The NIJ Conference
June 21, 2011 – Crystal City, VA

NIST Efforts and Future Perspectives with Y-Chromosome Markers

John M. Butler
NIST Applied Genetics Group

Presentation Outline

- **NIST ChrY research and activities**
  - New PCR primers & assay development (Y-STR 10plex/20plex)
  - U.S. population samples studied at ~100 Y-STR markers
  - Allele nomenclature defined
  - SRM 2395 (released in 2003, updated in 2008)
  - Kit beta testing (Reliagene Y-PLEX, PowerPlex Y, and Yfiler)
  - Duplications, deletions, mutations characterized
  - Training presentations prepared and workshops conducted

- **Future trends for ChrY use in human identity testing**
  - Aiding familial searching (similar to genetic genealogy)
  - Fast mutating loci to separate paternal lineage samples

Contributors to NIST ChrY work over the years

John Butler

Initial primer design and testing, duplication studies, STRBase & nomenclature

Margaret Kline

Y-STR allele sequencing & SRM 2395

Pete Vallone

Y-SNP typing

Air Force

Richard Schoske

Y-STR 20plex & SRM 2395

PhD dissertation

AFDIL

Amy Decker

Yfiler testing, additional loci & mutation rates

Pharma

Christian Ruitberg

Y-STR 10plex

NC SBI

Jill Appleby

Y-STR locus-specific brackets

Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards

Past collaborators on chrY projects

Michael Hammer (University of Arizona) – new marker information
Alan Redd (University of Arizona) – new markers & YCC samples
Tom Reid (DNA Diagnostics Center) – father/son samples
David Duewer (NIST) – data analysis support

NIJ National Institute of Justice

"State of the Y STR Assay" in June 2000

From J.M. Butler talk June 1, 2000 at CHI "DNA Forensics" meeting (Springfield, VA)

- A number of multiplex reactions have been reported in the literature but Y STR multiplexes have not reached their potential…

- Very little PCR optimization to-date (most work has been done with the original PCR primer sequences)

- No commercial Y STR kit exists yet (therefore these markers remain inaccessible to the general forensic DNA community)

- New Y STR markers are becoming available which will greatly improve the power of discrimination between unrelated individuals (e.g., DYS385) and these will need to be incorporated into future multiplex sets

What happened in the next few years…

- "Full" Y-chromosome sequence became available in June 2003; over 350 Y-STR loci identified (only ~20 in 2000)
- **Selection of core Y-STR loci** (SWGDAM Jan 2003)
- **Commercial Y-STR kits released**
  - ~~Y PLEX 6,5,12 (2001-03),~~ **PowerPlex Y** (9/03), **Yfiler** (12/04)
- Many population studies were performed and databases were generated with thousands of Y-STR haplotypes
- Forensic casework demonstrations showed the value of Y-STR testing along with court acceptance

NIST Activities with Y-STRs

- **Developed new PCR primers and assays**
  - Butler et al. (2002) *Forensic Sci. Int*. 129: 10-24 {20plex}
  - Schoske et al. (2003) *Anal. Bioanal. Chem.* 375: 333-343 {10plex}
- **Created SRM 2395** (Human Y Chromosome Standard)
  - http://www.cstl.nist.gov/biotech/strbase/SRM2395.htm
- **Characterized duplications and deletions**
  - Butler et al. (2005) *J. Forensic Sci.* 50(4): 853-859
- **Sequenced numerous Y-STR variant alleles**
  - Kline et al. (2011) *FSI Genetics* 5(4):329-332
- **Supplied ~20% of Yfiler 3561 database** (now YHRD & USYSTR)
  - http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm
- **Measured mutation rates with Yfiler loci**
  - Decker et al. (2008) *FSI Genetics* 2(3): e31-e35
- **Performed concordance studies between kits & NIST assays**
- **Produced hundreds of training slides for talks and workshops**

28 publications since 2002 on NIST Y-chromosome work

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

The Role of NIST Y-Chromosome Efforts

Information, Assays, and Standards

- **New Y-STR Primers and Assays** – improves commercial kits
- **Ranking Variable Markers** in U.S. populations – helps in selection of loci for kit design
- **Y-SNP Typing** – compares relative value against Y-STRs
- **NIST SRM 2395** – enables reliable typing with standard samples
- **Nomenclature Standardization** – prevents confusion
- **Duplication & Deletion Studies** – aids interpretation

ELSEVIER

Forensic Science International 129 (2002) 10–24

Forensic Science International

www.elsevier.com/locate/forsciint

A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers

John M. Butler[a,*], Richard Schoske[a,b], Peter M. Vallone[a], Margaret C. Kline[a], Alan J. Redd[c], Michael F. Hammer[c]

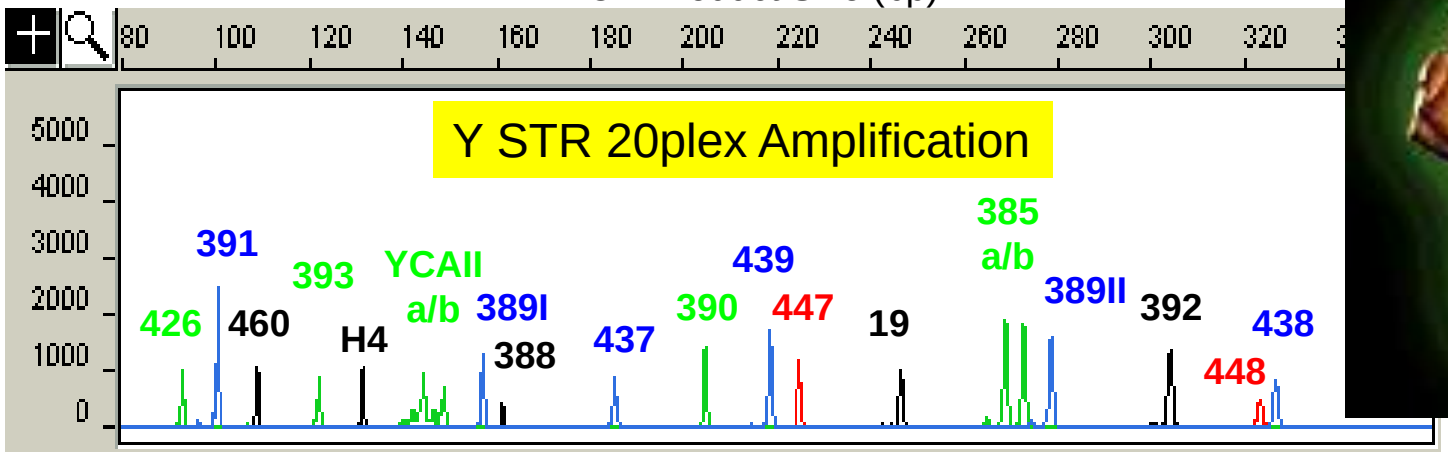
[a]*Biotechnology Division, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899, USA*

