National Institute of Standards and Technology
. . .working with industry to develop and apply technology, measurements and standards

# NIST Research Update

**John M. Butler**

Peter M. Vallone, Michael D. Coble, Amy E. Decker, Janette W. Redman, David L. Duewer, Margaret C. Kline

July 12, 2004

SWGDAM

## NIST Human Identity Project Team


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Funding:
Interagency Agreement between NIJ and NIST Office of Law Enforcement Standards

## Presentation Outline

- *Forensic DNA Typing*, 2nd Edition
  - to be available in Jan 2005
- NIST Research Projects
  - Y-chromosome information, kits, and standards
  - New loci under development
  - miniSTRs
  - Autosomal SNPs
  - Performance with degraded DNA samples including hair shafts
  - DNA quantitation interlaboratory performance across 80 labs (NIST QS04)
  - STRBase updates and other tools to aid state and local labs

## *Forensic DNA Typing*, 2nd Edition:
Biology, Technology, and Genetics of STR Markers

**John Butler (not NIST)**

| | |
|---|---|
| Chapter 1 | Overview & History of DNA Typing |
| Chapter 2 | DNA Biology Review |
| Chapter 3 | Sample Collection, Extraction, Quantitation |
| Chapter 4 | PCR Amplification |
| Chapter 5 | Common STRs and Commercial Kits |
| Chapter 6 | Biology of STRs |
| Chapter 7 | Forensic Issues |
| Chapter 8 | **Single Nucleotide Polymorphisms** |
| Chapter 9 | **Y-Chromosome DNA Tests** |
| Chapter 10 | **Mitochondrial DNA Analysis** |
| Chapter 11 | **Non-Human DNA and Microbial Forensics** |
| Chapter 12 | DNA Separation Methods |
| Chapter 13 | DNA Detection Methods |
| Chapter 14 | Instrumentation for STR Typing: ABI 310, ABI 3100, FMBIO |
| Chapter 15 | STR Genotyping Issues |
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| Chapter 17 | New Technologies, Automation, and Expert Systems |
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| Chapter 19 | **Basic Genetic Principles and Statistics** |
| Chapter 20 | **STR Database Analyses** |
| Chapter 21 | **Profile Frequency Estimates** |
| Chapter 22 | **Statistical Analysis of Mixtures and Degraded DNA** |
| Chapter 23 | **Kinship and Paternity Testing** |
| Chapter 24 | **Mass Disaster DNA Victim Identification** |
| Appendix I | Reported STR Alleles |
| Appendix II | **U.S. Population Data-STR Allele Frequencies** |
| Appendix III | Suppliers of DNA Analysis Equipment |
| Appendix IV | DAB QA Standards |
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| Appendix VI | **Application of NRC II to STR Typing** |
| Appendix VII | **Example DNA Cases** |

New Material:
**10 additional chapters**
Statistics (basics with examples)
Real-time PCR
Serology tests
Y-STRs and mtDNA
ABI 3100
Expert systems
Mass disasters including WTC
Example cases for training purposes

>500 new reference citations
50 new figures and 45 new tables
Manuscript is ~950 pages
*Approximately double the size of the first edition*

***Academic Press plans to have available by January 2005***

National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

## Current Areas of NIST Research Effort

- **Y-Chromosome** Information, Assays, and Standards
- Resources for "**Challenging Samples**" (SNPs and miniSTRs)
- **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)

# Y-Chromosome

Information, Assays, and Standards

*Forensic Science Communications* July 2004 – Volume 6 – Number 3
Standards and Guidelines

## Report on the Current Activities of the Scientific Working Group on DNA Analysis Methods Y-STR Subcommittee

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**Scientific Working Group on DNA Analysis Methods Y-STR Subcommittee**

**Introduction**

Detecting DNA from a male perpetrator is the goal in the forensic investigation of most sexual assault cases. Y-chromosome-specific STR typing targets the male DNA and is a useful additional tool in cases that often involve a mixture of male and female DNA. Although many technical aspects of Y-STR testing are parallel to autosomal STR testing, the unilateral (patrilineal) inheritance of the Y-chromosome alleles creates a haplotype of linked loci, and the statistical evaluation and reporting of the results differ significantly. Therefore, the SWGDAM Y-STR Subcommittee was established to deal with all aspects of Y-chromosome-specific testing in forensic casework.

