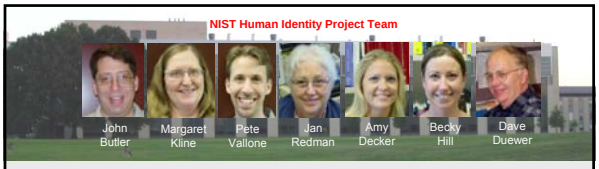


NIST Update

Dr. Peter M. Vallone
and Human Identity Project Team
National Institute of Standards and Technology

Centre of Forensic Sciences
Toronto Canada
June 10, 2008

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

National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Effort with Forensic DNA

- **Standards** http://www.cstl.nist.gov/biotech/strbase/
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

Outline

- SRM 2372
- STR allele sequencing
- STRBase updates
- Biomatrica stability study
- New autosomal STR loci
- Rapid PCR
- Workshops
- Other

SRM 2372 Now Available

- The NIST SRM Office began selling SRM 2372 Human DNA Quantitation standard on 10/05/07
- Cost is $338.00 per unit
- As of May 2008 100 units have been sold

SRM 2372
Human DNA Quantitation Standard

Components

A: Male/single donor/RNased/NIST
B: Female/multiple donors/NIST
C: Mixture/male & female/commercial

Quantities supplied:
110 µL of Human Genomic DNA ≈ 50ng/µL

Certification

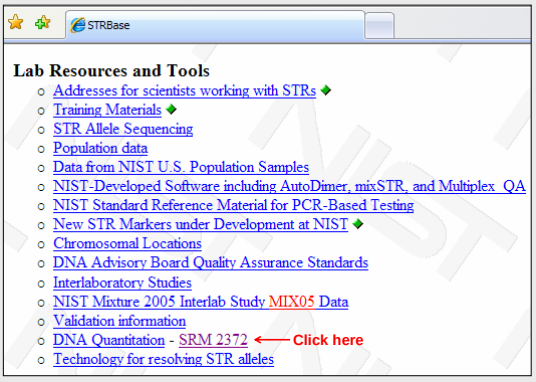
Decadic Attenuance (Absorbance) by a US National Reference Spectrophotometer
Homogeneity by a Cary 100 Bio Spectrophotometer
Validation of conventional [DNA] by Interlaboratory Study and NIST qPCR studies

Certified Values of Decadic Attenuance for SRM 2372

Component	260 nm	error at 260nm	Nominal [DNA], ng/µL
A	1.049	± 0.025	52.5
B	1.073	± 0.030	53.6
C	1.086	± 0.028	54.3

The nominal DNA concentration was estimated *Using 1 OD = 50 ng/µL double stranded DNA.* ***We do not know the uncertainty in this conversion.***

Information on SRM 2372 Now on STRBase



Additional Information

Supplemental data for SRM 2372 can be found on STRBase

Includes information on the production and characterization of the materials:

Homogeneity study

Interlaboratory study

Quantifiler, Alu, CFS assays

DNA standard calibration

http://www.cstl.nist.gov/biotech/strbase/srm2372.htm

Example of Calibrant Value Assignment

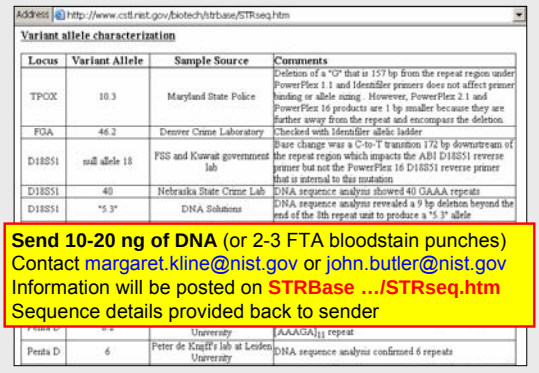
Commercial Standards	1		2		3		4	
Dilution	[DNA]	SD	[DNA]	SD	[DNA]	SD	[DNA]	SD
10x	105	3.2	122	1	126	5.8	256	10.1
50x	105	3.3	122	7.3	145	0.8	272	7.8
100x	99	6.2	113	11.6	138	0.5	270	10.5
200x	100	1.7	137	18.5	137	3.9	311	3.7
Average	102		123		136		277	
Stated	200		200		200		260	
Deviation	-49%		-38%		-32%		6%	

The table above is a summary of the results using SRM Component A as the calibrant.

