Evaluation of SNPs as Tools in Human Identity Testing

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Outline

Single Nucleotide Polymorphims

mtSNPs – coding region mtSNPs

Y-SNPs – 51 Y-SNPs typed for U.S. samples

Autosomal SNPs – Panel of 70 SNPs and 12-plex assay

SNPs

Why are we interested in using SNPs?

- Use on degraded samples (WTC), low copy number, or telogenic (shed) hairs
- Lower mutation rate (Paternity testing)
- Easier data interpretation (no microvariants or stutter)
- Amenable to high throughput analysis

SNPs

General issues that need to be addressed

- How many SNPs = STR
- Multiplexing (50-plex < 1ng DNA)
- Databases
- Platform for SNP typing?
- Unique interpretation issues – mixtures
- Validation
- Sensitivity
- Cost

SNP Typing Platforms

- RT-PCR (TaqMan, Light Cycler, Molecular Beacon)
- ASPE (SNaPshot, Orchid UHT, MALDI, FP)
- Mass Spectrometry (Electrospray)
- Sequencing
- Flow Cytometry (Luminex)
- Pyrosequencing
- Ligation (SNPplex, Illumina)
- Invader assay
- ARMS assay (FSS)
- RFLP

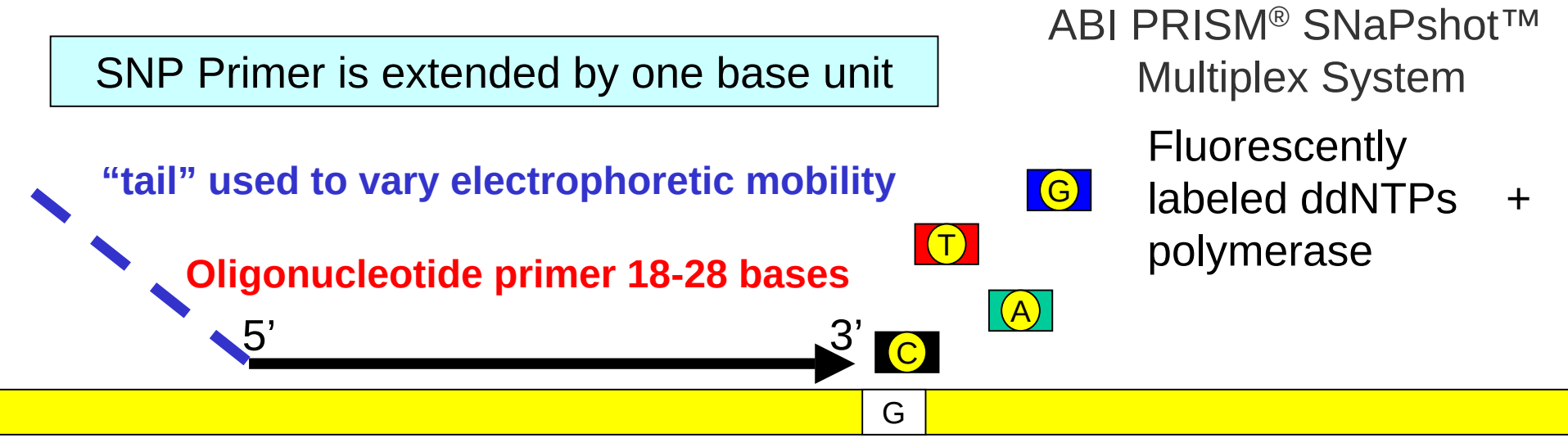
Sensitivity, multiplexing, accurate typing

Budowle 2004 FSI 139-142; Sobrino et al., 2005 FSI (epub); Dixon et al., 2005 FSI (epub)

Allele-Specific Primer Extension

SNP Primer is extended by one base unit

ABI PRISM® SNaPshot™ Multiplex System



Fluorescently labeled ddNTPs + polymerase

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

Utility of SNP Markers

Replace Autosomal STRs?

"It is unlikely that SNPs will replace STRs as the preferred method of testing of forensic samples in the near to medium future."

Specialized applications

mtDNA – coding region and linear arrays

Y-SNPs – lineage, population study, sample discrimination

Autosomal SNPs – highly degraded samples, shed hairs, physical characteristics, ethnic/geographical determination

Gill, P., Werret, D.J., Budowle, B., and Guerreri, R. Science and Justice 2004 44: 51-53

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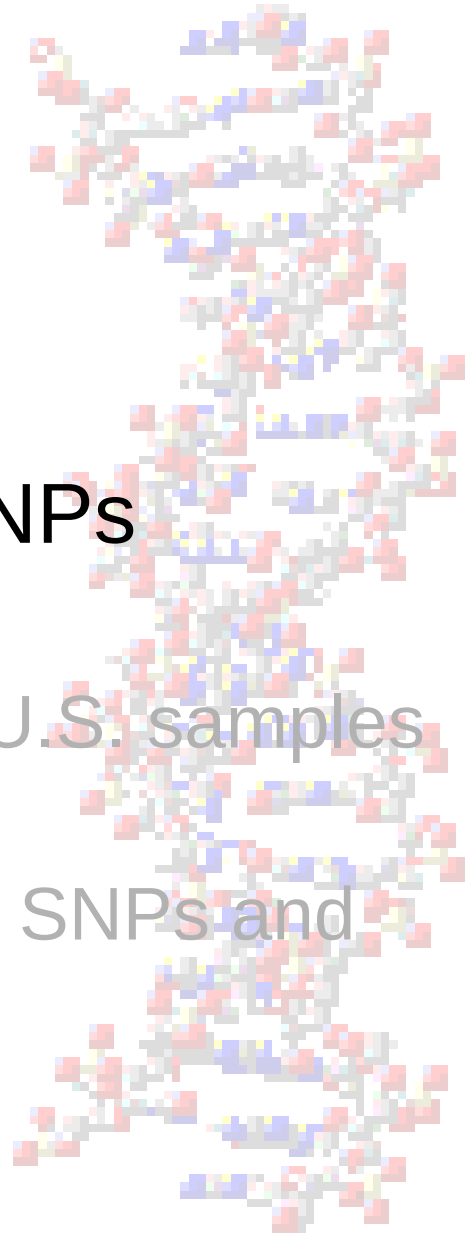
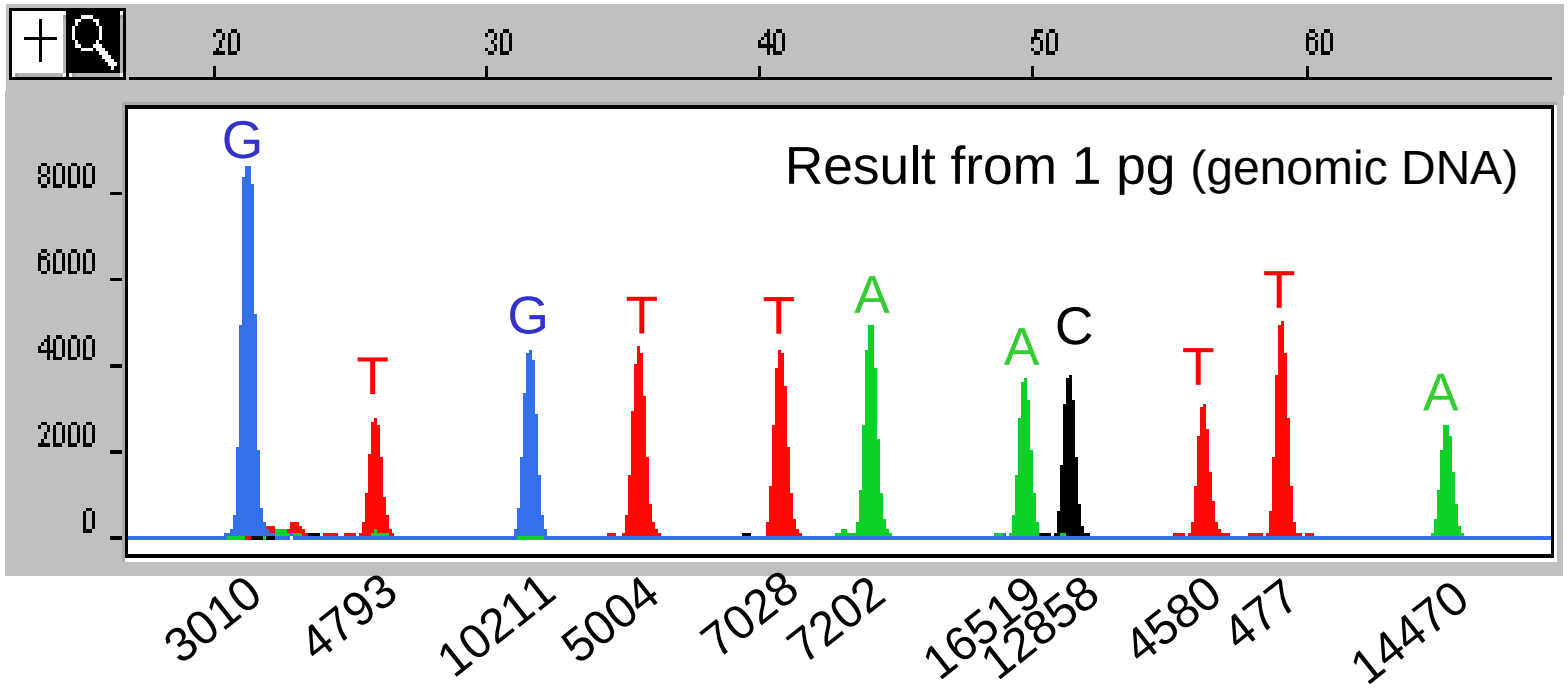
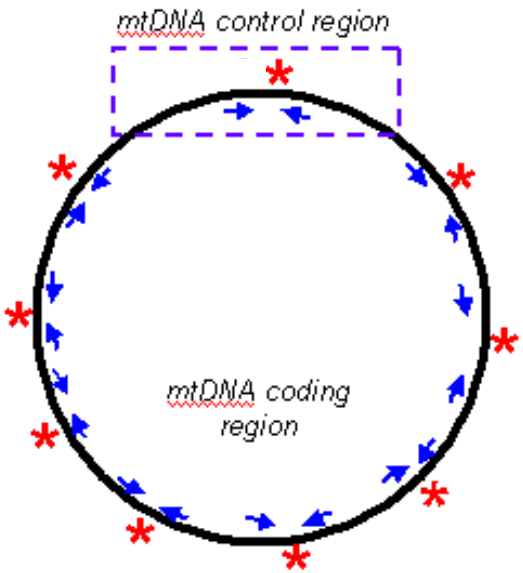


mtDNA Coding Region 11-plex ASPE Assay





11-plex PCR and 11-plex SNP detection

Sites are polymorphic in Caucasians (H1) and useful in resolving most common HV1/HV2 types

Multiplex PCR used to co-amplify all regions of interest at once

PCR product sizes kept under 200 bp to enable success with degraded DNA samples

Vallone et al., IJLM 2004 118: 147-57

Typing 51 samples with mt 11-plex assay

51 (47 cauc/4 hisp) samples were identical by Roche linear array assay (most common Haplogroup observed in NIST U.S. Caucasian population samples)

3010	G	A	G	G	G	G	A	G	G	G	G	A	G
4793	A	A	A	A	A	A	A	A	A	A	G	A	A
10211	C	C	C	C	C	C	C	C	C	C	C	C	C
5004	T	T	C	T	T	T	T	T	T	T	T	T	T
7028	C	C	C	C	T	T	C	T	C	T	C	C	C
7202	A	A	A	A	A	A	A	A	A	A	A	A	A
16519	T	C	T	C	C	T	C	C	T	T	C	C	C
12858	C	T	C	C	C	C	C	C	C	C	C	C	C
4580	G	G	G	G	G	G	G	A	G	A	G	G	G
477	T	C	T	T	T	T	C	T	T	T	T	T	T
14470	T	T	T	A	T	T	T	T	T	T	T	T	T
	rCRS	1	1	1	1	1	2	2	3	4	4	15	16

12 haplogroups were observed (5 unique)
2 of 11 sites did not vary
The 11-plex assay is currently in use at AFDIL

Kline et al., J. Forensic Sci., 2005 In Press (epub)

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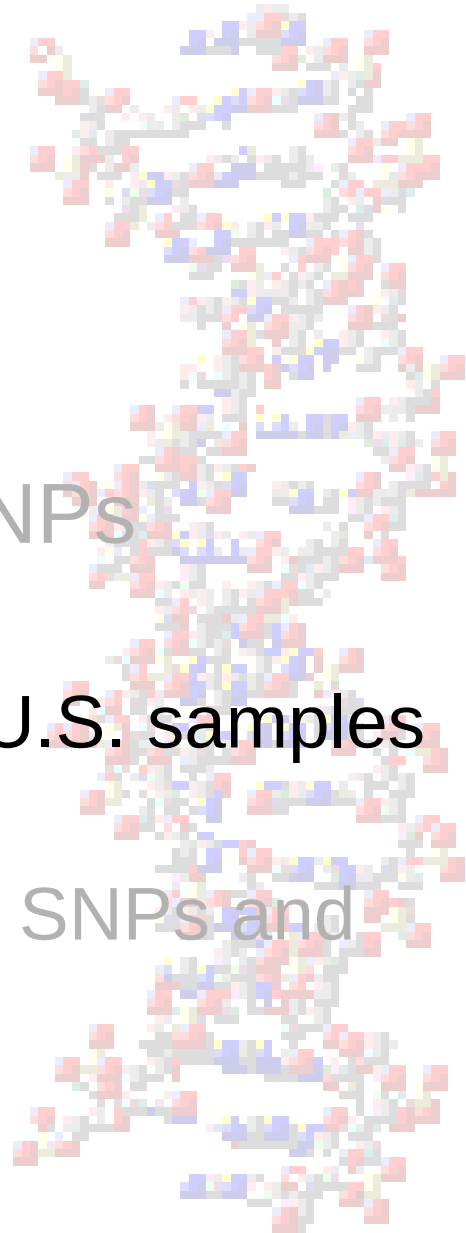


