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

Y-Chromosome and Mitochondrial DNA Work at the U.S. National Institute of Standards and Technology

John M. Butler

Amy E. Decker, Peter M. Vallone, Michael D. Coble,
Janette W. Redman, Margaret C. Kline, and Richard Schoske*

November 20, 2004
IV. International Forensic Y-User Workshop

NIST Human Identity Project Team


John Butler
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Jan Redman

Former Team Member

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Amy Decker


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Dave Duewer

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

Presentation Outline

- General NIST Projects
  - STRBase, etc.
- Y-Chromosome Work
  - SWGDAM Y-chromosome subcommittee
  - SRM 2395
  - New Y-STR loci under development
  - Y-SNPs
- Mitochondrial DNA Work
  - Coding region SNP assay development with AFDIL
  - LINEAR ARRAYs as an mtDNA screening tool
  - Standard Reference Material SRM 2392-I (Barbara Levin)
- Invitation to Participate in a New NIST Interlaboratory Study involving mixture interpretation

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

Current Areas of NIST Research Effort

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

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

STRBase

Short Tandem Repeat DNA Internet Database

Recent Additions

•**Validation** (summary sheets)
…/validation.htm

•**miniSTR information**
…/miniSTR.htm

We Regularly Update

•Reference List
•**Variant Alleles**
•Addresses for Scientists
•Links to Other Web Sites
•Y-STR Information

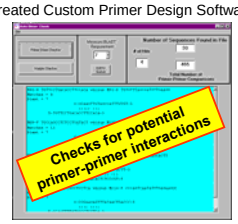
NIST publications and presentations as pdf files
…/NISTpub.htm

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

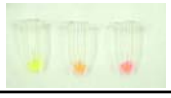
Technology Development Efforts

*Centered around **multiplex PCR**…*

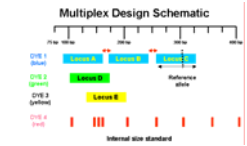
Created Custom Primer Design Software



Implemented Quality Control Methods for PCR Primers

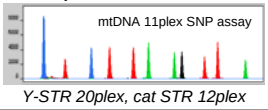


Standardized Assay Design Formats



Schoske *et al.* (2003) *Anal. Bioanal. Chem.* 375:333-343

Demonstrated Success with Multiple Projects and Collaborations



Y-STR 20plex, cat STR 12plex

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

Download Page

Home | Download | Tips for running | Examples Tested | Referencing AutoDimer | FAQ | Support

AutoDimer was packaged for installation using Visual Basic 6.0. I have tested the installation on PCs running Win98, 2000, XP and NT. However, I cannot guarantee installation success for each user's specific computer configuration.

By clicking the link below you will be downloading the file AutoDimer.zip. Once extracted (www.winzip.com), the files can be used to install the AutoDimer program (click setup.exe).

The end user is responsible for the installation and running of the program (this is done at your own risk). The author will not be held responsible for any subsequent computer/operating system issues due to conflicts with the AutoDimer software. AutoDimer is a general tool for screening sequences, we do not guarantee the success of your PCR/assay.

Please click here to download AutoDimer (~5 MB).

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

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

ORIGINAL PAPER

Describes how to build STR multiplex assays…

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

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

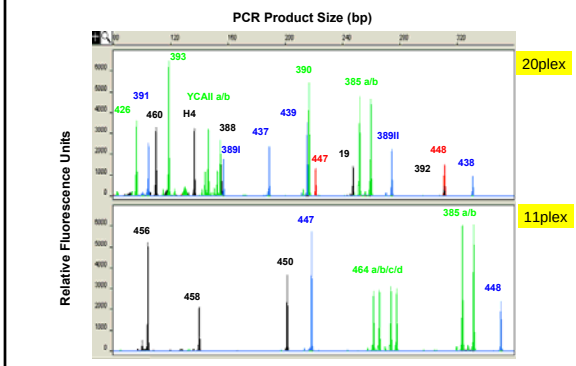
Stringent primer quality control

Dye labeled oligos

6FAM ("blue"), VIC ("green"), NED ("yellow")

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

NIST Multiplexes for High-Throughput Y STR Typing



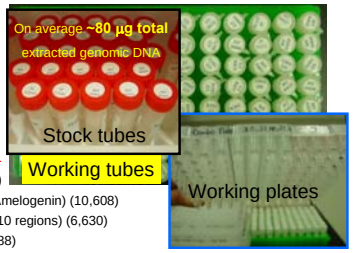
Schoske *et al.* (2004) High-throughput Y-STR typing of U.S. populations…, *Forensic Sci. Int.*, 139:107-121

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: (~85,000 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); 6 new autosomal STRs (2,844)
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

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

Standard U.S. Population Dataset

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

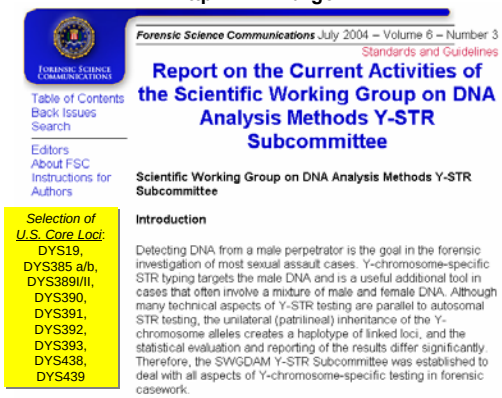
260 Caucasians, 260 African Americans, 140 Hispanics, 3 Asians = **663 males**

