Development of Multiplexed SNP Assays from Mitochondrial and Y Chromosome DNA for Human Identity Testing

NIJ Grantees Meeting

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DNA Technologies Group - NIST

Overview

Multiplexing

U.S. Population Samples

Assays and Instrumentation

Y Chromosome and Mitochondrial Markers

Results

- mtSNP 11 plex
- Y SNP multiplexes

Advantages of Multiplexing

Obtain more information per unit time

Reduce the amount of limited forensic sample used

Save on reagents; enzyme, buffers, DNA oligomers

Reduces labor

Streamlines data analysis

For certain markers it is essential (SNPs, YSTRs)

Coincides with high capacity instrumentation and new SNP typing technologies

Goals for Multiplex Assay Development

Working with collaborators who have markers of forensic interest

Evaluate the forensic utility of newly discovered markers (medium sized multiplexes 5 – 10 loci)

Further the understanding of developing multiplex assays (primer design, QC)

Publish assay details for others to evaluate (commercial and research)

NIST U.S. Population Samples

As of 06/2003 663 (males with self identified ethnicities)

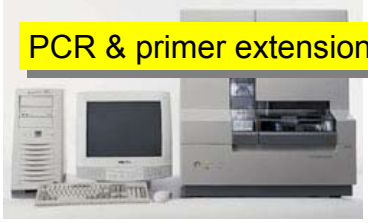
260 Caucasians
260 African Americans
140 Hispanic
3 Asian

~80 μg total extracted genomic DNA
Working plates 1 ng/uL

To date: (35,139 allele calls)
Identifiler (15 autosomal markers + Amelogenin) (10,608)
Roche Mito-strips (HV1 HV2 10 regions) (6,630)
Y STRs 27 markers (17,901)

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

Bio-Rad iCycler

Allele-Specific Primer Extension

SNP Primer is extended by one base unit

"tail" used to vary electrophoretic mobility

Oligonucleotide primer 18-28 bases

5' 3'

SNP

PCR Amplified DNA Template

ABI PRISM® SNaPshot™ Multiplex System

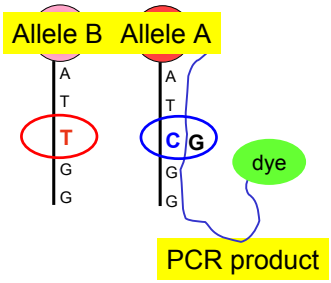
Fluorescently labeled ddNTPs + polymerase

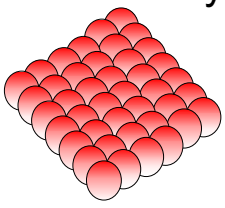
ddNTP	Dye label	Color
A	dR6G	Green
C	dTAMRA	Black
G	dR110	Blue
T	dROX	Red

25 Cycles
96°C 10s
50°C 5s
60°C 30s

Y SNP Detection by Hybridization

Luminex Bead Array Assay



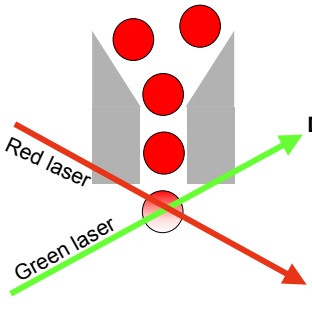


100 different colored beads are possible (potential for multiplexing 50 SNP markers)

Luminex 100 Flow Cytometer

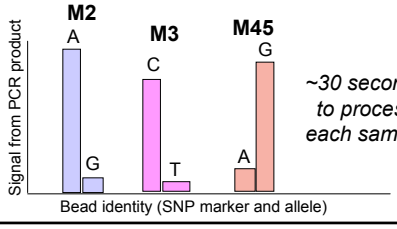
MARLIGEN BIOSCIENCE

Signet™ Y SNP Typing System (42 Y SNPs + AMEL)



Detects labeled PCR product

Identity of bead (probe)



~30 seconds to process each sample

Advantages of Typing SNPs?

