



NIST Update Applied Genetics Group

SWGAM
17 July 2014

Peter M. Vallone, Ph.D.
Applied Genetics Group
Biomolecular Measurement Division




Applied Genetics Group (06) Group Leader Peter Vallone




Advancing technology and traceability through quality genetic measurements to aid work in Forensic and Clinical Genetics


A core competency of our group is the application of *nucleic acid-based methods*
PCR – Genotyping – Sequencing – Real-time PCR – Digital PCR – DNA based SRMs

Forensic Genetics



Clinical Genetics





Simone Gittelson
Guest Researcher


Recent focus areas: DNA mixture interpretation, update of SRM2391c, digital PCR, RDNA, & next generation sequencing

Topics

- **SRM 2391c**
- **Candidate SRM 2372a**
- **Rapid DNA**
- **Next-generation sequencing**
- Y STRs
- DNA mixture interpretation
- Upcoming talks/workshops

SRM 2391c: PCR-Based DNA Profiling Standard

- Components A through D are DNA extracts in liquid form
- Components E and F are DNA spotted on 903 paper or FTA paper
- Certified values are for STR alleles based on length polymorphisms observed using capillary electrophoresis



Lab 1 ↔ Lab 2

Helps meet QAS Std. 9.5.5 and ISO 17025

Standard Reference Material

Calibration with SRMs enables confidence in comparisons of results between laboratories

Current price: \$626 USD

Genomic DNAs characterized for the expanded CODIS core loci and Y-STRs

Certified, Reference, & Information Values of SRM 2391c

- **Certified Values:** Value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account
2 or more methods are used to compare values (i.e. Sanger sequencing, genotyping with multiple sets of primers)
- **Reference Values:** High-confidence estimate of the true value but where all possible sources of bias have not been fully investigated by NIST
Genotyping with only 2 sets of primers to compare
- **Information Values:** Data that may be of interest and use to the SRM user, but insufficient information is available to access the confidence of the assignment
Genotyping of only 1 kit is available

Current Values for STR Loci

Certified Values			Reference Values			Information Values		
Autosomal STR (24)	Y STR (17)	X STR (0)	Autosomal STR (23)	Y STR (0)	X STR (0)	Autosomal STR (1)	Y STR (0)	X STR (0)
D1S1656	DYS19	None	D1GATA113	None	None	Penta C	None	None
D2S1338	DYS385a		D1S1627					
D2S441	DYS385b		D1S1677					
D3S1358	DYS390I		D2S1776					
D5S818	DYS389II		D3S3053					
D7S820	DY390		D3S4529					
D8S1179	DY391		D4S2364					
D8S1115	DYS392		D4S2408					
D10S1248	DY393		D5S2500					
D12S391	DY437		D6S1017					
D13S317	DY438		D6S474					
D18S539	DY439		D6S1122					
D18S51	DY448		D6S2157					
D19S433	DY456		D10S1435					
D21S11	DY458		D17S1301					
D22S1045	DY635		D17S974					
CSF1PO	Y GATA H4		D18S853					
FGA			D20S1082					
Penta D			D20S482					
Penta E			F13A01					
SE33			F13B					
TH01			FESFPS					
TPOX			LPL					
vWA								

STR Typing kits and Primer Mixes

100%
Concordance
with all kits



Kit Provider				Primer Mixes (2)	Current
Thermo Fisher (12)	Promega Corp. (8)	Qiagen Inc. (2)	Primer Mixes (2)		
Identifier	PowerPlex 16	ESplex	26plex	Update	
Identifier Plus	PowerPlex 16 HS	IDplex	miniSTRs		
NGM	PowerPlex ESX 17				
NGM SElect	PowerPlex ESI 17	ESplex SE	RM Y STRs		
COiler	PowerPlex ES	ESplex SE Plus			
Profiler	PowerPlex S5	ESplex SE GDI			
Profiler Plus	PowerPlex Y	IDplex Plus			
Profiler Plus ID	FFFL	IDplex GO!			
SGM Plus		Argus X-12			
SEiler		IDplex			
MiniFiler					
Yfiler					