*Selection of U.S. Core Loci*: DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439

## Commercial Y-STR Kits

(Minimal/extended haplotype): DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS385 a/b, YCAII a/b, DYS388, DYS425, DYS426, YCAIII a/b

(White et al.): A7.1 (DYS460), A7.2 (DYS461), A10, C4, H4

(Ayub et al.): DYS434, DYS435, DYS436, DYS437, DYS438, DYS439

(Iida et al.): DYS441, DYS442, DYS443, DYS444, DYS445

(Redd et al.): DYS446, DYS447, DYS448, DYS449, DYS450, DYS452, DYS453, DYS454, DYS455, DYS456, DYS458, DYS459 a/b, DYS463, DYS464 a/b/c/d

(Bosch et al.) G09411 (DYS462)

43 (51) Y-STRs (217 with Manfred's)

**Y-PLEX 6 (ReliaGene)**
**Y-PLEX 5 (ReliaGene)**
**Y-PLEX 12 (ReliaGene)**
**PowerPlex Y (Promega)**
**Yfiler (Applied Biosystems)**

DYS468-DYS645
**166 new Y STRs**
(Manfred Kayser GDB entries)

## New Y-STR paper

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

*Am. J. Hum. Genet.* 74:1183–1197, 2004

### A Comprehensive Survey of Human Y-Chromosomal Microsatellites

Manfred Kayser,[1,*] Ralf Kittler,[1,4] Axel Erler,[1,4] Minttu Hedman,[2] Andrew C. Lee,[3] Aisha Mohyuddin,[4,5] S. Qasim Mehdi,[5] Zoë Rosser,[3] Mark Stoneking,[1] Mark A. Jobling,[3] Antti Sajantila,[2] and Chris Tyler-Smith[4,6]

[1]Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig; [2]Department of Forensic Medicine, University of Helsinki, Helsinki; [3]Department of Genetics, University of Leicester, Leicester, United Kingdom; [4]Department of Biochemistry, University of Oxford, Oxford; [5]Biomedical and Genetic Engineering Laboratories, Islamabad; and [6]The Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom

- **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**

## U.S. Population Data on 22 Y-STRs

ELSEVIER
Available online at www.sciencedirect.com
SCIENCE DIRECT®
Forensic Science International 139 (2004) 107–121
Forensic Science International
www.elsevier.com/locate/forsciint

### High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays

Richard Schoske[a,b], Peter M. Vallone[a], Margaret C. Kline[a], Janette W. Redman[a], John M. Butler[a,*]

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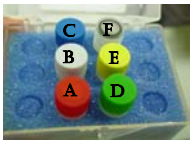
**pdf file available at http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm**

