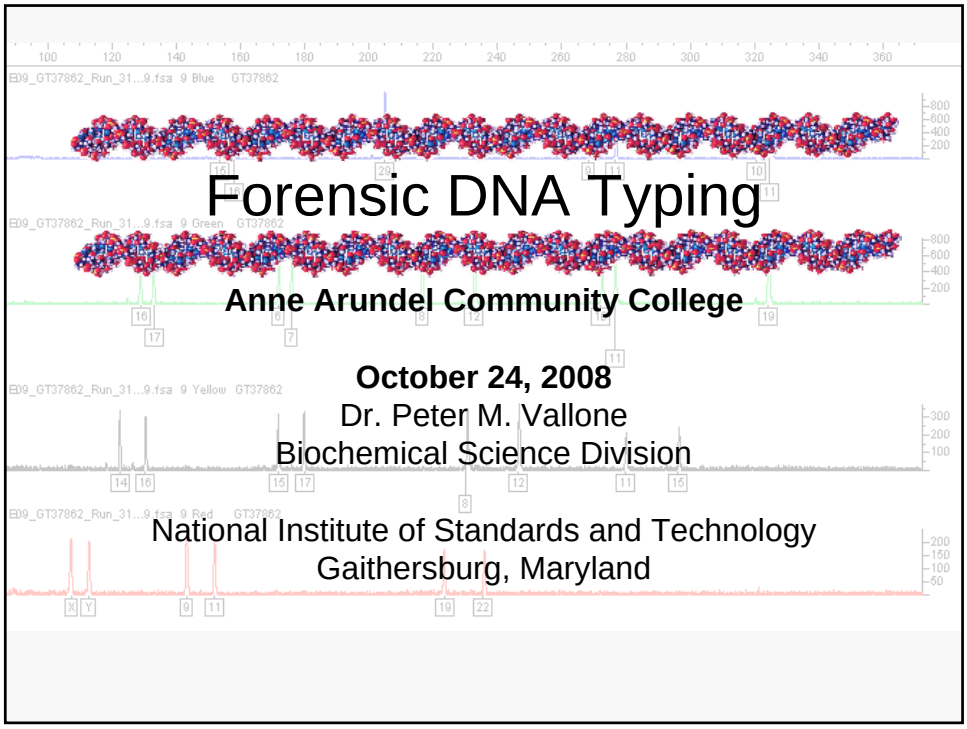


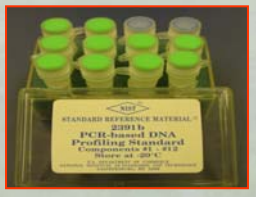

## Outline

- NIST
- Basics of DNA Typing
- Paternity Testing
- Newer DNA tests
- Everyday Examples

# NIST History and Mission

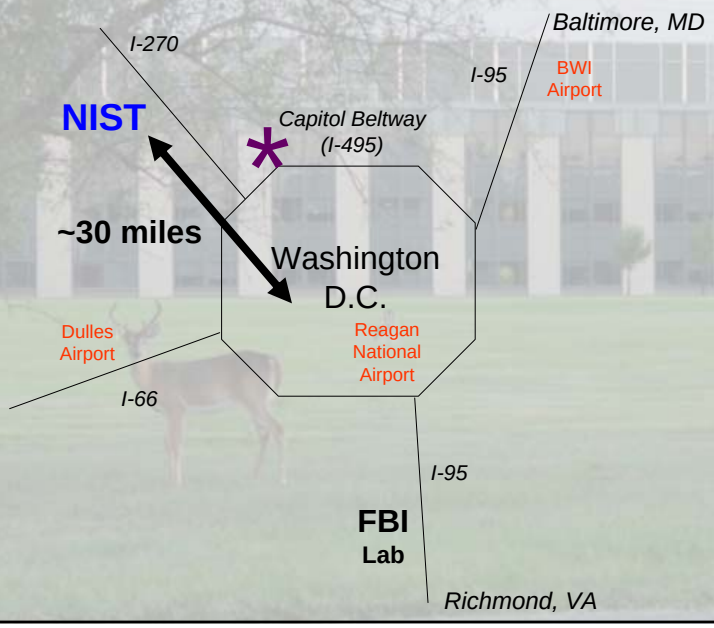
- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is **part of the U.S. Department of Commerce** with a mission to develop and promote measurement, standards, and technology to enhance productivity,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

# Location of NIST (Gaithersburg, MD)



## NIST Human Identity Project Team


John Butler
(Project Leader)


Margaret Kline


**Pete Vallone**


Dave Duewer
*Anal. Chem. Division*


Jan Redman


Amy Decker


Becky Hill

Funding: **Interagency Agreement 2003-IJ-R-029 between National Institute of Justice (NIJ) and NIST Office of Law Enforcement Standards (OLES)**

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

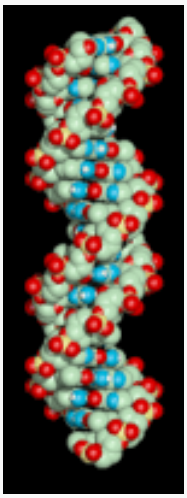
## Current Areas of Effort with Forensic DNA

- **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**

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

## Forensic Applications

- 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
- Genetic genealogy

## 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)

## Steps in DNA Analysis

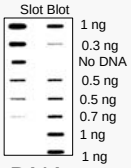
**Steps Involved**

- Collection
- Specimen Storage
- Extraction
- Quantitation
- Multiplex PCR
- STR Typing
- Interpretation of Results
- Database Storage & Searching
- Calculation of Match Probability

*Usually 1-2 day process (a minimum of ~5 hours)*

Blood Stain Buccal swab
Sample Collection & Storage

DNA Extraction




DNA Quantitation

Biology

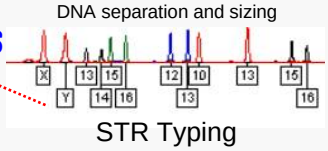
Multiplex PCR Amplification

Genetics

**If a match occurs**, comparison of DNA profile to population allele frequencies to generate a case report with probability of a random match to an unrelated individual

DNA Database Search

DNA separation and sizing



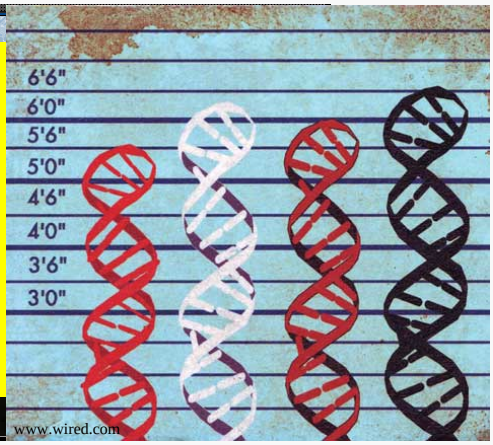

Technology

STR Typing
Interpretation of Results

CSI: CRIME SCENE INVESTIGATION

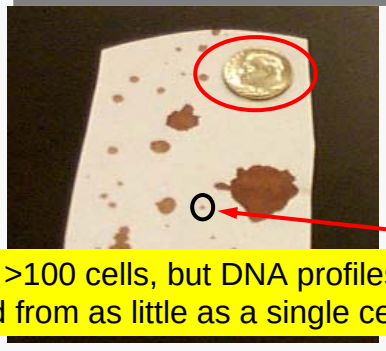
**Unfortunately, current DNA testing cannot be performed as quickly as a commercial break…**