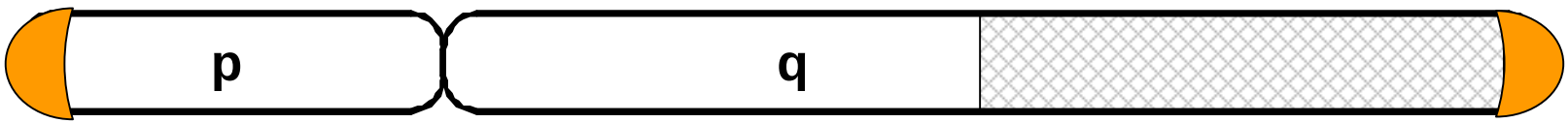
Forensic Utility of Y Chromosome SNPs

Y chromosome markers are useful in mixed male - female samples

Haplogroups are non-randomly distributed among populations therefore potential exists for predicting population of origin

Low mutation rate of SNPs 2 x 10^{-8} per base per generation

Typed 51 Y-SNPs using ASPE and Marligen (Luminex beads)



heterochromatin

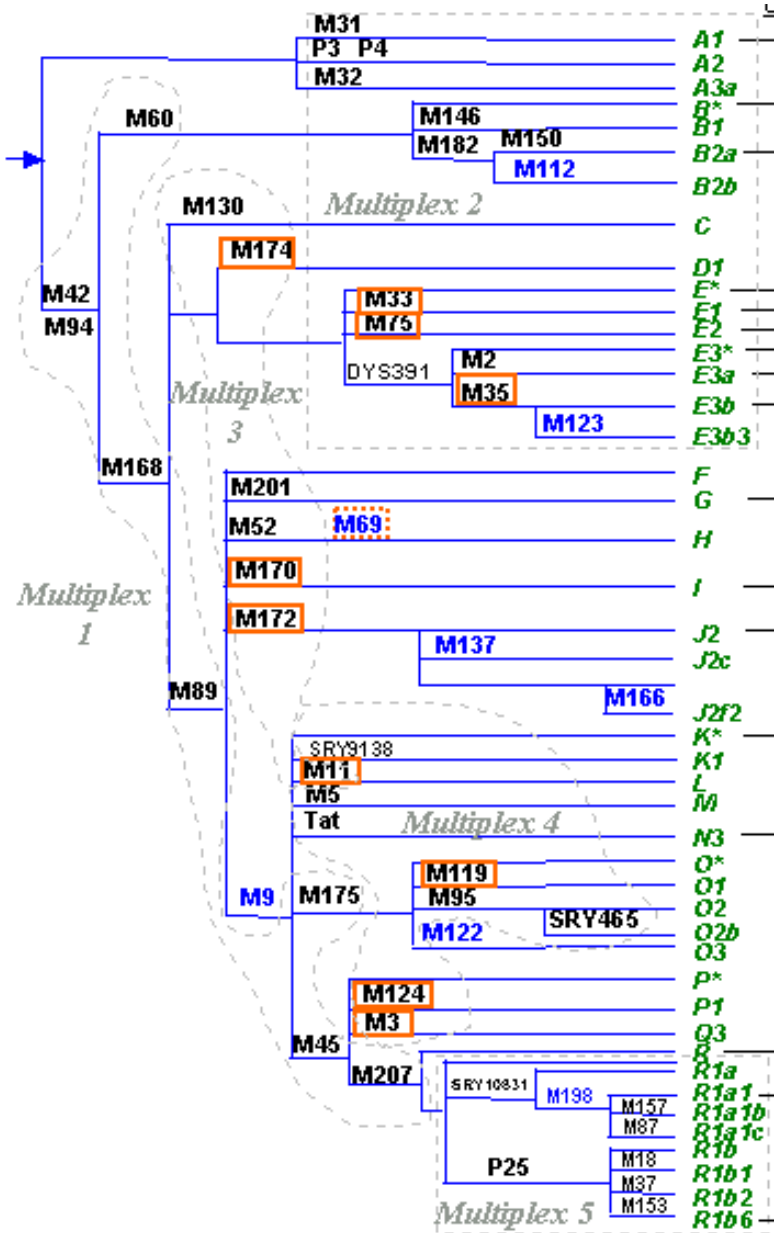
Pseudoautosomal region

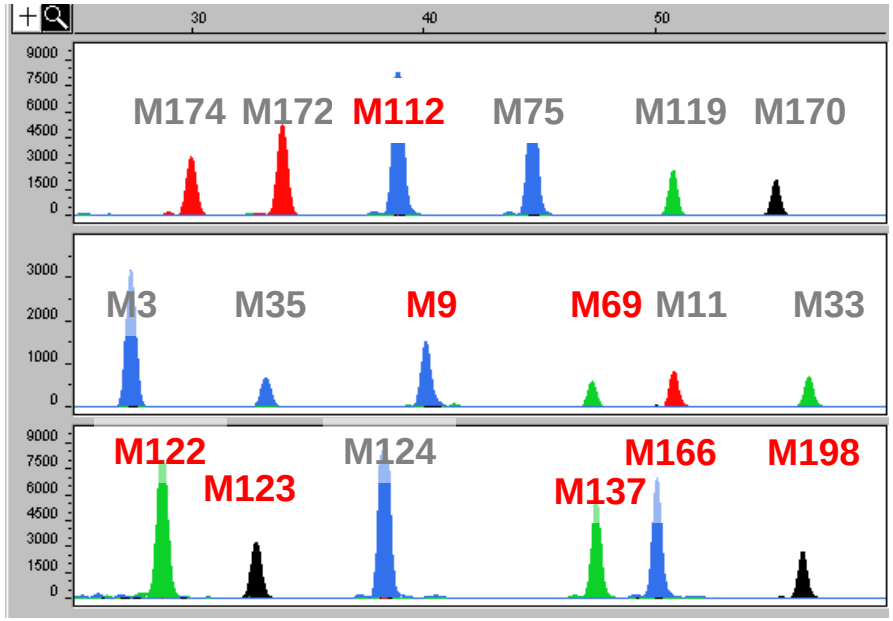
Pseudoautosomal region

>250 Y-SNPs described

The Y Chromosome Consortium Map (2003) Nat Rev Genet. 4 :598-612

Typing 51 Y-SNPs

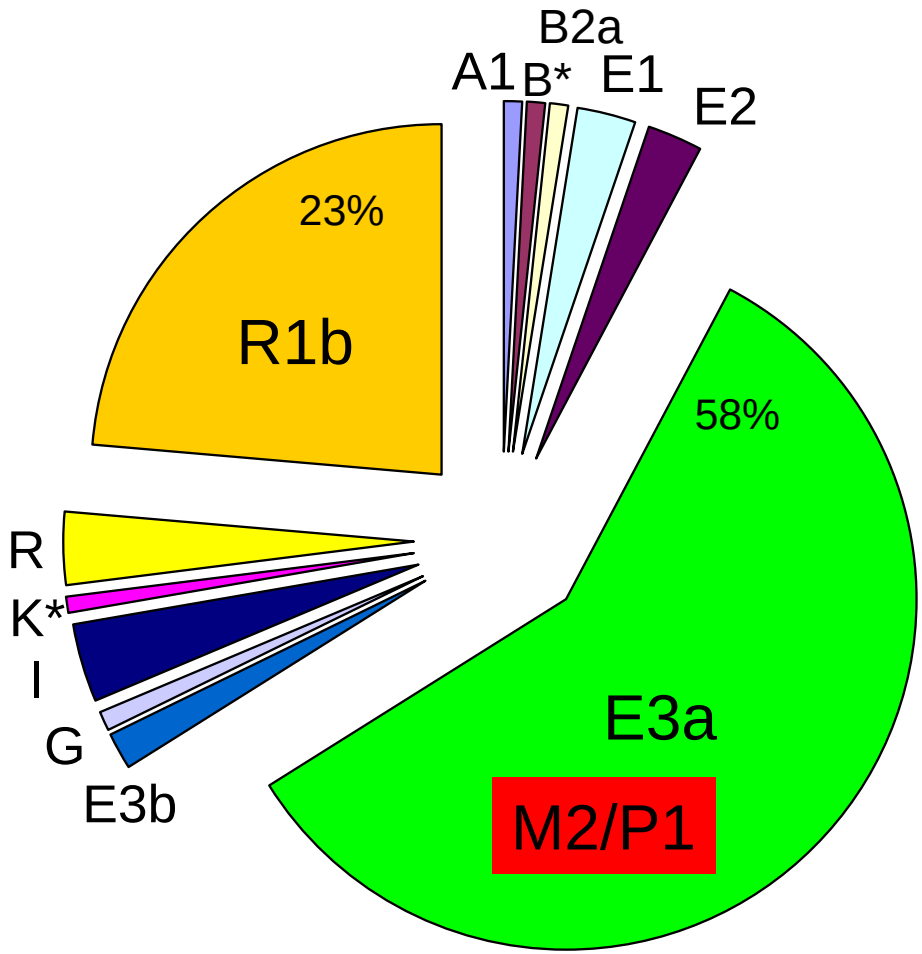




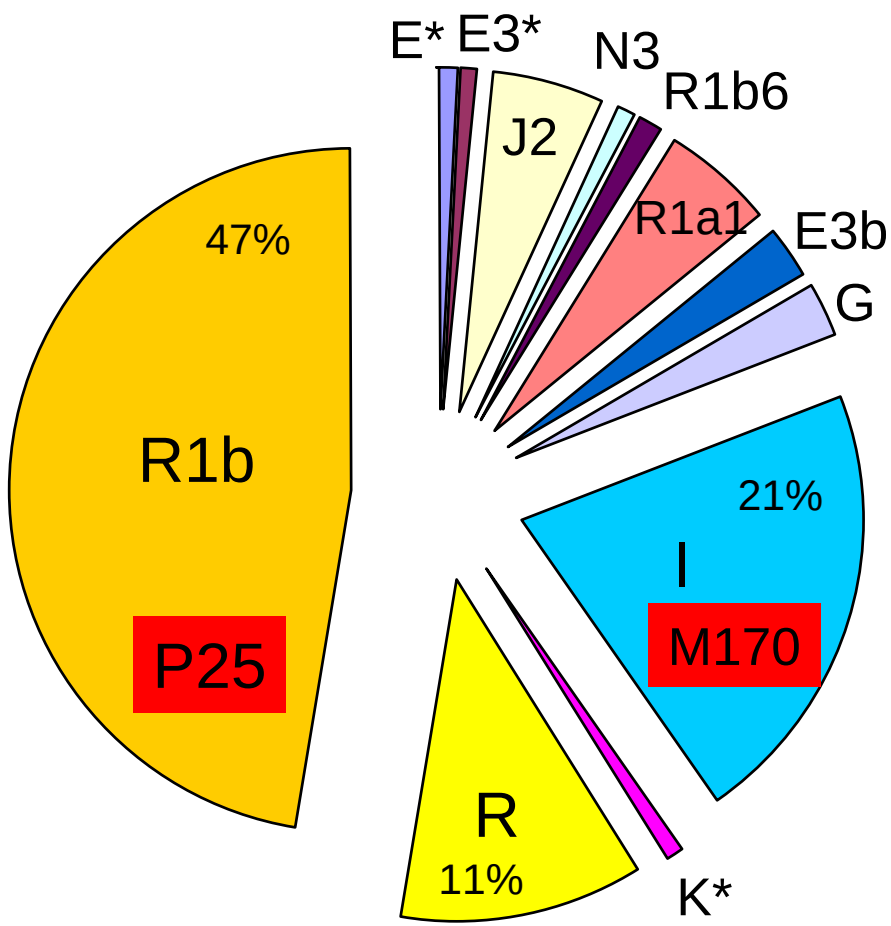
51 Y-SNPs
115 African Americans
114 Caucasians

Concordant typing results
for over 19 loci
(>3,800 allele calls)

Y-SNP haplogroups
for 115 African Americans



Y-SNP haplogroups
for 114 Caucasians



18 of 46 different haplogroups observed in 229 males

Variation was observed
in 24 of the 51 Y-SNPs

Vallone and Butler, J Forensic Sci. 2004 49: 723-32

Outline

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Y-SNPs – 51 Y-SNPs typed for U.S. samples

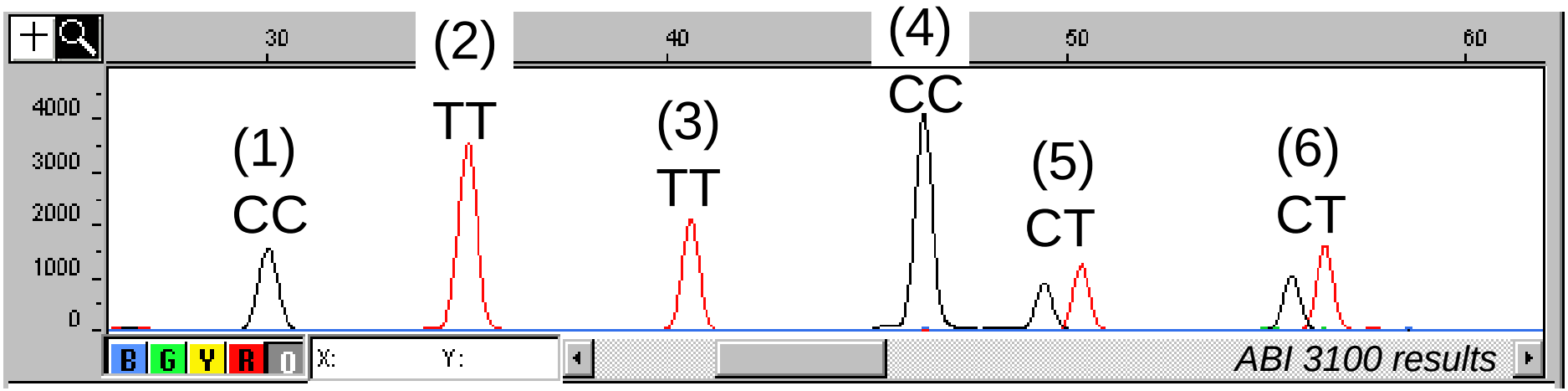
Autosomal SNPs – Panel of 70 SNPs and 12-plex assay

