
# Development of Multiplexed Assays for Evaluating SNP and STR Forensic Markers

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**Department of Biological Sciences**

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National Institute of Standards and Technology


DNA Technologies Group (4 projects)

Human Identity Project (funded by NIST and NIJ)

## Role of NIST in Forensic DNA Typing

- Develop new DNA tests which are more rapid and efficient than those currently used.
- Evaluation and development of new technologies.

- Develop DNA standards so that laboratories around the world may compare their results.
- Conduct tests of laboratories around the world to insure accurate results in DNA testing.
- Create useful information databases (STRBase) http://www.cstl.nist.gov/biotech/strbase.

## What Type of Genetic Variation?

•Length Variation

short tandem repeats (STRs)

CTAGTCGT**(GATA)(GATA)(GATA)**GCGATCGT

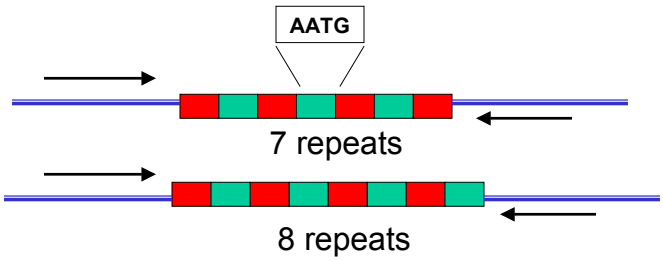
•Sequence Variation

single nucleotide polymorphisms (SNPs)

insertions/deletions

GCTAGTCGATGCTC**(G/A)**GCGTATGCTGTAGC

# Short Tandem Repeats (STRs)



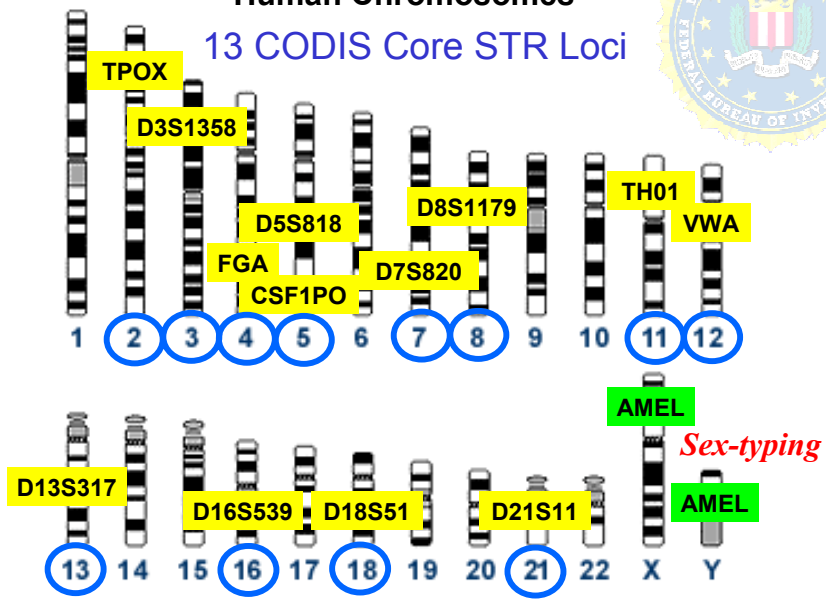

***the repeat region is variable between samples while the flanking regions where PCR primers bind are constant***

Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

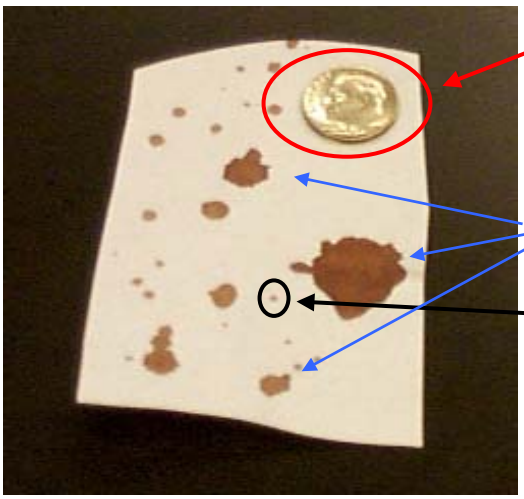
## Position of Forensic STR Markers on Human Chromosomes

13 CODIS Core STR Loci



## Sources of Biological Evidence

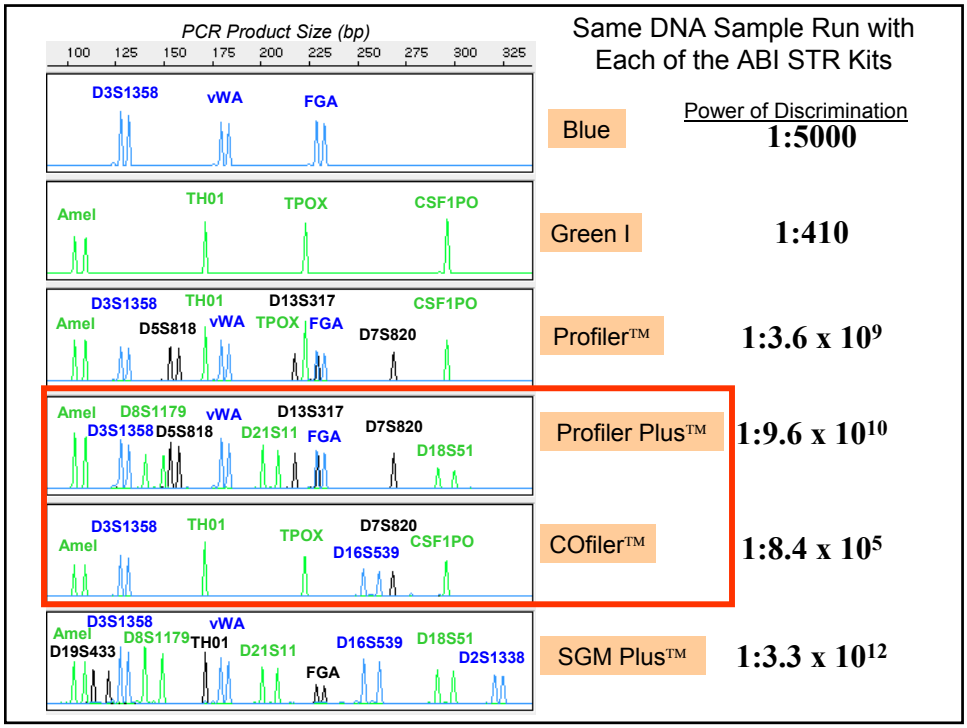
- Blood
- Semen
- Saliva
- Urine
- Hair
- Teeth
- Bone
- Tissue

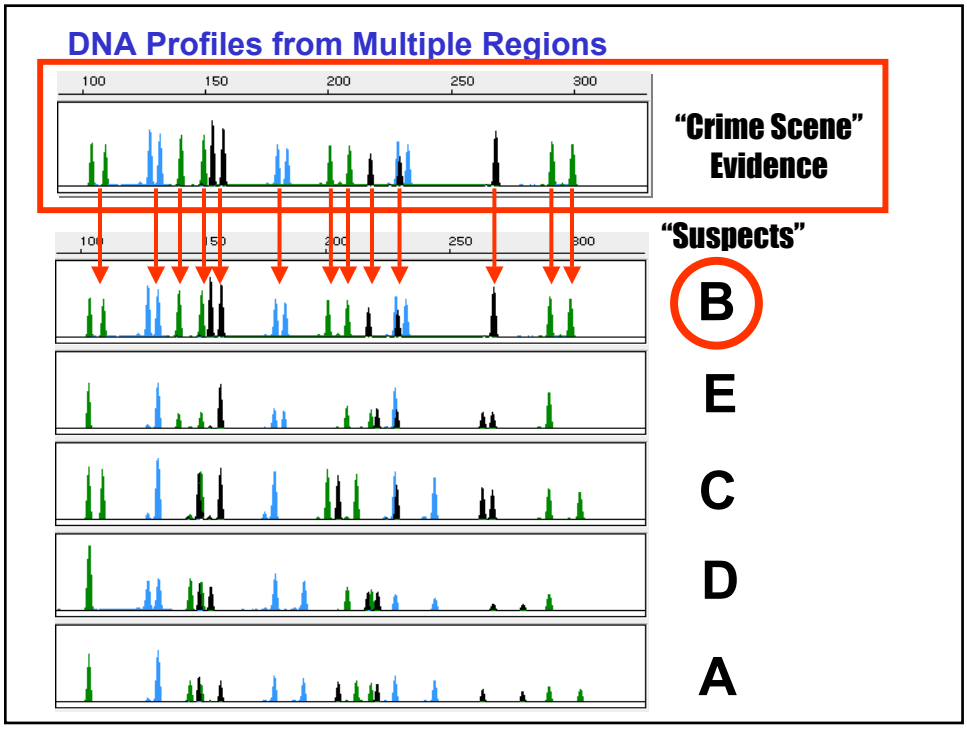


Dime

Blood sample

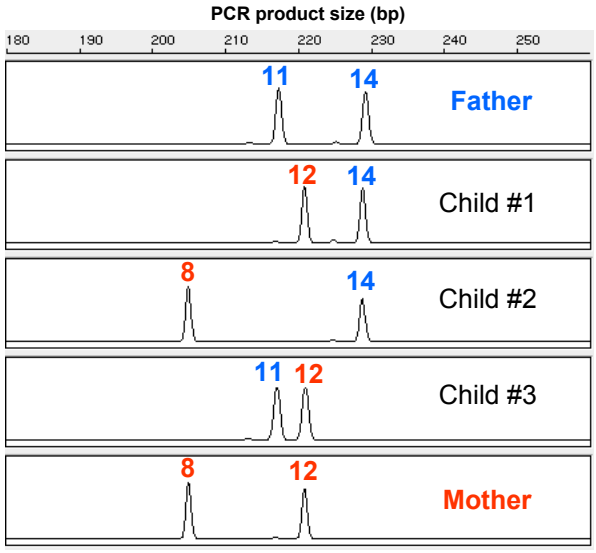
Only a very small amount of blood is needed to obtain a DNA profile


PCR Product Size (bp)
100 125 150 175 200 225 250 275 300 325
Same DNA Sample Run with Each of the ABI STR Kits
Power of Discrimination
Blue
1:5000
Green I
1:410
Profiler™
1:3.6 x 10^9
Profiler Plus™
1:9.6 x 10^10
COfiler™
1:8.4 x 10^5
SGM Plus™
1:3.3 x 10^12


DNA Profiles from Multiple Regions
100 150 200 250 300
"Crime Scene" Evidence
"Suspects"
B
E
C
D
A

## PATERNITY TESTING

Family Inheritance of STR Alleles (D13S317)




## Outline of Presentation

Multiplexing

Assays and Instrumentation

Y Chromosome and Mitochondrial DNA

Primer design strategy

Results

- mtSNP 11 plex
- Y SNP multiplexes
- Y STR multiplexes

## What are the 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

## What are the Challenges of Multiplexing?

- Only guidelines exist for designing multiplexes
- More markers = increased complexity
- Testing a robust multiplex
- Inclusion of useful markers in the multiplex
- Managing the volume of information obtained

## What Assays are we Multiplexing?

Polymerase chain reaction (PCR)

- Amplification of specific region of the human genome
- Typically used for STRs
- Use **Capillary Electrophoresis** for detection

Primer Extension reaction (minisequencing)

- Typically used for SNP markers
- Use **Capillary Electrophoresis** and **Mass Spectrometry** for detection

Multiplexing

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# Instrumentation

PCR&primer extension

Multi-Color Capillary Electrophoresis
(ABI 310 or 3100)