Kit Provider				Primer Mixes (3)	Update
Thermo Fisher (14)	Promega Corp. (16)	Qiagen Inc. (9)	Primer Mixes (3)		
Identifier	PowerPlex 16	ESplex	26plex	Update	
Identifier Plus	PowerPlex 16 HS	IDplex	miniSTRs		
NGM	PowerPlex ESX 17	ESplex SE	RM Y STRs		
NGM SElect	PowerPlex ESI 17	ESplex SE Plus			
COiler	PowerPlex ES	ESplex SE GDI			
Profiler	PowerPlex S5	IDplex Plus			
Profiler Plus	PowerPlex Y	IDplex GO!			
Profiler Plus ID	FFFL	Argus X-12			
SGM Plus	PowerPlex ESI 17 Pro	IDplex			
SEiler	PowerPlex ESX 17 Fast				
MiniFiler	PowerPlex ESI 17 Fast				
Yfiler	PowerPlex 18D				
GlobalFiler	PowerPlex 21				
Yfiler Plus	PowerPlex CS7				
	PowerPlex Fusion				
	PowerPlex V23				

Updated Values for STR Loci

All certified loci have been fully sequenced

Certified Values				Reference Values				Information Values			
Autosomal STR (25)	Y STR (29)	X STR (6)		Autosomal STR (23)	Y STR (0)	X STR (0)		Autosomal STR (1)	Y STR (0)	X STR (12)	
D1S1656	DY1518	None		D1GAT113	None	None		Penta C	None	DXS7132	
D2S1338	DYS385a			D1S1627						DXS7423	
D2S441	DYS385b			D1S1677						DXS8378	
D3S1358	DYS389I			D2S1776						DXS10074	
D5S818	DYS389II			D3S1053						DXS10079	
D6S1043	DYS390			D3S4529						DXS10101	
D7S820	DYS391			D4S2364						DXS10103	
D8S1179	DYS392			D4S2408						DXS10134	
D8S1115	DYS393			D1S2500						DXS10135	
D10S1248	DYS437			D6S1017						DXS10146	
D12S391	DYS438			D6S474						DXS10148	
D13S317	DYS439			D9S1122						HPRTB	
D16S539	DYS448			D9S2157							
D18S51	DYS456			D10S1435							
D19S433	DYS458			D17S1301							
D21S11	DYS635			D17S874							
D22S1045	Y GATA H4			D18S893							
CSF1PO	DYS449			D20S1082							
FGA	DYS460			D20S482							
Penta D	DYS481			F13A01							
Penta E	DYS518			F13B							
SE33	DYS533			FESFPS							
TH01	DYS549			LPL							
TPOX	DYS570										
vWA	DYS576										
	DYS627										
	DYS643										
	DYF387S1a										
	DYF387S1b										

Argus X-12 kit

*Other Information Value Considerations:
- DIPlex kit (InDel markers)
- PGM and MiSeq IISNPs

New Y-STR loci in commercial kits (Yfiler Plus & PPY23)

Update to be completed by Oct. 2014

Digital PCR (dPCR) and SRM 2372a

Next Certification Method

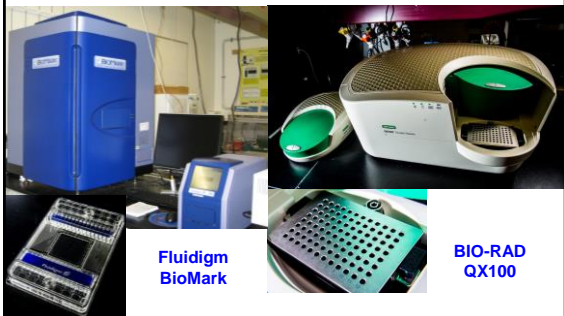
- The next generation of SRM 2372 (SRM 2372a) will be certified for "copy/target number"
- Digital PCR allows for the calculation of the absolute concentration without the use of an external calibrant

Migrating away from UV based measurements for DNA quantitation
- A sample is partitioned so that individual nucleic acid targets within the sample are localized
 - Microfluidic (Fluidigm BioMark)
 - Emulsion/droplet PCR (Bio-Rad QX100, RainDance)
- Each partition will contain a negative or positive PCR reaction

Instruments available for dPCR at NIST

Chamber Digital - cdPCR

Droplet Digital - ddPCR



Fluidigm
BioMark

BIO-RAD
QX100

Fluidigm BioMark

Chamber Digital PCR

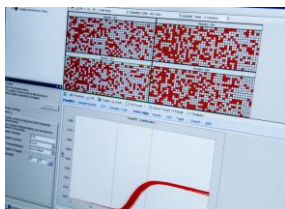


12.765

- Fluidic module transfers PCR mastermix onto chip
- 'Reader' performs thermal cycling and fluorescence detection (real-time PCR)

Fluidigm Digital Array

- 12.765 = 765 chambers x 12 panels (samples)
- 48.770 = 770 chambers x 48 panels (samples)



