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.

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.genesee.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…
“We got him!”

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

Our DNA Comes from our Parents

Father’s Sperm

Mother’s Egg

Child’s Cell

Inheritance Pattern of DNA Profiles

Father’s Sperm

Mother’s Egg

Child’s Cell

PATERNITY TESTING

Family Inheritance of STR Alleles (D13S317)

PCR product size (bp)

<table>
<thead>
<tr>
<th>Me</th>
<th>Child #1</th>
<th>Child #2</th>
<th>Child #3</th>
<th>Mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>12</td>
<td>14</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Historical Investigation DNA Study
(Matching Relatives to Remains or Relatives to Relatives)

Thomas Jefferson II

Field Jefferson

Punt Jefferson

President Thomas Jefferson

Eston Hemings

Jefferson Y Haplotype

Jefferson Y Haplotype

Amanda

Marshall

Katy

My Wife

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

Genetic Genealogy Companies
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, predisposition 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

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

- 22 pairs + XX or XY
- Double stranded DNA molecule
- ~3 billion total base pairs
- Target Region for PCR

What Type of Genetic Variation?
- **Length Variation**
  - short tandem repeats (STRs)
  - CTAGTCGT(GATA)(GATA)(GATA)GGCGATCGT
- **Sequence Variation**
  - single nucleotide polymorphisms (SNPs)
  - insertions/deletions
  - GCTAGTCGTGCTC(G/A)CGTATGCTGCTAGC

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 chromosome

Short Tandem Repeats (STRs)

- Fluorescent dye creates a labeled PCR product
- Fluorescent dye label
- AATG
  - 7 repeats
  - 8 repeats
- Primer positions define PCR product size

Position of Forensic STR Markers on Human Chromosomes

13 CODIS Core STR Loci

Sex-typing

AMEL
Capillary Electrophoresis System

36 cm
Capillary filled with polymer solution

Inlet Buffer
Outlet Buffer

Sample tray moves automatically through the cathode end of the capillary to deliver each sample in succession.

Labeled DNA fragments (PCR products)
Capillary or Gel Lane

Sampling Detection
CCD Panel

Color Separation

ABI Prism spectrograph

Principles of Sample Separation and Detection

Size Separation

Sample Detection

Abi Pram spectrograph

Scanned Gel Image

Capillary Electropherogram

Crime Scene - Two Suspects

Suspect 1

D3  vWA  FGA
S1  14,15  17,18  23.24
S2  15,18  17,19  23.24
E   15,18  17,19  23.24

Suspect 2

Evidence

Allelic Ladders

PCR Product Size (bp)

8 11 14

All heterozygous
**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

\[ P = 2(0.2315)(0.2118) = 0.0981 \text{ or } 1 \text{ in } 10.2 \]

For independent loci, the genotype frequencies can be combined through multiplication…

Profile Probability = \((P_1)(P_2)\ldots(P_n) = 1 \text{ in a very large number…}\)

**DNA Profile Frequency with all 13 CODIS STR loci**

<table>
<thead>
<tr>
<th>Locus</th>
<th>allele value</th>
<th>allele frequency</th>
<th>1 in N</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>16.0</td>
<td>0.2718</td>
<td>16.00</td>
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<tr>
<td>TH01</td>
<td>17.0</td>
<td>0.2590</td>
<td>0.25</td>
</tr>
<tr>
<td>TPOX</td>
<td>18.0</td>
<td>0.2015</td>
<td>18.17</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>19.0</td>
<td>0.1629</td>
<td>19.06</td>
</tr>
<tr>
<td>TH01</td>
<td>20.0</td>
<td>0.1589</td>
<td>20.00</td>
</tr>
<tr>
<td>TPOX</td>
<td>21.0</td>
<td>0.1442</td>
<td>21.06</td>
</tr>
<tr>
<td>D16S539</td>
<td>22.0</td>
<td>0.1369</td>
<td>22.00</td>
</tr>
<tr>
<td>D7S820</td>
<td>23.0</td>
<td>0.1247</td>
<td>23.06</td>
</tr>
<tr>
<td>D13S317</td>
<td>24.0</td>
<td>0.1125</td>
<td>24.00</td>
</tr>
<tr>
<td>D5S818</td>
<td>25.0</td>
<td>0.1027</td>
<td>25.06</td>
</tr>
<tr>
<td>D18S51</td>
<td>26.0</td>
<td>0.0924</td>
<td>26.00</td>
</tr>
<tr>
<td>D21S11</td>
<td>27.0</td>
<td>0.0825</td>
<td>27.06</td>
</tr>
<tr>
<td>D8S1179</td>
<td>28.0</td>
<td>0.0727</td>
<td>28.00</td>
</tr>
<tr>
<td>D5S518</td>
<td>29.0</td>
<td>0.0630</td>
<td>29.06</td>
</tr>
<tr>
<td>D19S433</td>
<td>30.0</td>
<td>0.0536</td>
<td>30.00</td>
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<tr>
<td>CSF1PO</td>
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<td>31.06</td>
</tr>
<tr>
<td>FGA</td>
<td>32.0</td>
<td>0.0354</td>
<td>32.00</td>
</tr>
</tbody>
</table>

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

**Steps in DNA Analysis**

- Collection
- Specimen Storage
- Extraction
- DNA Extraction (Blood Stain)
- Quantitation
- DNA Quantitation
- Genotyping
- STR Typing
- Interpretation of Results
- Database Storage & Searching

**Information is tied together with multiplex PCR and data analysis**

- AmpFlSTR® Identifiler™ (Applied Biosystems)
High-Throughput STR Typing on the ABI 3100 (16-capillary array)

256 data points in 45 minutes with STR 16plex and 16 capillaries

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)

When working with degraded samples it is difficult to generate longer PCR products

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

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.)

Using Family Members to Identify Remains

Victim's Profile? 11, 14

Biological Relatives are asked to donate DNA samples

DNA profile from toothbrush (believed to belong to victim)

DNA profile from mass disaster victim

Using Family Members to Identify Remains

Victim's Profile? 11, 14

Biological Relatives are asked to donate DNA samples

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DNA profile from mass disaster victim

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 Cammody, Ph.D. Carleton U.
- Ken Kidd, Ph.D. Yale
- Mike Conneally, Ph.D. Indiana U.
- Art Eisenberg, Ph.D. 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. NJI
- Benoit Lozeille, Ph.D. Myriad Genetics
- Steve Nieszgora, M.B.A NJI Contractor
- Tom Parsons, Ph.D. AFDL
- Elizabeth Pugh, Ph.D. NH/NIH
- Steve Sherry, Ph.D. NIH/NIH
- Mandy Sasser, Ph.D. NJI Contractor
- Lois Tully, Ph.D. NJI
- Charles Brommer, Ph.D. DNA View
- Mike Hennessy GeneCode Forensics
- Judy Nolan, Ph.D. GeneCode Forensics
- John Butler, Ph.D. NIST
- Robert Shaler, Ph.D., Sc.D. NYC OCME
- Howard Baum, Ph.D. NYC OCME
- Fred Bieber, M.D. Ph.D. Harvard Med
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- Joan Bailey-Wilson, Ph.D. NIH
- Leslie Biesecker, Ph.D. NIH

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

Efforts for WTC Victim Identification Using DNA Testing

Government/Corporate/University Participation

- OCME Staff
- NYPD
- FBI
- NIST
- NYSB
- AFDL
- 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
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

NIST SRM 2391b

Ensuring Accurate Forensic DNA Results

ASCLD-LAB Accreditation
Proficiency Testing of Analysts
Inspections/Audits
DAB Standards-SWGDAM Guidelines
NIST Standards (SRMs)

22 autosomal STRs characterized across 12 DNA samples
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)

Creating databases with useful information
- STRBase (http://www.cstl.nist.gov/biotech/strbase)

Evaluating and developing new technologies

Quality control testing for labs & companies

STRBase
Short Tandem Repeat DNA Internet Database

General Information
- Introduction to STRs (downloadable PowerPoint)
- STR Fact Sheets
- Sequence Information
- Multiplex STR Kits
- Variant Allele Reports

Forensic Interest Data
- FBI CODIS Core Loci
- DAB Standards
- NIST SRM 2391
- Published PCR Primers
- Y-Chromosome STRs
- Population Data
- Validation Studies

Supplemental Information
- Reference List
- Technology Review
- Addresses for Scientists
- Links to Other Web Sites

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

Technology Development Efforts
Centered around multiplex PCR...

Created Custom Primer Design Software

Implemented Quality Control Methods for PCR Primers

Demonstrated Success with Multiple Projects and Collaborations

Checks for potential primer-primer interactions

mtDNA 11plex SNP assay

Y-STR 20plex, cat STR 12plex

Cat STR 12plex ("MeowPlex") developed at NIST

- 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

Dr. John M. Butler
Participation in NIST Interlaboratory Study on DNA Quantitation

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

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

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

Non-forensic labs:
- NIH/NCI
- ATCC

36 states + Puerto Rico

8 DNA Samples in This NIST Study
- Single source DNA
- Mixed source DNA
- Teflon tube

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.

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
Improved Capabilities

COST to Change

Validation time & effort
Impact on legacy data

New multiplex STR kit
New detection technology
New DNA markers

Decision to Switch/Upgrade to New Technology

Hard to calculate

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.

Funding and Collaborations

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

Collaborators (also funded by NIJ):
- Mike Rehmeyer 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

Human Identity Project Team

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

NIST Human Identity Project Team

John Butler
Margaret Kline
Jan Redman
Pete Vallone
Dave Duewer
Amy Decker
Jill Appleby
Mike Coble

Former (Honorary) Project Team Members

Rich Schoske
Christian Rubberg

Dr. John M. Butler
Thank you for your attention...

NIST Project Team:
John Butler (leader)
Margaret Kline
Jan Redman
Pete Vallone
Dave Duewer
Jill Agresti
Amy Decker
Mike Coble

National Institute of Justice
Funding through NIST Office of Law Enforcement Standards