


Forensic DNA Typing: The Application of Nucleic Acid Based Technology to Human Identity Testing


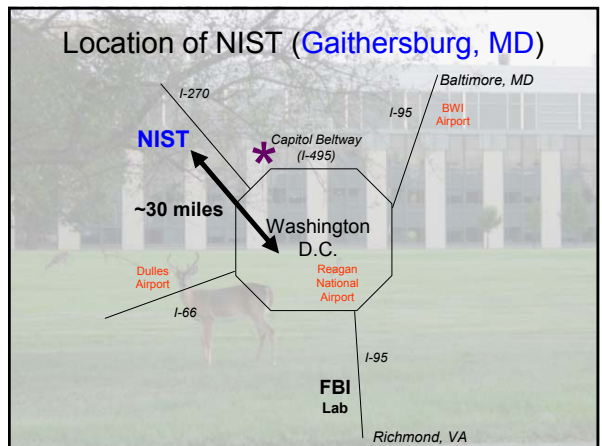
Guest Lecture Series
Science and Engineering Alliance
 Southern University and A&M College, Baton Rouge, LA

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




Outline

- NIST Campus
- DNA, Short Tandem Repeats (STRs) and the polymerase chain reaction (PCR)
- Uses of DNA testing
- Steps involved in sample typing
- NIST Standard Reference Materials (SRMs)
- Applications




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



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Working with DNA is relatively easy

Lengths of DNA up to 100 base pairs can be commercially synthesized and purified

DNA is relatively stable: can be stored in water or low salt buffer at 4°C for 6 months to a year

Single strands can be functionalized and attached to beads or a glass/silicon surface

Fluorescent dyes can be covalently attached on the 5' end of the molecule for detection purposes

Single strands of DNA have a strong affinity for their complement

Length	Sequence	Ka (M-1)
5mer	agctc	8.0E+01
10mer	acgtagctca	8.3E+05
15mer	acgtatcgatcgatc	1.4E+09
20mer	acgtactgcatcgatcgatc	5.5E+13
25mer	acgtatcgatcgatcgatctacgat	3.1E+16

Other binding constants: Streptavidin-biotin[10¹⁵], Drugs[~10⁶], Antibodies[10⁷ to 10¹¹]

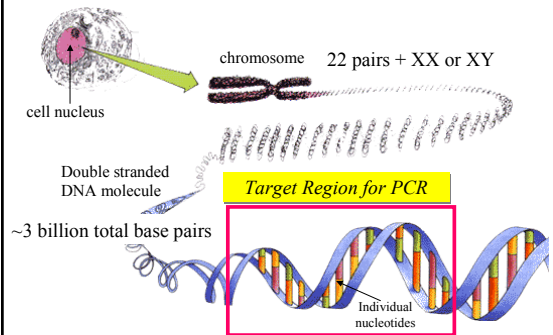
DNA is a biopolymer that consists of only 4 monomers units

Relationship: 4^N where N is the length of the sequence

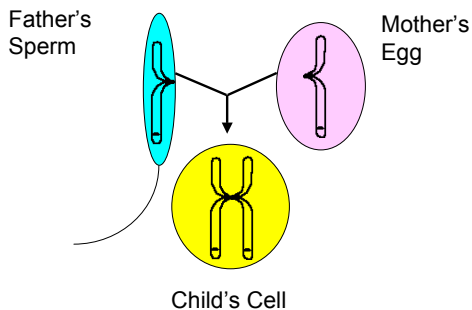
Length (nt)	Possible unique seqs	Base pairs in Human Genome
5	1024	3.2E+09
10	1.0E+06	3.2E+09
15	1.1E+09	3.2E+09
20	1.1E+12	3.2E+09
25	1.1E+15	3.2E+09

It is reasonable to assume that DNA molecules of ~15 units or greater are unique in the human genome (exceptions, repeats, duplicated regions etc)

DNA in the Cell



Our DNA Comes from our Parents



What Type of Genetic Variation?

