


Forensic DNA Typing


**Technology Focus Day on
Human Identification by DNA Fingerprinting**
In-Q-Tel
January 22nd, 2008
Arlington, VA

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


Outline

- NIST
- Basics of DNA Typing
- Paternity Testing
 - Y-STRs
 - miniSTRs
 - SNPs
 - Examples




NIST History and Mission

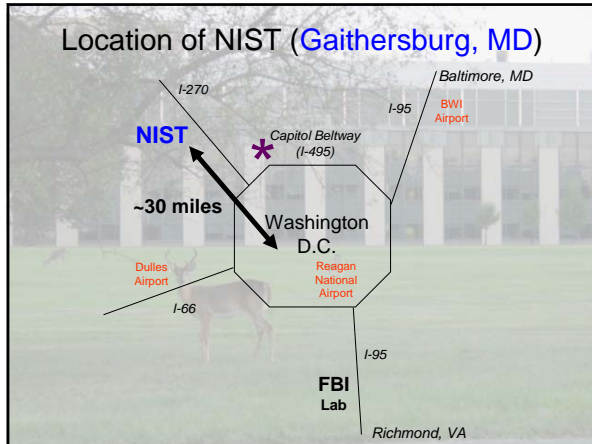
- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is **part of the U.S. Department of Commerce** with a mission to **develop and promote measurement, standards, and technology to enhance 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.



\$532 for 3 jars



DNA typing standard



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
- **Technology**
 - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
 - Assay and software development, expert system review
- **Training Materials**
 - Review articles and workshops on STRs, CE, validation
 - PowerPoint and pdf files available for download

<http://www.cstl.nist.gov/biotech/strbase/NJprojects.htm>

STRBase
National Institute of Standards and Technology
Short Tandem Repeat DNA Internet Database

... working with industry to develop and apply technology, measurements and standards

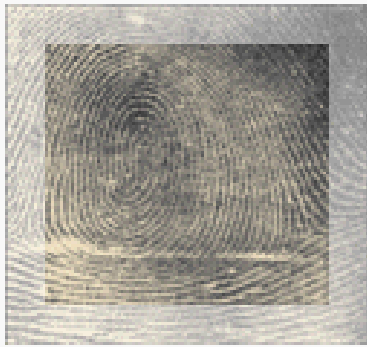
Recent Additions <ul style="list-style-type: none">• Forensic SNP Information (will be official site for ISFG SNP information) .../SNP.htm• NIST publications and presentations as pdf files .../NISTpub.htm	We Regularly Update <ul style="list-style-type: none">• Reference List• Variant Alleles• Addresses for Scientists• Links to Other Web Sites• Y-STR Information
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We will continue to add downloadable PowerPoint files that can be used for training purposes

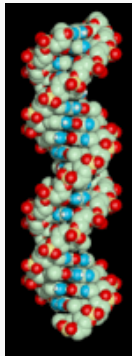
<http://www.cstl.nist.gov/biotech/strbase>

Basics of Forensic DNA Testing

Methods for Human Identification

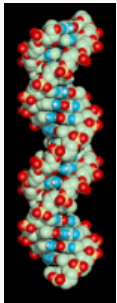


Fingerprints have been used since 1901



DNA since 1986

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

Applications of Human Identity Testing

- Forensic cases -- **matching suspect with evidence**
- Paternity testing -- **identifying father**
- Missing persons investigations
- Military DNA "dog tag"
- Convicted felon DNA databases
- Mass disasters -- **putting pieces back together**
- Historical investigations and genetic genealogy

Involves generation of DNA profiles usually with the same genetic markers and then **MATCHING TO REFERENCE SAMPLE**

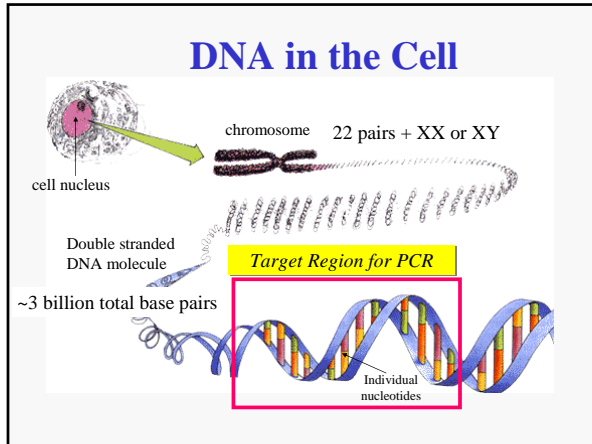
DNA Testing Requires a Reference Sample

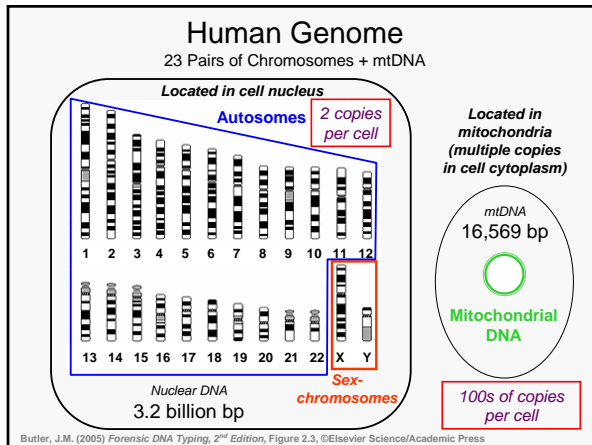
A DNA profile by itself is fairly useless because it has no context...



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

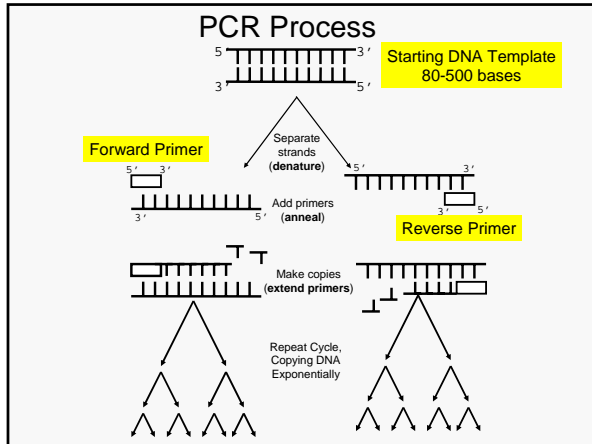
- Crime Scene Evidence** compared to **Suspect(s)** (Forensic Case)
- Child** compared to **Alleged Father** (Paternity Case)
- Victim's Remains** compared to **Biological Relative** (Mass Disaster ID)
- Soldier's Remains** compared to **Direct Reference Sample** (Armed Forces ID)

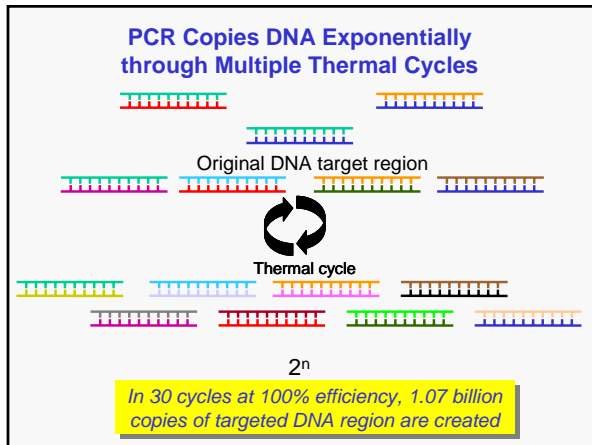


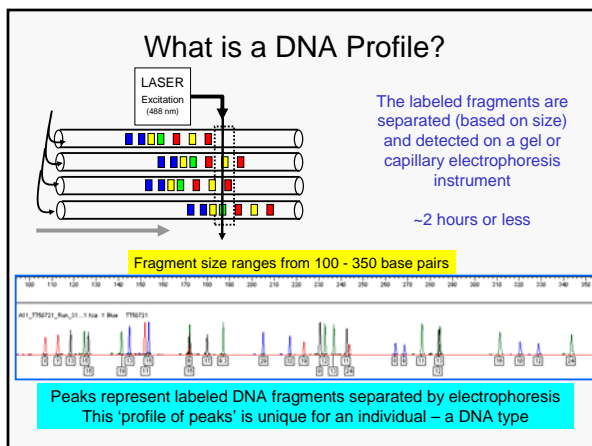


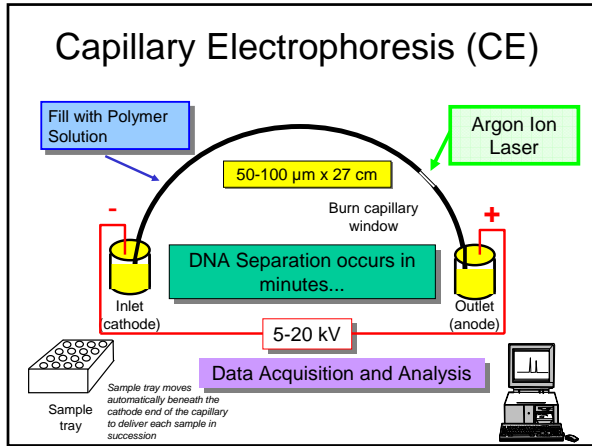
What Type of Genetic Variation?

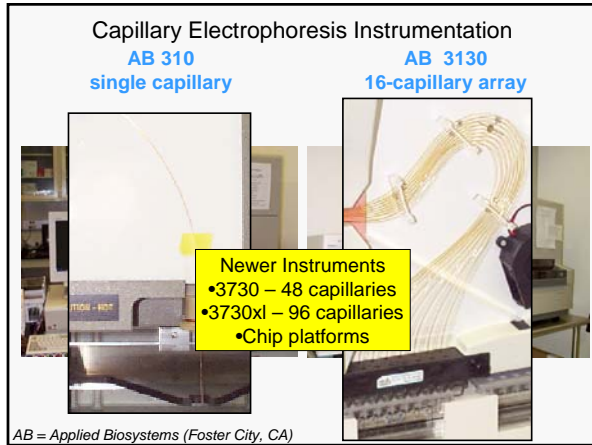
- Length Variation
 - short tandem repeats (STRs)
 - CTAGTCGT(GATA)(GATA)(GATA)GCGATCGT
- Sequence Variation
 - single nucleotide polymorphisms (SNPs)
 - insertions/deletions
 - GCTAGTCGATGCTC(G/A)GCGTATGCTGTAGC

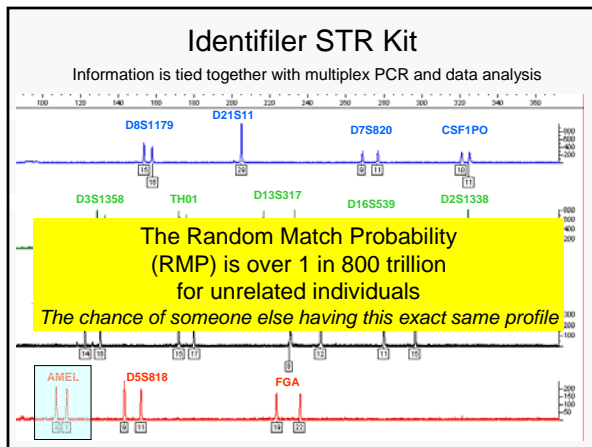












Product Rule

For heterozygous loci

$$P = 2pq$$

P = probability; p and q are frequencies of allele in a given population

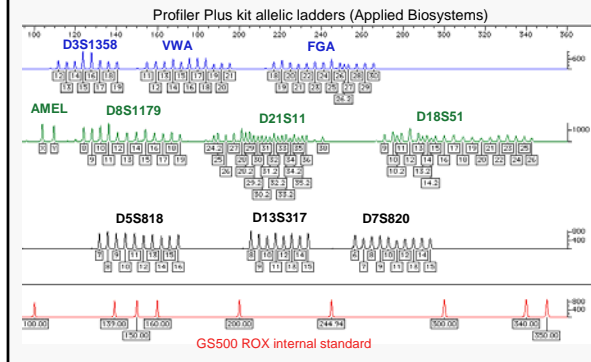
Example: For the locus D3S1358 an individual is 15,18 with frequencies of 0.2825 and 0.1450 respectively

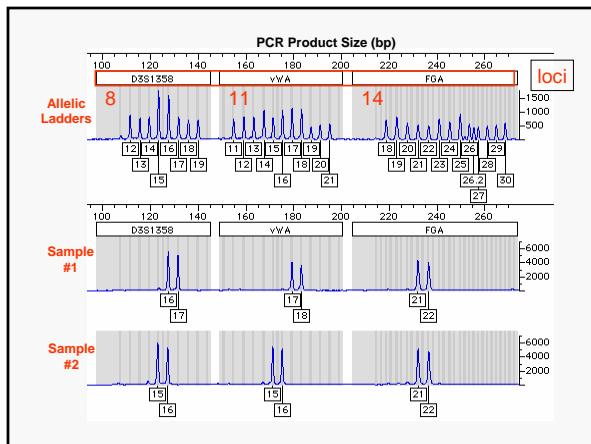
$$P = 2(0.2825)(0.1450) = 0.0819 \text{ or } 1 \text{ in } 12$$

For 5 loci the

$$\begin{aligned} \text{Profile Probability} &= (P_1)(P_2)\dots(P_n) \\ &= (0.0819)(0.0875)(0.0687)(0.0245)(0.0984) \\ &= 0.00001187 \text{ or } 1 \text{ in } 842,539 \end{aligned}$$

Companies Supply Allelic Ladders in STR Kits to Aid Interlaboratory Consistency





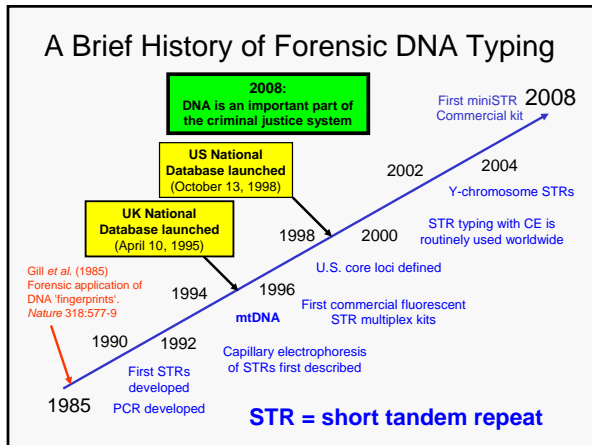
Data Format

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

The profiles are reviewed by analysts

The number of repeats observed for each locus is tabulated

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

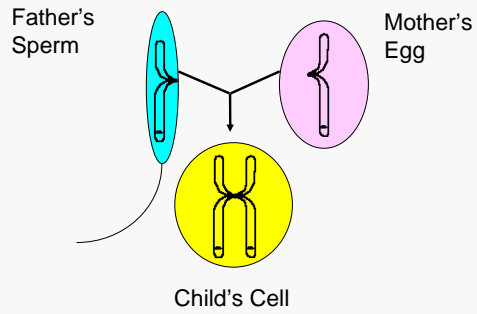


Paternity Testing

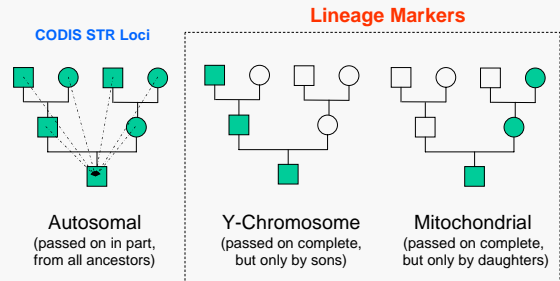
Paternity Testing

- The use of DNA testing methods for determining paternity also relates to:
 - Mass disasters
 - Missing persons investigations
 - Familial matching
 - Genetic Genealogy

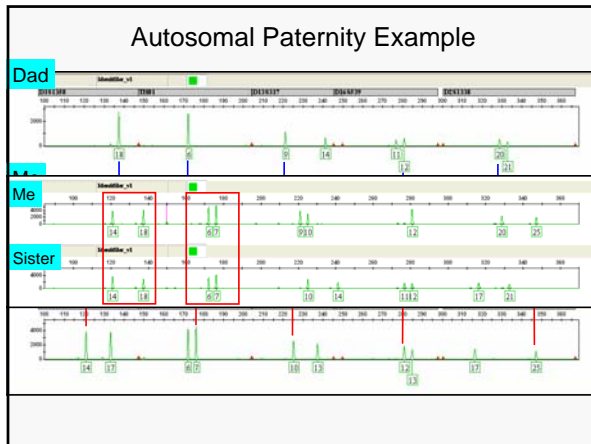
Our DNA Comes from our Parents



Different Inheritance Patterns



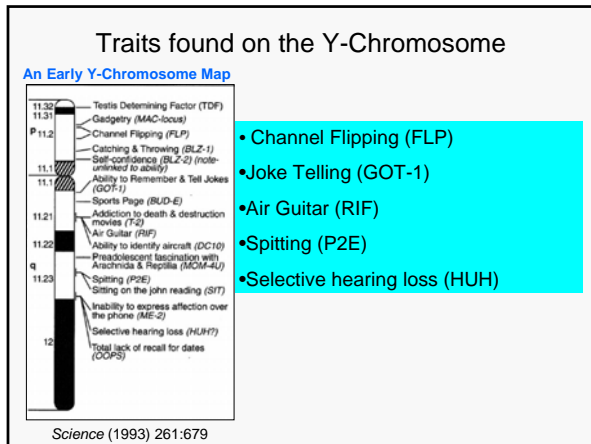
Butler, J.M. (2005) Forensic DNA Typing, 2nd Edition, Figure 9.1, ©Elsevier Science/Academic Press

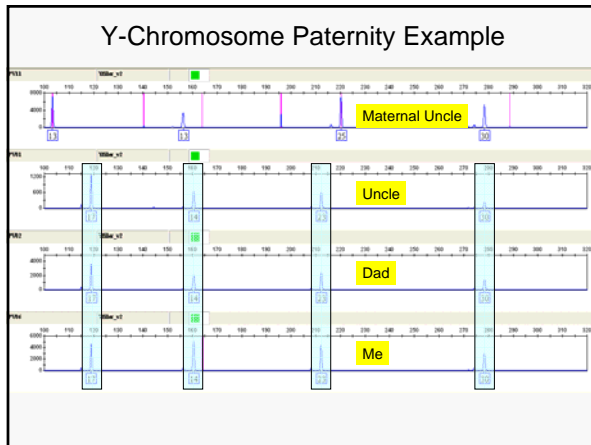


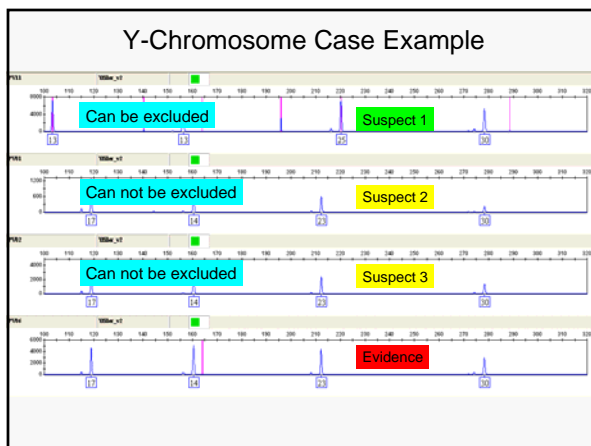
Y-STRs

Y-STRs

- Similar to autosomal STRs just located on the Y-Chromosome
- Since only males possess a Y-Chromosome these markers are useful in male-female mixtures (sexual assault cases)
- A limitation of the Y-STRs lies in that they do not have the discrimination capacity of autosomal STRs (no recombination)