US haplotype (Reliagene kits)
Yfiler (ABI)
PowerPlex Y (Promega)
+C4

| Y-STR | Pooled Population STR diversity (N=647) | Rank | African American STR diversity (N=260) | Rank | Caucasian STR diversity (N=244) | Rank | Hispanic STR diversity (N=143) | Rank |
|---|---|---|---|---|---|---|---|---|
| DYS464 a/b/c/d | 0.956 | 1 | 0.954 | 1 | 0.934 | 1 | 0.937 | 1 |
| **DYS385 a/b** | 0.912 | 2 | 0.942 | 2 | 0.838 | 2 | 0.901 | 2 |
| YCAII a/b | 0.790 | 3 | 0.797 | 3 | 0.701 | 5 | 0.772 | 4 |
| DYS458 | 0.765 | 4 | 0.758 | 5 | 0.743 | 3 | 0.793 | 3 |
| **DYS390** | 0.764 | 5 | 0.664 | 10 | 0.701 | 5 | 0.665 | 13 |
| DYS447 | 0.747 | 6 | 0.767 | 4 | 0.683 | 7 | 0.748 | 5 |
| **DYS389II** | 0.736 | 7 | 0.722 | 6 | 0.675 | 8 | 0.734 | 6 |
| DYS448 | 0.721 | 8 | 0.722 | 6 | 0.595 | 11 | 0.704 | 8 |
| DYS456 | 0.700 | 9 | 0.671 | 9 | 0.731 | 4 | 0.695 | 9 |
| DYS438 | 0.691 | 10 | 0.560 | 15 | 0.594 | 12 | 0.690 | 10 |
| **DYS19** | 0.676 | 11 | 0.722 | 6 | 0.498 | 19 | 0.672 | 12 |
| DYS439 | 0.656 | 12 | 0.636 | 11 | 0.639 | 9 | 0.717 | 7 |
| DYS437 | 0.637 | 13 | 0.499 | 17 | 0.583 | 13 | 0.624 | 14 |
| H4 | 0.611 | 14 | 0.612 | 12 | 0.562 | 14 | 0.609 | 15 |
| **DYS392** | 0.609 | 15 | 0.434 | 20 | 0.596 | 10 | 0.673 | 11 |
| DYS460 | 0.570 | 16 | 0.568 | 14 | 0.555 | 15 | 0.556 | 18 |
| **DYS389I** | 0.549 | 17 | 0.531 | 16 | 0.538 | 17 | 0.596 | 16 |
| **DYS391** | 0.534 | 18 | 0.447 | 19 | 0.552 | 16 | 0.577 | 17 |
| DYS426 | 0.519 | 19 | 0.375 | 21 | 0.482 | 20 | 0.522 | 19 |
| DYS450 | 0.489 | 20 | 0.487 | 18 | 0.177 | 22 | 0.414 | 21 |
| **DYS393** | 0.485 | 21 | 0.586 | 13 | 0.363 | 21 | 0.448 | 20 |
| DYS388 | 0.365 | 22 | 0.246 | 22 | 0.501 | 18 | 0.312 | 22 |

Schoske *et al.* (2004) High-throughput Y-STR typing of U.S. populations…, *Forensic Sci. Int.*, *139:107-121*

## 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

$248

Certified for all loci in commercial Y-STR kits:
Y-PLEX 6
Y-PLEX 5
Y-PLEX 12
PowerPlex Y

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

**Y-filer** - adds DYS635 (C4); now sequenced

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

## Y-STRs in Casework

*July 2004* issue of Journal of Forensic Sciences

*J Forensic Sci*, July 2004, Vol. 49, No. 4
Paper ID JFS2003246
Available online at: www.astm.org

*Sudhir K. Sinha,[1] Ph.D.; Bruce Budowle,[2] Ph.D.; Ranajit Chakraborty,[3] Ph.D.; Ana Paunovic,[1] B.S.; Robin DeVille Guidry,[1] B.S.; Chris Larsen,[1] M.S.; Amrita Lal,[1] M.S.F.S.; Megan Shaffer,[1] Ph.D.; Gina Pineda,[1] M.S.; Siddhartha K. Sinha,[1] B.S.; Elaine Schneida,[1] B.S.; Huma Nasir,[1] B.S.; and Jaiprakash G. Shewale,[1] Ph.D.*

Utility of the Y-STR Typing Systems Y-PLEX™ 6 and Y-PLEX™ 5 in Forensic Casework and 11 Y-STR Haplotype Database for Three Major Population Groups in the United States*

TABLE 1—*Y-STR cases using the Y-PLEX™ 6 and Y-PLEX™ 5 kits that have been accepted in U.S. courts.*

| Case | Date | Jurisdiction | Docket No. | Notes |
|---|---|---|---|---|
| State of LA vs. Samuel Williams | 10/23/01 | Orleans Parish | 416-355 | Criminal paternity case |
| State of MS vs. Leon Felder | 6/26/01 | Pike County | 00-537-KA | Sexual assault case-also had other STRs, Y-STR produced no result |
| State of GA vs. Ali R. Shabazz | 7/31/02 | Dekalb County | 01-CR-4002 | Sexual assault case |
| United States vs. Spc. Michael Kelly | 10/16/02 | Ft. Knox | ... | Sexual assault case |
| State of OH vs. Chuckie Unsworth | 4/16/03 | Lucas County | G-4801-CR-200301510 | Daubert Hearing |

## Thoughts on Y-Chromosome Issues

- Core loci are selected, commercial kits are now available
- *Y-STRs need to be put into greater use with forensic casework to demonstrate their value*

Research Issues

- Nomenclature for Y-STR alleles in new loci
- Impact of additional loci to resolve most-common types
- Publicly available databases for additional loci
- Statistical issues with combining autosomal and Y-STR information

## 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?

## 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."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

## 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."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

## 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

## Why evaluate new markers?

- Highly Degraded samples (fragmented, questionable DNA quantity, inhibitors?)
- Telogenic/shed hairs (few copies)
- Low copy number cases (few copies)
- Siblings/Closely related individuals (paternity)

The primary characteristic of the assays for typing these new markers is their short PCR amplicon size (60 –150 base pairs)

## STR Size Reduction

### Through Moving Primer Positions Closer to Repeat


Focus on previously characterized STR markers with:

- High Heterozygosity
- Relatively small allele range
- "Clean" flanking regions for primer design adjacent to target repeat

## 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

↓

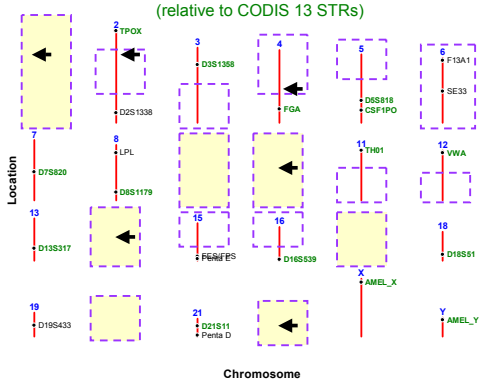
18 markers for initial testing

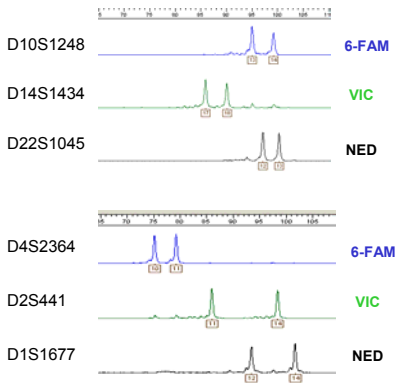
↓

2 three loci miniplexes

## Locations of Focus for New miniSTR Loci

(relative to CODIS 13 STRs)



## miniSTR characteristics

| STR Locus | Sequence Motif | Allele Range | Size Range (bp) | Observed Heterozygosity |
|---|---|---|---|---|
| D1S1677 | $(GGAA)_n$ | 9-18 | 81-117 | 0.75 |
| D2S441 | $(TCTA)_n$ | 9-17 | 78-110 | 0.76 |
| D4S2364 | $(GAAT)(GGAT)(GAAT)_n$ | 8-12 | 67-83 | 0.53 |
| D10S1248 | $(GGAA)_n$ | 10-20 | 83-123 | 0.78 |
| D14S1434 | $(GATA)_n(GACA)_n$ | 13-20 | 70-98 | 0.68 |
| D22S1045 | $(TAA)_n$ | 5-16 | 76-109 | 0.77 |

Coble and Butler, **JFS**, *manuscript submitted*

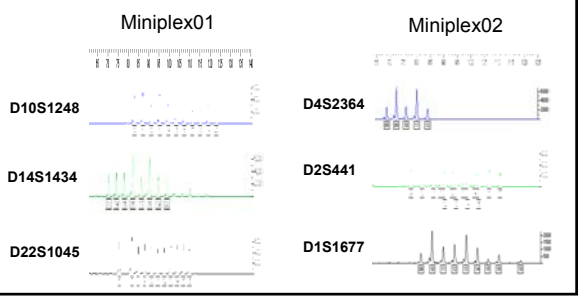
## Population Testing –Miniplexes vs. Identifiler

| Heterozygosity | Marker |
|---|---|
| 0.8784 | D2S1338 |
| 0.8753 | D18S51 |
| 0.8710 | FGA |
| 0.8393 | D21S11 |
| 0.8245 | vWA |
| 0.8076 | D7S820 |
| 0.7970 | D19S433 |
| 0.7759 | mD10S1248 - mini01 |
| 0.7759 | D16S539 |
| 0.7674 | mD22S1045 - mini01 |
| 0.7674 | D8S1179 |
| 0.7590 | mD2S441 - mini02 |
| 0.7548 | D3S1358 |
| 0.7526 | D13S317 |
| 0.7463 | mD1S1677 - mini02 |
| 0.7378 | CSF1PO |
| 0.7378 | TH01 |
| 0.7294 | D5S818 |
| 0.7146 | TPOX |
| 0.6765 | mD14S1434 - mini01 |
| 0.5307 | mD4S2364 - mini02 |

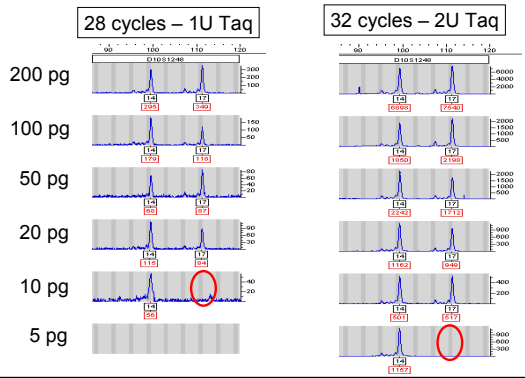
N = 474 Individuals
164 African Americans
170 Caucasians
140 Hispanics

## miniSTR Assay

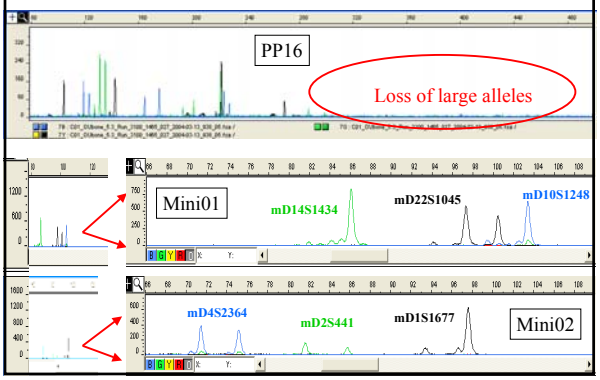
### Allelic Ladders




## miniSTR Assay Sensitivity (D10S1248)

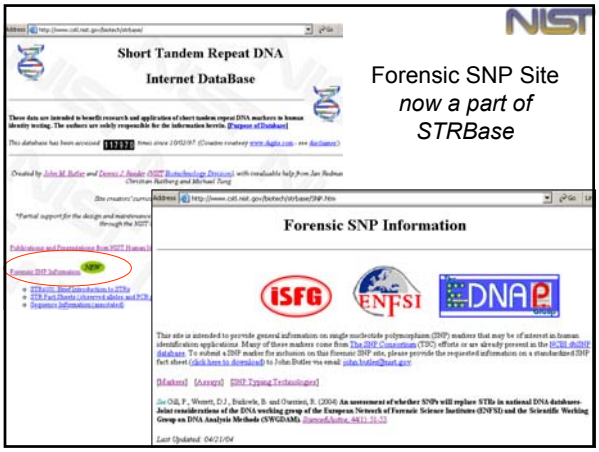



## Sensitivity - Degraded DNA from an OU Bone Sample




## SNP Typing at NIST

- STRBase is the official ISFG/EDNAP/ENFSI repository of forensic SNP information
  - Gill *et al. Science & Justice* 2004, 44, 51-53
  - **http://www.cstl.nist.gov/biotech/strbase/SNP.htm**
- 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

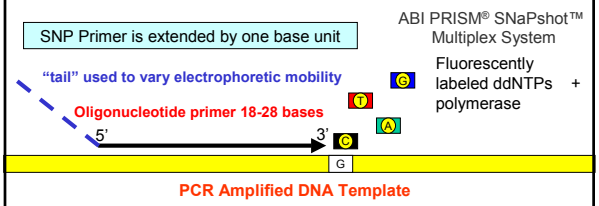



## SNP characteristics

- 70 Loci – sites from Orchid – C/T bi-allelic
- Present on 20 of 22 autosomal chromosomes (all but 3,16, and X,Y)
- Amplicon size range 59 - 108 bp (average 69 bp)
- Markers are typed by allele-specific primer extension assays (ABI SNaPshot)
- Level of multiplexing (6-12-plexes)
- Web page for SNP site info

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

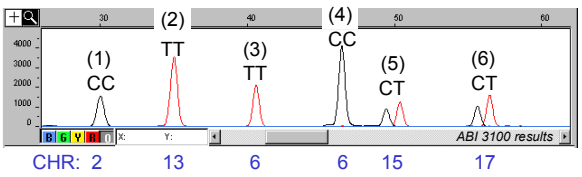
## Allele-Specific Primer Extension




6-plex PCR was used to amplify 2 ng of gDNA (0.5 U Taq Gold)

6-plex primer extension was used to type the loci

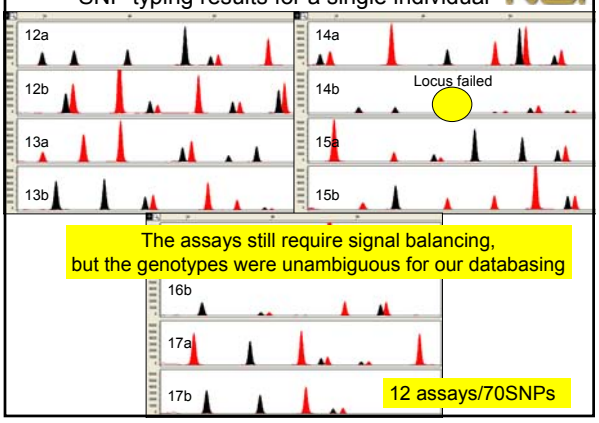
## 6-plex SNP Assay




Extension primers for 6-plex

(1) TTTTTAGCTCCTAATTTCTTGATGGG
(2) TTTTTTTTTTCATCTGATGCCATGAGAAAGC
(3) TTTTTTTTTTTTTTGTTCTGCTTTAATACAAAACCAG
(4) TTTTTTTTTTTTTTTTTTTTTTTTATAAAGGGCAGAATGAGGATTA
(5) TTTTTTTTTTTTTTTTTTTTTTTTAGAAAGTATCTTGCAAAAGGTCCA
(6) TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCATAATCACAGCTTTTTTCTCCCAA

## SNP typing results for a single individual




## SNP Assay Results

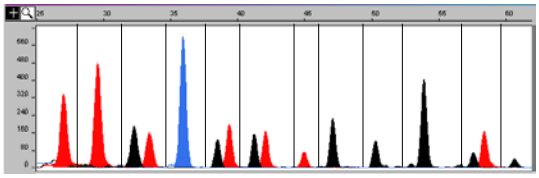
- 70 were typed for 189 U.S. samples (self identified ethnicities)
- 74 Caucasians + 71 African Americans AA + 44 Hispanics

- Total of 13,230 possible genotypes
- One marker failed across all samples (13,041-98.6%)

- 42 Samples were re-injected to confirm ambiguous results (99.7 %) success rate on first pass
- Results described in manuscript (*Forensic Sci. Int.*)

- We are in the process of optimizing a 12-plex panel of SNPs

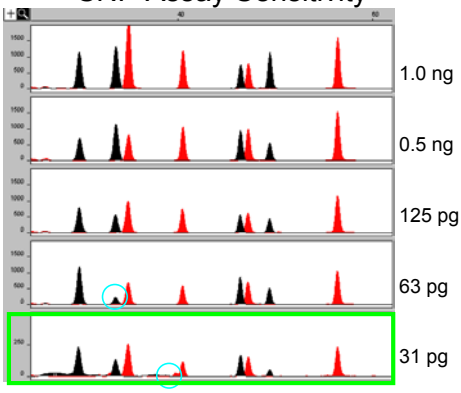
## Autosomal SNP 12-plex



CHR: 15 1 13 11 1 15 6 10 20 17 13 17

- A subset of the 70 SNP markers
- Observed heterozygosity of >0.45 in each of the 3 populations
- All 189 samples were individualized using these 12 markers

## SNP Assay Sensitivity




## Can we get nuclear DNA from hair shafts?

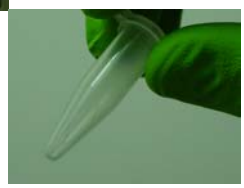
Yes…

But depends on the extraction method and assay used

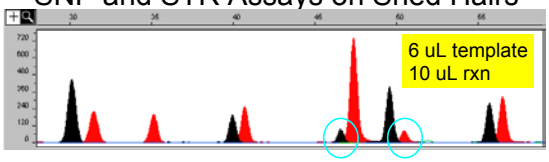
**$TN_{Ca}$ Buffer**
Tris
NaCl
$CaCl_2$
2%SDS
ProK
**DTT**

Complete digestion of hair in about 1 hour based on method by

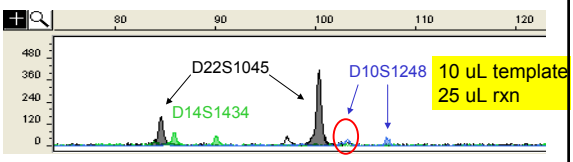
Hellmann A, Rohleder U, Schmitter H, Wittig M. (2001) STR typing of human telogen hairs--a new approach. *Int J Legal Med* 114(4-5): 269-273.



## SNP and STR Assays on Shed Hairs

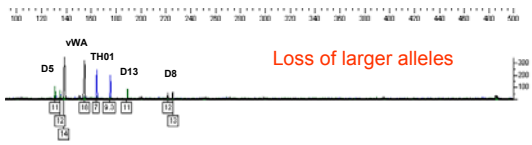



5 hairs (2.5 cm ea); 2 U Taq; 32 cycles



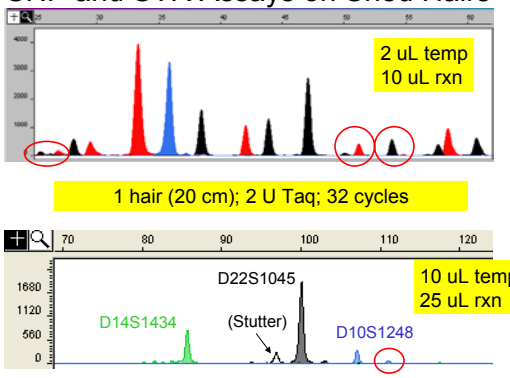

## PowerPlex 16 Genotype

5 Hair Shafts (2.5 cm each, no roots)




(5 markers typed – note: no Amelogenin )

## SNP and STR Assays on Shed Hairs




## Future directions with SNPs and miniSTRs

- Optimize 12-plex for SNPs
- Determine sensitivity of assays
- Examine data interpretation issues for LCN assays (eg allele drop out, RFU thresholds)
- Type on a "standard" degraded sample (compare to commercial kits)
- Mobility modifiers with miniSTRs (potential for greater multiplexing)

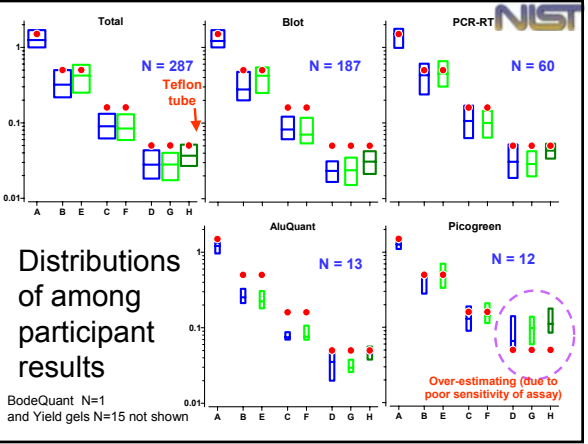
## *DNA Quantitation*

Interlaboratory Study Results
SRM 2372 : Human DNA Quantitation Standard

## NIST Quantitation Study 2004 (QS04)

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

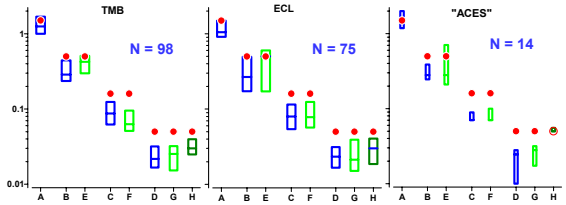
We received data from 80 Labs (95%)
**Total of 287 sets of data**
Participants used 19 different quantification methods (primarily variations on Quantiblot and Real-time PCR)




Distributions of among participant results

BodeQuant N=1 and Yield gels N=15 not shown

## Summary of the Blot based Methods

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## Summary of the RT-PCR Methods

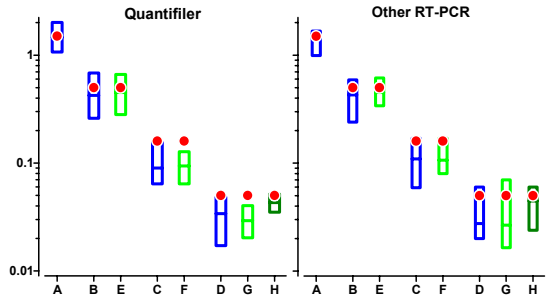


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Table 2. The percent success rate reported for a sample.

