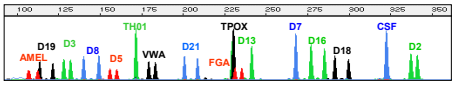


NIST Projects in Human Identity Testing

John Butler
Margaret Kline, Pete Vallone, Mike Coble
Jan Redman, Amy Decker, and Becky Hill
Dave Duewer (NIST Analytical Chemistry Division)

FBI Laboratory Presentation—May 19, 2005

NIST Human Identity Project Team


John Butler
(Project Leader)


Margaret Kline


Pete Vallone


Mike Coble


Dave Duewer
Anal. Chem. Division


Jan Redman


Amy Decker


Becky Hill

Funding: **Interagency Agreement 2003-IJ-R-029 between National Institute of Justice (NIJ) and NIST Office of Law Enforcement Standards (OLES)**

Disclaimers and Collaborations

Funding: Interagency Agreement 2003-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

Points of view are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

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

Past and Present Collaborators (also funded by NIJ):
Mike Hammer and Alan Redd (U. AZ) for Y-chromosome studies
Tom Parsons, Rebecca Just, Jodi Irwin (AFDIL) for mtDNA coding SNP work
Sandy Calloway (Roche) for mtDNA LINEAR ARRAYs
Bruce McCord and students (FL Int. U.) for miniSTR work
Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work
Artie Eisenberg and John Planz (U. North Texas)

Some Publications from Our Group this Past Year

Available as pdf files from http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA., *J. Forensic Sci.* 50(1): 43-53.

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

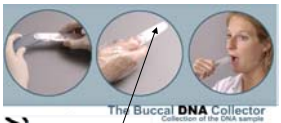
Kline, M.C., Vallone, P.M., Redman, J.W., Duewer, D.L., Calloway, C.D., Butler, J.M. (2005) Mitochondrial DNA typing screens with control region and coding region SNPs, *J. Forensic Sci.* 50(2): 377-385.

Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2005) Results from the NIST 2004 DNA Quantitation Study, *J. Forensic Sci.* 50(3): 571-578.

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, *J. Forensic Sci., in press.*

Butler, J.M. (2005) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci., in press.*

Our Research Impacts Commercial Products and Forensic Use



The Bode Technology Group, Inc.

Decision for which collection paper to use was based on these NIST storage studies

As of January 1, 2003, any individual arrested for a violent felony crime (Code of Virginia § 19.2-310.2:1) must provide a buccal sample for DNA analysis, with the resultant profile incorporated into the Virginia DNA Data Bank (Code of Virginia § 19.2-310.5).


Anal. Chem. (2002) 74:1863-1869
Polymerase Chain Reaction Amplification of DNA from Aged Blood Stains: Quantitative Evaluation of the "Suitability for Purpose" of Four Filter Papers as Archival Media
Margaret C. Kline,* David L. Duewer, Janette W. Redman, and John M. Butler
David A. Beyer

National Institute of Justice

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

Current Areas of NIST Research Effort

Mike
- **Resources for "Challenging Samples"** (miniSTRs)

Pete
- **Information on New Loci** (SNPs, Y-Chromosome, new STRs)

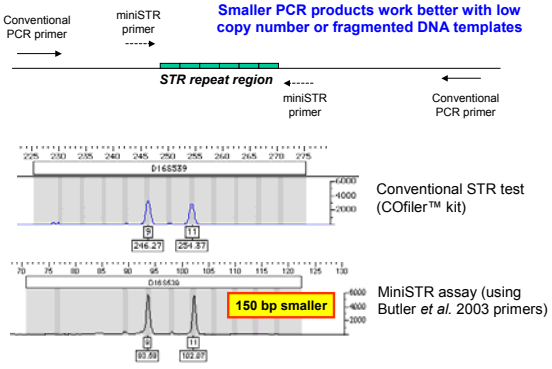
Margaret
- **Allele Sequencing and Interlaboratory Studies** (Real-time PCR, mixture interpretation)

John
- **Standard Information Resources** (STRBase website, training materials/review articles, validation standardization)

Mike Coble

miniSTRs for degraded DNA

miniSTRs: new tool for degraded DNA

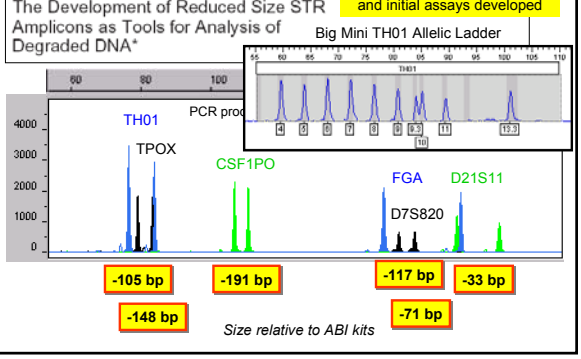


J. Forensic Sci. Sept 2003 issue

John M. Butler,[1] Ph.D.; Yin Shen,[2,3] Ph.D.; and Bruce R. McCord

The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA*

Describes new primer sequences for all CODIS loci and initial assays developed



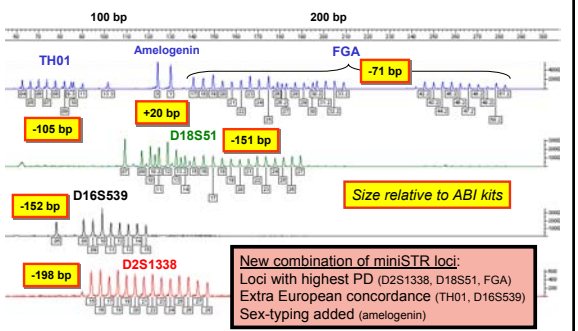
Recent Publications on miniSTRs

- 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.
- Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA., *J. Forensic Sci.*, 50: 43-53.

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

New miniSGM miniplex assay

Provided to EDNAP/ENFSI group for degraded DNA study (Fall 2004)



Retains same miniSTR primers from Butler et al. (2003) *J. Forensic Sci* 48(5): 1054-1064

Many CODIS Loci Make Poor miniSTRs

- Large allele range (e.g., FGA)
- Large alleles (e.g., D21S11 and FGA)
- Poor flanking regions prohibiting reliable primer annealing immediately adjacent to the repeat region (e.g., D7S820)

Why go beyond CODIS loci

"STRs have proven to be highly successful [for mass disasters] in the past e.g. Waco disaster and various air disasters. However, even if the DNA is high quality there are occasions when there are insufficient family members available to achieve a high level of confidence with an association."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

Why go beyond CODIS loci

"To achieve this purpose, either **new STRs could be developed**, or alternatively, existing STRs could be supplemented with a SNP panel."

"There are also efforts for modifying existing STR panels by decreasing the size amplicons by designing new primers."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

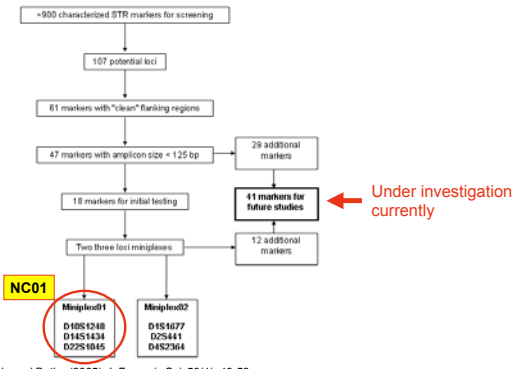
Why go beyond CODIS loci

- Desirable to have markers unlinked from CODIS loci (different chromosomes) for some applications
- Small size ranges to aid amplification from degraded DNA samples
- **New miniSTR loci will benefit missing persons investigations and paternity testing (and perhaps national databases in the future)**

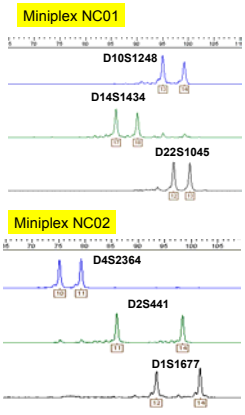
Characterization of New miniSTR Loci

- Candidate STR marker selection
- Chromosomal locations and marker characteristics
- PCR primer design
- Initial testing results
- Population testing
- Allelic ladder construction
- Miniplex assay performance

