



# Highly Multiplexed Assays for Measuring Polymorphisms on the Y-Chromosome

**International Society of Forensic Genetics**

**August 30, 2001**

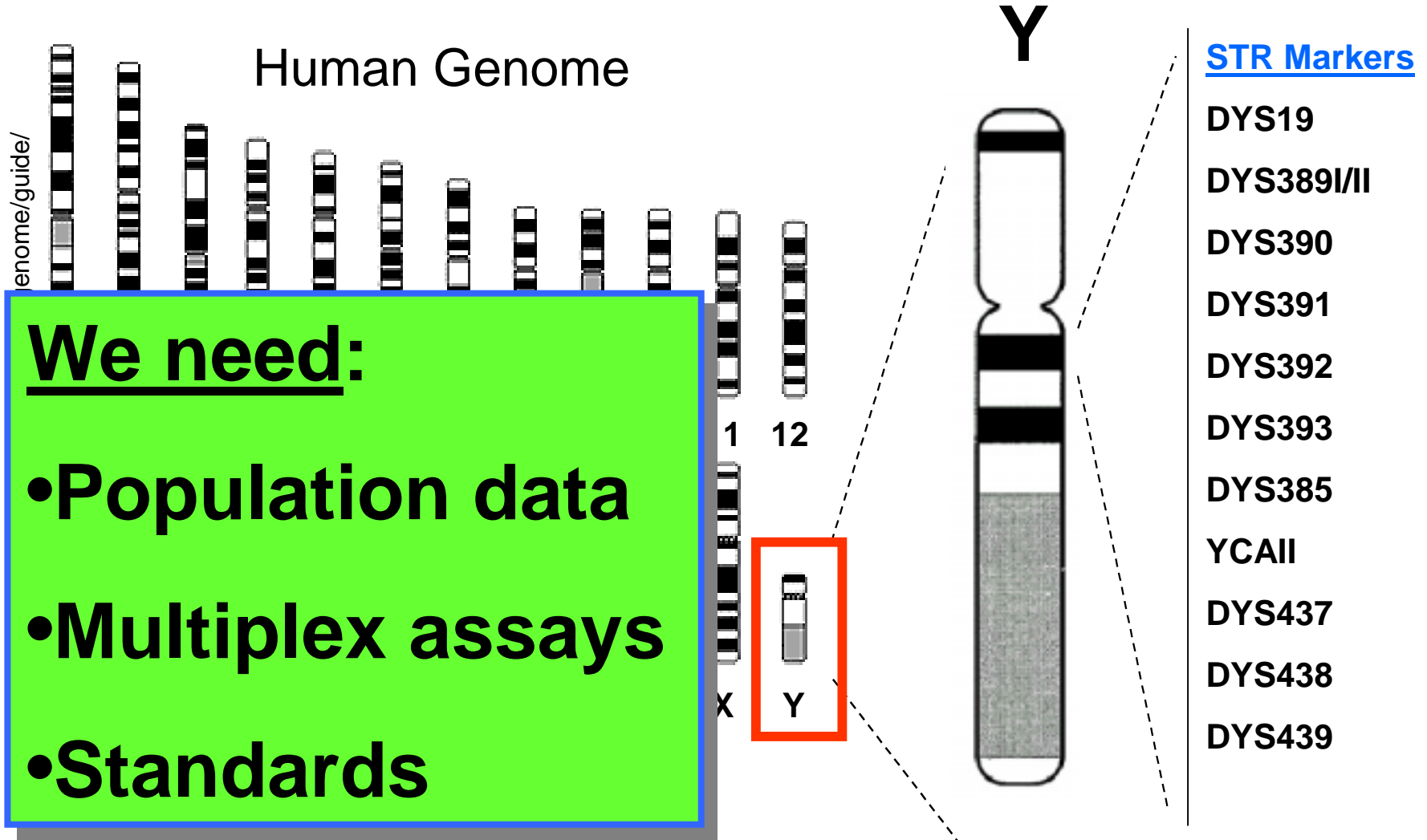
John Butler

Rich Schoske

Pete Vallone

**There is a growing interest in the Y-chromosome to aid forensic and paternity testing...**

(>50 presentations here at ISFG on Y markers)



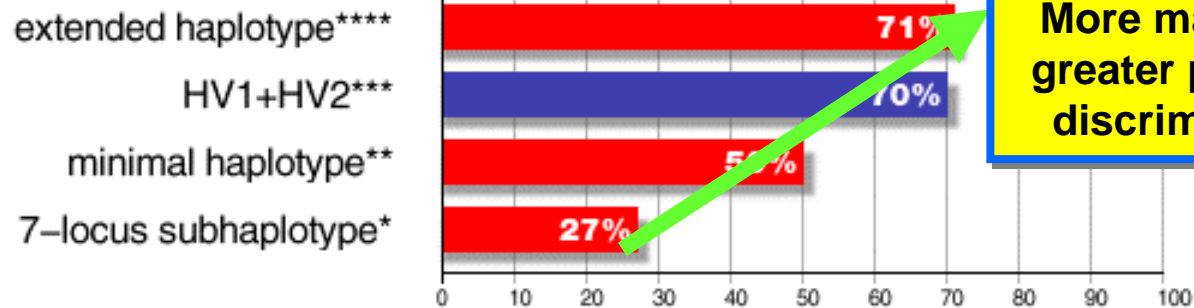
# European Y-STR Haplotype Reference Database

Created by Sascha Willuweit and Lutz Roewer  
Institute of Legal Medicine, Humboldt-Universität Berlin, Germany  
in cooperation with Michael Krawczak (Cardiff), Manfred Kayser (Leipzig) and Peter de Knijff (Leiden)

This database has been accessed **14809** times since 01/01/2000. Last haplotype entry **3/26/2001**

Current state of the database: **45** European population samples  
**5529** minimal haplotypes **2196** of these are extended haplotypes

DYS19    DYS389I    DYS389II    DYS390    DYS391    DYS392    DYS393    DYS385    YCAII



**More markers =  
greater power of  
discrimination**

n = 850 mt-DNA D-Loop sequences (data kindly provided by the  
Institute of Legal Medicine Magdeburg, Germany)

n = 2196 extended European haplotypes logged in the database



<http://www.ystr.org/europe/>

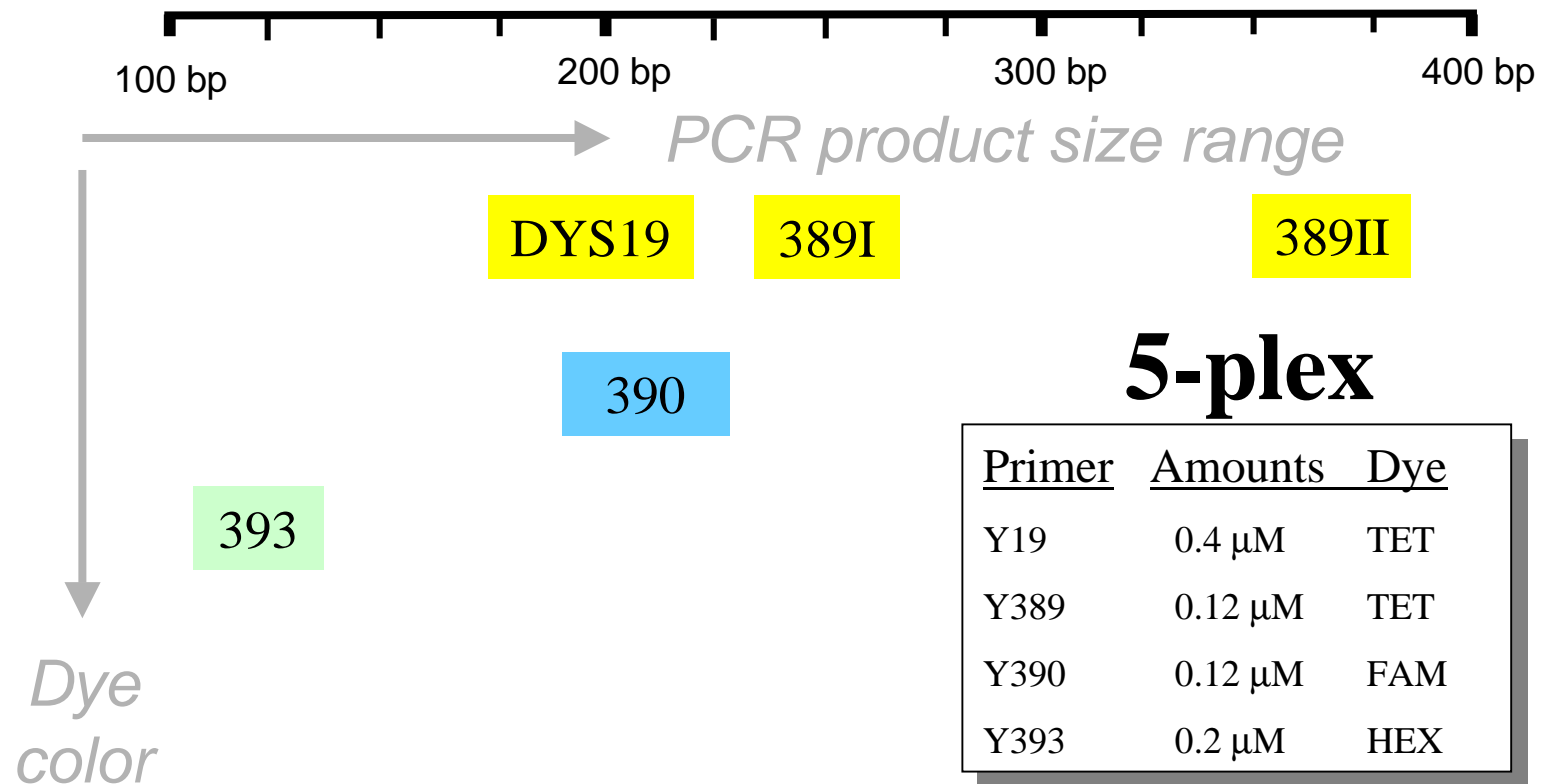
## Standard Way to Type “Extended Haplotype”

- GDB primers
- 2-3 multiplexes for minimal haplotype
- DYS385 run separately sometimes
- YCAII run separately to obtain extended haplotype
- Different PCR conditions
- Primers often require titrations (10 fold difference in amounts) in order to establish reasonable balance between Y STR PCR products

**Published primers were not designed to work together (originally used in single-plex PCRs)**

# Commonly Used Y STR Multiplex Assay

Higher level multiplexes are needed to improve the power of discrimination for Y chromosome DNA tests



Inefficient use of space across size range and dye color

# Assay Development Goals

*Discussion with M. Prinz December 2000*

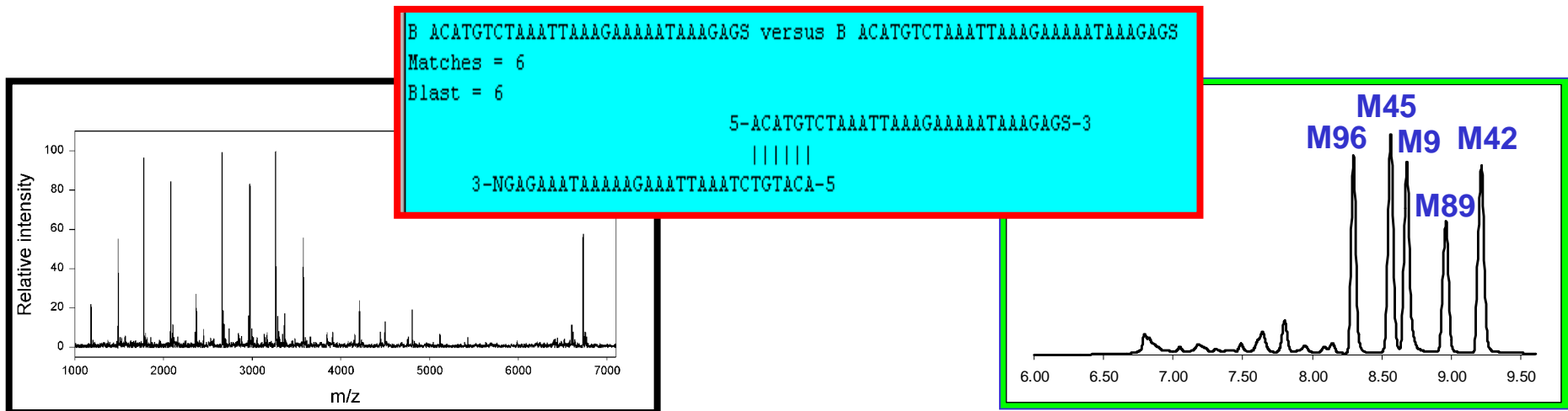
- **Single tube amplification** of all loci in minimal (9-loci) or extended (11-loci) European Y STR haplotypes
- **Incorporation of additional polymorphic markers** recently published
- **No female amplification products** (avoid X chromosome homology)
- Spacing between loci in same color to allow additional undiscovered alleles to be accommodated
- Similar concentration of primers to produce balanced amplification products
- Sensitivity to <100 pg male DNA with 28 cycle PCR

# Development Strategy for Y STR Multiplexes

- Careful definition of allele ranges
  - Literature searches of over 200 papers to locate all known alleles
  - Evaluation of diverse population samples to search for rare alleles (M. Hammer cell lines)
    - Permits markers to be packed together more closely in a single dye color
- Avoiding polymorphic nucleotides in primer binding sites
  - Alignment of multiple GenBank entries
    - Permits primers to be designed with less risk of null alleles
- Incorporation of newly developed NIST multiplex assay design and testing tools

# Tools for Multiplexing

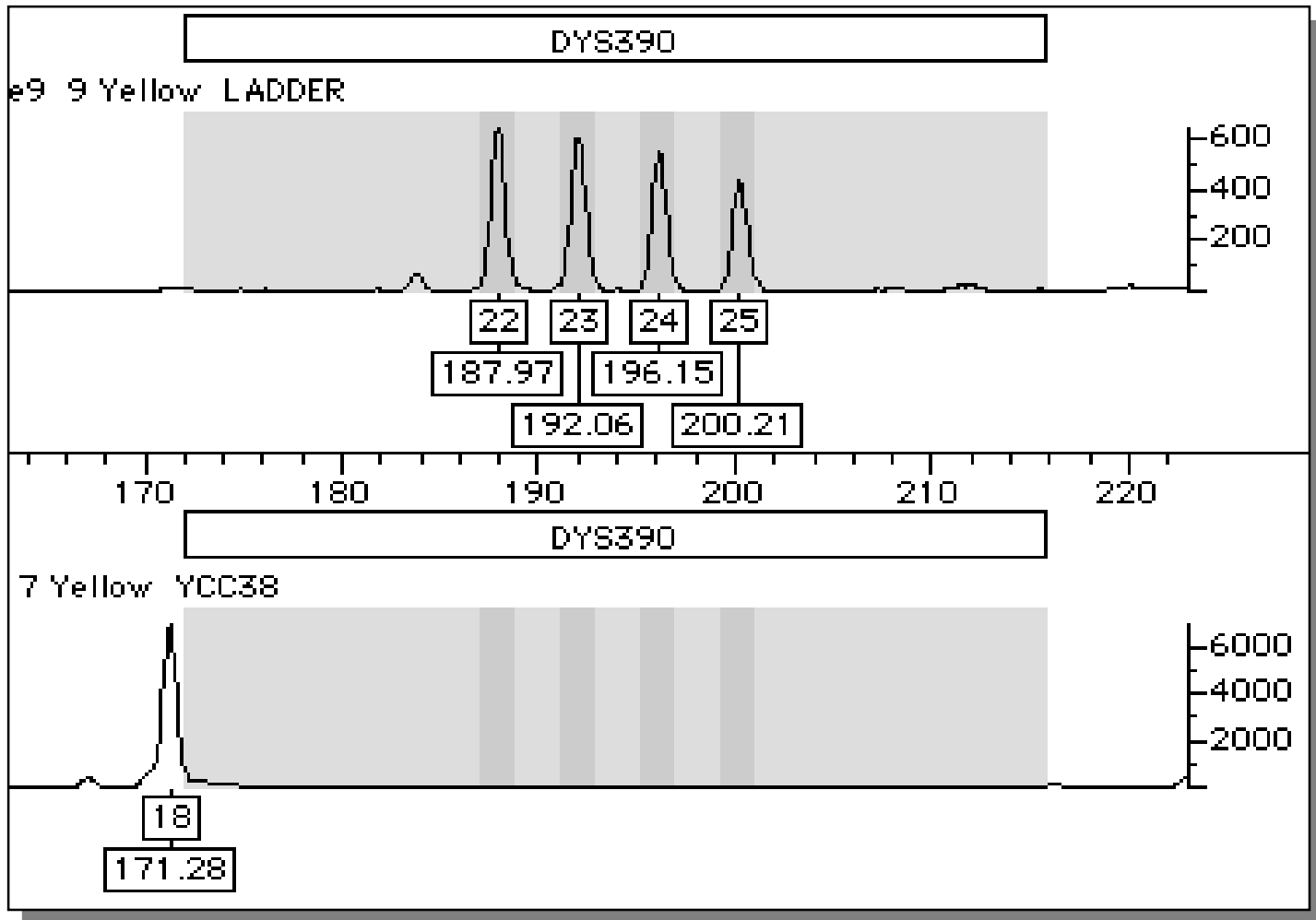
- Primer design software
  - Visual Basic programs to check potential primer dimer formation
- Quality control testing of primers
  - Butler *et al.* (2001) *Forensic Sci. Int.* 119: 87-96
- Rapid multiplex testing
  - Butler *et al.* (2001) *Fresenius J. Anal. Chem.* 369: 200-205





# YCC Panel Sample Typed with Reliagene Y STR Kit

Extreme "off-ladder" allele

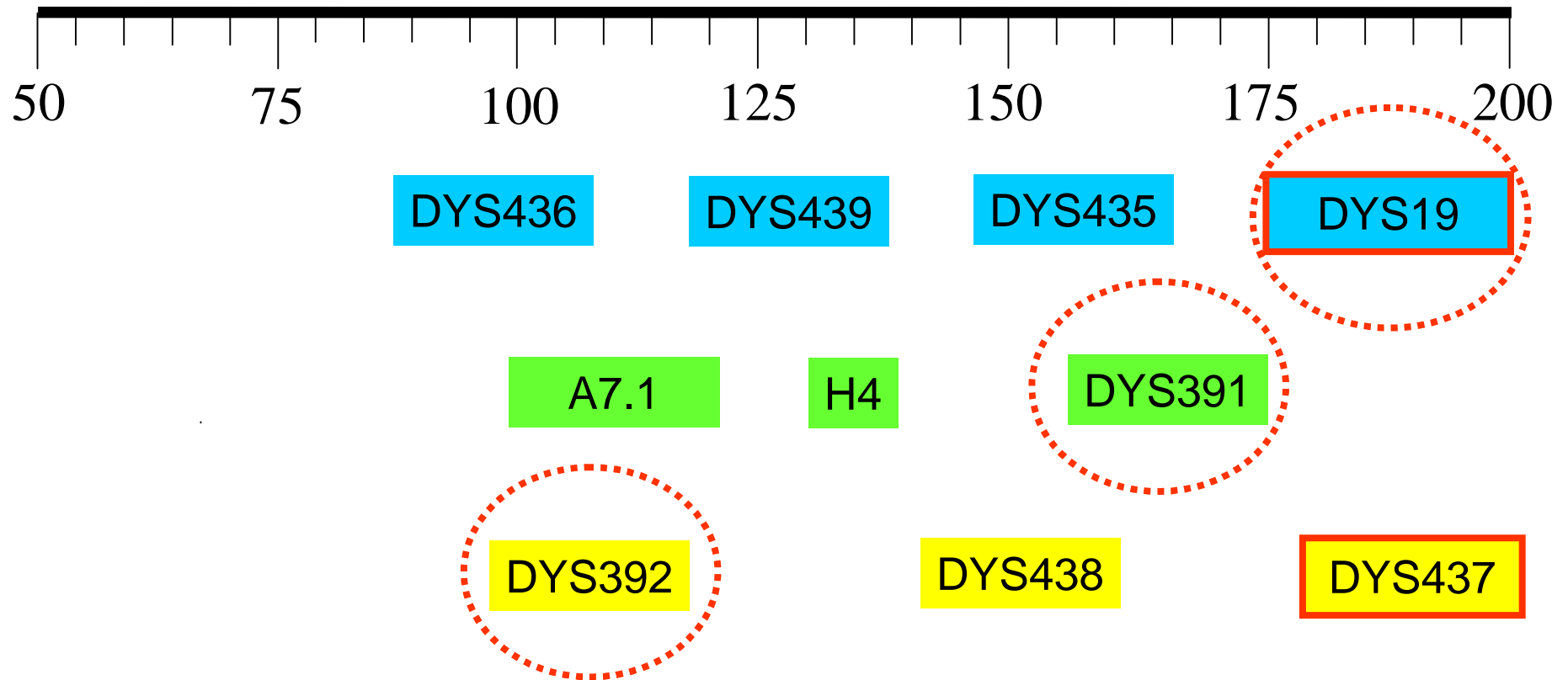


Presented at Promega meeting October 2000

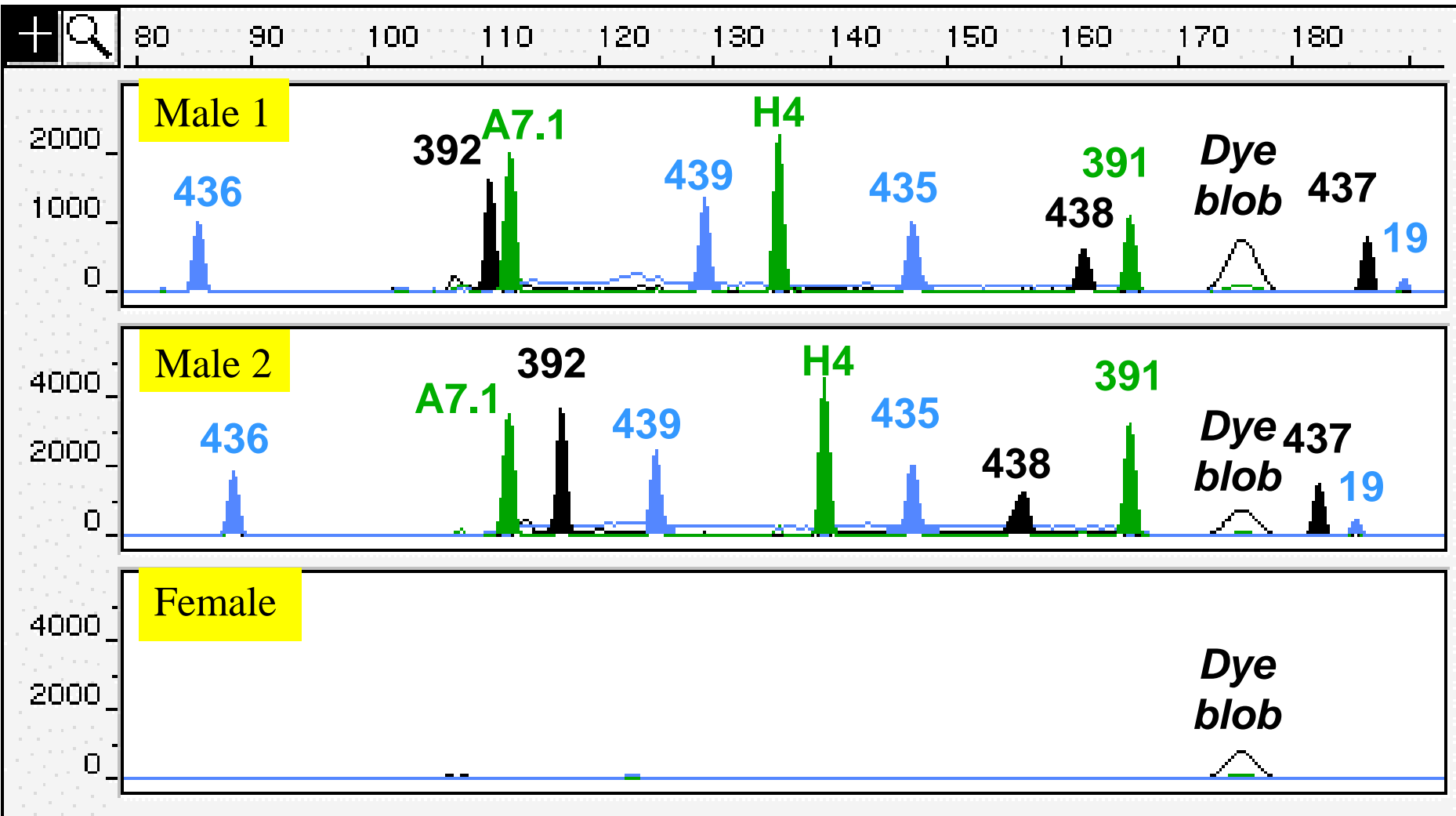
# NIST Y STR 10-plex

*Designed by Christian Ruitberg*

DNA Size (bp)

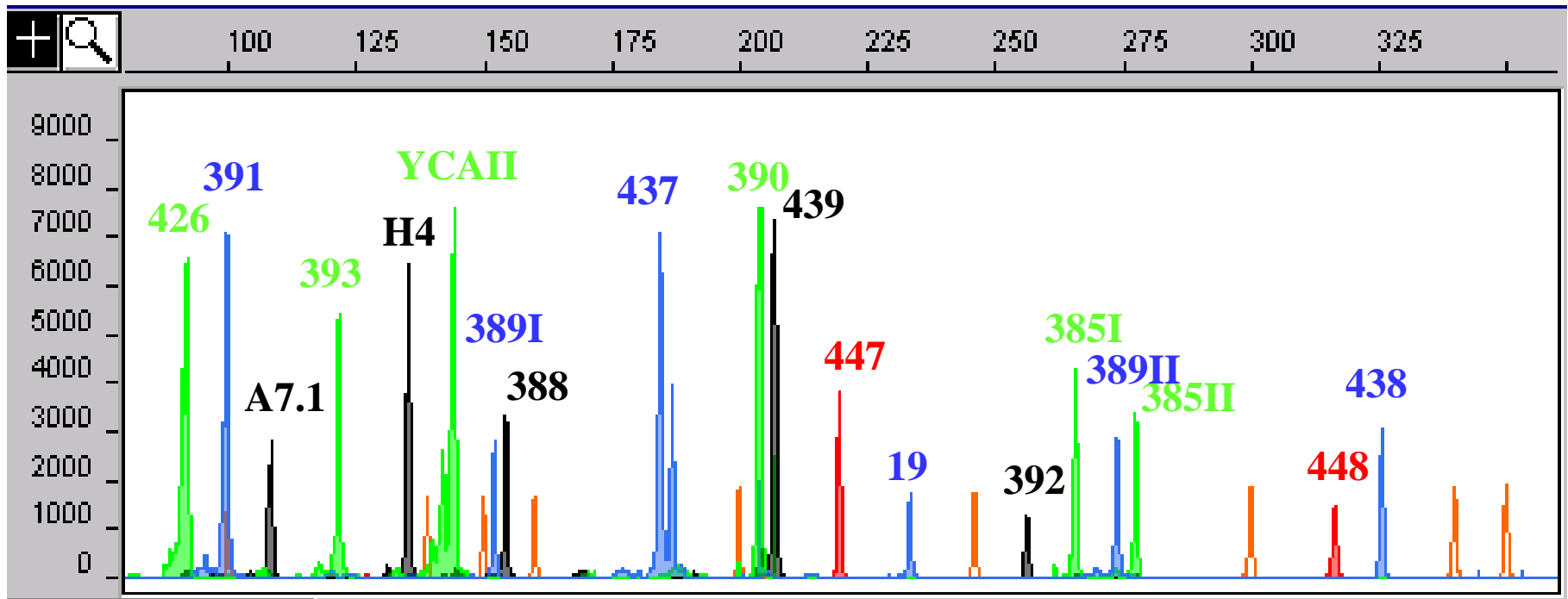


*Primers were redesigned for most of these loci in order to keep the PCR products under 200 bp so that degraded DNA could be more successfully typed.*



# NIST Y-STR 20plex

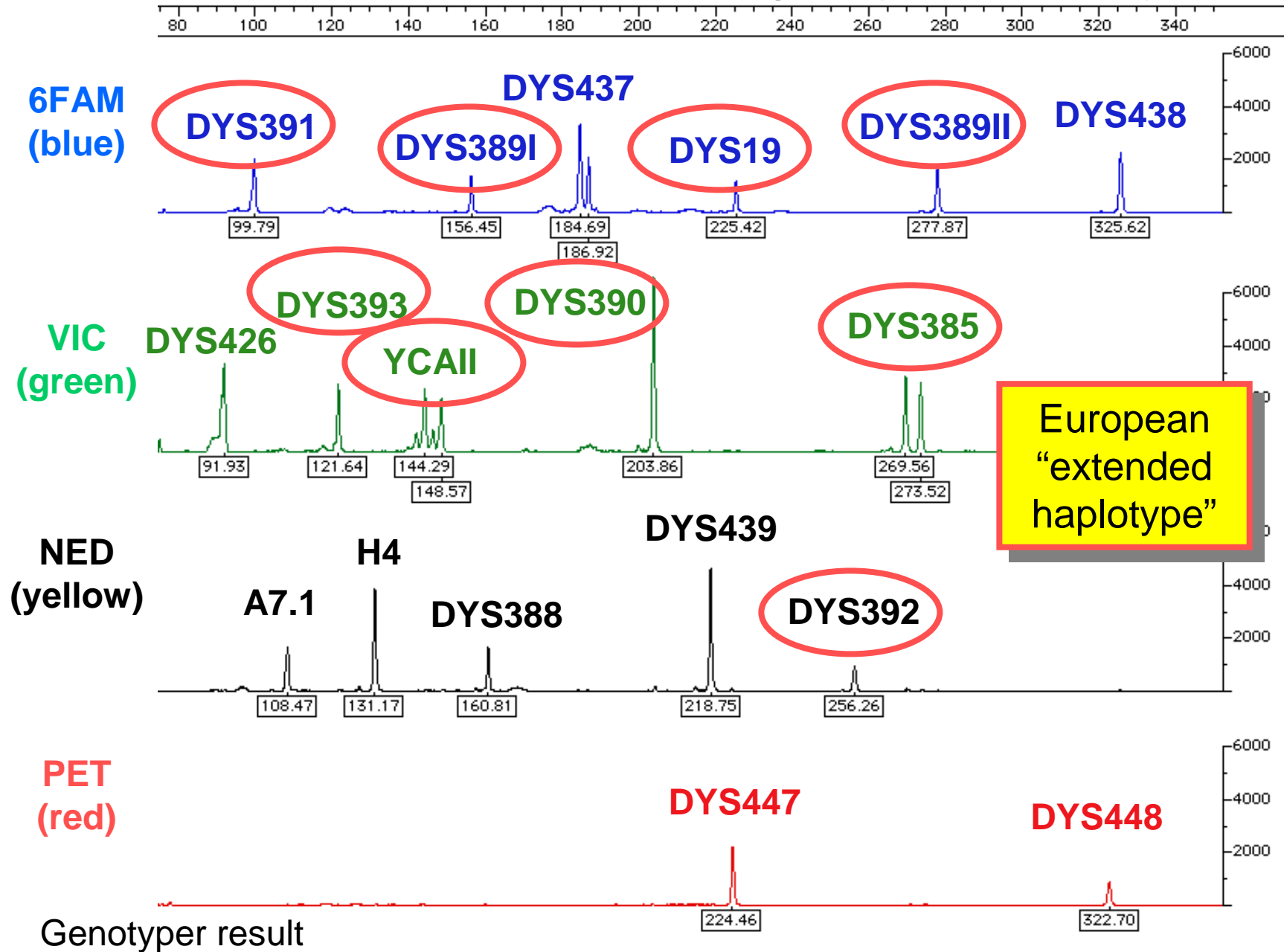
*Developed by Rich Schoske and John Butler*



GS500 LIZ internal sizing standard

**Incorporates all loci in the European Y STR “extended haplotype” plus a number of other useful markers**

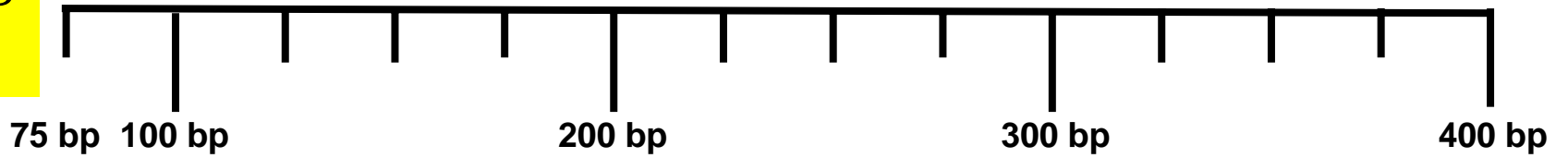
# Y STR 20plex utilizing 5 dye chemistry



# Schematic of Loci in NIST Y STR 20-plex

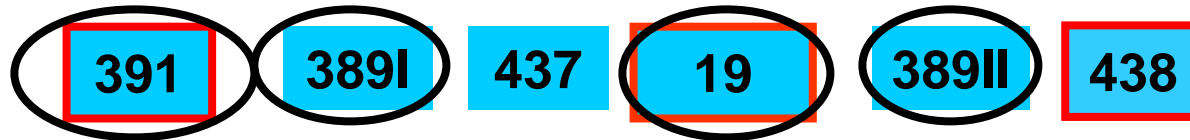
*Designed by Richard Schoske*

Utilizes  
5-dyes



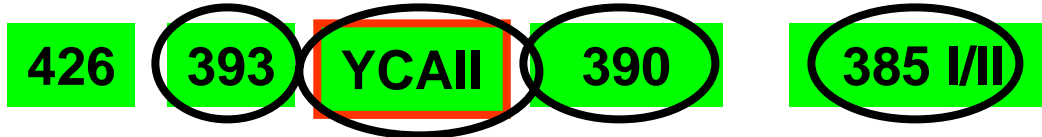
Same dyes as new ABI Identifier™ kit

6FAM  
(blue)



European  
"extended  
haplotype"

VIC  
(green)



***Loci outlined  
in red have  
new primers***

NED  
(yellow)



PET  
(red)



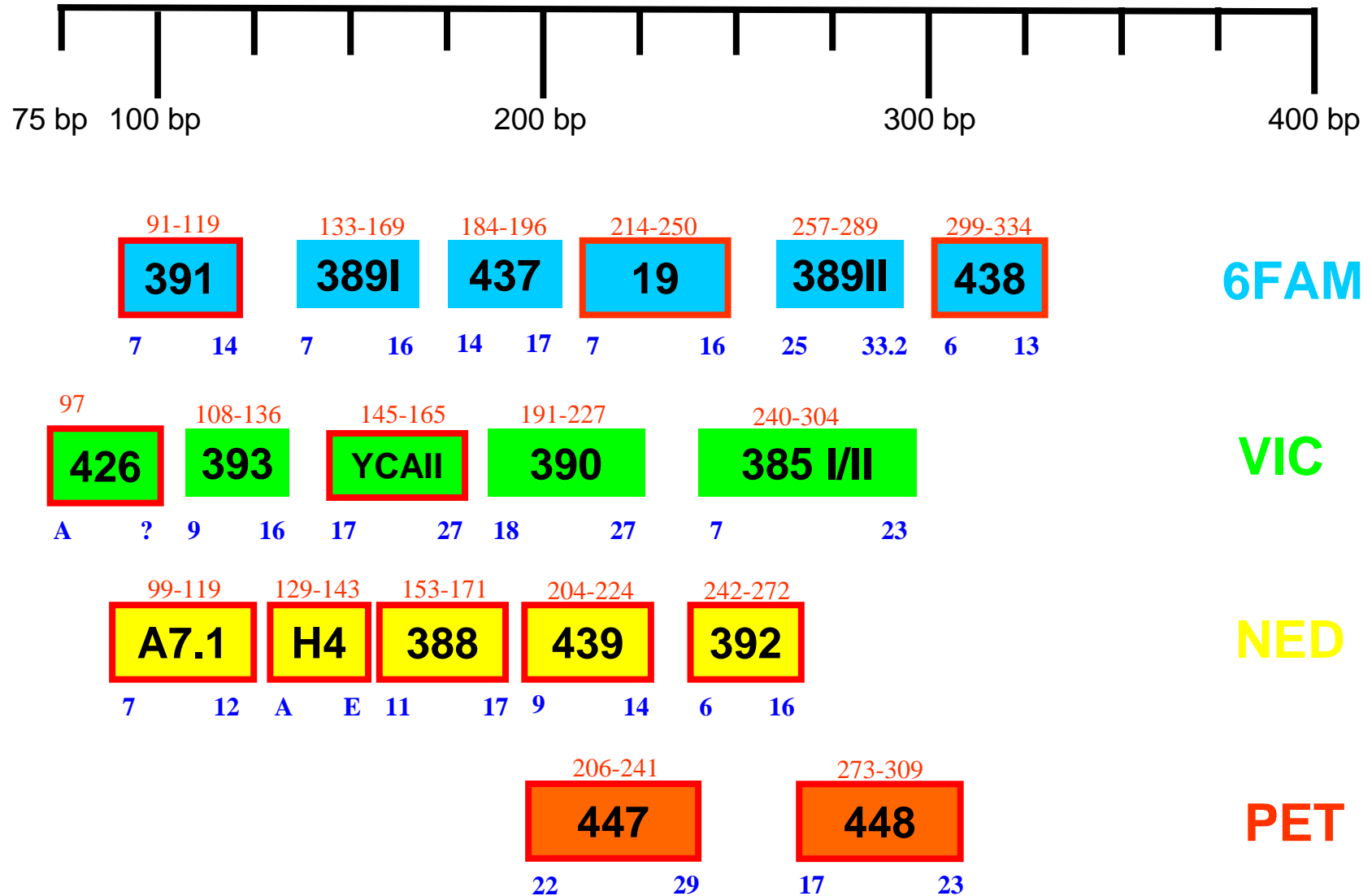
LIZ  
(orange)

LIZ GS500-internal size standard

*Details will be presented at the Promega meeting in October 2001*

# Schematic of Loci in NIST Y STR 20-plex

*Designed by Richard Schoske*



# Advantages of Y STR 20plex

- Single amplification of “extended haplotype”
  - European loci: 19, 385 I/II, 389I, 389II, 390, 391, 392, 393, YCAII a/b
  - Additional loci: 426, A7.1, H4, 388, 437, 438, 439, 447, 448
- Sensitive to <500 pg with 28 cycle PCR
- Male-specific with >100X female DNA
- 10 loci with amplicons less than 200 bp in size to aid results with degraded DNA



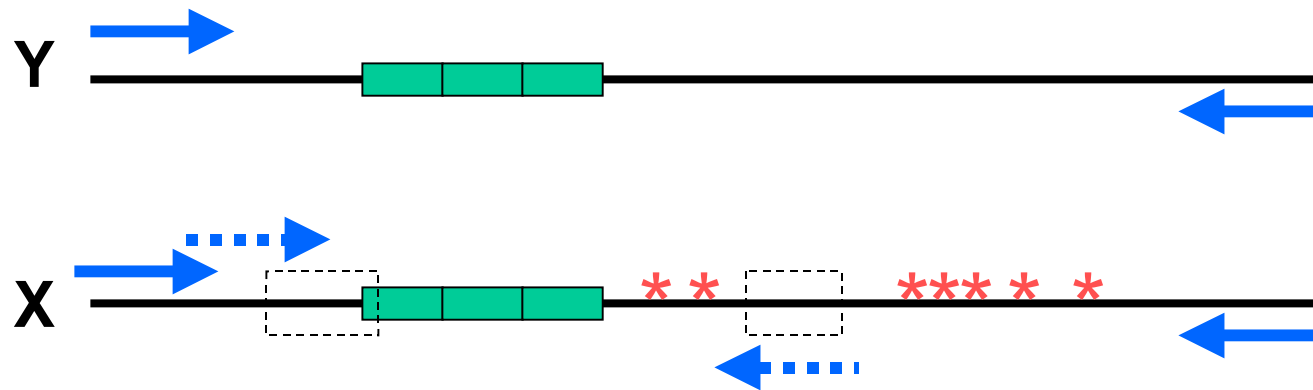
# Mass spec primers designed for DYS391

Allele with 9 TCTA repeats = 99 bp



**Regions deleted in X homolog**

# DYS391 Primer Improvements



Significant homology exists between X and Y

We have designed primers to anneal to regions that only appear on the Y chromosome (target X deletion regions)

Primers also work well to produce small PCR products that can be readily analyzed by mass spec

# Power of Discrimination

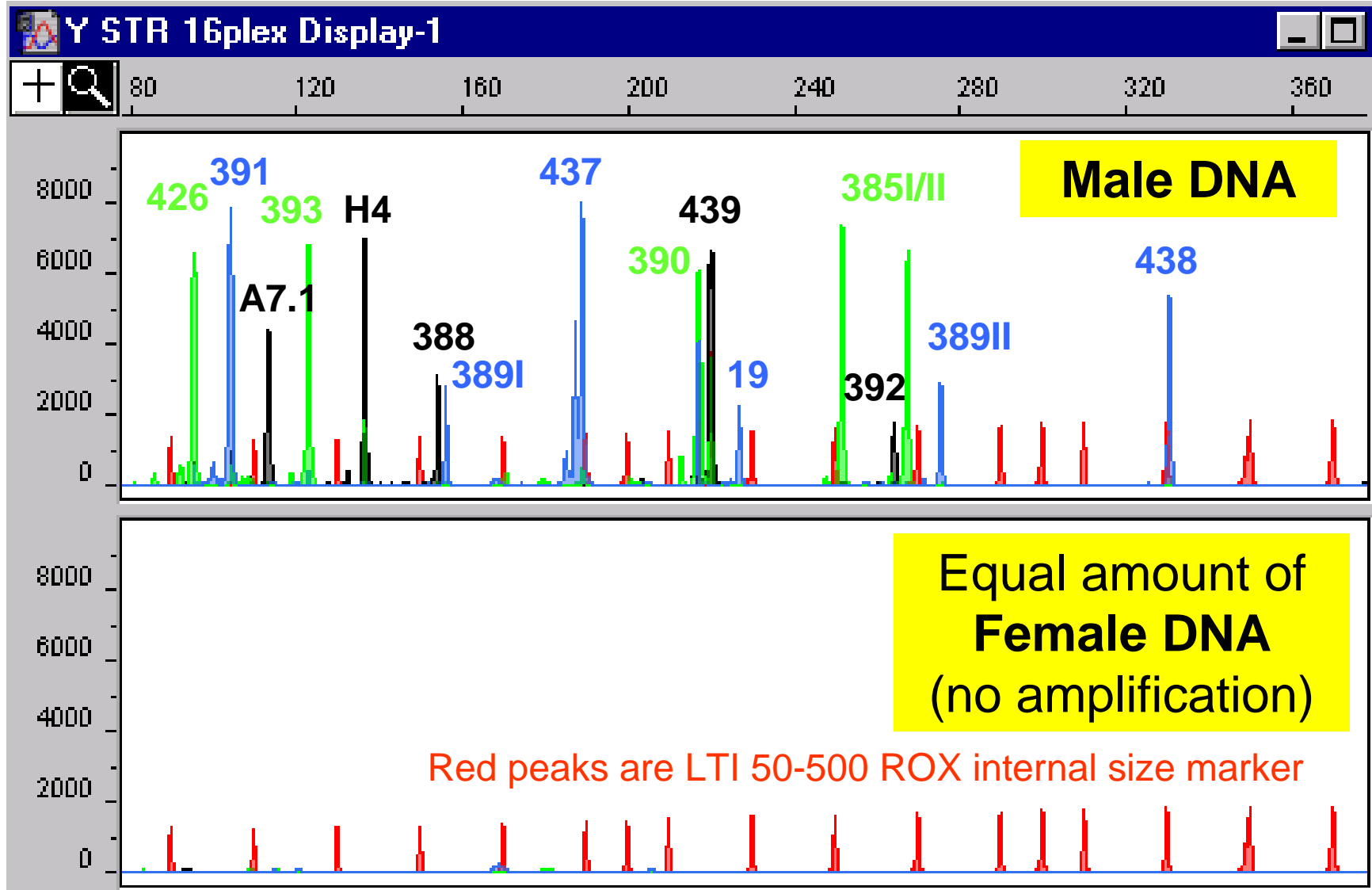
*Tested against 97 male DNA samples including YCC panel*

<u>Loci Tested</u>	<u>Unique Haplotypes</u>	<u>Discrimination*</u>
DYS385 (1)	18	~1:56
19, 389I/II, 390, 393 (5)	62	~1:210
Reliagene Y-Plex 6 kit (6)	79	~1:470
Minimal haplotype (9)	83	~1:600
Extended haplotype (11)	87	~1:900
NIST Y STR 20plex	89	<b>~1:1100</b>

\*results based on different combination from subsets from 20plex testing results...

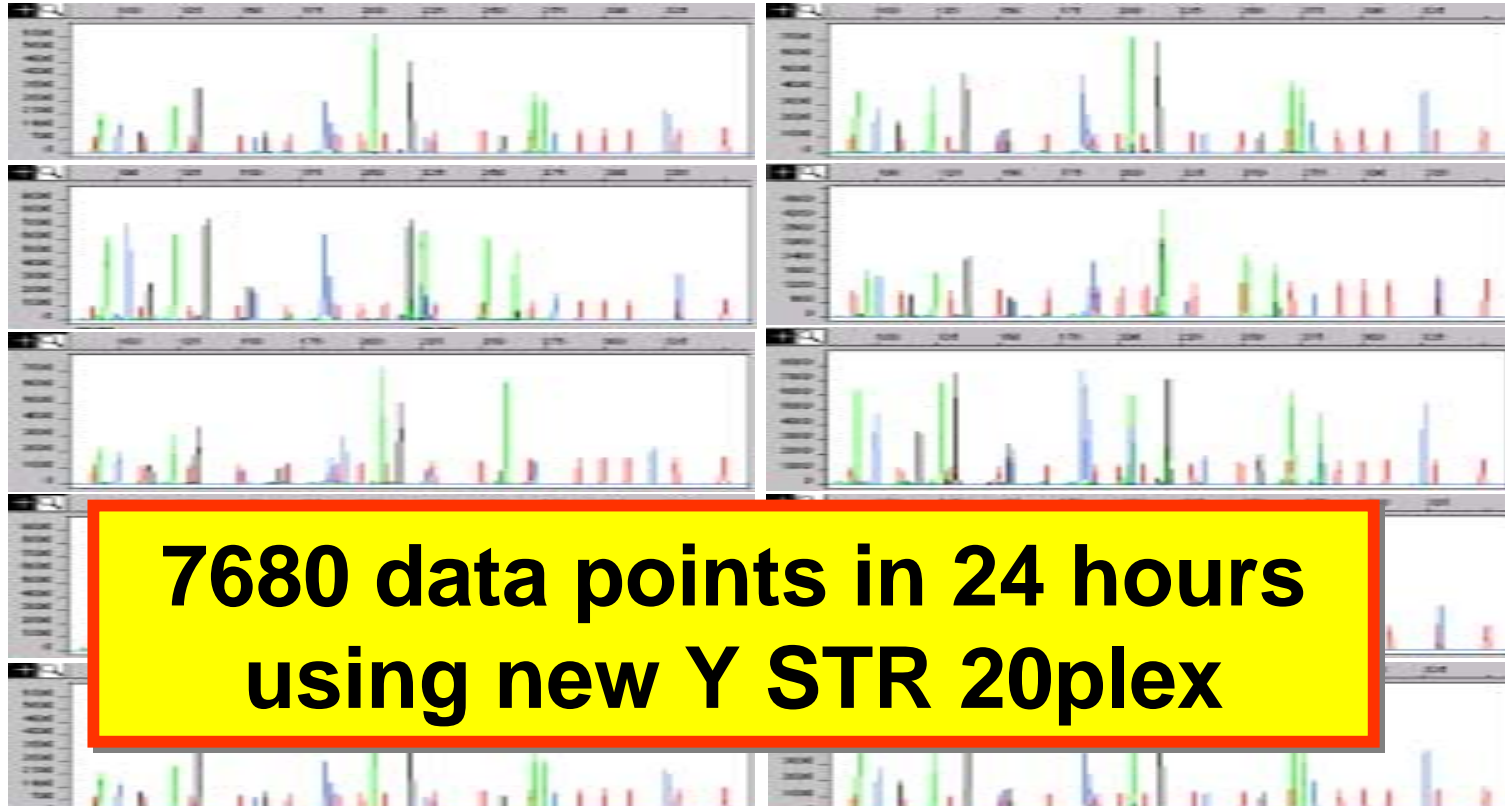
<http://dna-view.com/haplofreq.htm> (Charles Brenner; chance of obtaining a non-unique haplotype)

# Male-Specific Amplification with Y STR Megaplex (16plex with 4 dye chemistry)



Result obtained with ABI 3100 capillary array instrument

## High-throughput Y STR Typing on the ABI 3100 (16-capillary array)

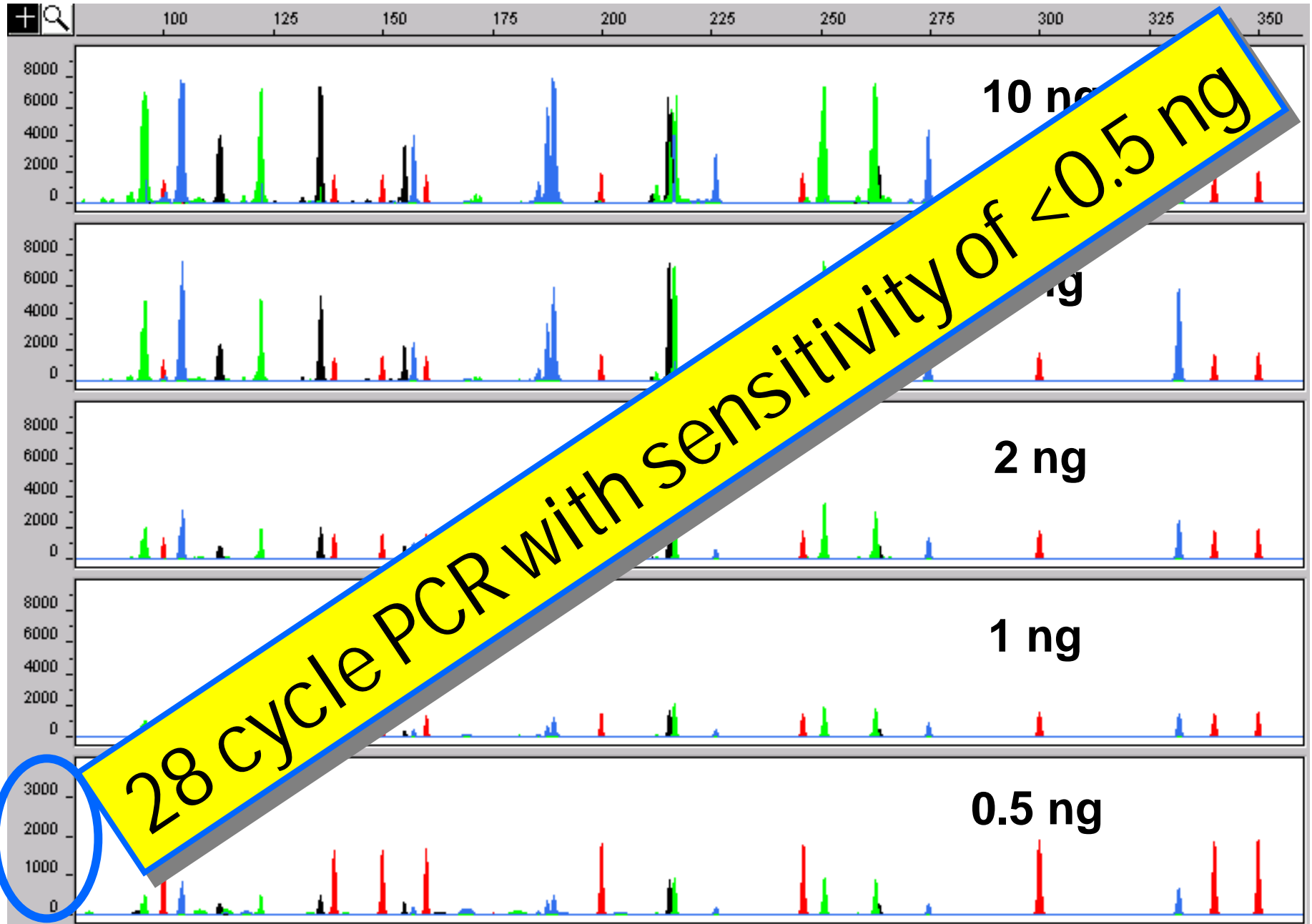


**7680 data points in 24 hours  
using new Y STR 20plex**

Capability to double current size of European Y STR database in less than one week with one instrument



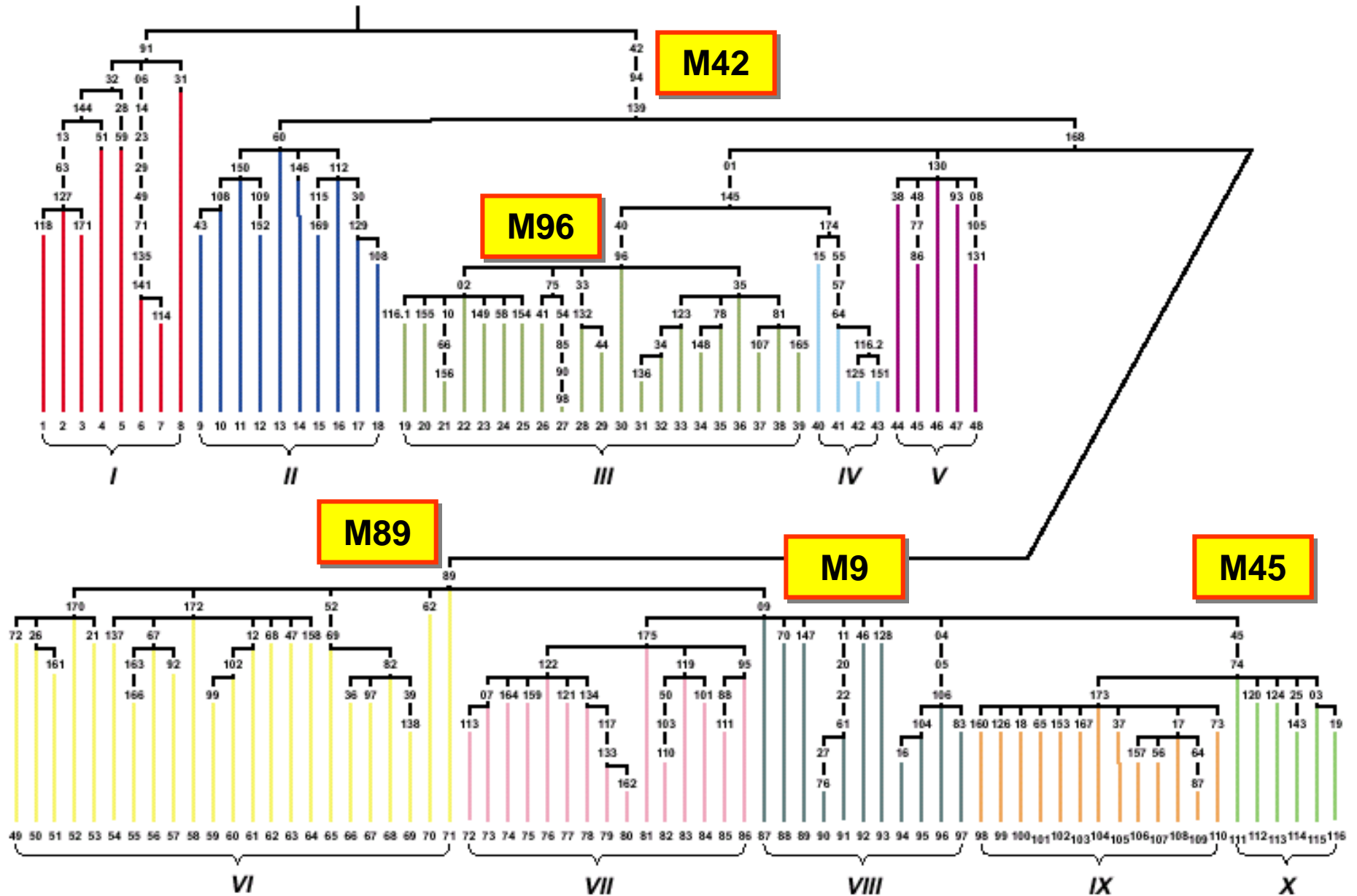
# Sensitivity Study with new NIST Y STR 16-plex



# Plans with our Y STR Megaplex

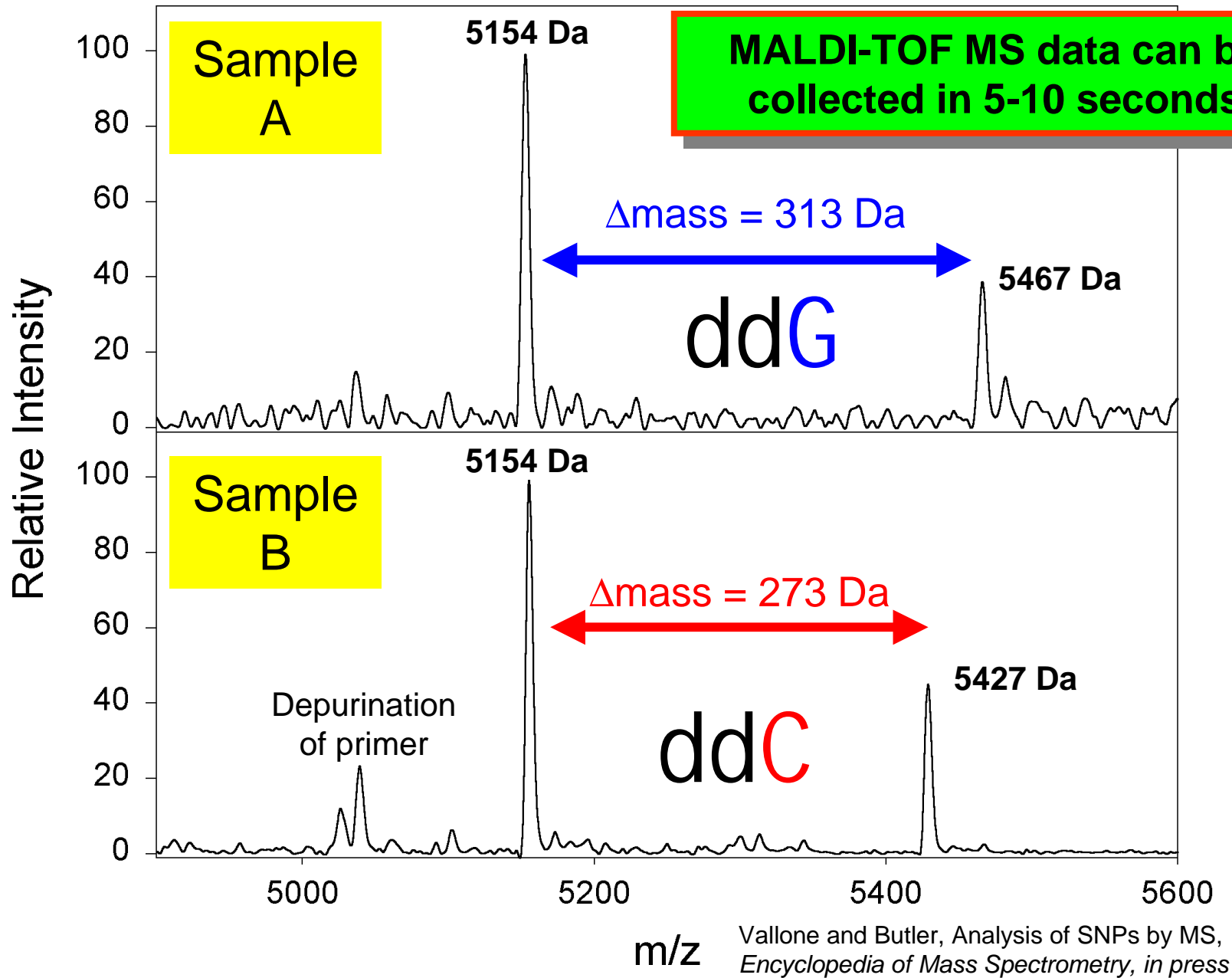
- Currently being tested in 2 other labs
  - **M. Prinz** -- forensic samples with 4 dye 16plex
  - **M. Hammer** -- population studies with 5 dye 20plex
- Concordance studies with YCC sample panel
- Allele sequencing...developmental validation
- Presentation at ISFG and Promega meeting
- Publications with complete details
- Commercializing into kit form?

# Haplogroups produced by Y SNP genotyping

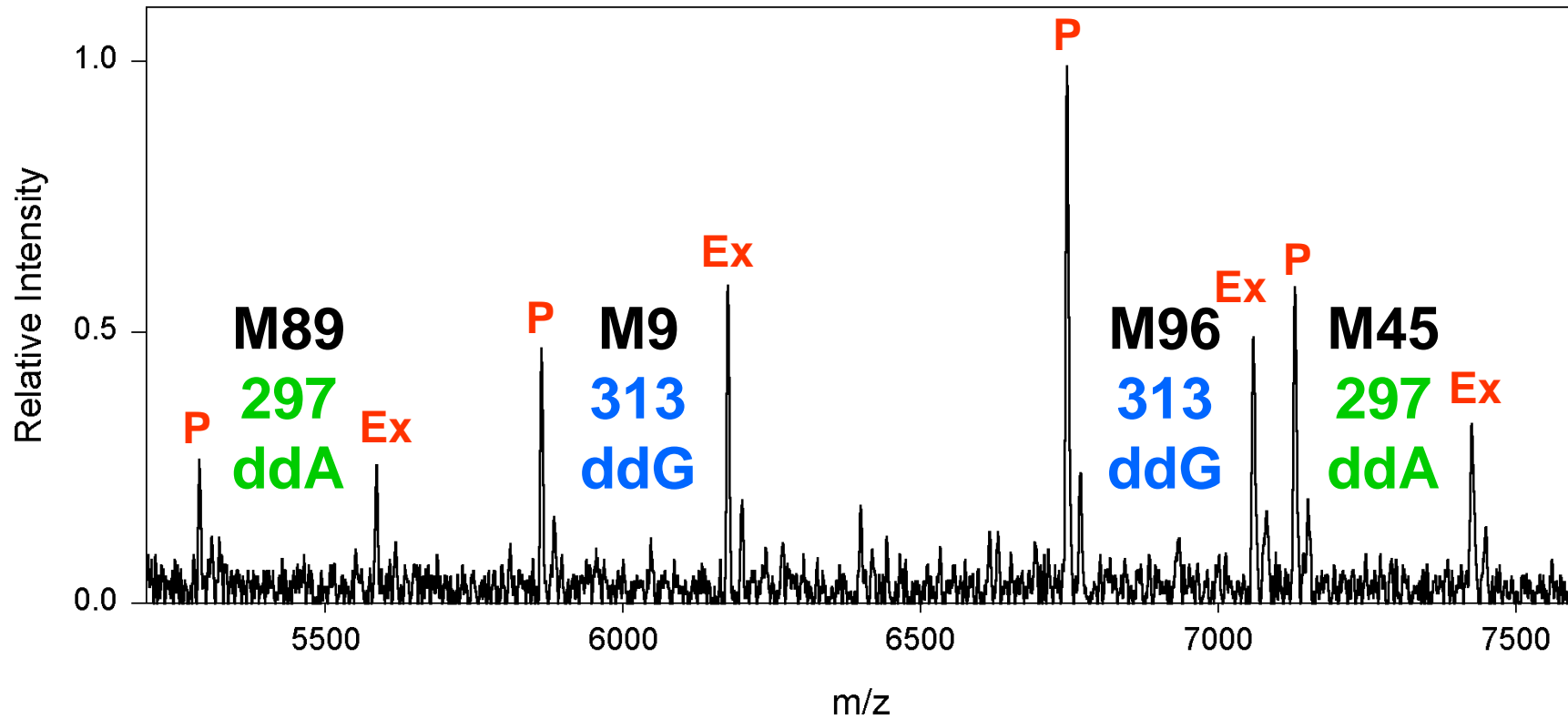




# MS Data from Y SNP Marker M96

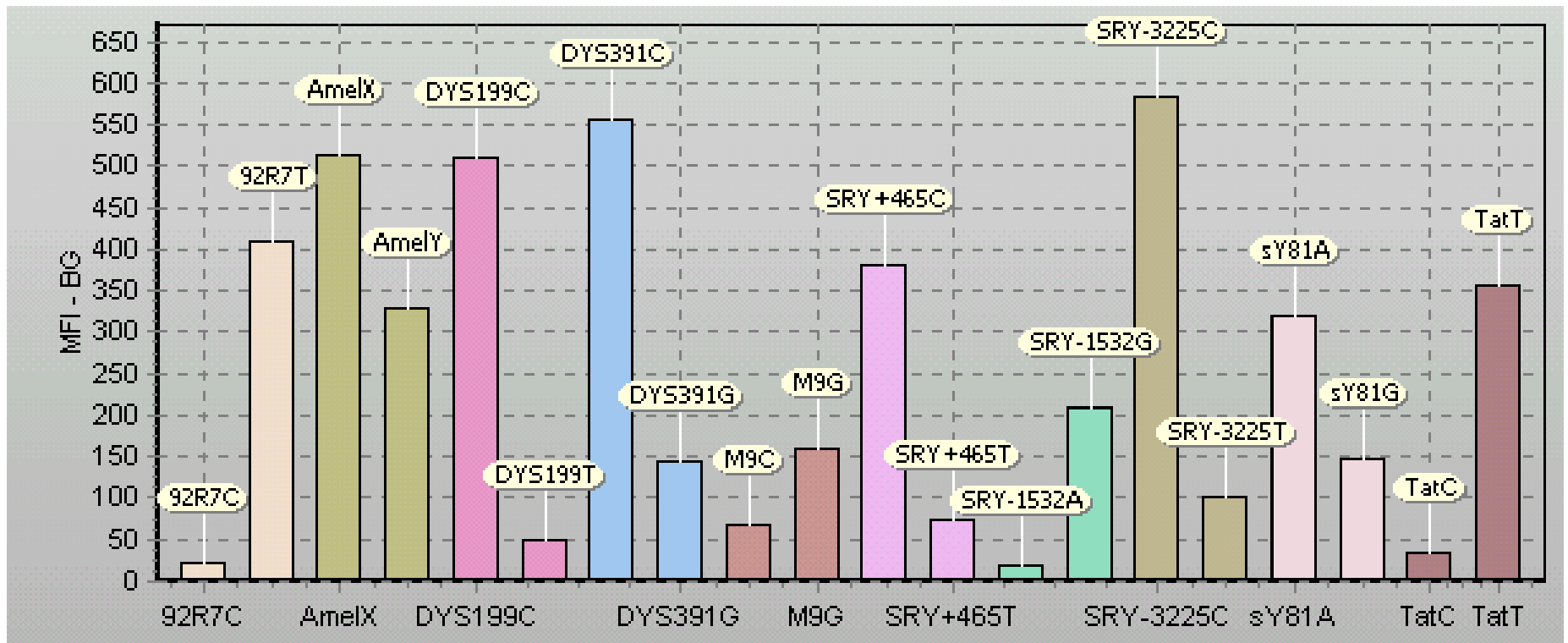


# Mass Spec Y SNP Multiplex Assay (M9, M45, M89, and M96)



# Luminex Bead Y SNP Multiplex Assay

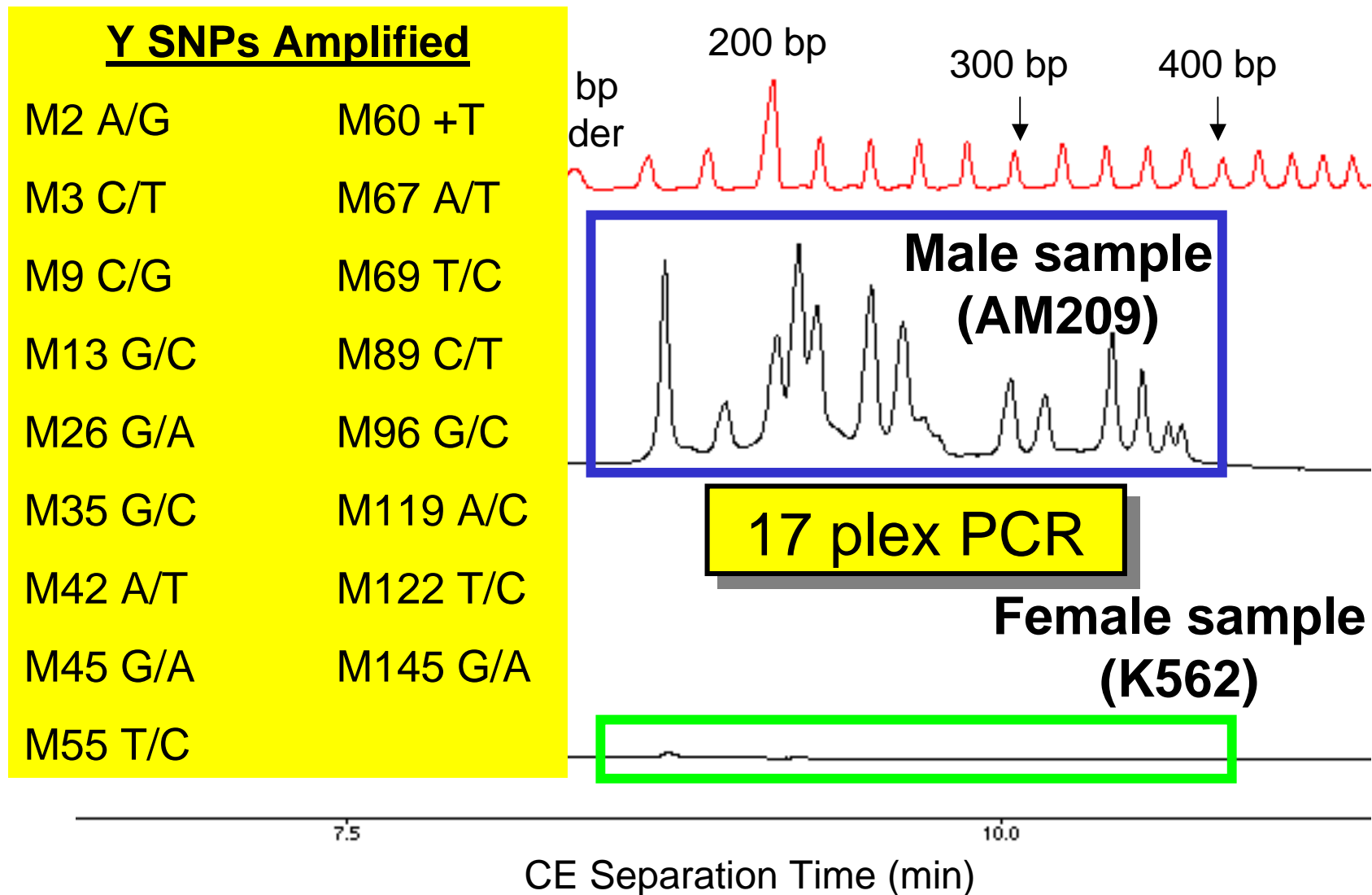
## (9 Y SNPs plus amelogenin)



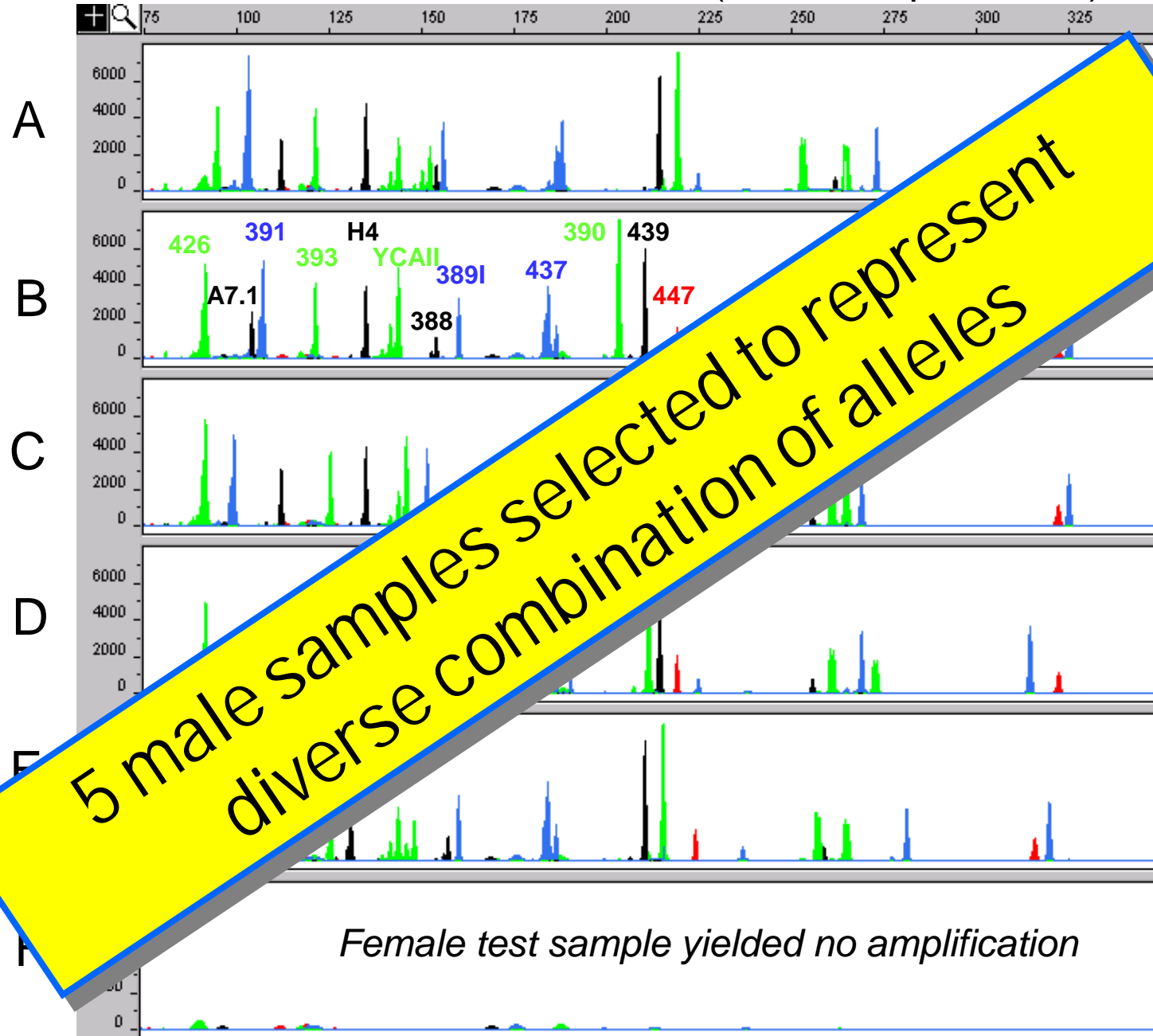
CM55 Test Sample (H1)

*Result from Invitrogen beta-test kit*

# Male-Specific Multiplex PCR at 17 Y SNP Loci



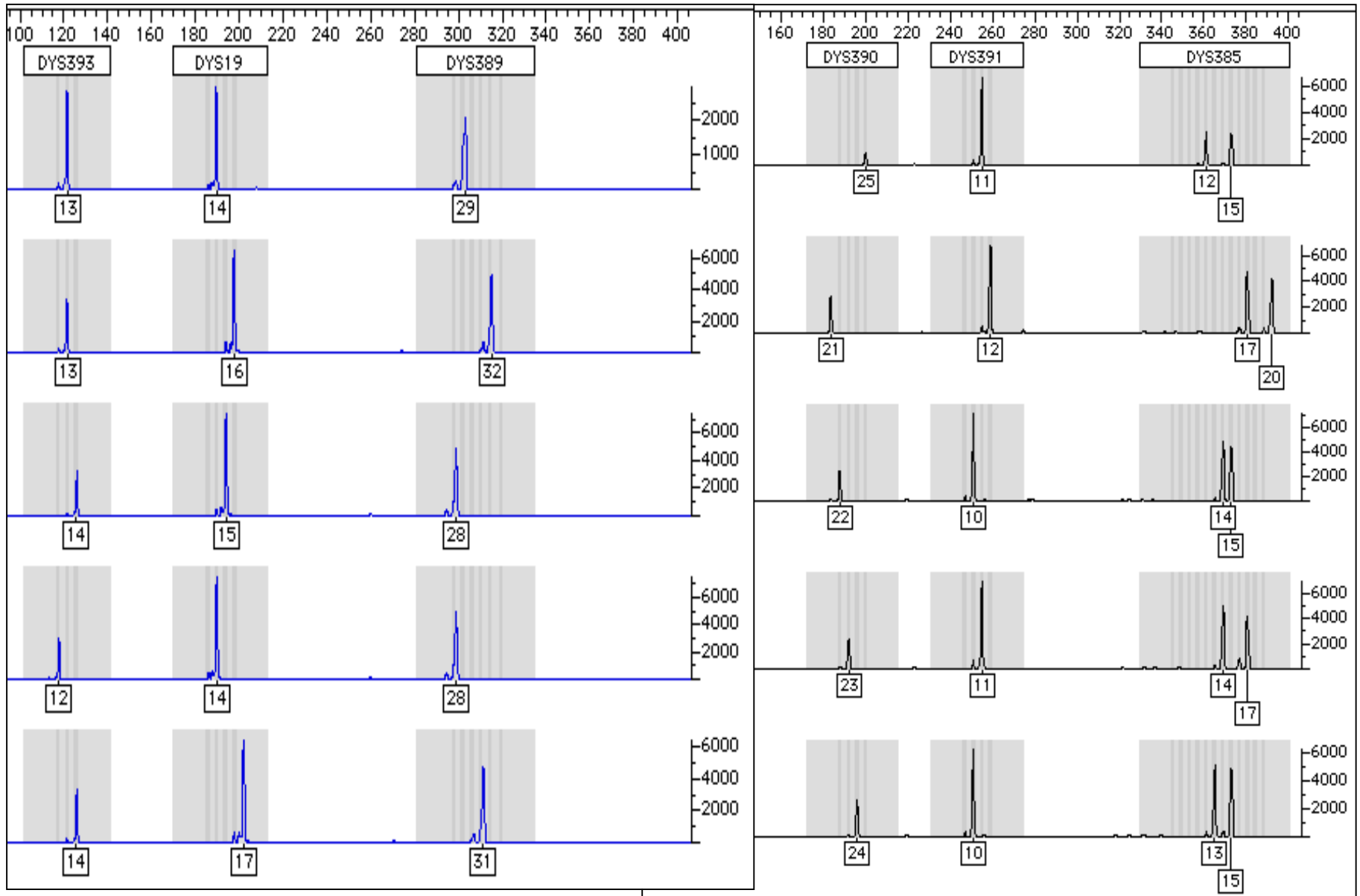
# SRM 2395 Candidates (Y STR 20plex Data)

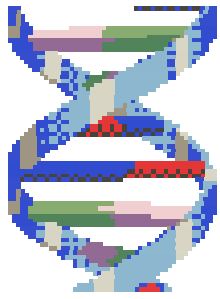


5 male samples selected to represent diverse combination of alleles

*Female test sample yielded no amplification*

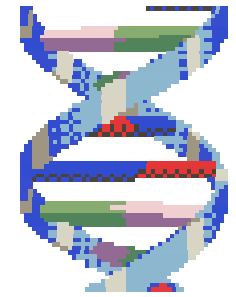
# Genotyper Results with Reliagene Y Plex 6 Kit on 5 male candidate samples for SRM 2395





# STRBase

## Short Tandem Repeat DNA Internet Database



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

### Y-Chromosome STR Information Available

- ❖ Over 200 publications on Y STRs & SNPs cataloged
- ❖ Allele information on 16 Y STR loci
- ❖ Downloadable PowerPoint on Y STRs and Y SNPs
- ❖ Links to other Y-chromosome sites
- ❖ Information on new Y STR multiplexes developed at NIST

# NIST Work with Y Markers

- Y STR Multiplex Assays ([Y STR 20plex](#))
- [Y SNP Multiplexes](#) and Marker Evaluation with MALDI-TOF MS and other technologies
- Development of Y Chromosome Standard Reference Material ([SRM 2395](#))
- Information on Y chromosome markers is being made available through [STRBase](#)



# Acknowledgments



## Funding:

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## NIST Personnel:

John Butler (Project Leader)

**Pete Vallone**

**Margaret Kline**

Jan Redman

**Rich Schoske** (AU)

Gordon Spangler (AU)

Christian Ruitberg (RPI)

Dave Duewer (Anal. Chem.)

## Collaborators:

**Mike Hammer** and **Alan Redd**  
(U. Arizona) for Y STR  
sequences and samples

**Mecki Prinz** (NYC OME)

Dave Carlson (Invitrogen) on Y  
SNP work with Luminex beads

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