- Well volumes
6 nL (12 samples)
0.85 nL (48 samples)
- TaqMan compatible chemistry
- FAM-VIC dye detection

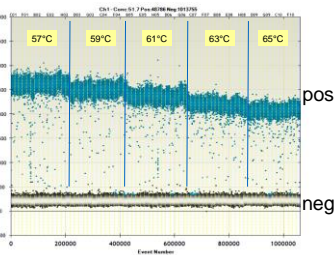
Bio-Rad QX-100

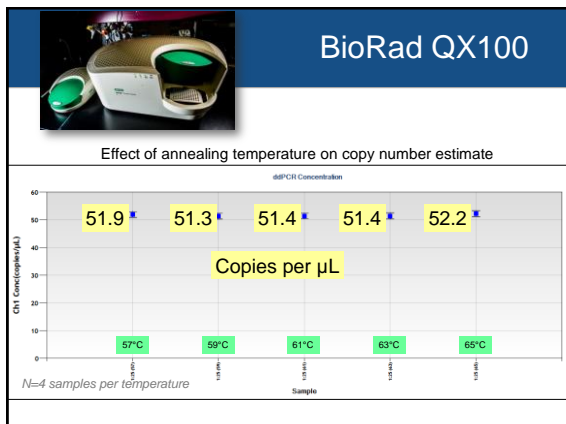
Droplet Digital PCR

- PCR master mix and DNA template are partitioned into droplets
- 8 strip tubes - up to 96 samples/run
- Thermal cycling is performed on a standard cycler (9700, Veriti)
- Fluorescence from up to 20,000 droplets are detected in the reader (3.5 h)



Validating annealing temperatures for the validation of a digital PCR assay





ddPCR Copies/µL original Sample to ng/µL

Convert copies/µL and calculate the DNA concentration as ng/µL:

Assay	Average ng/µL	sd
D6S474	56.3	1.2
D9S2157	55.2	0.7
HBB1	51.7	1.5
D5S2500	51.7	0.9
D14S1434	52.1	0.4
2PR4	50.6	0.9
22C3	50.0	1.2
EIF5	49.0	0.3
D1P32.3	39.7	0.8
All good assays	52.1	2.5

Rapid PCR Protocols Paper

- Invited to submit articles by Dr. Bruce McCord (FIU)
- Accepted for publication in Electrophoresis

ELECTROPHORESIS

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Editor-in-Chief: Ziad El Rassi

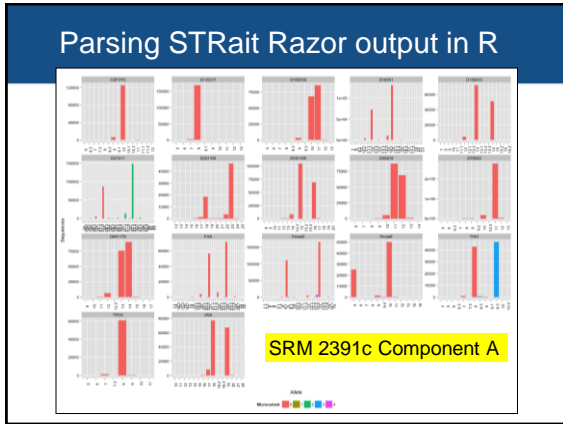
Impact Factor: 3.261

ISI Journal Citation Reports® Ranking 2012: 14/75 (Chemistry Analytical), 23/75 (BIOCHEMICAL RESEARCH METHODS)

Online ISSN: 1522-2683

"Rapid PCR Protocols for Forensic DNA Typing on Six Thermal Cycling Platforms"

Erica L.R. Butts and Peter M. Vallone



HID-Ion Ampliseq Identity Panel (version 2.3) Run on Ion Torrent PGM

- 90 autosomal SNPs
- From Pakstis 2010, Kidd 2012, and SNPforID panels
- 30 Y-chromosome SNPs
- Upper clade branches
- $RMP \approx 4 \times 10^{-36}$

SNPs for a universal individual identification panel

Andrew J. Pakstis¹, William C. Speed¹, Brian Fag², Fabien G. Schaller¹, Richard W. Krahl¹, Judith A. Kidd³, Kenneth R. Kidd³

Forensic Science International: Genetics

Expanding data and resources for forensic use of SNPs in individual identification

Mourad R. Kaddif¹, Judith R. Kidd¹, William C. Speed¹, Brian Fag², Mourad R. Kaddif¹, Fabien G. Schaller¹, Richard W. Krahl¹

