


**Advances in the Field of
Forensic DNA Typing**

**University of Rhode Island
April 27, 2007**
Dr. Peter M. Vallone, Biochemical Science Division,
National Institute of Standards and Technology
Gaithersburg, Maryland


Outline

- NIST
- Current State of DNA Typing
- MiniSTRs
- Y-STRs
- SNPs
- NIST Standard Reference Materials (SRMs)
- Examples




NIST History and Mission

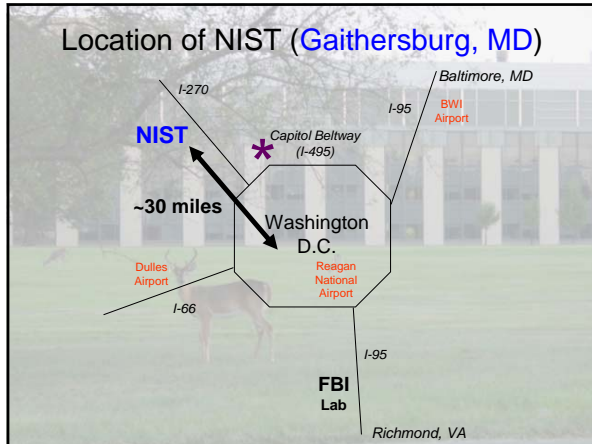
- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is **part of the U.S. Department of Commerce** with a mission to **develop and promote measurement, standards, and technology to enhance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

Support to the Forensic Community



...Bringing traceability and technology to the scales of justice...







- Conduct interlaboratory studies
- Perform beta-testing of new human identity testing products
- Provide input to
 - Scientific Working Group on DNA Analysis Methods (SWGDM)
 - Department of Defense Quality Assurance Oversight Committee for DNA Analysis
 - American Prosecutor's Research Institute (APRI) DNA Forensics Program "Course-in-a-Box" for training lawyers
 - WTC Kinship and Data Analysis Panel (KADAP)
 - 2005 Hurricane Victim DNA Identification Expert Group (HVDIEG)
 - NIJ Expert System Testbed (NEST) Project










Training Workshops Conducted

<http://www.cstl.nist.gov/biotech/strbase/training.htm>
John Butler (and Bruce McCord, Robyn Ragsdale, Pete Vallone, or Mike Coble)

Sept 29-30, 2004
Nov 1-2, 2006
May 3, 2006
February 20, 2006
June 13-14, 2005
April 27-28, 2006
May 10, 2006
August 17, 2006
October 11, 2006
April 4, 2006
August 17, 2006
May 19, 2005
June 8, 2005
June 6, 2006
Aug 7, 2006
Dec 5-6, 2006
Apr 3-4, 2007

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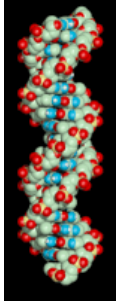
Outline

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

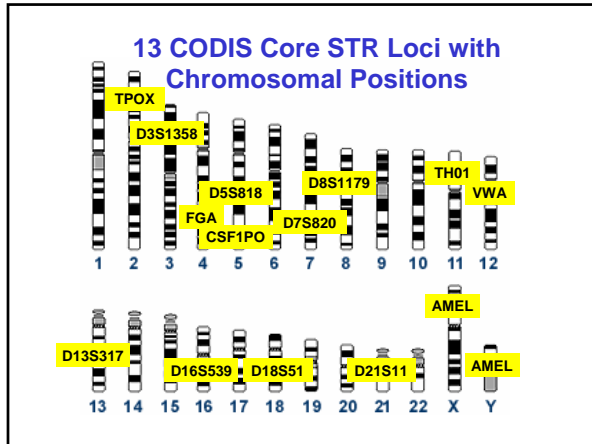
DNA typing must be done efficiently and reproducibly (information must hold up in court)

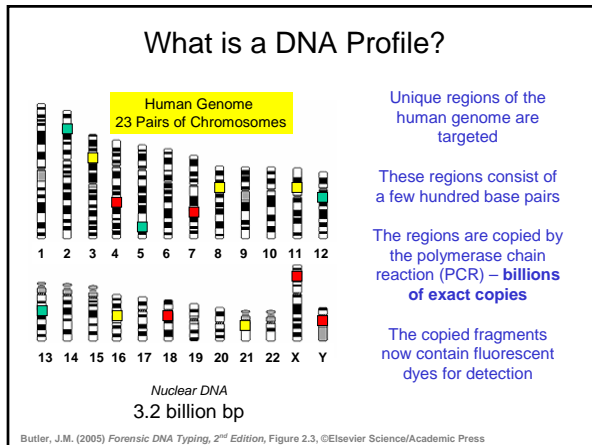
Typically, we are not looking at genes – little/no information about race, predisposal to disease, or phenotypical information (eye color, height, hair color) is obtained

