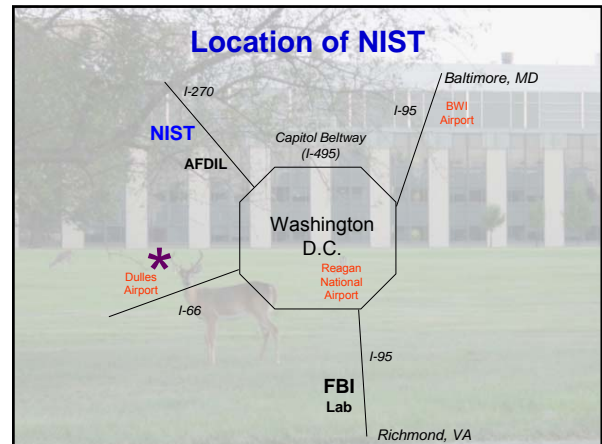


# NIST Update

**John M. Butler**  
and the Human Identity Project Team

National CODIS Conference  
November 2, 2009  
Reston, VA





## Presentation Outline


- **Team Members and Outputs for the Past Year**
- **Standards**
  - SRM 2391c: plans for next reference material
- **Technology**
  - Rapid PCR for DNA biometrics
  - New STR loci, assays, and kits evaluated
  - Variant alleles cataloged in STRBase
  - Low template DNA studies
- **Training**
  - Workshops and STRBase information available
  - New book: *Fundamentals of Forensic DNA Typing*


## NIST Human Identity Project Team


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

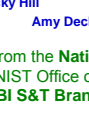



  
Becky Hill


  
John Butler  
*(aka Elvis)*


  
Margaret Kline

  
Amy Decker

  
Pete Vallone

  
Jan Redman

  
Erica Butts

  
Dave Duewer


Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards and the **FBI S&T Branch** for DNA Biometrics projects

## Communication with the Community

Since the Nov 2008 CODIS Conference...

	Totals	John	Pete	Margaret	Becky	Amy
<b>Presentations</b>	<b>51</b>	32	8	4	4	3
<b>Posters</b>	<b>8</b>	2	3	1	1	1
<b>Workshops</b>	<b>11</b>	11	1			1
<b>Totals</b>	<b>70</b>	45	12	5	5	5

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




## APPLIED GENETICS Group

Major Programs Currently Underway

- **Forensic DNA**
  - New loci and assays (26plex)
  - **STR kit testing**
  - Ancestry SNP assays
  - **Low-template DNA studies**
  - Mixture interpretation
  - STR nomenclature
  - **Variant allele cataloging** and sequencing
  - Expert systems review
  - Training workshops to forensic DNA laboratories
  - Validation information and **software tools**
  - **Textbook** – 3<sup>rd</sup> ed. (2 vol.)

- **Clinical Genetics**
  - CMV SRM
  - Huntington's SRM
- **Ag Biotech**
  - "universal" GMO detection/quantitation (35S promoter)
- **DNA Biometrics**
  - **Rapid PCR** methods
  - Efforts to standardize testing of future portable DNA systems
  - Kinship analysis
- **Cell Line Authentication**



Looking for a Ph.D. to Expand Our Team

- We are currently seeking a Ph.D. scientist to help with our forensic DNA and clinical DNA efforts
- Please email me if you or someone you know may be interested: [john.butler@nist.gov](mailto:john.butler@nist.gov)

# Standards

NIST SRM 2391b will need to be replaced within the next year

SRM	Name	FY06	FY07	FY08	FY09	Avg	Remaining	Current \$
2372	Human DNA Quantitation Std	0	0	160	147	153.5	1,078	\$372
2390	DNA Profiling	2	0	1	0	0.8	3	\$833
2391B	PCR-Based DNA Profiling	86	81	125	140	108	107	\$811
2392	Mitochondrial DNA Sequencing	8	6	0	12	6.8	165	\$883
2392a	Mitochondrial DNA Sequencing (Human HL-60 DNA)	6	32	20	19	19.3	176	\$365
2395	Human Y-Chromosome DNA Profiling	34	39	72	88	58.3	136	\$383

\*As of Oct 7, 2009

PCR-based DNA Profiling Standard

SRM 2391 (1995)    SRM 2391a (2000)    SRM 2391b (2003, 2008)    SRM 2391c (planned 2010)



Would like your input into the desired number of components & loci certified

Considering preparing DNA in different forms:  
3 liquid samples  
2 swabs  
1 paper punch

Plan to certify both autosomal and Y-STRs on SRM 2391c components

\*coverage for all commercially available kit STR loci at the time of release

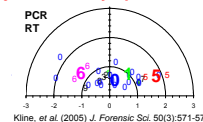
[http://www.cstl.nist.gov/biotech/strbase/pub\\_pres/Promega2009poster\\_SRM2391c.pdf](http://www.cstl.nist.gov/biotech/strbase/pub_pres/Promega2009poster_SRM2391c.pdf)

SRM 2372: Human DNA Quantitation Standard

Do NOT use Component C with Quantifiler Duo



- Released in Oct 2007  
- >300 units in use as of October 2009
- Used by more than 110 forensic laboratories worldwide
- Manuscript describing production published in *Anal. Bioanal. Chem.*
- Serves to adjust qPCR calibrants supplied by manufacturers and adjust for assay-specific bias



# Technology

- How fast can we go?
- How large of a multiplex can we build?
- Are there better loci to improve DNA testing?
- Can we speed up validation of new STR kits?
- How low can we go in terms of sensitivity?

