Development of Multiplexed Assays for Typing SNP and STR Forensic Markers

NCI - Advanced Technology Center

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National Institute of Standards and Technology

Outline of Presentation

- Multiplexing
- Assays and Instrumentation
- Y Chromosome and Mitochondrial DNA
- Primer design strategy
- Results
  - mtSNP 10 plex
  - Y SNP 5 plex
  - Y STR multiplexes
  - Other

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 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

What Assays are we Multiplexing?

Polymerase chain reaction (PCR)

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

Primer Extension reaction (minisequencing)

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

Goals for Multiplex Assay Development

Working with collaborators who have markers of forensic interest

By using our multiplex assays collaborators can type markers and evaluate forensic utility

Further understanding of multiplex assays

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

Multiplexing

Assays and Instrumentation

Y Chromosome and Mitochondrial DNA

Primer design strategy

Results

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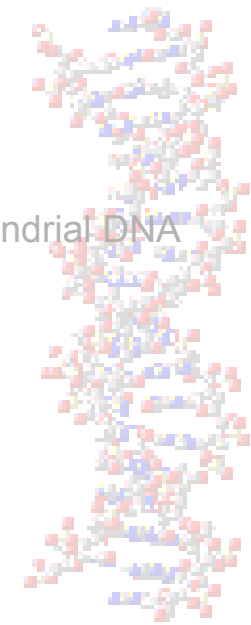
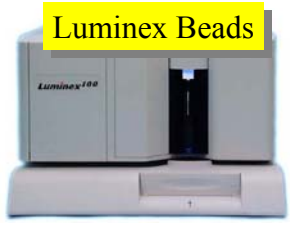


Instrumentation

SNaPshot/PCR

Multi-Color Capillary Electrophoresis
(ABI 310 or 3100)

Luminex Beads



Luminex 100 Flow Cytometer

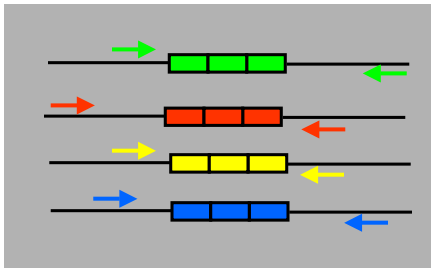
Primer Extension

Time-of-Flight Mass Spectrometer

TaqMan

Roche LightCycler

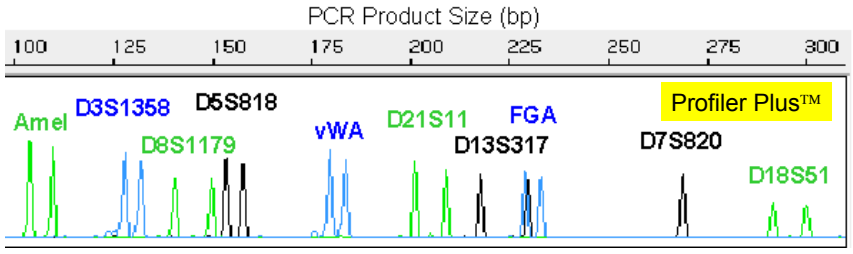
Multiplex PCR

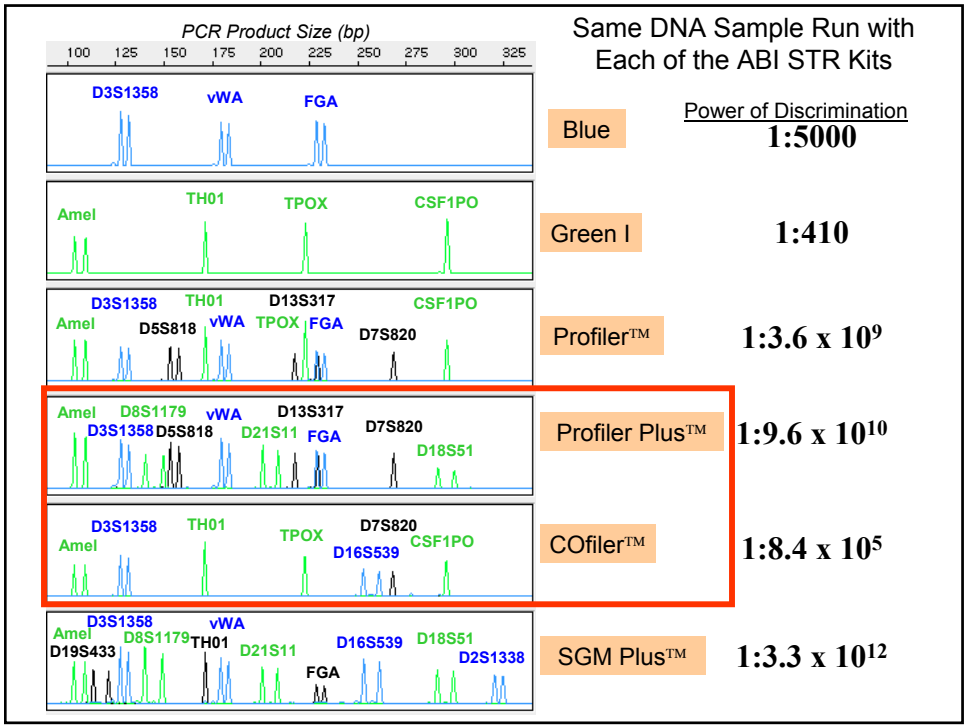


Multiple primer pairs target more than one specific site on the DNA strand

Compatible primers are the key to successful multiplex PCR

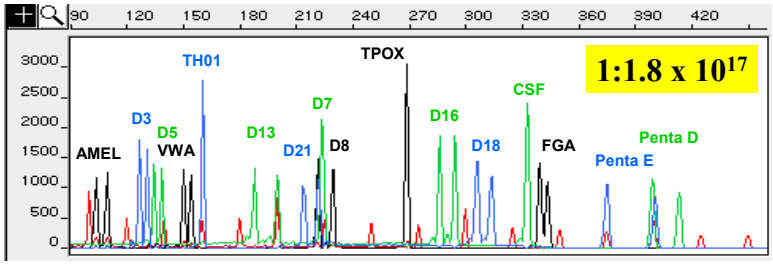
Commercial kits are available for targeting and simultaneously amplifying 16 markers



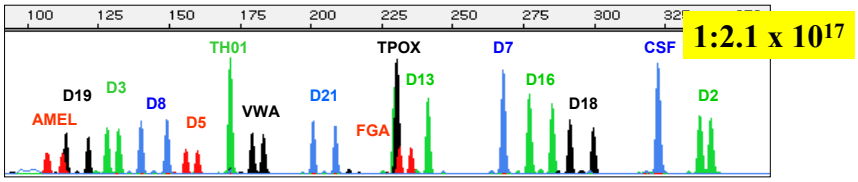


Commercial STR 16plex Kits

PowerPlex® 16 (Promega)

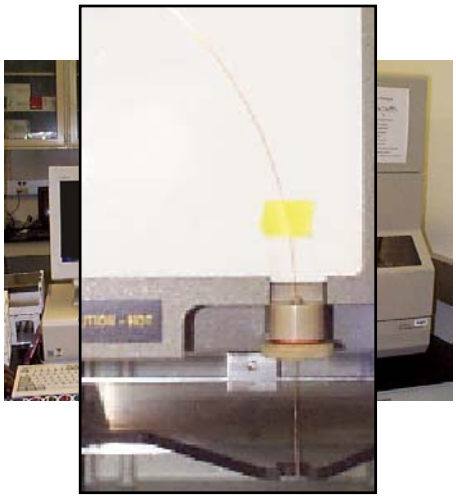


AmpFISTR® Identifiler™ (Applied Biosystems)



Capillary Electrophoresis Instrumentation

ABI 310
single capillary



ABI 3100
16-capillary array

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

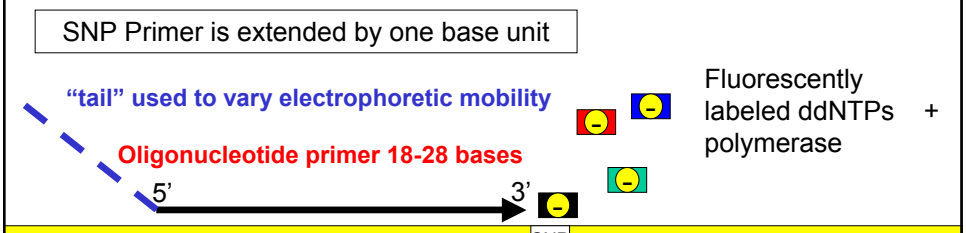
Primer extension assay that utilizes fluorescently labeled ddNTPs

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!

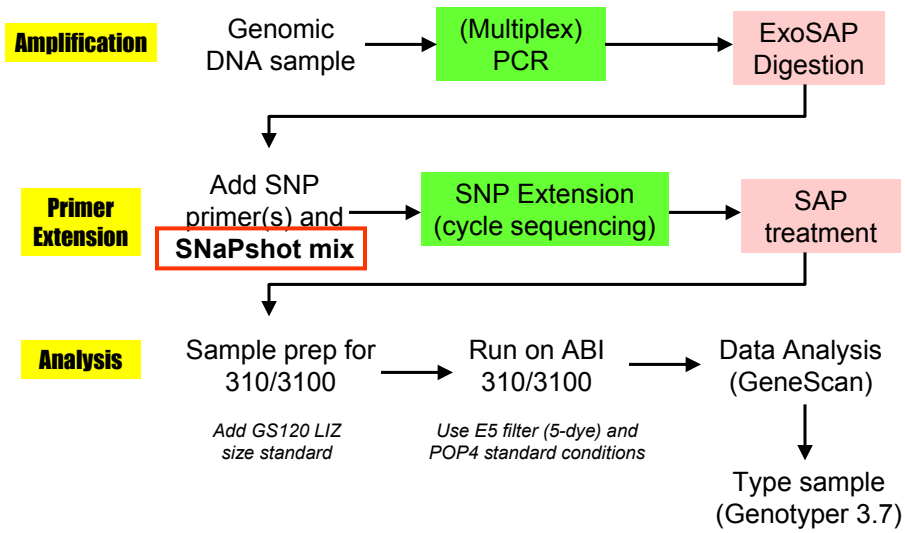
Primer Extension with SNaPshot™



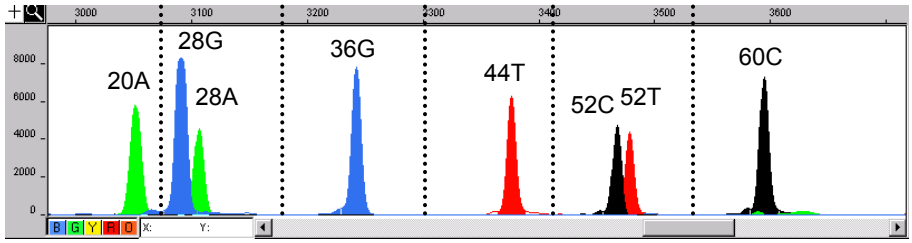
ddNTP	Dye label	Color
A	dR6G	Green
C	dTAMRA	Black
G	dR110	Blue
T	dROX	Red

