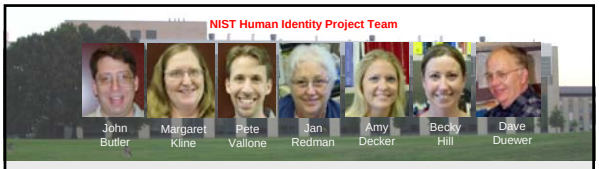


NIST Resources for the Forensic DNA Community

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

NIJ Conference
Crystal City, VA
July 22, 2008

Presentation Outline

- NIST projects overview (OLES)
- SRM update: SRM 2372, 2391b, 2392, 2395
- STR allele sequencing (Margaret Kline)
- Mixture work (Amy Decker, Angie Dolph & Michelle Burns)
- Additional STRs and 26plex (Becky Hill)
- Rapid PCR for DNA biometrics (Pete Vallone)
- Training workshops (John Butler/Pete Vallone)

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

National Institute of Standards and Technology
...working with industry to foster innovation, trade, security and jobs

Physics Laboratory
Defines U.S. Time
3 Nobel Prize Winners (97, 01, 05)

MSEL Materials Science and Engineering Laboratory

NIST Center for Neutron Research

NEL

CNST Center for Nanoscale Science & Technology

Chemical Science and Technology Laboratory
Human Identity Project Team

Technology Services
SRMs

Electronics and Electrical Engineering Laboratory
OLES
Microchip and microwave PCR

Information Technology Laboratory
Biometrics
Fingerprints
Iris scans
Other biometrics

Building and Fire Research Laboratory
WTC Investigation

Forensic DNA Work

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

Sue Ballou
(Forensics)

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

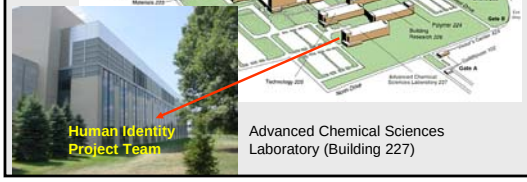
NIST Gaithersburg Campus

Located in Gaithersburg, Maryland, on approximately 234 hectares (578 acres) just off Interstate 270 about **25 miles northwest of Washington, D.C.**

http://www.nist.gov

~2,500 staff

OLES
(Building 220)



Advanced Chemical Sciences Laboratory (Building 227)

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

Forensics Research at NIST

- Computer (digital evidence) forensics
- Ballistics
- Fingerprints
- Arson investigation
- **DNA**

SRM 2461
Standard Casing

For more information, contact:
Susan Ballou
Program Manager for Forensic Sciences
susan.ballou@nist.gov
301-975-8750

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)

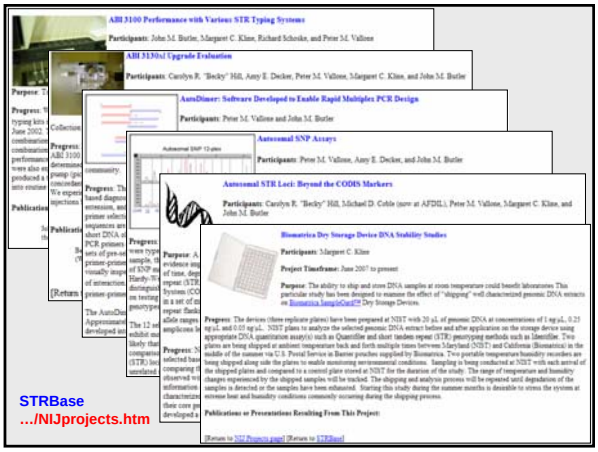
ABI 3130xl upgrade evaluation (Sept 2005-May 2006)

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

Autosomal SNP loci (July 2002-present)

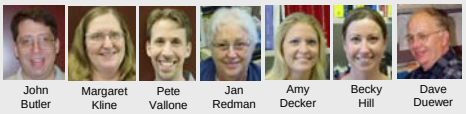
Autosomal STR loci beyond the CODIS markers (Jan 2004-present) [see also newSTRs.htm]

