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

NIST Research Summary for AFDIL

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Rockville, MD

NIST Human Identity Project Team


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Funding:
Interagency Agreement between NIJ and NIST Office of Law Enforcement Standards

Presentation Outline

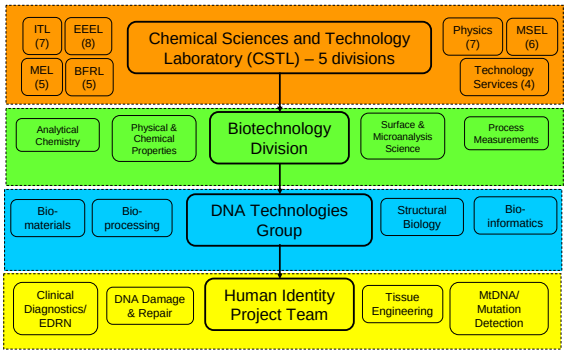
John
- Overview of projects and resources
- Y-chromosome information, kits, and standards

Pete
- mtSNPs
- Y-SNPs
- Autosomal SNPs

Mike
- miniSTR background
- New STR loci under investigation
- Work with degraded and LCN DNA samples including hair shafts

John
- Validation standardization efforts
- Training materials: NEAFS workshop, STRBase updates
- *Forensic DNA Typing*, 2nd Edition (expected Jan 2005)

Overview of NIST Organization



http://www.cstl.nist.gov/biotech/DNAtech/HumanIdentity.html

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

Current Areas of NIST Research Effort

- **Y-Chromosome** Information, Assays, and Standards
- Resources for "**Challenging Samples**" (SNPs and miniSTRs)
- **DNA Quantitation** (Interlab study, Real-time PCR comparisons)
- **Tools to Aid State and Local Laboratories** (e.g., STRBase)
- **Aid to or Completion of Other NIJ Projects** (e.g., LSBs)

Instrumentation at NIST

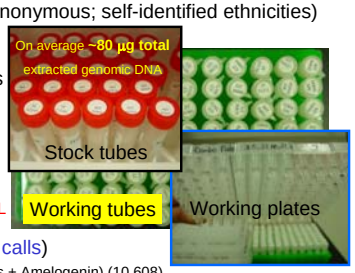
- ABI 3100
- ABI 310
- ABI 7000
- Agilent BioAnalyzer 2100
- FMBIO III+
- GeneAmp 9700s
- STR kits used: Identifiler, PowerPlex 16, PowerPlex Y, Y-PLEX 12 (6/5), SGM Plus, Profiler Plus, COfiler, Profiler

NIST U.S. Population Samples

As of 06/2003 663 males (anonymous; self-identified ethnicities)

260 Caucasians
260 African Americans
140 Hispanics
3 Asians

Whole blood received from Interstate Blood Bank (Memphis, TN)



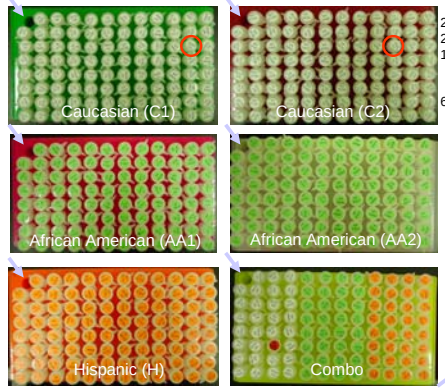
Working tubes/plates 1 ng/uL

To date: (~50,000 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 SNPs 50 markers on sub-set of samples (11,498)

Samples supplied to **OhioU** for miniSTR typing and **AFDIL** for whole mtGenome sequencing

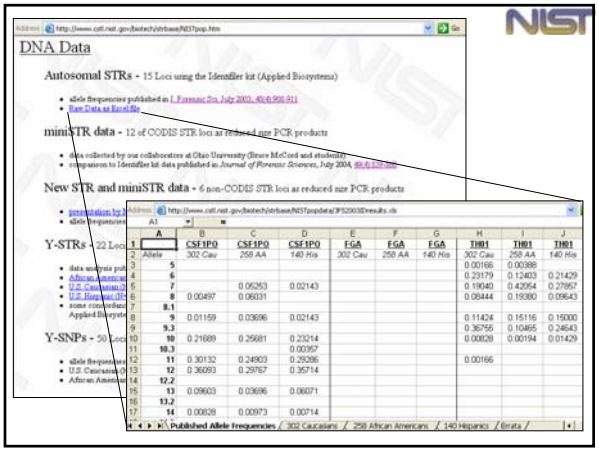
NIST U.S. Population Samples

Position A1 left open for controls or allelic ladder



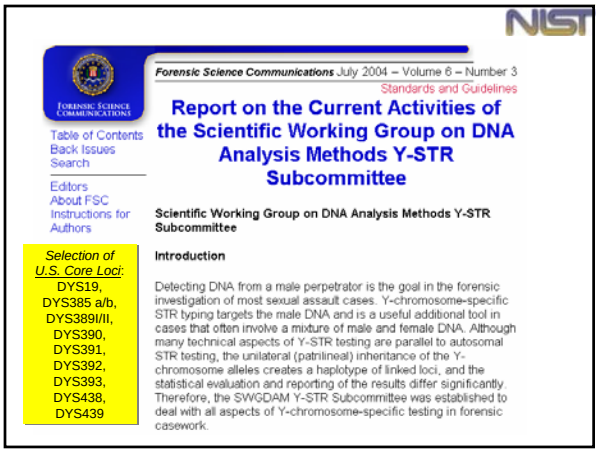
260 Caucasians
260 African Americans
140 Hispanic
3 Asian
2 females (Caucasians)
665 Samples in 7 plates

