

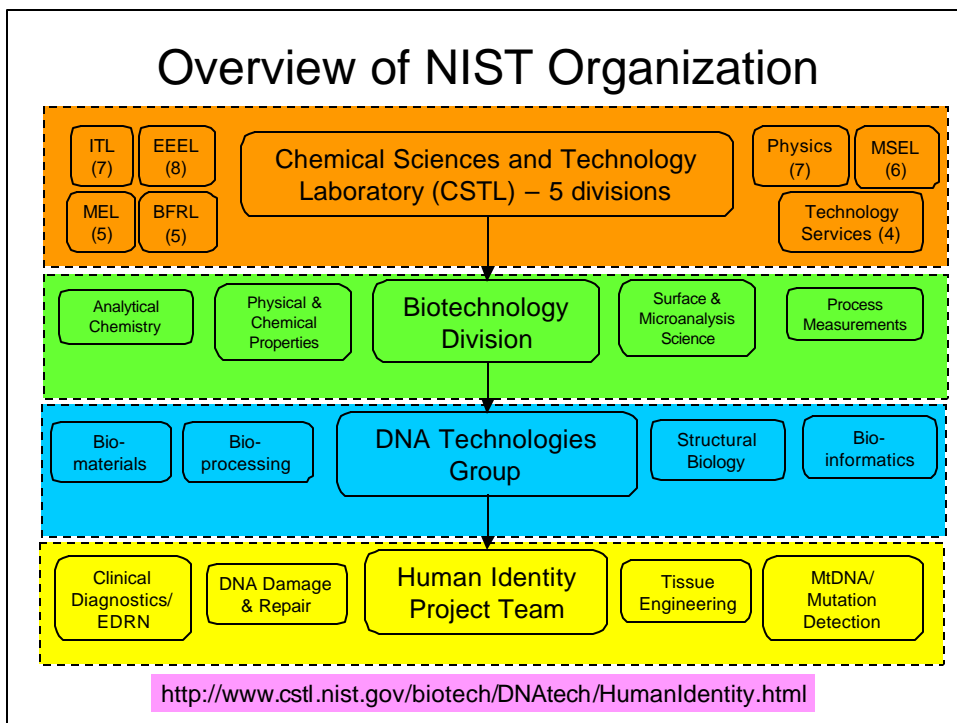
NIST
National
Institute of
Standards
and Technology

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

NIST Developments in Y-Chromosome Analysis

John Butler

Seminar for Relative Genetics and Myriad Genetics
August 29, 2003

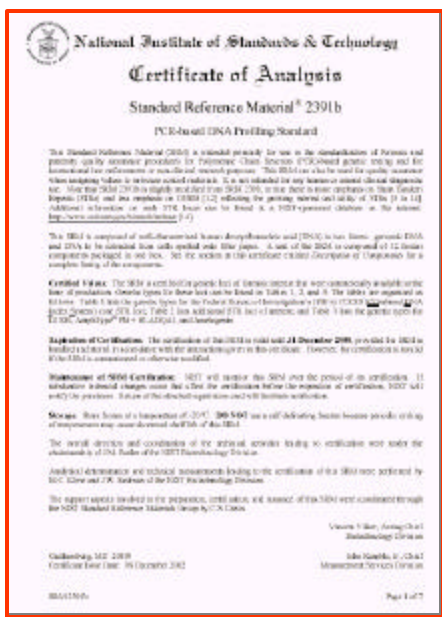




... working with industry to develop and apply technology, measurements and standards

- Standard Reference Materials
 - SRM 2391b PCR-based DNA Profiling Standard
 - SRM 2395 Human Y-Chromosome DNA Profiling Standard
- Creating databases with useful information
 - STRBase (<http://www.cstl.nist.gov/biotech/strbase>)
- Evaluating and developing new technologies
- Interlaboratory testing
- Quality control testing for labs & companies

NIST SRM 2391b



National Institute of Standards & Technology
Certificate of Analysis
 Standard Reference Material[®] 2391b
 PCR-based DNA Profiling Standard

The Standard Reference Material (SRM) is a standard provided for use in the identification of persons and paternity, quality assurance procedures for Polymerase Chain Reaction (PCR)-based genetic testing and for forensic and law enforcement or medical and research purposes. The SRM is also used for quality assurance when analyzing human DNA extracted from blood, hair, or other forensic or medical samples.

The SRM is composed of well-characterized human deoxyribonucleic acid (DNA) in two forms: genomic DNA and STRs to be obtained from cells stored under other pages. A set of the SRM is composed of 12 human samples prepared in duplicate in two sets. See the section in the certificate chart description of Components for a complete listing of the components.