Luminex Beads
-hybridization

Luminex 100 Flow Cytometer

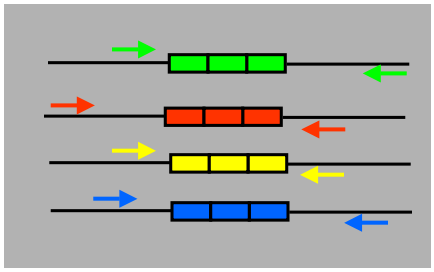
Primer Extension

Time-of-Flight Mass Spectrometer

TaqMan

Bio-Rad iCycler

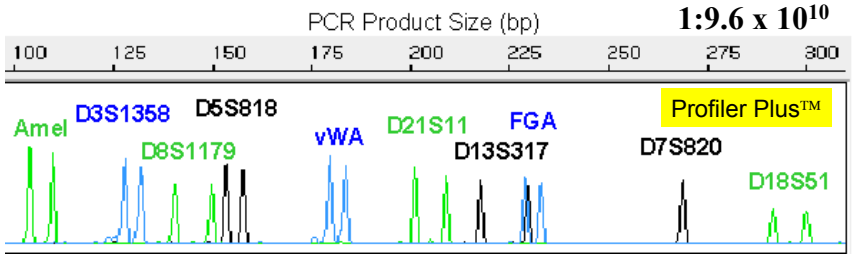
# Multiplex PCR



Multiple primer pairs target more than one specific site on the DNA strand

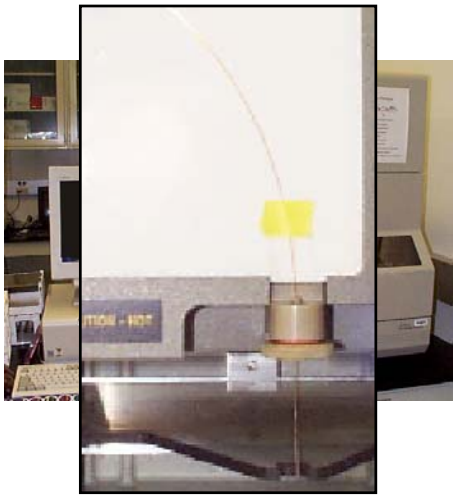
Compatible primers are the key to successful multiplex PCR

Commercial kits are available for targeting and simultaneously amplifying 16 markers



## Capillary Electrophoresis Instrumentation

**ABI 310**
**single capillary**



**ABI 3100**
**16-capillary array**

## Primer Extension Reaction Using the ABI PRISM® SNaPshot™ Multiplex System

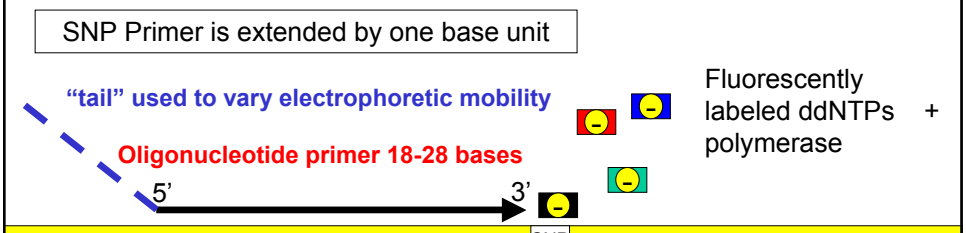
Primer extension assay that utilizes fluorescently labeled ddNTPs

Analysis of fragment size and fluorescent label identity by CE allows typing of multiple SNPs

Multiplexed amplicons or pooled singleplex PCR amplicons can be used as templates

**Primer design must be done by user!**

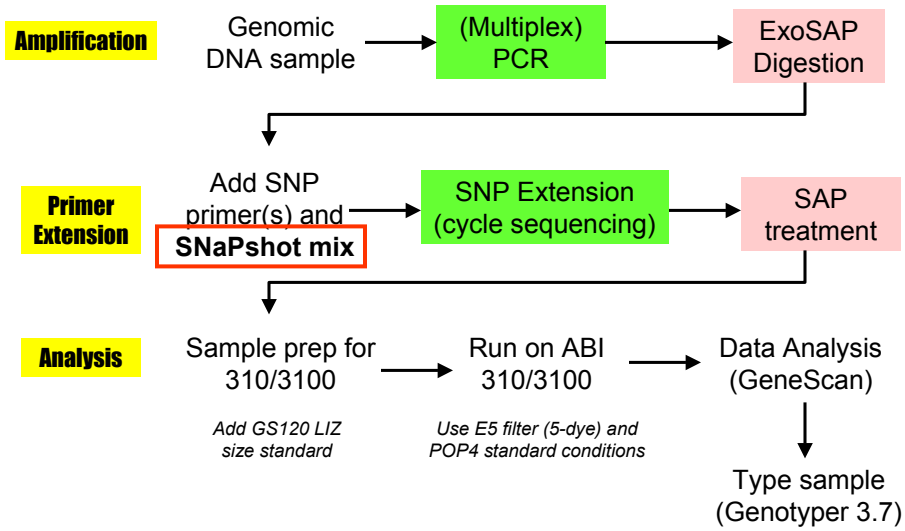
# Primer Extension with SNaPshot™



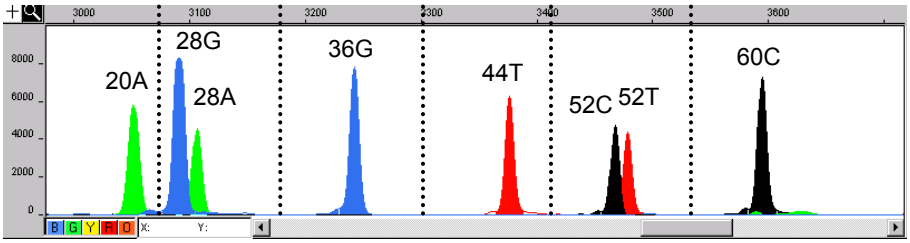

| ddNTP | Dye label | Color |
|---|---|---|
| A | dR6G | Green |
| C | dTAMRA | Black |
| G | dR110 | Blue |
| T | dROX | Red |

25 Cycles
96°C 10s
50°C 5s
60°C 30s

# Protocol with SNaPshot™ "Kit"



# Detection of SNPs with ABI 310/3100




SNaPshot™ CEPH Control Reaction

20 nucleotides — ddA

36 nucleotides — ddG

44 nucleotides — ddT

60 nucleotides — ddC

**Multiplexing possible by use of different length primers**

***Poly(T) tail or non-nucleotide linker to aid separation***

**Priming site**

# Primer Extension for MALDI TOF MS Analysis

SNP Primer is extended by one base unit

Natural non-labeled ddNTPs + polymerase

**Oligonucleotide primer 18-28 bases**

5’ 3’

SNP

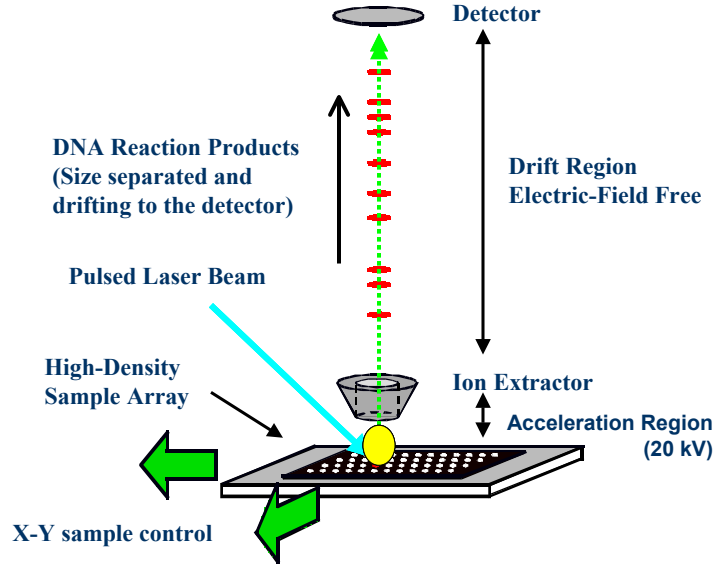
**PCR Amplified DNA Template**

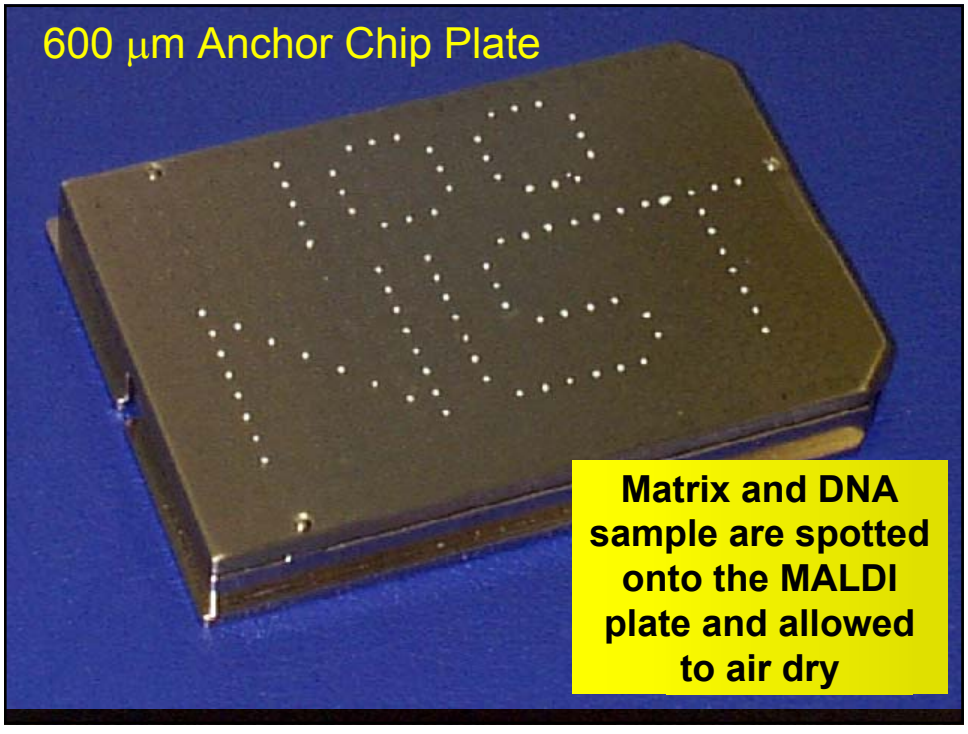
| ddNTP | Mass (Da) |
|---|---|
| A | 297 |
| C | 273 |
| G | 313 |
| T | 288 |

40 Cycles
96°C 10s
50°C 20s
72°C 30s

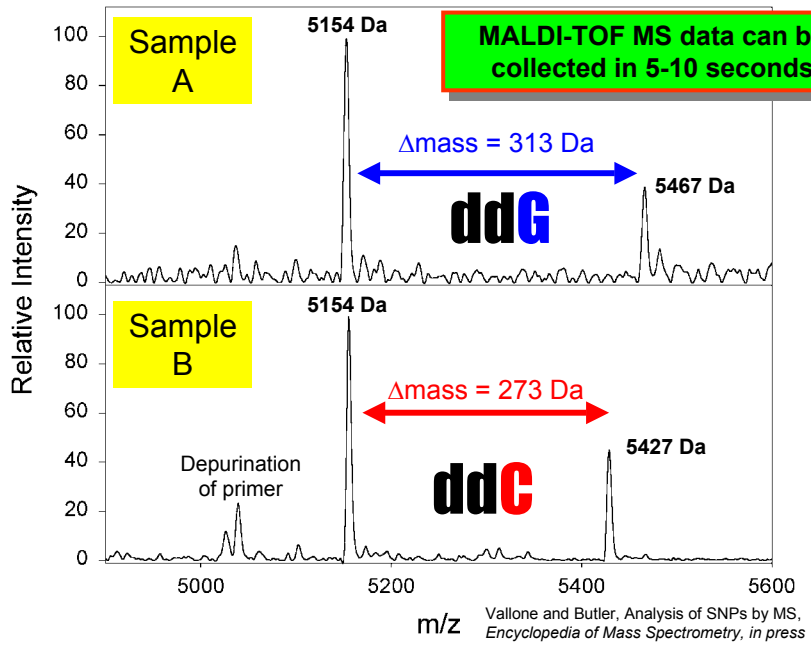
Mass difference between SNP primer and single base extension product provides genotype

