Applied Genetics

NIST Update for SWGDAM

John M. Butler
Fredericksburg, VA
July 15, 2010

NIST Human Identity Project Teams
within the Applied Genetics Group

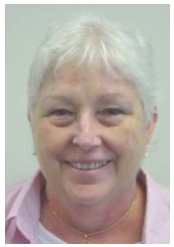
Forensic DNA Team


John Butler


Mike Coble


Becky Hill


Margaret Kline


Jan Redman

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

DNA Biometrics Team


Pete Vallone


Erica Butts


Kristen Lewis O'Connor

Funding from the **FBI S&T Branch** through NIST Information Access Division

Data Analysis Support


Dave Duewer

In March 2010, **Mike Coble returned to NIST** after 4 years at AFDIL

New Staff and Projects

Erica Butts – DNA extraction

Kristen Lewis - kinship analysis

Amy Decker left for AFDIL in Nov 2009

Applied Genetics

APPLIED GENETICS Group

Major Programs Currently Underway

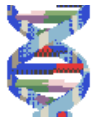
- **Forensic DNA**
  - New loci and assays (26plex)
  - **STR kit testing - concordance**
  - Ancestry SNP assays
  - Low-template DNA studies
  - Mixture interpretation
  - **SRM 2391c**
  - Variant allele cataloging and sequencing
  - Expert systems review
  - Training workshops to forensic DNA laboratories
  - Validation information and software tools
  - **Textbook** – 3rd 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
  - DNA swab extraction efficiencies
  - **Kinship analysis**

- **Cell Line Authentication**



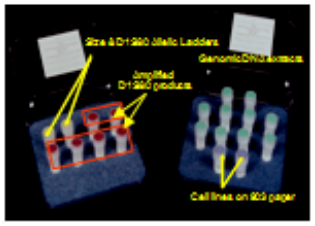
Forensic DNA Standard Reference Materials

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
2392-I	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

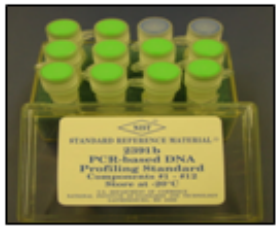
PCR-based DNA Profiling Standard

SRM 2391
(1995)



SRM 2391a
(2000)

SRM 2391b
(2003, r2008)



SRM 2391c
(planned 2010)

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

Will likely run out of SRM 2391b in mid-2011

*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

SRM 2391c Plans

- 6 components (different DNA samples)
- Inclusion of a mixture sample
- Genomic DNA + DNA spotted on paper
- Will certify both autosomal STRs and Y-STRs
- Will replace both SRM 2391b and SRM 2395
  - Goal to have lower cost and single STR SRM

- Have typed >25 DNA candidate samples to assess allele ranges and combinations
- Have studied different formats
  - buccal swab DNA, etc.

Opinion Poll on SRM 2391c

- Volume provided (20 uL or 50 uL)?
- Concentration (1 ng/uL, 2 ng/uL, other)?
- Mixture to be provided
  - supply reference samples as singles?
  - Mixture ratio (5:1, 3:1, other?)
- Genomic vs DNA spotted on paper
  - 5 genomic + 1 spot
  - 4 genomic + 2 spot
- Paper spots: 903 or FTA?
- Sequence information on all alleles?
  - **will have coverage for all STR kits available**

Commercially Available STR Kits

7 out of 17

Applied Biosystems

- ~~AmpFlSTR Blue (1996)~~
- ~~AmpFlSTR Green I (1997)~~
- Profiler (1997)
- Profiler Plus (1997)
- COfiler (1998)
- SGM Plus (1999)
- **Identifiler** (2001)
- Profiler Plus ID (2001)
- ~~SEfiler (2002)~~
- Yfiler (2004)
- MiniFiler (2007)
- SEfiler Plus (2007)
- Sinofiler (2008) – China only
- Identifiler Direct (2009)
- NGM (2009)
- **Identifiler Plus** (2010)
- NGM SElect (2010)

5 out of 12

Promega Corporation

- PowerPlex 1.1 (1997)
- PowerPlex 1.2 (1998)
- PowerPlex 2.1 (1999)
- **PowerPlex 16** (2000)
- PowerPlex ES (2002)
- PowerPlex Y (2003)
- PowerPlex S5 (2007)
- **PowerPlex 16 HS** (2009)
- PowerPlex ESX 16 (2009)
- PowerPlex ESX 17 (2009)
- PowerPlex ESI 16 (2009)
- PowerPlex ESI 17 (2009)

Qiagen (2010)

Primarily selling kits in Europe

Due to patent restrictions cannot sell in U.S.

- ESSplex
- ESSplex SE
- Decaplex SE
- IDplex
- Nonaplex ESS
- Hexaplex ESS
- HD (Chimera)
- Argus X-12
- Argus Y-12
- DIPlex (30 indels)

~1/3 of all STR kits were released in the last year

Improved buffer formulation to handle PCR inhibitors
(user no longer adds TaqGold DNA polymerase)

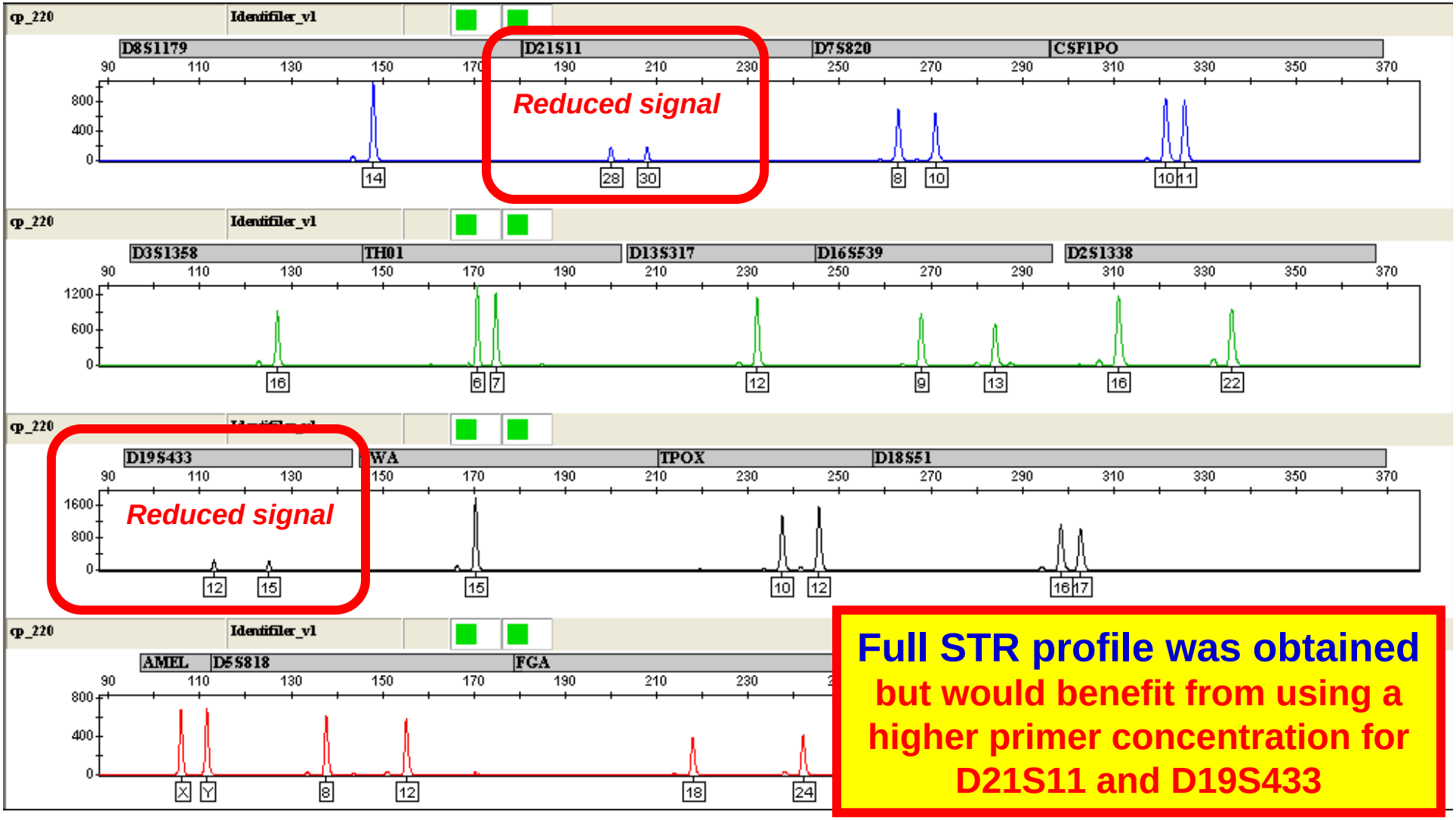
Goals with STR Kit Improvements

- **Better resistance to PCR inhibitors**
- **Higher sensitivity**
- **More rapid PCR amplification**

Can be achieved through changing:

- Primer concentration and chemistry
- Polymerase
- Buffer (components & concentrations)

