Technology Developments in Forensic DNA Typing

Dr. John M. Butler
NIST Biotechnology Division

January 13, 2004
Sigma Xi Lecture

Top Ten Ways to Know that You are a Forensic DNA Scientist

10. You have your children's DNA profiles framed on your desk instead of their pictures.
9. When your children hurt themselves, you are more interested in collecting their blood to generate a DNA profile than getting them cleaned up…
8. Your pockets are full of napkins with DNA sequences written on them.
7. You want to name your first four kids: Adenine, Thymine, Guanine, and Cytosine.
6. You wonder how jello would work as a separation medium…and have tried it when no one else was around…but were too afraid to publish the results.

Top Ten Ways to Know that You are a Forensic DNA Scientist

5. You want to start a paternity testing business for all those who appear on the Jerry Springer show.
4. You know that DNA stands for more than the National Dyslexics Association.
3. You want to do a study on the genetics of inbreeding…and have selected the NIST deer population for a case study.

Top Ten Ways to Know that You are a Forensic DNA Scientist

2. You know that "scientists" on the popular TV show *CSI: Crime Scene Investigation* cannot possibly get their DNA results within the timeframe of a single commercial break.

1. Your license plate reads: **OJ DID IT**!

Examples of DNA in the News

- Saddam Hussein Identification
- Source of Cow with "Mad cow" Disease
- Scot Peterson Murder Trial
- Identification of WTC Victims
- "Thomas Jefferson fathered slave's children"

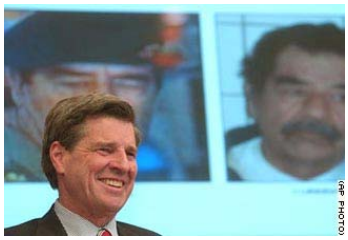
DNA Testing for U.S. Mad Cow Case

PRESS RELEASE from www.geneseek.com

GENESEEK PROVIDES DNA TESTING FOR U.S. MAD COW CASE

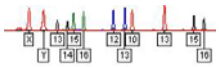
Lincoln, Nebraska. January 8, 2004. GeneSeek Inc. today announced that it had been contracted by the USDA to provide the DNA testing related to the recent case of mad cow disease (BSE) in the state of Washington. Working over a 24 hour period spanning New Year's Eve and New Year's Day, a team of scientists at GeneSeek evaluated the DNA extracted from the brain of the cow with BSE, DNA from suspected relatives of the cow, and many unrelated control DNA samples…

GeneSeek initially analyzed the DNA samples using an expanded set of short tandem repeat markers …



December 14, 2003

"We got him!"

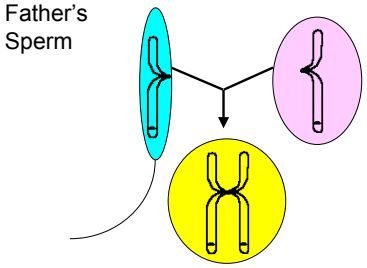


DNA Profile

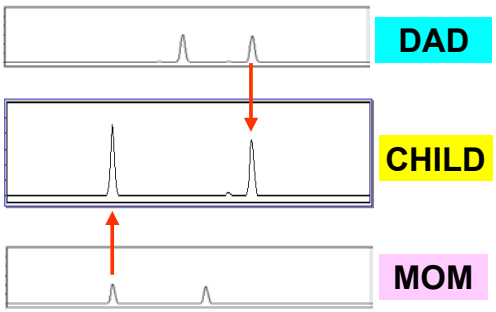
Saddam Hussein's capture verified with DNA testing conducted in Rockville at Armed Forces DNA Identification Laboratory

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

Our DNA Comes from our Parents

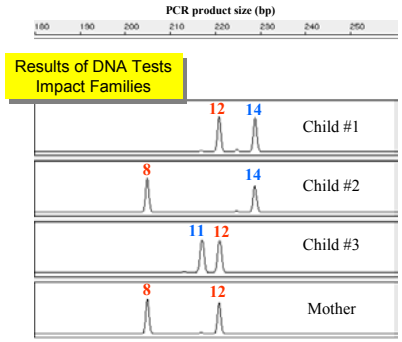


Inheritance Pattern of DNA Profiles



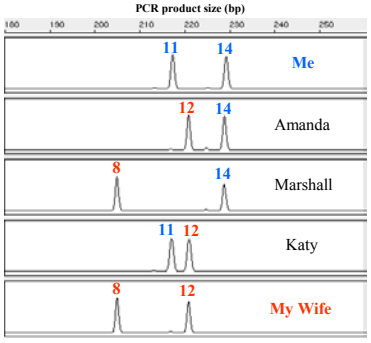
PATERNITY TESTING

Family Inheritance of STR Alleles (D13S317)



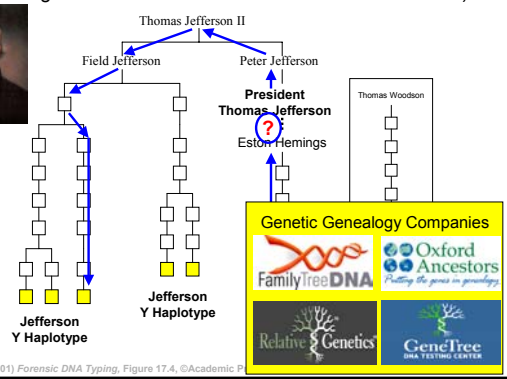
PATERNITY TESTING

Family Inheritance of STR Alleles (D13S317)



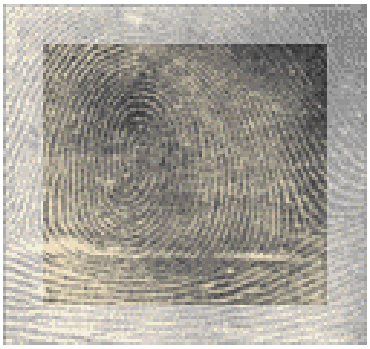
Historical Investigation DNA Study

(Matching Relatives to Remains or Relatives to Relatives)

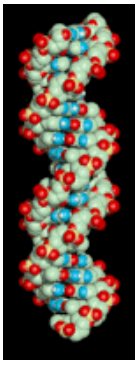


Butler, J.M. (2001) *Forensic DNA Typing*, Figure 17.4, ©Academic P

Methods for Human Identification



Fingerprints have been used since 1901



DNA since 1986

Forensic DNA Testing

The genome of each individual is unique (with the exception of identical twins)

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

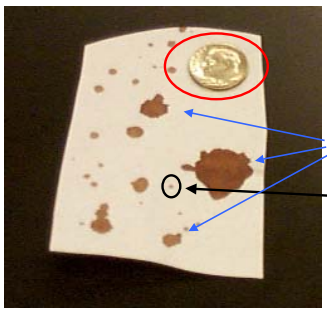
As DNA analysis has shown its usefulness, the number of samples gathered for testing purposes has gone up dramatically…

Roles of Biological Evidence in Criminal Investigation

Identify a person
Exclude a suspect – Innocence Project
Link suspect, victim and crime scene
Link weapon to victim
Link witness to scene
Prove or disprove an alibi
Reconstruct the scene
Provide investigative leads

Sources of Biological Evidence

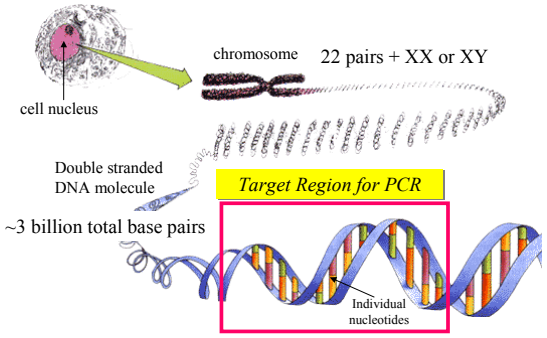
- **Blood**
- **Semen**
- **Saliva**
- **Urine**
- **Hair**
- **Teeth**
- **Bone**
- **Tissue**



Blood sample

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

DNA in the Cell



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

Basic Concepts

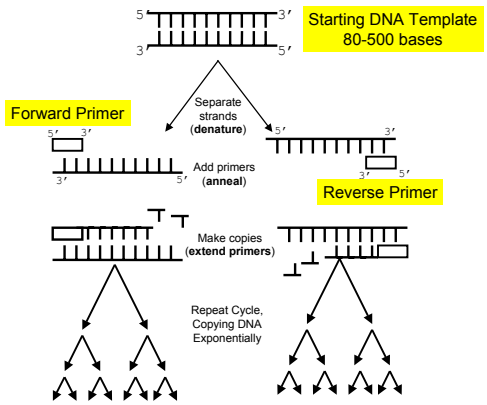
PCR polymerase chain reaction – method of amplifying a specific region of the genome – go from 1 to over a billion copies in about 2 hours

Locus region of the genome being examined

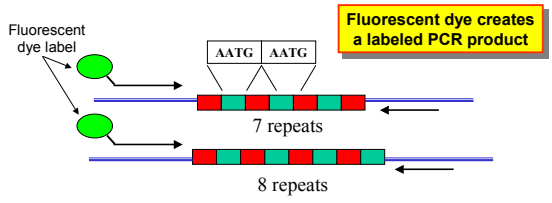
Allele the state of the genetic variation being examined
(STRs = number of repeat units)
(SNPs = base sequence at the site)

Chromosomes are paired so…
Homozygous – Alleles are identical on each chromosome
Heterozygous - Alleles differ on each on each chromosome

PCR Process



Short Tandem Repeats (STRs)

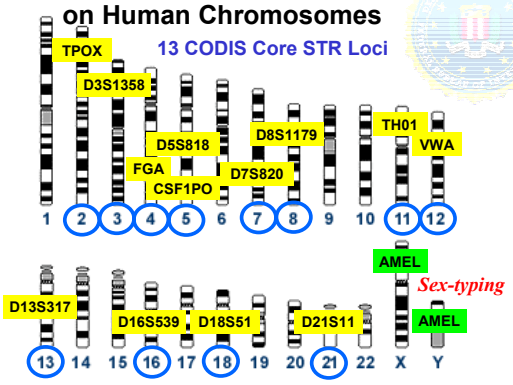


the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

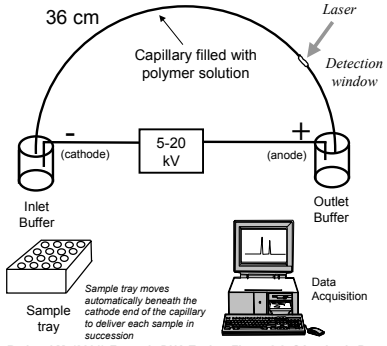
Homozygote = both alleles are the same length
Heterozygote = alleles differ and can be resolved from one another

Primer positions define PCR product size

Position of Forensic STR Markers on Human Chromosomes

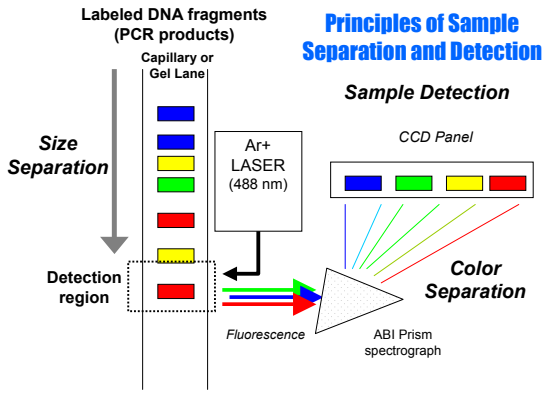


Capillary Electrophoresis System



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

Principles of Sample Separation and Detection

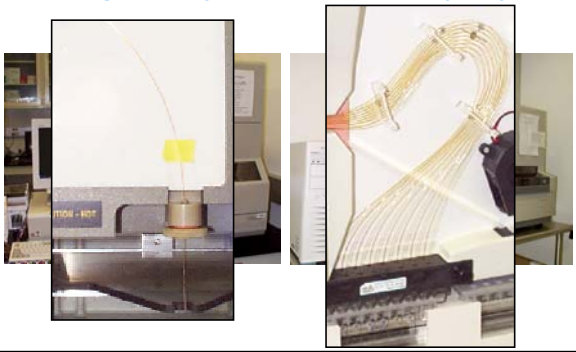


Butler, J.M. (2001) *Forensic DNA Typing*, Figure 10.8, ©Academic Press

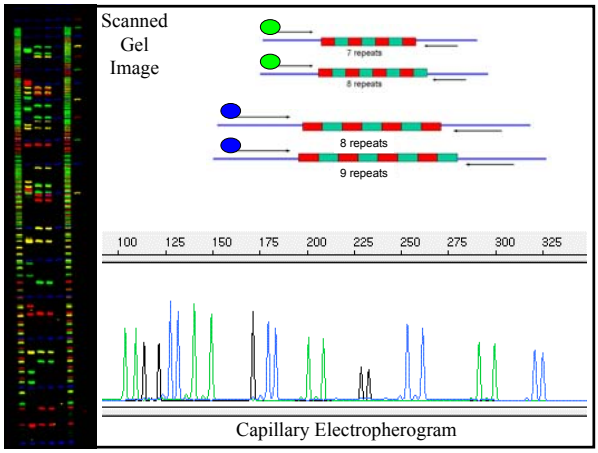
Capillary Electrophoresis Instrumentation

ABI 310 single capillary

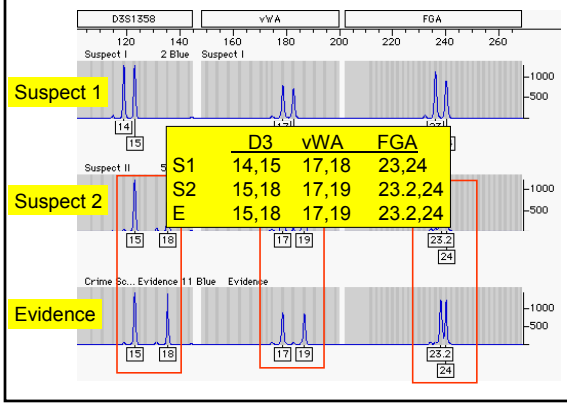
ABI 3100 16-capillary array



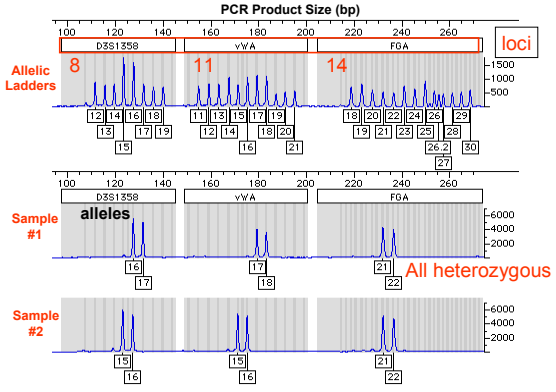
Scanned Gel Image



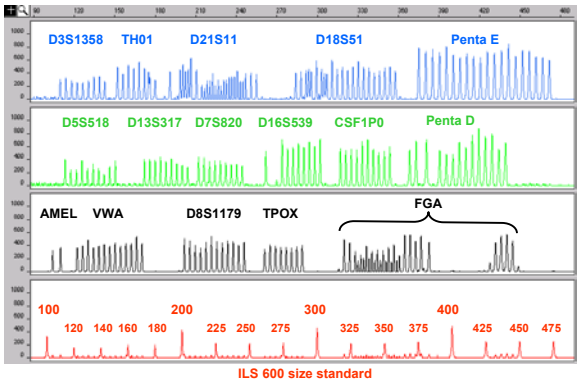
Crime Scene - Two Suspects



PCR Product Size (bp)



PowerPlex® 16 Ladders on NIST 3100

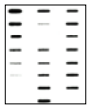


Steps in DNA Analysis

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


Collection & Storage (Blood Stain)

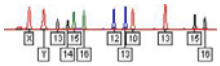

DNA Extraction


Slot Blot


PicoGreen

DNA Quantitation


Multiplex PCR Amplification


STR Typing

Male: 13,14-15,16-12,13-10,13-15,16
Interpretation of Results


DNA Database

Product Rule

For heterozygous loci

$$P = 2pq$$

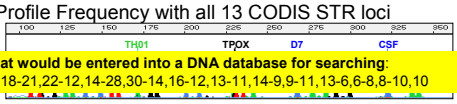
P = probability; p and q are frequencies of allele in a given population

Example: For the locus D3S1358 and individual is 16,17 with frequencies of 0.2315 and 0.2118 respectively

P = 2(0.2315)(0.2118) = 0.0981 or 1 in 10.2

For independent loci, the genotype frequencies can be combined through multiplication…
Profile Probability = (P1)(P2)…(Pn)
= *1 in a very large number…*

DNA Profile Frequency with all 13 CODIS STR loci



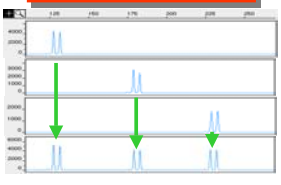
What would be entered into a DNA database for searching:
16,17-17,18-21,22-12,14-28,30-14,16-12,13-11,14-9,9-11,13-6,6-8,8-10,10

Locus	allele	value	allele	value	frequency, 1 in
D3S1358	16.0	0.2315	17.0	0.2118	10.20
VWA	17.0	0.2628	18.0	0.2219	8.57
FGA	21.0	0.1735	22.0	0.1888	15.26
D8S1179	12.0	0.1454	14.0	0.2015	17.07
D21S11	28.0	0.1658	30.0	0.2321	12.99
D18S51	14.0	0.1735	16.0	0.1071	26.91
D5S818	12.0	0.3539	13.0	0.1462	9.66
D13S317	11.0	0.3189	14.0	0.0357	43.92
D7S820	9.0	0.1478			43.28
D16S539	11.0	0.2723	13.0	0.1634	11.24
THO1	6.0	0.2266			18.83
TPOX	8.0	0.5443			3.35
CSF1PO	10.0	0.2537			15.09

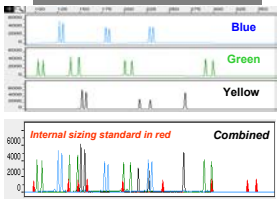
The Random Match Probability for this profile in the FBI Caucasian population is **1 in 1.56 quadrillion (10^{15})**

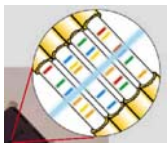
Methods for Parallel Sample Processing

Multiplex by Size



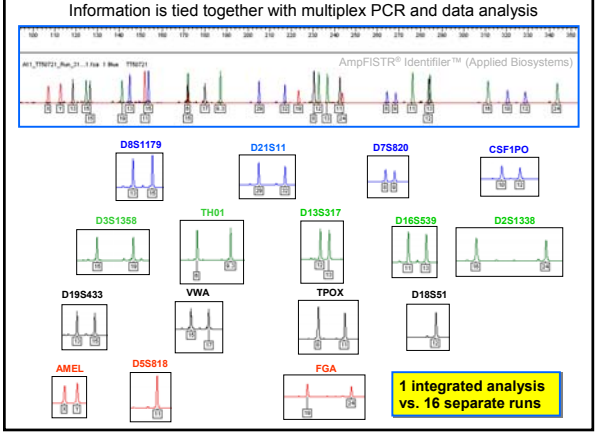
Multiplex by Dye Color



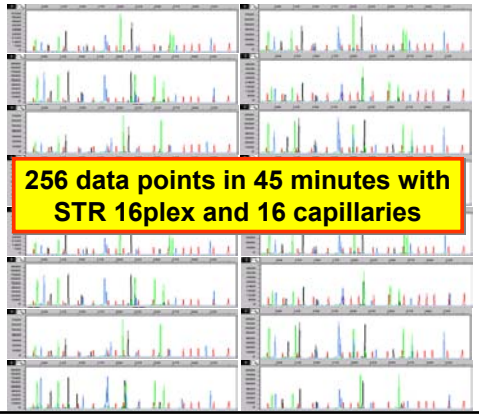


Multiplex by Number of Capillaries

Information is tied together with multiplex PCR and data analysis



High-Throughput STR Typing on the ABI 3100 (16-capillary array)



WTC DNA Identifications

World Trade Center Victim Identification Efforts

Without DNA
736 Victims Identified
Finished May 2002

Source: Mecki Prinz (OCME) ISFG presentation, Sept 11, 2003

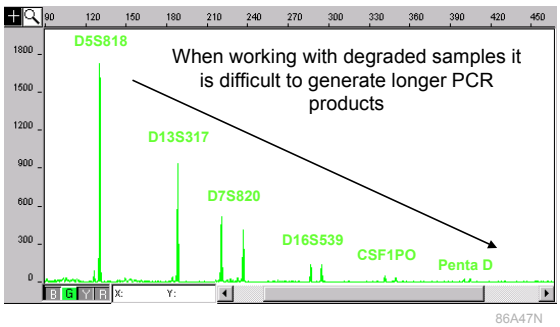
Special Circumstances

- **Destructive Energy of Attack**
  - Kinetic energy and fuel load of airplanes
  - Kinetic energy of collapse
- Two Boeing 767 airplanes (fueled with 10,000 gallons each) traveling at 429 to 586 mph
- Towers 110 floors each, 1362 ft high
- Towers reduced to 70 ft hill, 16 acres, 1.7 million tons debris
- Subterranean fires until December

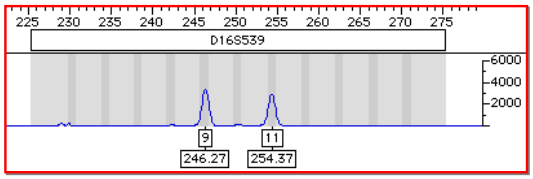
Source: Mecki Prinz (OCME) ISFG presentation, Sept 11, 2003

Typing Result on Aged Blood Stain

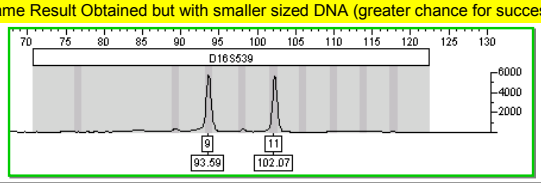
(15 years at room temperature storage)



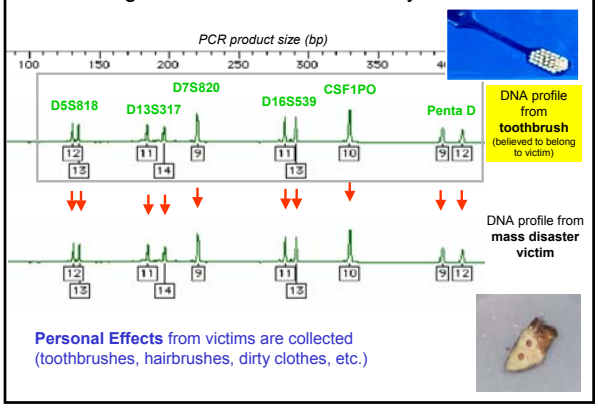
Development of **miniSTRs** to Aid Testing of Degraded DNA



Same Result Obtained but with smaller sized DNA (greater chance for success)

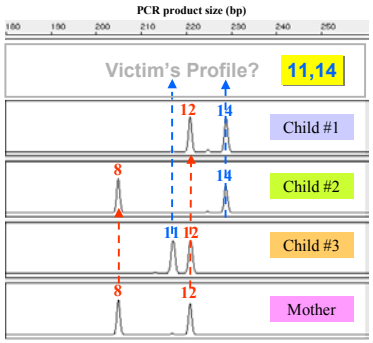


Using Personal Effects to Identify Remains

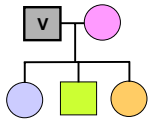


Personal Effects from victims are collected (toothbrushes, hairbrushes, dirty clothes, etc.)

Using Family Members to Identify Remains



Biological Relatives are asked to donate DNA samples



NIJ WTC KADAP (Kinship and Data Analysis Panel)

- Robert Shaler, Ph.D., Sc.D. NYC OCME
- Howard Baum, Ph.D. NYC OCME
- Fred Bieber, M.D, Ph.D. Harvard Med
- Bruce Budowle, Ph.D. FBI
- George Carmody, Ph.D. Carleton U.
- Ken Kidd, Ph.D. Yale
- Mike Conneally, Ph.D. Indiana U.
- Art Eisenberg, Ph.D. U. North Texas
- Mark Dale NY State Police
- Barry Duceman, Ph.D. NY State Police
- Dennis Gaige NY State Police
- Steve Swinton NY State Police
- Anne Walsh, Ph.D. NY State Dept Public Health
- Jack Ballantyne, Ph.D. U. Central Florida
- Joan Bailey-Wilson, Ph.D. NIH
- Leslie Biesecker, Ph.D. NIH
- Lisa Forman, Ph.D. NIJ
- Benoit Leclair, Ph.D. Myriad Genetics
- Steve Niezgoda, MBA NIJ Contractor
- Tom Parsons, Ph.D. AFDIL
- Elizabeth Pugh, Ph.D. NIH/CIDR
- Steve Sherry, Ph.D. NIH/NCBI
- Mandy Sozer, Ph.D. NIJ Contractor
- Lois Tully, Ph.D. NIJ
- Charles Brenner, Ph.D. DNA View
- Mike Hennessy GeneCode Forensics
- Judy Nolan, Ph.D. GeneCode Forensics
- John Butler, Ph.D. NIST

Met in NYC, Albany, DC, Baltimore Oct 2001, Dec 2001, Feb 2002, Apr 2002, July 2002, Sept 2002, Jan 2003, July 2003

A "Lessons Learned" Document is in preparation to aid work with future mass disasters…

Efforts for WTC Victim Identification Using DNA Testing

Government/Corporate/University Participation

- OCME Staff
- NYSP
- NYPD
- NIJ
- FBI
- NCBI
- NIH
- NIST
- NYSDOH
- AFDIL

- Myriad Genetics
- Bode Technology Group
- Gene Codes Forensics
- Celera Genomics
- Orchid Biosciences
- Johns Hopkins Univ
- SAIC
- Harvard University
- NYU Med. School
- Columbia Med. School
- Porter-Lee

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

>10,300 Investigations Aided through December 2003

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

Total number of profiles: 1,579,308
Total Forensic profiles: 78,276
Total Convicted Offender Profiles: 1,501,032

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

- Standard Reference Materials
  - SRM 2391b PCR-based DNA Profiling Standard
  - SRM 2395 Human Y-Chromosome DNA Profiling Standard
- Creating databases with useful information
  - STRBase (http://www.cstl.nist.gov/biotech/strbase)
- Evaluating and developing new technologies
- Interlaboratory testing
- Quality control testing for labs & companies

NIST DNA Standard Reference Materials

SRM 2390 - DNA Profiling Standard
Meets RFLP Needs

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

SRM 2392, 2392-I - Mitochondrial DNA
Standard Cell Lines

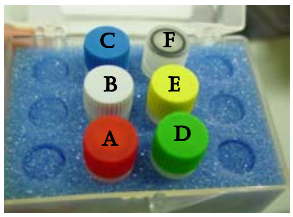
SRM 2395 – Y chromosome DNA standards

DAB Quality Assurance Standards for Forensic DNA Testing Laboratories

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.

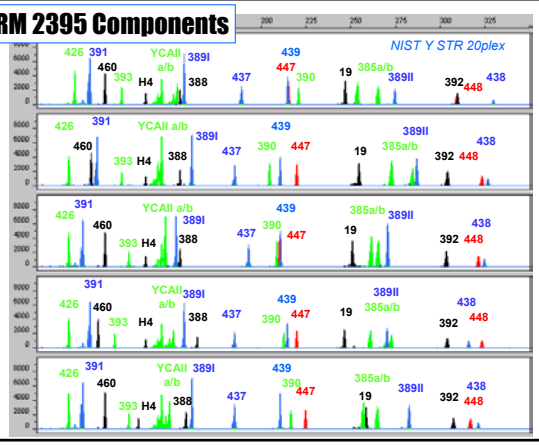
NIST Y Chromosome Standard



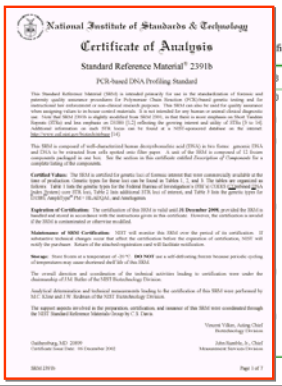
6 genomic DNA samples
5 male and 1 female
Typing Information on 27 Y STRs and 50 Y SNP markers

Available as of 07/2003

SRM 2395 Components



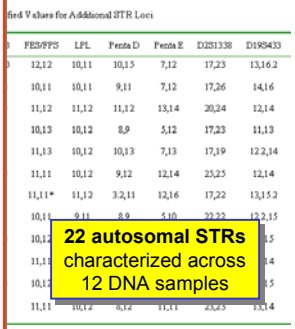
NIST SRM 2391b


National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2391b

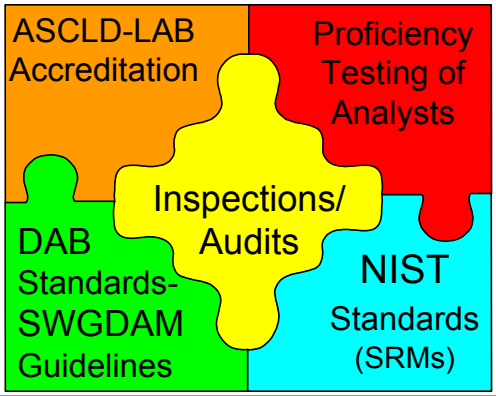
PCR-based DNA Profiling Standard


fied Values for Additional STR Loci

	FES/FPS	LPL	Penta D	Penta E	D2S1338	D19S433
	12,12	10,11	10,15	7,12	17,23	13,16.2
	10,11	10,11	9,11	7,12	17,26	14,16
	11,12	11,12	11,12	13,14	20,24	12,14
	10,13	10,12	8,9	5,12	17,23	11,13
	11,13	10,12	10,13	7,13	17,19	12.2,14
	11,11	10,12	9,12	12,14	25,25	12,14
	11,11*	11,12	3.2,11	12,16	17,22	13,15.2
	10,11	9,11	8,9	5,10	22,22	12.2,15
	10,12	10,12	8,12	11,11	23,23	13,14

22 autosomal STRs
characterized across
12 DNA samples

Ensuring Accurate Forensic DNA Results



NIST National Institute of Standards and Technology

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

- Standard Reference Materials
  - SRM 2391b PCR-based DNA Profiling Standard
  - SRM 2395 Human Y-Chromosome DNA Profiling Standard
- Creating databases with useful information
  - STRBase (http://www.cstl.nist.gov/biotech/strbase)
- Evaluating and developing new technologies
- Interlaboratory testing
- Quality control testing for labs & companies

STRBase

Short Tandem Repeat DNA Internet Database

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

General Information	Forensic Interest Data	Supplemental Info
•Intro to STRs (downloadable PowerPoint)	•FBI CODIS Core Loci	•Reference List
•**STR Fact Sheets**	•DAB Standards	•Technology Review
•Sequence Information	•NIST SRM 2391	•Addresses for Scientists
•Multiplex STR Kits	•Published PCR Primers	•Links to Other Web Sites
•Variant Allele Reports	•Y-Chromosome STRs	
	•Population Data	
	•Validation Studies	

Standardized information formats

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

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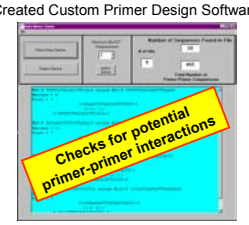
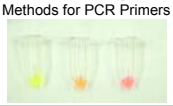
Technology Development Efforts

*Centered around **multiplex PCR**…*

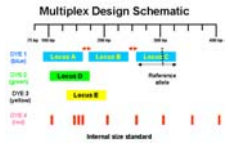
Created Custom Primer Design Software



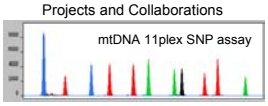
Implemented Quality Control Methods for PCR Primers



Standardized Assay Design Formats

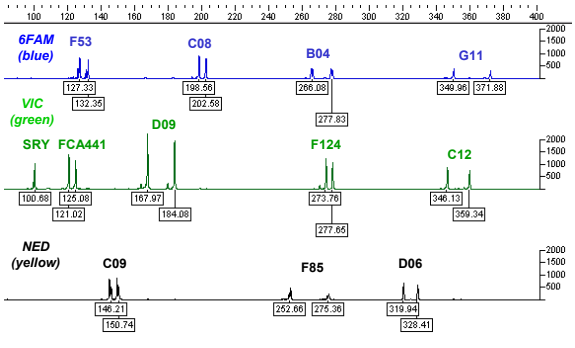


Demonstrated Success with Multiple Projects and Collaborations



Y-STR 20plex, cat STR 12plex

Cat STR 12plex (“MeowPlex”) developed at NIST



4 ng male cat DNA; Sample #5; 3/12/02

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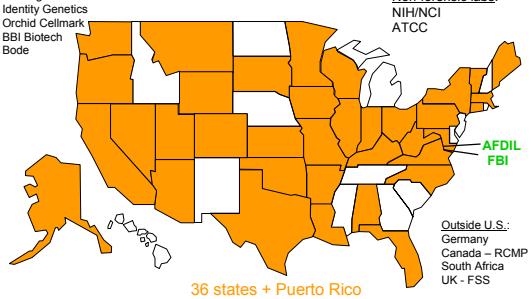
Participation in NIST Interlaboratory Study on DNA Quantitation

Companies:
Applied Biosystems
Promega
Identity Genetics
Orchid Cellmark
BBI Biotech
Bode

Non-forensic labs:
NIH/NCI
ATCC



Outside U.S.:
Germany
Canada – RCMP & CFS
South Africa
UK - FSS

36 states + Puerto Rico

As of 12-30-03, 80 laboratories been sent samples

8 DNA Samples in This NIST Study

Laboratories are only being asked to provide their quant values (no typing results expected)

Mixed source DNA

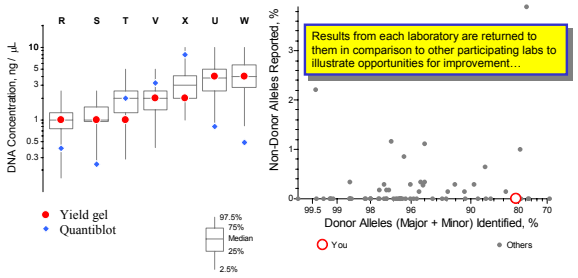
Single source DNA

Volume of each DNA sample provided = 100 µL

Individual Performance in an Interlaboratory Study

DNA Quantitation

Accuracy in STR Typing



Results from each laboratory are returned to them in comparison to other participating labs to illustrate opportunities for improvement…

See Kline, M.C., *et al.* (2003) *Anal. Chem.* 75: 2463-2469

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Our Recent Work with the Biotech Industry

Product Beta-Testing for…

- Applied Biosystems (Foster City, CA)
- Marligen Biosciences (Ijamsville, MD)
- Millipore Corporation (Bedford, MA)
- OligoTrail LLC (Evanston, IL)
- Promega Corporation (Madison, WI)
- ReliaGene Technologies, Inc. (New Orleans, LA)
- Roche Molecular Systems (Alameda, CA)
- Schleicher & Schuell, Inc. (Keene, NH)

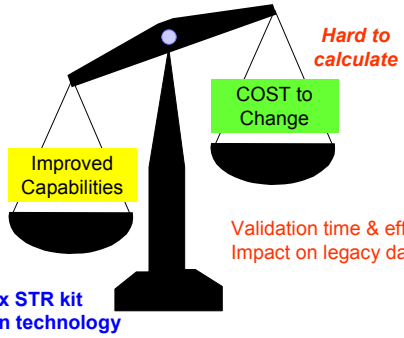
- Orchid GeneScreen (Dallas, TX) – validation of autosomal SNP typing markers
- Bode Technology Group (Springfield, VA) – supplied information for development of miniSTR assays

Future Methods Used in DNA Analysis

- Improved capabilities for multiplex analysis (parallel processing of genotypes)
- More rapid separation/detection technology (higher throughputs)
- More automated sample processing and data analysis
- Improved sensitivities and resolution
- Less expensive sample analysis

We must maintain accurate and robust methods

Decision to Switch/Upgrade to New Technology



New multiplex STR kit
New detection technology
New DNA markers

Decisions on Changing Technologies

- DNA technologies will continue to evolve *(just as computer systems become more powerful)*
- Decision to move to next technology must be carefully weighed as it takes time to validate new systems in forensic science
- New technologies will continue to impact our society for good

$1 Billion Proposed for DNA Testing

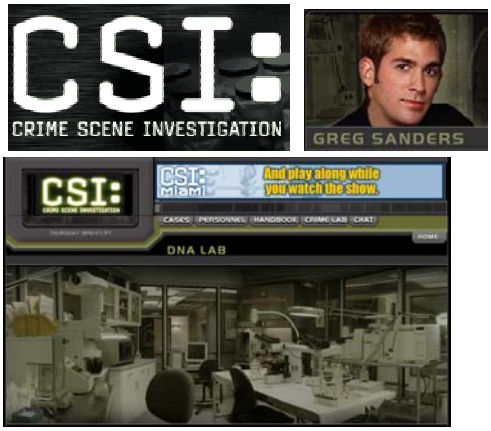
Administration Seeks to Clear Backlog of Analysis in Criminal Cases
By Dan Eggen
Washington Post Staff Writer
Wednesday, March 12, 2003; Page A03

Attorney General John D. Ashcroft yesterday proposed spending more than $1 billion on DNA analysis in criminal cases over the next five years, vowing to eliminate a massive backlog that has left hundreds of thousands of genetic samples untested nationwide.

The plan, first suggested in President Bush's 2004 budget proposal, envisions a dramatic expansion of an FBI database that contains DNA profiles from across the nation, a move that would improve chances of matching samples recovered at crime scenes. The government also would provide millions of dollars to state and local governments for DNA testing in criminal cases.

Attorney General John D. Ashcroft, holding a slide for DNA, hailed the technology as a tool in solving crimes. With him is Kellie Greene, whose attacker was found by DNA testing.

http://www.washingtonpost.com/wp-dyn/articles/A12570-2003Mar11.html



Funding and Collaborations

We are funded by an Interagency Agreement between National Institute of Justice and NIST Office of Law Enforcement Standards

Our publications and presentations are made available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Human Identity Project Team
John Butler (Project Leader)
Margaret Kline
Jan Redman
Peter Vallone
David Duewer
Jill Appleby
Amy Decker
Mike Coble

Collaborators (also funded by NIJ):
Mike Hammer and Alan Redd (U. AZ) for Y-chromosome studies
Tom Parsons (AFDIL) for mtDNA coding SNP work
Sandy Calloway (Roche) for mtDNA linear arrays
Bruce McCord and students (Ohio U) for miniSTR work
Steve Sherry and Jon Baker (NCBI) for STR data quality assurance software
Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work

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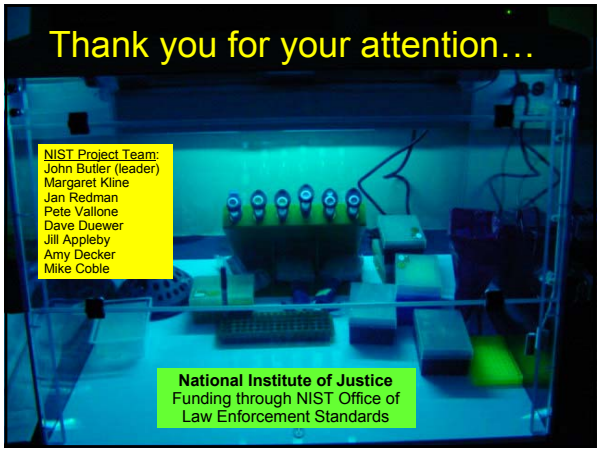
Mike Coble

Former (Honorary) Project Team Members

Rich Schoske

Christian Ruitberg


Thank you for your attention…
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Amy Decker
Mike Coble
National Institute of Justice
Funding through NIST Office of
Law Enforcement Standards