


Y-Chromosome and Mitochondrial DNA Analysis

The Human Y-Chromosome: Markers, Core Loci, and Kits

NEAFS 2006 Workshop
 Rye Brook, NY
 November 1, 2006

Dr. John M. Butler
 Dr. Michael D. Coble

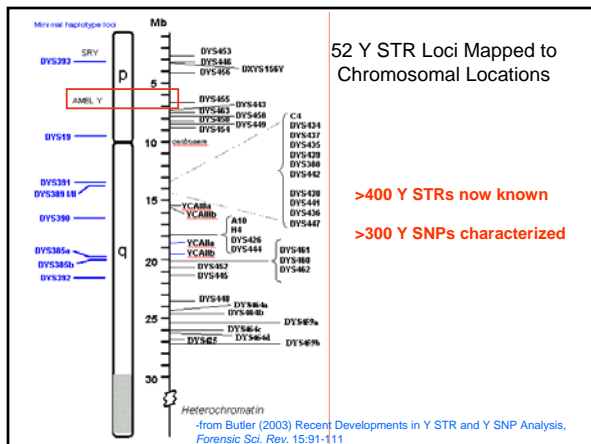


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 of
 Forensic Scientists

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Goals for NIST Y-STR Work

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



Physical Map of the Human Y-Chromosome
Hanson, E.K. and Ballantyne, J. (2006) *Legal Med* 8: 110-120

Describe the precise location of 417 Y-STRs
They note that not all will be useful due to low genetic variation or high X-chromosome homology

See also <http://ncfs.ucf.edu/ystar/ystar.html>

History of Y-STR Marker Discovery

- 1992 - **DYS19** (Roewer et al.) **"Extended Haplotype"**
- 1994 - YCAI a/b, YCAII a/b; YCAIII a/b, DXYS156 (Mathias et al.)
- 1996 - **DYS389I/II**, **DYS390**, **DYS391**, **DYS392**, **DYS393** (Roewer et al.)
- 1996 - DYF371, DYS425, DYS426 (Jobling et al.)
- 1997 - DYS288, DYS388 (Kayser et al.)
- 1998 - **DYS385 a/b** (Schneider et al.) **"Minimal Haplotype"**
- 1999 - A7.1 (DYS460), A7.2 (DYS461), A10, C4, H4 (White et al.)
- 2000 - DYS434, DYS435, DYS436, DYS437, **DYS438**, **DYS439** (Ayub et al.)
- 2000 - G09411 (DYS462), G10123 (de Knijff unpublished) **U.S. Haplotype**
- 2001 - DYS441, DYS442 (Iida et al.)
- 2002 - DYS443, DYS444, DYS445 (Iida et al.); DYS446, DYS447, DYS448, DYS449, DYS450, DYS452, DYS453, DYS454, DYS455, DYS456, DYS458, DYS459 a/b, DYS463, DYS464 a/b/c/d (Redd et al.)
- 2002 - DYS468-DYS596 (**129 new Y STRs**; Manfred Kayser GDB entries)
- 2003 - DYS597-DYS645 (**50 new Y STRs**; Manfred Kayser GDB entries)

From J.M. Butler (2003) Recent developments in Y-STR and Y-SNP analysis. *Forensic Sci. Rev.* 15:91-111

STR Markers with Low Stutter Products Benefit Forensic Analysis where Mixtures might be Present

YCAII a/b (dinucleotide repeat)