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



STRBase
.../NIJprojects.htm

NIST Human Identity Project Team



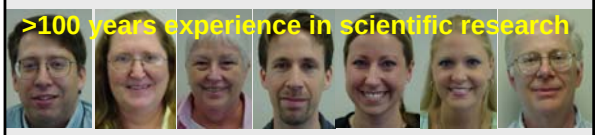
John Butler | Margaret Kline | Pete Vallone | Jan Redman | Amy Decker | Becky Hill | Dave Duewer

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

FY 2007 Achievements:
14 publications
44 presentations
9 workshops

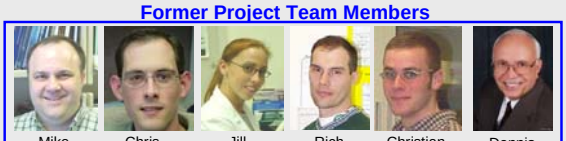
Since 2000:
98 publications
254 presentations
29 workshops

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

Why We Have Been Successful

- **Well-qualified and hard-working team members**
- Meet weekly and communicate daily on project specifics
- **Solid financial support from NIJ**
- Our own supply sergeant (Jan Redman)
- Publish and present often (maintains focus)
- Maintain a comprehensive literature collection for our field
- Share what we learn (STRBase, textbook)
- Know our customers' needs and work hard to meet them

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

- We provide input to (or have aided):
  - Scientific Working Group on DNA Analysis Methods (**SWGDAM**)
  - 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

STRBase: a Community Resource…

The **Revised Quality Assurance Standards** (which were recently approved by the FBI Director—to be effective July 1, 2009) will be posted on STRBase tomorrow.

Benefits of Website like STRBase

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

- Develops expertise when collecting information
- Requires me to stay up-to-date with field
- Provides transparency to our team's work
- Training tool and resource for the world
- Respected resource for >11 years
- ~10,000 pages of information available now
- >300,000 hits cumulative

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 **(>130 units in use)**
  - Updates to SRM 2391b (STRs), 2395 (Y-STRs), 2392 (mtDNA)
- **Technology Evaluation and Development**
  - Unusual STR allele characterization
  - Y-chromosome characterization (mutation rates, deletions)
  - New STR loci and assays (26plex)
  - Rapid multiplex PCR protocols (multiplex STR amplification in <35 min)
- **Training Materials**
  - AAFS workshops on DNA quantitation and mixture interpretation
  - Third edition of *Forensic DNA Typing* textbook

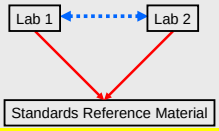
Standard Reference Materials

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

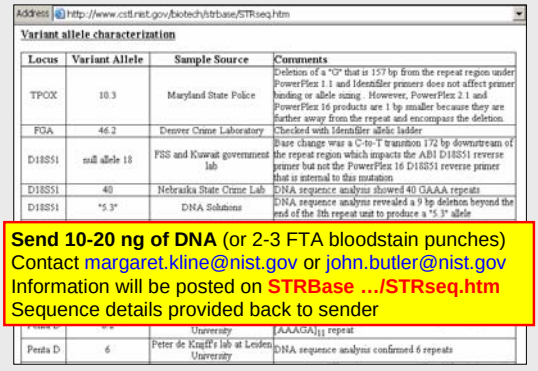


Calibration with SRMs enables confidence in comparisons of results between laboratories

Certificate Updates – new information (loci) added and **stability testing** performed to enable extension of expiration dates

Unusual STR Allele Characterization (Free)

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

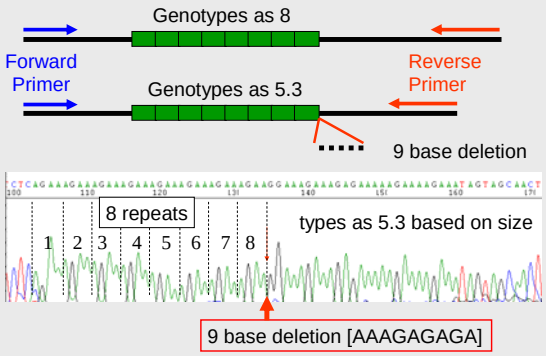


Variant allele characterization

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

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

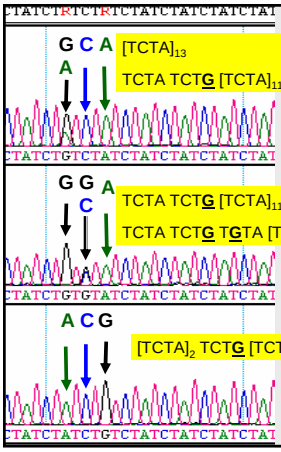
D18S51 deletion results in 5.3 Allele



STR Locus Sequence Variability

- Collaboration with Tom Hall (IBIS): has analyzed some of our NIST U.S. population samples by their mass spec methods
- In many samples the mass spec detected SNPs (base pair changes) within specific STR loci
- Margaret Kline has gone back and sequenced some of these samples to verify the mass spec results and determine where the SNPs are located

SNPs within the D8S1179 repeat



Repeat is TCTA
Three NIST samples have genotypes 13,13.

Analysis by Mass Spec indicates the presence of SNPs (Tom Hall, IBIS)
Confirmation of the Mass Spec by sequencing at NIST indicates:

There are 4 different "13" alleles in these three samples.

Base Pair difference between Repeats

D8S1179

$[TCTA]_{13}$
TCTA TCTG $[TCTA]_{11}$
TCTA TCTG TGTA $[TCTA]_{10}$
$[TCTA]_2$ TCTG $[TCTA]_{10}$

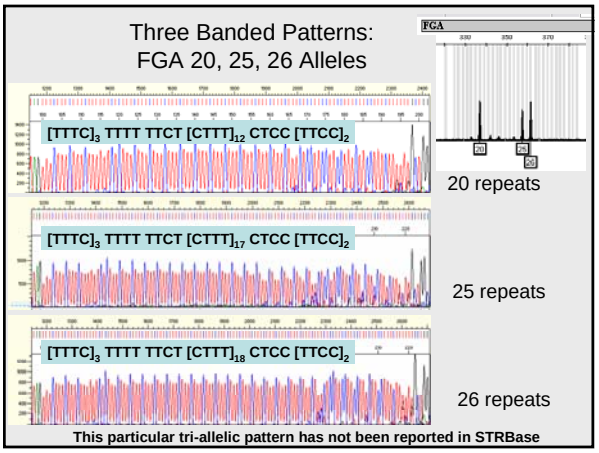
Allele	AVG	SD	N	
8	123.82	0.02	10	4.1
9	127.90	0.02	7	4.1
10	132.03	0.06	42	4.1
11	136.17	0.05	35	4.3
12	140.42	0.07	80	4.5
13	144.93	0.08	153	4.4
14	149.36	0.07	131	4.3
15	153.67	0.07	89	4.3
16	157.93	0.07	21	4.1
17	162.02	0.04	2	

D16S539

Mass Spec detected fewer SNPs in this locus.
Less average bp variability seen between repeat sizes.

Allele	AVG	SD	N	
5	252.08	0.01	2	4.0
8	264.09	0.11	17	4.0
9	268.14	0.11	93	4.1
10	272.20	0.12	56	4.0
11	276.18	0.14	162	4.1
12	280.25	0.14	158	4.1
13	284.30	0.12	72	4.1
14	288.35	0.07	8	

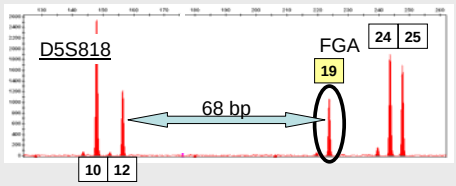
Three Banded Patterns: FGA 20, 25, 26 Alleles



This particular tri-allelic pattern has not been reported in STRBase

Is this an FGA - Tri-allelic pattern identified using Identifiler?

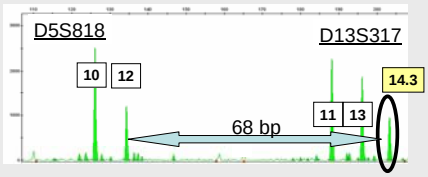
PK HT Ratio
12/10 - 0.48



PK HT Ratio
19/24 - 0.55
25/24 – 0.89

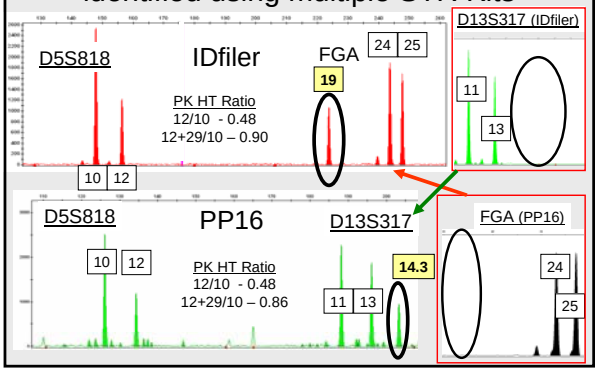
Or is this a D13S317 - Tri-allelic pattern identified using Powerplex 16?

PK HT Ratio
12/10 - 0.48

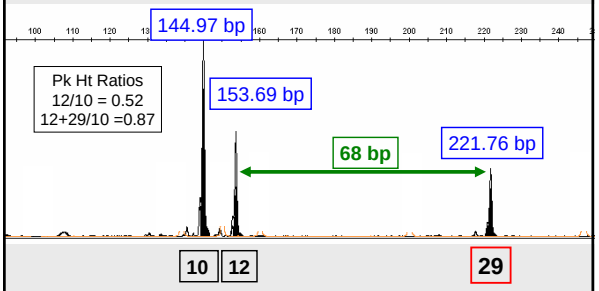


PK HT Ratio
13/11 - 0.83
14.3/11 – 0.42

It's really a D5S818 Tri-allelic pattern identified using multiple STR Kits

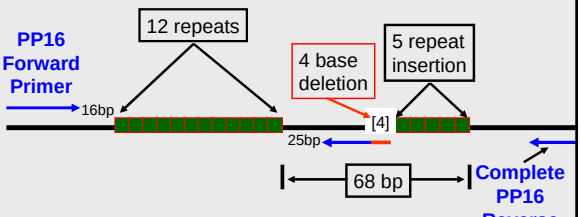


D5S818 monoplex results



The 68 bp size difference between the 12 allele and the variant allele sizing as an **"apparent 29"** allele.

D5S818 Apparent 29 Allele Sequencing Results



There is a 4 bp deletion, the last 4 bases of the PP16 reverse primer binding site, followed by an insertion of 5 repeats.
The 10 and 12 alleles of this sample have been sequenced and have the expected sequences.

Are there other large D5S818 alleles?

- STRBase Tri-allelic reports for FGA for 19,*,* patterns with AB amplification kits.
  - 5 reports :
  - 19,20,21; 19,20,23; 19,20,24; 19,22,23; 19,24,25
  - But there we have sequenced true tri-allelic FGA samples
- STRBase Tri-allelic reports for D13S317 for *,*, OL patterns with PP16 amplification kits.
  - NO tri-allelic patterns with Off-Ladder alleles reported

Y-STR Mutation Rates Measured at NIST

Decker, A.E., Kline, M.C., Redman, J.W., Reid, T.M., Butler, J.M. (2008) Analysis of mutations in father-son pairs with 17 Y-STR loci. *FSI Genetics* 2(3): e31-e35

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

Mixture Work

- Testing software tools
  - FSS-i3
  - DNA_DataAnalysis (US Army Crime Lab)
- Examining reproducibility of mixture replicates to see how well mixture ratios hold across loci
- Peak height ratio studies with multiple data sets to understand mixture ratio ranges

New STR Loci Characterized

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

J Forensic Sci, January 2008, Vol. 53, No. 1
doi: 10.1111/j.1556-4029.2008.00595.x
Available online at: www.blackwell-synergy.com

Carolyn R. Hill, M.S.; Margaret C. Kline, M.S.; Michael D. Coble,† Ph.D.; and John M. Butler, Ph.D.

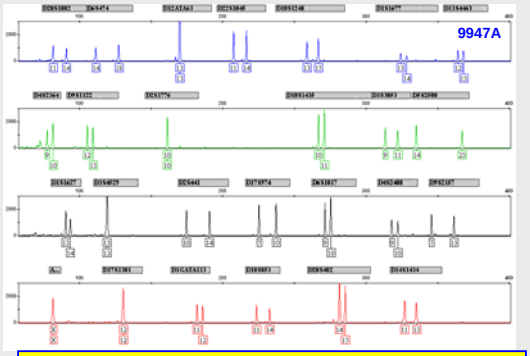
Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples

- Primer sequences, GeneMapper bins and panels, genotypes on common samples, and allele frequency information **available on STRBase**

http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_NC_loci_types.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_Panels_Panels.txt
http://www.cstl.nist.gov/biotech/strbase/miniSTR/miniSTR_Panels_NC_bins_bins.txt

"Autoplex" (26plex)

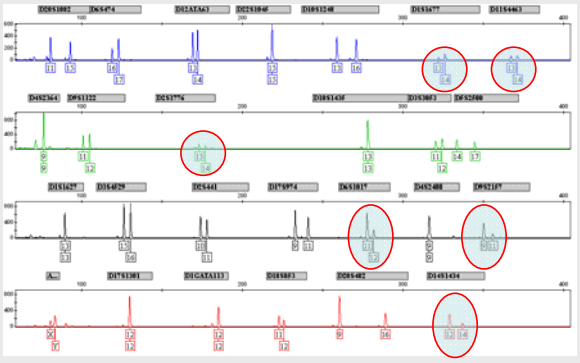
Hill et al. AAFS 2008 talk (Washington, DC) and poster at DNA in Forensics 2008 meeting (Ancona, Italy)



Gender identification + 25 autosomal STR loci in a single amplification

NIST 26plex

32 Cycles, 50 pg



Heterozygote peak imbalance due to stochastic effects with low DNA template

Promega Meeting Proceedings Paper

New Autosomal and Y-Chromosome STR Loci Butler *et al.*

New Autosomal and Y-Chromosome STR Loci:
Characterization and Potential Uses*

John M. Butler[†], Carolyn R. Hill[†], Amy E. Decker[†], Margaret C. Kline[†], Thomas M. Reid[‡], and Peter M. Vallone[†]

[†]Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg MD 20899
[‡]DNA Diagnostics Center, Fairfield, OH 45014

http://www.cstl.nist.gov/biotech/strbase/pub_pres/Promega2007_NewSTRloci.pdf

- **42 page article** available on STRBase and Promega site
- Describes 26 miniSTR loci
- Covers 23plex STR assay
- Includes world-wide Yfiler data review

The 26plex assay (**including primer sequences**) has been submitted for publication in the *Journal of Forensic Sciences*

STRs vs SNPs Article

Forensic Sci Med Pathol (2007) 3:200–205
DOI 10.1007/s12024-007-0018-1

ORIGINAL PAPER

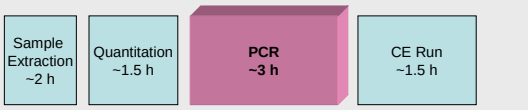
STRs vs. SNPs: thoughts on the future of forensic DNA testing

John M. Butler · Michael D. Coble · Peter M. Vallone

- Describes challenges with SNPs in terms of mixture detection and interpretation
- Most likely use of SNPs is as ancestry-informative markers (AIMs)

Butler *et al.* (2007) STRs vs SNPs: thoughts on the future of forensic DNA testing. *Forensic Science, Medicine and Pathology* 3:200-205.

Typical STR DNA Analysis Workflow



How can we reduce the time needed for cycling?

What happens when we alter cycling parameters?
How will existing commercial kits work?
How will different polymerases perform?
How robust will the results be?
Can we develop novel assays and further the understanding/limits of rapid multiplex PCR?

Rapid PCR Project at NIST

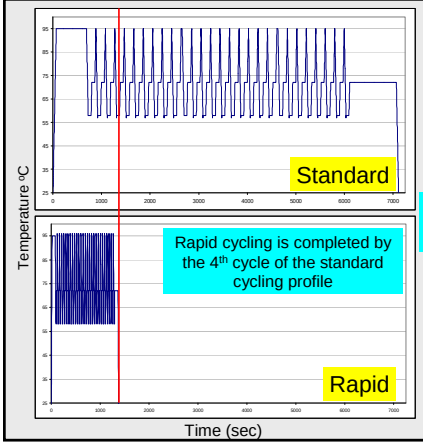
Rapid Thermal Cycling with ABI 9700

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

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

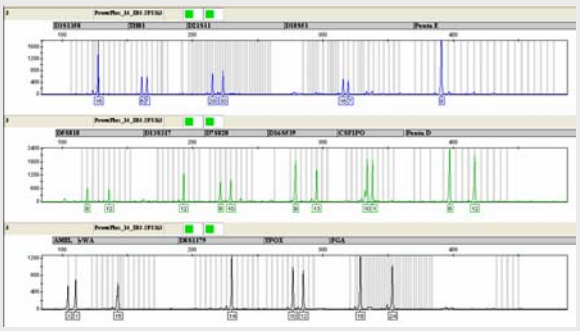
Evaluate robustness and reproducibility

Using different DNA polymerases besides TaqGold

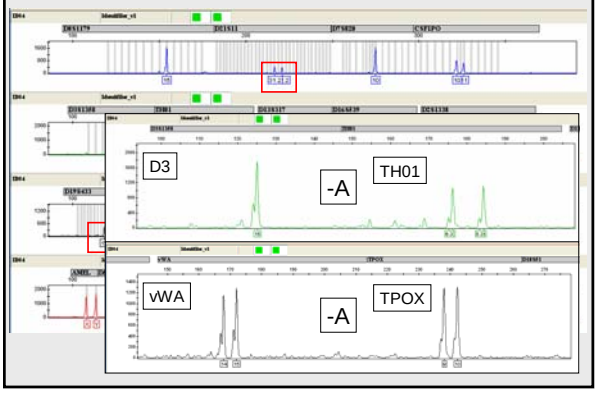


Comparison of Thermal Cycling Profiles on an AB 9700

PowerPlex 16 Rapid Cycling



Identifiler Rapid Cycling



Seminars and Training Workshops to Individual Forensic DNA Laboratories

Feb 3, 2005 | May 19, 2005 | June 8, 2005 | June 13-14, 2005

Apr 27-28, 2006 | June 6, 2006 | Aug 7, 2006 | Nov 15, 2006 | Dec 5-6, 2006

March 7, 2007 | March 14, 2007 | Apr 3-4, 2007 | Apr 5, 2007 | June 5, 2007

Training Workshops in the Past Year

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

- ISFG Meeting (**August 2007**, Copenhagen, Denmark)
  - **CE Fundaments and Troubleshooting**
  - **Validation**
- Int. Symposium on Human Identification (Promega) Meeting (**October 2007**, Hollywood, CA)
  - **Validation**
- NEAFS Meeting (**November 2007**, Bolton Landing, NY)
  - **Mixture Interpretation**
  - **Low-copy Number DNA Issues**
  - **miniSTRs**
- AAFS Meeting (**February 2008**, Washington, DC)
  - **DNA Quantitation by qPCR** ***(158 page handout)***
  - **Mixture Interpretation** ***(196 page handout)***

Planned Promega 2008 Meeting
Troubleshooting Workshop

- Title: **"Principles of Interpretation and Troubleshooting of Forensic DNA Typing Systems"**
- Instructors: **John Butler (NIST) and Bruce McCord (FIU)**
- Date: **October 16, 2008** with Promega Int. Symp. Human ID

The workshop will consist of three parts:

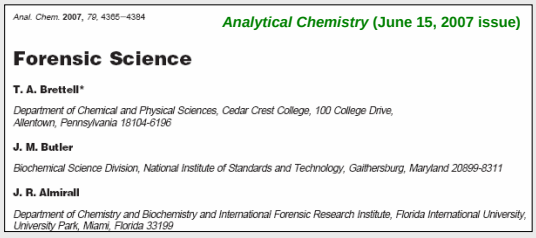
(1) a through examination of theoretical issues with capillary electrophoresis PCR amplification of short tandem repeat markers

(2) a discussion of how to properly set instrument parameters to interpret data (including mixtures), and

(3) **a review of specific problems seen by labs** submitting problematic data and commentary on possible troubleshooting solutions.

Seeking input of problems observed with CE systems

Forensic Science Review Article



Anal. Chem. **2007**, *79*, 4365–4384

Analytical Chemistry **(June 15, 2007 issue)**

Forensic Science

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560 references covering articles published in 2005-2006

181 articles on forensic DNA analysis

Brettell, T.A., Butler, J.M., Almirall, J.R. (2007) Forensic science. *Anal. Chem.* 79: 4365-4384.

Thank you for your attention…

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

Questions?

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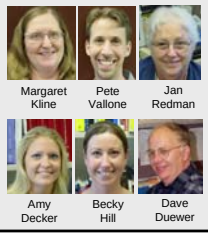

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