

NIST
National
Institute of
Standards
and Technology

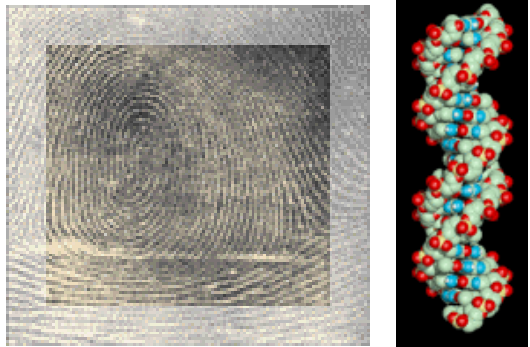
... working with industry to develop and apply technology, measurements and standards

Forensic DNA Identification and Standards Development

Margaret Kline
Research Biologist

Presented at: Shepherd University
THE COMMUNITY AND TECHNICAL COLLEGE OF SHEPHERD,
CJST 210. Introduction to Forensic Science (3) Martinsburg, WV.
28 September 2004

Methods for Human Identification

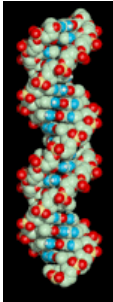


Fingerprints have been used since 1901
DNA since 1986

- DNA = Deoxyribo-Nucleic Acid
- It is in every cell of our bodies.
- Found in a long strand, like a piece of rope.
- Made up of a simple alphabet containing four letters: A, T, C, G


The order of these letters is what makes everyone different.


Characteristics of DNA





- Each person has a unique DNA profile (except identical twins).
- Each person's DNA is the same in every cell.
- An individual's DNA profile remains the same throughout life.
- Half of your DNA comes from your mother and half from your father.


Where can you find DNA?


Blood → 


Hair → 

Bone & Teeth → 


Urine → 

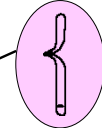
Sperm cells → 


Muscle & Tissue → 

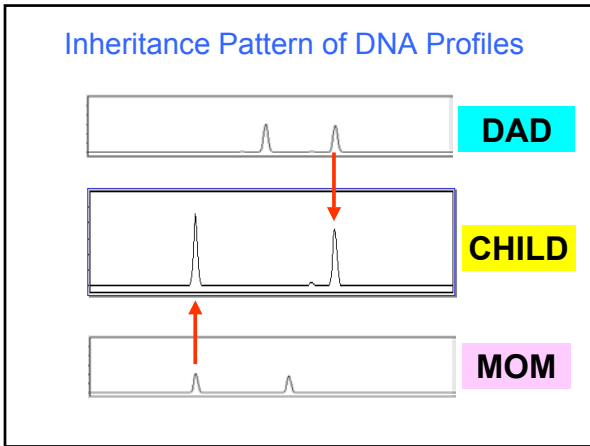
Saliva (spit contains cheek cells) → 

Our DNA Comes from our Parents

Father's Sperm → 

Mother's Egg → 

Child's Cell → 



DNA in the News

Charlottesville, Virginia (Fall 1999): DNA from a rape case matched to a suspect through a DNA profile database

World Trade Center Towers (Sept 11, 2001)

DNA typing being used as only possible method to identify ~2,800 victims of this tragedy

Wreckage at Ground Zero

DNA identification efforts are on-going with over 1,500 victims now identified

Applications for Human Identity Testing

- Crime solving – matching suspect with evidence...
- Accident victims – after airplane crashes...
- Soldiers in war – who is the “unknown” soldier...
- Paternity testing – who is the father...
- Inheritance claims – who gets the money...
- Missing persons investigations – who’s body...
- Convicted felons databases – cold cases solved...

All uses involve accurate measurement of DNA profiles and PATTERN MATCHING

Armed Forces DNA Repository

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

Being used to identify remains in case of combat casualties (e.g., Operation Iraqi Freedom)

Located off Gaither Rd. in Gaithersburg, MD

At NIST, we make DNA standards to help crime labs analyze DNA accurately.

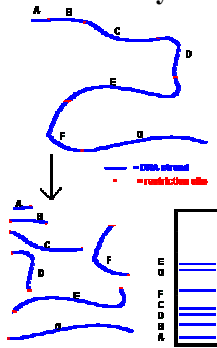
Historical Perspective - and Molecular Biology Jargon

- 1980 - Ray White describes first polymorphic RFLP marker
- 1985 - Alec Jeffreys discovers multilocus VNTR probes
- 1985 - first paper on PCR
- 1988 - FBI starts DNA casework
- 1991 - first STR paper
- 1995 - FSS starts UK DNA database
- 1998 - FBI launches CODIS database

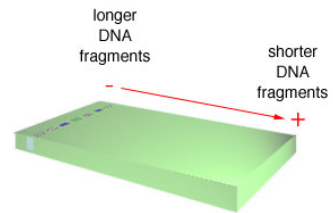
What are the Tools of DNA Typing?

- RFLP Testing (Late 1980's)
 - Radioactive Based
 - Chemiluminescent Based
- PCR-Based Testing (Mid 1990's)
 - Dot-Blot
 - VNTR
 - STR (Fluorescent markers used today)
- DNA Sequencing (Late 1990's)
 - Mitochondrial DNA

Restriction Enzyme Cut Sites



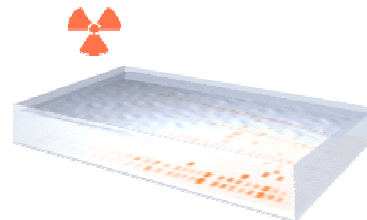
Electrophoresis of DNA Fragments



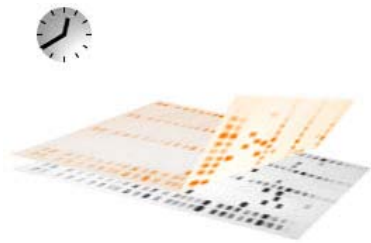
Transfer of DNA Fragments to a Nylon Membrane - “Southern Blotting”



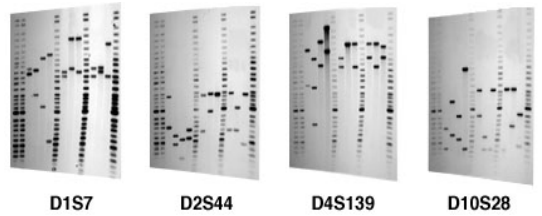
Probing of Membrane with Radioactive or Labeled DNA Probes



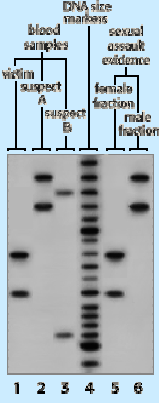
Exposure of Labeled Membrane to Autoradiographic Film



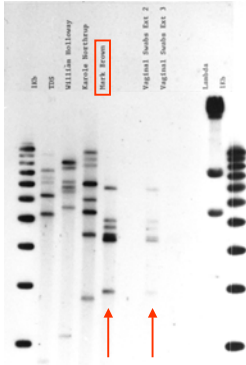
Repeated Probing of Same Membrane to Yield a Series of Autoradiograms



Which Suspect, A or B, cannot be excluded from potential perpetrators of this assault?



1985: Jeffreys' multi-locus RFLP

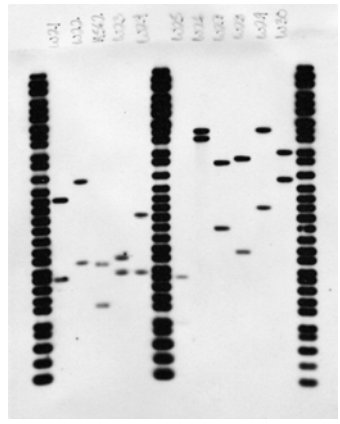


Restriction Fragment Length Polymorphism

Introduced in the U.K. in by the Forensic Science Service (FSS)

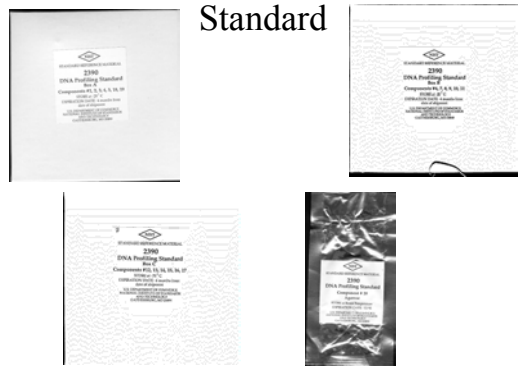
Used the *HinfI* restriction Enzyme

1990 Single Locus RFLP



FBI promotes single locus RFLP using *Hae III* restriction enzyme, in US, many laboratories have slight variations. Worldwide sharing of RFLP data not possible.

1992: SRM 2390 DNA Profiling Standard



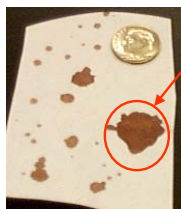
1992: SRM 2390 DNA Profiling Standard

<p>Box A</p> <p>Molecular Weight Marker DNA Molecular Weight Marker Dilution Molecular Weight Marker Probe DNA Klenow Fragment (For labeling Marker Probe) Stop Solution Adenovirus Visible Ladder 10X Buffer</p> <p>Box C</p> <p>K562 Cell Pellet K562 Undigested DNA K562 DNA <i>Hae</i>III Digested TAW Male Cell Pellet TAW Male Undigested DNA TAW Male DNA, <i>Hae</i>III Digested</p>	<p>Box B</p> <p>250 ng DNA standard 100 ng DNA standard 50 ng DNA standard 25 ng DNA standard 12.5 ng DNA standard 6 ng DNA standard</p> <p>For evaluating extracted DNA on a Yield Gel</p> <p>Agarose low electroendosmosis</p>
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1992: SRM 2390 DNA Profiling Standard

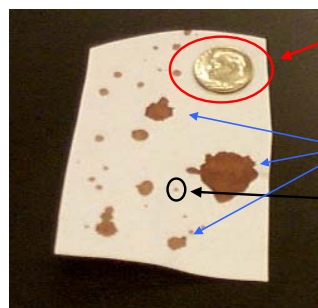
Each step of the RFLP process could be checked with these components. At the time of release, ³²P labeling was the most common practice. The certificate contained quantitative allele band sizes with uncertainty expressed as a 95% tolerance. In 2001 the 2390 certificate was updated to include Chemiluminescent practices

RFLP Drawbacks:



- Requires 100 ng to 1 µg of DNA (stain the size of a dime)
- The DNA must be relatively intact 1000-20,000 bp in size (not always possible to obtain)
- ³²P visualization requires 3 – 7 days @ – 80 °C
- 5 – 7 probes required for matching
- Time required weeks to months

Technology moves forward



Dime

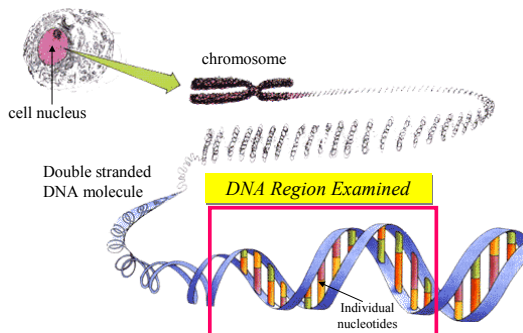
Blood sample

Only a very small amount of blood is needed to obtain a persons DNA profile

Progression of DNA Typing Markers

- RFLP
 - multilocus VNTR probes
 - single locus VNTR probes (³²P and chemiluminescence)
- PCR
 - D1S80 (AMP-FLPs)
 - DQ-alpha (reverse dot blot)
 - PolyMarker (6 plex PCR; dots for SNPs)
 - singleplex STRs with silver staining
 - multiplex STRs with fluorescent dyes

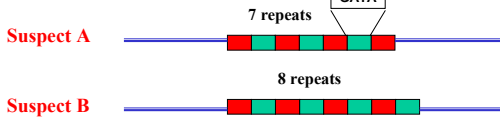
DNA in the Cell



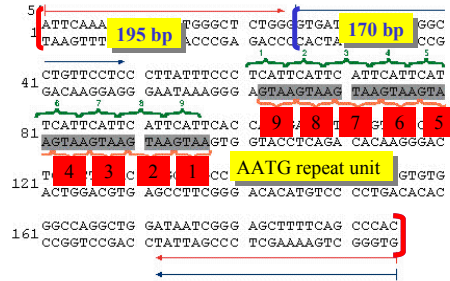
How is DNA Analyzed?

- Length Variation
short tandem repeats (STRs)

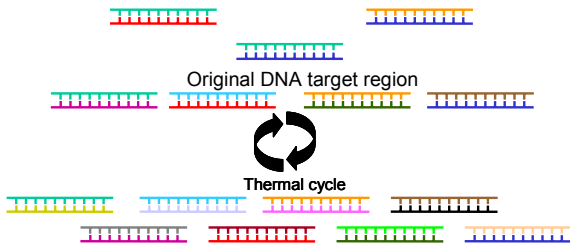
CTAGTCGT(GATA)(GATA)(GATA)GCGATCGT



HUMTH01 Sequence from GenBank
(Accession D00269)

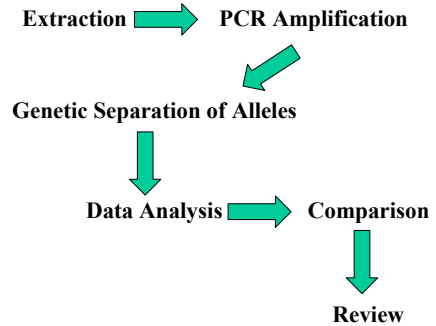


PCR Copies DNA Exponentially through Multiple Thermal Cycles

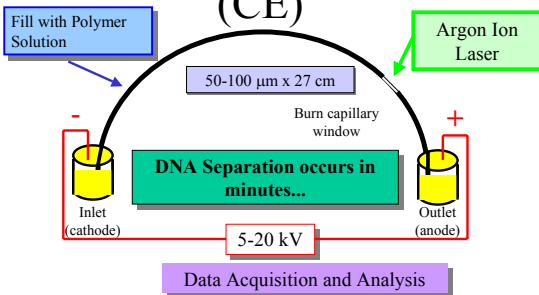


In 32 cycles at 100% efficiency, 1.07 billion copies of targeted DNA region are created

How is DNA Analyzed?



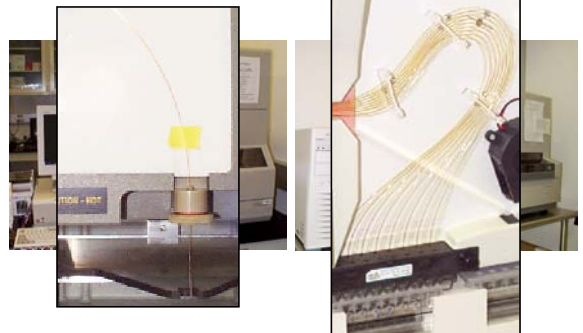
Capillary Electrophoresis (CE)



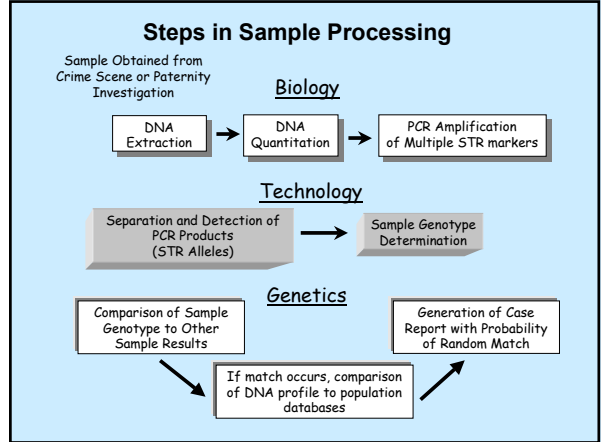
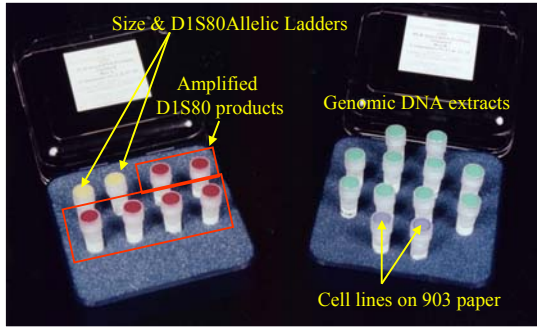
Capillary Electrophoresis Instrumentation

