The Use of Forensic DNA Typing as a Biometric Tool

IEEE Third International Conference on
Biometrics: Theory, Applications and Systems

September 28th, 2009
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National Institute of Standards and Technology
Gaithersburg, MD

Outline

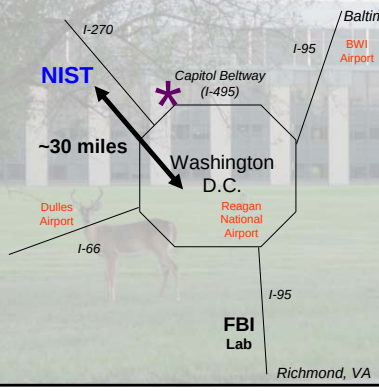
- Basics of DNA Typing
- Paternity Testing
- Rapid PCR
- DNA as a Biometric?

NIST History and Mission

- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is **part of the U.S. Department of Commerce** with a mission to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.
- NIST supplies over 1,300 Standard Reference Materials (SRMs) for industry, academia, and government **use in calibration of measurements**.
- NIST defines time for the U.S.

DNA typing standard

Location of NIST (Gaithersburg, MD)



National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of Effort with Forensic DNA

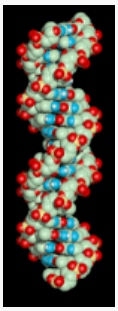
- **Standards**
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Tec**
  - R
  - Assay and software development, expert system review
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

Supporting the Forensic DNA Typing community for over 15 years

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

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 ancestry, predisposal to disease, or phenotypical information (eye color, height, hair color) is obtained

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

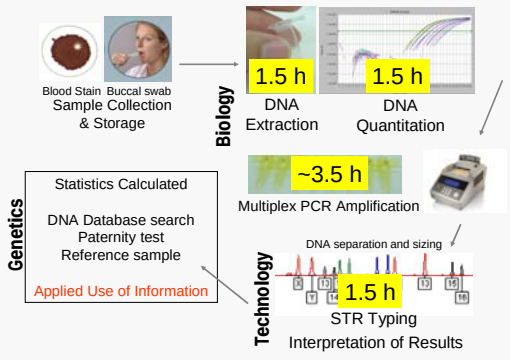
A DNA profile by itself is fairly useless because it has no context…

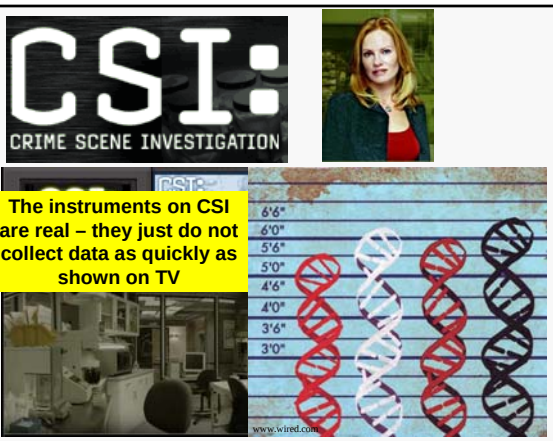
DNA analysis for identity only works by comparison – **you need a reference sample**

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

Steps in Forensic DNA Analysis

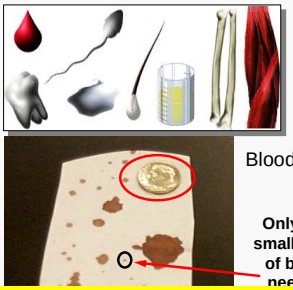
Usually 1-2 day process (a minimum of ~8 hours)





Sources of Biological Evidence

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

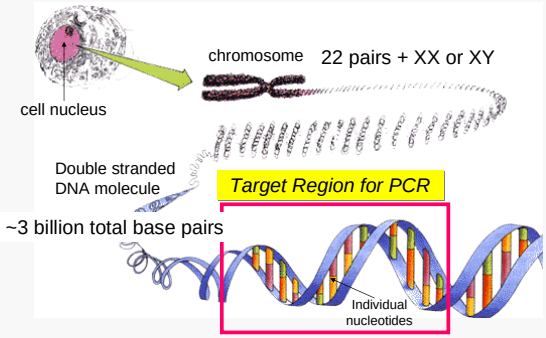


Blood Sample

Only a very small amount of blood is needed to

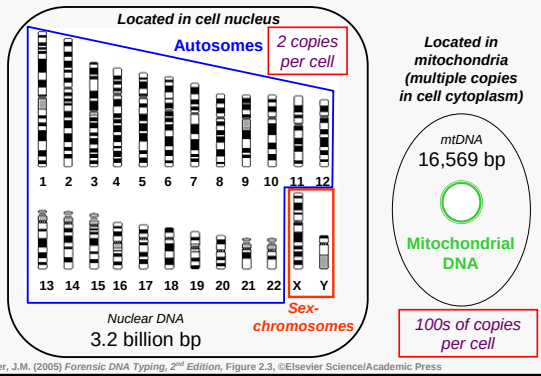
best results with >100 cells, but DNA profiles can be recovered from as little as a single cell

DNA in the Cell



Human Genome

23 Pairs of Chromosomes + mtDNA



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 2.3, ©Elsevier Science/Academic Press

What Type of Genetic Variation?

- Length Variation
  - **short tandem repeats** (STRs)

CTAGTCGT(GATA)(GATA)(GATA)GCGATCGT

- Sequence Variation
  - single nucleotide polymorphisms (SNPs)
  - insertions/deletions

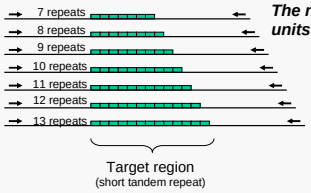
GCTAGTCGATGCTC(G/A)GCGTATGCTGTAGC

Short Tandem Repeat (STR) Markers

An accordion-like DNA sequence that occurs between genes

TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACA
GGTGGATAGATAGATAGATAGATAGATAGATAGATAGATAGA
TAGATAGATATCATTGAAAGACAAAACAGAGATGGATGATAGAT
ACATGCTTACAGATGCACAC

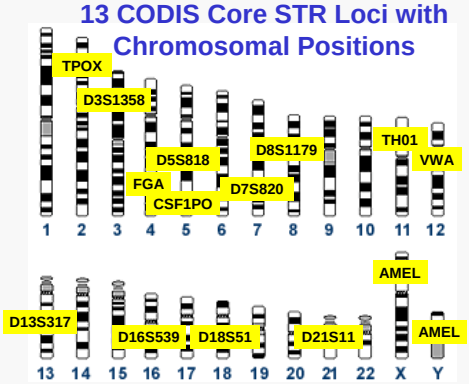
= 12 GATA repeats ("12" is all that is reported)



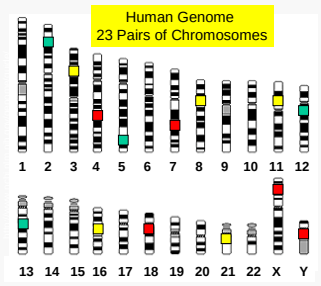
The number of consecutive repeat units can vary between people

The FBI has selected **13 core STR loci** that must be run in all DNA tests in order to provide a common currency with DNA profiles

13 CODIS Core STR Loci with Chromosomal Positions



What is a DNA Profile?



Nuclear DNA
3.2 billion bp

Unique regions of the human genome are targeted

These regions consist of a few hundred base pairs

The regions are copied by the polymerase chain reaction (PCR) – **billions of exact copies**

The copied fragments now contain fluorescent dyes for detection

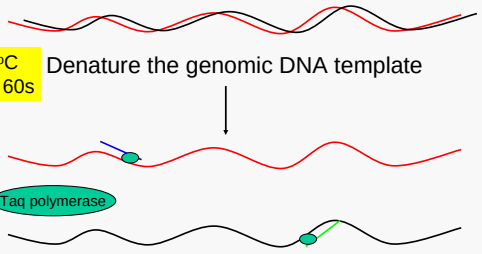
Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 2.3, ©Elsevier Science/Academic Press