**The instruments on CSI are real – they just do not collect data as quickly as shown on TV**




## Sources of Biological Evidence

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



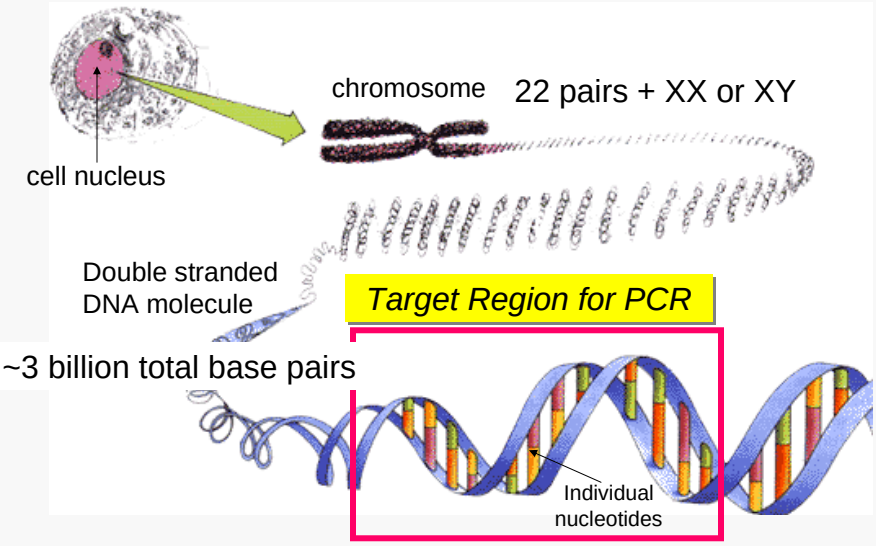
Blood Sample

**Only a very small amount of blood is needed to**

**DNA**

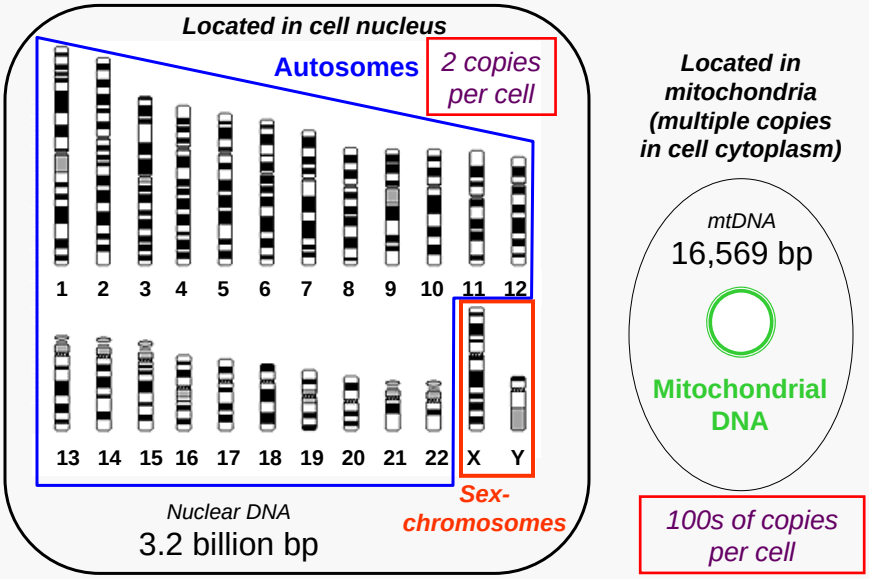
best results with >100 cells, but DNA profiles can be recovered from as little as a single cell

# 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

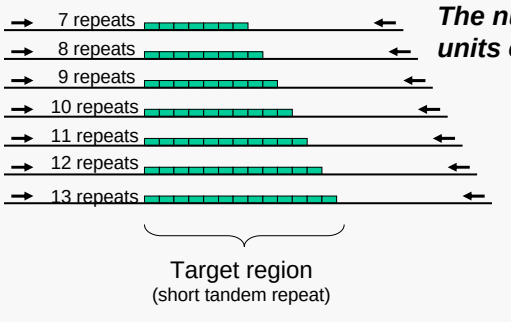
GCTAGTCGATGCTC**(G/A)**GCGTATGCTGTAGC

# Short Tandem Repeat (STR) Markers

***An accordion-like DNA sequence that occurs between genes***

TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACA
GGTG**GATAGATAGATAGATAGATAGATAGATAGATAGATAGA**
**TAGATAGATA**TCATTGAAAGACAAAACAGAGATGGATGATAGAT
ACATGCTTACAGATGCACAC

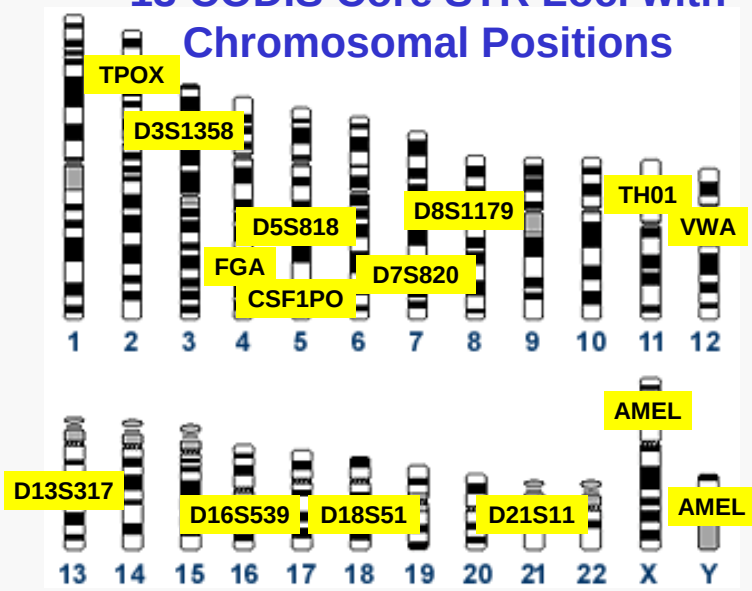
**= 12 GATA repeats ("12" is all that is reported)**



