6.1	1.9	12.0	2.8	12.3	3.2	12.7	3.1	12.4	3.2
D3S1358	7.6	4.1	11.7	4.7	10.4	2.3	11.4	3.1	10.4	2.4
D5S818	4.8	3.6	8.8	4.7	8.0	2.6	9.1	3.8	8.3	2.9
D7S820	3.7	1.3	5.9	2.6	5.9	2.0	6.5	2.2	5.8	2.0
D8S1179	5.7	3.6	9.3	4.4	8.5	2.5	8.9	2.2	8.6	2.8
FGA	7.0	3.7	9.9	4.6	10.0	3.8	10.2	3.5	9.6	3.2
TH01	3.0	5.0	5.8	6.5	3.8	3.9	4.8	6.2	5.1	6.6
TPOX	2.6	3.8	4.7	4.1	4.5	3.5	4.1	2.0	4.8	4.0
vWA	6.5	5.0	11.0	4.9	10.5	3.1	10.6	3.6	10.8	4.0

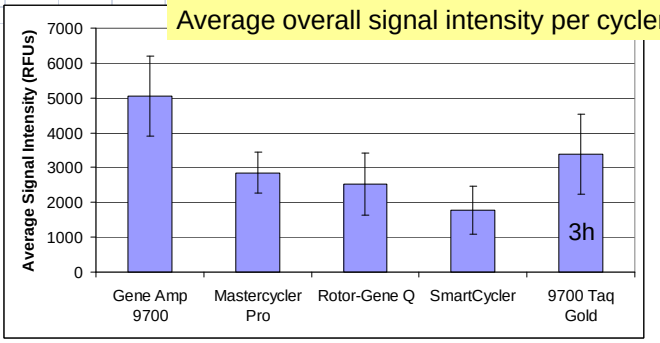
On average, stutter peak intensity for rapid protocol is 30-40 % higher than for PCR standard conditions

Signal Intensity

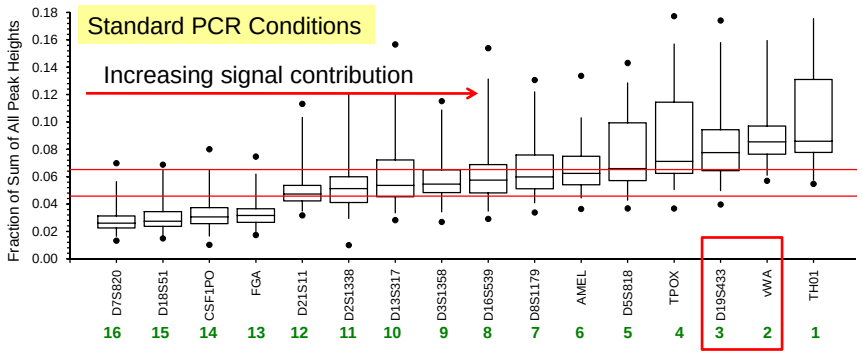
Average signal intensity per locus (RFUs)