Modern Use of Y-STR Testing

Captured December 13, 2003

Matching Y-STR Haplotype Used to Confirm Identity
(along with allele sharing from autosomal STRs)

Uday and Qusay Hussein
Killed July 22, 2003

Is this man really Sadaam Hussein?

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Box 23.1, p. 534

Historical Investigation DNA Study (Matching Relatives to Remains or Relatives to Relatives)

Thomas Jefferson II

Field Jefferson (Uncle) Peter Jefferson

President Thomas Jefferson

Eston Hemings

Same Y Haplotype

Different Y Haplotype

Jefferson Y Haplotype

Jefferson Y Haplotype

Thomas Woodson

SOURCE: Foster et al. (1998) *Nature* 396:27-28

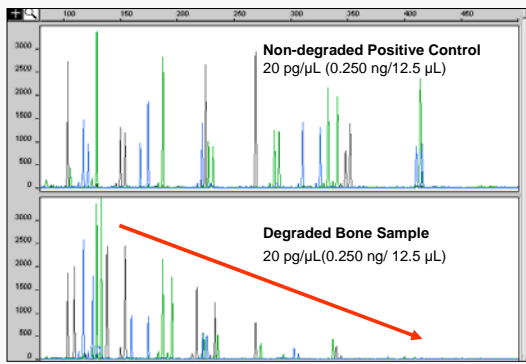
Butler, J.M. (2001) *Forensic DNA Typing*, Figure 17.4, ©Academic Press

miniSTRs

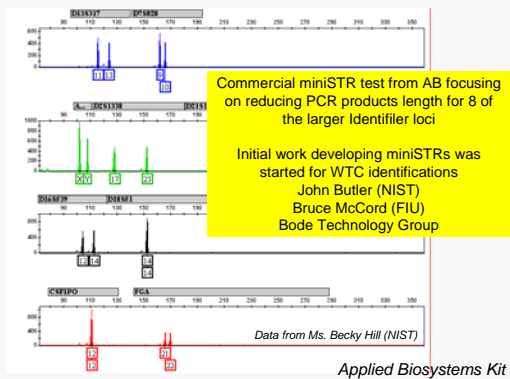
miniSTRs

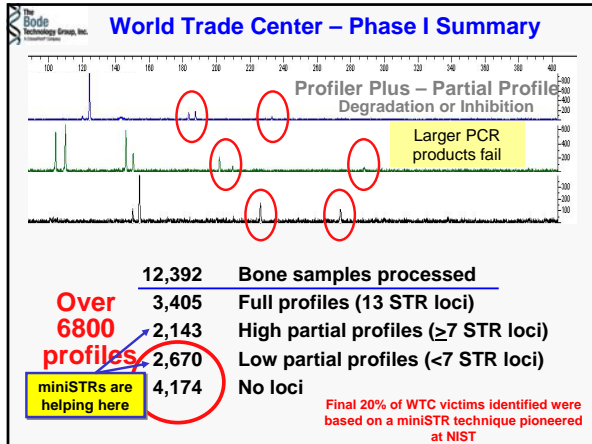
- Simply a smaller PCR product size
- Typically less than ~200 base pairs
- Contains the **same information** as a traditional STR (repeat length)
- Useful for typing degraded DNA samples
- New loci helpful for **missing persons**
paternity testing/mass disasters

DNA Degradation



A MiniFiler Kit Profile





SNPs

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

Potential Use of SNPs in Forensic DNA Testing

- Human Identification (need 50+ loci)
- Predicting Geographical Origin
- Prediction of Phenotypical Information
 - Hair color, eye color etc
- Evolutionary studies

- May be cheaper/faster
- Can be run on higher throughput platforms
- Current databases are for the 13 CODIS loci

Rapid PCR

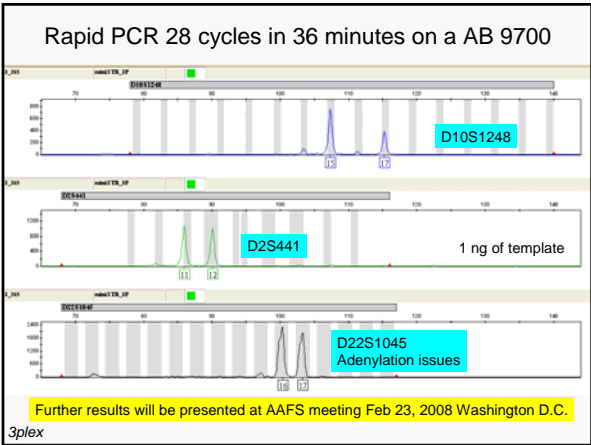
Rapid PCR

- Existing commercial STR typing kits are not optimized for rapid PCR
- Challenge for miniaturize STR typing platforms – since they are tied into the commercial kits/loci
- Fewer loci and smaller amplicon size favor rapid multiplex PCR
- We have well characterized miniSTR panels

Collaborations with:
Dr. Michael Gaitan (NIST) – microwave thermal heating
Dr. Eugene Tan (Network Biosystems) – chip platforms

Rapid Thermal Cycling

- Evaluate faster polymerases
- Test with miniSTRs
 - primer concentrations can be adjusted and PCR primer sequences are known
- Use standard cycler (GeneAmp 9700), tubes
- Examine shortened dwell times and adenylation soak
- Study limitations in terms of PCR amplification speed when examining multiplex STR assays



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
- The City will lobby Albany to require DNA fingerprinting from everyone who is arrested

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

Forensic DNA in the News

The screenshot shows the Innocence Project website. At the top, the URL <http://www.innocenceproject.org> is highlighted. Below the navigation bar, there is a news article titled "Michael A. Williams Exonerated" with a date of "March 11, 2005". A summary states "163 exonerated as of March 11, 2005" and "167 EXONERATED of October 12, 2005". Below the article is a bar chart titled "DNA EXONERATIONS BY YEAR IN THE U.S." showing the number of exonerations per year from 1989 to 2005. The chart shows a general upward trend, peaking in 2001 and 2002.

Year	Exonerations
'89	1
'90	2
'91	3
'92	4
'93	5
'94	6
'95	7
'96	8
'97	9
'98	10
'99	11
'00	12
'01	13
'02	14
'03	15
'04	16
'05	17

The screenshot shows the Genographic Project website. The title "The Genographic Project" is prominent, along with the URL <https://www3.nationalgeographic.com/genographic/>. Below the title is a list of bullet points:

- Different populations carry distinct markers. Following them through the generations reveals a genetic tree on which today's many diverse branches may be followed ever backward to their common African root
- Our genes allow us to chart the ancient human migrations from Africa across the continents
- Funded \$50 million for 5 years by IBM and National Geographic
- Will gather and run DNA samples from ~100,000 people around the world with Y-SNPs and mtDNA

Tsunami Survivor "Baby 81" Connected to His Parents with DNA

Wednesday, March 2, 2005 Posted: 9:27 AM EST (1427 GMT)

NEW YORK (AP) -- The parents of the infant tsunami survivor nicknamed "Baby 81" say they found it difficult to feel overjoyed about their reunion in the midst of so much tragedy. The 4-month-old Sri Lankan baby and his parents, who were reunited after court-ordered [DNA tests proved their relationship](#), appeared on ABC's "Good Morning America" Wednesday, a day after their 20-hour-long flight landed in New York.

'Baby 81,' parents make TV appearance



<http://www.cnn.com/2005/US/03/02/baby.81.ap/index.html>

Identification of Remains from Former Yugoslavia

>90,000 family reference samples collected
>17,000 bones identified as of April 2007

DNA testing is performed on 100s of bones collected each week from mass graves in Bosnia and Croatia to help in the re-association of remains

The New York Times April 11, 2006 Hurricane Katrina Victim Identification Being Performed with DNA

In Attics and Rubble, More Bodies and Questions

August Blanchard and an aunt, Shirley, looking into the living room where the decomposed remains of his mother, Charlene, were found in February on Reynes Street in the Lower Ninth Ward of New Orleans.

DNA Testing Assists Immigration

DNA Tests Offer Immigrants Hope or Despair



Sandra and Balfour Francis of Brooklyn, with a photograph of Nickiesha, who is in Jamaica. Last year, DNA tests showed she is not his daughter.

The New York Times

By RACHEL L. SWARNS
Published: April 10, 2007

<http://www.nytimes.com/2007/04/10/us/10dna.html>

- Mary K. Mount, a DNA testing expert for the A.A.B.B. — formerly known as the American Association of Blood Banks — estimates that about 75,000 of the 390,000 DNA cases that involved families in 2004 were immigration cases. Of those, she estimates, 15 percent to 20 percent do not produce a match.
- Negative results can suggest an effort to bring in illegal immigrants or distant relatives, officials say, though they note that requests for DNA tests deter illicit activities.

The New York Times April 11, 2006

Lawyers for Duke Players Say DNA Clears Team



http://www.nytimes.com/2006/04/11/sports/othersports/11duke.html?_r=Top%2fReference%2fTimes%20Topics%2fOrganizations%2fD%2fDuke%20University%20

Armed Forces DNA Repository





>4.5 million bloodstain cards on file from members of U.S. military

Are being used to identify remains from combat casualties

Located in Gaithersburg, Maryland




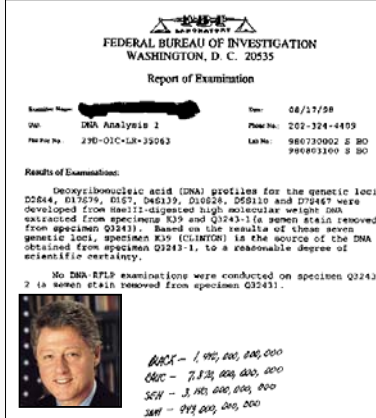
Tomb of the Unknown Soldier

- **Armed Forces DNA Identification Laboratory (AFDIL)** (Rockville, MD)
- In June 1998 AFDIL identified Michael J. Blassie as the Vietnam Unknown in the Tomb of the Unknown Soldier (located in Arlington National Cemetery)
- **There will be no more “unknown” soldiers.**

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Box 10.1, pp. 250-251

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Box 1.3, p. 9

August 17, 1998 FBI Report on Analysis of Stain on Monica Lewinsky's Blue Dress

Results of Examination:
Deoxyribonucleic acid (DNA) profiles for the genetic loci D2S1338, D3S1378, D15S11, D16S1131, D18S11, D19S11 and D21S11 were developed from HaeIII-digested high molecular weight DNA extracted from specimens K33 and Q1243-1 (a semen stain removed from specimen Q1243). Based on the results of these seven genetic loci, specimen K19 (CLINTON) is the source of the DNA obtained from specimen Q1243-1, to a reasonable degree of scientific certainty.

No DNA RFLP examinations were conducted on specimen Q1243-2 (a semen stain removed from specimen Q1243).

Handwritten notes:
K19 - 1,000,000,000,000
K19 - 7,830,000,000,000
K19 - 3,100,000,000,000
K19 - 999,000,000,000

<http://www.law.umkc.edu/faculty/projects/frtrial/clinton/lewinsskydress.html>

Results Announced in Anna-Nicole Smith DNA Parentage Test




Ten Aylen, AP
Christine Aylen, AP

Larry Birkhead receives a handshake from DNA expert Dr. Michael Baird outside the court after a paternity hearing in Nassau, Tuesday, April 10, 2007.