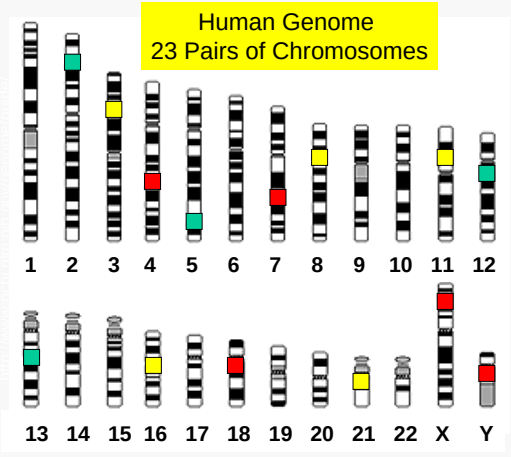

***The number of consecutive repeat units can vary between people***

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

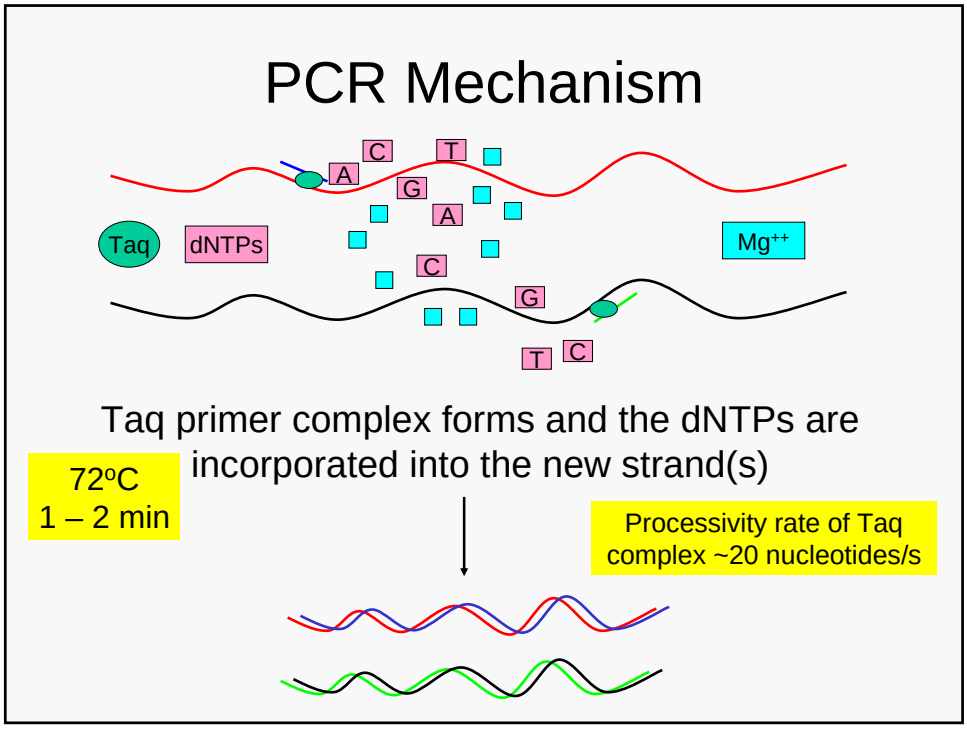
# PCR Mechanism

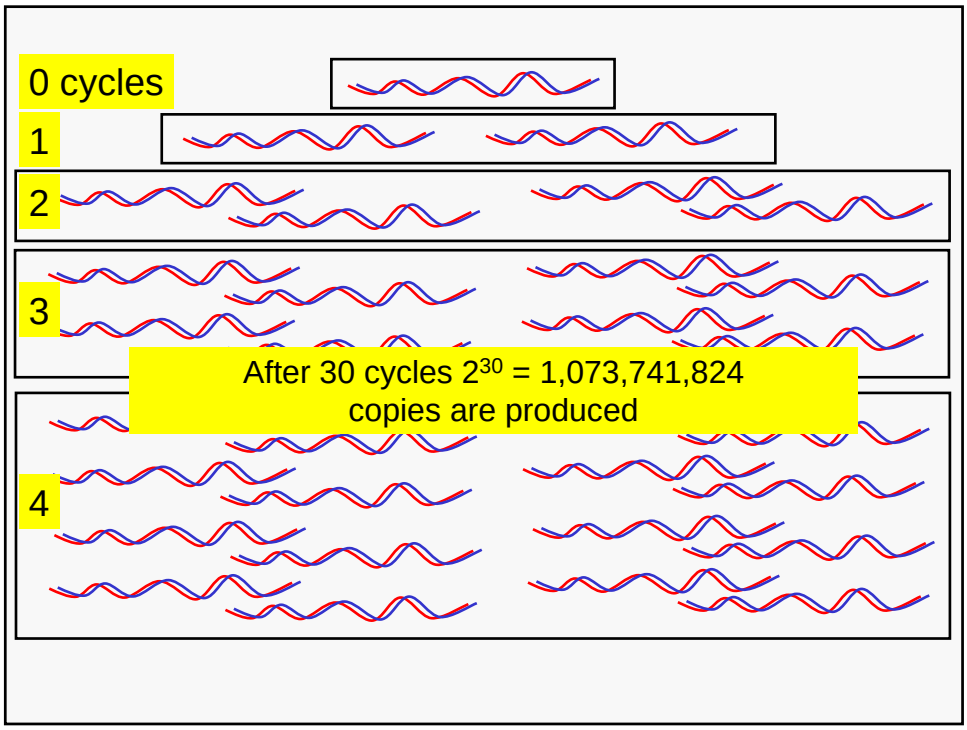
95°C
30 – 60s

Denature the genomic DNA template

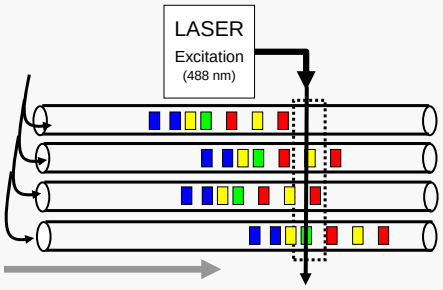

~60°C
1 – 2 min

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


PCR Mechanism
C
T
A
G
A
Taq
dNTPs
Mg++
C
G
T
C
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


0 cycles
1
2
3
After 30 cycles $2^{30}$ = 1,073,741,824 copies are produced
4

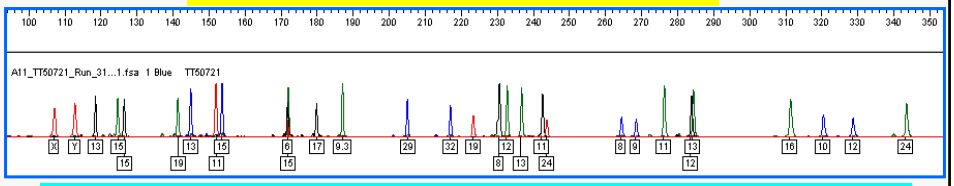
## What is a DNA Profile?