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**

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

Priming site

Multiplexing possible by use of different length primers

Primer Extension for MALDI TOF MS Analysis

SNP Primer is extended by one base unit

Oligonucleotide primer 18-28 bases

5’ 3’

SNP

PCR Amplified DNA Template

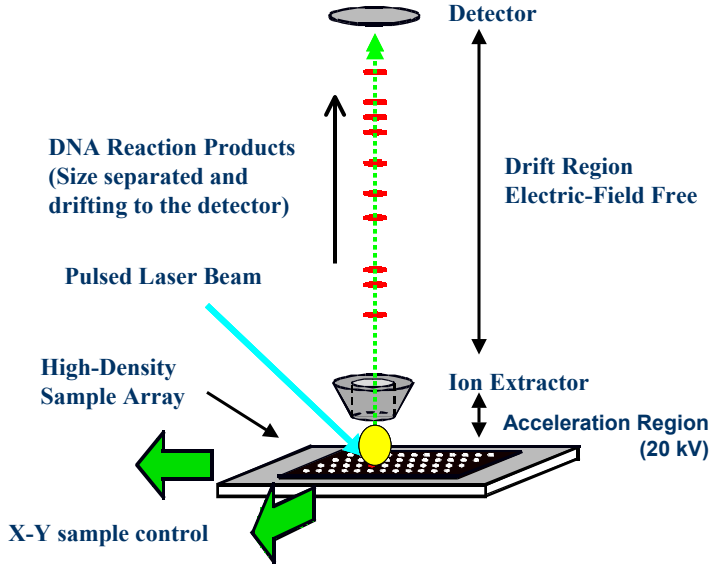
Natural non-labeled ddNTPs + polymerase

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)



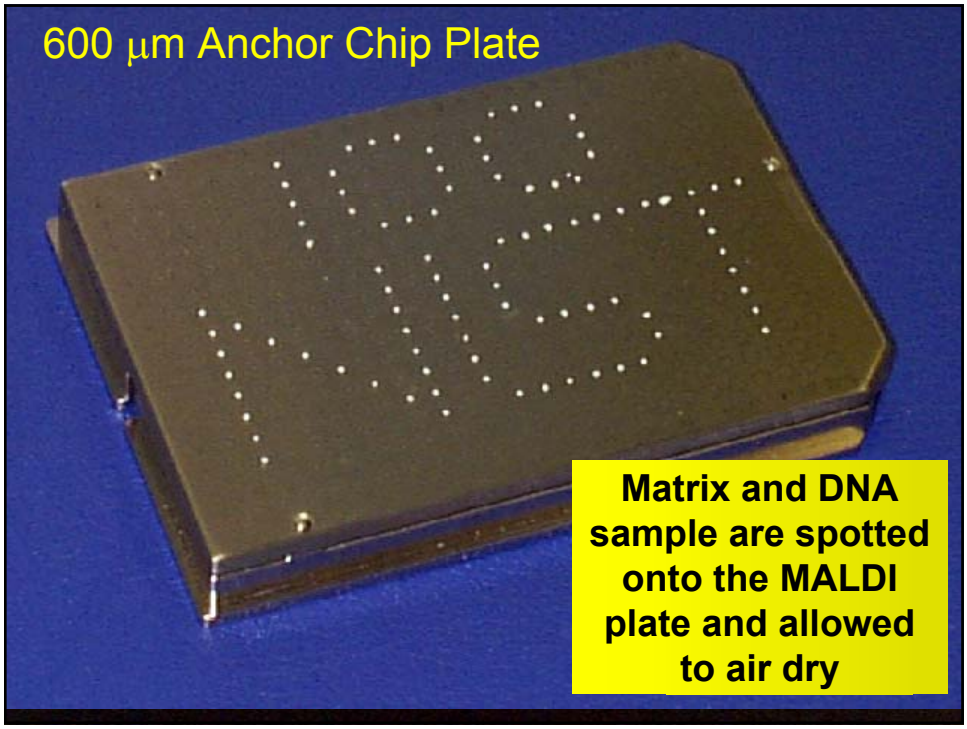
MWG Robotic Sample Preparation Workstation

Bruker BIFLEX III MALDI-TOF MS

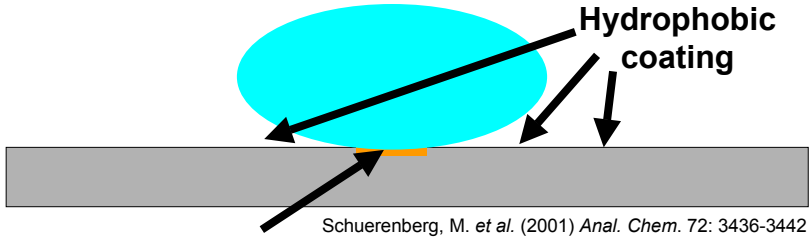
Instrumentation at NIST

•MWG Biotech RoboAmp 4200 (Ebersberg, Germany) capable of on-board PCR thermal cycling with non-cross contamination 96-well plates

•Bruker BIFLEX III MALDI-TOF mass spectrometer (Bremen, Germany) capable of operation in both linear and reflectron mode

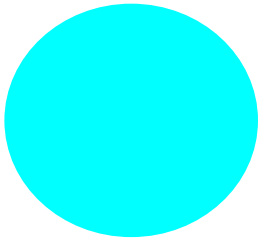


Treated Surfaces Improve Sample Preparation



Schuerenberg, M. *et al.* (2001) *Anal. Chem.* 72: 3436-3442

Top View of Spot Drying



Sample is concentrated and focused to an exact location as it dries

Different Types of Matrix Spots on MALDI Targets

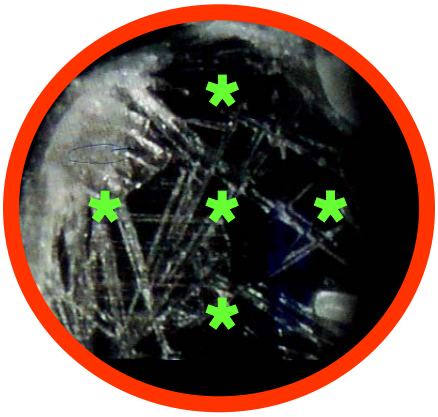
3 HPA Matrix Dried on 600 µm Anchor Spot

Concentrated spot (greater sensitivity)

Uniform spot (impacts automation)

Specific Location

3 HPA Matrix Dried on Stainless Steel Surface



Non-uniform crystallization produces "sweet spots"

Desalting Primer Extension Reactions

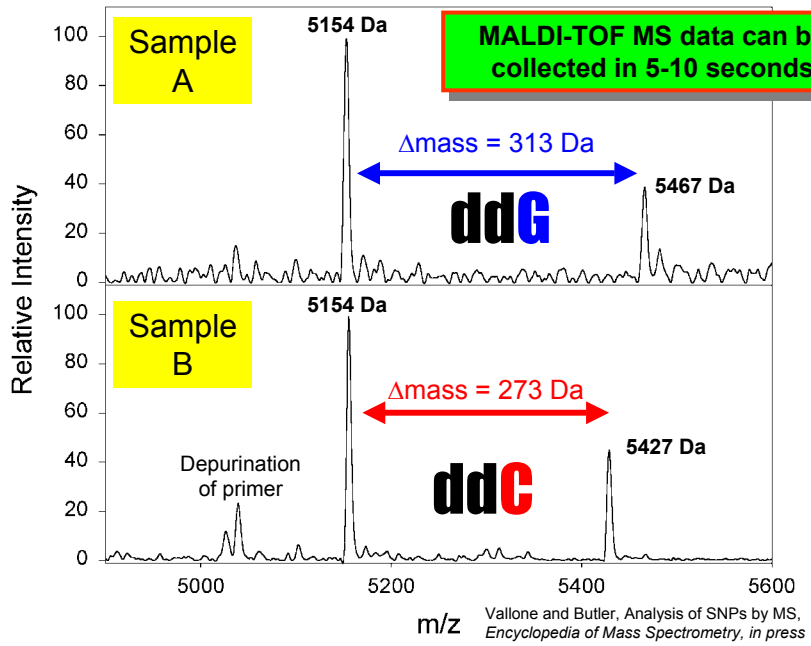
Genopure Beads (single stranded DNA)

8 strip or 96 well format

Single stranded DNA oligomers are bound and washed free from salts, enzyme, ddNTPs

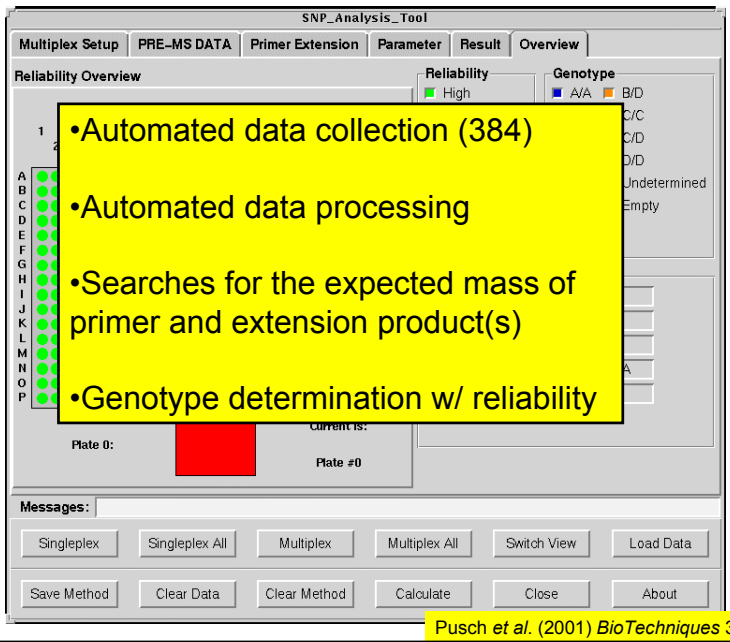
Salt free sample is eluted from the bead, spotted on the MALDI target