•Length Variation

short tandem repeats (STRs)

CTAGTCGT(GATA)(GATA)(GATA)GCGATCGT

•Sequence Variation

single nucleotide polymorphisms (SNPs)

insertions/deletions

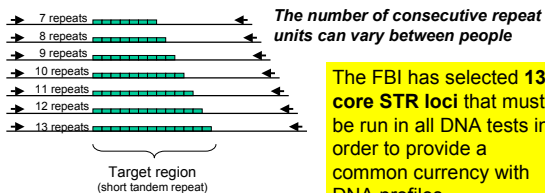
GCTAGTCGATGCTC(G/A)GCGTATGCTGTAGC

Short Tandem Repeat (STR) Markers

An accordion-like DNA sequence that occurs between genes

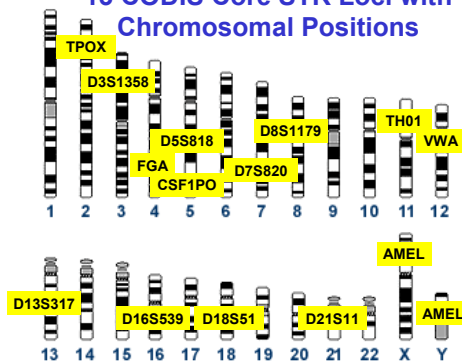
TCCCAAGCCTTCCTCTCCCTAGATCAATACAGACAGAAGACA
GGTGATAGATAGATAGATAGATAGATAGATAGATAGATAGA
TAGATAGATATCATTGAAAGACAAAACAGAGATGGATGATAGAT
ACATGCTTACAGATGCACAC

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



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



Basic Concepts

PCR polymerase chain reaction – method of amplifying a specific region of the genome – go from 1 to over a billion copies in about 2 hours

Locus region of the genome being examined

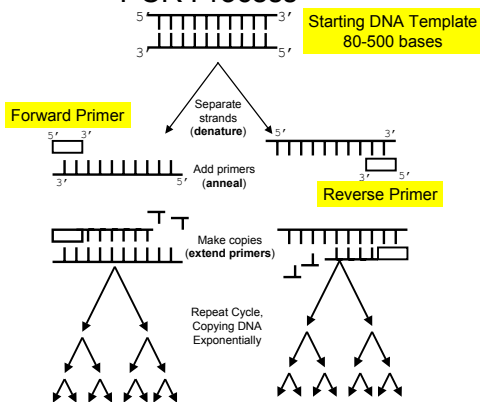
Allele the state of the genetic variation being examined (STRs = number of repeat units)

Chromosomes are paired so...

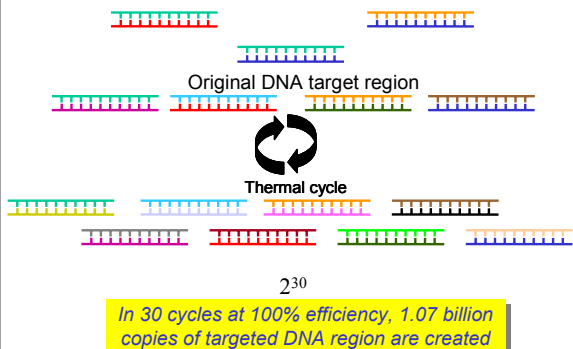
Homozygous – Alleles are identical on each chromosome

Heterozygous - Alleles differ on each on each chromosome

PCR Process



PCR Copies DNA Exponentially through Multiple Thermal Cycles

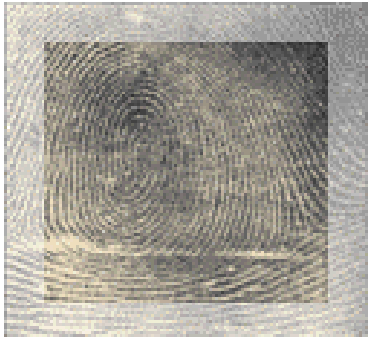


Outline

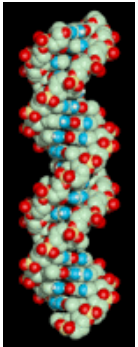
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Methods for Human Identification

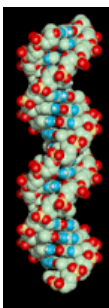


Fingerprints have been used since 1901



DNA since 1986

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)

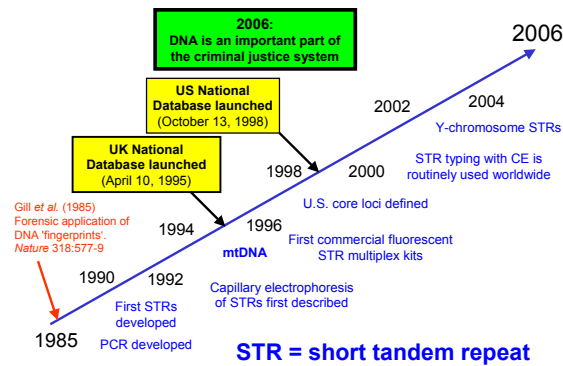
Typically, we are not looking at genes – little/no information about race, predisposal to disease, or phenotypical information (eye color, height, hair color) is obtained

Applications for Human Identity Testing


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

As DNA analysis has shown its usefulness, the number of samples gathered for testing purposes has gone up dramatically...

A Brief History of Forensic DNA Typing



STR = short tandem repeat




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

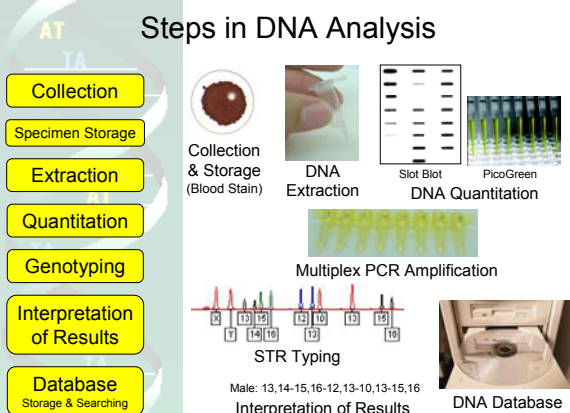
The instruments on CSI are real – they just do not collect data as quickly as shown on TV.

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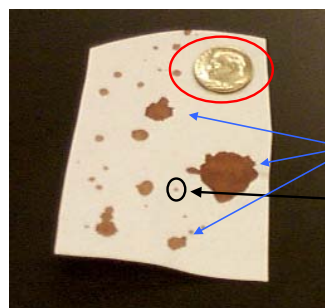
Steps in DNA Analysis



- Collection
- Specimen Storage
- Extraction
- Quantitation
- Genotyping
- Interpretation of Results
- Database Storage & Searching

Sources of Biological Evidence

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


Blood sample

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

Steps in DNA Analysis

[Steps in DNA Analysis](#)

- Collection
- Extraction
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The Buccal DNA Collector
Collection of the DNA sample

The Bode Technology Group, Inc.
A ChoicePoint® Company

Decision for which collection paper to use based on NIST storage studies

SIMPLE EFFICIENT AUTOMATABLE ARCHIVAL



Steps in DNA Analysis

[Steps in DNA Analysis](#)

- Collection
- Extraction
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A small punch taken from the buccal device and is incubated in an extraction buffer

It is important to remove the heme from blood sample as it will inhibit the PCR

Commercial chromatography columns are available for the extraction process

Steps in DNA Analysis

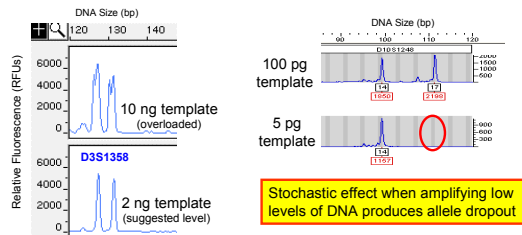
[Steps in DNA Analysis](#)