20 Minute PCR Amplification
on Cepheid Cycler

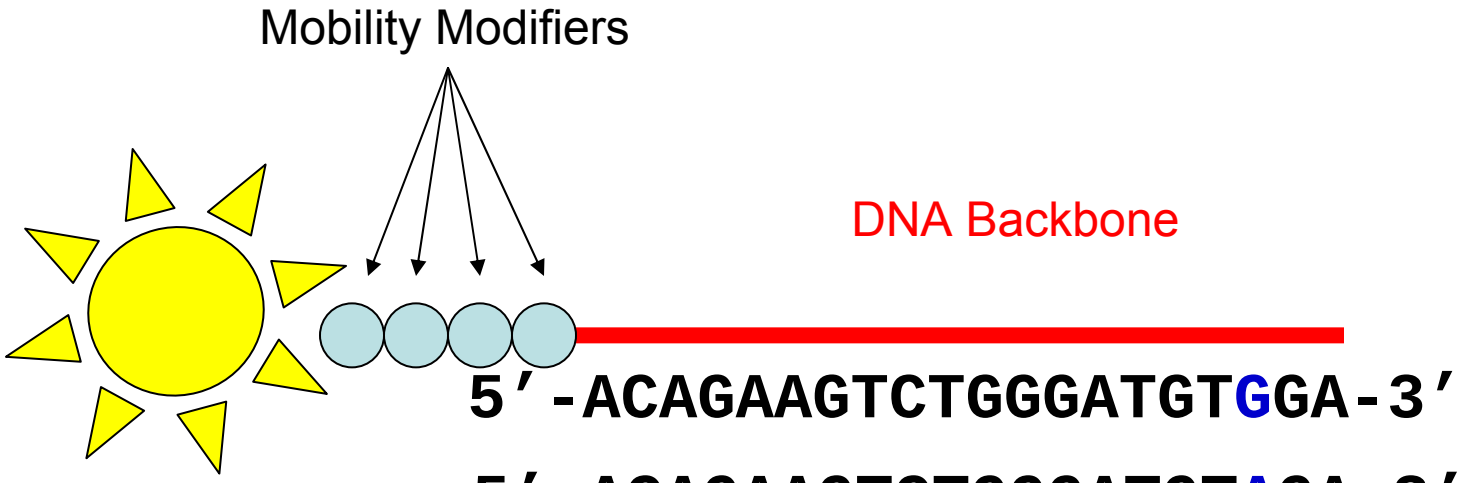


Full STR profile was obtained but would benefit from using a higher primer concentration for D21S11 and D19S433

Using fast cycler and new DNA polymerases

28 cycles, Identifiler STR kit, 1 ng of DNA

Potential Primer Modifications



DNA Base Sequence

Primer Concentration (relative to other primers in the PCR reaction mix)

The base sequence determines the primer binding position while the primer concentration helps produce the PCR product yield

Recent ABI Patent Applications

- "Method for Direct Amplification from Crude Nucleic Acid Samples" (Identifiler Direct?)
  - **Chang et al. (Jan 21, 2010) US 2010/0015621**
  - Buffer includes 0.2-0.9% polysorbate, 3%-8% glycerol, and 1000-3000 µg/mL BSA

- "Method and Composition for Nucleic Acid Amplification" (Identifiler Plus?)
  - **Mulero & Hennessy (Jan 1, 2009) US 2009/0004662**
  - Utilize a high stability nucleic acid analog (e.g., LNA or locked nucleic acid) to overcome PCR inhibitors

To obtain free copies of patent applications, see: http://www.pat2pdf.org/

Locked Nucleic Acids (LNAs)

G Model
FSIGEN-594; No. of Pages 5

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Forensic Science International: Genetics xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

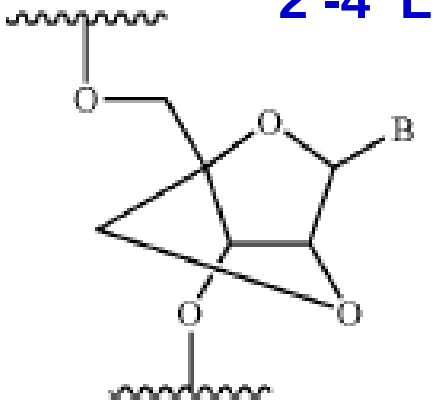
Forensic Science International: Genetics

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

Increased amplification success from forensic samples with locked nucleic acids

Kaye N. Ballantyne [a,b,*], Roland A.H. van Oorschot [a], R. John Mitchell [b]

[a] Biology Division, Victoria Police Forensic Services Department, Macleod, Victoria, Australia



2'-4' LNA

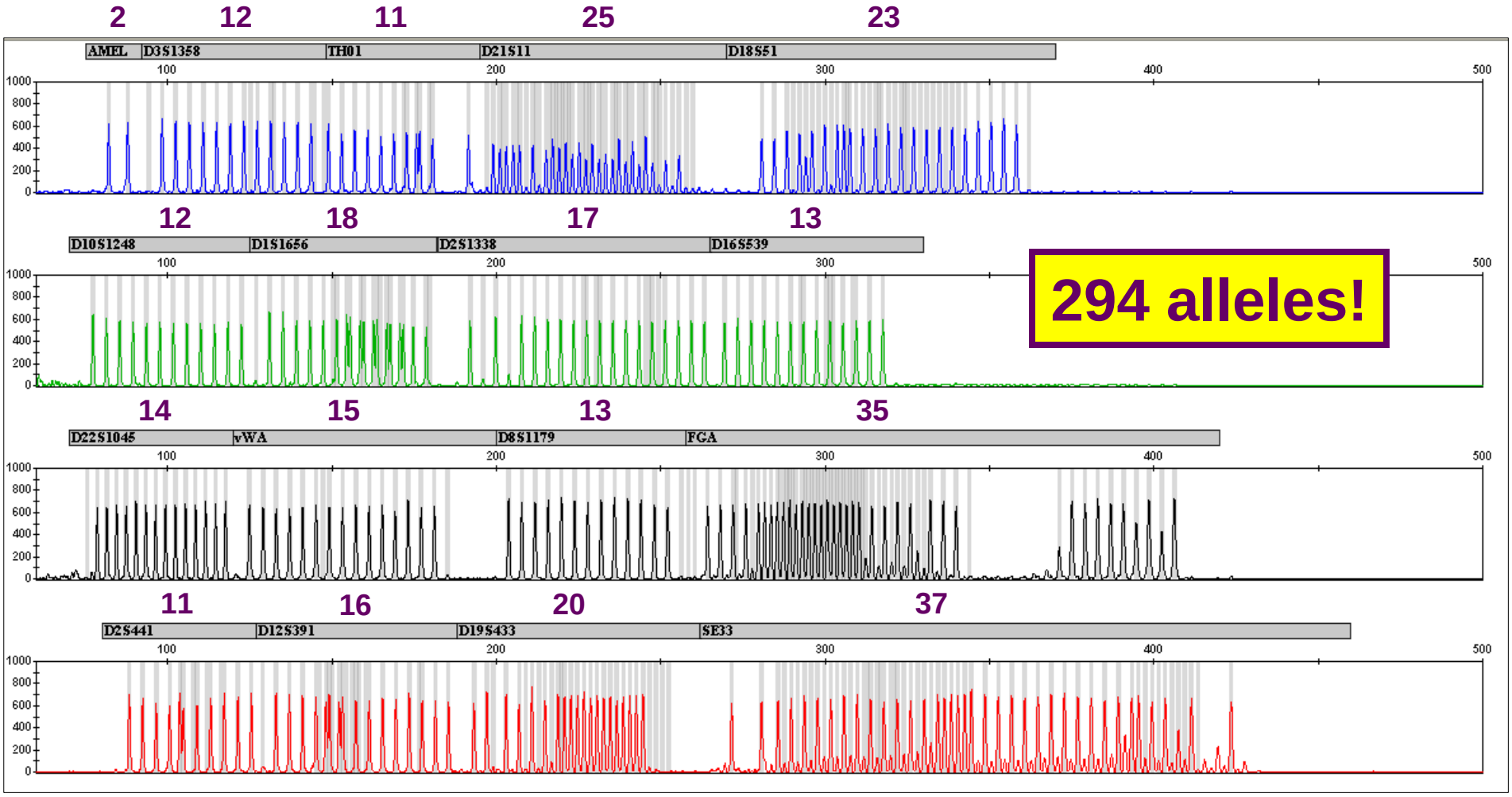
LNAs are nucleic acid analogues that **form standard Watson-Crick base pairs but with increased thermal stability** and improved capability for discriminating mismatches – **and are more tolerate to PCR inhibitors…**

NIST Pipeline for STR Kit Analysis

Work by Becky Hill and Dave Duewer

- Concordance testing with standard samples
  - Sequence analysis of any null alleles to understand differences
- Locus characteristics
  - Heterozygote peak height ratios
  - Stutter percentages (including allele-specific)
- Allele frequencies for all new loci
  - Across U.S. Caucasian, Hispanic, African American, and Asian
- Probability of identity for different locus sets

PowerPlex® ESX 17 Allelic Ladders



Virtual bins added in for fairly commonly observed micro-variants (i.e. >4 mentions on STRBase)

ESX/ESI 17 kits provided by Promega Corporation

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. Duewer and John M. Butler
National Institute of Standards and Technology, Biochemical Science Division, Gaithersburg, Maryland, USA

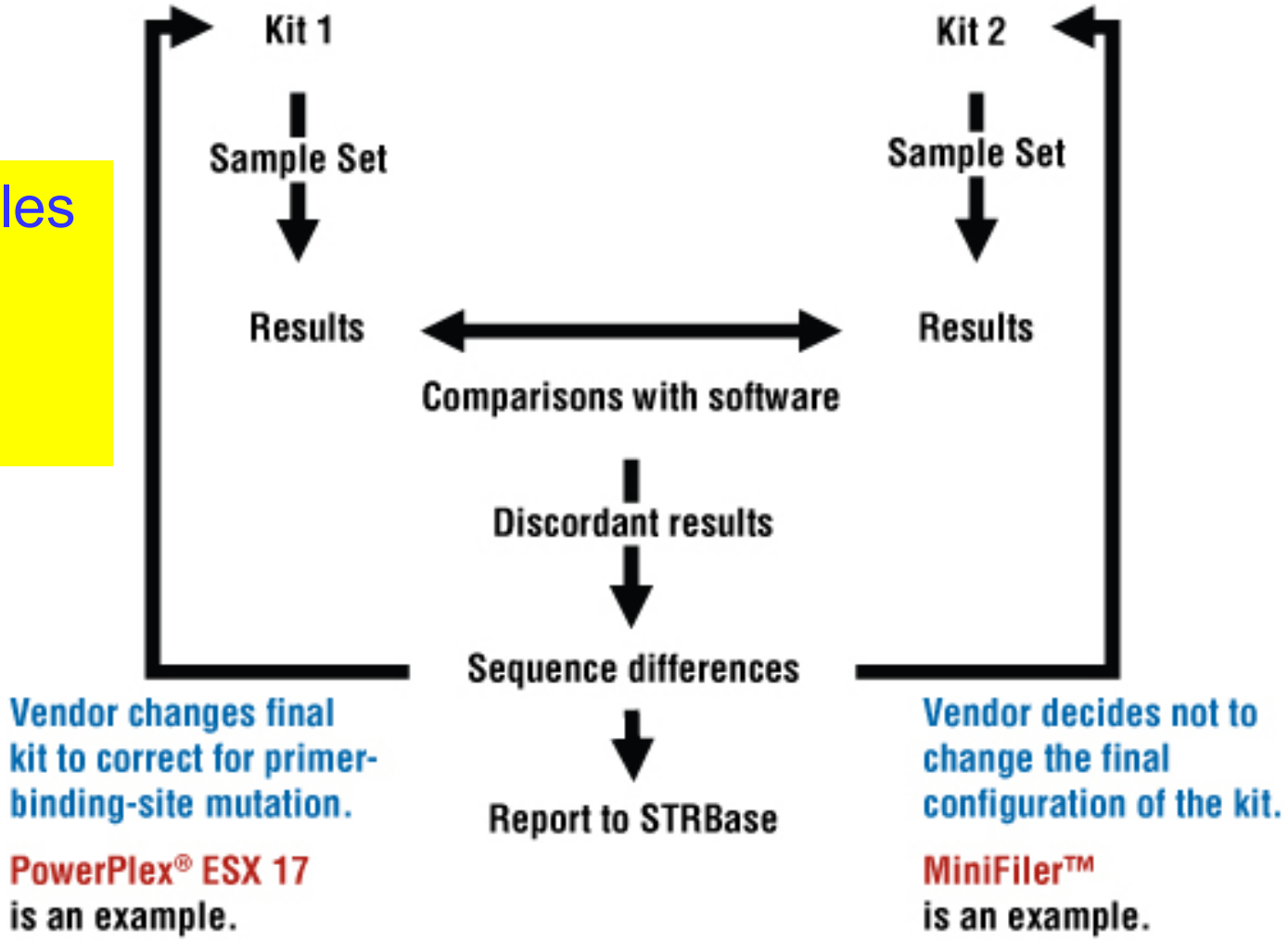
Concordance evaluations are important to conduct to determine if there are any allelic dropout or "null alleles" present in a data set. These studies are performed because there are a variety of commercial short tandem repeat (STR) multiplex kits with different configurations of STR markers available 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 allele 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

Strategies for Concordance Testing

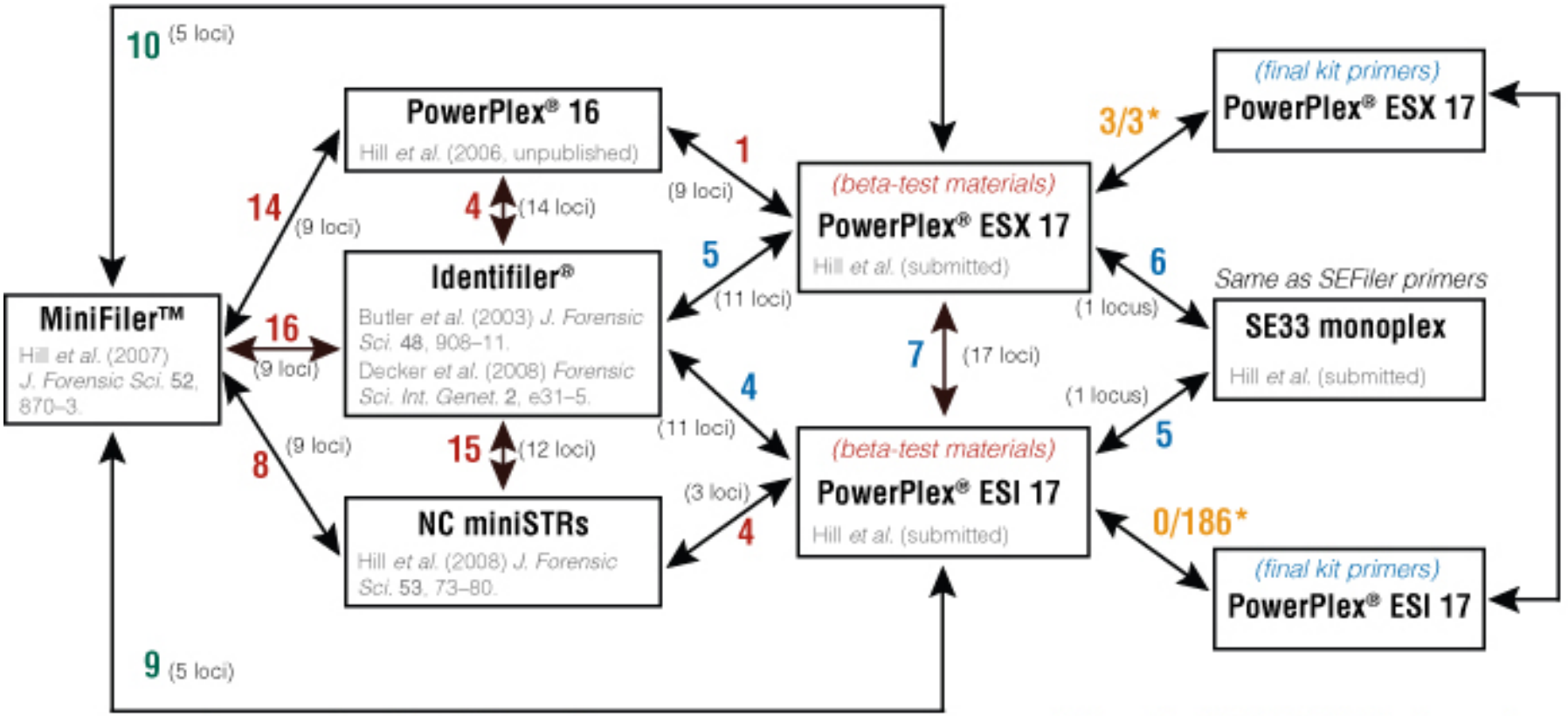
the four S's of concordance studies

Standard samples
Software
Sequencing
STRBase



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

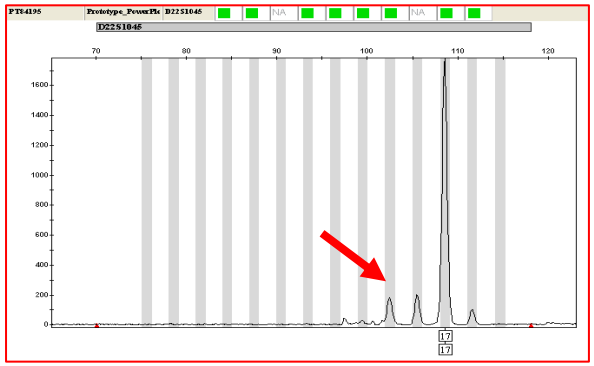
Concordance Comparisons



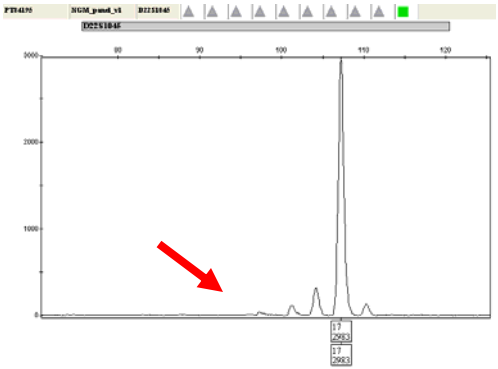
http://www.promega.com/profiles/1301/1301_08.html

D22S1045 Discordance

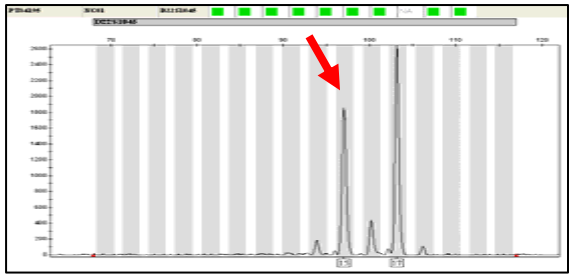
ESX 17 (prototype) = 17,17



NGM = 17,17

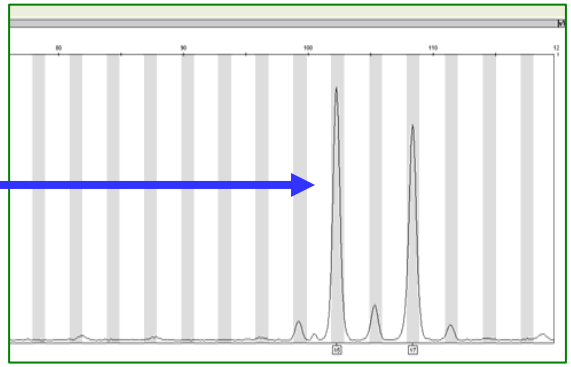


NIST NC01 = 15,17

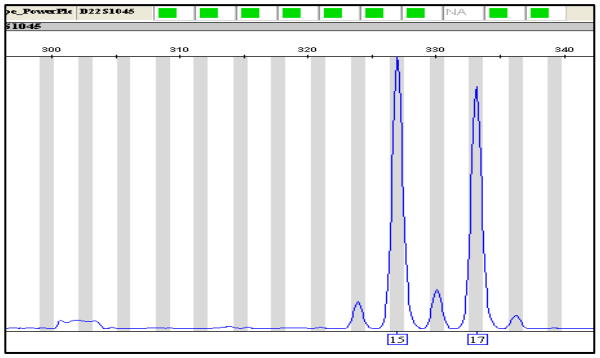


Destabilized some (but uses lower annealing temperature with fewer amplicons in multiplex)

