Forensic DNA Standard Reference Materials

Margaret C. Kline and John Butler
National Institute of Standards and Technology

2006 PITTCON Workshop: Standard Reference Materials (SRMs) for Environmental, Food, Metal, fossil Fuel, and Forensic DNA Analysis. March 12,2006; Olando, FL.

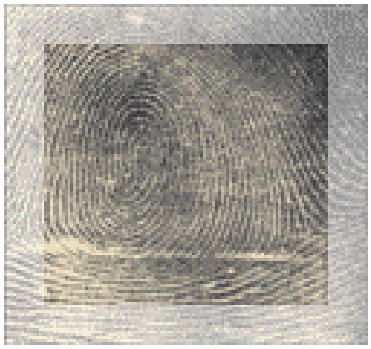
Disclaimers

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

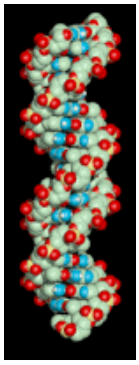
Points of view are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

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

Methods for Human Identification



Fingerprints have been used since 1901

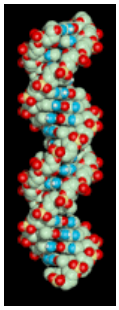


DNA since 1986

- 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

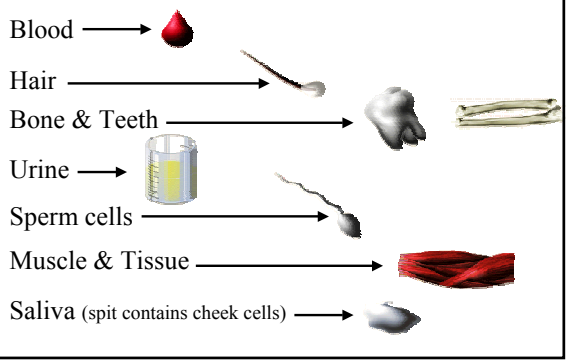
The order of these letters is what makes everyone different.

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?



DNA in the News


DNA nets capture of suspected rapist

By ERIC SWENSEN
Daily Progress staff writer

A DNA match — and two pieces of luck — led to the arrest of a 26-year-old Norfolk man Tuesday in connection with the Aug. 28 rape of a University of Virginia student.

First, the DNA sample of Montarel D. Davis happened to be one of 4,000 added to Virginia's DNA database this month as the state attempts to catch up with a backlog of more than 100,000 unanalyzed samples. State law requires all felons and juveniles 14 and older convicted of serious crimes to provide blood samples.

Davis "has convictions for some violent crimes," said Lt. J.E. "Chip" Harding of the Charlottesville Police Department.Davis' sample matched DNA in semen found in the bedroom of the Venable neighborhood house where the rape occurred and saliva found on a half-empty can of beer outside, Harding said. The results, Harding added, make it virtually certain that Davis is the attacker.

"Without the database, we would have absolutely no idea who our man was," Harding said. "Just

See CAPTURE on A7

How police tense

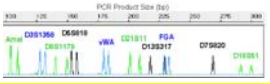
Davis was charged with rape

Authorities escort rape suspect Montarel D. Davis, 26, of Norfolk, out of a police car on Water Street in downtown Charlottesville.

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



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


Wreckage at Ground Zero

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)

Vietnam Veterans Memorial

Tomb of the Unknown Soldier

- **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.**

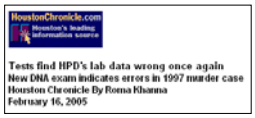
Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Box 10.1, pp. 250-251

Quality Is Essential in Forensic DNA Testing


Chicago Tribune

FORENSICS UNDER THE MICROSCOPE

Unproven techniques sway courts, erode justice


HoustonChronicle.com

Tests find HPD's lab data wrong once again
New DNA exam indicates errors in 1997 murder case
Houston Chronicle By Roma Khanna
February 16, 2005

- **DNA results impact lives** – the guilty can be implicated in a crime and the innocent can be exonerated
- Scientific attacks against the science behind DNA testing are rare in court now. Rather **the focus is on demonstrating that quality results were obtained**.
- **DNA databases involve comparisons** of DNA profiles analyzed at different times or in different locations

DNA Testing Requires a Reference Sample

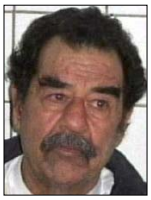
A DNA profile by itself is fairly useless because it has no context…

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

Is this man really Sadaam Hussein?