~50% stutter

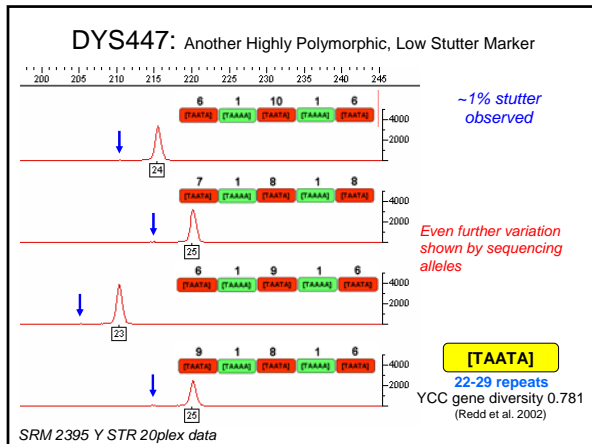
[CA]
11-24 repeats
YCC gene diversity 0.908
(Redd et al. 2002)

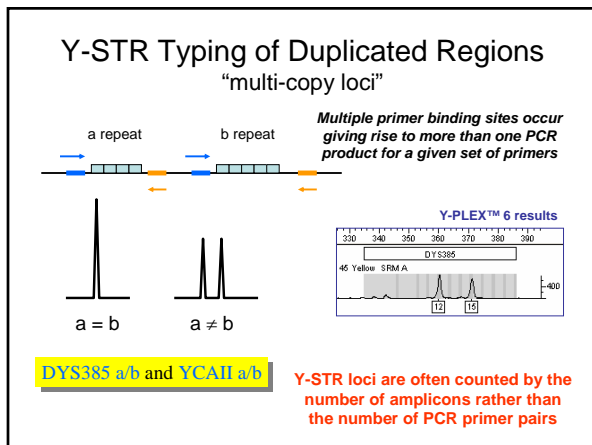
DYS448 (hexanucleotide repeat)

Almost no stutter observed

<2% stutter

[AGAGAT]
20-26 repeats
YCC gene diversity 0.782
(Redd et al. 2002)





- SWGAM 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)
 - Jill Smerick (FBI)
 - Sam Baechtel (FBI)
 - Roger Frappier (CFS)
 - Phil Kinsey (OR now MT)
 - Gary Sims (CA DOJ)
 - George Carmody (retired)
 - Mike Adamowicz (CT)
 - Bruce Budowle (FBI) – removed in 2004
 - **U.S. CORE Y-STR LOCI selected in January 2003**
 - Discussed setting up of national consolidated Y-STR database
 - Evaluation of commercially available kits (primer concordance)
 - Developing Y-STR interpretation guidelines
 - Examining value of additional loci
 - Discuss potential addition of ~~Y-STRs~~ ~~to CODIS~~

Report on the Current Activities of the 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 (patrilial) 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.

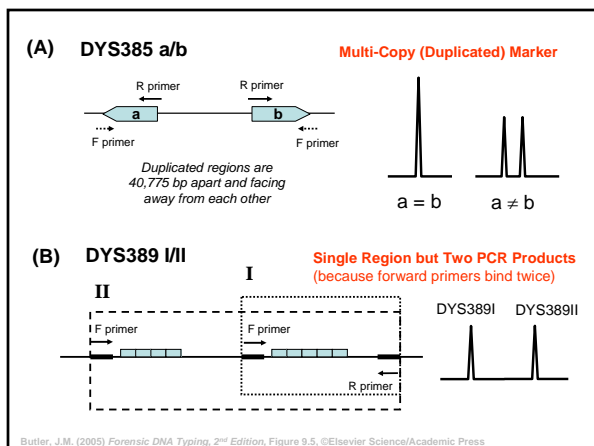
**11 PCR products
9 primer sets**


Core Y-STR Characteristics

STR Marker	Position (Mb)	Repeat Motif	Allele Range	Mutation Rate
DYS393	3.17	AGAT	8-17	0.05%
DYS19	10.12	TAGA	10-19	0.20%
DYS391	12.54	TCTA	6-14	0.40%
DYS439	12.95	AGAT	8-15	0.38%
DYS389 I/II	13.05	[TCTG] [TCTA]	9-17 / 24-34	0.20% / 0.31%
DYS438	13.38	TTTTTC	6-14	0.09%
DYS390	15.71	[TCTA] [TCTG]	17-28	0.32%
DYS385 a/b	19.19, 19.23	GAAA	7-28	0.23%
DYS392	20.97	TAT	6-20	0.05%

Positions in megabases (Mb) along the Y-chromosome were determined with NCBI build 35 (May 2004) using BLAT. Allele ranges represent the full range of alleles reported in the literature. Mutation rates summarized from YHRD (<http://www.yhrd.org>; accessed 6 Apr 2005).


Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci.* 51(2): 253-265





Forensic Sci. Int. (2001) 124: 5-10

Forensic Science International 124 (2001) 5-10
www.elsevier.com/locate/forensic



DNA Commission of the International Society of Forensic Genetics: recommendations on forensic analysis using Y-chromosome STRs

P. Gill^a, C. Brenner^b, B. Brinkmann^c, B. Budowle^d, A. Carracedo^e, M.A. Jobling^f, P. de Knijff^g, M. Kayser^h, M. Krawczakⁱ, W.R. Mayr^j, N. Morling^k, B. Olaisen^l, V. Pascali^m, M. Prinzⁿ, L. Roewer^o, P.M. Schneider^p, A. Sajantila^q, C. Tyler-Smith^r

ISFG Guidelines for Y-STRs

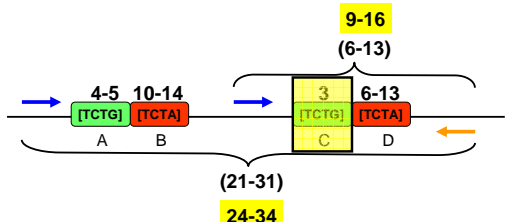
- Locus nomenclature should be DYS number if possible
- Allelic ladders should be used
- Allele nomenclature discussed...

ISFG Guidelines for Y STR Allele Nomenclature

Gill et al. (2001) *Forensic Sci. Int.* 124: 5-10

- Number of complete repeats
- A partial repeat (variant allele) is designated by number of complete repeats separated by a dot followed by the number of bases in the incomplete repeat (e.g., 17.3)
- Some locus nomenclatures take into account the **total number** of repetitive units (non-variant plus variant) while others have taken into account **only the variable repetitive stretches**
 - "If a nomenclature is already in use, it is recommended that it should be continued. However, to encourage consistency for newly reported STRs, it is recommended that alleles should be named according to the **total number of repeat units** of the DNA that comprises **both variant and non-variant repeats**"
- Duplicated systems such as DYS385 have to be treated as genotypes and alleles should be separated by a hyphen (e.g., "11-14")

Nomenclature Issues with DYS389 I/II



DYS389I = 3+11 = **14**; [TCTG]₃[TCTA]₁₁

DYS389II = 5+12+3+11 = **31**; [TCTG]₅[TCTA]₁₂[TCTG]₃[TCTA]₁₁

Original paper (Kayser et al. (1997) *Int. J. Legal Med.* 110:141-149) defines allele nomenclature without repeat segment "C"; it has now been added in more recent nomenclatures thus making alleles +3 repeats larger

Issues with DYS439 Nomenclature

© 2000 Oxford University Press Nucleic Acids Research, 2000, Vol. 28, No. 2 e8

Identification and characterisation of novel human Y-chromosomal microsatellites from sequence database information

Qasim Ayub^{1,2}, Aisha Mohyuddin^{1,2}, Raheel Qamar^{1,2}, Kehkashan Mazhar², Tatiana Zerjal¹, S. Qasim Mehd² and Chris Tyler-Smith^{1*}

¹Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK and ²Biomedical and Genetic Engineering Laboratories, 25 Mauve Area, PO Box 2891, Islamabad, Pakistan

Received October 7, 1999. Revised and Accepted November 26, 1999

Original description of DYS439 (only variable repeat used)

