NIST Human Identity Project Team – *Leading the Way in Forensic DNA…*


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NIST Update: Projects that the Human Identity Team are Working on.

Margaret C. Kline and the NIST HID Project Team

National Institute of Standards and Technology

Seventh Annual Advanced DNA Technical Workshop - East

Captiva Island, Florida, May 20, 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

Current Activities

- **Standard Reference Materials**
  - SRM 2372 (DNA quant) released Oct 2007 **(>100 units sold in 6 months)**
  - SRM 2391b (STRs), 2395 (Y-STRs), 2392 (mtDNA) updates
- **Technology Evaluation and Development**
  - Unusual STR allele characterization
  - New STR loci and assays (26plex)
  - Y-chromosome characterization (mutation rates, deletions)
  - Rapid multiplex PCR protocols (Identifiler amplification in 35 min)
- **Training Materials**
  - Workshops on DNA quantitation and mixture interpretation
  - Third edition of *Forensic DNA Typing* textbook

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

Cost $338 per unit

Overview of SRM 2372 Values and Use

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

NIST

Attenuance (λ_{260})

1 OD
= 50 ng/µL

DNA Concentration (ng/µL)

Certified Values → **Informational Values**

SRM 2372 Components A B C

Quantifiler

Alu qPCR

Other assays

Interlab Study Confirms Assay Relative Bias

Different Assays
Different Calibrants

Adjust calibrant values for each lot

Forensic Labs

"Calibrated" NIST-Traceable Calibrant for Use in Daily Work

Measure Unknown DNA Samples

HAS II 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

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 ng/μL double stranded DNA.* ***We do not know the uncertainty in this conversion.***

Information on SRM 2372 Now on STRBase

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

Lab Resources and Tools

- Addresses for scientists working with STRs
- Training Materials
- STR Allele Sequencing
- Population data
- Data from NIST U.S. Population Samples
- NIST-Developed Software including AutoDimer, mixSTR, and Multiplex QA
- NIST Standard Reference Material for PCR-Based Testing
- New STR Markers under Development at NIST
- Chromosomal Locations
- DNA Advisory Board Quality Assurance Standards
- Interlaboratory Studies
- NIST Mixture 2005 Interlab Study MIX05 Data
- Validation information
- DNA Quantitation - SRM 2372 ← **Click here**
- Technology for resolving STR alleles

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

Technology: Research Programs

- **Characterization of unusual alleles**
- **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

Unusual STR Allele Characterization (Free)

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

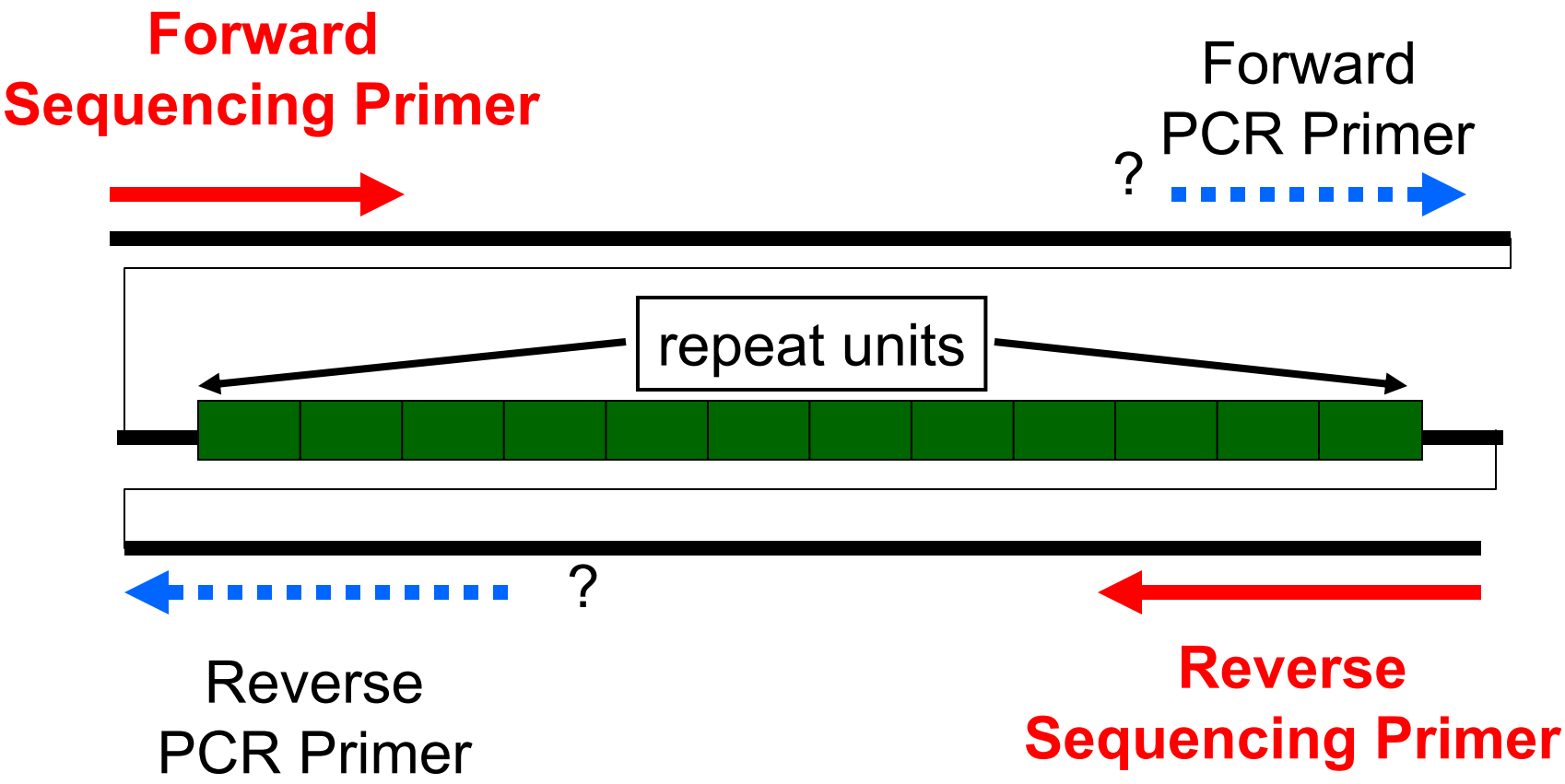
Address http://www.cstl.nist.gov/biotech/strbase/STRseq.htm

Variant allele characterization

Locus	Variant Allele	Sample Source	Comments
TPOX	10.3	Maryland State Police	Deletion of a "G" that is 157 bp from the repeat region under PowerPlex 1.1 and Identifiler primers does not affect primer binding or allele sizing . However, PowerPlex 2.1 and PowerPlex 16 products are 1 bp smaller because they are further away from the repeat and encompass the deletion.
FGA	46.2	Denver Crime Laboratory	Checked with Identifiler allelic ladder
D18S51	null allele 18	FSS and Kuwait government lab	Base change was a C-to-T transition 172 bp downstream of the repeat region which impacts the ABI D18S51 reverse primer but not the PowerPlex 16 D18S51 reverse primer that is internal to this mutation
D18S51	40	Nebraska State Crime Lab	DNA sequence analysis showed 40 GAAA repeats
D18S51	"5.3"	DNA Solutions	DNA sequence analysis revealed a 9 bp deletion beyond the end of the 8th repeat unit to produce a "5.3" allele
		University	$[AAAGA]_{11}$ repeat
Penta D	6	Peter de Knijff's lab at Leiden University	DNA sequence analysis confirmed 6 repeats

Send 10-20 ng of DNA (or 2-3 FTA bloodstain punches)
Contact margaret.kline@nist.gov or john.butler@nist.gov
Information will be posted on **STRBase …/STRseq.htm**
Sequence details provided back to sender