Genetic Markers	Loci Examined	Publications
Common STRs	Identifiler kit 15 STRs (CODIS + D2S1338 & D19S433)	Butler et al. (2003) JFS
miniSTRs	All CODIS loci except D3S1358	Drabek et al. (2004) JFS
New autosomal STRs	New 6 loci for miniSTRs	Coble et al. (2005) JFS
Autosomal SNPs	70 C/T SNPs (Orchid panel)	Vallone et al. (2004) FSI
Common Y-STRs	22 loci (27 regions)	Schoske et al. (2004) FSI
	Yfiler concordance study	*Data in ABI Yfiler database*
New Y-STRs	**27 additional loci**	**Butler et al., submitted**
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*

Y-Chromosome

SWGDAM, SRM 2395,
New Y-STR Loci, and Y-SNPs

http://www.fbi.gov



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

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

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.

SWGDAM Sub-Committee on the Y Chromosome

- Formed in July 2002
- Members
  - Jack Ballantyne (UCF) – chair
  - Mecki Prinz (NYC) – co-chair
  - **John Butler (NIST)**
  - Ann Gross (MN)
  - John Hartmann (Orange Co.)
  - Sam Baechtel (FBI Lab)
  - Jill Smerick (FBI Lab)
  - Debra Figarelli (Phoenix)
  - Carll Ladd (CT)
  - Demris Lee (AFDIL)
  - Jonathan Newman (CFS-Toronto)
  - Phil Kinsey (OR)
  - Gary Sims (CA DOJ)
- **U.S. CORE Y-STR LOCI selected in January 2003**
- 60 sample set selected for screening markers and initial testing
- Testing of Y-PLEX 6 and Y-PLEX 5 kits in all labs
  - *All results completed agreed with NIST results sent to participating labs in Dec 2002*
- **Jack Ballantyne's lab and John Butler's lab to examine additional Y-STR and Y-SNP markers in the same sample set**

European and U.S. Core Y-STR Loci

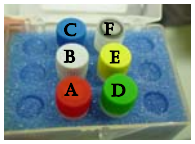
Marker Name	Allele Range (repeat numbers)	Repeat Motif
DYS19	10-19	TAGA
DYS385 a/b	7-28	GAAA
DYS389 I **DYS389 II**	I: 9-17 II: 24-34	(TCTG) (TCTA) (TCTG) (TCTA)
DYS390	17-28	(TCTA) (TCTG)
DYS391	6-14	TCTA
DYS392	6-18	TAT
DYS393	8-17	AGAT
YCAII a/b	11-25	CA
DYS438	6-14	TTTTC
DYS439	8-15	AGAT

Minimal haplotype (Europe)

U.S. haplotype

Extended haplotype (Europe)

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

$248

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

Sequence Summaries for SRM 2395

DYS19	A	14	$[TAGA]_3$ tagg$[TAGA]_{11}$
	B	14	$[TAGA]_3$ tagg$[TAGA]_{11}$
	C	16	$[TAGA]_3$ tagg$[TAGA]_{13}$
	D	15	$[TAGA]_3$ tagg$[TAGA]_{12}$
	E	17	$[TAGA]_3$ tagg$[TAGA]_{14}$

DYS438	A	12	$[TTTTC]_{12}$
	B	9	$[TTTTC]_9$
	C	11	$[TTTTC]_{11}$
	D	11	$[TTTTC]_{11}$
	E	10	$[TTTTC]_{10}$

DYS390	A	25	$[TCTG]_4$ $[TCTA]_{12}$ $[TCTG]_1$ $[TCTA]_4$
	B	23	$[TCTG]_4$ $[TCTA]_{10}$ $[TCTG]_1$ $[TCTA]_4$
	C	21	$[TCTG]_4$ $[TCTA]_8$ ACTA $[TCTA]_2$ $[TCTG]_1$ $[TCTA]_4$
	D	22	$[TCTG]_4$ $[TCTA]_9$ $[TCTG]_1$ $[TCTA]_4$
	E	24	$[TCTG]_4$ $[TCTA]_{11}$ $[TCTG]_1$ $[TCTA]_4$

We will continue to add information on new Y-STR loci as they are adopted by the community and put into commercial kits

Y-SNP Results on SRM 2395

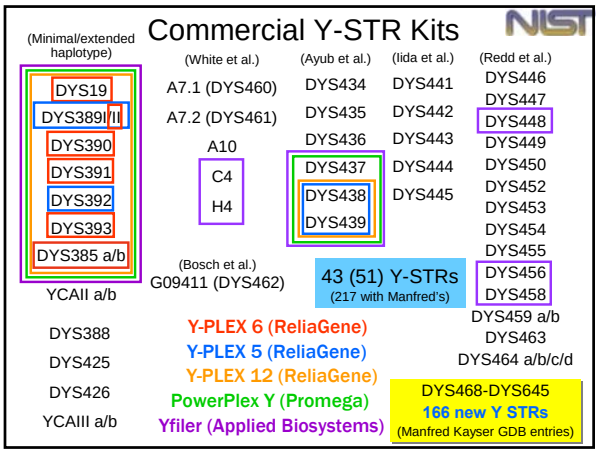
from Marligen Signet™ Multiplexes (Luminex bead assay)

