Y-Chromosome and Mitochondrial DNA Analysis

The Human Y-Chromosome:
Value of Additional Loci

NEAFS 2006 Workshop
Rye Brook, NY
November 1, 2006

Northeastern Association of Forensic Scientists

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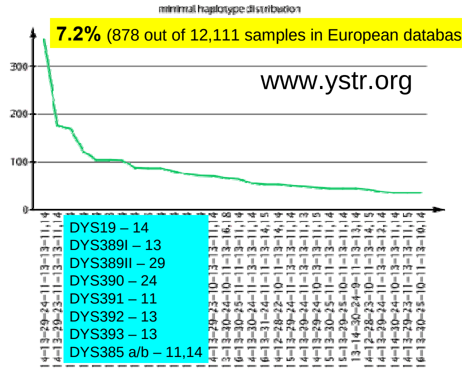
Dr. Michael D. Coble Michael.Coble@afip.osd.mil

New Y-STRs

More than 150 new Y-STR loci were characterized in June 2004. These new loci need to be studied in common sample sets including U.S. population groups in order to understand their ability to differentiate most common types and closely related individuals.

Problems with Most Common Type (MCT)



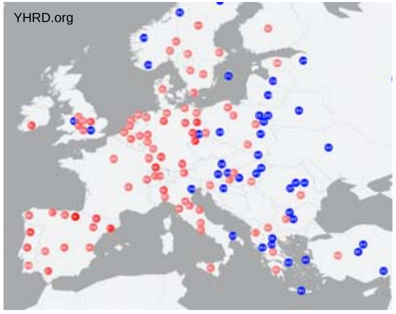
7.2% (878 out of 12,111 samples in European database)

www.ystr.org

DYS19 – 14
DYS389I – 13
DYS389II – 29
DYS390 – 24
DYS391 – 11
DYS392 – 13
DYS393 – 13
DYS385 a/b – 11,14

Most Common Type in Europeans

1,116 matches in 28,650 samples without DYS385 (3.9%)



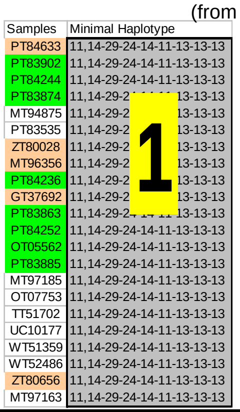
Most common type

DYS19 – 14
DYS389I – 13
DYS389II – 29
DYS390 – 24
DYS391 – 11
DYS392 – 13
DYS393 – 13
DYS385 a/b – 11,14

Need more Y-STR loci to separate…

606 matches in 27,773 samples (2.1%)

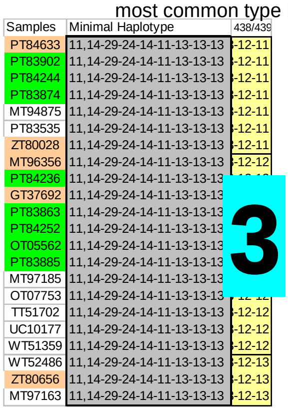
Most Common Type seen in 22 NIST samples (3.7%) (from all 3 populations)



Samples	Minimal Haplotype
PT84633	11,14-29-24-14-11-13-13-13
PT83902	11,14-29-24-14-11-13-13-13
PT84244	11,14-29-24-14-11-13-13-13
PT83874	11,14-29-2 13-13-13
MT94875	11,14-29-2 13-13-13
PT83535	11,14-29-2 13-13-13
ZT80028	11,14-29-2 13-13-13
MT96356	11,14-29-2 13-13-13
PT84236	11,14-29-2 13-13-13
GT37692	11,14-29-2 13-13-13
PT83863	11,14-29-2 13-13-13
PT84252	11,14-29-24-14-11-13-13-13
OT05562	11,14-29-24-14-11-13-13-13
PT83885	11,14-29-24-14-11-13-13-13
MT97185	11,14-29-24-14-11-13-13-13
OT07753	11,14-29-24-14-11-13-13-13
TT51702	11,14-29-24-14-11-13-13-13
UC10177	11,14-29-24-14-11-13-13-13
WT51359	11,14-29-24-14-11-13-13-13
WT52486	11,14-29-24-14-11-13-13-13
ZT80656	11,14-29-24-14-11-13-13-13
MT97163	11,14-29-24-14-11-13-13-13

1

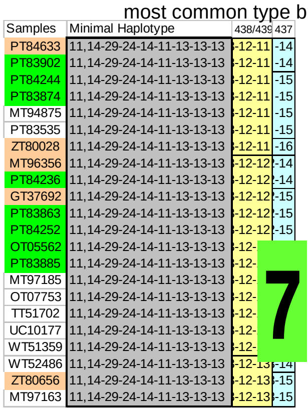
With SWGDAM US core loci (minimal haplotype+438,439) most common type breaks into 3 groups



Samples	Minimal Haplotype	438/439
PT84633	11,14-29-24-14-11-13-13-13	-12-11
PT83902	11,14-29-24-14-11-13-13-13	-12-11
PT84244	11,14-29-24-14-11-13-13-13	-12-11
PT83874	11,14-29-24-14-11-13-13-13	-12-11
MT94875	11,14-29-24-14-11-13-13-13	-12-11
PT83535	11,14-29-24-14-11-13-13-13	-12-11
ZT80028	11,14-29-24-14-11-13-13-13	-12-11
MT96356	11,14-29-24-14-11-13-13-13	-12-12
PT84236	11,14-29-24-14-11-13-13-13	
GT37692	11,14-29-24-14-11-13-13-13	
PT83863	11,14-29-24-14-11-13-13-13	
PT84252	11,14-29-24-14-11-13-13-13	
OT05562	11,14-29-24-14-11-13-13-13	
PT83885	11,14-29-24-14-11-13-13-13	
MT97185	11,14-29-24-14-11-13-13-13	
OT07753	11,14-29-24-14-11-13-13-13	
TT51702	11,14-29-24-14-11-13-13-13	-12-12
UC10177	11,14-29-24-14-11-13-13-13	-12-12
WT51359	11,14-29-24-14-11-13-13-13	-12-12
WT52486	11,14-29-24-14-11-13-13-13	-12-13
ZT80656	11,14-29-24-14-11-13-13-13	-12-13
MT97163	11,14-29-24-14-11-13-13-13	-12-13