Initial Testing Results with Potential miniSTR Loci



Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

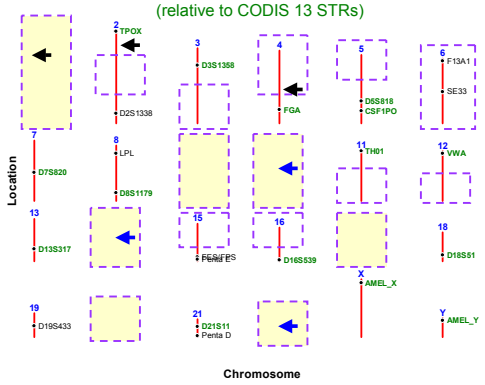


Some Marker Characteristics

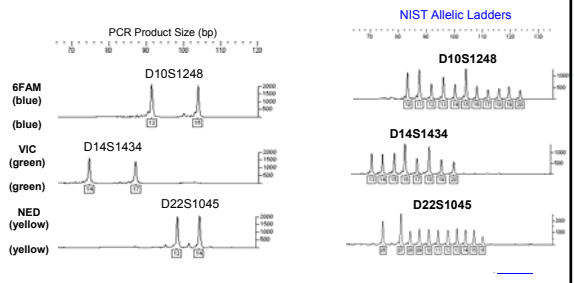
Chr.	Marker Name	(Motif)	Ref. Repeat	Amplicon Size	Primer distance from repeat
10	D10S1248	TETRA	13	102	1
	GGAA23C09N	GGAA			0
14	D14S1434	TETRA	10	88	1
	GATA168F06	GATA			0
22	D22S1045	TRI	13	105	3
	ATA37D06	ATA			6
1	D1S1677	TETRA	15	103	0
	GGAA22G10N	GGAA			0
2	D2S441	TETRA	12	92	0
	GATA8F03	GATA			0
4	D4S2364	TETRA	7	78	2
	GAAT1F09	GAAT			1

Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

Locations of Focus for New miniSTR Loci
(relative to CODIS 13 STRs)



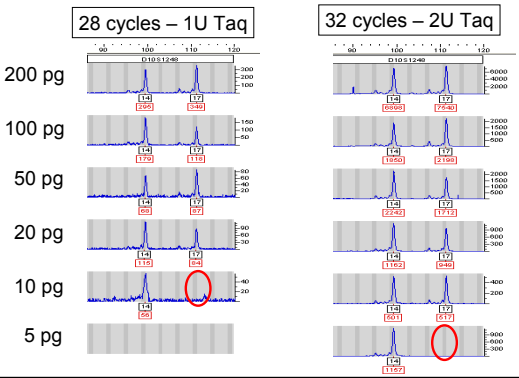
Miniplex "NC01"



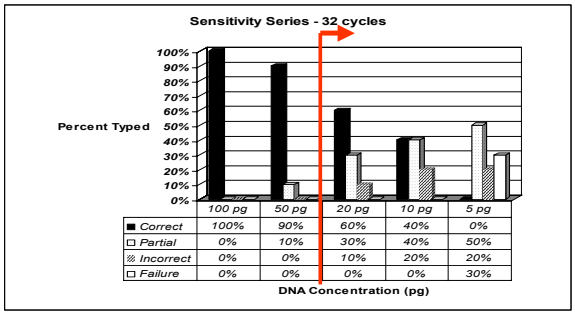
http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm

Coble and Butler (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA *J. Forensic Sci.* 50(1): 43-53

miniSTR Assay Sensitivity (D10S1248)



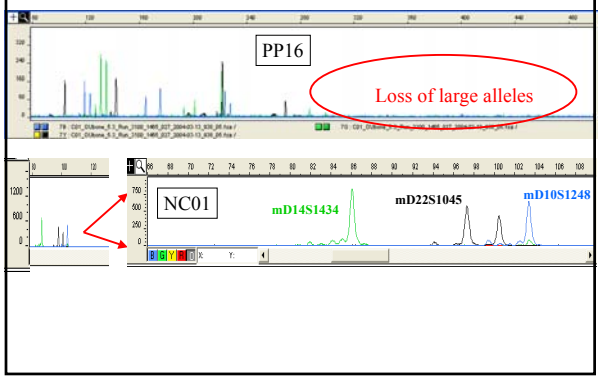
Problems with Obtaining Correct Allele Calls at Low DNA Levels



	100 pg	50 pg	20 pg	10 pg	5 pg
■ Correct	100%	90%	60%	40%	0%
□ Partial	0%	10%	30%	40%	50%
▨ Incorrect	0%	0%	10%	20%	20%
□ Failure	0%	0%	0%	0%	30%

Coble, M.D. and Butler, J.M. (2005) *J. Forensic Sci.* 50: 43-53

Sensitivity - Degraded DNA from an OU Bone Sample



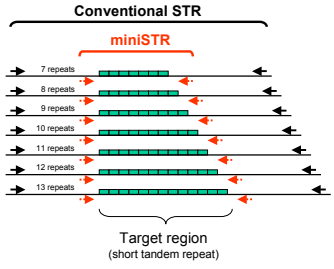
Peter Gill Recommendations to EDNAP and ENFSI (April 2005, Scotland)

- **"miniSTRs are the best way forward for stain work for the foreseeable future…"**
- miniSTRs and 34 cycle PCR seems to be the best option to maximise sensitivity (note importance of minimising cycle number to avoid stochastic effects).
- Recommended to the ENFSI group that miniSTRs are the best way forward.
- Suggested NIST NC01 loci as additional European markers that are being advocated to manufacturers for future STR kits.

Status of Additional STR Loci

- **D10S1248, D14S1434, D22S1045** are chromosomally unlinked to all CODIS STR loci
- Full locus characterization, allelic ladders constructed, population studies completed and published (Coble and Butler JFS Jan 2005)
- Demonstrated success in EDNAP degraded DNA interlab study coordinated by Peter Gill
- EDNAP/ENFSI newly recommended loci to commercial manufacturers for future STR kits
- Being adopted in multiple U.S. paternity testing labs (BRT Labs and Orchid Cellmark East Lansing)

Comparison of STRs and SNPs



Larger target region (miniSTR targets same region)
More possible variants than SNPs
Only need a moderate number of STR markers
Range of sizes examined (e.g., 28 bp spread if 4 bp/repeat)

Smaller target region
Fewer possible variants
Need more SNP markers
Constant size examined

Pete Vallone

Information on new loci

Information on New Loci

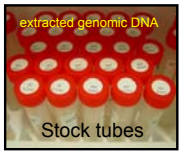
- NIST U.S. population data set
- Tools for multiplex assay development
- Autosomal SNPs
- mtDNA coding region SNPs
- Y-STRs

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**

DNA extracted from whole blood (anonymous; self-identified ethnicities) received from Interstate Blood Bank (Memphis, TN) and Millennium Biotech Inc. (Ft. Lauderdale, FL)



To date: (~95,000 allele calls)
Identifiler (15 autosomal markers + Amelogenin) (10,608)
Roche Linear Arrays (HV1/HV2 10 regions) (6,630)
Y STRs 22 loci—27 amplicons (17,388)
Y STRs 27 new loci (14,535)
Yfiler kit 17 loci (11,237)
Y SNPs 50 markers on sub-set of samples (11,498)
Orchid 70 autosomal SNPs on sub-set (13,230)
miniSTR testing-new loci and CODIS concordance (9,228)
mtDNA full control region sequences by AFDIL

Genotypes with various human identity testing markers

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	**D2S1338 and D19S433** information has been provided to the FBI for inclusion in PopStats to aid statistical calculations	Butler et al. (2003) JFS
miniSTRs		Drabek et al. (2004) JFS
New autosomal STRs		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. (2005) 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*