663 males



Y-Chromosome

Information, Assays, and Standards



Forensic Science Communications July 2004 – Volume 6 – Number 3

Standards and Guidelines

Report on the Current Activities of the Scientific Working Group on DNA Analysis Methods Y-STR Subcommittee

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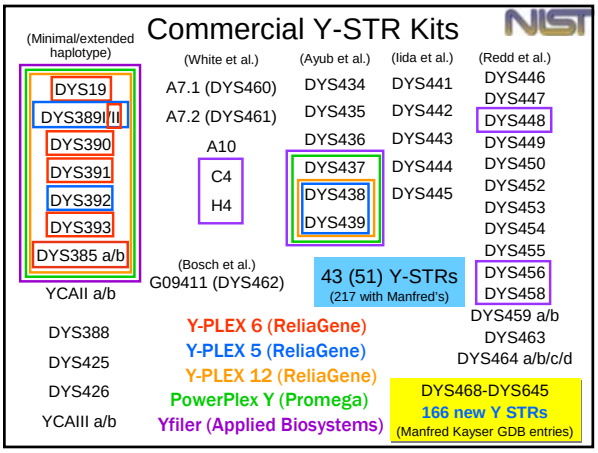
Scientific Working Group on DNA Analysis Methods Y-STR Subcommittee

Introduction

Detecting DNA from a male perpetrator is the goal in the forensic investigation of most sexual assault cases. Y-chromosome-specific STR typing targets the male DNA and is a useful additional tool in cases that often involve a mixture of male and female DNA. Although many technical aspects of Y-STR testing are parallel to autosomal STR testing, the unilateral (patrilineal) inheritance of the Y-chromosome alleles creates a haplotype of linked loci, and the statistical evaluation and reporting of the results differ significantly. Therefore, the SWGDAM Y-STR Subcommittee was established to deal with all aspects of Y-chromosome-specific testing in forensic casework.

Selection of U.S. Core Loci:
DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439

Commercial Y-STR Kits



U.S. Population Data on 22 Y-STRs

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Forensic Science International 139 (2004) 107-121
Forensic Science International
www.elsevier.com/locate/forsciint

High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays

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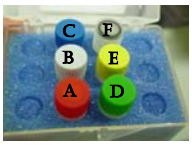
pdf file available at http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

US haplotype (Reliagene kits)
Yfiler (ABI)
PowerPlex Y (Promega)
+C4

Y-STR	Pooled Population STR diversity (N=647)	Rank	African American STR diversity (N=260)	Rank	Caucasian STR diversity (N=244)	Rank	Hispanic STR diversity (N=143)	Rank
DYS464 a/b/c/d	0.956	1	0.954	1	0.934	1	0.937	1
DYS385 a/b	0.912	2	0.942	2	0.838	2	0.901	2
YCAII a/b	0.790	3	0.797	3	0.701	5	0.772	4
DYS458	0.765	4	0.758	5	0.743	3	0.793	3
DYS390	0.764	5	0.664	10	0.701	5	0.665	13
DYS447	0.747	6	0.767	4	0.683	7	0.748	5
DYS389II	0.736	7	0.722	6	0.675	8	0.734	6
DYS448	0.721	8	0.722	6	0.595	11	0.704	8
DYS456	0.700	9	0.671	9	0.731	4	0.695	9
DYS438	0.691	10	0.560	15	0.594	12	0.690	10
DYS19	0.676	11	0.722	6	0.498	19	0.672	12
DYS439	0.656	12	0.636	11	0.639	9	0.717	7
DYS437	0.637	13	0.499	17	0.583	13	0.624	14
H4	0.611	14	0.612	12	0.562	14	0.609	15
DYS392	0.609	15	0.434	20	0.596	10	0.673	11
DYS460	0.570	16	0.568	14	0.555	15	0.556	18
DYS389I	0.549	17	0.531	16	0.538	17	0.596	16
DYS391	0.534	18	0.447	19	0.552	16	0.577	17
DYS426	0.519	19	0.375	21	0.482	20	0.522	19
DYS450	0.489	20	0.487	18	0.177	22	0.414	21
DYS393	0.485	21	0.586	13	0.363	21	0.448	20
DYS388	0.365	22	0.246	22	0.501	18	0.312	22

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

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)** $248
- **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 DAB Standard 9.5 (and ISO 17025)…traceability to a national standard

Pete's Section

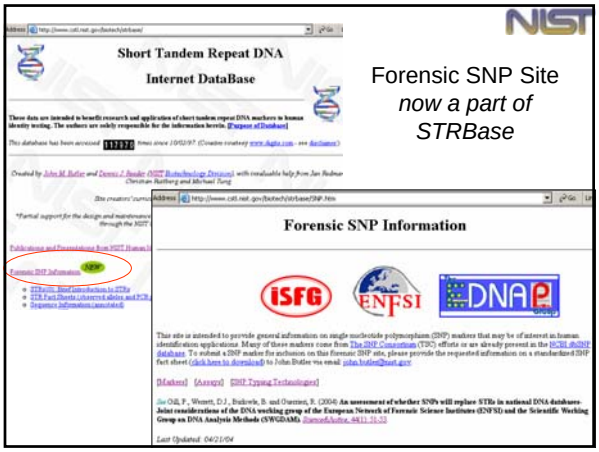
Why evaluate new markers?

