

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




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

NIST Human Identity Project Team

John M. Butler, Ph.D.
 National Institute of Standards and Technology
NIJ Applied Technology Conference
 April 5, 2007

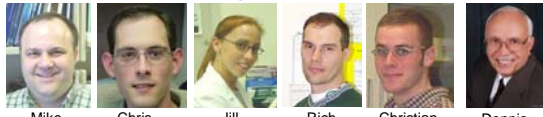
NIST Human Identity Project Team

>90 years experience in scientific research



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

Scope of Our Work (Team Mission Statement)

- The NIST Human Identity Project Team is trying **to lead the way in forensic DNA...** through research that helps bring traceability and technology to the scales of justice.


Support to the Community

...Bringing traceability and technology to the scales of justice...

- Conduct interlaboratory studies
- Perform beta-testing of new human identity testing products
- Provide input to
 - Scientific Working Group on DNA Analysis Methods (SWGDM)
 - Department of Defense Quality Assurance Oversight Committee for DNA Analysis
 - American Prosecutor's Research Institute (APRI) DNA Forensics Program "Course-in-a-Box" for training lawyers
 - WTC Kinship and Data Analysis Panel (KADAP)
 - 2005 Hurricane Victim DNA Identification Expert Group (HVDIEG)
 - NIJ Expert System Testbed (NEST) Project



Team Impact



- 26 publications** from Jan-Dec 2006
- 45 presentations** and **10 workshops** to the community from Jan-Dec 2006
- Training workshops:** AAFS, MAAFS, MAFS, OCME, MASP, NYSP, MN BCA (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>

Training Workshops Conducted

<http://www.cstl.nist.gov/biotech/strbase/training.htm>
 John Butler (and Bruce McCord, Robyn Ragsdale, Pete Vallone, or Mike Coble)



Training Resources on STRBase...

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

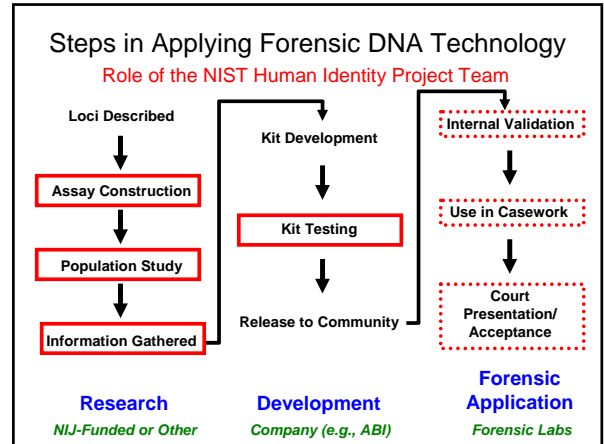
STR Training Materials

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

4.2.7 *STR Base Training Manual* (2.5 Mb self file) for the Massman DNA Highplex Panel Forensic Laboratory... as an example of information might required reading and expectations for 25M analysis and applications in training... provided by *John Montgomery of the Massman DNA Highplex Panel Core Laboratory*

PowerPoint Presentations and Slide Shows

- Background Information (20 slides)
- STR Technology (12 slides)
- Y-Chromosomal STRs (40 slides)
- Short tandem repeats by John Butler at Cambridge Healthtech Institute's Fourth Annual DNA Forensic Meeting June 1, 2003
- STR 2011 Lab (20 slides)
- STR 2011 Lab (20 slides)
- Training on STR Typing Using Commercial Kits and ABI 3100/3100i *Part 1* (44 slides) *Part 2* (44 slides)
- Margaret C. Kline, Lucetta W. Jordan, 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
 - STR Biology, Markers, and Methods (19 slides, 5.4 Mb file)
 - Y-Chromosomal STRs: Fundamentals, Theory and Applications (73 slides, 5.4 Mb file)
 - Y-STRs: A Practical Guide to Interpreting a Y-STR STR Kit "On-Line" (91 slides, 9.1 Mb file)
 - STR Technology (12 slides, 3.3 Mb file)
 - STR Markers Interpretation (46 slides, 2.1 Mb file)
 - STRs: Commercial Kits, Biology, PCR, and Low-Copy Number Issues (93 slides, 3.3 Mb file)
 - Y-STRs and mtDNA (27 slides, 2.3 Mb file)



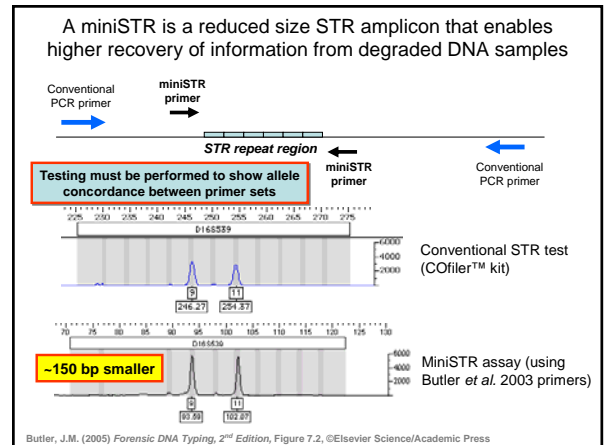
Assay Development with Collaborators

(Usually Other NIJ-Funded Researchers)

Pete Vallone | John Butler

PCR Product Size (bp)

- Y-STR 20plex** (Mike Hammer and Alan Redd)
 - Butler et al. (2002) FSI
- Cat STRs** (Marilyn Raymond and Victor David)
 - Butler et al. (2002) Profiles in DNA
 - Raymond et al. (2005) JFS
- miniSTRs** (Bruce McCord)
 - Butler et al. (2003) JFS
- mtDNA coding region SNPs** (AFDIL)
 - Vallone et al. (2004) IJLM
- Others:** Dog STRs, ancestry SNPs, phenotype SNPs, additional autosomal STRs...



miniSTRs: Past, Present, and Future

Applied Biosystems Forensic News

October 2006 Customer Corner

MiniSTRs: Past, Present, and Future
By John M. Butler, National Institute of Standards and Technology

DNA molecules that are exposed to water and/or heat will over time begin to break down into smaller pieces. This degradation occurs due to bacterial, biochemical or oxidative processes. A number of studies have demonstrated that successful analysis of degraded DNA specimens from mass disasters or compromised forensic evidence improves with smaller sized PCR products. For example, in 1994 the Forensic Science Service noted that smaller STR loci worked more often on biological remains recovered from the Branch Davidian fire. The first major effort to purposefully reduce STR amplicon sizes was for use in time-of-flight mass spectrometry, where detection sensitivity improved dramatically with PCR products less than 100 bp in size. Later many of these "miniSTR" primers were labeled with fluorescent dyes and used to aid identification of World Trade Center victims. A timeline covering the development of miniSTRs may be found at <http://www.cstl.nist.gov/biotech/strbase/miniSTRtimeline.htm>.

http://marketing.appliedbiosystems.com/images/news/ForensicNews_Vol7/PDF/02A_CustomerCorner_Butler.pdf

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
- NIJ 1997 – New primers developed for time-of-flight mass spectrometry to make small STR amplicons
- NIJ 2001 – Work at NIST and OhioU with CODIS STRs; BodePlexes used in WTC investigation starting 2002
- NIJ 2004 – Work at NIST with non-CODIS (NC) miniSTRs
- 2007 – Applied Biosystems releases 9plex MiniFiler

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

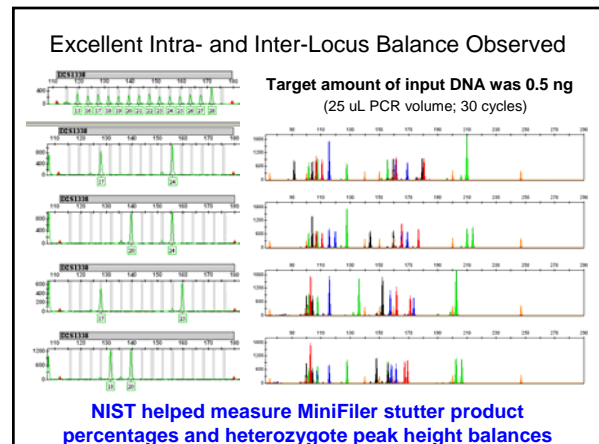
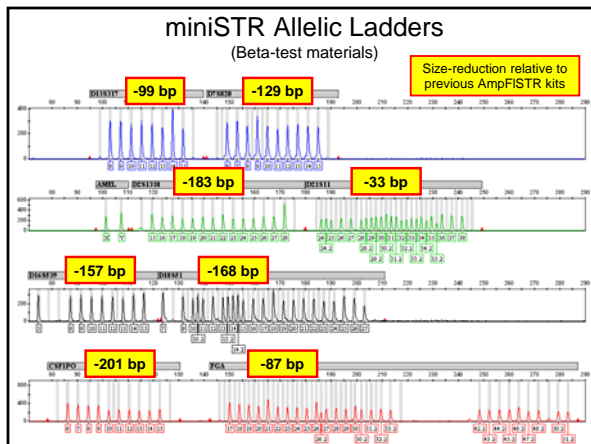
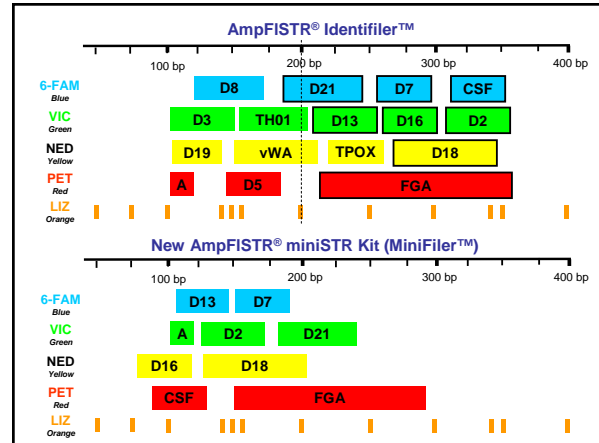
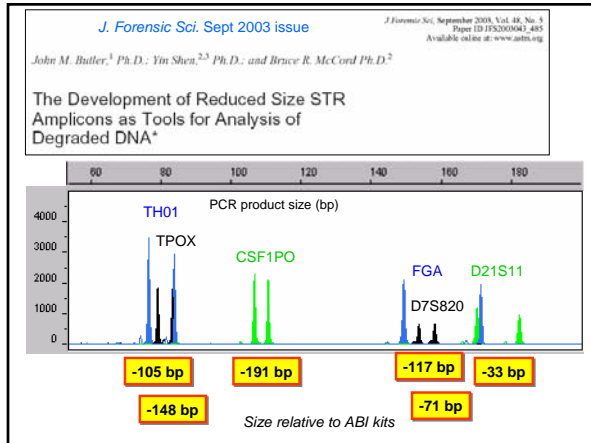
Our Publications on miniSTRs

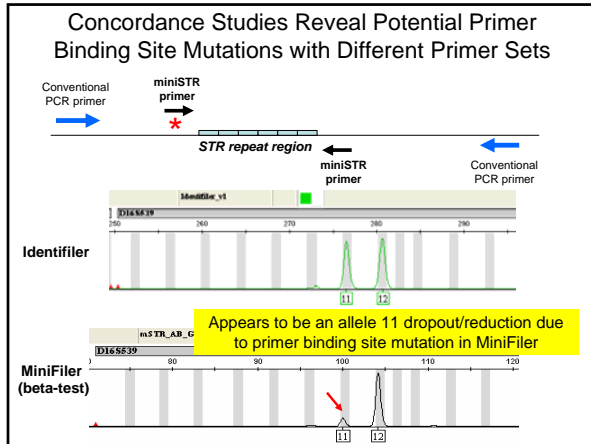
- Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5): 1054-1064.
- Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.
- Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. (2004) Concordance study between miniplex STR assays and a commercial STR typing kit. *J. Forensic Sci.* 49(4): 859-860.
- Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. *J. Forensic Sci.*, 50: 43-53.

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

More Recent miniSTR Publications

- Butler, J.M. (2006) MiniSTRs: past, present, and future. *Forensic News* (Applied Biosystems), October 2006
- Dixon, L.A., Dobbins, A.E., Pulker, H., Butler, J.M., Vallone, P.M., Coble, M.D., Parson, W., Berger, B., Grubweiser, P., Mogensen, H.S., Morling, N., Nielsen, K., Sanchez, J.J., Petkovski, E., Carracedo, A., Sanchez-Diz, P., Brion, M., Irwin, J.A., Just, R.S., Loreille, O., Parsons, T.J., Syndercombe-Court, D., Schmitter, H., Gill, P. (2006) Analysis of artificially degraded DNA using STRs and SNPs--results of a collaborative European (EDNAP) exercise. *Forensic Sci. Int.* 164: 33-44.
- Hill, C.R., Kline, M.C., Mulero, J.J., Lagace, R.E., Chang, C.-W., Hennessy, L.K., Butler, J.M. (2007) Concordance study between the AmpFISTR MiniFiler PCR Amplification Kit and conventional STR typing kits. *J. Forensic Sci.*, in press.
- Hill, C.R., Coble, M.D., Butler, J.M. (2007) Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples. *submitted.*





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**

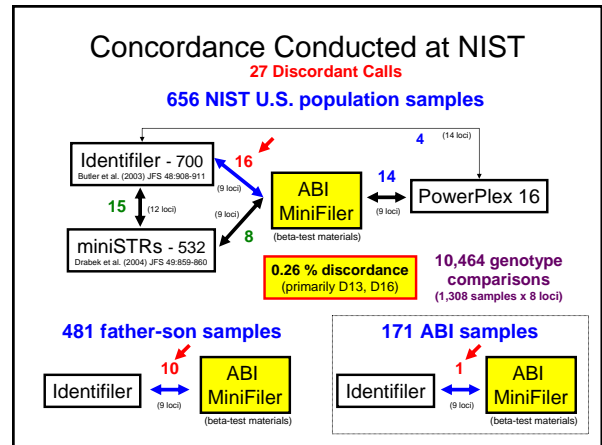
DNA extracted from whole blood (anonymous; self-identified ethnicities) received from Interstate Blood Bank (Memphis, TN) and Millennium Biotech Inc. (Ft. Lauderdale, FL)

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) MiniFiler (8 STRs + amelogenin)
 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

Summary of Samples Typed with ABI MiniFiler kit at NIST and ABI

- Primarily only population samples examined – no extensive sensitivity or degraded DNA tests were performed
- 1,308 samples** Allele concordance = 10,437/10,464 = 99.7%
- 656 NIST U.S. population samples**
 - 260 Caucasian, 253 African American, 140 Hispanic, 3 Asian
 - Previously examined with Identifiler; also with PowerPlex 16
 - Also tested with Butler et al. (2003) published miniSTR primers
 - <http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm>
- 481 father-son pairs**
 - 184 Caucasian, 196 African American, 101 Asian samples (provided by paternity testing company DDC)
 - Previously examined with Identifiler
- 171 samples from Applied Biosystems**



Apparent Null Alleles Observed During Concordance Studies

10/13 CODIS loci affected so far

Locus	STR Kit/Assay	Results	Reference
New Section of STRBase (launched to track MiniFiler discordance and allele dropout frequency): http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm			
D13S317	Identifiler vs miniplexes	Shift of alleles 10 and 11 due to deletion outside of miniplex assay	Butler et al. (2003), Drabek et al. (2004)
D16S539	PP1.1 vs PP16 vs COfiler	Loss of alleles with PP1.1; fine with PP16 and COfiler	Nelson et al. (2002)
D8S1179	PP16 vs ProPlus	Loss of alleles 15, 16, 17, and 18 with ProPlus; fine with PP16	Budowle et al. (2001)
FGA	PP16 vs ProPlus	Loss of allele 22 with ProPlus; fine with PP16	Budowle and Sprecher (2001)
D18S51	SGM vs SGM Plus	Loss of alleles 17, 18, 19, and 20 with SGM Plus; fine with SGM	Clayton et al. (2004)
CSF1PO	PP16 vs COfiler	Loss of allele 14 with COfiler; fine with PP16	Budowle et al. (2001)
TH01	PP16 vs COfiler	Loss of allele 9 with COfiler; fine with PP16	Budowle et al. (2001)
D21S11	PP16 vs ProPlus	Loss of allele 32.2 with PP16; fine with ProPlus	Budowle et al. (2001)

From Table 6.2 in J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, p. 136

Standard Reference Materials

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

Traceable standards to ensure accurate measurements in our nation's crime laboratories


Helps meet DAB Std. 9.5 and ISO 17025

SRM 2391b – CODIS STRs
 SRM 2392-I – mtDNA
 SRM 2395 – Y-STRs
 SRM 2372 – DNA quantitation

Working to update 2391b with new miniSTRs and 2395 with new Y-STRs

Calibration with SRMs enables confidence in comparisons of results between laboratories

Information Resource
<http://www.cstl.nist.gov/biotech/strbase>



Includes information on:
 Core STR loci
 Validation
 STR reference list
 NIST publications
miniSTRs
 Forensic SNPs
 Variant STR alleles
 Population data resources
 Addresses of scientists

Provides up-to-date information and has been used in court cases to support application of DNA technology

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 (Promega Corporation), vol. 9(2), pp. 3-6 / PROFILES IN DNA

VALIDATION

http://www.promega.com/profiles/902/ProfilesInDNA_902_03.pdf

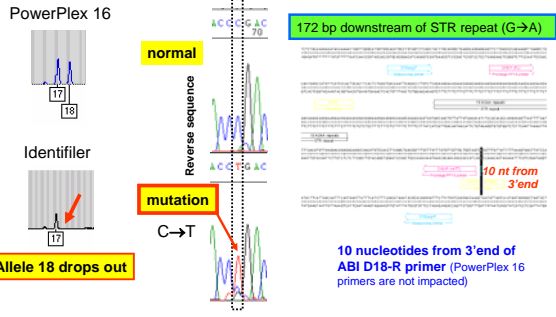
Debunking Some Urban Legends Surrounding Validation Within the Forensic DNA Community
 By John Butler
 National Institute of Standards and Technology, Gaithersburg, Maryland, USA

Variant Allele Sequencing Service (Free)
Send us any unusual variant or null alleles and we will sequence them...

Address: <http://www.cstl.nist.gov/biotech/strbase/STRSeq.htm>

Locus	Variant Allele	Sample Source	Comments
TPOX	10.3	Maryland State Police	Presence of a "G" that is 157 bp from the repeat region under PowerPlex 1.1 and Identifier 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 Identifier allele 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 CAAA repeats
D18S51	"5.3"	DNA Solutions	DNA sequence analysis revealed a 9 bp deletion beyond the end of the 18th repeat unit to produce a "5.3" allele
DYS392	"10.2"	AFDIL	DNA sequence analysis revealed a C-to-G transversion 100 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 47-8.5 bp genotyping bin.
DYS365	21.3	NIST U.S. population samples	DNA sequence analysis revealed a deletion of a "T" in the repeat region; full repeat was TCTAAGTGTATCTCTAAGTGTAA, TCTAAGTGTATCTCTAAGTGTAA
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 [AAAGAA] ₁₁ repeat
Penta D	6	Peter de Knijff's lab at Leiden University	DNA sequence analysis confirmed 6 repeats

D18S51 Null Allele from Kuwait Samples with ABI Primers



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

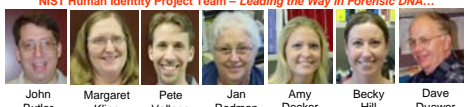
National Institute of Justice
 The Research, Development, and Evaluation Agency of the U.S. Department of Justice

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, expert system review
- **Training Materials**
 - Review articles and workshops on STRs, CE, validation
 - PowerPoint and pdf files available for download

<http://www.cstl.nist.gov/biotech/strbase/NIJprojects.htm>

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



Past and Present Collaborators (also funded by NIJ):
 Mike Hammer and Alan Redd (U. AZ) for Y-chromosome studies
 Tom Parsons, Rebecca Just, Jodi Irwin (AFDIL) for mtDNA coding SNP work
 Sandy Calloway (Roche) for mtDNA LINEAR ARRAYS
 Bruce McCord and students (FL Int. U.) for miniSTR work
 Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work
 Arnie Eisenberg and John Planz (U. North Texas) for miniSTR testing on bones
 Murray Brilliant (U. AZ) for phenotype markers
 Ken Kidd (Yale U.) for SNP typing population samples
 Sree Kanthaswamy (UC Davis) for dog STR multiplex assay
 Tom Reid (DNA Diagnostics Center) for father-son samples

Disclaimer: 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.

Thank you for your attention...

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

Questions?



See also <http://www.dna.gov/research/nist>
<http://www.cstl.nist.gov/biotech/strbase>
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