3

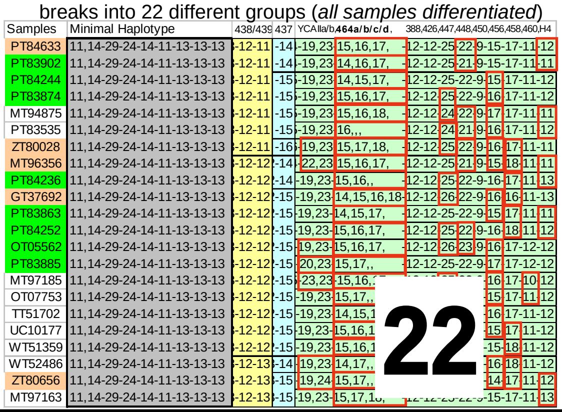
With Promega's loci (minimal haplotype+438,439,437) most common type breaks into 7 groups



Samples	Minimal Haplotype	438/439	437
PT84633	11,14-29-24-14-11-13-13-13	-12-11	-14
PT83902	11,14-29-24-14-11-13-13-13	-12-11	-14
PT84244	11,14-29-24-14-11-13-13-13	-12-11	-15
PT83874	11,14-29-24-14-11-13-13-13	-12-11	-15
MT94875	11,14-29-24-14-11-13-13-13	-12-11	-15
PT83535	11,14-29-24-14-11-13-13-13	-12-11	-15
ZT80028	11,14-29-24-14-11-13-13-13	-12-11	-16
MT96356	11,14-29-24-14-11-13-13-13	-12-12	-14
PT84236	11,14-29-24-14-11-13-13-13	-12-12	-14
GT37692	11,14-29-24-14-11-13-13-13	-12-12	-15
PT83863	11,14-29-24-14-11-13-13-13	-12-12	-15
PT84252	11,14-29-24-14-11-13-13-13	-12-12	-15
OT05562	11,14-29-24-14-11-13-13-13	-12-	
PT83885	11,14-29-24-14-11-13-13-13	-12-	
MT97185	11,14-29-24-14-11-13-13-13	-12-	
OT07753	11,14-29-24-14-11-13-13-13	-12-	
TT51702	11,14-29-24-14-11-13-13-13	-12-	
UC10177	11,14-29-24-14-11-13-13-13	-12-	
WT51359	11,14-29-24-14-11-13-13-13	-12-	
WT52486	11,14-29-24-14-11-13-13-13	-12-13	-14
ZT80656	11,14-29-24-14-11-13-13-13	-12-13	-15
MT97163	11,14-29-24-14-11-13-13-13	-12-13	-15

7

With NIST 27 Y STRs (2 multiplexes) most common type breaks into 22 different groups (*all samples differentiated*)



Samples	Minimal Haplotype	438/439	437	YCAIIa/b,	**464a/b/c/d,**	388,426,447,448,450,456,458,460,H4
PT84633	11,14-29-24-14-11-13-13-13	-12-11	-14	-19,23	15,16,17,	-12-12-25-22-9-15-17-11-12
PT83902	11,14-29-24-14-11-13-13-13	-12-11	-14	-19,23	14,16,17,	-12-12-25-21-9-15-17-11-11
PT84244	11,14-29-24-14-11-13-13-13	-12-11	-15	-19,23	14,15,17,	-12-12-25-22-9-15-17-11-12
PT83874	11,14-29-24-14-11-13-13-13	-12-11	-15	-19,23	15,16,17,	-12-12-25-22-9-16-17-11-12
MT94875	11,14-29-24-14-11-13-13-13	-12-11	-15	-19,23	15,16,18,	-12-12-24-22-9-17-17-11-11
PT83535	11,14-29-24-14-11-13-13-13	-12-11	-15	-19,23	16,,,	-12-12-24-21-9-16-17-11-12
ZT80028	11,14-29-24-14-11-13-13-13	-12-11	-16	-19,23	15,17,18,	-12-12-25-22-9-16-17-11-11
MT96356	11,14-29-24-14-11-13-13-13	-12-12	-14	-22,23	15,16,17,	-12-12-25-21-9-15-18-11-11
PT84236	11,14-29-24-14-11-13-13-13	-12-12	-14	-19,23	15,16,,	-12-12-25-22-9-16-17-11-13
GT37692	11,14-29-24-14-11-13-13-13	-12-12	-15	-19,23	14,15,16,18	-12-12-26-22-9-16-16-11-13
PT83863	11,14-29-24-14-11-13-13-13	-12-12	-15	-19,23	14,15,17,	-12-12-25-22-9-15-17-11-11
PT84252	11,14-29-24-14-11-13-13-13	-12-12	-15	-19,23	15,16,17,	-12-12-25-22-9-16-18-11-12
OT05562	11,14-29-24-14-11-13-13-13	-12-12	-15	-19,23	15,16,17,	-12-12-26-23-9-16-17-12-12
PT83885	11,14-29-24-14-11-13-13-13	-12-12	-15	-20,23	15,17,,	-12-12-25-22-9-17-17-12-12
MT97185	11,14-29-24-14-11-13-13-13	-12-12	-15	-23,23	15,16,	-16-17-10-12
OT07753	11,14-29-24-14-11-13-13-13	-12-12	-15	-19,23	15,17,,	-15-17-11-12
TT51702	11,14-29-24-14-11-13-13-13	-12-12	-15	-19,23	14,15,1	-16-17-11-12
UC10177	11,14-29-24-14-11-13-13-13	-12-12	-15	-19,23	15,16,1	-15-17-11-12
WT51359	11,14-29-24-14-11-13-13-13	-12-12	-15	-19,23	15,16,1	-15-18-11-12
WT52486	11,14-29-24-14-11-13-13-13	-12-13	-14	-19,23	14,17,,	-16-18-11-12
ZT80656	11,14-29-24-14-11-13-13-13	-12-13	-15	-19,24	15,17,,	-14-17-11-12
MT97163	11,14-29-24-14-11-13-13-13	-12-13	-15	-19,23	15,17,18,	-12-12-25-22-9-15-17-11-13

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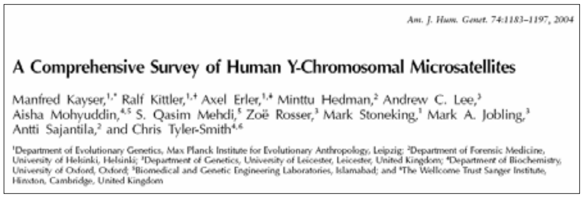
New Y-STR paper

***June 2004 issue** of American Journal of Human Genetics*



Am. J. Hum. Genet. 74:1183–1197, 2004

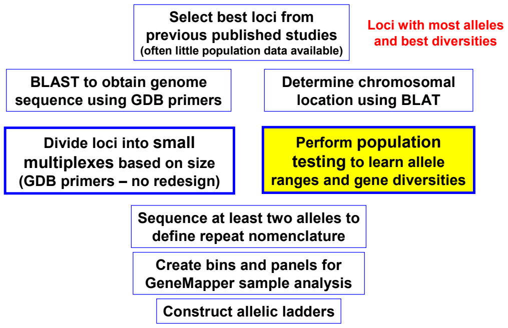
A Comprehensive Survey of Human Y-Chromosomal Microsatellites

Manfred Kayser,[1,*] Ralf Kittler,[1,+] Axel Erler,[1,4] Minttu Hedman,[2] Andrew C. Lee,[3] Aisha Mohyuddin,[4,5] S. Qasim Mehdi,[5] Zoë Rosser,[3] Mark Stoneking,[1] 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**

Strategy for Y-STR Locus Selection



Select best loci from previous published studies (often little population data available)

Loci with most alleles and best diversities

BLAST to obtain genome sequence using GDB primers

Determine chromosomal location using BLAT

Divide loci into small multiplexes based on size (GDB primers – no redesign)

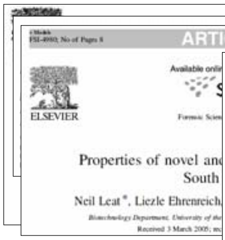
Perform population testing to learn allele ranges and gene diversities

Sequence at least two alleles to define repeat nomenclature

Create bins and panels for GeneMapper sample analysis

Construct allelic ladders

Develop large multiplex reactions with best Y-STRs



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Properties of novel an… South

Neil Leat *, Liezle Ehrenreich,

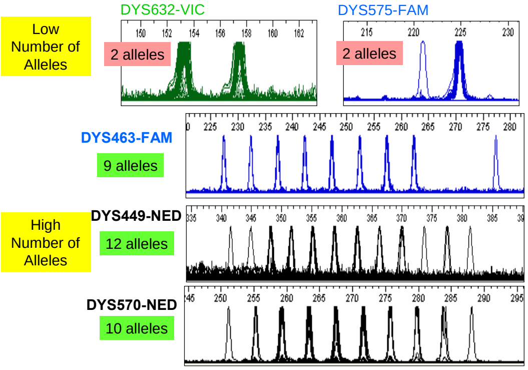
Table 2
Gene diversity values for 19 loci selected from Y-chromosome sequence data

Locus	Number of alleles	Gene diversity
DYS710[a]	**15**	**0.90**
DYS711[a]	**12**	**0.89**
DYS626	**10**	**0.85**
DYS712[a]	**11**	**0.85**
DYS713[a]	**10**	**0.80**
DYS481	**7**	**0.80**
DYS518	**7**	**0.79**
DYS570	**8**	**0.78**
DYS714[a]	**8**	**0.77**
DYS557	**7**	**0.75**
DYS614	**8**	**0.74**
DYS612	**6**	**0.74**
DYS607	**7**	**0.70**
DYS644	**9**	**0.70**
DYS715[a]	5	0.64
DYS485	6	0.56
DYS716[a]	3	0.55
DYS521	5	0.28
DYS717[a]	3	0.24

Samples from 46 Caucasians were analyzed using uniplex PCR followed by PAGE and silver staining. Gene diversity was calculated as $1 - \sum P_i^2$, where P_i is the allele frequency. The 14 loci in bold were selected for further analysis using dye-labeled primers and multiplex PCR.

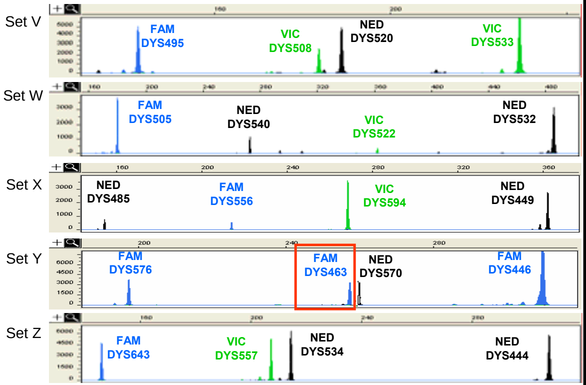
Loci selected from:
Redd et al. 2002
Kayser et al. 2004
Leat et al. 2006

Screening for Number of Y-STR Alleles with 95 Sample Plate

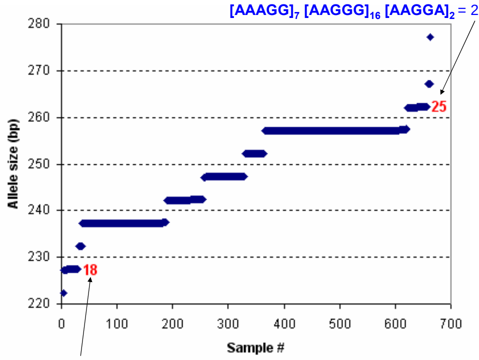


5 Panels Used to Type 20 New Y-STRs

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



DYS463 Alleles Observed



$[AAAGG]_7\ [AAGGG]_{16}\ [AAGGA]_2$ = 25

$[AAAGG]_6\ [AAGGG]_{10}\ [AAGGA]_2$ = 18

Conversion to Allele Frequency Information

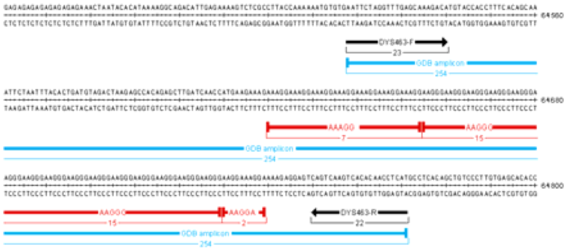
Locus	Allele	Size Range (bp)	Count	Combined Freq (N = 661)
DYS463	17	222.45	1	0.0015
	18	227.34-227.44	27	0.0408
	19	232.30-232.39	7	0.0106
	20	237.24-237.44	151	0.2284
	21	242.21-242.41	67	0.1014
	22	247.12-247.40	74	0.1120
	23	252.13-252.33	35	0.0530
	24	257.05-257.49	256	**0.3873**
	25	262.01-262.26	37	0.0560
	26	267.05-267.21	5	0.0076
	28	277.22	1	0.0015
		failure	2	
				STR diversity
		TOTAL	**661**	**0.7684**

$D = (n/n-1)(1 - \sum x^2)$

DYS463

GenBank AC007275 reference allele = 24 (7+15+2) repeats

Nomenclature from Redd *et al.* (2002) *Forensic Sci. Int.* 130: 97-111



CAACCATGAAGAAAG **[AAAGG]$_{7}$ [AAGGG]$_{15}$ [AAGGA]$_{2}$** AAAGAGGAGT

(H09_GT37888) = 18 = **[AAAGG]$_{6}$ [AAGGG]$_{10}$ [AAGGA]$_{2}$**
(G10_TT51399) = 25 = **[AAAGG]$_{7}$ [AAGGG]$_{16}$ [AAGGA]$_{2}$**

Impact of Additional Y-STR Loci on Resolving Common Haplotypes and Closely Related Individuals

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Amy E. Decker, Margaret C. Kline, John M. Butler

U.S. National Institute of Standards and Technology

September 30, 2006
V. International Forensic Y-User Workshop
Innsbruck, Austria

Going Beyond Commercial Y-STR Kits

- Most forensic DNA laboratories (certainly in the U.S.) will **only use commercially available kits** due to quality control issues
- Using these kits as a starting point, **are there additional loci that would be beneficial in separating samples with common types**, which could be advocated to companies for possible future adoption in Y-STR kits?
- Is it possible to regularly **resolve individuals from the same paternal lineage** (e.g., fathers and sons) if enough Y-STRs are examined?

Data Set Used to Examine Common Types

- Yfiler kit (**17 Y-STR loci**) run on all NIST male U.S. population samples
  - makes up ~20% of Applied Biosystems database
  - **submitted to the YHRD**
- Additional **20 Y-STR loci** run on full set of NIST population samples *(and several less polymorphic ones only on subset of samples)*
  - Butler, J.M., Decker, A.E., Vallone, P.M., Kline, M.C. (2006) Allele frequencies for 27 Y-STR Loci with U.S. Caucasian, African American, and Hispanic samples. *Forensic Sci. Int.* 156:250-260.

NIST Contribution to the YHRD

658 U.S. samples run with the Yfiler kit

Search or list the YHRD contributors

Contact	**John M. Butler, Amy E. Decker, Peter M. Vallone**
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Phone	(301) 975-4049
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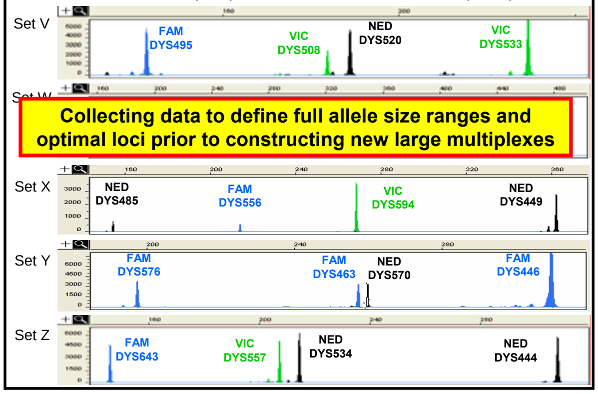
Haploytpe count	658
Contrib. Populations	USA [African American] USA [European American] USA [Hispanic American]
QC since	20.12.2005
Publication(s)	Butler JM, Decker AE, Vallone PM, Kline MC (2005) Allele Caucasian, African American, and Hispanic samples. For

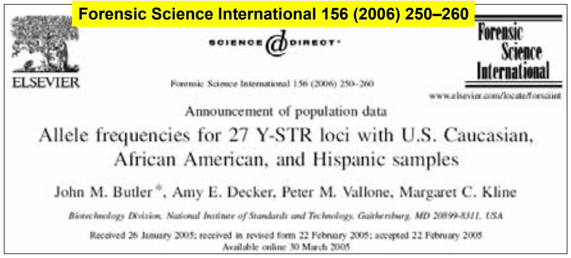
5 Panels Used to Type 20 New Y-STRs

Across 656 U.S. Samples (Caucasians, African Americans, Hispanics)



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

Publication with Additional Y-STR Loci



Forensic Science International 156 (2006) 250–260

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Announcement of population data

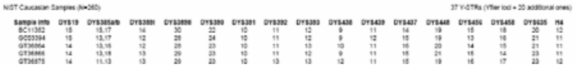
Allele frequencies for 27 Y-STR loci with U.S. Caucasian, African American, and Hispanic samples

John M. Butler*, Amy E. Decker, Peter M. Vallone, Margaret C. Kline

Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899-8311, USA

Received 26 January 2005; received in revised form 22 February 2005; accepted 22 February 2005
Available online 30 March 2005

Full Haplotypes Available on http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm



NIST Caucasian Samples (N=260)

37 Y-STRs (Yfiler loci + 20 additional ones)

# times haplotype observed	9 MHL
1	429
2	34
3	13
4	4
5	3
6	1
7	1
8	1
9	2
10	.
11	1
12	.
13	1
15	.
26	**1**
HD	0.996644
%DC	0.748476
# HT	491

429 of the 656 had a unique haplotype with the MHL loci, 34 sample haplotypes were observed twice in the sample set, 13 sample haplotypes were observed three times, etc.