- Collection**
 - Extraction**
 - Quantitation**
 - Genotyping**
 - Interpretation of Results**
 - Database Storage & Searching**
- It is important that the optimal amount of DNA is added to the PCR
- Typically 0.5 ng to 2 ng works well
- Too much or too little DNA will result in artifacts obscuring data interpretation
- DNA can be quantitated using UV spectroscopy, fluorescence (after the addition of an intercalation dye), hybridization with a probe, and qRT-PCR

Impact of DNA Amount into PCR

Reason that DNA Quantitation is Important Prior to Multiplex Amplification

- Too much DNA
 - Off-scale peaks
 - Split peaks (+/-A)
 - Locus-to-locus imbalance
- Too little DNA
 - Heterozygote peak imbalance
 - Allele drop-out
 - Locus-to-locus imbalance



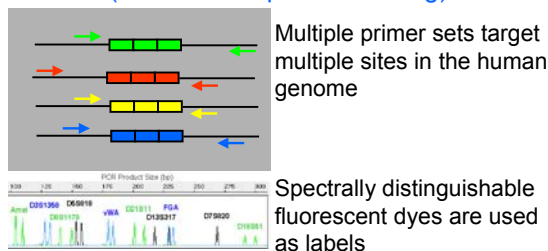
Steps in DNA Analysis

[Steps in DNA Analysis](#)

- Collection**
 - Extraction**
 - Quantitation**
 - Genotyping**
 - Interpretation of Results**
 - Database Storage & Searching**
- The quantitated extract is then amplified (PCR)
- Various commercial kits containing fluorescently labeled PCR primers exist
- Up to 16 loci are simultaneously amplified
- Amplification takes approximately 2 hours
- The PCR products are diluted and separated/detected on a gel or capillary platform

Multiplex PCR

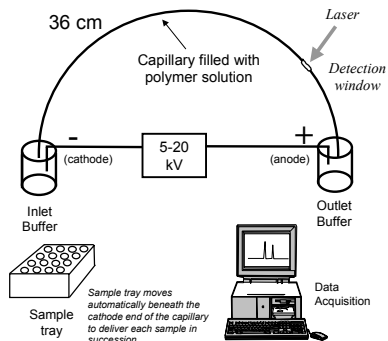
(Parallel Sample Processing)



Advantages of Multiplex PCR

- Increases information obtained per unit time (increases power of discrimination)
- Reduces labor to obtain results
- Reduces template required (smaller sample consumed)

Capillary Electrophoresis System

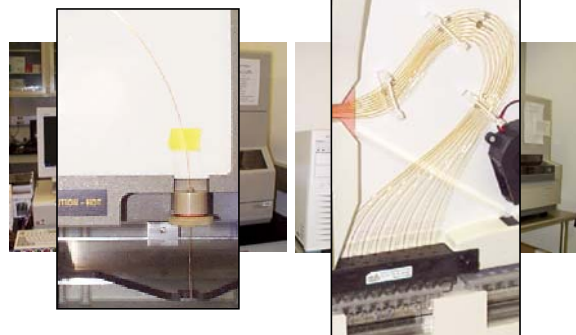


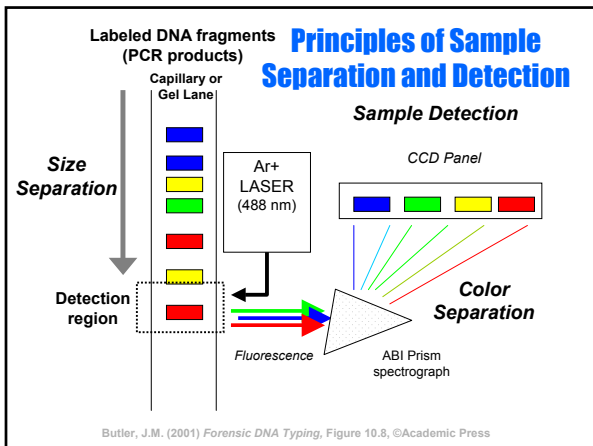
Butler, J.M. (2001) *Forensic DNA Typing*, Figure 9.3, ©Academic Press

Capillary Electrophoresis Instrumentation

ABI 310
single capillary

ABI 3100
16-capillary array

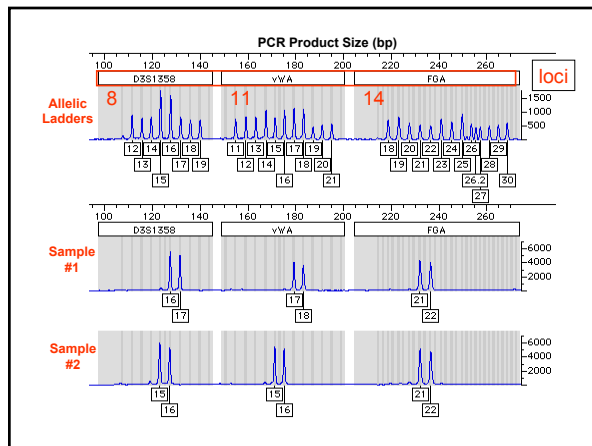
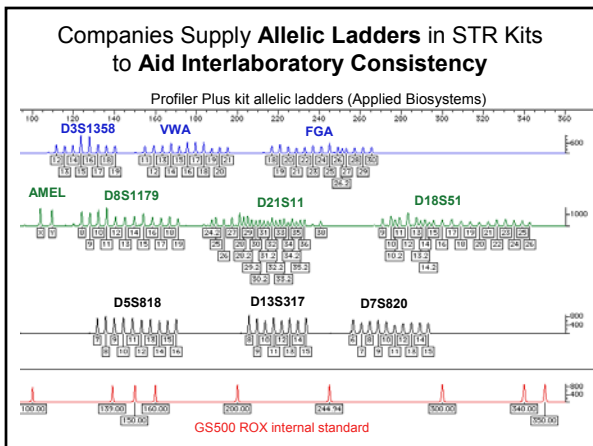
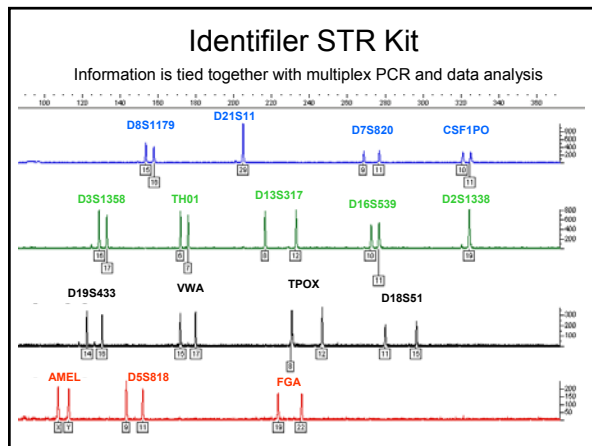


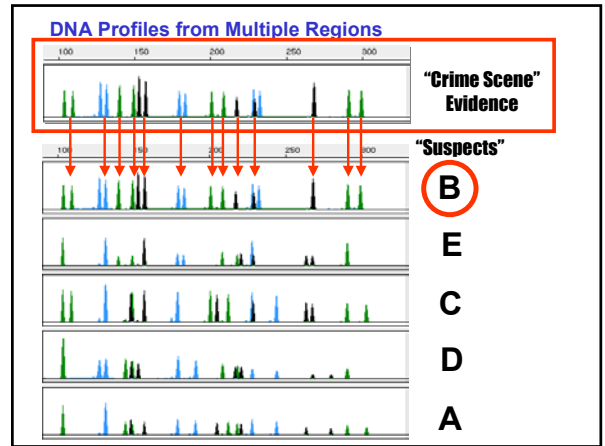
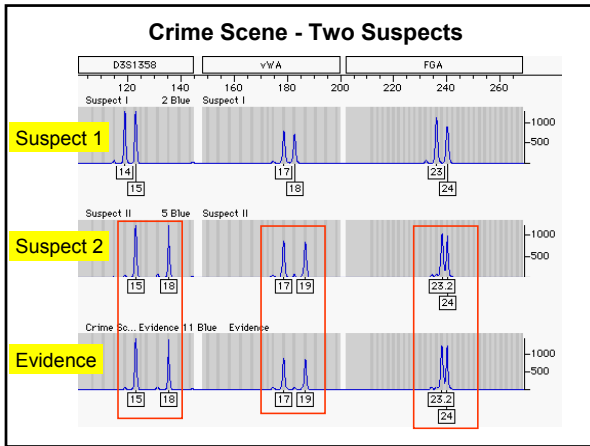


Steps in DNA Analysis

Steps in DNA Analysis

- Collection** The peak(s) for each locus are compared to an allelic ladder which contains all the possible alleles
- Extraction**
- Quantitation** A repeat number is assigned for each observed peak
- Genotyping** The repeat values are tabulated for each locus
- Interpretation of Results** Visual comparisons between data can be helpful
- Database Storage & Searching**



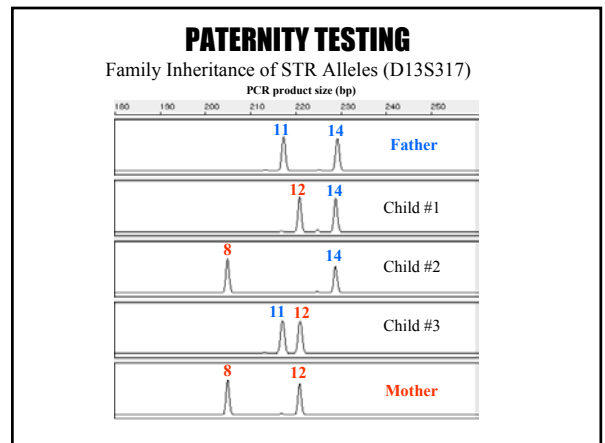
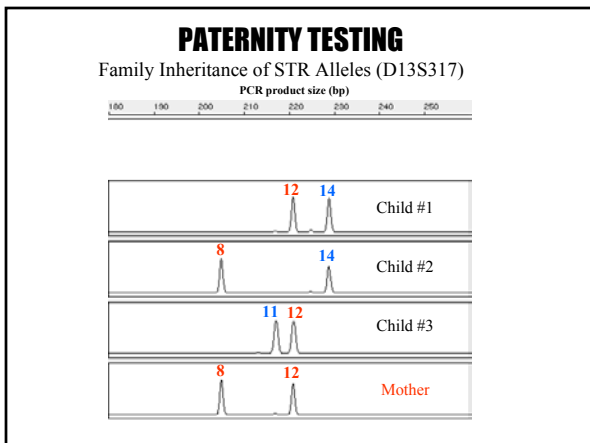
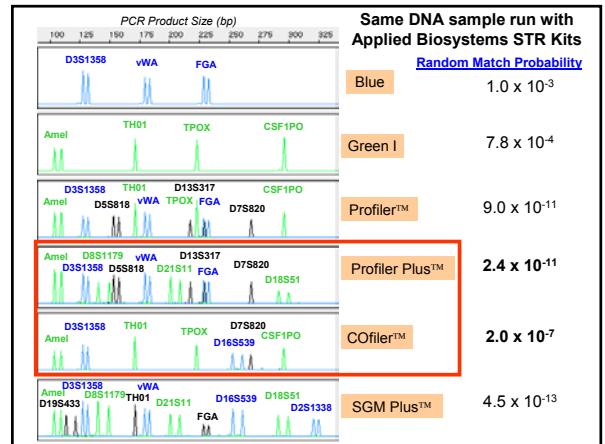


Data Format

	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 number of repeats observed for each locus is tabulated

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



Steps in DNA Analysis

[Steps in DNA Analysis](#)

Collection


Extraction

Quantitation


Genotyping

Interpretation of Results

Database Storage & Searching



FBI LABORATORY



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



>27,079 Investigations Aided through August 2005

As of August 2005 the total profile composition of the National DNA Index System (NDIS) is as follows:

Total number of profiles: 2,695,885
Total Forensic profiles: 117,255
Total Convicted Offender Profiles: 2,578,630

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

- 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>

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NIST Standard Reference Materials (SRMs)

Standard Reference Materials Program

SRM 2390 - DNA Profiling Standard Meets RFLP Needs

NIST SRMs are used to help calibrate forensic genotyping laboratories


SRM 2392 - Mitochondrial DNA Standard Cell Lines and Cloned HV1 Plasmid

SRM 2393 - mtDNA heteroplasmy

SRM 2395 - Y chromosome DNA standards

NIST SRM 2391: DNA Profiling Standard for Forensic and Paternity Testing Laboratories

- Human identity testing using Polymerase Chain Reaction (PCR) technologies growing rapidly
- PCR techniques diverse, requiring DNA standards that perform under various amplification and electrophoretic methods
- SRM 2391 certified for genetic locus D1S80; expanding for other loci as needs evolve




CHROME LABORATORY

ANALYTICAL PRODUCTS

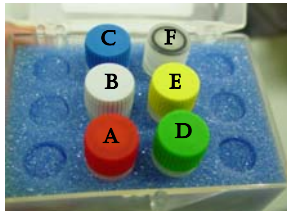
DNA Quality Assurance recommendations by the DNA Advisory Board were signed by the FBI director on July 15, 1998.

Beginning October 1, 1998, all federally-funded laboratories that conduct DNA testing must verify procedures using NIST SRMs



Chemical Science and Technology Laboratory

NIST Y Chromosome Standard



6 genomic DNA samples
 5 male and 1 female
 Typing Information on 27 Y STRs and 50 Y SNP markers

Available as of 07/2003

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

Innocence Project

<http://www.innocenceproject.org>

Search Go

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

Michael A. Williams
Exonerated

Kenneth Wyniemko
Year of Incarceration: 1994
Jurisdiction: Michigan
Sentence: 40-60 years
Year of Exoneration: March 11, 2005
163 exonerated as of October 12, 2005

DNA EXONERATIONS BY YEAR IN THE U.S.

The Innocence Project at the University of North Carolina is a non-profit legal clinic through postconviction DNA testing, and cases where postconviction DNA testing would likely exonerate a person.

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

Examination Number: [REDACTED] Date: 06/27/98

Unit: DNA Analysis 1 Phone No.: 202-324-4409

File No.: 290-DIC-LR-35063 Lab No.: 980735902 S BO 980803100 S BO

Results of Examination:

Deoxyribonucleic acid (DNA) profiles for the genetic loci D2S44, D17S79, D1S7, D6S1329, D16S62A, D5S116 and F13A47 were developed from BamHI-digested high molecular weight DNA extracted from specimens K39 and Q243-1 (semen stain removed from specimen Q243). Based on the results of these genetic loci, specimen K39 (CLINTON) is the source of the DNA obtained from specimen Q243-1, to a reasonable degree of scientific certainty.

No DNA-RFLP examinations were conducted on specimen Q243-2 (a semen stain removed from specimen Q243).

BACK - 1,999,000,000,000
CLINTON - 7,839,000,000,000
SCY - 3,185,000,000,000
SMITH - 493,000,000,000

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

December 14, 2003

"We got him!"

DNA Profile

Saddam was known to have many "stunt doubles" that acted as decoys for his own safety

Saddam Hussein's capture was verified with DNA testing conducted in Rockville, MD at the Armed Forces DNA Identification Laboratory

Source: www.cnn.com, The Scientist Dec 19, 2003

Biological Relatives Served as References

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

Tomb of the Unknown Soldier



Vietnam Veterans Memorial

Butler, J.M. (2006) *Forensic DNA Typing, 2nd Edition*, Box 10.1, pp. 250-251

- **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.**

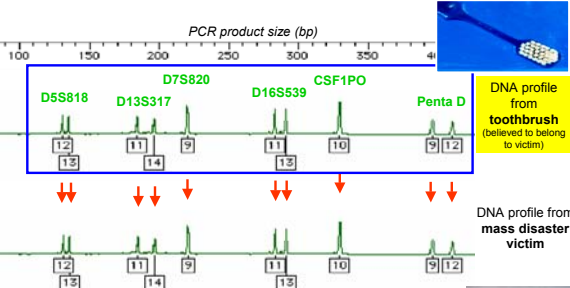
World Trade Center Towers (Sept 11, 2001)



Wreckage at Ground Zero

Highly degraded DNA was recovered

Using Personal Effects to Identify Remains



PCR product size (bp)

D5S818 D13S317 D7S820 D16S539 CSF1PO Penta D

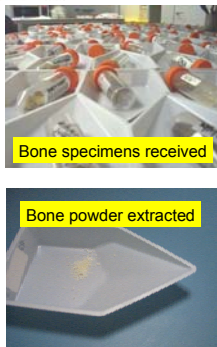
DNA profile from toothbrush (believed to belong to victim)

DNA profile from mass disaster victim

Personal Effects from victims are collected (toothbrushes, hairbrushes, dirty clothes, etc.)

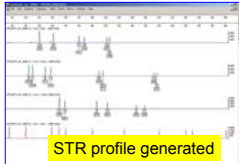
DNA Testing on Bones from WTC Site

The Bode Technology Group, Inc. A ChoicePoint® Company



Bone specimens received

Bone powder extracted

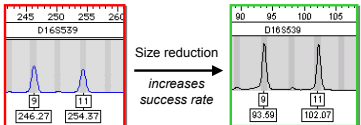


STR profile generated

Typing data uploaded to WTC CODIS database to search for match

12,13-11,14-9,9-11,13-10,10-9,12...

New DNA Tests Pioneered at NIST



Size reduction increases success rate

Same result but with smaller region of DNA

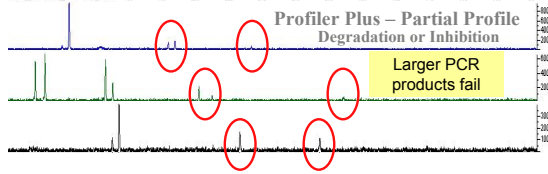
New test developed to aid in identification of World Trade Center victims of 9/11/01 terrorist attacks

OFFICE OF CHIEF MEDICAL EXAMINER NEW YORK CITY DEPARTMENT OF FORENSIC BIOLOGY

NIST The Bode Technology Group, Inc. A ChoicePoint® Company Springfield, Virginia

Has resulted in an increase in the number of WTC victims identified

World Trade Center – Phase I Summary



Profiler Plus – Partial Profile Degradation or Inhibition

Larger PCR products fail

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

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>

Hurricane Katrina Victims Will Be Identified with Forensic DNA Testing Methods



NIST Human Identity Project Team



John Butler
(Project Leader)



Margaret Kline



Pete Vallone



Mike Coble



Dave Duewer
Anal. Chem. Division



Jan Redman



Amy Decker



Becky Hill



Chris DeAngelis
computer programmer

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