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National  
Institute of  
Standards  
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## Multiplexed Assays for Evaluation of Y-SNP Markers in U.S. Populations

### International Congress ISFG Arcachon, France September 12<sup>th</sup> 2003

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## 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

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Commercial kit (Marligen) 42 Y-SNPs

Medium sized multiplexes developed in-house  
(CE or MS)

## NIST U.S. Population Samples

As of 08/2003 **663** males (anonymous; self-identified ethnicities)

260 Caucasians  
260 African Americans  
140 Hispanic  
3 Asian

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

~80  $\mu$ g total extracted  
genomic DNA  
Working plates 1 ng/uL



To date: (35,139 allele calls)

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

Roche Linear Arrays (HV1 HV2 10 regions) (6,630)

**Y-STRs 27 markers (17,901)**

## 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 were redundant between the methodologies

### **Resulting in a total of 51 Y-SNPs**

115 African Americans


114 Caucasians

**95 Hispanics (presently typed for 10 Y-SNPs)**




## Instrumentation

PCR & primer extension




**Multi-Color Capillary Electrophoresis  
(ABI 310 or 3100)**

Luminex Beads hybridization




**Luminex 100 Flow Cytometer**



Primer Extension

**Time-of-Flight Mass Spectrometer**

TaqMan



**ABI 7000 SDS**

## Allele-Specific Primer Extension (ASPE)

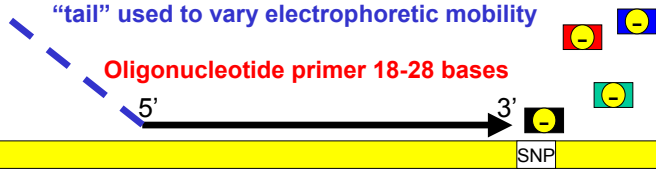
SNP Primer is extended by one base unit

ABI PRISM® SNaPshot™ Multiplex System

Fluorescently labeled ddNTPs + polymerase

“tail” used to vary electrophoretic mobility

Oligonucleotide primer 18-28 bases

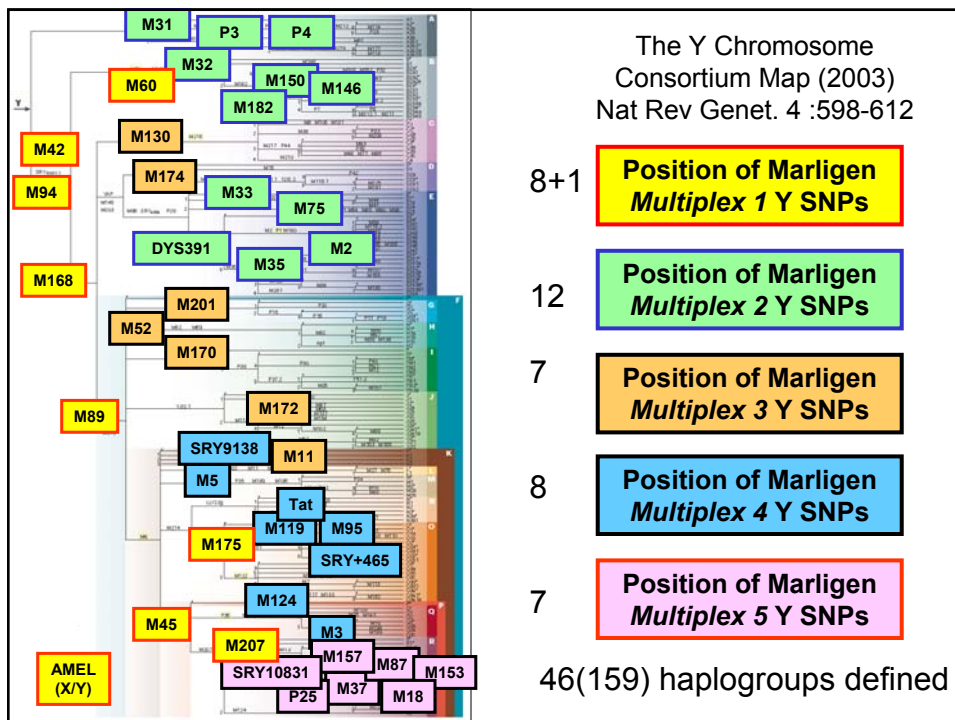
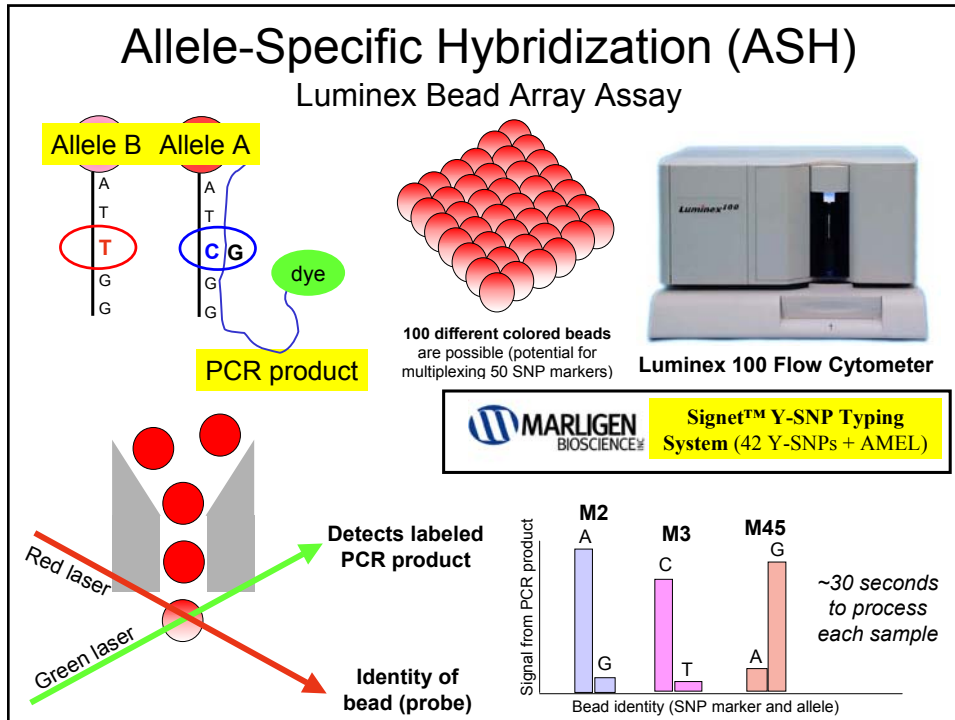


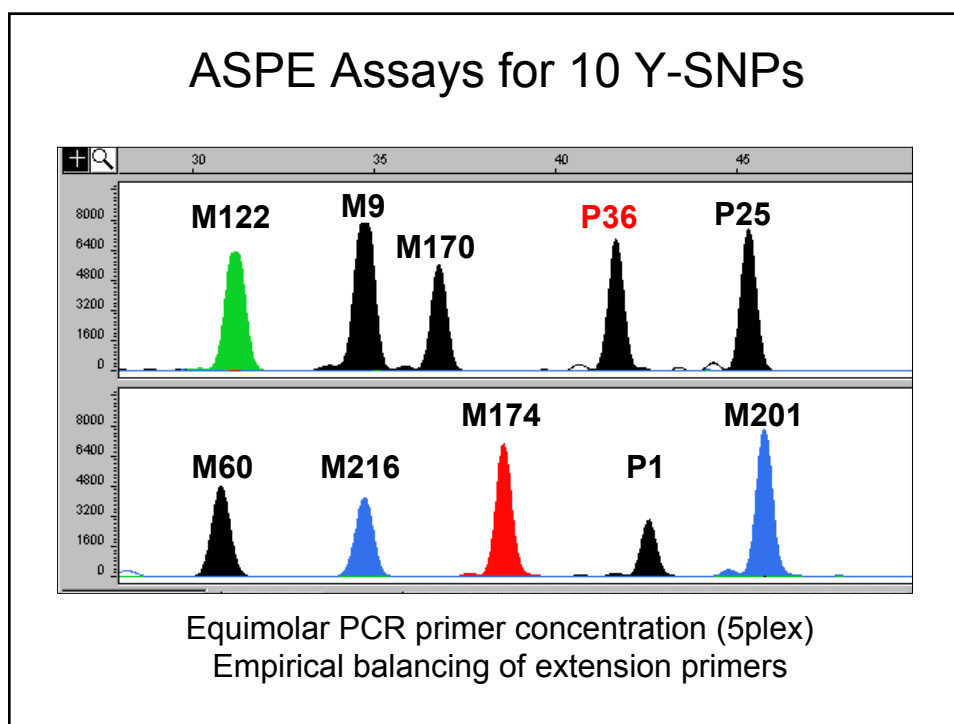
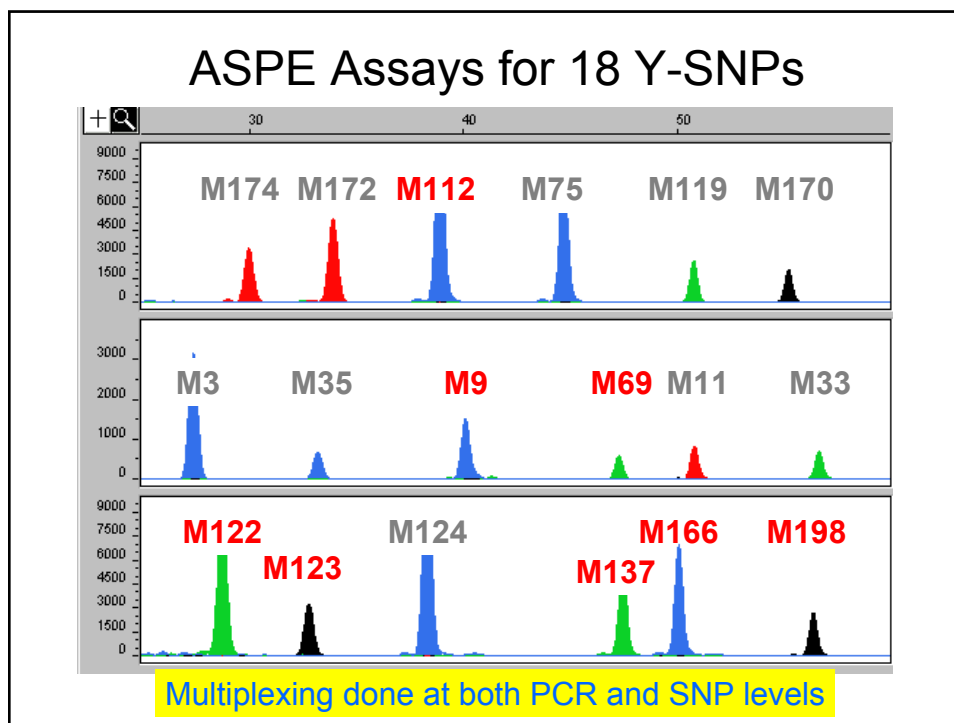
**PCR Amplified DNA Template**

ddNTP	Dye label	Color
A	dR6G	Green
C	dTAMRA	Black
G	dR110	Blue
T	dROX	Red

25 Cycles

96°C 10s  
50°C 5s  
60°C 30s





## 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

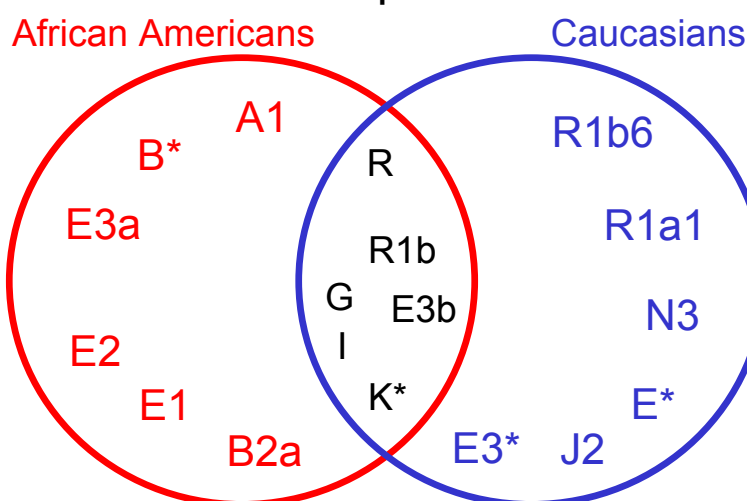
18 out of 46 haplogroups observed

Over 99 % success rate for allele calls (both methods)

Variation was observed in 24 of the 51 Y-SNPs

100% concordance for the 18 overlapping markers (>3,800 allele calls)

## Observed Haplogroups in Two U.S. Populations

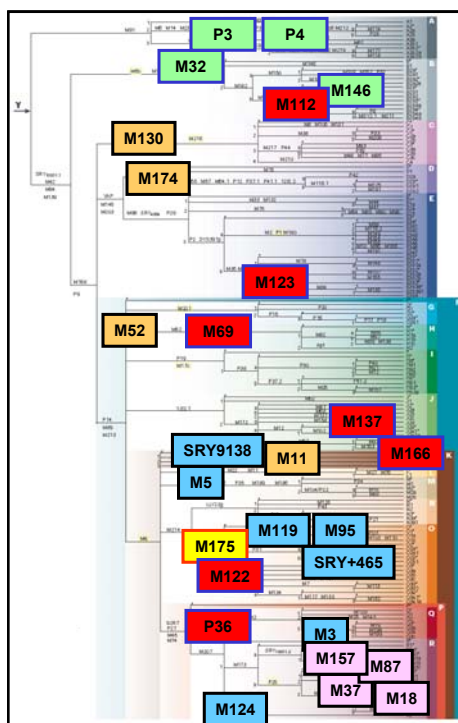


18 total Hgs; 6 shared

## Variation was not observed for 27 Y SNPs

(in AA and CAUC populations)

<u>M175 +/-</u>	<u>M119 A/C</u>	<u>M37 C/T</u>
<u>M146 A/C</u>	<u>M124 C/T</u>	<u>M87 T/C</u>
<u>M32 T/C</u>	<u>M3 C/T</u>	<u>M69 T/C</u>
<u>P3 (C/T)</u>	<u>M5 C/T</u>	<u>M112 G/A</u>
<u>P4 (G/A)</u>	<u>M95 C/T</u>	<u>M122 T/C</u>
<u>M11 A/G</u>	<u>SRY465 C/T</u>	<u>M123 G/A</u>
<u>M130 C/T</u>	<u>SRY9138 C/T</u>	<u>M137 T/C</u>
<u>M174 T/C</u>	<u>M157 A/C</u>	<u>M166 G/A</u>
<u>M52 A/C</u>	<u>M18 +/-</u>	<u>P36</u>



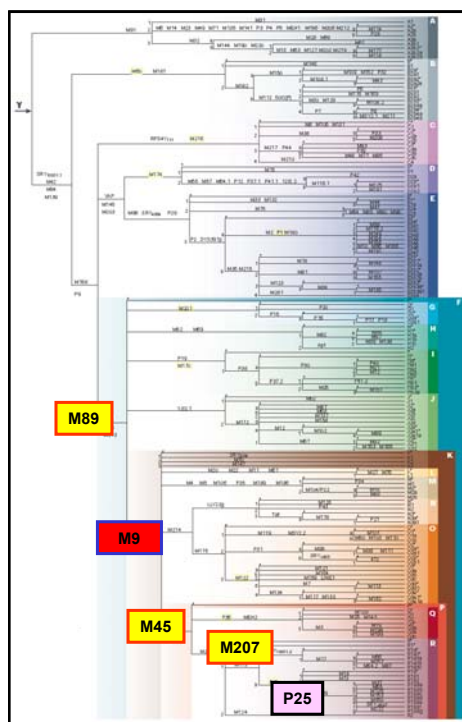
## Variation was not observed for 27 Y-SNPs

(in AA and CAUC populations)

P3 (A2)	M5 (M)
P4 (A2)	M175 (O)
M32 (A3a)	M119 (O1)
M146 (B1)	M95 (O2a)
M112 (B2b)	SRY+465 (O2b)
M130 (C)	M122 (O3)
M174 (D)	P36 (Q)
M123 (E3b)	M3 (Q3)
M52 (H)	M124 (R2)
M69 (H)	M157 (R1a1b)
M137 (J2c)	M87 (R1a1c)
M166 (J2f2)	M37 (R1b3a)
SRY9138 (K1)	M18 (R1b1)
M11 (L)	

## Y-SNPs derived at greater than 25 % in more than one population

Locus	All	AA	Cauc	Hisp	Hap
<u>M207 A/G</u>	0.46	0.27	0.65	na	R
<u>M45 G/A</u>	0.46	0.27	0.64	na	P-R
<u>M89 C/T</u>	0.64	0.32	0.96	na	F-R
<u>P25 C/A</u>	0.47	0.30	0.57	0.53	R1b
<u>M9 C/G</u>	0.53	0.31	0.65	0.64	K-R



Y SNPs observed at  
greater than 25 %  
in more than one  
population

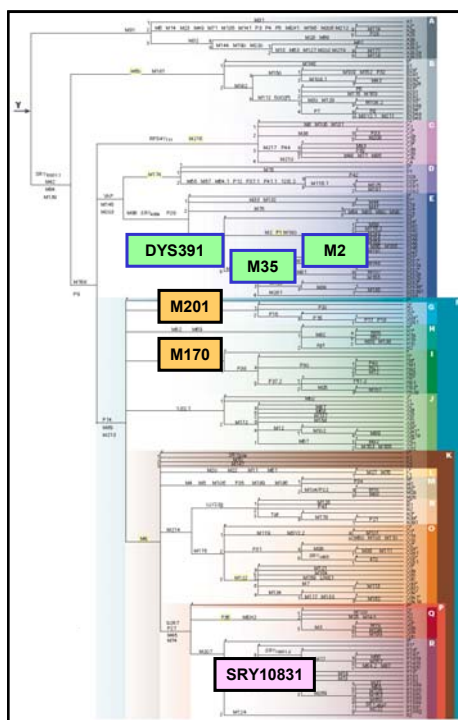
M45 (P-R)  
M89 (F-R)  
M9 (K-R)

M207 (R)  
P25 (R1b)



### Derived in more than one population

Locus	All	AA	Cauc	Hisp	Hap
<u>M2 A/G</u>	0.23	0.58	not obs	0.08	E3a
<u>DYS391 C/G</u>	0.31	0.60	0.04	na	E3
<u>M170 A/C</u>	0.10	0.04	0.21	0.04	I
<u>M35 G/C</u>	0.02	0.02	0.03	na	E3b
<u>M201 G/T</u>	0.03	0.01	0.03	0.05	G
<u>SRY10831 A/G</u>	0.03	0.01	0.05	na	R1a

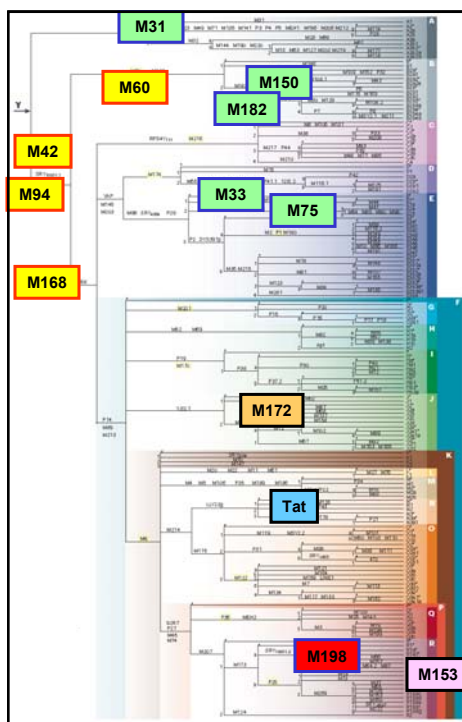


### Derived in more than one population

- M2 (E3a)
- DYS391 (E3)
- M170 (I)
- M35 (E3b)
- M201 (G)
- SRY10831 (R1a)

### Low frequency and population specific

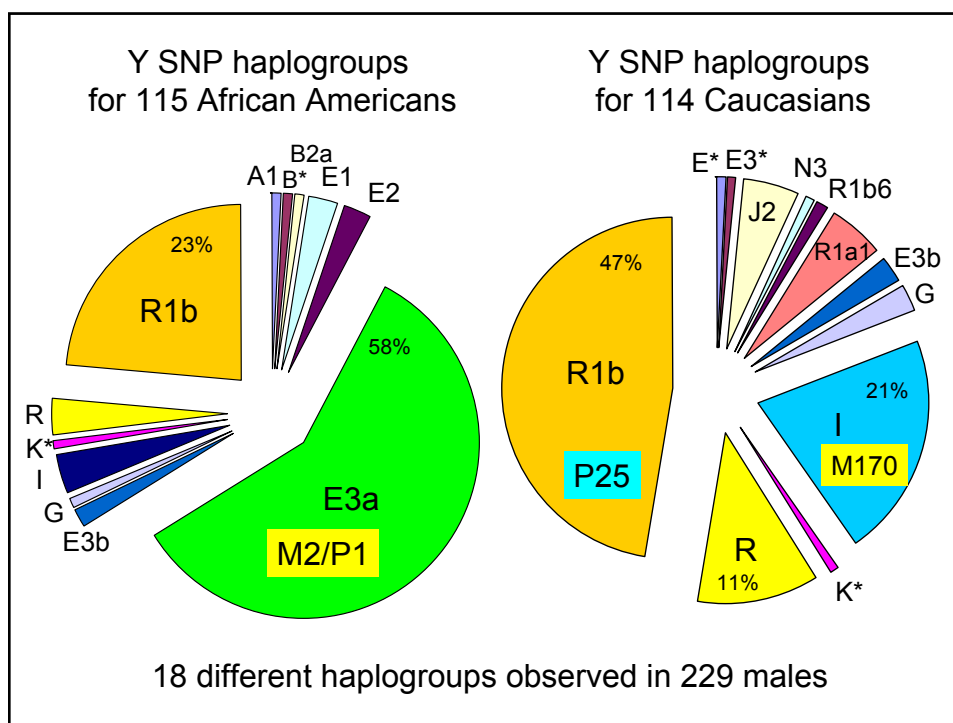
Locus	All	AA	Cauc	Hisp
<u>M168 C/T</u>	0.01	0.03	not obs	na
<u>M42 A/T</u>	0.04	0.01	not obs	na
<u>M60 -/+</u>	0.01	0.02	not obs	not obs
<u>M94 C/A</u>	0.01	0.01	not obs	na
<u>M150 C/T</u>	0.01	0.01	not obs	na
<u>M182 C/T</u>	0.01	0.01	not obs	na
<u>M31 G/C</u>	0.01	0.01	not obs	na
<u>M33 A/C</u>	0.01	0.03	not obs	na
<u>M75 G/A</u>	0.01	0.03	not obs	na
<u>M172 T/G</u>	0.03	not obs	0.05	na
<u>M198 C/T</u>	0.03	not obs	0.05	na
<u>Tat T/C</u>	0.01	not obs	0.01	na
<u>M153 T/A</u>	0.01	not obs	0.01	na



### Low frequency and population specific

- | <u>AA</u>  | <u>Cauc</u>  |
|------------|--------------|
| M168 (C-E) | M172 (J2)    |
| M42 (B)    | M198 (R1a1)  |
| M60 (B)    | TaT (N3)     |
| M94 (B)    | M153 (R1b3d) |
| M150 (B2a) |              |
| M182 (B2a) |              |
| M31(A1)    |              |
| M33 (E1)   |              |
| M75 (E2a)  |              |

Derived less than 5 %

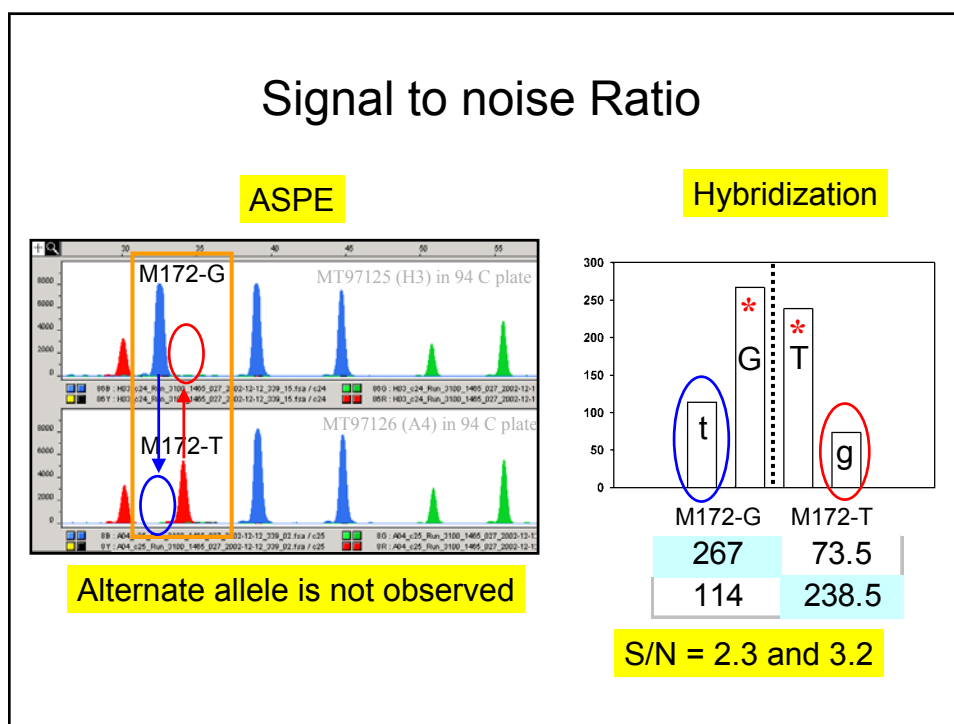
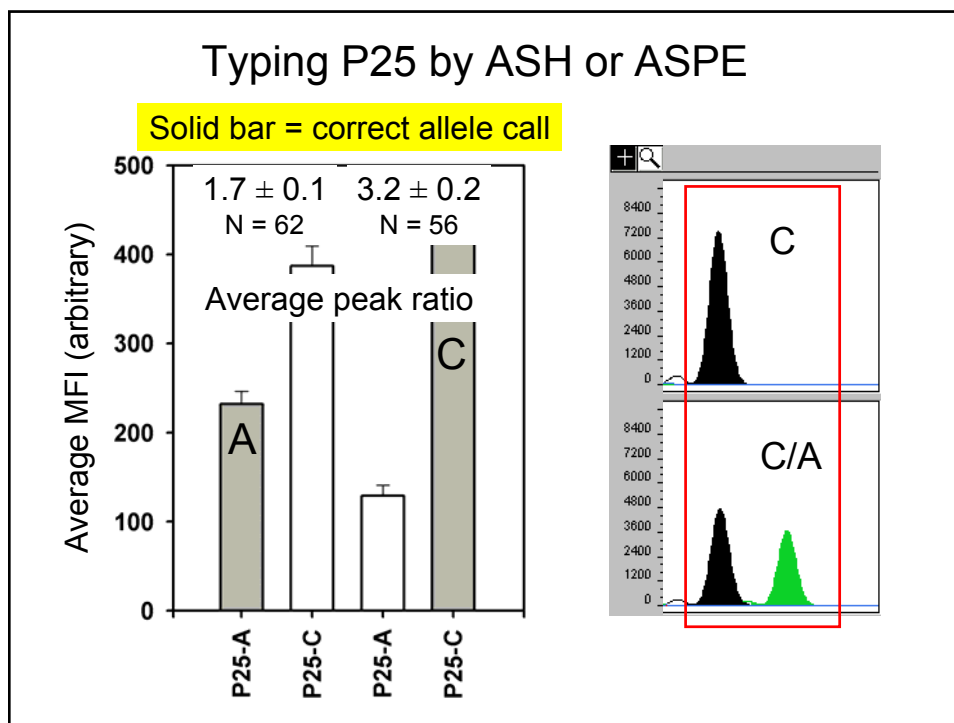


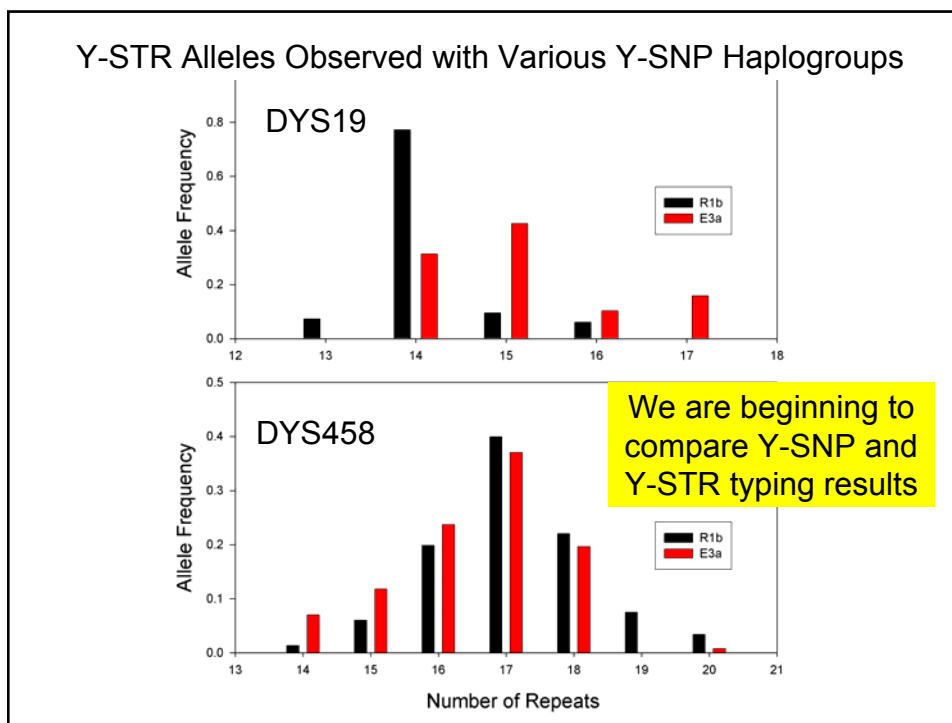
## Multi-Copy Y-SNP P25

Initially when typing P25 with the Marligen kit the derived allele (A) was not called

BLAST results indicate that the region surrounding P25 is present 3 times on the Y chromosome

After further review of our data we were able to make correct allele call for the P25 marker based on **signal intensity ratio**





## 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

## Forensic Utility 51 Y-SNPs versus 1 Y-STR

For N = 211 male samples

	<u>51Y-SNPs</u>	<u>Y-STR DYS464</u>
Amount of sample consumed	10ng	1ng
Number for types observed	<b>18</b>	<b>62</b>
Analysis	Multiple	1 reaction
Degraded samples	+	?

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### Collaborators

David Carlson (Marligen)

Mike Hammer and Alan Redd (U of AZ)



Jan Redman

Margaret Kline