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

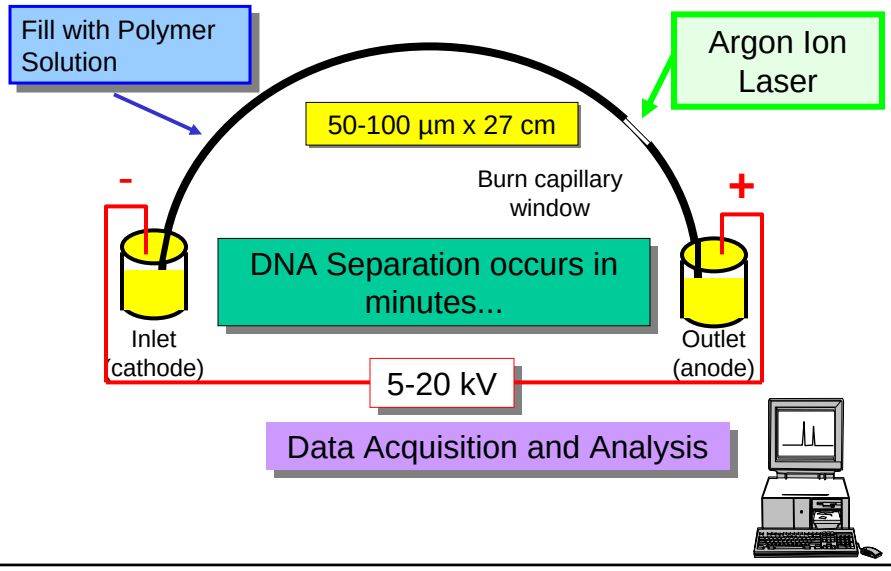
~2 hours or less

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

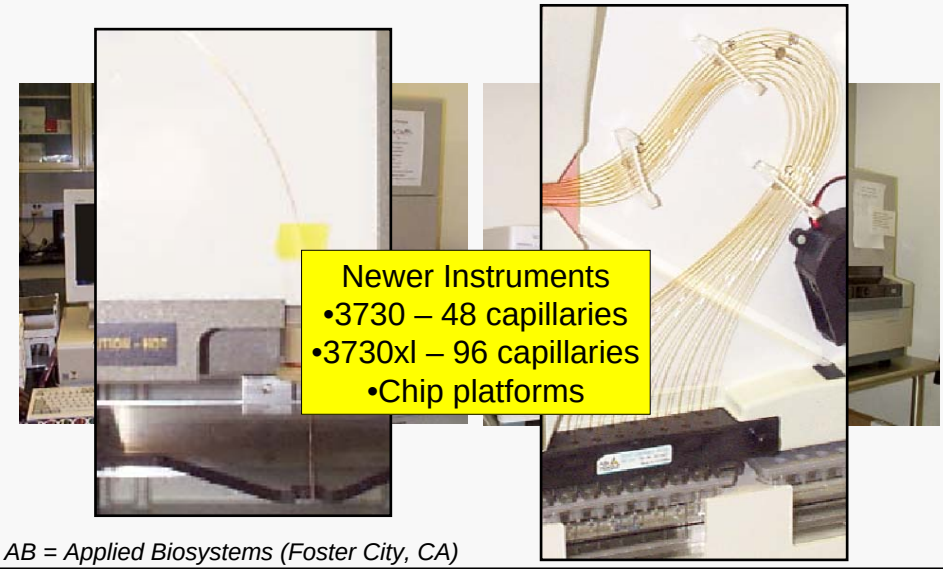
## Capillary Electrophoresis (CE)



## Capillary Electrophoresis Instrumentation

**AB 310**
**single capillary**

**AB 3130**
**16-capillary array**

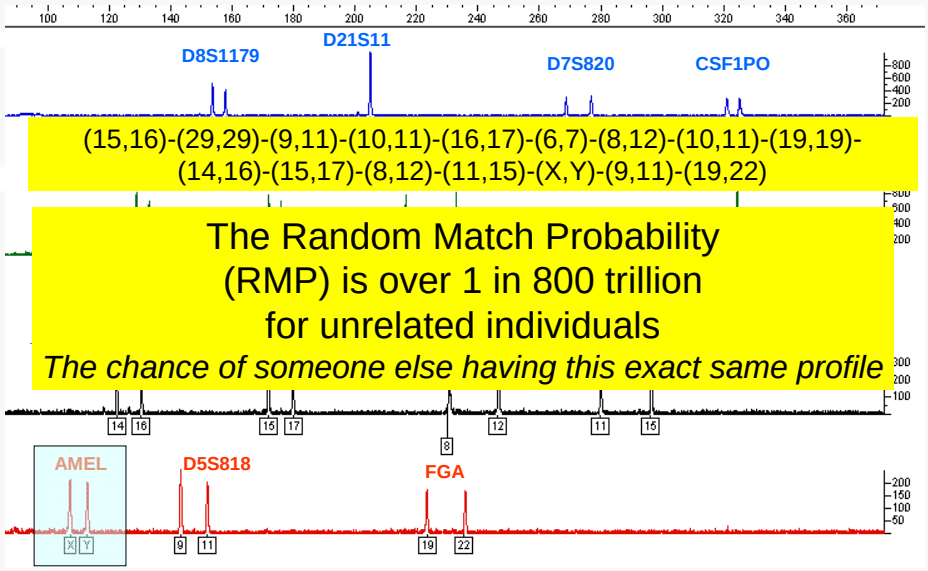


Newer Instruments
•3730 – 48 capillaries
•3730xl – 96 capillaries
•Chip platforms

*AB = Applied Biosystems (Foster City, CA)*

## Identifiler STR Kit

Information is tied together with multiplex PCR and data analysis

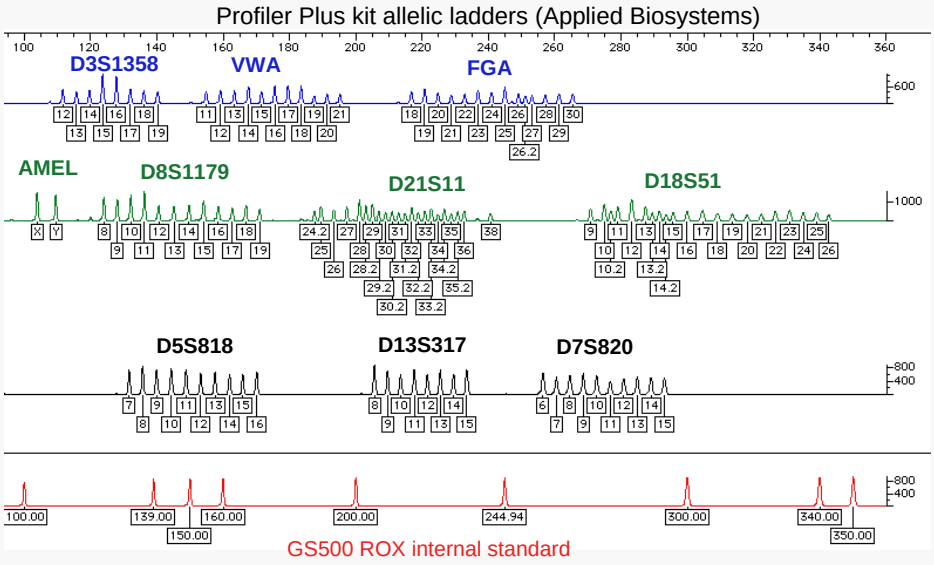


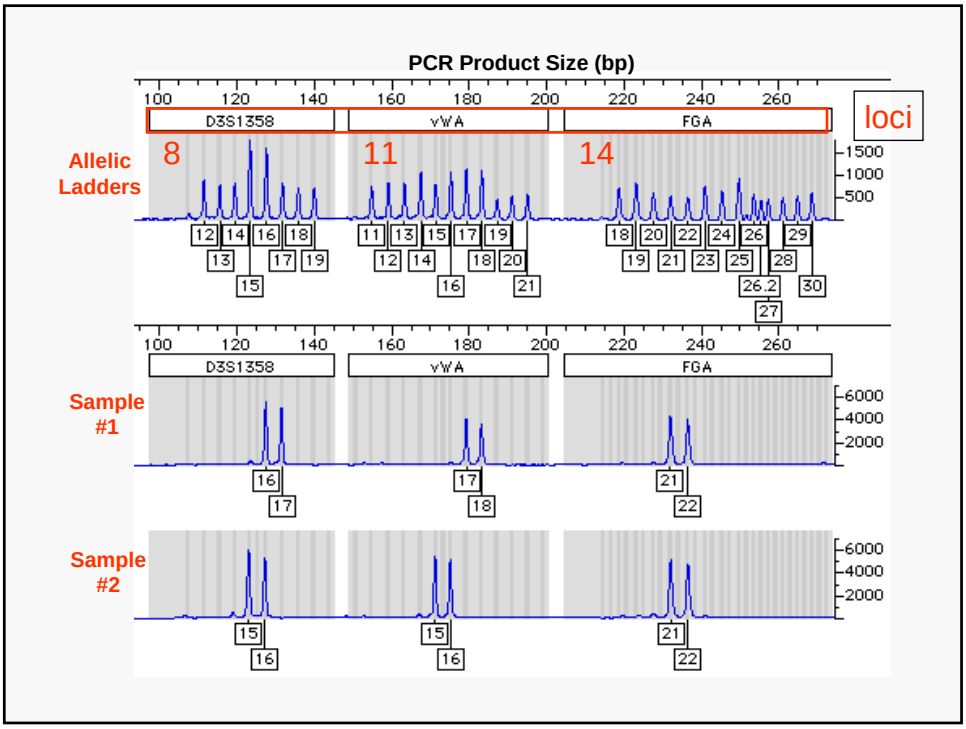

(15,16)-(29,29)-(9,11)-(10,11)-(16,17)-(6,7)-(8,12)-(10,11)-(19,19)-(14,16)-(15,17)-(8,12)-(11,15)-(X,Y)-(9,11)-(19,22)

The Random Match Probability (RMP) is over 1 in 800 trillion for unrelated individuals

*The chance of someone else having this exact same profile*

## Companies Supply **Allelic Ladders** in STR Kits to **Aid Interlaboratory Consistency**



## Data Format

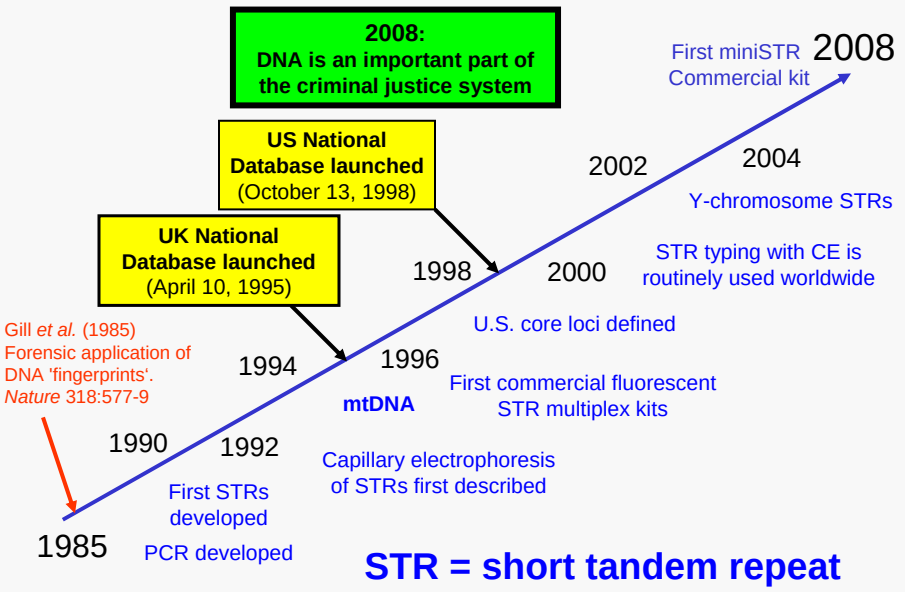
| | AMEL | CSF1PO | FGA | TH01 | TPOX | VWA | D3S1358 | D5S818 |
|---|---|---|---|---|---|---|---|---|
| Ind(1) | X,Y | 11,12 | 19,21 | 6,7 | 8,8 | 15,18 | 14,18 | 10,13 |

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

## A Brief History of Forensic DNA Typing



# CODIS

**Combined DNA Index System (CODIS)**

- 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


>74,700 Investigations Aided through August 2008

**Maryland**

| Statistical Information | Total |
|---|---|
| Offender Profiles | 69,896 |
| Forensic Samples | 4,584 |
| Number of CODIS Labs | 6 |
| NDIS Participating Labs | 5 |
| Investigations Aided | 1,119 |

Back to top

**Total number of profiles: ~6.4 million**
**Total Forensic profiles: 233,454**
**Total Convicted Offender Profiles: 6,220,372**

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

## Standard Reference Materials

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

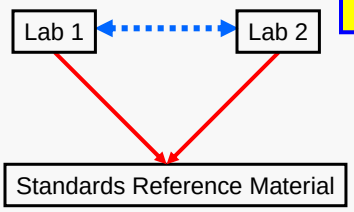
*Traceable standards to ensure accurate measurements in our nation's crime laboratories*


National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2391b
PCR-based DNA Profiling Standard

**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***



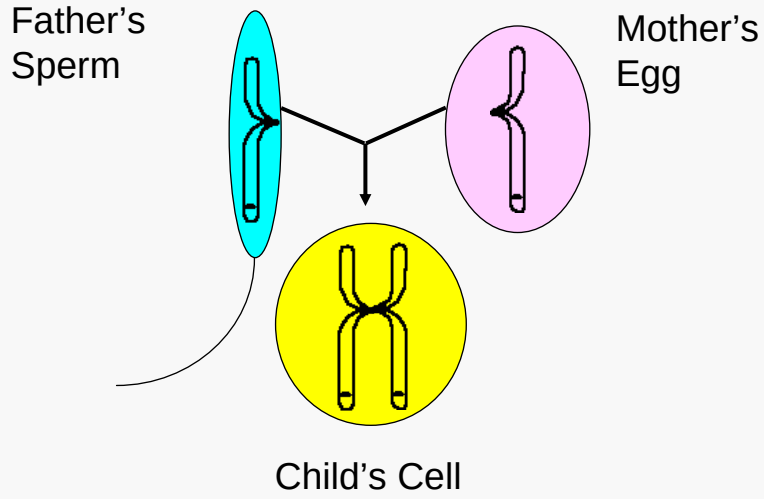

**Calibration with SRMs enables confidence in comparisons of results between laboratories**

## Paternity Testing

## Paternity Testing

- DNA testing methods for determining paternity also relate to:
  - Mass disasters
  - Missing persons investigations
  - Familial matching
  - Genetic Genealogy

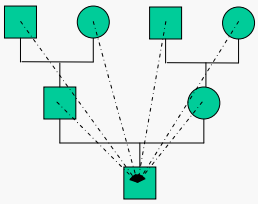
## Our DNA Comes from our Parents



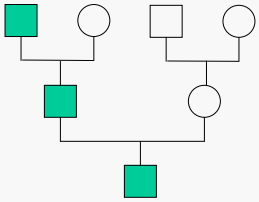
# Different Inheritance Patterns

**Lineage Markers**

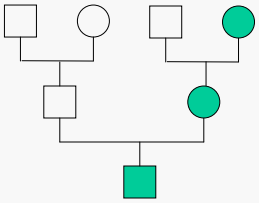
**CODIS STR Loci**



Autosomal
(passed on in part, from all ancestors)



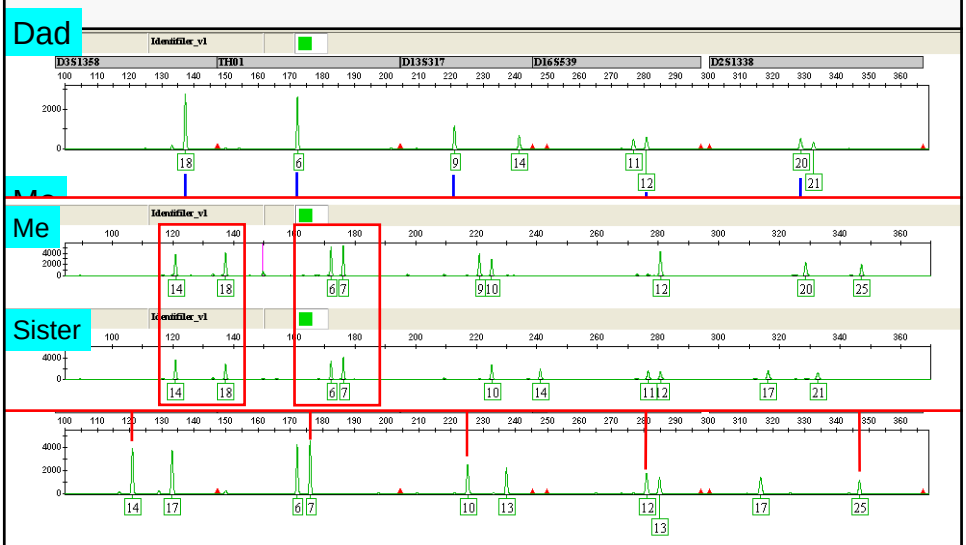
Y-Chromosome
(passed on complete, but only by sons)



Mitochondrial
(passed on complete, but only by daughters)

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

# Autosomal Paternity Example




Only looking at 5 markers…

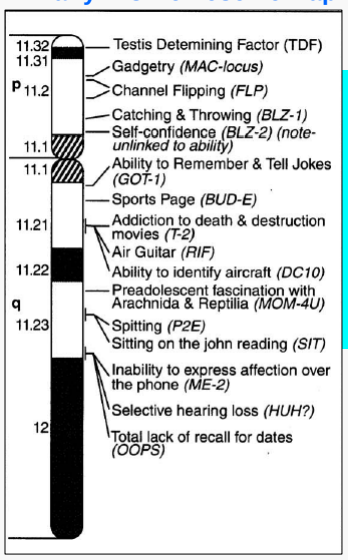
# Y-STRs

# Y-STRs

- Similar to autosomal STRs just located on the Y-Chromosome
- Since only males posses 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)

# Traits found on the Y-Chromosome

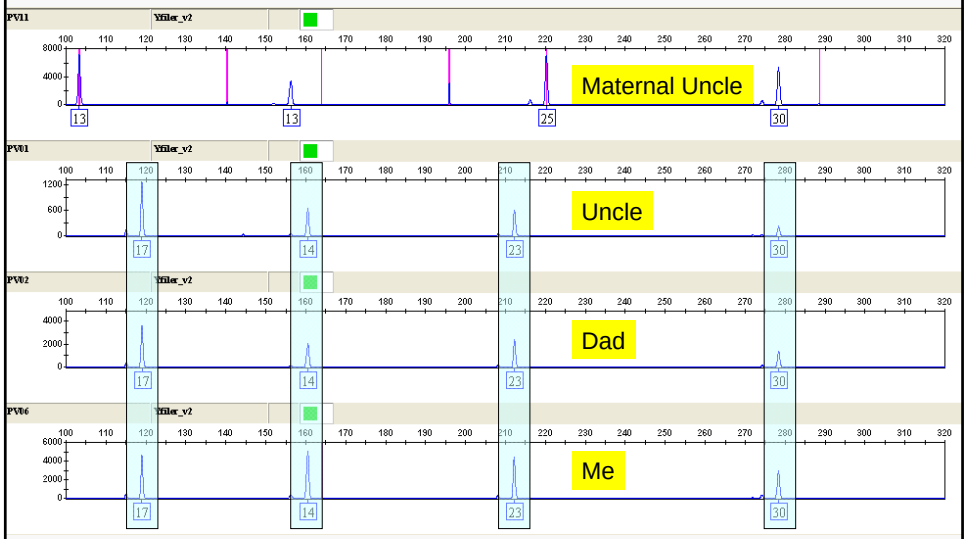
**An Early Y-Chromosome Map**




*Science* (1993) 261:679

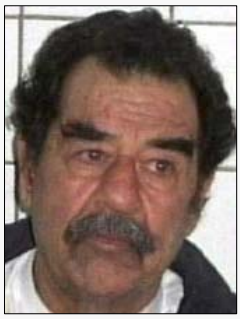
- Channel Flipping (FLP)
- Joke Telling (GOT-1)
- Air Guitar (RIF)
- Spitting (P2E)
- Selective hearing loss (HUH)

# Y-Chromosome Paternity Example



# 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

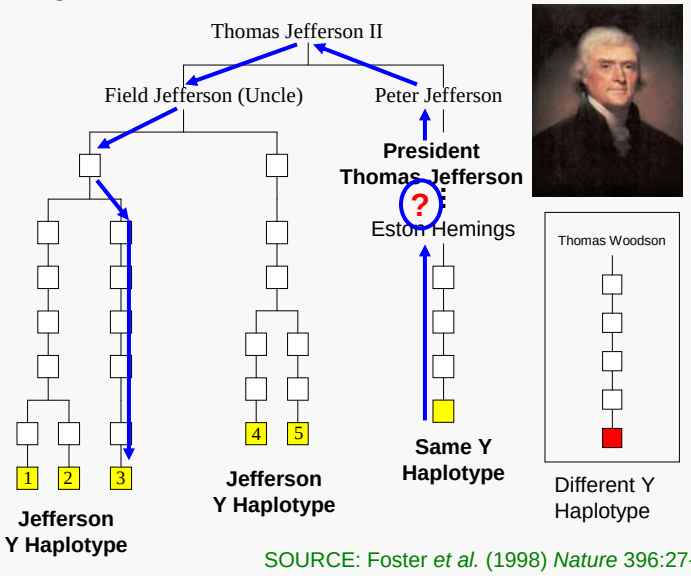
**Is this man really Sadaam Hussein?**

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)