Journal of Forensic Sciences

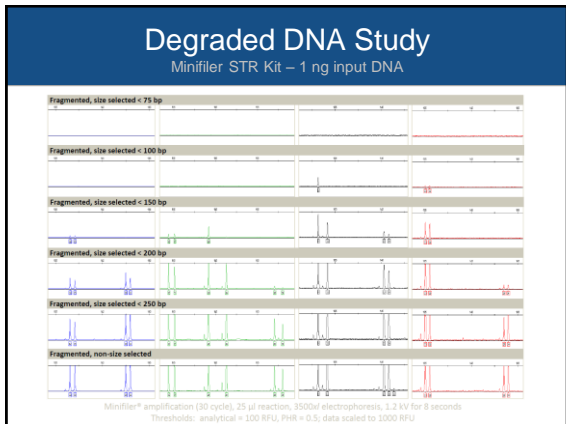
Forensic validation of the SNPforID 52-plex assay

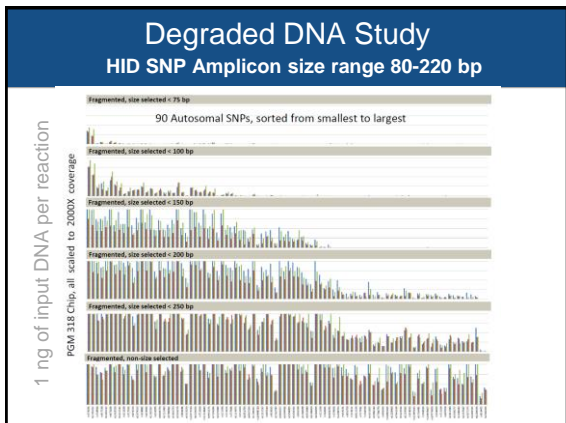
Fallah Moghreb-Browns^{1,2}, David Ballant¹, Kung Bokang³, Klaus Bentske⁴, Bernhard Berger⁵, Magdalene Beyer⁶, Chris Harcourt⁷, Maria Bensch⁸, Marco Fosteschi⁹, Christa Harman¹⁰, Cristian Oprea¹¹, Walter Pinner¹², Chris Phillipps¹³, Carsten Pohl¹⁴, Eva Rostová¹⁵, Hans-Joachim Schuster¹⁶, Paula Slomová¹⁷, Ana-Jelena Smej¹⁸, Boris Strobaner-Bodilopoulos¹⁹, Catherine Thacker²⁰, Angel Carrascosa²¹, Nilsa Montag²², Richard Schrottman²³, Peter M. Schneider²⁴, Christa Dierker-Gruber-Czap²⁵

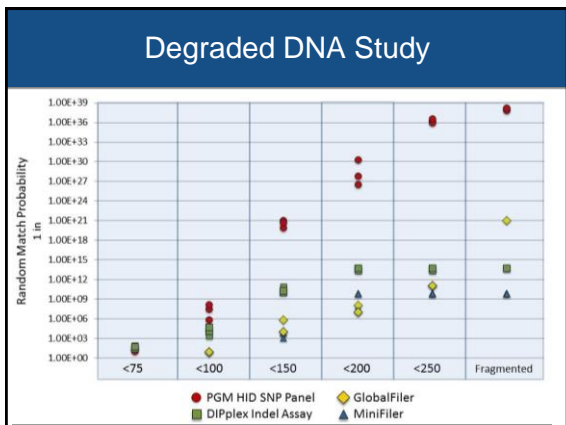
Degraded DNA Study

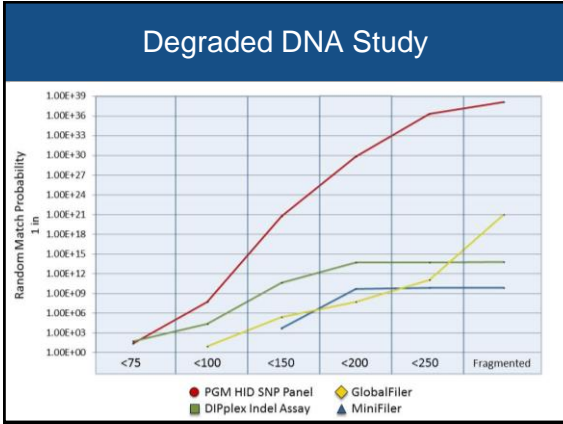
Assays	PGM IISNPs	MiniFiler	Identifiler Plus	GlobalFiler	DIPplex
DEGRADED DNA SAMPLES					
35-250 bp	X	X		X	X
35-200 bp	X	X		X	X
35-150 bp	X	X		X	X
35-100 bp	X	X		X	X
35-75 bp	X	X		X	X

