

EDNAP and 32th ENFSI DNA WG Meeting
April 13-15, 2010 – The Hague, The Netherlands



NIST Update

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

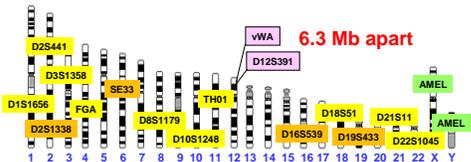


Topics to Address

- Potential linkage with D12S391 and vWA
- Concordance studies with ESX/ESI and NGM kits
- New LT-DNA section of STRBase website
- *Fundamentals* book published & *Advanced Topics* book underway

For additional information on our research activities, see our EDNAP presentation available on STRBase:
<http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm>

Chromosomal Positions for the European Standard Set and Other Common STR Markers Used



European Standard Set + D16S539, D2S1338, D19S433, SE33

Evaluation of New European STR Loci

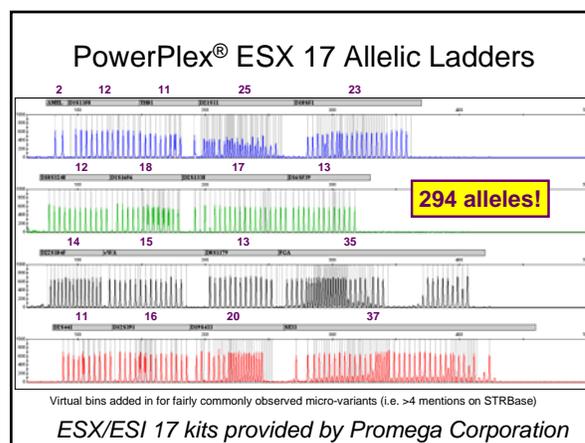
- U.S. population data collected using multiple kits
 - Examined **U.S. population data from 1443 individuals** (Caucasian, African American, Hispanic, Asian)
 - **PowerPlex ESX 17 & ESI 17 Systems (Promega)**
 - **AmpFISTR NGM Kit (Applied Biosystems)**
- Linkage analysis of vWA and D12S391
 - Located 6.3 Mb apart on chromosome 12
 - With unrelated individuals, no significant linkage in agreement with Phillips, C., et al. (2010)
 - **With related individuals, linkage observed**
 - Recommending use of diplotypes with relatives – see Lewis, K.E., et al. (submitted)