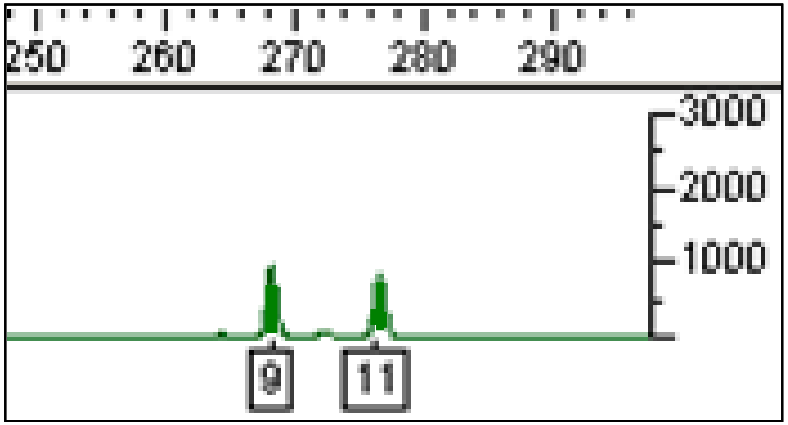
Sequencing Primers



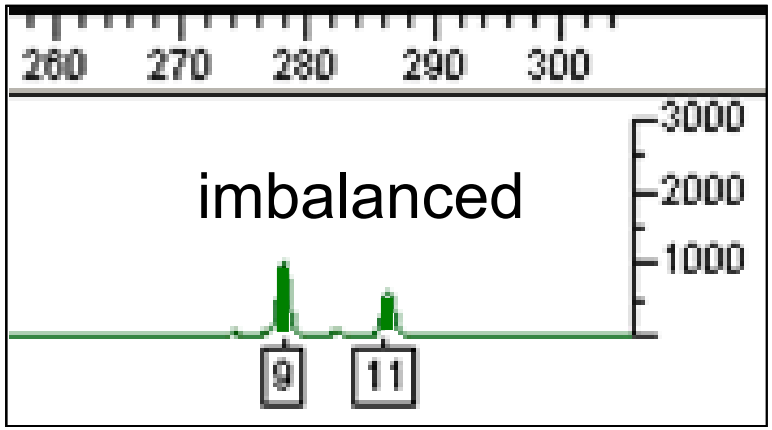
Sequencing primers are designed to encompass the normal PCR primer locations based on PCR product sizes, or published primer sets.

D16S539 SRM 2391b Genomic 8

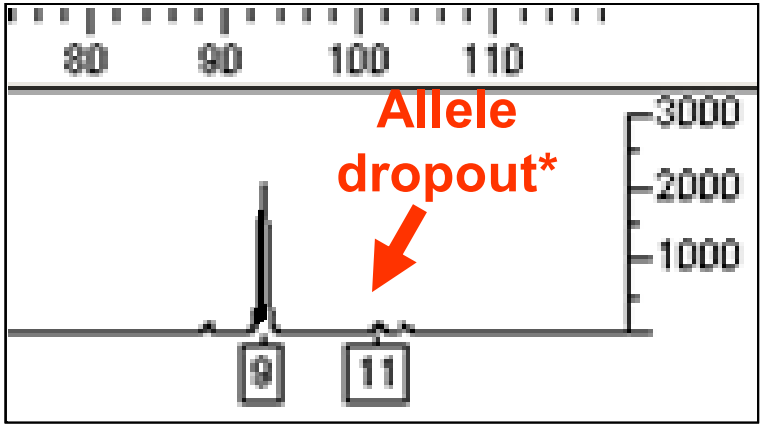
Identifiler



PowerPlex 16



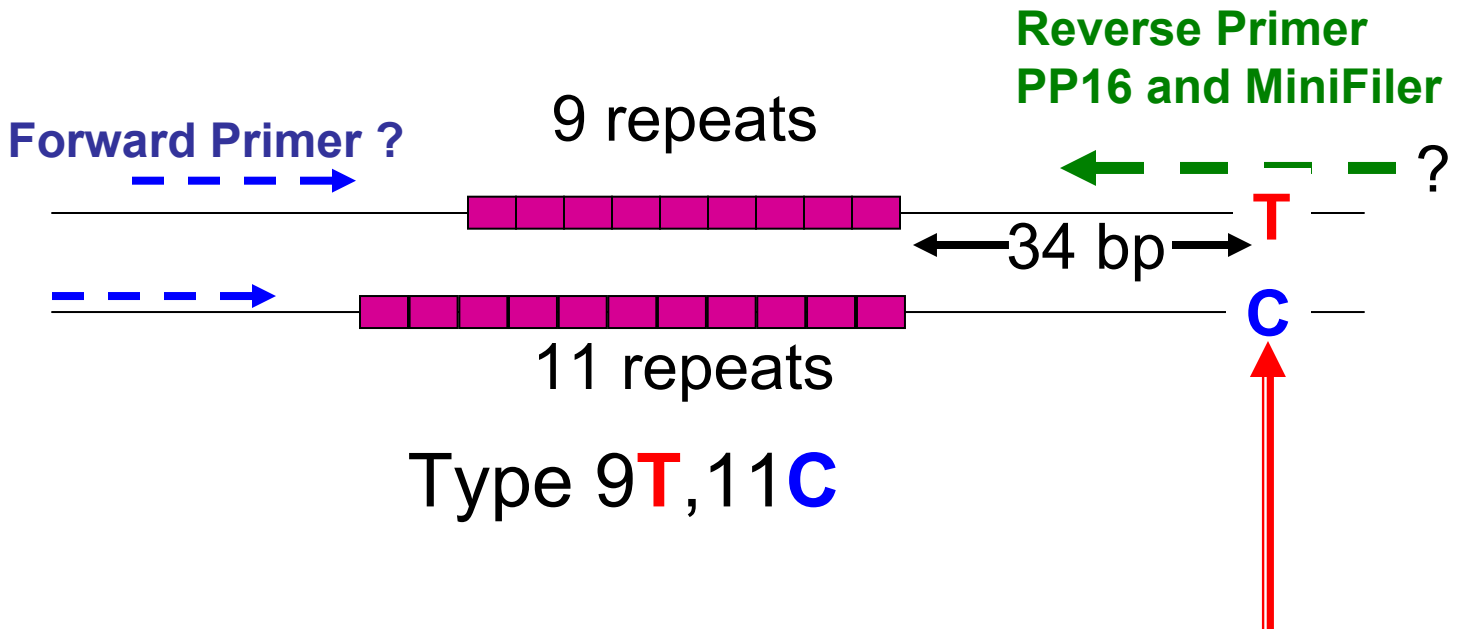
MiniFiler



**Due to primer binding site mutation*

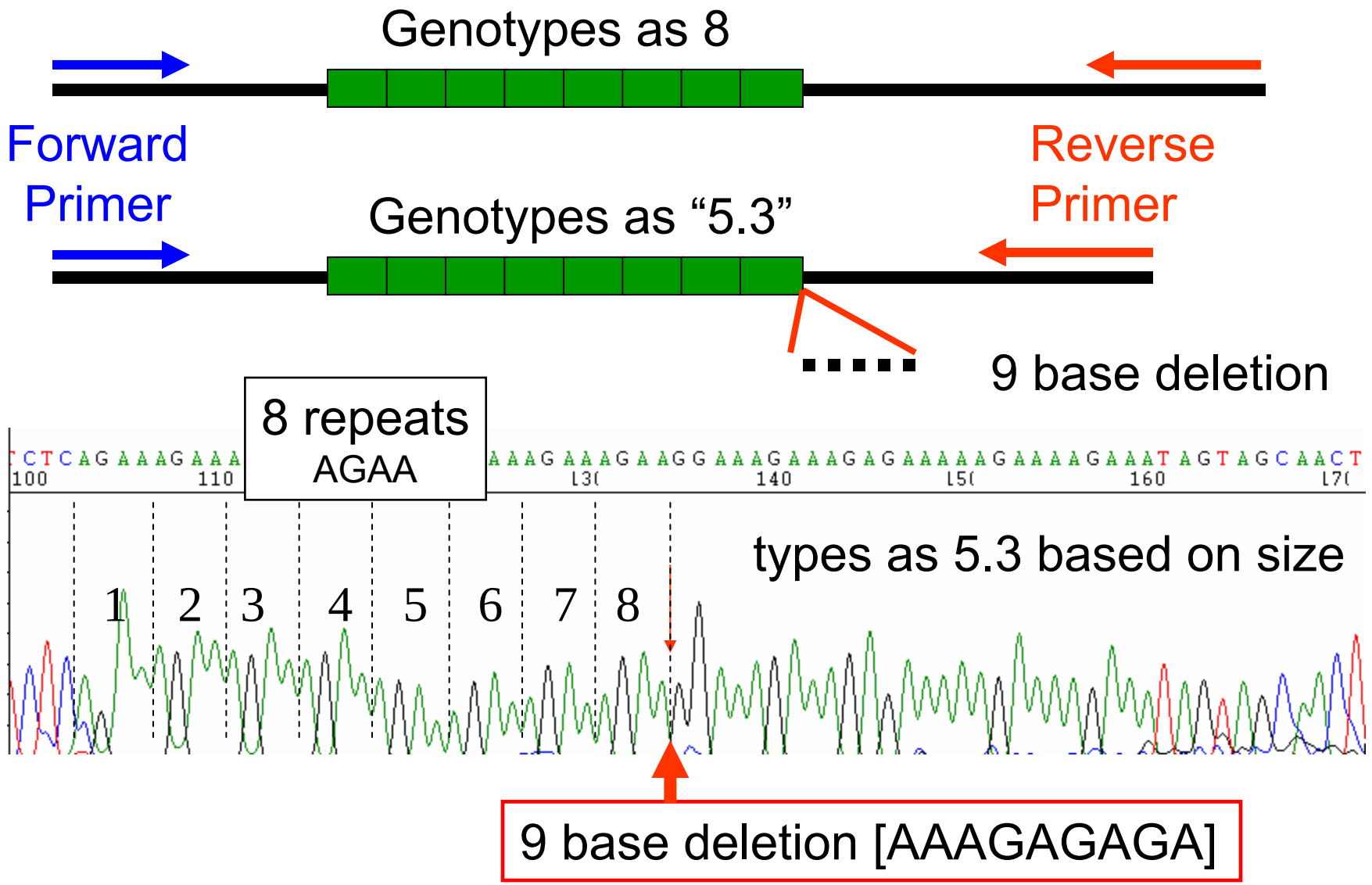
Example of a SNP in a primer region causing peak imbalances and Allele dropout

D16S539 SRM 2391b Genomic 8



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

D18S51 deletion results in “5.3” Allele



DNA Storage Study with Biomatrica

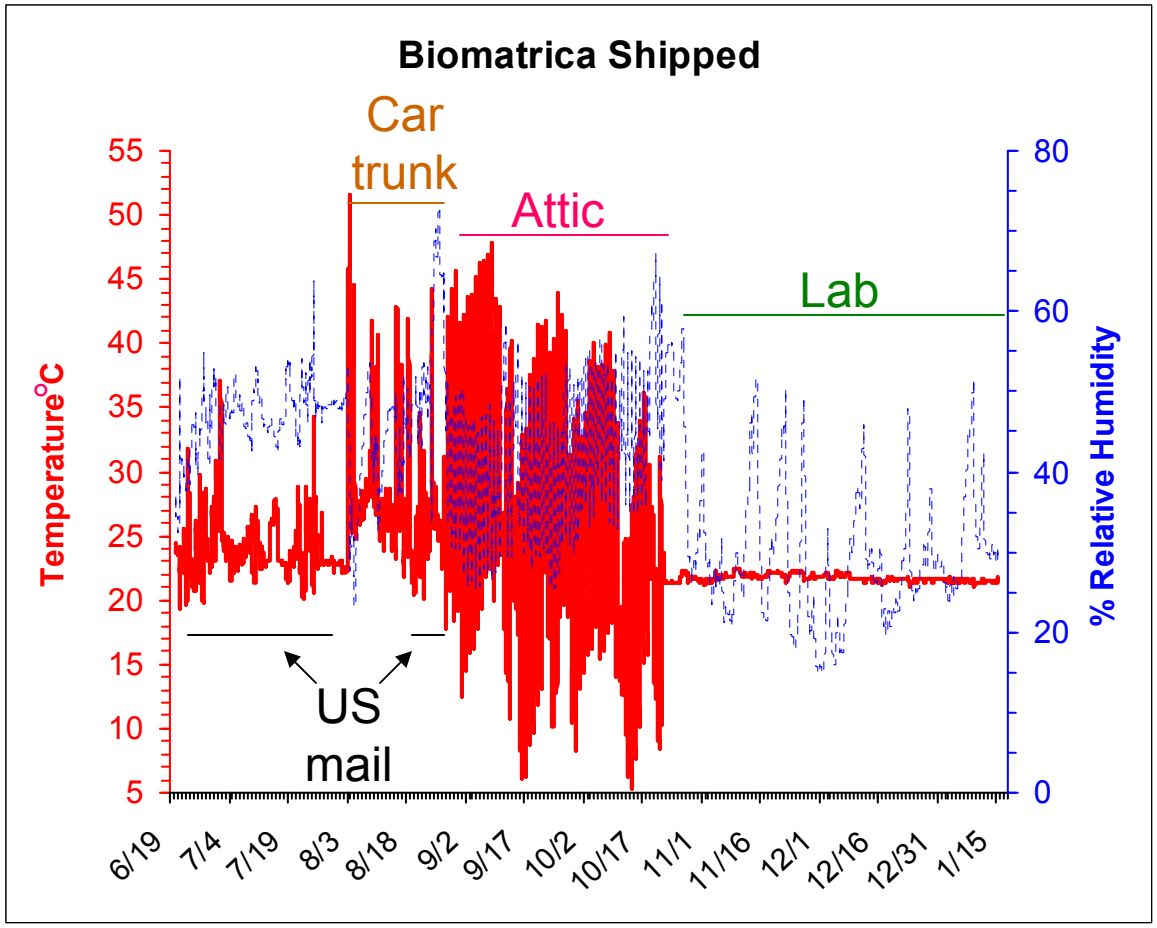
DNA SampleMatrix

- 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

- Prepare several plates 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 quantified by qPCR and STR profiles obtained using Identifiler

“Shipped/Stressed” Temperature & % Relative Humidity Profile, 208 days



Two Biomatrica SampleGard plates were “shipped” back and forth between MD and CA during the Summer of 2007.

After 6 cross country trips the plates were placed in a Car trunk for 14 days.

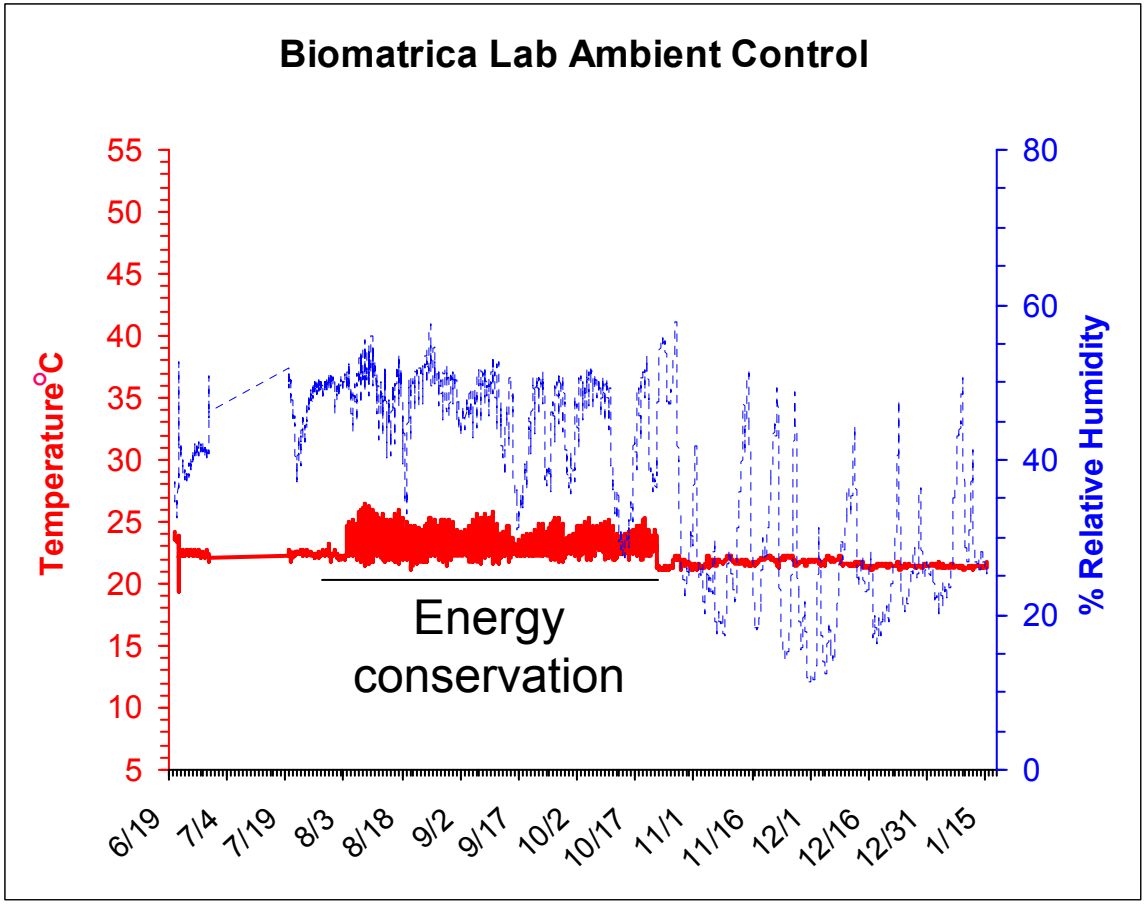
Two more cross country trips.

Followed by exposure to ambient Attic temperatures for 56 days.

Finally plates were placed at lab ambient conditions.

Max:	51.6 °C, 73 % RH	Median:	22.1 °C, 40 % RH
Min:	5.3 °C, 15 % RH	Avg:	23.6 °C, 39 % RH

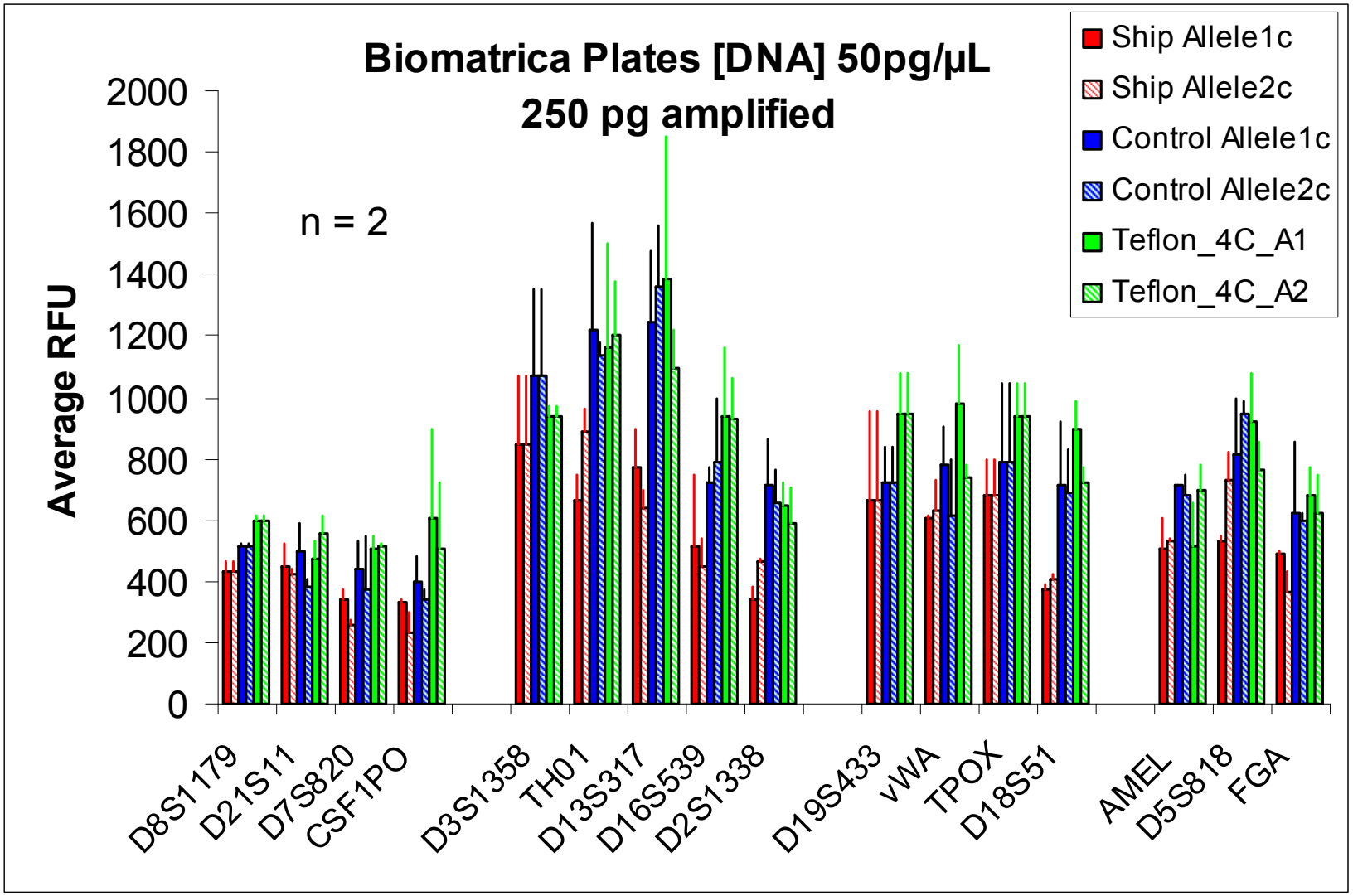
Lab Ambient Temperature & % Relative Humidity Profile, 208 days



Two Biomatrica SampleGard plates were stored in a office/lab during the Summer of 2007. Materials were transferred to the lab when it became apparent that summer energy conservation efforts were measurable.

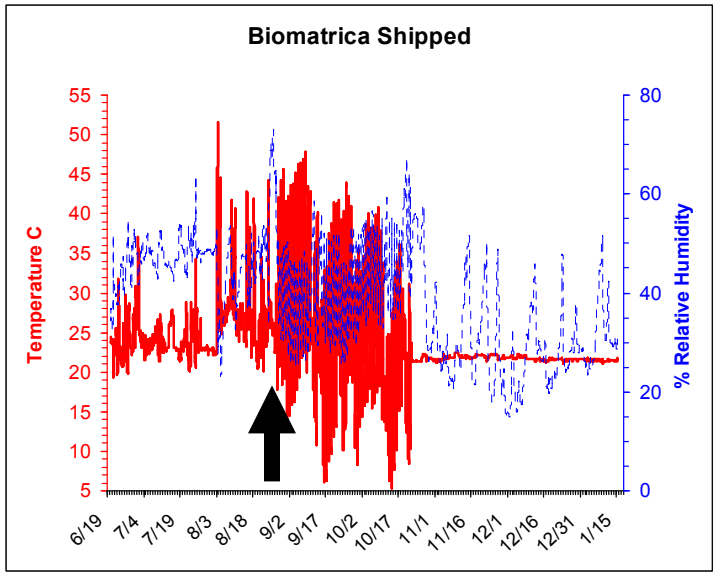
Max:	26.4 °C, 58 % RH
Min:	19.4 °C, 11 % RH
Median:	22.2 °C, 41 % RH
Avg:	22.4 °C, 38 % RH

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

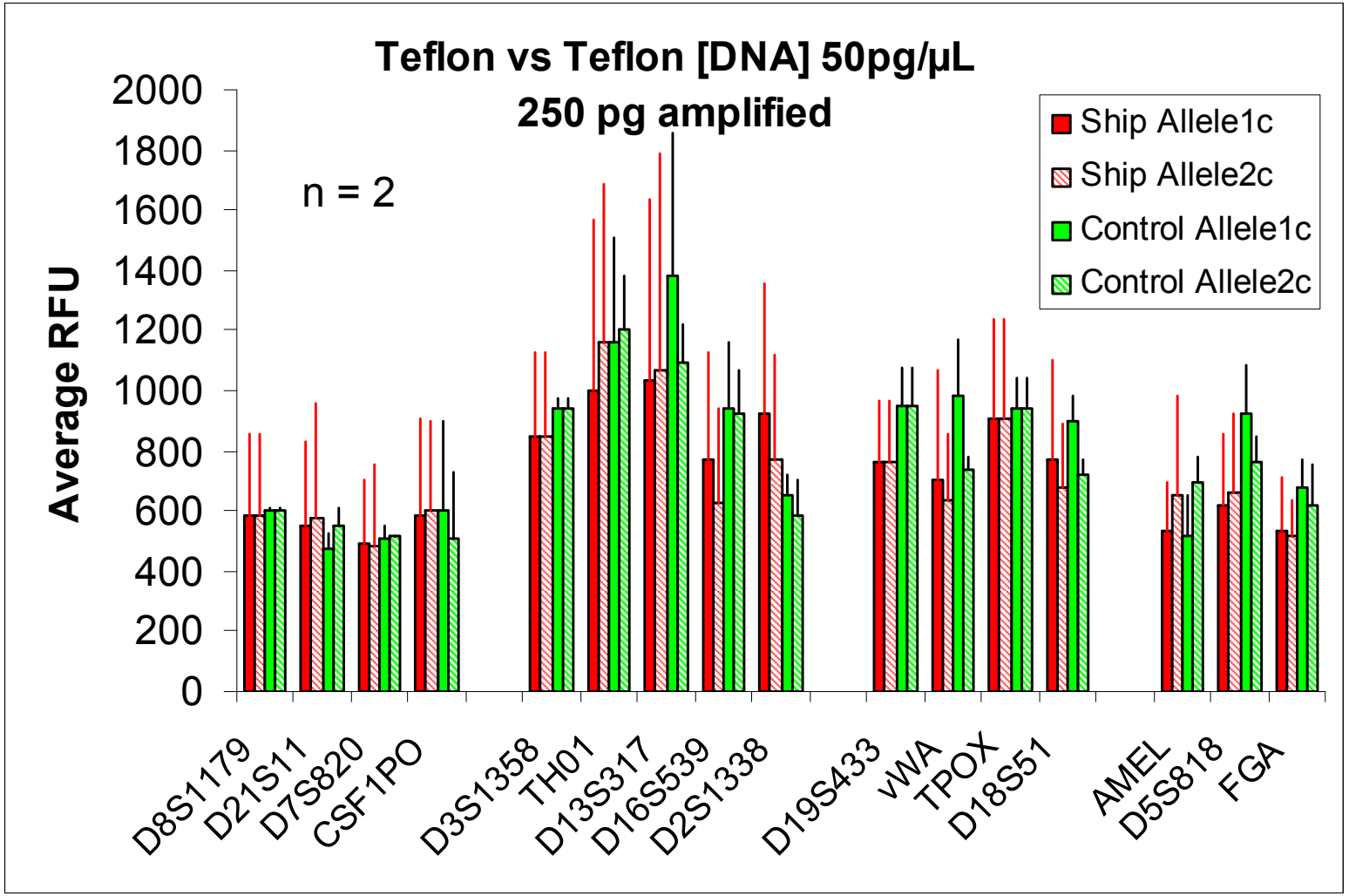


Additional information

- On August 26, 2007 100 µL aliquots from the Control 4 °C Teflon containers were removed and placed in sterile labeled Teflon vials.
- The new Teflon vials were placed with the “shipped/stressed” Biomatrica SampleGard plates.
- The Shipped/stress boxed was then placed in an attic for 8 weeks then moved to Lab ambient temperature.
- At this analysis time the Teflon vials have been stressed for 147 days out of the total 208 days of this study.



Identifiler results [DNA] 0.05 ng/µL after 147 days storage

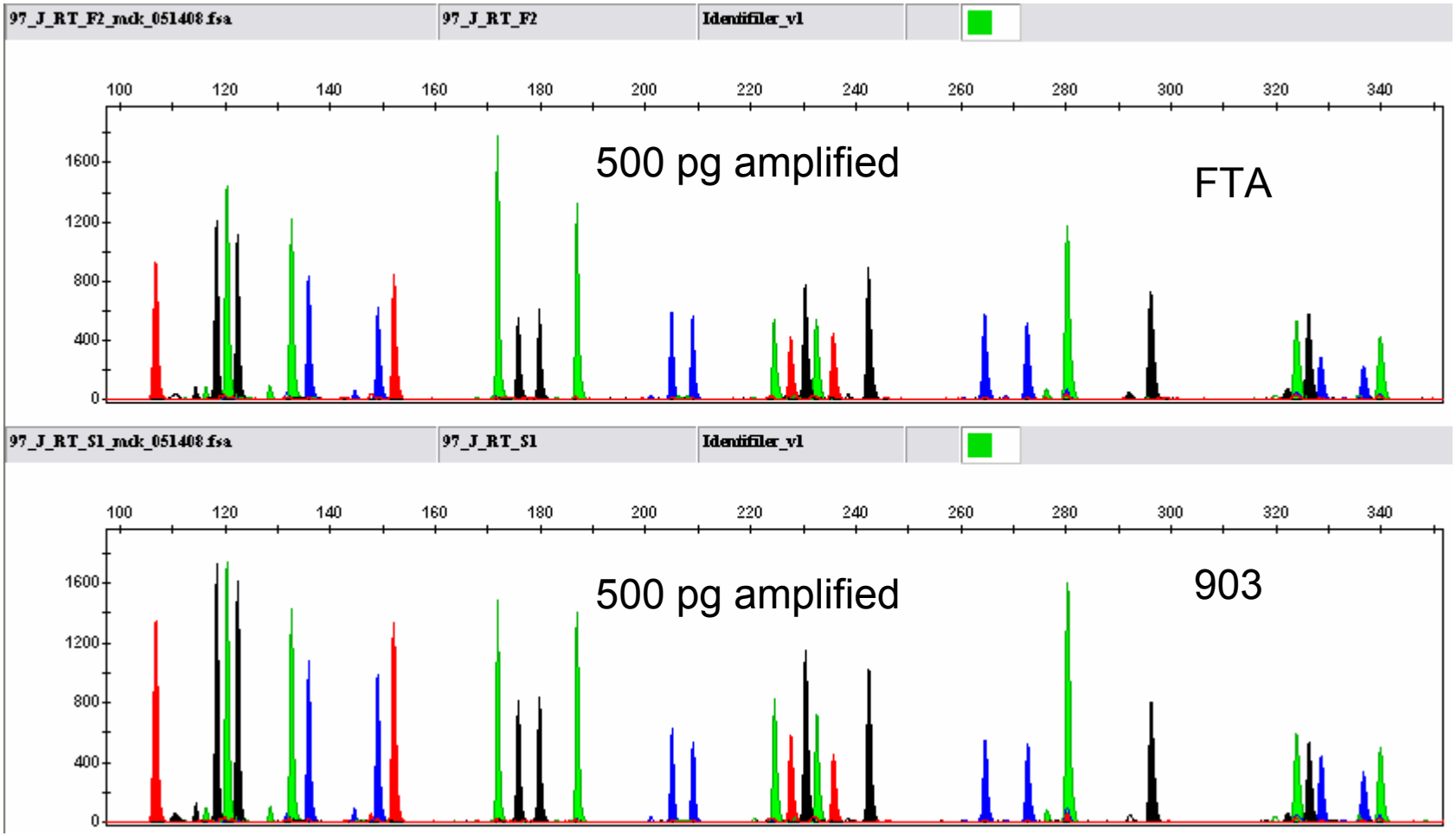


11 year Stability Study

FTA and 903 papers

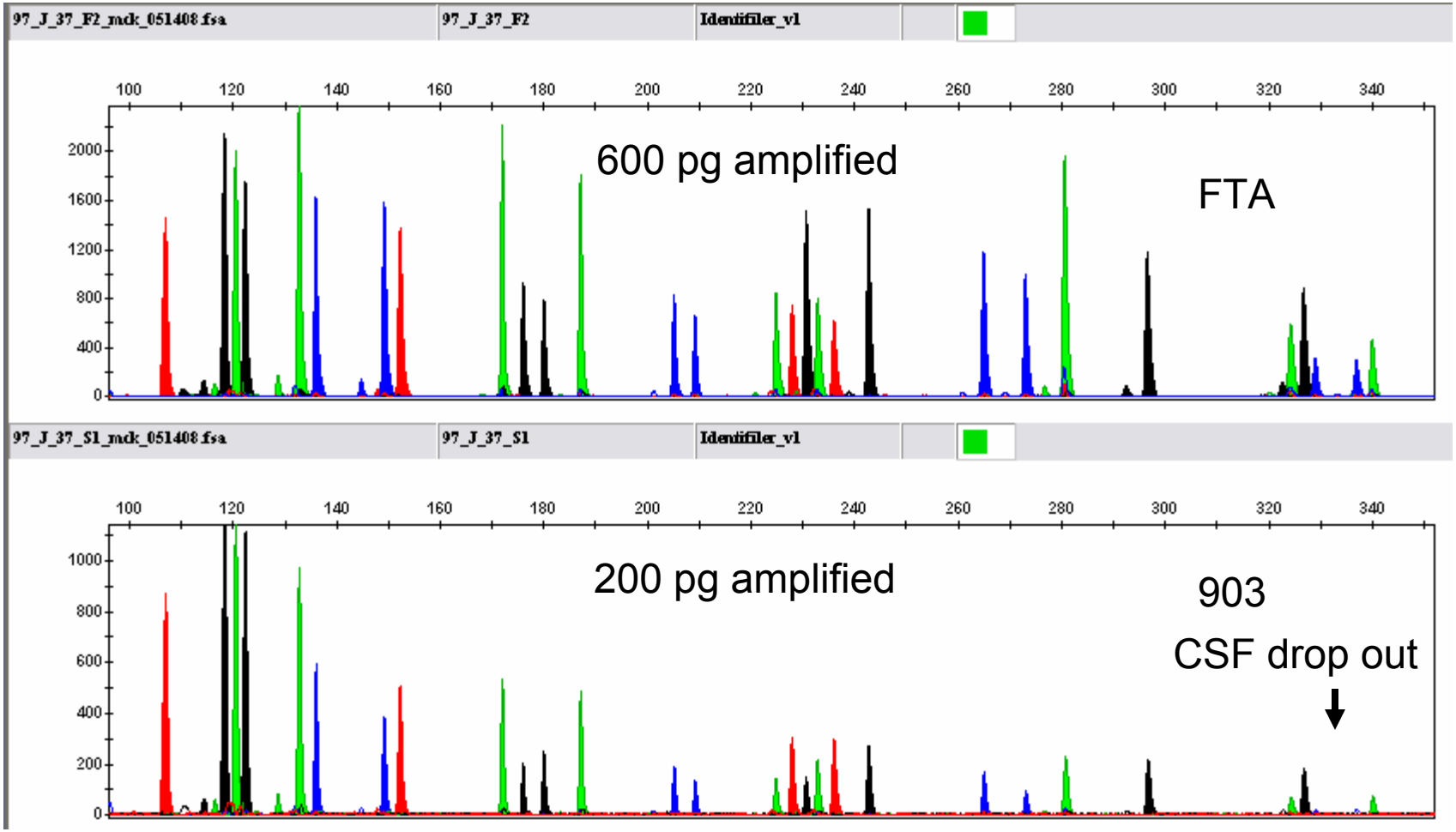
Room Temperature Storage
11 year time point

Identifiler profiles after DNA IQ Extract



+ 37 °C Storage 11 year time point

Identifiler profiles after DNA IQ Extract



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.

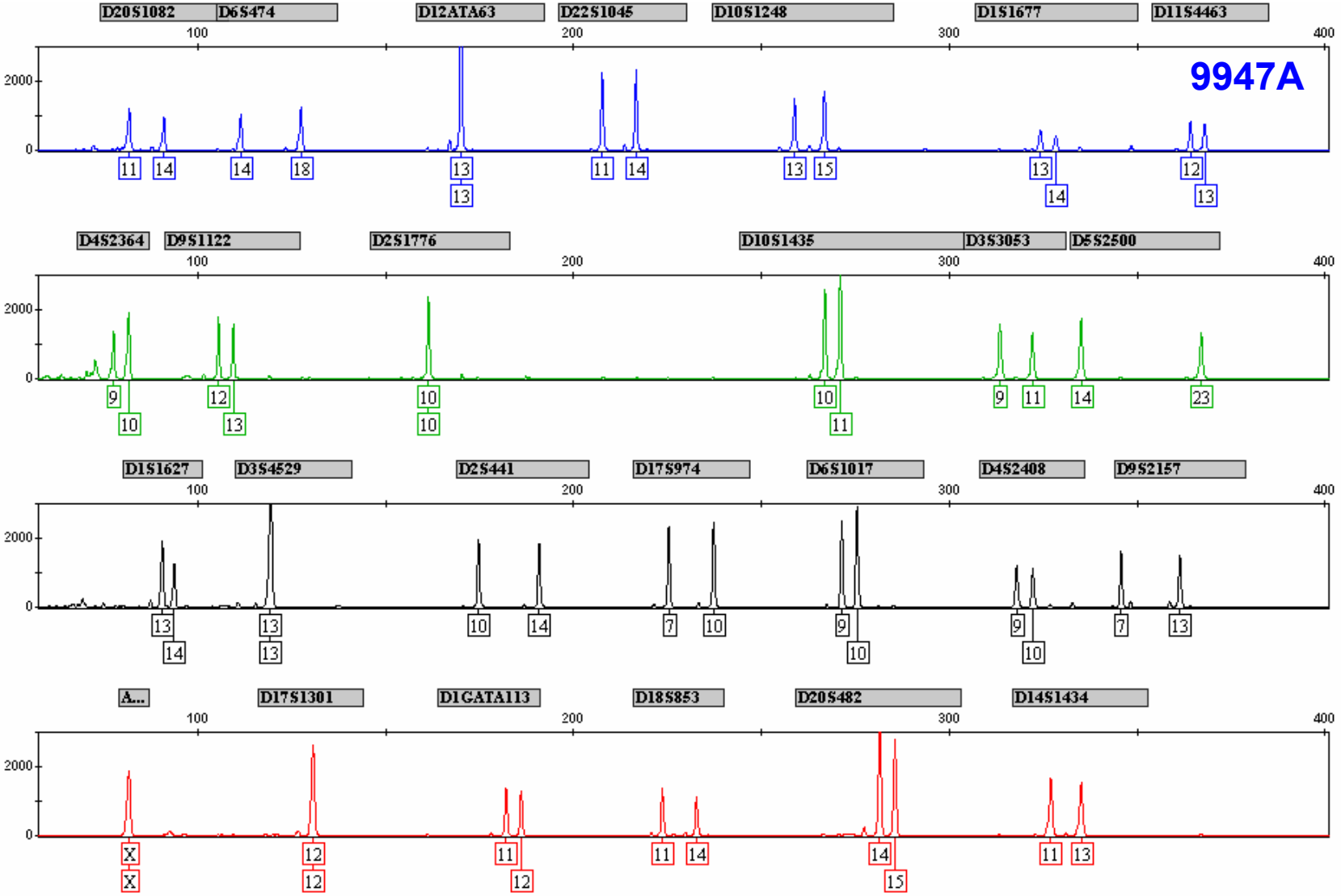
Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples

- 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

Yfiler Loci Mutation Rates Measured at NIST

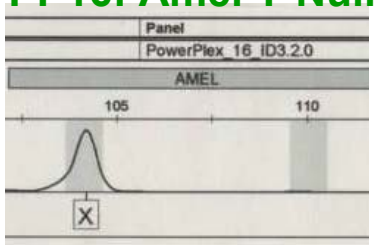
- **389 father/son sample pairs**
  - 788 samples with full profiles
- **17 Y-STR loci** in the Yfiler kit
- **24 differences** between father and son
  - 13 mutations resulted in the gain of a repeat in the son
  - 11 resulted in a loss of a repeat
- All single step repeat mutations
  - except a two repeat loss at Y-GATA-H4
- **2 sample pairs were found to have two mutations**
  - African American pair: mutations at DYS458 and DYS635
  - Asian pair: mutations at DYS439 and Y-GATA-H4
- Also observed 4 duplications, 1 triplication, and 4 deletions that were seen in both father and son

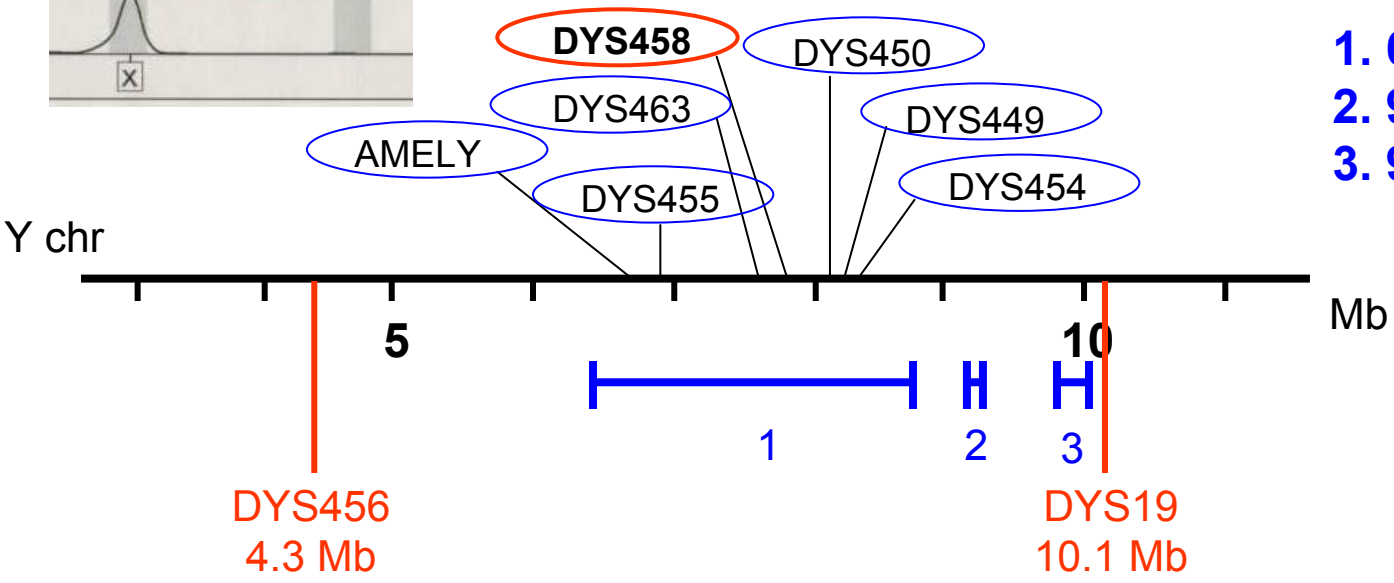
Decker, A.E., Kline, M.C., Redman, J.W., Reid, T.M., Butler, J.M. (2008) Analysis of mutations in father-son pairs with 17 Y-STR loci. *Forensic Science International: Genetics 2 (2008) e31 – e35.*

Y Chromosome Deletions

Deleted region at Yp11.2

PP16: Amel Y Null

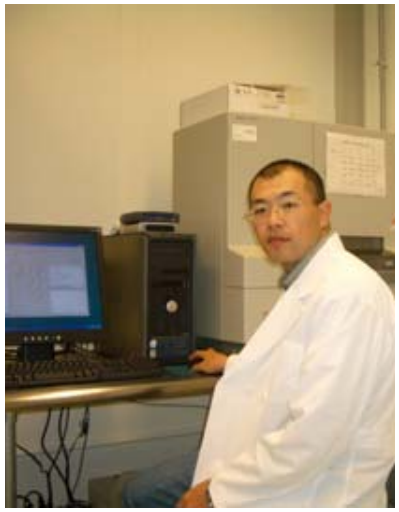




1. 6.44Mb - 8.96Mb
2. 9.23Mb
3. 9.99Mb - 10.01Mb

Examined 60 loci for Y deletion

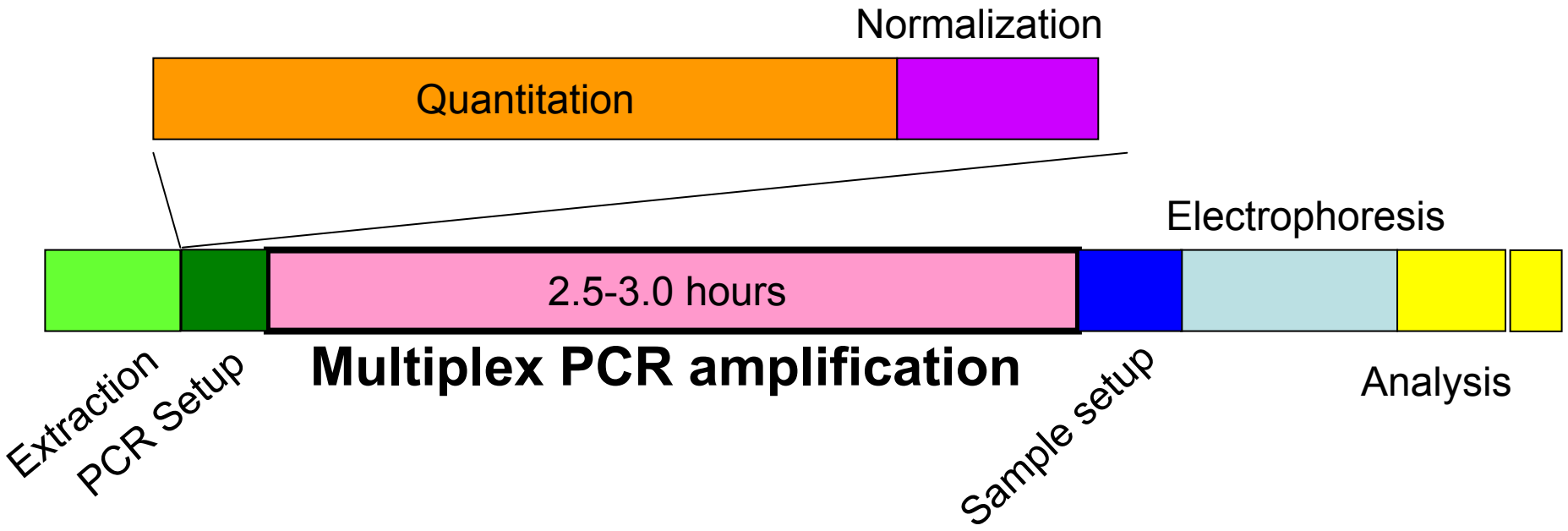
- Identified 3 deleted regions in an Amel Y negative male from Gifu, Japan
- This pattern has not been reported before



Tomohiro Takayama Ph.D. - Research Specialist from Criminal Investigation Laboratory, Gifu Pref. Police H.Q., Japan
Worked at NIST from Sept-Nov. 2007

Rapid PCR

Relative Time for Overall DNA Process



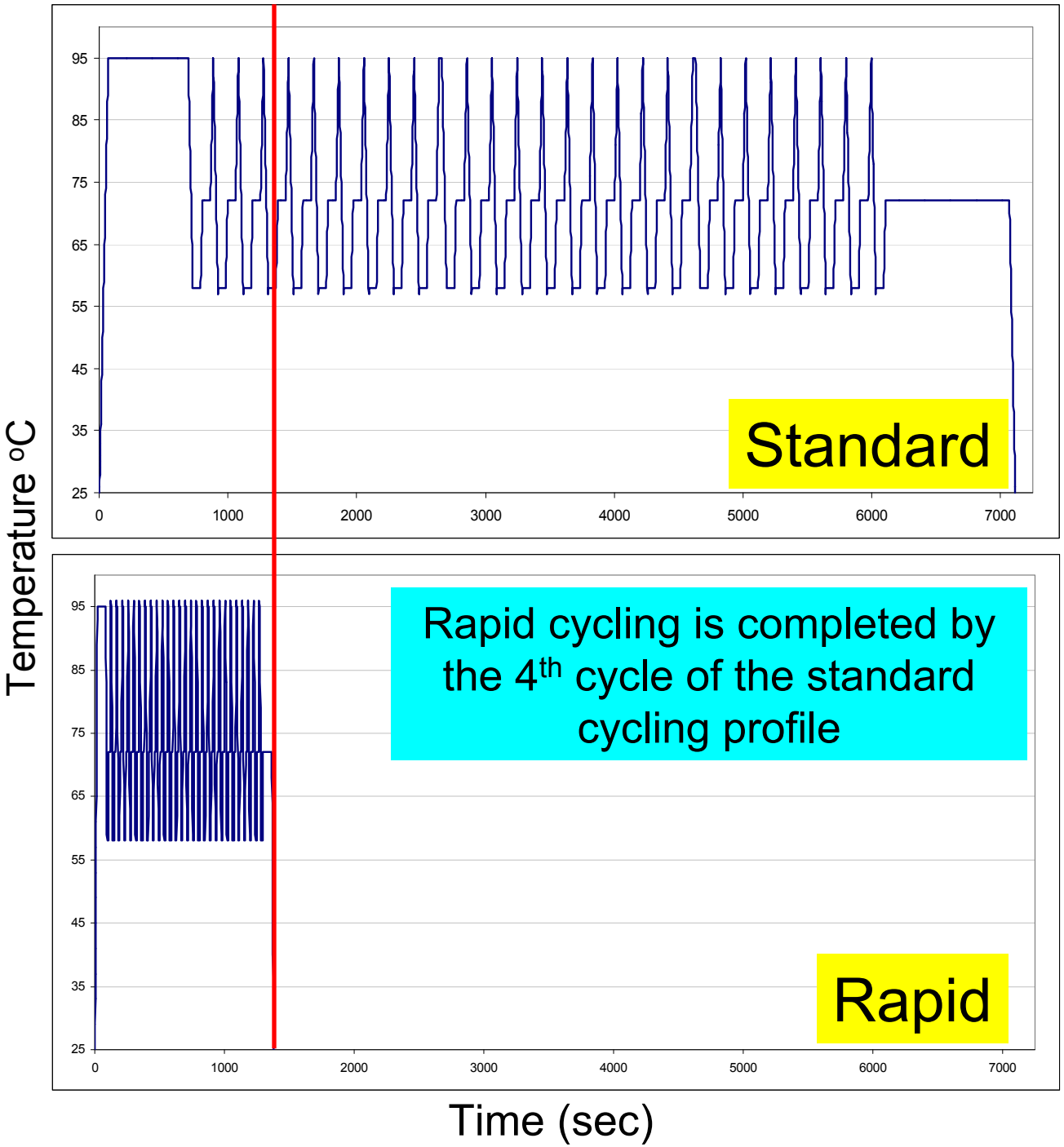
Innovations and Improvements in Speed

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

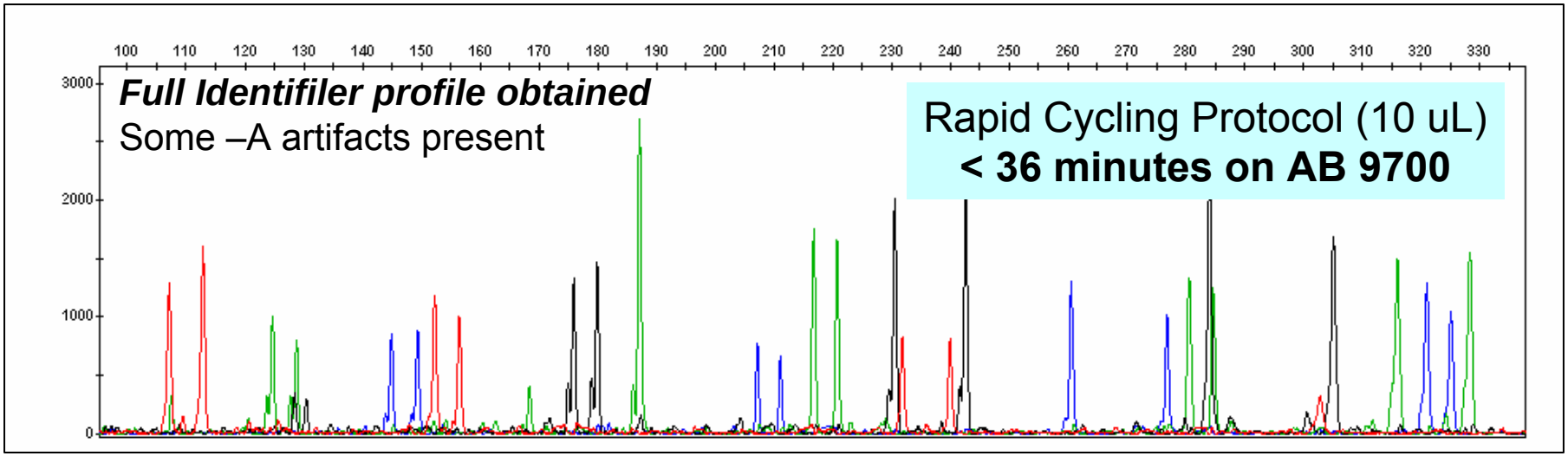
Evaluate robustness and reproducibility



Comparison of Thermal Cycling Profiles

Rapid Multiplex PCR Protocols

Testing the potential of rapid multiplex PCR methods
Utilizing AB 9700 cycler and 'fast' commercial enzymes
Manuscript in preparation

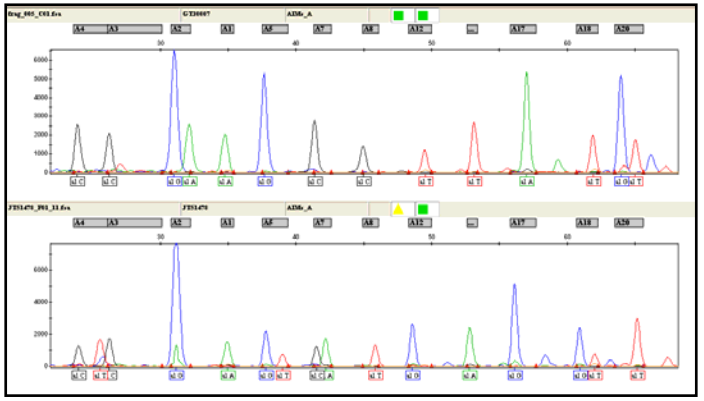


Identifiler STR kit
28 cycles, 1ng template DNA

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

SNP Work

- Working with Dr. Manfred Kayser (Netherlands)
  - Panel of Ancestry Informative Markers (AIMs)
  - NIST developed multiplex assays for typing SNPs
  - Typed over 600 + of our population samples
- Dr. Peter deKnijff (Netherlands)
  - Performing Y SNP typing
- Dr. Michael Coble (AFDIL)
  - mitochondrial control region sequencing



SNP assays

- Data will be presented in Ancona, Italy May 29, 2008

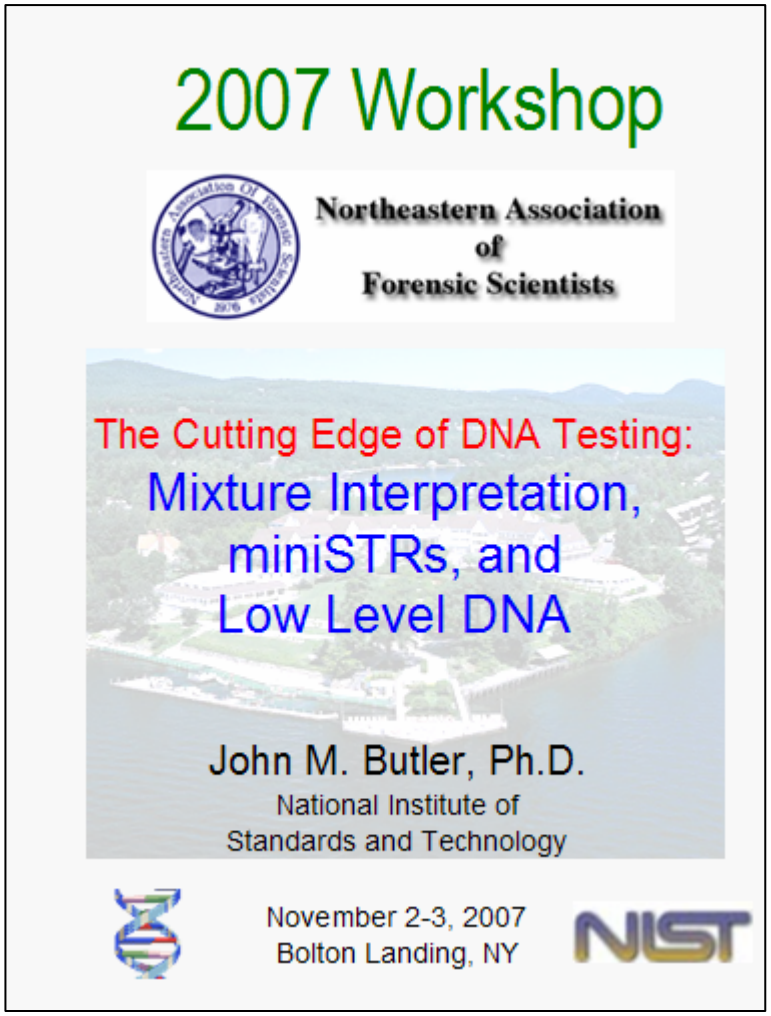
Peter Vallone et al. "Informativity of ancestry-sensitive markers from autosomes, Y-chromosome and mitochondrial DNA in U.S. populations" (OP42)

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)

158 page handout prepared

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

Florida Statewide DNA Training Workshop

STRs, CE, and Mixtures

Two-day workshop for Statewide DNA Training
***May 12-13, 2008** - Indian Rocks Beach, FL*

John Butler (NIST)
[full workshop handouts-82 pages] [full workshop with articles - 135 pages]

STRs and Molecular Biology Artifacts

CE Fundamentals and Troubleshooting

Principles of Mixture Interpretation

Variability between Labs in Approaches and summaries of Mixture Interlaboratory Studies

http://www.cstl.nist.gov/biotech/strbase/training/FL-May2008-Workshop.htm

For More Information

- Email: margaret.kline@nist.gov

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

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