[GATA]₂N₄[GATA]₃N₄[GATA]₁N₃[GATA]₁N₁[GATA]₉₋₁₄

Alleles 9-14

Issues with DYS439 Nomenclature

Int J Legal Med (2000) 114:125–129 © Springer-Verlag 2000

SHORT COMMUNICATION

P. Grignani - G. Peloso - P. Fattorini - C. Previderè

Highly informative Y-chromosomal haplotypes by the addition of three new STRs DYS437, DYS438 and DYS439

Received: 29 November 1999 / Accepted: 17 March 2000

[GATA]₂N₄[GATA]₃N₄[GATA]₁N₃[GATA]₁N₁[GATA]₉₋₁₄

+7 repeats

Alleles 16-21

Issues with DYS439 Nomenclature

Elsevier Forensic Science International

Elssevier Science International 175 (2000) 19–26 www.elsevier.com/locate/forensic

Sequence structure of 12 novel Y chromosome microsatellites and PCR amplification strategies

Amalshah González-Neira^a, Mike Elmentariño^b, María Victoria Latorre^a, Paula Sánchez-Diz^a, Leonor Gusmão^c, Mechthild Prinz^d, Angel Carmacido^{e*}

^aInstituto de Medicina Legal, Universidad Complutense de Madrid, Madrid, Spain; ^bDepartamento de Genética, Universidad Complutense de Madrid, Madrid, Spain; ^cInstituto de Medicina Legal, Universidad Complutense de Madrid, Madrid, Spain; ^dDepartment of Forensic Biology, Office of the Chief Medical Examiner, New York, NY 10022, USA; ^eUMHESP, University of Pernambuco, Brazil

Received 1 November 2000; accepted in revised form 17 January 2001; accepted 14 January 2001

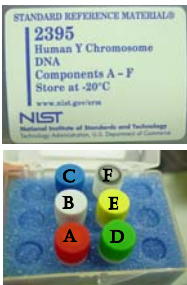
DYS439	Consensus structure	Primers
Allele (bp)		
19 (240)		PrA(24bp) 45bp(ATCT) ₂ 30bp(GATA) ₃ 30bp(AGAT) ₁ 40bp(AGAT) ₁ 10bp(AGAT) ₁₀ 30bp PrB(20bp)
20 (244)		PrA(24bp) 45bp(ATCT) ₂ 30bp(GATA) ₃ 30bp(AGAT) ₁ 40bp(AGAT) ₁ 10bp(AGAT) ₁₁ 30bp PrB(20bp)
21 (248)		PrA(24bp) 45bp(ATCT) ₂ 30bp(GATA) ₃ 30bp(AGAT) ₁ 40bp(AGAT) ₁ 10bp(AGAT) ₁₂ 30bp PrB(20bp)

+9 repeats

Alleles 18-23

Repeat designation changed to AGAT from previous GATA

Y-Chromosome Standard NIST SRM 2395



Human Y-Chromosome DNA Profiling Standard

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

Certified for all loci in commercial Y-STR kits:

Y-PLEX 6	
Y-PLEX 5	SWGAM recommended loci:
Y-PLEX 12	DYS19, DYS385 a/b, DYS389I/II,
PowerPlex Y	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 Determination of Y-STR Repeat Region for Each Component

DYS392 (forward) A 13 TAT repeats

DYS392 (forward) B 11 TAT repeats

DYS392 (forward) C 11 TAT repeats

DYS392 (forward) D 11 TAT repeats

DYS392 (forward) E 12 TAT repeats

Sequencing Performed

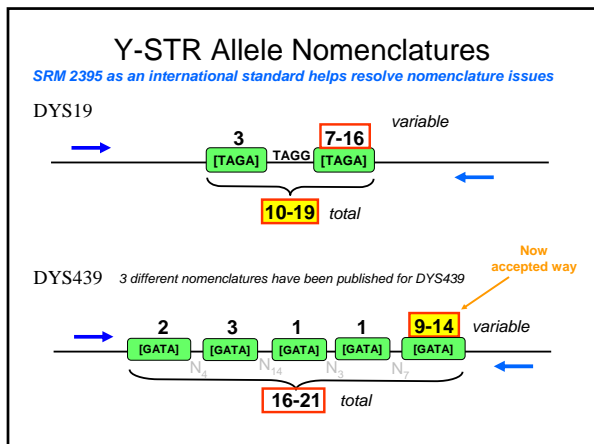
DYS19	DYS388
DYS385 a/b	DYS426
DYS389 I/II	
DYS390	DYS435
DYS391	DYS436
DYS392	DYS437
DYS393	DYS438
	DYS439
DYS447	DYS460 (A7.1)
DYS448	DYS461 (A7.2)
Y-GATA-H4	DYS462

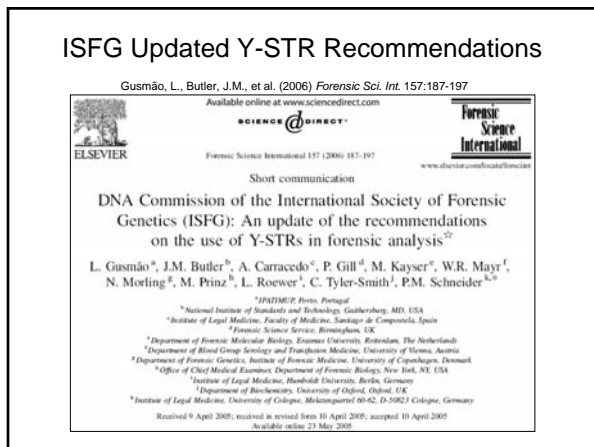
Sequence Summaries for SRM 2395

DYS19	A	14	[TAGA] ₁ tagg[TAGA] ₁	DYS438	A	12	[TTTTC] ₁₂
	B	14	[TAGA] ₁ tagg[TAGA] ₁		B	9	[TTTTC] ₉
	C	16	[TAGA] ₁ tagg[TAGA] ₂		C	11	[TTTTC] ₁₁
	D	15	[TAGA] ₁ tagg[TAGA] ₂		D	11	[TTTTC] ₁₁
	E	17	[TAGA] ₁ tagg[TAGA] ₄		E	10	[TTTTC] ₁₀

DYS390	A	25	[TCTG] ₁ [TCTA] ₂ [TCTG] ₁ [TCTA] ₁
	B	23	[TCTG] ₁ [TCTA] ₁ [TCTG] ₁ [TCTA] ₁
	C	21	[TCTG] ₁ [TCTA] ₁ ACTA [TCTA] ₁ [TCTG] ₁ [TCTA] ₁
	D	22	[TCTG] ₁ [TCTA] ₁ [TCTG] ₁ [TCTA] ₁
	E	24	[TCTG] ₁ [TCTA] ₁ [TCTG] ₁ [TCTA] ₁

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





Y-GATA-H4 Nomenclature Differences

- Mulero, J.J., Budowle, B., Butler, J.M., Gusmão, L. (2006) **Letter to the Editor--Nomenclature and allele repeat structure update for the Y-STR locus GATA H4.** *J. Forensic Sci.* 51(3): 694.
- "There are differences in allele designations at the GATA H4 marker between those recommended in the Applied Biosystems AmpFISTR Yfiler™ polymerase chain reaction amplification kit (Applied Biosystems, Foster City, CA) and the ISFG recommendations. The nomenclature for the GATA H4 marker in the Yfiler kit is based on the allele repeat structure defined by the National Institute of Standards and Technology Standard reference material (SRM) 2395 and the work of Butler et al. (2)."
- "Add a correction factor of nine to the Yfiler allele number... and refer to this marker as GATA H4.1. Employing the ISFG proposed allele designation for GATA H4.1 changes the Yfiler kit allelic ladder range from 8–13 to 17–22."

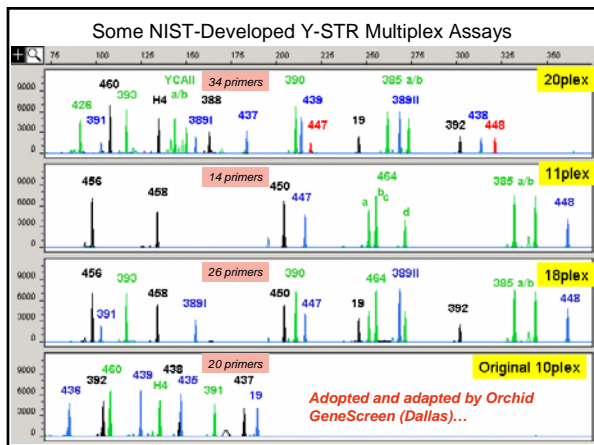
http://www.cstl.nist.gov/biotech/strbase/YSTRs/H4_nomenclature.htm

Y-GATA-H4

Reference sequence: GenBank accession G42676 (submitted May 1999 by White et al.)

ISFG = 20 repeats
Yfiler = 11 repeats

Unfortunately, use of TAGA vs. GATA repeat designation results in a difference of one repeat unit!
NIST SRM 2395 follows ISFG guidelines (for our primer pair):
first adjacent repeat starting from 5'end is TAGA



Orchid Cellmark Y-STR 10plex

J Forensic Sci, November 2003, Vol. 48, No. 6
 Paper ID JFS300314-046
 Available online at: www.jafmd.org

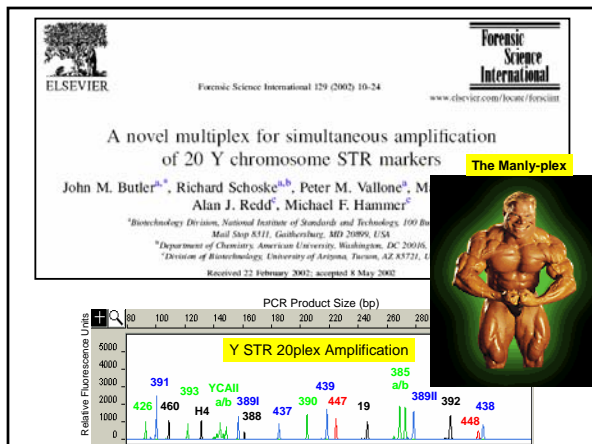
Cassie L. Johnson,¹ M.S.; Joseph H. Warren,¹ B.A.; Robert C. Giles,¹ Ph.D.; and Rick W. Stamb,¹ Ph.D.

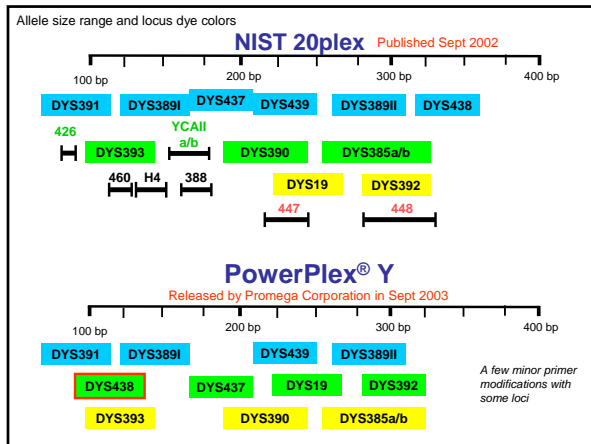
Validation and Uses of a Y-Chromosome STR 10-Plex for Forensic and Paternity Laboratories*

Y-STR Kits

Commercial Y-STR Kits

(Minimal/extended haplotype)	(White et al.)	(Ayub et al.)	(Iida et al.)	(Redd et al.)
DYS19	A7.1 (DYS460)	DYS434	DYS441	DYS446
DYS389I/II	A7.2 (DYS461)	DYS435	DYS442	DYS447
DYS390	A10	DYS436	DYS443	DYS449
DYS391	C4	DYS437	DYS444	DYS450
DYS392	H4	DYS438	DYS445	DYS452
DYS393		DYS439		DYS453
DYS385 a/b				DYS454
YCAII a/b	(Bosch et al.) G09411 (DYS462)	43 (51) Y-STRs (217 with Manfred's)		DYS455
DYS388	Y-PLEX 6 (ReliaGene)			DYS456
DYS425	Y-PLEX 5 (ReliaGene)			DYS458
DYS426	Y-PLEX 12 (ReliaGene)			DYS459 a/b
YCAIII a/b	PowerPlex Y (Promega)			DYS463
	Yfiler (Applied Biosystems)			DYS464 a/b/c/d
				DYS468-DYS645 166 new Y STRs (Manfred Kayser GDB entries)





PowerPlex Y Validation Studies

Available online at www.sciencedirect.com

SCIENCE @ DIRECT

Forensic Science International

Forensic Science International 148 (2005) 1-14
www.elsevier.com/locate/forensint

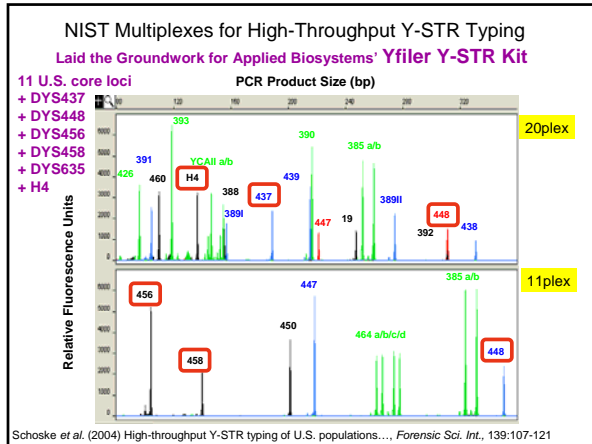
Validation of a male-specific, 12-locus fluorescent short tandem repeat (STR) multiplex[☆]

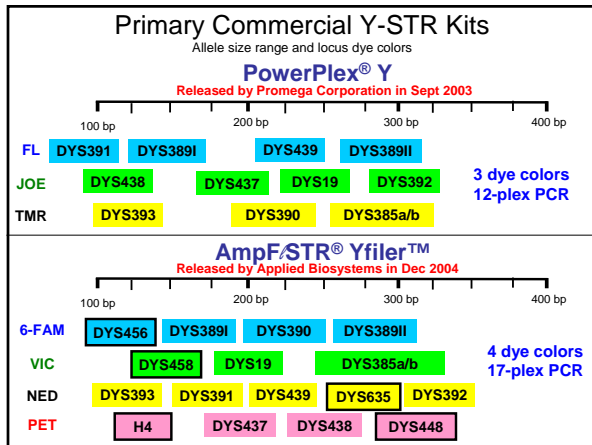
Benjamin E. Krenke^{a,*}, Lori Viculis^b, Melanie L. Richard^c, Mechthild Prinz^d,
Scott C. Milne^e, Carl Ladd^f, Ann Marie Gross^g, Tanis Gomall^h,
J. Roger H. Frappier^c, Arthur J. Eisenberg^b, Charles Barna^b,
Xavier G. Aranda^b, Michael S. Adamowiczⁱ, Bruce Budowleⁱ

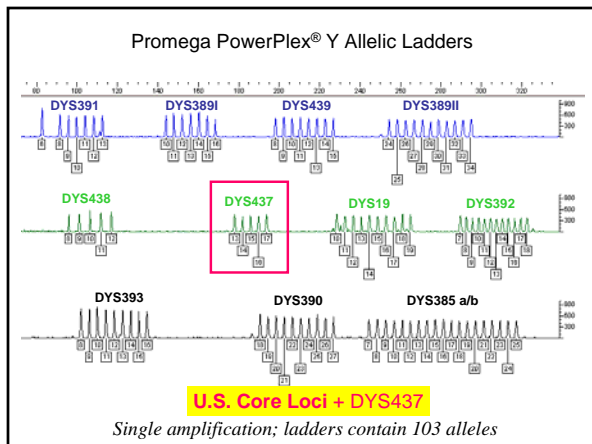
Validation Summary Sheet for PowerPlex Y

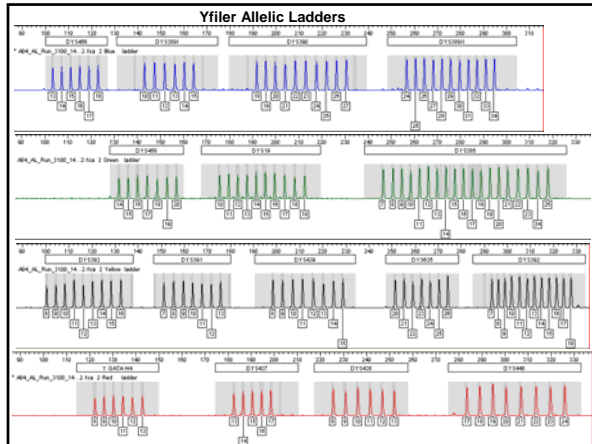
Study Completed (17 studies done)	Description of Samples Tested (performed in 7 labs and Promega)	# Run
Single Source (Concordance)	5 samples x 8 labs	40
Mixture Ratio (male:female)	6 labs x 2 MF mixture series x 11 ratios (1:0.1, 1:1, 1:10, 1:100, 1:300, 1:1000, 0.5:300, 0.25:300, 0.125:300, 0.0625:300, 0.03:300 ng MF)	132
Mixture Ratio (male:female)	6 labs x 2 MM mixtures series x 11 ratios (1:0, 19:1, 9:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:9, 1:19, 0:1)	132
Sensitivity	7 labs x 2 series x 6 amounts (1/0.5/0.25/0.125/0.06/0.03)	84
Non-Human	24 animals	24
NIST SRM	6 components of SRM 2395	6
Precision (ABI 3100 and ABI 377)	10 ladder replicates + 10 sample replicated + 8 ladders + 8 samples for 377	36
Non-Probative Cases	65 cases with 102 samples	102
Stutter	412 males used	412
Peak Height Ratio	N/A (except for DYS385 but no studies were noted)	
Cycling Parameters	5 cycles (28/27/26/25/24) x 8 punch sizes x 2 samples	80
Annealing Temperature	5 labs x 5 temperatures (54/58/60/62/64) x 1 sample	25
Reaction volume	5 volumes (50/25/15/12.5/6.25) x [5 amounts + 5 concentrations]	50
Thermal cycler test	4 models (4802/400/9600/9700) x 1 sample + [3 models x 3 sets x 12 samples]	76
Male-specificity	2 females x 1 titration series (0-500 ng female DNA) x 5 amounts each	10
TaqGold polymerase titration	5 amounts (1.38/2.06/2.75/3.44/4.13 IU) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Primer pair titration	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Magnesium titration	5 amounts (1:1, 25:1, 5:1, 7.5:2 mM Mg) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20

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









Thoughts on Y-Chromosome Issues

- Core loci are selected, commercial kits are now available
- *Y-STRs need to be put into greater use with forensic casework to demonstrate their value*

Research Issues

- Nomenclature for Y-STR alleles in new loci
- Impact of additional loci to resolve most-common types
- Publicly available databases for additional loci
- Statistical issues with combining autosomal and Y-STR information
