Providing SRMs for the Rapidly Evolving Forensic DNA & Human Identity Testing Communities

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National Institute of Standards and Technology

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Disclaimers

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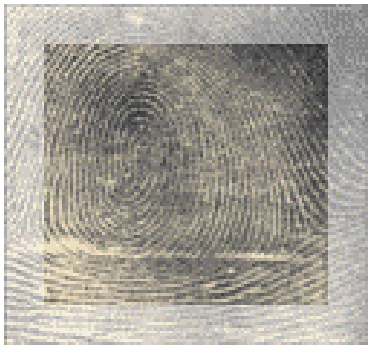
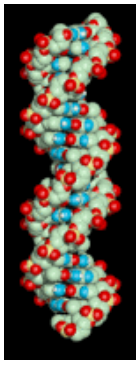
Methods for Human Identification

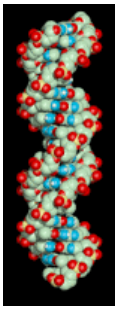


Fingerprints have been used since 1901



DNA since 1986

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.

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

DNA Testing Requires a Reference Sample

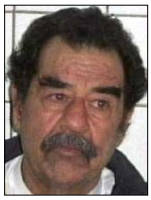
A DNA profile by itself is fairly useless because it has no context…

DNA analysis for identity only works by comparison – **you need a reference sample**

Crime Scene Evidence compared to **Suspect(s)** (Forensic Case)
Child compared to **Alleged Father** (Paternity Case)
Victim's Remains compared to **Biological Relative** (Mass Disaster ID)
Soldier's Remains compared to **Direct Reference Sample** (Armed Forces ID)

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

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

If appropriate reference samples can be found!

DNA Databases involve Comparison of DNA Profiles Collected at Different Times or in Different Locations

Total number of profiles: **3,072,083** (as of Feb 2006) **31,776 Investigations Aided**

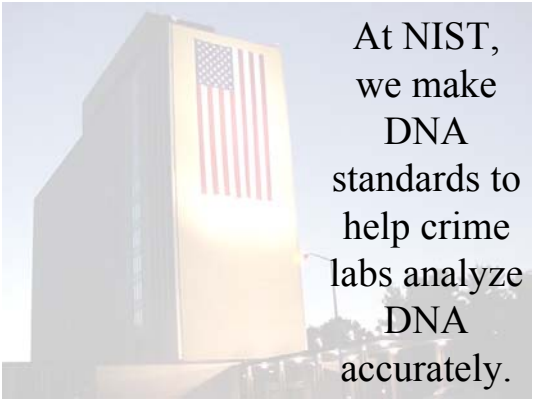
CODIS COMBINED DNA INDEX SYSTEM

Convicted Offenders 2,941,206
Forensic Casework 130,877
Missing Persons

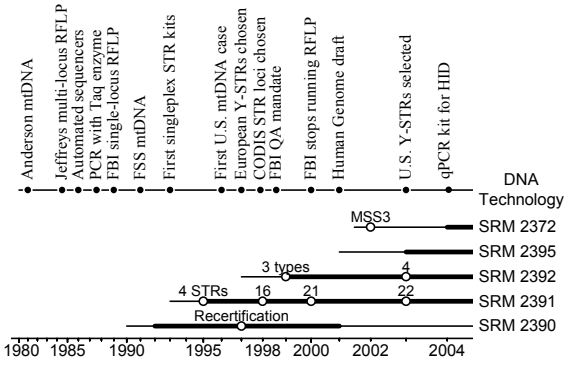
FBI Laboratory

State and Local Forensic Laboratories

	FBI	FDLE	VA	SLED
Investigations aided (thru Feb 2006)	3,903	3,196		409
Offender Profiles	287,590	238,458		46,495
Forensic Samples	10,353	5,627		1.704



Forensic DNA Timeline



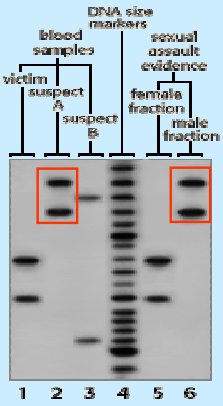
What are the Tools of DNA Typing?