| | | % Quantitative Results* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Target [DNA] ng/µL | | 1.5 | 0.5 | 0.5 | 0.16 | 0.16 | 0.05 | 0.05 | 0.05 |
| Method | $N_{test}$ | A | B | E | C | F | D | G | H |
| Quantifiler | 37 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Other RT-PCR | 23 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| "ACES" | 14 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| AluQuant | 13 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| PicoGreen | 12 | 100 | 100 | 92 | 100 | 100 | 92 | 83 | 83 |
| ECL | 75 | 100 | 99 | 99 | 93 | 95 | 84 | 77 | 87 |
| TMB | 98 | 100 | 100 | 99 | 93 | 94 | 59 | 62 | 63 |
| Yield gel | 14 | 57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 286 | | | | | | | | |

a Quantitative results are those that were reported as values, values reported as the range between contiguous calibration standards, values reported as less-than the lowest calibration standard if smaller than the target [DNA], or values reported as greater-than the highest calibration standard if larger than the target[DNA].

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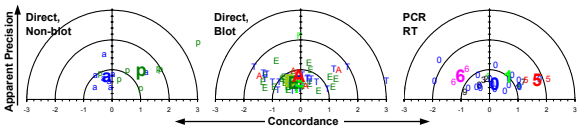
## Among Participate Results




Concordance – is a direct multi-material analogue of bias

Apparent precision – is analogous to precision but also incorporates sample-specific measurement differences or "matrix effects".

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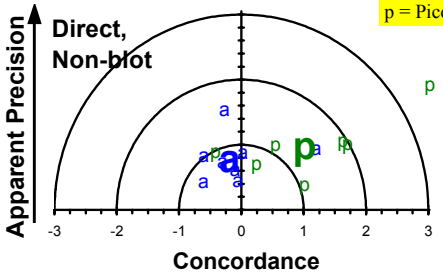


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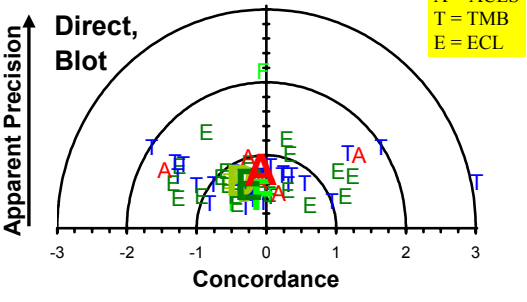


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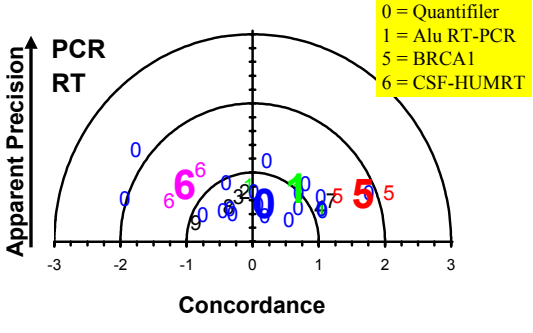


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## Publication of NIST QS04 Results

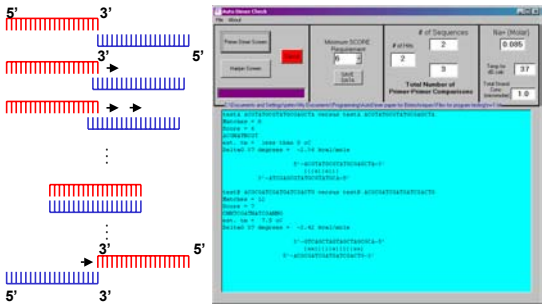
- Paper describing results is complete
- Being sent out for review by all 80 contributing laboratories along with certificates of participation
- Concurrently going through NIST internal review
- Following these reviews, the manuscript will be submitted to *J. Forensic Sci.*

*Results being used for SRM 2372 development*

## 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
  - *Forensic DNA Typing*, 2nd Edition (Dec 2004/Jan 2005)

AutoDimer – primer screening software is now freely available




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

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NIST Project Team:

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