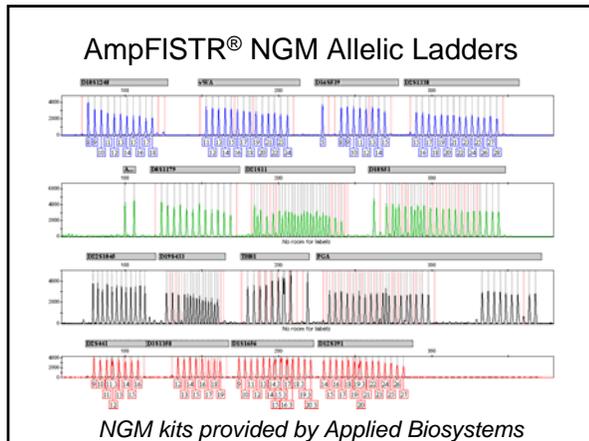
STR Loci Present in Commercial Kits

U.S.			Europe			
PP16	Identifiler	MiniFiler	ESX/ESI17	NGM	SEfiler	SGM Plus
TPOX	TPOX					
CSF1PO	CSF1PO	CSF1PO				
D5S818	D5S818					
D7S820	D7S820	D7S820				
D13S317	D13S317	D13S317				
FGA	FGA	FGA	FGA	FGA	FGA	FGA
vWA	vWA		vWA	vWA	vWA	vWA
D3S1358	D3S1358		D3S1358	D3S1358	D3S1358	D3S1358
D8S1179	D8S1179		D8S1179	D8S1179	D8S1179	D8S1179
D18S51	D18S51	D18S51	D18S51	D18S51	D18S51	D18S51
D21S11	D21S11	D21S11	D21S11	D21S11	D21S11	D21S11
TH01	TH01		TH01	TH01	TH01	TH01
D16S539	D16S539	D16S539	D16S539	D16S539	D16S539	D16S539
D2S1338	D2S1338	D2S1338	D2S1338	D2S1338	D2S1338	D2S1338
D19S433	D19S433		D19S433	D19S433	D19S433	D19S433
			D12S391	D12S391		
			D1S1656	D1S1656		
			D2S441	D2S441		
			D10S1248	D10S1248		
			D22S1045	D22S1045		
			SE33		SE33	

U.S. is looking to expand the core loci (18-20 total) to provide more international overlap

Penta D
Penta E





Summary of NIST Samples Evaluated

- **U.S. Population Samples (657 samples)**
 - Previously studied with Identifiler, MiniFiler, Yfiler, PP16, miniSTRs, and many additional assays (>200,000 allele calls)
 - 260 African Americans, 260 Caucasians, 140 Hispanics, and 3 Asians
 - <http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm>
- **U.S. Father/Son pairs (786 samples)**
 - Previously studied with Identifiler, MiniFiler, Yfiler
 - ~100 fathers/100 sons for each group: African Americans, Caucasians, Hispanics, and Asians
- **NIST SRM 2391b PCR DNA Profiling Standard (12 samples)**
 - Components 1-10 (includes 9947A and 9948): well characterized
 - ABI 007 and K562

Total number of samples attempted = 1455
1443 samples with complete profiles

FSI Genetics Forthcoming Article on PowerPlex ESX 17 and ESI 17 Systems

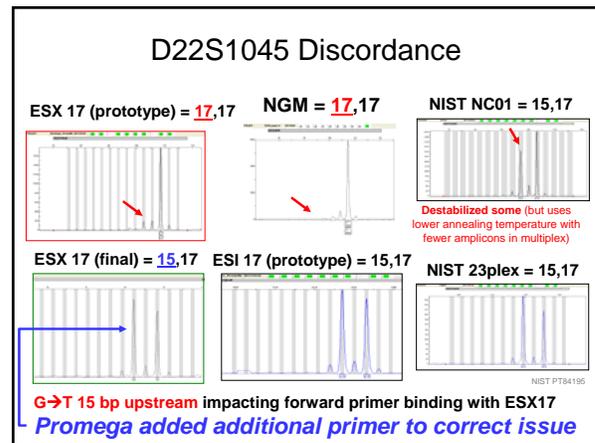
ARTICLE IN PRESS
 Forensic Science International: Genetics xxx (2010) xxx–xxx

Contents lists available at ScienceDirect
 Forensic Science International: Genetics
 journal homepage: www.elsevier.com/locate/fsig

Concordance and population studies along with **stutter and peak height ratio analysis** for the PowerPlex® ESX 17 and ESI 17 Systems

Carolyn R. Hill^{a,*}, David L. Dueder^a, Margaret C. Kline^a, Cynthia J. Sprecher^b, Robert S. McLaren^b, Dawn R. Rabbach^b, Benjamin E. Krenke^b, Martin G. Ensenberger^b, Patricia M. Falmer^b, Douglas R. Storts^b, John M. Butler^a

^aNational Institute of Standards and Technology, Chemical Science and Technology Laboratory, Gaithersburg, MD 20899-8312, USA
^bPromega Corporation, Madison, WI 53711-3399, USA



Profiles in DNA Article Published April 2010

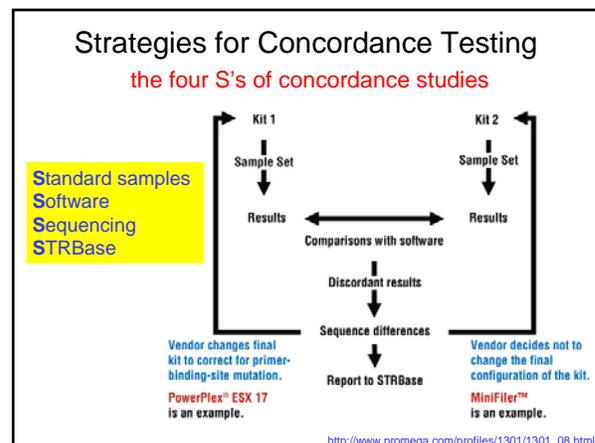
Article Type: Feature Volume 13 No. 1, April 2010

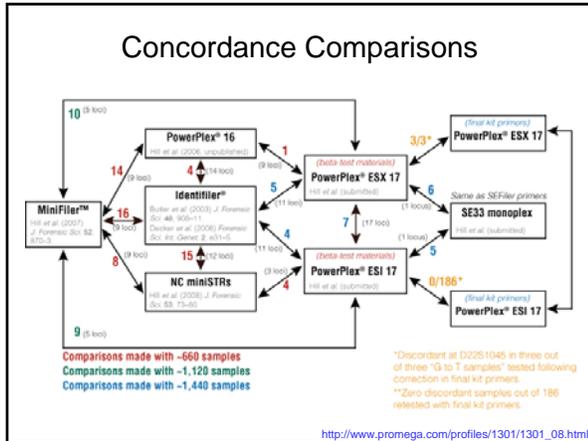
Strategies for Concordance Testing

Carolyn R. Hill, Margaret C. Kline, David L. Dueder and John M. Butler
 National Institute of Standards and Technology, Biochemical Science Division, Gaithersburg, Maryland, USA

Concordance evaluations are important to conduct to determine if there are any allelic dropout or "null alleles" present in a data set. These studies are performed because there are a variety of commercial short tandem repeat (STR) multiplex kits with different configurations of STR markers available to the forensic community. The placement of the markers can vary between kits because the primer sequences were designed to amplify different polymerase chain reaction (PCR) product sizes. When multiple primer sets are used, there is concern that allelic dropout may occur due to primer-binding-site mutations that affect one set of primers but not another.

http://www.promega.com/profiles/1301/1301_08.html





New NIST Software Tools Developed by Dave Duewer (NIST)

From NIST STRBase Website:

- Lab Resources and Tools
 - Addresses for scientists working with STRs
 - Training Materials
 - STR Allele Sequencing
 - Population data
 - Data from NIST U.S. Population Samples
 - NIST-Developed Software: Index AutoDimer, minSTR, 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 Mixture 2005 Interlab Study MIX05 Data
 - Linkage Inference
 - DNA Quantitation - SRM 2172 (available as of October 5, 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.

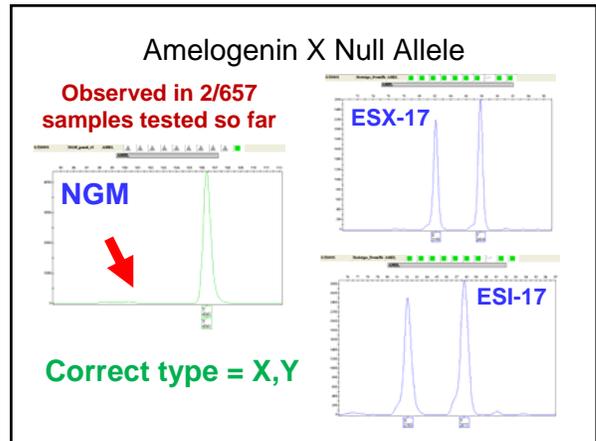
Tools under development (to aid validation studies)

- 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/software.htm>

- ### Concordance Testing with NGM Kit
- NGM results compared to ESX/ESI kits with **657 unrelated individuals** (NIST U.S. population set)
 - NGM and ESX/ESI 17 are **fully concordant** with **NIST SRM 2391b** certified values (including D2S441, D10S1248, D22S1045, D1S1656, D12S391)
 - NGM null alleles:**
 - AMEL X (2/657)
 - D18S51 (1/657)
 - D22S1045 (3/657)