Autosomal SNP characteristics

- 70 Loci – sites from Orchid – C/T bi-allelic
- Present on 20 of 22 autosomal CHR (3,16,X,Y)
- Amplicon size range 59 - 108 bp (average 69)
- Markers are typed by allele-specific primer extension assays (ABI SNaPshot)
- Level of multiplexing (6- 12-plexes)
- Web page for SNP site info

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

6-plex SNP Assay



CHR: 2 13 6 6 15 17

Extension primers for 6-plex

(1) TTTTTAGCTCCTAATTTCTTGATGGG
(2) TTTTTTTTTTCATCTGATGCCATGAGAAAGC
(3) TTTTTTTTTTTTTTGTTCTGCTTTAATACAAAACCAG
(4) TTTTTTTTTTTTTTTTTTTTTTTTATAAAGGGCAGAATGAGGATTA
(5) TTTTTTTTTTTTTTTTTTTTTTTTTAGAAAGTATCTTGCAAAAGGTCCA
(6) TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCATAATCACAGCTTTTTTCTCCCAA

SNP Assay Results

70 were typed for 189 U.S. samples (self identified ethnicities)
74 Caucasians + 71 African Americans AA + 44 Hispanics

Total of 13,230 possible genotypes

42 Samples were re-injected to confirm ambiguous results
(99.7 %) success rate on first pass

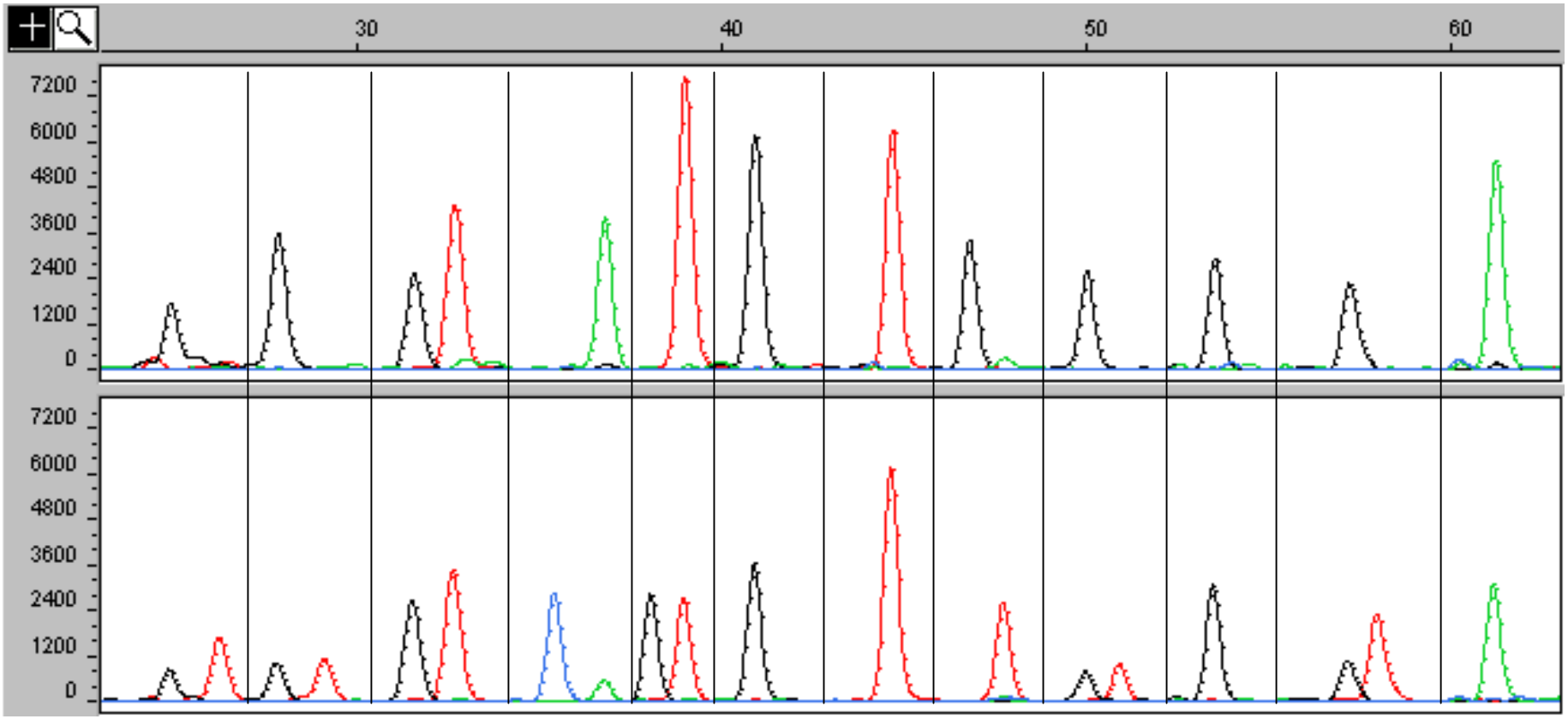
Allele distribution ranged from (0.25 – 0.74)

P-value was < 5% for 10 loci

Results described in manuscript *(Vallone, P.M., Decker, A.E., Butler, J.M. (2005) Forensic Sci. Int., in press.)*

Results on a 12-plex panel of SNPs to follow…

Autosomal SNP 12-plex



CHR: 13 15 10 01 17 13 17 01 06 11 20 15

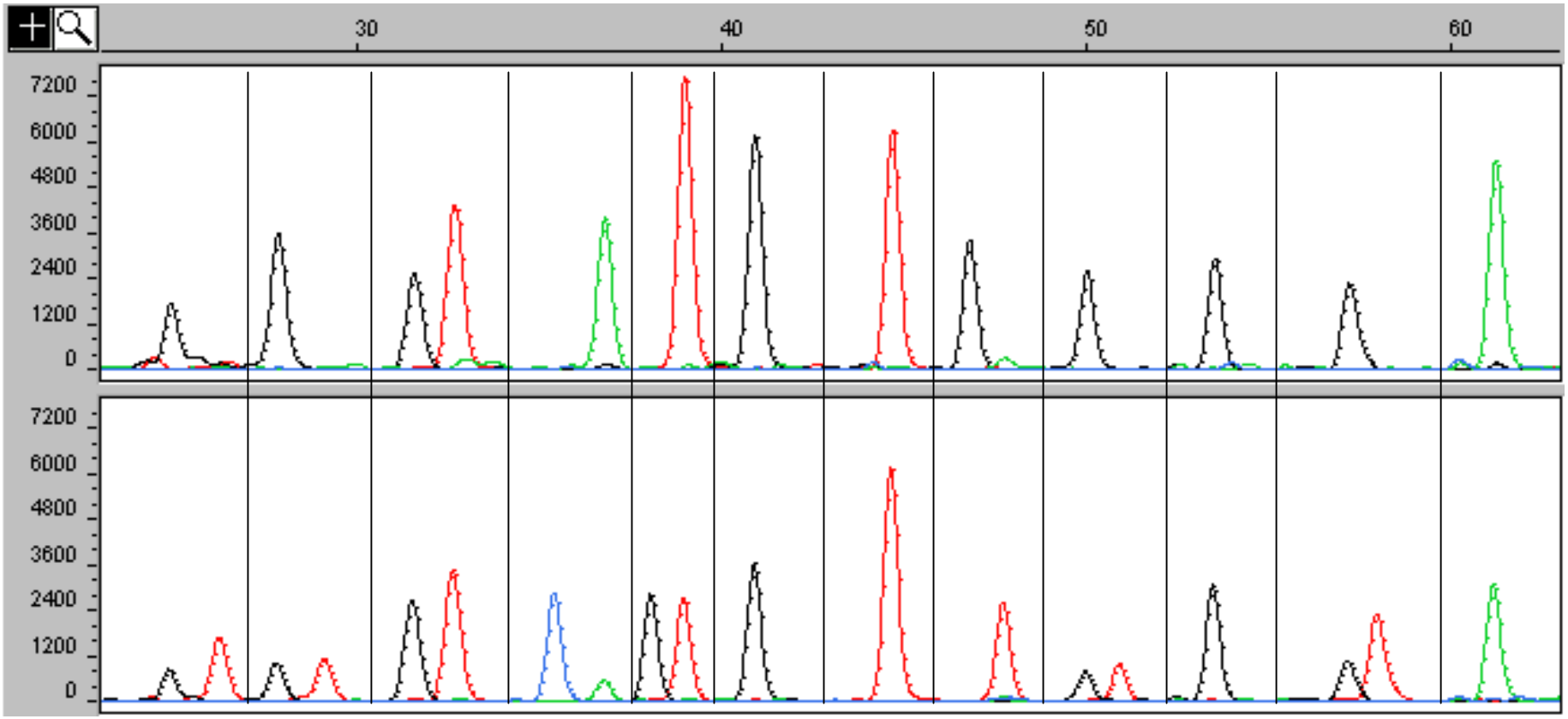
A subset of the 70 SNP markers

Observed heterozygosity of >0.45

in each of the 3 populations

32 cycle PCR
1.5 U Taq Gold
15 uL volume

Autosomal SNP 12-plex



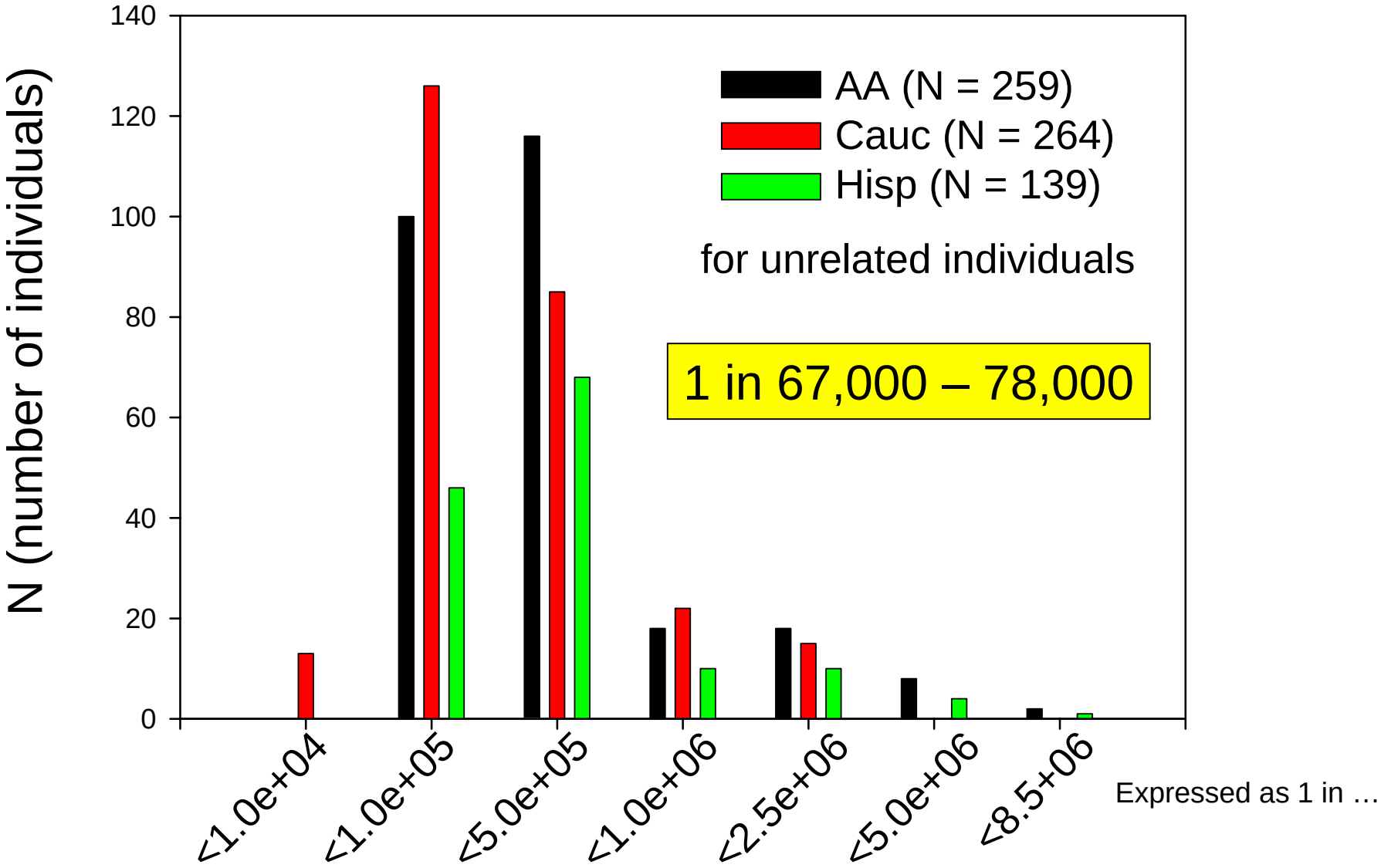
CHR: 13 15 10 01 17 13 17 01 06 11 20 15

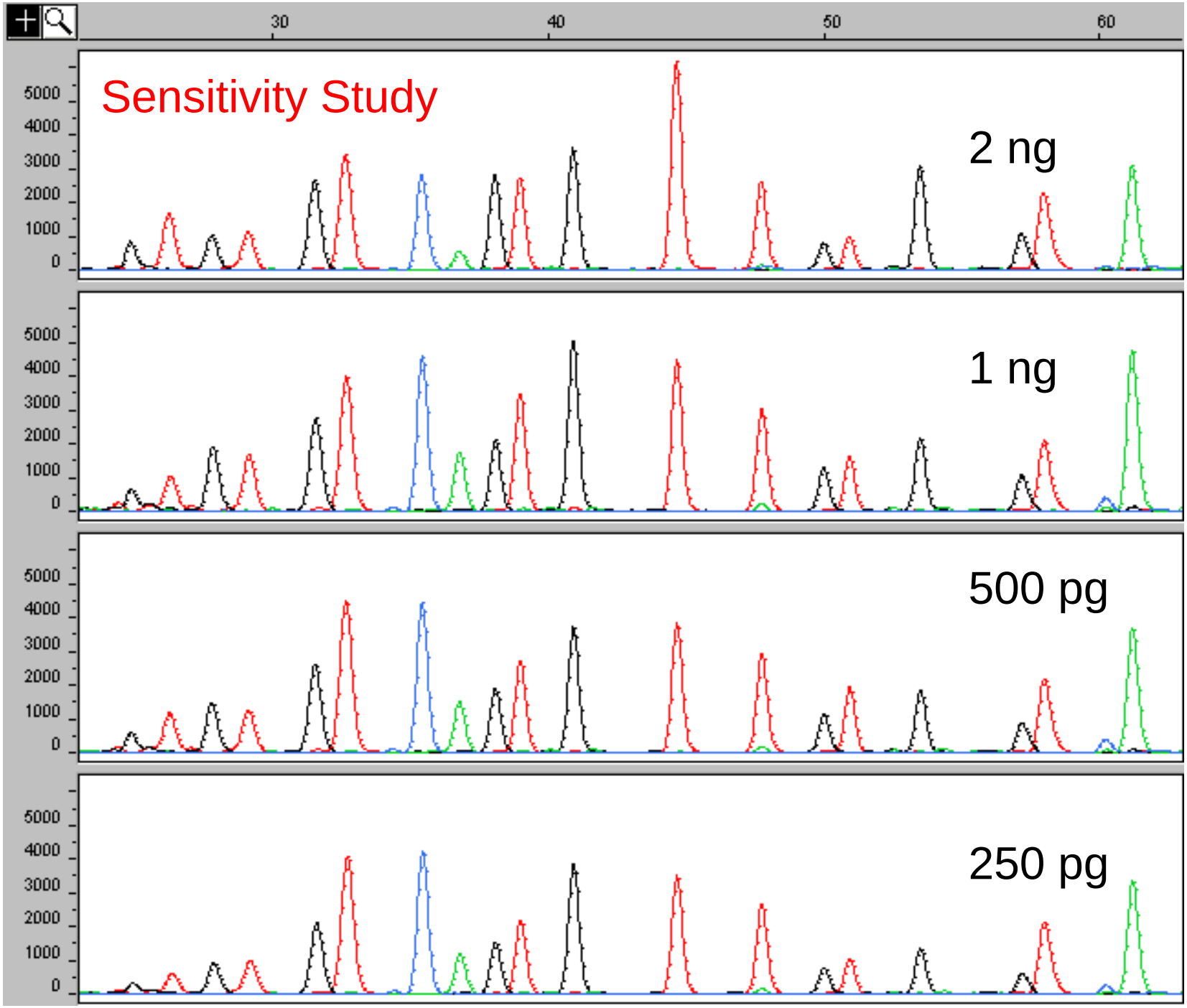
NIST population samples (N = 662) were typed using the 12-plex

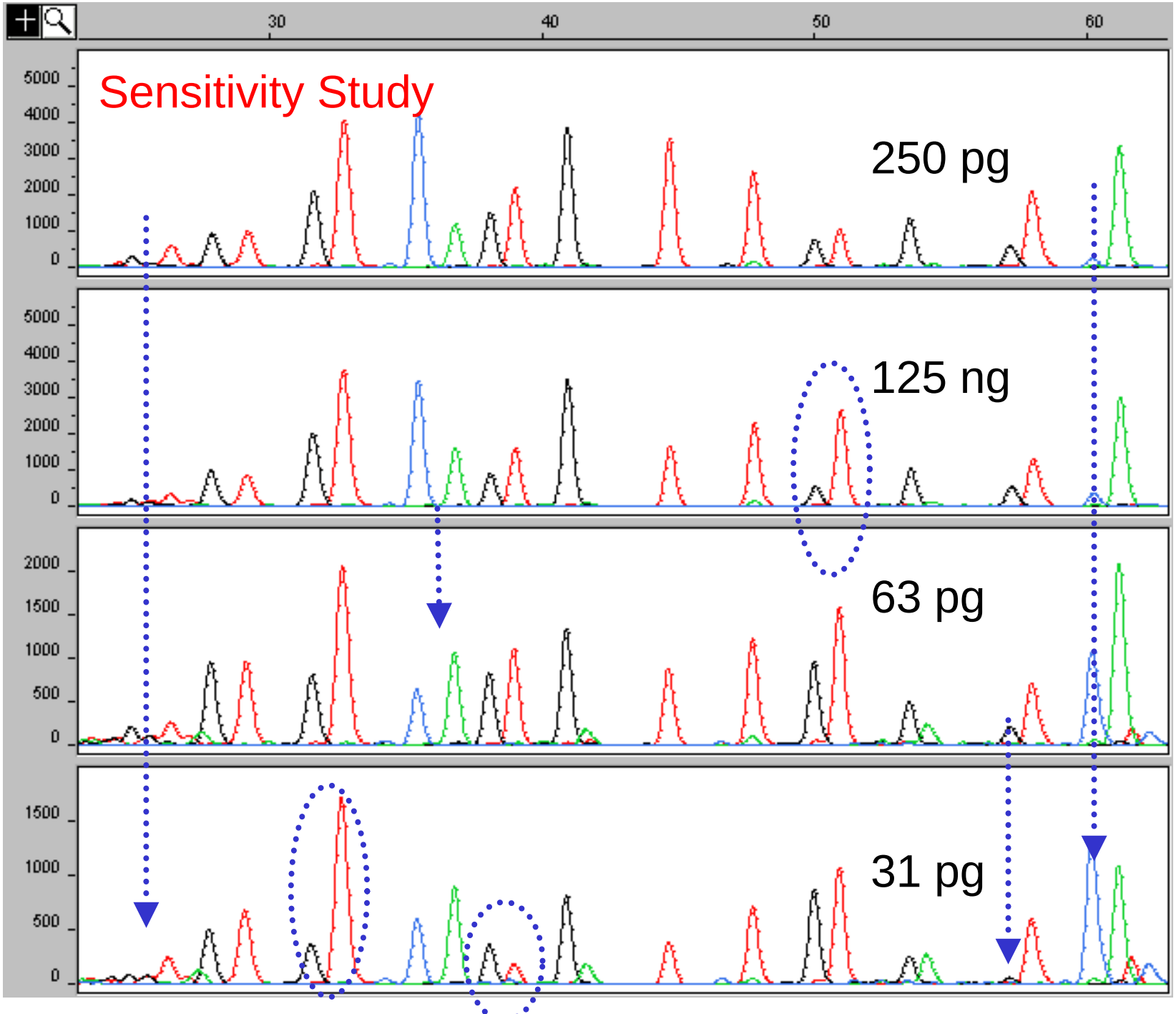
AA (N = 259); Caucasian (N = 264); Hispanic (N = 139)

12-plex typing results 100 % concordant with 6-plexes (N = 189)

Probability of a Random Match using 12-plex




Sensitivity Study
30
40
50
60
5000
4000
3000
2000
1000
0
2 ng
1 ng
500 pg
250 pg

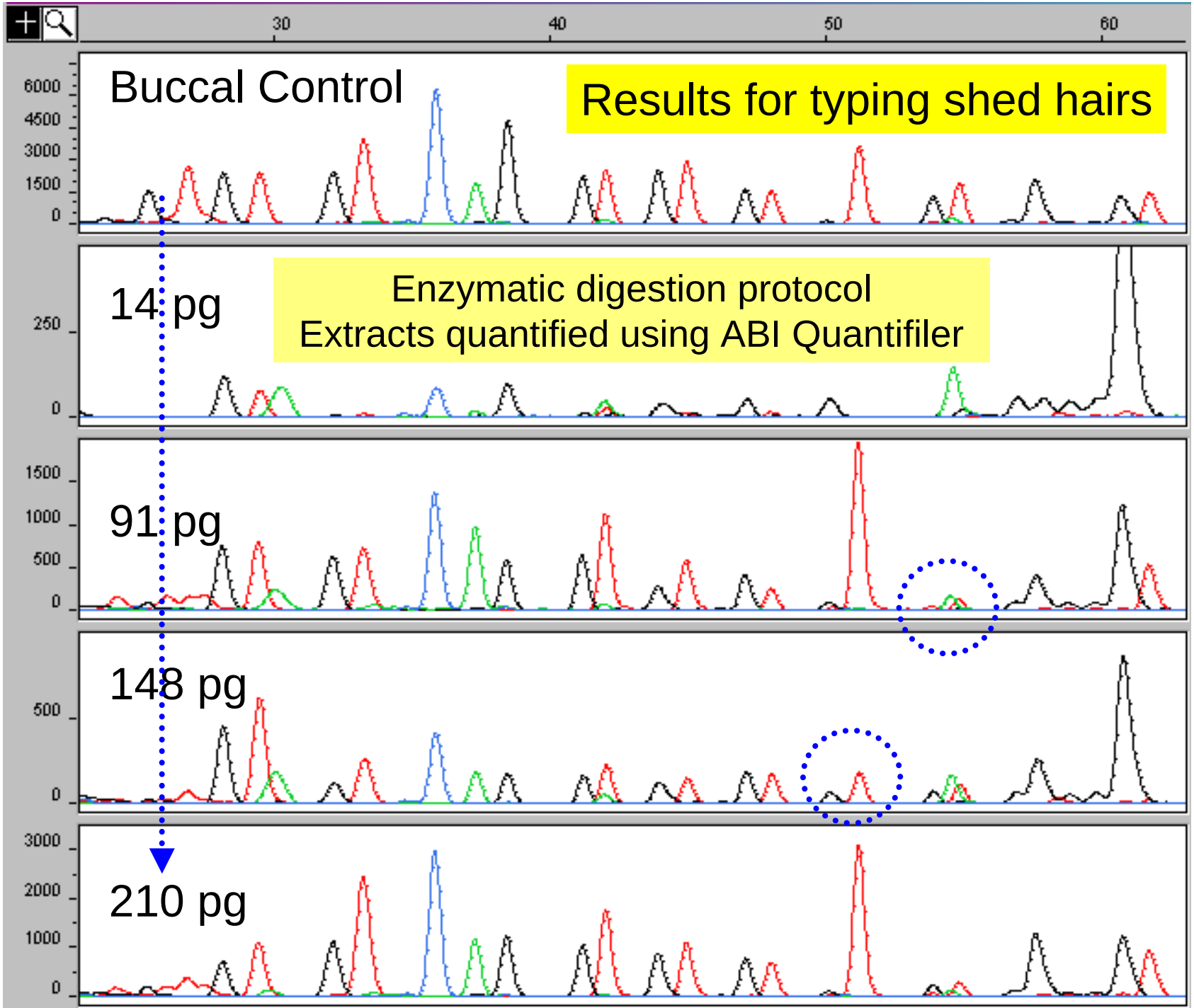

Sensitivity Study
250 pg
125 ng
63 pg
31 pg
30
40
50
60
5000
4000
3000
2000
1000
0
2000
1500
1000
500
0
1500
1000
500
0

Sensitivity Study

pg	1	2	3	4	5	6	7	8	9	10	11	12
Cntrl	CT	CT	CT	CT	CT	CC	TT	TT	CT	CC	CT	TT
2000	CT	CT	CT	CT	CT	CC	TT	TT	CT	CC	CT	TT
1000	CT	CT	CT	CT	CT	CC	TT	TT	CT	CC	CT	TT
500	CT	CT	CT	CT	CT	CC	TT	TT	CT	CC	CT	TT
250	CT	CT	CT	CT	CT	CC	TT	TT	CT	CC	CT	TT
125	ct	CT	CT	CT	CT	CC	TT	TT	Ct	CC	CT	CT
62.5	ct	CT	CT	CT	CT	CC	TT	TT	CT	CC	CT	CT
31.25	TT	CT	cT	CT	CT	CC	TT	TT	CT	CC	cT	Ct

Signal for locus 1 drops out first

An artifact in locus 12 obscures typing < 250 pg


30
40
50
60
Buccal Control
Results for typing shed hairs
6000
4500
3000
1500
0
14 pg
Enzymatic digestion protocol
Extracts quantified using ABI Quantifiler
250
0
91 pg
1500
1000
500
0
148 pg
500
0
210 pg
3000
2000
1000
0

SNP 12-plex typing results on a shed hair

	1	2	3	4	5	6	7	8	9	10	11	12		
N13	CT	CT	CT	CT	CC	CT	CT	CT	TT	CT	CC	CT	pg of DNA	cm
N13.2	X	CT	CT	CT	CC	CT	CC	CC	CC	CC	X	CC	14	15
N13.4	X	CT	CT	CT	CC	CT	CT	CT	TT	TT	CC	CT	91	20
N13.5	X	CT	CT	CT	CC	CT	CT	CT	CT	CT	CC	CC	148	22
N13.6	X	CT	CT	CT	CC	CT	CT	CT	TT	CT	CC	CT	182	32

Low signal for N13.2 (14 pg of DNA)
Signal for Locus 1 is too low to type
ASPE artifacts prohibit locus 12 from being typed

Conclusions

- mtSNPs: Coding region SNPs can fulfill a useful role for separating common HV1/HV2 mitotypes

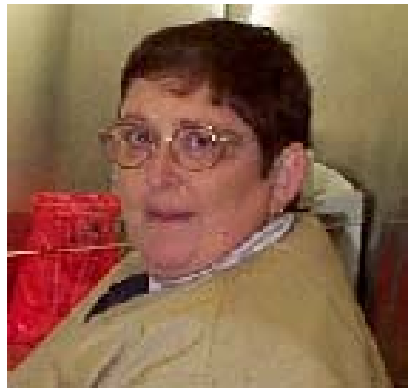
- Y-SNPs: Y-SNPs will have limited utility for individualizing a sample. Determination of ethnic origin may be challenging for U.S. samples

- Autosomal SNPs: 12-plex assay shows some promise for typing degraded samples and shed hairs
  Further work needs to be performed for full characterization of the 12-plex assay

NIST Human Identity Project Team

John Butler

Mike Coble



Margaret Kline

Amy Decker

Dave Duewer

Jan Redman

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