Anal Bioanal Chem (2003) 375 : 333–343
DOI 10.1007/s00216-002-1683-2

ORIGINAL PAPER

Richard Schoske · Pete M. Vallone
Christian M. Ruitberg · John M. Butler

Multiplex PCR design strategy used for the simultaneous amplification of 10 Y chromosome short tandem repeat (STR) loci

Received: 3 July 2002 / Revised: 24 October 2002 / Accepted: 29 October 2002 / Published online: 14 January 2003

Describes how to build STR multiplex assays…

Careful primer design

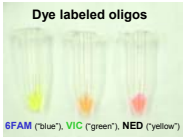
- Uniform annealing temperatures
- Checking for all potential primer-primer interactions

Potential Interaction

```
   3-TAGTAGATAGACAGAGGTGGATACA-5
     || |||||||
5-CCCCCTCCTCTCGTCTATCT-3
```

Butler *et al.* (2001) *Fresenius J. Anal. Chem.* 369:200-205

Stringent primer quality control



Butler *et al.* (2001) *Forensic Sci. Int.* 119: 87-96

AutoDimer Primer Screening Program

SHORT TECHNICAL REPORTS

Vallone, P.M. and Butler, J.M. (2004) *BioTechniques* 37:226-231

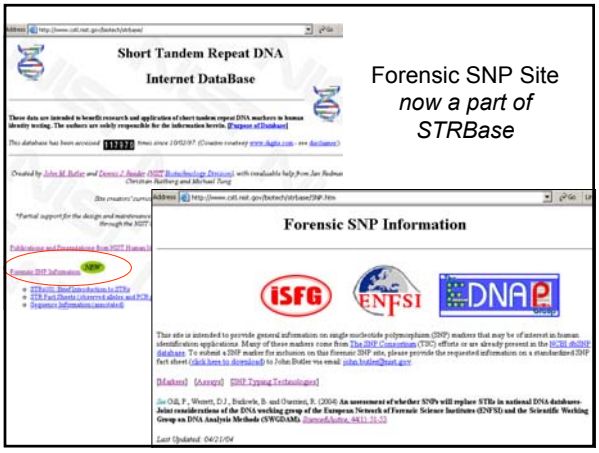
AutoDimer: a screening tool for primer-dimer and hairpin structures

Peter M. Vallone and John M. Butler
National Institute of Standards and Technology, Gaithersburg, MD, USA

BioTechniques 37:226-231 (August 2004)

Available for download from STRBase: http://www.cstl.nist.gov/biotech/strbase

A web-based interface is in development (similar to Primer3)



Forensic SNP Site *now a part of STRBase*

SNP Typing Instrumentation

PCR & primer extension

Multi-Color Capillary Electrophoresis (ABI 310 or 3100)

Luminex Beads hybridization

Luminex 100 Flow Cytometer

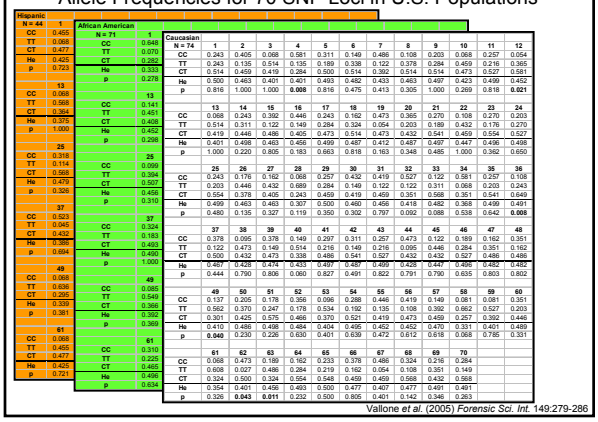
Primer Extension

Time-of-Flight Mass Spectrometer

TaqMan

ABI 7000 SDS

Allele Frequencies for 70 SNP Loci in U.S. Populations

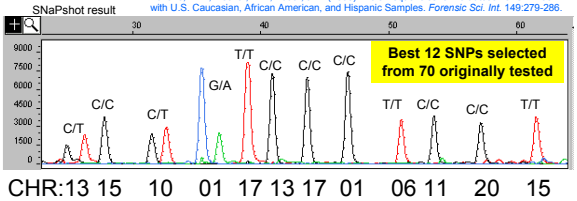


Vallone *et al.* (2005) *Forensic Sci. Int.* 149:279-286

NIST Autosomal 12plex SNP Assay

SNaPshot result

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.

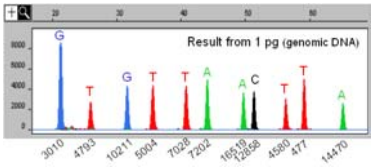


CHR:13 15 10 01 17 13 17 01 06 11 20 15

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

# of SNPs	# of Genotypes
1	3
2	9
3	27
4	64
5	107
6	145
7	160
8	175
9	182
10	186
11	188
12	189

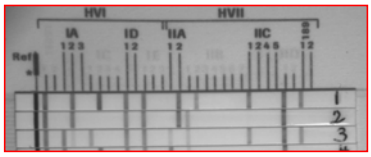
NIST mtDNA Work



Coding Region mtSNP 11plex (minisequencing assay)

Developed with AFDIL to resolve mtDNA most common types

Int. J. Legal Med., 2004; 118: 147-157



Roche Linear Arrays (probes for HVI/HVII)

J. Forensic Sci. 2005, 50(2): 377-385

Automated washing/ Population Study

Typing frequencies for 666 NIST population samples

# in Group	Freq	% Types	% People
1	185	65.6	27.8
2	46	16.3	13.8
3	18	6.4	8.1
4	4	1.4	2.4
5	3	1.1	2.3
6	4	1.4	3.6
7	1	0.4	1.1
8	9	3.2	10.8
9	2	0.7	2.7
10	4	1.4	6.0
11	1	0.4	1.7
12	1	0.4	1.8
18	1	0.4	2.7
23	1	0.4	3.5
28	1	0.4	4.2
51	1	0.4	7.7

Summary of Our Population Typing with Roche mtDNA LINEAR ARRAYS

LINEAR ARRAY summary
- 282 different types
- 185 were unique (occurred only once)
- 51 samples had "Most Common Type"

HV1/HV2 sequencing summary
- 518 different types
- 454 were unique (occurred only once)
- 17 samples had "Most Common Type"

"Most Common Type" evaluated further with mtDNA coding region SNP assay

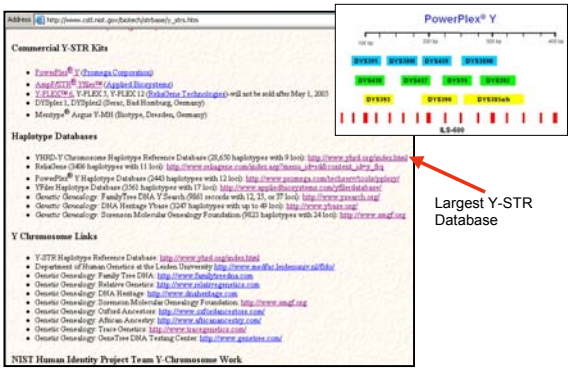
Value of Y-Chromosome Markers

Application	Advantage
Forensic casework on sexual assault evidence	**Male-specific amplification** (can avoid differential extraction to separate sperm and epithelial cells)
Paternity testing	Male children can be tied to fathers in motherless paternity cases
Missing persons investigations	**Patrilineal male relatives may be used for reference samples**
Human migration and evolutionary studies	Lack of recombination enables comparison of male individuals separated by large periods of time
Historical and genealogical research	Surnames usually retained by males; can make links where paper trail is limited

NIST Y-STR Work

- Standardize information resources on Y-STRs and nomenclature for alleles
- Understand variation in U.S. populations so the best loci can be selected for commercial kits
- Construct multiplex assays to quickly evaluate loci
- Provide reference material for laboratory calibration (SRM 2395)

New Y-Chromosome Information Resources on STRBase



Y-Chromosome Haplotype Reference Database (YHRD)

yhrd str database

http://www.yhrd.org

As of 12/17/04: **28,650 haplotypes**

6,281 haplotypes with all US required loci

Commercial Y-STR kits exist to amplify all of the core loci in a single reaction (plus a few additional markers)