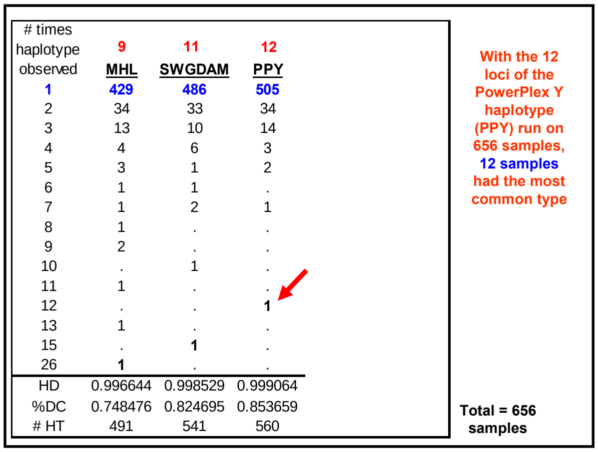
With the 9 loci of the minimal haplotype (MHL) run on 656 samples, 26 samples had the most common type

Total = 656 samples

# times haplotype observed	9 MHL	11 SWGDAM
1	429	486
2	34	33
3	13	10
4	4	6
5	3	1
6	1	1
7	1	2
8	1	.
9	2	.
10	.	1
11	1	.
12	.	.
13	1	.
15	.	**1**
26	**1**	.
HD	0.996644	0.998529
%DC	0.748476	0.824695
# HT	491	541

With the 11 loci of the SWGDAM haplotype run on 656 samples, 15 samples had the most common type

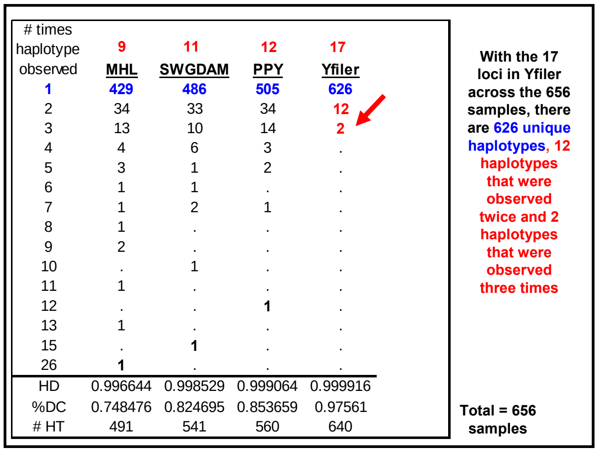
Total = 656 samples



# times haplotype observed	9 MHL	11 SWGDAM	12 PPY
1	429	486	505
2	34	33	34
3	13	10	14
4	4	6	3
5	3	1	2
6	1	1	.
7	1	2	1
8	1	.	.
9	2	.	.
10	.	1	.
11	1	.	.
12	.	.	**1**
13	1	.	.
15	.	**1**	.
26	**1**	.	.
HD	0.996644	0.998529	0.999064
%DC	0.748476	0.824695	0.853659
# HT	491	541	560

With the 12 loci of the PowerPlex Y haplotype (PPY) run on 656 samples, 12 samples had the most common type

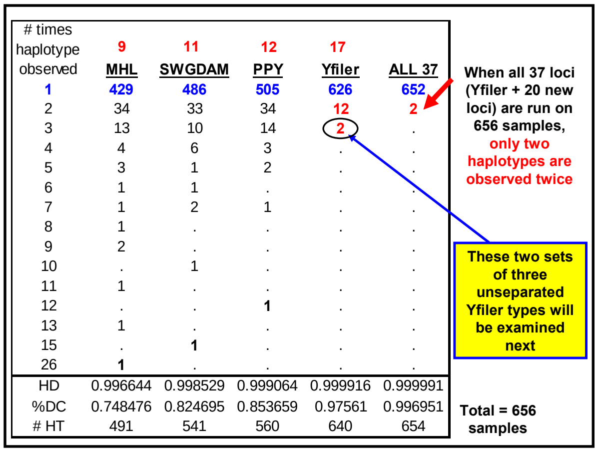
Total = 656 samples



# times haplotype observed	9 MHL	11 SWGDAM	12 PPY	17 Yfiler
1	429	486	505	626
2	34	33	34	12
3	13	10	14	2
4	4	6	3	.
5	3	1	2	.
6	1	1	.	.
7	1	2	1	.
8	1	.	.	.
9	2	.	.	.
10	.	1	.	.
11	1	.	.	.
12	.	.	**1**	.
13	1	.	.	.
15	.	**1**	.	.
26	**1**	.	.	.
HD	0.996644	0.998529	0.999064	0.999916
%DC	0.748476	0.824695	0.853659	0.97561
# HT	491	541	560	640

With the 17 loci in Yfiler across the 656 samples, there are 626 unique haplotypes, 12 haplotypes that were observed twice and 2 haplotypes that were observed three times

Total = 656 samples

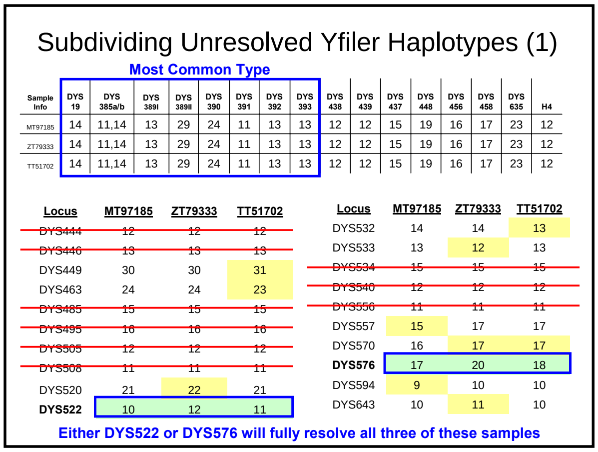


# times haplotype observed	9 MHL	11 SWGDAM	12 PPY	17 Yfiler	ALL 37
1	429	486	505	626	652
2	34	33	34	12	2
3	13	10	14	2	.
4	4	6	3	.	.
5	3	1	2	.	.
6	1	1	.	.	.
7	1	2	1	.	.
8	1	.	.	.	.
9	2	.	.	.	.
10	.	1	.	.	.
11	1	.	.	.	.
12	.	.	**1**	.	.
13	1	.	.	.	.
15	.	**1**	.	.	.
26	**1**	.	.	.	.
HD	0.996644	0.998529	0.999064	0.999916	0.999991
%DC	0.748476	0.824695	0.853659	0.97561	0.996951
# HT	491	541	560	640	654

When all 37 loci (Yfiler + 20 new loci) are run on 656 samples, only two haplotypes are observed twice

These two sets of three unseparated Yfiler types will be examined next

Total = 656 samples



Subdividing Unresolved Yfiler Haplotypes (1)

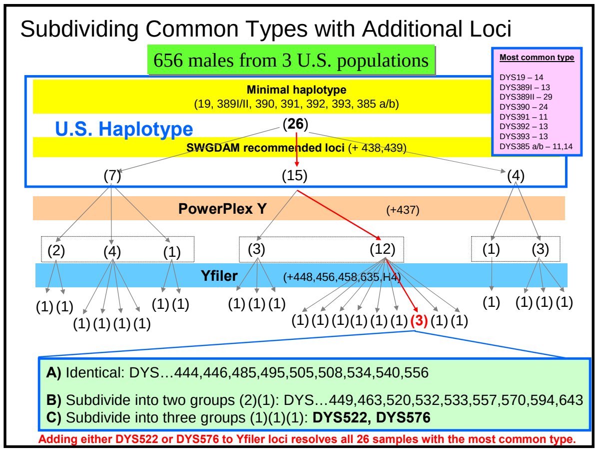
Most Common Type

Sample Info	DYS 19	DYS 385a/b	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 438	DYS 439	DYS 437	DYS 448	DYS 456	DYS 458	DYS 635	H4
MT97185	14	11,14	13	29	24	11	13	13	12	12	15	19	16	17	23	12
ZT79333	14	11,14	13	29	24	11	13	13	12	12	15	19	16	17	23	12
TT51702	14	11,14	13	29	24	11	13	13	12	12	15	19	16	17	23	12

Locus	MT97185	ZT79333	TT51702
~~DYS444~~	~~12~~	~~12~~	~~12~~
~~DYS446~~	~~13~~	~~13~~	~~13~~
DYS449	30	30	31
DYS463	24	24	23
~~DYS485~~	~~15~~	~~15~~	~~15~~
~~DYS495~~	~~16~~	~~16~~	~~16~~
~~DYS505~~	~~12~~	~~12~~	~~12~~
~~DYS508~~	~~11~~	~~11~~	~~11~~
DYS520	21	22	21
DYS522	10	12	11

Locus	MT97185	ZT79333	TT51702
DYS532	14	14	13
DYS533	13	12	13
~~DYS534~~	~~15~~	~~15~~	~~15~~
~~DYS540~~	~~12~~	~~12~~	~~12~~
~~DYS556~~	~~11~~	~~11~~	~~11~~
DYS557	15	17	17
DYS570	16	17	17
DYS576	17	20	18
DYS594	9	10	10
DYS643	10	11	10

Either DYS522 or DYS576 will fully resolve all three of these samples



Subdividing Common Types with Additional Loci

656 males from 3 U.S. populations

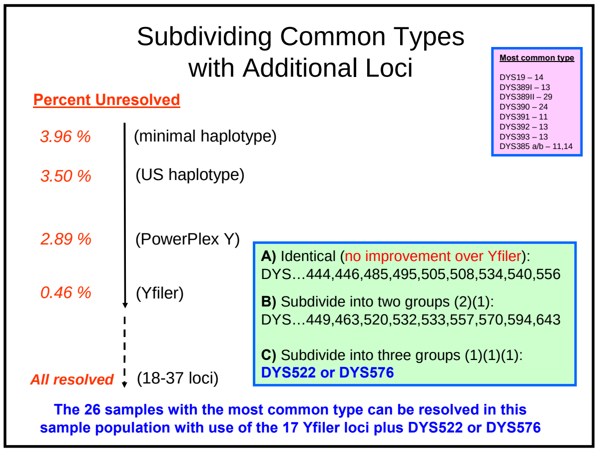
Most common type

DYS19 – 14
DYS389I – 13
DYS389II – 29
DYS390 – 24
DYS391 – 11
DYS392 – 13
DYS393 – 13
DYS385 a/b – 11,14

A) Identical: DYS…444,446,485,495,505,508,534,540,556

B) Subdivide into two groups (2)(1): DYS…449,463,520,532,533,557,570,594,643
C) Subdivide into three groups (1)(1)(1): **DYS522, DYS576**

Adding either DYS522 or DYS576 to Yfiler loci resolves all 26 samples with the most common type.



Subdividing Common Types with Additional Loci

Most common type

DYS19 – 14
DYS389I – 13
DYS389II – 29
DYS390 – 24
DYS391 – 11
DYS392 – 13
DYS393 – 13
DYS385 a/b – 11,14

Percent Unresolved

3.96 %	(minimal haplotype)
3.50 %	(US haplotype)
2.89 %	(PowerPlex Y)
0.46 %	(Yfiler)
All resolved	(18-37 loci)

A) Identical (no improvement over Yfiler):
DYS…444,446,485,495,505,508,534,540,556

B) Subdivide into two groups (2)(1):
DYS…449,463,520,532,533,557,570,594,643

C) Subdivide into three groups (1)(1)(1):
DYS522 or DYS576

The 26 samples with the most common type can be resolved in this sample population with use of the 17 Yfiler loci plus DYS522 or DYS576

Subdividing Unresolved Yfiler Haplotypes(2)