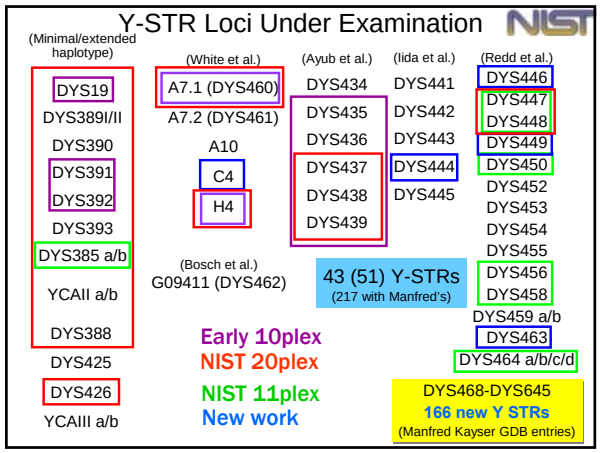
42 Y-SNPs measured across all samples

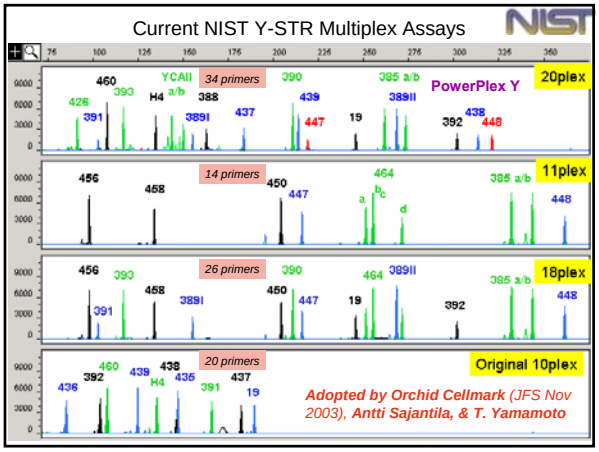
SRM 2395	AMEL	M207	M45	M89	DYS391	M2	M170	M172	M201
		(A/G)	(A/G)	(C/T)	(C/G)	(A/G)	(A/C)	(G/T)	(G/T)
Component A	XY	**G**	**A**	T	C	A	A	T	G
Component B	XY	A	G	T	C	A	A	**G**	G
Component C	XY	A	G	**C**	**G**	**G**	A	T	G
Component D	XY	A	G	T	C	A	A	T	**T**
Component E	XY	A	G	T	C	A	**C**	T	G
Component F	XX								

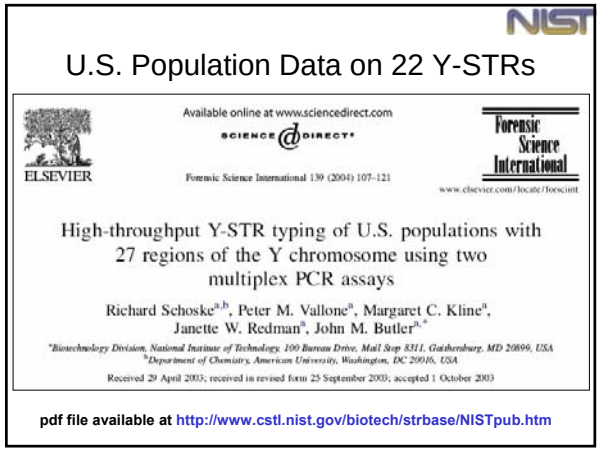
SRM components are all distinguishable from one another with these Y SNPs

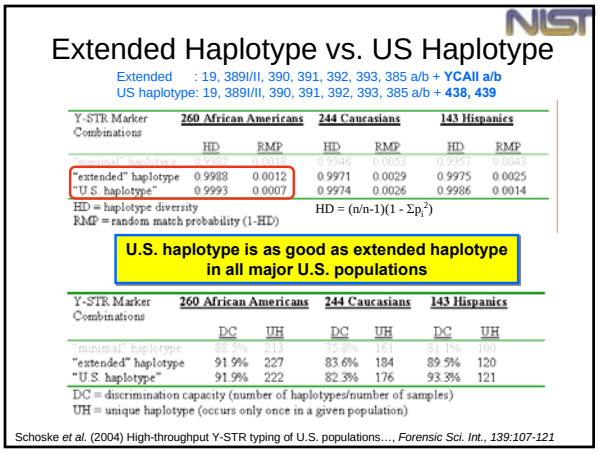
5 male components in SRM 2395 have 5 different Y-SNP backgrounds: **R1b, J2, E3a, G, and I**

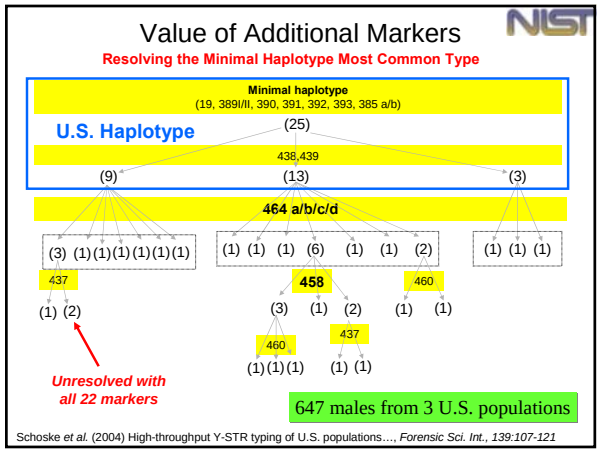

Commercial Y-STR Kits
(Minimal/extended haplotype)
DYS19
DYS389I/II
DYS390
DYS391
DYS392
DYS393
DYS385 a/b
YCAII a/b
DYS388
DYS425
DYS426
YCAIII a/b
(White et al.)
A7.1 (DYS460)
A7.2 (DYS461)
A10
C4
H4
(Bosch et al.)
G09411 (DYS462)
(Ayub et al.)
DYS434
DYS435
DYS436
DYS437
DYS438
DYS439
(Iida et al.)
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
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)


