National Institute of Standards and Technology

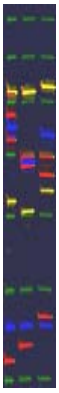
. . .working with industry to develop and apply technology, measurements and standards

NIST Research Update

John Butler

Jill Appleby, Mike Coble, Amy Decker, Dave Duewer, Margaret Kline, Jan Redman, and Pete Vallone

SWGDAM
January 8, 2004

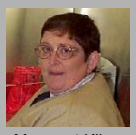


Outline for Presentation

- Project Team and Last Year's Progress
- SRM 2395 Y-Chromosome Standard
- mtDNA Typing Work
- miniSTR Work
- STR and SNP Marker Mapping Work
- NIST Interlaboratory Study on DNA Quantitation
- Real-time PCR Measurements
- Tools to Aid Forensic DNA Laboratories

NIST Human Identity Project Team


John Butler


Margaret Kline


Jan Redman


Pete Vallone


Dave Duewer


Amy Decker


Jill Appleby


Mike Coble
NRC Postdoc
(Dec 29, 2003)

Former (Honorary) Members


Rich Schoske


Christian Ruitberg

Funding and Collaborations

We are funded by an Interagency Agreement between National Institute of Justice and NIST Office of Law Enforcement Standards

We send quarterly reports to both NIJ and NIST-OLES

Our publications and presentations are made available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Collaborators (also funded by NIJ):

Mike Hammer and Alan Redd (U. AZ) for Y-chromosome studies

Tom Parsons and Mike Coble (AFDIL) for mtDNA coding SNP work

Sandy Calloway (Roche) for mtDNA linear arrays

Bruce McCord and students (Ohio U) for miniSTR work

Steve Sherry and Jon Baker (NCBI) for STR data quality assurance software

Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work

Publications in 2003
9 published
7 in press
5 submitted
5 in preparation
26 total

22 presentations
to forensic DNA community

Publications from Our Group this Past Year

Available as pdf files from http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Schoske, R., Vallone, P.M., Ruitberg, C.M., Butler, J.M. (2003) Multiplex PCR design strategy used for the simultaneous amplification of 10 Y chromosome short tandem repeat (STR) loci. *Anal. Bioanal. Chem.*, 375: 333-343.

Butler, J.M., Schoske, R., Vallone, P.M. Highly multiplexed assays for measuring polymorphisms on the Y-chromosome. (2003) *Progress in Forensic Genetics 9* (Brinkmann, B. and Carracedo, A., eds.), Elsevier Science: Amsterdam, The Netherlands, International Congress Series 1239, pp. 301-305.

Schoske, R. (2003) The design, optimization and testing of Y chromosome short tandem repeat megaplexes. PhD dissertation, American University, 270 pp.

Butler, J.M., Schoske, R., Vallone, P.M., Redman, J.W., Kline, M.C. (2003) Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J. Forensic Sci.* 48(4):908-911.

Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2003) NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. *Anal. Chem.* 75: 2463-2469.

Butler, J.M. (2003) Recent developments in Y-short tandem repeat and Y-single nucleotide polymorphism analysis. *Forensic Sci. Rev.* 15:91-111.

Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5) 1054-1064.

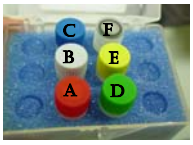
Butler, J.M. and Vallone, P.M. (2004) High-throughput genetic analysis through multiplexed PCR and multicapillary electrophoresis. *PCR Technologies: Current Innovations* (2nd edition), Weissensteiner, T., Griffin, H.G., Griffin, A. (Eds.), CRC Press: Boca Raton, Chapter 11, pp 111-120.

Schoske, R., Vallone, P.M., Kline, M.C., Redman, J.W., Butler, J.M. (2003) High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays, *Forensic Sci. Int., in press*

Butler, J.M. (2003) Constructing STR multiplex assays. *Methods in Molecular Biology: Forensic DNA Typing Protocols* (Carracedo, A., ed.), Humana Press: Totowa, New Jersey, *in press.*

Butler, J.M. (2003) Short tandem repeat analysis for human identity testing. *Current Protocols in Human Genetics*, John Wiley & Sons, Hoboken, NJ, Unit 14.8, *in press*

New Y-Chromosome NIST SRM



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

$245

Certified for all loci in commercial Y-STR kits:

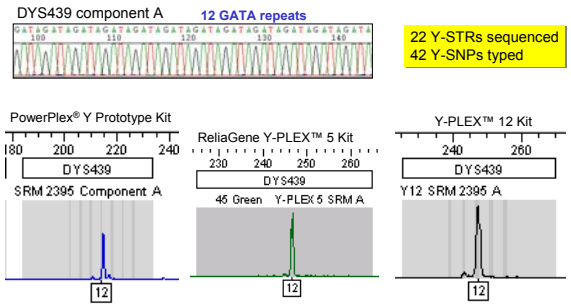
Y-PLEX 6
Y-PLEX 5
Y-PLEX 12
PowerPlex Y

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

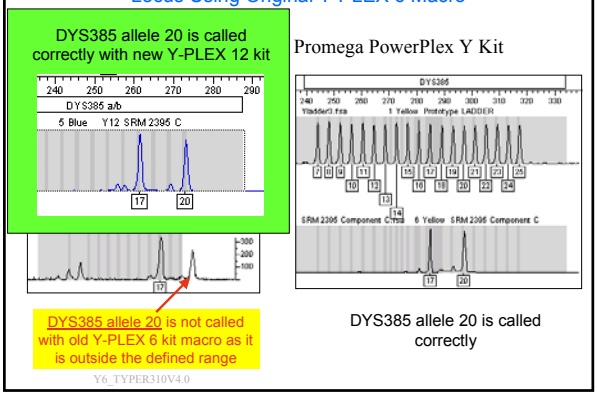
SRM 2395

NIST Y-Chromosome DNA Profiling Standard

Helps establish allele nomenclature and confirm/calibrate typing data



Warning with SRM 2395 Component C and DYS385 Locus Using Original Y-PLEX 6 Macro



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Forensic Science International

Forensic Science International xxx (2003) xxx–xxx

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,*]

[a]Biotechnology Division, National Institute of Technology, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899, USA

[b]Department of Chemistry, American University, Washington, DC 20016, USA

Received 29 April 2003; received in revised form 25 September 2003; accepted 1 October 2003

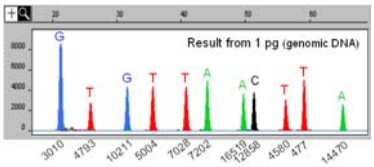
This study permitted an examination of currently used Y-STR markers vs. new loci on 647 U.S. Caucasians, African Americans, and Hispanics

Will be discussed in detail at AAFS Y-STR Workshop (Dallas, Feb 2004)

NIST mtDNA Work

Roche Linear Arrays
(probes for HVI/HVII)

Beta-test/Population Study



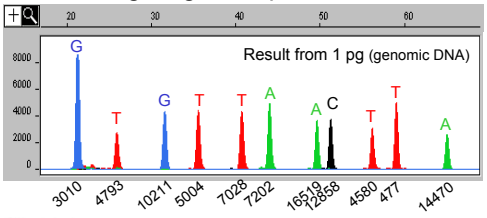
Coding Region
mtSNP 11plex
(minisequencing assay)

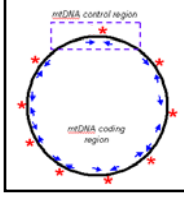
Developed with AFDIL to resolve mtDNA most common types

Int. J. Legal Med., in press

Will be discussed in detail at AAFS mtDNA Workshop (Dallas, Feb 2004)

mtDNA Coding Region 11plex SNaPshot Assay





11plex PCR and 11plex SNP detection

Sites are polymorphic in Caucasians (H1) and useful in resolving most common HV1/HV2 types

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

PCR product sizes kept under 200 bp to enable success with degraded DNA samples

STR Size Reduction

Through Moving Primer Positions Closer to Repeat

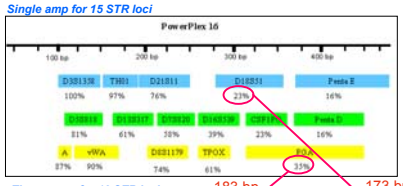
Primer positions define PCR product size
Repeat information is independent of amplicon size

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)

Comparison of PCR Amplification Success Rates with Commercial Kit vs. miniSTR Assays

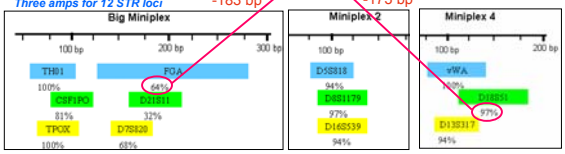
Single amp for 15 STR loci



Study with 31 bones from the "Body Farm" (Knoxville, TN) and Franklin County Coroner's Office (OH)

Three amps for 12 STR loci

-183 bp -173 bp



Chung, et al., The application of miniplex primer sets in the DNA profiling of human skeletal remains, submitted

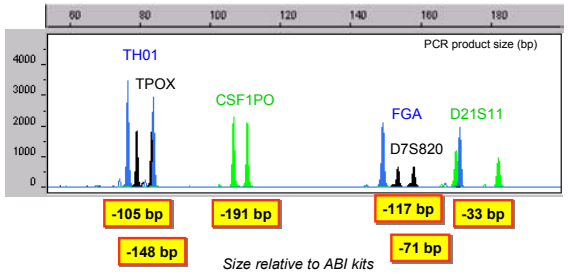
J. Forensic Sci. Sept 2003 issue

J Forensic Sci, September 2003, Vol. 48, No. 5
Paper ID JFS2003043_485
Available online at: www.astm.org

John M. Butler,[1] Ph.D.; Yin Shen,[2,3] Ph.D.; and Bruce R. McCord Ph.D.[2]

The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA*

Describes new primer sequences for all CODIS loci and initial assays developed



Size relative to ABI kits

Plans for Improved miniSTR Markers

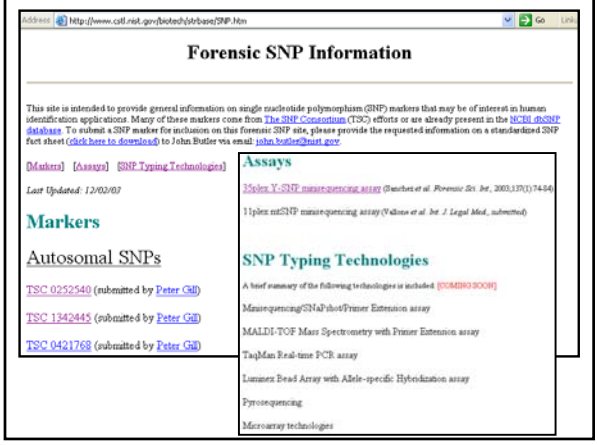
(going beyond the CODIS 13)

- New markers with smaller allele ranges, low stutter, and better characteristics for small PCR products (will make use of Human Genome Project information)
- Additional STRs to aid in large mass disasters to provide higher discrimination power than is possible with 13 CODIS loci
- Coverage of all chromosomes (22 autosomes + X/Y)
- Dual development of primer sets to enable null allele detection
  - large megaplex system for population data collection
  - miniplex systems to aid casework situations

Will be discussed in more detail at AAFS presentation (Dallas, Feb 2004)

SNP Typing at NIST

- STRBase is the official ISFG repository of forensic SNP information
  - 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
- We intend to evaluate SNP performance directly against miniSTRs for analysis of degraded DNA



Forensic SNP Information

This site is intended to provide general information on single nucleotide polymorphism (SNP) markers that may be of interest in human identification applications. Many of these markers come from The SNP Consortium (TSC) efforts or are already present in the NCBI dbSNP database. To submit a SNP marker for inclusion on this forensic SNP site, please provide the requested information on a standardized SNP fact sheet (click here to download) to John Butler via email: john.butler@nist.gov.

[Markers] [Assays] [SNP Typing Technologies]

Last Updated: 12/02/03

Markers

Autosomal SNPs

TSC 0252540 (submitted by Peter Gill)

TSC 1342445 (submitted by Peter Gill)

TSC 0421768 (submitted by Peter Gill)

Assays

35plex Y-SNP minisequencing assay (Sanchez et al. Forensic Sci. Int., 2003;137(1):74-84)

11plex mtSNP minisequencing assay (Vallone et al. Int. J. Legal Med., submitted)

SNP Typing Technologies

A brief summary of the following technologies is included [COMING SOON]

Minisequencing/SNaPshot/Primer Extension assay

MALDI-TOF Mass Spectrometry with Primer Extension assay

TaqMan Real-time PCR assay

Luminex Bead Array with Allele-specific Hybridization assay

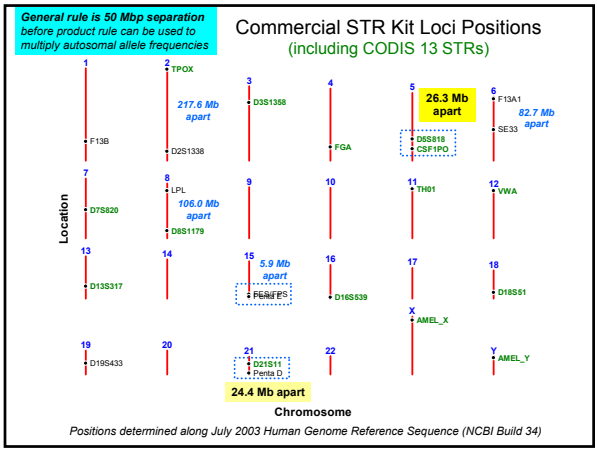
Pyrosequencing

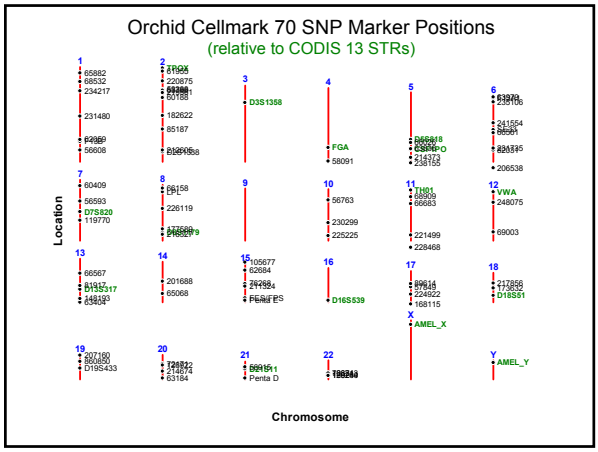
Microarray technologies

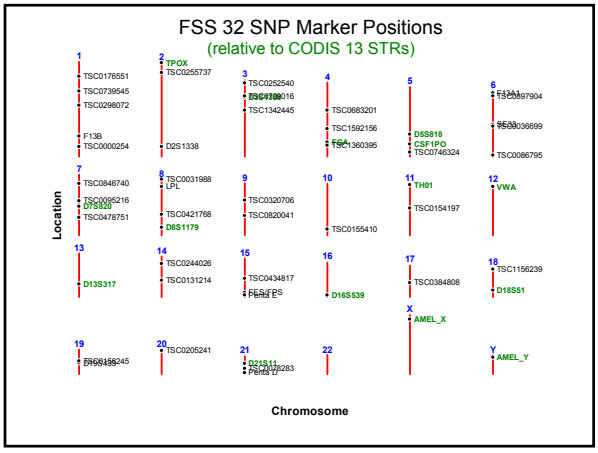
Chromosome Positions for Orchid SNPs, FSS SNPs, and CODIS and other kit STRs

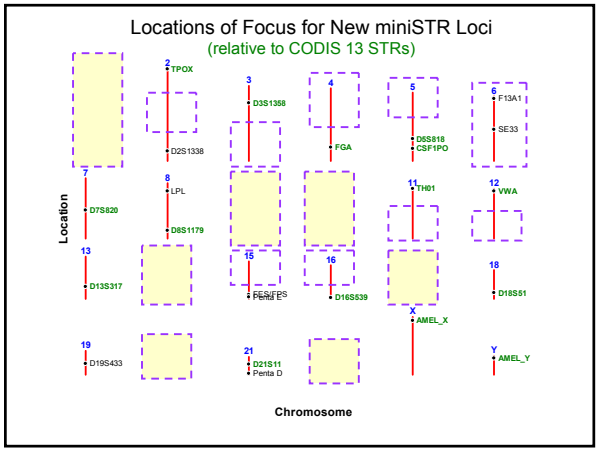
Multiplex	Polymorphism	SNP (STR)	Chr	Position (bp)	Delta Distance (bp)
Forensic 16	C/T	65882	1	15,083,389	
FSS 26plex	A/T	TSC0176551	1	37,237,110	22,153,721
Forensic 13	C/T	68532	1	37,373,821	136,711
Forensic 17D	C/T	234217	1	62,576,736	25,202,915
FSS 26plex	A/T	TSC0739545	1	76,045,653	13,468,917
FSS 26plex	A/T	TSC0298072	1	113,585,934	37,540,281
Forensic 15	C/T	231480	1	127,795,556	14,209,622
Forensic 13	C/T	62059	1	188,625,020	60,829,464
FFFL kit	$[AAAT]_{8-11}$	F13B	1	194,296,000	5,670,980
Forensic 13	C/T	56608	1	216,351,977	22,055,977
FSS 26plex	A/T	TSC0000254	1	220,649,838	4,297,861
CODIS STR	$[GAAT]_{6-13}$	TPOX	2	1,436,000	
Forensic 16	C/T	61955	2	8,262,536	6,826,536
FSS 26plex	C/G	TSC0255737	2	26,495,179	18,232,643
Forensic 13	C/T	220875	2	33,858,492	25,595,956
Forensic 14	C/T	58388	2	57,015,551	23,157,059
Forensic 12	C/T	63799	2	57,807,654	792,103
Forensic 14	C/T	219561	2	64,320,501	6,512,847
Forensic 15	C/T	60188	2	77,914,715	13,594,214
Forensic 14	C/T	182622	2	124,697,754	46,783,039
Forensic 15	C/T	85187	2	159,832,074	35,134,320
Forensic 16	C/T	212605	2	213,930,876	54,098,802
Identifiler kit	$[TKCC]_{15-28}$	D2S1338	2	219,082,000	5,151,124

General rule is 50 Mbp separation *before product rule can be used to multiply autosomal allele frequencies*









Why is Accurate DNA Quantitation Important in Forensic DNA Testing?

- Limited amount of DNA available
  - Usually cannot perform multiple tests for quantity
  - Want to preserve DNA for STR testing
- Optimal signal from multiplex STR reactions is only in a tight concentration range (usually 0.5-2 ng)
  - Too much DNA leads to split peaks, off-scale peaks, and bleed through between dye colors
  - Too little DNA leads to loss of loci or alleles due to stochastic effects

Goals for NIST 2004 DNA Quantitation Study

Samples already shipped to 80 laboratories

- Evaluate new real-time PCR assays
- Evaluate behavior of single-source vs. mixed source DNA
- Study shipping conditions for prototype human DNA quant standard (SRM 2372)
  - Teflon tube included in study

Enough sample provided for multiple technologies and analysts to try

Results due by March 15, 2004

8 DNA Samples in This NIST Study

Laboratories are only being asked to provide their quant values (no typing results expected)

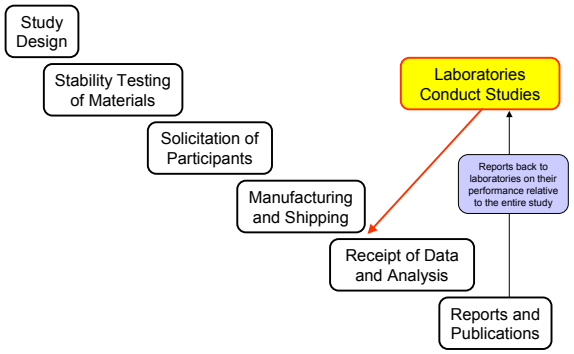
Mixed source DNA

Single source DNA

Teflon tube

Volume of each DNA sample provided = 100 µL

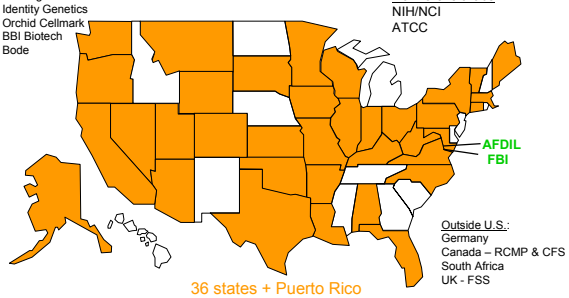
Process for Interlaboratory Study



Participation in NIST Interlaboratory Study on DNA Quantitation

Companies:
Applied Biosystems
Promega
Identity Genetics
Orchid Cellmark
BBI Biotech
Bode

Non-forensic labs:
NIH/NCI
ATCC



Outside U.S.:
Germany
Canada – RCMP & CFS
South Africa
UK - FSS

36 states + Puerto Rico

As of 12-30-03, 80 laboratories been sent samples

Real-Time PCR Efforts

- Marie Allen – nuclear and mtDNA assay (BioTechniques 2002, 33(2): 402-411)
- Eric Buel – Alu system (JFS 2003, 48(5):936-944)
- Centre for Forensic Sciences – nuclear; TH01 flanking region (JFS 2003, 48(5):1041-1046)
- John Hartmann – Alu system (SWGDAM Jan 2003)
- CA-DOJ – TH01 assay (NIJ DNA Grantees June 2003)

- SYBR Green assay – human-specific with right PCR
- Quantifiler kit (ABI) – separate nuclear and Y assays

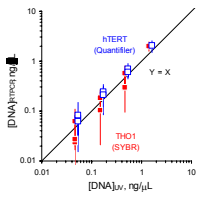
Some Lessons Learned from Real Time-PCR Assays

We are using ABI 7000 (some work also with Roche LightCycler)

- Results are RELATIVE to standards used
- Single source and mixed source samples with same UV concentrations differ with RT-PCR assays
- Need to keep instrument clean to avoid background fluorescence problems
- Assay reagent costs:
  - Quantifiler: $2.46/sample (only permits 2 µL/sample)
  - SYBR Green: $0.80/sample (up to 10 µL/sample)
  - QuantiBlot: $0.54/sample (5 µL/sample)

Comparison of Methods and DNA Quant Standards (UV vs. RTPCR in ng/µL)

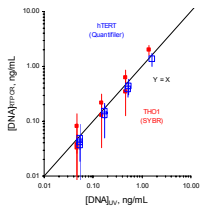
ABI Quant Standard



Qfiler **over** estimates [DNA]

$SYBR_{TH01}$ source dependent [DNA]

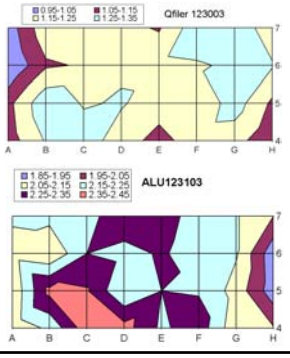
Promega Quant Standard



Qfiler **under** estimates [DNA]

$SYBR_{TH01}$ source dependent [DNA]

Measurement of Consistency Across a Plate Using the Same DNA Sample



Quantifiler Assay (Applied Biosystems)

1.20 +/- 0.09 ng/uL
(N=32; 7.5%RSD)

Against ABI standard
Std curve: 50 ng to 0.023 ng

SYBR Alu Assay (Nicklas and Buel)

2.18 +/- 0.13 ng/uL
(N=32; 6.0%RSD)

Against Promega standard (G3041)
Std curve: 10 ng to 0.021 ng

Information from Quantifiler Kit Manual

Table 6-10 Comparison with A_{260} and Quantiblot kit

Sample	Sex	A_{260} Result (ng/µL)	QB[a] Result (ng/µL)	Quantifiler Human Kit			Quantifiler Y Kit		
				Result (ng/µL)	% Diff. from A_{260}	% Diff. from QB	Result (ng/µL)	% Diff. from A_{260}	% Diff. from QB
1	M	17.5	20	6.69	61.7	66.6	10.13	41.9	49.4
2	M	15.4	20	14.3	7.1	28.5	16.78	9.0	16.1
3	M	13.9	30	15.48	11.4	48.4	14.30	2.9	52.3
4	M	11.4							37.8
5	M	10.3							45.0
6	M	13.9							32.2
7	M	11.5							69.3

The different methods produced similar quantification results.

Table 6-11 Average differences from A_{260} and Quantiblot kit

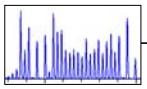
Method	Average Difference (%)	
	Quantifiler Human Kit	Quantifiler Y Kit
A_{260}	16.9	15.1
Quantiblot	42.0	35.5

Quantifiler Kits User's Manual 6-29

NIST QA/QC Software

Tool being developed by Dave Duewer for STR Process Control

Will track allelic ladders and positive controls



NCBI Program

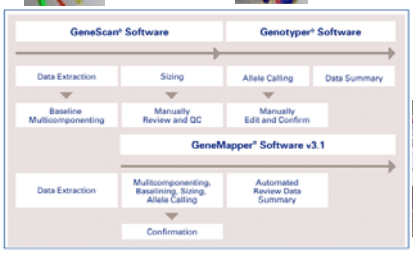
Peak Height, Area, Size

X | Y
103 | 436
104 | 569

NIST ControlCharter → Date vs Sensitivity, Resolution, Precision

NIST ControlCharter → Date vs Signal/Noise

GeneMapper ID v 3.1 Software



The above workflow demonstrates how GeneScan® and Genotyper® software functions are combined into the new, streamlined GeneMapper® ID Software v3.1.

Released in Nov 2003

NIST was a GeneMapper beta-test site

We participated in WebEx training with ABI on Dec 8, 2003

Applied Biosystems will no longer sell GeneScan and Genotyper after June 2004 (will support until June 2009)

STRBase

Short Tandem Repeat DNA Internet Database

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

Recent Additions

- **Forensic SNP Information** (will be official site for ISFG SNP information) .../SNP.htm
- **NIST publications** and presentations as pdf files .../NISTpub.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

Variant Alleles Cataloged in STRBase

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

Off-Ladder Alleles

201 total variants reported as of 09/05/03

- CSF1PO (9)
- D2S1338 (2)
- D3S1358 (15)
- D5S818 (5)
- D7S820 (19)
- D8S1179 (4)
- D13S317 (8)
- D16S539 (10)
- D18S51 (27)
- D19S433 (4)
- D21S11 (19)
- FESFPS (1)
- FGA (60)
- HUMTH01 (4)
- Penta E (2)
- TPOX (7)
- VWA (5)

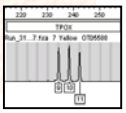
Currently **201** at 13/13 CODIS loci

Tri-Allelic Patterns

49 total patterns reported as of 09/15/03

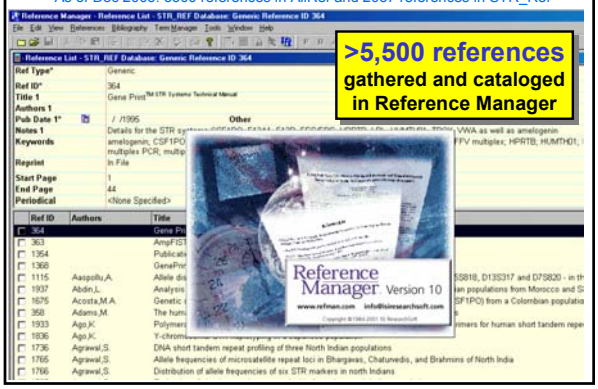
- CSF1PO (2)
- D3S1358 (4)
- D5S818 (1)
- D7S820 (0)
- D8S1179 (3)
- D13S317 (3)
- D16S539 (1)
- D18S51 (4)
- D21S11 (4)
- FGA (7)
- HUMTH01 (1)
- TPOX (12)
- VWA (7)

Currently **49** at 12/13 CODIS loci



Reference Manager Database

As of Dec 2003: 3500 references in AllRef and 2037 references in STR_Ref



Tools to Aid Forensic DNA Labs

- Quality assurance testing software (not for typing) to track peak resolution and S/N over time
  - Dave Duewer in collaboration with NCBI
- Interlaboratory DNA Quantitation Study
- Quantitation standard SRM 2372
- Further evaluation of real-time PCR methodologies and measurements for DNA quantitation
- More miniSTR work for degraded DNA
- Comparison of new SNP markers to miniSTRs on degraded DNA samples
- STRBase updates to standardize information for the community

Presentations at AAFS Feb 2004

- Forensic Human Mitochondrial DNA Analysis Workshop
  - February 16, 2004
  - John Butler: "SNPs and Strips"
- Y-STR Analysis on Forensic Casework Workshop
  - February 17, 2004
  - John Butler: "Going Beyond the U.S. Haplotype: A Look at Additional Y-STR and Y-SNP Loci in U.S. Populations"
- Criminalistics Section DNA III
  - February 20, 2004
  - Mike Coble: "Development of New miniSTR Loci for Improved Analysis of Degraded DNA Samples"