DNA Profiling and Quantitation of Human DNA

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NIST

CCQM BAWG (Sevres, France)
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National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Research Effort

- **Standard Information Resources** (STRBase information, training materials/review articles, validation standardization, calibration datasets)
- **Interlaboratory Studies** (Real-time PCR, mixture interpretation)
- **Resources for "Challenging Samples"** (miniSTRs for degraded DNA)
- **Information on New Loci** (Y-Chromosome, new STRs)

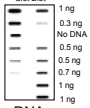
Steps in DNA Analysis

Usually 1-2 day process (a minimum of ~5 hours)

Collection
Specimen Storage
Extraction
Quantitation
Genotyping
Interpretation of Results
Database
Storage & Searching

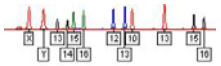

Blood Stain Buccal swab
Sample Collection & Storage


DNA Extraction



DNA Quantitation


Multiplex PCR
(Amplification of STR Loci)

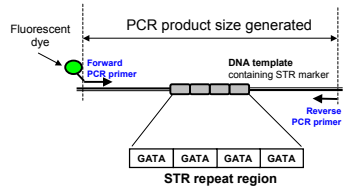

STR Typing (DNA separation)

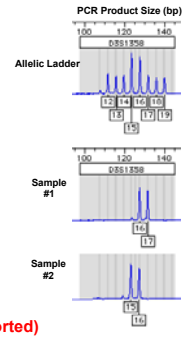
Male: 13,14-15,16-12,13-10,13-15,16
Interpretation of Results


DNA Database

Short Tandem Repeat (STR) Markers

PCR primers anneal to unique sequences bracketing the variable STR repeat region





TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGA
AGACAGGTG**GATAGATAGATAGATAGATAGATAGATA**
GATAGATAGATAGATATCATTGAAAGACAAAACAGAGA
TGGATGATAGATACATGCTTACAGATGCACAC

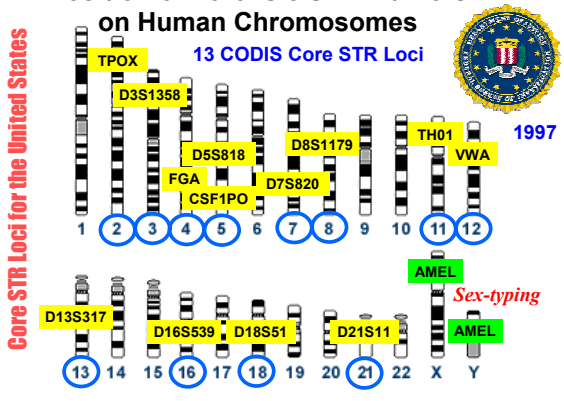
= 11 GATA repeats ("11" is all that is reported)

Position of Forensic STR Markers on Human Chromosomes

Core STR Loci for the United States

13 CODIS Core STR Loci

1997

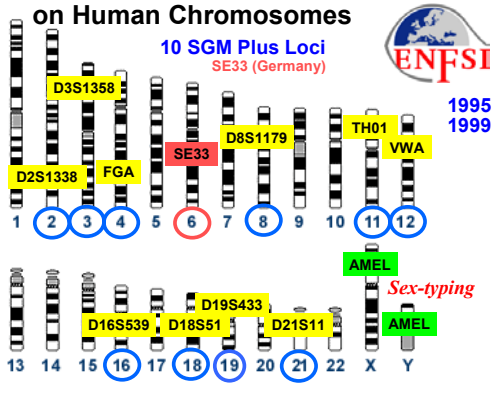


Position of Forensic STR Markers on Human Chromosomes

Core STR Loci for Europe

10 SGM Plus Loci
SE33 (Germany)

1995
1999



Congress Passed **the DNA Identification Act of 1994** (Public Law 103 322)

Formalized the FBI's authority to establish a national DNA index for law enforcement purposes.

FBI's DNA Advisory Board
Quality Assurance Standards for Forensic DNA Testing Laboratories

(October 1, 1998)

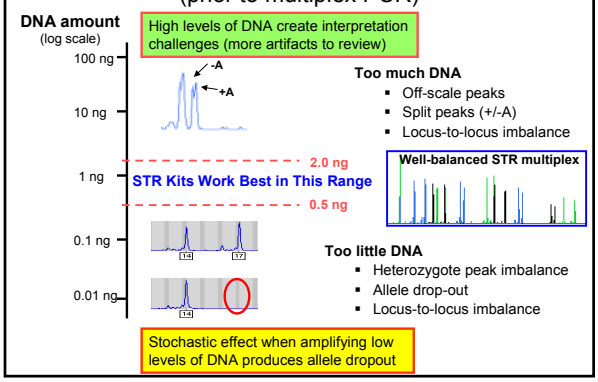
STANDARD 9.5

The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

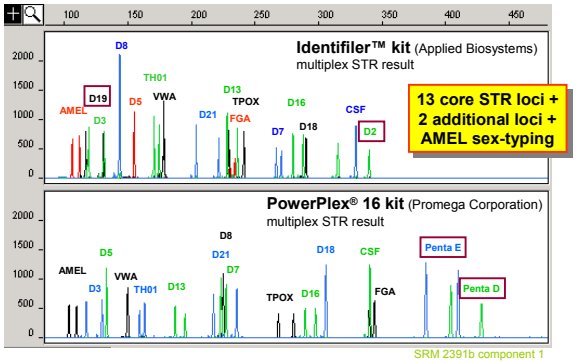
Human DNA Quantitation

- **FBI STANDARD 9.3** The laboratory shall have and follow a procedure for evaluating the quantity of the human DNA in the sample where possible.
- **No requirement on how accurate DNA quantitation needs to be…**
- Quantitation is important in order to determine how much DNA template to put into the PCR reaction

Importance of DNA Quantitation
(prior to multiplex PCR)

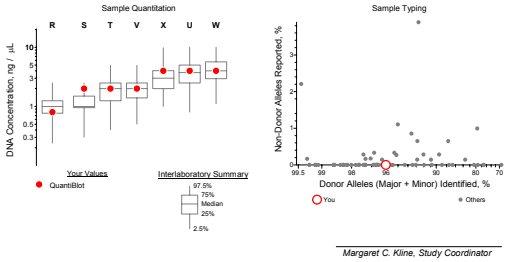


Commercial STR 16plex Kits



The National Institute of Standards and Technology
Gratefully Acknowledges the Participation of the

This feedback can be helpful to a laboratory to know where they stand relative to other labs to illustrate opportunities for improvement.



Margaret C. Kline, Study Coordinator

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)

Article will be published next month

To be published in the *Journal of Forensic Sciences* (May 2005)

TECHNICAL NOTE

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Results from the NIST 2004 DNA Quantitation Study*

8 DNA Samples in NIST QS04 Study

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

Mixed source DNA

Single source DNA

Volume of each DNA sample provided = 100 µL

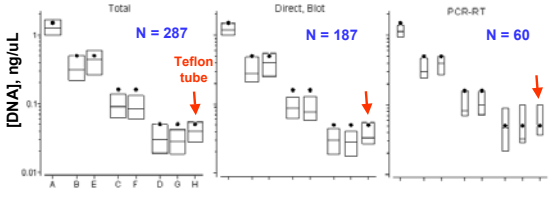
Table 2. The percent success rate reported for a sample.

		% Quantitative Results[a]								
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	Real-time PCR
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	Quantiblot
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].

Kline, *et al., J. Forensic Sci., in press (May 2005)*

NIST QS 04 Results-Box plots



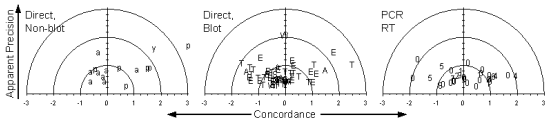
Width of the box is proportional to the number data points.

Line in the box is the median value.
The box represents 50% of the data submitted.

Dot is the target [DNA].

Kline, *et al., J. Forensic Sci., in press (May 2005)*

NIST QS 04 Results-Target Plots



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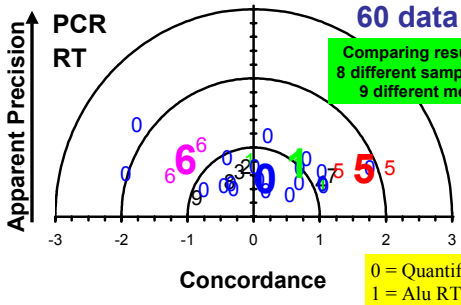
Interlaboratory Comparisons

Laboratory Performances with Real-Time PCR Methods



60 data sets

Comparing results from 8 different samples using 9 different methods

0 = Quantifiler
1 = Alu RT-PCR
5 = BRCA1
6 = CFS-HUMRT

Kline, *et al., J. Forensic Sci., in press (May 2005)*

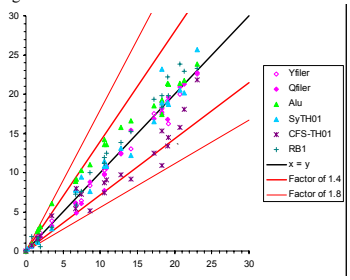
Comparison of real-time qPCR assays

Following Published Protocols

Series of NIST population samples with a range of [DNA] from 40 pg to 23 ng

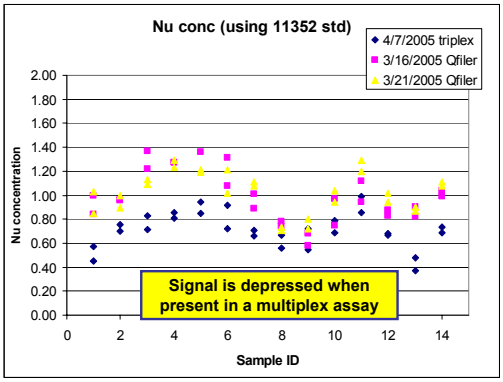
The same "Standard" was used for all methods (8 dilutions).

Time for the assay:
Alu-RT-PCR ~ 1.25 h
(fewer cycles required)
The rest ~ 1.75 h



X = median value for all methods
Y = measured value for the method

Recent Examination of Intra-Lab Reproducibility



On-going Studies

- DNA stability in various types of tubes
- Examination of impact of DNA stability at different DNA concentrations
- SRM 2372 Human DNA Quantitation standard is under development (genomic DNA)

Additional Resources

- *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers (2nd Edition)* by John M. Butler, Elsevier Academic Press, 2005
- Butler, J.M., *et al.* (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. *Electrophoresis* 25: 1397-1412.
- NIST website: **http://www.cstl.nist.gov/biotech/strbase**