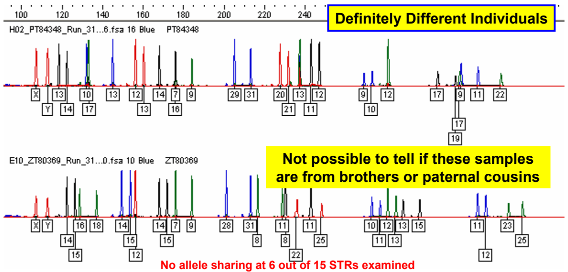
Sample Info	DYS 19	DYS 385a/b	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 438	DYS 439	DYS 437	DYS 448	DYS 456	DYS 458	DYS 635	H4
PT83904	13	13,14	15	31	24	9	11	13	10	10	14	20	16	18	21	12
PT84348	13	13,14	15	31	24	9	11	13	10	10	14	20	16	18	21	12
ZT80369	13	13,14	15	31	24	9	11	13	10	10	14	20	16	18	21	12

Locus	PT83904	PT84348	ZT80369
~~DYS444~~	~~12~~	~~12~~	~~12~~
~~DYS446~~	~~12~~	~~12~~	~~12~~
~~DYS449~~	~~31~~	~~31~~	~~31~~
~~DYS463~~	~~18~~	~~18~~	~~18~~
~~DYS485~~	~~15~~	~~15~~	~~15~~
~~DYS495~~	~~12~~	~~12~~	~~12~~
~~DYS505~~	~~11~~	~~11~~	~~11~~
~~DYS508~~	~~11~~	~~11~~	~~11~~
~~DYS520~~	~~19~~	~~19~~	~~19~~
~~DYS522~~	~~12~~	~~12~~	~~12~~

Locus	PT83904	PT84348	ZT80369
~~DYS532~~	~~14~~	~~14~~	~~14~~
~~DYS533~~	~~11~~	~~11~~	~~11~~
DYS534	16	17	17
~~DYS540~~	~~11~~	~~11~~	~~11~~
~~DYS556~~	~~12~~	~~12~~	~~12~~
~~DYS557~~	~~18~~	~~18~~	~~18~~
~~DYS570~~	~~22~~	~~22~~	~~22~~
~~DYS576~~	~~18~~	~~18~~	~~18~~
~~DYS594~~	~~11~~	~~11~~	~~11~~
~~DYS643~~	~~12~~	~~12~~	~~12~~

PT84348 and ZT80369 are unresolved even with 37 Y-STRs

Identifiler Results from Two Unresolved Hispanic Males Following Typing with 37 Y-STR Loci



Definitely Different Individuals

Not possible to tell if these samples are from brothers or paternal cousins

No allele sharing at 6 out of 15 STRs examined

Sample	D8	D21	D7	CSF	D3	TH	D13	D16	D2	D19	VWA	TPO	D18	Am	D5	FGA
PT84348	10,13	29,31	9,10	9,11	17,17	7,9	13,13	12,12	17,22	13,14	14,16	11,12	17,19	X,Y	12,13	20,21
ZT80369	14,15	28,31	10,11	11,12	16,18	7,9	8,11	12,13	23,25	14,15	14,15	8,11	13,15	X,Y	12,12	22,25

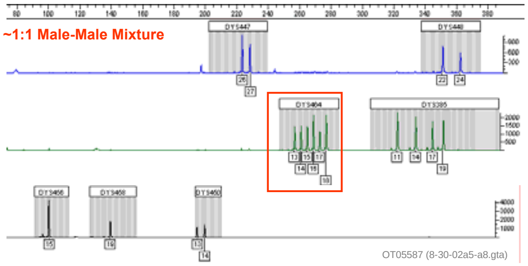
PT84348 and ZT80369 are unresolved even with 37 Y-STRs

Summary on Subdividing Common Types

- 640 haplotypes were observed in the 656 U.S. population samples with the Yfiler loci: 626 were unique, 2 were observed 3 times, and 12 haplotypes were observed twice.
- With the addition of 20 new Y-STR loci, all but two sample pairs are resolved.
- In this sample set, the 7 Y-STRs (DYS532, **DYS522, DYS576**, DYS570, DYS505, DYS449, DYS534) have the same ability to resolve the sample haplotypes as all 20 new loci.
- **These 7 loci will be the focus of future studies and multiplex assays**.

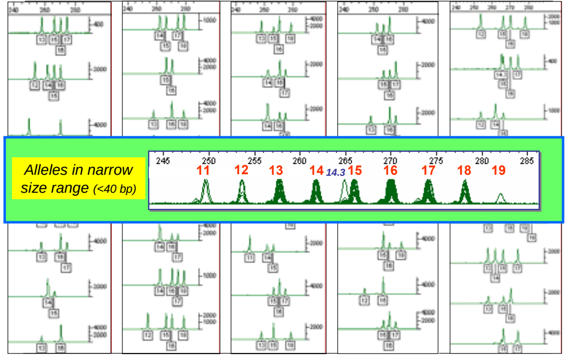
Multi-Copy Markers

- Should they be considered for use in forensic testing?
  - mixture interpretation will be complicated…



Some DYS464 Peak Patterns

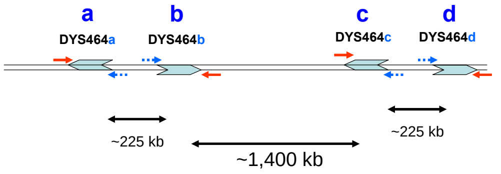
38 different haplotypes *in 61 African American samples*



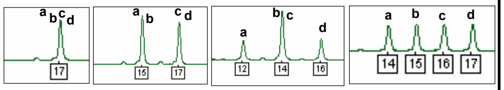
DYS464: A Highly Polymorphic, Multi-Copy Y STR Marker

has four copies of $[CCTT]_n$ repeat *(usually)*

A single primer pair produces various combinations of alleles with 9-20 repeats



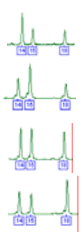
Methods for Allele Calling



Expanded *(E-type)*	17,17,17,17	15,15,17,17	12,14,14,16	14,15,16,17
Conservative *(C-type)*	17	15,17	12,14,16	14,15,16,17

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

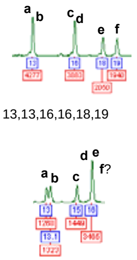
14,15,18,-

14,15,18,18

Conservative "C-type"