Y-STR Loci Under Examination
(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
(Bosch et al.)
G09411 (DYS462)
(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
43 (51) Y-STRs
(217 with Manfred's)
Early 10plex
NIST 20plex
NIST 11plex
New work
DYS468-DYS645
166 new Y STRs
(Manfred Kayser GDB entries)


Current NIST Y-STR Multiplex Assays
34 primers
PowerPlex Y
20plex
14 primers
11plex
26 primers
18plex
20 primers
Original 10plex
Adopted by Orchid Cellmark (JFS Nov 2003), Antti Sajantila, & T. Yamamoto

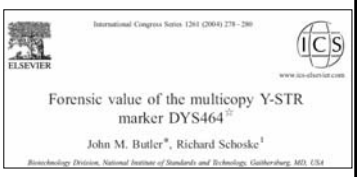

U.S. Population Data on 22 Y-STRs
Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Forensic Science International
High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays
Richard Schoske, Peter M. Vallone, Margaret C. Kline, Janette W. Redman, John M. Butler
pdf file available at http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm


Extended Haplotype vs. US Haplotype
Extended : 19, 389I/II, 390, 391, 392, 393, 385 a/b + YCAII a/b
US haplotype: 19, 389I/II, 390, 391, 392, 393, 385 a/b + 438, 439
Y-STR Marker Combinations
260 African Americans
244 Caucasians
143 Hispanics
HD
RMP
"extended" haplotype 0.9988 0.0012 0.9971 0.0029 0.9975 0.0025
"U.S. haplotype" 0.9993 0.0007 0.9974 0.0026 0.9986 0.0014
HD = haplotype diversity
RMP = random match probability (1-HD)
HD = (n/n-1)(1 - Σp_i^2)
U.S. haplotype is as good as extended haplotype in all major U.S. populations
DC
UH
"extended" haplotype 91.9% 227 83.6% 184 89.5% 120
"U.S. haplotype" 91.9% 222 82.3% 176 93.3% 121
DC = discrimination capacity (number of haplotypes/number of samples)
UH = unique haplotype (occurs only once in a given population)
Schoske et al. (2004) High-throughput Y-STR typing of U.S. populations..., Forensic Sci. Int., 139:107-121


Value of Additional Markers
Resolving the Minimal Haplotype Most Common Type
Minimal haplotype
(19, 389I/II, 390, 391, 392, 393, 385 a/b)
U.S. Haplotype
(25)
438,439
(9)
(13)
(3)
464 a/b/c/d
Unresolved with all 22 markers
647 males from 3 U.S. populations
Schoske et al. (2004) High-throughput Y-STR typing of U.S. populations..., Forensic Sci. Int., 139:107-121

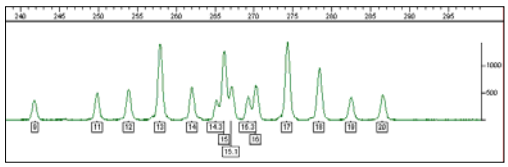
Poster at ISFG 2003

Progress in Forensic Genetics 10, pp. 278-290


Forensic value of the multicopy Y-STR marker DYS464

John M. Butler*, Richard Schoske

DYS464 Allelic Ladder



NIST allelic ladder for DYS464 produced from 8 different DNA samples. The variant alleles 14.3, 15.1, and 15.3 are helpful as a tool for measuring single base resolution in electrophoretic systems.

Example of some samples with same C-type that can be separated by E-type

C-type: 14,15,18

E-type


14,14,15,18


14,15,15,18


14,15,18,-

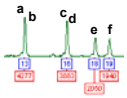

14,15,18,18

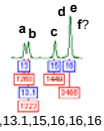
Butler and Schoske (2004) *Progress in Forensic Genetics 10, pp. 278-290*

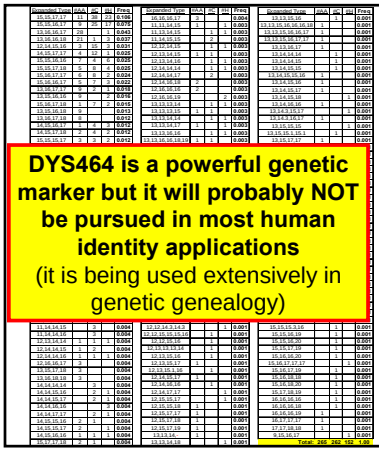
Some Complex Patterns from Single Source Samples (>4 copies)

Conservative "C-type"

Expanded "E-type"


13,13,16,16,18,19


13,13.1,15,16,16,16



All DYS464 Expanded Types Observed with 679 Samples

113 Different C-Types

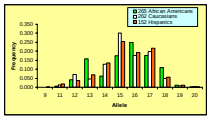
179 Different E-Types

92 occur in only a single sample

DYS464 is a powerful genetic marker but it will probably NOT be pursued in most human identity applications (it is being used extensively in genetic genealogy)