- Highly Degraded samples (fragmented, questionable DNA quantity, inhibitors?)
- Telogenic/shed hairs (few copies)
- Low copy number cases (few copies)
- Siblings/Closely related individuals (paternity)

The primary characteristic of the assays for typing these new markers is their short PCR amplicon size (60 –150 base pairs)

SNP Typing at NIST

- STRBase is the official ISFG/EDNAP/ENFSI repository of forensic SNP information
  - Gill *et al. Science & Justice* 2004, 44, 51-53
  - **http://www.cstl.nist.gov/biotech/strbase/SNP.htm**
- We are cataloging SNP information with the goal to standardize assays and speed validation of markers
- We will continue to explore various SNP typing technologies to provide information to the forensic DNA typing community – primary focus on SNaPshot/primer extension
- **We are beginning to evaluate SNP performance directly against miniSTRs for analysis of degraded DNA -** collaborative study planned with EDNAP



Forensic SNP Site
now a part of STRBase

SNP Markers

Coding region mtSNPs expand the power of control region sequencing for common HV1/HV2 types (Collaboration with Tom Parsons).

Y-SNPs Evaluate their ability to discriminate between individuals and population specificity (typing U.S. sample groups).

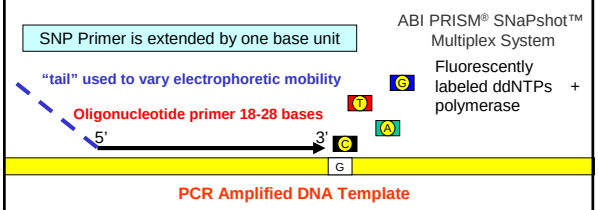
Autosomal SNPs
Type NIST population samples. Evaluate and test on degraded samples. Make relevant comparisons to STR markers.

Typing mtSNPs

Collaboration with AFDIL (Tom Parsons)
Develop an 11-plex assay for typing SNPs outside the control region
The 11 SNP sites are thought to help resolve Caucasians with the most common mitotype (~7%)

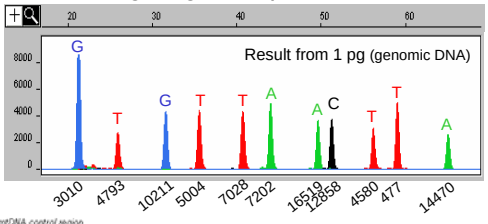
Typing population samples with Roche linear arrays (Cassandra Calloway)
Probe 8 regions within HVI and HVII + 189 and 16093
Evaluate assay performance and ability to separate NIST population samples

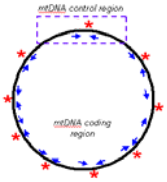
Allele-Specific Primer Extension



ABI PRISM® SNaPshot™ Multiplex System
Fluorescently labeled ddNTPs + polymerase

mtDNA Coding Region 11plex SNaPshot Assay





11plex PCR and 11plex SNP detection

Sites are polymorphic in Caucasians (H1) and useful in resolving most common HV1/HV2 types

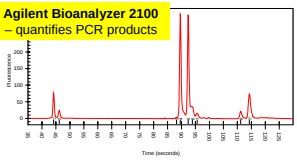
Multiplex PCR used to co-amplify all regions of interest at once

PCR product sizes kept under 200 bp to enable success with degraded DNA samples

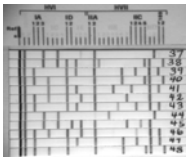
Vallone et al., *Int. J. Legal Med., 118: 147-157*

Semi-Automation of mtDNA LINEAR ARRAYS

Agilent Bioanalyzer 2100 – quantifies PCR products



Tecan Profiblot – processes sample through wash steps



Analysis of probe results is still manual!

Typing frequencies for 666 NIST population samples

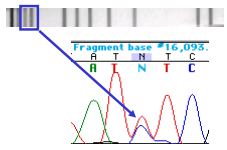
#*	Freq	% Types	% People
1	185	65.6	27.8
2	46	16.3	13.8
3	18	6.4	8.1
4	4	1.4	2.4
5	3	1.1	2.3
6	4	1.4	3.6
7	1	0.4	1.1
8	9	3.2	10.8
9	2	0.7	2.7
10	4	1.4	6.0
11	1	0.4	1.7
12	1	0.4	1.8
18	1	0.4	2.7
23	1	0.4	3.5
28	1	0.4	4.2
51	1	0.4	7.7

Kline et al., Submitted 2004

Results with Roche mtDNA LINEAR ARRAYS

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

Accurate Detection of Heteroplasmy at 16093



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

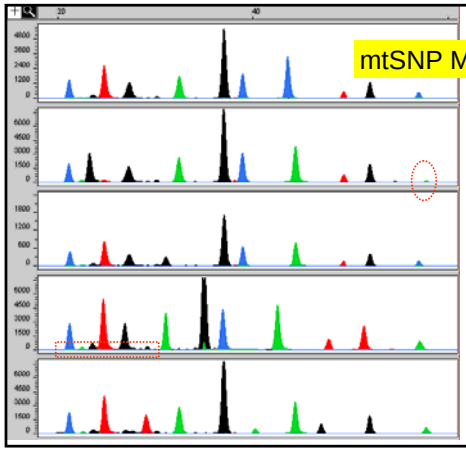
Typing 51 samples with 11plex assay

51 (47 cauc/4 hisp) samples were identical by Roche linear array assay (most common Haplogroup observed in NIST U.S. Caucasian population samples)

