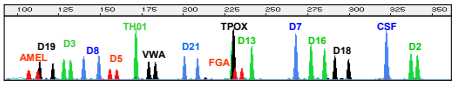


NIST Research Update

John Butler
Margaret Kline, Pete Vallone,
Jan Redman, Amy Decker, Becky Hill,
Dave Duewer (NIST Analytical Chemistry Division)

SWGDAM Meeting – July 13, 2006

Disclaimers and Collaborations

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

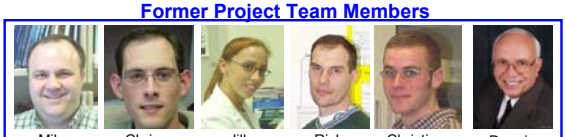
Points of view are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such 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

NIST Human Identity Project Team

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

Former Project Team Members



Mike Coble	Chris DeAngelis	Jill Appleby	Rich Schoske	Christian Ruitberg	Dennis Reeder
AFDIL	Medical School	NC SBI	Air Force	Pharma	Retired/ABI

Team Impact

- **32 publications** since Jan 2005
- **65 presentations** and **7 workshops** to the community since Jan 2005
- **Training workshops**: **AAFS, MASP, MAAFS, OCME (slides available on STRBase)**
- **PDI Workshops: Validation, mtDNA, qPCR**

All NIST publications and presentations available on STRBase:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Presentation Outline

- Overview of Research Efforts
- **New miniSTR Loci Characterized**
- STR Allele Sequencing
- Training Information
- Validation Information
- DNA Quantitation (qPCR)

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

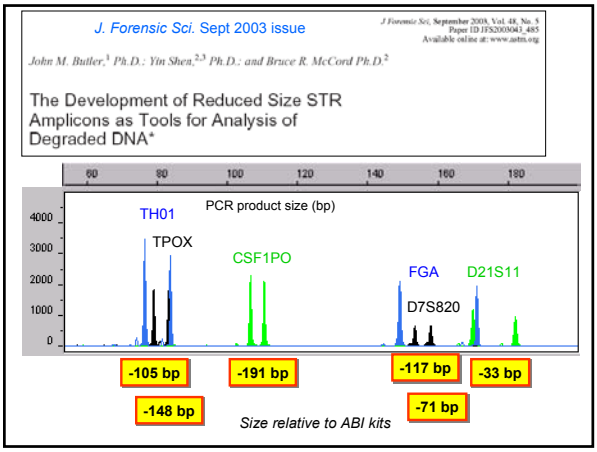
- **Standards**
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development
- **Training Materials**
  - Review articles and workshops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

Timeline for miniSTRs

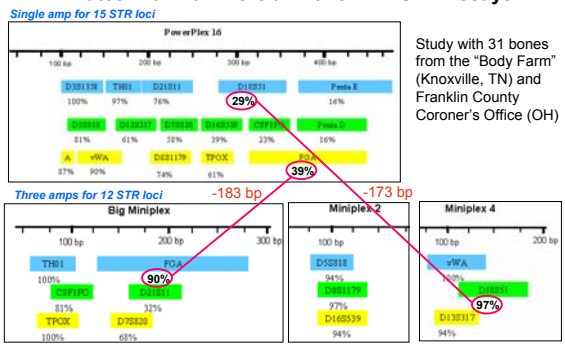
and Demonstrating the Value of Using Reduced Size Amplicons for Degraded DNA

- 1994 – FSS finds that smaller STR loci work best with burned bone and tissue from Branch Davidian fire
- 1997 – New primers developed for time-of-flight mass spectrometry to make small STR amplicons
- 2001 – Work at NIST and OhioU with CODIS STRs; BodePlexes used in WTC investigation starting 2002
- 2004 – Work at NIST with **non-CODIS (NC) miniSTRs**
- 2006 – Applied Biosystems to release a 9plex miniSTR kit

http://www.cstl.nist.gov/biotech/strbase/miniSTR/timeline.htm



Comparison of PCR Amplification Success Rates with Commercial Kit vs. miniSTR Assays

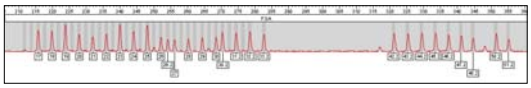


Study with 31 bones from the "Body Farm" (Knoxville, TN) and Franklin County Coroner's Office (OH)

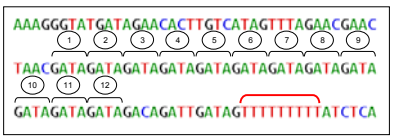
Opel K. L.; Chung, D. T.; Drábek, J.; Tatarek, N. E.; Jantz, L. M.; McCord, B.R. (2006) The Application of Miniplex Primer Sets in the Analysis of Degraded DNA from Human Skeletal Remains. *J. Forensic Sci.* 51(2): 351-356.

Why Go Beyond the CODIS Loci?

(1) Large Allele Ranges (e.g. FGA)



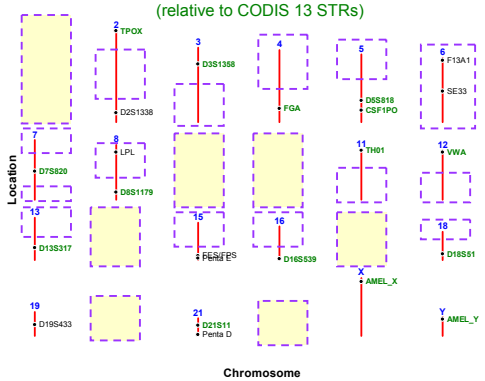
(2) "Unclean" Flanking Sequences (e.g. D7S820)



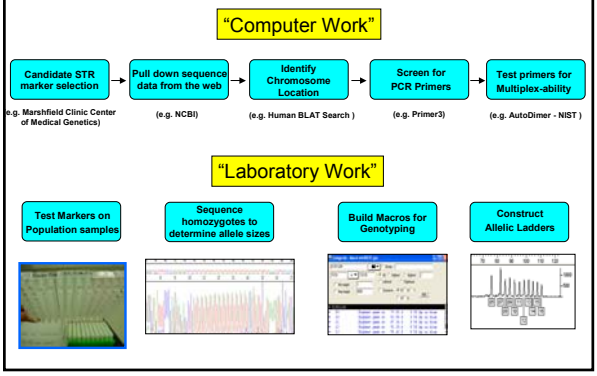
Butler, JM, Shen, Y., McCord, BR (2003) *JFS* 48(5): 1054-1064

Locations of Focus for New miniSTR Loci

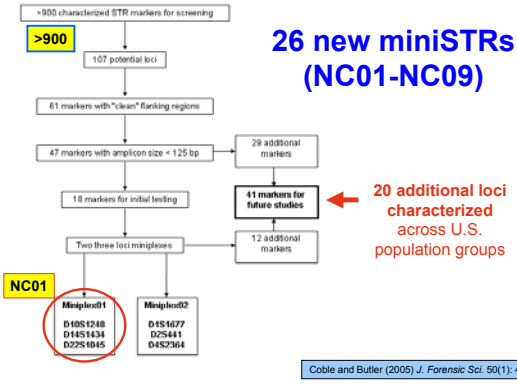
(relative to CODIS 13 STRs)



Characterization of New miniSTR Loci



Initial Testing Results with Potential miniSTR Loci



26 new miniSTRs (NC01-NC09)

20 additional loci characterized across U.S. population groups

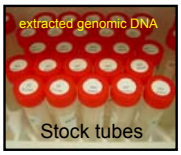
Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

Standard U.S. Population Dataset

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

260 Caucasians, 260 African Americans, 140 Hispanics, 3 Asians = **663 males**

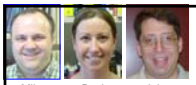
DNA extracted from whole blood (anonymous; self-identified ethnicities) received from Interstate Blood Bank (Memphis, TN) and Millennium Biotech Inc. (Ft. Lauderdale, FL)



To date: (>100,000 allele calls)

Identifiler (15 autosomal markers + Amelogenin) (10,608)
Roche Linear Arrays (HV1/HV2 10 regions) (6,630)
Y STRs 22 loci—27 amplicons (17,388)
Y STRs 27 new loci (14,535)
Yfiler kit 17 loci (11,237)
Y SNPs 50 markers on sub-set of samples (11,498)
Orchid 70 autosomal SNPs on sub-set (13,230)
miniSTR testing-new loci and CODIS concordance (9,228)
New miniSTR loci – for 26 loci, 17,238 genotypes
mtDNA full control region sequences by AFDIL

Genotypes with various human identity testing markers



Mike Coble, Becky Hill, John Butler

New miniSTR Loci

- 32 STR loci tested on NIST **665 U.S. population samples**
- **26 STR loci with allele sizes below 140 bp and good heterozygosities (above TPOX level)**
- All new STR loci are **physically unlinked** to the 13 CODIS core loci
- **Allelic ladders created**, GeneMapper*ID* bins & panels
- **Submitted articles** regarding primer sequences and locus characterization including population statistics

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

EDNAP Exercise on Degraded DNA

ARTICLE IN PRESS

Available online at www.sciencedirect.com SCIENCE DIRECT®

ELSEVIER

Forensic Science International

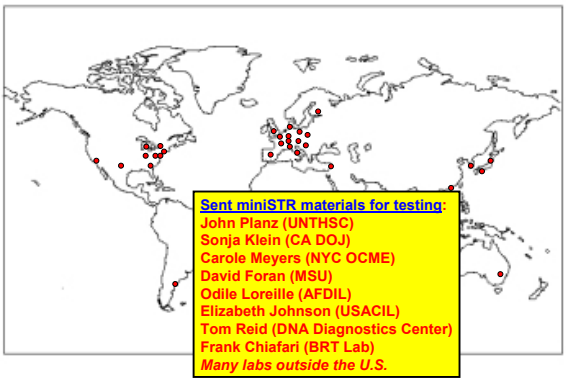
Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise

L.A. Dixon [a,*], A.E. Dobbins [a], H.K. Pulker [a], J.M. Butler [b], P.M. Vallone [b], M.D. Coble [b], W. Parson [c], B. Berger [c], P. Grubweiser [c], H.S. Mogensen [d], N. Morling [d], K. Nielsen [d], J.J. Sanchez [d], E. Petkovski [e], A. Carracedo [f], P. Sanchez-Diz [f], E. Ramos-Luis [f], M. Brión [f], J.A. Irwin [g], R.S. Just [g], O. Loreille [g], T.J. Parsons [g], D. Syndercombe-Court [h], H. Schmitter [i], B. Stradmann-Bellinghausen [j], K. Bender [j], P. Gill [a]

Conducted in the Fall of 2004

MiniSTR primer mixes and allelic ladders were provided by NIST

Global Impact of NC miniSTRs



Sent miniSTR materials for testing:
John Planz (UNTHSC)
Sonja Klein (CA DOJ)
Carole Meyers (NYC OCME)
David Foran (MSU)
Odile Loreille (AFDIL)
Elizabeth Johnson (USACIL)
Tom Reid (DNA Diagnostics Center)
Frank Chiafari (BRT Lab)
Many labs outside the U.S.

The International Commission on Missing Persons (ICMP) is Now Using miniSTRs

100s of bones are tested each week with miniSTRs to help in the re-association of remains

Miniplex 02
D21S11, D13S317, D7S820, CSF1PO, vWA and D8S1179

(Tom Parsons, personal communication)

European Labs Have Adopted the NIST-Developed NC miniSTRs

FSI (2006) **156(2):** 242-244

Short communication

The evolution of DNA databases—Recommendations for new European STR loci

Peter Gill [a,*], Lyn Fereday [b], Niels Morling [c], Peter M. Schneider [d]

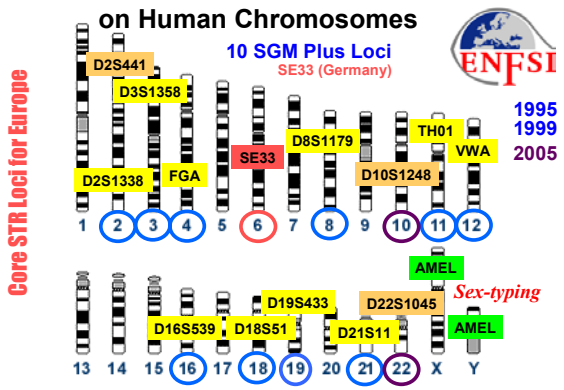
[a] Forensic Science Service, Birmingham, UK
[b] Forensic Science Service, London, UK
[c] Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark
[d] Institute of Legal Medicine, University of Cologne, Germany

Received 25 May 2005; accepted 26 May 2005

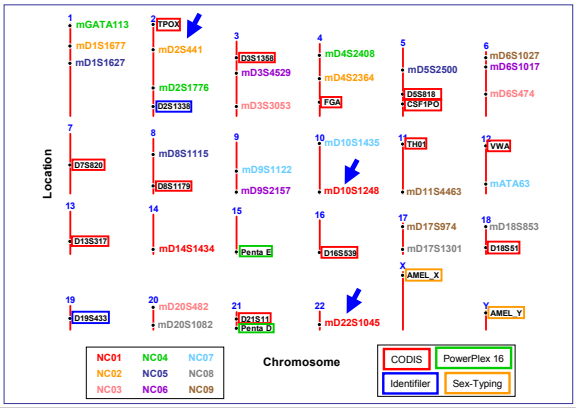
…recommended that existing multiplexes are re-engineered to enable small amplicon detection, and that three new mini-STR loci with alleles <130 bp (D10S1248, D14S1434 and D22S1045) are adopted as universal. This will increase the number of European standard Interpol loci from 7 to 10.

(D14 has been replaced with D2S441 from NC02)

Position of Forensic STR Markers on Human Chromosomes



Chromosomal Locations of New miniSTR Loci

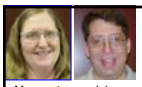


Comparison of heterozygosity values on 26 non-CODIS loci across the U.S. samples examined in this study.

Locus	N	Heterozygosity (Overall)	Rank	African American	Caucasian	Hispanic
D9S2157	661	0.844	1	0.884	0.840	0.779
ATA63 (D12)	659	0.829	2	0.788	0.842	0.879
D10S1248 (NC01)	663	0.792	3	0.825	0.785	0.743
D22S1045 (NC01)	663	0.784	4	0.817	0.785	0.721
D2S441 (NC02)	660	0.774	5	0.798	0.780	0.721
D10S1435	663	0.766	6	0.798	0.770	0.700
D2S1776	654	0.763	7	0.740	0.801	0.734
D3S4529	660	0.761	8	0.752	0.723	0.829
D6S474	648	0.761	9	0.765	0.802	0.679
D5S2500	664	0.747	10	0.757	0.747	0.729
D1S1627	660	0.746	11	0.783	0.737	0.693
D1S1677 (NC02)	660	0.746	12	0.743	0.749	0.743
D6S1017	664	0.740	13	0.807	0.698	0.693
D3S3053	648	0.739	14	0.713	0.724	0.814
D9S1122	659	0.734	15	0.753	0.742	0.686
D17S974	664	0.732	16	0.757	0.702	0.743
D11S4463	664	0.730	17	0.780	0.676	0.743
D4S2408	654	0.722	18	0.752	0.709	0.691
D18S853	664	0.711	19	0.772	0.645	0.721
D20S1082	664	0.696	20	0.792	0.653	0.600
D14S1434 (NC01)	663	0.696	21	0.685	0.721	0.650
D20S482	648	0.691	22	0.673	0.689	0.729
GATA113 (D1)	654	0.668	23	0.673	0.632	0.727
D8S1115	664	0.663	24	0.629	0.660	0.729
D17S1301	664	0.649	25	0.626	0.717	0.564
D4S2364 (NC02)	660	0.511	26	0.385	0.551	0.664

Past/Future Publications on New miniSTR Loci

- **Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. *J. Forensic Sci.* 50(1):43-53**
- Coble, M.D., Hill, C.R., Vallone, P.M., Butler, J.M. (2006) Characterization and performance of new miniSTR loci for typing degraded samples. *Progress in Forensic Genetics 11*, Elsevier Science: Amsterdam, The Netherlands, International Congress Series 1288, 504-506.
- Dixon, L.A., Dobbins, A.E., Pulker, H., Butler, J.M., Vallone, P.M., Coble, M.D., et al. (2006) Analysis of artificially degraded DNA using STRs and SNPs--results of a collaborative European (EDNAP) exercise. *Forensic Sci. Int., in press*.
- Yong, R.Y.Y., Gan, L.S.H., Coble, M.D., Yap, E.P.H. (2006) Allele frequencies of six miniSTR loci of three ethnic populations in Singapore. *Forensic Sci. Int., in press*.
- Hill, C.R., Butler, J.M., Coble, M.D. (2006) Allele frequencies for 27 new miniSTR loci with U.S. Caucasian, African American, and Hispanic populations. *J. Forensic Sci., in press*.
- Hill, C.R., Coble, M.D., Butler, J.M. (2006) Development of additional new miniSTR loci for improved analysis of degraded DNA samples. *submitted*.



Margaret Kline John Butler

STR Allele Sequencing and Characterization

- Variant characterization
  - TPOX 10.3 (Maryland State Police)
  - D18S51 null alleles (FSS and Kuwait govt)
  - D18S51 allele 40 (Nebraska State Crime Lab)
  - D18S51 allele 5.3 (DNA Solutions)
  - FGA allele 46.2 (Denver Crime Lab)
  - DYS392 allele "10.3" (AFDIL)
- Locus duplication or deletion
  - DYS390 (CFS Toronto)
  - DYS392 (MN BCA)
- **Send us your unusual STR alleles for sequence characterization**

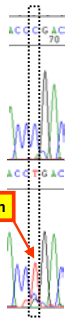
http://www.cstl.nist.gov/biotech/strbase/STRseq.htm

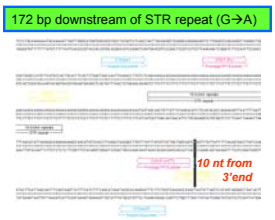
D18S51 Null Allele from Kuwait Samples with ABI Primers

PowerPlex 16

Identifiler

Allele 18 drops out





10 nucleotides from 3'end of ABI D18-R primer (PowerPlex 16 primers are not impacted)

Clayton et al. (2004) **Primer binding site mutations affecting the typing of STR loci contained within the AMPFISTR SGM Plus kit.** *Forensic Sci Int.* 139(2-3): 255-259

Sequencing Summaries of Unusual STR Alleles

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
DYS392	"10.2"	AFDIL	DNA sequence analysis revealed a C-to-G transversion 180 bp upstream of the STR repeat region, the mutation causes an apparent mobility shift of approximately 0.75 bp such that the allele falls outside of the +/-0.5 bp genotyping bin
DYS635	21.3	NIST U.S. population samples	DNA sequence analysis revealed a deletion of a "T" in the repeat region, full repeat was [TCTA]$_4$(TGTA)$_4$[TCTA]$_4$(TGTA)$_3$ [TCTA]$_3$(TGTA)$_{10}$[TCTA]$_3$ TC-A [TCTA]$_3$
Penta D	18	DNA Solutions	DNA sequence analysis confirmed 18 repeats
Penta D	"8.2"	Peter de Knijff's lab at Leiden University	DNA sequence analysis revealed a 13 bp deletion prior to a [AAAGA]$_{11}$ repeat
Penta D	6	Peter de Knijff's lab at Leiden University	DNA sequence analysis confirmed 6 repeats

Training Materials and Review Articles

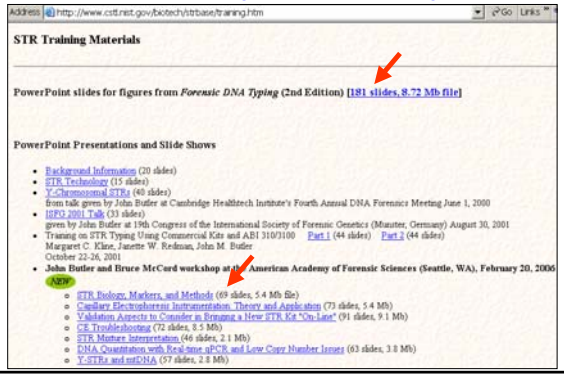
John Butler

- Workshops on STRs and CE (ABI 310/3100)
  - **Taught with Bruce McCord (Florida Int. Univ.)**
  - AAFS Workshop (February 20, 2006)
  - U. Albany DNA Academy II (April 27-28, 2006)
- PowerPoint slides from *Forensic DNA Typing*, 2nd Edition
- Review articles
  - ABI 310 and 3100 chemistry – Electrophoresis 2004, 25, 1397-1412
  - Forensic DNA analysis – Anal. Chem. 2005, 77, 3839-3860
  - STR core loci – J. Forensic Sci. 2006, 51(2): 253-265

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

Training Materials Available on STRBase

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



STR Training Materials

PowerPoint slides for figures from *Forensic DNA Typing* (2nd Edition) [181 slides, 8.72 Mb file]

PowerPoint Presentations and Slide Shows

- Background Information (20 slides)
- STR Technology (15 slides)
- Y-Chromosomal STRs (40 slides)
  from talk given by John Butler at Cambridge Healthtech Institute's Fourth Annual DNA Forensics Meeting June 1, 2000
- ISFG 2001 Talk (33 slides)
  given by John Butler at 19th Congress of the International Society of Forensic Genetics (Münster, Germany) August 30, 2001
- Training on STR Typing Using Commercial Kits and ABI 310/3100 Part 1 (44 slides) Part 2 (44 slides)
  Margaret C. Kline, Janette W. Redman, John M. Butler
  October 22-26, 2001
- **John Butler and Bruce McCord workshop at the American Academy of Forensic Sciences (Seattle, WA), February 20, 2006**
  AAFS
  - STR Biology, Markers, and Methods (69 slides, 5.4 Mb file)
  - Capillary Electrophoresis Instrumentation: Theory and Application (73 slides, 5.4 Mb)
  - Validation Aspects to Consider in Bringing a New STR Kit "On-Line" (91 slides, 9.1 Mb)
  - CE Troubleshooting (72 slides, 8.5 Mb)
  - STR Mixture Interpretation (46 slides, 2.1 Mb)
  - DNA Quantitation with Real-time qPCR and Low Copy Number Issues (63 slides, 3.8 Mb)
  - Y-STRs and mtDNA (57 slides, 2.8 Mb)

Forensic Bioinformatics 5th Annual Conference
The Science of DNA Profiling: A National Expert Forum

August 11 - 13, 2006 (Dayton, OH)

http://www.bioforensics.com/conference06/index.html

Defense Expert Training

Friday, August 11

Session I: Mitochondrial DNA profiling: Dan Krane, Mitch Holland, Norah Rudin, Bill Shields, Jason Eshleman

Session II: Objective characterization of STR testing results: Keith Inman, Carrie Rowland, Simon Ford, Dan Krane, Bill Shields, Thaddeus Tarpey, Jason Gilder

Saturday, August 12

Session III: DNA profile database statistics: Larry Mueller, Fred Bieber, David Balding, Jason Gilder, Dan Krane

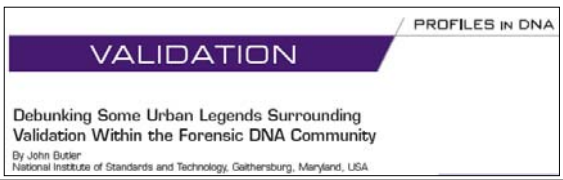
Session IV: Laboratory oversight and reform: Michael Saks, Paul Giannelli, Norah Rudin, Fred Bieber, Dan Krane, Bill Thompson

Sunday, August 13

Session V: Psychological aspects of DNA evidence: Keith Inman, Bill Thompson, Michael Saks, David Balding, Jay Koehler

Validation Information

- President's DNA Initiative: **Validation Workshop (Aug 2005) with Robyn Ragsdale** – slides on STRBase; NFSTC working on DVD
- **ABI Roadshow/HID University: Validation Workshop (May 2006)** – slides available on STRBase
- We would love to have **more internal validation information for STRBase Validation Section** (e.g., Y-STRs)

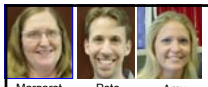


PROFILES IN DNA

VALIDATION

Debunking Some Urban Legends Surrounding Validation Within the Forensic DNA Community

By John Butler
National Institute of Standards and Technology, Gaithersburg, Maryland, USA


Margaret Kline | Pete Vallone | Amy Decker

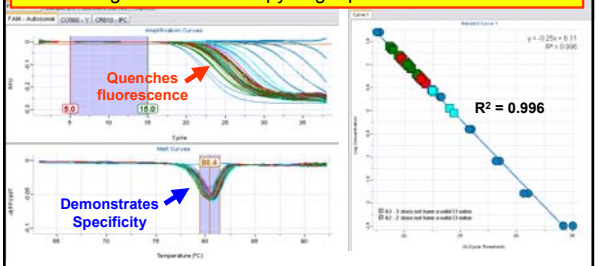
DNA Quantitation (qPCR)

- Production of SRM 2372 (Human DNA Quant Std)
  - Planned Aug 2006 interlab study; contact Margaret if interested
- PDI Training: qPCR Course (July 25-26, 2006)
  - Slides will be made available on STRBase
- Evaluation of published assays on same samples
  - Promega 2005 and AAFS 2006 presentations
  - Trying to educate ABI on quality control of their materials (Quantifiler lot-to-lot variation)
- Additional studies under way utilizing qPCR:
  - Evaluation of Promega's Plexor Genetic Identity prototype assay

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

Plexor GI Autosomal Prototype Assay

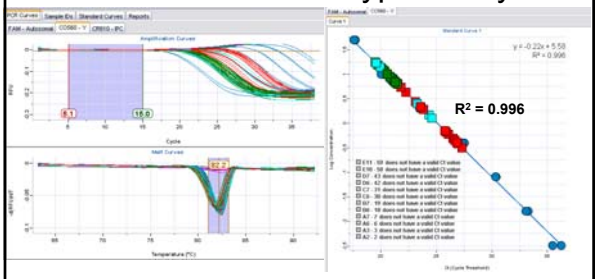
Enables simultaneous detection of total human DNA and male DNA through use of multi-copy target probes



Amplification Curve and Melt Curve | Generated Standard Curve

Calibration curve generated using a 1:5 dilution scheme from **50 ng/µL to 0.0032 ng/µL**

Plexor GI Y Prototype Assay

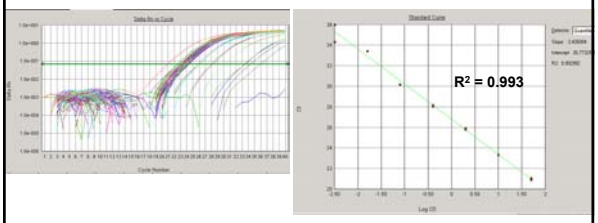


Amplification Curve and Melt Curve | Generated Standard Curve

Calibration curve generated using a 1:5 dilution scheme from **50 ng/µL to 0.0032 ng/µL**

Run in a different dye channel on ABI 7500

Quantifiler Human



Standard curve generated using Plexor Genetic Identity calibrant

Requirements for NIST SRM 2372 Human DNA Quantitation Standard

Material must be fit for purpose:

- **Homogeneity**
  - All tubes are the same
- **Stability**
  - Will withstand shipping and normal storage
- **Recoverability**
  - What went in the tubes comes out
- **Traceability**
  - Values assigned are traceable to the designated certification method.

NIST SRM 2372
Human DNA Quantitation Standard

In progress

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

Quantities
110 µL of Human Genomic DNA
Absorbance of 1.0 OD
Conventional [DNA] 50 ng/µL.

Certification
Absorbance by US National Spectrophotometer
Homogeneity by Cary 100 Bio
Validation of conventional [DNA] by Interlaboratory Study

What is Delaying Release?

- **Need to extract more DNA** in order to reach goal of producing >1,500 units (there is a great deal of interest in SRM 2372 outside of the forensic community—e.g., pharmaceutical industry)
  - ~30 units (3 mL) are required by the NIST National-Reference Spectrophotometer for its measurements
- **Additional studies to be performed**: interlaboratory (performed by multiple forensic labs), homogeneity (monitored by NIST statisticians), and continual stability testing for the life of the product

An Interlaboratory Study Will Be Performed to Demonstrate Commutability of SRM 2372

You will have 3 weeks to return your data once we ship the final packaged material (≈August 2006).

Any Volunteers?

Contact: margaret.kline@nist.gov

Thank you for your attention…

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

Questions?

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