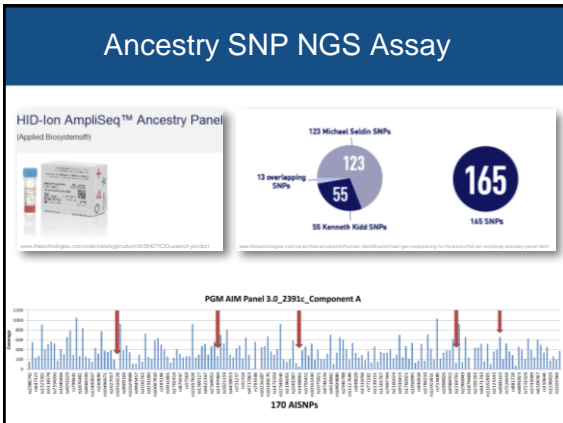
DNA fragmented using Covaris instrument

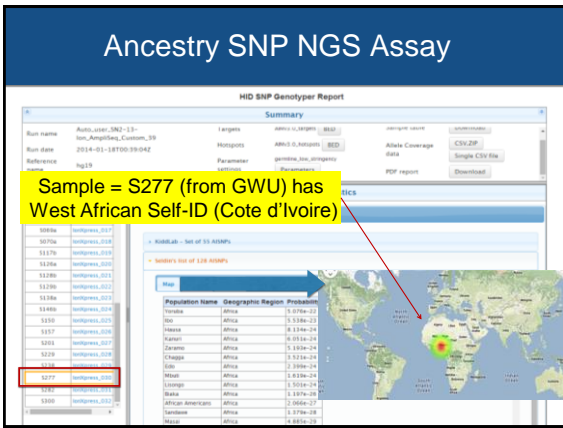






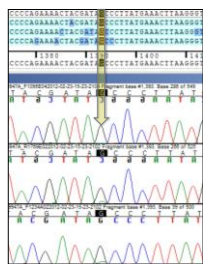






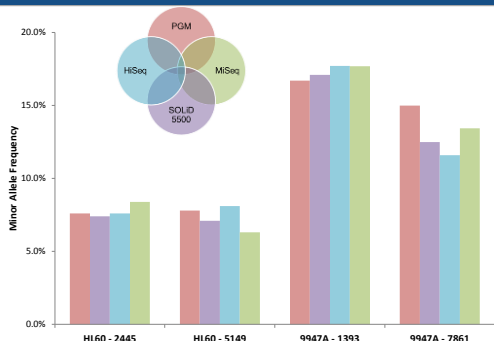
NGS Support for mtDNA Analysis

- NIST mtDNA SRM 2392 / 2392-I
- PGM & MiSeq analysis
 - 5% SNP calling threshold
 - Concordance across platforms
 - Two heteroplasmies in two components, not in previous certificates
- FSIG short communication
- Certificate update Fall 2014

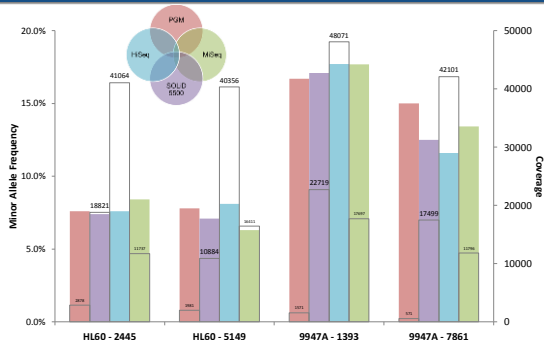


Site 1,393 (G/A)