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

Multiplexing

Assays and Instrumentation

Y Chromosome and Mitochondrial DNA

Primer design strategy

Results

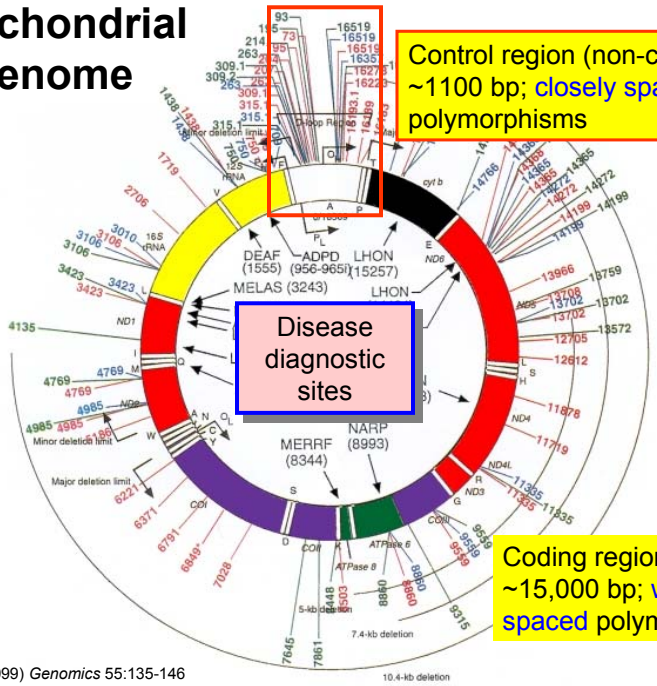
- mtSNP 10 plex
- Y SNP 5 plex
- Y STR multiplexes
- Other

Markers of Interest

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

Require large databases because recombination does not occur

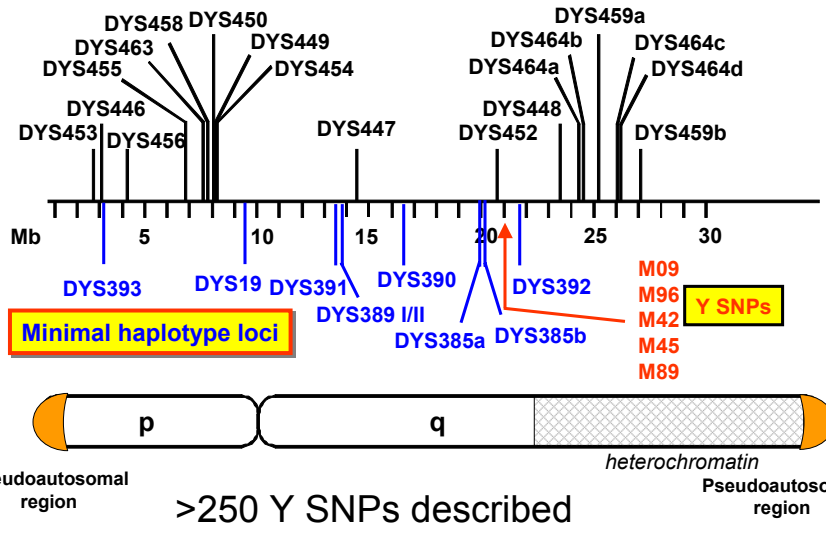
Mitochondrial Genome



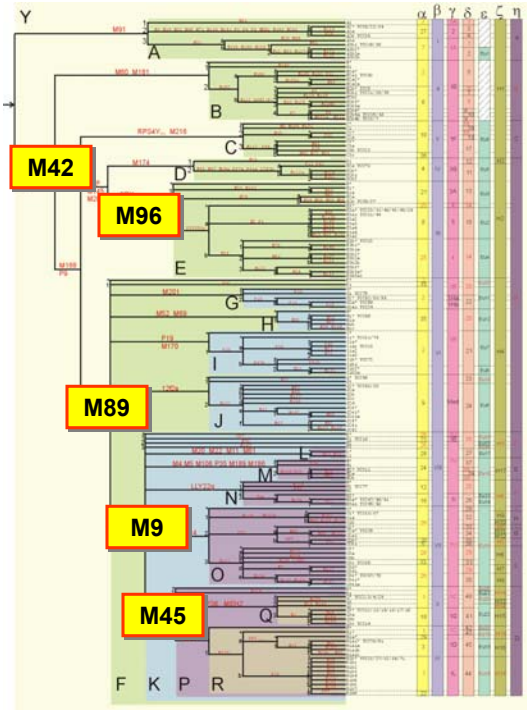
Levin *et al* (1999) *Genomics* 55:135-146

There is a growing interest in the Y-chromosome to aid forensic, paternity, and missing persons testing…

New Y STRs from Mike Hammer's group



>250 Y SNPs described



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

5 Y SNPs typed define major haplogroups

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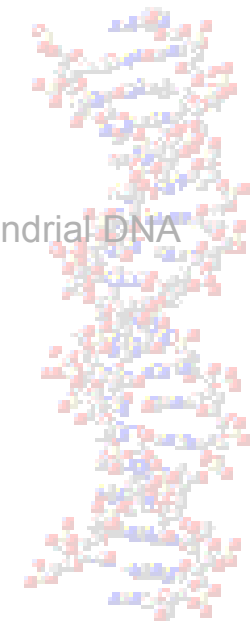


Multiplex PCR Primer Selection

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

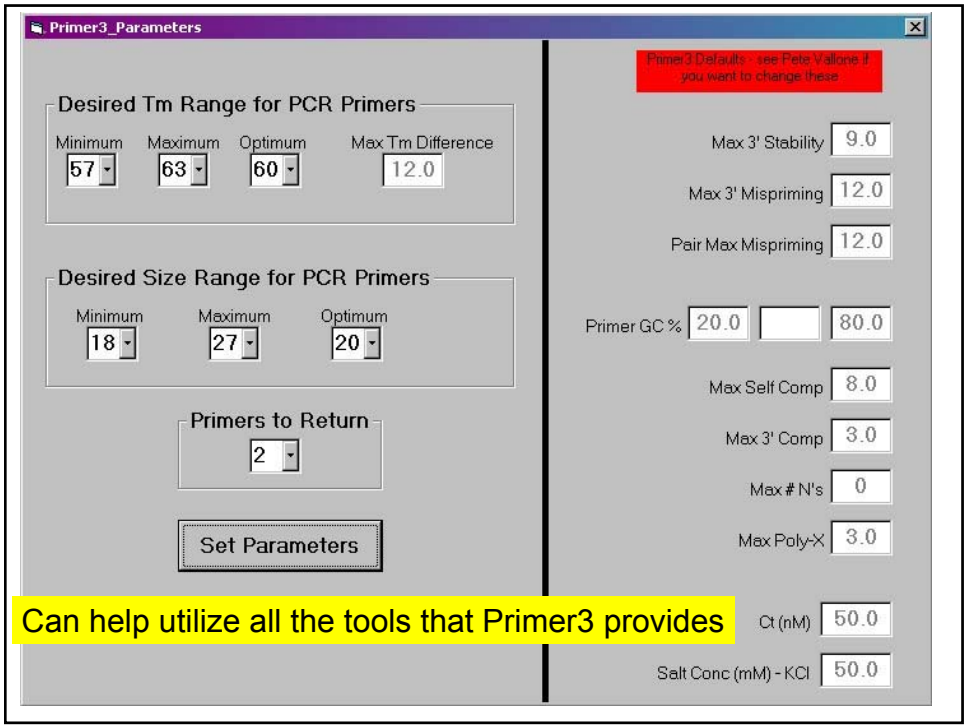
Primer3 is publicly available and can be run (in batch!) on a Unix, PC (Linux), or Mac (OSX) computer

Developed a program that formats files for Primer3 input

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

Example input format for Primer3

```
PRIMER_SEQUENCE_ID=M9
SEQUENCE=GCAGCATATAAAACTTTCAGGACCCTGAAATACAGAACTG
CAAAGAAACGGCCTAAGATGGTTGAATNCTCTTTATTTTTCTTTAATTTAG
ACATGTTCAAACGTTCAATGTCTTACATACTTAGTTATGTAAGTAAGGTAG
CGCTTACTTCATTATGCATTTCAATACTCAAAAAAAATTCCTTTGTGAAAT
GTTGAAATATTTTTCTAATCTGTTTCACGAGCTTCAAAAATGAGGAAAAAA
GATTCAGTTTACATTTCAGCAAAATGCCTCTTTTTAATCGGATTTATGTTT
ACTTAACATTTACAGTACATTTACGCTTGAGCAAAGTTAGGTTTT
PRIMER_COMMENT=(340 bp); G to C at position 68
PRIMER_MISPRIMING_LIBRARY=/Users/vallone/Desktop/primer3/misprM9
PRIMER_MAX_MISPRIMING=8
PRIMER_PAIR_MAX_MISPRIMING=20
EXCLUDED_REGION=38,60
PRIMER_PRODUCT_SIZE_RANGE=90-150
PRIMER_PRODUCT_OPT_SIZE=105
PRIMER_MIN_SIZE=18
PRIMER_MAX_SIZE=27
PRIMER_OPT_SIZE=20
PRIMER_OPT_TM=60
PRIMER_MIN_TM=57
PRIMER_MAX_TM=63
PRIMER_NUM_RETURN=1
PRIMER_EXPLAIN_FLAG=1
PRIMER_LIBERAL_BASE=1
=
PRIMER_SEQUENCE_ID=M42
SEQUENCE=AAAGCGAGAGATTCAATCCAGGATGACAGAATGCGTTCAC
CTTTAAAGGGATTAAAAGAAGTATAATACAGTCTGTATTATTAGATCACCC
AGAGACACACAAAACAAGAACCGTGAATTGAATTAGTGGTATACTAATAG
```