## Time-of-Flight Mass Spectrometry (TOF-MS)



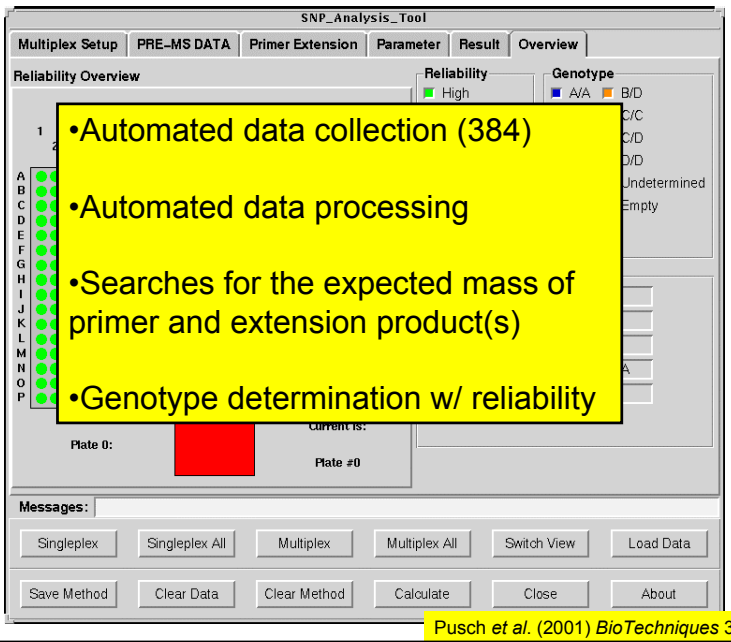



## MS Data from Y SNP Marker M96




Vallone and Butler, Analysis of SNPs by MS, *Encyclopedia of Mass Spectrometry, in press*

## Bruker SNP Manager Genotyping Software

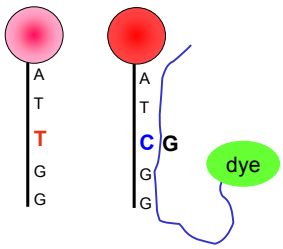


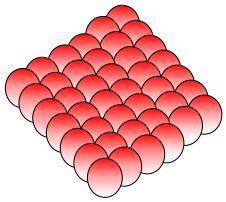

- •Automated data collection (384)
- •Automated data processing
- •Searches for the expected mass of primer and extension product(s)
- •Genotype determination w/ reliability

Pusch *et al.* (2001) *BioTechniques* 30: 210-215

# Y SNP Detection by Hybridization

## Luminex Bead Array Assay

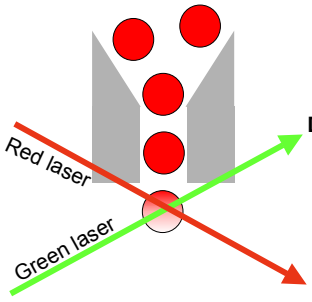


**100 different colored beads** are possible (potential for multiplexing 50 SNP markers)

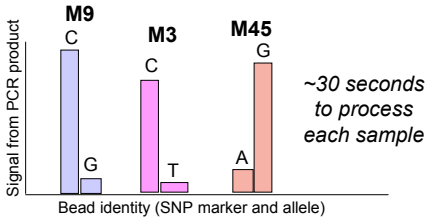
**Luminex 100 Flow Cytometer**

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*

Multiplexing

Assays and Instrumentation

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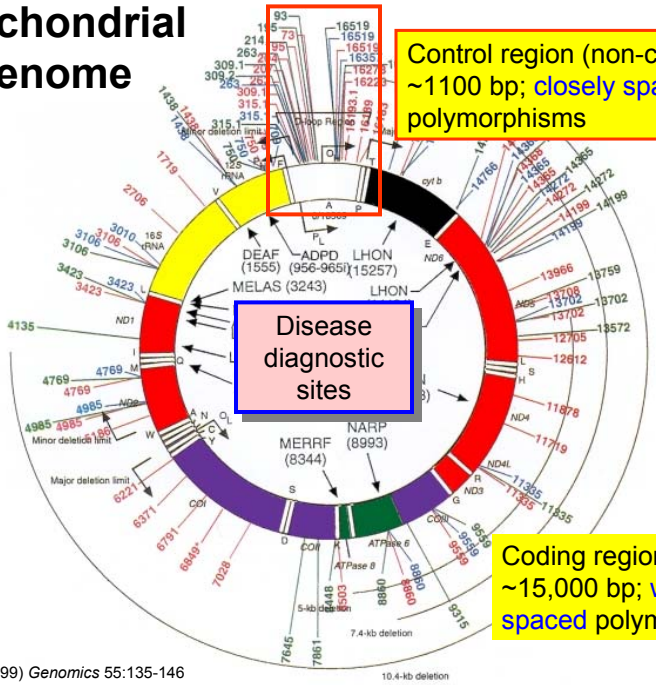
# Markers of Interest

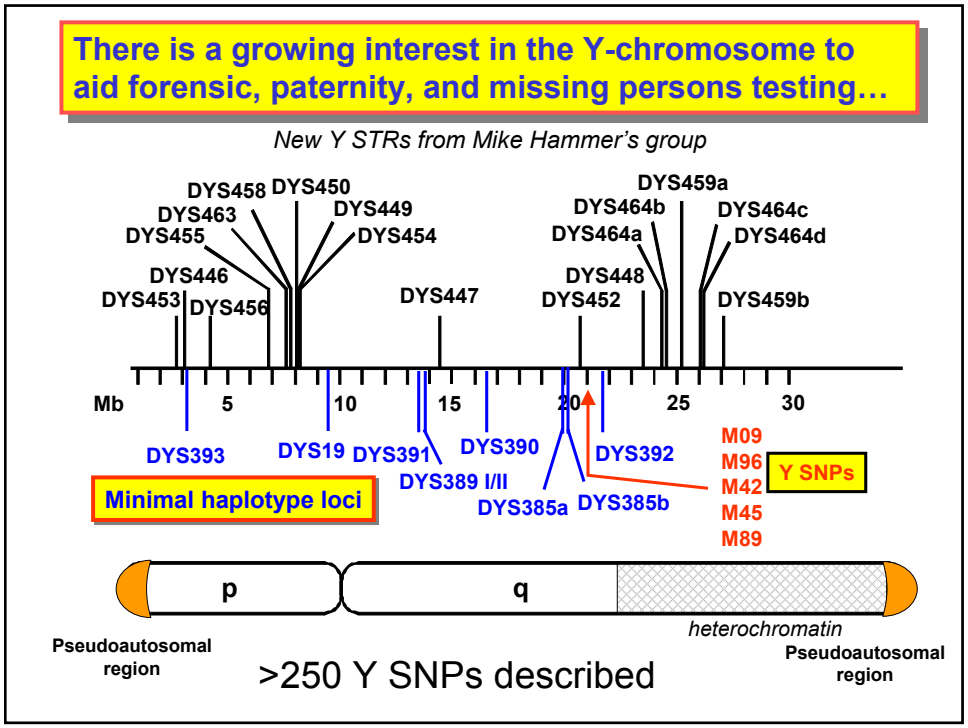
- Mitochondrial DNA (mtDNA)
  - maternally inherited
  - polymorphic control region (D-loop)
  - ~1000's of copies per cell
  - coding region
- Y chromosome
  - paternally inherited
  - variety of Y STR and Y SNP markers
  - ***haplotype rather than genotype***

Require large databases because recombination does not occur

# Mitochondrial Genome




Levin *et al* (1999) *Genomics* 55:135-146




# Y SNPs

Low mutation rate of SNPs $2e^{-8}$ per base per generation

Over 3,700 SNP found on the Y so far *(http://www.ncbi.nlm.nih.gov)*

245 SNPs validated in population studies

Sites discovered using DHPLC (Underhill et.al., Nat Genet 2000 26:358-61)

Y SNP validation and nomenclature (Y chromosome consortium, Genome Res. 2002 12:339-348)

Paracchini et. al., have designed 20 multiplexes for typing 118 Y SNPs by MALDI TOF MS (Paracchini et. al., Nucleic Acids Res. 2002 30:e27)

NIST Y Chromosome SRM material 2395 will include haplotypes including 5-10 SNP sites UPDATE

# Forensic Utility of Y Chromosome SNPs

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

Y chromosome markers are useful in mixed male - female samples

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

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

Improve multiplex assay development (both PCR and SNP detection)

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

Multiplexing

Assays and Instrumentation

Y Chromosome and Mitochondrial DNA

Primer design strategy

Results

- mtSNP 11 plex
- Y SNP multiplexes
- Y STR multiplexes

# Multiplex PCR Primer Selection

Identify markers of interest (collaborations, literature, research)

Obtain reference sequences containing the sites of interest (Genbank) with approximately 500 bases of sequence information upstream and downstream of the marker

Decide upon a desired PCR product size
- Short amplicons for degraded samples, SNPs
- Longer amplicons for STRs

Use software for selecting singleplex primer pairs

Primer3
www-genome.wi.mit.edu/genome_software/other/primer3.html

# Multiplex PCR Design

Select singleplex PCR primers for each amplicon using Primer 3 software

```
OLIGO            start  len      tm      gc%    any    3'  seq
LEFT PRIMER         27   20   60.06    50.00   4.00   2.00 GGGATAACAGCGCAATCCTA
RIGHT PRIMER       174   22   60.31    50.00   8.00   3.00 CGGTCTGAACTCAGATCACGTA
SEQUENCE SIZE: 205
INCLUDED REGION SIZE: 205

PRODUCT SIZE: 148, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 2.00
EXCLUDED REGIONS (start, len)*: 70,65

    1 CTTGACCAACGGAACAAGTTACCCTAGGGATAACAGCGCAATCCTATTCTAGAGTCCATA
                                >>>>>>>>>>>>>>>>>>>>>

   61 TCAACAATAGGGTTTACGACCTCGATGTTGGATCAGGACATCCCRATGGTGCAGCCGCTA
               XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

  121 TTAAAGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACCGGAGTAA
      XXXXXXXXXXXXXX                    <<<<<<<<<<<<<<<<<<<<<<<<

  181 TCCAGGTCGGTTTCTATCTACCTTC
```

## Stand Alone Primer3

Sending multiple sequences over the web for primer selection can be tedious

The Primer3 web output is fine for the screen viewing or printing but not for organizing in spreadsheets

Primer3 is publicly available and can be run (in batch!) on a Unix, PC (Linux), or Mac (OSX) computer

Developed a program that formats files for Primer3 input

Reference sequences that are stored in Excel can be quickly formatted for Primer3

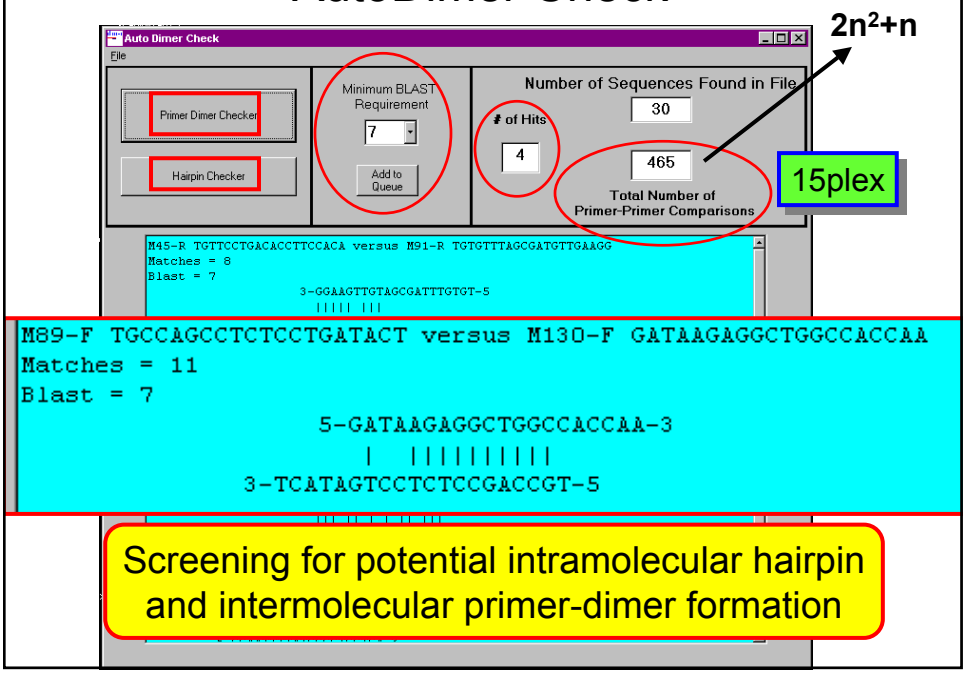
## Non-Specific Interactions

Primers that interact with non-specific (undesired) regions of a genome OR with each other can degrade PCR performance

Screening for alternate genomic binding regions can be accomplished using **BLAST** *http://www.ncbi.nlm.nih.gov*

Screening for potential primer-dimer interactions is accomplished using in house software - **AutoDimer**

# AutoDimer Check

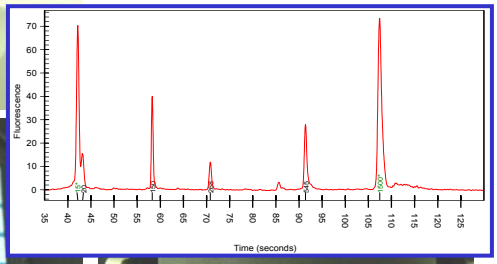



# PCR Assay Design

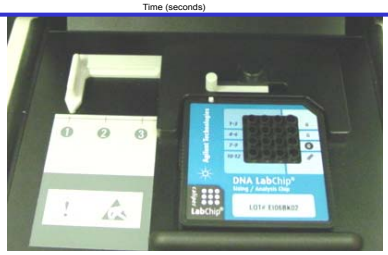
If primer pairs meet criteria

Obtain primer pairs and test singleplex PCR
(QC all primers with MS/CE/HPLC)

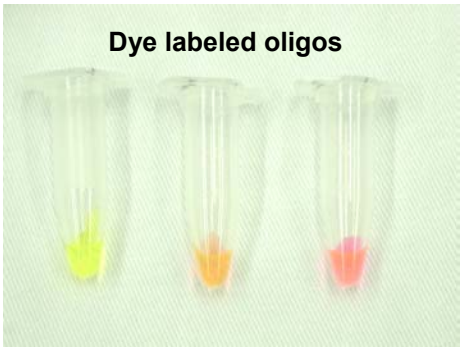

Agilent Bioanalyzer 2100
DNA chip for rapid testing of PCR product yields (singleplex and multiplex)







# PCR Primer Quality Control

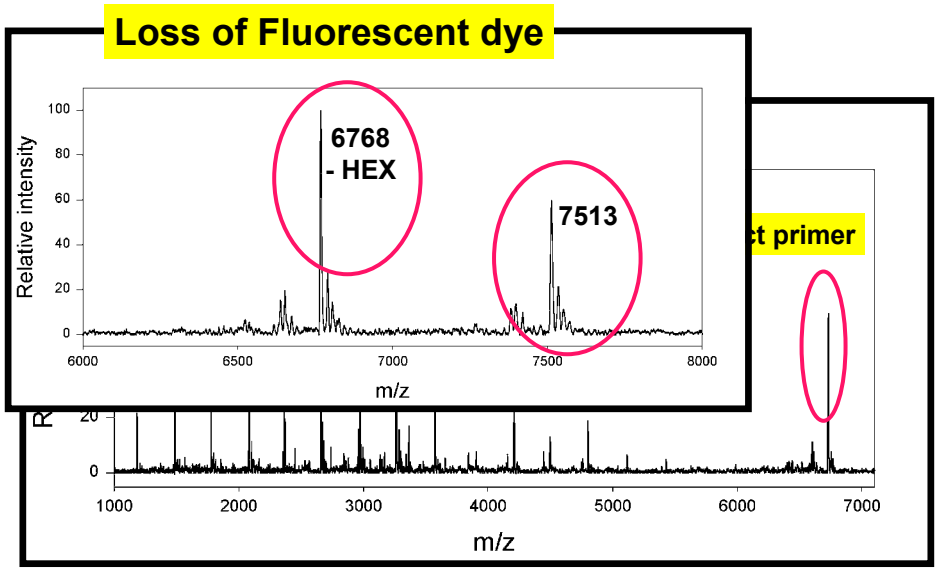



**6FAM** (yellow), **VIC** (orange), **NED** (red)

- UV Spec to determine concentration
- HPLC to evaluate purity
- TOF-MS to confirm correct sequence

Butler *et al.* (2001) *Forensic Sci. Int.* 119: 87-96

# MALDI QC of Commercial Oligos




Vallone and Butler (Oct 2000) *International Symposium on Human Identification* (Biloxi, MS)

# PCR Assay Design

If primer pairs meet criteria

Obtain primer pairs and test singleplex PCR
(QC all primers with MS/CE/HPLC)

Begin initial testing of multiplex PCR
Start with a PCR mix containing
0.5 μM of each primer pair

Evaluate amplicon yields, presence and balance

Variables: primer pair concentrations, [polymerase], number of cycles, [$Mg^{++}$], [dNTPs], BSA

Redesign and retest failing loci

Multiplexing

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Y Chromosome and Mitochondrial DNA

Primer design strategy

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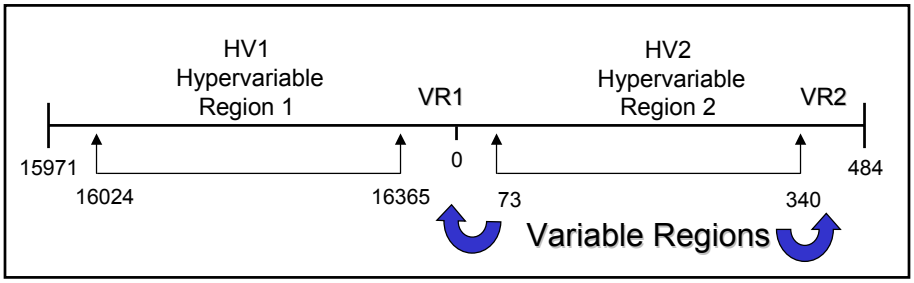
## The Current mtDNA Amplification & Sequencing Strategy Focuses on the Hypervariable Regions of the mitochondrial genome HV1 and HV2

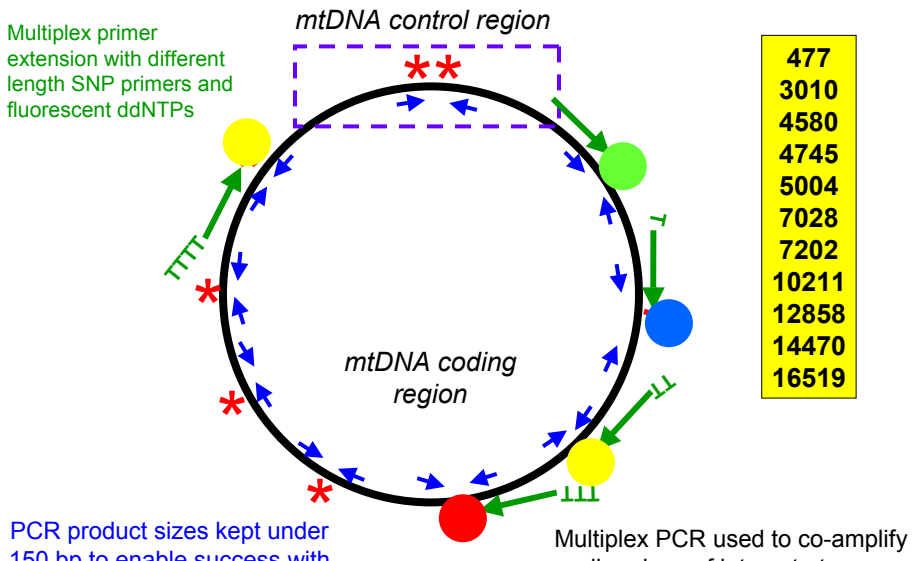



However, the greatest limitation for mtDNA testing lies with the small number of common types for which the power of discrimination is low.

## The Use of Full mtGenome Polymorphisms

- Sequence data from mtDNA genome coding region reveals numerous SNPs that can nearly distinguish Caucasians sharing common HV types (Tom Parsons and Mike Coble AFDIL)
- 11 SNP sites are being evaluated to resolve Caucasian individuals having the most common HV type

## mtSNP 11-plex Assay

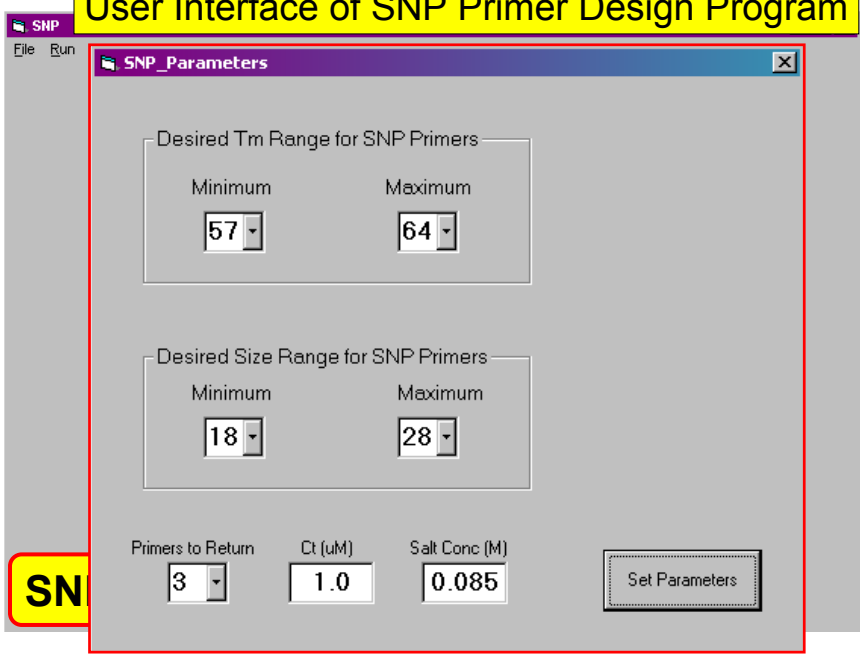


# Tailed SNP primers allows for multiplexing in the SNaPshot assay

## Sequences for 11 extension primers

| | | |
|---|---|---|
| 3010-F | TGTTGGATCAGGACATCCC | 19 19 |
| 4793-R | $(T)_4$ – TCAGAAGTGAAAGGGGGC | 18 22 |
| 10211-R | $(T)_{10}$ – ACTAAGAAGAATTTTATGGA | 20 30 |
| 5004-F | $(T)_{14}$ – AGACCCAGCTACGCAAAATC | 20 34 |
| 7028-F | $(T)_{18}$ –GACACGTACTACGTTGTAGC | 20 38 |
| 7202-F | $(T)_{22}$ –CCACAACACTTTCTCGGCCT | 20 42 |
| 16519-R | $(T)_{24}$ –TGTGGGCTATTTAGGCTTTATG | 22 46 |
| 12858-F | $(T)_{27}$ –GCAGCCATTCAAGCAATCCTATA | 23 50 |
| 4580-R | $(T)_{29}$ –TGGTTAGAACTGGAATAAAAGCTAG | 25 54 |
| 477-F | $(T)_{38}$ –CCCTCCCACTCCCATACTAC | 20 58 |
| 14470-R | $(T)_{41}$ –GGGAATGATGGTTGTCTTTGG | 21 62 |

## User Interface of SNP Primer Design Program



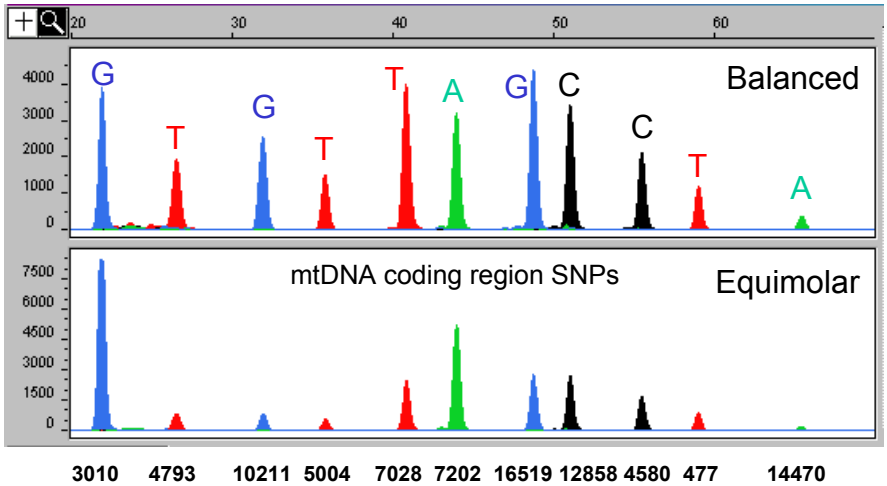
## Program Output

| Label | Length | Sequence | Position | Tm |
|---|---|---|---|---|
| Forward Primers Salt = 0.3Ct = 10 | | | | |
| M42 340 bp (A/T 297 W) AC010889 | 18 | ATTTAGGACACAAAAGCW | 280 | 60.65398 |
| M42 340 bp (A/T 297 W) AC010889 | 19 | GATTTAGGACACAAAAGCW | 279 | 61.96716 |
| M42 340 bp (A/T 297 W) AC010889 | 20 | AGATTTAGGACACAAAAGCW | 278 | 63.67808 |
| Reverse Primers | | | | |
| M42 340 bp (A/T 297 W) AC010889 | 23 | GCTCTCTTTTTCATTATGTAGTW | 319 | 63.5462 |
| M42 340 bp (A/T 297 W) AC010889 | 21 | TCTCTTTTTCATTATGTAGTW | 317 | 59.28964 |
| M42 340 bp (A/T 297 W) AC010889 | 20 | CTCTTTTTCATTATGTAGTW | 316 | 57.50257 |

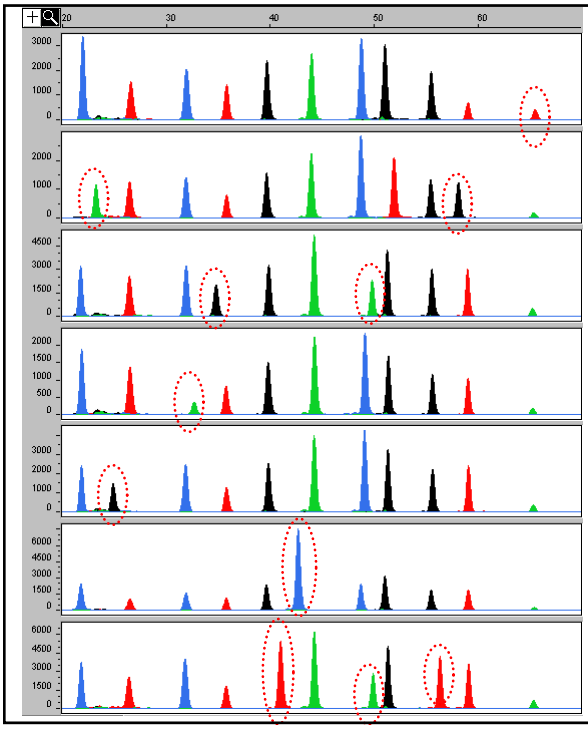
| Hairpin | Dimer | Template | Mass | Rank | Mutation | +ddC | +ddT | +ddA | +ddG |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| 4 | 8 | 10 | 5273.48 | 2.133333 | W | N/A | 5561.67998 | 5570.68998 | N/A |
| 5 | 10 | 10 | 5602.69 | 2 | W | N/A | 5890.889941 | 5899.899941 | N/A |
| 5 | 10 | 11 | 5915.9 | 2 | W | N/A | 6204.099902 | 6213.109902 | N/A |
| | | | | | | | | | |
| 4 | 8 | 22 | 6734.42 | 2.133333 | W | N/A | 7022.619922 | 7031.629922 | N/A |
| 4 | 8 | 20 | 6116.02 | 2.133333 | W | N/A | 6404.22002 | 6413.23002 | N/A |
| 4 | 8 | 19 | 5811.82 | 2.133333 | W | N/A | 6100.019824 | 6109.029824 | N/A |

## mtSNP 11-plex run on ABI 3100

Multiplex PCR and Multiplex SNP Detection




*Sizing performed by comparison to GS120 LIZ internal size standard (not shown)*

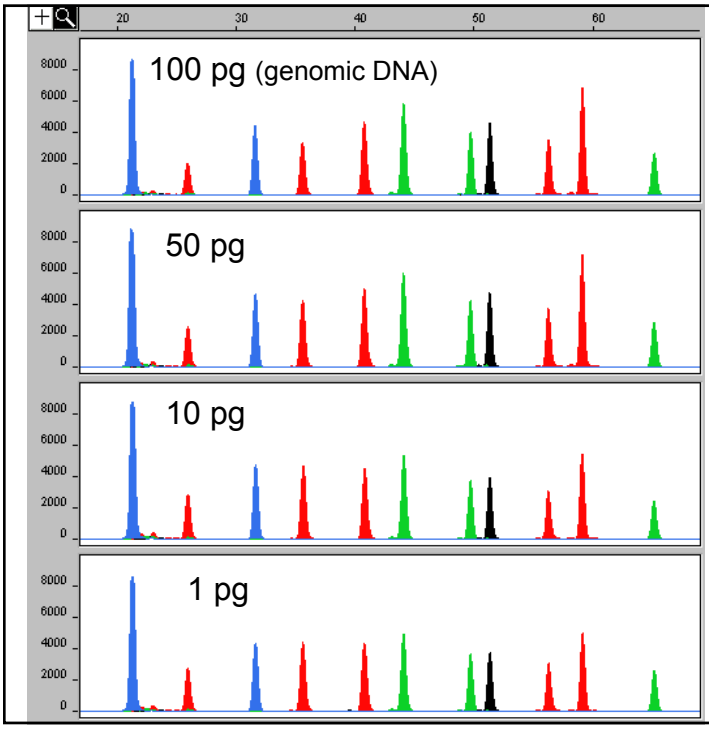


## 11 plex run on 7 unique samples

All allele variations are represented in these 7 samples

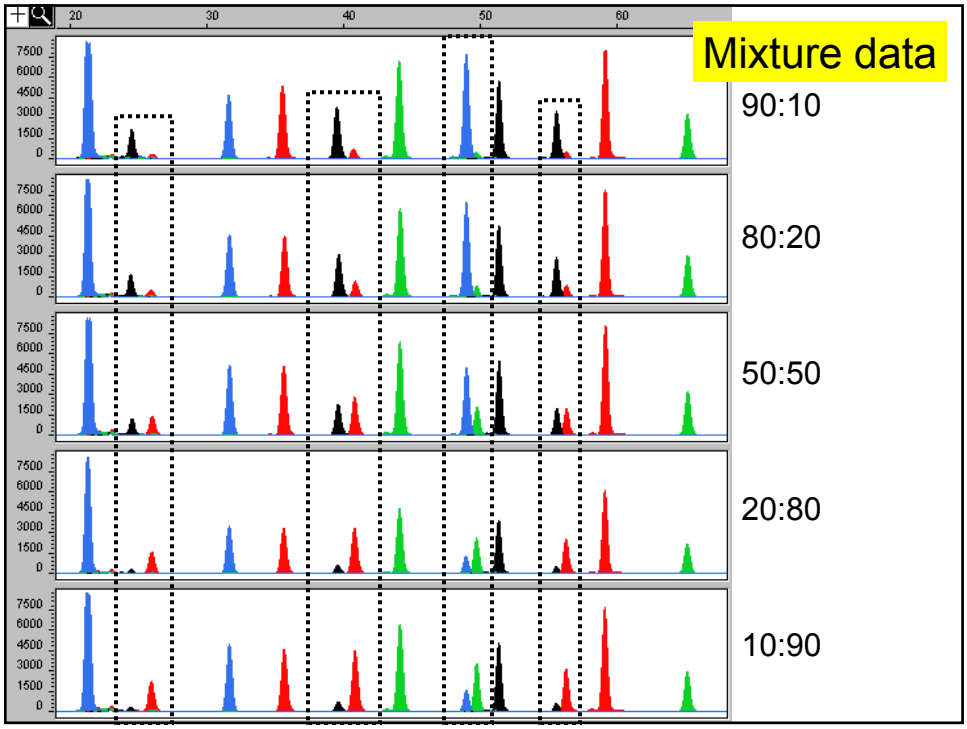
The assay accurately detects each variant

Sizing can be used to develop a macro for automated typing




## Sensitivity Study

Assay performs down to 1 pg of genomic DNA




## Status of 11plex mtSNP assay

Currently the 11plex assay is being validated for case work samples at AFDIL

Manuscript is in preparation

Further multiplexes are being developed for other common HV1/HV2 types in collaboration with AFDIL

Multiplexing

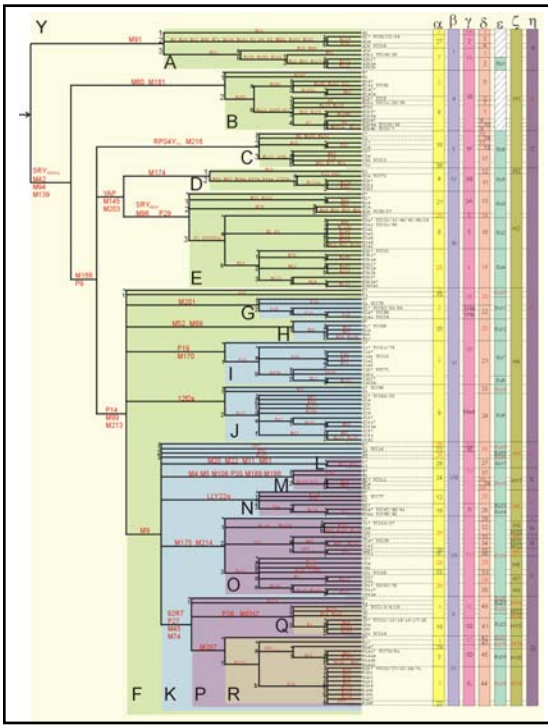
Assays and Instrumentation

Y Chromosome and Mitochondrial DNA

Primer design strategy

Results

- mtSNP 10 plex
- Y SNP multiplexes
- Y STR multiplexes



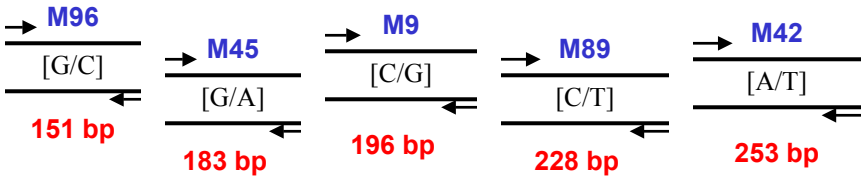

The Y Chromosome Consortium (2002) *Genome Res*. 12: 339-348

**245 Y SNPs typed**
74 males (YCC cell lines)
**153 haplogroups observed**

*This paper unifies previous haplogroup nomenclatures*

Primers and other information for all 245 markers are included in supplementary material

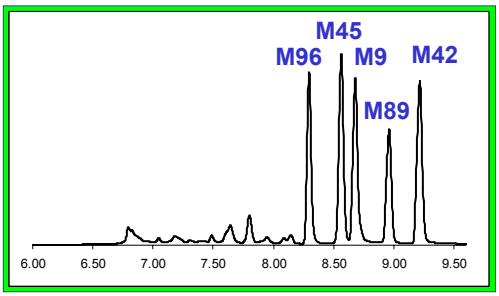
# Multiplex PCR with Y-Chromosome SNP Markers




**5-plex PCR**

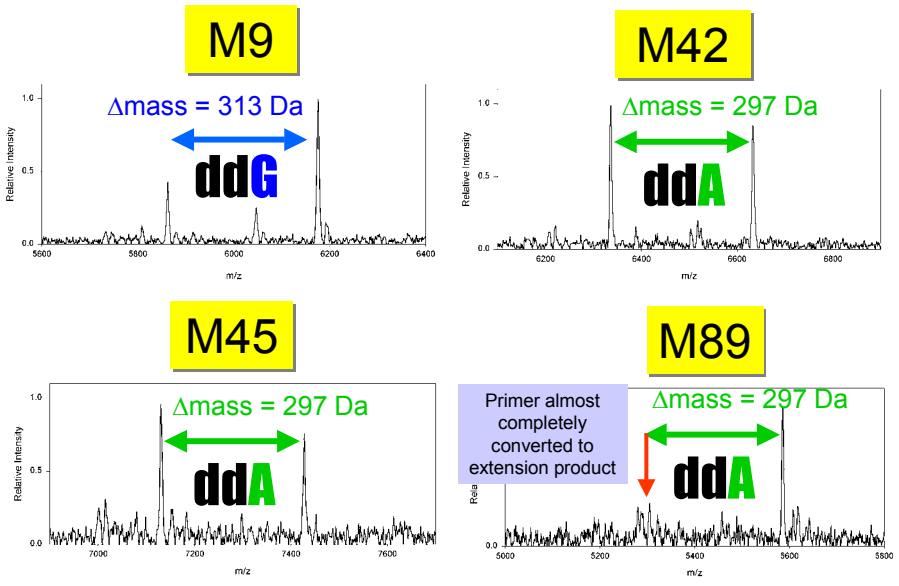
**Rapid CE Separation and Quantitation of Multiplex PCR Products**

*Intercalating dye used to fluorescently label amplicons*

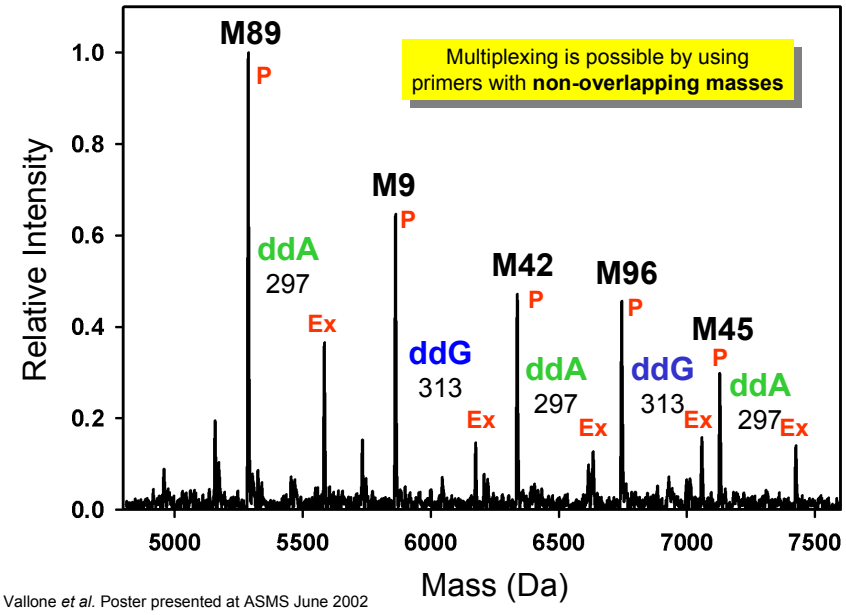



Butler *et al. (2001) Fresenius J. Anal. Chem.* 369: 200-205

# Y Chromosome SNP Results by Probing PCR Products through Primer Extension and TOF-MS Detection

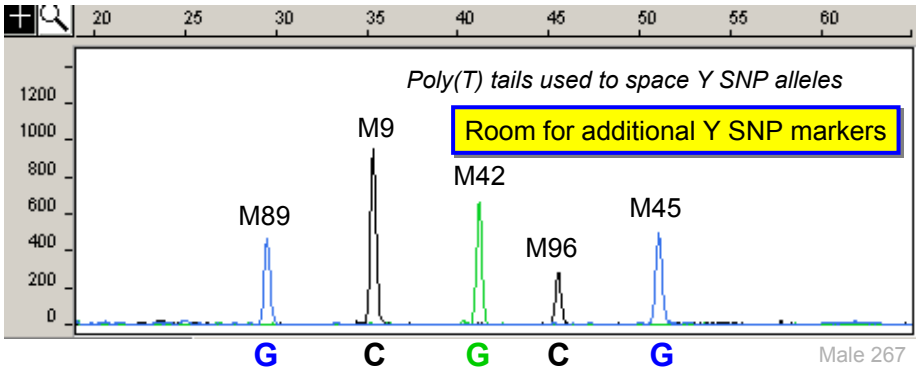


## Multiplexed Y SNPs (5-plex) Analyzed by TOF-MS




Vallone *et al.* Poster presented at ASMS June 2002

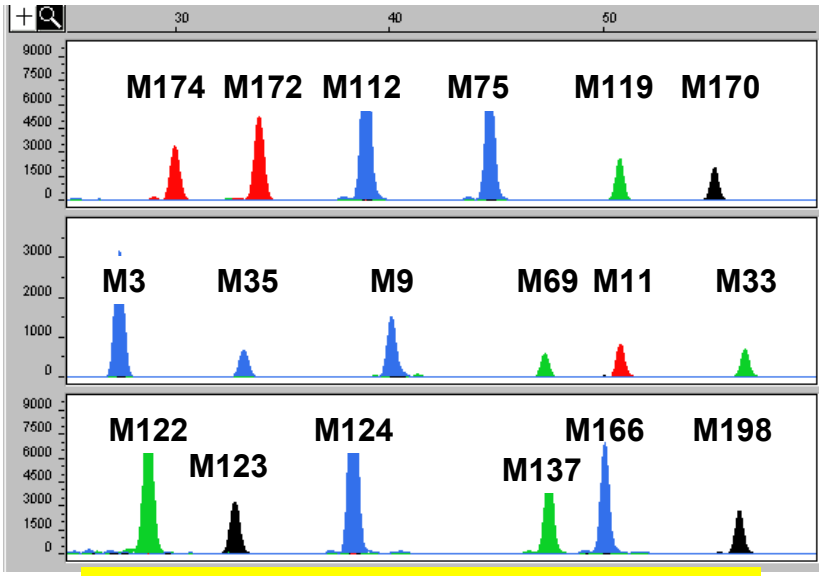
## Y SNP Results with SNaPshot Assay



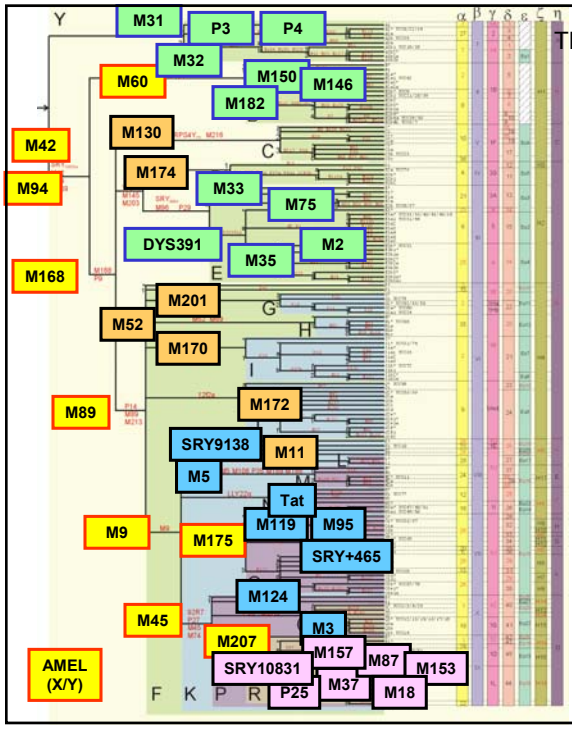

| | |
|---|---|
| TTTTTTTTTCAGGCAAAGTGAGAGAT | 17/26 |
| TTTTTTTTTTTTCGGCCTAAGATGGTTGAAT | 19/32 |
| TTTTTTTTTTTTTTTTTTGCTCTCTTTTTCATTATGTAGT | 22/38 |
| TTTTTTTTTTTTTTTTTTTTTTTTGGAAAACAGGTCTCTCATAATA | 22/44 |
| TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGCAGTGAAAAATTATAGATA | 22/50 |

*Data obtained by Gordon Spangler (graduate student at American University)*

# Y SNaPshot Assays = 18 YSNPs




Multiplexing done at both PCR and SNP levels




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

*18 major haplogroups A-R*

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

**Position of Marligen *Multiplex 2* Y SNPs**

**Position of Marligen *Multiplex 3* Y SNPs**

**Position of Marligen *Multiplex 4* Y SNPs**

**Position of Marligen *Multiplex 5* Y SNPs**

# 42 Y SNPs Typed with Luminex Assay

MARLIGEN BIOSCIENCE

**Multiplex 1**

| AMEL | M168 | M175 | M207 | M42 | M45 | M60 | M89 | M94 |
|---|---|---|---|---|---|---|---|---|
| XX or XY | (C/T) | (+/-) | (A/G) | (A/T) | (A/G) | (-/+) | (C/T) | (A/C) |

**Multiplex 2**

| DYS391 | M146 | M150 | M182 | M2 | M31 | M32 | M33 | M35 | M75 | P3 | P4 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (C/G) | (A/C) | (C/T) | (C/T) | (A/G) | (C/G) | (C/T) | (A/C) | (C/G) | (A/G) | (C/T) | (A/G) |

**Multiplex 3**

| M11 | M130 | M170 | M172 | M174 | M201 | M52 |
|---|---|---|---|---|---|---|
| (A/G) | (C/T) | (A/C) | (G/T) | (C/T) | (G/T) | (A/C) |

**Multiplex 4**

| M119 | M124 | M3 | M5 | M95 | SRY465 | SRY9138 | Tat |
|---|---|---|---|---|---|---|---|
| (A/C) | (C/T) | (C/T) | (C/T) | (C/T) | (C/T) | (C/T) | (C/T) |

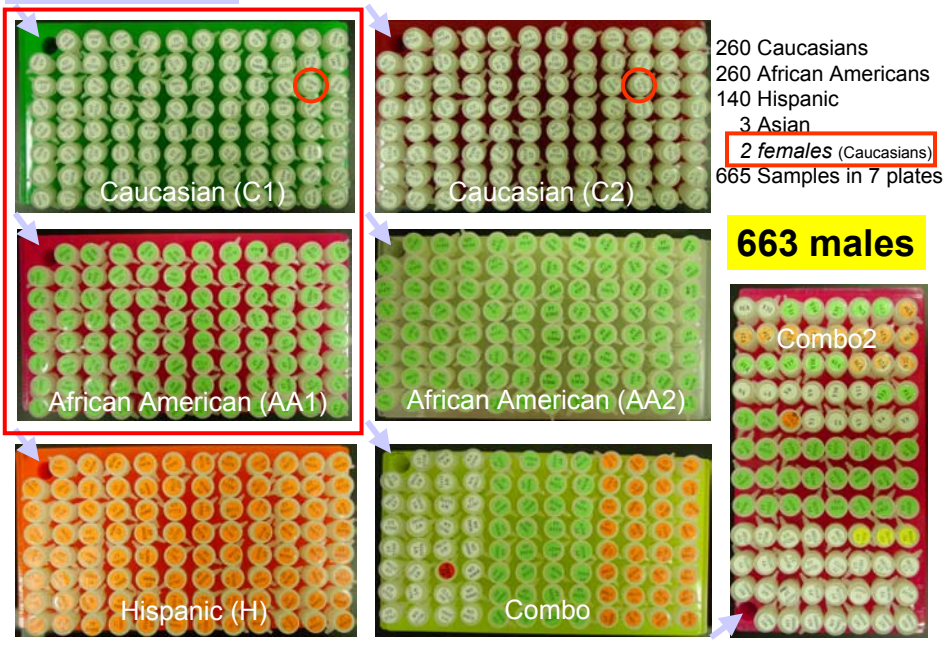
**Multiplex 5**

| M153 | M157 | M18 | M37 | M87 | P25 | SRY10831 |
|---|---|---|---|---|---|---|
| (A/T) | (A/C) | (-/+) | (C/T) | (C/T) | (A/C) | (A/G) |

***17 Y SNPs overlap with current SNaPshot assays***

*Position A1 left open for controls or allelic ladder*

## NIST U.S. Population Samples




260 Caucasians
260 African Americans
140 Hispanic
3 Asian
*2 females* (Caucasians)
665 Samples in 7 plates

**663 males**

# Summary of YSNP Data

•Excellent success with Signet Y SNP kits using ~10 ng of each NIST population sample (5 multiplexes used; 2 ng each)

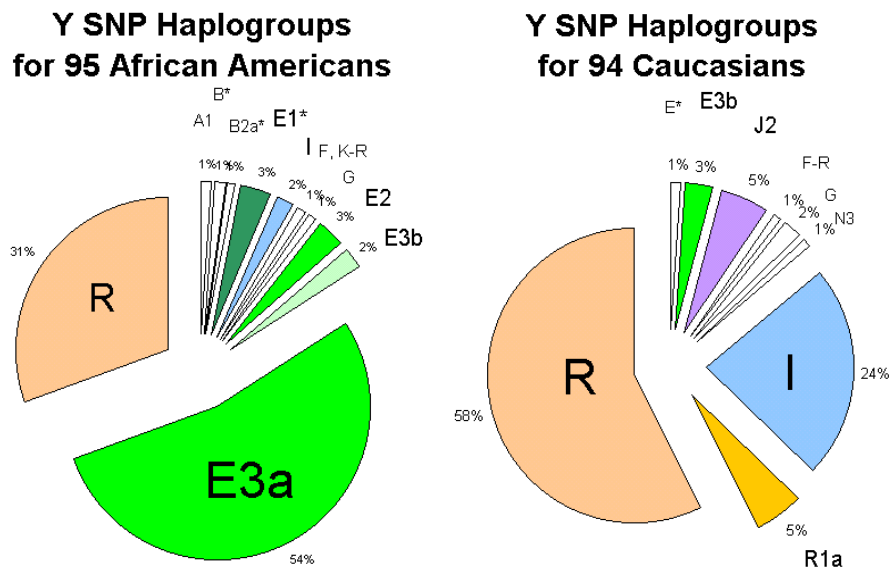
•A total of 8,109 allele calls out of 8,170 attempts on first pass (99.3% success rate)

•Single female sample gave "no calls" at all loci except amelogenin X,X

•Variation was only observed in 19 of the 42 YSNPs

Number of haplogroups

| | No. of Markers | AA(95) | Cau(94) |
|---|---|---|---|
| Y-SNPs | 42 | 11 | 9 |




15 different haplogroups seen in 189 males
(11 in 95 AA and 9 in 94 C)

# Y SNP Concordance Summary

- Comparison of Luminex (Signet Y SNP kit) and SNaPshot assays developed at NIST
- **2,090 allele calls compared**
- **Complete Concordance Seen!**

SNaPshot data (ABI 3100)


Signet kit data (Luminex)

| M172-G | M172-T |
|---|---|
| 267 | 73.5 |
| 114 | 238.5 |

Multiplexing

Assays and Instrumentation

Y Chromosome and Mitochondrial DNA

Primer design strategy

Results

- mtSNP 10 plex
- Y SNP multiplexes
- Y STR multiplexes

# Chromosomal Positions of Y STRs

Short Arm (p)

DYS393

DYS19

Collaboration with Michael Hammer (U of Arizona)

*Based on BLAT search from Aug 6, 2001 Human Genome Working Draft http://genome.ucsc.edu/*

Long Arm (q)

DYS391
DYS437
DYS439
DYS389 I/II
DYS388
DYS438
DYS447
DYS390
H4
DYS426
YCAII a
YCAII b
DYS385 a
DYS385 b
A7.1
DYS392
DYS448

| Y STR Marker | Sequence Position |
|---|---|
| DYS393 | 3,038,729 |
| DYS19 | 9,437,335 |
| DYS391 | 13,413,353 |
| DYS437 | 13,777,618 |
| DYS439 | 13,825,798 |
| DYS389 I/II | 13,922,787 |
| DYS388 | 14,057,445 |
| DYS438 | 14,247,805 |
| DYS447 | 14,588,695 |
| DYS390 | 16,521,407 |
| H4 | 17,990,102 |
| DYS426 | 18,381,316 |
| YCAII a | 18,868,535 |
| YCAII b | 19,754,090 |
| DYS385 I | 19,998,053 |
| DYS385 II | 20,038,828 |
| A7.1 | 20,247,345 |
| DYS392 | 21,780,328 |
| DYS448 | 23,511,495 |

# New Y STR Markers (Redd et al.)

ELSEVIER

Published *Forensic Sci. Int.* (Dec 2002)

Forensic Science International

Forensic Science International 130 (2002) 97–111

www.elsevier.com/locate/forsciint

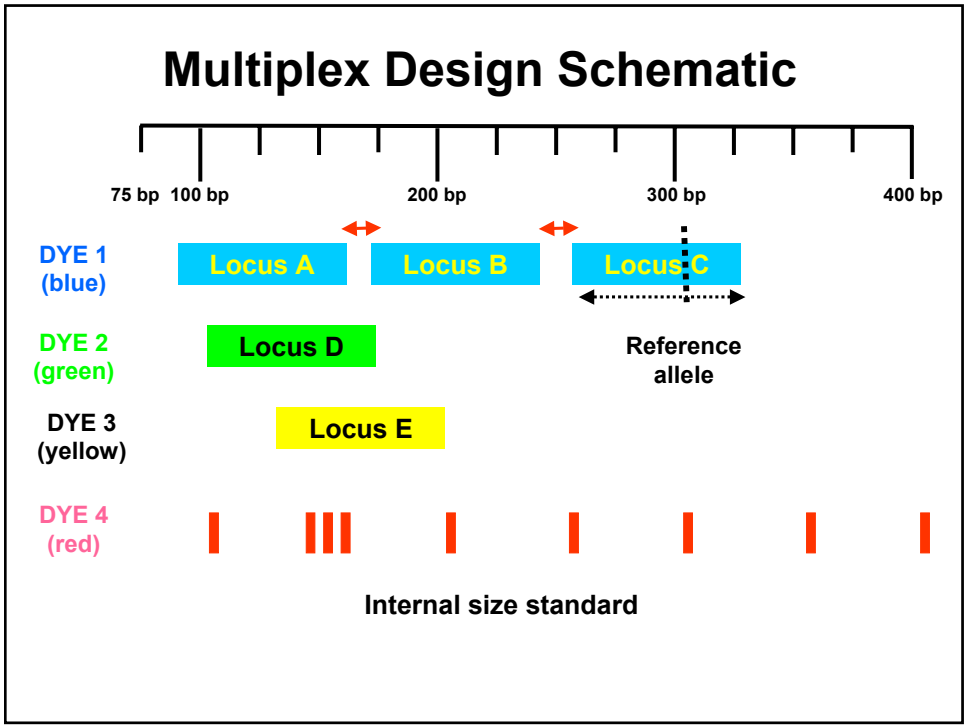
## Forensic value of 14 novel STRs on the human Y chromosome

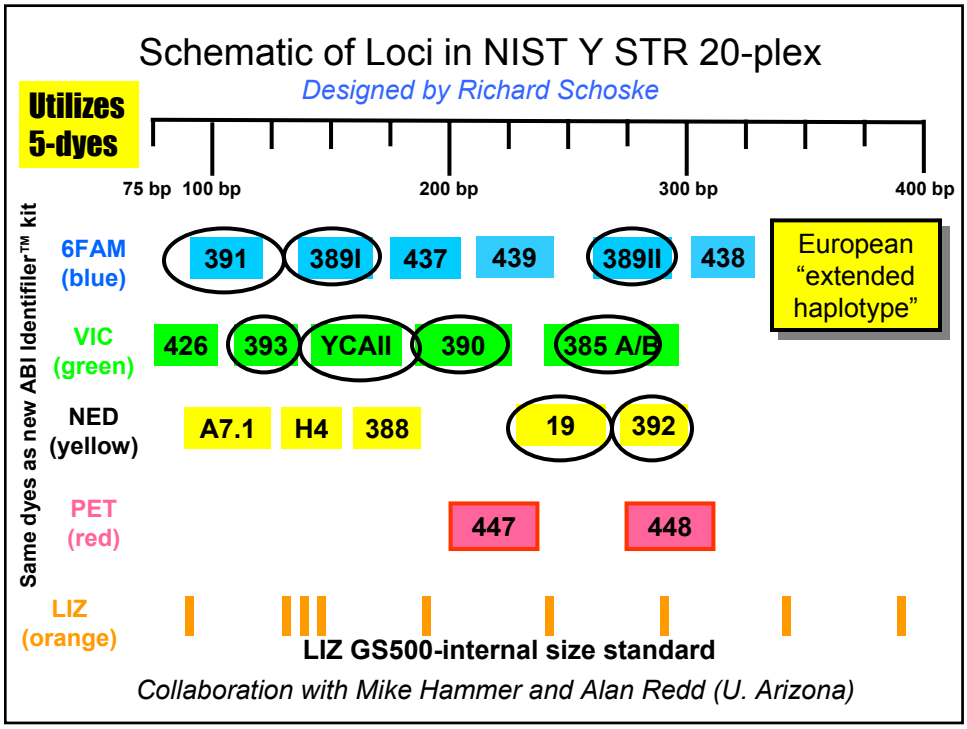
Alan J. Redd[a,*], Al B. Agellon[a], Veronica A. Kearney[a], Veronica A. Contreras[a], Tatiana Karafet[a], Hwayong Park[a,c], Peter de Knijff[b], John M. Butler[d], Michael F. Hammer[a]

[a]*Division of Biotechnology, University of Arizona, Biosciences West room 239, Tucson, AZ 85721, USA*
[b]*Forensic Laboratory for DNA Research, MGC-Department of Human and Clinical Genetics, Leiden University Medical Center, RA Leiden 2300, The Netherlands*
[c]*Genome Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejon 305-333, South Korea*
[d]*National Institute of Standards and Technology, Biotechnology Division, Gaithersburg, MD 20899, USA*

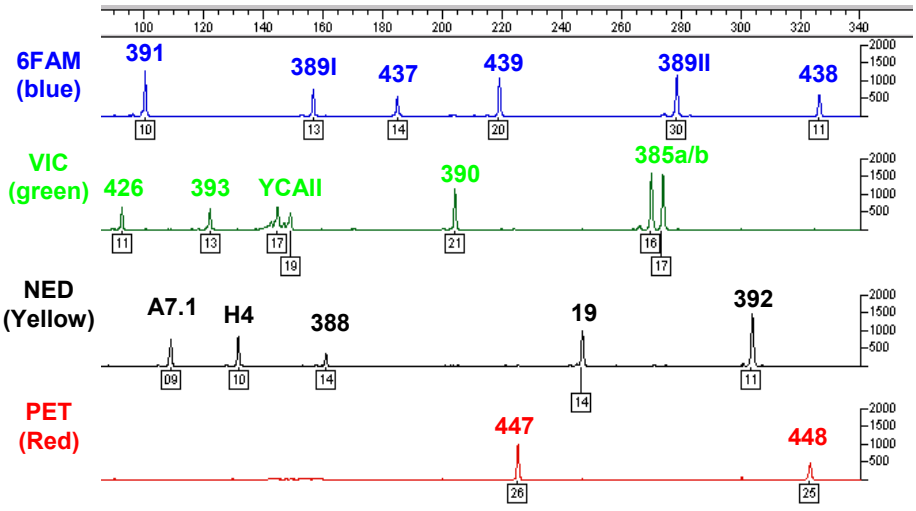
Received 11 February 2002; accepted 10 September 2002

eight tetranucleotide repeats (DYS449, DYS453, DYS454, DYS455, **DYS456**, **DYS458**, DYS459, and **DYS464**), five pentanucleotide repeats (DYS446, **DYS447**, **DYS450**, DYS452, and DYS463), and one hexanucleotide repeat (**DYS448**)


Multiplex Design Schematic
75 bp
100 bp
200 bp
300 bp
400 bp
DYE 1 (blue)
Locus A
Locus B
Locus C
DYE 2 (green)
Locus D
Reference allele
DYE 3 (yellow)
Locus E
DYE 4 (red)
Internal size standard


Schematic of Loci in NIST Y STR 20-plex
Designed by Richard Schoske
Utilizes 5-dyes
75 bp
100 bp
200 bp
300 bp
400 bp
Same dyes as new ABI Identifiler™ kit
6FAM (blue)
391
389I
437
439
389II
438
European "extended haplotype"
VIC (green)
426
393
YCAII
390
385 A/B
NED (yellow)
A7.1
H4
388
19
392
PET (red)
447
448
LIZ (orange)
LIZ GS500-internal size standard
Collaboration with Mike Hammer and Alan Redd (U. Arizona)

# Y STR 20-plex Assay




Recently Published Paper

ELSEVIER

Forensic Science International 129 (2002) 10–24

Forensic Science International

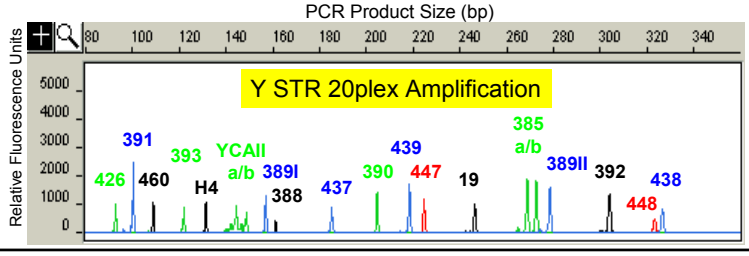
www.elsevier.com/locate/forsciint

## A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers

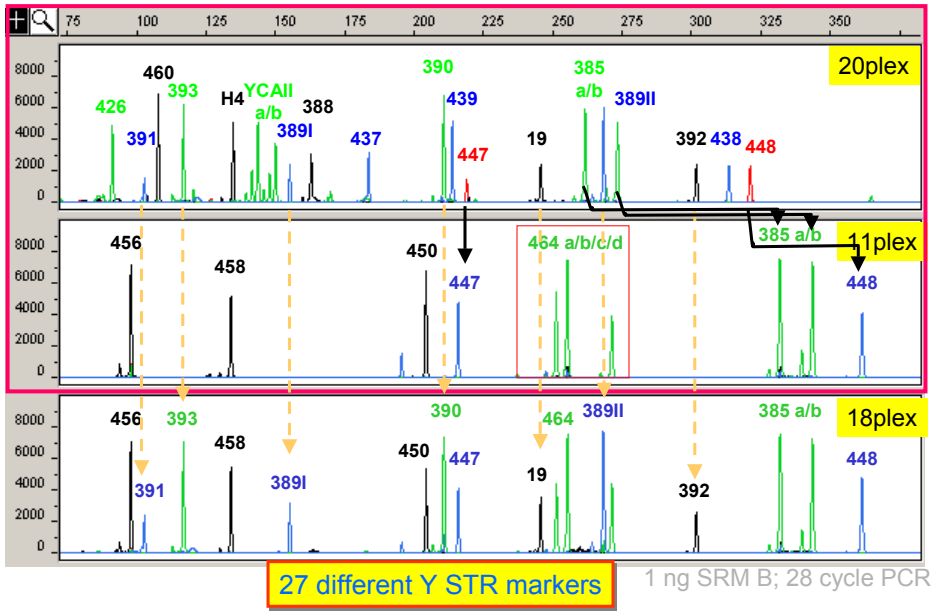
John M. Butler[a,*], Richard Schoske[a,b], Peter M. Vallone[a], Margaret C. Kline[a], Alan J. Redd[c], Michael F. Hammer[c]

[a]Biotechnology Division, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899, USA
[b]Department of Chemistry, American University, Washington, DC 20016, USA
[c]Division of Biotechnology, University of Arizona, Tucson, AZ 85721, USA

Received 22 February 2002; accepted 8 May 2002



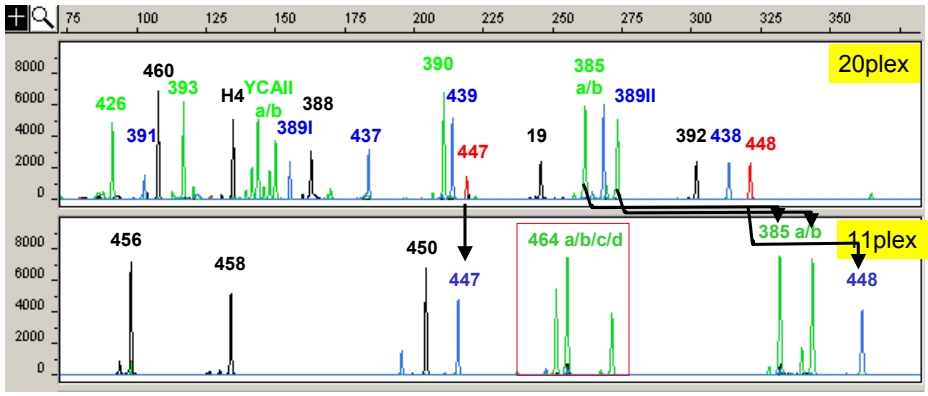
## NIST Multiplexes for High-Throughput Y STR Typing




27 different Y STR markers

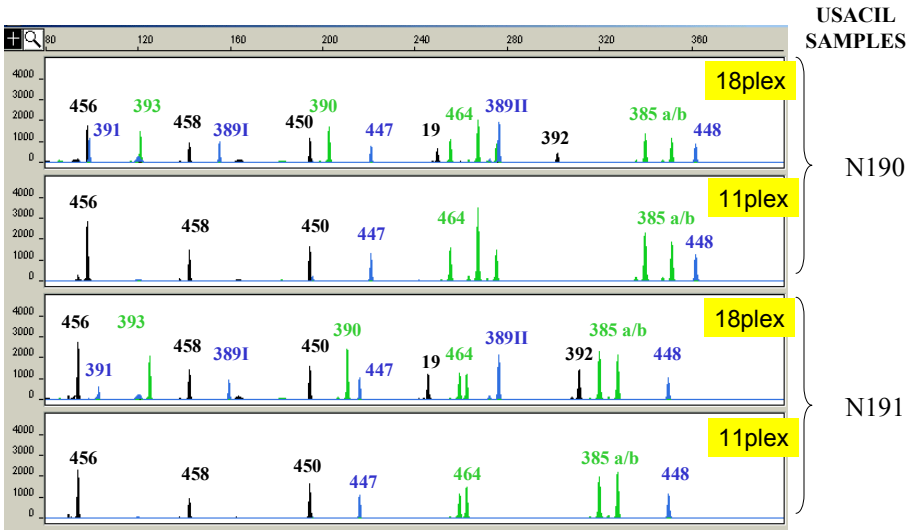
1 ng SRM B; 28 cycle PCR

## 27 Different Y STR Markers Typed in 2 Multiplexes




1 ng SRM B; 28 cycle PCR

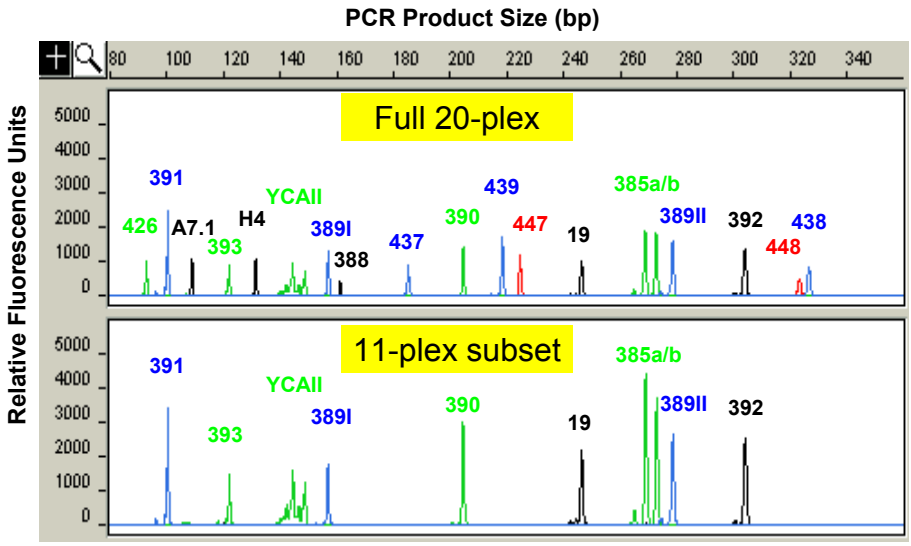
# Comparison of NIST 18plex & 11plex



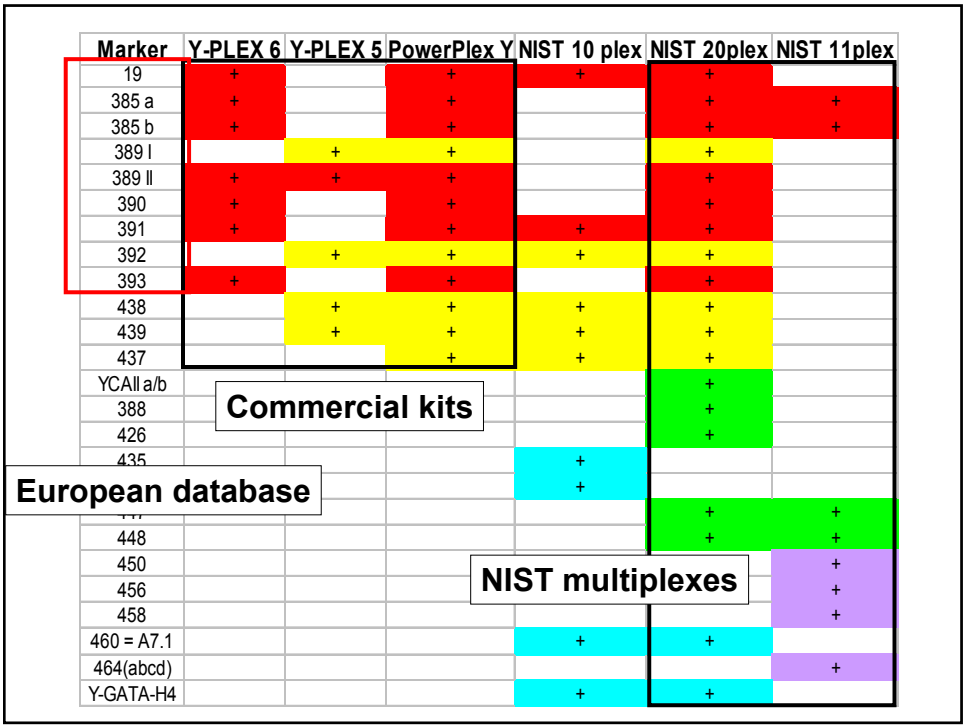

USACIL 8-21-02 Run on USACIL 3100 POP4

1 ng DNA; 28 cycle PCR

# Y STR 20-plex & Y STR 11-plex




*Butler et al. (2002) Forensic Sci. Int., in press*




| Marker | Y-PLEX 6 | Y-PLEX 5 | PowerPlex Y | NIST 10 plex | NIST 20plex | NIST 11plex |
|---|---|---|---|---|---|---|
| 19 | + | | + | + | + | |
| 385 a | + | | + | | + | + |
| 385 b | + | | + | | + | + |
| 389 I | | + | + | | + | |
| 389 II | + | + | + | | + | |
| 390 | + | | + | | + | |
| 391 | + | | + | + | + | |
| 392 | | + | + | + | + | |
| 393 | + | | + | | + | |
| 438 | | + | + | + | + | |
| 439 | | + | + | + | + | |
| 437 | | | + | + | + | |
| YCAII a/b | | | | | + | |
| 388 | | | | | + | |
| 426 | | | | | + | |
| 435 | | | | + | | |
| | | | | + | | |
| | | | | | + | + |
| 448 | | | | | + | + |
| 450 | | | | | | + |
| 456 | | | | | | + |
| 458 | | | | | | + |
| 460 = A7.1 | | | | + | + | |
| 464(abcd) | | | | | | + |
| Y-GATA-H4 | | | | + | + | |

# Summary of Typing Y-STRs

260 AA, 244 Cauc, 143 HIS samples were typed from the NIST U.S. population samples (647 total)

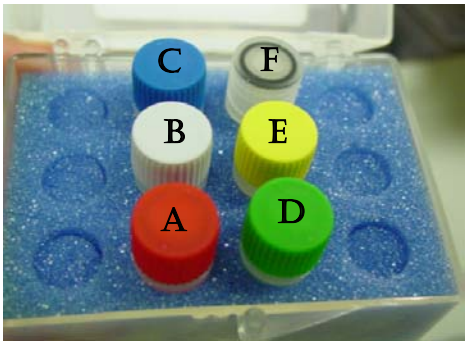
Number of haplogroups

| | No. of Markers | AA(260) | Cau(244) | HIS(143) |
|---|---|---|---|---|
| Y-PLEX 5&6 | 11 | 239 | 201 | 133 |
| NIST 20 and 11 plex | 27 | 257 | 243 | 142 |
| 10 best loci | 10 | 252 | 238 | 142 |

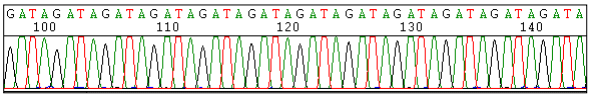
Multiplexing results in 13 fold reduction
647 x 27 = 17,469
2 x 647 = 1294

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





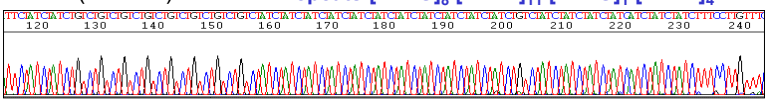

DYS439 (forward) A **12 GATA repeats**




Sequencing Results for
23 Y STR Loci

50 Y SNP Loci Typed

DYS390 (forward) E **24 repeats $[TCTG]_8$ $[TCTA]_{11}$ $[TCTG]_1$ $[TCTA]_4$**



# Acknowledgments

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Thomas Parsons, Rebecca Hamm and Mike Coble (AFDIL)

Mike Hammer and Alan Redd (U. of Arizona)