

Outline

- · SRM 2372 status
- Updated certificates for SRM 2391b and 2395
- · Biomatrica stability study
- · New autosomal STR loci
- · Y-STR and autosomal SNP work
- · Training workshops
- Other topics

SRM 2372 Now Available

- The NIST SRM Office began selling SRM 2372 Human DNA Quantitation Standard on October 5, 2007
- · Cost is \$316 per unit
- >50 units in use already

SRM 2372 Human DNA Quantitation Standard

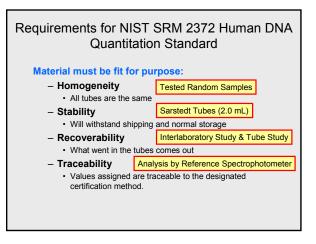
A: Male/single donor/RNased/NIST B: Female/multiple donors/NIST C: Mixture/male & female/commercial

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

Certification

Decadic Attenuance (Absorbance) by a US National Reference Spectrophotometer

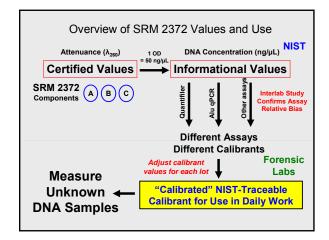
Homogeneity by a Cary 100 Bio Spectrophotometer Validation of conventional [DNA] by Interlaboratory Study and NIST qPCR studies

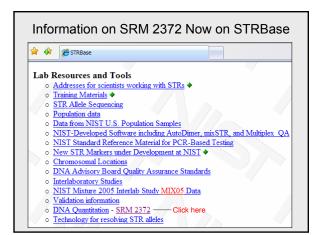


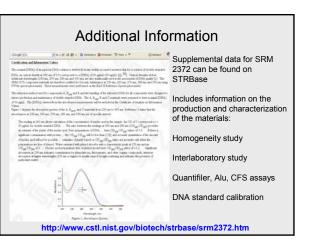
HAS II Certified Values of Decadic Attenuance for SRM 2372				
Component	260 nm	error at 260nm	Nominal [DNA], ng/µL	
Α	1.049	± 0.025	52.5	
В	1.073	± 0.030	53.6	
С	1.086	± 0.028	54.3	

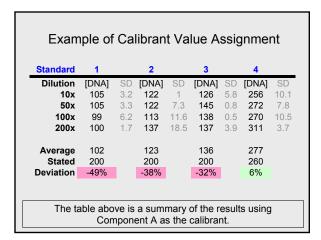
5 mL were required to fill 2 cuvettes per component, each run in duplicate (4 replicate measurements).

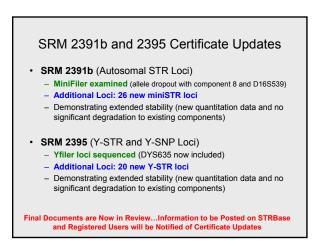
The nominal DNA concentration was estimated Using 1 $OD = 50 \text{ ng/}\mu\text{L}$ double stranded DNA. We do not know the uncertainty in this conversion.











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

Technology: Research Programs

- DNA stability studies Biomatrica tests
- miniSTRs new STR loci and megaplex
- Y-chromosome STRs worldwide Yfiler studies
- SNPs comparison to STRs; efforts with AIMs
 Rapid PCR to speed multiplex amplification
- mtDNA
- qPCR for DNA quantitation
- Variant allele characterization and sequencing
- Software tools
- · Expert System review
- Assay development with collaborators

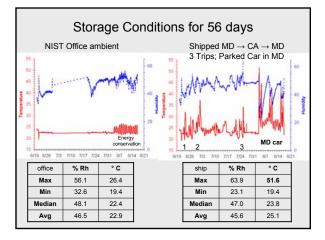
DNA Storage Study with Biomatrica

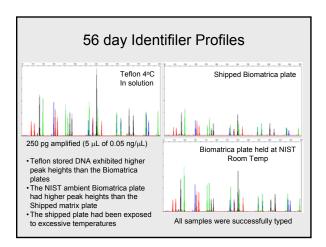
DNA SampleMatrix

- Preservation of genomic and plasmid DNA at room temperature
- Biomatrica SampleGuard[™] 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





miniSTRs				
<u>Advantages</u>	Disadvantages			
 Better success with degraded DNA (compared to larger PCR products present in commercial STR kits) 	 Not all commonly used STRs can be made significantly smaller—thus new loci will be needed 			
 Better success with low amounts of DNA (due to more efficient PCR amplification compared to larger PCR products) 	Cannot multiplex as many loci due to size constraints			
 Better capacity for handling mixed DNA samples than SNPs (due to more alleles being possible) Concordance to STR loci in commercial kits is possible 	 No commercial kit available yet STR flanking region mutations may make results disconcordant (e.g., D13 and VWA deletions) 			

