The Future of Forensic DNA Typing: Y-STRs, SNPs, and Real-time PCR

John M. Butler
CODIS State Administrators
FBI Academy
May 25, 2004

Presentation Outline
- Some general interest information
  - OmniPop 150.4.2 (166 Profiler Plus, 97 full CODIS loci pops)
  - Forensic DNA Typing, 2nd Edition (to be available in Jan 2005)
- Y-STRs
  - Advantages of Y-chromosome
  - Available Y-STR kits
  - Comparison to Y-SNPs
- Autosomal SNPs
  - Forensic SNP web page (http://www.cstl.nist.gov/biotech/strbase/SNP.htm)
  - Challenges ahead for SNPs
- miniSTRs
  - CODIS loci made small
  - New loci under development
  - Performance with degraded DNA samples
- DNA Quantitation
  - Real-time PCR methods
  - Performance across 80 laboratories (NIST interlab study QS04)

OmniPop 150.4.2
- Published allele frequencies
  - from 97 populations containing all 13 CODIS loci
  - From 166 populations with 9 loci (Profiler Plus)
- From 64 publications
- Available from Brian Burritt (San Diego Police Dept)
  - (619) 531-2215
  - bburritt@pd.sandiego.gov

Forensic DNA Typing, 2nd Edition
- Resources for “Challenging Samples” (degraded DNA or mixtures)
- Y-Chromosome Information, Assays, and Standards
- DNA Quantitation (Interlab study, Real-time PCR comparisons)
- Tools to Aid State and Local Laboratories (e.g., STRBase)
- Aid to or Completion of Other NIJ Projects (e.g., LSBs)

CODIS State Administrators meeting
(Quantico, VA)
Resources for “Challenging Samples”
(degraded DNA or mixtures)

- minISTRs
  - CODIS loci (JFS 2003, 48, 1054-1064) – “BodePlexes”; WTC IDs; McCord collaboration
  - New loci (Coble, AAFS Feb 2004) – non-CODIS loci; unlinked; optimal for small amplicons and size ranges; <120 bp

- Autosomal SNPs
  - Validated Orchid 70 SNP markers (60-80 bp); population typing

- Mitochondrial DNA SNP Assays
  - Improve ease of use – Roche LINEAR ARRAY testing
  - Improve power of discrimination – AFDIL coding region SNPs

- Y-STRs
  - Improve evaluation of some extreme female-male mixtures?

Tools to Aid State and Local Laboratories

- STRBase – standard information source
- Variant Alleles – cataloging variants and tri-allelic patterns
- NIST U.S. Population Samples and Database
- Quality Assurance Tool – resolution monitor to track analytical performance over time
- Validation Standardization Information
- Training Materials
  - Downloadable PowerPoint files from STRBase
  - Current Protocols in Human Genetics, Electrophoresis review article on STR analysis with ABI 310 and ABI 3100

Variant Alleles Cataloged in STRBase

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

Off-Ladder Alleles

Tri-Allelic Patterns

Currently 224
at 13/13 CODIS loci

Currently 56
at 13/13 CODIS loci

Variant Alleles Cataloged in STRBase

- Large D18 Allele Characterized at NIST
  - Sample from Christine Moraczewski, NE State Patrol Crime Lab
  - PowerPlex 16
  - D18
  - FGA
  - CSF
  - Penta E

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

We regularly update...

- Reference List
- Variant Alleles
- Addresses for Scientists
- Links to Other Web Sites
- Y-STR Information

We will continue to add downloadable PowerPoint files that can be used for training purposes

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

CODIS State Administrators meeting
(Quantico, VA)
**Y-Chromosome STRs**

**Male-specific amplification**
(can handle extreme mixtures of male and female DNA)

**Enables tracing paternal lineages**
(permits extension of possible reference samples)

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**SWGDAM Sub-Committee on the Y Chromosome**

- **Formed in July 2002**
- **Members**
  - Jack Ballantyne (UCF) – chair
  - Mecki Prinz (NYC) – co-chair
  - Bruce Budowle (FBI)
  - John Butler (NIST)
  - Ann Gross (MN)
  - John Hartmann (Orange Co.)
  - Laura Kienker (FBI Academy)
  - Carl Ladd (CT)
  - Dennis Lee (AFDIL)
  - Phil Kinsey (OR)
  - Barb Koons (FBI Academy)
  - Tim Kupferschmid (ME)
  - Gary Sims (CA DOJ)
  - Jack Ballantyne’s lab and John Butler’s lab to examine additional Y STR and Y SNP markers in the same sample set