NGS Support for mtDNA Analysis



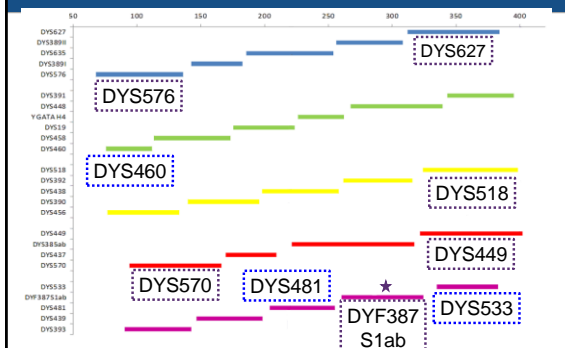
NGS Support for mtDNA Analysis



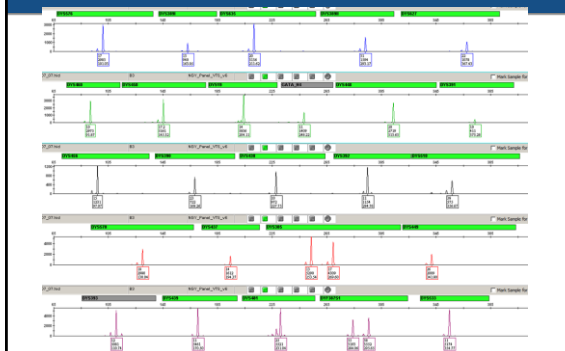
Topics

- SRM 2391c
- Candidate SRM 2372a
- Rapid DNA
- Next-generation sequencing
- **Y STRs**
- **DNA mixture interpretation**
- **Upcoming talks/workshops**

Yfiler Plus (10 new loci)



Yfiler Plus



Sensitivity

Full reaction volumes (25 ul):

- 0.5 ng single amp
- 0.25 ng single amp
- 0.125 ng duplicate amp
- 0.0625 ng duplication amp
- 0.03125 ng duplicate amp

Half reaction volumes (12.5 ul):

- 0.5 ng duplicate amp
- 0.25 ng duplicate amp
- 0.125 ng duplicate amp
- 0.0625 ng duplication amp
- 0.03125 ng duplicate amp

Sensitivity – 25µL

Marker	FULL REACTION VOLUME (25 ul)				
	0.03125 ng	0.0625 ng	0.125 ng	0.25 ng	0.5 ng
DVS576	184	222	257	491	574
DVS389I	86	151	344	318	1081
DVS635	198	153	198	152	179
DVS389II	115	279	174	725	648
DVS627	142	373	165	147	1257
DVS460	463	463	304	128	263
DVS458	404	531	261	469	581
DVS19	173	220	143	335	525
GATA_H4	97	159	226	482	153
DVS448	93	151	204	504	196
DVS391	342	231	53	1369	723
DVS456	330	180	269	131	732
DVS390	232	123	87	225	331
DVS438	144	166	207	203	501
DVS392	209	53	124	700	154
DVS518	232	114	145	181	638
DVS570	92	163	301	987	327
DVS437	81	248	368	152	749
DVS382	109	364	303	134	327
DVS385		130		383	
DVS449	57	91	321	238	713
DVS393	465	124	472	386	
DVS439	278	130	108	411	313
DVS481	254	375	234	1023	588
DVF3875I	203	208	165	413	487
DVF3875II		197	193	686	426
DVS531	208	280	381	814	656

>175









<175

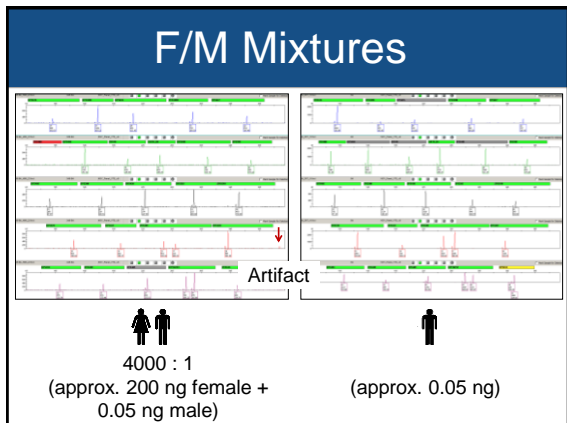
Sensitivity – 12.5µL

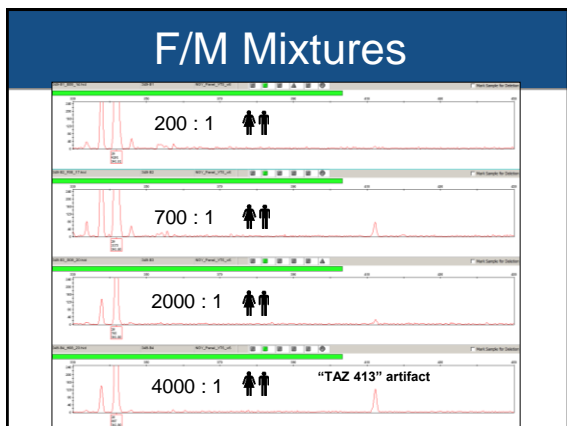
Marker	HALF REACTION VOLUME (12.5 ul)				
	0.03125 ng	0.0625 ng	0.125 ng	0.25 ng	0.5 ng
DVS576	84	404	740	488	2271
DVS389I	1089	1265	2442	1854	2389
DVS635	331	911	1540	1950	3991
DVS389II	1053	717	2561	1026	1885
DVS627		1175	870	1346	2054
DVS460	763	655	1462	2661	2146
DVS458	304	262	2099	2610	1013
DVS19	833	351	1283	1888	1464
GATA_H4	245	475	1690	1766	1274
DVS448		594	1842	1131	1611
DVS391	199	398	1140	398	2862
DVS456	122	121	901	1280	1391
DVS390	214	672	771	1428	2059
DVS438	321	656	731	2162	2261
DVS392	153	1306	444	1853	858
DVS518	367	262	844	281	771
DVS570	196	592	376	1163	1754
DVS437	145	1162	808	321	1424
DVS385	93	613	630	1672	2218
DVS385	197	726	582	1434	894
DVS449	79	112	420	1727	2309
DVS393	72	824	719	621	2248
DVS439	280	841	1260	1449	2793
DVS481	83	618	689	1368	3258
DVF3875I	51	294	842	999	2175
DVF3875II	448	899	1441	653	2845
DVS531	527	484	4038	7388	961

>175

<175

F/M Mixtures		
200 : 1	 200 ng + 1 ng	 1 ng
700 : 1	 200 ng + 0.3 ng	 0.3 ng
2000 : 1	 200 ng + 0.1 ng	 0.1 ng
4000 : 1	 200 ng + 0.05 ng	 0.05 ng





Performance with unrelated males

NIST U.S. Population Samples

N = 948 males		PowerPlex Y	Number of unique and shared haplotypes observed with various combinations of Y-STR loci across 948 U.S. population samples
# haplotypes	816		
discrimination capacity	0.8608	PPY	
# times haplotype observed	(12 loci)		
1	751		
2	42		
3	12		
4	4		
5	2		
6	2		
7	.		
8	1		
9	.		
10	.		
11	1		
12	.		
13	.		
14	.		
15	.		
16	.		
17	.		
18	.		
19	.		
20	1		

N = 948 males		Yfiler	New Loci*	Yfiler Plus*	The new loci alone perform slightly better than Yfiler (Note: Ignoring DYS460)
# haplotypes	930	945	946		
discrimination capacity	0.9810	0.9842	0.9979		
# times haplotype observed	(17 loci)	(10 loci)	(27 loci)		
1	916	918	944		
2	11	15	2		
3	2	.	.		
4	1	.	.		
5	.	.	.		
6	.	.	.		
7	.	.	.		
8	.	.	.		
9	.	.	.		
10	.	.	.		
11	.	.	.		
12	.	.	.		
13	.	.	.		
14	.	.	.		
15	.	.	.		
16	.	.	.		
17	.	.	.		
18	.	.	.		
19	.	.	.		
20	.	.	.		

DNA Mixture Interpretation

- LR mix Studio (Haned and Gill)
- DNA-View Mixture Solution (Charles Brenner)
- STRmix (ESR and S. Australia collaboration)
- LikeLTD (Balding)
- Lab Retriever (Lohmueller, Rudin and Inman)
- TrueAllele (Cybergenetics)

DNA Mixture Interpretation

The screenshot shows the LRmixStudio interface with a table of samples and a table of loci data. A red box highlights the text "Same functionality as LRmix in a user-friendly GUI".

Active	Sample	Source File
<input checked="" type="checkbox"/>	Rep1	sample.csv
<input checked="" type="checkbox"/>	Rep2	sample.csv
<input checked="" type="checkbox"/>	Rep3	sample.csv

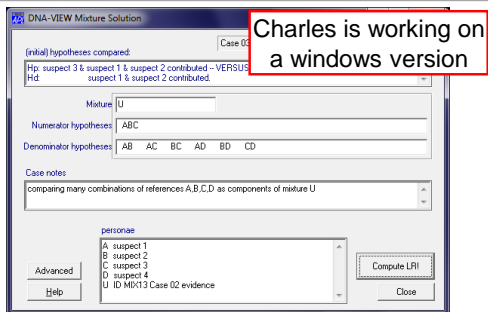
Case Number: case example

Locus	Rep1	Rep2
D10S1248	13 14 16	13 14 15 16
VWA	15 17 18 19	15 16 17 18 19
D16S539	11 13	10 11 12 13
D2S1338	17 18 19	17 18
D8S1179	10 12 14 15	10 12 14 15

DNA Mixture Interpretation

- LR mix Studio (Haned and Gill)
- DNA-View Mixture Solution (Charles Brenner)
- STRmix (ESR and S. Australia collaboration)
- LikeLTD (Balding)
- Lab Retriever (Lohmueller, Rudin and Inman)
- TrueAllele (Cybergenetics)

DNA Mixture Interpretation



Charles is working on a windows version

Slide courtesy of Charles Brenner

Webcasts

NIST DNA Analyst Webinar Series: Probabilistic Genotyping and Software Programs (Part 1)

Purpose:

NIST will host a series of free webinars focused on topics of interest to DNA analysts. This four-hour webinar will be held on May 28, 2014, from 1:00 pm – 5:00 pm (EDT), and will focus on probabilistic genotyping for complex low-level DNA mixtures.

Watch a recording of the webinar on this page.

Details:

Start Date: Wednesday, May 28, 2014

End Date: Wednesday, May 28, 2014

Audience: Industry, Government, Academia

<http://www.nist.gov/forensics/nist-dna-analyst-webinar-series-pt1.cfm>

Susan Berdine (Denver PD) – Lab Retriever

Todd Bille (ATF) – STRmix

Ate Kloosterman (Netherlands Forensic Institute) – LRmix

Craig O'Connor (NY OCME) – FST

Webcasts

Probabilistic Genotyping – Part 2
(Tentative date – September 18 (Thursday))

Planned Talks - Confirmed

TrueAllele

LikeLTD

DNA-View

(Armed Expert)

(LiRa/LiRaHT)

Webcasts

NIST DNA Analyst Webinar Series: Validation Concepts and Resources – Part 1

Purpose:
NIST will host a series of free webinars focused on topics of interest to DNA analysts. This four-hour webinar will be held on August 6, 2014, from 1:00 pm – 5:00 pm (EDT), and will focus on validation concepts and NIST software tools to assist in the validation process.

Agenda:
.....

<http://www.nist.gov/forensics/nist-dna-analyst-webinar-series-validation-concepts-and-resources-part-1.cfm>

Robin Cotton, *Boston University*
John Butler, *NIST*
Mark Timken, *California Department of Justice*
Catherine Grgicak, *Boston University*
Becky Hill, *NIST*

Aug. 6th

Details:
Start Date: Wednesday, August 6, 2014
End Date: Wednesday, August 6, 2014
Audience: Industry, Government, Academia
Format: Webinar

Workshops

Almost Everything You Wanted to Know About Probabilistic Software (But Were Afraid to Ask)

Workshop Chairs:

- Charlotte Word
- Michael Coble (National Institute of Standards and Technology)

Additional Speakers:

- Dr. Charles Brenner (DNA-VIEW & UC Berkeley) DNA-VIEW Mature Solution
- Dr. David Balding (UCL Genetics Institute) LikelTD
- Dr. Roberto Puch-Solis (LOC Forensics) LRa and LRaH
- Dr. Norah Rudin (SCEG) Lab Retriever
- Dr. Hinda Hamed (Netherlands Forensic Institute) LRmix studio
- Luigi Aronogida (NicheVision) ArnedOpen™
- Jo-Anne Bright (ESR Ltd) STRmix™
- Dr. Mark Perlin (Cybergenetics) TrueAllele® Casework



INTERNATIONAL SYMPOSIUM ON HUMAN IDENTIFICATION
PHOENIX, AZ - SEP. 29-OCT. 2, 2014

Upcoming Talks / Workshops

NIST Presentations @ ISHI

Almost Everything You Wanted to Know About Probabilistic Software (But Were Afraid to Ask)
Emerging Forensic Genomic Applications – Genome ID Forum 2014

Workshops:
Greenville, North Carolina – The Center for Advanced Forensic DNA Analysis (CAFDA) is pleased to announce the Genome ID Forum 2014 – Emerging Forensic Genomic Applications, a collaborative conference to take place on September 9 and 10 at the Hilton Greenville, North Carolina.

NGS Advances in Human Forensic Genomics
Workshop Presentation: Vallone & Gettings

STR Sequence Diversity in Population Samples and Applications
Panel: Rapid DNA and CODIS integration
Callaghan, Wendel, Vallone, Selden, Jovanovich

2014 "Future trends in forensic DNA technology" seminar series
7/30/14 Burlington, VT
8/6/14 Crystal City, VA

HID NIST presenting on Yfiler Plus and PGM SNP assays



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Vallone (NGS, digital PCR)



Global Identity Summit
Rapid DNA Session Sept 17, 2014

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