Probing a single base change usually only requires PCR amplification of the region surrounding the site

Shorter PCR amplicons may result in success with degraded samples and possibly higher sensitivity

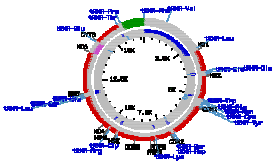
Simplicity in testing – typically bi-allelic markers (versus length polymorphisms)

Improve multiplex assay development (both PCR and SNP detection)

For serious forensic usage parallel high-throughput methods will be required for typing

Markers of Interest

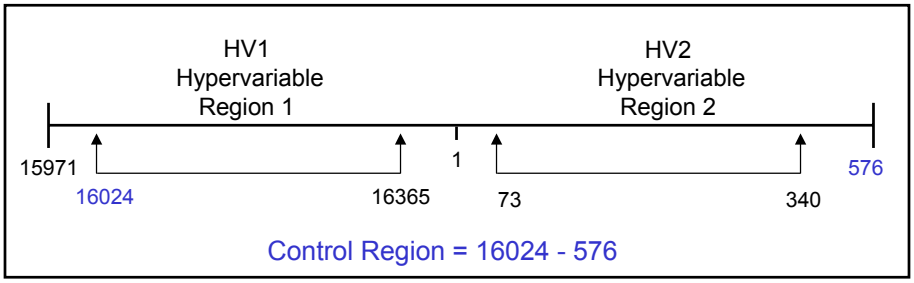
- Mitochondrial DNA (mtDNA)
  - maternally inherited
  - polymorphic control region (D-loop)
  - ~500-2000 copies per cell
  - coding region



- Y chromosome
  - paternally inherited
  - variety of Y STR and Y SNP markers
  - ***haplotype rather than genotype***

Require large databases because recombination does not occur

The Current mtDNA Amplification & Sequencing Strategy Focuses on the Hypervariable Regions of the mitochondrial genome HV1 and HV2



In Caucasians, approximately 7% of HV1 and HV2 sequences are identical

The Use of Full mtGenome Polymorphisms

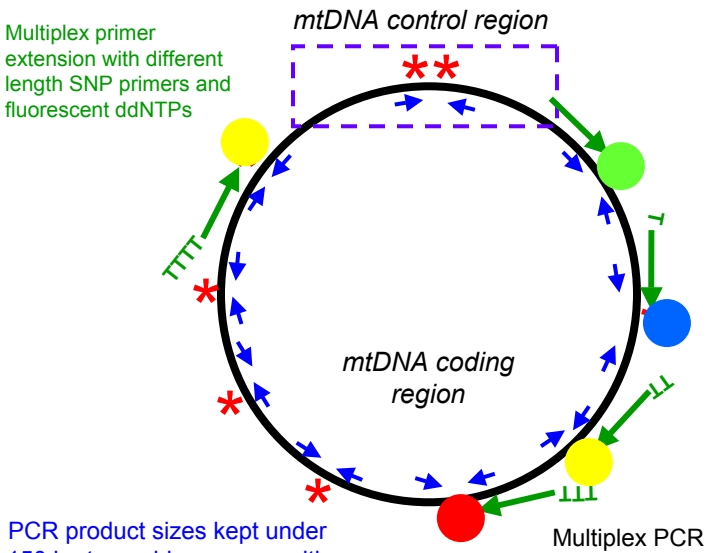
mtGenome sequencing data reveals numerous SNPs that can help distinguish Caucasians sharing common HV types (Tom Parsons and Mike Coble AFDIL)

11 SNP sites were selected to help resolve Caucasian individuals having the most common HV1/HV2 type

mtSNPs: Silent and at third codon positions or fall in the short non-coding regions between genes in the coding region

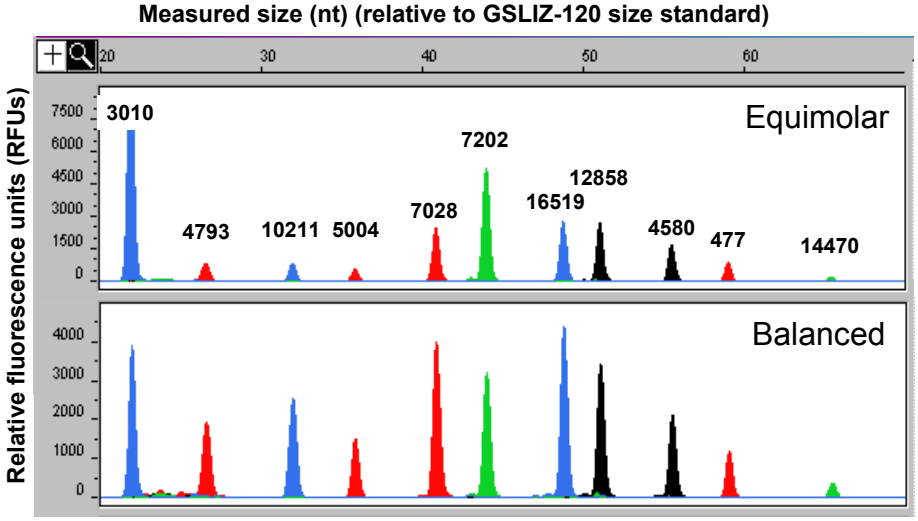
Detect in a multiplex assay run on a common forensic instrumental platform

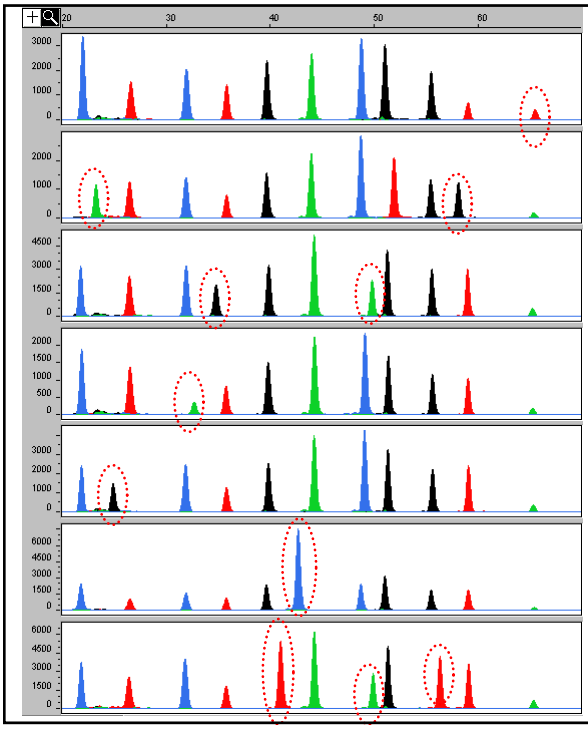
mtSNP 11-plex Assay



mtSNP 11-plex run on ABI 3100

Multiplex PCR and Multiplex SNP Detection



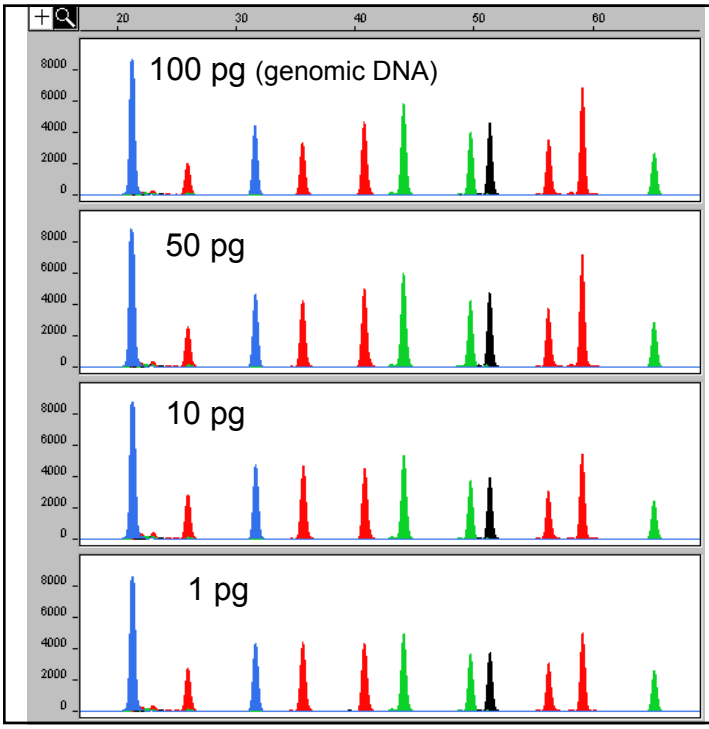


11 plex run on 7 unique samples

All allele variations are represented in these 7 samples

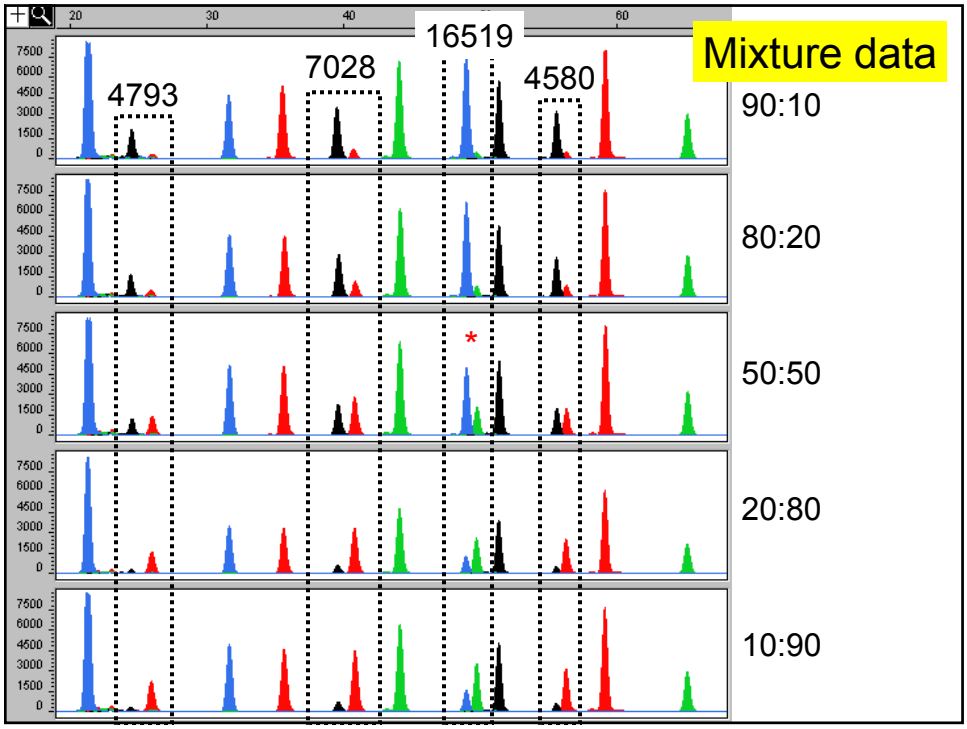
The assay accurately detects each variant

Sizing can be used to develop a macro for automated typing

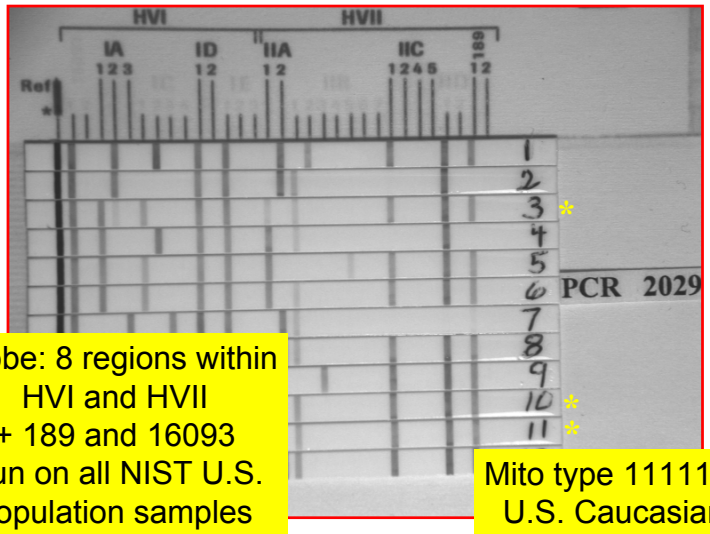


Sensitivity Study

Assay performs down to 1 pg of genomic DNA



Roche Mito Strips



Probe: 8 regions within HVI and HVII + 189 and 16093 Run on all NIST U.S. population samples

Mito type 11111111AT
U.S. Caucasian pop
44 / 286 = 15.4%

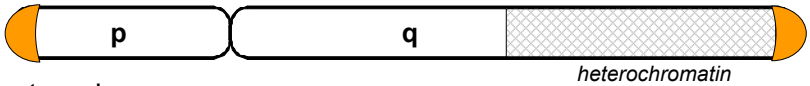
Forensic Utility of Y Chromosome SNPs

Human identification purposes (criminal, paternity, evolutionary, population studies)

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 $2e^{-8}$ per base per generation



heterochromatin

Pseudoautosomal region

Pseudoautosomal region

>250 Y SNPs described

Y SNPs Typed at NIST

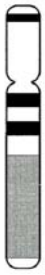
18 SNPs in 3 NIST designed 6 plexes (ASPE)

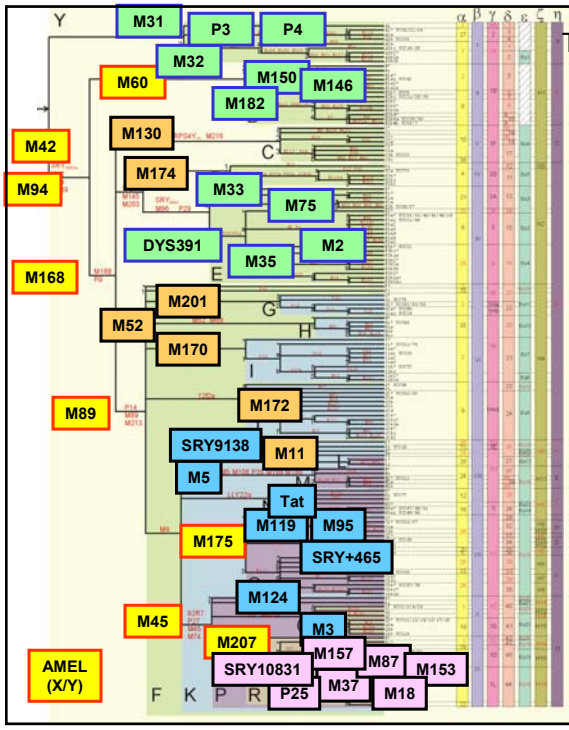
42 SNPs + Amelogenin present in 5 multiplexes (ASH) commercially available kit from Marligen

10 of the SNP sites were redundant between the two methodologies

Resulting in a total of 50 Y SNPs

115 African Americans
114 Caucasians





The Y Chromosome Consortium Map (2002) *Genome Res.* 12: 339-348

Position of Marligen *Multiplex 1* Y SNPs 8+1

Position of Marligen *Multiplex 2* Y SNPs 12

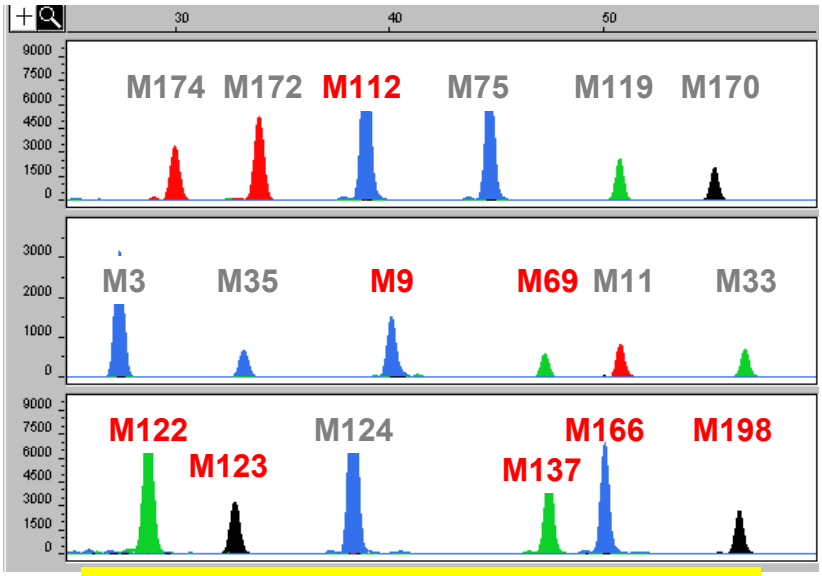
Position of Marligen *Multiplex 3* Y SNPs 7

Position of Marligen *Multiplex 4* Y SNPs 8

Position of Marligen *Multiplex 5* Y SNPs 7

45 haplogroups defined

ASPE Assays for 18 YSNPs



Multiplexing done at both PCR and SNP levels

Summary of YSNP Data

A total of 16 ng of genomic was consumed for the 8 multiplexes

18 out of 45 haplogroups observed (n=229)

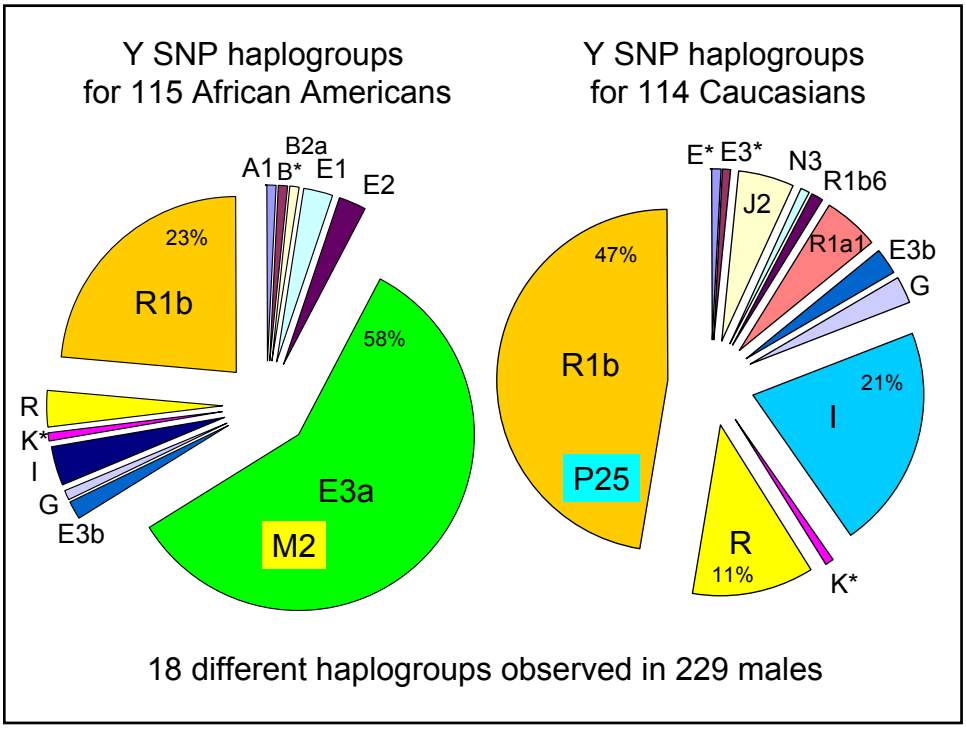
Over 99 % success rate for allele calls (both methods)
Variation was only observed in 24 of the 50 YSNPs

100% concordance for the 10 overlapping markers (>2,000 allele calls)

Number of haplogroups

	No. of Markers	AA	Cau
Y-SNPs	50	12(6)	12(6)

6 of the haplogroups were shared



18 different haplogroups observed in 229 males

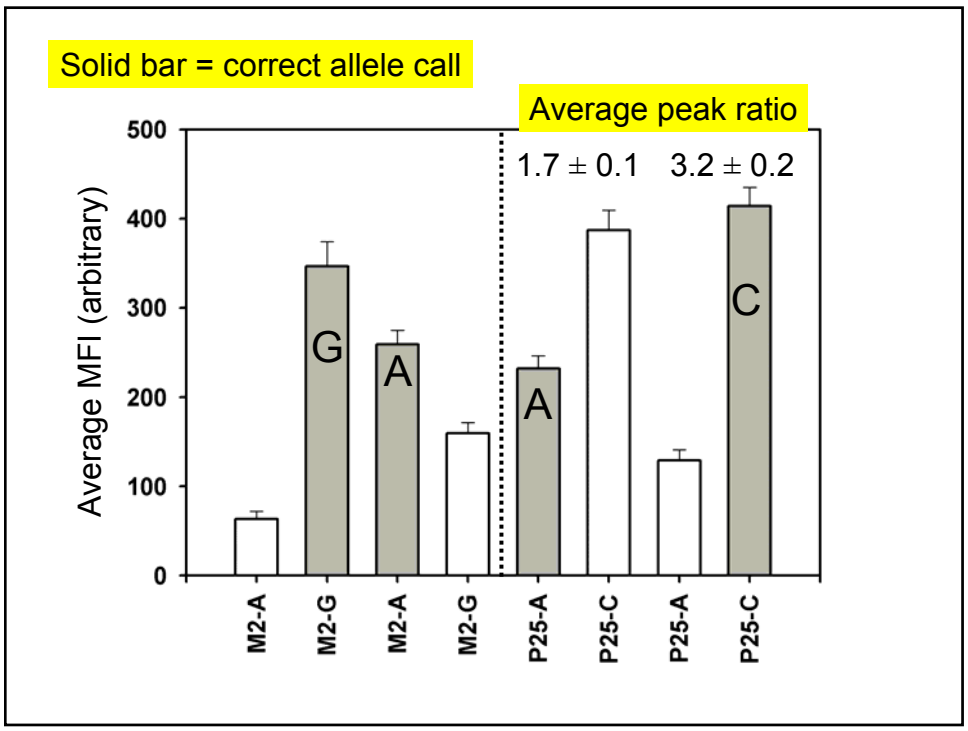
Issues with Y SNP P25

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

Alan Redd informed us that P25 is a multi copy locus

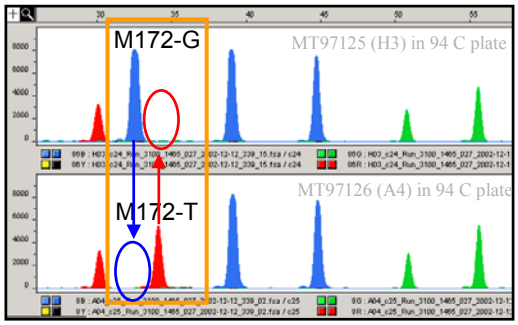
After further review of our data we were able to make correct allele call for the P25 marker based on signal intensity ratio

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



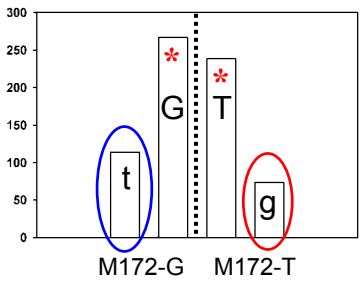
Signal to noise Ratio

ASPE



Alternate allele is not observed

Hybridization



267	73.5
114	238.5

S/N = 2.3 and 3.2

Conclusions

<u>mtSNP 11plex</u>

Capable of accurately detecting 11 mtSNPs in a single assay

Assay development described in manuscript

Development of additional multiplex mtSNP assays for other common HV1/HV2 types (AFDIL)

<u>YSNP Assays</u>

Additional Y SNP markers

ASPE with Luminex beads

Typing additional NIST population samples

Results for typing the 50 Y SNPs detailed in manuscript

Acknowledgments

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Margaret Kline and Jan Redman

Collaborators

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Mike Hammer and Alan Redd (U of AZ)

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