Before we can dive into the specific content of the document, let's first understand the text's structure and context. The document appears to be a presentation slide titled "Going Beyond the U.S. Haplotype: A Look at Additional Y-STR and Y-SNP Loci in U.S. Populations." The author is John Butler, and the presentation is associated with the AAFS (American Academy of Forensic Sciences) Workshop on Y-STR Analysis on Forensic Casework held on February 17, 2004.

### Background Information
- **History of Y STR Marker Discovery**
  - 1992: DYS19 (Roewer et al.)
  - 1994: YCAI a/b, YCAII 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.)
  - 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  - 2000: G09411 (DYS462), G10123 (de Knijff unpublished)
  - 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 (Hadj et al.)
  - 2002: DYS468-DYS465 (59 new Y STRs Manfred Kayser GDB entries)
  - 2003: DYS597-DYS645 (50 new Y STRs Manfred Kayser GDB entries)

### Commercial Y-STR Kits Available
- **ReliaGene Technologies (New Orleans, LA):**
  - Y-PLEX™ 6: DYS19, DYS389I, DYS390, DYS391, DYS393, DYS385 a/b
  - Y-PLEX™ 5: DYS389I/II, DYS392, DYS438, DYS439
  - Y-PLEX™ 12: DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, amelogenin

- **Promega Corporation (Madison, WI):**
  - PowerPlex® Y: DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439

- **Serac (Germany):**
  - genRES® DYSplex-1: DYS389I/II, DYS390, DYS391, DYS385 a/b, amelogenin
  - genRES® DYSplex-2: DYS19, DYS389I/II, DYS392, DYS393

- **GKT Inc. (South Korea):**
  - silver-stain kits
    - GeneKin® Y-STR Systems I: DYS388, DYS19, DYS392
    - GeneKin® Y-STR Systems II: DYS393, DYS390, DYS391, DXX391X
    - GeneKin® Y-STR Systems III: DYS385/6X, DYS385/6Y, DYS388II

### Y-STR Kits on ABI 310
- **ReliaGene Y STR Kits on ABI 310**
  - Y-PLEX™ 6
  - Y-PLEX™ 5

### Results with Y-PLEX™ 12
- **ReliaGene Y STR Kits on ABI 310**
  - Single amplification of U.S. core loci with amelogenin
  - U.S. Core Loci Provided with 2 Amplifications

Y STR Markers and NIST Multiplexes

Promega Prototype PowerPlex® Y Allelic Ladders

U.S. Core Loc + DYS437

Single amplification; ladders contain 103 alleles

Current NIST Y-STR Multiplex Assays

Adopted and adapted by Orchid Cellmark (Dallas), see JFS Nov 2003

High Sensitivity (50 pg male DNA)

High Male Specificity with Excess Female DNA

Describes how to build STR multiplex assays...

Careful primer design

- Uniform annealing temperatures
- Checking for all potential primer-primer interactions

Stringent primer quality control

- Dye labeled oligos


Uniform annealing temperatures
Checking for all potential primer-primer interactions

High sensitivity (50 pg male DNA)

High male specificity with excess female DNA

- 0.5 ng male
- 1:1600 mixture (0.5 ng male/2000 ng female)

Non-specific products (but none interfere with expected allele ranges)
Relative Fluorescence Units

PCR Product Size (bp)

426
385 a/b
389I
H4
388
19
392
437
390
439
391
447 448
438
393
458
450
448
456
464 a/b/c/d


Describes multiplex PCR primer sequences for NIST 11plex

ARTICLE IN PRESS

High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays

Richard Schoske1,2,3, Petr M. Valtaov,2,3,4, Marguerite C. Kline2,3,4, Janne W. Rollman2,3 and John M. Butler2,3,4

1Department of Chemistry, University of Wyoming, Laramie, WY, USA
2Department of Chemistry, Arizona State University, Tempe, AZ, USA
3Office of the Chief Medical Examiner, State of Arizona, Tempe, AZ, USA
4Office of the Chief Medical Examiner, State of New Mexico, Santa Fe, NM, USA


As of 06/2003 666 males (anonymous; self-identified ethnicities)

286 Caucasians
252 African Americans
128 Hispanics

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

Working tubes/plates 1 ng/uL

NIST U.S. Population Samples

To date: (~50,000 allele calls)