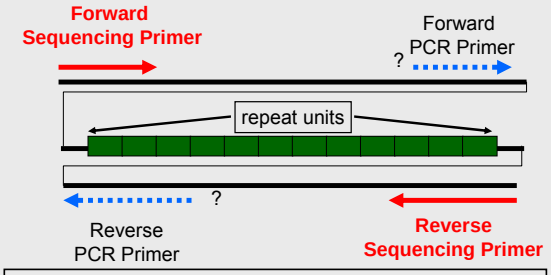
STR Allele Sequencing

Unusual STR Allele Characterization (Free)

Send us any unusual variant or null alleles and we will sequence them…

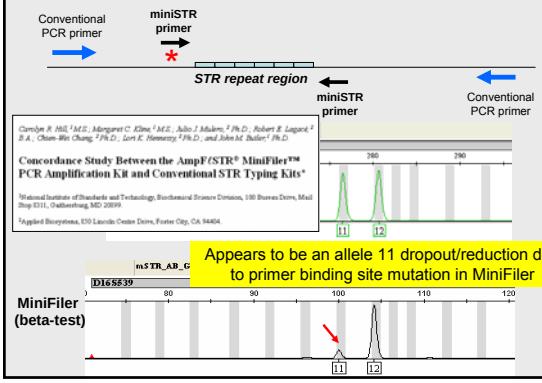


Sequencing Primers

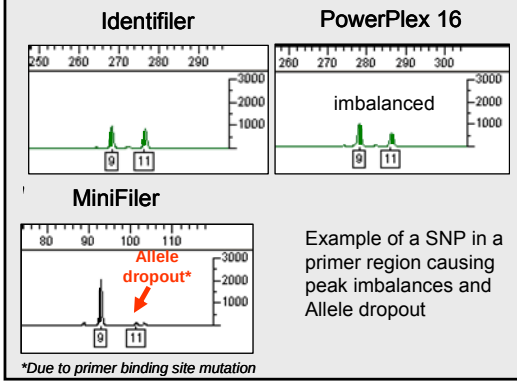


Sequencing primers are designed to encompass the normal PCR primer locations based on PCR product sizes, or published primer sets.

Concordance Studies Reveal Potential Primer Binding Site Mutations with Different Primer Sets



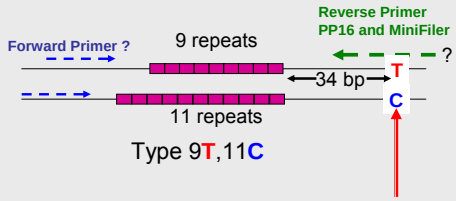
D16S539 SRM 2391b Genomic 8



Example of a SNP in a primer region causing peak imbalances and Allele dropout

Due to primer binding site mutation

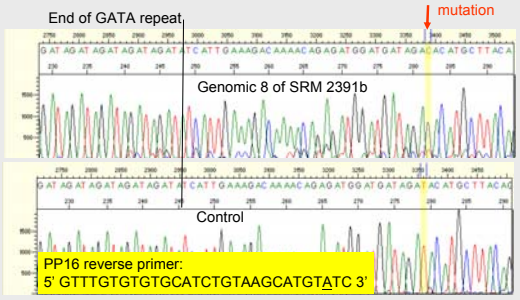
D16S539 SRM 2391b Genomic 8



This T→C mutation 34 bp downstream of the repeats causes allelic dropout!

D16S539 SRM 2391b Genomic 8

T→C mutation 34 bp downstream of the repeat



Position of the T→C probably affects the reverse primer of Minifiler and is the 3rd base found the 3' end of the Reverse PP16 primer. This could explain the imbalance of the allele seen when using PP16.