Aren't the Current STR Loci Good Enough?

- Depends on the question being asked...
- For general forensic matching of evidence to suspect, the 13 CODIS STR loci are sufficient
- For other human identity/relationship testing questions, more autosomal or Y-STR loci can be beneficial or even necessary

How Would Additional STR Loci Be Useful?

- Databases: More loci to help resolve relatives in growing national DNA databases (UK went from 6 to 10 STRs in 1999; future Pan-European database will include >10 loci)
- Casework: Obtaining additional information with degraded DNA samples (miniSTRs); rapid screening of multiple crime scene samples
- Identity/Relationship Testing: Kinship analysis, parentage testing, complex criminal paternity, missing persons/mass disasters, immigration testing

Call for More Loci in Situations Involving Relatives

- **Missing Persons** and Disaster Victim Identification (kinship analysis)
- Immigration Testing (often limited references)
 Recommendations for 25 STR loci
- Deficient Parentage Testing – often needed if only one parent and child are tested

Relationship testing labs are being pushed to answer more difficult genetic questions...and we want to make sure the right tools are in place

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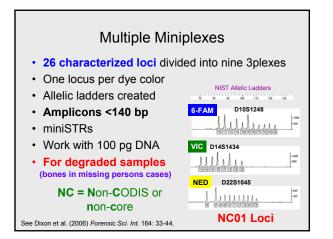
Carolyn R. Hill, M.S.; Margaret C. Kline, M.S.; Michael D. Coble,[†] Ph.D.; and John M. Butler, Ph.D.

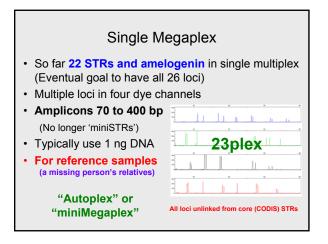
Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples

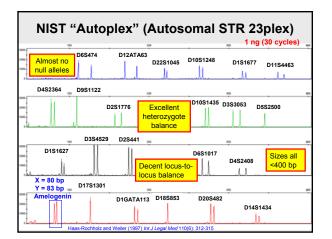
 Primer sequences, GeneMapper bins and panels, genotypes on common samples, and allele frequency information already available on STRBase

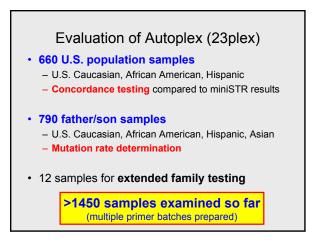
Assay Performance

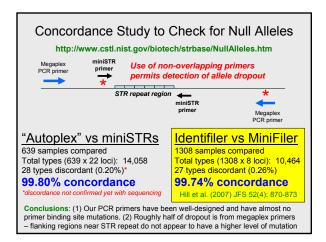
- Our multiplex assays are designed to perform similarly to commercial kits
 - PCR Reaction (buffer, fluorescent dyes, volume)
 - PCR thermal cycling conditions
 - Work robustly on 0.5 to 1 ng of template DNA (or lower)
- Multiple miniplexes and a single megaplex developed to study 26 autosomal STRs

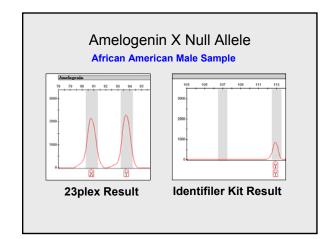


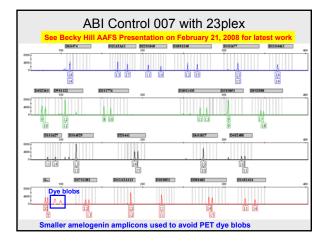


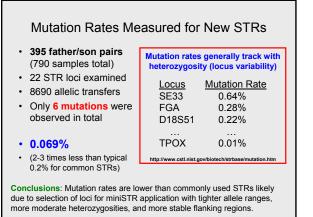


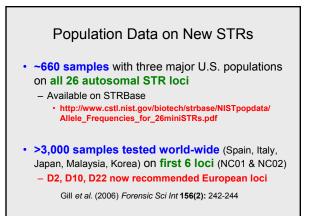


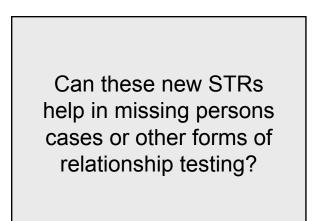


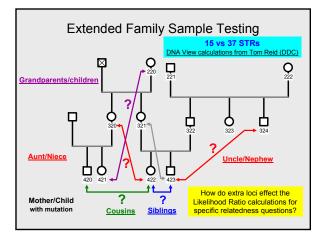




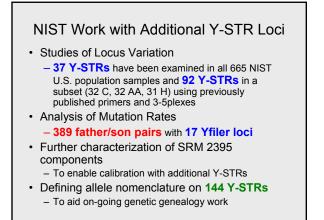


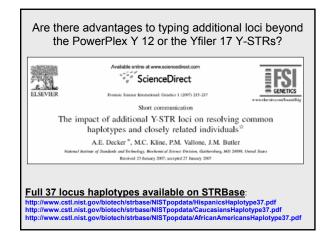


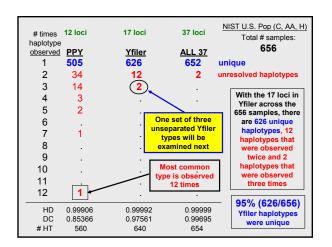


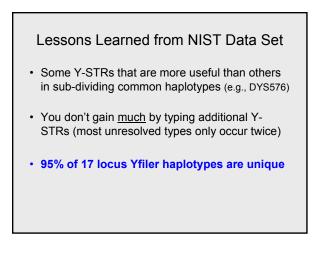


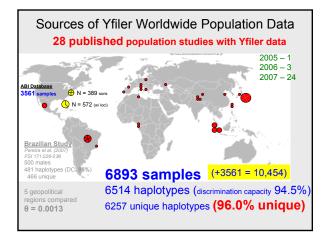
Relationship Examined	15 STRs (Identifiler, ID15)	ID15 + Autoplex 22 STRs = 37 loci (A37)
Mother/Child* (*with single mutation)	0.214	5,200,000 Extra loci help
Siblings	477	113,000 Extra loci help
Uncle/Nephew	824	247,000 Extra loci help
Cousins	0.45	2.25
Grandparents/ Grandchildren	0.53	1.42

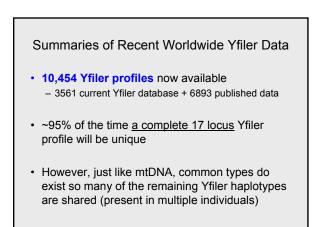


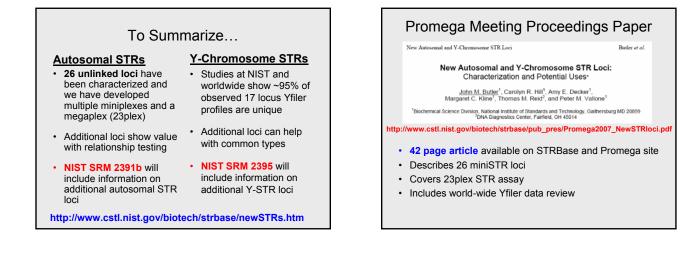




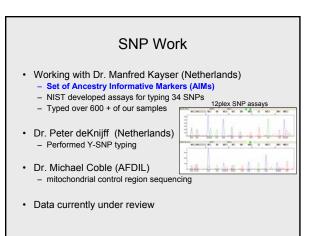








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Rapid PCR

- Existing commercial STR typing kits are not optimized for rapid PCR
- Challenge for miniaturize STR typing platforms since they are tied into the commercial kits/loci
- Fewer loci and smaller amplicon size favor rapid multiplex PCR
- · We have well characterized miniSTR panels

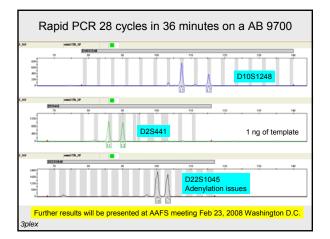
Informal collaborations with:

Dr. Michael Gaitan (NIST) – microwave thermal heating Dr. Eugene Tan (Network Biosystems) – chip platforms

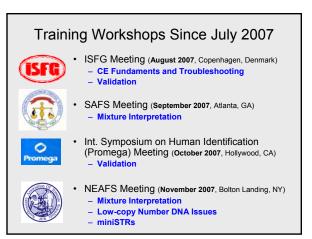
Rapid Thermal Cycling

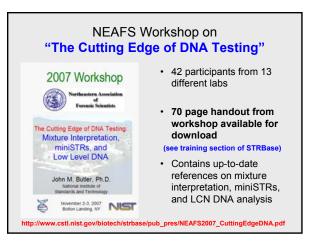
- · Evaluate faster polymerases
- Test with miniSTRs
 - primer concentrations can be adjusted and PCR primer sequences are known
- Use standard cycler (GeneAmp 9700), tubes, ...
- Examine shortened dwell times and adenylation soak
- Study limitations in terms of PCR amplification speed when examining multiplex STR assays

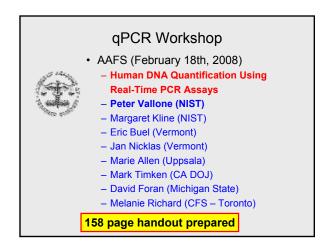
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

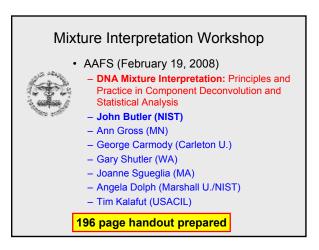






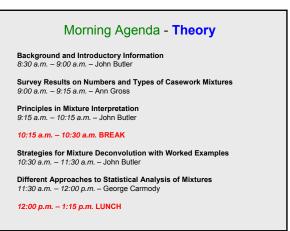






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Afternoon Agenda – Practical Application

Real Case Example – Importance of Properly Stating Your Conclusions 1:15 p.m. – 1:30 p.m. – Gary Shutler

Variability between Labs in Approaches & Mixture Interlaboratory Studies 1:30 p.m. - 2:15 p.m. - John Butler

Validation Studies and Preparing Mixture Interpretation Guidelines 2:15 p.m. - 2:45 p.m. - Joanne Sgueglia

2:45 p.m. - 3:00 p.m. BREAK

Testing of Mixture Software Programs 3:00 p.m. – 3:15 p.m. – Angela Dolph

DNA_DataAnalysis Software Demonstration 3:15 p.m. - 4:00 p.m. - Tim Kalafut

Training Your Staff to Consistently Interpret Mixtures 4:00 p.m. - 4:45 p.m. - Panel Discussion with Ann Gross, Gary Shutler, Joanne Sgueglia

4:45 p.m. - 5:00 p.m. - Questions and Answers as needed

Upcoming Lawyer Training...

- NY State Prosecutors in West Point, NY
 - March 5, 2008
 - Will address "Emerging Issues" similar to NDAA talk in May 2007
- Defense Attorneys in Richmond, VA - April 25, 2008
 - Invited to address subject of mixture interpretation

Planned Promega 2008 Meeting Troubleshooting Workshop

- Title: "Principles of Interpretation and Troubleshooting of Forensic DNA Typing Systems"
- Instructors: John Butler (NIST) and Bruce McCord (FIU)
- · Date: October 16, 2008 with Promega Int. Symp. Human ID
- The workshop will consist of three parts: (1) a through examination of theoretical issues with capillary electrophoresis PCR amplification of short tandem repeat markers (2) a discussion of how to properly set instrument parameters to interpret data (including mixtures), and (3) a review of specific problems seen by labs submitting problematic data and commentary on possible troubleshooting solutions.

Seeking input of problems observed with CE systems



Angel Carracedo (Spain)

Peter M. Schneider (Germany)

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