3010	G	A	G	G	G	G	A	G	G	G	G	A	G
4793	A	A	A	A	A	A	A	A	A	A	G	A	A
10211	C	C	C	C	C	C	C	C	C	C	C	C	C
5004	T	T	C	T	T	T	T	T	T	T	T	T	T
7028	C	C	C	C	T	T	C	T	C	T	C	C	C
7202	A	A	A	A	A	A	A	A	A	A	A	A	A
16519	T	C	T	C	C	T	C	C	T	T	C	C	C
12858	C	T	C	C	C	C	C	C	C	C	C	C	C
4580	G	G	G	G	G	G	G	A	G	A	G	G	G
477	T	C	T	T	T	T	C	T	T	T	T	T	T
14470	T	T	T	A	T	T	T	T	T	T	T	T	T
	rCRS	1	1	1	1	1	2	2	3	4	4	15	16

12 haplogroups were observed
5 haplogroups were unique
2 of 11 sites did not vary

Kline et al., Submitted 2004



mtSNP Multiplex D

J1,J2,K2,K3

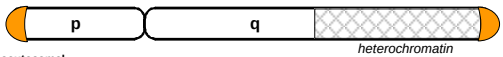
9548
5198
6260
9635
15355
15884
16368
11485
11914
482

Forensic Utility of Y Chromosome SNPs

Y chromosome markers are useful in mixed male - female samples

Haplogroups are non-randomly distributed among populations therefore potential exists for predicting population of origin

Low mutation rate of SNPs $2e^{-8}$ per base per generation



>250 Y-SNPs described

J Forensic Sci, July 2004, Vol. 49, No. 4
Paper ID JFS2003303
Available online at: www.astm.org

Peter M. Vallone,[1] Ph.D. and John M. Butler,[1] Ph.D.

Y-SNP Typing of U.S. African American and Caucasian Samples Using Allele-Specific Hybridization and Primer Extension*

Summary

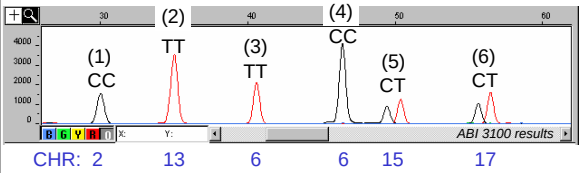
- Different technologies yield the same Y-SNP type
  - Full concordance was observed between hybridization and primer extension technologies on 18 different Y-SNPs (>3,800 allele calls)
- Y-SNPs will have limited value for individualizing a sample
  - 18 different types observed in 229 individuals
- Current Y-SNPs appear to have limited value for ethnic differentiation in U.S. populations
  - One exception: M2 only in African Americans; not in Caucasians

Autosomal SNP characteristics

- 70 Loci – sites from Orchid – C/T bi-allelic
- Present on 20 of 22 autosomal CHR (3,16,X,Y)
- Amplicon size range 59 - 108 bp (average 69)
- Markers are typed by allele-specific primer extension assays (ABI SNaPshot)
- Level of multiplexing (6- 12-plexes)
- Web page for SNP site info

http://www.cstl.nist.gov/biotech/strbase/SNPs/OrchidSNPinfo.htm

6-plex SNP Assay

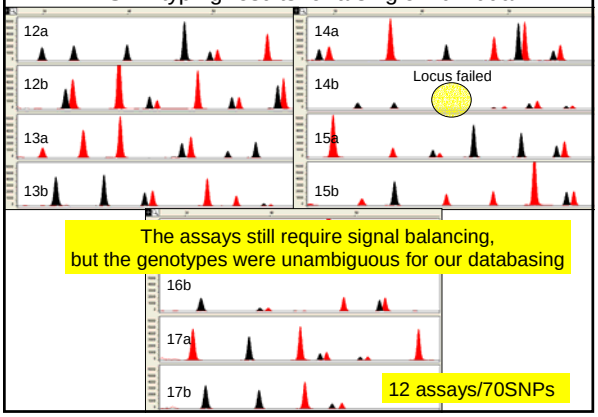


CHR: 2 13 6 6 15 17

Extension primers for 6-plex

(1) TTTTTAGCTCCTAATTTCTTGATGGG
(2) TTTTTTTTTTCATCTGATGCCATGAGAAAGC
(3) TTTTTTTTTTTTTTTGTTCTGCTTTAATACAAAACCAG
(4) TTTTTTTTTTTTTTTTTTTTTTTTATAAAGGGCAGAATGAGGATTA
(5) TTTTTTTTTTTTTTTTTTTTTTTTTAGAAAGTATCTTGCAAAAGGTCCA
(6) TTTTTTTTTTTTTTTTTTTTTTTTTTTTTCATAATCACAGCTTTTTTCTCCCAA

SNP typing results for a single individual



The assays still require signal balancing, but the genotypes were unambiguous for our databasing

12 assays/70SNPs

SNP Assay Results

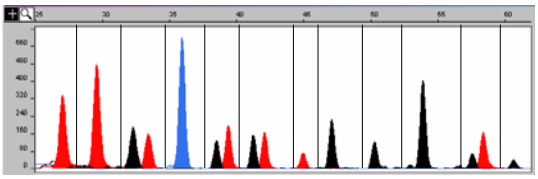
- 70 were typed for 189 U.S. samples (self identified ethnicities)
- 74 Caucasians + 71 African Americans AA + 44 Hispanics

- Total of 13,230 possible genotypes
- One marker failed across all samples (13,041-98.6%)