- RFLP Testing (Late 1980's)
  - Radioactive Based
  - Chemiluminescent Based

  SRM 2390
- PCR-Based Testing (Mid 1990's)
  - Dot-Blot
  - VNTR
  - STR (Fluorescent markers used today)

  SRM 2391..a..b
- DNA Sequencing (Late 1990's)
  - Mitochondrial DNA

  SRM 2392, 2392-I
- Y-Chromosome Testing (early 2000's) **SRM 2395**



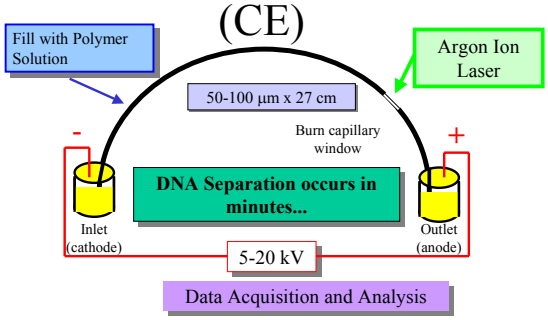
Which Suspect, A or B, cannot be excluded from potential perpetrators of this assault?

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)
- ^{32}P visualization requires 3 – 7 days @ – 80 ºC
- 5 – 7 probes required for matching
- Time required weeks to months

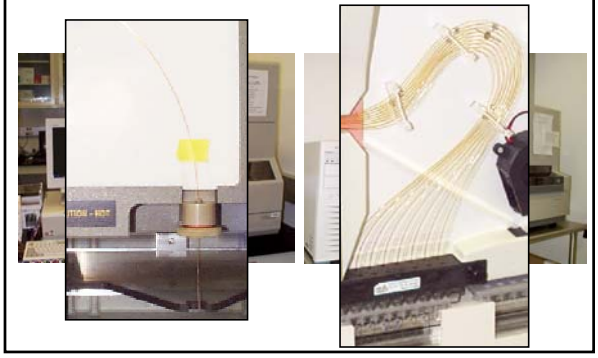
Technology moves forward

Capillary Electrophoresis (CE)



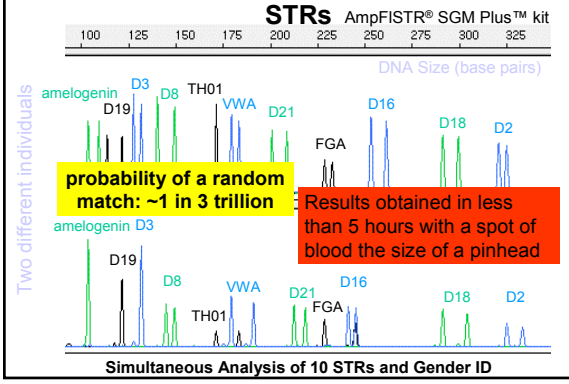
Capillary Electrophoresis Instrumentation

ABI 310 single capillary | **ABI 3100 16-capillary array**



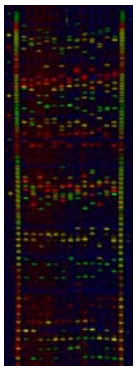
Human Identity Testing with Multiplex STRs

AmpFISTR® SGM Plus™ kit

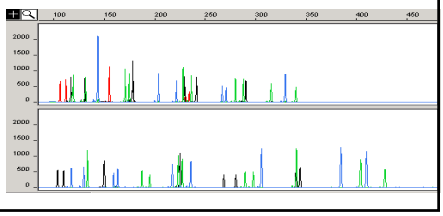


Simultaneous Analysis of 10 STRs and Gender ID

2003: SRM 2391b

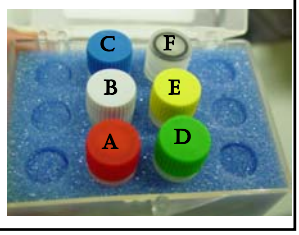


- 22 STR Loci,
- D1S80, and DQa1/PM
- Many labs using 16-plexes
- 0.5 – 1 ng DNA



2003: SRM 2395 Human Y-Chromosome DNA Profiling Standard

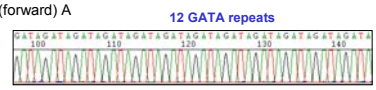
Extracted genomic DNAs: 5 Male, 1 Female (neg Control)



SRM 2395 Certificate Information

For the 5 male samples :
22 Y-chromosome STR markers sequenced
5 Y-chromosome STR markers typed/not sequenced
42 Y-chromosome biallelic SNPs

