National Institute of Standards and Technology
. . .working with industry to develop and apply technology, measurements and standards

NIST Research Update

Michael Coble

Peter M. Vallone, Margaret C. Kline, Amy E. Decker, Janette W. Redman, Becky Hill, David L. Duewer, John Butler

April 6, 2005

ENFSI Meeting – Glasgow, Scotland

NIST Human Identity Project Team


John Butler
(Project Leader)


Pete Vallone


Margaret Kline


Jan Redman


Amy Decker


Mike Coble


Dave Duewer

Becky Hill
(new hire)

Funding:
Interagency Agreement between National Institute of Justice and NIST Office of Law Enforcement Standards

National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Research Effort

- **Standard Information Resources** (STRBase information, training materials/review articles, validation standardization, calibration datasets)
- **Interlaboratory Studies** (Real-time PCR, mixture interpretation)
- **Resources for "Challenging Samples"** (miniSTRs for degraded DNA)
- **Information on New Loci** (Y-Chromosome, new STRs)

Standard Information Resources

STRBase, training materials, variant allele sequencing etc.

STRBase Updates
(since July 2004)

- Validation section
- miniSTR section
- Y-chromosome information (multiplexes & databases)
- Population data summary & OmniPop program download (courtesy of Brian Burritt)
- Reference Sequences for Commonly Used STR Markers

More minor additions

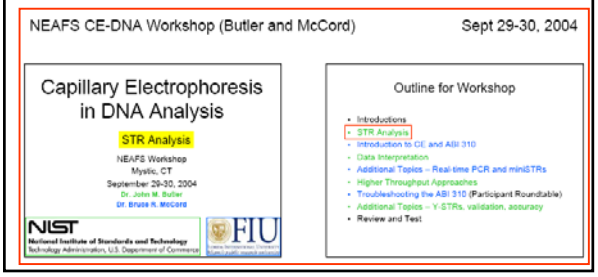
- Additional commercial STR kit schematics (Yfiler, PowerPlex Y)
- Published Promega primers (added PP16)
- Additional NIST publications/presentations (14 new talks, 12 new papers)
- Additional variant alleles & scientist addresses

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

NEAFS Workshop Slide Handouts

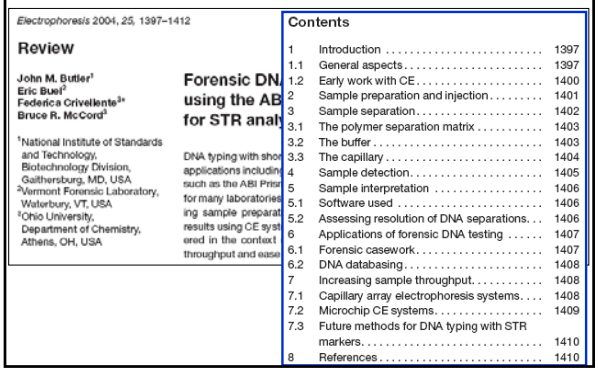
Handouts available as downloadable pdf files from
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm#NEAFSworkshop

2 day workshop with **>500 slides** describing STRs and CE (ABI 310 and ABI 3100)



Review Article on STRs and CE

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



Electrophoresis 2004, 25, 1397–1412

Review

John M. Butler[1]
Eric Buel[2]
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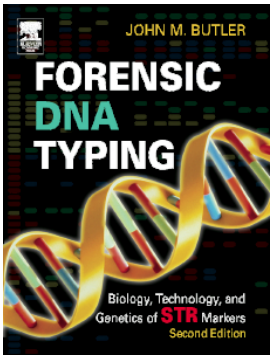
[3]Ohio University, Department of Chemistry, Athens, OH, USA

Forensic DN
using the AB
for STR anal

DNA typing with shor
applications including
such as the ABI Prism
for many laboratories
ing sample preparat
results using CE syst
ered in the context
throughput and ease

Contents



FORENSIC DNA TYPING
Biology and Technology behind STR Markers
- Second Edition

John M. Butler

Listed on amazon.com

Published February 2005

ELSEVIER ACADEMIC PRESS Forensic Science

Validation Standardization Efforts

Presentation at Promega meeting (October 2004)

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

Validation Standardization Questionnaire (conducted June-August 2004)

Can Validation be Standardized?

Statements from survey responders…

Over 86% (45/52) said yes

Those who responded "no" said

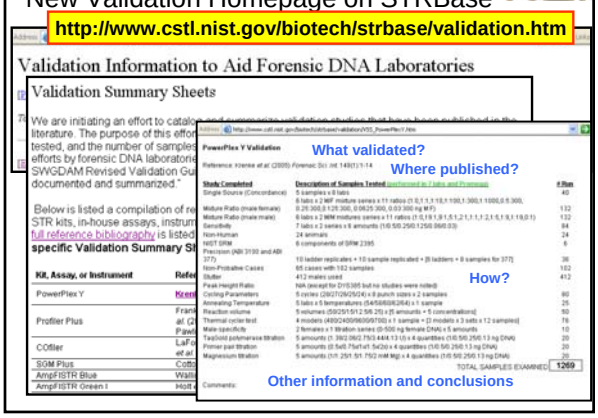
- "to some degree it can be, however, validation is specific to the platform, kits, …",
- "a start-up lab should do much more than an experienced lab…",
- "validation builds on previous work by lab or published data",
- "parts of it can be standardized; I don't think the non-probative cases could be", and
- "only in a general way, as with the SWGDAM guidelines. The uniqueness of each new procedure would make standardization difficult."

Our Conclusion…

to a certain extent it can…but everyone will always have a different comfort level…and **inflexible, absolute numbers for defined studies will not likely be widely accepted**

New Validation Homepage on STRBase

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

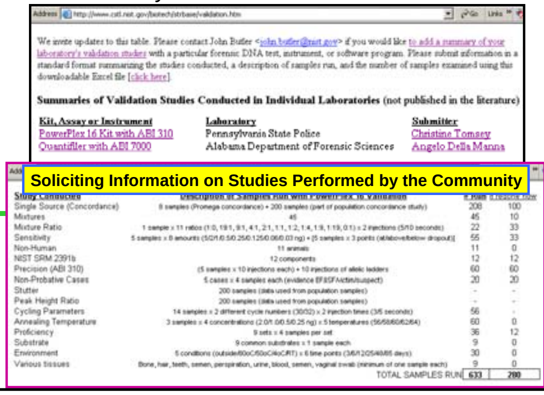


Validation Summary Sheet for PowerPlex Y

Study Completed (17 studies done)	Description of Samples Tested (performed in 7 labs and Promega)	# Run
Single Source (Concordance)	5 samples x 8 labs	40
Mixture Ratio (male:female)	6 labs x 2 M/F mixture series x 11 ratios (1:0,1:1,1:10,1:100,1:300,1:1000,0.5:300, 0.25:300,0.125:300, 0.0625:300, 0.03:300 ng M:F)	132
Mixture Ratio (male:male)	6 labs x 2 M/M mixtures series x 11 ratios (1:0, 19:1, 9:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:9, 1:19, 0:1)	132
Sensitivity	7 labs x 2 series x 6 amounts (1/0.5/0.25/0.125/0.06/0.03)	84
Non-Human	24 animals	24
NIST SRM	6 components of SRM 2395	6
Precision (ABI 3100 and ABI 377)	10 ladder replicates + 10 sample replicated + [8 ladders + 8 samples for 377]	36
Non-Probative Cases	65 cases with 102 samples	102
Stutter	412 males used	412
Peak Height Ratio	N/A (except for DYS385 but no studies were noted)	
Cycling Parameters	5 cycles (28/27/26/25/24) x 8 punch sizes x 2 samples	80
Annealing Temperature	5 labs x 5 temperatures (54/58/60/62/64) x 1 sample	25
Reaction volume	5 volumes (50/25/15/12.5/6.25) x [5 amounts + 5 concentrations]	50
Thermal cycler test	4 models (480/2400/9600/9700) x 1 sample + [3 models x 3 sets x 12 samples]	76
Male-specificity	2 females x 1 titration series (0-500 ng female DNA) x 5 amounts each	10
TaqGold polymerase titration	5 amounts (1.38/2.06/2.75/3.44/4.13 U) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Primer pair titration	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Magnesium titration	5 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20

Krenke *et al.* (2005) *Forensic Sci. Int.* 148: 1-14 TOTAL SAMPLES EXAMINED 1269

Laboratory Internal Validation Summaries



Address http://www.cstl.nist.gov/biotech/strbase/validation.htm

We invite updates to this table. Please contact John Butler <john.butler@nist.gov> if you would like to add a summary of your laboratory's validation studies with a particular forensic DNA test, instrument, or software program. Please submit information in a standard format summarizing the studies conducted, a description of samples run, and the number of samples examined using this downloadable Excel file [click here].

Summaries of Validation Studies Conducted in Individual Laboratories (not published in the literature)

Kit, Assay or Instrument	Laboratory	Submitter
PowerPlex 16 Kit with ABI 310	Pennsylvania State Police	Christine Tomsey
Quantifiler with ABI 7000	Alabama Department of Forensic Sciences	Angelo Della Manna

Soliciting Information on Studies Performed by the Community

Study Conducted	Description of Samples Run with PowerPlex 16 Validation	# Run	[illegible]
Single Source (Concordance)	8 samples (Promega concordance) + 200 samples (part of population concordance study)	208	100
Mixtures	45	45	10
Mixture Ratio	1 sample x 11 ratios (1:0, 19:1, 9:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:9, 1:19, 0:1) x 2 injections (5/10 seconds)	22	33
Sensitivity	5 samples x 8 amounts (5/2/1/0.5/0.25/0.125/0.06/0.03 ng) + [5 samples x 3 points (at/above/below dropout)]	55	33
Non-Human	11 animals	11	0
NIST SRM 2391b	12 components	12	12
Precision (ABI 310)	(5 samples x 10 injections each) + 10 injections of allelic ladders	60	60
Non-Probative Cases	5 cases x 4 samples each (evidence EF/SF/victim/suspect)	20	20
Stutter	200 samples (data used from population samples)	-	-
Peak Height Ratio	200 samples (data used from population samples)	-	-
Cycling Parameters	14 samples x 2 different cycle numbers (30/32) x 2 injection times (3/5 seconds)	56	-
Annealing Temperature	3 samples x 4 concentrations (2.0/1.0/0.5/0.25 ng) x 5 temperatures (56/58/60/62/64)	60	0
Proficiency	9 sets x 4 samples per set	36	12
Substrate	9 common substrates x 1 sample each	9	0
Environment	5 conditions (outside/80oC/50oC/4oC/RT) x 6 time points (3/6/12/25/48/85 days)	30	0
Various tissues	Bone, hair, teeth, semen, perspiration, urine, blood, semen, vaginal swab (minimum of one sample each)	9	0
	TOTAL SAMPLES RUN	633	280

Goals of this Validation Standardization Project

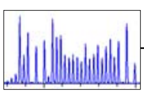
- To help the community gain a better understanding of the validation process and how others have implemented validation in their labs so that validation in one's own lab may be performed more quickly
- To help with establishing uniformity throughout the field to aid auditors in their inspections

NIST QA/QC Software

Tool being developed by Dave Duewer for STR Process Control

Tracks internal size standard in samples

Will be available soon for beta-testing; *still working on user's manual (and will need NCBI file conversion program to be more easily accessible)*



NCBI Program → Peak Height, Area, Size → NIST **Multiplex QAC** Software → Date vs Resolution (or Signal/Noise)

X | Y
103 | 436
104 | 569

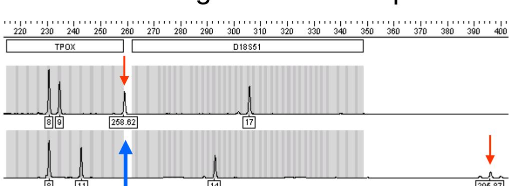
We welcome data sets to help calibrate this software tool

This software does not perform genotyping.
It merely permits a view of analytical parameters over time.

Variant Allele Sequencing

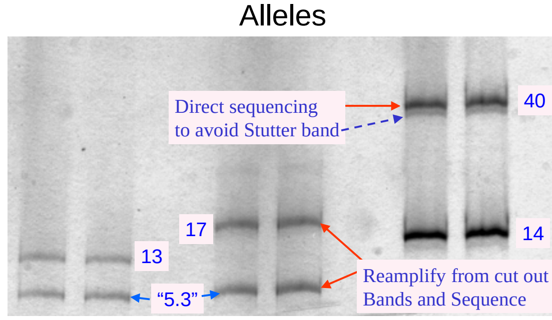
- AAFS talk (Feb 26, 2005) by Margaret Kline on sequencing methods and applications
- We are happy to sequence unusual variant alleles for laboratories

Extreme D18S51 Variants Range 258 – 396 bp

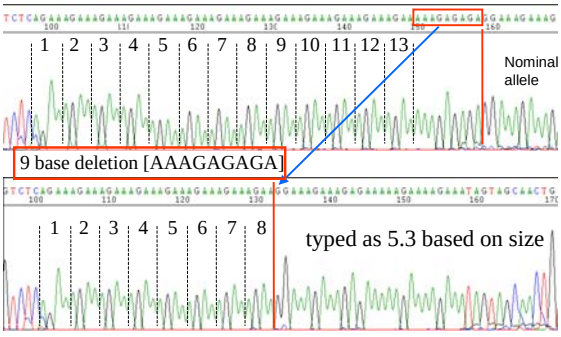


Samples amplified with Identifiler. Nominal range 8 - 28
Small D18 allele just outside of the TPOX allele range.
Large D18 is 46 bp larger than the D18 allele range.

Gel Separation Of D18 Variant Alleles



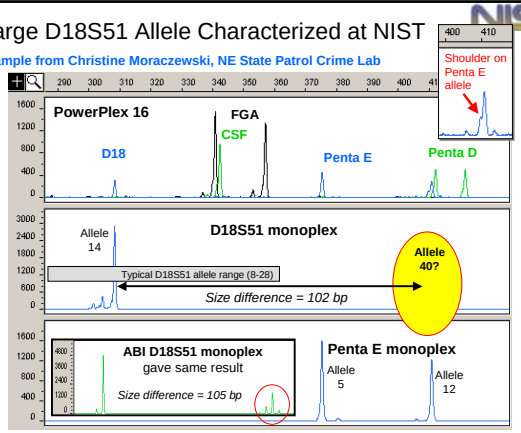
D18S51 sequencing Sample 188



Nominal allele range 8-27 repeats. Sample courtesy DNA Solutions

Large D18S51 Allele Characterized at NIST

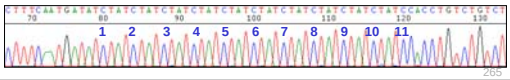
Sample from Christine Moraczewski, NE State Patrol Crime Lab



Analysis of Common STR Variant Alleles

- We have monoplex primers for all common STR loci and kits
- We have sequencing primers that bind outside of STR kit primer sequence positions to enable view of polymorphic nucleotides that cause primer binding site mutations
- NIJ has funded us to characterize STR variants for the forensic DNA community

D16S539 (bottom strand)



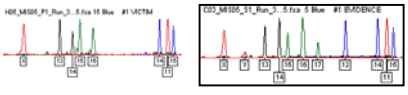
Interlaboratory Studies

DNA Quantitation (2004) – in press (May issue JFS)

Mixture Interpretation (2005) – under evaluation

Mixture Interpretation Interlab Study (MIX05)

- **Only involves interpretation of data**
- As of early March, ~97 labs are enrolled for participation (22 from overseas) – Data are currently being evaluated.
- Four mock cases supplied with "victim" and "evidence" electropherograms (GeneScan .fsa files – that can be converted for Mac or GeneMapper; gel files MAC & NT made available to FMBIO labs)
- Data available with Profiler Plus, COfiler, SGM Plus, PowerPlex 16, Identifiler, PowerPlex 16 BIO (FMBIO) kits
- Summary of results with involve training materials to illustrate various approaches to solving mixtures



Perpetrator Profile(s) ??

Along with reasons for making calls and any stats that would be reported

Resources for Challenging Samples

Degraded DNA and Mixtures

Degraded DNA work

- ENFSI study participation
  - compared STRs, miniSTRs, and autosomal SNPs on same set of degraded DNA samples provided by Peter Gill
- miniSTR website
  - http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
- New miniSTR loci published
  - http://www.cstl.nist.gov/biotech/strbase/pub_pres/Coble2005miniSTR.pdf
- SNP markers and assays
  - http://www.cstl.nist.gov/biotech/strbase/SNP.htm
- Performance of miniSTRs on shed hairs
  - Mike Coble spoke at AAFS (Feb 25, 2005)

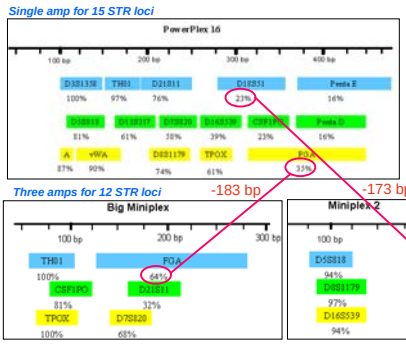
STR Size Reduction

Through Moving Primer Positions Closer to Repeat

Primer positions define PCR product size
Repeat information is independent of amplicon size

Advantages of Approach:
Size reduction enhances success rate with degraded DNA
Retains same marker information (database compatibility)
Uses highly polymorphic STR loci (high discriminatory power)

Comparison of PCR Amplification Success Rates with Commercial Kit vs. miniSTR Assays



Study with 31 bones from the "Body Farm" (Knoxville, TN) and Franklin County Coroner's Office (OH)

Bruce McCord – Florida International University

Characterization of New miniSTR Loci

Rosenberg *et al.* 2002 – 1062 samples; 377 STRs; diverse populations

Locus name	Alternate name	Heterozygosity	Number of alleles	Chromosome
D6S1017	GCAT3H10	0.748	9	6
D6S1009	GATA32B03	0.748	13	6
D8S261	AFM123XG5	0.747	17	8
D18S1390	18QTEL11	0.747	15	18
D13S1807	GATA11C08	0.747	9	13
D12S1052	GATA26D02	0.747	8	12
D16S2616	ATA41E04	0.746	11	16
D2S1780	GATA72G11	0.746	13	2
NA-D1S-3	GATA133A08	0.745	12	1
NA-D8S-2	GAATA4	0.745	9	8
D4S1625	GATA107	0.745	10	4
D18S851	GATA6D09	0.745	12	18
D8S1136	GATA41A01	0.745	11	8
NA-D5S-1	ATA52D02	0.744	27	5
D3S3038	GATA73E01	0.743	14	3
D15S1515	GATA109B10	0.743	9	15
D16S2624	GATA81D12	0.742	8	16
D2S2972	GATA176C01	0.741	14	2
D13S895	GGAA22G01	0.740	11	13
D11S1998	GATA23E06	0.740	9	11

Focus on:

High Heterozygosity
Small # of Alleles
Tetranucleotide Repeats

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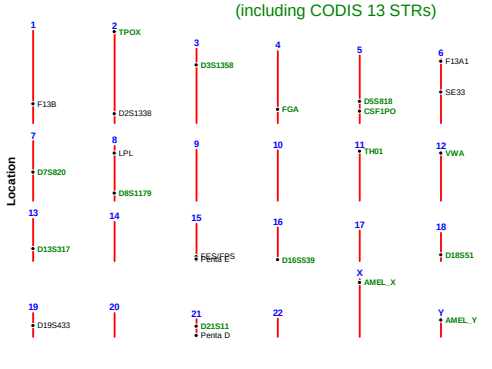
D8S261	AFM123XG5	0.747	17	8
D18S1390	18QTEL11	0.747	15	18
D13S1807	GATA11C08	0.747	9	13
D12S1052	GATA26D02	0.747	8	12
D16S2616	ATA41E04	0.746	11	16
D2S1780	GATA72G11	0.746	13	2
NA-D1S-3	GATA133A08	0.745	12	1
NA-D8S-2	GAATA4	0.745	9	8
D4S1625	GATA107	0.745	10	4
D18S851	GATA6D09	0.745	12	18
D8S1136	GATA41A01	0.745	11	8
NA-D5S-1	ATA52D02	0.744	27	5
D3S3038	GATA73E01	0.743	14	3
D15S1515	GATA109B10	0.743	9	15
D16S2624	GATA81D12	0.742	8	16
D2S2972	GATA176C01	0.741	14	2
D13S895	GGAA22G01	0.740	11	13
D11S1998	GATA23E06	0.740	9	11

Focus on:

High Heterozygosity
Small # of Alleles
Tetranucleotide Repeats

Commercial STR Kit Loci Positions

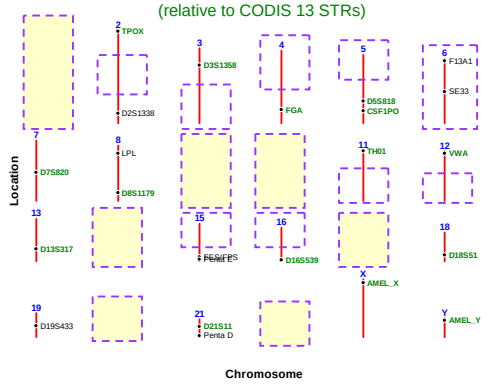
(including CODIS 13 STRs)



Positions determined along July 2003 Human Genome Reference Sequence (NCBI Build 34)

Locations of Focus for New miniSTR Loci

(relative to CODIS 13 STRs)



Initial Testing Results

107 potential markers

↓

61 markers with “clean” flanking regions

↓

43 markers with amplicon size < 125bp

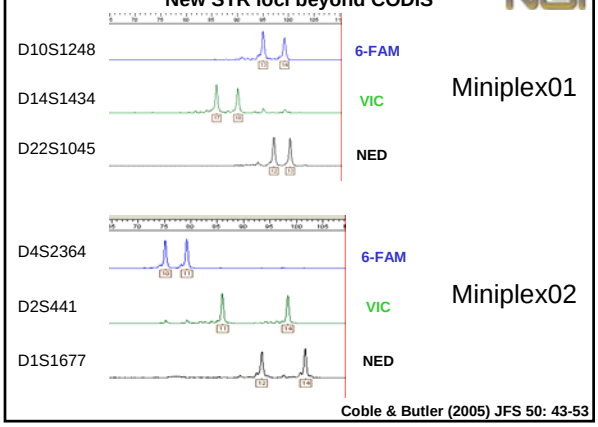
↓

18 markers for initial testing

↓

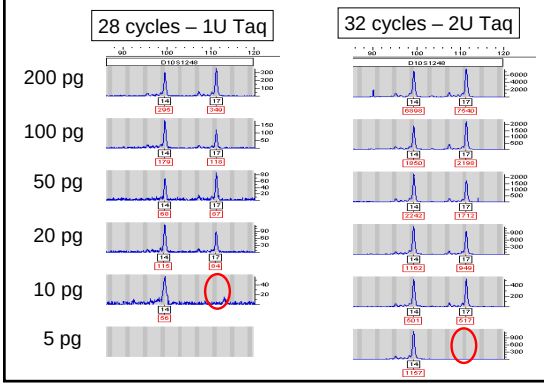
2 three loci miniplexes

New STR loci beyond CODIS



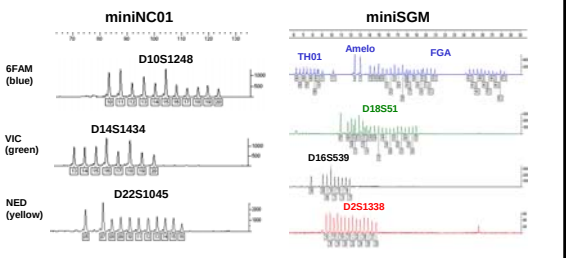
Coble & Butler (2005) JFS 50: 43-53

miniSTR Assay Sensitivity (D10S1248)



miniSTR Assays Used in the ENFSI Study

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



Recovered Volumes from DNA Extractions – Sample 02

Sample	Recovered Volume (uL)	Approx. Quantity (ng/uL)	Approx. Final Conc.(ng/uL)
02 blood - day 0	22	3.8	
02 blood - day 0	26	4.36	5.161
02 blood - day 0	22	7.47	
02 blood - week 2	31	0.741	
02 blood - week 2	21	0.884	0.921
02 blood - week 2	16	1.32	
02 blood - week 8	37	0.188	
02 blood - week 8	25	0.237	0.249
02 blood - week 8	21	0.372	
02 blood - week 16	29	1.16	
02 blood - week 16	25	0.224	0.455
02 blood - week 16	38	0.068	
Reagent Blank	18	0	0.000

Sample	Recovered Volume (uL)	Approx. Quantity (ng/uL)	Approx. Final Conc.(ng/uL)
02 saliva - day 0	17	6.45	
02 saliva - day 0	17	17.74	8.952
02 saliva - day 0	24	4.5	
02 saliva - week 2	22	0.104	
02 saliva - week 2	26	0.04	0.057
02 saliva - week 2	21	0.029	
02 saliva - week 8	26	0.01	
02 saliva - week 8	17	0.015	0.013
02 saliva - week 8	22	0.014	
02 saliva - week 12	33	0.004	
02 saliva - week 12	34	0.006	0.034
02 saliva - week 12	27	0.106	
Reagent Blank	42	0	0.000

ENFSI Degraded DNA Collaborative Study

Summary of Results for Sample 02 Blood (Weeks 2, 8, 16)

	Week 2			Week 8			Week 16	
	SGM+	miniSGM		SGM+	miniSGM		SGM+	miniSGM
Amelo	++	++	Amelo	++	++	Amelo	++	++
TH01	++	++	TH01	++	++	TH01	++	++
FGA	failed	++	FGA	failed	failed	FGA	failed	failed
D18	drop out	drop out	D18	failed	failed	D18	failed	failed
D16	++	++	D16	failed	drop out	D16	++	++
D2	++	++	D2	failed	drop out	D2	++	++
	miniNC01			miniNC01			miniNC01	
D10	++		D10	++		D10	++	
D14	++		D14	++		D14	++	
D22	++		D22	++		D22	++	

ENFSI Degraded DNA Collaborative Study

Recent Publications on miniSTRs

- Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5) 1054-1064.
- Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.
- Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. (2004) Concordance study between miniplex STR assays and a commercial STR typing kit, *J. Forensic Sci.* 49(4): 859-860.
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http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/timeline.htm

Information on New Loci

Autosomal SNPs, Y-Chromosome

Standard U.S. Population Dataset

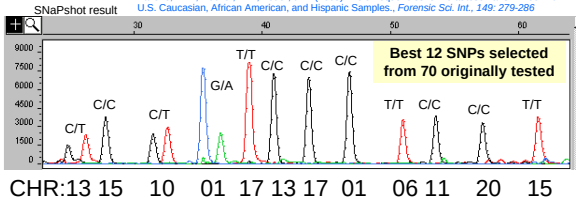
http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm

260 Caucasians, 260 African Americans, 140 Hispanics, 3 Asians = **663 males**

Genetic Markers	Loci Examined	Publications
Common STRs	Identifiler kit 15 STRs (CODIS + D2S1338 & D19S433)	Butler et al. (2003) JFS
miniSTRs	All CODIS loci except D3S1358	Drabek et al. (2004) JFS
New autosomal STRs	New 6 loci for miniSTRs	Coble et al. (2005) JFS
Autosomal SNPs	**70 C/T SNPs** (Orchid panel)	Vallone et al. (2004) FSI
Common Y-STRs	22 loci (27 regions)	Schoske et al. (2004) FSI
	Yfiler concordance study	***Data in ABI Yfiler database***
New Y-STRs	**27 additional loci**	**Butler et al., in press FSI**
Y-SNPs	50 loci spanning haplogroups A-R	Vallone et al. (2004) JFS
mtDNA	LINEAR ARRAY and coding mtSNPs	Kline et al. (2005) JFS
	Full control regions by **AFDIL**	***inclusion in EMPOP***

NIST Autosomal 12plex SNP Assay

Vallone, P.M., Decker, A.E., Butler, J.M. (2005) Allele frequencies for 70 autosomal SNP loci with U.S. Caucasian, African American, and Hispanic Samples., *Forensic Sci. Int., 149: 279-286*



12plex PCR followed by 12-plex ASPE
Fragments separated on a ABI 3100 in 35 minutes
A Genotyper macro has been developed to type data
The 12plex assay has been run on over 600 samples
Works well on 1-2 ng of template
Sensitivity studies are underway along with degraded DNA

New Y-STR Loci, Issues, and Assays

- Updates on Y-chromosome information
  - http://www.cstl.nist.gov/biotech/strbase/y_strs.htm
- Testing on 27 new Y-STR loci
  - Butler, J.M., Decker, A.E., Vallone, P.M., Kline, M.C. (2005) Allele frequencies for 27 Y-STR loci with U.S. Caucasian, African American, and Hispanic samples, *in press FSI*
- Chromosomal duplication issues
  - Butler, J.M., Decker, A.E., Kline, M.C., Vallone, P.M. (2005) Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation, *in press JFS*

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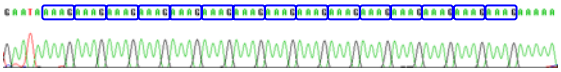
DYS576

15 AAAG repeats



Allele	Combined Freq (N = 659)	Cau freq (N = 261)	Afr Am freq (N = 258)	Hisp freq (N = 140)
13	0.0030	0.0038	0.0039	0.0000
14	0.0273	0.0115	0.0426	0.0286
15	0.1047	0.0421	0.1977	0.0500
16	0.1745	0.1762	0.2016	0.1214
17	0.2640	0.2835	0.2209	**0.3071**
18	**0.2822**	**0.3257**	**0.2403**	0.2786
19	0.1077	0.1188	0.0698	0.1571
20	0.0288	0.0307	0.0194	0.0429
21	0.0076	0.0077	0.0039	0.0143

Butler *et al.* (2005) *in press FSI*

Our Recent Y-Chromosome Work

pdf files available at http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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- Butler, J.M. and Schoske, R. (2005) U.S. population data for the multi-copy Y-STR locus DYS464, *J. Forensic Sci., in press.*

Mitochondrial DNA Work

- Evaluation of Roche LINEAR ARRAY screening assay. Kline et al. (2005) JFS 50: 377-385
- Comparison of LINEAR ARRAY resolution to control region sequencing performed by AFDIL
- Collaboration with AFDIL (Tom Parsons) for developing coding SNP assays using SNaPshot

Coble, M.D., Just, R.S., O'Callaghan, J.E., Letmanyi, I.H., Peterson, C.T., Irwin, J.A., Parsons, T.J. (2004) Single nucleotide polymorphisms over the entire mtDNA genome that increase the power of forensic testing in Caucasians. *Int. J. Legal Med.,* 118: 137-146.

Vallone, P.M., Just, R.S., Coble, M.D., Butler, J.M., Parsons, T.J. (2004) A multiplex allele-specific primer extension assay for forensically informative SNPs distributed throughout the mitochondrial genome. *Int. J. Legal Med.,* 118: 147-157.

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