Expanded "E-type"

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



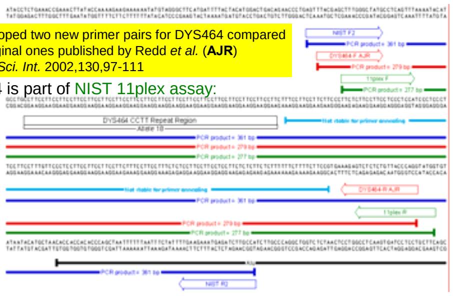
13,13,16,16,18,19

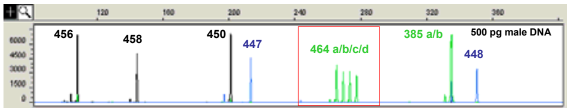
13,13.1,15,16,16,16

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

We developed two new primer pairs for DYS464 compared to the original ones published by Redd *et al.* (**AJR**) *Forensic Sci. Int.* 2002,130,97-111

DYS464 is part of NIST 11plex assay:



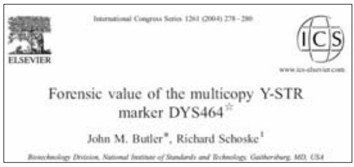


Schoske et al. (2003) *Forensic Sci. Int., in press*

NIST 11plex (32 cycles)

Poster at ISFG 2003

Progress in Forensic Genetics 10, pp. 278-290


International Congress Series 1261 (2004) 278 – 290

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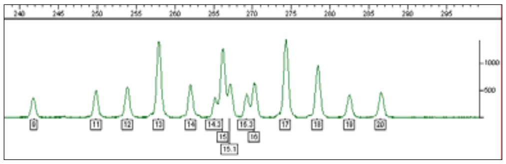
Forensic value of the multicopy Y-STR marker DYS464☆

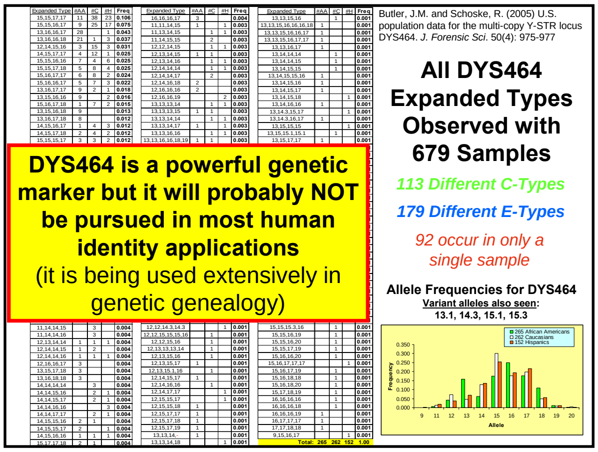
John M. Butler*, Richard Schoske1

Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD, USA

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.



Butler, J.M. and Schoske, R. (2005) U.S. population data for the multi-copy Y-STR locus DYS464. *J. Forensic Sci.* 50(4): 975-977

All DYS464 Expanded Types Observed with 679 Samples

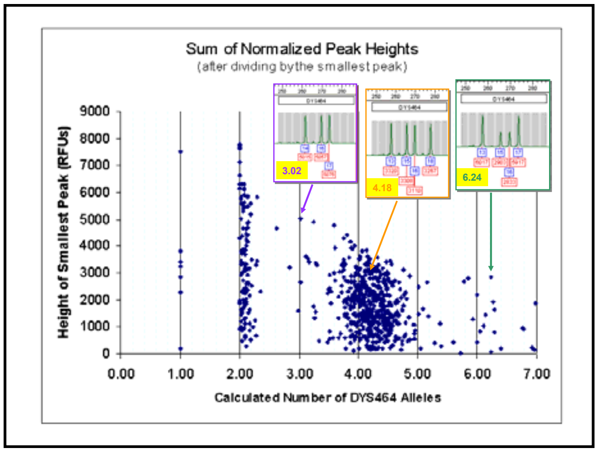
113 Different C-Types

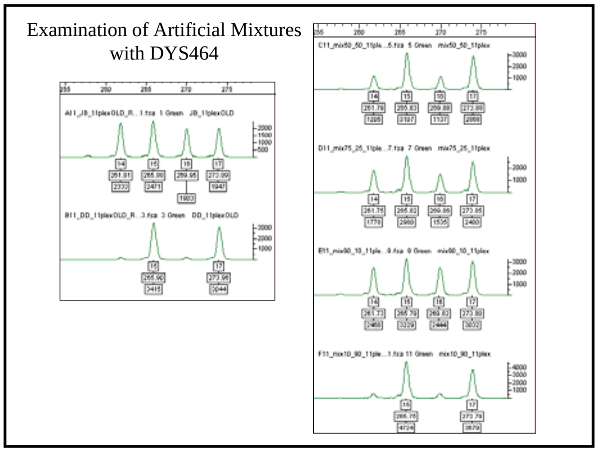
179 Different E-Types

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)

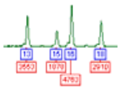
Allele Frequencies for DYS464
Variant alleles also seen: 13.1, 14.3, 15.1, 15.3





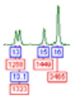
Examination of Artificial Mixtures with DYS464

DYS464 Samples with more than 4 copies

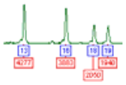


PT84206 (10-30-02_224-226)

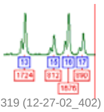
PT84348 (12-6-02_305-308)



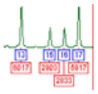
TT51696 (12-24-02_388-390)
PT84213 (12-24-02_380-381)



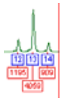
TT50923 (10-30-02_224-226)
UA16903 (12-24-02_391-393)



ZT79319 (12-27-02_402)



OT05582 (8-30-02a5-a8)



MT97179 (12-24-02_388-390)

Conclusions

- As expected, **more Y-STR loci increase the ability to resolve samples from one another** particularly those with a most common type.
- **Studies with father and son sample pairs are on-going** to measure mutation rates and to assist understanding which and how many Y-STRs may be optimal for differentiating between closely related individuals.