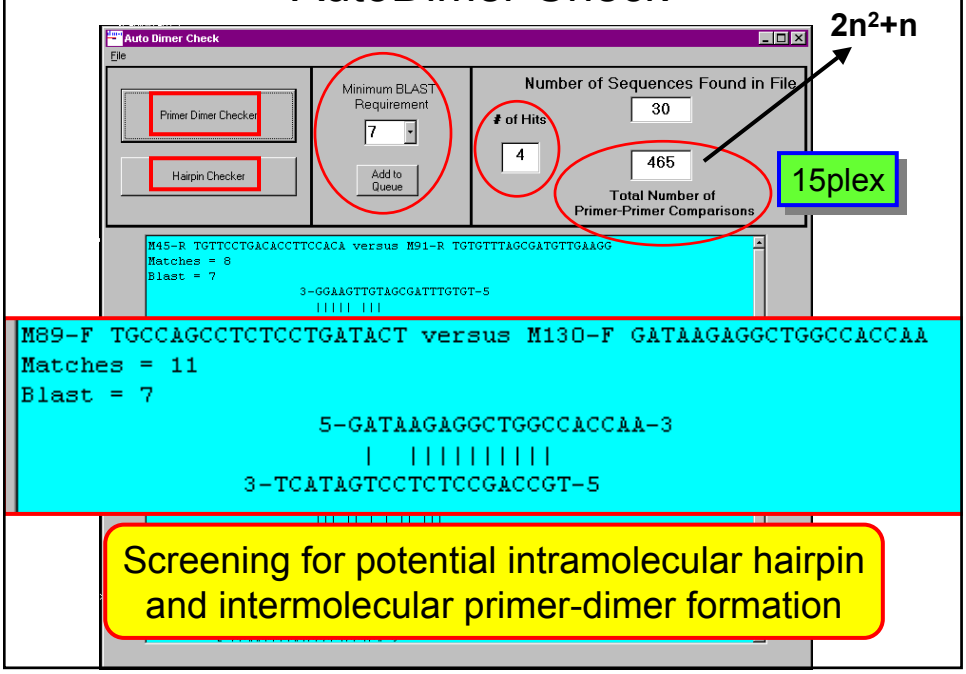
Non-Specific Interactions

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

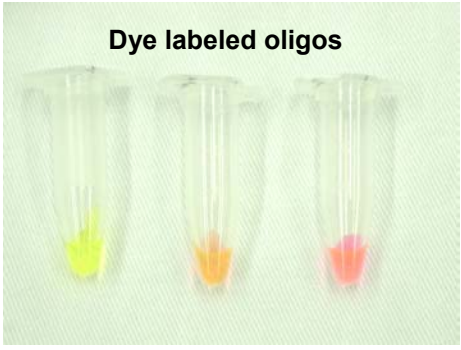


PCR Assay Design

If primer pairs meet criteria

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

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

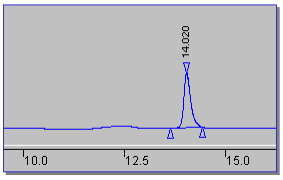
Varian Helix DHPLC System

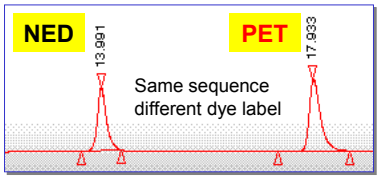
Oligo QC

Oligo Purification

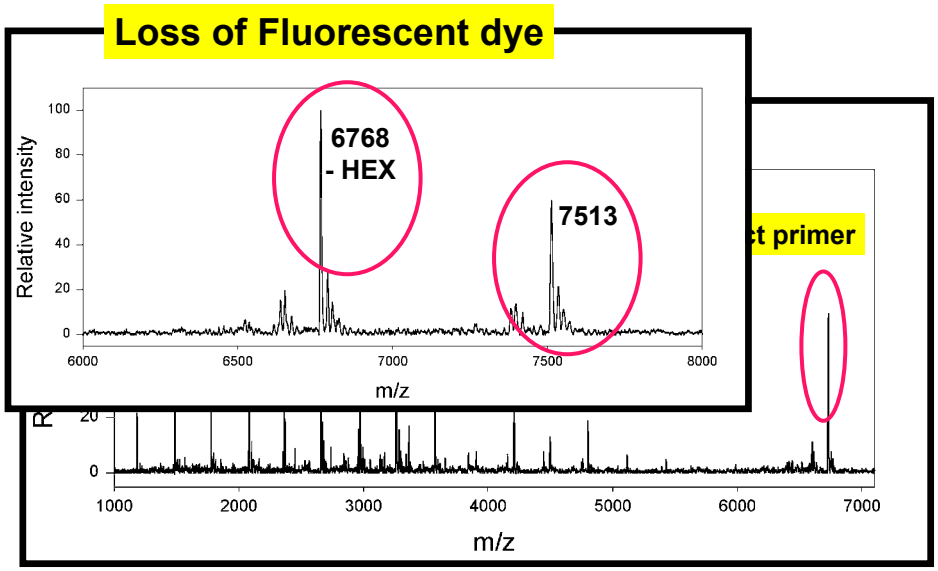
STR allele isolation for sequencing purposes

Fluorescent dye studies (excess dye removal)





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
1.0 μM of each primer pair

Evaluate amplicon yields, presence and balance

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

Redesign and retest failing loci

Multiplexing

Assays and Instrumentation

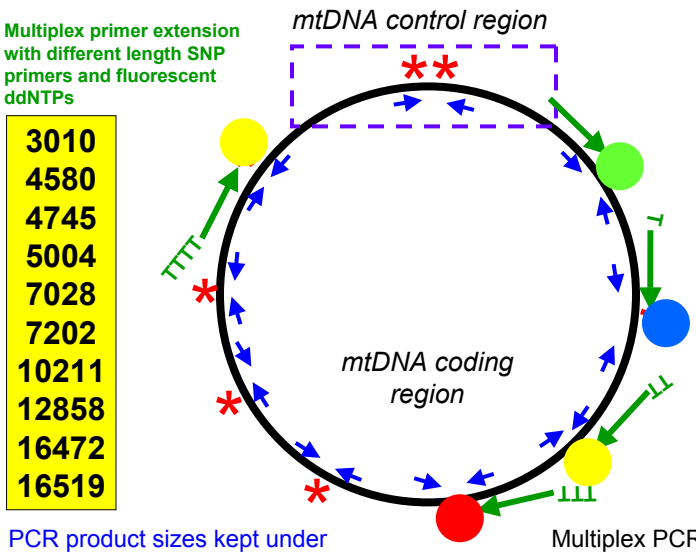
Y Chromosome and Mitochondrial DNA

Primer design strategy

Results

- mtSNP 10 plex
- Y SNP 5 plex
- Y STR multiplexes
- Other

Coding Region mtSNP 10-plex Assay



Collaboration with Tom Parsons (AFDIL) provided sequence variation sites located in the coding region

PCR product sizes kept under 150 bp to enable success with degraded DNA samples

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

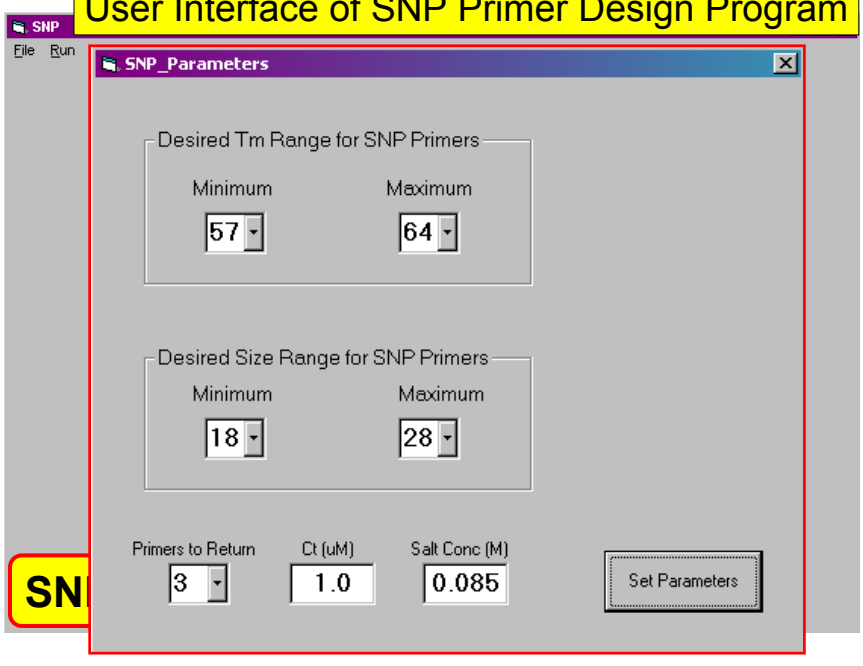
Tailed SNP primers allows for multiplexing in the SNaPshot assay

Sequences for 10 SNP primers

TCAGAAGTGAAAGGGGGC	18/na
TTTTTTTGTTGGATCAGGACATCCC	19/26
TTTTTTTTTTACTAAGAAGAATTTTATGGA	20/30
TTTTTTTTTTTTTTAGACCCAGCTACGCAAAATC	20/34
TTTTTTTTTTTTTTTTTTGACACGTACTACGTTGTAGC	20/38
TTTTTTTTTTTTTTTTTTTTTTCCACAACACTTTCTCGGCCT	20/42
TTTTTTTTTTTTTTTTTTTTTTTTTTGTGGGCTATTTAGGCTTTATG	22/46
TTTTTTTTTTTTTTTTTTTTTTTTTTTTGCAGCCATTCAAGCAATCCTATA	23/50
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGTTAGAACTGGAATAAAAGCTAG	25/54
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGAACCATAACCAATACTACCAATCA	25/58

Template binding sequence – black
Tailed sequence for fragment separation - red

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

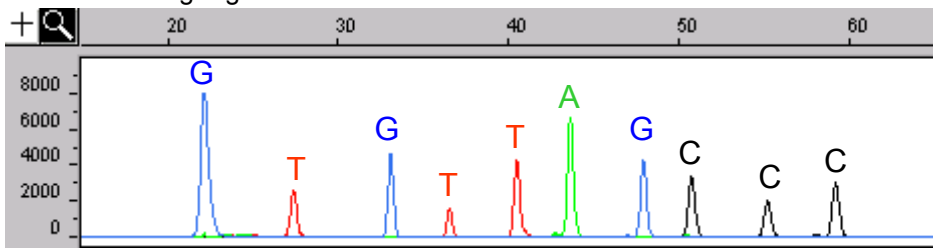
Hairpin	Dimer	Template	Mass	Rank	Mutation	+ddC	+ddT	+ddA	+ddG
4	8	10	5273.48	2.133333	W	N/A	5561.67998	5570.68998	N/A
5	10	10	5602.69	2	W	N/A	5890.889941	5899.899941	N/A
5	10	11	5915.9	2	W	N/A	6204.099902	6213.109902	N/A
4	8	22	6734.42	2.133333	W	N/A	7022.619922	7031.629922	N/A
4	8	20	6116.02	2.133333	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 10-plex run on ABI 3100

(SNapShot™ assay)

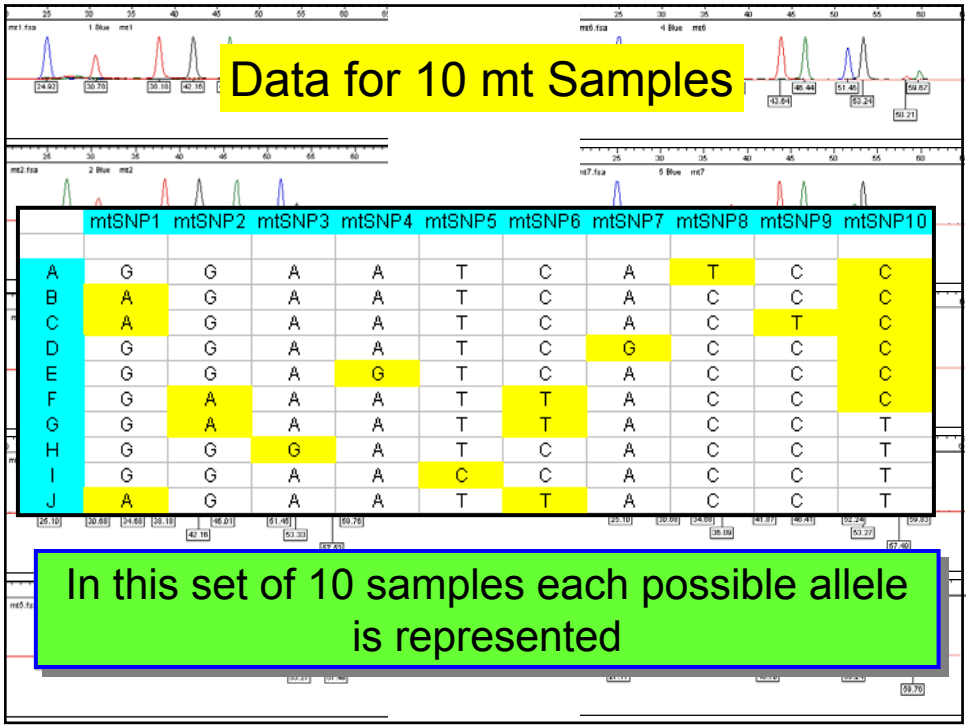
Multiplex PCR and Multiplex SNP Detection

mtDNA coding region SNPs



SNP types

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



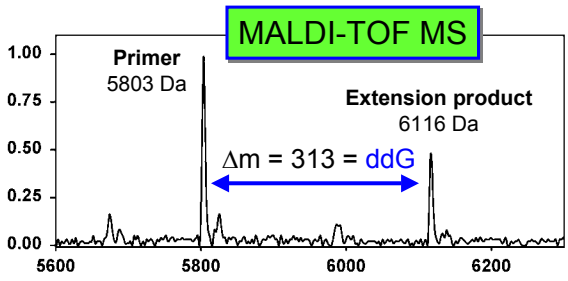
Sizing of Fragments in mtSNP 10plex

Actual versus observed

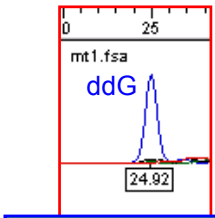
Actual length (bases)	alelle 1	allele 2	Δ allele1	Δ allele 2
18	25.0	27.1	-7.0	-9.1
26	28.6	30.7	-2.6	-4.7
30	34.7	35.6	-4.7	-5.6
34	36.9	38.2	-2.9	-4.2
38	42.2	43.7	-4.2	-5.7
42	45.0	46.4	-3.0	-4.4
46	51.4	52.2	-5.4	-6.2
50	53.3	54.2	-3.3	-4.2
54	57.5	58.3	-3.5	-4.3
58	59.2	59.7	-1.2	-1.7

Sizing differences vary with sequence, length and fluorescent dye attachment

Comparison of Results between Techniques



Mass difference between primer and extension product



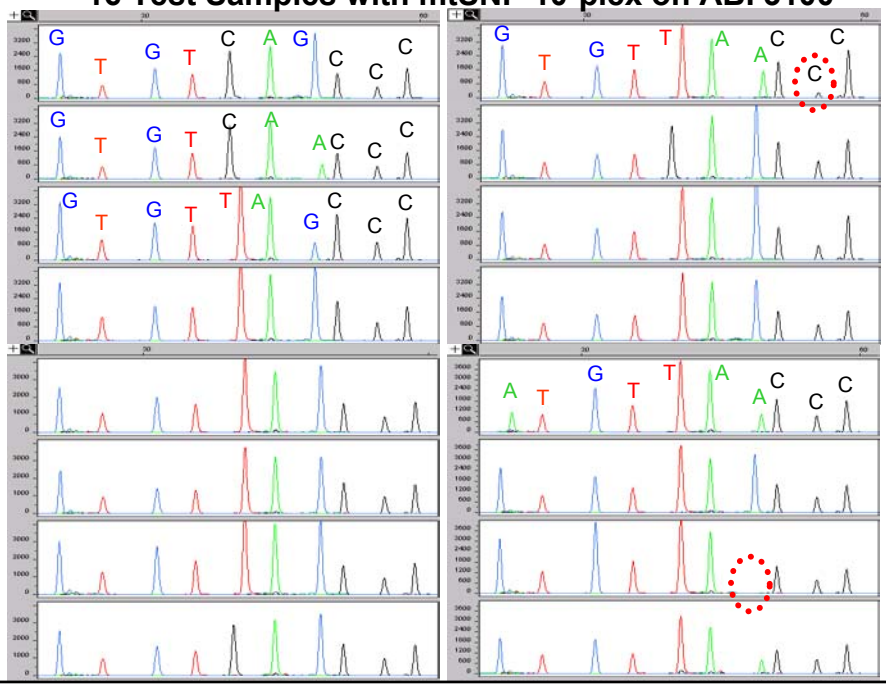
Fluorescent dye color of peak

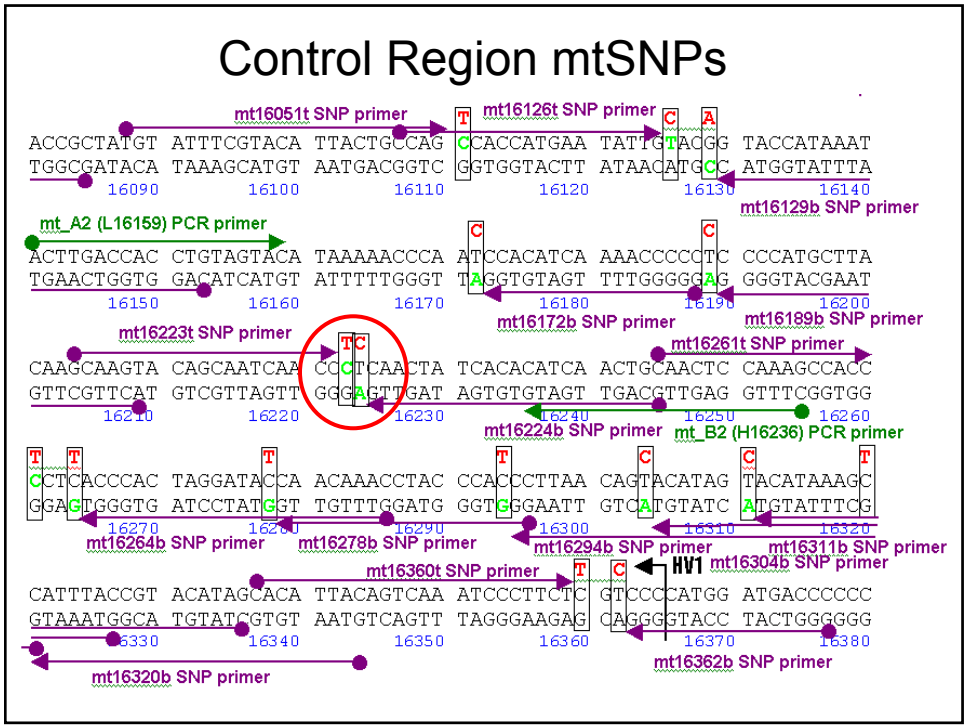
SNaPshot (ABI 310)

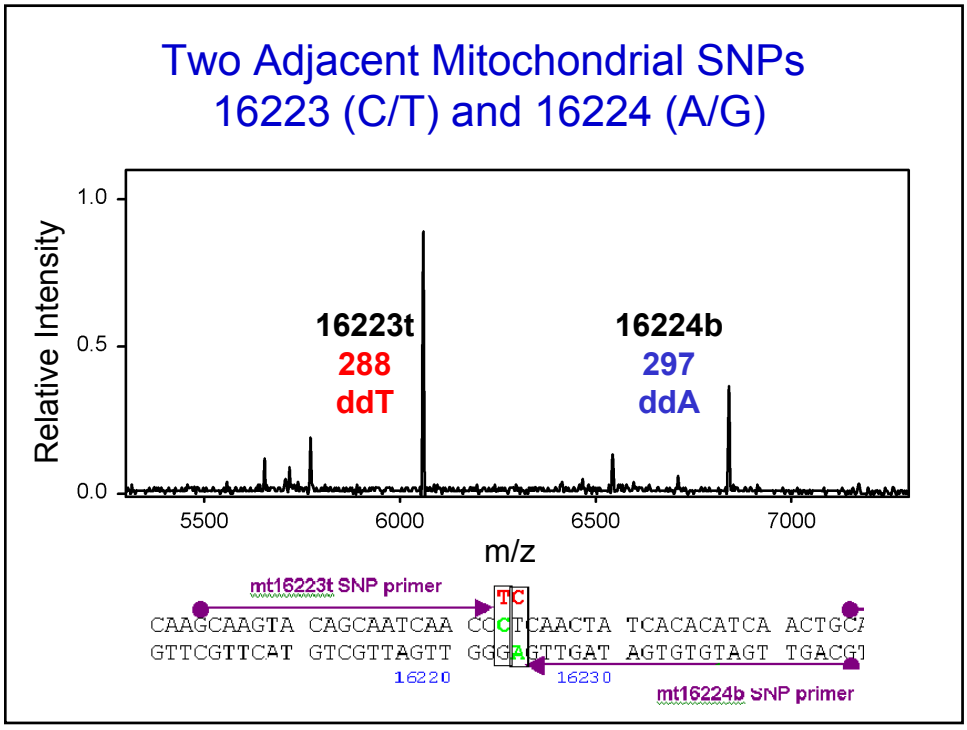
	mtSNP1 SNaPshot	mtSNP1 MALDI
A	G	G
B	A	A
C	A	A
D	G	G
E	G	G
F	G	G
G	G	G
H	G	G
I	G	G
J	A	A

Complete agreement in results

16 Test Samples with mtSNP 10-plex on ABI 3100

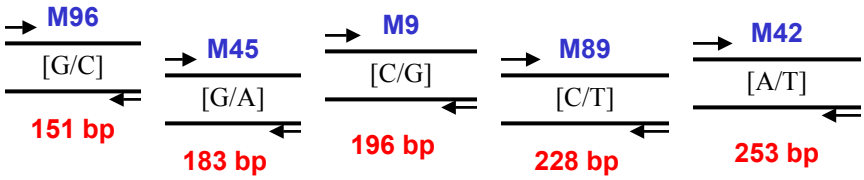



Control Region mtSNPs
mt16051t SNP primer
mt16126t SNP primer
mt16129b SNP primer
mt_A2 (L16159) PCR primer
mt16172b SNP primer
mt16189b SNP primer
mt16223t SNP primer
mt16261t SNP primer
mt16224b SNP primer
mt_B2 (H16236) PCR primer
mt16264b SNP primer
mt16278b SNP primer
mt16294b SNP primer
mt16311b SNP primer
mt16360t SNP primer
HV1
mt16304b SNP primer
mt16362b SNP primer
mt16320b SNP primer


Two Adjacent Mitochondrial SNPs
16223 (C/T) and 16224 (A/G)
Relative Intensity
1.0
0.5
0.0
16223t
288
ddT
16224b
297
ddA
5500
6000
6500
7000
m/z
mt16223t SNP primer
mt16224b SNP primer

- Multiplexing
- Assays and Instrumentation
- Y Chromosome and Mitochondrial DNA
- Primer design strategy
- Results
  - mtSNP 10 plex
  - Y SNP 5 plex
  - Y STR multiplexes
  - Other