- **U.S. CORE Y-STR LOCI**
  - Selected in January 2003
  - 60 sample set selected for screening markers and initial testing
  - Testing of Y-PLEX 6 and Y-PLEX 5 kits in all labs
    - All results completed agreed with NIST results sent to participating labs in Dec 2002

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**European and U.S. Core Y-STR Loci**

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>Allele Range (repeat count)</th>
<th>Repeat Motif</th>
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<tr>
<td>DYS19</td>
<td>10-19</td>
<td>TATA</td>
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<tr>
<td>DYS385 a/b</td>
<td>7-28</td>
<td>GAAA</td>
</tr>
<tr>
<td>DYS389 I</td>
<td>1, 9-17</td>
<td>(TCTG) (TCTA)</td>
</tr>
<tr>
<td>DYS390</td>
<td>17-28</td>
<td>(TCTG) (TCTG)</td>
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<tr>
<td>DYS391</td>
<td>6-14</td>
<td>TCTA</td>
</tr>
<tr>
<td>DYS392</td>
<td>6-18</td>
<td>TAT</td>
</tr>
<tr>
<td>DYS393</td>
<td>8-17</td>
<td>AGAT</td>
</tr>
<tr>
<td>YCAII a/b</td>
<td>11-25</td>
<td>CA</td>
</tr>
<tr>
<td>DYS438</td>
<td>6-14</td>
<td>TTTTC</td>
</tr>
<tr>
<td>DYS439</td>
<td>8-15</td>
<td>AGAT</td>
</tr>
</tbody>
</table>

**U.S. Haplotype**

**Extended Haplotype (Europe)**

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**NIST QA/QC Software**

- Tool being developed by Dave Duewer for STR Process Control
- Will track allelic ladders and positive controls
- NCBI Program
- Peak Height, Area, Size
- Date vs Sensitivity, Resolution, Precision
- Date vs Signal/Noise

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**PowerPlex Y Performance in Our Hands**

- 2 ng male
- 2 ng male: 15 ng female
- 500 pg male: 408 ng female
- 800X female DNA
- 1 ng male: 816 ng female
**New Y-STR paper**

**June 2004 issue of American Journal of Human Genetics**

A Comprehensive Survey of Human Y-Chromosomal Microsatellites

- Searched for all regions with ≥8 consecutive repeats and 2,3,4,5,or 6 bp repeat units
- Discovered 139 new polymorphic Y-STR loci (166 male-specific)
- Only studied so far in 8 different samples

**Commercial Y-STR Kits Available**

- ReliaGene Technologies (New Orleans, LA)
  - Y-PLEX™ 6: DYS19, DYS389II, DYS390, DYS391, DYS393, DYS385 a/b
  - Y-PLEX™ 5: DYS389II, DYS392, DYS438, DYS439
  - Y-PLEX™ 12: DYS19, DYS385 a/b, DYS389II, DYS390, DYS391, DYS392, DYS383, DYS438, DYS439, amelogenin
- Promega Corporation (Madison, WI)
  - PowerPlex® Y: DYS19, DYS389II, DYS392, DYS393
- Serac (Germany)
  - genRES® DYSplex-1: DYS390, DYS391, DYS385 a/b, amelogenin
  - genRES® DYSplex-2: DYS19, DYS390, DYS392
- GKT Inc. (South Korea); silver-stain kits
  - GeneKin® Y-STR Systems I: DYS388, DYS19, DYS392
  - GeneKin® Y-STR Systems II: DYS393, DYS390, DYS391, DYS391X
  - GeneKin® Y-STR Systems III: DXYS156X, DXYS156Y, DYS389I/II
  - GeneKin® Y-STR Systems IV: DXYS156X, DXYS156Y, DYS385 a/b
- Applied Biosystems (Y-filer: 17plex with 5-dye chemistry)

**Commercial Y-STR Kits Available**

**Y-Chromosome Standard NIST SRM 2395**

**Human Y-Chromosome DNA Profiling Standard**
- 5 male samples + 1 female sample (neg. control)
- 100 ng of each (50 µL at ~2 ng/µL)
- 22 Y STR markers sequenced
- 9 additional Y STR markers typed
- 42 Y SNPs typed with Marligen kit

Certified for all loci in commercial Y-STR kits:
- Y-PLEX 6
- Y-PLEX 5
- Y-PLEX 12
- PowerPlex Y
- FMBIO III and ABI 3100 Results with Y-PLEX™ 12

**SWGDAM recommended loci**
- DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439

**Helps meet DAB Standard 9.5 (and ISO 17025)**…traceability to a national standard

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**CODIS State Administrators meeting**

(Quantico, VA)
The future of forensic DNA typing may refer to advancements in techniques, technologies, and methodologies that improve the accuracy, efficiency, and reliability of DNA analysis. This could include new types of STRs or SNPs, improved sample collection, and novel approaches to data interpretation. The specific focus of this document appears to be on Y-STRs, which are particularly useful in forensic investigations involving paternity testing, lineage tracing, and other genetic studies.

The document mentions the collection and analysis of U.S. population data on 22 Y-STRs. This data is available as a PDF file at a specified URL. The data includes information on the distribution of Y-STRs across different ethnic groups in the U.S., such as 286 Caucasians, 252 African Americans, and 128 Hispanics. The data is derived from whole blood samples obtained from the Interstate Blood Bank and includes samples supplied to Ohio University for miniSTR typing and the Armed Forces DNA Identification Laboratory (AFDIL) for whole mtGenome sequencing.

The document also discusses the resolution of commonly used Y-STRs and the implementation of additional markers to enhance typing accuracy. It references a study by Schoske et al. (2004) which details high-throughput Y-STR typing of U.S. populations. This study may be found in the journal Forensic Science International, volume 139, pages 107-121.
Single Nucleotide Polymorphisms (SNPs)

Autosomal SNPs
Y-SNPs
mtDNA SNPs (control region & coding region)

Forensic Utility of SNPs

The short PCR amplicons required for typing SNPs may result in success with degraded samples and possibly higher sensitivity – but this has not been demonstrated yet in real-world samples...

For serious forensic usage, parallel high-throughput methods and multiplex amplification will be required for typing low amounts of DNA

Forensic Utility of SNPs

Short tandem repeat (STR)
CTAGTCTAGA(GATA)nGACTACTTA
n = 5 to 15 = 66 possible allelic combinations

Single Nucleotide Polymorphism (SNP)
CTAGTCTAGA(G/A)GACTACTTA
3 possible allelic combinations
For human ID purposes more SNPs would be needed than STRs
Multiplexing is essential

SNP Typing at NIST

- STRBase is the official ISFG repository of forensic SNP information
- We are cataloging SNP information with the goal to standardize assays and speed validation of markers
- We will continue to explore various SNP typing technologies to provide information to the forensic DNA typing community – primary focus on SNaPshot
- We are beginning to evaluate SNP performance directly against miniSTRs for analysis of degraded DNA - collaborative study planned with EDNAP

Autosomal SNPs

Orchid Cellmark provided their panel of 70 SNPs (C/T) located throughout the human genome

We validated these markers with SNaPshot assays for 8 CEPH samples in July 2001 for the WTC investigation as part of KADAP (Kinship and Data Analysis Panel)

We are evaluating these markers in U.S. populations (N=189 so far)
Marker info now on STRBase forensic SNP site: http://www.cstl.nist.gov/biotech/strbase/SNP.htm
Y-SNP Typing Conclusions

- Different technologies yield the same Y-SNP type
  - Full concordance was observed between hybridization and primer extension technologies on 18 different Y-SNPs (>3,800 allele calls)
- Y-SNPs will have limited value for individualizing a sample
  - 18 different types observed in 229 individuals
- Y-SNPs appear to have limited value for ethnic differentiation in U.S. populations
  - One exception: M2 only in African Americans; not in Caucasians


Typing mtSNPs

Coding Region SNPs
Collaboration with AFDIL (Tom Parsons and Mike Coble)
Develop an 11-plex assay for typing SNPs outside the control region
The 11 SNP sites are thought to help resolve Caucasians with the most common mitotype (~7%)