Rapid PCR work published in *FSI Genetics* (Dec 2008)

Full STR profiles in 36 minutes (instead of 3 hour PCR)



Short communication

Demonstration of rapid multiplex PCR amplification involving 16 genetic loci<sup>®</sup>  
Peter M. Vallone<sup>1</sup>, Carolyn R. Hill, John M. Butler

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.

Pete Vallone has described additional work on rapid PCR in two recent posters

**"Rapid Amplification of Commercial STR Typing Kits"**

- International Society of Forensic Genetics (ISFG) meeting (Buenos Aires, Argentina), September 16-17, 2009
- 20th International Symposium on Human Identification (Las Vegas, NV), October 13-14, 2009

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

**Rapid PCR Work wins ISFG Poster Contest**

**Rapid Amplification of Commercial STR Typing Kits**  
 Peter M. Vallone<sup>1</sup>, Carolyn R. Hill<sup>1</sup>, Daniele Podini<sup>2</sup>, and John M. Butler<sup>1</sup>

<sup>1</sup> U.S. National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-8111  
<sup>2</sup> Department of Forensic Sciences, The George Washington University, 2526 H St NW Washington, DC 20052

Selected from >300 posters

INTERNATIONAL SOCIETY FOR FORENSIC GENETICS  
 The Prize for the best poster presentation at the 23rd Congress of the International Society for Forensic Genetics has been awarded to  
**Peter VALLONE**  
 for his work  
 "Rapid Amplification of Commercial STR Typing Kits"  
 (co authors: C. Hill, C. Podini and J. Butler)

**Further Rapid PCR Work**

Rapid PCR Thermal Cycling Profile

- Much shorter hold times at each temperature
- Faster ramp rates between temperatures

Maximum heating rate of ~4°C/s on a GeneAmp 9700 (Applied Biosystems)

- Examination of different enzyme mixes
  - 0.5 x master mix PyroStart (Fermentas) (\$0.14/rxn)
  - 0.5 x master mix Premix Ex Taq (Takara) (\$0.22/rxn)
  - 0.25 µL = 1.25 units of SpeedStar (Takara) (\$1.09/rxn)
- Evaluation of additional kits
  - Identifiler, PP16, Yfiler, MiniFiler and Promega S5
- Testing thermal cyclers with faster ramp rates

**Four Thermal Cyclers Being Evaluated**

**How fast can we run 28 cycles?**

<b>GeneAmp 9700 (Applied Biosystems)</b> • Heating rate: 4°C/s • Heating mechanism: Peltier block (Al) • Tube format: 0.2 mL, 96 well plate • 28 cycles = 36 min (w/ the general heat ramp cycling profile shown on the left) <b>36 minutes</b>	<b>SmartCycler (Cepheid)</b> • Heating rate: 10°C/s • Heating mechanism: heating plates and air circulating fan • Tube format: proprietary 25 µL tubes • 15 reactions per instrument, safety to run 16 independent thermal cycling profiles • Can also be used for real time PCR • 28 cycles = 20 min <b>20 minutes</b>
<b>Mastercycler pro (Eppendorf)</b> • Heating rate: 6°C/s • Heating mechanism: Peltier block (Ag) • Tube format: 0.2 mL, 96 well plate • 28 cycles = 19 min <b>19 minutes</b>	<b>Rotor-Gene Q (Qiagen)</b> • Heating rate: 15°C/s • Heating mechanism: Air chamber (spinning rotor) • Tube format: 0.1 mL, 72 tube/rotor • 28 cycles = 36 min <b>36 minutes</b>

[http://www.cstl.nist.gov/biotech/strbase/pub\\_pres/VallonePromega2009poster.pdf](http://www.cstl.nist.gov/biotech/strbase/pub_pres/VallonePromega2009poster.pdf)

**How Fast Can We Go?**

Steps Involved

- Collection
- Extraction → **Better chemistry has potential to lead to ability to routinely obtain results in < 1 hour with commercially available instruments**
- Quantitation
- Amplification → **Direct PCR (new enzymes & master mix to overcome PCR inhibitors from blood)**
- Separation/ Detection → **Rapid PCR (new enzymes & thermal cyclers)**
- Data Interpretation → **Improved CE systems (ABI 3500?)**  
**Expert system software**

**Direct PCR (no DNA extraction) using PP16 HS from a 23 year old blood stain (room temperature storage)**

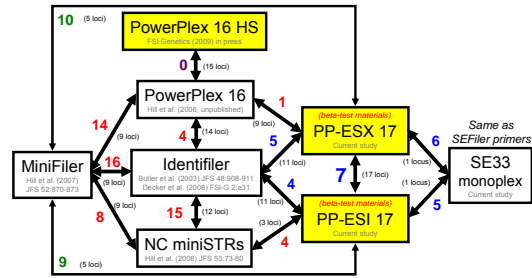
1.2 mm punch (untreated blood stain card S&S 903) and PP16 HS (28 cycles)

### NIST Studies with New STR Kits

- Over the past year, our NIST team has worked with Promega to evaluate their new STR kits
  - PowerPlex ESX 17 & ESI 17 - with new European loci
  - PowerPlex 16 HS – with new enzyme & buffer system
- Concordance studies**, population data analysis, and additional validation information
- Plan to do similar studies with Applied Biosystems on their NGM and other new kits

### Summary of Allele Discordance Observed

Number of Discordant Results Observed between Various STR Assays



Comparison made with 186 samples  
 Comparisons made with ~660 samples  
 Comparisons made with ~1120 samples  
 Comparisons made with ~1440 samples

### Features of These New STR Kits

- Improved sensitivity** (“turbo-charged engines”)
  - New PCR buffer and polymerase master mix
  - Better fluorescent dye labels (5-dye chemistry)
- Greater tolerance for PCR inhibitors**
  - New PCR buffer and polymerase master mix
- Different primer sequences** for amplifying core STRs
  - Requires concordance checks to confirm no null alleles
- Additional STR loci** with high heterozygosities to improve power of discrimination for entire profile
  - Addresses requirements for extended European Standard Set

### PowerPlex® ESI 17 Population Data (N=1443)

Marker	Number of Alleles	Theoretical Genotypes	Genotypes Observed	Heterozygosity	PIC
Amelogenin	2	3	3	--	--
TH01	8	36	25	0.7479	0.7572
D3S1358	11	66	31	0.7493	0.7305
D22S1045	11	66	45	0.7548	0.7318
D2S441	15	120	47	0.7729	0.7499
D16S539	9	45	30	0.7791	0.7650
D10S1248	12	78	41	0.7805	0.7460
D8S1179	11	66	48	0.7971	0.7961
vWA	11	66	42	0.7999	0.7866
D19S433	16	136	83	0.8089	0.7984
D21S11	28	406	95	0.8296	0.8293
D12S391	24	300	120	0.8650	0.8651
FGA	29	435	111	0.8691	0.8598
D18S51	23	276	103	0.8698	0.8699
D2S1338	13	91	73	0.8726	0.8821
D1S1656	17	153	101	0.8837	0.8806
SE33	58	1711	343	0.9377	0.9426