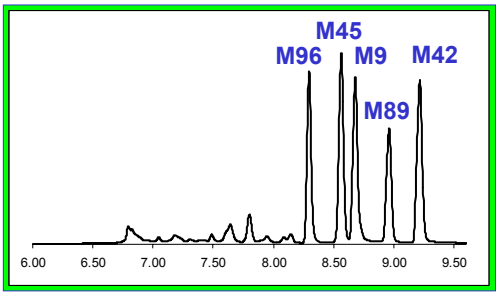
Multiplex PCR with Y-Chromosome SNP Markers



5-plex PCR

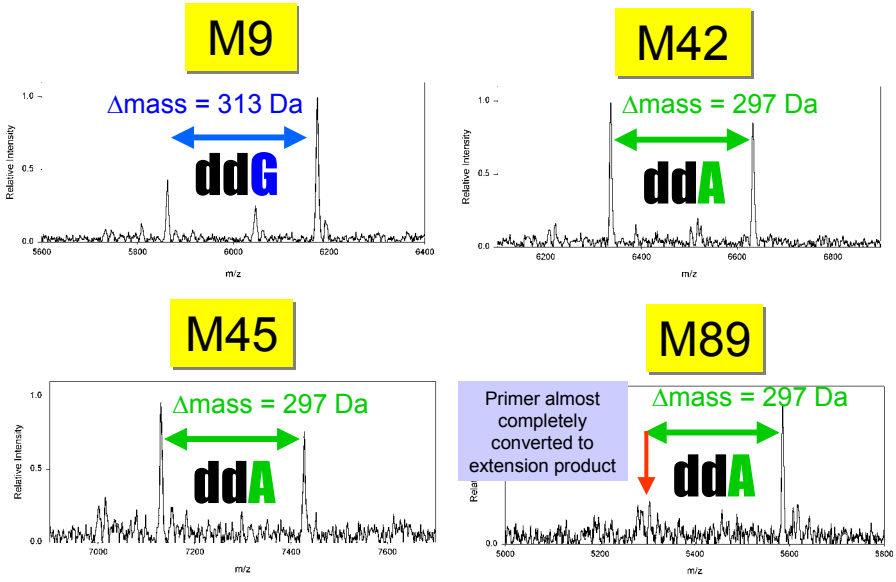
Rapid CE Separation and Quantitation of Multiplex PCR Products

Intercalating dye used to fluorescently label amplicons

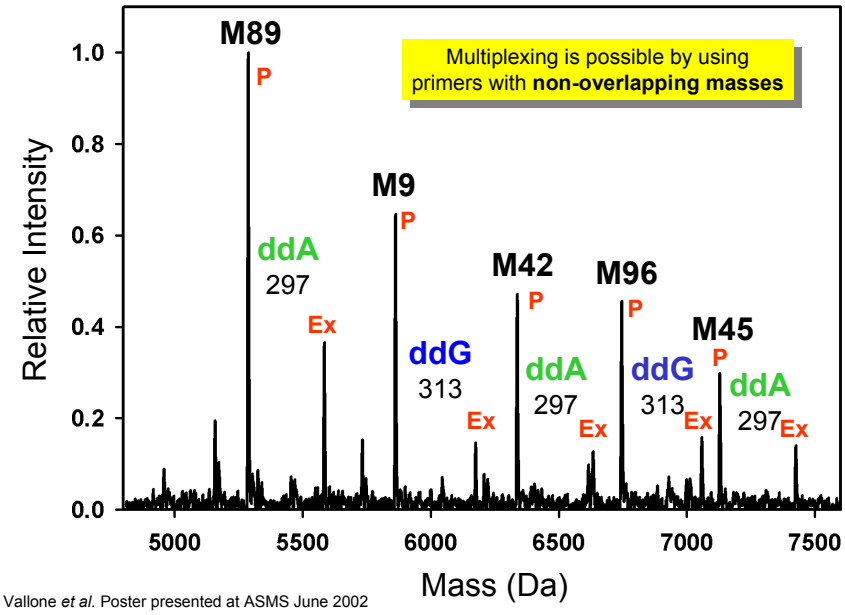


Butler *et al. (2001) Fresenius J. Anal. Chem.* 369: 200-205

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

SRM 2395 Candidate Sample Testing

Y SNP Results with Primer Extension and MALDI-TOF MS

	M9(C/G)	M42(A/T)	M45(G/A)	M89(C/T)	M96(G/C)
A	G	T	A	T	C
B	C	T	G	T	C
C	C	T	G	T	C
D	C	T	G	C	G
E	C	T	G	T	C
F	--	--	--	--	--

Primer Extension Using a UV Photocleavable Analyte

A standard primer extension assay (mini-sequencing) is performed using an extension primer that contains a UV photocleavable linker

After the extension reaction is completed, the linker is cleaved (λ = 366 nm) resulting in a ~5 base oligonucleotide for MALDI TOF MS analysis

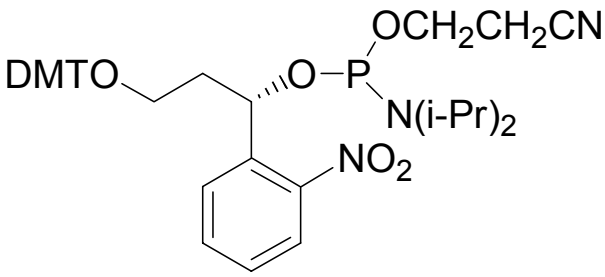
An analyte of reduced mass results in higher sensitivity, resolution, and more uniform ionization for multiplexing

Example M42

5' CCAGCTCTCTTTTTCATTATLTAGT 3' mass = 7492.9

5' TAGT 3' mass = 1268.8

Structure of Cleavable Moiety



Ordoukhanian, P. and Taylor, J.S., J. Am. Chem. Soc 1995 117:9570-9571

Collaboration with Jay Stoerker and Markus Kostrzewa at Bruker Daltonics

UV Cleavable SNP Primers

Locus	Extension Primer	Mass
M9	ACATGTCTAAATTAAAGAAAAATA**A**A^{OMe}GA^{OMe}G	1362.9
M42	CCAGCTCTCTTTTTCATTAT**G**TAGT	1268.8
M45	GCAGTGAAAAATTAT**A**G^{OMe}ATA	1307.8
M89	CTCTTCCTAAGGTTATGTACAAA**A**ATCT	1228.8
M96	AACTTGGAAAACAGGTCTCTCA**T**AATA	1261.8

Underlined base = position of UV photocleavable moiety

A^{OMe} and G^{OMe} are 2'-O-methyladenosine and 2'-O-methylguanosine, respectively

Desalting Primer Extension Reactions

Biotin-Strepavdin

96 or 384 well format

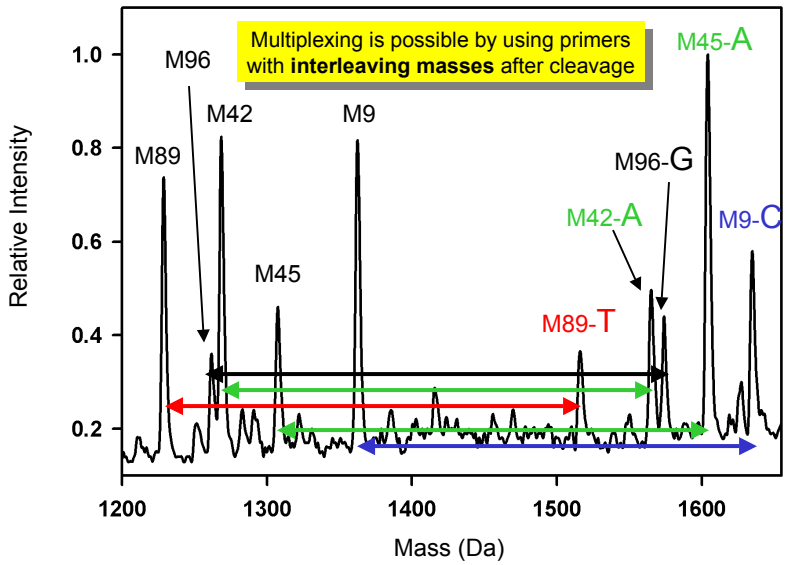
5’ Biotin labeled extension primers are required

Primers are bound to a 384 well plate with strepavidin coated wells

Salt is washed off as DNA remains anchored to plate surface

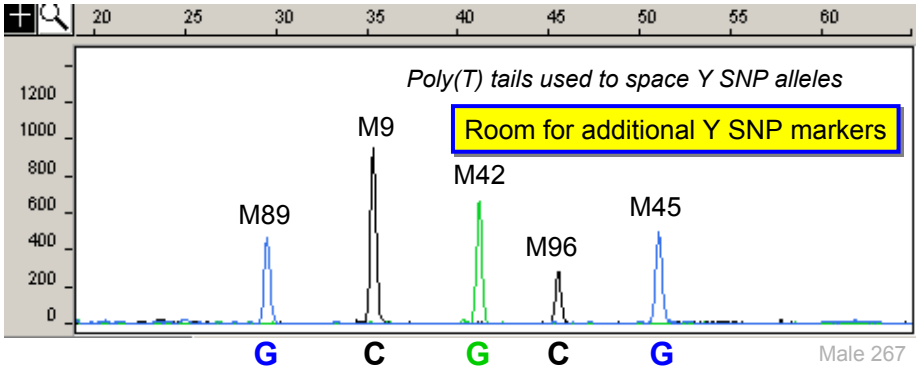
Salt free DNA is cleaved by UV light in solution on the plate and the fragment is spotted onto the MALDI target

Y SNP 5plex using UV Photocleavable Extension Primers



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

Y SNP Results with SNaPshot Assay



TTTTTTTTTCAGGCAAAGTGAGAGAT	17/26
TTTTTTTTTTTTTCGGCCTAAGATGGTTGAAT	19/32
TTTTTTTTTTTTTTTTTTTTGCTCTCTTTTTCATTATGTAGT	22/38
TTTTTTTTTTTTTTTTTTTTTTTTTGGAAAACAGGTCTCTCATAATA	22/44
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGCAGTGAAAAATTATAGATA	22/50

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

Y SNP Haplotypes for 16 Test Samples

Sample ID	M9 (C/G)	M42 (A/T)	M45 (G/A)	M89 (C/T)	M96 (G/C)
Male 1	G	T	A	T	C
Male 2	G	T	A	T	C
Male 3	G	T	A	T	C
Male 4	G	T	A	T	C
Male 5	G	T	A	T	C
Male 6	G	T	A	T	C
Male 7	G	T	A	T	C
Male 8	C	T	G	C	G
Male 9	C				
Male 10	C				
Male 11	C				
Male 12	C	T	G	C	G
Male 13	C	T	G	C	G
Male 14	G	T	G	T	C
Male 15	C	T	G	T	C
Female	-	-	-	-	-

Typing Results Obtained from SNaPshot and MS techniques Agree

Advantages/Disadvantages of the Basic Primer Extension Assay (TOF MS)

- Uses readily available reagents
- Synthetic primers (no modifications)
- ddNTPs
- Automation of assay

- Limited multiplexing capabilities
- As mass range increases, resolution decreases
- Heterozygous samples may be difficult to resolve
- Salt adducts may interfere with data interpretation
  - products must be purified
- 3HPA matrix
  - non-homogeneous crystal formation

Multiplexing

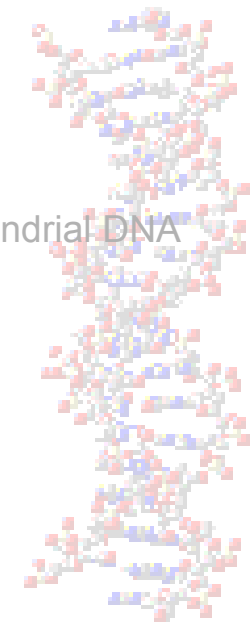
Assays and Instrumentation

Y Chromosome and Mitochondrial DNA

Primer design strategy

Results

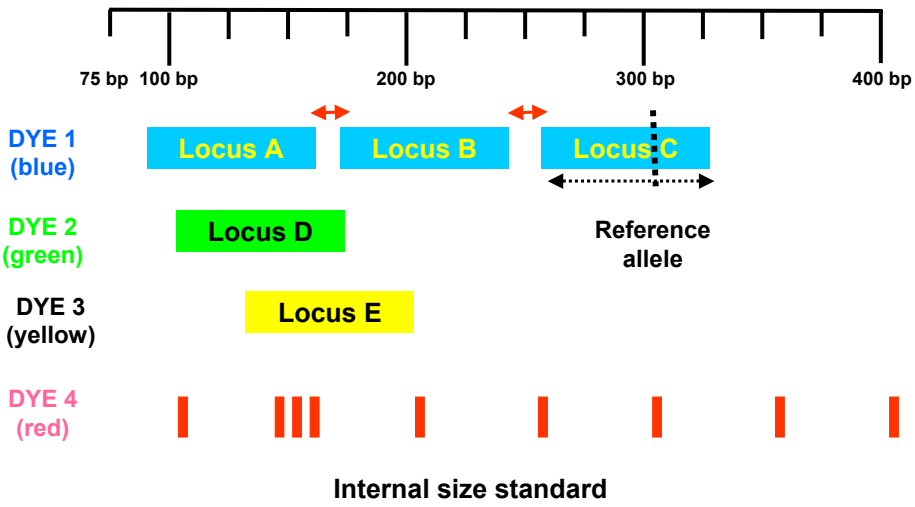
- mtSNP 10 plex
- Y SNP 5 plex
- Y STR multiplexes
- Other

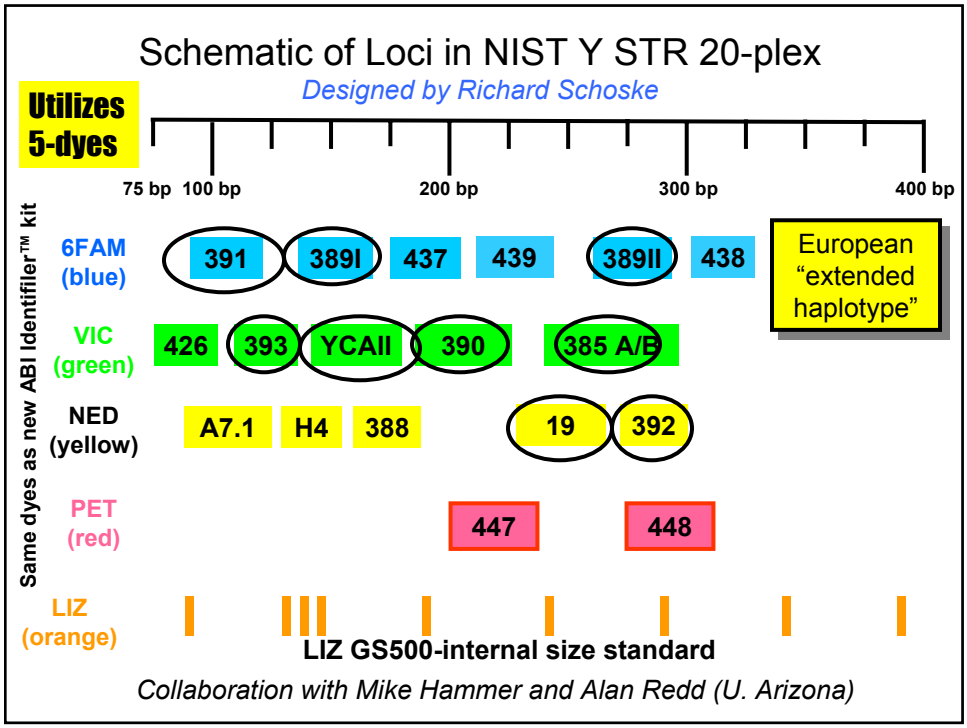


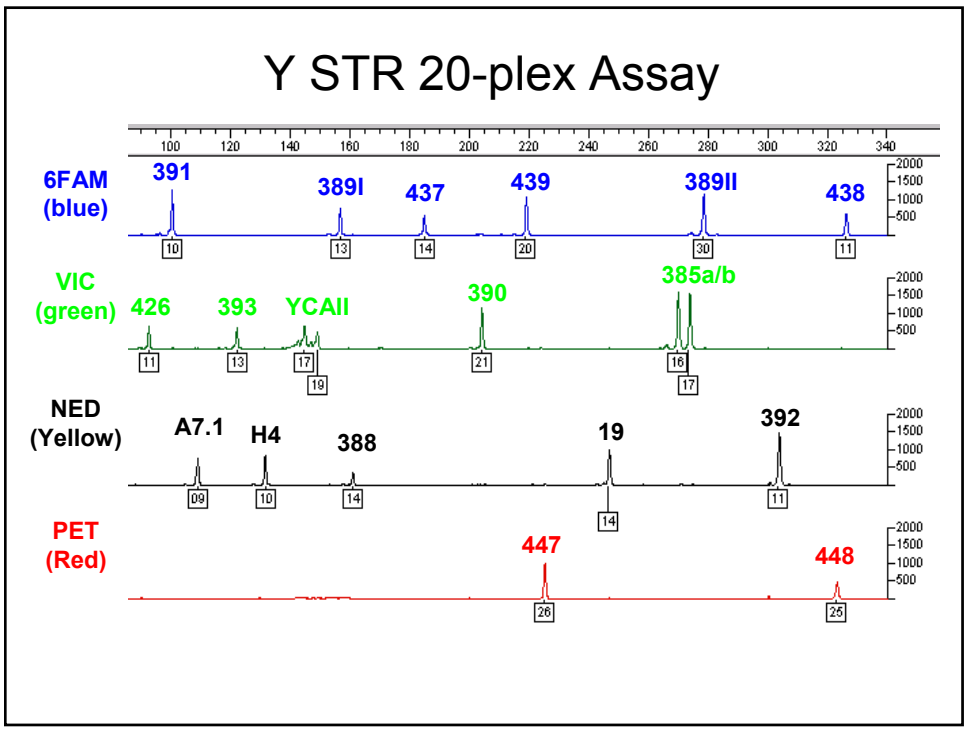
Chromosomal Positions of Y STRs

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

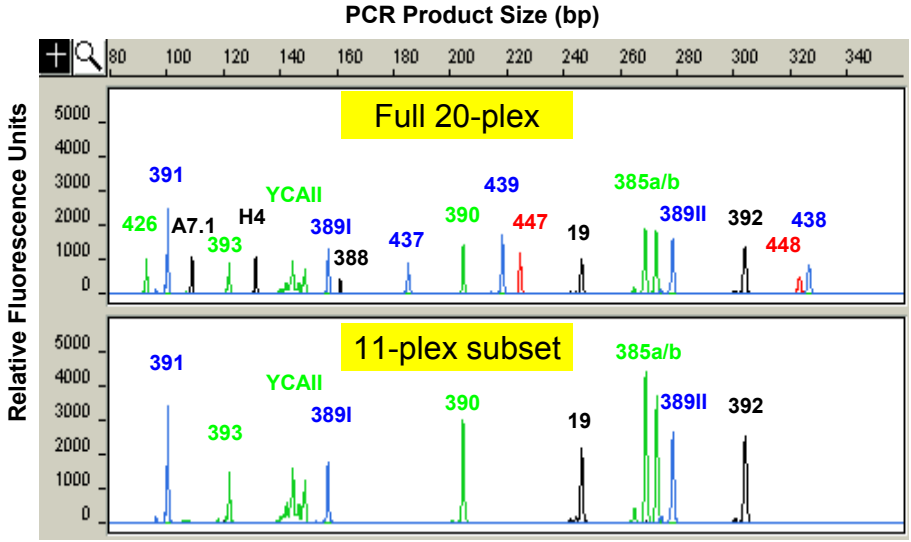
Multiplex Design Schematic




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)

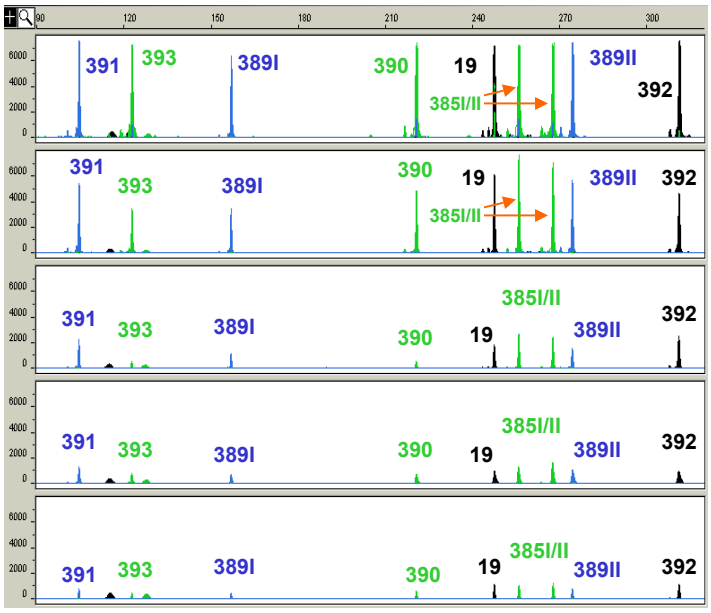

Y STR 20-plex Assay
6FAM (blue)
391
389I
437
439
389II
438
VIC (green)
426
393
YCAII
390
385a/b
NED (Yellow)
A7.1
H4
388
19
392
PET (Red)
447
448

Y STR 20-plex & Y STR 11-plex



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

Y STR 9PLEX – SENSITIVITY STUDY



DNA Amounts

6.7 ng

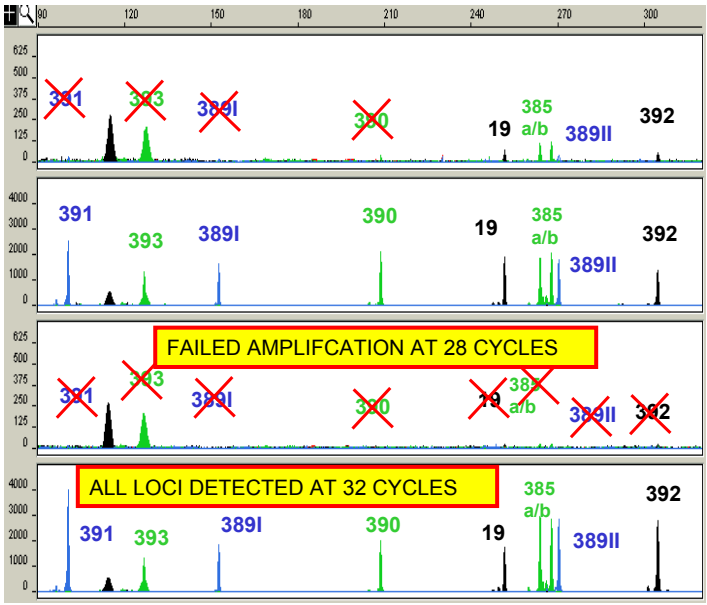
3.4 ng

1.3 ng

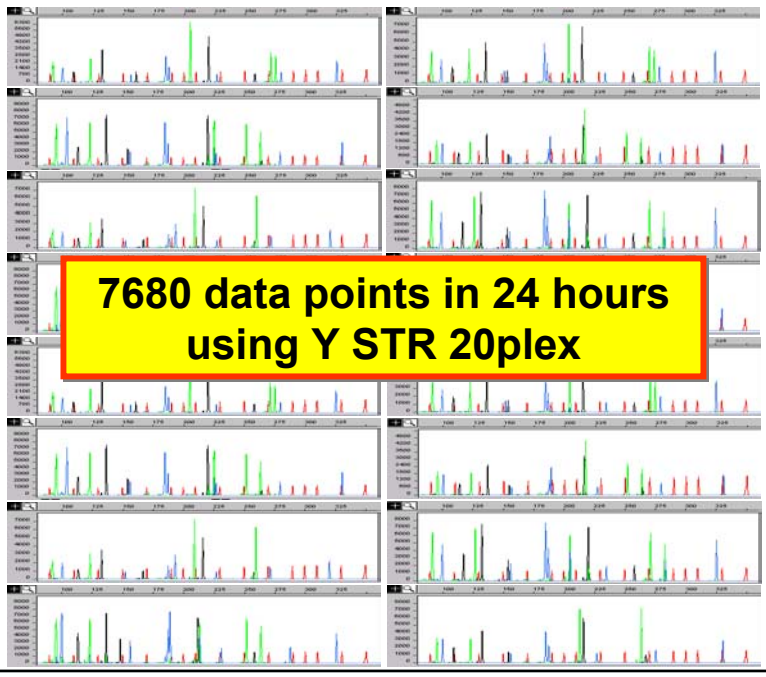
0.7 ng

330 pg

Y STR 9-plex – Sensitivity Study



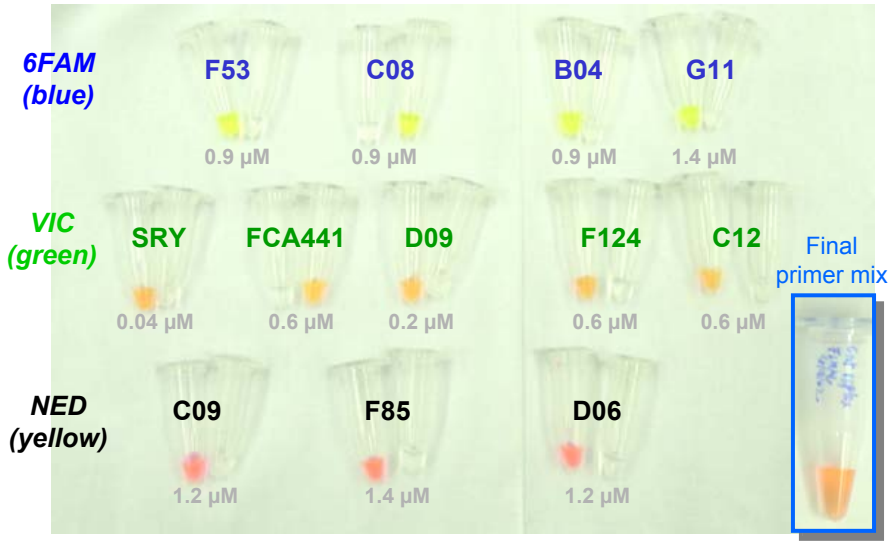
High-throughput Y STR Typing on the ABI 3100 (16-capillary array)



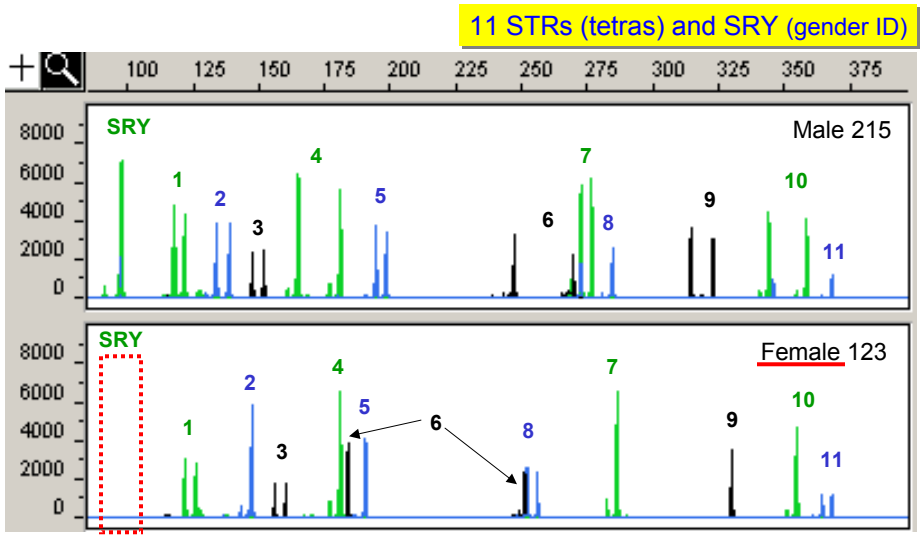
- Multiplexing
- Assays and Instrumentation
- Y Chromosome and Mitochondrial DNA
- Primer design strategy
- Results
  - mtSNP 10 plex
  - Y SNP 5 plex
  - Y STR multiplexes
  - Other

Primers Used in Cat STR 12plex

(MeowPlex)

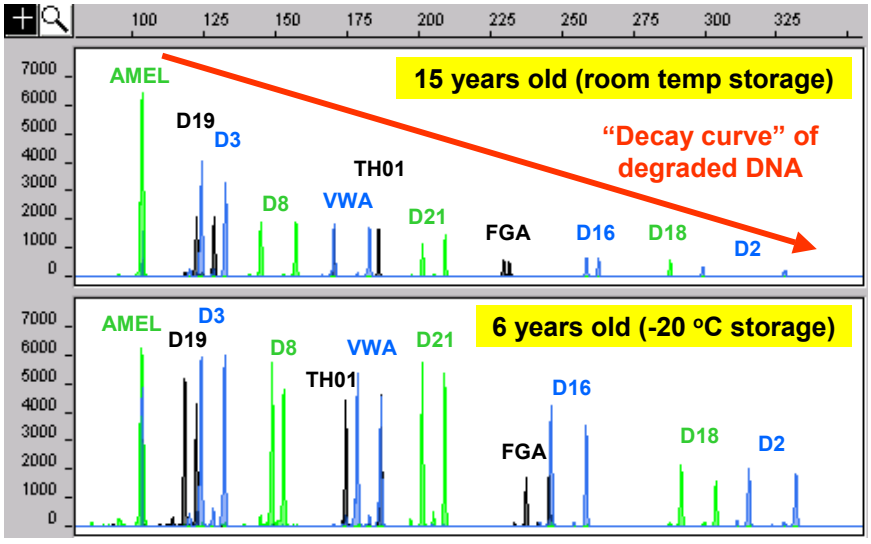


Cat STR Results (The "MeowPlex"—12plex)



Collaboration with M. Raymond and V. David (NCI-Frederick)

Degraded DNA Results



Results with SGM Plus STR kit (Applied Biosystems)

STR Size Reduction

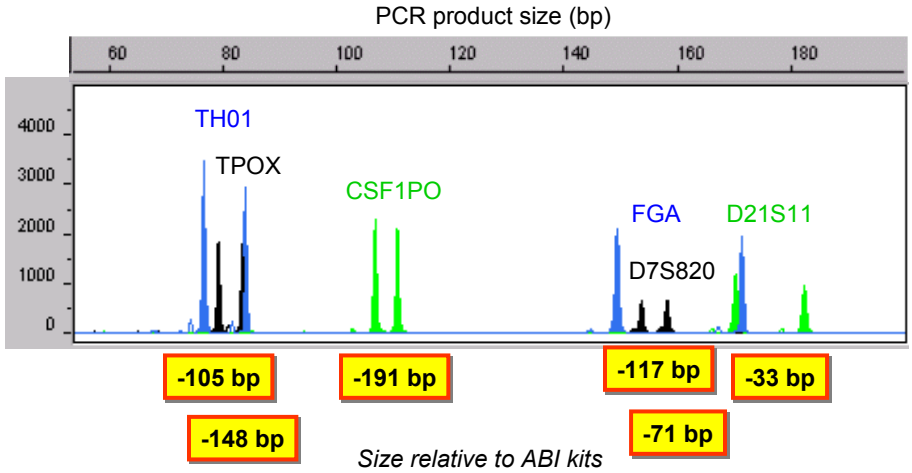
Through Moving Primer Positions Closer to Repeat

Primer positions define PCR product size
Repeat information is independent of amplicon size

Advantages of Approach:
Size reduction enhances success rate with degraded DNA
Retains same marker information (database compatibility)
Uses highly polymorphic STR loci (high discriminatory power)

miniSTR Systems:

Size Reduction to Aid with Typing Degraded DNA Samples



Future Directions

- Collaborations
- Continue comparisons with various SNP chemistries and technologies on the same model Y SNP and mtSNP markers
- Optimize automation of assays/data analysis to permit high throughput typing
- Type population samples with forensic markers
- Further understanding of multiplex assay design
- Informatics

Acknowledgments

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Rich Schoske

Margaret Kline

Gordon Spangler

Collaborators

Thomas Parsons and Mike Coble (AFDIL)
Mike Hammer and Alan Redd (U. of Arizona)
Jay Stoerker and Markus Kostrzewa (Bruker)