Identifier (15 autosomal markers + Amelogenin) (10,608)

Roche Linear Arrays (H1/H2 10 regions) (6,630)

Y STRs 22 loci—27 amplicons (17,888)

Y SNPs 50 markers on sub-set of samples (11,498)

Types generated at NIST for 22 Y-STRs and 50 Y-SNPs

Performance of U.S. haplotype vs. European "extended" haplotype (DYS438 and DYS439 vs. stutter-prone dinucleotide YCAII a/b)

Resolution of most common types with additional markers

Conservative Expanded Methods for Allele Calling

Schoske J.M. Butler AAFS 2004 Y-STR Workshop February 17, 2004

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

Example of some samples (3) (1) (1) (1)(1)(1)(1)(1)

Unresolved with all 22 markers

DYS464, a quadruplicated locus, occurs in the DAZ region near 25 Mb. This region was demonstrated to have paternome sequence in the recent publication of the human Y-chromosome sequence (Skepner et al., 2003 Science 260:520-527).

We developed two new primer pairs for DYS464 compared to the original ones published by Rusti et al. (AJR: Am J Roentgenol, 2002, 178:911-)

DYS464 is part of NIST 1plex assay

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

All DYS464 Expanded Types Observed with 679 Samples

113 Different C-Types
179 Different E-Types
92 occur in only a single sample
Y-STR Conclusions

- Additional markers beyond the European minimal haplotype and SWGDAM-recommended U.S. haplotype do help resolve common lineages
- The multi-copy marker DYS464 is extremely polymorphic and would be beneficial to use

Y-SNPs

SNP Typing Instrumentation at NIST

- **PCR & primer extension**
- **Luminex Beads hybridization**
- **Time-of-Flight Mass Spectrometer**
- **Luminex 100 Flow Cytometer**
- **Primer Extension**
- **ABI 7000 SDS**

Allele-Specific Primer Extension (ASPE)

<table>
<thead>
<tr>
<th>Electrophoretic Run Time (sec)</th>
<th>RFUs</th>
</tr>
</thead>
<tbody>
<tr>
<td>20A</td>
<td>28G</td>
</tr>
<tr>
<td>28G</td>
<td>36G</td>
</tr>
<tr>
<td>36G</td>
<td>44T</td>
</tr>
<tr>
<td>52C 52T</td>
<td>60C</td>
</tr>
</tbody>
</table>

They advocate using DYS464 as a screening tool.
SNP Detection by Hybridization
Luminex Bead Array Assay

Luminex 100 Flow Cytometer
100 different colored beads are possible (potential for multiplexing 50 SNP markers)

Signal from PCR product
Bead identity (SNP marker and allele)

~30 seconds to process each sample
Detects labeled PCR product

Green laser
Identity of bead (probe)

Red laser
PCR product
green dye

Nat Rev Genet. 4:598-612

250 Y-SNPs
Samples were typed for 48 world populations
18 main groups A-R
159 haplogroups defined

Global Distribution of Y Haplogroups

Y-SNPs have been primarily typed in world populations
What haplogroups will be observed in U.S. populations?

Y-SNPs in U.S. populations
What haplogroups will be observed?
How specific will certain Y-SNPs be for a U.S. population group?
Forensic utility in comparison/addition to Y-STRs
Commercial kit (Marligen) 42 Y-SNPs
Medium sized multiplexes developed in-house
(CE or MS)

Y-SNPs Typed at NIST
42 SNPs + Amelogenin present in 5 multiplexes
(commercially available kit from Marligen)
18 SNPs in 3 NIST-designed 6plexes (8 unique)
10 SNPs in 2 NIST-designed 5plexes (1 unique)
19 of the SNP sites overlapped...
Resulting in a total of 51 Y-SNPs

115 African Americans
114 Caucasians
95 Hispanics (presently typed for 10 Y-SNPs)
50 Y SNPs Typed

- 114 U.S. Caucasians
- 115 African Americans

10 overlapping loci (boxed)

- 42 Y SNPs + amelogenin with Marligen Signet Y SNP Identification kit

- 18 Y SNPs with SNaPshot assays developed at NIST

- >2,000 allele calls compared between two methods

Complete Concordance Seen!