Run only with minimal haplotype

DYS19
DYS389I/II
DYS390
DYS391
DYS392
DYS393
DYS385 a/b

US haplotype requires 2 additional loci:
DYS438
DYS439

Commercial Y-STR Kits

(Minimal/extended haplotype)

DYS19
DYS389I/II
DYS390
DYS391
DYS392
DYS393
DYS385 a/b
YCAII a/b
DYS388
DYS425
DYS426
YCAIII a/b

(White et al.)
A7.1 (DYS460)
A7.2 (DYS461)
A10
C4
H4

(Ayub et al.)
DYS434
DYS435
DYS436
DYS437
DYS438
DYS439

(Iida et al.)
DYS441
DYS442
DYS443
DYS444
DYS445

(Redd et al.)
DYS446
DYS447
DYS448
DYS449
DYS450
DYS452
DYS453
DYS454
DYS455
DYS456
DYS458
DYS459 a/b
DYS463
DYS464 a/b/c/d

(Bosch et al.)
G09411 (DYS462)

43 (51) Y-STRs
(217 with Manfred's)

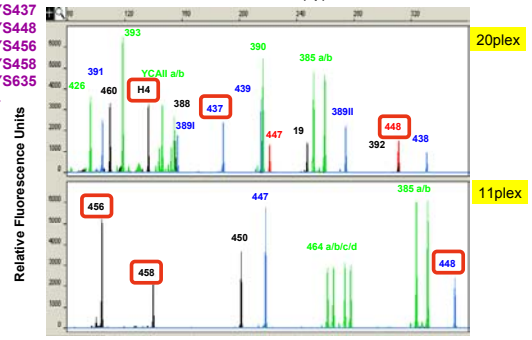
~~Y-PLEX 6 (ReliaGene)~~
~~Y-PLEX 5 (ReliaGene)~~
~~Y-PLEX 12 (ReliaGene)~~
PowerPlex Y (Promega)
Yfiler (Applied Biosystems)

DYS468-DYS645
166 new Y STRs
(Manfred Kayser GDB entries)

NIST Multiplexes for High-Throughput Y STR Typing

Laid the Groundwork for Applied Biosystems' Yfiler Y-STR Kit

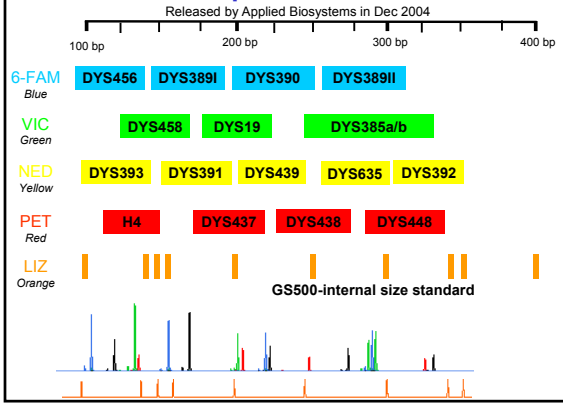
11 U.S. core loci
+ DYS437
+ DYS448
+ DYS456
+ DYS458
+ DYS635
+ H4



Schoske *et al.* (2004) High-throughput Y-STR typing of U.S. populations…, *Forensic Sci. Int.*, 139:107-121

AmpFℓSTR® Yfiler™

Released by Applied Biosystems in Dec 2004



Yfiler Haplotype Database

http://www.appliedbiosystems.com/yfilerdatabase/

Population	# Haplotypes
African American	985
Asian	330
Caucasian	1276
Filipino	105
Hispanic	597
Native American	106
Sub-saharan African	59
Vietnamese	103
All	3561

#Samples Contributed by NIST

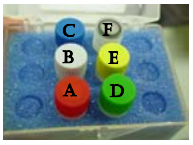
259 African Americans
3 Asians
262 Caucasians

139 Hispanics

Data provided by NIST

663/3561 = **18.6%**

Y-Chromosome Standard NIST SRM 2395



Human Y-Chromosome DNA Profiling Standard

- **5 male samples** + 1 female sample (neg. control)
- **100 ng of each (50 µL at ~2 ng/µL)**
- **22 Y STR markers sequenced**
- **9 additional Y STR markers typed**
- **42 Y SNPs typed** with Marligen kit

Certified for all loci in commercial Y-STR kits:
Y-PLEX 6
Y-PLEX 5
Y-PLEX 12
PowerPlex Y

SWGDAM recommended loci:
DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439

Y-filer - adds DYS635 (C4); now sequenced

Helps meet FBI Standard 9.5 (and ISO 17025)…traceability to a national standard

Margaret Kline

Variant allele sequencing
Interlaboratory studies

Practical Aspects of DNA Typing

- Variant allele sequencing
- NIST initiated interlab studies
  - DNA Quantitation Study 2004 (QS04)
  - Mixture Interpretation Study 2005 (MIX05)
- Real-time qPCR efforts at NIST to improve DNA quantitation

We want to understand the basis for allele dropout

Apparent Null Alleles Observed During Concordance Studies

10/13 CODIS loci affected so far

Locus	STR Kits/Assays Compared	Results	Reference
VWA	PP1.1 vs ProPlus	Loss of allele 19 with **ProPlus**; fine with PP1.1	Kline et al. (1998)
D5S818	PP16 vs ProPlus	Loss of alleles 10 and 11 with **PP16**; fine with ProPlus	Alves et al. (2003)
D13S317	Identifiler vs miniplexes	Shift of alleles 10 and 11 due to deletion outside of **miniplex assay**	Butler et al. (2003), Drabek et al. (2004)
D16S539	PP1.1 vs PP16 vs COfiler	Loss of alleles with **PP1.1**; fine with PP16 and COfiler	Nelson et al. (2002)
D8S1179	PP16 vs ProPlus	Loss of alleles 15, 16, 17, and 18 with **ProPlus**; fine with PP16	Budowle et al. (2001)
FGA	PP16 vs ProPlus	Loss of allele 22 with **ProPlus**; fine with PP16	Budowle and Sprecher (2001)
D18S51	SGM vs SGM Plus	Loss of alleles 17, 18, 19, and 20 with **SGM Plus**; fine with SGM	Clayton et al. (2004)
CSF1PO	PP16 vs COfiler	Loss of allele 14 with **COfiler**; fine with PP16	Budowle et al. (2001)
TH01	PP16 vs COfiler	Loss of allele 9 with **COfiler**; fine with PP16	Budowle et al. (2001)
D21S11	PP16 vs ProPlus	Loss of allele 32.2 with **PP16**; fine with ProPlus	Budowle et al. (2001)

From Table 6.2 in J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, p. 136

Steps in STR Allele Sequencing

DNA Extraction

Amplification with primers external to kit primers

Gel Cutouts with Heterozygotes

Re-Amplification

Amplicon Quantitation

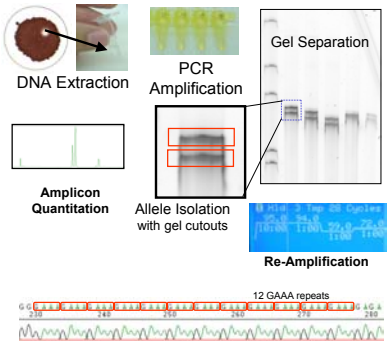
ExoSAP

Cycle Sequencing

Dye Terminator Removal

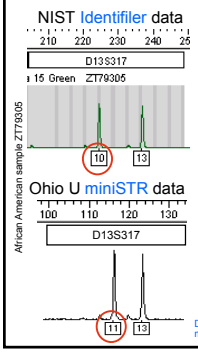
F/R Sequence Alignment to Reference Sequence

Samples provided by collaborators or forensic practioners

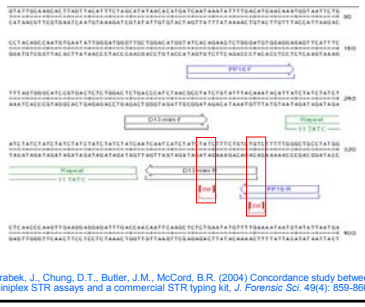


D13S317 Flanking Region Deletion

A deletion *outside* the miniSTR primers causes the commercial kit produced allele to appear one repeat smaller…



Sequence analysis identified two regions where 4 bp deletions occur to cause this 1 repeat variation



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.