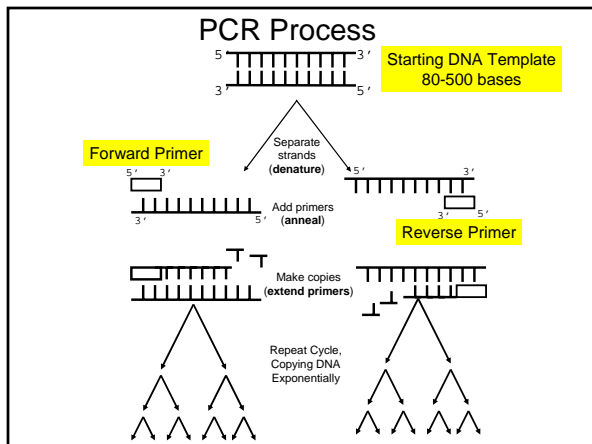
Applications of Human Identity Testing

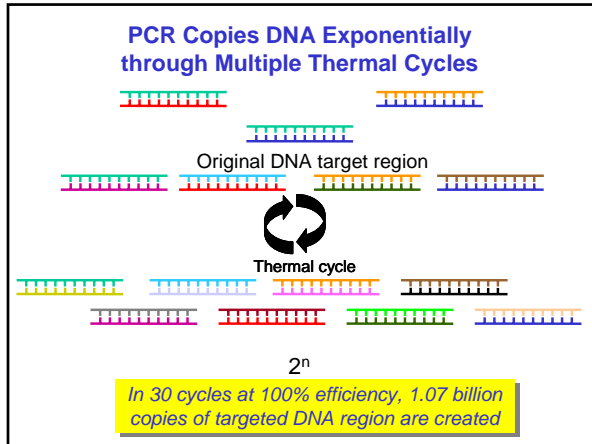
- Forensic cases -- **matching suspect with evidence**
- Paternity testing -- **identifying father**
- Missing persons investigations
- Military DNA “dog tag”
- Convicted felon DNA databases
- Mass disasters -- **putting pieces back together**
- Historical investigations and genetic genealogy

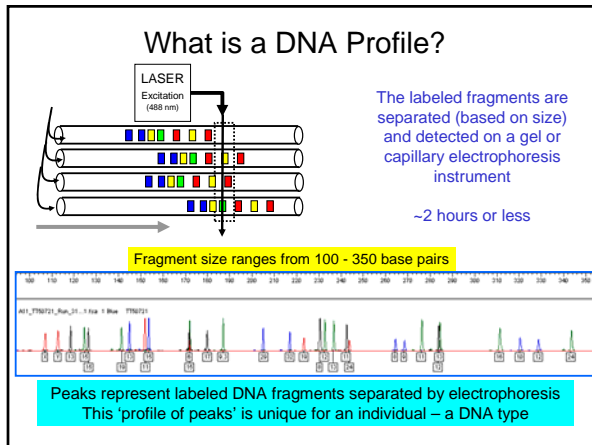
Involves generation of DNA profiles usually with the same genetic markers and then **MATCHING TO REFERENCE SAMPLE**