New STRBase Website on LT-DNA (LCN)

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

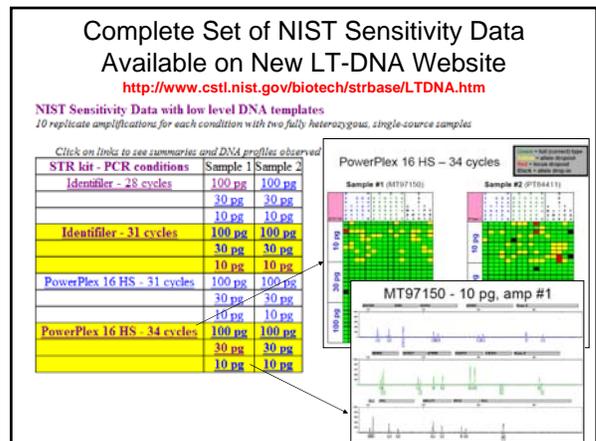
Information on Low Template / Low Copy Number DNA Testing

Scientific Issues with Analysis of Low Amounts of DNA

Presentation Prepared for the LT-DNA Panel

Theresa Caragine 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/CMLL in their entirety.



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*)

LT-DNA References

Peer-reviewed literature (containing data)

Links to papers when freely available

Buckleton, J. (2000) Validation issues around DNA typing of low level DNA. *Forensic Sci. Int. Genet.* 3: 235-240.

Craigino, T., Mikolajczyk, R., Terenzi, J., Baska, E., Schmittner, J., Bann, H., Peto, M. (2000) Validation of forensic and interpretation protocols for low template DNA samples using AmpFISTR Identifiler. *Criminal Med. J.* 50: 250-261. [\[link to paper\]](#)

Freidly, J., Taylor, A., Quake, P., Frasier, R., and Orban, A. (1997) DNA sequencing from single cells. *Nature* 389(6612): 555-558.

Oh, P., Whitaker, J., Flanagan, C., Brown, N., and Buckleton, J. (2005) An evaluation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci. Int.* 152(1): 1-45.

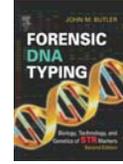
The Expansion of *Forensic DNA Typing*

1st Edition



Jan 2001
335 pp.
17 chapters

2nd Edition

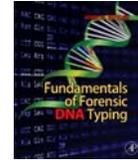


Feb 2005
688 pp.
24 chapters

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

Lazarek, K., Walsh, P.S., Gals, G., Gilbert, D., Rasmussen, B.B., Minchen, L., Schellberg, D., Wang, H.M., Harris, C., and Watts, J. (1998) *Electrophoresis*, 19, 86-93.

Li, A.M., Miska, K.A., Sprafkin, C.J., Tashir, J.A., Bachler, J.W., Ralbach, D., Bawa, R.A., Conway, S., and Schmittner, J.W. (1998) *Journal of Forensic Sciences*, 43, 1163-1180.

Marolf, E.S., Vianer, M., Doad, S., Barker, D.L., Harris, D., Rappaport, E., and Fortina, P. (1998) *Genome Research*, 8, 850-863.

Marolf, E.S., Robinson, J.M., Vianer, M., Isomberg, A.R., Frasier, R.B., Ferguson, K., Chen, S., Harris, D.W., Barker, D.L., Gil, F.D., Ralbach, S., and McGee, S.A. (1998) *Electrophoresis*, 19, 183-197.

McGee, S.R., Jung, J.M., and Holloway, E.A. (1993a) *Journal of Liquid Chromatography*, 16, 1963-1981.

McGee, S.R., McKen, D.L., and Jung, J.M. (1993b) *Journal of Chromatography A*, 602, 79-82.

McGee, S.R. (2003) Troubleshooting reagent electrophoresis systems. *Profile in DNA*, 4(2). Available at: http://www.genome.gov/genetics/fundamentals/ProfileDNA_S02_10.pdf

Makishima, K. (1998) *Electrophoresis*, 19, 224-226.

Fundamentals
(3rd Edition)

Subdivided by subject
with article title provided

Instrument Platforms
ABI 377 and 377
Harris, E. E., et al. (1996) Validation of the Applied Biosystems Prism™ 177 automated sequencer for forensic short tandem repeat analysis. *Electrophoresis*, 17, 1556-1557.

Ferguson, C. J., et al. (1999) Validation of highly polymorphic Bioscience Analytix short tandem repeat system using two generations of DNA sequences. *Journal of Forensic Sciences*, 44, 133-140.

FMBIO Gel Imager
Gomberg, S. A., et al. (2004) Validation and implementation of the PowerPlex 16-880 forensic STR multiplex for forensic casework. *Journal of Forensic Sciences*, 49, 7-10.

>1500 references total
(>600 new since 2nd edition)

ABI Prism 310 Genetic Analyzer
Bost, E., et al. (1999) Capillary electrophoresis STR analysis: Comparison to gel-based systems. *Journal of Forensic Sciences*, 44, 104-110.

Banks, J. M., et al. (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. *Electrophoresis*, 25, 1397-1402.

Lawlor, K., et al. (1998) Genotyping of forensic short tandem repeat (STR) systems based on using precision in a capillary electrophoresis instrument. *Electrophoresis*, 19, 461-50.

Chapters Re-ordered to Reflect DNA Testing Process

Steps Involved

Serology

Biology

Technology

Genetics

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

Fundamentals of Forensic DNA Typing (2009)

CHAPTER 1	Overview and History of DNA Typing	1
CHAPTER 2	Basics of DNA Biology and Genetics	19
CHAPTER 3	Historical Methods	41
CHAPTER 4	Sample Collection, Storage, and Characterization	79
CHAPTER 5	DNA Extraction	99
CHAPTER 6	DNA Quantitation	111
CHAPTER 7	DNA Amplification (The Polymerase Chain Reaction)	125
CHAPTER 8	Short Tandem Repeat Markers	147
CHAPTER 9	Fundamentals of DNA Separation and Detection	175
CHAPTER 10	STR Genotyping and Data Interpretation	205
CHAPTER 11	Statistical Interpretation: Evaluating the Strength of Forensic DNA Evidence	229
CHAPTER 12	DNA Databases	259
CHAPTER 13	Quality Assurance	291
CHAPTER 14	Forensic Challenges: Degraded DNA, Mixtures, and LCN	315
CHAPTER 15	Useful Loci and Nonhuman DNA Testing	341
CHAPTER 16	Lineage Markers: Y Chromosome and mtDNA Testing	363
CHAPTER 17	Applications of DNA Typing	397
CHAPTER 18	Future Trends	421
APPENDIX 1	Glossary of Terms	439
APPENDIX 2	Useful Web Sites	471
APPENDIX 3	Probability and Statistics	475
INDEX		485

References are provided at the end of each chapter by subject (but without direct citation within the text).

The NIST Human Identity Project Team
(Forensic DNA & DNA Biometrics)

Funding from the **National Institute of Justice (NIJ)** through the NIST Office of Law Enforcement Standards and the **FBI S&T Branch** through the NIST Information Access Division

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

John Butler
Project Leader, Forensic DNA

Erica Butts

Mike Coble

Dave Diewer

Becky Hill

Margaret Kline

Kristen Lewis

Jan Redman

Pete Vallone
Project Leader, DNA Biometrics

Workshops & Textbooks

- DNA Extraction Efficiency
- Mixtures, mtDNA & Y
- Software Tools & Data Analysis
- Concordance & LT-DNA
- Variant alleles & Cell Line ID
- Kinship Analysis
- Rapid PCR & Biometrics
- STRBase Support

<http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm>
john.butler@nist.gov
001-301-975-4049

Questions?