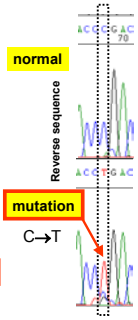
D18S51 Null Allele from Kuwait Samples with ABI Primers

DNA sample supplied by Kuwait government lab

PowerPlex 16

Identifiler

Allele 18 drops out



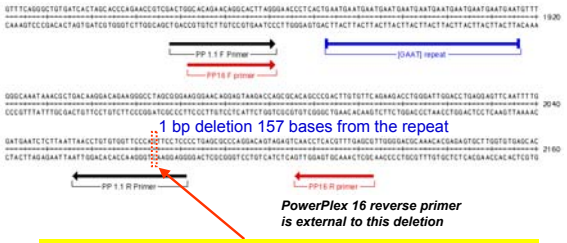
172 bp downstream of STR repeat (G→A)

10 nt from 3'end

10 nucleotides from 3'end of ABI D18-R primer (PowerPlex 16 primers are not impacted)

Clayton et al. (2004) **Primer binding site mutations affecting the typing of STR loci contained within the AMPFISTR SGM Plus kit.** *Forensic Sci Int.* 139(2-3): 255-259

TPOX Flanking Region Deletion Impacting Calls with Different Kits

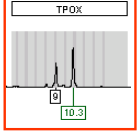


PowerPlex 16 reverse primer is external to this deletion

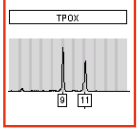
Deletion results in a 10.3 allele call with PP 16 but an allele 11 call with COfiler/Identifiler/PP1.1.

DNA sample supplied by Amy Kelly (MD State Police)

PowerPlex 16

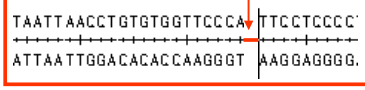


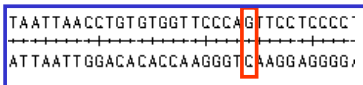
Identifiler



Sequence of the Variant TPOX allele

deletion





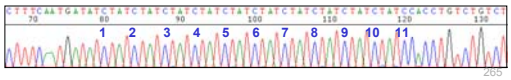
PP 1.1 R Primer

Sequence of the Nominal TPOX allele

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)

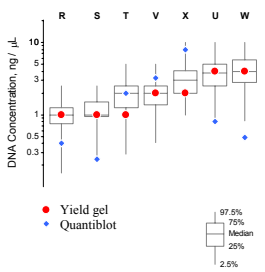


NIST Initiated Interlaboratory Studies

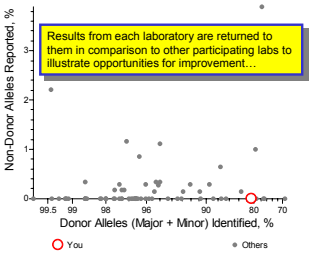
Studies involving STRs	# Labs	Publications
Evaluation of CSF1PO, TPOX, and TH01	34	Kline MC, Duewer DL, Newall P, Redman JW, Reeder DJ, Richard M. (1997) Interlaboratory evaluation of STR triplex CTT. *J. Forensic Sci.* 42: 897-906
Mixed Stain Studies #1 and #2 (Apr–Nov 1997 and Jan–May 1999)	45	Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. (2001) NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples. *J. Forensic Sci.* 46: 1199-1210
MSS3 Mixed Stain Study #3 (Oct 2000-May 2001)	74	Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2003) NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. *Anal. Chem.* 75: 2463-2469. Duewer, D.L., Kline, M.C., Redman, J.W., Butler, J.M. (2004) NIST Mixed Stain Study #3: signal intensity balance in commercial short tandem repeat multiplexes, *Anal. Chem.* 76: 6928-6934.
DNA Quantitation Study (Jan-Mar 2004) QS04	80	Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2005) Results from the NIST 2004 DNA Quantitation Study, *J. Forensic Sci.* 50(3):571-578
MIX05 Mixture Interpretation Study (Jan-Mar 2005)	**64**	***Data analysis currently on-going ...*** Will be presented at NIJ Grantees and SWGDAM (June 2005) and ISFG (Sept 2005)

Individual Performance in an Interlaboratory Study

DNA Quantitation



Accuracy in STR Typing



See Kline, M.C., *et al.* (2003) *Anal. Chem.* 75: 2463-2469

NIST Quantitation Study 2004 (QS04)

Consisted of:
- 8 DNA extracts labeled A – H
- Shipped Dec 2003 –Jan 2004 to 84 laboratories for quantification; data received back by April 2004
- Labs were requested to use multiple methods / multiple analysts

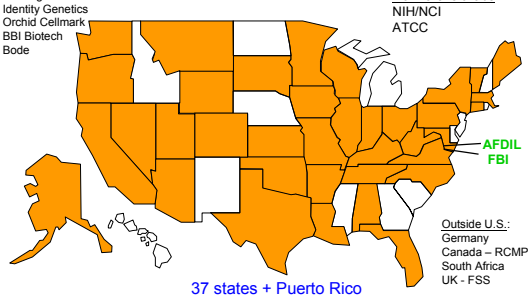
We received data from 80 Labs (95%)
Total of 287 sets of data
Participants used 19 different quantification methods (primarily variations on Quantiblot and Real-time PCR)

Information from this interlab study is being used to help construct SRM 2372 (Human DNA Quantitation Standard)

Participation in NIST Interlaboratory Study on DNA Quantitation (QS04)

Companies:
Applied Biosystems
Promega
Identity Genetics
Orchid Cellmark
BBI Biotech
Bode

Non-forensic labs:
NIH/NCI
ATCC



Outside U.S.:
Germany
Canada – RCMP & CFS
South Africa
UK - FSS

37 states + Puerto Rico

84 laboratories were sent samples (80 returned results)

8 DNA Samples in This NIST Study

Laboratories are only being asked to provide their quant values (no typing results expected)

Mixed source DNA

Single source DNA

Teflon tube

Volume of each DNA sample provided = 100 µL

Table 2. The percent success rate reported for a sample.

		% Quantitative Results*							
Target [DNA] ng/µL		1.5	0.5	0.5	0.16	0.16	0.05	0.05	0.05
Method	N_{total}	A	B	E	C	F	D	G	H
Quantifiler	37	100	100	100	100	100	100	100	100
Other RT-PCR	23	100	100	100	100	100	100	100	100
"ACES"	14	100	100	100	100	100	100	100	100
AluQuant	13	100	100	100	100	100	100	100	100
PicoGreen	12	100	100	92	100	100	92	83	83
ECL	75	100	99	99	93	95	84	77	87
TMB	98	100	100	99	93	94	59	62	63
Yield gel	14	57	0	0	0	0	0	0	0
	286								

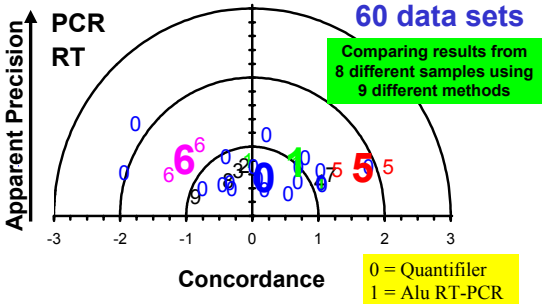
a Quantitative results are those that were reported as values between contiguous calibration standards, values reported standard if smaller than the target [DNA], or values repo calibration standard if larger than the target[DNA].

At least one lab used poor performance of their Quantiblot with low level samples to justify purchase of qPCR instrumentation and conversion to Quantifiler kit DNA quantitation

Kline, *et al.*, *J. Forensic Sci.* 50(3): 571-578

Interlaboratory Comparisons

Laboratory Performances with Real-Time PCR Methods



0 = Quantifiler
1 = Alu RT-PCR
5 = BRCA1
6 = CFS-HUMRT

Kline, *et al.* (2005) *J. Forensic Sci.* 50(3):571-578

Real-time qPCR Work at NIST

- Careful examination of published assays on the same set of DNA samples
- Lot-to-lot variability with Quantifiler "standard"
  - *qPCR is a relative measurement that depends on the quality of the material used to generate the standard curve*

Variability of Quantifiler DNA Standards

Two lots of ABI "standards" using Quantifiler Human assay

Sample (n = 4)	Standard Lot 1 (ng/mL)	Standard Lot 2 (ng/mL)
1	4*	2.91 ± 0.04
2	7.26 ± 0.79	4*
3	2.93 ± 0.27	1.88 ± 0.09
4	3.46 ± 0.30	2.22 ± 0.08
5	2.99 ± 0.28	1.91 ± 0.08
6	2.62 ± 0.22	1.70 ± 0.03

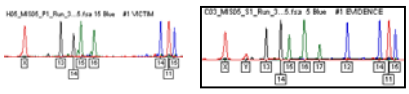
* - indicates "standard" value based on starting material provided by the manufacturer

Samples 1-3 = commercially available kit standards

Samples 4-6 = in-house standards based on UV absorbance

Mixture Interpretation Interlab Study (MIX05)

- **Only involves interpretation of data**
- **91 labs enrolled** for participation (20 from overseas)
- **64 labs have returned results**
- Four mock cases supplied with "victim" and "evidence" electropherograms (GeneScan .fsa files – that can be converted for Mac or GeneMapper; gel files 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



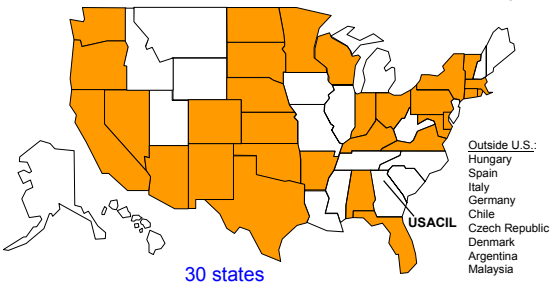
Perpetrator Profile(s) ??

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

Participation in NIST Interlaboratory Study on Mixture Interpretation (MIX05)

Companies:
Myriad Genetics

20 labs outside of U.S. signed up



30 states

91 laboratories signed up for study (64 returned results so far)

http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm

Forensic SNP Information

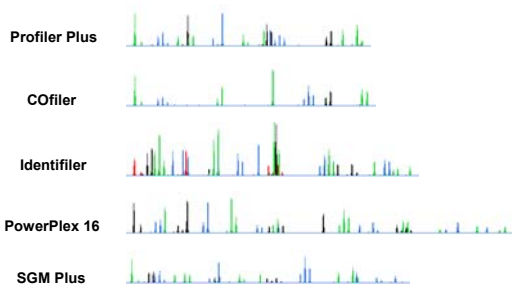
- STRs101: Brief Introduction to STRs
- STR Fact Sheets (observed alleles and PCR product sizes)
- Sequence Information (annotated)
- Multiplex STR sets
- STR Training Materials
- Variant Allele Reports
- Tri-Allelic Patterns
- FBI CODIS Core STR Loci
- DNA Advisory Board Quality Assurance Standards
- NIST Standard Reference Material for PCR-Based Testing
- Chromosomal Locations
- Mutation Rates for Common Loci
- Published PCR primers
- Validation information (Updated)
- Interlaboratory Studies (NEW) **NIST Interlab Study MIX05 Data**
- Population data (Updated)
- Data from NIST U.S. Population Samples
- Y-chromosome STRs (Updated)
- miniSTRs (short amplicons) (NEW)
- Sex-typing markers
- Technology for resolving STR alleles

Interlab Study MIX05 Data Available for Download from STRBase

ABI 3100 Generated Data was also supplied on CD-ROM to all labs as either .fsa files (for Genotyper NT or GeneMapperID) or Mac-converted files for Genotyper Mac

MIX05 Results on Multiple Kits

Case 1 evidence (mixture)



Plans for Dissemination of MIX05 Results

- Data shipped in mid-January 2005
- Responses due before March 15, 2005 (but still open)
- **Goal is to understand the "lay of the land" regarding mixture analysis across the DNA typing community**
- Results to be discussed at NIJ DNA Grantees Meeting (June 2005), SWGDAM (June 2005), and ISFG (Sept 2005)
- We plan to develop training materials to aid in mixture interpretation with available software tools and to help in standardizing reports involving mixture analysis

John Butler

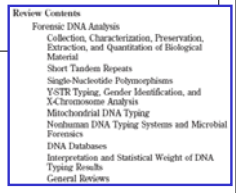
Standard Information Resources

Analytical Chemistry Application Review

June 15, 2005 issue of *Analytical Chemistry*

Forensic Science

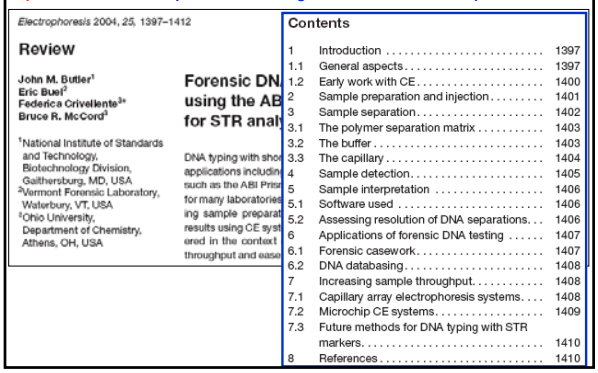
T. A. Brettell*
Office of Forensic Sciences, New Jersey State Police, New Jersey Forensic Science and Technology Complex, 1200 Negron Road, Horizon Center, Hamilton, New Jersey 08691
J. M. Butler
National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311
R. Saferstein
Box 1334, Mount Laurel, New Jersey 08054


Review Contents
Forensic DNA Analysis
Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material
Short Tandem Repeats
Single-Nucleotide Polymorphisms
Y-STR Typing, Gender Identification, and X-Chromosome Analysis
Mitochondrial DNA Typing
Nonhuman DNA Typing Systems and Microbial Forensics
DNA Databases
Interpretation and Statistical Weight of DNA Typing Results
General Reviews

250 articles referenced covering forensic DNA analysis during 2003-2004

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]
Federica Crivellente[3]*
Bruce R. McCord[3]

[1]National Institute of Standards and Technology, Biotechnology Division, Gaithersburg, MD, USA
[2]Vermont Forensic Laboratory, Waterbury, VT, USA
[3]Ohio University, Department of Chemistry, Athens, OH, USA

Forensic DN
using the AB
for STR anal

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

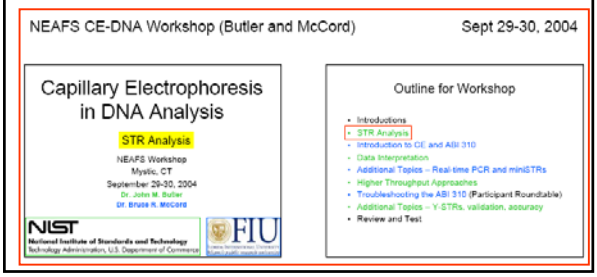
Contents

1	Introduction	1397
1.1	General aspects	1397
1.2	Early work with CE	1400
2	Sample preparation and injection	1401
3	Sample separation	1402
3.1	The polymer separation matrix	1403
3.2	The buffer	1403
3.3	The capillary	1404
4	Sample detection	1405
5	Sample interpretation	1406
5.1	Software used	1406
5.2	Assessing resolution of DNA separations	1406
6	Applications of forensic DNA testing	1407
6.1	Forensic casework	1407
6.2	DNA databasing	1408
7	Increasing sample throughput	1408
7.1	Capillary array electrophoresis systems	1408
7.2	Microchip CE systems	1409
7.3	Future methods for DNA typing with STR markers	1410
8	References	1410