Equimolar PCR primer concentration (5plex)
Empirical balancing of extension primers

35plex Y-SNP assay (25plex PCR)

Summary of Y-SNP Data
(115 African Americans and 114 Caucasians)

A total of 20 ng of genomic DNA was consumed for the 10 multiplexes

18 out of 46 haplogroups observed

Over 99% success rate for allele calls (both methods)
Variation was observed in 24 of the 51 Y-SNPs
100% concordance for the 19 overlapping markers (>3,800 allele calls)

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**

- **African Americans**
  - E1, E2, E3a, B2a, B*
  - 18 total Hgs, 5 shared

- **Caucasians**
  - A1, R1a1, R1b, R*

---

**Derived in more than one population**

<table>
<thead>
<tr>
<th>Locus</th>
<th>All</th>
<th>AA</th>
<th>Cau</th>
<th>Hisp</th>
<th>Hap</th>
<th>Hap</th>
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</thead>
<tbody>
<tr>
<td>M2 A/G</td>
<td>0.23</td>
<td>0.58</td>
<td>not</td>
<td>0.08</td>
<td>E3a</td>
<td></td>
</tr>
<tr>
<td>DYS391 C/G</td>
<td>0.31</td>
<td>0.60</td>
<td>0.04</td>
<td>na</td>
<td>E3</td>
<td></td>
</tr>
<tr>
<td>M170 A/C</td>
<td>0.10</td>
<td>0.04</td>
<td>0.21</td>
<td>0.04</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>M35 G/C</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>na</td>
<td>E3b</td>
<td></td>
</tr>
<tr>
<td>M261 G/T</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.05</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>SRY10831 A/G</td>
<td>0.03</td>
<td>0.01</td>
<td>0.05</td>
<td>na</td>
<td>R1a</td>
<td></td>
</tr>
</tbody>
</table>

M2 is primarily derived in the African American pop

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**Signal-to-Noise Ratio**

**Primer Extension (ASPE)**

<table>
<thead>
<tr>
<th>Locus</th>
<th>All</th>
<th>AA</th>
<th>Cau</th>
<th>Hisp</th>
<th>Hap</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2</td>
<td>0.23</td>
<td>0.58</td>
<td>not</td>
<td>0.08</td>
<td>E3a</td>
<td>109</td>
</tr>
<tr>
<td>DYS391</td>
<td>0.31</td>
<td>0.60</td>
<td>0.04</td>
<td>na</td>
<td>E3</td>
<td>238</td>
</tr>
<tr>
<td>M170</td>
<td>0.10</td>
<td>0.04</td>
<td>0.21</td>
<td>0.04</td>
<td>I</td>
<td>73</td>
</tr>
<tr>
<td>M35</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>na</td>
<td>E3b</td>
<td>267</td>
</tr>
<tr>
<td>M261</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.05</td>
<td>G</td>
<td>114</td>
</tr>
<tr>
<td>SRY10831</td>
<td>0.03</td>
<td>0.01</td>
<td>0.05</td>
<td>na</td>
<td>R1a</td>
<td>54</td>
</tr>
</tbody>
</table>

**Hybridization (ASH)**

- S/N > 1,000
- S/N = 2.3 and 3.2

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**Allele Calling with Multi-Copy Y-SNP P25**

**ASH**

<table>
<thead>
<tr>
<th>Locus</th>
<th>All</th>
<th>AA</th>
<th>Cau</th>
<th>Hisp</th>
<th>Hap</th>
<th>S/N</th>
</tr>
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<tbody>
<tr>
<td>M2</td>
<td>0.23</td>
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<td>not</td>
<td>0.08</td>
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<td>na</td>
<td>E3b</td>
<td>267</td>
</tr>
<tr>
<td>M261</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.05</td>
<td>G</td>
<td>114</td>
</tr>
<tr>
<td>SRY10831</td>
<td>0.03</td>
<td>0.01</td>
<td>0.05</td>
<td>na</td>
<td>R1a</td>
<td>54</td>
</tr>
</tbody>
</table>

- Solid bar = correct allele call

**ASPE**

- Average peak ratio
- C/A

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**Forensic Utility**

51 Y-SNPs versus 1 Y-STR

- For N = 211 male samples

<table>
<thead>
<tr>
<th>Comparison</th>
<th>51Y-SNPs</th>
<th>Y-STR DYS464</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of sample consumed</td>
<td>20ng</td>
<td>&lt;1ng</td>
</tr>
<tr>
<td>Number for types observed</td>
<td>18</td>
<td>62</td>
</tr>
<tr>
<td>Analysis</td>
<td>Multiple</td>
<td>1 reaction</td>
</tr>
<tr>
<td>Degraded samples</td>
<td>+</td>
<td>?</td>
</tr>
</tbody>
</table>
Y-SNP Conclusions

- Full concordance was observed between hybridization and primer extension technologies on 18 different Y-SNPs (>3,800 allele calls)
- Caucasian admixture was observed with our African American population (Hg R and R1b in ~30%)—agrees with Kayser et al. (2003) (Genome Res. 13:624-634 done with 9 Y-STRs)
- Y-SNPs may have limited value for ethnic differentiation in U.S. populations
  - One exception: M2 not in Caucasians
- Y-SNPs are not a useful stand-alone assay for forensic purposes, but may be helpful in combination with Y-STRs

NIST SRM 2395
Human Y-Chromosome DNA Profiling Standard

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

- Now available from NIST Standard Reference Material office (www.nist.gov/srm)
- Can be used to verify results with any primer sets
- Will help U.S. labs meet DAB/FBI Standards

SRM 2395 Now Available…

http://www.nist.gov/srm

Sequence Determination of Y STR Repeat Region for Each Component

<table>
<thead>
<tr>
<th>Component</th>
<th>DYS392 (forward) A</th>
<th>DYS392 (forward) B</th>
<th>DYS392 (forward) C</th>
<th>DYS392 (forward) D</th>
<th>DYS392 (forward) E</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13 TAT repeats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>11 TAT repeats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td>11 TAT repeats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td>11 TAT repeats</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11 TAT repeats</td>
</tr>
</tbody>
</table>

Y SNP Results on SRM 2395
from Marligen Signet™ Multiplexes (Luminex bead assay)

<table>
<thead>
<tr>
<th>Component</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component A</td>
<td>Y</td>
<td>G</td>
<td>A</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>Component B</td>
<td>Y</td>
<td>A</td>
<td>G</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>Component C</td>
<td>Y</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>Component D</td>
<td>Y</td>
<td>A</td>
<td>G</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>Component E</td>
<td>Y</td>
<td>A</td>
<td>G</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>Component F</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

42 Y SNPs measured across all samples
STRBase
Short Tandem Repeat DNA
Internet Database

General Information
• Intro to STRs
  (downloadable PowerPoint)
• STR Fact Sheets
• Sequence Information
• Multiplex STR Kits
• Variant Allele Reports

Forensic Interest Data
• FBI CODIS Core Loci
• DAB Standards
• NIST SRM 2391
• Published PCR Primers
• Y-Chromosome STRs
• Population Data
• Validation Studies

Supplemental Info
• Reference List
• Technology Review
• Addresses for Scientists
• Links to Other Web Sites

Standardized information formats

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

Example Y STR Fact Sheet from STRBase

We would like to collect variant alleles for Y STRs as they are discovered...

Y-Chromosome STR Information Available
• Over 200 publications on Y STRs & SNPs cataloged
• Allele information on over 20 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 (published 20plex primers)
• Y STR mapped positions along chromosome

Summary of NIST Y Chromosome Work
• Development of new Y STR multiplex assays (Y STR 20plex, etc.)
• Evaluation of SNP typing methodologies and development of Y SNP assays involving primer extension and the SNaPshot kit
• Creation of a Y Chromosome Standard Reference Material (SRM 2395)
• Standardization of information on Y chromosome markers with internet accessibility (STRBase)

>30,000 Y chromosome allele calls generated to aid studies on optimal markers for U.S. populations

Publications from Our Group on Y Chromosome Assays

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NIST Project Team:
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Margaret Kline
Jan Fedman
Rich Schoske (American U)
Dave Duewer

Collaborators:
Mike Hammer and Alan Redd (U. AZ) for Y STR sequences

This presentation available as pdf file from http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm