NIST Update

John M. Butler
and Human Identity Project Team
National Institute of Standards and Technology

National CODIS Conference
Crystal City, VA
November 10, 2008

Mark Stolorow is now Director of the NIST Office of Law Enforcement Standards (OLES)

Mark Stolorow
(formerly of Orchid Cellmark)

http://www.eeel.nist.gov/oles/directory.html

Sue Ballou
(Forensics)

Robert Thompson
(recent hire for forensics)

Forensics Research at NIST
Computer (digital) forensics
Ballistics
Fingerprints
Arson investigation
DNA

http://www.eeel.nist.gov/oles/forensics.html

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+)

Current Collaborators
Mike Coble (AFDIL) **Tom Hall** (IBIS)
Bruce McCord (FIU) **Danielle Podini** (GWU)
Tom Reid (DDC)
Manfred Kayser (The Netherlands)

Since CODIS 2007 Conf:
12 publications
25 presentations
8 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

NIST Human Identity Team Projects

Funded by the National Institute of Justice

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

Projects

33 different projects are described

[Human DNA Quantitation] [Mitochondrial DNA] [Y Chromosome] [Compromised DNA Evidence] [Miniaturization and Automation] [General Tools and Information] [Non-Human DNA] [Alternative Forensic DNA Markers]

Alphabetical Listing of Projects

ABI 3100 performance with various STR typing systems (April 2001-June 2003)

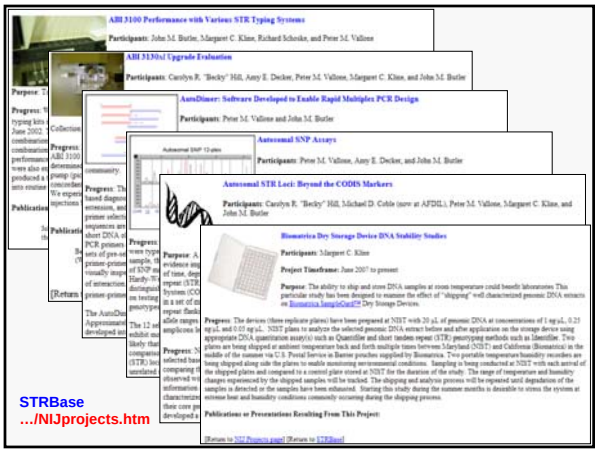
ABI 3130xl upgrade evaluation (Sept 2005-May 2006)

AutoDimer: software to enable rapid multiplex PCR design (2000-2005) [see also software.htm]

Autosomal SNP loci (July 2002-present)

Autosomal STR loci: beyond the CODIS markers (Jan 2004-present) [see also newSTRs.htm]

Biomatrica dry storage device DNA stability studies (June 2007-present)



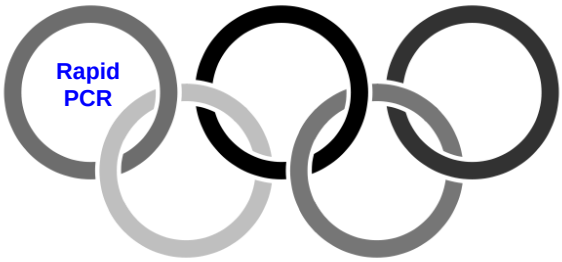
STRBase
…/NIJprojects.htm

Our Research Efforts are Similar to the Olympic Motto of "Swifter, Higher, Stronger"

Better, faster, cheaper…DNA results to aid the forensic community


Pete Vallone


Michelle Burns
(summer 2008+)



What is "Rapid PCR"?

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

- Use of new commercial DNA polymerases

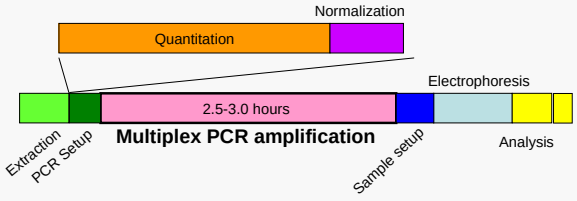
  - **Replace the current standard polymerase** (AmpliTaq Gold) and buffer but **keep commercial STR kit primer mixes**
    - rapid hot start (save ~10min)
    - 'faster' nucleotide incorporation (processivity >100 bases/sec)
- Use with common thermal cycler (GeneAmp 9700)

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

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

Relative Time for Overall DNA Process

Current Time is ~8-10 hours (with 5-6 hours if no quantitation performed)



Innovations and Improvements in Speed

Potential Applications with Rapid PCR Capabilities

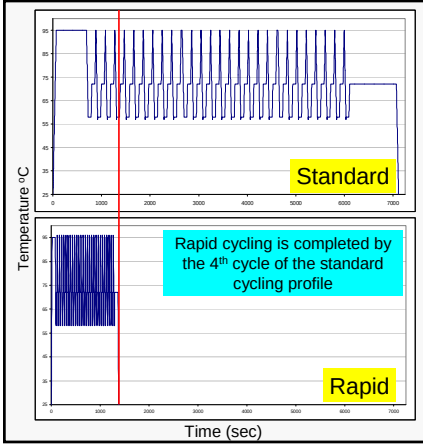
- **Improve overall laboratory throughput**
  - Multiplex PCR amplification is already in many situations the longest part of the DNA analysis process (depending on DNA extraction and DNA quantitation methods)
  - With increased use of robotic sample preparation and expert system data analysis, bottleneck 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

NYC Forensic DNA "X-Prize"

January 17, 2008 Press Release
From Mayor Bloomberg's **STATE OF THE CITY ADDRESS**

"The City will establish **a six-figure prize** for anyone who can invent **a device** tailored to the NYPD **which analyzes the DNA of potential suspects right at the crime scene** - so that officers can release innocent suspects before they are arrested, and track down promising leads more quickly"

http://home2.nyc.gov/html/om/html/2008a/pr017-08.html



Comparison of Temperature Tracking during thermal cycling on a AB 9700

Comparison of Thermal Cycling Times

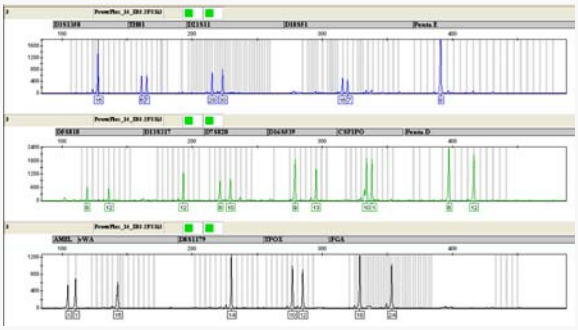
Parameter	Unit	Trad	Rapid	Difference (min)	%
Hot Start	Min	10	1	9.0	6.3
Hold	Sec	60	5/10	72.3	50.6
Soak	Min	60	1	59.0	41.2
Ramp rate	(deg/sec)	1	4	22.4	15.7
				Saving 2 hours, 23 minutes	
Cycles		28	28		
Time		2:58:41	0:35:38	2:23:03	

Overall time reduction on GeneAmp 9700 from 3 hours to 35 minutes

Parameter	Purpose
Hot Start	Primer Dimer, non-specific amplification
Hold	Denature, annealing, elongation, Inter and intra locus balance
Soak	Full adenylation of PCR products

Rapid DNA Polymerases used: **PyroStart** (Fermentas, Glen Burnie, MD) and **SpeedSTAR** (Takara Bio USA, Madison, WI)

PowerPlex 16 Rapid Cycling

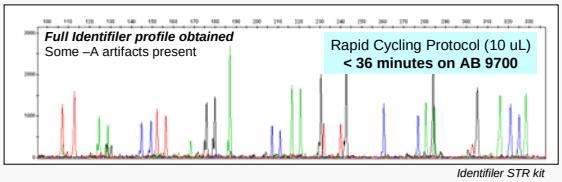


Rapid DNA Polymerases used: **PyroStart** (Fermentas, Glen Burnie, MD) and **SpeedSTAR** (Takara Bio USA, Madison, WI)

< 36 minutes on AB 9700

Rapid Multiplex PCR Protocols Under Development

Utilizing AB 9700 cycler and 'fast' commercial enzymes
Manuscript in press with FSI Genetics

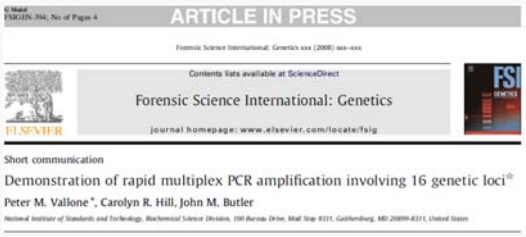


Identifiler STR kit
28 cycles, 1ng template DNA

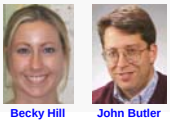
Initial results presented by at 60th Annual Meeting of the American Academy of Forensic Sciences (Washington, DC), February 23, 2008, "Developing Rapid PCR Multiplex Assays with miniSTR Loci"

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

Rapid PCR Article

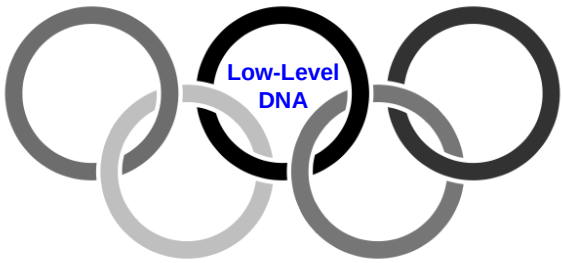


Complete concordance of STR allele calls (for 60 samples) between the rapid and standard thermal cycling protocols were observed although there was incomplete adenylation at several of the loci examined and some PCR artifacts were detected. Using less than **750 pg of template DNA and 28 cycles, STR 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.



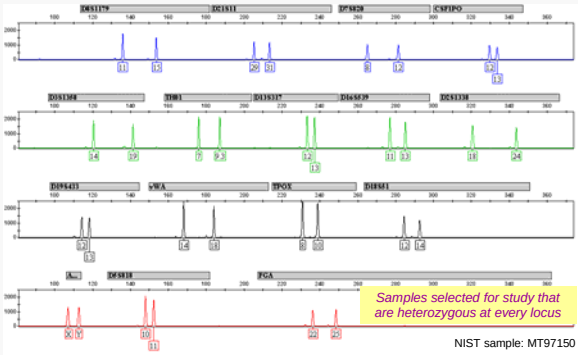
Becky Hill

John Butler



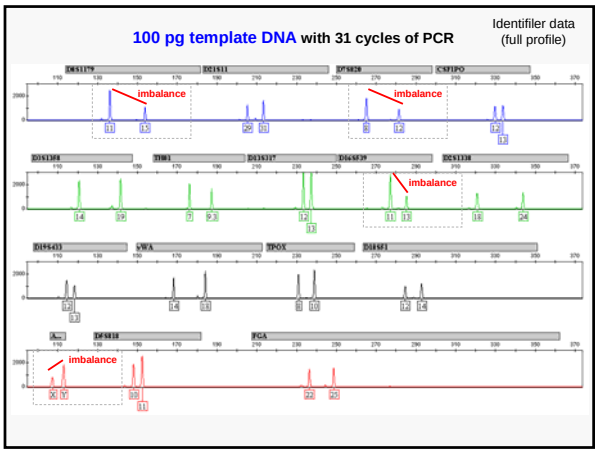
1 ng template DNA, 28 cycles

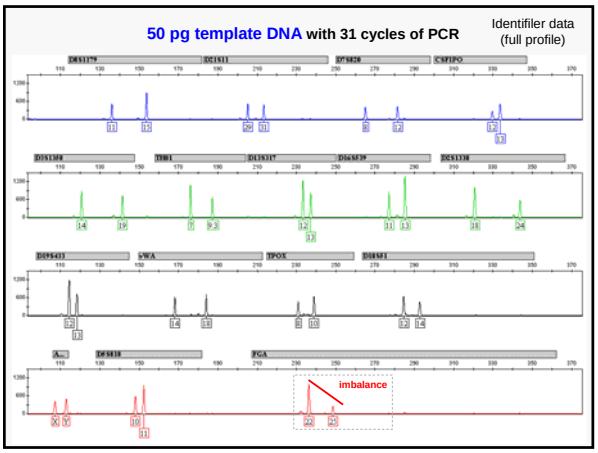
Identifiler data (full profile)

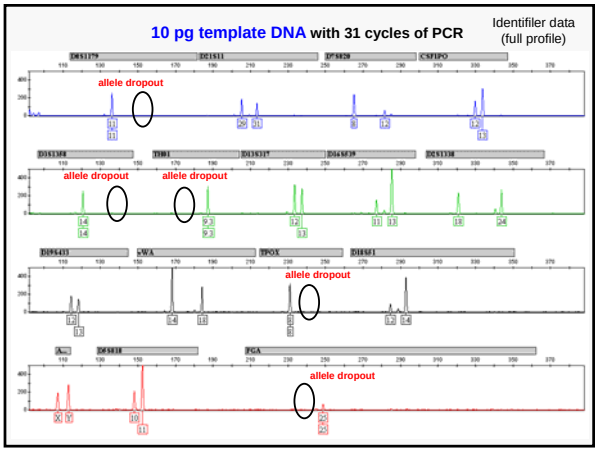


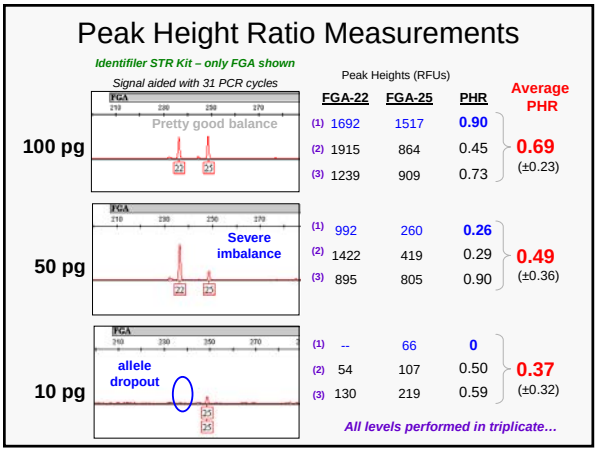
NIST sample: MT97150

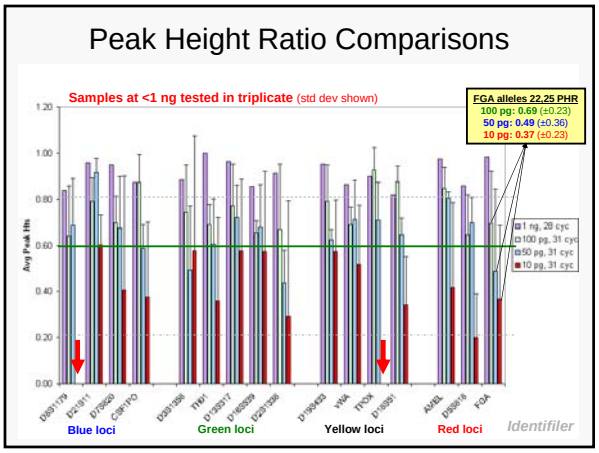
Peak Height Ratios (PHRs) all >0.80

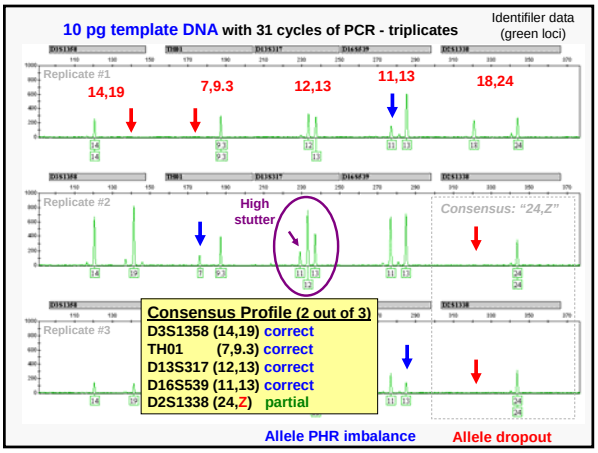

100 pg template DNA with 31 cycles of PCR
Identifiler data (full profile)
imbalance
imbalance
imbalance
imbalance

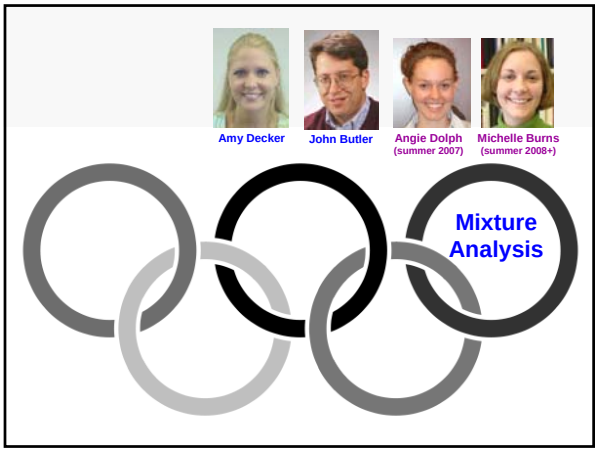

50 pg template DNA with 31 cycles of PCR
Identifiler data (full profile)
imbalance


