EDNAP and 30th ENFSI DNA WG Meeting
April 22-24, 2009 – Lisbon, Portugal

NIST Update

John M. Butler
and the NIST Human Identity Project Team
National Institute of Standards and Technology
Gaithersburg, Maryland USA

NIST Human Identity Project Team

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


John Butler
Group Leader


Amy Decker


Becky Hill


Margaret Kline


Jan Redman


Pete Vallone


Dave Duewer
(data analysis)


Angie Dolph
(summer 2007)


Michelle Burns
(summer 2008+)

And many wonderful collaborators...

Since 2000:
>100 publications
>250 presentations
>30 training workshops

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

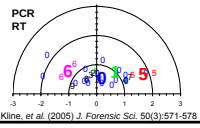
Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Current Activities at NIST

- **Standard Reference Materials**
  - SRM 2372 (DNA quantitation standard) (>225 units in use since Oct 2007)
  - Adding extended ESS loci (D1, D12, D2, D10, D22) to SRM 2391b
- **Technology Evaluation and Development**
  - Rapid multiplex PCR protocols (multiplex STR amplification in <35 min)
  - Low-level DNA studies underway
  - Mixture interpretation – research and training materials
  - Unusual STR allele characterization
  - New STR loci and assays (STR 26plex, kit concordance, SNP testing)
- **Training Materials**
  - Workshops on mixture interpretation and CE troubleshooting
  - Third edition of *Forensic DNA Typing* textbook (2009 & 2011)

SRM 2372: Human DNA Quantitation Standard

- Released in Oct 2007
  - >225 units in use as of April 2009
- Used by more than 110 forensic laboratories worldwide
- Manuscript describing production is in press with *Anal. Bioanal. Chem.*
- **Serves to adjust qPCR calibrants supplied by manufacturers and adjust for assay-specific bias**



Kline, *et al.* (2005) *J. Forensic Sci.* 50(3):571-578

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)

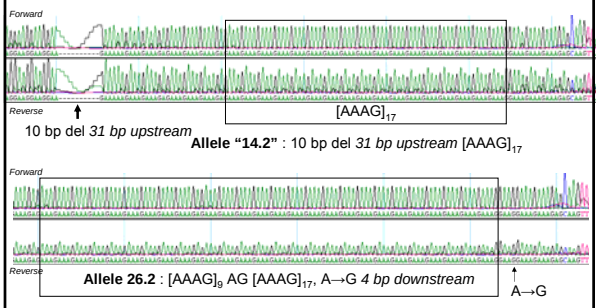
  http://www.cstl.nist.gov/biotech/strbase/srm2395.htm

Revised Certificates available since September 5, 2008

D1S1656 and D12S391 Results with SRM 2391b Components

	D1S1656 Type	D1S1656 Repeat motif		D12S391 Type	D12S391 Repeat motif
1	13	[TAGA]13[TG]5	1	15	[AGAT]8 [AGAC]6 AGAT
	14	[TAGA]13 TAGG [TG]5		18	[AGAT]11 [AGAC]6 AGAT
2	12	[TAGA]12[TG]5	2	17	[AGAT]10 [AGAC]6 AGAT
	17.3	[TAGA]4 TGA [TAGA]12 TAGG [TG]5		22	[AGAT]13 [AGAC]9
3	14	[TAGA]13 TAGG [TG]5	3	15	[AGAT]8 [AGAC]6 AGAT
	15	[TAGA]14 TAGG [TG]5		21	[AGAT]12 [AGAC]9
4	15	[TAGA]14 TAGG [TG]5	4	17	[AGAT]11 [AGAC]5 AGAT
	17.3	[TAGA]4 TGA [TAGA]12 TAGG [TG]5		17	[AGAT]10 [AGAC]6 AGAT
5	11	[TAGA]11[TG]5	5	18	[AGAT]11 [AGAC]6 AGAT
	16.3	[TAGA]4 TGA [TAGA]11 TAGG [TG]5			
6	11	[TAGA]11[TG]5	6	21	[AGAT]11 [AGAC]10
	17	[TAGA]16 TAGG [TG]5		22	[AGAT]12 [AGAC]10
7	12	[TAGA]12[TG]5	7	17	[AGAT]10 [AGAC]6 AGAT
	17.3	[TAGA]4 TGA [TAGA]12 TAGG [TG]5		20	[AGAT]10 [AGAC]9 AGAT
8	14	[TAGA]13 TAGG [TG]5	8	18	[AGAT]11 [AGAC]6 AGAT
	16.3	[TAGA]4 TGA [TAGA]11 TAGG [TG]5		24	[AGAT]15 [AGAC]9
9	18.3	[TAGA]4 TGA [TAGA]13 TAGG [TG]5	9	18	[AGAT]11 [AGAC]6 AGAT
				20	[AGAT]12 [AGAC]7 AGAT
10	14	[TAGA]13 TAGG [TG]5	10	18	[AGAT]11 [AGAC]6 AGAT
	17	[TAGA]16 TAGG [TG]5		24	[AGAT]13 [AGAC]6

SE33 Sequence Data for SRM 2391b Component 3



SE33 Genotype = 14.2, 26.2

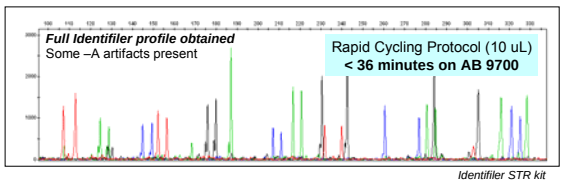
SE 33 Results for SRM 2391b Components

Component #	Genotype	Sequencing Results
1	20	$[AAAG]_{20}$
	30.2	$[AAAG]_{13}$ AAAAAG $[AAAG]_{16}$
2	23.2	$[AAAG]_7$ AA $[AAAG]_{16}$
	28.2	$[AAAG]_9$ AAAAAG $[AAAG]_{18}$
3	"14.2"	10 bp del 31 bp us $[AAAG]_{17}$ G?A 4 bp ds
	26.2	$[AAAG]_9$ AG $[AAAG]_{17}$
4	"22"	$[AAAG]_{21}$ AAAG ins13 bp ds
	28.2	$[AAAG]_9$ AAAAAG $[AAAG]_{18}$
5	14	$[AAAG]_{14}$
	30.2	$[AAAG]_{11}$ AAAAAG $[AAAG]_{18}$
6	20	$[AAAG]_{20}$
	21	*Inconclusive; not completed yet*
7	"13.2"	14 bp del 11 bp us $[AAAG]_{17}$ G?A 4 b p ds
	20	$[AAAG]_{20}$ G?A 4 b p ds
8	16	$[AAAG]_{16}$
	27.2	$[AAAG]_{12}$ AAAAAG $[AAAG]_{14}$
9	19	$[AAAG]_{19}$
	29.2	$[AAAG]_{13}$ AAAAAG $[AAAG]_{15}$
10	23.2	$[AAAG]_{12}$ AAAAAG $[AAAG]_{10}$
	26.2	$[AAAG]_{11}$ AG $[AAAG]_{15}$

us : upstream from the repeat ds : downstream from the repeat

Rapid Multiplex PCR Protocols Under Development

Utilizing AB 9700 cycler and 'fast' commercial enzymes



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"

More recent results on a different thermal cycler in as little as ~15 minutes…

What is "Rapid PCR"?

Current STR kits were optimized by manufacturers for slower PCR (~3 hours)

- Use of new commercial DNA polymerases

  - **Replace the current standard polymerase** (AmpliTaq Gold) and buffer but **keep commercial STR kit primer mixes**
    - rapid hot start (save ~10min)
    - 'faster' nucleotide incorporation (processivity >100 bases/sec)
- Use with common thermal cycler (GeneAmp 9700)

  - **Utilize maximum ramp rate of 4 °C/sec with 9700**
  - Shorten cycling hold times (to 1-5 sec vs 1 min)
  - Eliminate 60 °C adenylation soak (to save ~30-60 min)
- **Explore possibilities with faster thermal cyclers** (e.g., 10 °C/sec ramp) and possibly new primer mixes

Goal: to obtain full STR profiles in as little time as possible (<30 min?)

Rapid PCR Article

Forensic Science International: Genetics 3 (2008) 42–45

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Short communication

Demonstration of rapid multiplex PCR amplification involving 16 genetic loci☆

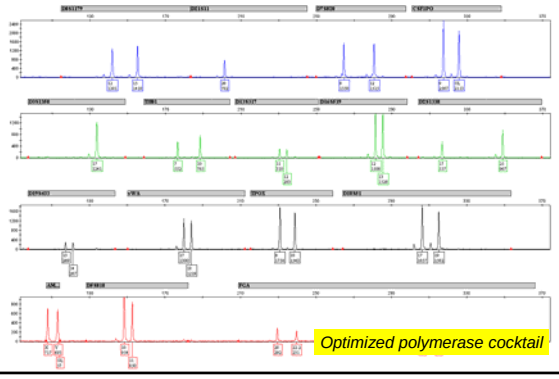
Peter M. Vallone *, Carolyn R. Hill, John M. Butler

National Institute of Standards and Technology, Biochemical Science Division, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899-8311, United States

Complete concordance of STR allele calls (for 60 samples) between the rapid and standard thermal cycling protocols were observed although there was incomplete adenylation at several of the loci examined and some PCR artifacts were detected. Using less than **750 pg of template DNA and 28 cycles, STR peaks for all loci were above a 150 relative fluorescent unit (RFU) detection threshold** with fully adequate inter-locus balance and heterozygote peak height ratios of greater than 0.84.

Identifiler Profile from 17 minute PCR

Further details will be given at the ISFG meeting in Buenos Aires

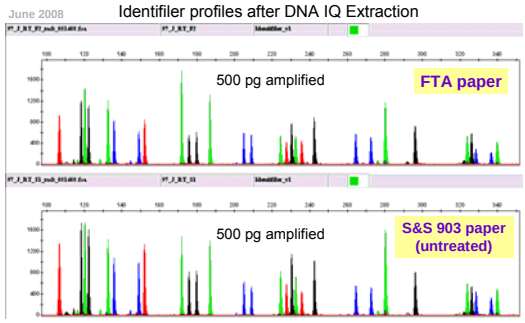


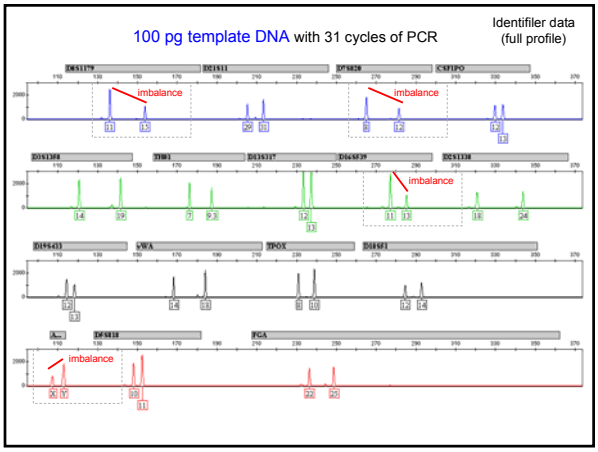
Optimized polymerase cocktail

Potential Applications with Rapid PCR Capabilities

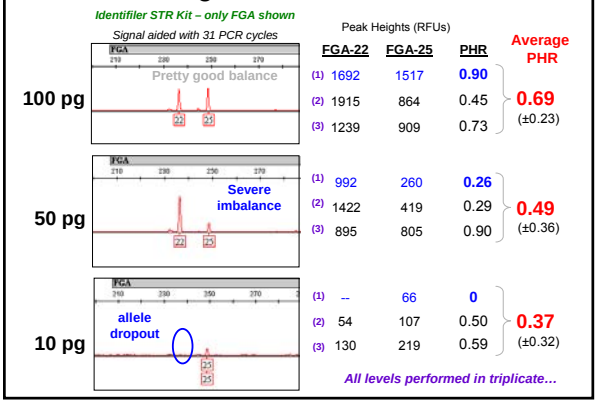
- **Improve overall laboratory throughput**
  - Multiplex PCR amplification is already in many situations the longest part of the DNA analysis process (depending on DNA extraction and DNA quantitation methods)
  - With increased use of robotic sample preparation and expert system data analysis, bottleneck for sample processing will shift to time for PCR amplification…
- **Enable new potential DNA biometric applications** (because the overall DNA analysis process is faster)
  - Permit analysis of individuals at a point of interest such as an embassy, an airport, or a country border

Room Temperature Storage of Dried Bloodstains 11 year time point

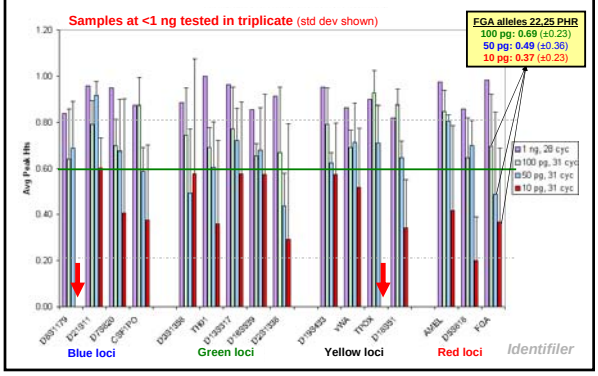


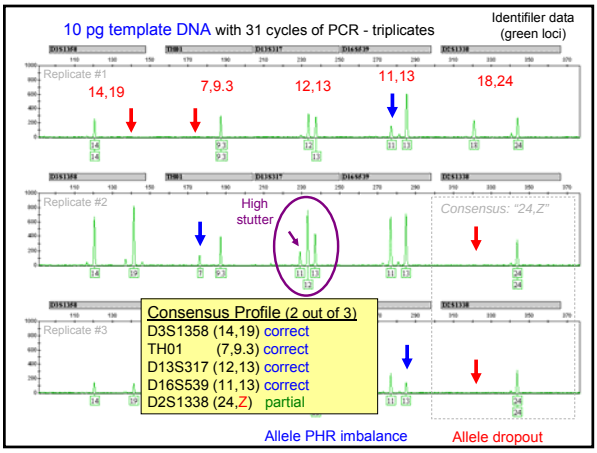


Peak Height Ratio Measurements



Peak Height Ratio Comparisons





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

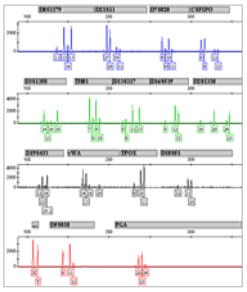
Mixture Analysis Efforts at NIST

- Interlaboratory Studies: MSS1,2,3 and MIX05
  - **Future ones planned** when software tools and guidelines are available
- Software testing (see posters from AAFS 2008 and Promega 2008)
  - DNA_DataAnalysis (USACIL) – user's manual written
  - FSS-i3 (Promega)
  - Web-LSD (UTenn)
  - GeneMapper *ID-X* v1.1 (ABI)
  - GenoProof Mixture 1.0 (Qualitype)

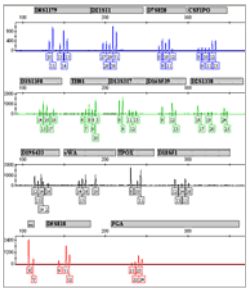
  Some conversations with Mark Perlin regarding TrueAllele 3 software
  Some work coordinated with NEST Project (Marshall University)
- Work with SWGDAM Mixture Committee
  - Case summaries
- Training workshops and discussion groups
  - AAFS Feb 2008, MD Apr 2008, FDLE May 2008, MD Dec 2008, AFDIL Jan 2009, Houston Jan 2009, NYC Mar 2009

Creating Known Mixtures for Testing Software Tools

NIST 2-person mixture
(Identifiler data, 1ng DNA, **1:5**)



NIST 3-person mixture
(Identifiler data, 1ng DNA, **5:2:1**)



Mixtures were created for research purposes and are synthetic mixtures of extracted DNA created in a controlled environment without PCR inhibitors or an unknown amount of degraded DNA as may be found in forensic casework.

Mixture Interpretation Workshop

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

AAFS (February 19, 2008)

DNA Mixture Interpretation: Principles and Practice in Component Deconvolution and Statistical Analysis

196 page handout

- **John Butler (NIST)**
- Ann Gross (MN)
- George Carmody (Carleton U.)
- Gary Shutler (WA)
- Joanne Sgueglia (MA)
- Angela Dolph (Marshall U./NIST)
- Tim Kalafut (USACIL)

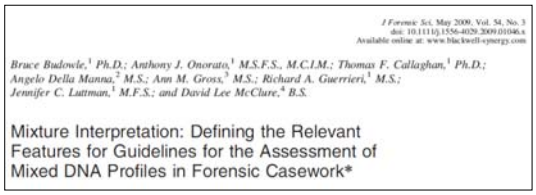
Some Recent Mixture Workshops

60 DNA analysts from 16 labs (all MD labs, DE, NMS)

25 DNA analysts from AFDIL

Mixture Interpretation and Other Topics January 27-28, 2009
50 DNA analysts from Harris County, Texas (HPD, MEO, TX DPS)

Budowle/FBI Mixture Paper



J Forensic Sci, May 2009, Vol. 54, No. 3
doi: 10.1111/j.1556-4029.2009.01046.x
Available online at: www.blackwell-synergy.com

Bruce Budowle,[1] Ph.D.; Anthony J. Onorato,[1] M.S.F.S., M.C.I.M.; Thomas F. Callaghan,[1] Ph.D.; Angelo Della Manna,[2] M.S.; Ann M. Gross,[3] M.S.; Richard A. Guerrieri,[1] M.S.; Jennifer C. Luttman,[1] M.F.S.; and David Lee McClure,[4] B.S.

Mixture Interpretation: Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles in Forensic Casework*

Does NOT represent the opinion of the SWGDAM Mixture Committee as guidelines are still in discussion and development *(will be at least Jan 2010 before anything will be voted on by SWGDAM)*

Mixture Case Summaries

During 2007 and early 2008, **Ann Gross** (MN BCA) from the SWGDAM Mixture Interpretation Committee **coordinated the collection of case summary data** from **14 different forensic labs** who collectively reported on **4780 samples**. A preliminary summary of this information is shown below divided by crime classifications: sexual assault, major crime (homicide), and high volume (burglary). **Over half of the samples examined were single source and ~75% of all reported mixtures were 2-person.**

	minimum # of contributors					
Crime Class	**1**	**2**	**3**	**4**	**>4**	**N**
Sexual Assault	884	787	145	11	0	1827
Major Crime	1261	519	182	32	0	1994
High Volume	344	220	140	11	5	720
Total	2489	1526	467	54	5	4541
Single source	54.8%	33.6%	10.3%	1.2%	0.1%	mixtures

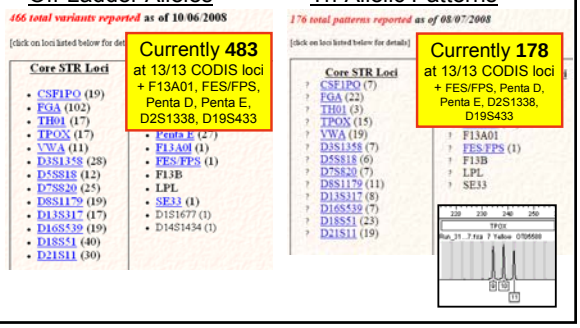
http://www.cstl.nist.gov/biotech/strbase/pub_pres/Promega2008poster.pdf

Variant Alleles Cataloged in STRBase

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

Off-Ladder Alleles

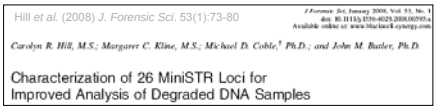
Tri-Allelic Patterns



New STR Loci and Assays

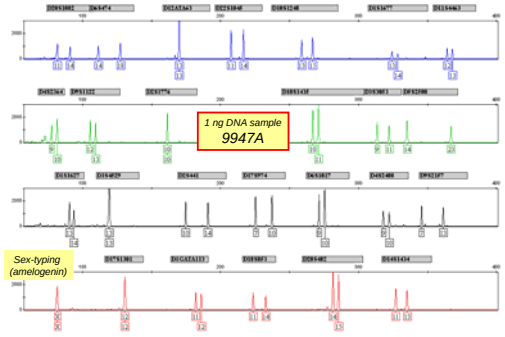
Usefulness of new STR loci:

- **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 (25 STRs are recommended)



NIST 26plex

Gender identification + 25 autosomal STR loci in a single amplification



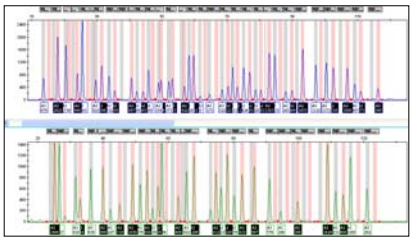
Hill, C.R., et al. (2009) A new STR 26plex assay for human identity testing. *J. Forensic Sci.* (in press)

Primer sequences and GM bins & panels available: **http://www.cstl.nist.gov/biotech/strbase/str26plex.htm**

SNPlex Interlaboratory Study

- In the process of running 48plex assay
- Samples and reagents sent from Niels' lab
- AB SNPlex typing platform
- SNP*for*ID loci

500 pg DNA from Copenhagen



Ancestry Informative Markers

- Working with **Manfred Kayser** (Netherlands)
  - Set of Ancestry Informative Markers (AIMs)
  - NIST developed assays for typing 24 SNPs
  - Typed 711 NIST US population samples

24 SNPs in two 12plex SNP assays

- **Peter deKnijff** (Netherlands)
  - Performing Y SNP typing

28 defined Y haplogroups

- **Michael Coble** (AFDIL)
  - mitochondrial control region + selective coding region sequencing

128 defined mito haplogroups

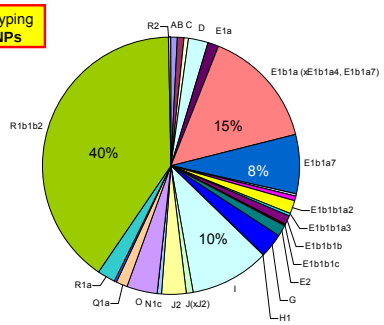
Correlation between AIMs & lineage markers with Self-identified ancestry in NIST US samples

Frequencies of Y and Mito haplogroups observed in NIST US samples

Y-haplogroups for 711 U.S. Samples

Self-declared African Americans, Caucasians, Hispanics, Asians

Based on typing **~35 Y-SNPs**



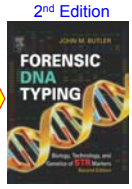
Forensic DNA Typing Textbook

1st Edition

Jan 2001
335 pp.
17 chapters


With Y. Fukuma
(Japanese translator)

2nd Edition



Feb 2005
688 pp.
24 chapters

Now available in **Chinese**
(Yiping Hou)

Japanese in preparation
(Yoshiya Fukuma)

3rd Edition

Fundamentals
Chapters 1-18
Sept 2009

Advanced Topics
Chapters 1-25
Feb 2011

Planned for 2009 & 2011
~1000 pages


With Y. Hou (Chinese translator)

Forensic Science Review Article

See June 15, 2009 issue of *Analytical Chemistry*

Anal. Chem. **2007**, *79*, 4365–4384

Forensic Science

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Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311

J. R. Almirall
Department of Chemistry and Biochemistry and International Forensic Research Institute, Florida International University, University Park, Miami, Florida 33199

2009 review article covers 160 DNA articles published in 2007-2008

A new group was formed in Oct 2008 with an expanded mission

NIST
Applied Genetics

Applied Genetics Group
Mission Statement

Advancing technology and traceability through quality genetic measurements to aid work in

- **forensic DNA testing,**
- **clinical genetics,**
- **agricultural biotechnology,** and
- **DNA biometrics.**


Harry T. Edwards
U.S. Court of Appeals (DC)
Co-Chair, Forensic Science Committee

National Academies Report on Forensic Science

- Released February 18, 2009
- Entitled "Strengthening Forensic Science in the United States: A Path Forward"
- 13 recommendations provided to Congress
- **Recommends establishing a National Institute of Forensic Science (NIFS)**
- **NIST will have a role in NIFS and our group has been asked to contribute expertise** regarding validation and testing of DNA systems as a model for other forensic disciplines

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

The Future of Forensic DNA

is Similar to the Olympic Motto of
"Swifter, Higher, Stronger"

Resources Training Action

Thank you for your attention!

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


Margaret Kline
STR allele sequencing


Jan Redman
Variant allele cataloging


Becky Hill
miniSTRs, LCN, and 26plex work


Amy Decker
Mixtures & Y-STRs


Pete Vallone
SNPs & Rapid PCR


Dave Duewer
Data analysis tools

http://www.cstl.nist.gov/biotech/strbase
john.butler@nist.gov
301-975-4049