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

New Autosomal Forensic Markers and a Review of the NIST Interlaboratory Quantitation Study

5th Annual NIJ Grantees Meeting

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DNA Technologies Group
NIST

Current Areas of NIST Research Effort

- 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)

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 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)

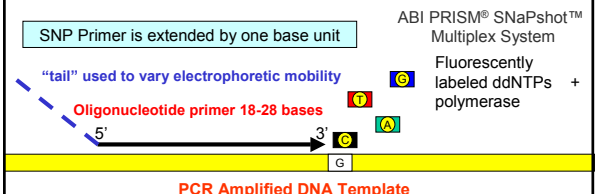
The primary characteristic of the assays for typing these new markers is their short PCR amplicon size (60 –150 base pairs)

SNP characteristics

- 70 Loci – sites from Orchid – C/T bi-allelic
- Present on 20 of 22 autosomal CHR (3,16,X,Y)
- Amplicon size range 59 - 108 bp (average 69)
- 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

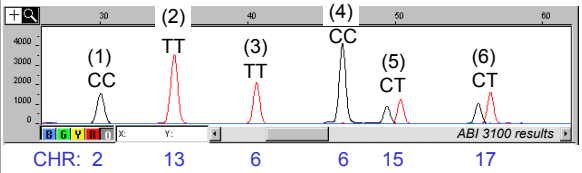


PCR Amplified DNA Template

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

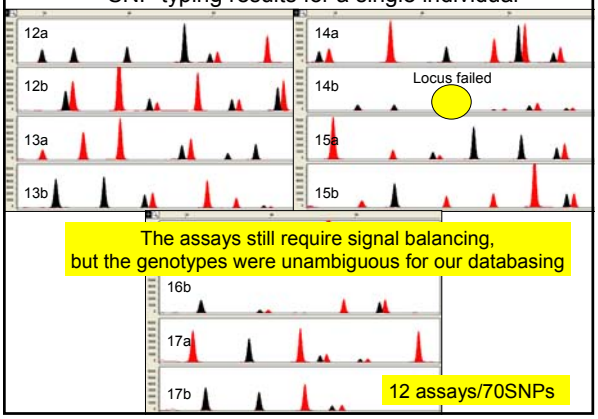


CHR: 2 13 6 6 15 17

Extension primers for 6-plex

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

SNP typing results for a single individual



The assays still require signal balancing, but the genotypes were unambiguous for our databasing

12 assays/70SNPs

SNP Assay Results

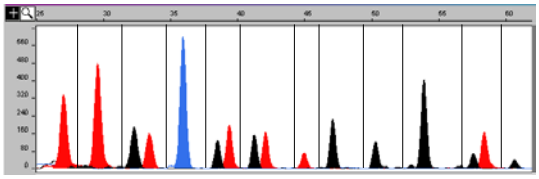
- 70 were typed for 189 U.S. samples (self identified ethnicities)
- 74 Caucasians + 71 African Americans AA + 44 Hispanics

- 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

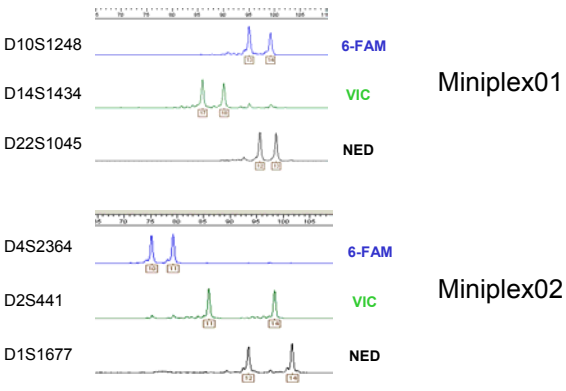
- A subset of the 70 SNP markers
- Observed heterozygosity of >0.45 in each of the 3 populations
- All 189 samples were individualized using these 12 markers

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

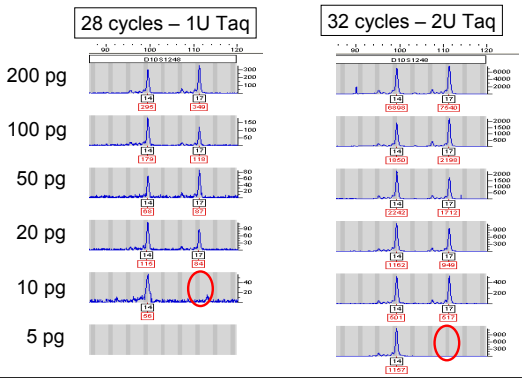


miniSTR characteristics

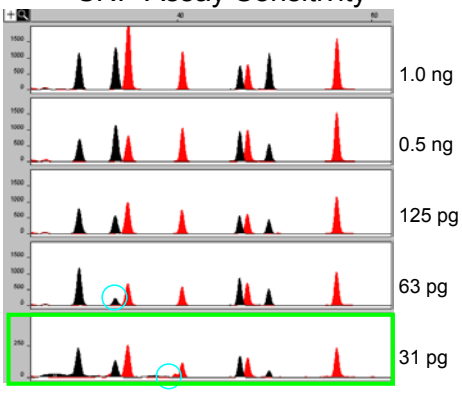
STR Locus	Sequence Motif	Allele Range	Size Range (bp)	Observed Heterozygosity
D1S1677	$(GGAA)_n$	9-18	81-117	0.75
D2S441	$(TCTA)_n$	9-17	78-110	0.76
D4S2364	$(GAAT)(GGAT)(GAAT)_n$	8-12	67-83	0.53
D10S1248	$(GGAA)_n$	10-20	83-123	0.78
D14S1434	$(GATA)_n(GACA)_n$	13-20	70-98	0.68
D22S1045	$(TAA)_n$	5-16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

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

miniSTR Assay Sensitivity (D10S1248)



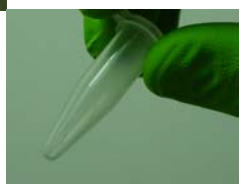
SNP Assay Sensitivity



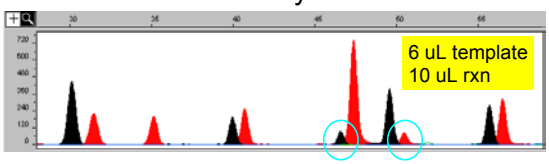
TN_{Ca} Buffer
Tris
NaCl
$CaCl_2$
2%SDS
ProK
DTT

Complete Digestion of hair in about 1 hour.

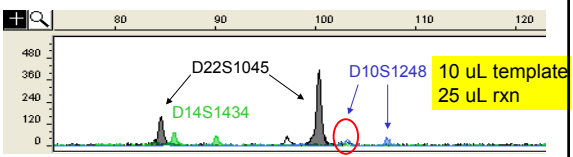
Hellmann et al., IJLM 2001



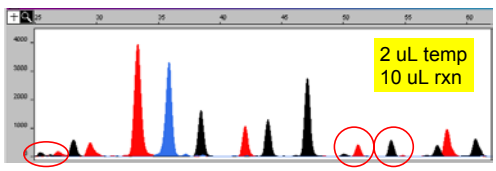
SNP and STR Assays on Shed Hairs



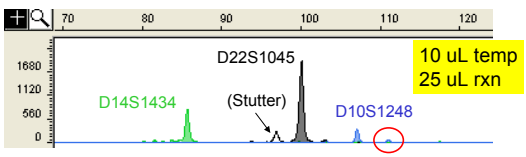
5 hairs (2.5 cm ea); 2 U Taq; 32 cycles



SNP and STR Assays on Shed Hairs



1 hair (20 cm); 2 U Taq; 32 cycles



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

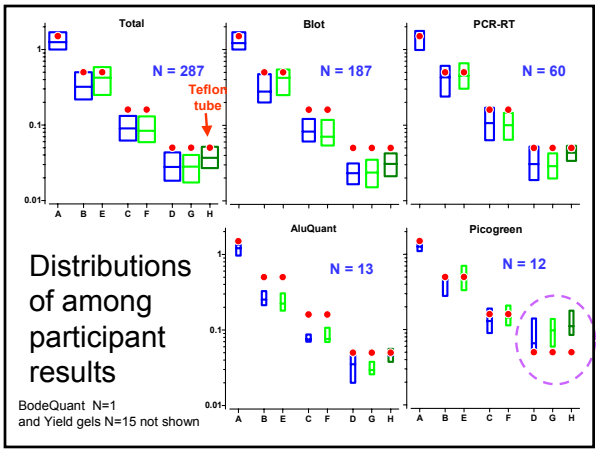
DNA Quantitation

- **Interlaboratory Study** -- to compare methods across many forensic laboratories and prototype a "gold-standard" DNA quantitation material
- **Development of SRM 2372** – to aid reproducible DNA quantitation and provide NIST-traceable materials

Results of NIST Quantitation Study 04

- 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

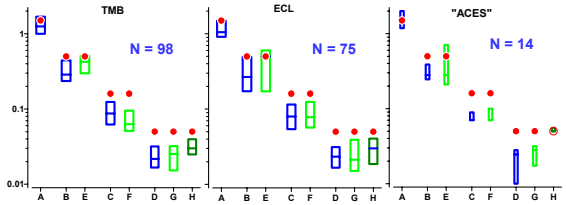
We received data from 80 Labs (95%)
Total of 287 sets of data
Participants used 19 different quantification methods (primarily variations on Quantiblot and Real-time PCR)



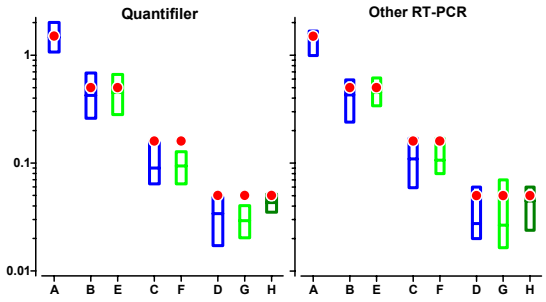
Distributions of among participant results

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

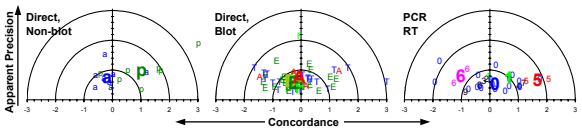
Summary of the Blot based Methods



Summary of the RT-PCR Methods

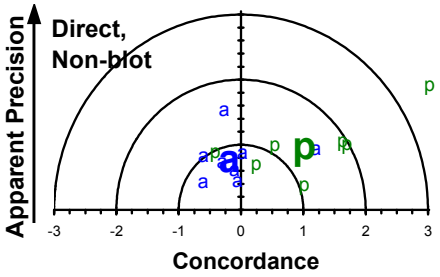


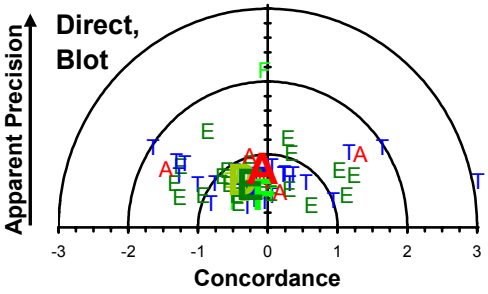
Among Participate Results

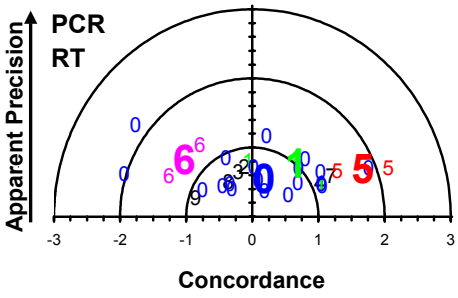


Concordance – is a direct multi-material analogue of bias

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







SRM 2372 : Human DNA Quantitation Standard

How do we determine 1 ng of Human Genomic DNA and relate it to SI units? (which we have to do for it to be a true Standard)

UV measurements are based on "standard data" (obtained in 1950). It is now known that measurements are influenced by:
1) the presence of common chemicals used in the extraction procedure (phenol, EtOH).
2) The "condition" of the DNA (double stranded, single stranded, or the length).

This methodology great for getting relative values, but not values traceable to the SI.

SRM 2372 : Human DNA Quantitation Standard

Mass measurements are dependent on water and counter-ion (removed these and the DNA is permanently denatured).

Can we do it by count?

Number of basepairs per genome is known within a few percent.
Can we count base pairs? LGC is working on it.

Can we count phosphorus? NIST is working on it.

Can we count chromosomes?
Chromosomes have been counted for 48 yrs … and now it can be done from solutions!

NIST Human Identity Project Team


John Butler


Margaret Kline


Jan Redman


Pete Vallone


Dave Duewer

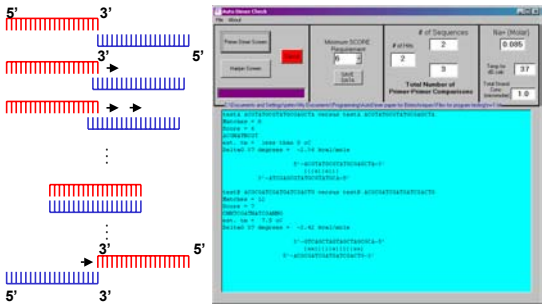

Amy Decker


Jill Appleby


Mike Coble

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

AutoDimer – primer screening software is now freely available at:



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

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Validation Standardization Questionnaire

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Margaret Kline