Killed July 22, 2003

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

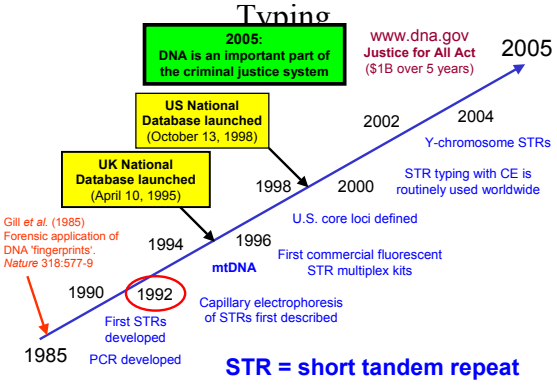
http://www.cnn.com/2005/US/03/02/baby.81.ap/index.html

Hurricane Katrina Victims Will Be Identified with Forensic DNA Testing Methods

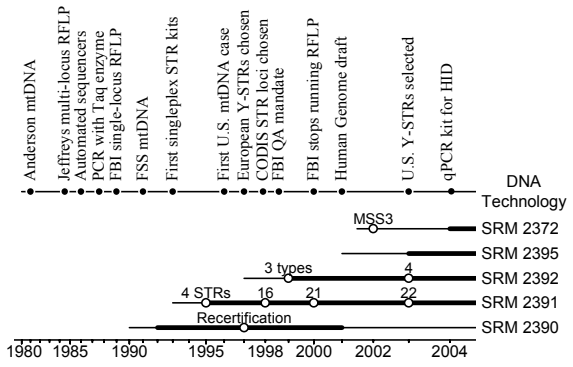
If appropriate reference samples can be found!

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

A Brief History of Forensic DNA Typing

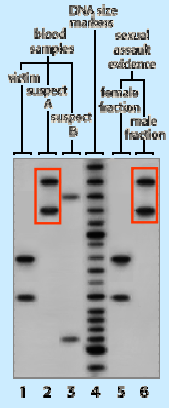


Forensic DNA Timeline



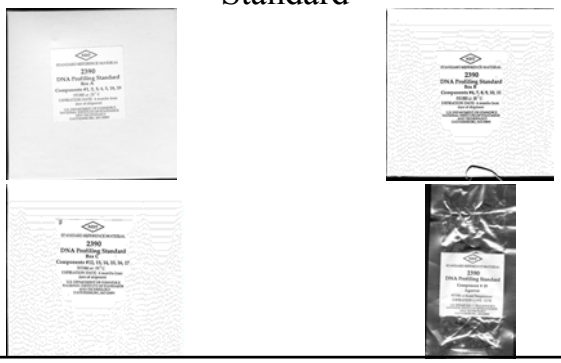
What are the Tools of DNA Typing?

- 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
- Y-Chromosome Testing (early 2000's)



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

1992: SRM 2390 DNA Profiling Standard



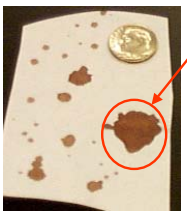
1992: SRM 2390 DNA Profiling Standard

Each step of the RFLP process could be checked with these components. At the time of release, ^{32}P 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:



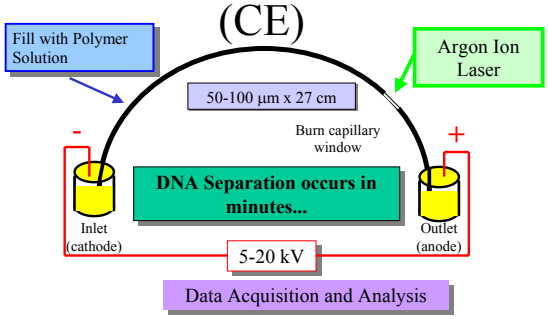
- Requires 100 ng to 1 µg of DNA (stain the size of a dime)
- The DNA must be relatively intact 1000-20,000 bp in size (not always possible to obtain)
- ^{32}P visualization requires 3 – 7 days @ – 80 ºC
- 5 – 7 probes required for matching
- Time required weeks to months

Technology moves forward

Progression of DNA Typing Markers

- RFLP
  - multilocus VNTR probes
  - single locus VNTR probes **(^{32}P 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

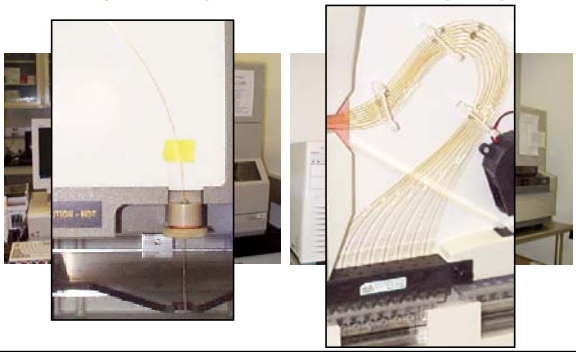
Capillary Electrophoresis (CE)



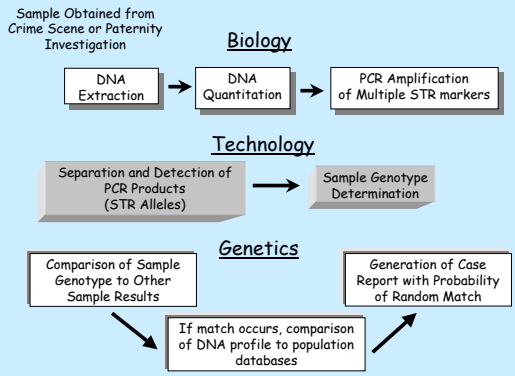
Capillary Electrophoresis Instrumentation

ABI 310 single capillary

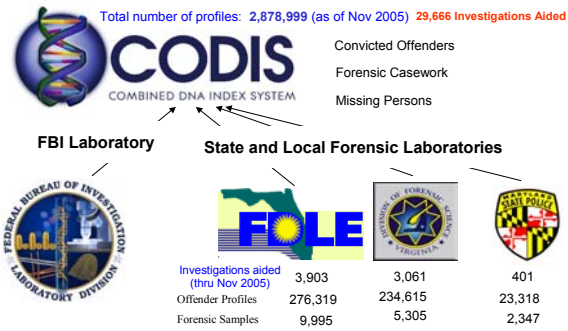
ABI 3100 16-capillary array



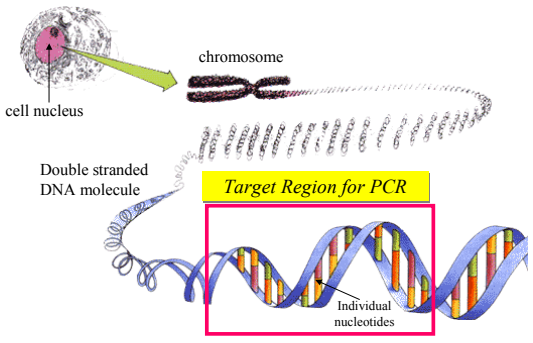
Steps in Sample Processing



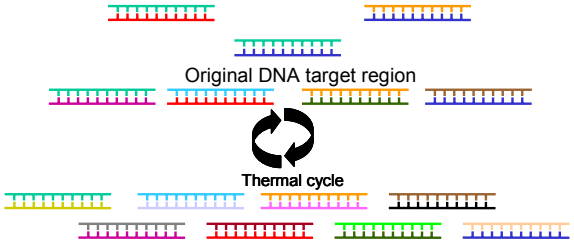
DNA Databases involve Comparison of DNA Profiles Collected at Different Times or in Different Locations



DNA in the Cell

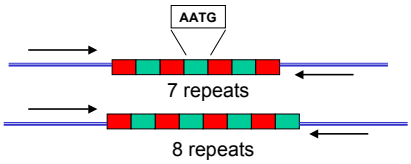


PCR Copies DNA Exponentially through Multiple Thermal Cycles



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

Short Tandem Repeats (STRs)

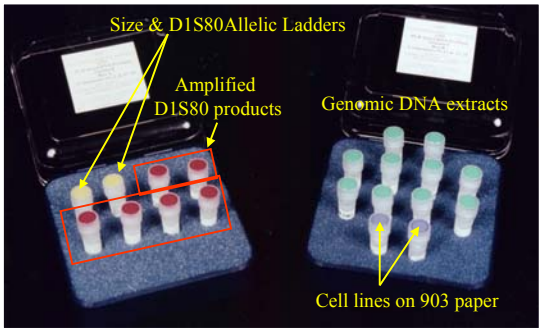


the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

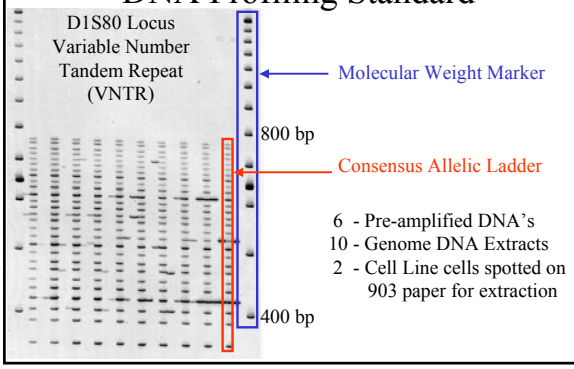
Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

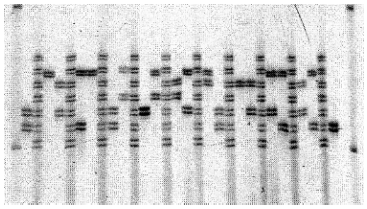
1995: SRM 2391 PCR-based DNA Profiling Standard



1995: SRM 2391 PCR-based DNA Profiling Standard



DQ alpha & Polymarker Reverse Dot Blot Hybridization



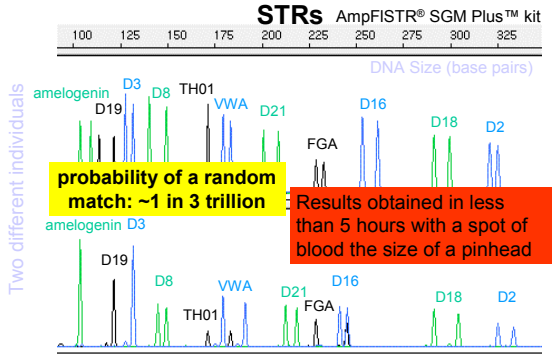
Silver stained gels for STR monoplexes

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."

Human Identity Testing with Multiplex STRs

AmpFISTR® SGM Plus™ kit

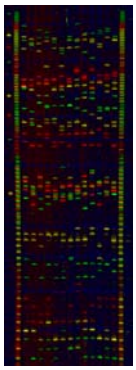


probability of a random match: ~1 in 3 trillion

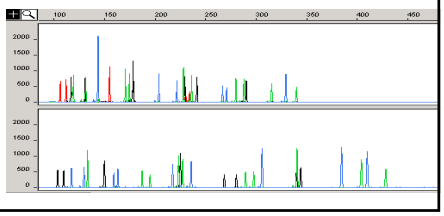
Results obtained in less than 5 hours with a spot of blood the size of a pinhead

Simultaneous Analysis of 10 STRs and Gender ID

2003: SRM 2391b

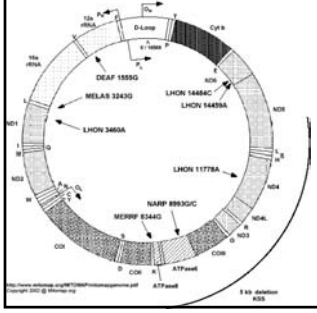


- 22 STR Loci,
- D1S80, and DQa1/PM
- Many labs using 16-plexes
- 0.5 – 1 ng DNA



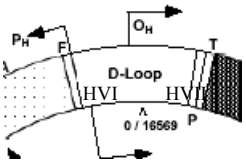
Human Mitochondrial Genome

1981: sequenced by Anderson et al.



1991: FSS uses the non-coding region, control region for casework

1996: FBI began mtDNA casework



Hypervariable Region I & II

Forensic usefulness of mtDNA

Can be obtained from highly degraded samples
- skeletal remains
- shed hairs

Any maternal relative can serve as a reference

Forensic drawbacks of mtDNA

Extreme care must be taken to avoid contamination when working with limited quantities.

Power of discrimination is limited to database size.

1999: SRM 2392 Mitochondrial DNA Sequencing Standard

- SRM 2392 certifies the entire mtDNA sequence information for apparently normal cell lines : CHR, GM09947a, and GM09948.
- Included with SRM 2392:
  - DNA extracts of CHR and GM09947a
  - Cloned DNA from CHR HVI region
- 2003: SRM 2392I, Cell line HL-60 extract and sequence information.

History of Y STR Marker Discovery

European Y markers 1997

US Core Y loci: 2003

1992 - DYS19 (Roewer et al.)
1994 - YCAI a/b, YCAII a/b, YCAIII a/b, DXYS156 (Mathias et al.)
1996 - DYS389I/II, DYS390, DYS391, DYS392, DYS393 (Roewer et al.)
1996 - DYF371, DYS425, DYS426 (Jobling et al.)
1997 - DYS288, DYS388 (Kayser et al.)
1998 - DYS385 a/b (Schneider et al.)
1999 - A7.1, A7.2, A10, C4, H4 (White et al.)
2000 - DYS434, DYS435, DYS436, DYS437, DYS438, DYS439 (Ayub et al.)
2000 - G09411, G10123 (de Knijff unpublished)
2001 - DYS441, DYS442 (Iida et al.)
2002 - DYS443, DYS444, DYS445 (Iida et al.); DYS446, DYS447, DYS448, DYS449, DYS450, DYS452, DYS453, DYS454, DYS455, DYS456, DYS458, DYS459, DYS463, DYS464 (Redd et al.)
2002 – DYS468-DYS596 (129 new Y STRs; Manfred Kayser GDB entries)

Why is the the Y chromosome useful?

- Lack of recombination
- Paternal inheritance
- Many polymorphic markers: slow evolving and fast evolving

Applications using the Y chromosome

- Genetic genealogy
- Evolutionary studies regarding population origins
- Forensic casework

When will a male-specific forensic typing system will be particularly useful?

- Rape cases with mixtures of semen from the assailant and cells from the victim, in which the male component is at low frequency and/or is degraded.
- Multiple male donors
- Cases involving vasectomized or azoospermic men
- Other mixtures where the differential lysis technique cannot be performed (blood-blood, blood-saliva, skin-saliva, etc.)

Y-SNPs and Y-STRs

Single Nucleotide Polymorphisms

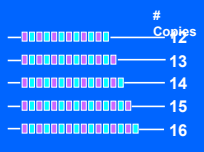
–CGATG–

–CGGTG–

Insertion/deletions (indels)

Short Tandem Repeats

–GATAGATAGATAGATA–

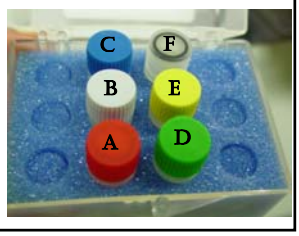


Binary characters
Slowly evolving (~10^{-8}/gen)
-> Low resolution haplogroups

Multi-state characters
Quickly evolving (2 x 10^{-3}/gen)
-> High resolution haplotypes

2003: SRM 2395 Human Y-Chromosome DNA Profiling Standard

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

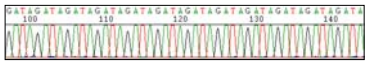


SRM 2395 Certificate Information

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 $[TCTG]_8$ $[TCTA]_{11}$ $[TCTG]_1$ $[TCTA]_4$

The Next Task:

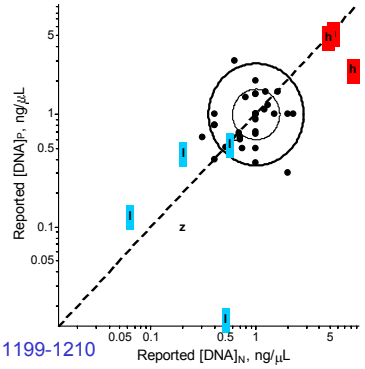
SRM 2372: Human DNA Quantitation Standard

Challenge:
What is a nanogram of genomic DNA ?

From interlaboratory studies we know there is a factor of 1.6 in the measurement systems currently in use. But the range is 20 fold.

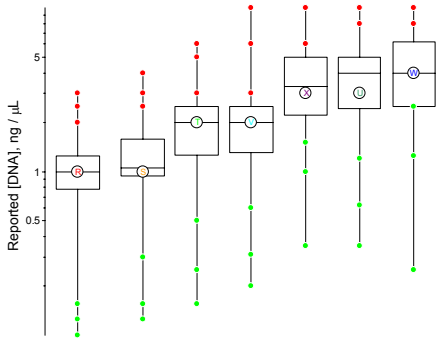
Quantitation Issue from Mixed Stain 2 (1999)

Target plot of replicate samples N and P.

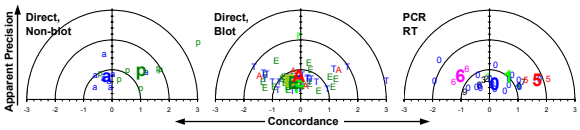


J. Forensic Sci. 46: 1199-1210

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



Among Participate Results for QS04



Concordance – is a direct multi-material analogue of bias

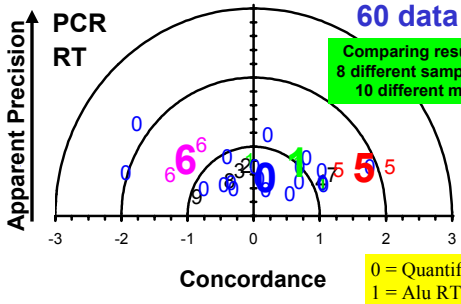
Apparent precision – is analogous to precision but also incorporates sample-specific measurement differences or "matrix effects".

"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



60 data sets

Comparing results from 8 different samples using 10 different methods

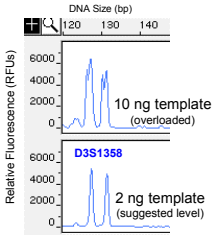
0 = Quantifiler
1 = Alu RT-PCR
5 = BRCA1
6 = CFS-HUMRT

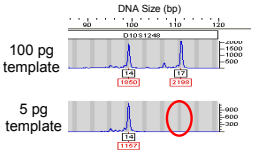
Kline, *et al.* (2005) *J. Forensic Sci.* 50(3):571-578

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





Stochastic effect when amplifying low levels of DNA produces allele dropout

Why Real Time qPCR?

- 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

- "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?

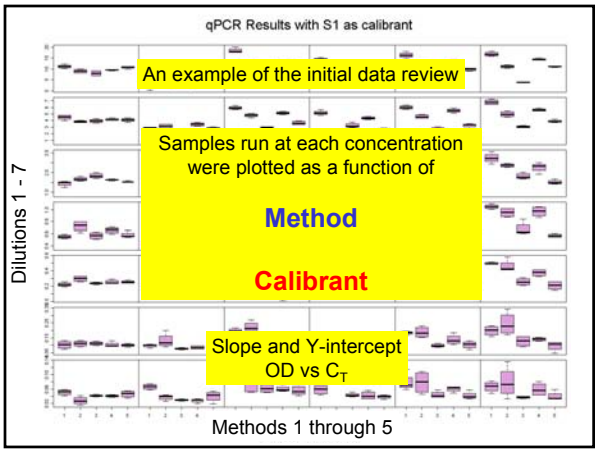
Things to Consider with Calibrants

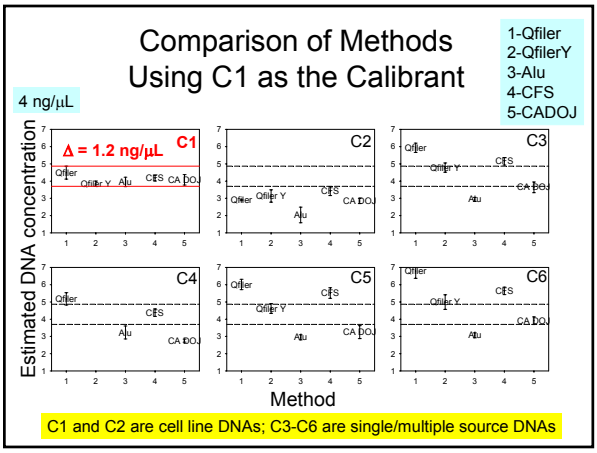
- Will the calibrant have inherent characteristics that may bias results?
- If probing a multi copy locus (Alu) will different calibrants have significantly different numbers of copies (cell line vs single source)?
- If using UV spectroscopy for quantitation: do the OD measurements correlate with qPCR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

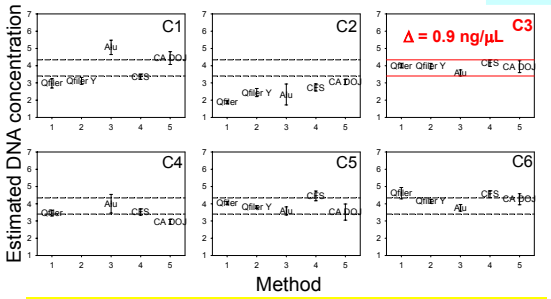




Comparison of Methods using C3 as the Calibrant

1-Qfiler
2-QfilerY
3-Alu
4-CFS
5-CADOJ

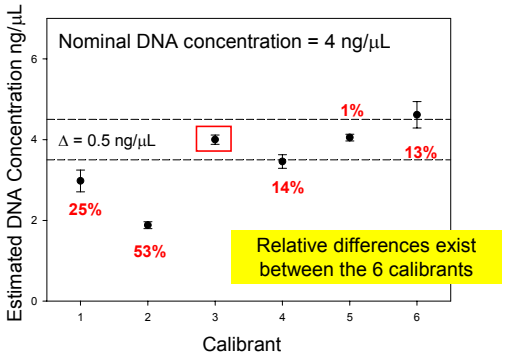
4 ng/μL



C1 and C2 are cell line DNAs; C3-C6 are single/multiple source DNAs

Six Calibrants quantitated using Quantifiler

(Calibrant Curve was generated with C3)



Relative differences exist between the 6 calibrants

Thank you for your Attention!!

Acknowledgements

Funding:

Interagency Agreement between National Institute of Justice and NIST Office of Law Enforcement Standards

NIST Project Team:

John Butler	Pete Vallone
Margaret Kline	Jan Redman
Amy Decker	Mike Coble
Becky Hill	Dave Duewer

Email: Margaret.Kline@nist.gov