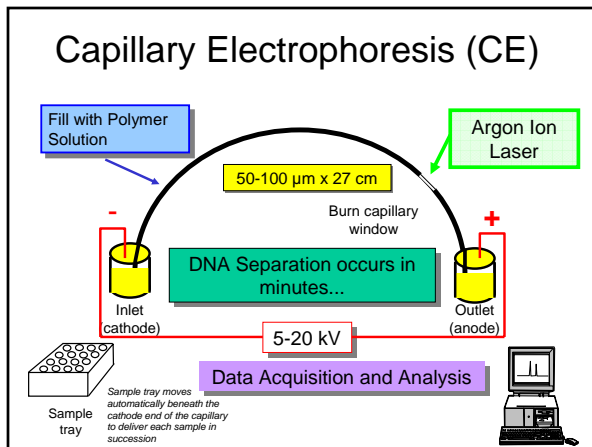














Capillary Electrophoresis Instrumentation

AB 310
single capillary



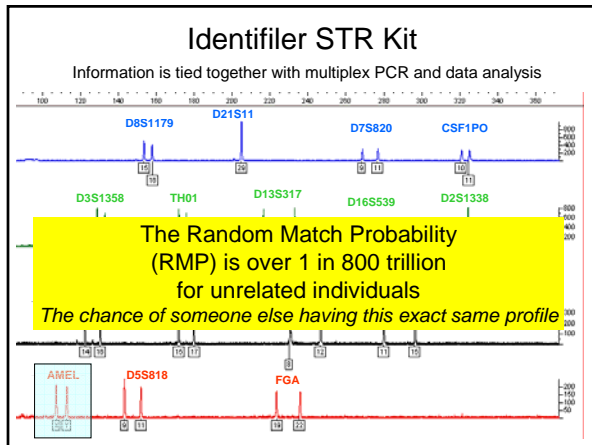
AB 3130
16-capillary array

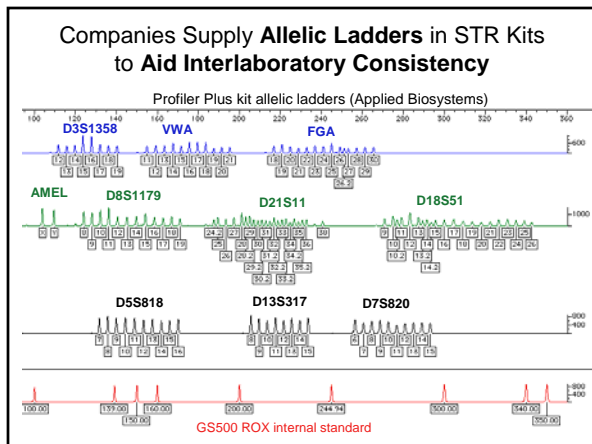


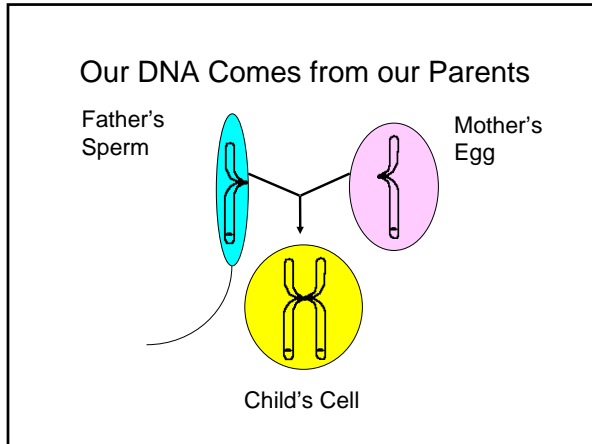
Newer Instruments

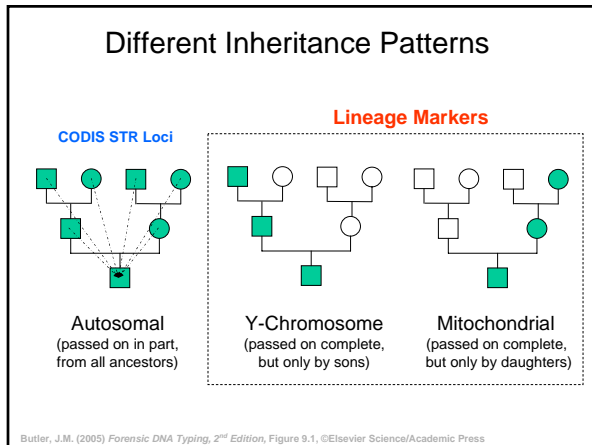
- 3730 – 48 capillaries
- 3730xl – 96 capillaries
- Chip platforms

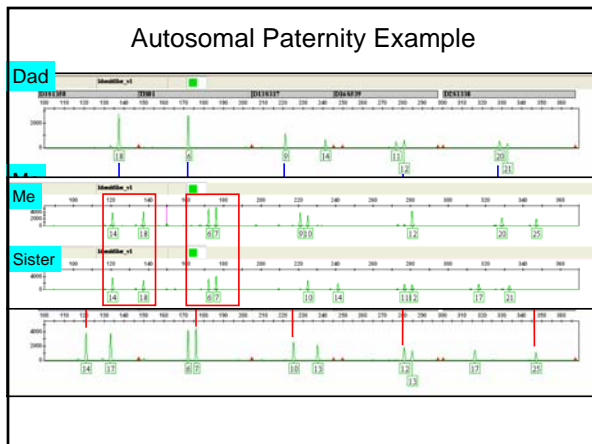
AB = Applied Biosystems (Foster City, CA)