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)



NEAFS CE-DNA Workshop (Butler and McCord) Sept 29-30, 2004

Capillary Electrophoresis in DNA Analysis

STR Analysis

NEAFS Workshop
Mystic, CT
September 29-30, 2004
Dr. John M. Butler
Dr. Bruce R. McCord

NIST National Institute of Standards and Technology Technology Administration, U.S. Department of Commerce

FIU

Outline for Workshop

- Introductions
- STR Analysis
- Introduction to CE and ABI 310
- Data Interpretation
- Additional Topics – Real-time PCR and miniSTRs
- Higher Throughput Approaches
- Troubleshooting the ABI 310 (Participant Roundtable)
- Additional Topics – Y-STRs, validation, accuracy
- Review and Test

Content of STRBase Website

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

- …/str_fact.htm STR Fact Sheets on Core Loci
- …/multiplx.htm Multiplex STR Kit Information
- …/y_strs.htm Y-Chromosome Information
- …/var_tab.htm Variant Alleles Reported
- …/mutation.htm Mutation Rates for Common STRs
- …/str_ref.htm Reference List with ~2,300 Papers
- …/training.htm Downloadable PowerPoints for Training
- …/validation.htm Validation Information
- …/miniSTR.htm miniSTR Information
- …/address.htm Addresses for Scientists
- …/NISTpub.htm Publications & Presentations from NIST

Forensic DNA Typing, 2nd Edition:

Biology, Technology, and Genetics of STR Markers

John Butler (not NIST)

Chapter 1	Overview & History of DNA Typing
Chapter 2	DNA Biology Review
Chapter 3	Sample Collection, Extraction, Quantitation
Chapter 4	PCR Amplification
Chapter 5	Common STRs and Commercial Kits
Chapter 6	Biology of STRs
Chapter 7	Forensic Issues
Chapter 8	**Single Nucleotide Polymorphisms**
Chapter 9	**Y-Chromosome DNA Tests**
Chapter 10	**Mitochondrial DNA Analysis**
Chapter 11	**Non-Human DNA and Microbial Forensics**
Chapter 12	DNA Separation Methods
Chapter 13	DNA Detection Methods
Chapter 14	Instrumentation for STR Typing: ABI 310, ABI 3100, FMBIO
Chapter 15	STR Genotyping Issues
Chapter 16	Lab Validation
Chapter 17	New Technologies, Automation, and Expert Systems
Chapter 18	CODIS and DNA Databases
Chapter 19	**Basic Genetic Principles and Statistics**
Chapter 20	**STR Database Analyses**
Chapter 21	**Profile Frequency Estimates**
Chapter 22	**Statistical Analysis of Mixtures and Degraded DNA**
Chapter 23	**Kinship and Paternity Testing**
Chapter 24	**Mass Disaster DNA Victim Identification**
Appendix I	Reported STR Alleles
Appendix II	**U.S. Population Data-STR Allele Frequencies**
Appendix III	Suppliers of DNA Analysis Equipment
Appendix IV	DAB QA Standards
Appendix V	**DAB Recommendations on Statistics**
Appendix VI	**Application of NRC II to STR Typing**
Appendix VII	**Example DNA Cases**

New Material:
10 additional chapters
Statistics (basics with examples)
Real-time PCR
Serology tests
Y-STRs and mtDNA
ABI 3100
Expert systems
Mass disasters including WTC
Example cases for training purposes

>500 new reference citations
50 new figures and 45 new tables
688 pages; $79.95

1st Edition 2nd Edition

Jan 2001 Feb 2005

Locus Name	Chromosomal Location	Physical Position[a]
CSF1PO	5q33.1 c-fms proto-oncogene, 6th intron	Chr 5 149.484 Mb
FGA	4q31.3 alpha fibrinogen, 3rd intron	Chr 4 156.086 Mb
TH01	11p15.5 tyrosine hydroxylase, 1st intron	Chr 11 2.156 Mb
TPOX	2p25.3 thyroid peroxidase, 10th intron	Chr 2 1.436 Mb
VWA	12p13.31 von Willebrand Factor, 40th intron	Chr 12 19.826 Mb
D3S1358	3p21.31	Chr 3 45.543 Mb
D5S818	5q23.2	Chr 5 123.187 Mb
D7S820	7q21.11	Chr 7 83.401 Mb
D8S1179	8q24.13	Chr 8 125.863 Mb
D13S317	13q31.1	Chr 13 80.52 Mb
D16S539	16q24.1	Chr 16 86.168 Mb
D18S51	18q21.33	Chr 18 59.098 Mb
D21S11	21q21.1	Chr 21 19.476 Mb

Position of Each CODIS STR Locus in Human Genome

Review article on core STR loci genetics and genomics to be published this fall

From Table 5.2, *Forensic DNA Typing*, 2nd Edition, p. 96 (J.M. Butler, 2005)

Mutation Rates for Common STR Loci

J.M. Butler (2005) *J. Forensic Sci., in press*
http://www.aabb.org/About_the_AABB/Stds_and_Accred/plannrpt03.pdf, Appendix 2

STR System	Maternal Meioses (%)	Paternal Meioses (%)	Number from either	Total Number of Mutations	Mutation Rate
CSF1PO	95/304,307 (0.03)	982/643,118 (0.15)	410	1,487/947,425	**0.16%**
FGA	205/408,230 (0.05)	2,210/692,776 (0.32)	710	3,125/1,101,006	**0.28%**
TH01	31/327,172 (0.009)	41/452,382 (0.009)	28	100/779,554	**0.01%**
TPOX	18/400,061 (0.004)	54/457,420 (0.012)	28	100/857,481	**0.01%**
VWA	184/564,398 (0.03)	1,482/873,547 (0.17)	814	2,480/1,437,945	**0.17%**
D3S1358	60/405,452 (0.015)	713/558,836 (0.13)	379	1,152/964,288	**0.12%**
D5S818	111/451,736 (0.025)	763/655,603 (0.12)	385	1,259/1,107,339	**0.11%**
D7S820	59/440,562 (0.013)	745/644,743 (0.12)	285	1,089/1,085,305	**0.10%**
D8S1179	96/409,869 (0.02)	779/489,968 (0.16)	364	1,239/899,837	**0.14%**
D13S317	192/482,136 (0.04)	881/621,146 (0.14)	485	1,558/1,103,282	**0.14%**
D16S539	129/467,774 (0.03)	540/494,465 (0.11)	372	1,041/962,239	**0.11%**
D18S51	186/296,244 (0.06)	1,094/494,098 (0.22)	466	1,746/790,342	**0.22%**
D21S11	464/435,388 (0.11)	772/526,708 (0.15)	580	1,816/962,096	**0.19%**
Penta D	12/18,701 (0.06)	21/22,501 (0.09)	24	57/41,202	**0.14%**
Penta E	29/44,311 (0.065)	75/55,719 (0.135)	59	163/100,030	**0.16%**
D2S1338	15/72,830 (0.021)	157/152,310 (0.10)	90	262/225,140	**0.12%**
D19S433	38/70,001 (0.05)	78/103,489 (0.075)	71	187/173,490	**0.11%**
SE33 (ACTBP2)	0/330 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	**0.64%**