**SE33 (58 alleles observed)**

Total						Populations, %							
Allele	#	%	Af Am	Asian	Cauc	Hisp	Allele	#	%	Af Am	Asian	Cauc	Hisp
6.3	23	12.04	0.6	1.0	0.2	0.1	23.2	91	3.22	2.2	4.2	4.3	2.1
7	24	11.04	1.0	0.0	0.1	0.1	24	1	0.0	0.1	0.1	0.1	0.1
8	24.2	74.26	1.3	6.2	2.2	2.5	25.2	109	3.8	2.6	6.9	4.0	3.1
10.2	26	11.04	0.1	0.0	0.1	0.1	27.2	225	7.8	4.3	10.4	9.5	8.6
11	27.3	2	0.1	0.1	0.2	0.3	28.2	180	6.2	4.4	7.9	7.4	6.1
11.2	28.3	2	0.1	0.1	0.1	0.1	29	1	0.0	0.2	0.2	0.1	0.1
12	29	11.04	0.2	0.3	0.3	0.3	29.2	147	5.1	2.7	5.7	6.3	6.3
12.2	30	11.04	0.2	0.3	0.3	0.3	30	1	0.0	0.2	0.2	0.1	0.1
13	31	11.1	1.1	1.5	1.0	1.0	30.2	111	3.8	1.6	3.2	5.8	4.6
13.2	31.2	9	0.3	1.0	0.7	0.7	31	3	0.1	0.1	0.1	0.2	0.2
14	32	11.04	0.4	0.4	0.3	0.3	31.2	52	1.8	1.5	2.5	2.2	1.3
14.2	32.2	10	0.3	0.4	0.3	0.3	32	1	0.0	0.1	0.1	0.1	0.1
15	33	11.04	3.9	1.2	3.9	3.9	32.2	25	0.9	0.4	0.7	1.3	0.9
15.2	33.2	8	0.3	0.3	0.7	0.7	33	2	0.1	0.1	0.1	0.1	0.1
16	34	11.04	4.8	4.7	4.0	6.7	33.2	11	0.4	0.3	0.5	0.4	0.4
16.2	34.2	5	0.2	0.3	0.1	0.1	34	9	0.3	0.3	0.7	0.7	0.7
16.3	35	11.04	0.3	0.3	0.3	0.3	34.2	1	0.0	0.1	0.1	0.1	0.1
17	36	11.04	9.3	4.0	6.2	7.3	35	1	0.0	0.1	0.1	0.1	0.1
17.2	36.2	1	0.0	0.1	0.1	0.1	36	2	0.1	0.2	0.1	0.1	0.1
17.3	36.3	5	0.2	0.1	0.2	0.3	36	2	0.1	0.2	0.1	0.1	0.1
18	37	11.04	12.1	5.0	7.2	11.0	36	2	0.1	0.2	0.1	0.1	0.1
18.3	37.2	1	0.0	0.1	0.1	0.1	36	2	0.1	0.2	0.1	0.1	0.1
19	38	11.04	6.2	6.6	8.0	8.0	36	2	0.1	0.2	0.1	0.1	0.1
19.2	38.2	8	0.3	0.2	0.4	0.4	36	2	0.1	0.2	0.1	0.1	0.1
20	39	11.04	10.9	9.2	5.4	4.8	36	2	0.1	0.2	0.1	0.1	0.1
20.2	39.2	20	0.7	0.3	1.2	1.1	0.3	36	2	0.1	0.2	0.1	0.1
21	40	11.04	4.6	6.7	2.4	2.7	36	2	0.1	0.2	0.1	0.1	0.1
21.2	40.2	48	1.7	1.1	1.7	2.4	1.3	36	2	0.1	0.2	0.1	0.1
22	41	11.04	1.3	1.7	1.5	1.3	36	2	0.1	0.2	0.1	0.1	0.1
22.2	41.2	42	1.5	1.3	1.7	1.5	1.3	36	2	0.1	0.2	0.1	0.1
22.2	41.2	65	2.3	0.4	3.2	3.8	1.9	36	2	0.1	0.2	0.1	0.1

**343 genotypes observed**  
**Heterozygosity = 0.9377**

### Software Tools to Support STR Kit Evaluation and Validation Studies

### New NIST Software Tools Developed by Dave Duewer (NIST)

**From NIST STRBase Website:**

**Lab Resources and Tools**

- Addresses for scientists working with STRs
- Traceable Materials
- STR Allele Sequencing
- Population data
- Data from NIST U.S. Biotech Center
- **NIST-Developed Software** (including Auto-Denier, miniSTR, and Multiplex, QA)
- NIST Standard Reference Material for PCR-Based Testing
- New STR Markers under Development at NIST
- Chromosomal Locations
- DNA Advisory Board Quality Assurance Standards
- Interlaboratory Studies
- NIST Montage 2005 Interlab Study: MIX04 Data
- Validation information
- DNA Quantitation - SRM 2172 (available as of October 8, 2007)
- Technology for resolving STR alleles

**STR\_MatchSamples**

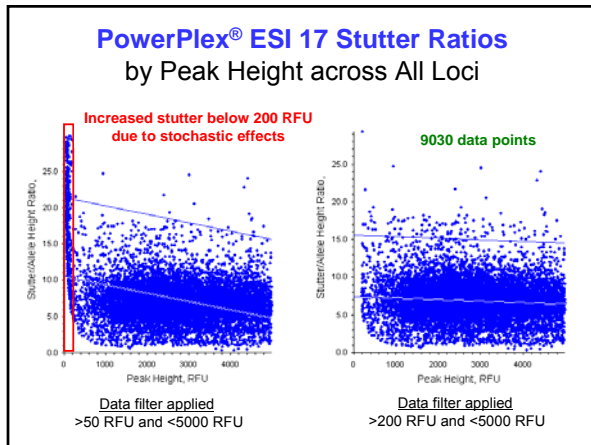
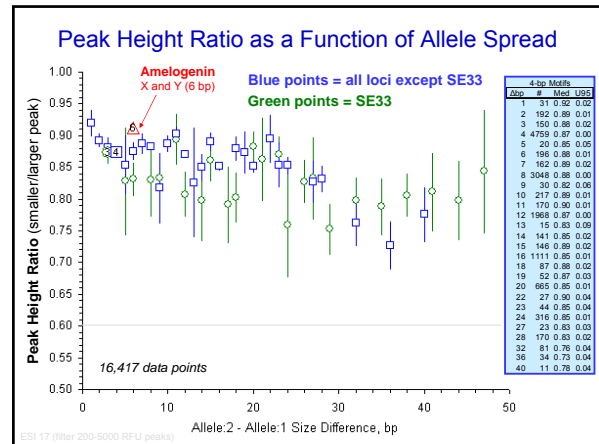
- An Excel-based tool developed to aid comparison of STR genotypes from two or more data sets.

**Tools under development (to aid validation studies)**

- Peak height ratio
- Inter-locus balance
- Stutter percentages
- Allele frequency

[http://www.cstl.nist.gov/biotech/strbase/tools/STR\\_MatchSamples.xls](http://www.cstl.nist.gov/biotech/strbase/tools/STR_MatchSamples.xls)

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



### Trinucleotide D2S1045 Allele-Specific Stutter Percentages

ESX 17				ESI 17			
Allele	Size	#	Median	Allele	Size	#	Median
10	84.5	21	1.8	10	308.7	22	1.9
11	87.4	134	3.0	11	311.8	98	2.8
12	90.4	37	4.2	12	314.8	32	4.5
14	96.4	51	7.2	14	321.0	36	6.1
15	99.4	165	8.9	15	324.0	150	9.9
16	102.4	120	10.5	16	327.1	94	9.8
17	105.5	105	14.7	17	330.1	95	14.2
Avg		633	7.2	Avg		527	7.0
SD			4.6	SD			4.4

633 data points      Avg + 3SD 21.0%

527 data points      Avg + 3SD 20.2%

### OSIRIS Software for STR Typing

**Open Source Independent Review and Interpretation System**

- Being developed at NCBI (NIH) by a team led by Lisa Forman
- Input: .fsa files
- Output: CODIS CMF
- Files are fully editable with audit trail
- Currently works with Identifier, PP16, ProPlus, COfiler
- NIST has supplied test data and feedback on performance

**Free download available at NCBI website:**  
<http://www.ncbi.nlm.nih.gov/projects/SNP/osiris/>

# Variant Alleles

### 524 Variant Alleles on STRBase

[http://www.cstl.nist.gov/biotech/strbase/var\\_tab.htm](http://www.cstl.nist.gov/biotech/strbase/var_tab.htm)

524 total variants reported as of 10/26/2009

[click on loci listed below for details]

New Addition

Core STR Loci (363)	Other Common STR Loci (122)	Y-STR Loci (39)
<ul style="list-style-type: none"> <li>• CSF1PO (20)</li> <li>• FGA (103)</li> <li>• TH01 (19)</li> <li>• TPOX (17)</li> <li>• YWA (13)</li> <li>• D3S1358 (21)</li> <li>• D5S818 (15)</li> <li>• D7S820 (26)</li> <li>• D8S1179 (21)</li> <li>• D13S317 (17)</li> <li>• D16S539 (21)</li> <li>• D18S51 (41)</li> <li>• D21S11 (32)</li> </ul>	<ul style="list-style-type: none"> <li>• D2S1338 (23)</li> <li>• D19S433 (27)</li> <li>• Penta D (37)</li> <li>• Penta E (30)</li> <li>• F13A01 (1)</li> <li>• FES-FPS (1)</li> <li>• F13B</li> <li>• LPL</li> <li>• SE33 (1)</li> <li>• D1S1677 (1)</li> <li>• D14S1434 (1)</li> </ul>	<ul style="list-style-type: none"> <li>• DYS19 (2)</li> <li>• DYS389I (2)</li> <li>• DYS389II (1)</li> <li>• DYS390 (2)</li> <li>• DYS391</li> <li>• DYS392 (4)</li> <li>• DYS392 (1)</li> <li>• DYS385 a,b (16)</li> <li>• DYS438 (2)</li> <li>• DYS439 (4)</li> <li>• DYS437 (3)</li> <li>• DYS448</li> <li>• DYS456</li> <li>• DYS458 (2)</li> <li>• DYS635 (1)</li> <li>• Y-GATA-H4</li> </ul>

### Many New Y-STR Variants Being Reported by Missouri State Highway Patrol

#### DYS439 Variants

At time of submission had run over 41,000 samples!

To compare to this table, [click here](#)

Allele Designation	Allele Size (bp)	Instrument	Amp Kit*	Contributor	Verification/Confirmation Method(s)	Notes	Frequency
7 (<8)	194.26	ABI 3130d	PPY	Jackie Johnson, MO State Hwy Patrol	redo sample		1 in 41635
10.1	207.20	ABI 3130d	PPY	Jackie Johnson, MO State Hwy Patrol	redo sample		2 in 41635
11.1	211.22	ABI 3130d	PPY	Jackie Johnson, MO State Hwy Patrol	redo sample		1 in 41635
16 (>15)	230.44	ABI 3130d	PPY	Jackie Johnson, MO State Hwy Patrol	redo sample		1 in 41635

### 189 Tri-Allelic Patterns on STRBase

[http://www.cstl.nist.gov/biotech/strbase/tri\\_tab.htm](http://www.cstl.nist.gov/biotech/strbase/tri_tab.htm)

189 total patterns reported as of 10/20/2009

[click on loci listed below for details]

Core STR Loci (165)	Other Common STR Loci (23)	Y-STR Loci (1) <i>duplications or triplications</i>
<ul style="list-style-type: none"> <li>• CSF1PO (7)</li> <li>• FGA (26)</li> <li>• TH01 (3)</li> <li>• TPOX (15)</li> <li>• YWA (20)</li> <li>• D3S1358 (7)</li> <li>• D5S818 (7)</li> <li>• D7S820 (1)</li> <li>• D8S1179 (11)</li> <li>• D13S317 (9)</li> <li>• D16S539 (8)</li> <li>• D18S51 (26)</li> <li>• D21S11 (19)</li> </ul>	<ul style="list-style-type: none"> <li>• D2S1338 (3)</li> <li>• D19S433 (3)</li> <li>• Penta D (6)</li> <li>• Penta E (11)</li> <li>• F13A01</li> <li>• FES-FPS (1)</li> <li>• F13B</li> <li>• LPL</li> <li>• SE33</li> </ul>	<ul style="list-style-type: none"> <li>• DYS19</li> <li>• DYS389I</li> <li>• DYS389II</li> <li>• DYS390</li> <li>• DYS391</li> <li>• DYS392</li> <li>• DYS393</li> <li>• DYS385 a,b</li> <li>• DYS438</li> <li>• DYS439</li> <li>• DYS437</li> <li>• DYS448</li> <li>• DYS456 (1)</li> <li>• DYS458</li> <li>• DYS635 GATA-C4</li> <li>• Y-GATA-H4</li> </ul>

# Low-Level DNA Studies

### New Section of STRBase on This Issue

- Recently launched webpage
  - <http://www.cstl.nist.gov/biotech/strbase/LTDNA.htm>
  - Low-template DNA = LTDNA (not LCN!)
- The LTDNA section includes:
  - Presentations from the Promega LCN Panel**
  - Validation data from our sensitivity studies** to illustrate problems and consensus profile solution to low levels of DNA testing
  - Literature listing of pertinent articles** to help explain the issues involved in this topic

### New STRBase Website on LT-DNA (LCN)

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

Information on Low Template / Low Copy Number DNA Testing

Low Copy Number (LCN) DNA Panel Discussion

#### Scientific Issues with Analysis of Low Amounts of DNA

Presentation Prepared for the LT-DNA Panel

Theresa Caviglio, Ph.D.  
Deputy Director  
October 16, 2009

The allotted time for each question was brief; thus, this presentation does not represent the practices and protocols of the NIST CODIS in their entirety.

General Information

- Purpose of STRBase
- Publications and Press
- NIF Funded Projects
- Training Materials
- Links to other web sites
- Glossary of common

Forensic STR Informa

- STRs101: Brief Intro
- Core Loci: FBI COD
- STR Fact Sheets (cbs)
- Multiplex STR kits
- Sequence Information
- Variant Allele Reports
- Tri-Allelic Patterns
- Mutation Rates for Co
- Published PCR prime
- Y-chromosome STRs

Presentations on LTDNA

- John Butler - ISHI (Promega)
- Becky Hill - DSM (Promega)
- Theresa Caviglio - ISHI (NIST)

LTDNA Validation Data

- Links having validation data on
- John Butler - nist.gov

NIST Sensitivity Data with low level DNA

- 10 replicate amplifications for each condition

Low-template DNA Information

- mainSTRs (short amplicons)
- Null Alleles - discordance observed between STR kits
- STR Reference List - now 2002 references

### Complete Set of NIST Sensitivity Data Available on New LT-DNA Website

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

**NIST Sensitivity Data with low level DNA templates**  
*10 replicate amplifications for each condition with two fully heterozygous, single-source samples*

Click on links to see summaries and DNA profiles observed

STR kit - PCR conditions	Sample 1	Sample 2
Identifiler - 28 cycles	100 pg	100 pg
	30 pg	30 pg
	10 pg	10 pg
Identifiler - 31 cycles	100 pg	100 pg
	30 pg	30 pg
	10 pg	10 pg
PowerPlex 16 HS - 31 cycles	100 pg	100 pg
	30 pg	30 pg
	10 pg	10 pg
PowerPlex 16 HS - 34 cycles	100 pg	100 pg
	30 pg	30 pg
	10 pg	10 pg

### Literature Listing on LT-DNA (LCN)

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

**Subdivided into categories**

- Peer-reviewed literature (*containing data*)
- Reports (*evaluating the methodology*)
- Review articles (*commenting on other's data*)
- Non-peer reviewed literature (*representing the authors' opinions only*)

**LTDNA References**

Peer-reviewed literature (containing data)

Buckleton, J. (2009) Validation issues around DNA typing of low level DNA. *Forensic Sci. Int. Genet.* 3: 255-260.

Carpino, T., Madsen-Rich, R., Tamoto, J., Baika, E., Selverston, J., Bann, H., Poon, M. (2008) Validation of triallelic and tetraallelic protocols for low template DNA samples using AmpFISTR Identifiler. *Criminal Med. J.* 50: 250-267. [Link to paper](#)

Fendley, J., Taylor, A., Quake, P., Fraine, R., and Orghian, A. (1997) DNA fingerprinting from single cells. *Nature* 389(6872): 555-556.

Oh, P., Whittaker, J., Flanagan, C., Brown, N., and Buckleton, J. (2005) An investigation of the effect of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci. Int.* 152(1): 17-40.

**Links to papers when freely available**

### Framing the Issues

- Forensic science methods often **must work close to the edge** of a technique due to the limited nature of the evidence
  - perpetrators are usually not willing to go back and add more biological material to a crime scene...
- **Validation studies** are performed in order to **define the limits of a technique**
  - sensitivity studies to determine at what point a lab cannot obtain reliable results anymore

**We would always like improved sensitivity to enable results where ever possible**

### “Enhanced Interrogation” Techniques to Improve Sensitivity

- **Increased PCR cycle number**  
 With 100% efficiency:
  - 28 cycles = 67 million copies
  - 31 cycles = 1 billion copies (x16)
  - 34 cycles = 4 billion copies (x64)
- Reduced volume PCR
- Sample desalting (e.g., MinElute) prior to CE
- Extended CE injections

Are you “waterboarding” your DNA trying to get more information from the sample?

**Requires validation to determine appropriate thresholds for reliability**

### Low Template DNA Testing

- **Every lab faces samples with low template DNA**
  - Do you choose to attempt an “enhanced interrogation technique” such as increasing the cycle number, desalting samples, etc.?
  - **Next generation kits coming from manufacturers are capable of greater sensitivity – will they be misused without appropriate caution and validation?**
- **At what point do you draw a line and not attempt to analyze data below this line?**
  - A certain amount of input DNA (based on what data?)
  - A pre-determined stochastic threshold (based on what data?)

### Stochastic (Random) Effects with Low [DNA] When Combined with Higher Sensitivity Techniques

**Loss of True Signal (False Negative)**

Heterozygote Peak Imbalance: Identifiler, 30 pg DNA, 31 cycles

Allelic Drop-out: Identifiler, 30 pg DNA, 31 cycles (14 allele drop-out)

**Gain of False Signal (False Positive)**

Higher Stutter: Identifiler, 10 pg DNA, 31 cycles (64% stutter)

Allelic Drop-in: Identifiler, 10 pg DNA, 31 cycles (16 allele drop-in)

### Early Work on Replicate Testing with Low Levels of DNA

© 1996 Oxford University Press. *Nucleic Acid Research*, 1996, Vol. 24, No. 18, 3189-3194

**Reliable genotyping of samples with very low DNA quantities using PCR**

Pierre Taberlet\*, Sally Griffin, Benoit Goossens, Sophie Questiau, Valérie Manceau, Nathalie Escaravage, Lisette P. Waits and Jean Bouvet

Laboratoire de Biologie des Populations d'Altitude, CNRS UMR 5553, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 9, France

Received May 1, 1995; Revised and Accepted July 2, 1995

**Replicate testing introduced (up to 7 times) to account for allele dropout and avoid miscalling allele drop-in**

**In conjunction with interpretation rules, duplication of observed alleles in replicates was shown to correctly define the original sample**

### Comparison of Approaches

**Replicate Amplification with Consensus Profile**

Low amount of DNA examined

↓ Stochastic effects

**Amplification #1  
Amplification #2  
Amplification #3**

**Consensus Profile Developed** (from repeated alleles observed)

**Interpretation Rules Applied** (based on validation experience) e.g., specific loci may dropout more

**Result can be and usually is Reliable & Reproducible**

**Single Amplification**

Low amount of DNA examined

↓ Stochastic effects

**Amplification #1 (only a single test)**

**Result can be Unreliable**

What "LCN Labs" Are Doing

**Individual results may vary but a consensus profile is reproducible (based on our experience with sensitivity studies and replicate amplifications)**

### Impact of "Unreliable" Results

- Allele drop-out can be dealt with using moderate stringency searches in CODIS algorithms
  - a homozygote "14" would hit to a heterozygote "11,14"
- Allele drop-in is most problematic for DNA database searches
  - this can be corrected for with replicate testing and consensus profiles to eliminate incorrect alleles

### The Expansion of Forensic DNA Typing

1st Edition

Jan 2001  
335 pp.  
17 chapters

2nd Edition

Feb 2005  
688 pp.  
24 chapters

Chinese Translation (2007) Y. Hou, translator

Japanese Translation (2009) Y. Fukuma, translator

3rd Edition

Sept 2009

**Fundamentals**  
18 chapters (504 pp.)

**Advanced Topics**  
25 chapters (~600 pp.)

**Planned for Spring 2011**

### Improved Reference Format

**Forensic DNA Typing (2nd Edition)**

*Full list of authors but no article title*

Lesarick, K., Walsh, P.S., Oatis, S., Gilbert, D., Rowlandson, B.B., Mochales, L., Schablik, D., West, M.M., Hark, C., and Wain, J. (1996) *Electrophoresis*, 19, 96-93.

Lin, A.M., Miska, K.A., Sanchez, C.J., Taylor, J.A., Butler, J.M., Ballantyne, D., Benn, R.A., Cooney, S., and Schonen, J.B. (1996) *Journal of Forensic Sciences*, 41, 1168-1180.

Martfeld, E.S., Varner, M., End, S., Barker, D.L., Harris, D., Reggipart, E., and Fortina, P. (1996) *Genome Research*, 6, 893-903.

Martfeld, E.S., Robertson, J.M., Varner, M., Isenberg, A.R., Frazer, K.R., Ferguson, K., Oatis, S., Harris, D.W., Barker, D.L., Gels, P.D., Buttrick, E., and McCord, B.R. (1998) *Electrophoresis*, 19, 191-195.

McCord, B.R., Jung, J.M. and Hillman, E.A. (1995a) *Journal of Liquid Chromatography*, 18, 1963-1981.

McCord, B.R., McClure, D.L. and Jung, J.M. (1995b) *Journal of Chromatography A*, 682, 75-82.

McCord, B.R. (2003) Troubleshooting capillary electrophoresis systems. *Practical DNA*, 4 (2). Available at: <http://www.promega.com/practicalDNA/>. Product#DNA\_402\_10.pdf.

Matthias, R.J. (1998) *Electrophoresis*, 19, 224-226.

**Fundamentals (3rd Edition)**

**Subdivided by subject with article title provided**

**Instrument Platforms**

**ABI 372 and 377**

Fraser, E. F. T., et al. (1996) Validation of the Applied Biosystems (ABI) 377 automated sequencer for forensic short tandem repeat analysis. *Electrophoresis*, 17, 1745-1752.

Ferguson, C. J., et al. (1999) Validation of highly polymorphic fluorescent multiplex short tandem repeat systems using two generations of DNA sequencers. *Journal of Forensic Sciences*, 44, 113-120.

**FM100 Gel Imager**

Compton, S. N., et al. (2004) Validation and implementation of the Promega FM100 System 770 imager for forensic casework. *Journal of Forensic Sciences*, 49, 71-80.

**ABI Prism 310 Genetic Analyzer**

Ball, S. M., et al. (2004) Capillary electrophoresis-STR analysis: Comparison to gel-based systems. *Journal of Forensic Sciences*, 49, 104-110.

Ball, S. M., et al. (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. *Electrophoresis*, 25, 1217-1222.

Lesarick, K., et al. (1996) Genotyping of forensic short tandem repeat (STR) systems based on using precision in a capillary electrophoresis instrument. *Electrophoresis*, 19, 93-102.

### Chapters Re-ordered to Reflect DNA Testing Process

**Steps Involved**

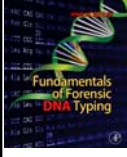
- Collection
- Sample Storage
- Characterization
- Extraction
- Quantification
- Amplification
- STR Markers
- Separation/Detection
- Data Interpretation
- Statistical Interpretation

**Fundamentals of Forensic DNA Typing (2009)**

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(no royalties to be received)**

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**Entire book was subjected to NIST review process for approval prior to publication**

**Forensic Science Review Article**  
June 15, 2009 issue of *Analytical Chemistry*  
Anal. Chem. 2009, 81, 4695-4711

**Forensic Science**


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**Review Contents**  
Forensic DNA Analysis  
Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material  
Short Tandem Repeats  
Single Nucleotide Polymorphisms  
Y-Chromosome and Y-Chromosome Analysis  
Mitochondrial DNA Typing  
Nonhuman DNA Typing Systems  
DNA Databases, Missing Persons, and Disaster Victim Identification  
Interpretation and Statistical Weight of DNA Typing Results  
General Reviews

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



**Overview of NIST Efforts**

- Communication with the Community
- Other Genetic Markers & Software
- DNA Biometrics (rapid PCR)
- International Impact (European loci/kits)
- STRBase Resources and SRMs

**Thank you for your attention!**

Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards and the **FBI S&T Branch** for DNA Biometrics projects

					
<b>Margaret Kline</b> STR allele sequencing	<b>Jan Redman</b> Variant allele cataloging	<b>Becky Hill</b> Kit analysis, miniSTRs, LCN, and 26plex work	<b>Amy Decker</b> Mixtures & Y-STRs	<b>Pete Vallone</b> Rapid PCR, SNPs, & DNA Biometrics	<b>Erica Butts</b> DNA Biometrics

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