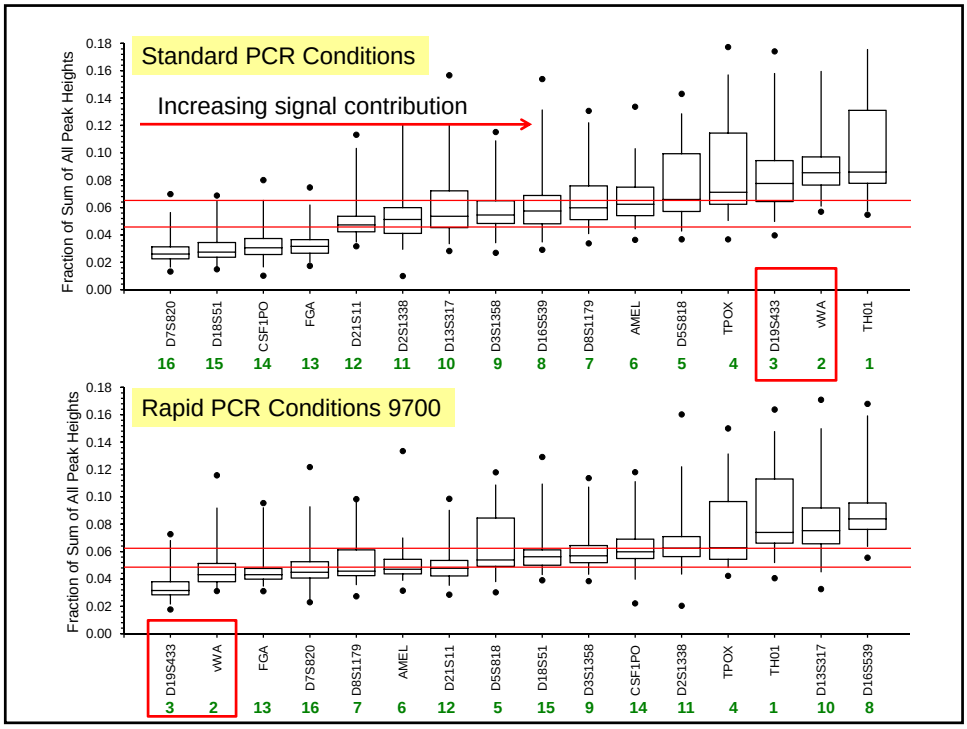
	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
9700	4289	4322	4068	5395	5243	6348	6541	7582	5896	2923	4042	5324	5209	4812	4723	4069
SmartCycler	1377	1036	1603	2133	1712	2625	2564	2409	2514	976	1175	2304	1888	1756	1530	1040
Mastercycler Pro	2488	2461	2783	3948	2005	2175	2081	4150	4431	2286	2807	3321	3927	3042	2466	1260
Rotor-Gene Q	2292	1751	1845	2458	2564	3568	3476	3520	3486	1503	1939	2885	2476	2358	2608	1775
9700 Taq Gold	3554	2958	1601	1840	3221	5166	3309	3501	3228	4631	5155	4241	1804	4146	3699	1952

Average overall signal intensity per cycler (RFUs)



Overall multiplex balance comparable to standard conditions…





Sensitivity Study on 9700

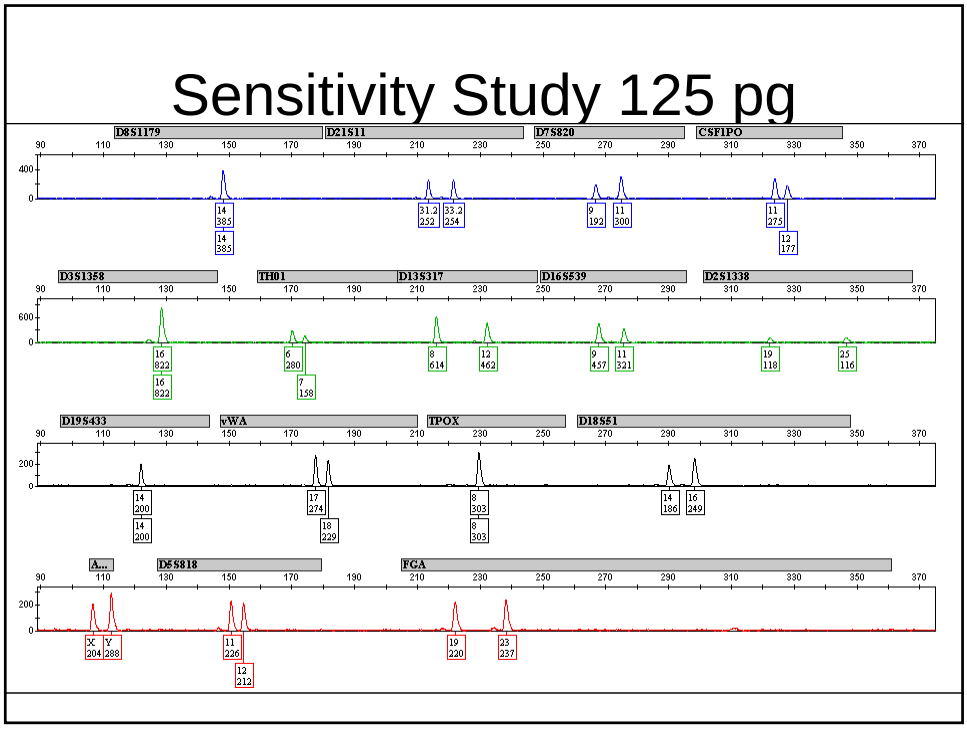
- Titration of highly characterized DNA
  - Stock at 52.44 ng/µL
- 7 concentrations amplified in duplicate
- Injected on the 3130
  - 3 kV for 10 s
- 50 RFU detection cutoff

Standard Injection

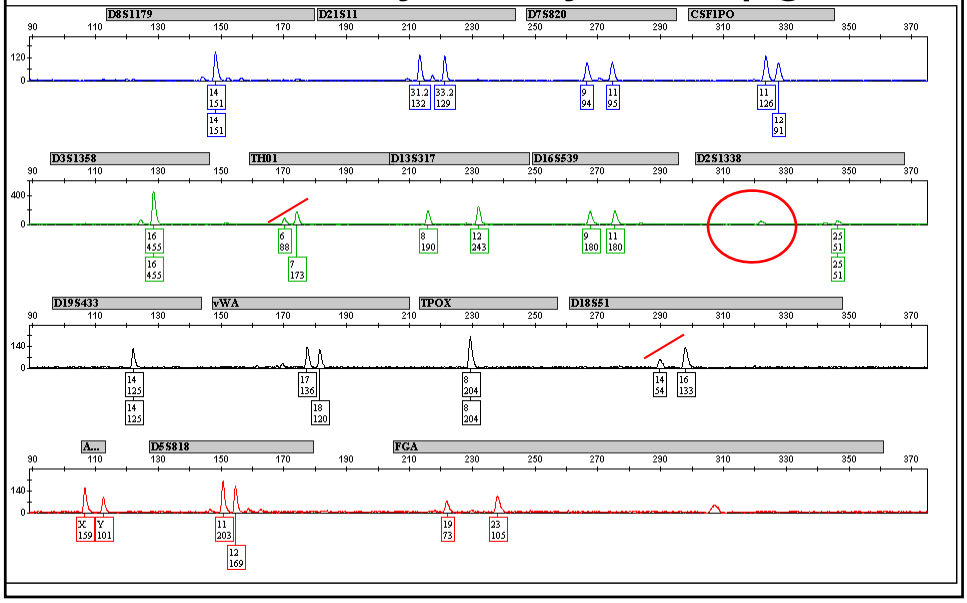
9700 36 min	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
1000 pg																
750 pg																
500 pg																
250 pg																
125 pg																
62.5 pg									51							
									180							
31.3 pg						85	71				60			134		56
		61		77		50	75	140						54		

full profile
full drop out
one allele
drop in/high stutter
*number corresponds to the height of the peak that is called