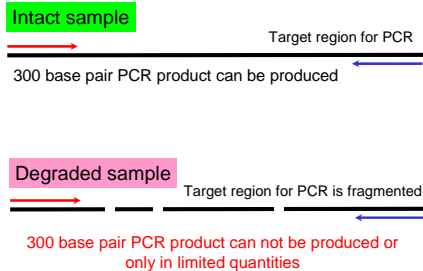
miniSTRs

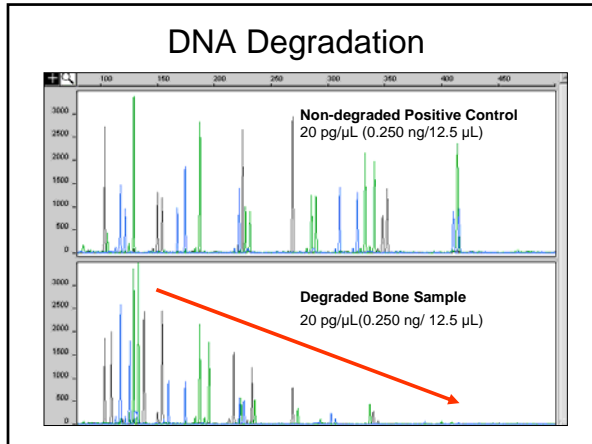
- Simply a smaller PCR product size
- Typically less than ~200 base pairs
- Contains the **same information** as a traditional STR (repeat length)
- Useful for typing degraded DNA samples
- New loci helpful for **missing persons paternity testing/mass disasters**

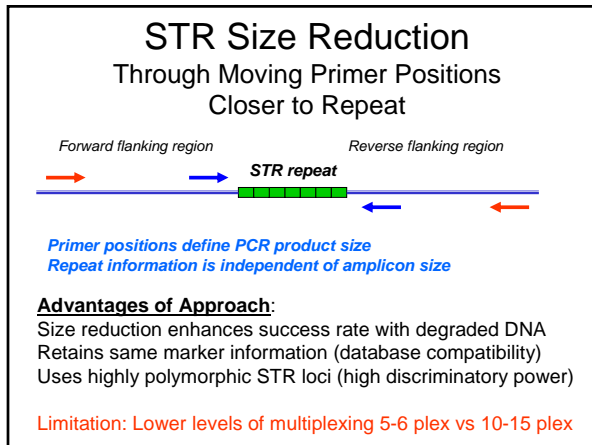
DNA Degradation

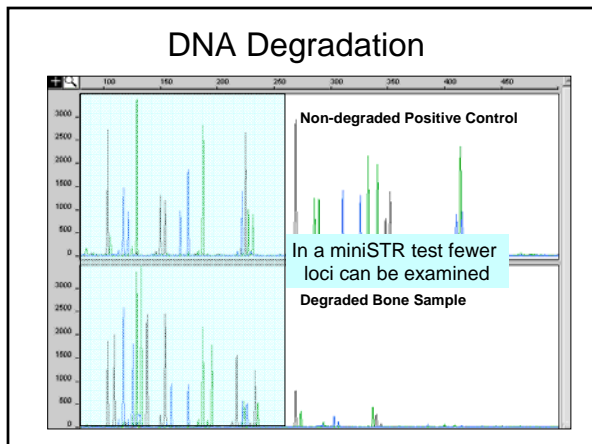
- What causes DNA degradation?
 - Heat, humidity, long term exposure to the elements
 - DNA breaks down into small fragments; smaller than the targeted PCR product size
- Mass disasters (aviation, WTC)
- Aged samples (missing persons, remains of soldiers, ancient DNA)

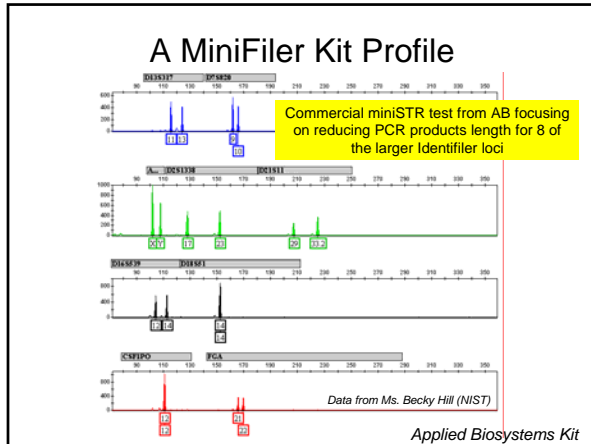
DNA Degradation

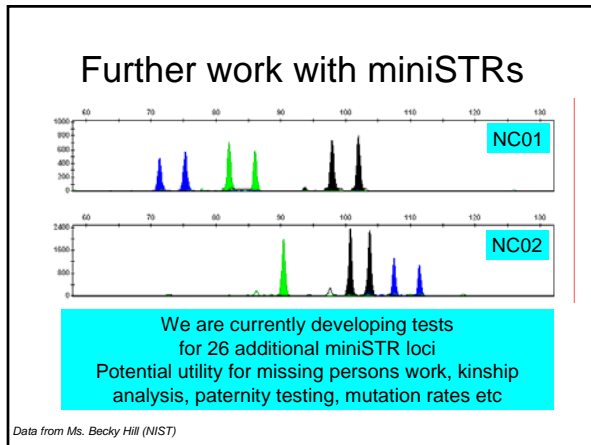


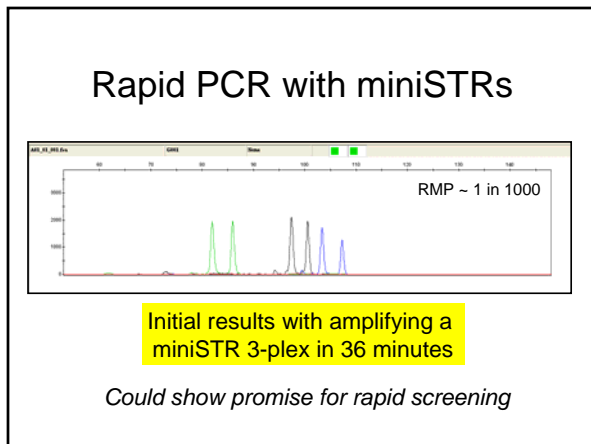












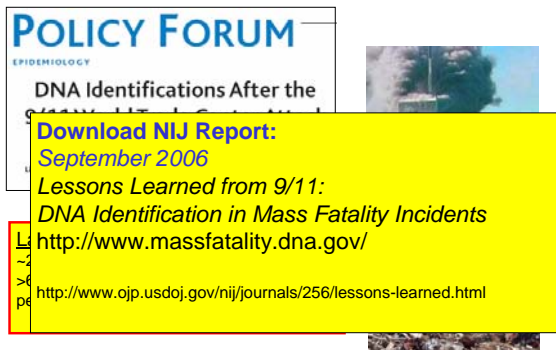
Identifying Victims of Mass Disasters

Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Chapter 24

POLICY FORUM
EPIDEMIOLOGY


DNA Identifications After the
September 11, 2001 Terrorist Attacks

Download NIJ Report:
September 2006
Lessons Learned from 9/11:
DNA Identification in Mass Fatality Incidents
<http://www.massfatality.dna.gov/>
<http://www.ojp.usdoj.gov/nij/journals/256/lessons-learned.html>



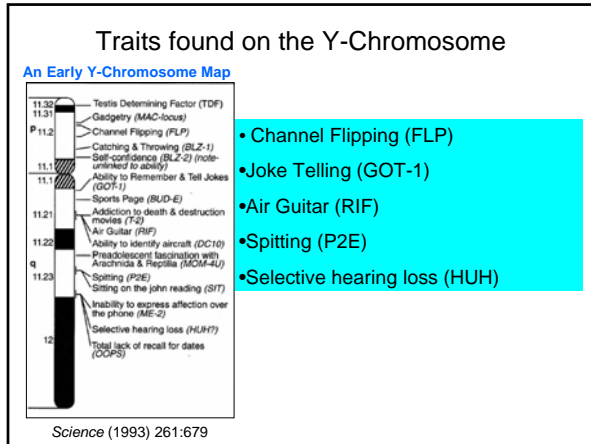
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Y-STRs

- 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)

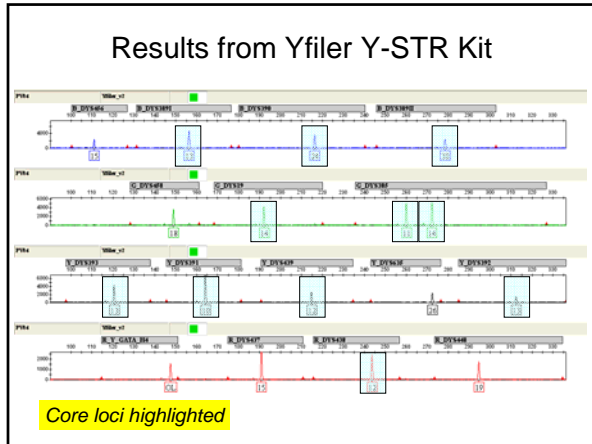


Value of Y-Chromosome Markers

<u>Application</u>	<u>Advantage</u>
Forensic casework on sexual assault evidence	Male-specific amplification (can avoid differential extraction to separate sperm and epithelial cells)
Paternity testing	Male children can be tied to fathers in motherless paternity cases
Missing persons investigations	Patrilineal male relatives may be used for reference samples
Human migration and evolutionary studies	Lack of recombination enables comparison of male individuals separated by large periods of time
Historical and genealogical research	Surnames usually retained by males; can make links where paper trail is limited