PCR

- Polymerase Chain Reaction
- In vitro enzymatic replication
- Saiki et al., (1985) *Science* 20: 1350-1354
- Targets a specific region of a genome
- 2^N amplification (N = number of cycles)
- 50 – 10,000 base pair fragments
- Products can be used for downstream applications

A means to create billions of exact copies of a specific region of the genome

PCR Mechanism



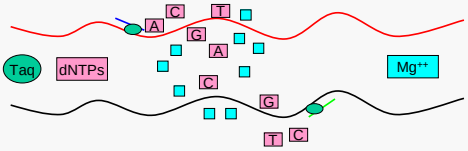
95°C
30 – 60s

Denature the genomic DNA template

~60°C
1 – 2 min

PCR primers bind to the DNA template; defines the product size

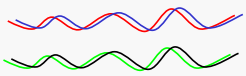
PCR Mechanism

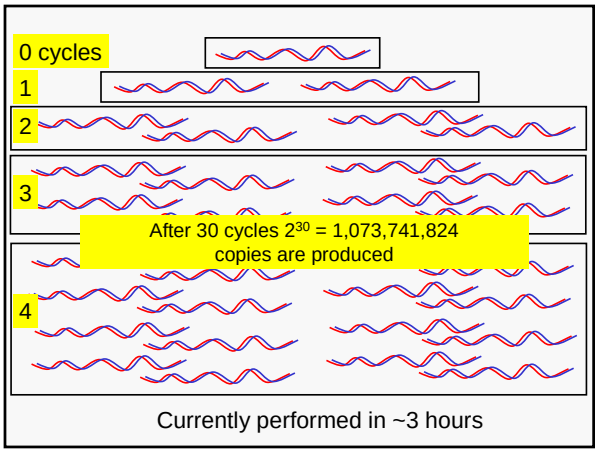


Taq primer complex forms and the dNTPs are incorporated into the new strand(s)

72°C
1 – 2 min

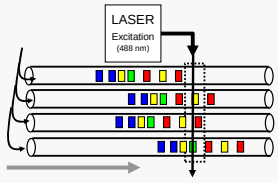
Processivity rate of Taq complex ~20 nucleotides/s





Currently performed in ~3 hours

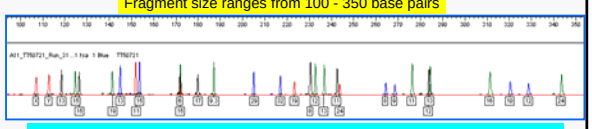
What is a DNA Profile?



The labeled fragments are separated (based on size) and detected on a gel or capillary electrophoresis instrument

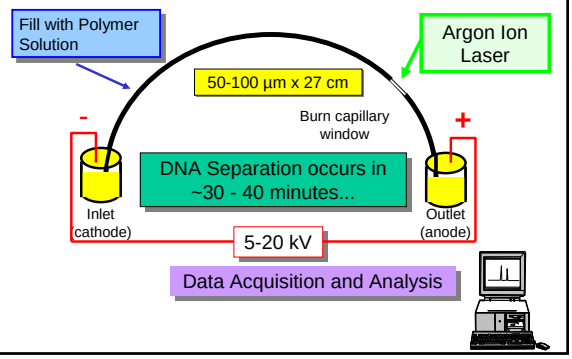
~2 hours or less

Fragment size ranges from 100 - 350 base pairs



Peaks represent labeled DNA fragments separated by electrophoresis
This 'profile of peaks' is unique for an individual – a DNA type

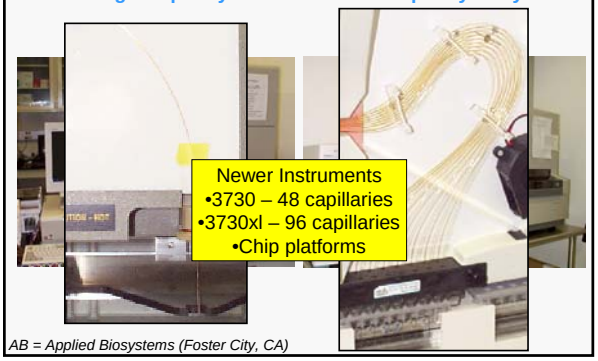
Capillary Electrophoresis (CE)



Capillary Electrophoresis Instrumentation

AB 310
single capillary

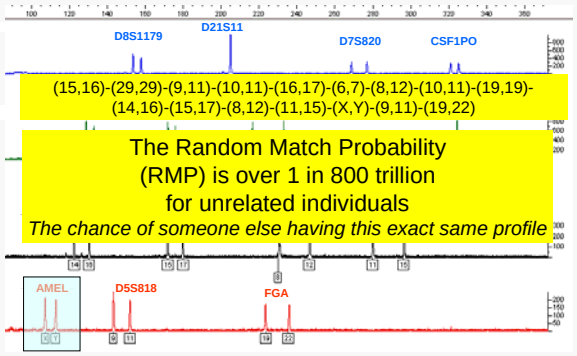
AB 3130
16-capillary array



AB = Applied Biosystems (Foster City, CA)

Identifiler (Applied Biosystems) 15 STR Loci Kit

Information is tied together with multiplex PCR and data analysis



Data Format

Genotype (consisting of two alleles)

	AMEL	CSF1PO	FGA	TH01	TPOX	VWA	D3S1358	D5S818
Ind(1)	X,Y	11,12	19,21	6,7	8,8	15,18	14,18	10,13

Profiles are reviewed by an analyst

The alleles observed at each locus are tabulated

This data format is stored in databases and used for comparisons/matches

Question

How are these extremely large Random Match Probability values determined?

Product Rule

Observed allele frequencies for the TH01 locus

Allele	Caucasian n = 302 TH01	Afr Amer n = 258 TH01	US Hisp n = 140 TH01
5	0.00166	0.00388	NA
6	0.23179	0.12403	0.21429
7	0.19040	0.42054	0.27857
8	0.08444	0.19380	0.09643
9	0.11424	0.15116	0.15000
9.3	0.36755	0.10465	0.24643
10	0.00828	0.00194	0.01429
11	0.00166	NA	NA

For heterozygous loci
$P = 2pq$
P = probability; p and q are frequencies of allele in a given population

Example: For the locus TH01 an individual is 6,9.3 with frequencies of 0.23179 and 0.36755 respectively

$P = 2(0.23179)(0.36755) = 0.17039$
or ~1 in 6

For multiple loci the
Profile Probability = (P1)(P2)…(Pn)
for n = 5 (5 loci)
(0.17039)(0.0875)(0.0687)(0.0245)(0.0984)
2.469×10^{-6} or 1 in 404,976

CODIS & NDIS

Combined DNA Index System (CODIS)

National DNA Index System (NDIS)

- Used for linking serial crimes and unsolved cases with repeat offenders
- Convicted offender and forensic case samples
- Launched October 1998
- Requires 13 core STR markers
- Annual Results with NIST SRM required for submission of data to CODIS

All 50 states now require convicted offenders to submit a sample for DNA testing purposes

>93,000 Investigations Aided through July 2009

Virginia

Statistical Information	Total
Offender Profiles	292,869
Forensic Samples	10,765
Number of CODIS Labs	4
NDIS Participating Labs	4
Investigations Aided	5,364

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Total number of profiles: ~7.6 million
Total Forensic profiles: 277,215
Total Convicted Offender Profiles: 7,261,604

http://www.fbi.gov/hq/lab/codis/clickmap.htm

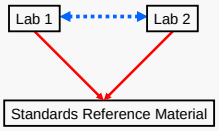
Standard Reference Materials

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

Traceable standards to ensure accurate measurements in our nation's crime laboratories

Helps meet DAB Std. 9.5 and ISO 17025

SRM 2391b – CODIS STRs
SRM 2392-I – mtDNA
SRM 2395 – Y-STRs
SRM 2372 – DNA quantitation



Calibration with SRMs enables confidence in comparisons of results between laboratories

Paternity Testing

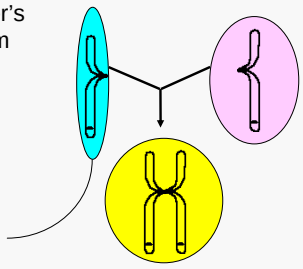
Paternity Testing

- DNA testing methods for determining paternity also relate to:
  - Mass fatalities
  - Missing persons investigations
  - Kinship testing
  - Genetic genealogy

Our DNA Comes from our Parents

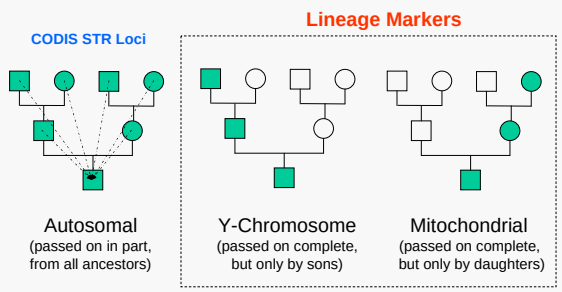
Father's Sperm

Mother's Egg



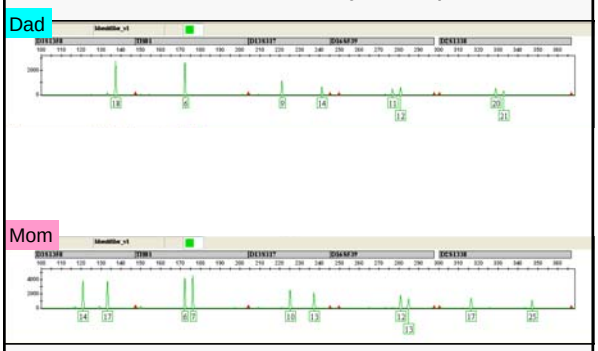
Child's Cell

Different Inheritance Patterns



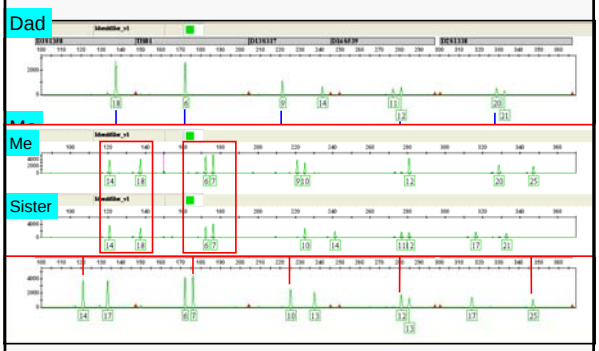
Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 9.1, ©Elsevier Science/Academic Press

Autosomal Paternity Example



Focusing on 5 markers…

Autosomal Paternity Example



Focusing on 5 markers…

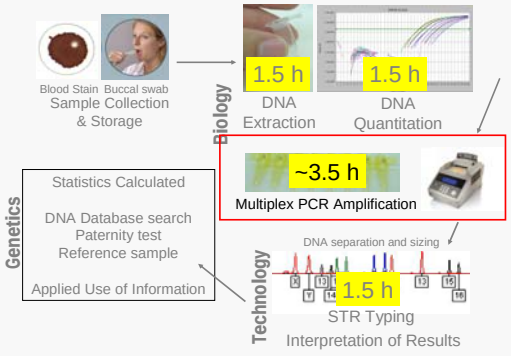
Kinship Testing

- As a familial relationship becomes more distant the ability of DNA to confirm the likelihood of that relationship decreases
  - Parent-offspring
  - Siblings
  - Half siblings | niece/nephew | cousins

Rapid PCR

Steps in Forensic DNA Analysis

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



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

Growing Interest in DNA for Biometrics

01-17-2008

NYC Prize

- In the months ahead, we will also challenge the private sector to speed up DNA fingerprinting so that when DNA is left behind, officers can identify suspects more quickly and avoid wrongful arrests. And to do this, we will establish **a six-figure prize for anyone who can invent a device tailored to the NYPD that analyzes DNA right at the crime scene**. It's just one more way we are trying to bring private sector innovation into the public sector

http://nyc.gov/portal/site/nycgov/?front_door=true

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

Efforts towards Portable/Mobile DNA Devices

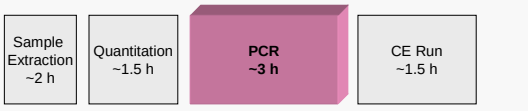
- Network Biosystems (Woburn, MA) http://www.netbio.com
- Landers group at UVA and MicroLab Diagnostics (Charlottesville,VA) http://www.microlabdiagnostics.com
- Mathies group at UC-Berkeley and Microchip Biotech (Dublin, CA) http://www.microchipbiotech.com
- Az State Univ./Forensic Science Service MiDAS 1 effort

Interest/End User Communities

- DHS (immigration, border testing)
- DoD (intelligence, military zone)
- FBI (booking stations, reference samples)
- AFDIL (Armed Forces DNA Identification Lab) (mass fatalities, remote DNA testing)
- State and local DNA labs

Typical STR Typing Workflow

Can the time required for PCR thermal cycling be reduced?



Alter thermal cycling parameters
Evaluate faster polymerases
Evaluate faster thermal cyclers
Test commercial STR typing kits

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

DNA Polymerases for Evaluation

Polymerase	Vendor	MasterMix	Hot Start
TaqGold	Applied Biosystems	no	10 min
GeneAmp	Applied Biosystems	yes (2x)	1 min
SpeedSTAR	Takara	no	1 min
PyroStart	Fermentas	yes (2x)	1 min
Qiagen Fast Cycling PCR Kit	Qiagen	yes (2x)	5 min

Brief survey of 'fast' commercial polymerases

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 GeneAmp9700
Ramp rate = 4°C/s

PCR Thermal Cycling Profile

28-32 cycles of PCR

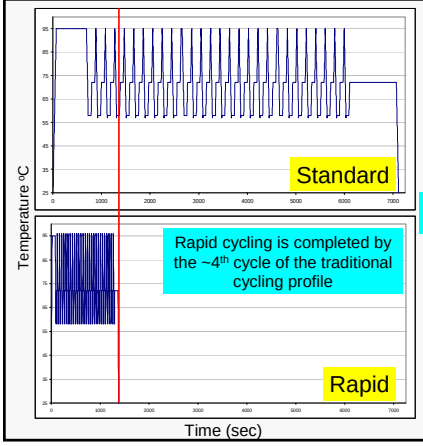
Currenty used
~3 hour run time

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

~36 min run time

Heating/Cooling rate of ~4°C/s

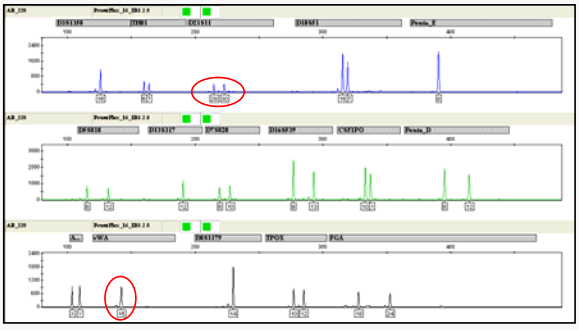


Comparison of Thermal Cycling Profiles

36 Minute PCR Amplification on AB 9700 Cycler

28 cycles, Identifiler STR kit, 1 ng of DNA

36 Minute PCR Amplification on AB 9700 Cycler



28 cycles, PowerPlex 16 STR kit, 1 ng of DNA

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 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)

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 Summary

- Fast 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

Summary

- DNA typing is a robust and reproducible means of identifying an individual
- DNA can be used to determine kinship between family members
- The time required for typing must be reduced for DNA to be used as a biometric (at least < 1 hour)

Thank you for your attention!

Questions?

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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