J.M. Butler (2005) *J. Forensic Sci., in press*

STR Locus	Number Reported	**264 variant alleles** reported as of Apr 2005 on STRBase
CSF1PO	11	5, 7.3, 8.3, 9.1, 9.3, 10.1, 10.2, 10.3, 11.1, 12.1, 16
FGA	69	12.2, 13.2, 14, 14.3, 15, 15.3, 16, 16.1, 16.2, "<17", 17, 17.2, 18.2, 19.1, 19.2, 19.3, 20.1, 20.2, 20.3, 21.1, 21.2, 21.3, 22.1, 22.2, 22.3, 23.1, 23.2, 23.3, 24.1, 24.2, 24.3, 25.1, 25.2, 25.3, 26.1, 26.2, 26.3, 27.3, 29.2, 30.2, 31, 31.2, 32.1, 32.2, 33.1, 34.1, 34.2, 35.2, 41.1, 41.2, 42.1, 42.2, 43.1, 43.2, 44, 44.1, 44.2, 44.3, 45.1, 45.2, 46.1, 46.2, 47.2, 48.2, 49, 49.1, 49.2, 50.2, 50.3
TH01	7	4, 7.3, 8.3, 9.1, 10.3, 11, 13.3
TPOX	7	4, 5, 7.3, 13.1, 14, 15, 16
VWA	6	16.1, 18.3, 22, 23, 24, 25
D3S1358	18	8, 8.3, 9, 10, 11, 15.1, 15.2, 15.3, 16.2, 17.1, 17.2, 18.1, 18.2, 18.3, ">19", 20, 20.1, 21.1
D5S818	5	10.1, 11.1, 12.3, 17, 18
D7S820	22	5, 5.2, 6.3, 7.1, 7.3, 8.1, 8.2, 8.3, 9.1, 9.2, 9.3, 10.1, 10.3, 11.1, 11.3, 12.1, 12.2, 12.3, 13.1, 14.1, 15, 16
D8S1179	4	7, 15.3, 18, 20
D13S317	10	5, 6, 7, 7.1, 8.1, 11.1, 11.3, 13.3, 14.3, 16
D16S539	10	6, 7, 9.3, 11.3, 12.1, 12.2, 13.1, 13.3, 14.3, 16
D18S51	30	7, 8, 9, 11.2, 12.2, 12.3, 13.1, 13.3, 14.2, 15.1, 15.2, 16.1, 16.2, 16.3, 17.2, 17.3, 18.1, 18.2, 19.2, 20.1, 20.2, 21.2, 22.1, 22.2, 23.2, 24.2, 27, 28.1, 28.3, 40
D21S11	24	24.3, 25.1, 25.2, 25.3, 26.2, 27.1, 27.2, 28.1, 28.3, 29.1, 29.3, 30.3, 31.1, 31.3, 32.1, 33.1, 34.1, 34.3, 35.1, 36.1, 36.2, 37, 37.2, 39
Penta D	14	6, 6.4, 7.1, 7.4, 9.4, 10.3, 11.1, 11.2, 12.2, 12.4, 13.2, 13.4, 14.1, 14.4
Penta E	13	9.4, 11.4, 12.1, 12.2, 13.2, 14.4, 15.2, 15.4, 16.4, 17.4, 18.4, 19.4, 23.4
D2S1338	3	13, 23.2, 23.3
D19S433	11	6.2, 7, 8, "<9", 11.1, 12.1, 13.2, 18, 18.2, 19.2, 20
SE33	0	None reported yet in STRBase

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

J.M. Butler (2005) *J. Forensic Sci., in press*

STR Locus	Number Reported	**62 tri-allelic patterns** reported as of April 2005 on STRBase
CSF1PO	2	9/11/12; 10/11/12
FGA	10	19/20/21; 19/22/23; 19/24/25; 20/21/22; 20/21/24; 20/23/24; 21/22/23; 21/25/26; 22/24/25; 22.2/23/23.2
TH01	1	7/8/9
TPOX	13	6/8/10; 6/9/10; 6/10/11; 6/10/12; 7/9/10; 7/10/11; 8/9/10; 8/10/11; 8/10/12; 8/11/12; 9/10/11; 9/10/12; 10/11/12
VWA	8	11/16/17; 12/18/19; 14/15/17; 14/15/18; 14/16/18; 14/17/18; 15/16/17; 18/19/20
D3S1358	4	15/16/17; 15/17/18; 16/17/19; 17/18/19
D5S818	2	10/11/12; 11/12/13
D7S820	2	8/9/12; 8/10/11
D8S1179	5	10/12/13; 10/12/15; 12/13/14; 12/13/15; 13/15/16
D13S317	3	8/11/12; 10/11/12; 10/12/13
D16S539	1	12/13/14
D18S51	7	12/13/15; 12/14/15; 12/16/17; 14/15/22; 15/16/20; 16/17/20; 19/22.2/23.2
D21S11	4	28/29/30; 28/30.2/31.2; 29/31/32; 30/30.2/31
Penta D	0	None reported yet in STRBase
Penta E	0	None reported yet in STRBase
D2S1338	0	None reported yet in STRBase
D19S433	0	None reported yet in STRBase
SE33	0	None reported yet in STRBase

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

Validation Project Purpose

- Review validation practices currently in use and available standards and guidelines (revised SWGDAM guidelines are too general)
- 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
- Attempt to define a minimum number of samples that could be recommended for various validation scenarios
- Help with establishing uniformity throughout the field to aid auditors in their inspections

Pathway to Improved DNA Validation

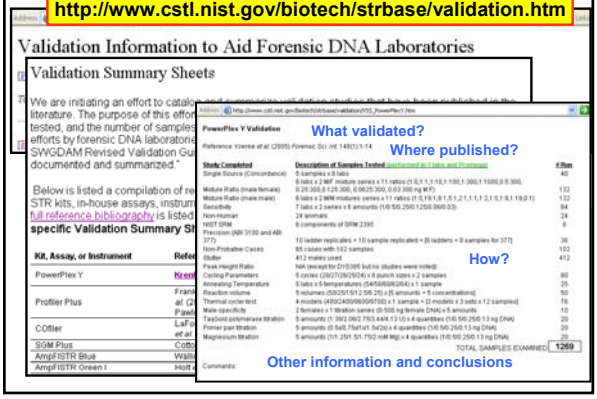
- Collection of Current Philosophy on Validation
  - Community survey
  - Interviews
  - Literature summary
- Training
  - Auditors must be consistent in treatment of labs
- Providing Tools to Enable Improved Validation
  - Sample set(s)
  - Workbook – provide specific examples
  - Standard report form – documentation standardization
- Collection of Validation Data from Labs
  - NIJ-funded labs to submit data to STRBase validation website

Pathway to Improved DNA Validation

- Collection of Current Philosophy on Validation
  - Community survey
  - Interviews
  - Literature summary
- Training
  - Auditors must be consistent in treatment of labs
- Providing Tools to Enable Improved Validation
  - Sample set(s)
  - Workbook – provide specific examples
  - Standard report form – documentation standardization
- Collection of Validation Data from Labs
  - NIJ-funded labs to submit data to STRBase validation website

New Validation Homepage on STRBase

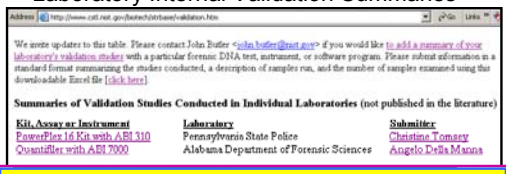
http://www.cstl.nist.gov/biotech/strbase/validation.htm



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

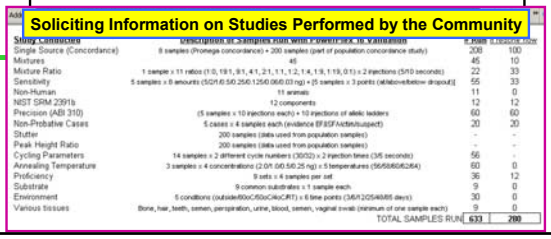


We invite updates to this table. Please contact John Butler <john.butler@nist.gov> if you would like to add a summary of your laboratory's validation studies with a particular forensic DNA test, instrument, or software program. Please submit information in a standard format summarizing the studies conducted, a description of samples run, and the number of samples examined using the 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 Conditions	Description of Samples Run with PowerPlex 16 Validation	# Male	# Female
Single Source (Concordance)	8 samples (Promega concordance) + 200 samples (part of population concordance study)	208	100
Mixtures	46	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 6 amounts (50/0.6:50:250:1250:060:03 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 EFS/FA/stn/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/60oC/60oC/40oC/RT) x 6 time points (3/6/12/24/48/85 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

Acknowledgements

Funding:

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

john.butler@nist.gov
margaret.kline@nist.gov
peter.vallone@nist.gov
michael.coble@nist.gov

This presentation available as pdf file from
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm