Development of Multiplexed Assays for Typing SNP and STR Forensic Markers

NCI - Advanced Technology Center
July 10th 2002
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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

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

- **SNaPshot/PCR**
- **Luminex Beads**
- **TaqMan**
- **Primer Extension**
- **Time-of-Flight Mass Spectrometer**
- **Multi-Color Capillary Electrophoresis (ABI 310 or 3100)**
- **Luminex 100 Flow Cytometer**
- **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

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- **Amel**
- **D3S1358**
- **D6S618**
- **D8S1179**
- **vWA**
- **D21S11**
- **FGA**
- **D13S317**
- **Profiler Plus™**
- **D7S820**
- **D18S61**
Same DNA Sample Run with Each of the ABI STR Kits

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Commercial STR 16plex Kits

PowerPlex® 16 (Promega)

AmpFISTR® Identifiler™ (Applied Biosystems)
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™**

- SNP Primer is extended by one base unit
- "tail" used to vary electrophoretic mobility
- Oligonucleotide primer 18-28 bases
- 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 |

**PCR Amplified DNA Template**

**Protocol with SNaPshot™ “Kit”**

1. **Amplification**
   - Genomic DNA sample
   - (Multiplex) PCR
   - ExoSAP Digestion

2. ** Primer Extension**
   - Add SNP primer(s) and SNaPshot mix
   - SNP Extension (cycle sequencing)
   - SAP treatment

3. **Analysis**
   - Sample prep for 310/3100
   - Add GS120 LIZ size standard
   - Run on ABI 310/3100
   - Use E5 filter (5-dye) and POP4 standard conditions
   - Data Analysis (GeneScan)
   - Type sample (Genotyper 3.7)
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

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

PCR Amplified DNA Template

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)

DNA Reaction Products (Size separated and drifting to the detector)

Pulsed Laser Beam

High-Density Sample Array

X-Y sample control

Drift Region Electric-Field Free

Ion Extractor

Acceleration Region (20 kV)

Detector

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
Completed spotting of a Bruker 384 MALDI plate.

Matrix and DNA sample are spotted onto the MALDI plate and allowed to air dry.

Treated Surfaces Improve Sample Preparation

Hydrophobic coating

Hydrophilic center

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
- 3 HPA Matrix Dried on Stainless Steel Surface

Concentrated spot (greater sensitivity)
Uniform spot (impacts automation)
Specific Location

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
MALDI-TOF MS data can be collected in 5-10 seconds

MALDI-TOF MS data can be collected in 5-10 seconds

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
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

- Control region (non-coding)
  - ~1100 bp: closely spaced polymorphisms

- Coding region
  - ~15,000 bp: widely spaced polymorphisms

Disease diagnostic sites


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

Pseudoautosomal region

heterochromatin

Pseudoautosomal region

Dr. Peter M. Vallone

**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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**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 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

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<tr>
<th>OLIPO</th>
<th>start</th>
<th>1en</th>
<th>ca</th>
<th>gc3</th>
<th>any</th>
<th>3' seq</th>
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<td>50.00</td>
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<td>2.00</td>
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<tr>
<td>RIGHT PRIMER</td>
<td>174</td>
<td>22</td>
<td>50.31</td>
<td>50.00</td>
<td>8.00</td>
<td>3.00</td>
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</table>

**SEQUENCE SIZE:** 205

**INCLUDED REGION SIZE:** 205

**PRODUCT SIZE:** 149, **PAIR ANY COMPL:** 5.00, **PAIR 3' COMPL:** 2.00

**EXCLUDED REGIONS**

- **SEQUENCE:** CTTGGCAAGGCGACAGATCACCTACGAGTAGAAACCGAAAATCTTCTCAAGGATCTCATA
- **SEQUENCE:** TCAAGCAGGCTTACGACCTGGATGTTGGATGACGATGGATGCTCA
- **SEQUENCE:** TTGAAGCATTACCTGCTGATCAAGGGTGCATGCTAAGGAG
- **SEQUENCE:** TCGAGCTGCTCATATCAGTCA

Dr. Peter M. Vallone
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=GCAAGACGCTATAAATACTTCAGGACCTGAAAATACAGAACTG
CAAAGAAAACGCTTGTAAGATGATGTTGAAATNCTTCCTTTATTTTTTTTTTAATTAG
ACATGTTCAAGATGTTAGATCTACTTATTTATTTTTTTTTAAATTTTAG
CGCATTACTCAGTATGATTTCAATACACTCAAAAAATCTTTTTGGAATAT
GGTAATTATTTTTCTCTAATCTGTTTCACGAGCTTCAAAAATGAGGAAAAAG
GATTCAGTTTACATTTCAGCAGAAAAATGCTCTTTTTTTTTTTTTATCGGATTTATTTTT
ACTTAACATTACATTTACGCTTGAAGAAAGTTAGTTTT
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=AAAGCGAGAGATTCAATCCAGGATGACAGAATGCGTTCACCTTTAAAGGGATTAAATAGTATAATACAGTCTGTATTATTAGATCACCC
AGAGACACACAAAACAAGAACCGTGAATTGAATTAGTGGTATACTAATAGGTGGTTTTACCTGAAATATTTACACATCAATCCTACTGAATTCTTACAAC
```

Example input format for Primer3
Can help utilize all the tools that Primer3 provides

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

Screening for potential intramolecular hairpin and intermolecular primer-dimer formation

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

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


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

Loss of Fluorescent dye

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

Coding Region mtSNP 10-plex Assay

Multiplex primer extension with different length SNP primers and fluorescent ddNTPs

3010
4580
4745
5004
7028
7202
10211
12858
16472
16519

mtDNA control region

mtDNA coding region

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

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<thead>
<tr>
<th>Sequence</th>
<th>ID Position</th>
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<tr>
<td>TCAGAAGTGAAAGGGGGC</td>
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<td>TTTTTTTTGTGGATCGGACATCCC</td>
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</tr>
<tr>
<td>TTTTTTTTACTAAGAAATTTATGGA</td>
<td>20/30</td>
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<tr>
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<td>TTTTTTTTTTTTTTTTTTGTGGGCTATTTAGGCTTTATG</td>
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<td>TTTTTTTTTTTTTTTTTTGGCCATTCAAGCAATCCTATA</td>
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<tr>
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<td>TTTTTTTTTTTTTTTTTTTTTTTGGACACGTACTACGTTGTC</td>
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</table>

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

User Interface of SNP Primer Design Program

Desired Tm Range for SNP Primers
Minimum 57 °C  Maximum 64 °C

Desired Size Range for SNP Primers
Minimum 18 bp  Maximum 28 bp

Parameters to Return
3 - 3 umM  1.0 - 0.085 M

Set Parameters
**Program Output**

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<tr>
<th>Label</th>
<th>Length</th>
<th>Sequence</th>
<th>Position</th>
<th>Tm</th>
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<td>Reverse Primers</td>
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**Hairpin Dimer Template**

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**mtSNP 10-plex run on ABI 3100**

(SNapShot™ assay)

**Multiplex PCR and Multiplex SNP Detection**

mtDNA coding region SNPs

*Sizing performed by comparison to GS120 LIZ internal size standard (not shown)*
### Data for 10 mt Samples

In this set of 10 samples each possible allele is represented.

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### Sizing of Fragments in mtSNP 10plex

*Actual versus observed*

<table>
<thead>
<tr>
<th>Actual length (bases)</th>
<th>allele 1</th>
<th>allele 2</th>
<th>Δ allele1</th>
<th>Δ allele 2</th>
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<td>-4.3</td>
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<td>-1.7</td>
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</table>

Sizing differences vary with sequence, length and fluorescent dye attachment.
Comparison of Results between Techniques

MALDI-TOF MS

Mass difference between primer and extension product

Extension product 6116 Da

Primer 5803 Da

Δm = 313 = ddG

SNaPshot (ABI 310)

Fluorescent dye color of peak

Complete agreement in results

16 Test Samples with mtSNP 10-plex on ABI 3100

ddG

Complete agreement in results

SNaPshot

MALDI-TOF MS
Control Region mtSNPs

Two Adjacent Mitochondrial SNPs
16223 (C/T) and 16224 (A/G)
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

Rapid CE Separation and Quantitation of Multiplex PCR Products

*Intercalating dye used to fluorescently label amplicons*

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

**M9**

\[ \Delta \text{mass} = 313 \text{ Da} \]

\[ \text{ddG} \]

**M42**

\[ \Delta \text{mass} = 297 \text{ Da} \]

\[ \text{ddA} \]

**M45**

\[ \Delta \text{mass} = 297 \text{ Da} \]

\[ \text{ddA} \]

**M89**

\[ \Delta \text{mass} = 297 \text{ Da} \]

Primer almost completely converted to extension product

\[ \text{ddA} \]

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

Multiplexing is possible by using primers with non-overlapping masses

Vallone et al. Poster presented at ASMS June 2002
SRM 2395 Candidate Sample Testing

Y SNP Results with Primer Extension and MALDI-TOF MS

<table>
<thead>
<tr>
<th></th>
<th>M9(C/G)</th>
<th>M42(A/T)</th>
<th>M45(G/A)</th>
<th>M89(C/T)</th>
<th>M96(G/C)</th>
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</thead>
<tbody>
<tr>
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<td>T</td>
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</tr>
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<td>T</td>
<td>C</td>
</tr>
<tr>
<td>F</td>
<td>--</td>
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<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

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 ($\lambda = 366$ 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’ CCAGCTCTCTTTTTTATTATLTAGT 3’ mass = 7492.9
5’ TAGT 3’ mass = 1268.8
Structure of Cleavable Moiety

DMTO\textbf{O-}P_{\text{N(i-Pr)}_2}\textbf{OCH}_2\textbf{CH}_2\textbf{CN}


Collaboration with Jay Stoerker and Markus Kostrzewa at Bruker Daltonics

UV Cleavable SNP Primers

<table>
<thead>
<tr>
<th>Locus</th>
<th>Extension Primer</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>M9</td>
<td>ACATGCTAAATTAAGAAAAATAA_{\text{OMe}}G_{\text{OMe}}G</td>
<td>1362.9</td>
</tr>
<tr>
<td>M42</td>
<td>CCAGCTCTCTTTTTCATTATGATG</td>
<td>1268.8</td>
</tr>
<tr>
<td>M45</td>
<td>GCAGTCGAAAAATTATA_{\text{OMe}}ATA</td>
<td>1307.8</td>
</tr>
<tr>
<td>M89</td>
<td>CTCTTTCTAGGTATGTACAAA_{\text{OMe}}ATA</td>
<td>1228.8</td>
</tr>
<tr>
<td>M96</td>
<td>AACTTGGAACAGGTCTCTCATATA</td>
<td>1261.8</td>
</tr>
</tbody>
</table>

Underlined base = position of UV photocleavable moiety

_{\text{OMe}}A and {\text{OMe}}G are 2'-O-methyladenosine and 2'-O-methylguanosine, respectively
Desalting Primer Extension Reactions

Biotin-Strepavidin

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

Multiplexing is possible by using primers with interleaving masses after cleavage

Vallone et al. Poster presented at ASMS June 2002
### Y SNP Results with SNaPshot Assay

![Y SNP Results with SNaPshot Assay](image)

- **Poly(T) tails used to space Y SNP alleles**
- **Room for additional Y SNP markers**
- **Data obtained by Gordon Spangler (graduate student at American University)**

### Y SNP Haplotypes for 16 Test Samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>M9 (C/G)</th>
<th>M42 (A/T)</th>
<th>M45 (G/A)</th>
<th>M89 (C/T)</th>
<th>M96 (G/C)</th>
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</thead>
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<td>T</td>
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<tr>
<td>Female</td>
<td>-</td>
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</table>

**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

<table>
<thead>
<tr>
<th>Y STR Marker</th>
<th>Sequence Position</th>
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<td>DYS393</td>
<td>3,038,729</td>
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<td>DYS19</td>
<td>9,437,335</td>
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<td>13,413,353</td>
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<td>DYS439</td>
<td>13,825,798</td>
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<td>DYS389 I/II</td>
<td>13,922,787</td>
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<tr>
<td>DYS388</td>
<td>14,057,445</td>
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<tr>
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<td>DYS426</td>
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<td>23,511,495</td>
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</tbody>
</table>

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

Multiplex Design Schematic

Internal size standard
Utilizes 5-dyes

Schematic of Loci in NIST Y STR 20-plex

Designed by Richard Schoske

6FAM (blue)

VIC (green)

NED (yellow)

PET (red)

LIZ (orange)

Same dyes as new ABI Identifier™ kit

LIZ GS500-internal size standard

Collaboration with Mike Hammer and Alan Redd (U. Arizona)

Y STR 20-plex Assay

Europe

European "extended haplotype"

Designed by Richard Schoske

Collaboration with Mike Hammer and Alan Redd (U. Arizona)
Y STR 20-plex & Y STR 11-plex

PCR Product Size (bp)

---

Full 20-plex

11-plex subset


---

Y STR 9PLEX – SENSITIVITY STUDY

DNA Amounts

- 6.7 ng
- 3.4 ng
- 1.3 ng
- 0.7 ng
- 330 pg

DNA Amounts

- 391
- 393
- 389I
- 390
- 19
- 385I/II
- 389II
- 392

- 391
- 393
- 389I
- 390
- 19
- 385I/II
- 389II
- 392

- 391
- 393
- 389I
- 390
- 19
- 385I/II
- 389II
- 392

- 391
- 393
- 389I
- 390
- 19
- 385I/II
- 389II
- 392
Y STR 9-plex – Sensitivity Study

DNA Amounts

- 75 pg (28 cycles)
- 75 pg (32 cycles)
- 60 pg (28 cycles)
- 60 pg (32 cycles)

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

- 7680 data points in 24 hours using Y STR 20plex
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)

6FAM (blue)

VIC (green)

NED (yellow)

Final primer mix
Cat STR Results (The “MeowPlex”—12plex)

11 STRs (tetras) and SRY (gender ID)

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

Degraded DNA Results

15 years old (room temp storage)

“Decay curve” of degraded DNA

6 years old (-20 °C storage)

Results with SGM Plus STR kit (Applied Biosystems)
STR Size Reduction
Through Moving Primer Positions Closer to Repeat

Forward flanking region  Reverse flanking region

STR 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

PCR product size (bp)

TH01  TPOX  CSF1PO  FGA  D21S11  D7S820

-105 bp  -191 bp  -117 bp  -105 bp  -33 bp  -148 bp

Size relative to ABI kits
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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Collaborators
Thomas Parsons and Mike Coble (AFDIL)
Mike Hammer and Alan Redd (U. of Arizona)
Jay Stoerker and Markus Kostrzewa (Bruker)

John Butler
petev@nist.gov

Rich Schoske
Margaret Kline
Gordon Spangler