DYS439 (forward) A — 12 GATA repeats

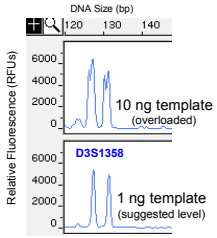


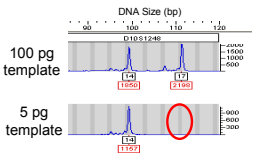
DYS390 (forward) E — 24 repeats $[TCTG]_8$ $[TCTA]_{11}$ $[TCTG]_1$ $[TCTA]_4$

Impact of the Amount of DNA used in PCR Reaction

Why 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





Stochastic effect when amplifying low levels of DNA produces allele dropout

The Next Task:

SRM 2372: Human DNA Quantitation Standard

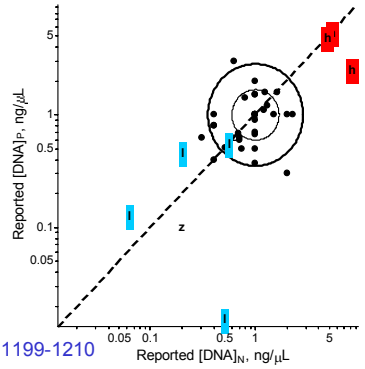
Challenges:
What is a nanogram of genomic DNA ?

How do we assure what goes into the tube is what the customer gets out of the tube ?

From interlaboratory studies we know there is ≈1.6-fold variability in the measurement systems currently in use… but the range is ≈ 20-fold.

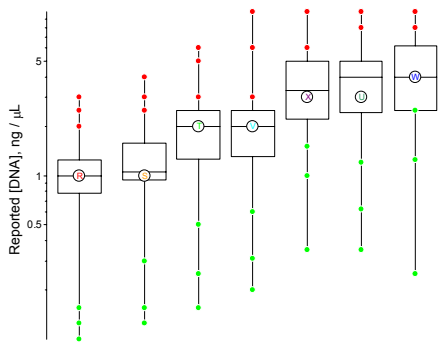
Quantitation Issue from Mixed Stain 2 (1999)

Target plot of replicate samples N and P.

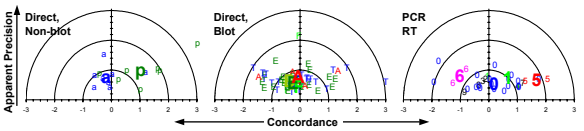


J. Forensic Sci. 46: 1199-1210

NMSS#3 Reported DNA concentrations of the samples, ng/µL



Among Participate Results for QS04



Concordance – is a direct multi-material analogue of bias

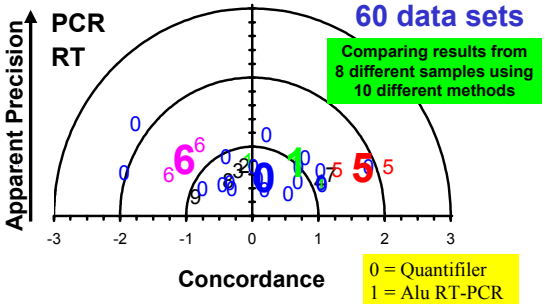
Apparent precision – is analogous to precision but also incorporates sample-specific measurement differences or "matrix effects".

"Bold" characters represent the median performance for all results submitted for a particular method

The semi-circles delimit 1, 2 and 3 standard deviations of total comparability.

Interlaboratory Comparisons

Laboratory Performances with Real-Time PCR Methods



Kline, *et al.* (2005) *J. Forensic Sci.* 50(3):571-578

Why Real Time qPCR?

- Forensic Labs are beginning to switch over to this method
- Higher throughput and reduced user intervention
- Experimental data rapidly analyzed in software; interpolating into the calibration curve
- qPCR be sensitive to same inhibitors as faced in traditional STR test (both PCR based)
- Inquiries from the community

General qPCR Comments from the Forensic Community

For one commercial kit

- "I have feel that the calibrant may exhibit a two-fold difference from the "true" value"
- "In practice we have found that utilizing a target range of 1-2 ng based on a method X result oftentimes yields STR data below our rfu threshold"
- "There appears to be an obvious difference between the two lots of a calibrant"
- "We have not had any problems with the lot_X calibrant and our results have been relatively stable"

Developing a Calibrant

- Some sources of genomic DNA
  - Single source
  - Multiple source
  - Cell line
- How is the concentration of the Calibrant determined?
  - UV, fluorescence, phosphorus, others
- Since qPCR is relative to the DNA calibrant used, different calibrants may give different results
  - Are these within error?
  - Can this be controlled?
  - Is the error acceptable for our purpose?

Things to Consider with Calibrants

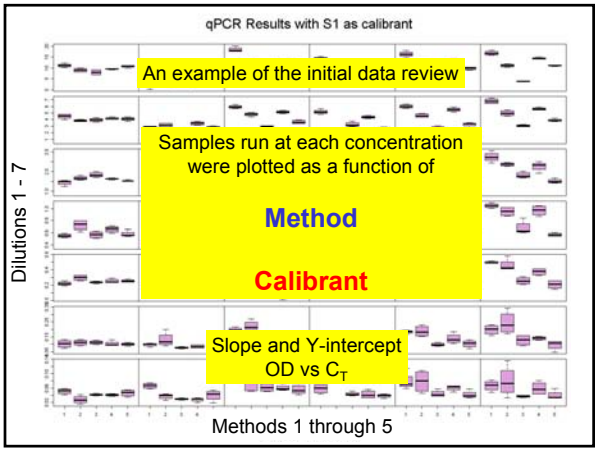
- Will the calibrant have inherent characteristics that may bias results?
- If probing a multi copy locus (Alu) will different calibrants have significantly different numbers of copies (cell line vs single source)?
- If using UV spectroscopy for quantitation: do the OD measurements correlate with qPCR results?

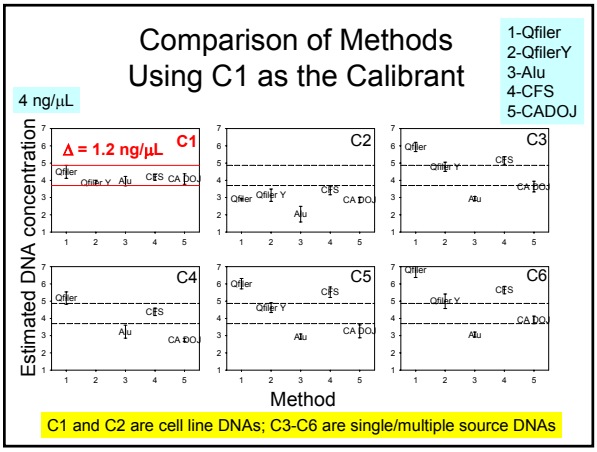
(1 OD = 50 ng/µL double stranded DNA)

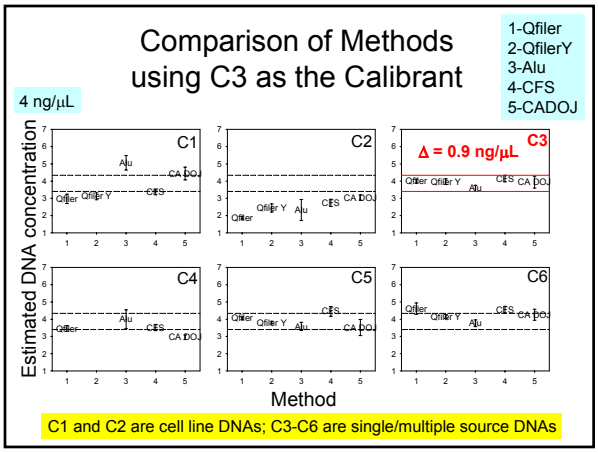
qPCR Method Evaluation Protocol

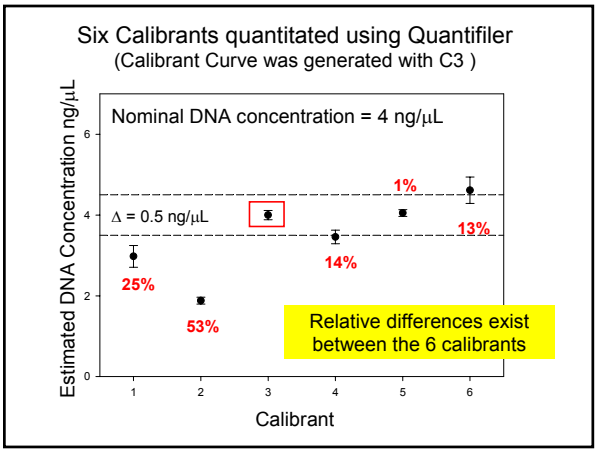
- 6 different calibrants:
  - 3 commercial (2 cell lines, one multiple source)
  - 3 purified at NIST (single source; one female, two males)
- Where possible, [DNA] was assigned from UV absorption at 260 nm; otherwise used manufacturer's values.
- Stocks of the candidates were diluted to:
  - 10.0, 4.0, 1.6, 0.64, 0.26, 0.1, and 0.04 ng/µL daily.
- Each candidate sample was run in duplicate on duplicate plates with each of the 5 qPCR methods.

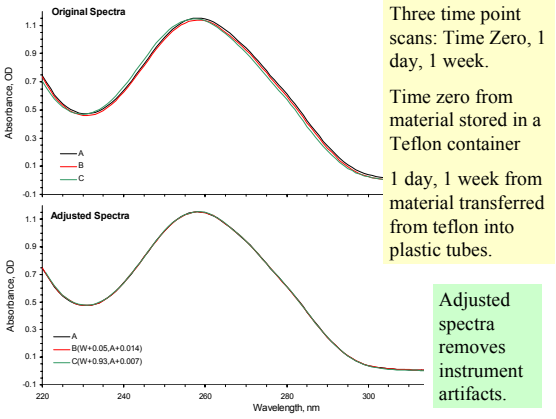
Samples run on ABI 7500









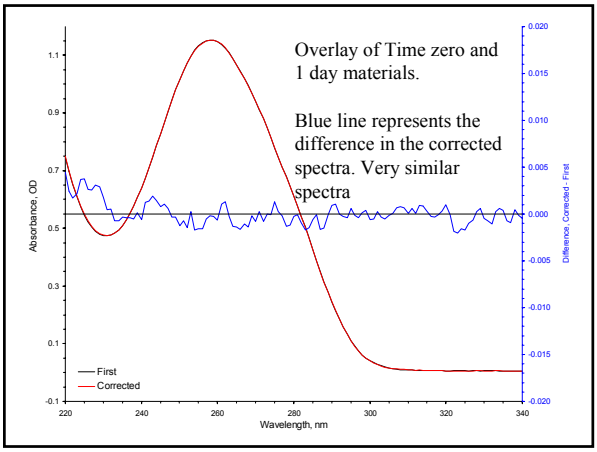


Three time point scans: Time Zero, 1 day, 1 week.

Time zero from material stored in a Teflon container

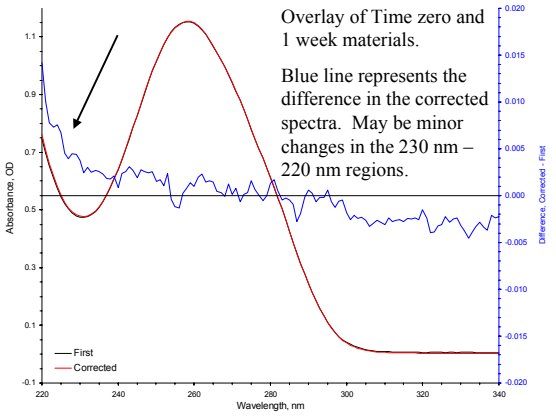
1 day, 1 week from material transferred from teflon into plastic tubes.

Adjusted spectra removes instrument artifacts.



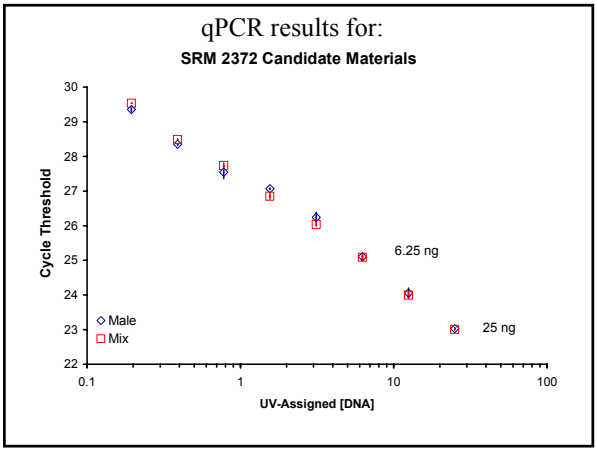
Overlay of Time zero and 1 day materials.

Blue line represents the difference in the corrected spectra. Very similar spectra



Overlay of Time zero and 1 week materials.

Blue line represents the difference in the corrected spectra. May be minor changes in the 230 nm – 220 nm regions.



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