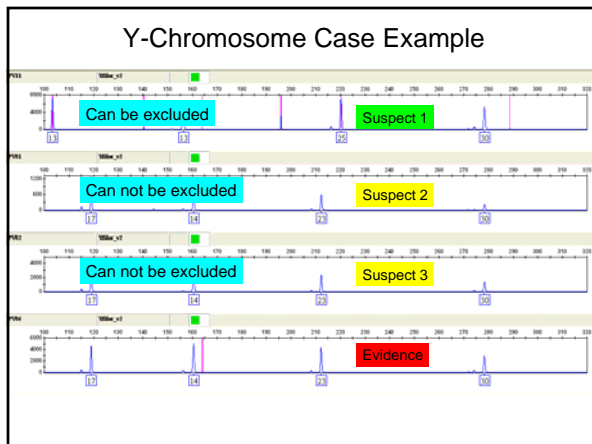
J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition; Table 9.1

- ### Disadvantages of the Y-Chromosome
- Loci are not independent of one another and therefore rare random match probabilities cannot be generated with the product rule; must use haplotypes (combination of alleles observed at all tested loci)
 - **Paternal lineages possess the same Y-STR haplotype** (barring mutation) and thus fathers, sons, brothers, uncles, and paternal cousins cannot be distinguished from one another
 - **Not as informative as autosomal STR results**
 - More like addition ($10 + 10 + 10 = 30$) than multiplication ($10 \times 10 \times 10 = 1,000$)



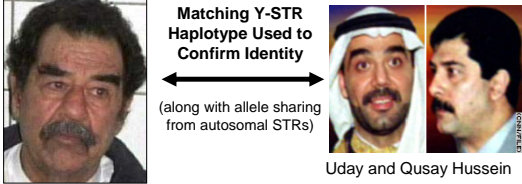
The Meaning of a Y-Chromosome Match

Conservative statement for a match report:
The Y-STR profile of the crime sample matches the Y-STR profile of the suspect (at xxx number of loci examined). Therefore, **we cannot exclude the suspect** as being the donor of the crime sample. In addition, we cannot exclude all patrilineal related male relatives and an unknown number of unrelated males as being the donor of the crime sample.



Modern Use of Y-STR Testing

Captured December 13, 2003



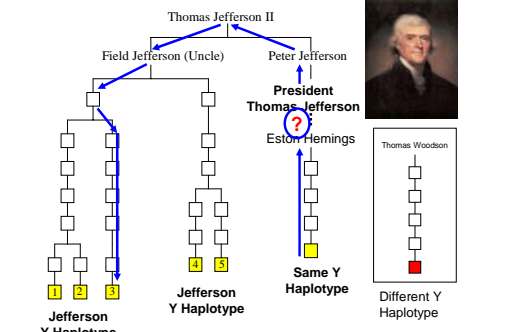
Is this man really Sadaam Hussein?

Killed July 22, 2003

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)




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

What has happened in the past few years...

- "Full" Y-chromosome sequence became available in June 2003; over 350 Y-STR loci identified (only ~20 in 2000)
- **Selection of core Y-STR loci** (SWGAM Jan 2003)
- **Commercial Y-STR kits released**
 - PowerPlex Y (9/03), Yfiler (12/04)
- Many population studies performed and databases generated with thousands of Y-STR haplotypes
- Forensic casework demonstration of value of Y-STR testing along with court acceptance

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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

SNPs

- More abundant than STRs (one very 1000 bases)
- Typically bi-allelic versus multi-allelic STRs
- Used for disease association studies
- Autosomal, Y-chromosome and mitochondrial SNPs
- Lower mutation rate than STRs

Utility of SNPs

- Similar to STRs – identification (but more are needed (50 versus 15))
- Work with degraded DNA (small PCR products < 100 bp)
- In lineage markers Y-chromosome and mitochondrial DNA
- To estimate population of origin – ancestry
- Possible phenotypic information hair and eye color

Allele-Specific Primer Extension

SNP Primer is extended by one base unit

“tail” used to vary electrophoretic mobility

Oligonucleotide primer 18-28 bases

ABI PRISM® SNaPshot™ Multiplex System

Fluorescently labeled ddNTPs + polymerase

PCR Amplified DNA Template

ddNTP	Dye label	Color
A	dR6G	Green
C	dTAMRA	Black
G	dR110	Blue
T	dROX	Red

25 Cycles	
96°C	10s
50°C	5s
60°C	30s

6-plex SNP Assay

Extension primers for 6-plex

```

(1) TTTT TAGCTCCTAATTTCTTGATGGG
(2) TTTT TTTT CATCTGATGCCATGAGAAAGC
(3) TTTT TTTT GTTCTGCTTTAATACAAAACCAG
(4) TTTT TTTT ATAAAGGCAGAATGAGGATTA
(5) TTTT TTTT AGAAAGTATCTTGCAAAGGTCCA
(6) TTTT TTTT CATAATCACAGCTTTTTTCTCCCAA
    
```

The Current mtDNA Amplification & Sequencing Strategy Focuses on the Hypervariable Regions of the mitochondrial genome HV1 and HV2

In Caucasians, approximately 7% of HV1 and HV2 sequences are identical

mtDNA Coding Region 11-plex ASPE Assay

11-plex PCR and 11-plex SNP detection
Sites are polymorphic in Caucasians (H1) and useful in resolving most common HV1/HV2 types
Multiplex PCR used to co-amplify all regions of interest at once
PCR product sizes kept under 200 bp to enable success with degraded DNA samples

Vallone et al., IJLM 2004 118: 147-57

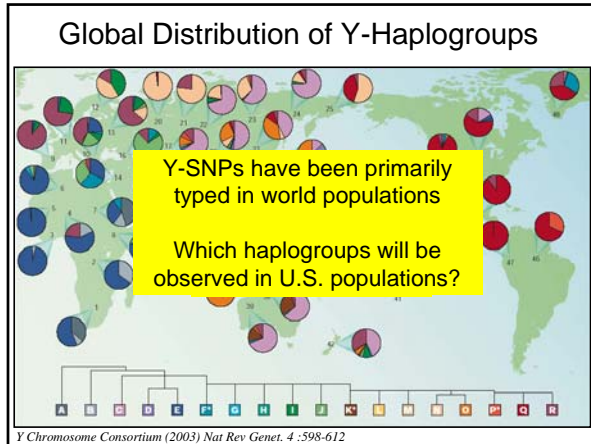
Utility of Y-Chromosome SNPs

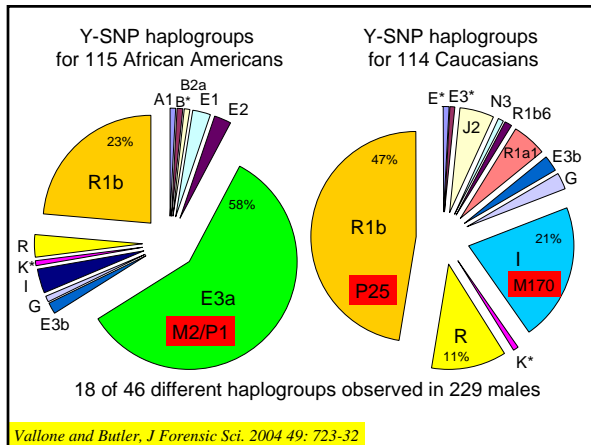
Y chromosome markers are useful in mixed male - female samples

Haplogroups are non-randomly distributed among populations therefore potential exists for predicting population of origin

Low mutation rate of SNPs 2×10^{-8} per base per generation

The Y Chromosome Consortium Map (2003) Nat Rev Genet. 4 :598-612

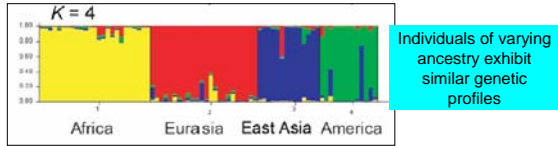




Ancestry Informative Markers (AIMs)

- AIMs are polymorphisms that relate information about population structure
- Exhibit different frequencies in specific population groups
- Could be used to estimate the population of origin for an individual (Caucasian, Hispanic, African American, Asian etc)

Ancestry Informative Markers (AIMs)



With an appropriate set of 10 SNPs genetic ancestry can be estimated

Data from Lao et al. AJHG 2006 78: 680-690

Pigmentation Genetics & Golden Gene

- Lighter variations of pigmentation in humans are associated with diminished number, size, and density of melanocytes. The pigmented

The evolutionarily conserved ancestral allele of a human coding polymorphism predominates in African and East Asian populations. In contrast, the variant allele is nearly fixed in European populations...and correlates with lighter skin pigmentation in admixed populations

heterozygosity, and correlates with lighter skin pigmentation in admixed populations, suggesting a key role for the SLC24A5 GENE in human pigmentation.

Science 16 December 2005:Vol. 310, no. 5755, pp. 1782 - 1786

Commercially Available Tests




ANCESTRYbyDNA 2.5

Skin Pigmentation
Eye color
Hair color
Earlobe attachment

http://www.dnaprint.com

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NIST Standard Reference Materials (SRMs)
Standard Reference Materials Program


SRM 2390 - DNA Profiling Standard
Meets RFLP Needs

SRM 2391 - PCR-Based DNA Standard
Cell Lines and Genomics

SRM 2395 – Y-chromosome DNA standards

NIST SRMs are used to help calibrate forensic genotyping laboratories


Soon: SRM 2372 Human DNA Quantitation




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


Working to update 2391b with new miniSTRs and 2395 with new Y-STRs

Lab 1

Lab 2

Standards Reference Material


Calibration with SRMs enables confidence in comparisons of results between laboratories



... 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>

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Results Announced in Anna-Nicole Smith DNA Parentage Test



Tim 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.