Allele Frequencies for DYS464

Variant alleles also seen: 13.1, 14.3, 15.1, 15.3



New Y-STR paper

June 2004 issue of American Journal of Human Genetics

Am. J. Hum. Genet. 74:1183–1197, 2004

A Comprehensive Survey of Human Y-Chromosomal Microsatellites

Manfred Kayser,[1,*] Ralf Kittler,[1,4] Axel Erler,[1,4] Minttu Hedman,[2] Andrew C. Lee,[3] Aisha Mohyuddin,[4,5] S. Qasim Mehdi,[5] Zoë Rosser,[3] Mark Stoneking,[3] Mark A. Jobling,[3] Antti Sajantila,[2] and Chris Tyler-Smith[4,6]

[1]Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig; [2]Department of Forensic Medicine, University of Helsinki, Helsinki; [3]Department of Genetics, University of Leicester, Leicester, United Kingdom; [4]Department of Biochemistry, University of Oxford, Oxford; [5]Biomedical and Genetic Engineering Laboratories, Islamabad; and [6]The Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom

- **Searched for all regions with ≥8 consecutive repeats and 2,3,4,5,or 6 bp repeat units**
- **Discovered 139 new polymorphic Y-STR loci (166 male-specific)**
- **Only studied so far in 8 different samples**

Y-STR Loci Under Examination

(Kayser et al.)	(Kayser et al.)	(Kayser et al.)
DYS485	DYS522	DYS557
DYS490	DYS525	DYS570
DYS495	DYS532	DYS575
DYS504	DYS533	DYS576
DYS505	DYS534	DYS594
DYS508	DYS540	DYS632
DYS520	DYS556	DYS641
		DYS643

These 22 loci have been examined in U.S. populations

New work

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

Y-STR Loci Under Examination

(Kayser et al.)	(Kayser et al.)	(Kayser et al.)
→ DYS485 ←	→ DYS522	**DYS557** ←
→ DYS490	→ DYS525	→ DYS570 ←
→ DYS495	**DYS532**	→ DYS575
DYS504	→ DYS533	→ DYS576
→ DYS505	**DYS534**	→ DYS594
→ DYS508	→ DYS540	**DYS632**
DYS520	→ DYS556	→ DYS641
		→ DYS643

These 22 loci have been examined in U.S. populations

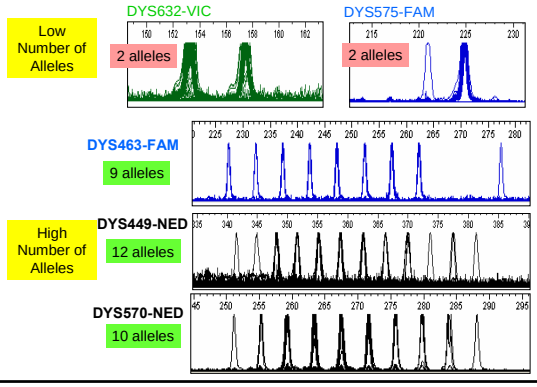
Poster #18 Neil Leat

Poster #24 Chris Tyler-Smith

New work

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

First Screen with 95 Samples (Combo1 plate)



Number of Alleles Seen with Various Y-STR Loci in Same Set of 95 U.S. Samples

Set	Locus ID	Dye	Length Range (bp)	# of alleles
B	DYS632	VIC	153-157	2
G	DYS575	FAM	221-225	2
E	DYS556	FAM	214-222	3
E	DYS641	NED	222-230	**3**
G	DYS540	NED	265-277	4
J	DYS522	VIC	359-370	4
A	DYS495	FAM	133-146	5
G	DYS594	VIC	264-289	5
C	DYS505	FAM	168-187	**6**
C	DYS508	VIC	180-200	**6**
C	DYS520	NED	181-201	6
E	DYS533	VIC	209-230	6
J	DYS446	FAM	298-325	**6**
H	DYS444	NED	299-325	6
B	DYS643	FAM	135-167	7
D	DYS534	NED	202-227	**7**
D	DYS557	VIC	196-219	7
H	DYS525	VIC	303-328	7
J	DYS532	NED	466-490	7
D	DYS576	FAM	178-205	8
A	DYS485	NED	138-164	8
H	DYS504	FAM	270-303	9
F	DYS463	FAM	227-277	9
F	DYS570	NED	251-288	10
A	DYS490	VIC	126-158	10
B	DYS449	NED	341-381	12

Loci Not Pursued Further…

Low Number of Alleles
DYS632
DYS575

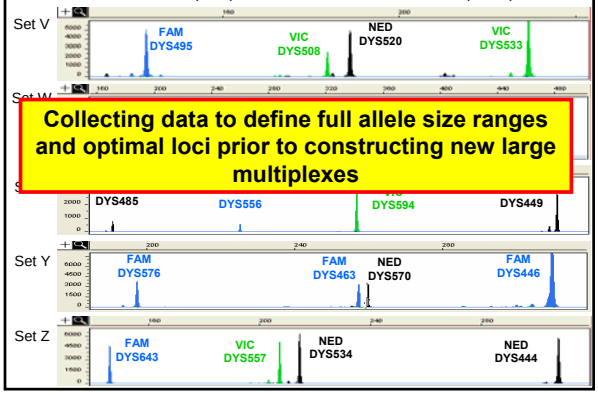
Primers Gave Artifacts in Female
DYS490 – duplicated and on chr X
DYS504
DYS525
DYS557