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

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

## miniSTRs

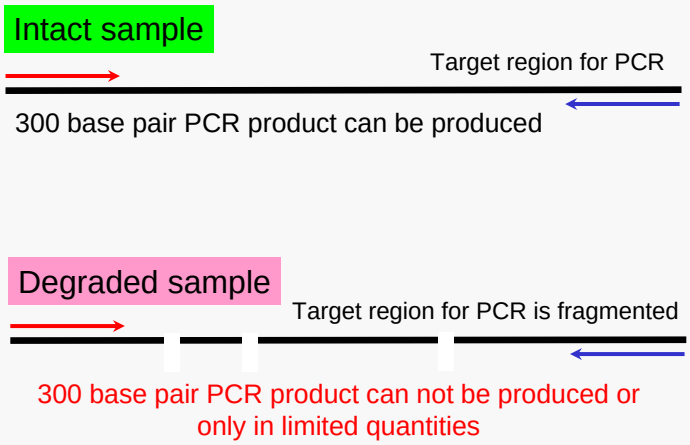
## miniSTRs

- 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

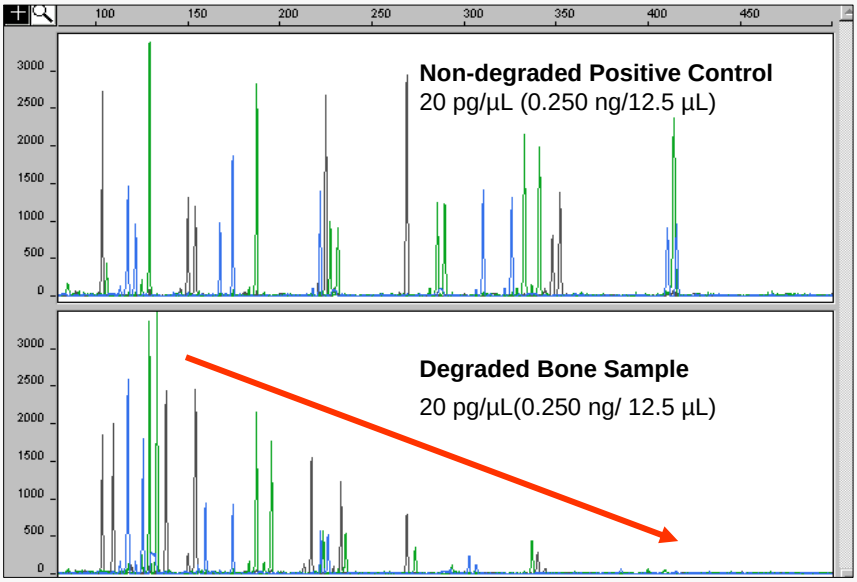
# DNA Degradation

- 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)

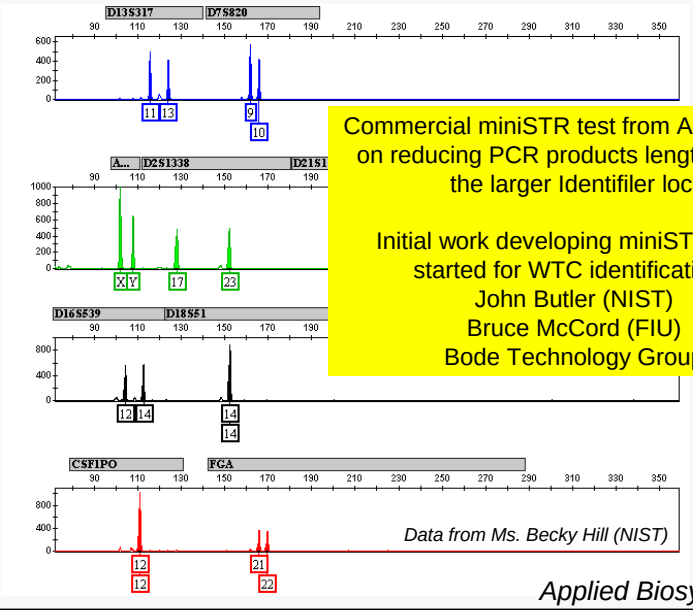
# DNA Degradation



# DNA Degradation




# A MiniFiler Kit Profile

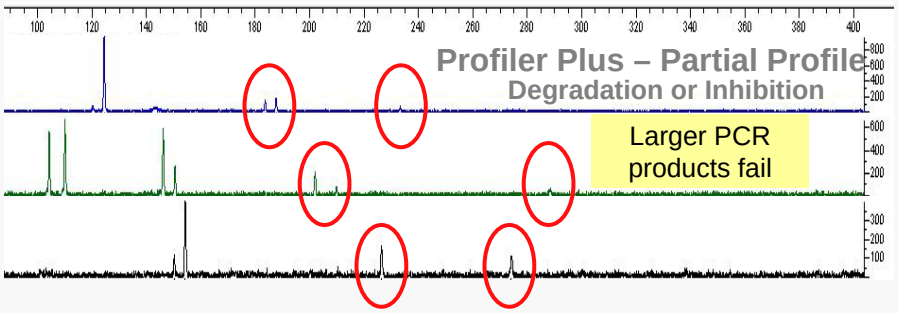



Commercial miniSTR test from AB focusing on reducing PCR products length for 8 of the larger Identifiler loci

Initial work developing miniSTRs was started for WTC identifications
John Butler (NIST)
Bruce McCord (FIU)
Bode Technology Group

*Applied Biosystems Kit*

## World Trade Center – Phase I Summary




| | | |
|---|---|---|
| | 12,392 | Bone samples processed |
| Over 6800 profiles | 3,405 | Full profiles (13 STR loci) |
| | 2,143 | High partial profiles (≥7 STR loci) |
| | 2,670 | Low partial profiles (<7 STR loci) |
| | 4,174 | No loci |

miniSTRs are helping here

Final 20% of WTC victims identified were based on a miniSTR technique pioneered at NIST

## 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

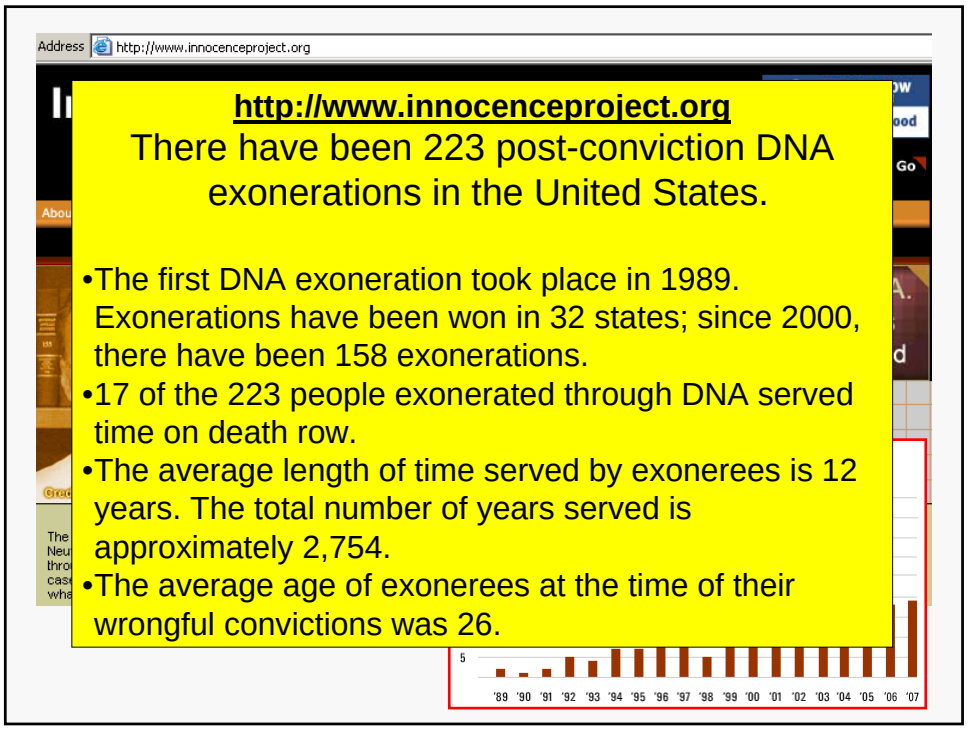
GCTAGTCGATGCTC**(G/A)**GCGTATGCTGTAGC

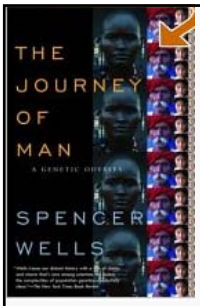
## Potential Use of SNPs in Forensic DNA Testing

- 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

# Forensic DNA in the News

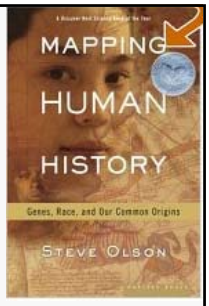





## The Genographic Project

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

THE GENOGRAPHIC PROJECT




- 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)

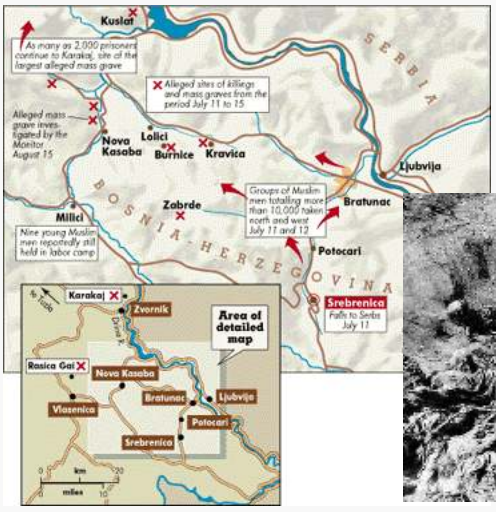
**NEW YORK (AP) -- The parents of the infant tsunami survivor nicknamed "Baby 81" say they found it difficult to feel overjoyed about their reunion in the midst of so much tragedy.**
The 4-month-old Sri Lankan baby and his parents, who were reunited after court-ordered **DNA tests proved their relationship**, 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**



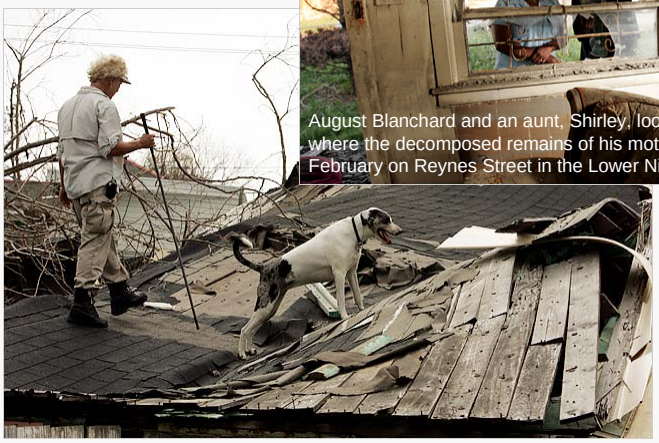

DNA testing is performed on 100s of bones collected each week from mass graves in Bosnia and Croatia to help in the re-association of remains

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.



Ozier Muhammad/The New York Times

# 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

- 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.

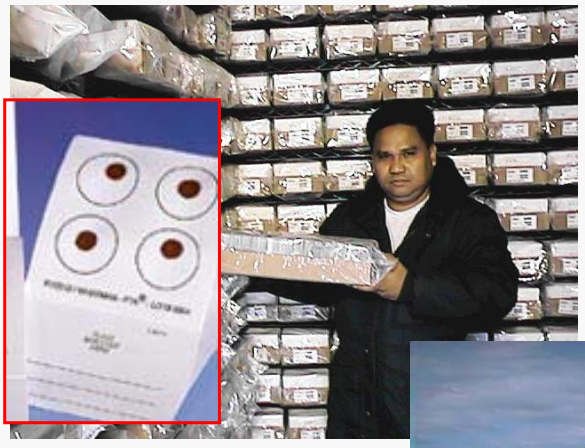
http://www.nytimes.com/2007/04/10/us/10dna.html

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%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

FEDERAL BUREAU OF INVESTIGATION
WASHINGTON, D. C. 20535

Report of Examination

Examiner Name: [redacted] Date: 08/17/98
Unit: DNA Analysis 2 Phone No.: 202-324-4409
FBI File No.: 29D-OIC-LR-35063 Lab No.: 980730002 S BO
980803100 S BO

Results of Examinations:

Deoxyribonucleic acid (DNA) profiles for the genetic loci D2S44, D17S79, D1S7, D4S139, D10S28, D5S110 and D7S467 were developed from HaeIII-digested high molecular weight DNA extracted from specimens K39 and Q3243-1(a semen stain removed from specimen Q3243). Based on the results of these seven genetic loci, specimen K39 (CLINTON) is the source of the DNA obtained from specimen Q3243-1, to a reasonable degree of scientific certainty.

No DNA-RFLP examinations were conducted on specimen Q3243-2 (a semen stain removed from specimen Q3243).

BLACK – 1,440,000,000,000
CAUC – 7,870,000,000,000
SEH – 3,140,000,000,000
SEH – 943,000,000,000

http://www.law.umkc.edu/faculty/projects/ftrials/clinton/lewinskydress.html

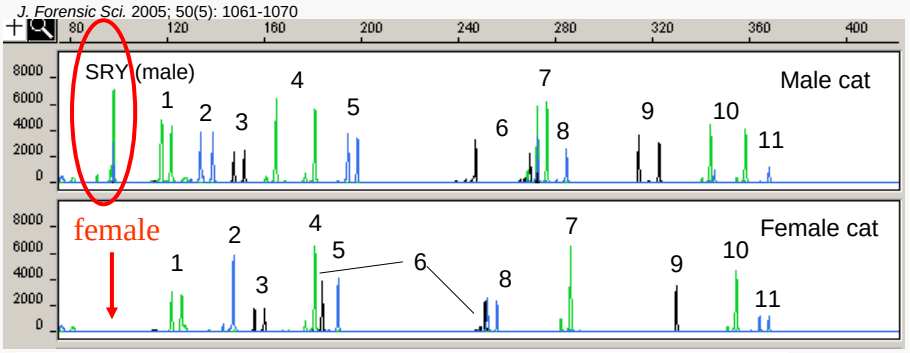
# Results Announced in Anna-Nicole Smith DNA Parentage Test

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.

## New DNA Test for Cats Developed at NIST




## Genetic Genealogy

# Resources

- American Academy of Forensic Science
  - www.aafs.org
  - http://www.aafs.org/?section_id=resources&page_id=colleges_and_universities
- Mid-Atlantic Association of Forensic Scientists
  - http://www.maafs.org
  - Hunt Valley, MD (May 4-8, 2009)

# STRBase

## Short Tandem Repeat DNA Internet Database

NIST National Institute of Standards and Technology
. . .working with industry to develop and apply technology, measurements and standards

Recent Additions

- **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

- Reference List
- Variant Alleles
- Addresses for Scientists
- Links to Other Web Sites
- Y-STR Information

We will continue to add downloadable PowerPoint files that can be used for training purposes

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