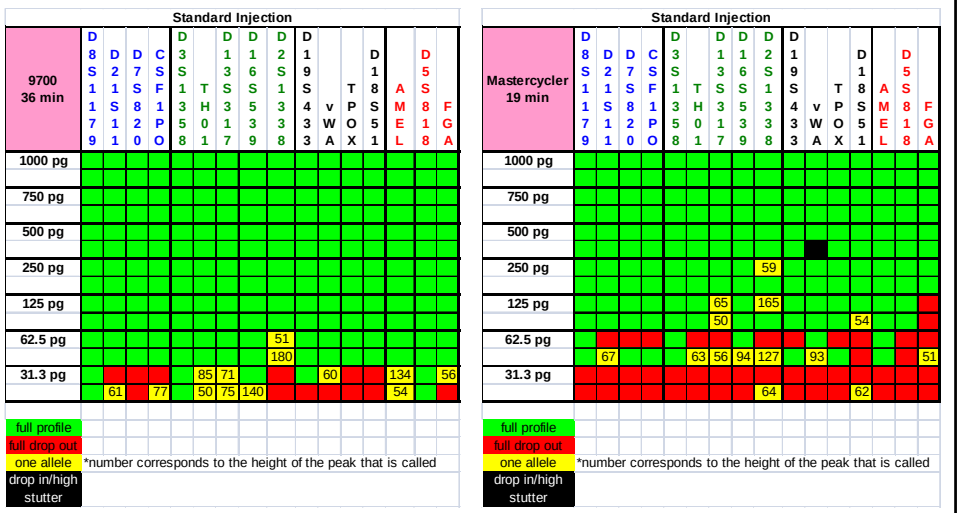
Sensitivity Study 125 pg



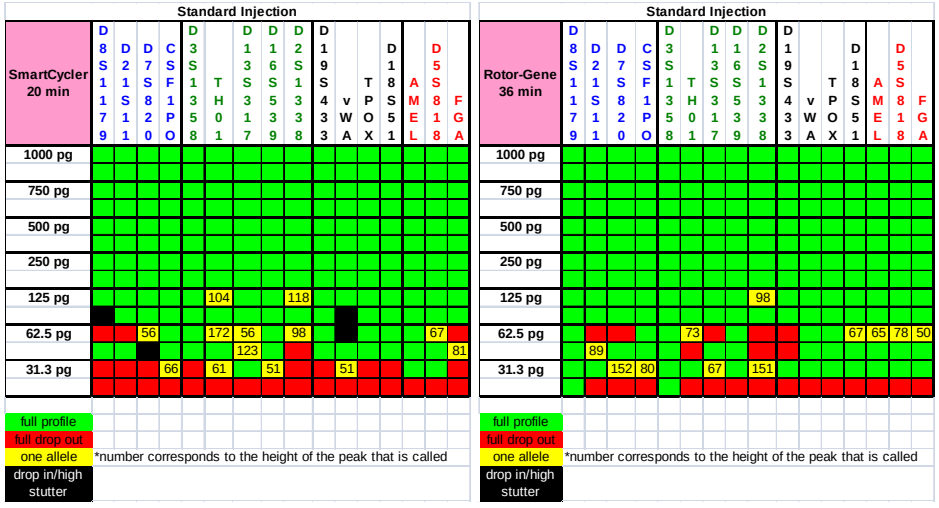
Sensitivity Study 62.5 pg



Peltier Cyclers: 9700 and Mastercycler Pro S



SmartCycler and Rotor-Gene Q



Changing Volumes and Cycling Times

- Increasing PCR volume (10 µL to 25 µL)
  - Slight decrease in signal intensity of PCR artifacts
  - Heterozygote peak height ratios similar
  - No decrease in signal for stutter peaks
  - Decrease in signal intensity (due to higher volume)
- Effects of increasing cycling hold times for the rapid protocol (36 min, 60 min, 3 hour)
  - Signal intensity of PCR artifacts increased
  - Heterozygote peak height ratios similar
  - No decrease in signal for stutter peaks

Summary of Rapid PCR Protocols

- Rapid PCR protocols can successfully amplify 15 STR loci in 19 to 36 minutes
  - Utility for reference samples, integrated typing systems
- PCR artifacts did not affect allele calls
- Stutter is 30-40% greater
  - Test different 'fast' polymerases
  - High stutter may affect DNA mixture interpretation
- Sensitivity varies by cycler (250 - 500 pg)
- Thermal cycler characteristics affect the quality of an STR profile (faster = fewer artifacts, less signal)

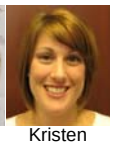
Acknowledgements

Erica Butts

John Butler

Dave Duewer

Becky Hill



Kristen O'Connor

NRC Postdoctoral fellow

DNA Biometrics Project Team

Funding from the FBI S&T Branch through the NIST Information Access Division

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