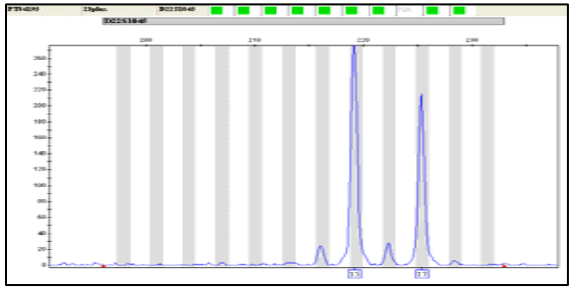
ESX 17 (final) = 15,17



ESI 17 (prototype) = 15,17



NIST 23plex = 15,17

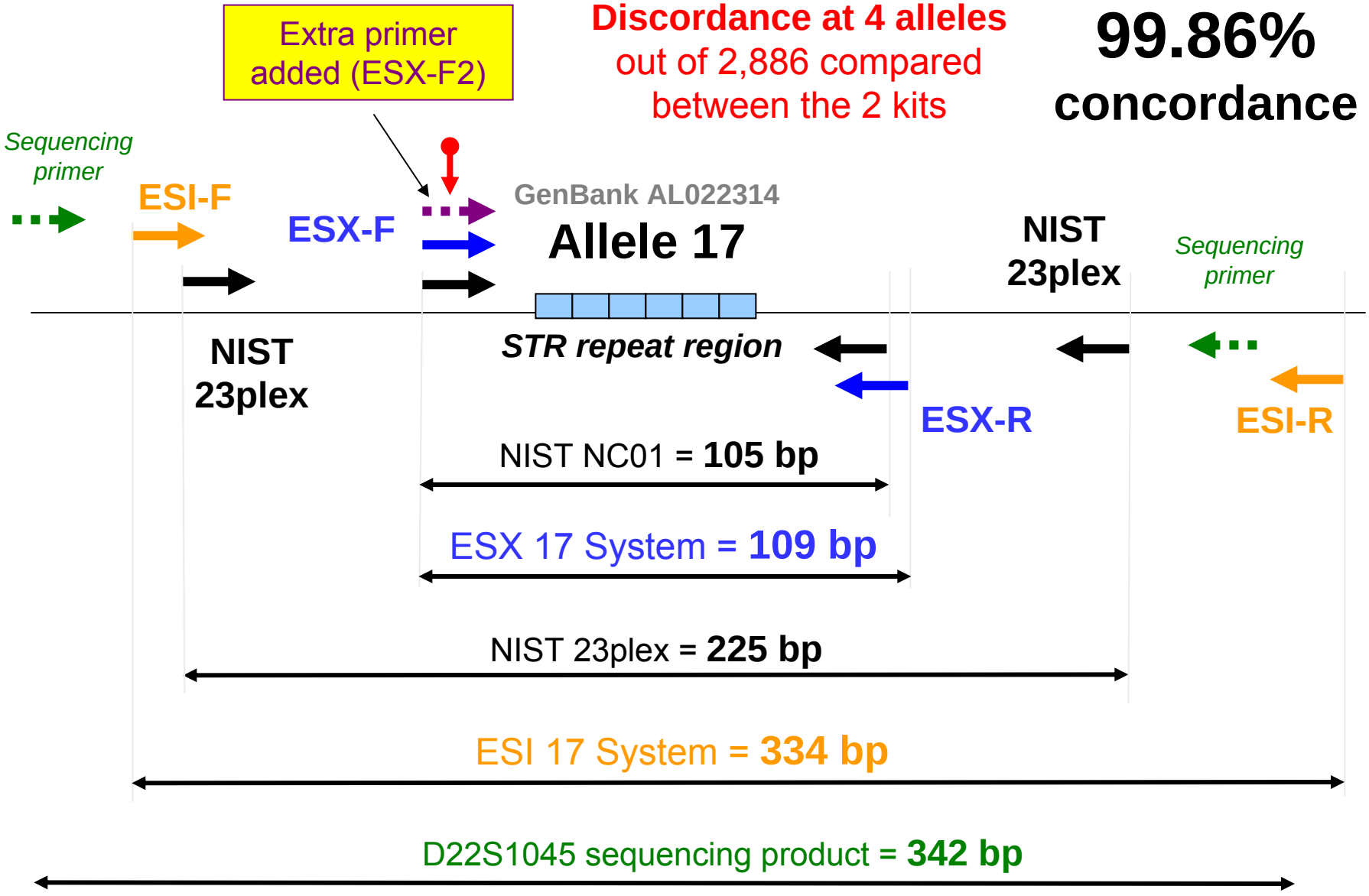


NIST PT84195

G→T 15 bp upstream impacting forward primer binding with ESX17

Promega added additional primer to correct issue

D22S1045 Relative PCR Primer Positions



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

G Model
FSIGEN-590; No. of Pages 7

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. Duewer [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. Fulmer [b], Douglas R. Storts [b], John M. Butler [a]

[a] *National Institute of Standards and Technology, Chemical Science and Technology Laboratory, Gaithersburg, MD 20899-8312, USA*
[b] *Promega Corporation, Madison, WI 53711-5399, USA*

Available online 23 April 2010

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. Popula[illegible] Samples
- NIST-Developed Software including AutoDimer, mixSTR, 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
- Validation information
- DNA Quantitation - SRM 2372 **(available as of October 5, 2007)**
- Technology for resolving STR alleles

http://www.cstl.nist.gov/biotech/strbase/tools/STR_MatchSamples.xls

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/software.htm

Stutter Thresholds for Each Locus

	PP-ESX17						PP-ESI17			
Locus	**50%**	**90%**	**95%**	**100%**		**Locus**	**50%**	**90%**	**95%**	**100%**
TH01	1.8	3.1	3.4	5.2		TH01	1.7	3.2	3.5	5.5
D2S441	4.8	6.7	7.2	9.8		D2S441	4.2	6.1	6.5	9.6
D16S539	5.8	8.6	9.0	11.1		D16S539	5.3	7.5	7.9	9.3
D8S1179	6.1	7.9	8.7	15.8		FGA	5.7	8.5	9.1	24.5
D19S433	6.2	8.2	8.8	11.4		D19S433	5.7	8.5	9.3	13.6
FGA	6.5	9.1	9.7	11.9		D8S1179	6.0	8.0	8.6	11.0
D18S51	6.9	9.8	10.4	13.3		D10S1248	6.7	8.6	9.2	11.8
D21S11	7.0	8.9	9.5	15.5		vWA	6.8	8.8	9.4	15.6
vWA	7.1	9.3	9.7	14.4		SE33	6.9	9.4	10.1	18.7
D2S1338	7.3	9.4	10.0	13.2		D1S1656	6.9	10.2	11.3	16.2
D3S1358	8.2	10.0	10.5	12.3		D21S11	6.9	9.8	11.6	20.5
D10S1248	8.2	10.4	11.0	16.4		D18S51	7.4	10.4	11.3	24.0
D12S391	8.2	11.9	13.0	16.1		D12S391	7.7	12.1	13.3	28.9
SE33	8.3	10.6	11.5	15.2		D2S1338	8.0	10.4	11.5	17.2
D1S1656	8.5	11.5	12.3	18.1		D3S1358	8.1	9.9	10.4	13.9
D22S1045	8.9	14.5	16.5	21.0		D22S1045	9.3	15.8	16.9	24.7

Allele-Specific Stutter Percentages

Trinucleotide **D22S1045**

ESX 17

Allele	Size	Stutter #	Stutter Median
10	84.5	21	1.8
11	87.4	134	3.0
12	90.4	37	4.2
14	96.4	51	7.2
15	99.4	165	8.9
16	102.4	120	10.5
17	105.5	105	14.7
	Avg	633	7.2
	SD		4.6

633 data points

Avg + 3SD

21.0%

ESI 17

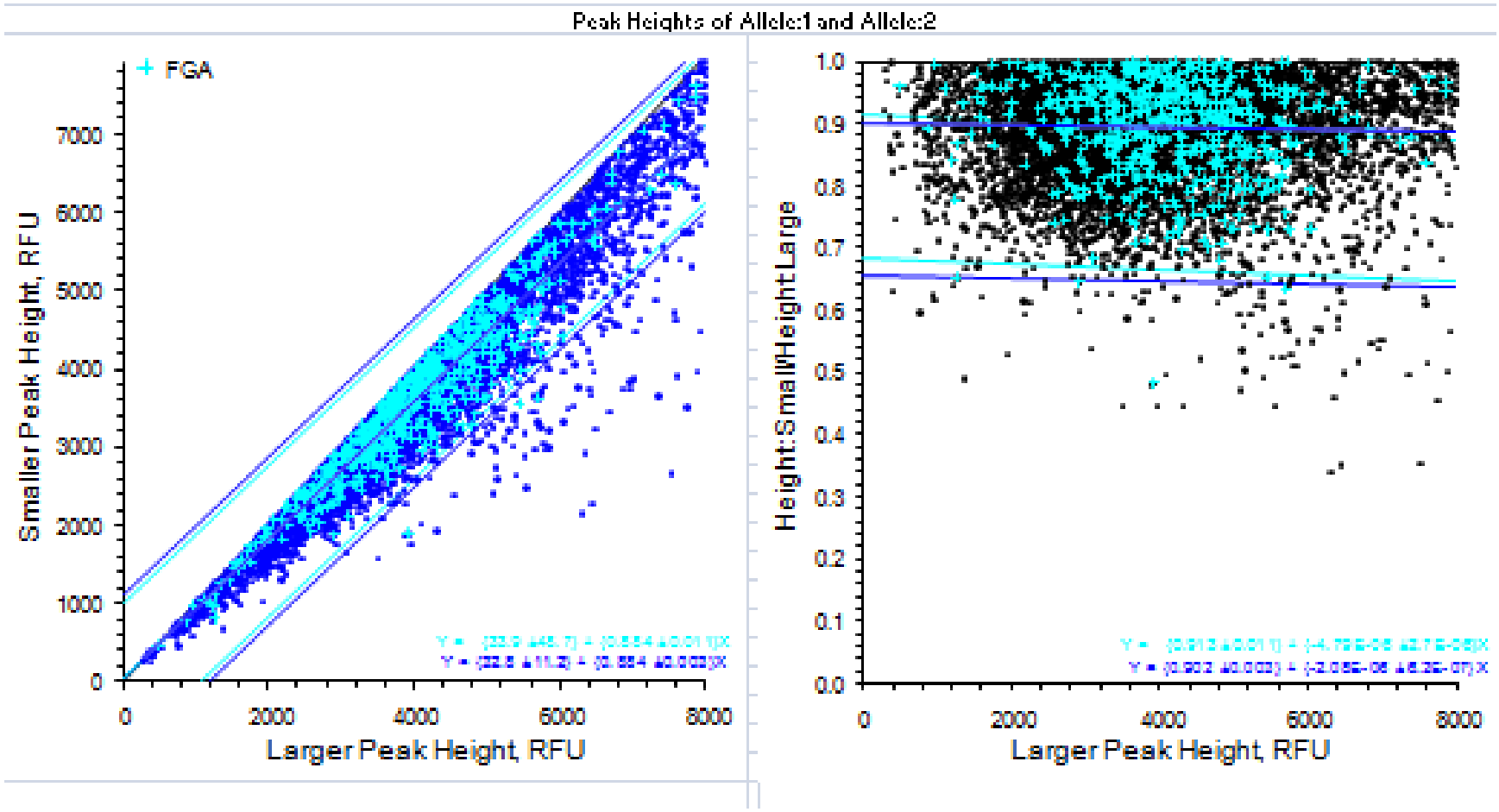
Allele	Size	Stutter #	Stutter Median
10	308.7	22	1.9
11	311.8	98	2.8
12	314.8	32	4.5
14	321.0	36	6.1
15	324.0	150	9.9
16	327.1	94	9.8
17	330.1	95	14.2
	Avg	527	7.0
	SD		4.4