- 42 Samples were re-injected to confirm ambiguous results (99.7 %) success rate on first pass
- Results described in manuscript (*Forensic Sci. Int. In Press*)

- We are in the process of optimizing a 12-plex panel of SNPs

Autosomal SNP 12-plex

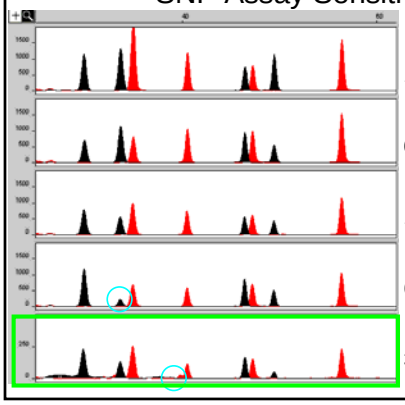


CHR: 15 1 13 11 1 15 6 10 20 17 13 17

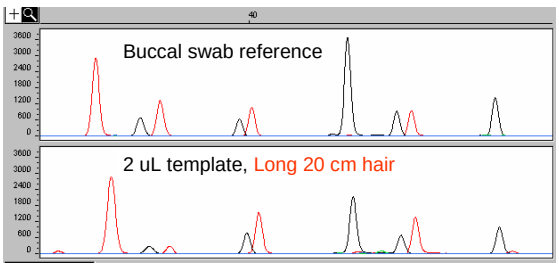
- A subset of the 70 SNP markers
- Observed heterozygosity of >0.45 in each of the 3 populations
- All 189 samples were individualized using these 12 markers

SNP Assay Sensitivity

Serial dilution of high quality gDNA

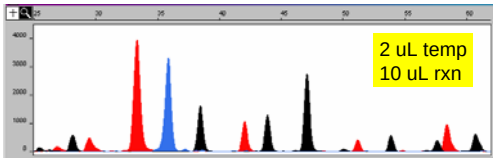


SNP Typing on a Shed Hair

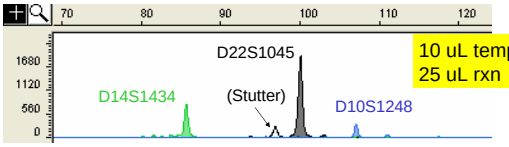


2 U Taq – 32 cycles

SNP and STR Assays on Shed Hairs



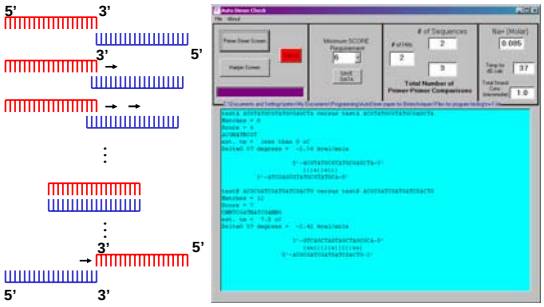
1 hair (20 cm); 2 U Taq – 32 cycles



Future directions with Autosomal SNPs

- Optimize 12-plex assay for SNPs
- Determine sensitivity of assays
- Examine data interpretation issues for LCN assays (eg allele drop out, RFU thresholds)
- Type on a "standard" degraded sample (compare to commercial kits)
- qRT-PCR

AutoDimer – primer screening software is now freely available



http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm BioTechniques (2004) 37: 226-231

DNA Analysis Tools

We have developed several stand alone software modules to assist efforts in designing multiplex PCR assays

Primer Stat: calculates values based on DNA sequence (Tm, fGC, mass, extinction coefficient etc)

AutoDimer: screens for primer-dimers and hairpins

ASPE (allele specific primer extension): design of probes for primer extension based SNP assays

With the help of NIJ funding we are in process of providing these applications on a web-based server

Mike's Section

Resources for "Challenging Samples"

(degraded DNA or mixtures)

- **Autosomal SNPs**
  - Validated Orchid 70 SNP markers (60-80 bp); population typing
- **Mitochondrial DNA SNP Assays**
  - Improve ease of use – Roche LINEAR ARRAY testing
  - Improve power of discrimination – AFDIL coding region SNPs
- **miniSTRs**
  - CODIS loci (JFS 2003, 48, 1054-1064) – "BodePlexes"; WTC IDs; McCord collaboration
  - New loci (Coble, AAFS Feb 2004) – non-CODIS loci; unlinked; optimal for small amplicons and size ranges; <120 bp

STR Size Reduction

Through Moving Primer Positions Closer to Repeat

Focus on previously characterized STR markers with:

- High Heterozygosity
- Relatively small allele range
- "Clean" flanking regions for primer design adjacent to target repeat

Why evaluate new markers?

•Highly Degraded samples (fragmented, questionable DNA quantity, inhibitors?)