Control Region SNPs
Typing population samples with Roche linear arrays
(Cassandra Calloway)
Probe 10 regions (18 SNPs) within HVI and HVII
Evaluate assay performance and ability to resolve U.S. population samples

miniSTRs
(Reduced Size Amplicons)
CODIS loci
New miniSTR loci

CODIS State Administrators meeting
(Quantico, VA)
Future of Forensic DNA Typing

May 25, 2004

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**Reduction in PCR Product Size**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Size Difference (relative to ABI kits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH01</td>
<td>-105 bp</td>
</tr>
<tr>
<td>FGA</td>
<td>-71 bp</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>-191 bp</td>
</tr>
<tr>
<td>D21S11</td>
<td>-33 bp</td>
</tr>
<tr>
<td>TPOX</td>
<td>-148 bp</td>
</tr>
<tr>
<td>D7S820</td>
<td>-117 bp</td>
</tr>
</tbody>
</table>

*Not as much size reduction as other STR loci...*

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**Problems with Large Allele Spreads**

<table>
<thead>
<tr>
<th>STR Locus</th>
<th>GenBank Accession</th>
<th>GenBank Allele</th>
<th>Allele Range</th>
<th>Allele Spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH01</td>
<td>D00269</td>
<td>9</td>
<td>3–14</td>
<td>44 bp</td>
</tr>
<tr>
<td>TPOX</td>
<td>M66565</td>
<td>11</td>
<td>5–14</td>
<td>36 bp</td>
</tr>
<tr>
<td>vWA</td>
<td>M25855</td>
<td>18</td>
<td>10–25</td>
<td>60 bp</td>
</tr>
<tr>
<td>D5S818</td>
<td>AC085312</td>
<td>11</td>
<td>7–16</td>
<td>36 bp</td>
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<tr>
<td>D7S820</td>
<td>AC094848</td>
<td>13</td>
<td>5–15</td>
<td>49 bp</td>
</tr>
<tr>
<td>D8S1179</td>
<td>AC216671</td>
<td>13</td>
<td>7–19</td>
<td>49 bp</td>
</tr>
<tr>
<td>D1S1817</td>
<td>AC353628</td>
<td>11</td>
<td>5–16</td>
<td>44 bp</td>
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<td>D1S515</td>
<td>AC074691</td>
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<td>5–15</td>
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<tr>
<td>D2S1338</td>
<td>AC090433</td>
<td>29</td>
<td>24–38.2</td>
<td>58 bp</td>
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<tr>
<td>Penta D</td>
<td>AP001752</td>
<td>13</td>
<td>2.2–17</td>
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<td>Penta E</td>
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<td>95 bp</td>
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<td>20</td>
<td>15–28</td>
<td>52 bp</td>
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**How Close Can a Stable Primer be Designed to the STR Repeat Region?**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Distance 3'end from Repeat</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1PO</td>
<td>14</td>
<td>partial repeat just 5' of repeat</td>
</tr>
<tr>
<td>FGA</td>
<td>23</td>
<td>partial repeat just 3' of repeat</td>
</tr>
<tr>
<td>TH01</td>
<td>4</td>
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<tr>
<td>TPOX</td>
<td>-4</td>
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<td>vWA</td>
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<tr>
<td>D8S1179</td>
<td>-5</td>
<td>polyA stretch just 3' of repeat</td>
</tr>
</tbody>
</table>

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**Why go beyond CODIS loci**

"STRs have proven to be highly successful [for mass disasters] in the past e.g. Waco disaster and various air disasters. However, even if the DNA is high quality there are occasions when there are insufficient family members available to achieve a high level of confidence with an association."

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**Why go beyond CODIS loci**

"To achieve this purpose, either new STRs could be developed, or alternatively, existing STRs could be supplemented with a SNP panel."

"There also efforts for modifying existing STR panels by decreasing the size amplicons by designing new primers."

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(Quantico, VA)

8
**Why go beyond CODIS loci**

- Desirable to have markers unlinked from CODIS loci (different chromosomes) for some applications
- Small size ranges to aid amplification from degraded DNA samples

**Characterization of New miniSTR Loci**

- Candidate STR marker selection
- Chromosomal locations and marker characteristics
- PCR primer design
- Initial testing results
- Population testing
- Allelic ladder construction
- Miniplex assay performance

**Initial Testing Results**

- >900 potential markers
- 61 markers with “clean” flanking regions
- 43 markers with amplicon size < 125bp
- 18 markers for initial testing
- 2 three loci miniplexes

**Locations of Focus for New miniSTR Loci**

(relative to CODIS 13 STRs)

<table>
<thead>
<tr>
<th>Chr.</th>
<th>Marker Name</th>
<th>Repeat</th>
<th>Size</th>
<th>Primer distance</th>
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<tbody>
<tr>
<td>10</td>
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<td>TETRA</td>
<td>102</td>
<td>1</td>
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<td>TETRA</td>
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<td>2</td>
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</table>

**CODIS State Administrators meeting**

(Quantico, VA)
Future of Forensic DNA Typing

CODIS State Administrators meeting
(Quantico, VA)
DNA Quantitation
Real-time PCR methods
Interlaboratory Study Results

$ Cost per sample (20 µL – 25 µL)

<table>
<thead>
<tr>
<th>Assay</th>
<th>$ PCR Master Mix</th>
<th>$ Primers</th>
<th>$ TaqMan probe</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>Au</td>
<td>0.80</td>
<td>0.0025</td>
<td>NA</td>
<td>$0.8025</td>
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<tr>
<td>TH01</td>
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<td>0.0025</td>
<td>NA</td>
<td>$0.8025</td>
</tr>
<tr>
<td>CFS-HUMRT</td>
<td>0.73*</td>
<td>0.0025</td>
<td>0.17</td>
<td>$0.9025</td>
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<tr>
<td>RB1</td>
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<td>0.0025</td>
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<td>$0.9025</td>
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<tr>
<td>mtDNA</td>
<td>0.73*</td>
<td>0.0025</td>
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<td>Qfiler Human</td>
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<td>Qfiler Y Male</td>
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<td>NA</td>
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<td>$2.50</td>
</tr>
</tbody>
</table>

* Platinum SYBR® Green qPCR SuperMix UDG (Invitrogen, Carlsbad, CA)
# Platinum Quantitative PCR SuperMix – UDG (Invitrogen, Carlsbad, CA)

Assay specifications tried at NIST
TaqMan Probes

<table>
<thead>
<tr>
<th>Assay</th>
<th>amplicon</th>
<th>Gene/Target</th>
<th>probe</th>
<th>#Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFS-HUMRT</td>
<td>62 bp</td>
<td>Human tyrosine hydroxylase gene</td>
<td>15 bp VIC</td>
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<tr>
<td>11p15.5</td>
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<tr>
<td>RB1</td>
<td>79 bp</td>
<td>Human retinoblastoma susceptibility gene</td>
<td>26 bp FAM</td>
<td>50</td>
</tr>
<tr>
<td>13</td>
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<td></td>
</tr>
<tr>
<td>mtDNA</td>
<td>143 bp</td>
<td>RNA lysate &amp; ATP synthase B, Coding Region</td>
<td>29 bp VIC</td>
<td>50</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>Qfiler Human</td>
<td>62 bp</td>
<td>Human telomerase reverse transcriptase (hTERT)</td>
<td>FAM</td>
<td>40</td>
</tr>
<tr>
<td>Sp15.33</td>
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<tr>
<td>Qfiler Y Male</td>
<td>64 or 61 bp</td>
<td>Sex-determining region Y gene (SRY)</td>
<td>FAM</td>
<td>40</td>
</tr>
<tr>
<td>Yp11.3</td>
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</table>

Comparison of RT-PCR assays
Following Published Protocols

Series of NIST population samples with a range of [DNA] from 40 pg to 23 ng
The same “Standard” was used for all methods (8 dilutions).
Time for the assay:
Alu-RT-PCR ~ 1.25 h (fewer cycles required)
The rest ~ 1.75 h

Results of NIST Quantitation Study 04
Consisted of:
8 DNA extracts labeled A – H Shipped Dec 2003 – Jan 2004
Shipped to 84 laboratories for quantification.
Labs asked to use multiple methods / multiple analysts
Last day for submission extended from 15 March to 5 April 2004

We received data from 80 Labs (95%)
Total of 287 sets of data
Participants used 19 different quantification methods
(primarily variations on Quantiblot and Real-time PCR)
Comparison of the Blot Methods

NIST QS 04 Results-Target Plots

AluQuant and PicoGreen

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