[b]*Department of Chemistry, American University, Washington, DC 20016, USA*

[c]*Division of Biotechnology, University of Arizona, Tucson, AZ 85721, USA*

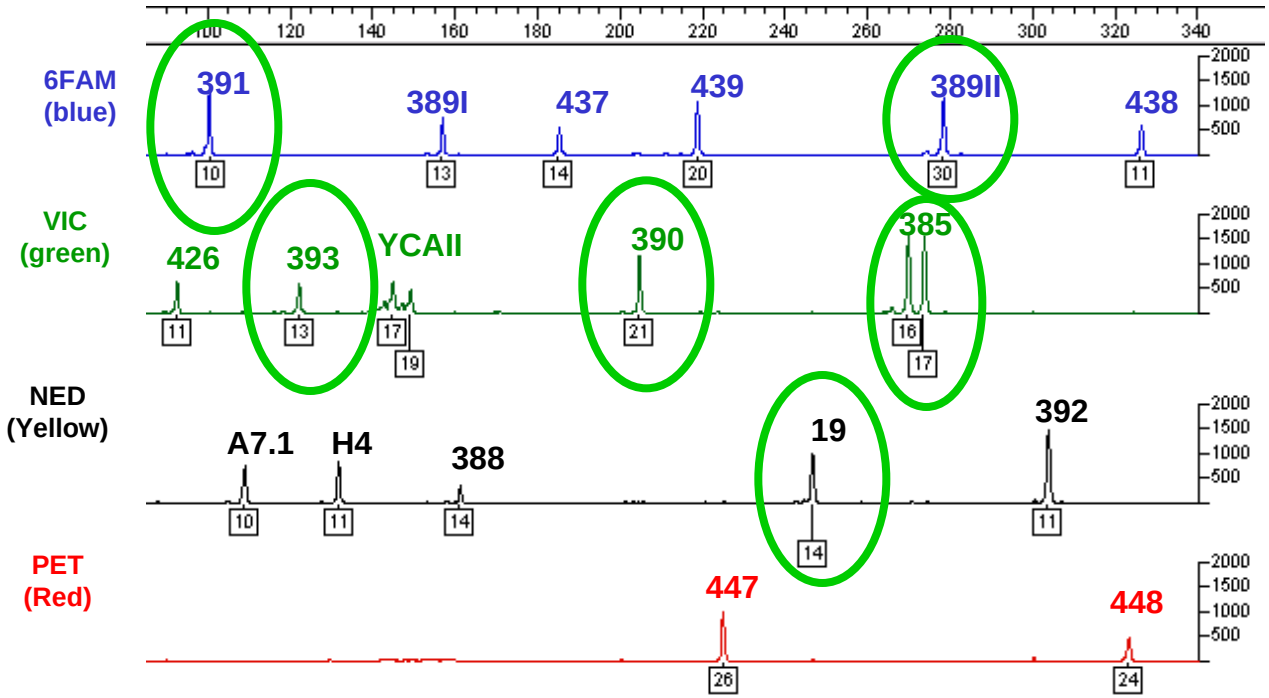
Received 22 February 2002; accepted 8 May 2002

The Manly-plex



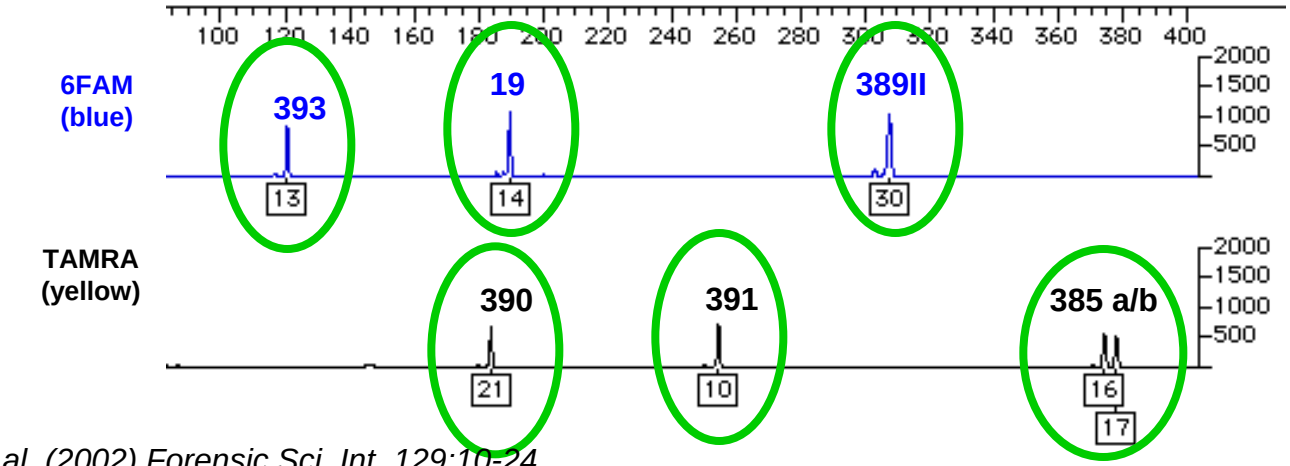
Concordance Studies with Different Primer Sets

Comparison of NIST Y-STR 20plex to Commercial Y-Plex™ 6 Kit (ReliaGene)



Full concordance with >200 samples

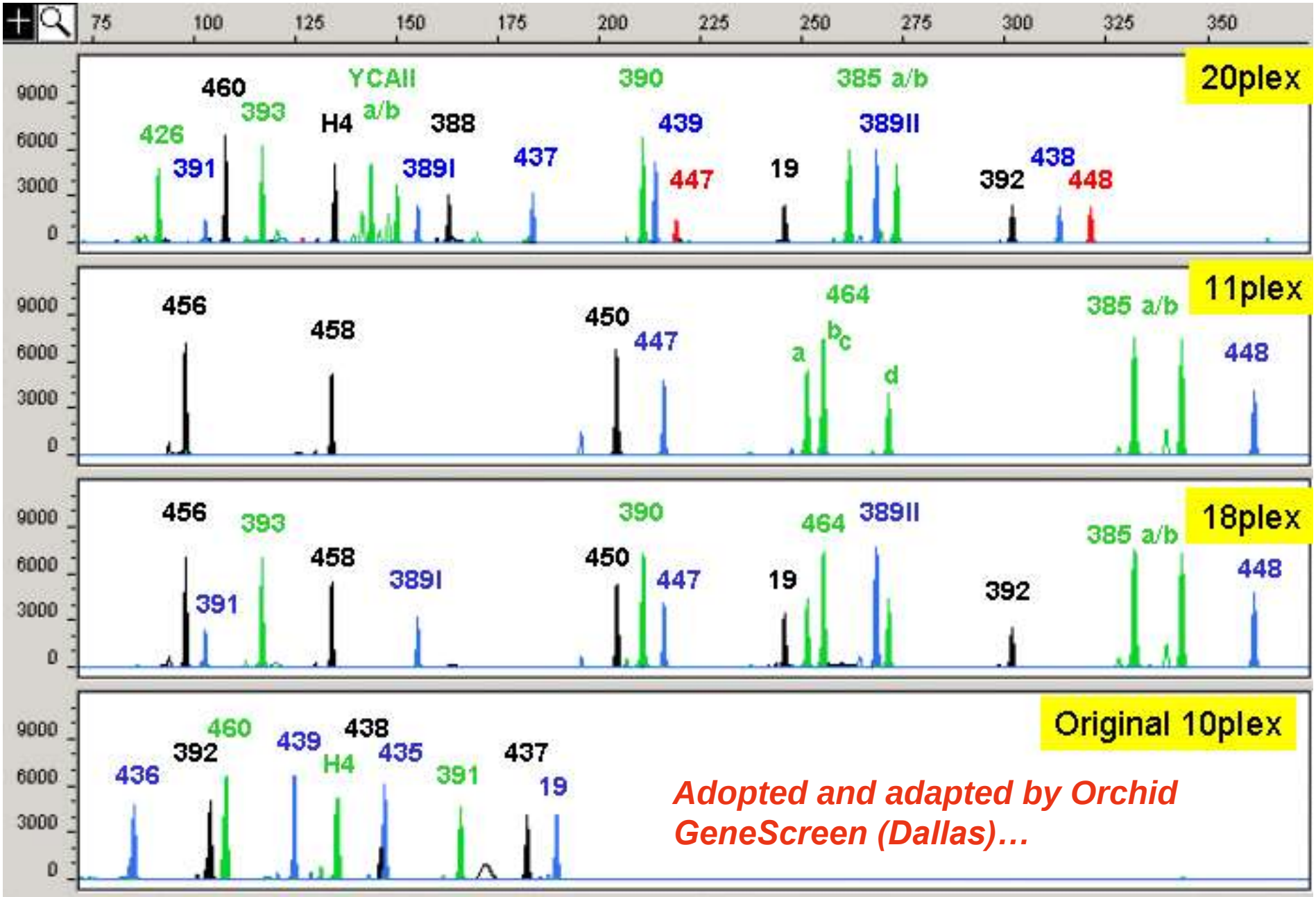
NIST 20plex (in-house assay)



ReliaGene Y-PLEX 6 (commercial kit)

Butler et al. (2002) Forensic Sci. Int. 129:10-24

Some NIST-Developed Y-STR Multiplex Assays



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

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

Our 10plex was used by Orchid Cellmark (Dallas) until other Commercial Y-STR Multiplexes became Available

J. Forensic Sci. 2003; 48(6):1260-1268

J Forensic Sci, November 2003, Vol. 48, No. 6
Paper ID JFS2003114_486
Available online at: www.astm.org

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

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

"The present study validated a Y-STR multiplex originally developed by NIST (the National Institute of Standards and Technology)."

J. Forensic Sci. 2005; 50(5):1116-1118

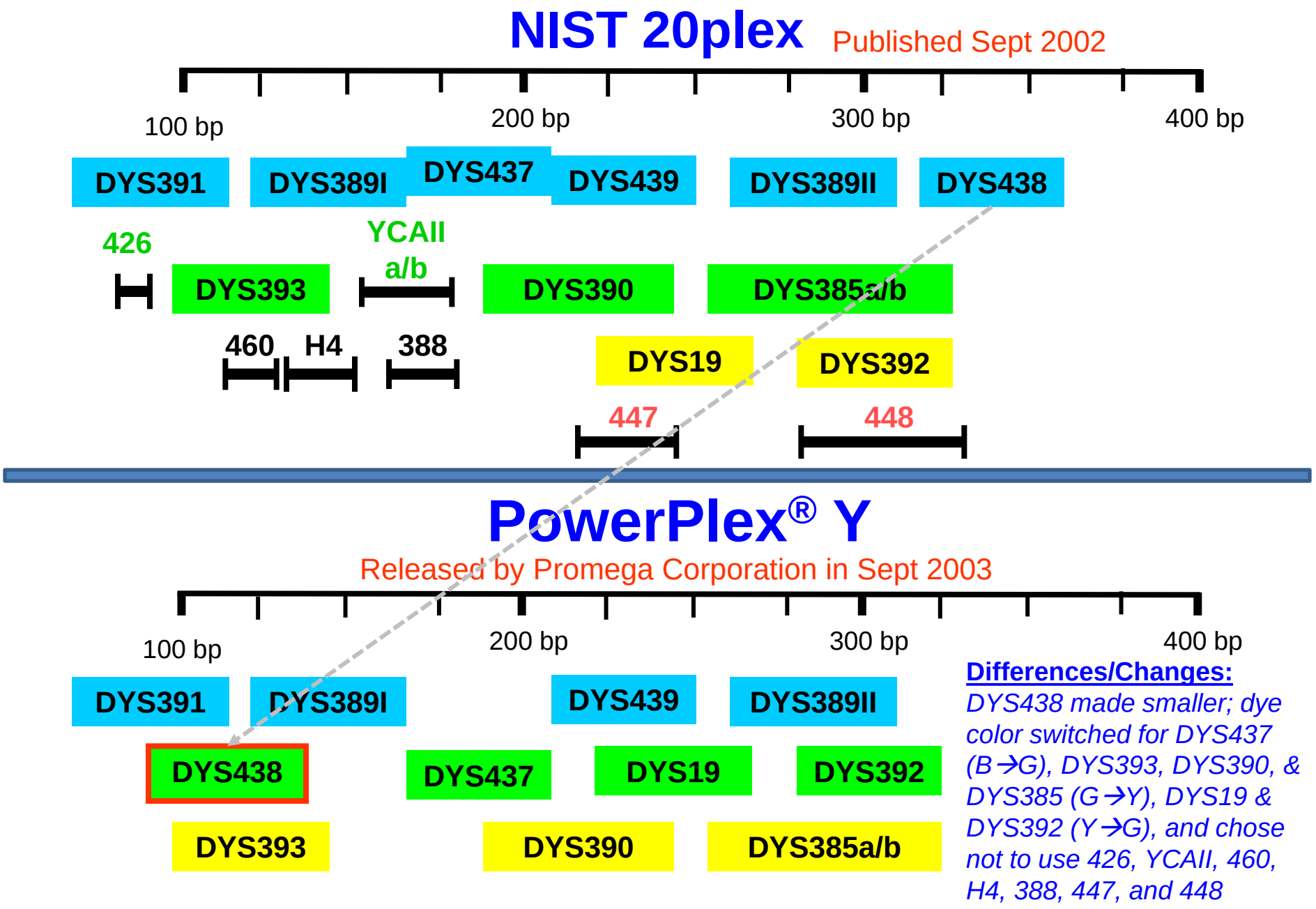
J Forensic Sci, Sept. 2005, Vol. 50, No. 5
Paper ID JFS2005052
Available online at: www.astm.org

TECHNICAL NOTE

Cassie L. Johnson,[1] M.S.; Robert C. Giles,[1] Ph.D.; Joseph H. Warren,[1] B.A.; Judith I. Floyd,[1] B.S.; and Rick W. Staub,[1] Ph.D.

Analysis of Non-Suspect Samples Lacking Visually Identifiable Sperm Using a Y-STR 10-Plex

Promega used our primers or ones very similar (except for DYS438)



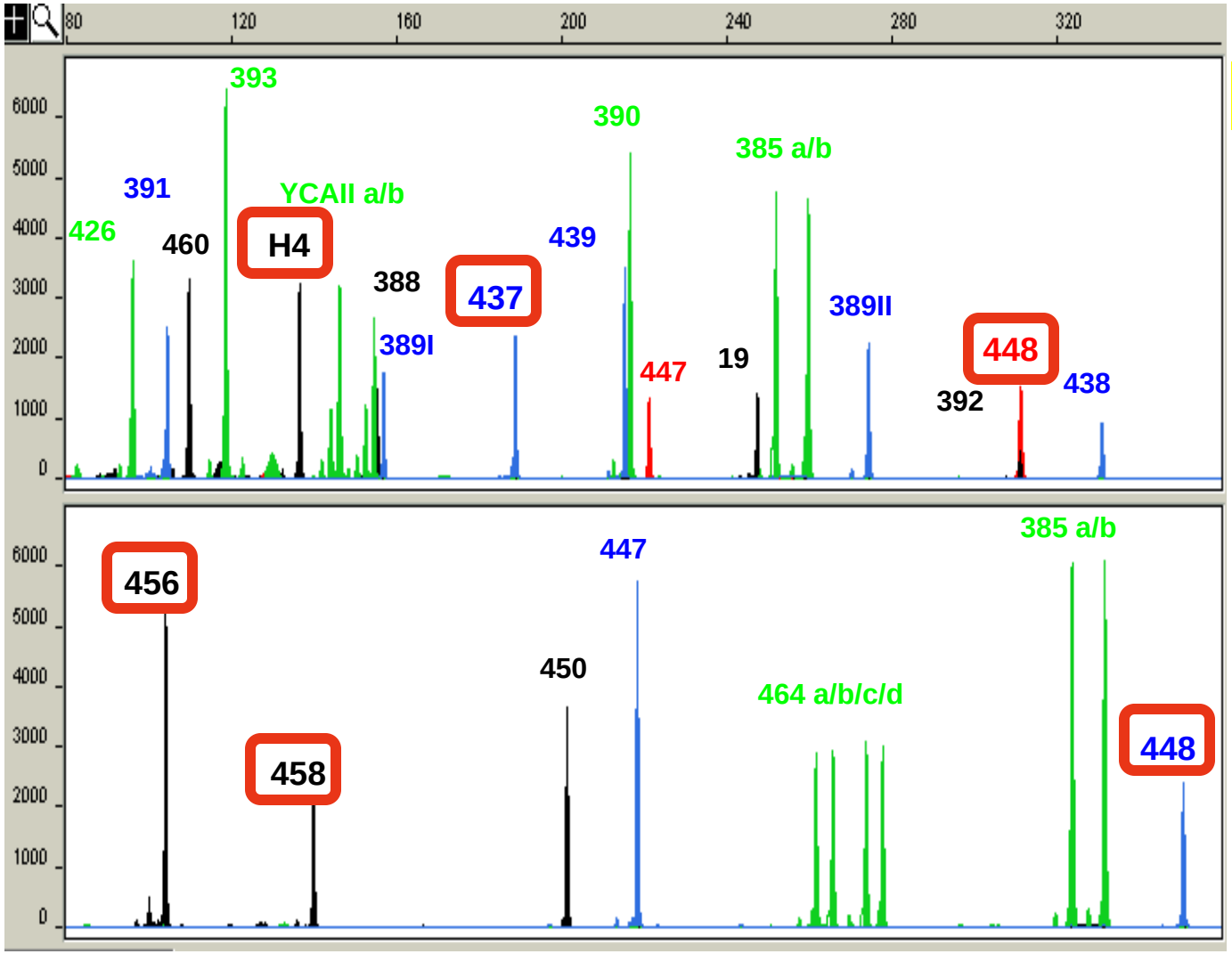
Differences/Changes:

DYS438 made smaller; dye color switched for DYS437 (B→G), DYS393, DYS390, & DYS385 (G→Y), DYS19 & DYS392 (Y→G), and chose not to use 426, YCAII, 460, H4, 388, 447, and 448

NIST Multiplexes for High-Throughput Y STR Typing

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

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



20plex

11plex

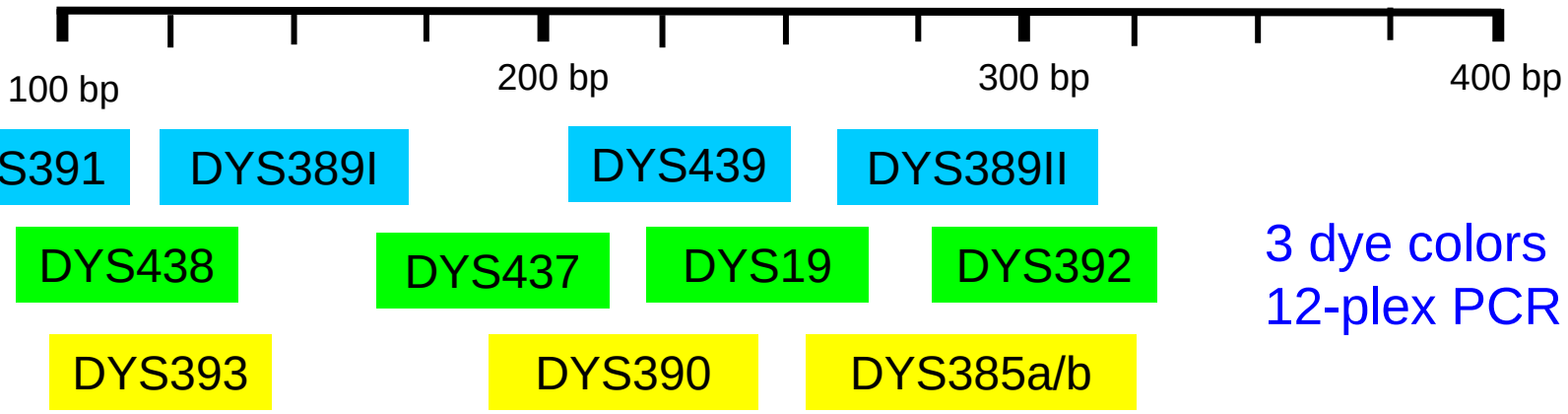
Schoske *et al.* (2004) High-throughput Y-STR typing of U.S. populations…, *Forensic Sci. Int.*, 139:107-121

Today's Primary Commercial Y-STR Kits

Allele size range and locus dye colors

PowerPlex® Y

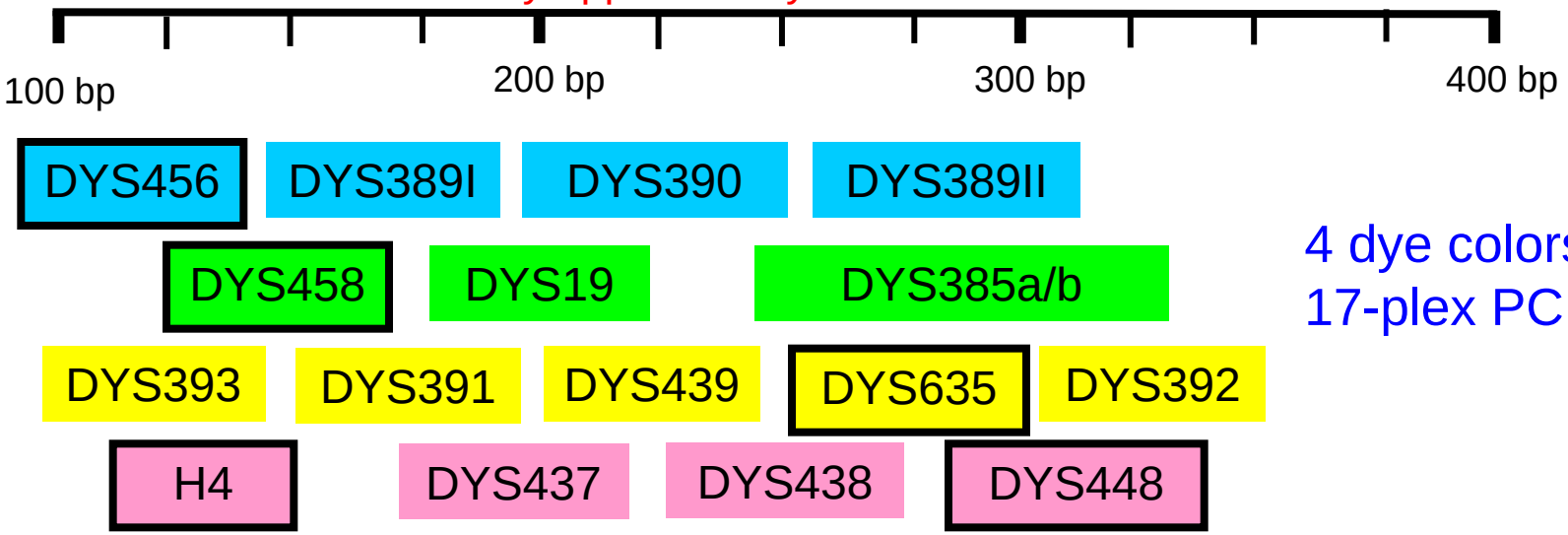
Released by Promega Corporation in Sept 2003



3 dye colors
12-plex PCR

AmpFℓSTR® Yfiler™

Released by Applied Biosystems in Dec 2004

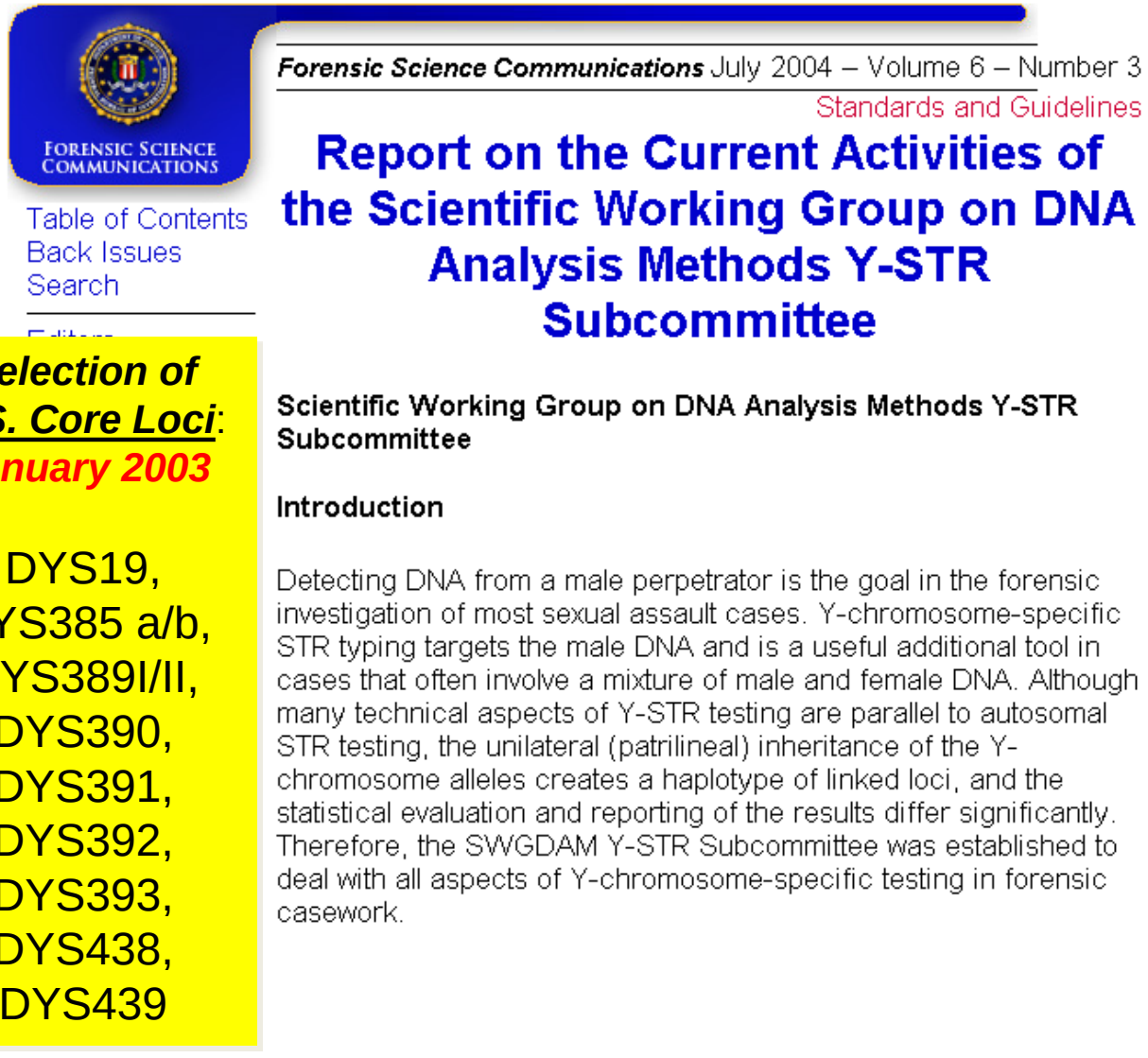


4 dye colors
17-plex PCR

Standardization is Critical for Success and Data Sharing

Needs	How/When Accomplished
Core Y-STR loci	SWGDAM Y-STR Committee selected 11-loci in January 2003
Consistent allele nomenclature	NIST SRM 2395 (2003); kit allelic ladders; ISFG (2006) and NIST (2008) publications
Commercially available Y-STR kits	Early ReliaGene kits (2001-2003); **PowerPlex Y** (2003) and **Yfiler** (2004)
Accessible, searchable population databases for haplotype frequency estimations	**YHRD** (64,237 11-locus haplotypes from 720 worldwide populations) **US YSTR** (18,537 11-locus haplotypes from primarily U.S. population groups)
Interpretation guidelines	SWGDAM Y-STR Interpretation Guidelines published in January 2009 (*will likely be revised soon*)

SWGDAM Y-STR Subcommittee



FORENSIC SCIENCE COMMUNICATIONS

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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

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*: *January 2003

DYS19,
DYS385 a/b,
DYS389I/II,
DYS390,
DYS391,
DYS392,
DYS393,
DYS438,
DYS439

Committee Members

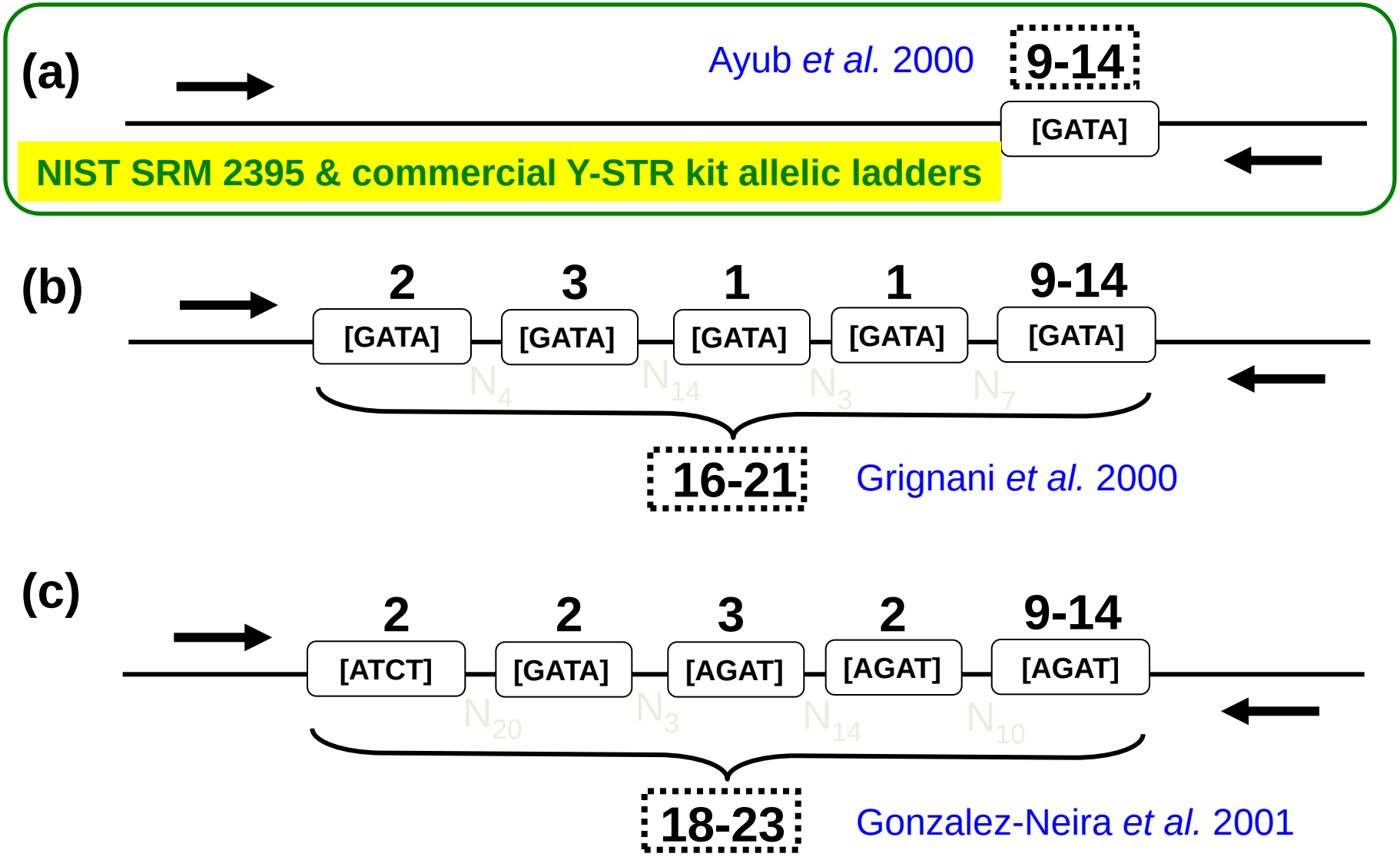
Not all were present for all meetings

July 2002 – Jan 2008

Jack Ballantyne (UCF) – chair
Mecki Prinz (NYC) – co-chair
John Butler (NIST)
Ann Gross (MN)
Jill Smerick (FBI)
Sam Baechtel (FBI)
John Hartmann (Orange Co.)
Jonathan Newman (CFS)
Phil Kinsey (OR)
Gary Sims (CA DOJ)
Demris Lee (AFDIL)
Carl Ladd (CT)
Charles Barna (MI)
Debbie Figarelli (Phoenix PD)

http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/standards/2004_03_standards03.htm

Published Allele Nomenclatures for DYS439



ISFG 2006 DNA Commission on Y-STRs

Defined allele nomenclature for **11 core Y-STRs** and **63 additional loci** *that were known at the time*

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Forensic Science International 157 (2006) 187–197
Forensic Science International
www.elsevier.com/locate/forsciint

Short communication

DNA Commission of the International Society of Forensic Genetics (ISFG): An update of the recommendations on the use of Y-STRs in forensic analysis☆

L. Gusmão [a], J.M. Butler [b], A. Carracedo [c], P. Gill [d], M. Kayser [e], W.R. Mayr [f], N. Morling [g], M. Prinz [h], L. Roewer [i], C. Tyler-Smith [j], P.M. Schneider [k,*]

Subject Matter Experts

Leonor Gusmão (Portugal)
John Butler (USA)
Peter Gill (UK)
Manfred Kayser (Netherlands)
Lutz Roewer (Germany)
Chris Tyler-Smith (UK)

ISFG Board Members

Angel Carracedo (Spain)
Wolfgang Mayr (Austria)
Niels Morling (Denmark)
Mecki Prinz (USA)
Peter Schneider (Germany)

Y-STR Allele Nomenclature Further Addressed

http://www.jogg.info/42/files/butler.pdf

Journal of Genetic Genealogy, 4(2):125-148, 2008

Addressing Y-Chromosome Short Tandem Repeat Allele Nomenclature

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

Abstract

A total of about 120 different Y-chromosome short tandem repeat (Y-STR) markers are currently used by different genetic genealogy testing laboratories. In some cases, different laboratories may designate the same Y-STR allele with two different nomenclatures, making data comparison difficult and frustrating due to needed conversion factors. This article explains how STR allele nomenclatures are typically determined, where ambiguity may exist, and how measurement accuracy and consistency can be promoted through use of common reference materials across all genetic genealogy testing laboratories. Comparisons are made to forensic DNA testing and the benefits of a common set of loci and STR allele nomenclatures.

Some differences in Y-STR allele nomenclature were noted across 8 different genetic genealogy labs in 2008

	Genetic Genealogy Test Providers Result Conversions Needed							
Marker	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
DYS389I	=	=	=	=	=	=	+3	=
DYS389II	=	=	=	=	=	Add DYS389I value	Add DYS389I value +3	=
GATA-H4	-10	-9	-10	+1	=	NT	NT	+1

NIST U.S. Population Samples

663 males (anonymous; self-identified ethnicities)

260 Caucasians
260 African Americans
140 Hispanics
3 Asians

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

Stock tubes

On average ~80 μg total extracted genomic DNA

Working tubes

Working plates

Working tubes/plates 0.5 ng/μL or 1 ng/μL

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

To date: (**>500,000 allele calls across these samples**)

STRs: **24 autosomal STR loci with commercial kits** including Identifiler, PP16, MiniFiler, ESI/ESX 17, NGM, ESSplex, IDplex, and some forthcoming kits
Y-STRs: Yfiler, NIST 20plex & 11plex; **~100 Y-STRs on a sub-set**
SNPs: Orchid 70 autosomal SNPs on a sub-set
Y-SNPs: 50 markers on a subset of the samples
mtDNA: full control region sequences (AFDIL), whole genomes (AFDIL)
mtDNA SNPs: Roche LINEAR ARRAYs
miniSTRs: MiniFiler; CODIS miniSTRs (Ohio U); NIST NC01-NC09 assays; NIST 23plex

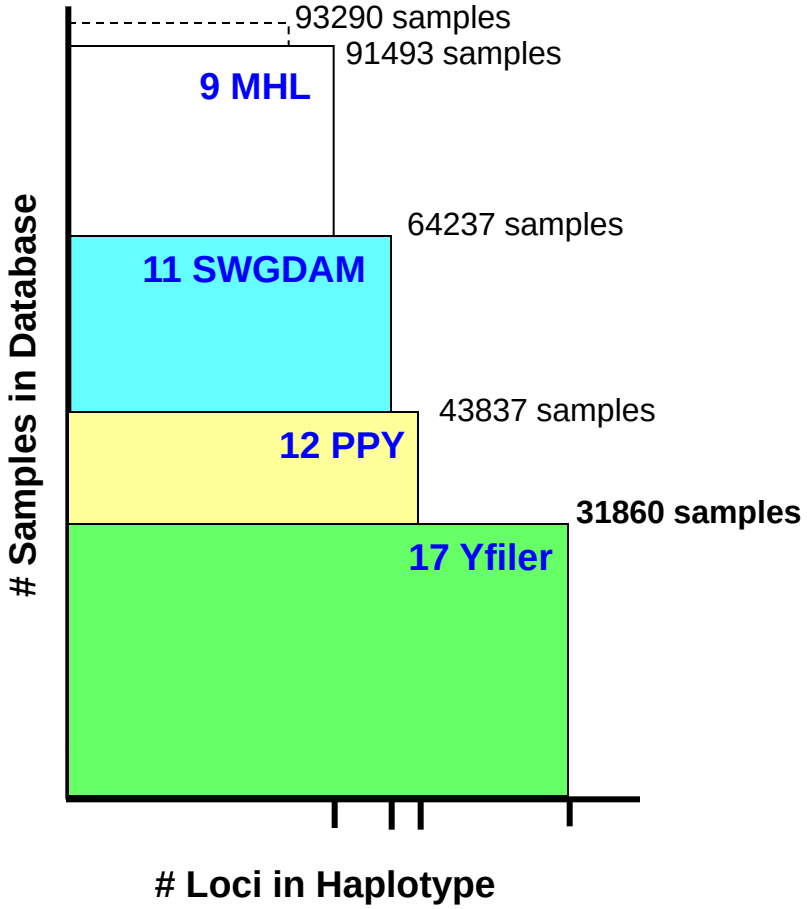
http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm

YHRD

http://www.yhrd.org

Release 36

720 Populations (106 countries)

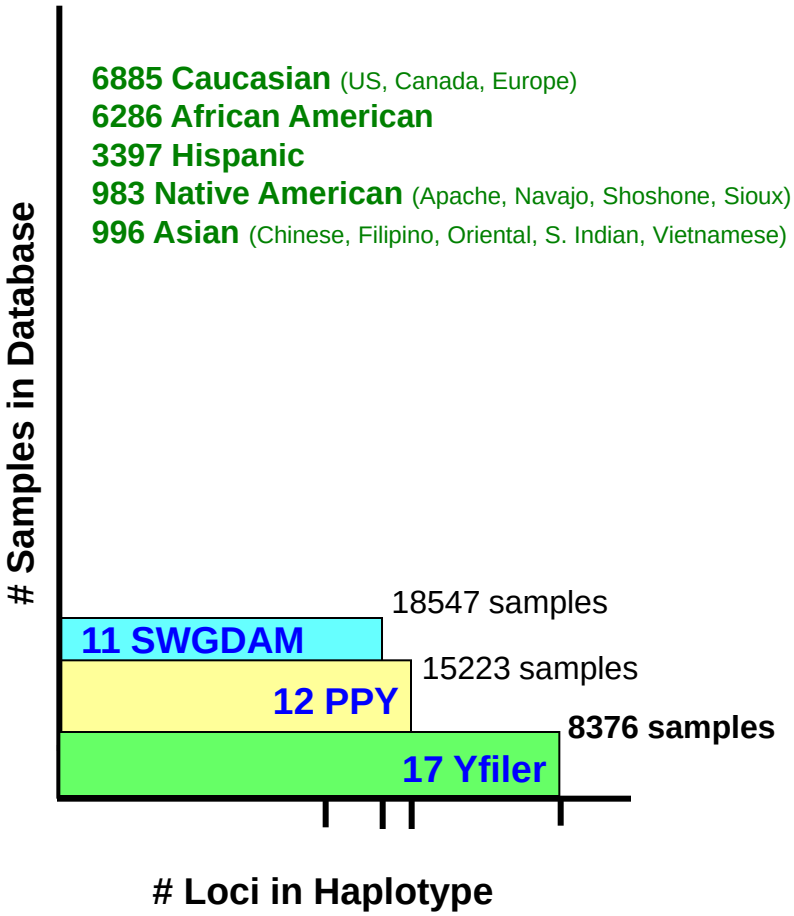


US YSTR

http://www.usystrdatabase.org

Release 2.4

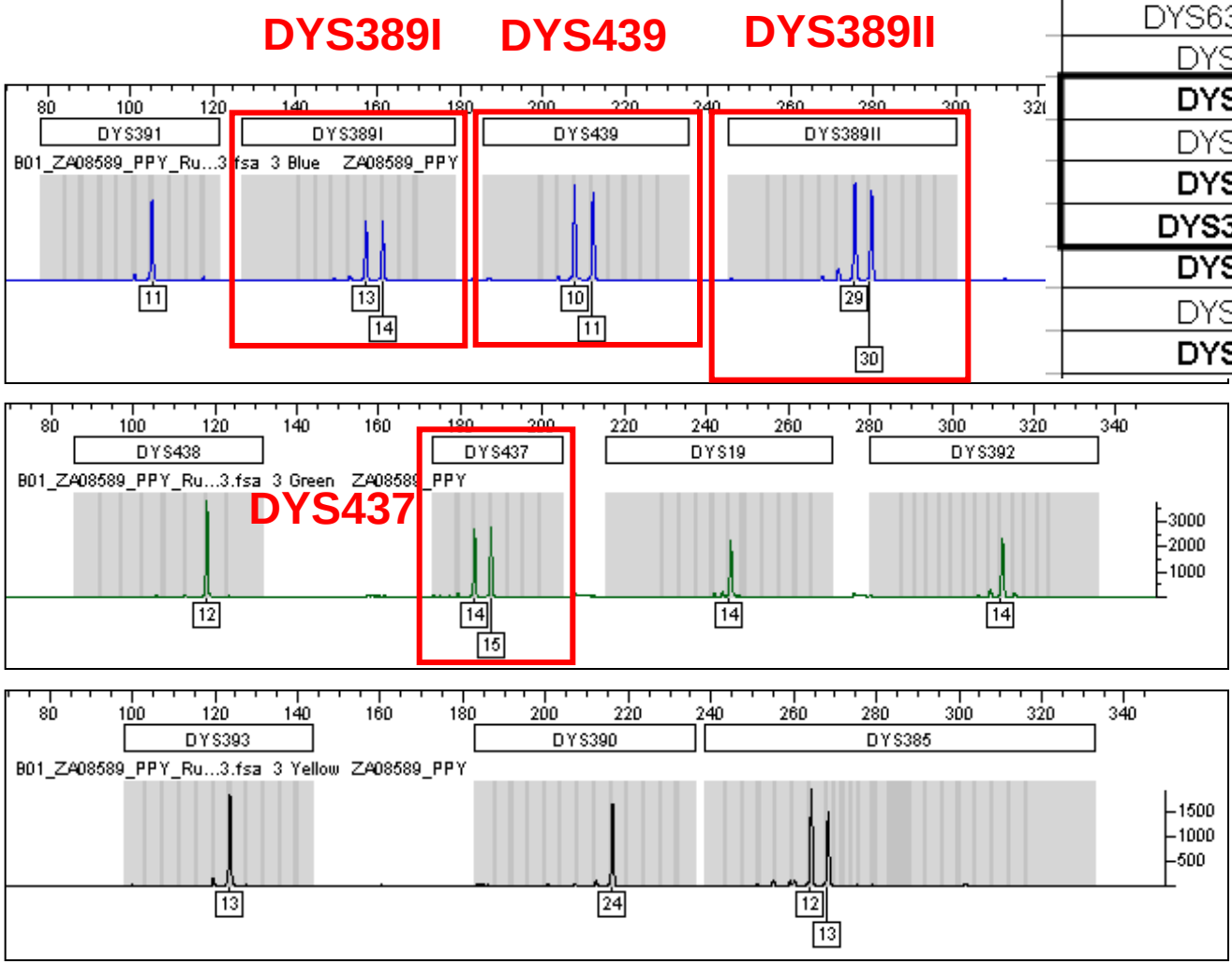
Focus is on U.S. samples



Updated from J.M. Butler (2011) *Advanced Topics in Forensic DNA Typing: Methodology*, Figure 13.10

Duplication at Multiple Loci with Single-Source Sample

PowerPlex Y data



Y-chromosome mapping

q-arm

Y STR Marker	Position (Mb)
DYS391	13.413
DYS635 (C4)	13.690
DYS434	13.777
DYS437	13.778
DYS435	13.807
DYS439	13.826
DYS389 I/II	13.923
DYS388	14.057
DYS442	14.071
DYS438	14.248

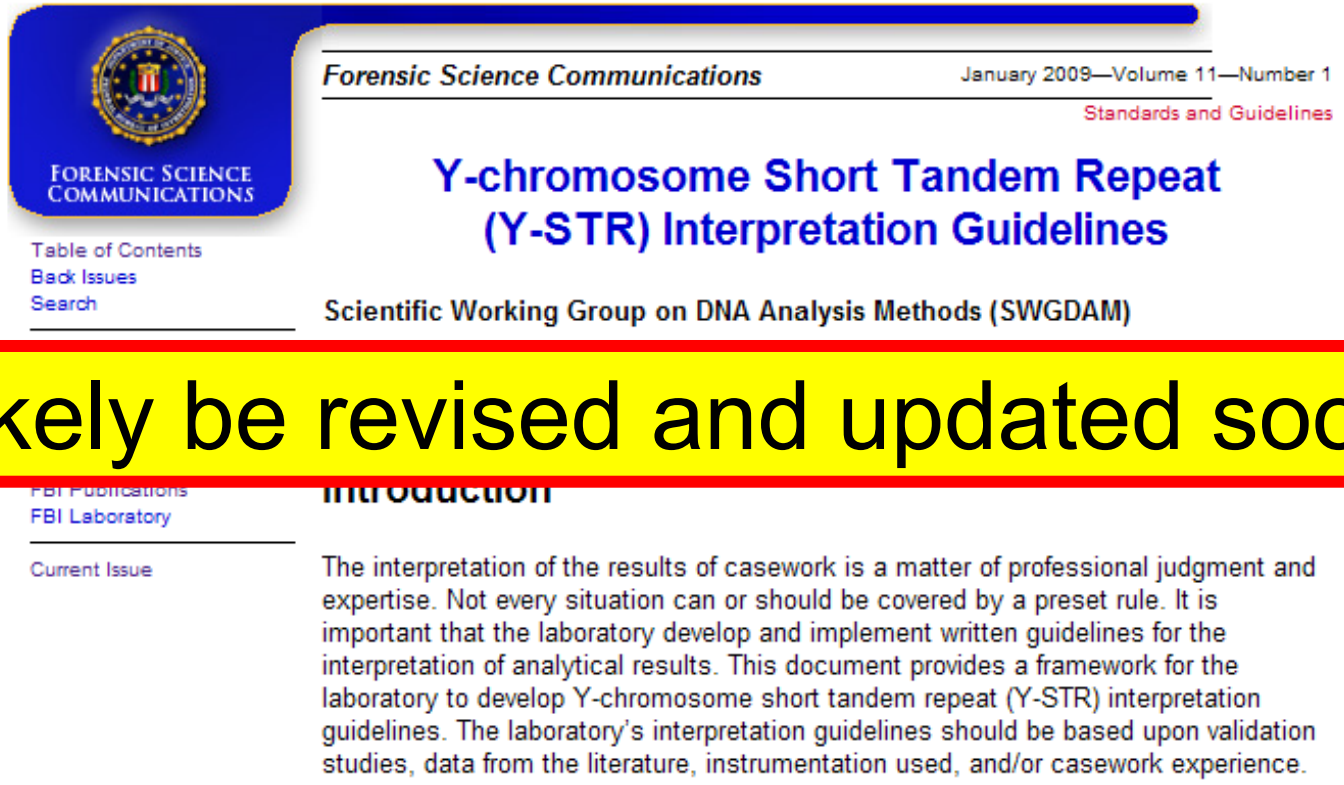
Entire region of Y-chromosome has likely been duplicated and then diverged

Most duplications have a single repeat spread in allele patterns

Butler et al. (2005) Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation *J. Forensic Sci.* 50(4): 853-859

SWGDAM Y-STR Interpretation Guidelines

- **Approved July 15, 2008 by SWGDAM**
- Published in *Forensic Sci. Comm.* Jan 2009 issue



FORENSIC SCIENCE COMMUNICATIONS

Forensic Science Communications January 2009—Volume 11—Number 1

Standards and Guidelines

Y-chromosome Short Tandem Repeat (Y-STR) Interpretation Guidelines

Scientific Working Group on DNA Analysis Methods (SWGDAM)

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Current Issue

Introduction

The interpretation of the results of casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a preset rule. It is important that the laboratory develop and implement written guidelines for the interpretation of analytical results. This document provides a framework for the laboratory to develop Y-chromosome short tandem repeat (Y-STR) interpretation guidelines. The laboratory's interpretation guidelines should be based upon validation studies, data from the literature, instrumentation used, and/or casework experience.

Will likely be revised and updated soon…

http://www.fbi.gov/hq/lab/fsc/backissu/jan2009/standards/2009_01_standards01.htm

New Y-STR Interpretation Information

Forensic Science International: Genetics 5 (2011) 78–83

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

The interpretation of lineage markers in forensic DNA testing

J.S. Buckleton [a], M. Krawczak [b], B.S. Weir [c,*]

[a] ESR Ltd, Private Bag 92021, Auckland, New Zealand
[b] Institute of Medical Informatics and Statistics, Christian-Albrechts University, 24105 Kiel, Germany
[c] Department of Biostatistics, University of Washington, Box 357232, Seattle, WA 98195-7232, USA

This article reviews and discusses a number of highly relevant topics:

- **Normal vs. binomial (Clopper-Pearson) sampling distributions**
- **Theta corrections**
- **Handling rare haplotypes (Charles Brenner approach)**
- **Combination of lineage and autosomal markers**

Predictions for **the Future** of Y-STR Analysis

- Continued use with casework (with excess female DNA)
- Improved frequency estimates with growing Y-STR databases
  - YHRD now at **64,237 11-locus profiles** (31,860 Yfiler)
  - USYSTR has **18,547 11-locus profiles** (8,376 Yfiler)
- New Y-STR kits with additional loci?
- Use with familial searching to eliminate false positives
  - Myers, S.P. et al. (2010) *FSI Genetics* (*in press*) – describes CA DOJ familial searching
- Use of fast mutating loci to help resolve paternal lineages (e.g., to separate brothers or father/son haplotypes)
  - Ballantyne, K.N. et al. (2011) *FSI Genetics* (*in press*)

Rapidly Mutating Y-STRs for Separating Male Relatives

Locus (average mutation rate)

DYS449 (1.2%)
DYS518 (1.8%)
DYS547 (2.4%)
DYS570 (1.2%)
DYS576 (1.4%)
DYS612 (1.4%)
DYS626 (1.2%)
DYS627 (1.2%)

G Model
FSIGEN-744; No. of Pages 11
ARTICLE IN PRESS
Forensic Science International: Genetics xxx (2011) xxx–xxx
Contents lists available at ScienceDirect
Forensic Science International: Genetics
journal homepage: www.elsevier.com/locate/fsig

A new future of forensic Y-chromosome analysis: Rapidly mutating Y-STRs for differentiating male relatives and paternal lineages

Kaye N. Ballantyne[a,1,2], Victoria Keerl[a,1,3], Andreas Wollstein[a,b], Ying Choi[a], Sofia B. Zuniga[c], Arwin Ralf[a], Mark Vermeulen[a], Peter de Knijff[c], Manfred Kayser[a,*]

[a] Department of Forensic Molecular Biology, Erasmus MC University Medical Center Rotterdam, 3000 CA Rotterdam, The Netherlands
[b] Cologne Center for Genomics, University of Cologne, D-50674 Cologne, Germany
[c] Department of Human Genetics, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

DYF387S1 (1.6%)
DYF399S1 (7.7%)
DYF403S1 a/b (3.1/1.2%)
DYF404S1 (1.3%)
DYS526 a/b (1.3%)

DYS458 (0.64%) is highest in Yfiler loci where average is ~0.2%

The American Journal of Human Genetics 87, 341–353, September 10, 2010 ARTICLE

Mutability of Y-Chromosomal Microsatellites: Rates, Characteristics, Molecular Bases, and Forensic Implications

Kaye N. Ballantyne,[1] Miriam Goedbloed,[1] Rixun Fang,[2] Onno Schaap,[1] Oscar Lao,[1] Andreas Wollstein,[1,3] Ying Choi,[1] Kate van Duijn,[1] Mark Vermeulen,[1] Silke Brauer,[1,4] Ronny Decorte,[5] Micaela Poetsch,[6] Nicole von Wurmb-Schwark,[7] Peter de Knijff,[8] Damian Labuda,[9] Hélène Vézina,[10] Hans Knoblauch,[11] Rüdiger Lessig,[12] Lutz Roewer,[13] Rafal Ploski,[14] Tadeusz Dobosz,[15] Lotte Henke,[16] Jürgen Henke,[16] Manohar R. Furtado,[2] and Manfred Kayser[1,*]

Using Y-STRs with a higher mutation rate, father-son and brother pairs can sometimes be distinguished

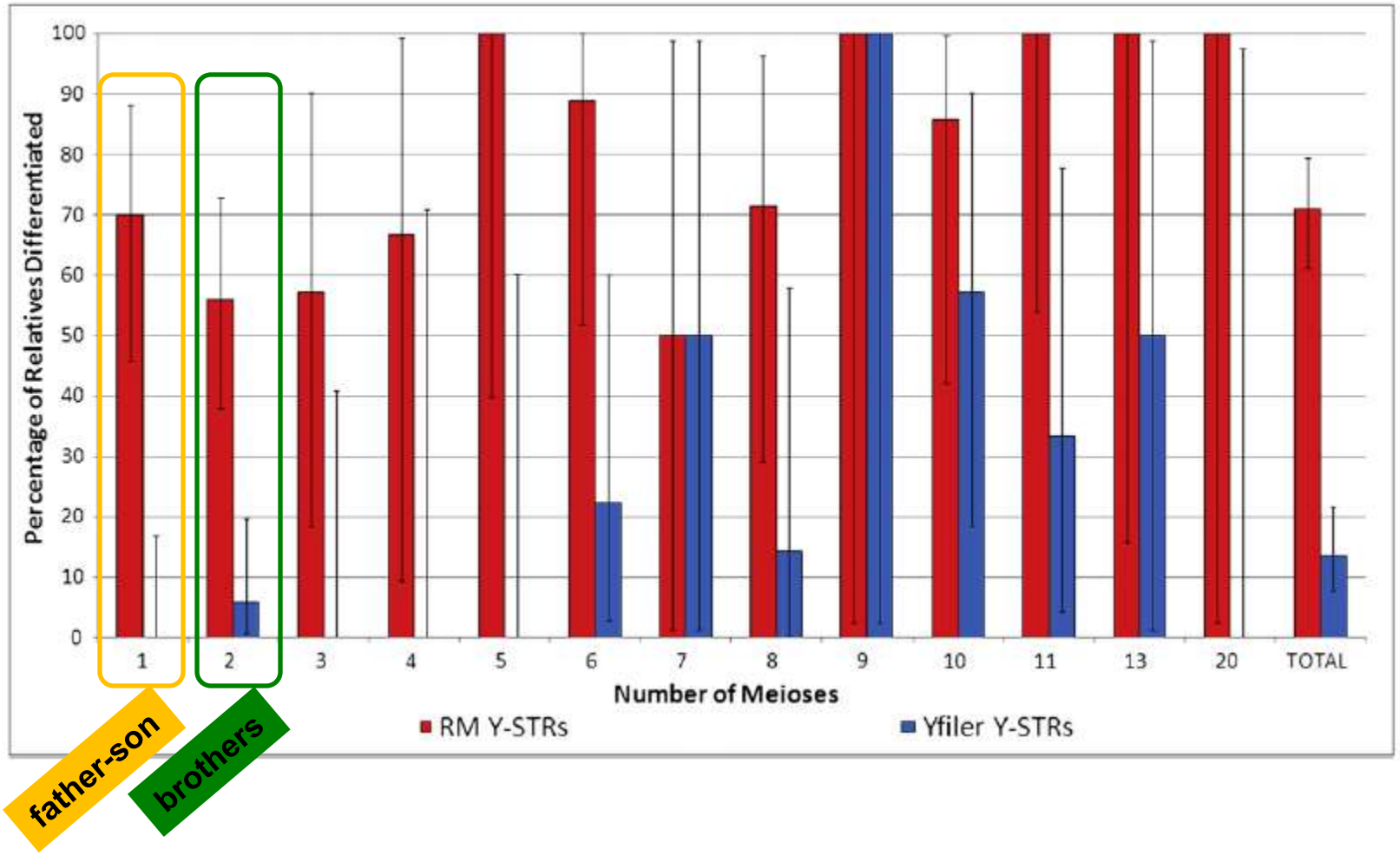

Figure 4. Male Relative Differentiation with Newly Identified 13 RM Y-STRs and Commonly Used 17 Yfiler Y-STRs

Results from differentiating between male relatives from analyzing 103 pairs from 80 male pedigrees, sorted according to the number of generations separating pedigree members, based on 13 RM Y-STRs (in red) and 17 Yfiler Y-STRs (in blue). Error bars represent 95% binomial confidence intervals. Note that these samples are independent from the father-son pairs initially used to establish the Y-STR mutation rates.

The American Journal of Human Genetics 87, 341–353, September 10, 2010

Summary and Lessons Learned

- Y-STR analysis can aid forensic casework and eliminate false positives in familial DNA searches (might help reduce false negatives with familial searching if entire database was run with Y-STRs)

- Careful primer design is important to avoid X-chromosome homology or Y-chromosome duplications

- Regions of the Y-chromosome can be duplicated or deleted causing Y-STRs to be duplicated or deleted

- Standardization of core loci, consistent allele nomenclature, and central haplotype frequency databases along with commercial Y-STR kits and SWGDAM interpretation guidelines has benefited the forensic DNA community's application of Y-chromosome markers

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

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