10 pg template DNA with 31 cycles of PCR
Identifiler data (full profile)
allele dropout
allele dropout
allele dropout
allele dropout
allele dropout


Peak Height Ratio Measurements
Identifiler STR Kit – only FGA shown
Signal aided with 31 PCR cycles
Peak Heights (RFUs)
FGA-22 FGA-25 PHR
Average PHR
100 pg
Pretty good balance
(1) 1692 1517 0.90
(2) 1915 864 0.45
(3) 1239 909 0.73
0.69 (±0.23)
50 pg
Severe imbalance
(1) 992 260 0.26
(2) 1422 419 0.29
(3) 895 805 0.90
0.49 (±0.36)
10 pg
allele dropout
(1) -- 66 0
(2) 54 107 0.50
(3) 130 219 0.59
0.37 (±0.32)
All levels performed in triplicate...


Peak Height Ratio Comparisons
Samples at <1 ng tested in triplicate (std dev shown)
FGA alleles 22,25 PHR
100 pg: 0.69 (±0.23)
50 pg: 0.49 (±0.36)
10 pg: 0.37 (±0.32)
Avg Peak Hts
1 ng, 28 cyc
100 pg, 31 cyc
50 pg, 31 cyc
10 pg, 31 cyc
Blue loci
Green loci
Yellow loci
Red loci
Identifiler


10 pg template DNA with 31 cycles of PCR - triplicates
Identifiler data (green loci)
Replicate #1
14,19
7,9.3
12,13
11,13
18,24
Replicate #2
High stutter
Consensus: "24,Z"
Replicate #3
Consensus Profile (2 out of 3)
D3S1358 (14,19) correct
TH01 (7,9.3) correct
D13S317 (12,13) correct
D16S539 (11,13) correct
D2S1338 (24,Z) partial
Allele PHR imbalance
Allele dropout



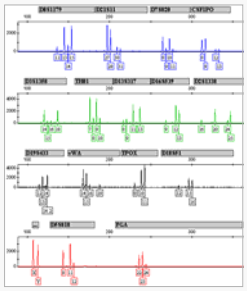
Mixture Analysis Efforts at NIST

- 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)

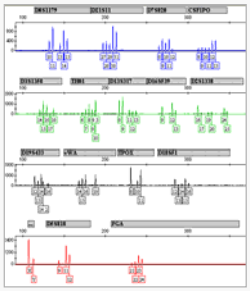
  Some conversations with Mark Perlin regarding TrueAllele 3 software
  Some work coordinated with NEST Project (Marshall University)
- Work with SWGDAM Mixture Committee
  - Case summaries
- Training workshops and discussion groups
  - AAFS Feb 2008, MD Apr 2008, FDLE May 2008

Creating Known Mixtures for Testing Software Tools

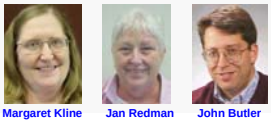
NIST 2-person mixture
(Identifiler data, 1ng DNA, **1:5**)

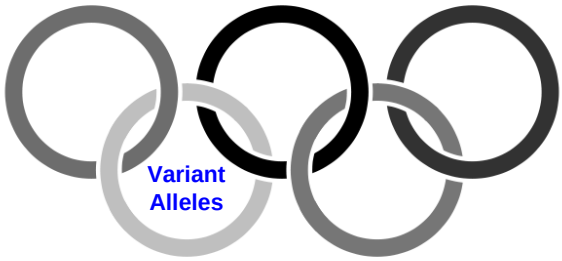


NIST 3-person mixture
(Identifiler data, 1ng DNA, **5:2:1**)



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.



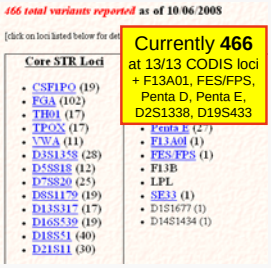


Variant Alleles Cataloged in STRBase

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

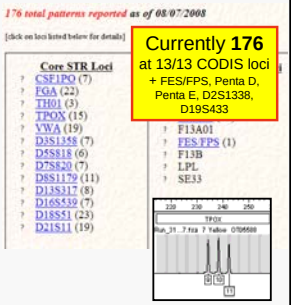
Off-Ladder Alleles

Currently **466** at 13/13 CODIS loci + F13A01, FES/FPS, Penta D, Penta E, D2S1338, D19S433



Tri-Allelic Patterns

Currently **176** at 13/13 CODIS loci + FES/FPS, Penta D, Penta E, D2S1338, D19S433



Sample Submissions

- For those that desire more assurances of confidentiality we can have MOUs signed.
- We generally re-type the samples at NIST prior to starting sequencing.
- We may run a monoplex assay (single locus).
- We return results as PowerPoint slides.
- We thank all of those agencies that have used this free service (thanks to NIJ)!
- Contact Margaret Kline: **margaret.kline@nist.gov**

Penta D 10, Variant Allele 19

All sequenced bases align before and after the repeat region. The 19 allele has been previously reported in STRBase. The Penta D ladder has Alleles 2.2, 3.2, 5, 7 – 17 represented.

In FY2008, **21 variant allele samples** were submitted for NIST sequence analysis

Summary of Variant Alleles Sequenced *(only 15 shown)*

Locus	Allele	Repeat Motif
D2S1338	12	$[TGCC]_4[TTCC]_8$
D2S1338	31	$[TGCC]_7[TTCC]_6$**TTAC** $[TTCC]_{14}$ **GTCC** $[TTCC]_2$
D3S1358	16.2	TCTA $[TCTG]_3$ **TC** $[TCTA]_{12}$
D3S1358	20	TCTA $[TCTG]_3$ $[TCTA]_{16}$
D3S1358	23	TCTA $[TCTG]_3$ $[TCTA]_{19}$
D5S818	10.1	**A**$[AGAT]_{10}$
D5S818	"29"	$[AGAT]_{12}$ **+68 bp**
D7S820	8.3	$[GATA]_9$ del **A** 22 bp DS
D16S539	11	$[GATA]_{11}$ (U83**T→C**) results in an anomalous migration 11/10.3
D18S51	15.2	$[AGAA]_7$ **AA** $[AGAA]_8$
D21S11	"24.3"	$[TCTA]_5$ $[TCTG]_6$ $[TCTA]_3$ TA $[TCTA]_3$ TCA $[TCTA]_2$ TCCATA $[TCTA]_9$ del 13 bp, 11 bp DS (28 allele -13 bp)
D21S11	28.1	$[TCTA]_5$ $[TCTG]_6$ $[TCTA]_3$ TA $[TCTA]_3$ TCA $[TCTA]_2$ TCCATA $[TCTA]_9$ **+T**
FGA	50	$TTTC]_4$ TTTT $[TTCT]_6$ TTTT $[CTTT]_{12}$ CTGT $[CTTT]_{14}$ $[CTTC]_3[CTTT]_3$ CTCC $[TTCC]_4$
Penta E	27	$[AAAGA]_{27}$
DYS389II	29.1	$[TCTG]_4$ $[TCTA]_{13}$ $\mathbf{N_{30}}$ $[TCTG]_3$ $[TCTA]_9$ or $[TCTG]_4$ $[TCTA]_{14}$ $\mathbf{N_{30}}$ $[TCTG]_3$ $[TCTA]_9$ (D3Tins = **+T** 3bp downstream)

D7S820 "8.3",10

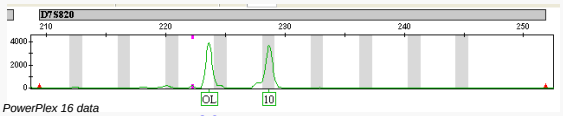
"8.3" allele
9 repeat units with a deletion 22 bases downstream from the repeat region

Deletion A

GATA GATA GATA GATA GATA GATA GATA GATA GACAGATTGATAGTTTTTTTTA_TCTC

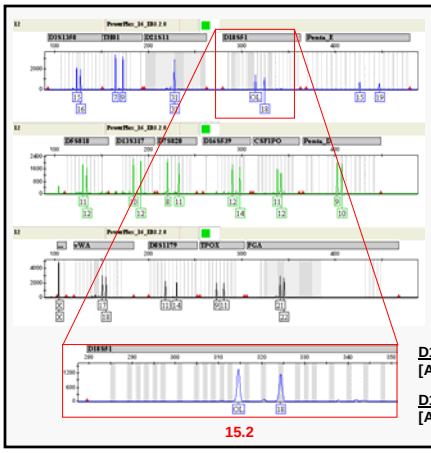
Normal 10 allele

GATA GATA GATA GATA GATA GATA GATA GATA GATA GACAGATTGATAGTTTTTTTTAATCTC



PowerPlex 16 data

8.3



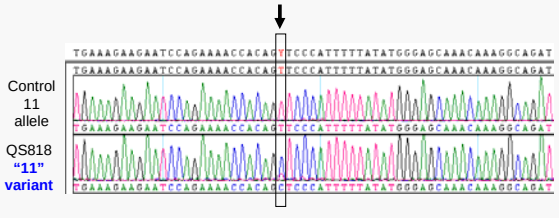
D18S51
15.2,18

D18S51 Allele 15.2
[AGAA]$_7$ AA [AGAA]$_8$

D18S51 Allele 18
[AGAA]$_{18}$

15.2

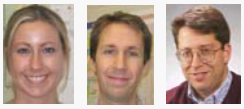
D16S539 Variant Causing Problems during a Recent Proficiency Test



An "11" variant allele that flip-flops between an 11 and "off-ladder" allele depending on electrophoresis conditions
(a sample in Collaborative Testing Services Test No. 07-572: Forensic Biology)

Sample has repeat region with $[GATA]_{11}$ but a base change of T→C 83 nucleotides upstream from the repeat (sequence shown above).

Second allele is a nominal 13 allele, $[GATA]_{13}$

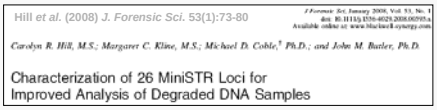


Becky Hill Pete Vallone John Butler

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)



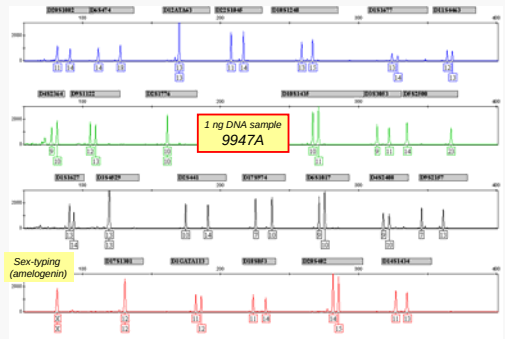
Hill *et al.* (2008) *J. Forensic Sci.* 53(1):73-80

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

NIST 26plex

Gender identification + 25 autosomal STR loci in a single amplification



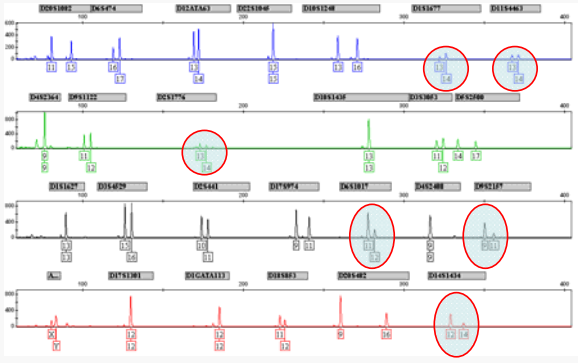
Hill, C.R., et al. (2009) A new STR 26plex assay for human identity testing. *J. Forensic Sci.* (in press)

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

NIST 26plex

Similar performance as commercial STR kits

32 Cycles, 50 pg



Heterozygote peak imbalance due to stochastic effects with low DNA template

Other Topics

- SRM updates
  - SRM 2372
  - SRM 2391b, 2395, 2392
- DNA stability testing (on-going)
- Training workshops
- SNPs for ethnicity estimation
- NIST group re-organization
  - Human Identity Project Team now part of Applied Genetics Group that also covers clinical genetics and agricultural biotechnology areas

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

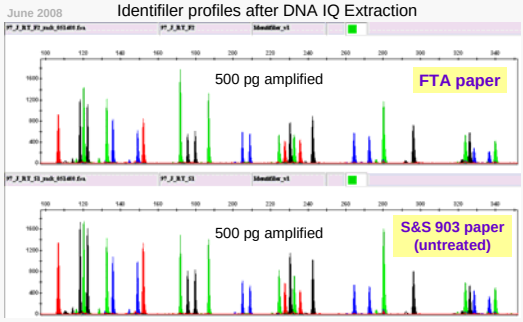
- **SRM 2395** (Y-STR and Y-SNP Loci)
  - **Yfiler loci sequenced** (DYS635 now included)
  - **Additional Loci: 20 new Y-STR loci**
  - Demonstrating extended stability (new quantitation data and no significant degradation to existing components)

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

Revised Certificates available since September 5, 2008

Room Temperature Storage of Dried Bloodstains
11 year time point

June 2008 Identifiler profiles after DNA IQ Extraction



Training Workshops in the Past Year
http://www.cstl.nist.gov/biotech/strbase/training.htm

- AAFS Meeting (**February 2008**, Washington, DC)
  - **DNA Quantitation by qPCR** ***(158 page handout)***
  - **Mixture Interpretation** ***(196 page handout)***
- Florida Statewide Training Meeting (**May 2008**, Indian Rocks Beach, FL)
  - **STRs and CE**
  - **Mixture Interpretation**
- Int. Symposium on Human Identification (Promega) Meeting (**October 2008**, Hollywood, CA)
  - **Troubleshooting CE and PCR Systems**

qPCR for DNA Quantitation Workshop
http://www.cstl.nist.gov/biotech/strbase/training/AAFS2008_qPCRworkshop.htm

AAFS (February 18th, 2008)

Human DNA Quantification Using Real-Time PCR Assays

158 page handout

- **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)
- Toni Diegoli (AFDIL)

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)

Lawyer Training…

- Virginia Defense Attorneys in Richmond, VA
  - **April 25, 2008**
  - Addressed general topics with DNA and mixtures
- New Jersey County Prosecutors in Jersey City, NJ
  - **September 27, 2008**
  - Spoke on past, present, and future issues with DNA

Presentations available at **http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm**

DNA Biometrics
and Other Applications of Human Identity Testing

- Interactions with **intelligence community and homeland security** personnel (e.g., DoD, DHS) seeking to learn more about forensic DNA challenges and opportunities
- Aid to **international humanitarian efforts** (e.g., International Committee of the Red Cross) seeking to use DNA to aid remains identification in natural or man-made disasters

Our Team Provides **Support to Other NIJ Grantees** and Commercial Collaborations

Support to NIJ-Funded Projects

- Akonni Biosystems (microchip SNPs)
- Network Biosystems (microchip CE)
- Roche (mtDNA strips)
- IBIS (mass spec of STRs)
- Marshall University (NEST Project)
- Florida International University (miniSTRs)

We welcome new collaborations…

Recent Commercial Collaborations

- Applied Biosystems – MiniFiler concordance
- Biomatrica – testing new DNA storage materials

Supplying U.S. population samples, multiplex assays, or evaluation of materials

Current Activities at NIST Enabled by Our NIJ Partnership

- **Standard Reference Materials**
  - SRM 2372 (DNA quant) released Oct 2007 (>150 units in use)
  - Updates to SRM 2391b (STRs), 2395 (Y-STRs), 2392 (mtDNA)
- **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, SNP testing)
  - Y-chromosome characterization (mutation rates, deletions, nomenclature)
- **Training Materials**
  - AAFS workshops on DNA quantitation and mixture interpretation
  - Third edition of *Forensic DNA Typing* textbook

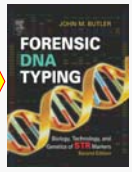
Forensic DNA Typing Textbook

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

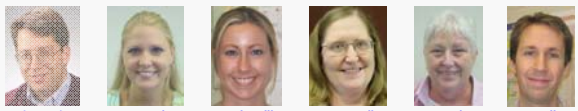
Fundamentals
Chapters 1-18

Advanced Topics
Chapters 1-25

In progress

Planned for fall 2009
~1000 pages


With Y. Hou (Chinese translator)


John Butler
Group Leader
Amy Decker
Becky Hill
Margaret Kline
Jan Redman
Pete Vallone

On November 18, 2008,
the NIST Human Identity Testing Project Team will receive
the Department of Commerce Gold Medal Award
for efforts in advancing forensic DNA science

Variant Alleles

New Loci & Assays


Dave Duewer

Thank you for your attention…

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

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

Questions?

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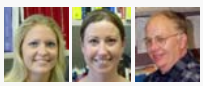
301-975-4049


Margaret Kline
Pete Vallone
Jan Redman


Amy Decker
Becky Hill
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Summer Interns
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Angela Gorman ('07)
Michelle Burns ('08)

Collaborators
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Bruce McCord (FIU)
Tom Reid (DDC)