•Telogenic/shed hairs (few copies)

•Low copy number cases (few copies)

•Siblings/Closely related individuals (paternity)

The primary characteristic of the assays for typing these new markers is their short PCR amplicon size (60 –150 base pairs)

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 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

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

>900 potential markers

↓

61 markers with “clean” flanking regions

↓

43 markers with amplicon size < 125bp

↓

18 markers for initial testing

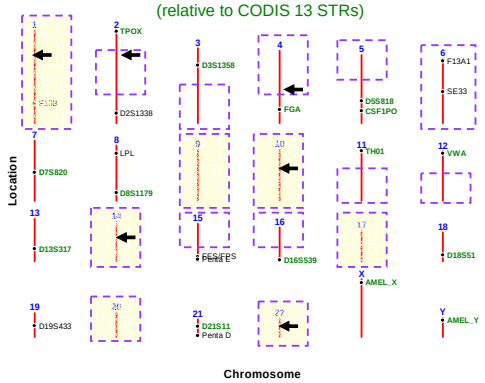
↓

2 three loci miniplexes

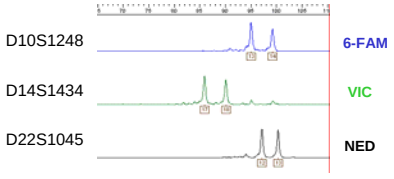
Coble and Butler, *J. Forensic Sci, submitted*

Locations of Focus for New miniSTR Loci

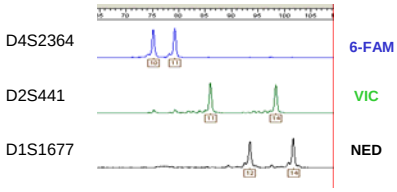
(relative to CODIS 13 STRs)



Miniplex01



Miniplex02



Coble and Butler, *J. Forensic Sci, submitted*

miniSTR characteristics

STR Locus	Sequence Motif	Allele Range	Size Range (bp)	Observed Heterozygosity
D1S1677	$(GGAA)_n$	9-18	81-117	0.75
D2S441	$(TCTA)_n$	9-17	78-110	0.76
D4S2364	$(GAAT)(GGAT)(GAAT)_n$	8-12	67-83	0.53
D10S1248	$(GGAA)_n$	10-20	83-123	0.78
D14S1434	$(GATA)_n(GACA)_n$	13-20	70-98	0.68
D22S1045	$(TAA)_n$	5-16	76-109	0.77

Coble and Butler, **JFS**, *manuscript submitted*

Population Testing –Miniplexes vs. Identifiler

Heterozygosity	Marker
0.8784	D2S1338
0.8753	D18S51
0.8710	FGA
0.8393	D21S11
0.8245	vWA
0.8076	D7S820
0.7970	D19S433
0.7759	mD10S1248 - mini01
0.7759	D16S539
0.7674	mD22S1045 - mini01
0.7674	D8S1179
0.7590	mD2S441 - mini02
0.7548	D3S1358
0.7526	D13S317
0.7463	mD1S1677 - mini02
0.7378	CSF1PO
0.7378	TH01
0.7294	D5S818
0.7146	TPOX
0.6765	mD14S1434 - mini01
0.5307	mD4S2364 - mini02

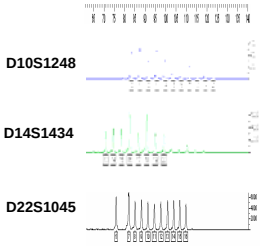
N = 474 Individuals
164 African Americans
170 Caucasians
140 Hispanics

Coble and Butler, *J. Forensic Sci, submitted*

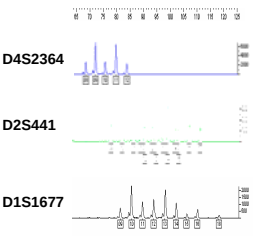
miniSTR Assay

Allelic Ladders

Miniplex01

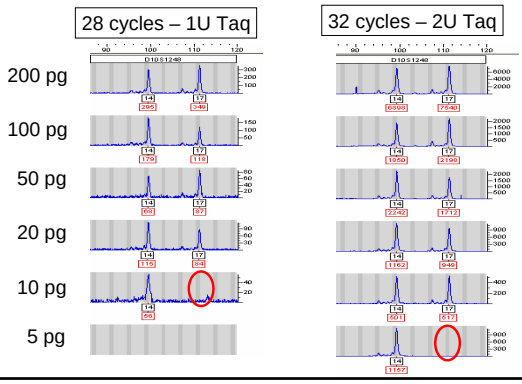


Miniplex02

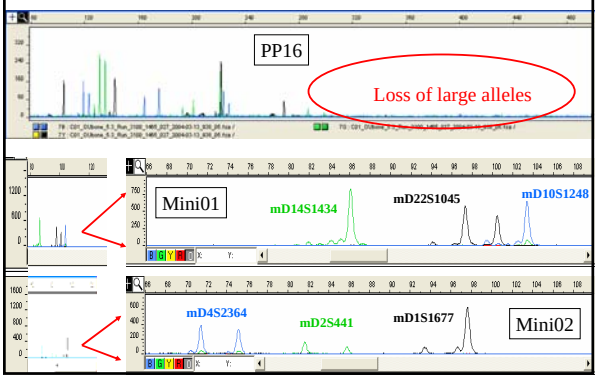


Coble and Butler, *J. Forensic Sci, submitted*

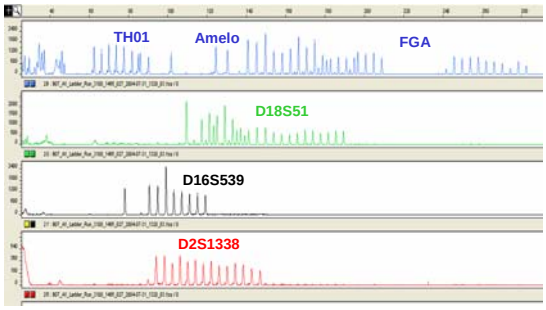
miniSTR Assay Sensitivity (D10S1248)



Sensitivity - Degraded DNA from an OU Bone Sample



miniSTR Multiplex with CODIS Markers



For potential collaborations

Can we get nuclear DNA from hair shafts?

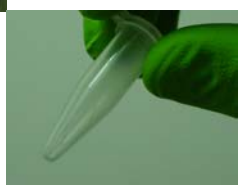
Yes…

But depends on the extraction method and assay used

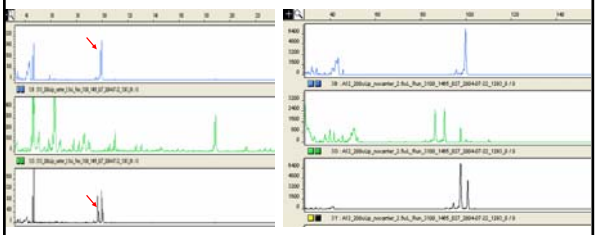
TN_{Ca} Buffer
Tris
NaCl
$CaCl_2$
2%SDS
ProK
DTT

Complete digestion of hair in about 1 hour based on method by

Hellmann A, Rohleder U, Schmitter H, Wittig M. (2001) STR typing of human telogen hairs--a new approach. *Int J Legal Med* 114(4-5): 269-273.



miniSTR Typing of Hairs



With Carrier Without Carrier

200pg input DNA – 2.5µL DNA in PCR

Complete Digestion

		D10S	D14S	D22S
	10ul	-	-	08, 13
PV 2.5cm	5ul	-	-	08, 08
	2.5ul	-	-	-
	10ul	-	-	-
PV 2.5cm	5ul	-	-	-
	2.5ul	-	-	-
	10ul	-	-	-
PV 2.7cm	5ul	-	-	-
	2.5ul	-	-	-
	10ul	-	-	08, 13 (<50)
PV 2.7cm	5ul	-	-	13, 13
	2.5ul	-	-	-
	10ul	16, 16	17, 18	08, 13
PV 5 hairs	5ul	16, 16	17, 18	08, 08
	2.5ul	15, 15	-	08, 13
	10ul	-	-	08, 13
PV 5 hairs	5ul	-	-	08, 13
	2.5ul	-	-	13, 13
	10ul	16, 17	17	13
KEC 20cm	5ul	16, 17	17	13
	2.5ul	16, 17	17	13
	10ul	14, 15	17, 18	08, 12
MK 18cm	5ul	14, 15	17, 18	08, 12
	2.5ul	14, 15	17, 18	08, 12

Genotypes	D10S	D14S	D22S
PV	15, 16	17, 18	08, 13
KEC	16, 17	17, 17	13, 13
MK	14, 15	17, 18	08, 12

miniSTR Typing of Hairs

Complete Digestion Protocol

32 cycles; 2U Taq

"Longer" Hairs – greater success

MicroTissue Grinding

		D10S	D14S	D22S
	10ul	-	-	-
PV 2.5cm	5ul	-	-	-
	2.5ul	-	-	-
	10ul	-	-	-
PV 2.5cm	5ul	-	-	-
	2.5ul	-	-	-
	10ul	-	-	-
PV 2.7cm	5ul	-	-	-
	2.5ul	-	-	-
	10ul	-	-	-
PV 2.7cm	5ul	-	-	-
	2.5ul	-	-	-
	10ul	-	-	-
PV 5 hairs	5ul	-	-	-
	2.5ul	-	-	08, 08
	10ul	-	-	-
PV 5 hairs	5ul	-	-	-
	2.5ul	-	-	-
	10ul	-	-	-
KEC 20cm	5ul	-	-	-
	2.5ul	-	-	13, 13
	10ul	-	-	-
MK 18cm	5ul	-	-	-
	2.5ul	14, 15 (<50)	17, 18	08, 12

miniSTR Typing of Hairs

MicroTissue Grinding Protocol

32 cycles; 2U Taq

miniSTR Typing of Hairs

Hair Color - Dark vs. Gray

Hair Length

Extraction Protocol – Complete Digestion vs. MicroTissue Grinding – Phenol/Chloroform vs. Qiagen

		32 cycles 5 ul	32 cycles 2.5 ul	36 cycles 2.5 ul (5U Taq)
	JB01 (1.5cm)	18, 18	18, 18	-
	JB02 (1.3cm)	-	-	13, 14
Dark Hair	JB03 (1.5cm)	-	-	08, 08
(Phenol)	JB04 (1.5cm)	17, 17	-	-
	JB07 (1.3cm)	-	-	-
	JB09 (1.0cm)	-	16, 16	-
				22, 22; 13, 13
	JB10 (3.3cm)	-	16, 16; 17, 17; 13, 13	
	JB01 (1.1cm)	14,14	-	-
	JB02 (1.2cm)	14, 16	-	13,13
Dark Hair	JB03 (1.8cm)	-	13,13	21,21
(Qiagen)	JB04 (1.4cm)	-	-	-
	JB05 (1.2cm)	-	-	16, 16; 13,13
	JB06 (1.5cm)	-	-	-
	JB01 (1.8cm)	14, 16	14, 15; 17,17	14, 16; 18,18; 13,13
	JB02 (2.0cm)	16, 16	16,16; 18,18, 13,13	16, 16; 13,13
Gray Hair	JB03 (1.1cm)	16, 16	17,17	13,13
(Phenol)	JB04 (1.4cm)	-	-	16, 16; 18,18
	JB05 (1.7cm)	15, 15	16, 16	18,18; 13,13
	JB06 (1.0cm)	-	18,18	-
	JB11 (1.0cm)	13, 13	-	-
				16, 16; 18, 18
	JB14 (1.5cm)	18, 18 13, 13	16, 16	
Gray Hair	JB15 (1.7cm)	-	-	13, 13
(Qiagen)	JB16 (1.1cm)	-	13, 13	13, 13
	JB17 (0.8cm)	18, 18	-	13, 13
	JB19 (1.4cm)	16, 16	-	16, 16
				14, 14; 18, 18
	JB20 (2.0cm)	-	-	

1.8 cm hair

miniSTR Typing of Hairs

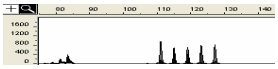
26 hairs (0.8 cm – 3.3 cm)

miniSTR Typing of Hairs

	"Correct"	"Partial"	"Incorrect"	"Did Not Type"
Dark Phenol (7)	4/63	2/63	5/63	52/63
%	0.06	0.03	0.08	0.83
Dark Qiagen (6)	4/54	1/54	2/54	47/54
%	0.07	0.02	0.04	0.87
Gray Phenol (6)	12/54	6/54	4/54	32/54
%	0.22	0.11	0.07	0.59
Gray Qiagen (7)	9/63	4/63	2/63	48/63
%	0.14	0.06	0.03	0.76

Potential New Miniplex

mD6S474

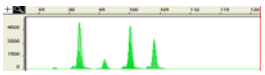


Tetranucleotide repeat – GATA

Exp. Het. – 0.77

Distance to Repeat
F – 0
R – 7

mD3S3053

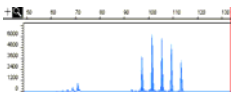


Tetranucleotide repeat – GATA

Exp. Het. – 0.74

Distance to Repeat
F – 14
R – 0

mD20S482



Tetranucleotide repeat – GATA

Exp. Het. – 0.85

Distance to Repeat
F – 3
R – 6

Future directions with miniSTRs

- Development of new loci
- Collaborations with labs working with LCN or degraded DNA
- Mobility modifiers to increase multiplexing level
- Further testing with hair shaft samples

John's Section

Tools to Aid State and Local Laboratories

- **STRBase** – standard information source
- **Variant Alleles** – cataloging variants and tri-allelic patterns
- **NIST U.S. Population Samples and Database**
- **Quality Assurance Tool** – resolution monitor to track analytical performance over time
- Validation Standardization Information
- **Training Materials**
  - Downloadable PowerPoint files from STRBase
  - *Current Protocols in Human Genetics*, *Electrophoresis* review article on STR analysis with ABI 310 and ABI 3100
  - *Forensic DNA Typing*, 2nd Edition (Dec 2004/Jan 2005)

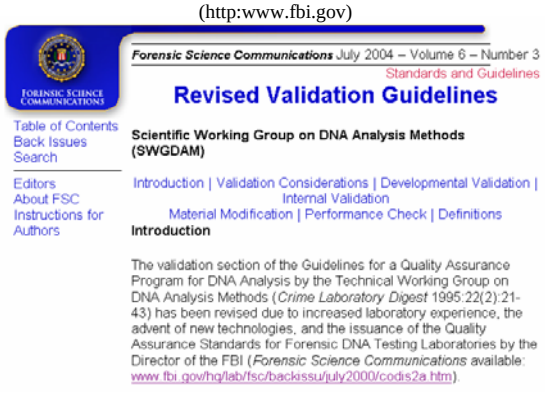
Validation Standardization Effort

John Butler (NIST), Christine Tomsey (PA State Police), Margaret Kline (NIST)

- **Survey of laboratory practices with questionnaire**
- Literature Review
- Lab notes review/interviews of a few laboratories
- Recommendations for minimum sample numbers
  - *an effort to define the minimum number of samples needed to reliably validate DNA typing procedures*
  - *through a survey of standard practices currently used by practitioners in forensic DNA laboratories*
  - *results will be summarized at the Promega meeting in October 2004 and made available on the NIST STRBase web site.*
- There is a lot of interest from the companies to have guidance in developmental validation and from practitioners for internal validation

SWGDAM Revised Validation Guidelines

(http:www.fbi.gov)



We would love to have your input on the

Validation Standardization Questionnaire

Fax (301-975-8505) or email responses to **john.butler@nist.gov**

Electrophoresis 2004, *25*, 1397–1412 1397

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 DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis

DNA typing with short tandem repeat (STR) markers is now widely used for a variety of applications including human identification. Capillary electrophoresis (CE) instruments, such as the ABI Prism 310 and ABI 3100 Genetic Analyzers, are the method of choice for many laboratories performing STR analysis. This review discusses issues surrounding sample preparation, injection, separation, detection, and interpretation of STR results using CE systems. Requirements for accurate typing of STR alleles are considered in the context of what future analysis platforms will need to increase sample throughput and ease of use.

Covers ABI 310 and ABI 3100 hardware, software, chemistry, and STR kits

.pdf file can be downloaded from http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

NEAFS Workshop being conducted September 29-30, 2004 covering STRs and CE in detail (handouts will be made available on the STRBase website)

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
Manuscript is ~950 pages
Approximately double the size of the first edition

Academic Press plans to have available by January 2005

Acknowledgements

Funding:

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

NIST Project Team:

John Butler Pete Vallone
Margaret Kline Jan Redman
Jill Appleby Amy Decker
Mike Coble Dave Duewer

Points for Discussion

- Progress with mtDNA coding SNP assays
- Supply of matching hair and buccal swabs (anonymous to meet NIST IRB)
- Status of AFDIL mtDNA control region sequencing with NIST population samples and plans for data release
- Validation review to aid our standardization efforts
- Further collaborations (beyond mtSNP work)?