DNA Storage Study with Biomatrica

DNA SampleMatrix

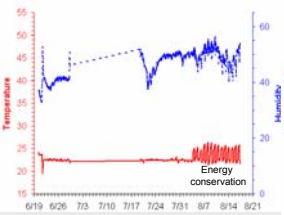
- Preservation of genomic and plasmid DNA at room temperature
- **Biomatrica SampleGuard™ (Now known as QIAsafe matrix)** is a novel sample storage medium ideal for (dry) shipping and long-term storage of DNA at room temperature.
- Eliminates the need to send samples overnight in costly dry ice containers

Experimental

- Margaret Kline (NIST)
- Prepare a plate of DNA extracts with varying concentrations (0.05, 0.25, and 1 ng/μL)
- Sample plates mailed back and forth from NIST and Biomatrica (CA)
- Monitor temperature and relative humidity
- Samples quantitated by qPCR and STR profiles obtained using Identifiler

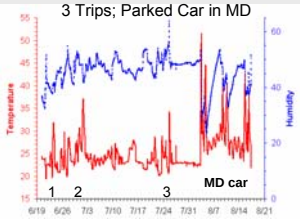
Storage Conditions for 56 days

NIST Office ambient



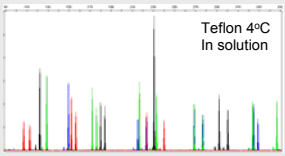
office	% Rh	° C
Max	56.1	26.4
Min	32.6	19.4
Median	48.1	22.4
Avg	46.5	22.9

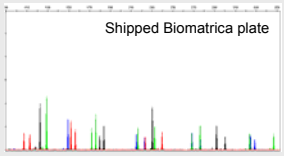
Shipped MD → CA → MD
3 Trips; Parked Car in MD



ship	% Rh	° C
Max	63.9	**51.6**
Min	23.1	19.4
Median	47.0	23.8
Avg	45.6	25.1

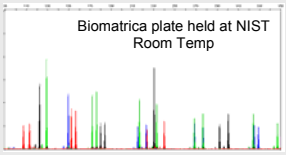
56 day Identifiler Profiles





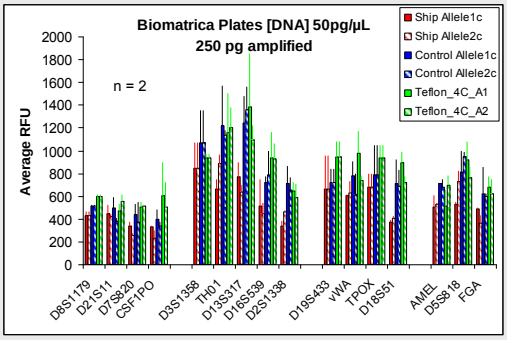
250 pg amplified (5 μL of 0.05 ng/μL)

- Teflon stored DNA exhibited higher peak heights than the Biomatrica plates
- The NIST ambient Biomatrica plate had higher peak heights than the Shipped matrix plate
- The shipped plate had been exposed to excessive temperatures



All samples were successfully typed

Identifiler results [DNA] 0.05 ng/μL after 208 days storage



STRBase Updates

Expanded NIJ Projects Section

33 different projects are described

Projects

[Human DNA Quantitation] [Mitochondrial DNA] [Y Chromosome] [Compromised DNA Evidence] [Miniaturization and Automation] [General Tools and Information] [Non-Human DNA] [Alternative Forensic DNA Markers]

Alphabetical Listing of Projects

ABI 3100 performance with various STR typing systems (April 2001-June 2003)

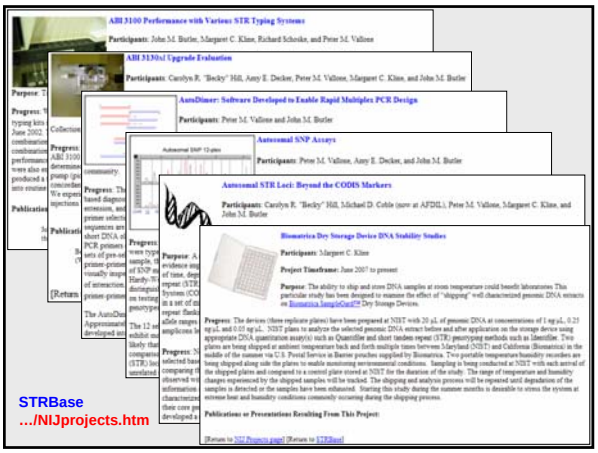
ABI 3130xl upgrade evaluation (Sept 2005-May 2006)

AutoDimer: software to enable rapid multiplex PCR design (2000-2005) [see also software.htm]

Autosomal SNP loci (July 2002-present)

Autosomal STR loci: beyond the CODIS markers (Jan 2004-present) [see also newSTRs.htm]

Biomatrica dry storage device DNA stability studies (June 2007-present)



STRBase
.../NIJprojects.htm

SRM 2391b and 2395 Certificate Updates

- **SRM 2391b** (Autosomal STR Loci)
  - **MiniFiler examined** (allele dropout with component 8 and D16S539)
  - **Additional Loci: 26 new miniSTR loci**
  - Demonstrating extended stability (new quantitation data and no significant degradation to existing components)

  http://www.cstl.nist.gov/biotech/strbase/srm2391b.htm
- **SRM 2395** (Y-STR and Y-SNP Loci)
  - **Yfiler loci sequenced** (DYS635 now included)
  - **Additional Loci: 20 new Y-STR loci**
  - Demonstrating extended stability (new quantitation data and no significant degradation to existing components)

  http://www.cstl.nist.gov/biotech/strbase/srm2395.htm

Final Documents Submitted, Information Posted on STRBase and Registered Users will be Notified of Certificate Updates

New Autosomal STR Loci

New STR Loci Characterized

Hill *et al.* (2008) *J. Forensic Sci.* 53(1):73-80

J Forensic Sci, January 2008, Vol. 53, No. 1
doi: 10.1111/j.1556-4029.2008.00595.x
Available online at: www.blackwell-synergy.com

Carolyn R. Hill, M.S.; Margaret C. Kline, M.S.; Michael D. Coble,† Ph.D.; and John M. Butler, Ph.D.

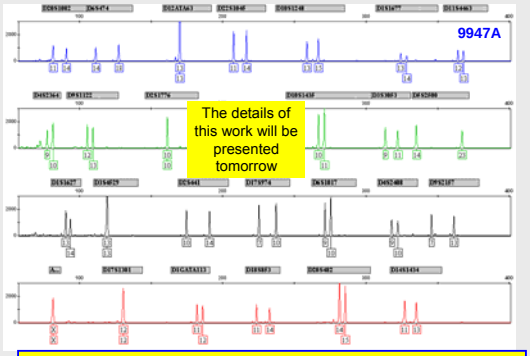
Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples

- Primer sequences, GeneMapper bins and panels, genotypes on common samples, and allele frequency information **available on STRBase**

http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_NC_loci_types.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_Panels_Panels.txt
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_Panels_NC_bins_bins.txt

"Autoplex" (26plex)

See Hill et al. AAFS 2008 talk (Washington, DC) and poster PP50 at DNA in Forensics 2008 meeting (Ancona)



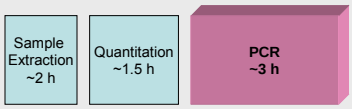
Gender identification + 25 autosomal STR loci in a single amplification

Rapid PCR

Rapid PCR

- Challenge for miniaturize STR typing platforms since they are tied into the commercial kits/loci
- Do fewer loci and smaller amplicon size favor rapid multiplex PCR?
- We have well characterized miniSTR multiplex PCR panels (26 loci)

Typical STR Workflow



Data Review ?

How can we reduce the time needed for cycling?

What happens when we alter cycling parameters?
How will existing commercial kits work?
How will different polymerases perform?
How robust will the results be?
Can we develop novel assays and further the understanding/limits of rapid multiplex PCR?

Rapid PCR

- What do we mean by rapid PCR?
  - Rapid hot start polymerases (save ~10min)
  - Shortening cycling hold times (5 sec vs 1 min)
  - Utilizing existing thermal cycling technology (AB 9700)
    - Eliminating 1 ºC/sec ramp rate (9600 emulation)
    - Utilize the 9700 4 ºC/sec ramp rate
  - Using commercial polymerases that are 'faster'