527 data points

Avg + 3SD

20.2%

Peak Height Ratios Across All Loci in a New STR Kit



Probability of Identity with Various Populations and Sets of STR Loci

		Probability of Identity				
Set of STR Loci	**# STR Loci**	**Total**	**Caucasian**	**African American**	**Hispanic**	**Asian**
ESS	7	7.51×10^{-10}	1.75×10^{-9}	1.51×10^{-9}	1.61×10^{-9}	8.10×10^{-9}
SGM Plus	10	6.47×10^{-14}	3.90×10^{-13}	8.80×10^{-14}	2.27×10^{-13}	1.74×10^{-12}
CODIS 13	13	4.58×10^{-16}	3.00×10^{-15}	8.90×10^{-16}	1.53×10^{-15}	8.18×10^{-15}
PP16*	15	2.87×10^{-19}	5.92×10^{-18}	8.85×10^{-19}	--	--
Identifiler	15	5.46×10^{-19}	6.91×10^{-18}	7.03×10^{-19}	2.86×10^{-18}	1.89×10^{-17}
ESX or ESI 16	15	**2.58×10^{-20}**	2.02×10^{-19}	3.87×10^{-20}	4.53×10^{-19}	3.14×10^{-18}
ESX or ESI 17	16	**1.61×10^{-22}**	1.43×10^{-21}	4.04×10^{-22}	3.86×10^{-21}	3.63×10^{-20}

Probability of Identity (P_I): Probability that two individuals selected at random will have an identical genotype at the tested locus (Sensabaugh 1982)

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
	D19S433		D19S433	D19S433	D19S433	D19S433
			D12S391	D12S391		
			D1S1656	D1S1656		
			D2S441	D2S441		
			D10S1248	D10S1248		
			D22S1045	D22S1045		
			SE33		SE33	
Penta D						
Penta E						

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

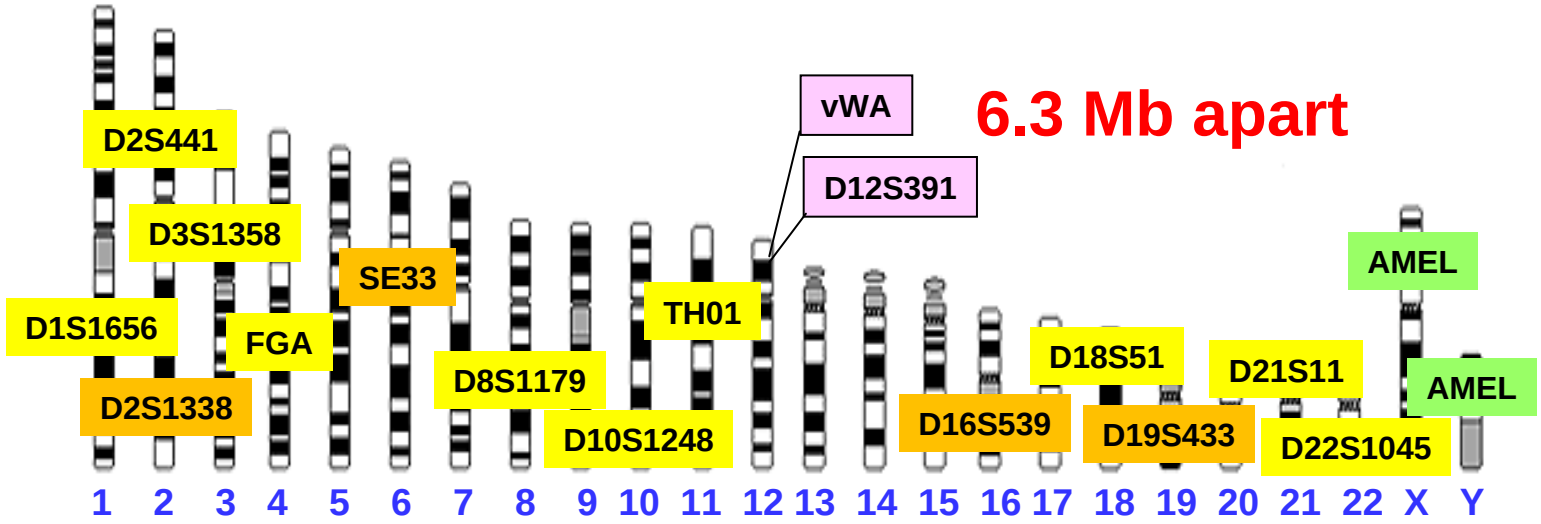
New STR Loci Adopted In Europe

- Approved November 30th, 2009
- Expand the European Standard Set (ESS)
  - FGA, vWA, D3S1358, D8S1179, D18S51, D21S11, TH01
- Additional 5 STR loci
  - D1S1656 and D12S391
  - D2S441, D10S1248, D22S1045
- Extra loci to be considered?
  - D16S539 (CODIS locus)
  - D2S1338 and D19S433
  - Penta D and Penta E
  - SE33

May impact future U.S. expansion of loci

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)**
  - **AmpFlSTR 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)

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

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)

343 genotypes observed
Heterozygosity = 0.9377

Allele	Total #	Total %	Populations, % Af Am	Asian	Cauc	Hisp
6.3						
7						
8						
10.2						
11						
11.2	2	0.1	0.2			
12	11	0.4	0.3		0.5	0.4
12.2	4	0.1	0.2			0.3
13	31	1.1	1.1		1.5	1.0
13.2	9	0.3	1.0			
14	85	2.9	5.1	0.2	2.5	2.4
14.2	10	0.3	0.4		0.4	0.3
15	102	3.5	3.9	1.2	3.9	3.9
15.2	8	0.3	0.3			0.7
16	144	5.0	4.8	4.7	4.0	6.7
16.2	5	0.2	0.3		0.1	0.1
16.3	2	0.1				0.3
17	205	7.1	9.3	4.0	6.2	7.3
17.2	1	0.0	0.1			
17.3	5	0.2	0.1		0.2	0.3
18	268	9.3	12.1	5.0	7.2	**11.0**
18.3	1	0.0			0.1	
19	250	8.7	**12.4**	6.2	6.6	8.0
19.2	8	0.3		0.2	0.4	0.4
20	216	7.5	10.9	9.2	5.4	4.8
20.2	20	0.7	0.3	1.2	1.1	0.3
21	108	3.7	4.6	6.7	2.4	2.7
21.2	48	1.7	1.1	1.7	2.4	1.3
22	42	1.5	1.3	1.7	1.5	1.3
22.2	65	2.3	0.4	3.2	3.8	1.9
23	12	0.4	0.6	1.0	0.2	0.1
23.2	91	3.2	2.2	4.2	4.3	2.1
24	1	0.0			0.1	
24.2	74	2.6	1.3	6.2	2.2	2.5
25.2	109	3.8	2.6	6.9	4.0	3.1
26	1	0.0	0.1			
26.2	163	5.6	6.1	5.2	4.3	7.1
27	1	0.0				0.1
27.2	225	7.8	4.3	**10.4**	**9.5**	8.6
27.3	2	0.1				0.3
28	2	0.1	0.1	0.2		
28.2	180	6.2	4.4	7.9	7.4	6.1
28.3	2	0.1	0.1		0.1	
29	1	0.0		0.2		
29.2	147	5.1	2.7	5.7	6.3	6.3
29.3	1	0.0		0.2		
30	1	0.0				0.1
30.2	111	3.8	1.6	3.2	5.8	4.6
31	3	0.1	0.1		0.2	
31.2	52	1.8	1.5	2.5	2.2	1.3
32	1	0.0			0.1	
32.2	25	0.9	0.4	0.7	1.3	0.9
33	2	0.1			0.1	0.1
33.2	11	0.4	0.3		0.5	0.4
34	9	0.3	0.3		0.7	
34.2	1	0.0			0.1	
35	1	0.0	0.1			
36	2	0.1	0.2			

D12S391

(#allele: 24)

24 alleles &
120 genotypes observed

Heterozygosities

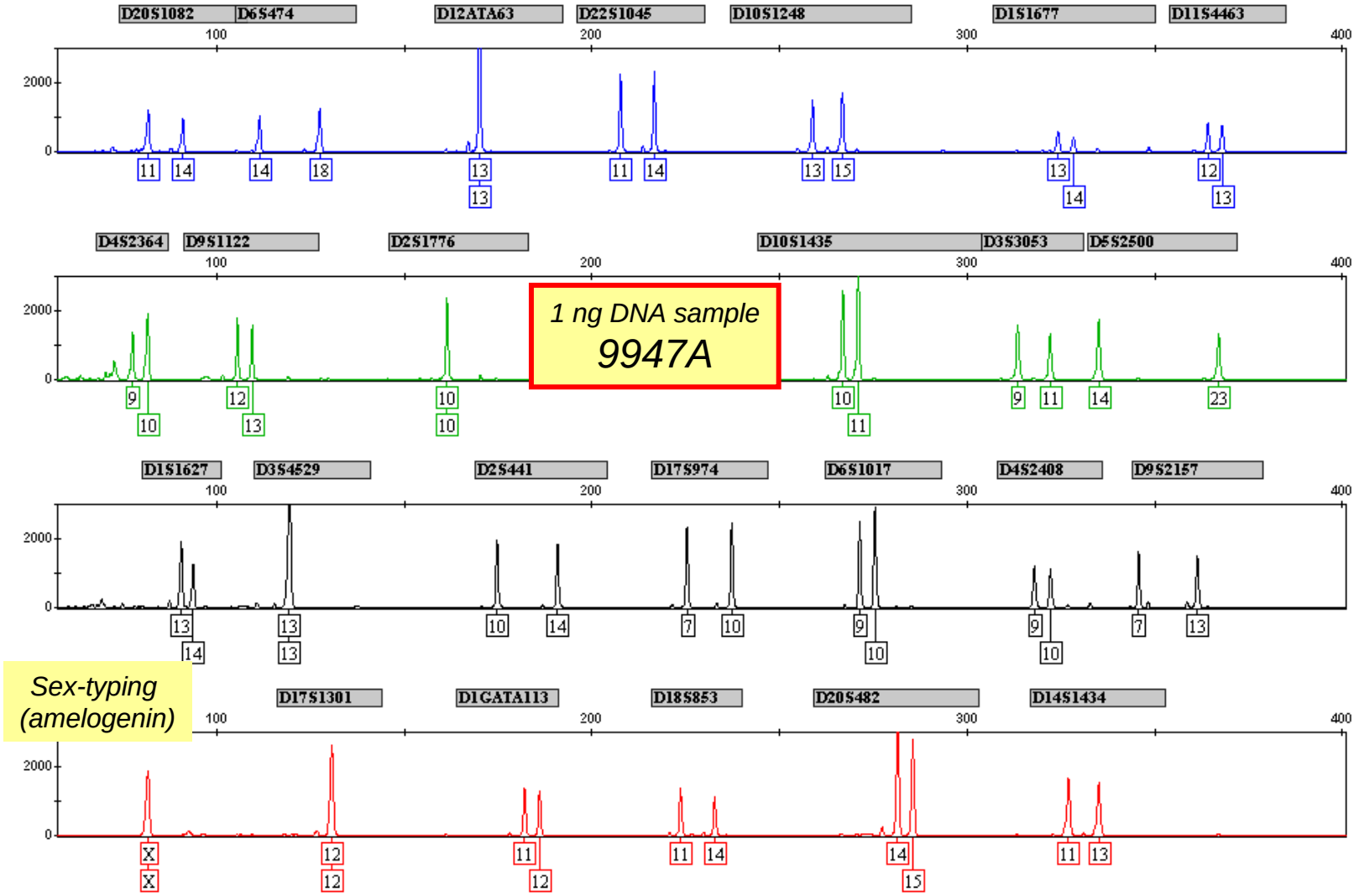
Asian	0.8424
African Am	0.8453
Hispanic	0.8487
Caucasian	0.9002

Locus	Allele	Total #	Total %	Populations, % Af Am	Asian	Cauc	Hisp
	14	1	0.0	0.1			
	15	148	5.1	8.0	4.0	3.5	4.3
	16	106	3.7	6.3	1.0	2.3	3.7
	17	327	11.3	14.6	9.2	12.5	6.7
	17.1	4	0.1	0.4			
	17.3	29	1.0	0.4		1.8	1.2
	18	616	21.3	27.0	24.5	17.4	17.4
	18.1	1	0.0	0.1			
	18.3	34	1.2	0.3		2.4	1.3
	19	447	15.5	14.7	17.3	12.4	19.6
	19.1	7	0.2	0.7			0.1
	19.3	14	0.5	0.3	0.2	0.5	0.7
	20	390	13.5	10.7	19.1	11.1	17.3
	20.1	2	0.1	0.2			
	20.3	1	0.0				0.1
	21	296	10.3	6.6	9.2	12.6	12.5
	22	200	6.9	4.0	7.4	10.0	6.3
	22.2	1	0.0				0.1
	23	141	4.9	3.3	3.7	6.7	5.2
	24	72	2.5	1.2	1.2	4.9	1.6
	24.3	1	0.0		0.2		
	25	33	1.1	0.9	1.7	1.5	0.6
	26	9	0.3		0.7	0.2	0.6
	27	6	0.2		0.5	0.1	0.4

NIST 26plex published in *J. Forensic Sci.* (Sept 2009)

25 new STR loci (unlinked from 13 CODIS core loci)

Gender identification + 25 autosomal STRs in a single amplification



Hill, C.R., et al. (2009) A new STR 26plex assay for human identity testing. *J. Forensic Sci.* 54: 1008-1015

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

Kinship Analysis

Work by Kristen Lewis O'Connor, NIST NRC Postdoc

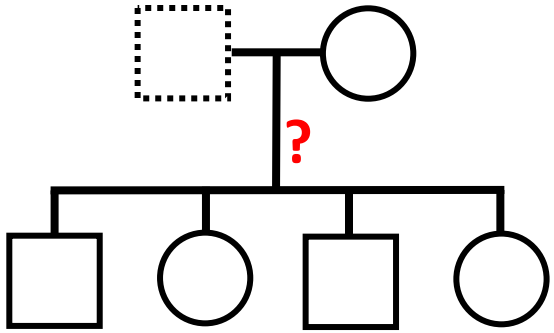
(PhD research with Bruce Weir at University of Washington on familial search issues)

- Provide technical expertise and advice to DHS and other federal agencies as needed
- Examine impact of additional STR loci (and other genetic markers) on addressing specific kinship questions
- Simulate likelihood ratio distributions with different sets of STR loci and different potential relationships
- Examine different software programs (and develop approaches for lab validation including investigating possible standard data sets for software testing)

US Citizenship and Immigration Services (USCIS)

- Immigration cases
  - 1,107,126 obtained legal permanent residence in US in 2008
  - 103,456 were relatives of US alien resident

- Refugee/asylum cases
  - 400 applications processed per day
  - 60,108 refugees admitted in 2008
  - 34,753 were relatives of applicant



- Support relationship claim with interview and documents
- Fraudulent claims (79%)

Chris Miles, DHS

Currently: Optional DNA Testing for Immigration

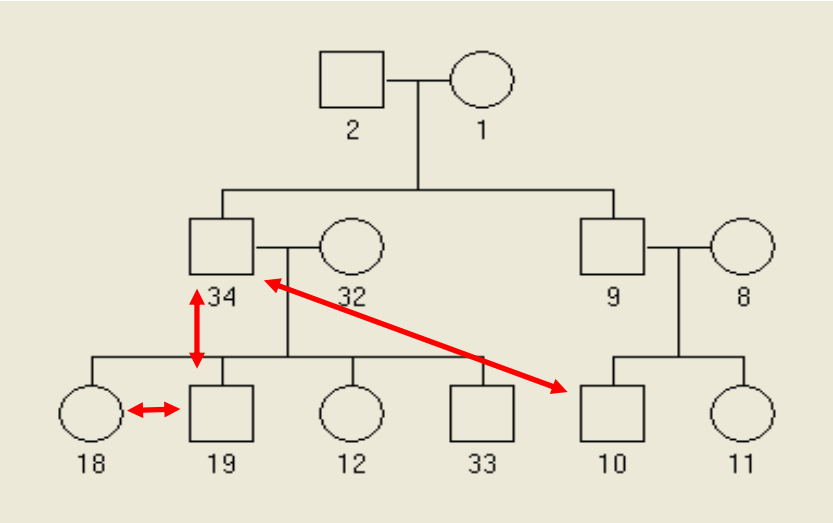
- DNA may be used if interview and documents are insufficient

- Number of cases:
  ~ One dozen accredited labs in US
  ~ 3,500/yr for small lab, ~10,000/yr large lab

- Time: Minimum two-day lab analysis;
  up to six months to coordinate with embassy

- Cost: $600-1500 for private lab testing
  – Applicant pays (usually US resident, “anchor”)

Likelihood Ratios with 15 Loci



Comparison	LR for 34 & 19	LR for 18 & 19	LR for 34 & 10
Parent-Child	1.28E+06	9.08E+05	0.00E+00
Full Siblings	3.22E+04	2.76E+07	6.07E-03
Half Siblings	7.38E+03	4.89E+04	6.65E-01
Cousins	1.95E+02	8.96E+02	1.52E+00
Uncle-Nephew	7.38E+03	4.89E+04	6.65E-01
Grandparent-Grandchild	7.38E+03	4.89E+04	6.65E-01
	Parent/ Child	Full Sibs	Uncle/ Nephew

LR calculations were performed with GeneMarker® HIDv1.90

Benefit of Additional Loci

Likelihood Ratios with 40 Loci

	15	40	15	40	15	40
Comparison	**LR for 34 & 19**	**LR for 34 & 19**	**LR for 18 & 19**	**LR for 18 & 19**	**LR for 34 & 10**	**LR for 34 & 10**
Parent-Child	1.28E+06	6.68E+16	9.08E+05	0.00E+00	0.00E+00	0.00E+00
Full Siblings	3.22E+04	5.73E+12	2.76E+07	1.57E+19	6.07E-03	3.30E+03
Half Siblings	7.38E+03	8.63E+11	4.89E+04	4.99E+12	6.65E-01	8.98E+05
Cousins	1.95E+02	1.32E+08	8.96E+02	1.05E+09	1.52E+00	2.17E+04
Uncle-Nephew	7.38E+03	8.63E+11	4.89E+04	4.99E+12	**6.65E-01**	**8.98E+05**
Grandparent-Grandchild	7.38E+03	8.63E+11	4.89E+04	4.99E+12	6.65E-01	8.98E+05

Parent/ Child

Full Sibs

Uncle/ Nephew

LR calculations were performed with GeneMarker® HIDv1.90

15 STR loci typed with commercial Identifiler kit
25 STR loci typed with an in-house NIST assay
Hill, C.R., Butler, J.M., Vallone, P.M. (2009) A 26plex autosomal STR assay to aid human identity testing. J. Forensic Sci. 54(5): 1008-1015.

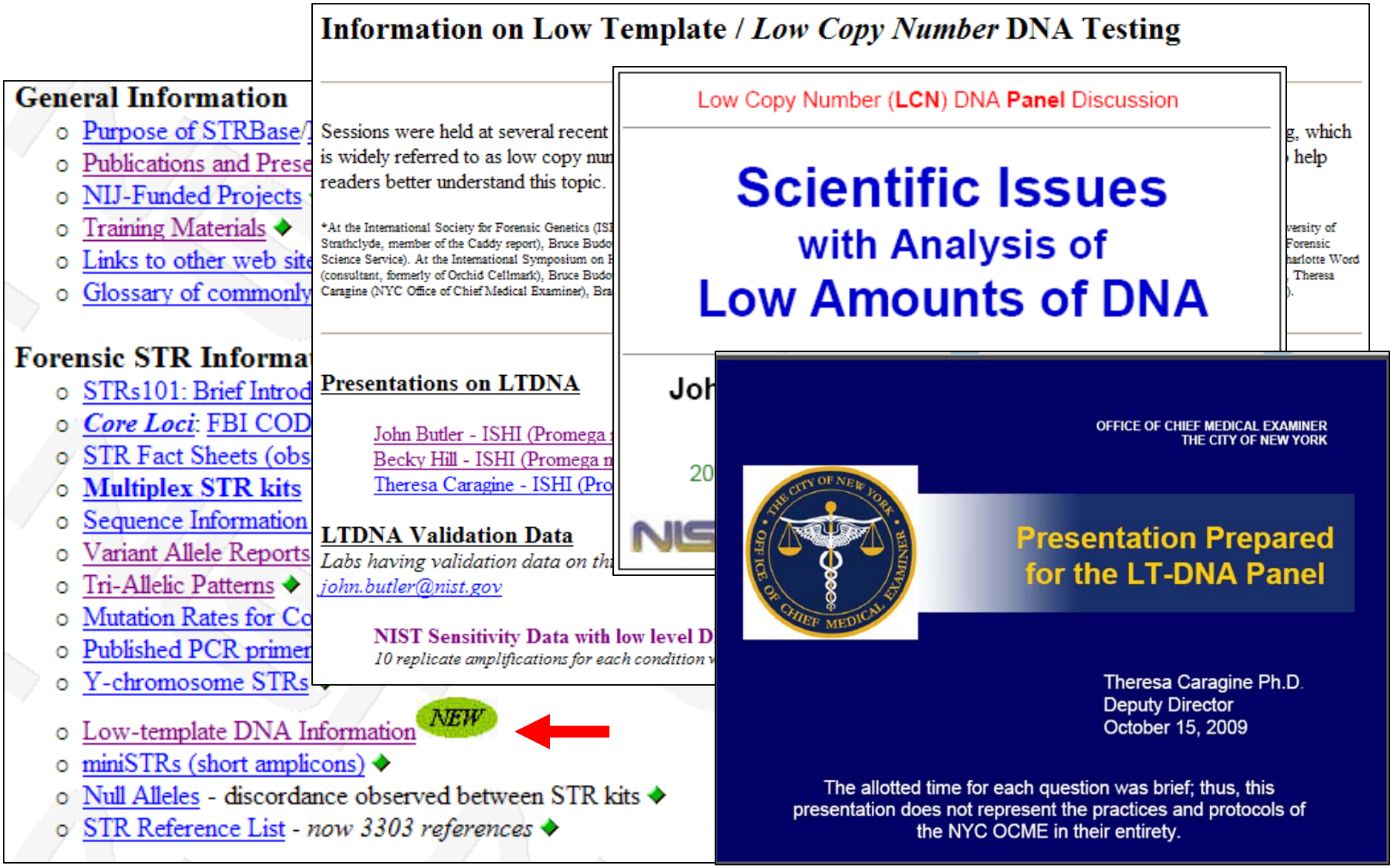
Kinship Analysis Software

- Public/commercial availability
  1. GeneMarker® HID v1.90 (SoftGenetics)
  2. DNA-VIEW™ v29.23 (Charles Brenner)
  3. LISA (Future Technologies Inc.)
  4. KIn CALc v4.0 (CA DOJ)
  5. FSS-ibd v.0.10.13 (Forensic Science Service)

- Restricted availability
  6. CODIS 6.0 (FBI)

New STRBase Website on LT-DNA (LCN)

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



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

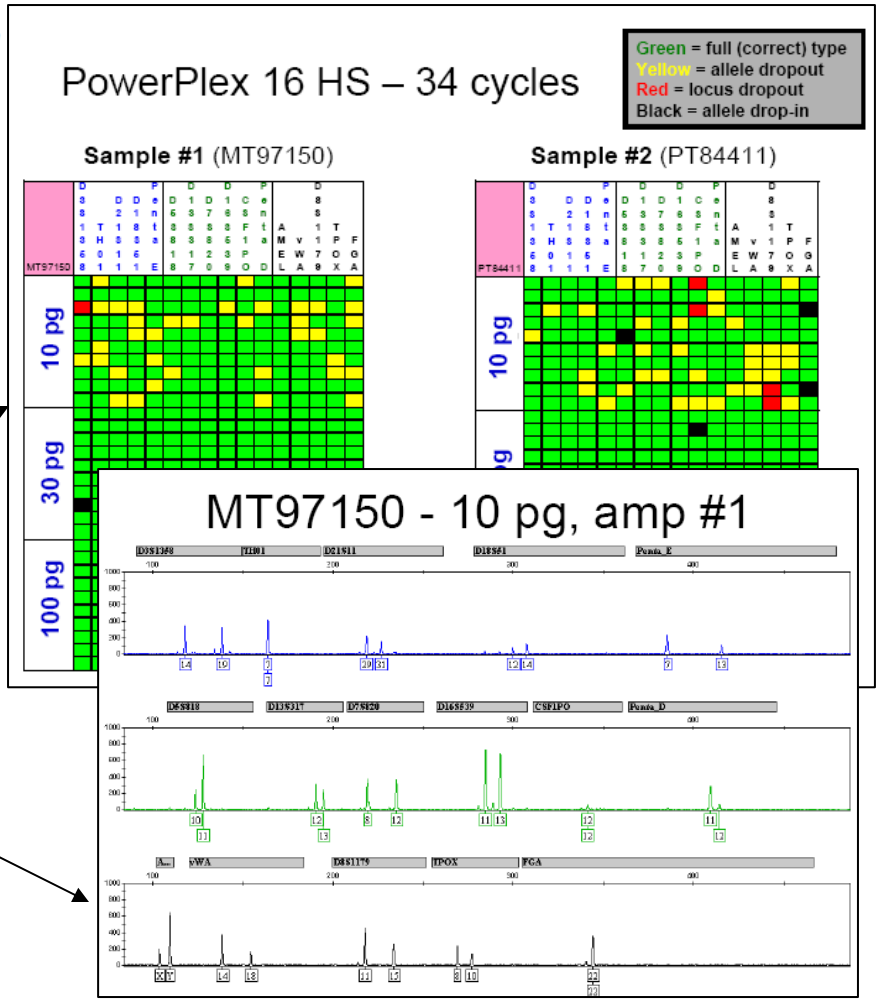
http://www.cstl.nist.gov/biotech/strbase/LTDNA.htm

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**

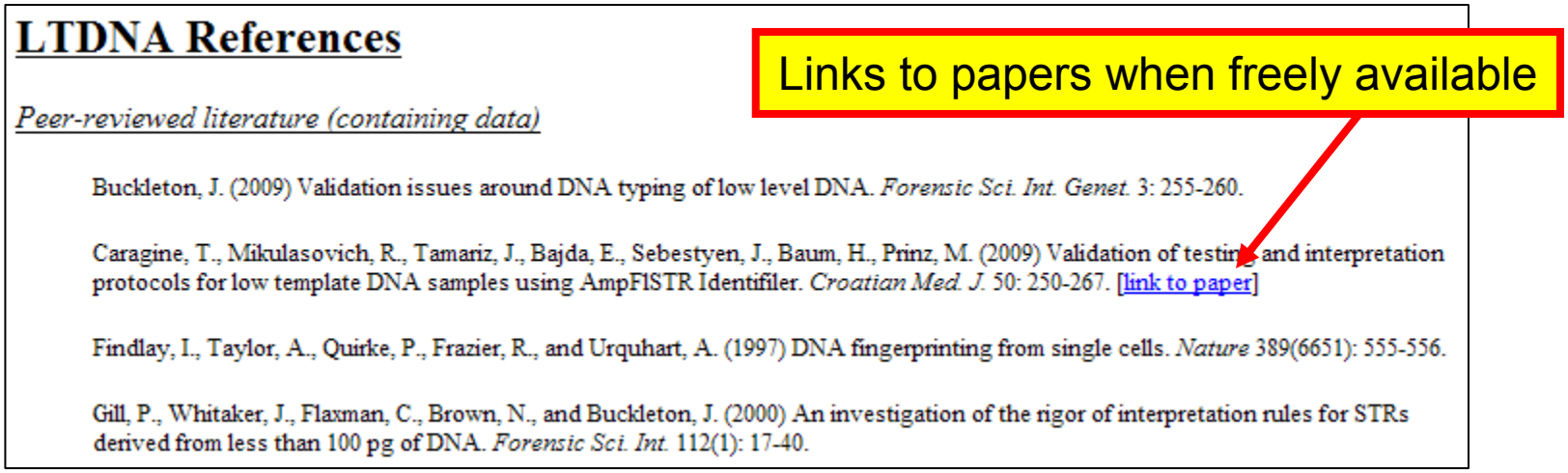


Literature Listing on LT-DNA (LCN)

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

Links to papers when freely available

Peer-reviewed literature (containing data)

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

Caragine, T., Mikulasovich, R., Tamariz, J., Bajda, E., Sebestyen, J., Baum, H., Prinz, M. (2009) Validation of testing and interpretation protocols for low template DNA samples using AmpFlSTR Identifiler. *Croatian Med. J.* 50: 250-267. [link to paper]

Findlay, I., Taylor, A., Quirke, P., Frazier, R., and Urquhart, A. (1997) DNA fingerprinting from single cells. *Nature* 389(6651): 555-556.

Gill, P., Whitaker, J., Flaxman, C., Brown, N., and Buckleton, J. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci. Int.* 112(1): 17-40.

Profiles in DNA (April 2010)

http://www.promega.com/profiles/

Profiles in DNA

Each issue provides news and information for researchers and analysts working in the field of genetic identity testing. Topics include forensic casework, database samples, paternity analyses, legal issues, technical tips, Promega genetic identity product updates, interesting cases and more.

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- Features
- Spotlight
- Products
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- Automation
- Meetings
- Technical Tips

All Products
All Technical Resources

VOLUME 13 NO. 1, APRIL 2010

SUMMARY OF THE LOW COPY NUMBER SESSION AT THE 20TH INTERNATIONAL SYMPOSIUM ON HUMAN IDENTIFICATION

At the 20th International Symposium on Human Identification, prominent figures in the DNA-typing field shared their view of low copy number (LCN) analysis by responding to a subset of LCN-related questions. Charlotte Word and John Butler provided introductions to the topic. Links to the introductory material and individual responses are provided below. Note: All of the responses are not yet available. The other LCN summaries will be published in *Profiles in DNA* as they become available.

A list of questions can be found here.

MEETINGS

What is LCN?—Definitions and Challenges

Charlotte Word presents a summary of her presentation during the LCN session. She helps us sort through some of the confusion about what constitutes LCN analysis.

MEETINGS

Scientific Issues with Analysis of Low Amounts of DNA

John Butler and Carolyn Hill discuss technical issues and challenges that can arise in low template DNA analyses.

MEETINGS

Low Copy Number Analysis From a Legal Perspective

Brad Leventhal from the Queens County District Attorney's Office shares his views on the biggest challenges with LCN analysis and his advice for forensic scientists working with attorneys on cases that could be considered LCN.

New *Profiles in DNA* Article on Low Level DNA

http://www.promega.com/profiles/1301/1301_02.html

Profiles in DNA

Article Type: Meetings

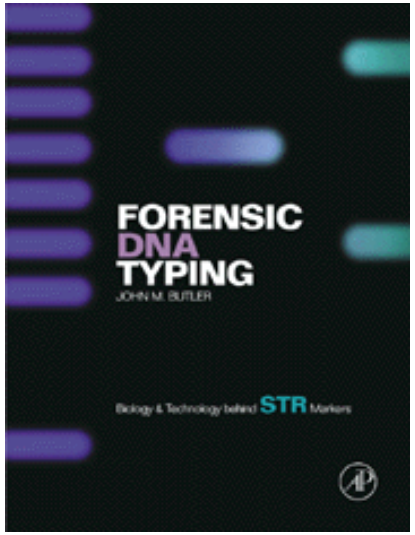
Scientific Issues with Analysis of Low Amounts of DNA

John M. Butler* and Carolyn R. Hill
National Institute of Standards and Technology, Biochemical Science Division, Gaithersburg, Maryland, USA
***Corresponding author: 301-975-4049; john.butler@nist.gov**

Faced with limited evidence that yield low amounts of DNA, forensic analysts will continually have to confront the question of how far to push DNA-testing techniques. Low copy number (LCN) analysis, also known as low template DNA (LT-DNA) testing, involves enhancing detection sensitivity usually through increasing the number of PCR cycles. Stochastic effects inherent with analysis of low amounts of DNA yield allele or locus drop-out. Additionally, increasing detection sensitivity can result in a greater potential for contamination or allele drop-in. Validation studies with replicate testing of low amounts of DNA were performed to assess the level of allele and locus drop-out and allele drop-in using 10, 30 and 100 picograms with several commercially available STR-typing kits under both standard and increased number of PCR cycles. The results with pristine, fully heterozygous samples demonstrate that a replicate testing approach can produce reliable information with single-source samples when consensus profiles are created.

The Expansion of *Forensic DNA Typing*

1st Edition

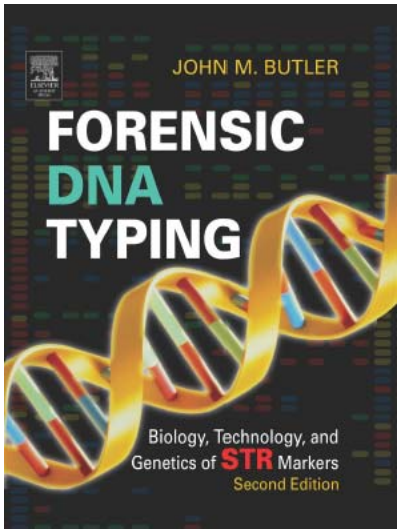


Jan 2001

335 pp.

17 chapters

2nd Edition



Feb 2005

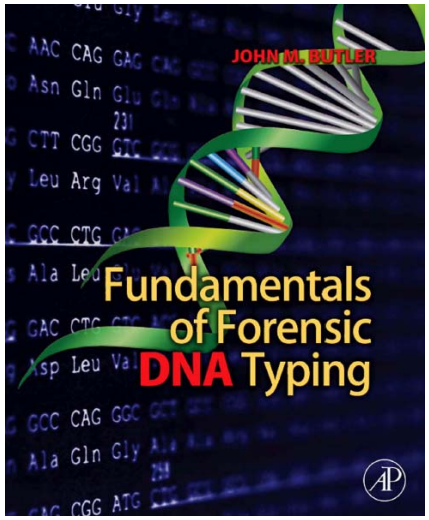
688 pp.

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 2011

Improved Reference Format

Forensic DNA Typing (2nd Edition)

Full list of authors but no article title

Lazaruk, K., Walsh, P.S., Oaks, F., Gilbert, D., Rosenblum, B.B., Menchen, S., Scheibler, D., Wenz, H.M., Holt, C. and Wallin, J. (1998) *Electrophoresis*, 19, 86–93.

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Mansfield, E.S., Vainer, M., Enad, S., Barker, D.L., Harris, D., Rappaport, E. and Fortina, P. (1996) *Genome Research*, 6, 893–903.

Mansfield, E.S., Robertson, J.M., Vainer, M., Isenberg, A.R., Frazier, R.R., Ferguson, K., Chow, S., Harris, D.W., Barker, D.L., Gill, P.D., Budowle, B. and McCord, B.R. (1998) *Electrophoresis*, 19, 101–107.

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McCord, B.R., McClure, D.L. and Jung, J.M. (1993b) *Journal of Chromatography A*, 652, 75–82.

McCord, B.R. (2003) Troubleshooting capillary electrophoresis systems. *Profiles in DNA*, 6 (2); Available at: http://www.promega.com/profiles/602/ProfilesInDNA_602_10.pdf.

Madabhushi, R.S. (1998) *Electrophoresis*, 19, 224–230.

Fundamentals (3rd Edition)

Subdivided by subject with article title provided

Instrument Platforms

ABI 373 and 377

Frazier, R. R. E., et al. (1996). Validation of the Applied Biosystems Prism™ 377 automated sequencer for forensic short tandem repeat analysis. *Electrophoresis, 17,* 1550–1552.

Fregeau, 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,* 133–166.

FMBIO Gel Imager

Greenspoon, S. A., et al. (2004). Validation and implementation of the PowerPlex 16 BIO System STR multiplex for forensic casework. *Journal of Forensic Sciences, 49,* 71–80.

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

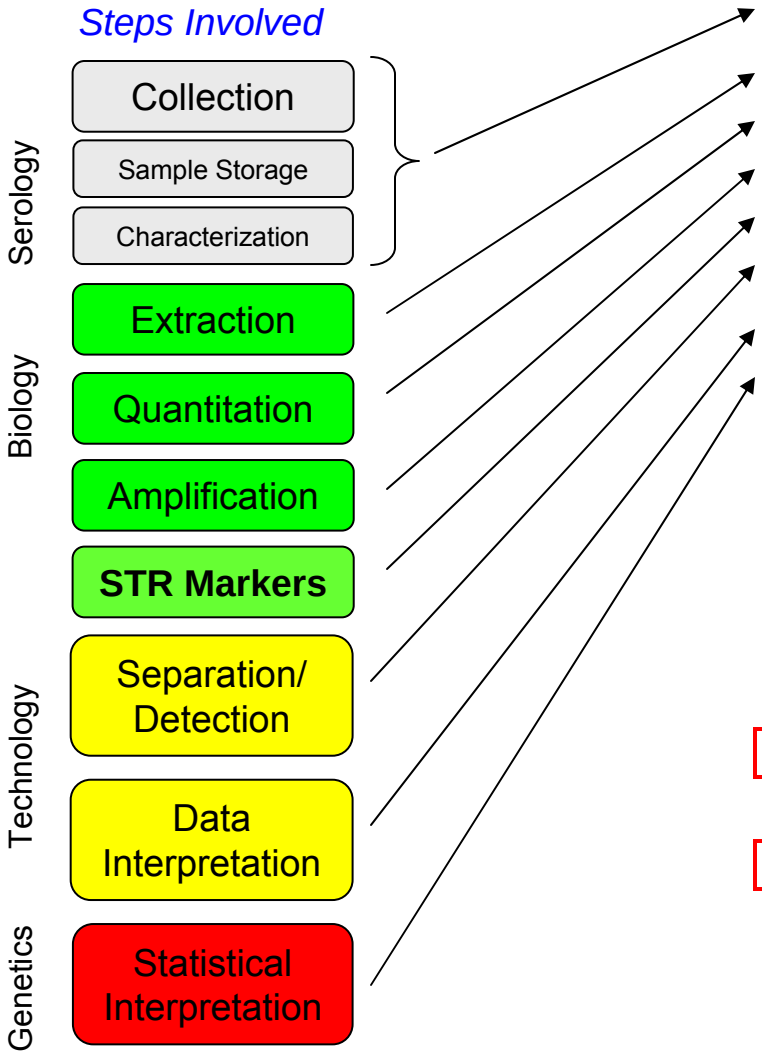
ABI Prism 310 Genetic Analyzer

Buel, E., et al. (1998). Capillary electrophoresis STR analysis: Comparison to gel-based systems. *Journal of Forensic Sciences, 43,* 164–170.

Butler, 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,* 1397–1412.

Lazaruk, K., et al. (1998). Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. *Electrophoresis, 19,* 86–93.

Chapters Re-ordered to Reflect DNA Testing Process



Fundamentals of Forensic DNA Typing (2009)

References are provided at the end of each chapter by subtopic (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…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Mike Coble


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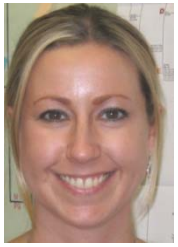
Becky Hill


Margaret Kline


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Jan Redman


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Workshops & Textbooks

Mixtures, mtDNA & Y

Concordance & LT-DNA

Kinship Analysis

Rapid PCR & Biometrics

DNA Extraction Efficiency

Software Tools & Data Analysis

Variant alleles & Cell Line ID

STRBase Support

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

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Questions?