ABI 310
single capillary

ABI 3100
16-capillary array



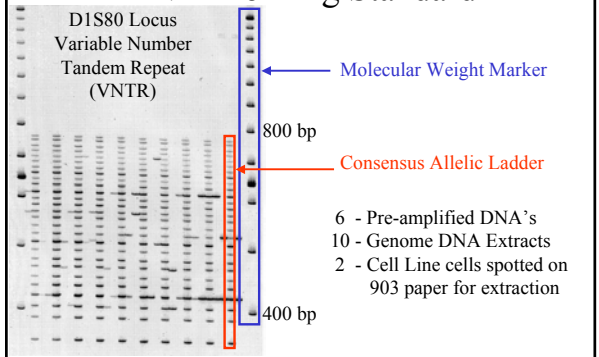
1995: SRM 2391 PCR-based DNA Profiling Standard



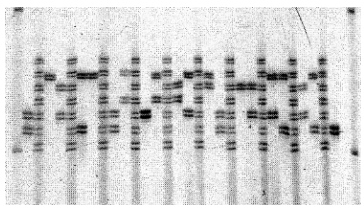
NIST SRM 2391a Certificate of Analysis



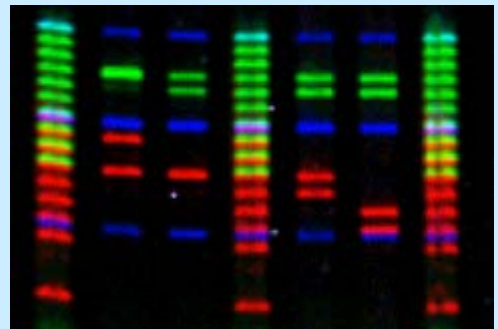
1995: SRM 2391 PCR-based DNA Profiling Standard




DQ alpha & Polymarker Reverse Dot Blot Hybridization



Silver stained gels for STR monoplexes



CODIS DNA Database



Combined DNA Index System

- Launched in October 1998
- Used for linking serial offenders and cold cases with repeat offenders
- CODIS requires labs putting samples into the FBI database to run the NIST DNA standard forensic case samples
- As of April 2004: **1,692,004 DNA profiles**
- Requires 13 core STR markers
- Some states moving to all felons and even arrestees

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



7,788 Investigations Aided through April 2003

As of April 2003 the total profile composition of the National DNA Index System (NDIS) is as follows:

Total number of profiles: 1,376,749
Total Forensic profiles: 54,895
Total Convicted Offender Profiles: 1,321,854

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

1998: FBI QA Standards for Forensic DNA Testing Laboratories

Federal Bureau of Investigation (FBI) Standard 9.5 “The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST Standard Reference Material or standard traceable to a NIST standard.”

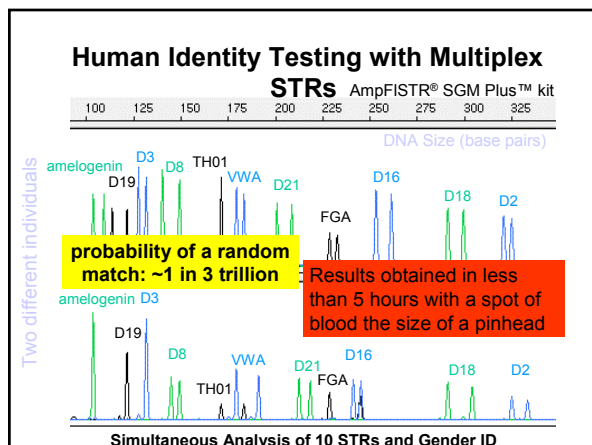
1998: SRM 2391 Certificate Update

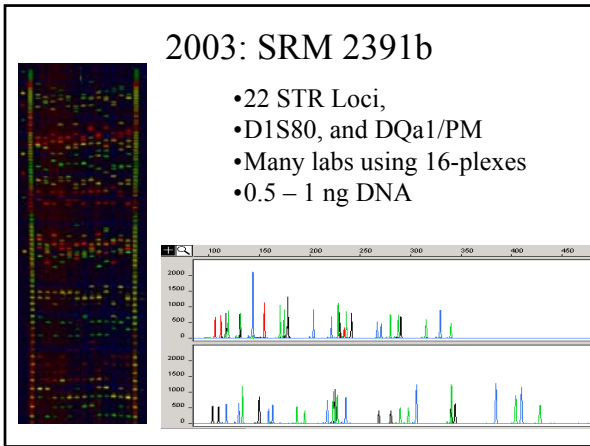
In November of 1997 the FBI’s STR working group selected 13 Core STR Loci for the CODIS National Database.

The update for SRM 2391 had 17 loci

2000: SRM 2391a

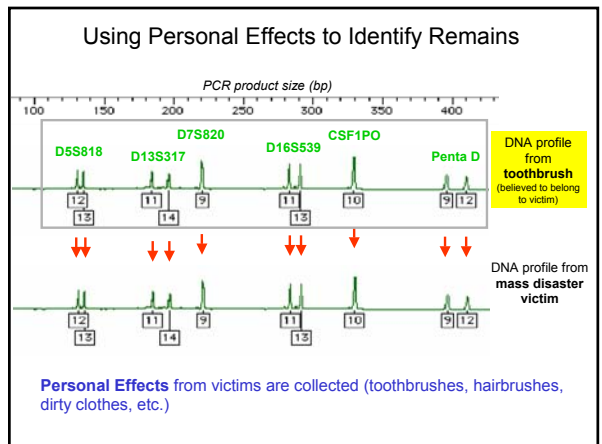
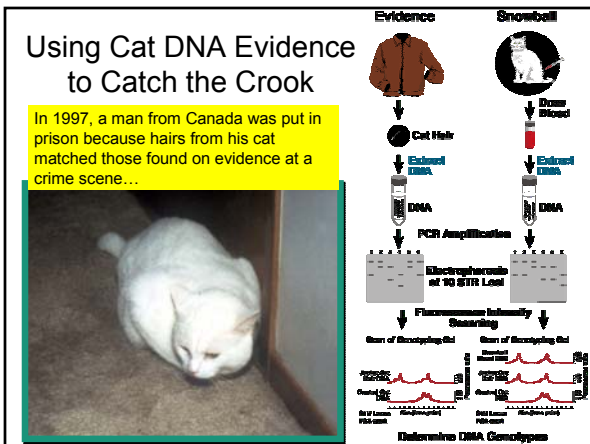
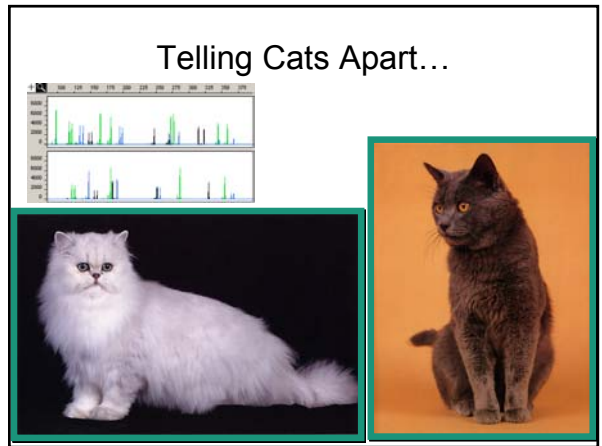
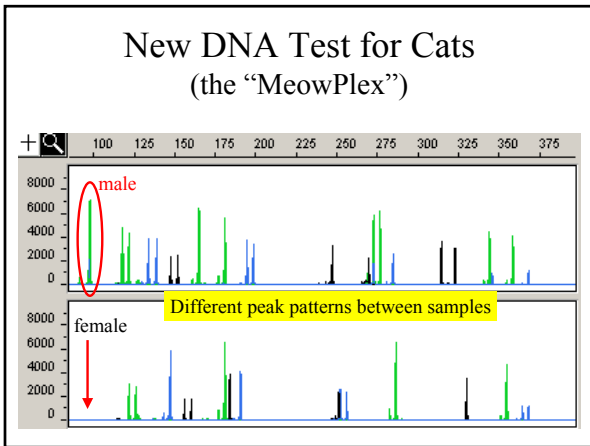
- The renewal of 2391, STR’s loci are the forensic DNA focus
- 21- STR loci are included in the certificate.
- D1S80 amplified products are no longer supplied
- Fluorescent labeling of the PCR products enables new analysis technologies and multiplexing

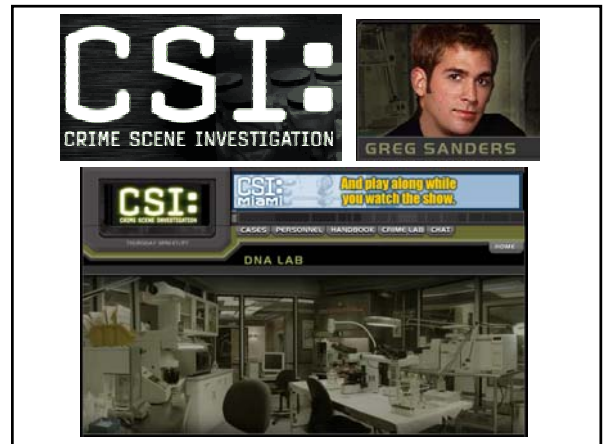
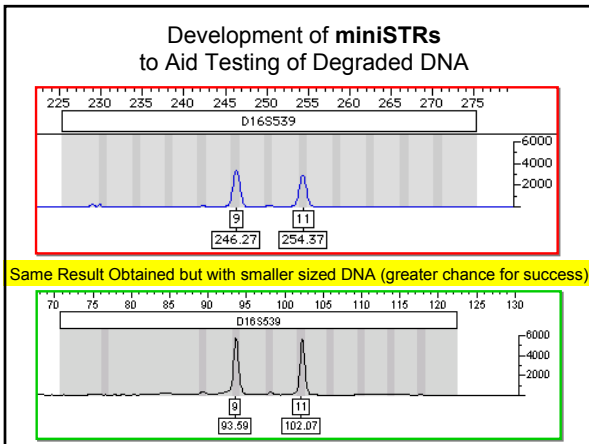
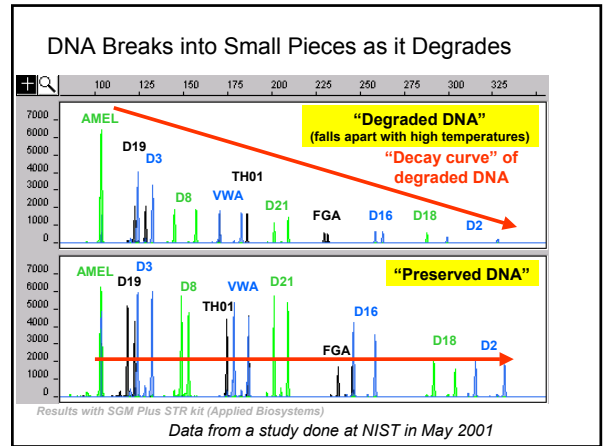
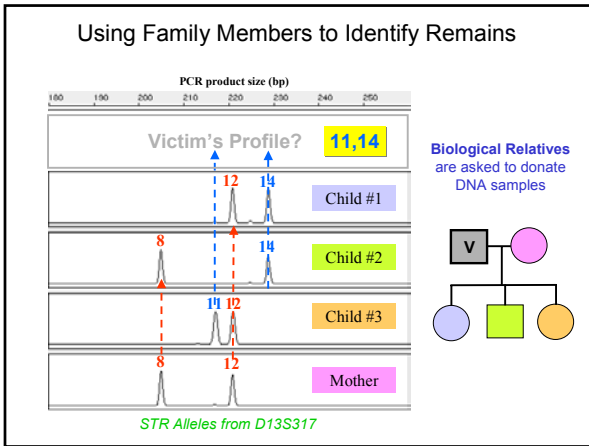




The Role of NIST Scientists

- **Develop DNA standards** so that laboratories around the world may compare their results.
- **Conduct tests of laboratories** around the world to insure accurate results in DNA testing.
- **Develop new DNA tests** which are more rapid and efficient than those currently used.

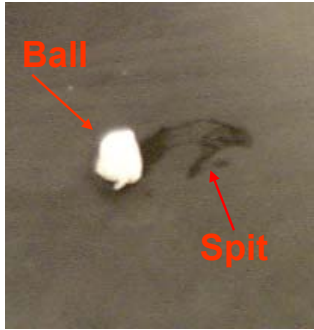




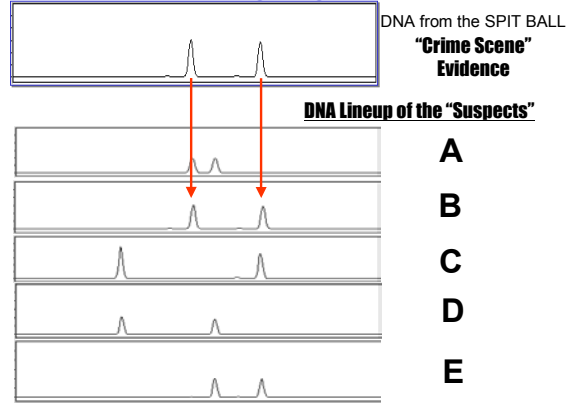
- ### Using DNA to Solve a Case
- A **spit ball** was shot from the back of the classroom and hit the teacher in the eye
 - It could have come from any one of five different students
 - DNA was obtained from the saliva on the spit ball and used to produce a DNA profile
 - Each of the 5 students (“suspects”) were asked to give blood in order to obtain a DNA profile for comparison purposes to the spit ball (“crime scene evidence”)



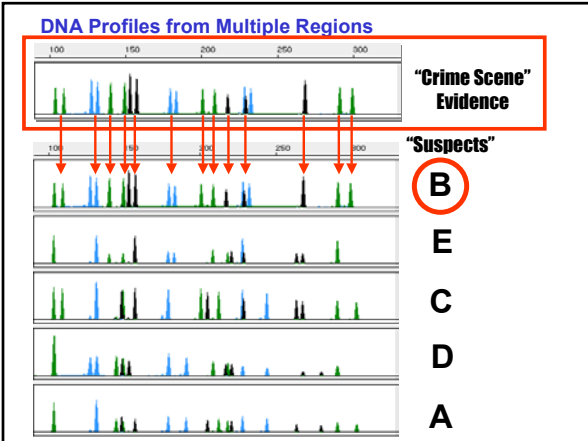
Close up of Evidence



DNA Profiles from a Single Region

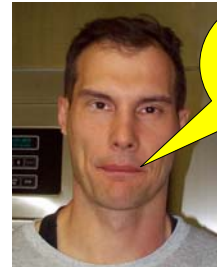


DNA Profiles from Multiple Regions

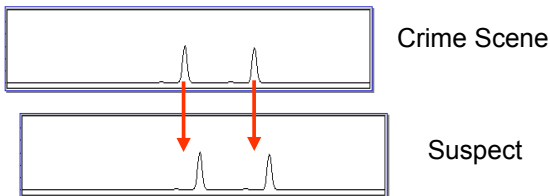


Conclusion of Case

- Student B was brought to justice and was asked to clean the teacher's glasses (& apologize)




What if the measurement of the DNA profile had not been performed correctly?



Failure to match the Perpetrator could result in the guilty going free...

Correct Measurements Helps Identify the Guilty and Free the Innocent





Short Tandem Repeat DNA Internet DataBase

These data are intended to benefit research and application of short tandem repeat DNA markers to human identity testing. The authors are solely responsible for the information herein. [\[Purpose of Database\]](#)



This database has been accessed **13311** times since 10/02/97. (Counter courtesy www.digits.com - see disclaimer.)

Created by [John M. Butler](#) and [Dennis J. Reeder](#) (NIST Biotechnology Division), with invaluable help from [Jan Redman](#), [Christian Ruitberg](#) and [Michael Tung](#).


Site creators' curriculum vitae available using links above.

Partial support for the design and maintenance of this website is being provided by [The National Institute of Justice](#) through the NIST Office of Law Enforcement Standards.

[Publications and Presentations from NIST Human Identity Project Team](#)

[AutoDimer program](#)  

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




FORENSIC DNA TYPING

Biology and Technology behind STR Markers

John M. Butler



Listed on amazon.com
Published January 2001



ACADEMIC PRESS
A Harcourt Science and Technology Company

http://www.apnet.com/aps/forensics/for_bio.html

Thank you for your Attention!!

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Margaret Kline	Jan Redman
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	Dave Duewer