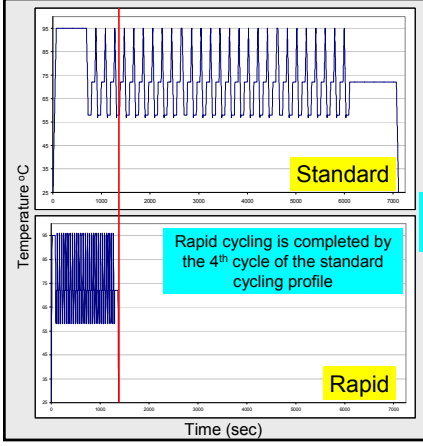
Obtain results in less than 45 minutes
Trying simple things first…

Thermal Cycling

Parameter	Unit	Trad	Rapid	Difference (min)	%
Hot Start	Min	10	1	9.0	6.3
Hold	Sec	60	5/10	72.3	50.6
Soak	Min	60	1	59.0	41.2
Ramp rate	(deg/sec)	1	4	22.4	15.7
Cycles		28	28		
Time		2:58:41	0:35:38	2:23:03	

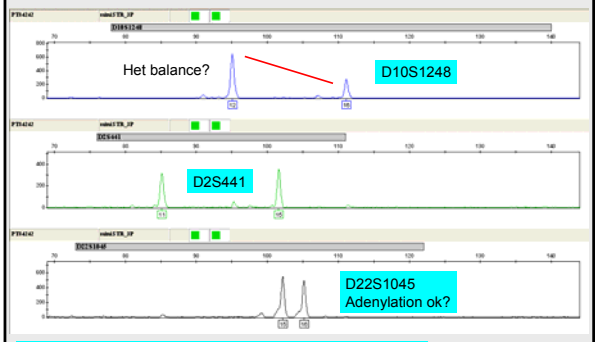
Parameter	Purpose
Hot Start	Primer Dimer, non-specific amplification
Hold	Denature, annealing, elongation, Inter and intra locus balance
Soak	Full adenylation of PCR products

Evaluate robustness and reproducibility



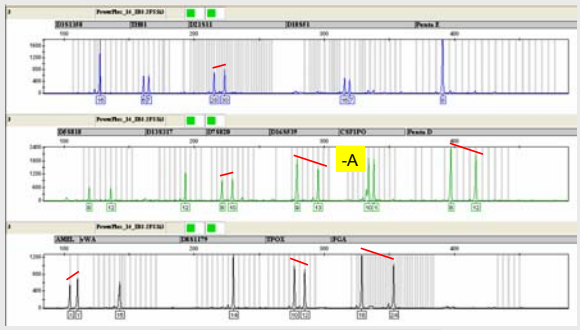
Comparison of Thermal Cycling Profiles On a AB 9700

Rapid PCR 28 cycles in 36 minutes



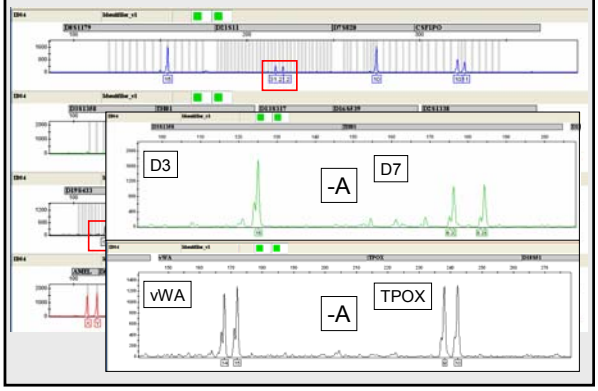
750 pg template, 28 cycles, PyroStart, 36 minute cycling time

PowerPlex 16 Rapid Cycling



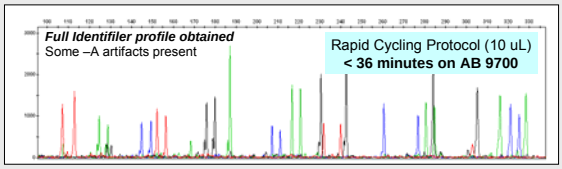
Some potential heterozygote issues?

Identifiler Rapid Cycling



Rapid Multiplex PCR Protocols

Testing the potential of rapid multiplex PCR methods
Utilizing AB 9700 cycler and 'fast' commercial enzymes
Manuscript submitted to FSI Genetics in March 2008



Identifiler STR kit
28 cycles, 1ng template DNA

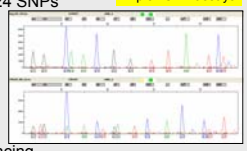
Initial results presented by at 60th Annual Meeting of the American Academy of Forensic Sciences (Washington, DC), February 23, 2008, "Developing Rapid PCR Multiplex Assays with miniSTR Loci"

SNPs

Ancestry Informative Markers
SNP Work

- Working with Dr. Manfred Kayser (Netherlands)
  - Set of Ancestry Informative Markers (AIMs)
  - NIST developed assays for typing 24 SNPs
  - Typed over 700 of our samples
- Dr. Peter deKnijff (Netherlands)
  - Performing Y SNP typing
- Dr. Michael Coble (AFDIL)
  - mitochondrial control region sequencing
- How do ancestry markers correlate with self identified ancestry in NIST U.S. samples?

12plex SNP assays



Work presented in Ancona, Italy May 29th 2008
Talk http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm#Presentations

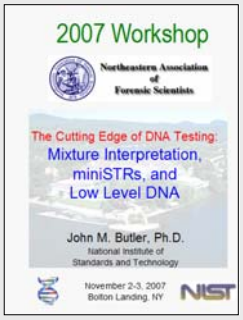
Training Workshops

Training Workshops in the Past Year

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

- ISFG Meeting (**August 2007**, Copenhagen, Denmark)
  - **CE Fundaments and Troubleshooting**
  - **Validation**
- Int. Symposium on Human Identification (Promega) Meeting (**October 2007**, Hollywood, CA)
  - **Validation**
- NEAFS Meeting (**November 2007**, Bolton Landing, NY)
  - **Mixture Interpretation**
  - **Low-copy Number DNA Issues**
  - **miniSTRs**
- AAFS Meeting (**February 2008**, Washington, DC)
  - **DNA Quantitation by qPCR** *(158 page handout)*
  - **Mixture Interpretation** *(196 page handout)*

NEAFS Workshop on "The Cutting Edge of DNA Testing"



- 42 participants from 13 different labs
- **70 page handout from workshop available for download**
  (see training section of STRBase)
- Contains up-to-date references on mixture interpretation, miniSTRs, and LCN DNA analysis

http://www.cstl.nist.gov/biotech/strbase/pub_pres/NEAFS2007_CuttingEdgeDNA.pdf

qPCR Workshop

- AAFS (February 18th, 2008)
  - **Human DNA Quantification Using Real-Time PCR Assays**
  - **Peter Vallone (NIST)**
  - Margaret Kline (NIST)
  - Eric Buel (Vermont)
  - Jan Nicklas (Vermont)
  - Marie Allen (Uppsala)
  - Mark Timken (CA DOJ)
  - David Foran (Michigan State)
  - Melanie Richard (CFS – Toronto)
  - Toni Diegoli (AFDIL)

158 page handout prepared

http://www.cstl.nist.gov/biotech/strbase/training/AAFS2008_qPCRworkshop.htm

Mixture Interpretation Workshop

- AAFS (February 19, 2008)
  - **DNA Mixture Interpretation:** Principles and Practice in Component Deconvolution and Statistical Analysis
  - **John Butler (NIST)**
  - Ann Gross (MN)
  - George Carmody (Carleton U.)
  - Gary Shutler (WA)
  - Joanne Sgueglia (MA)
  - Angela Dolph (Marshall U./NIST)
  - Tim Kalafut (USACIL)

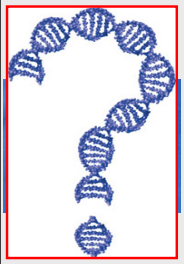
196 page handout prepared

http://www.cstl.nist.gov/biotech/strbase/training/AAFS2008_MixtureWorkshop.htm

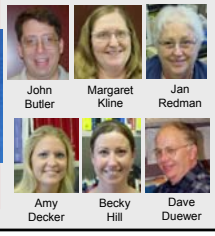
Thank you for your attention…

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

Questions?



http://www.cstl.nist.gov/biotech/strbase
peter.vallone@nist.gov
301-975-4872



Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards

Collaborators

Mike Coble (now AFDIL) – early miniSTR work

Tom Reid (DDC) - father/son samples