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?n=Top%2fReference%2fTimes%20Topics%2fOrganizations%2fDuke%2fUniversity%20>

address <http://www.innocenceproject.org>

Innocence Project <http://www.innocenceproject.org>

Search

About This Innocence Project | Case Profiles | Causes & Remedies | Support Us | Legislation | DNA News | Links

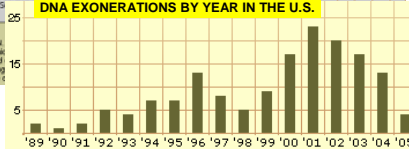


Michael A. Williams Exonerated

Kenneth Wyniemo
Year of Incarceration: 1994
Jurisdiction: Michigan
Sentence: 40-80 years
Year of Exoneration: 2005

March 11, 2005 **163 exonerated as**
167 EXONERATED of October 12, 2005

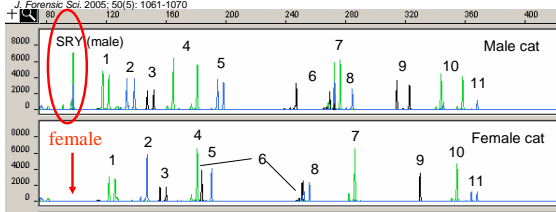
DNA EXONERATIONS BY YEAR IN THE U.S.



The Innocence Project at the University of North Carolina at Chapel Hill is a non-profit legal clinic through postconviction DNA testing, and cases where postconviction DNA testing vindicates innocent people.

New DNA Test for Cats Developed at NIST

J. Forensic Sci. 2005; 50(5): 1061-1070



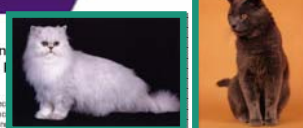
PROFILES IN DNA

MEOWPLEX

The MeowPlex: A New DNA Test Using Tetranucleotide STR Markers for the Identification of Individual Cats

By John M. Butler*, Victor A. David†, Stephen J. O'Brien*, and Marilyn Menotti-Raymond†

*Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, Maryland, and †Laboratory of Genomic Diversity, National Cancer Institute, Frederick, Maryland



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



Vietnam Veterans Memorial


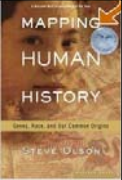
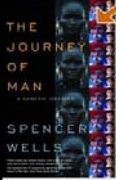


- **Armed Forces DNA Identification Laboratory (AFDIL)** (Rockville, MD)
- In June 1998 AFDIL identified Michael J. Blassie as the Vietnam Unknown in the Tomb of the Unknown Soldier (located in Arlington National Cemetery)
- **There will be no more "unknown" soldiers.**

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

The Genographic Project

<https://www3.nationalgeographic.com/genographic/>



- 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

The New York Times Genetic Genealogy

THE DNA AGE
Stalking Strangers' DNA to Fill in the Family Tree




David Calvert for The New York Times

Melissa Roberts has collected thousands of documents on the Springes, her father's family, and spent over \$1,000 on DNA testing of potential relatives.

By AMY HARMON
Published: April 2, 2007

EMAIL

The New York Times Genetic Genealogy



Derrell Teat, 63, a wastewater coordinator, recently found herself staking out a McDonald's. The man she believed was the last male descendant of her great-great-great grandfather's brother had refused to give her his DNA. So she decided to get it another way. "I was going to take his coffee cup out of the garbage can," said Ms. Teat, who traveled to the Georgia mountains from Tampa, Fla., with her test kit. "I was willing to do whatever it took."

Derrell Teat, determined in her research, once waited outside a restaurant with a test kit hoping to capture a reluctant would-be relative's DNA on a coffee cup.

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



COLLECTIVE

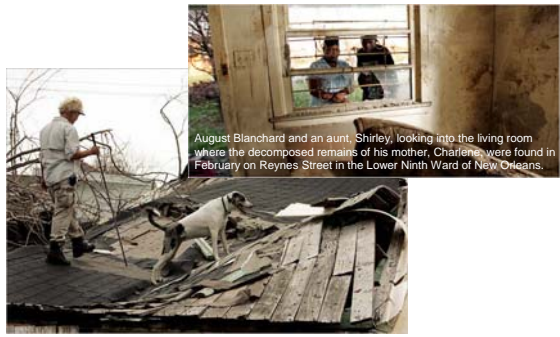
<http://www.cnn.com/2005/US/03/02/baby.81.ap/index.html>

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.

NIST Human Identity Project Team



John Butler (Project Leader) Margaret Kline Pete Vallone
Dave Duewer (Anal. Chem. Division) Jan Redman Amy Decker Becky Hill

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