Characterization: The SRM is a set of 16 autosomal STR loci for forensic use. The non-complexity analysis of the loci of resolution cluster types for these loci can be found in Tables 1, 2, and 3. The SRM is organized as follows: Table 1 lists the genetic types for the Polymerase Chain Reaction (PCR)-based genotyping (STRs); Table 2 lists the genetic types for the Polymerase Chain Reaction (PCR)-based genotyping (STRs); Table 3 lists the genetic types for the Polymerase Chain Reaction (PCR)-based genotyping (STRs).

Stability of the SRM: The stability of the SRM is maintained as of December 2000, provided the SRM is stored in the SRM. It is subject to the same conditions as the SRM. However, the conditions listed in the SRM is maintained as otherwise modified.

Stability of SRM Certification: NIST will maintain the SRM over the period of its certification. It anticipates additional changes over the SRM. The certification for the SRM will be maintained as of December 2000. The SRM will be maintained as of December 2000. The SRM will be maintained as of December 2000.

Storage: This form is a component of the SRM. The SRM is a self-declaring human genome profile of 16 autosomal STRs. The SRM is a component of the SRM. The SRM is a component of the SRM.

The serial numbers and coordinates of the serial number leading to certification were under the authority of the Director of the NIST Technology Division.

Additional information and technical assistance leading to the certification of the SRM may be obtained by NIST. For more information, contact the NIST Technology Division.

The support services provided in the preparation, certification, and storage of the SRM were conducted through the NIST Standard Reference Materials Group by NIST.

Challenging SRM 2391b
 Certificate Date: 05 December 2002

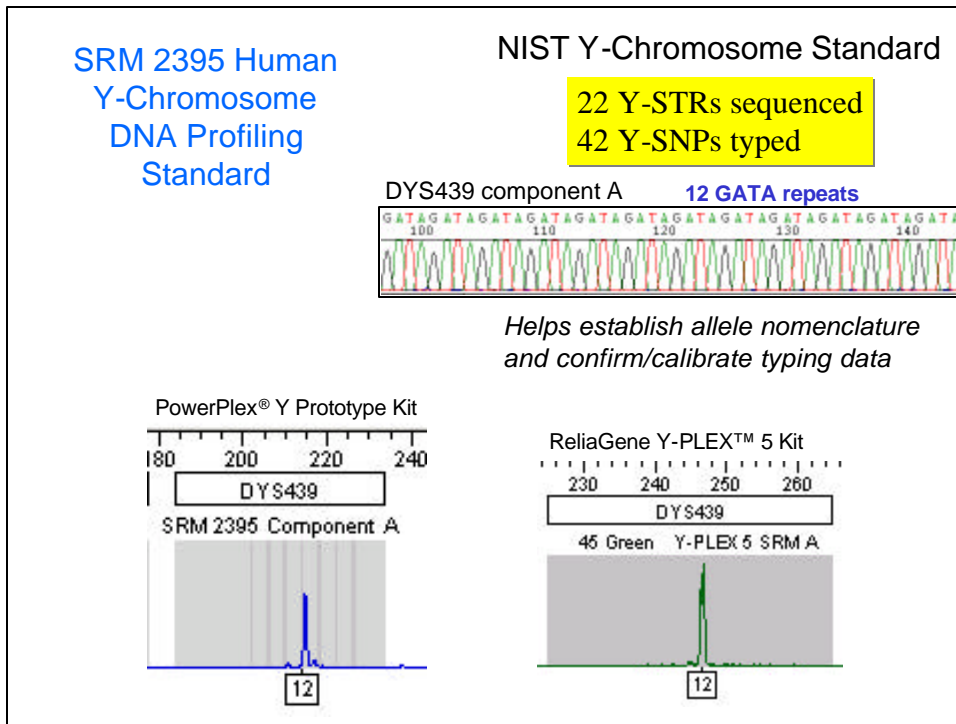
Vernon J. Allen, Group Chief
 Technology Division
 John W. Smith, Jr., Chief
 Management Services Group

SRM1390a Page 1 of 7

Fixed Values for Additional STR Loci

FES/FPS	LPL	Penta D	Penta E	D2S1338	D19S433
12,12	10,11	10,15	7,12	17,23	13,16,2
10,11	10,11	9,11	7,12	17,26	14,16
11,12	11,12	11,12	13,14	20,24	12,14
10,13	10,12	8,9	5,12	17,23	11,13
11,13	10,12	10,13	7,13	17,19	12,2,14
11,11	10,12	9,12	12,14	25,25	12,14
11,11*	11,12	3,2,11	12,16	17,22	13,15,2
10,11	9,11	8,9	5,10	22,22	12,2,15
10,12					1,5
11,11					1,4
10,12					1,5
11,11	10,12	8,12	11,11	25,25	13,14

22 autosomal STRs characterized across 12 DNA samples



STRBase

Short Tandem Repeat DNA Internet Database

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

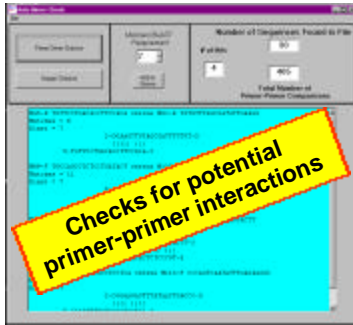
<p><u>General Information</u></p> <ul style="list-style-type: none"> •Intro to STRs (downloadable PowerPoint) •STR Fact Sheets •Sequence Information •Multiplex STR Kits •Variant Allele Reports 	<p><u>Forensic Interest Data</u></p> <ul style="list-style-type: none"> •FBI CODIS Core Loci •DAB Standards •NIST SRM 2391 •Published PCR Primers •Y-Chromosome STRs •Population Data •Validation Studies 	<p><u>Supplemental Info</u></p> <ul style="list-style-type: none"> •Reference List •Technology Review •Addresses for Scientists •Links to Other Web Sites <p>Standardized information formats</p>
---	--	---

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

Technology Development Efforts

Centered around multiplex PCR...

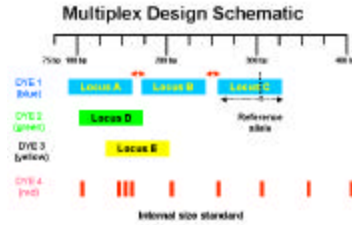
Created Custom Primer Design Software



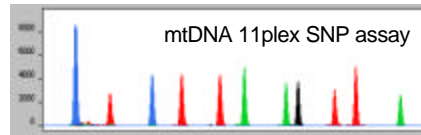
Implemented Quality Control Methods for PCR Primers



Standardized Assay Design Formats



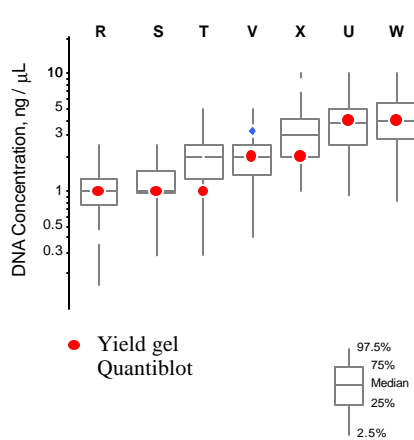
Demonstrated Success with Multiple Projects and Collaborations



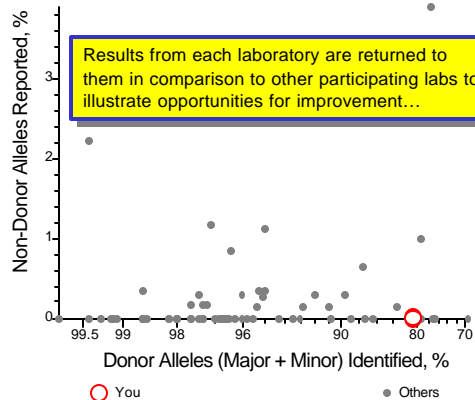
Y-STR 20plex, cat STR 12plex

Individual Performance in an Interlaboratory Study

DNA Quantitation



Accuracy in STR Typing



See Kline, M.C., et al. (2003) *Anal. Chem.* 75: 2463-2469

Steps in DNA Analysis

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

Collection & Storage (Blood Stain)
DNA Extraction
Slot Blot
PicoGreen
DNA Quantitation
Multiplex PCR Amplification
STR Typing
Male: 13,14-15,16-12,13-10,13-15,16
Interpretation of Results
DNA Database

NIST Involvement in DNA Analysis

[Steps in DNA Analysis](#)

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

Forensic Sci. Int. (2002) 129:10-24
John M. Butler*, Richard S..., Alan J...

Genetic Genealogy Companies
FamilyTreeDNA
Oxford Ancestors
Relative Genetics
GeneTree DNA TESTING CENTER

- Improving multiplex PCR
- Increasing capabilities for Y-chromosome DNA testing

PCR Product Size (bp)
Relative Fluorescence Units

426 460 H4 393 YCAII a/b 389I 388 437 390 439 447 19 385 a/b 389I 392 448 438

Why Use Y?

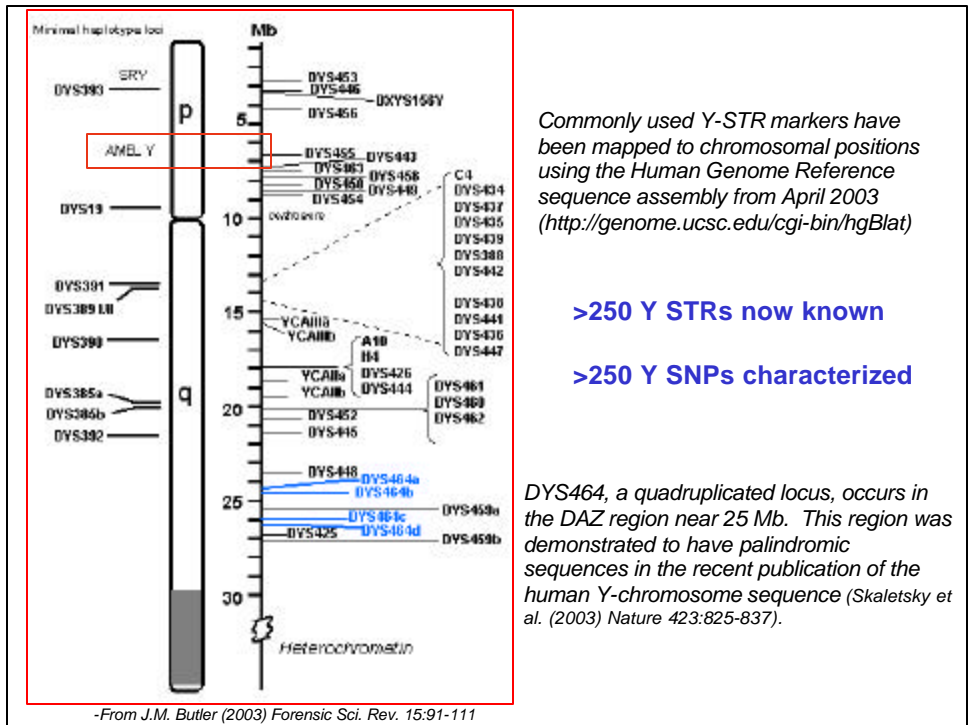
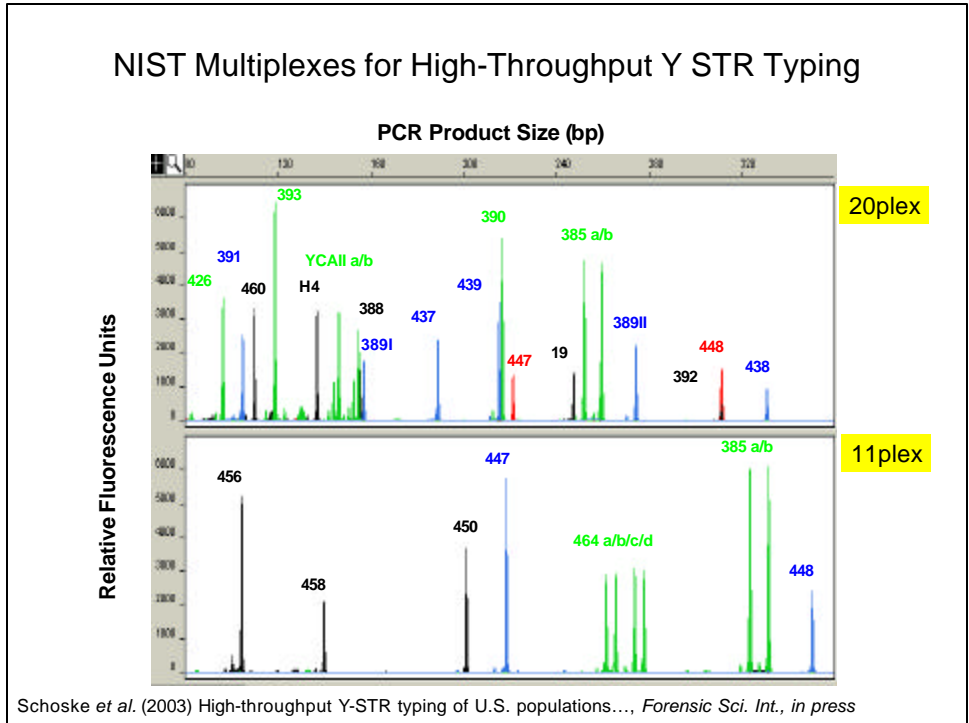
Application

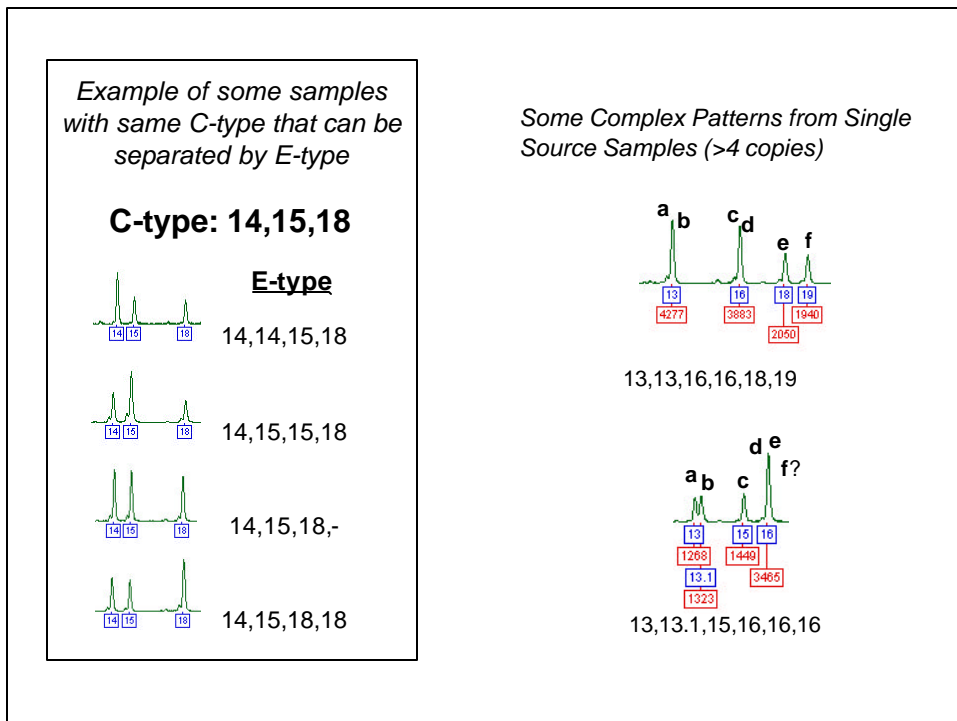
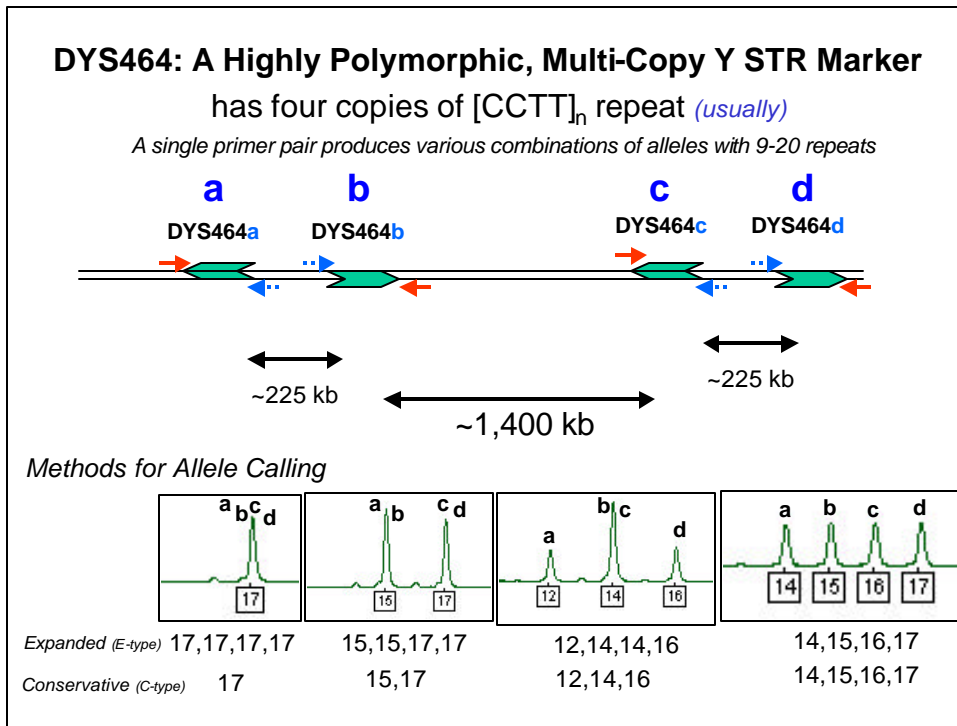
Advantage

Forensic casework on sexual assault evidence	Male-specific amplification (can avoid differential extraction to separate sperm and epithelial cells)
Paternity testing	Male children can be tied to fathers in motherless paternity cases
Missing persons investigations	Patrilineal male relatives may be used for reference samples
Human migration and evolutionary studies	Lack of recombination enables comparison of male individuals separated by large periods of time
Historical and genealogical research	Surnames usually retained by males; can make links where paper trail is limited

History of Y STR Marker Discovery

- 1992 - **DYS19** (Roewer et al.) "Extended Haplotype"
- 1994 - YCAI a/b, **YCAII a/b**, YCAIII a/b, DXYS156 (Mathias et al.)
- 1996 - **DYS389I/II**, **DYS390**, **DYS391**, **DYS392**, **DYS393** (Roewer et al.)
- 1996 - DYF371, DYS425, DYS426 (Jobling et al.)
- 1997 - DYS288, DYS388 (Kayser et al.)
- 1998 - **DYS385 a/b** (Schneider et al.) "Minimal Haplotype"
- 1999 - A7.1 (DYS460), A7.2 (DYS461), A10, C4, H4 (White et al.)
- 2000 - DYS434, DYS435, DYS436, DYS437, DYS438, DYS439 (Ayub et al.)
- 2000 - G09411 (DYS462), G10123 (de Knijff unpublished)
- 2001 - DYS441, DYS442 (Iida et al.)
- 2002 - DYS443, DYS444, DYS445 (Iida et al.); DYS446, DYS447, DYS448, DYS449, DYS450, DYS452, DYS453, DYS454, DYS455, DYS456, DYS458, DYS459 a/b, DYS463, DYS464 a/b/c/d (Redd et al.)
- 2002 - DYS468-DYS596 ([129 new Y STRs](#); Manfred Kayser GDB entries)
- 2003 - DYS597-DYS645 ([50 new Y STRs](#); Manfred Kayser GDB entries)

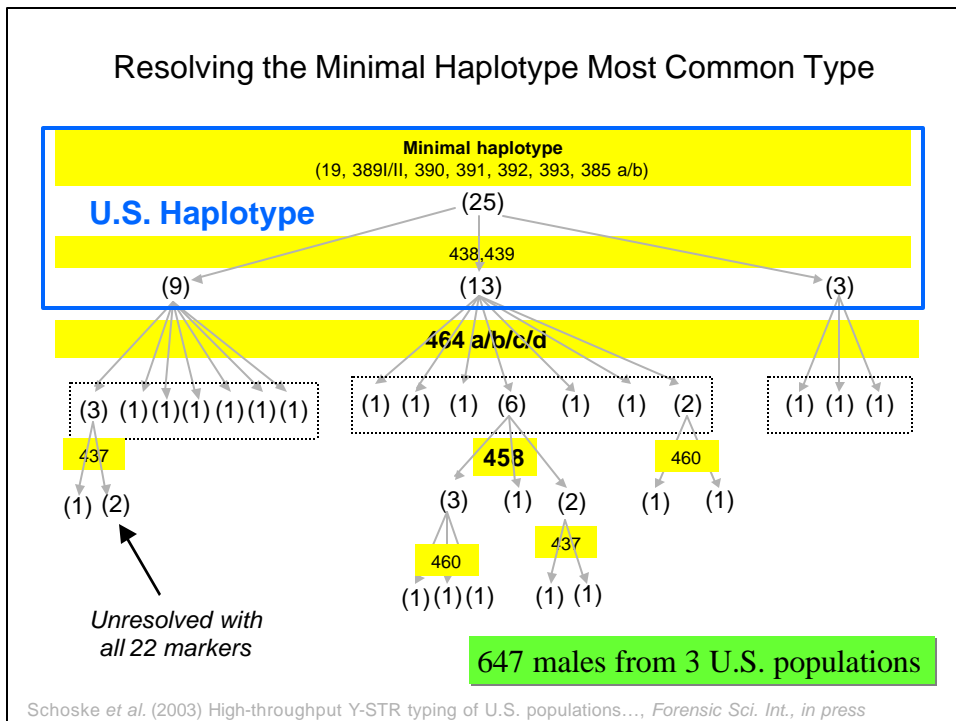


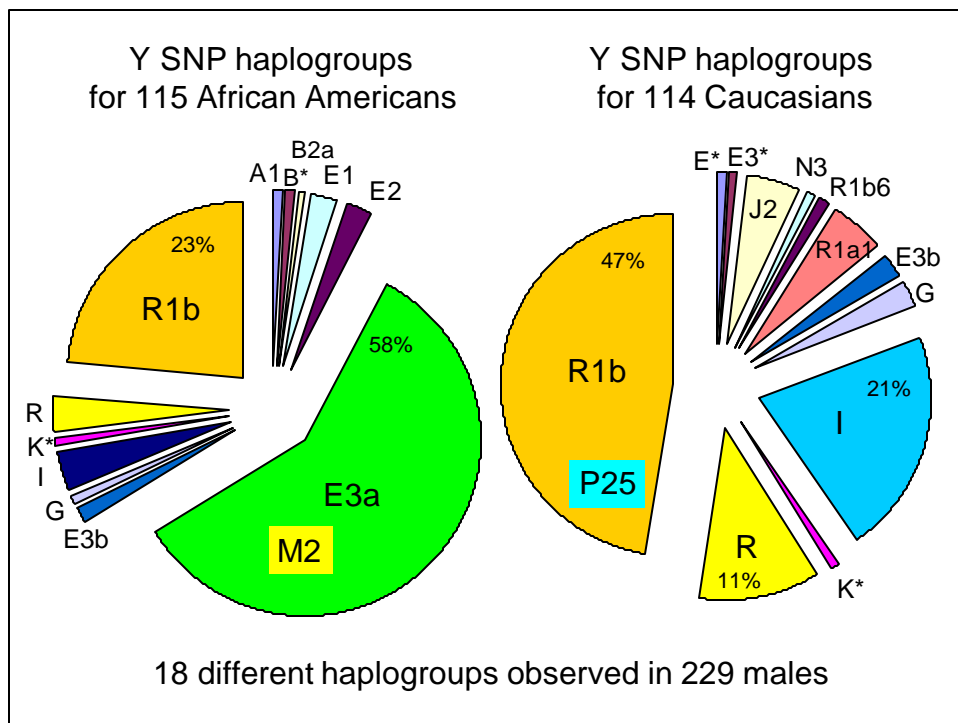
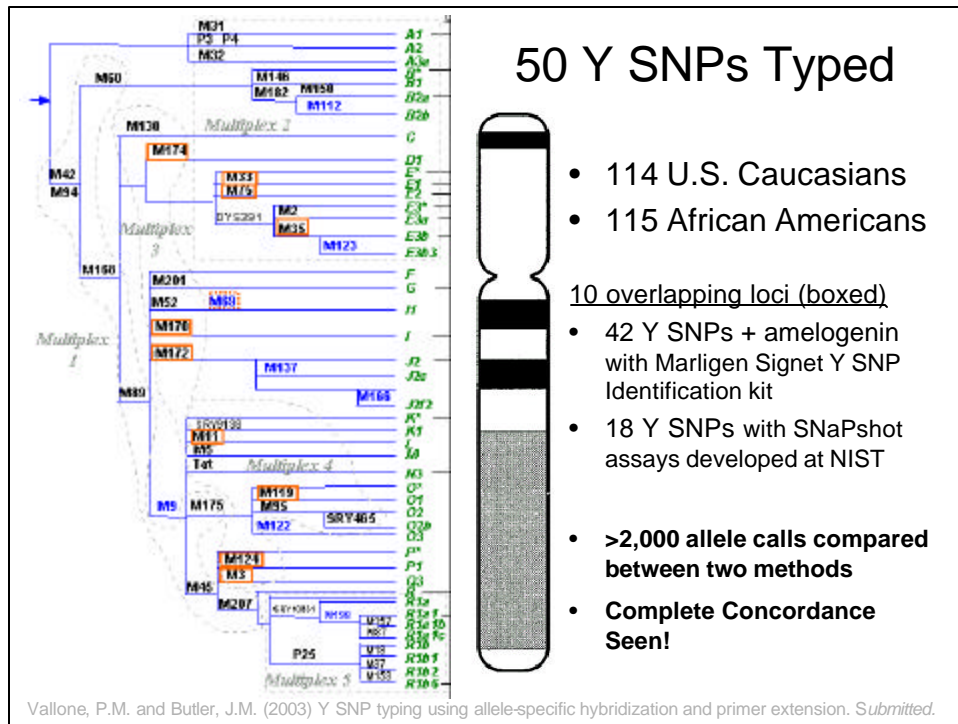


113 (179) different types in 679 males with DYS464

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
YCALL 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. (2003) High-throughput Y-STR typing of U.S. populations..., *Forensic Sci. Int.*, in press





Anal. Bioanal. Chem. (2003) 375: 333–343
DOI 10.1007/s00216-002-1683-2

ORIGINAL PAPER

Richard Schoske · Pete M. Vallone
Christian M. Ruitberg · John M. Butler

Multiplex PCR design strategy used for the simultaneous amplification of 10 Y chromosome short tandem repeat (STR) loci

Received: 3 July 2002 / Revised: 24 October 2002 / Accepted: 29 October 2002 / Published online: 14 January 2003
© Springer-Verlag 2003

Describes how to build STR multiplex assays...

Careful primer design

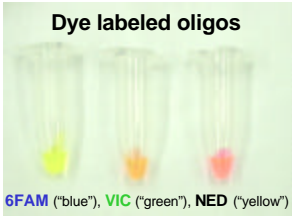
- Uniform annealing temperatures
- Checking for all potential primer-primer interactions

Potential Interaction
3-TAGTÀGATÀGÀCÀGÀGGTGGATÀCÀ-5
5-CCCCCTCCTCTCGTCTÀTCT-3

Butler et al. (2001) *Fresenius J. Anal. Chem.* 369:200-205

Stringent primer quality control

Dye labeled oligos



6FAM ("blue"), VIC ("green"), NED ("yellow")

Butler et al. (2001) *Forensic Sci. Int.* 119: 87-96

Acknowledgments



Funding:

Interagency Agreement between **National Institute of Justice** and **NIST Office of Law Enforcement Standards**

NIST Project Team:

Pete Vallone
Margaret Kline
Jan Redman
Rich Schoske (American U)
Dave Duewer

Collaborators:

Mike Hammer and **Alan Redd** (U. AZ)
for Y STR sequences

Publications from Our Group this Past Year

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

1. Kline, M.C., et al. (2002) Polymerase chain reaction amplification of DNA from aged blood stains: quantitative evaluation of the "suitability for purpose" of four filter papers as archival media. *Anal. Chem.* 74: 1863-1869.
2. Vallone, P.M., et al. (2002) A strategy for examining complex mixtures of deoxyoligonucleotides using IP-RP HPLC, MALDI-TOF MS, and informatics. *Anal. Biochem.* 304: 257-265.
3. Butler, J.M., et al. (2002) A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers. *Forensic Sci. Int.* 129: 10-24.
4. Butler, J.M., et al. (2002) The MeowPlex: a new DNA test using tetranucleotide STR markers for the domestic cat. *Profiles in DNA*, Promega Corporation, Volume 5, No. 2, pp. 7-10.
5. Redd, A.J., et al. (2002) Forensic value of fourteen novel STRs on the human Y chromosome. *Forensic Sci. Int.* 130: 97-111.
6. Schoske, R., et al. (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.
7. Butler, J.M., et al. (2003) Highly multiplexed assays for measuring polymorphisms on the Y-chromosome. *Progress in Forensic Genetics 9* (Brinkmann, B. and Carracedo, A., eds.), Elsevier Science: Amsterdam, The Netherlands, International Congress Series 1239, pp. 301-305.
8. Schoske, R. (2003) The design, optimization and testing of Y chromosome short tandem repeat megaplexes. Ph.D. dissertation, American University, 270 pp.
9. Kline, M.C., et al. (2003) NIST Mixed Stain Study 3: DNA quantitation accuracy and its influence on short tandem repeat multiple x signal intensity. *Anal. Chem.* 75:2463-2469.
10. Butler, J.M. (2003) Recent developments in Y STR and Y SNP analysis. *Forensic Sci. Rev.*, 15:91-111.
11. Butler, J.M., et al. (2003) Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J. Forensic Sci.*, 48(4): 908-911.
12. Butler, J.M., et al. (2003) The development of reduced size STR amplicons as tools for degraded DNA. *J. Forensic Sci.*, 48(5): 954-966.
13. Butler, J.M. and Vallone, P.M. (2003) High throughput genetic analysis through multiplexed PCR and multi-capillary electrophoresis. *PCR Technologies: Current Innovations* (2nd edition), *in press*.
14. Schoske R., et al. (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*.