14,535 types generated across 27 loci

Combo 1 plate
31 Caucasians
32 African Americans
32 Hispanics

5 Panels Used to Type 20 New Y-STRs

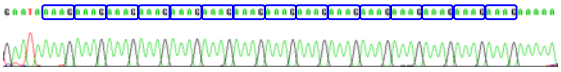
Across 660 U.S. Samples (Caucasians, African Americans, Hispanics)



Collecting data to define full allele size ranges and optimal loci prior to constructing new large multiplexes

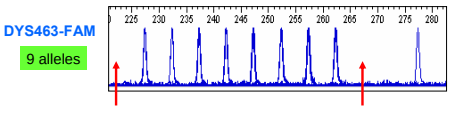
DYS576

15 AAAG repeats



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

Comparison of Different Datasets to Evaluate Variability in Locus



9 Alleles Observed

Combo 1 plate (95 samples)
31 Caucasians
32 African Americans
32 Hispanics

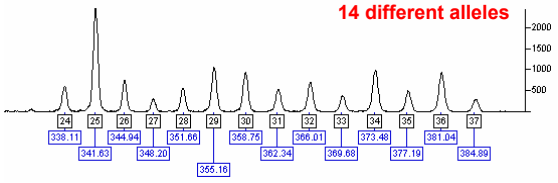
11 Alleles Observed

7 plates (663 samples)
260 Caucasians
260 African Americans
140 Hispanics
3 Asians

Our allele ranges are well defined after a 95 sample screen

DYS449 Allelic Ladder

14 different alleles



Crude **allelic ladders are being created** simply by mixing genomic DNA samples possessing each of the observed alleles

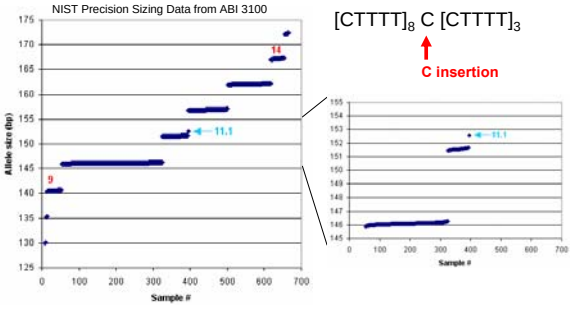
We plan to construct new Y-STR megaplexes with the best loci (based on haplotype discrimination not gene diversity ranking)

Characterizing Variant Alleles for Y-STRs

A new section of STRBase will be created soon to catalog and characterize these variants

DYS643 Variant

Allele 11.1

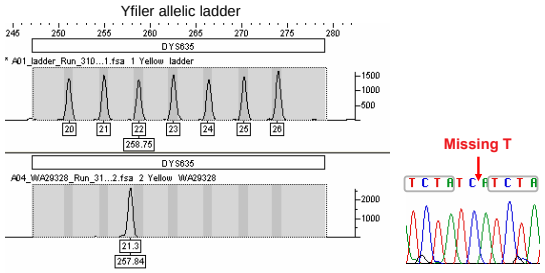


$[CTTTT]_8$ C $[CTTTT]_3$

C insertion

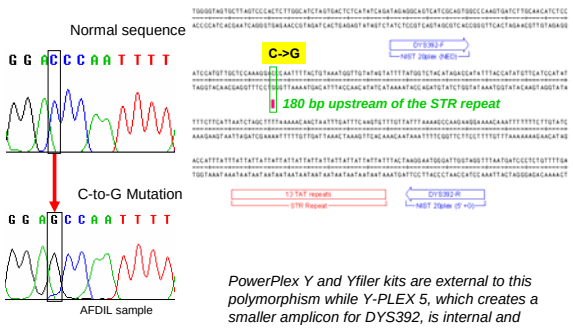
DYS635 Variant Allele 21.3

(Y-GATA-C4)



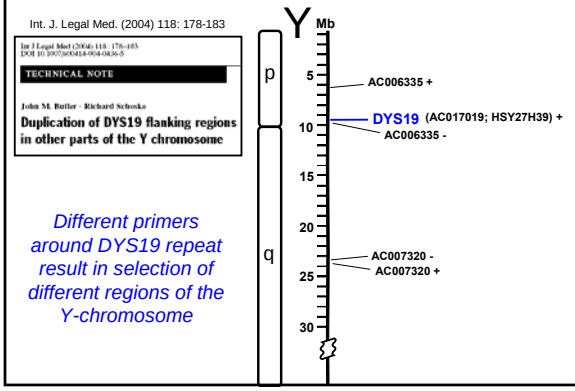
$[TCTA]_4(TGTA)_2[TCTA]_2(TGTA)_2[TCTA]_2(TGTA)_2[TCTA]_5$ TC-A $[TCTA]_2$

DYS392 Flanking Region Polymorphism



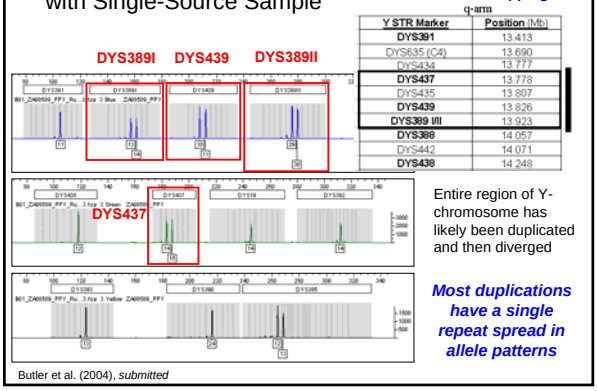
PowerPlex Y and Yfiler kits are external to this polymorphism while Y-PLEX 5, which creates a smaller amplicon for DYS392, is internal and therefore not impacted…

DYS19 Flanking Region Duplication



Different primers around DYS19 repeat result in selection of different regions of the Y-chromosome

Duplication at Multiple Loci with Single-Source Sample

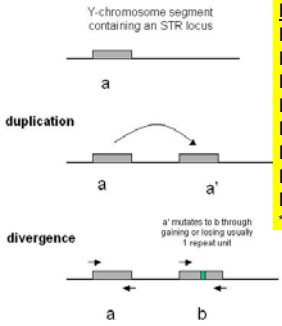


Entire region of Y-chromosome has likely been duplicated and then diverged

Most duplications have a single repeat spread in allele patterns

Butler et al. (2004), *submitted*

Duplication and Divergence Model



Locus	# dup*	>1 repeat
DYS19	23	2
DYS389I	5	0
DYS389II	9	2
DYS390	1	0
DYS391	3	1
DYS392	0	0
DYS393	3	0
DYS385a/b	17	0

*from www.yhrd.org, literature, and our work

92% have single repeat difference

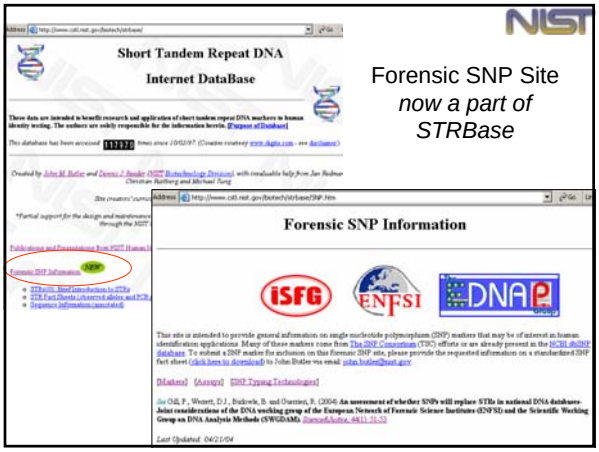
Since single-step mutations are most common, then single repeat spacing in duplicated alleles is expected

Butler et al. (2004), *submitted*

Y-SNPs

- U.S. population sample data collected on 229 individuals (115 African Americans + 114 Caucasians)
- Published in *J. Forensic Sci.* (July 2004)
- Mapped 50 SNPs on Y-chromosome
- Compared SNaPshot and Luminex assays
- Certified values for 42 loci are available with SRM 2395

Forensic SNP Site *now a part of STRBase*



Y-SNP Information Cataloged on STRBase Website

http://www.cstl.nist.gov/biotech/strbase/SNPs/YSNPs50.htm

Y position (Mb)	SNP Name	YCC Hg Defined	Multiplex	Polymorphism	African American (N=115)	Caucasian (N=114)
2,562,931	SRY+465	O2b	4	C->T	1.00/0.00	1.00/0.00
2,564,927	$SRY_{10831a,b}$	B-R, R1a	5	A->G, G->A	0.01/0.99, 1.00/0.00	0.00/1.00, 0.95/0.05
2,566,620	SRY_{9138}	K1	4	C->T	1.00/0.00	1.00/0.00
2,642,605	M130 (RPS4Y)	C	3	C->T	1.00/0.00	1.00/0.00
13,407,330	M2	E3a	2	A->G	0.42/0.58	1.00/0.00
13,413,670	DYS391	E3	2	C->G	0.40/0.60	0.96/0.04
14,124,138	M168	C-R	1	C->T	0.03/0.97	0.00/1.00
14,157,939	M170	I	3, A	A->C	0.97/0.03	0.79/0.21
14,179,223	M182	B2	2	C->T	0.99/0.01	1.00/0.00
14,232,730	Tat	N3	4	T->C	1.00/0.00	0.99/0.01
14,264,427	M174	D	3, A	T->C	1.00/0.00	1.00/0.00

Positions mapped against Human Genome reference sequence (July 2003) using BLAT

Vallone, P.M. and Butler, J.M. (2004) Y SNP typing…. *J. Forensic Sci.*, 49(4): 723-732

Publication on U.S. Groups with Y-SNPs

J. Forensic Sci. 2004; 49(4): 723-732

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

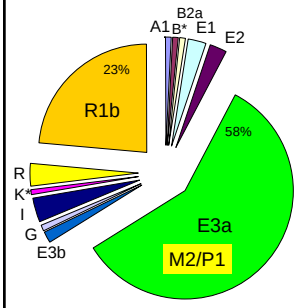
Y-SNP Typing of U.S. African American and Caucasian Samples Using Allele-Specific Hybridization and Primer Extension*

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)

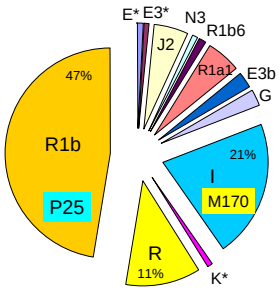
Y-SNPs will have limited value for individualizing a sample
- 18 different types observed in 229 individuals

Current Y-SNPs appear to have limited value for ethnic differentiation in U.S. populations (with the exception of M2 that is only found in African Americans and not in Caucasians)

Y-SNP haplogroups for 115 African Americans

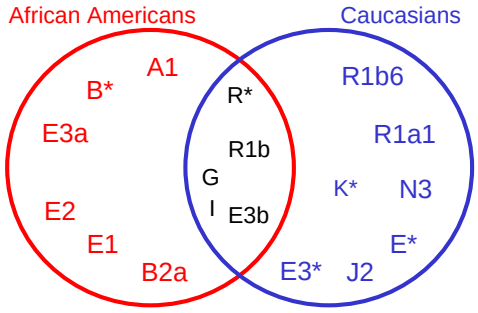


Y-SNP haplogroups for 114 Caucasians



18 different haplogroups observed in 229 males

Observed Haplogroups in Two U.S. Populations



18 total Hgs; 5 shared

Vallone, P.M. and Butler, J.M. (2004) Y SNP typing.... *Progress in Forensic Genetics 10., pp 85-87*

Summary of NIST Y-Chromosome Work

- Development of new Y-STR multiplex assays (**Y STR 20plex, 11plex, etc.**)
- Evaluation of new Y-STR loci in U.S. populations
- Evaluation of SNP typing methodologies and development of **Y-SNP assays** involving primer extension and the SNaPshot kit
- Creation of a Human Y-Chromosome Standard Reference Material (**SRM 2395**)
- Standardization of information on Y-chromosome markers with internet accessibility (**STRBase**)

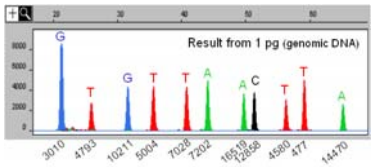
>45,000 Y-chromosome allele calls generated to aid studies on optimal markers for U.S. populations

Our Recent Y-Chromosome Work

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

- Schoske, R., Vallone, P.M., Kline, M.C., Redman, J.W., Butler, J.M. (2004) High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays, *Forensic Sci. Int.* 139: 107-121.
- Vallone, P.M. and Butler, J.M. (2004) Multiplexed assays for evaluation of Y-SNP markers in U.S. populations. *Progress in Forensic Genetics 10*, Elsevier Science: Amsterdam, The Netherlands, International Congress Series 1261, 85-87.
- Butler, J.M. and Schoske, R. (2004) Forensic value of the multi-copy Y-STR marker DYS464. *Progress in Forensic Genetics 10*, Elsevier Science: Amsterdam, The Netherlands, International Congress Series 1261, 278-280.
- Butler, J.M. and Schoske, R. (2004) Duplication of DYS19 flanking regions in other parts of the Y chromosome. *Int. J. Legal Med.*, 118: 178-183.
- Vallone, P.M. and Butler, J.M. (2004) Y-SNP typing of U.S. African American and Caucasian samples using allele-specific hybridization and primer extension. *J. Forensic Sci.* 49(4): 723-732.
- Butler, J.M., Decker, A.E., Kline, M.C., Vallone, P.M. (2004) Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation, *J. Forensic Sci., submitted.*
- Butler, J.M., Decker, A.E., Vallone, P.M., Kline, M.C. (2004) Allele Frequencies for 27 Y-STR Loci with U.S. Caucasian, African American, and Hispanic Samples, *Forensic Sci. Int., submitted.*

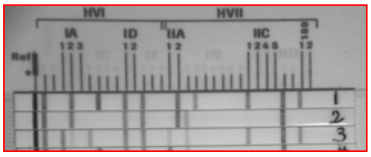
NIST mtDNA Work



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., in press (March 2005)

Automated washing/ Population Study

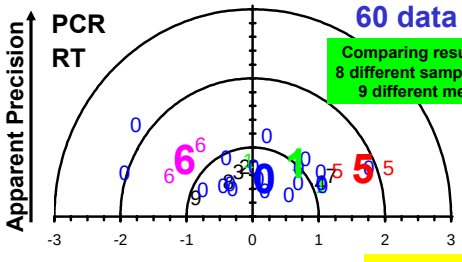
New Interlaboratory Study

Mixture Interpretation Study 2005 (**MIX05**)

- "Paper challenge" (no lab work required)
- Purpose to determine "lay of the land" for current practices in solving STR profile mixtures and reporting the results
- Results will be discussed at ISFG (Sept 2005)
- Please pick up a handout pertaining to our SOLICITATION FOR PARTICIPATION…

Interlaboratory Comparisons

Laboratory Performances with Real-Time PCR Methods

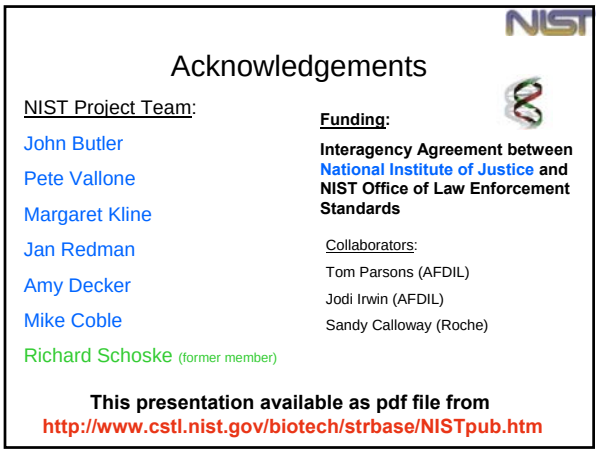


60 data sets

Comparing results from 8 different samples using 9 different methods

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

Kline, *et al., J. Forensic Sci., in press (May 2005)*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Margaret Kline
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Amy Decker
Mike Coble
Richard Schoske (former member)
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Collaborators:
Tom Parsons (AFDIL)
Jodi Irwin (AFDIL)
Sandy Calloway (Roche)
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