Capillary Electrophoresis in DNA Analysis

Additional Topics: Y-STRs, etc.

NEAFS Workshop
Mystic, CT
September 29-30, 2004

Dr. John M. Butler
Dr. Bruce R. McCord

NIST National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

FIU Florida International University
Miami's public research university

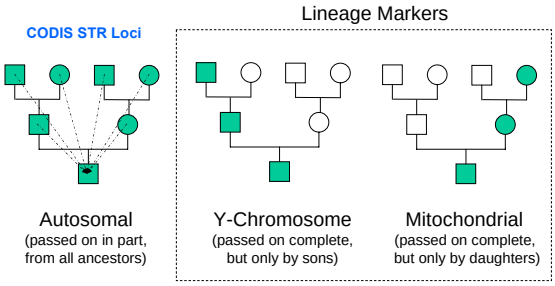
Outline for Workshop

- Introductions
- STR Analysis
- Introduction to CE and ABI 310
- Data Interpretation
- Additional Topics – Real-time PCR and miniSTRs
- Higher Throughput Approaches
- Troubleshooting the ABI 310 (Participant Roundtable)
- Additional Topics – Y-STRs, validation, accuracy
- Review and Test

Y-Chromosome

Information, Assays, and Standards

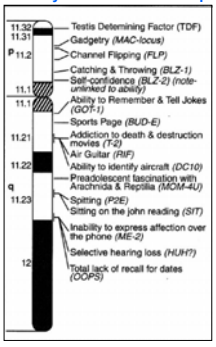
Different Inheritance Patterns



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 9.1, ©Elsevier Science/Academic Press

Traits found on the Y - Chromosome

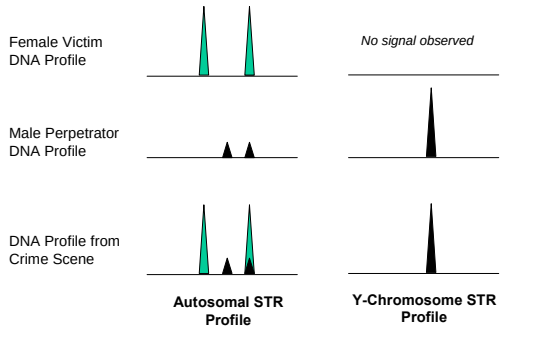
An Early Y-Chromosome Map



Science (1993) 261:679

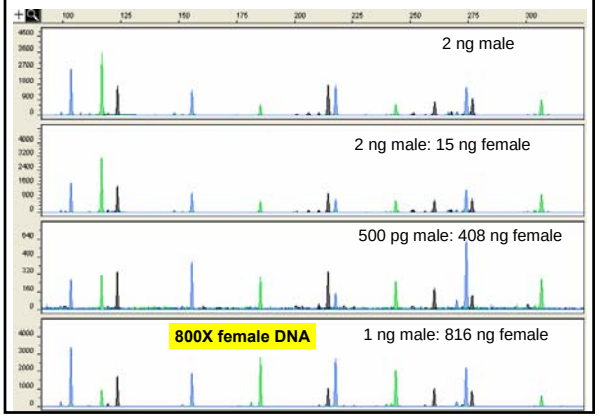
- spitting
- incessant use of TV remote buttons
- if lost, cannot stop and ask for directions
- ability to recall facts about baseball/basketball/hockey/golf/etc.
- male pattern baldness
- congregates with other Y-chromosome bearers to do "guy things"
- Source of "Testosterone poisoning"

Y-STRs can permit simplification of male DNA identification in sexual assault cases

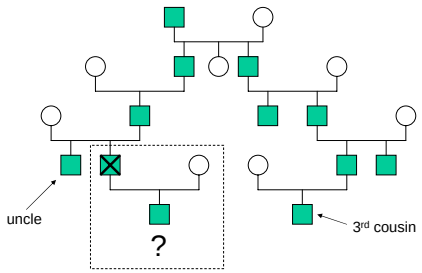


Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 9.2, ©Elsevier Science/Academic Press

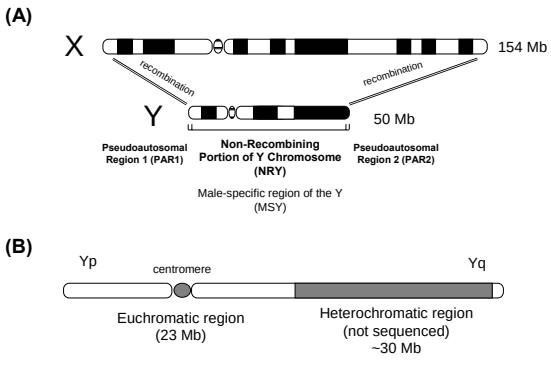
PowerPlex Y Performance in Our Hands



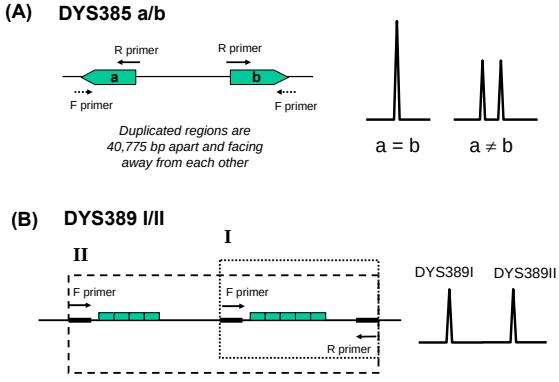
Y-STRs permit extension of possible reference samples in missing persons cases



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 9.3, ©Elsevier Science/Academic Press



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 9.4, ©Elsevier Science/Academic Press

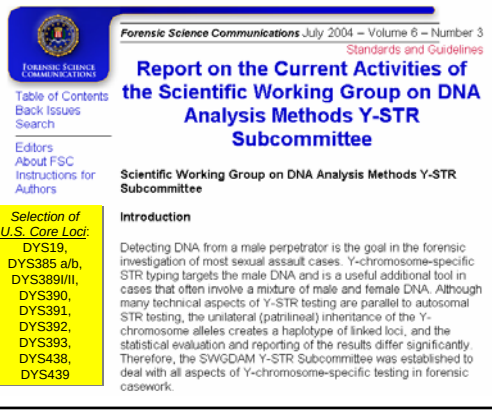


Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 9.5, ©Elsevier Science/Academic Press

SWGDAM Sub-Committee on the Y Chromosome

- Formed in July 2002
- Members
  - Jack Ballantyne (UCF) – chair
  - Mecki Prinz (NYC) – co-chair
  - Bruce Budowle (FBI)
  - **John Butler (NIST)**
  - Ann Gross (MN)
  - John Hartmann (Orange Co.)
  - Laura Kienker (FBI Academy)
  - Carll Ladd (CT)
  - Demris Lee (AFDIL)
  - Phil Kinsey (OR)
  - Barb Koons (FBI Academy)
  - Tim Kupferschmid (ME)
  - Gary Sims (CA DOJ)

- **U.S. CORE Y-STR LOCI selected in January 2003**
- 60 sample set selected for screening markers and initial testing
- Testing of Y-PLEX 6 and Y-PLEX 5 kits in all labs
  - *All results completed agreed with NIST results sent to participating labs in Dec 2002*
- Jack Ballantyne's lab and John Butler's lab to examine additional Y-STR and Y-SNP markers



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

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

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.

European and U.S. Core Y-STR Loci

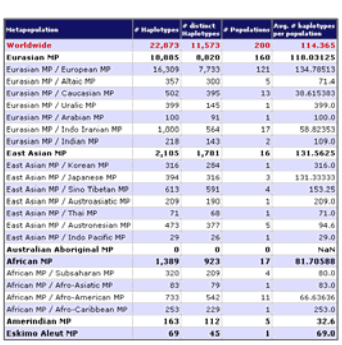
Marker Name	Allele Range (repeat numbers)	Repeat Motif
DYS19	10-19	TAGA
DYS385 a/b	7-28	GAAA
DYS389 I DYS389 II	I: 9-17 II: 24-34	(TCTG) (TCTA) (TCTG) (TCTA)
DYS390	17-28	(TCTA) (TCTG)
DYS391	6-14	TCTA
DYS392	6-18	TAT
DYS393	8-17	AGAT
YCAII a/b	11-25	CA
DYS438	6-14	TTTTC
DYS439	8-15	AGAT

Minimal haplotype (Europe)

U.S. haplotype

Extended haplotype (Europe)

http://www.yhrd.org



As of 5/24/04: 22,872 haplotypes

Run only with minimal haplotype

DYS19
DYS389I/II
DYS390
DYS391
DYS392
DYS393
DYS385 a/b

US haplotype requires 2 additional loci:
DYS438
DYS439

Commercial Y-STR Kits Available

- ReliaGene Technologies (New Orleans, LA)
  - **Y-PLEX™ 6**: DYS19, DYS389II, DYS390, DYS391, DYS393, DYS385 a/b
  - **Y-PLEX™ 5**: DYS389I/II, DYS392, DYS438, DYS439
  - **Y-PLEX™ 12**: DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, amelogenin
- Promega Corporation (Madison, WI)
  - **PowerPlex® Y**: DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, DYS437
- Applied Biosystems (Foster City, CA)
  - **Yfiler™**: DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS635 (Y-GATA-C4), Y-GATA-H4
- Serac (Bad Homburg, Germany)
  - **genRES® DYSplex-1**: DYS389I/II, DYS390, DYS391, DYS385 a/b, amelogenin
  - **genRES® DYSplex-2**: DYS19, DYS389I/II, DYS392, DYS393
- Biotype (Dresden, Germany)
  - **Mentype® Argus Y-MH**: DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393

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

(Bosch et al.)
G09411 (DYS462)

(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

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

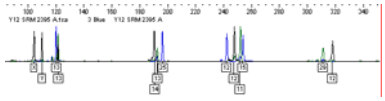
Y-PLEX 6 (ReliaGene)
Y-PLEX 5 (ReliaGene)
Y-PLEX 12 (ReliaGene)
PowerPlex Y (Promega)
Yfiler (Applied Biosystems)

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

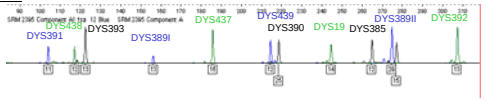
Y-STR Kit Results with SRM 2395

Component A

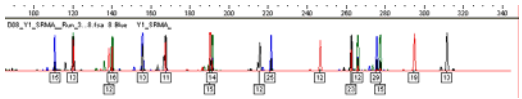
Y-PLEX 12



PowerPlex Y

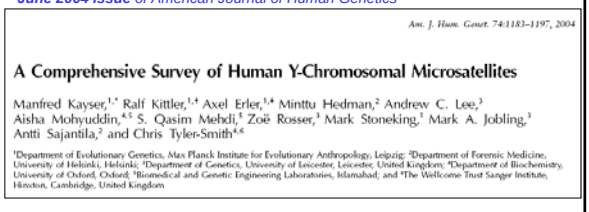


Yfiler



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

Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
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,*]
[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

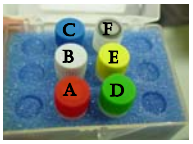
pdf file available at http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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:

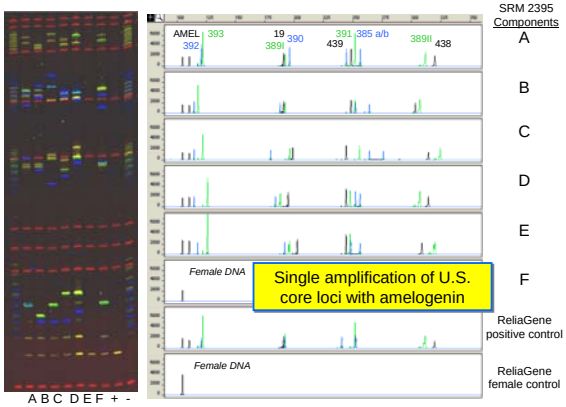
Y-PLEX 6
Y-PLEX 5
Y-PLEX 12
PowerPlex Y

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

FMBIO III and ABI 3100 Results with Y-PLEX™ 12



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	00-557-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. Chackie 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

Small Volume PCR

using STR kits with single source samples

References on Reduced Volume PCR

- Gaines ML, Wojtkiewicz PW, Valentine JA, Brown CL. Reduced volume PCR amplification reactions using the AmpFlSTR Profiler Plus kit. *J Forensic Sci* 2002; 47(6):1224-1237.
- Leclair B, Sgueglia JB, Wojtowicz PC, Juston AC, Fregeau CJ, Fourney RM. STR DNA typing: increased sensitivity and efficient sample consumption using reduced PCR reaction volumes. *J Forensic Sci* 2003; 48(5):1001-1013.
- Fregeau CJ, Bowen KL, Leclair B, Trudel I, Bishop L, Fourney RM. AmpFlSTR profiler Plus short tandem repeat DNA analysis of casework samples, mixture samples, and nonhuman DNA samples amplified under reduced PCR volume conditions (25 µL). *J Forensic Sci* 2003; 48(5):1014-1034.
- Butler JM, Schoske R, Vallone PM, Redman JW, Kline MC. Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J Forensic Sci* 2003; 48(4):908-911.

Identifiler 5 µL PCR Protocol

Identifiler PCR amplification was carried out on a GeneAmp® 9700 using 1 ng of DNA according to kit protocols with the exception of reduced volume reactions (5 µL instead of 25 µL) and reduced cycles (26 instead of 28).

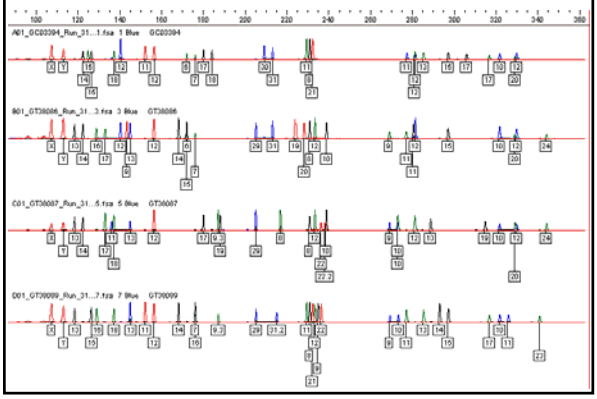
Amplification products were diluted 1:15 in Hi-Di™ formamide and GS500-LIZ internal size standard (0.3 uL) and analyzed on the 16-capillary ABI Prism® 3100 Genetic Analyzer without prior denaturation of samples.

POP™-6 (3700 POP6) rather than POP™-4 was utilized for higher resolution separations.

Allele calls were made in Genotyper® 3.7 by comparison with kit allelic ladders using the Kazaam macro (20% filter).

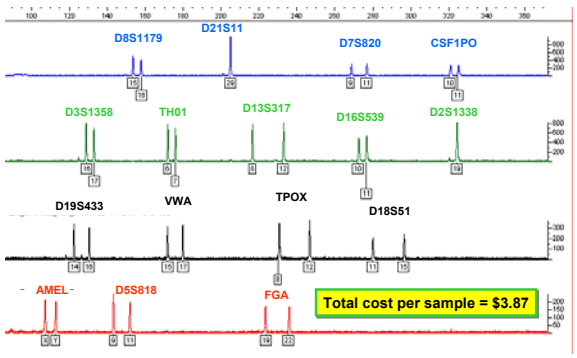
Butler JM, Schoske R, Vallone PM, Redman JW, Kline MC. Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J Forensic Sci* 2003; 48(4):908-911.

Some Example Data



Identifiler 5 uL PCR

(lower 3100 injection; 5s@2kV instead of 10s@3kV)



ABI 310 Reagents and Operating Costs

ABI 310 Reagent Costs		*for 500 samples*		factor for 500	Total Cost
	Part Number	Quantity Provided	Cost	*1000 runs with P+C*	
Capillaries	402839	5/pk (47cm x 50 um uncoated)	$294	2	$588
POP-4 polymer	402838	5 mL	$196	2	$392
Buffer, Genetic Analyzer 10X	402824	25 mL	$78	1	$78
Sample tubes (0.5 mL)	401957	500/pk	$52	2	$104
Septa for tubes	401956	500/pk	$163	2	$326
Formamide, Hi-Di	4311320	25 mL (for ~1000-1500 samples)	$29	1	$29
GS500-ROX size standard	401734	800 tests/pk	$260	1.25	$325
Matrix standards	4312131	5FAM, JOE, NED, ROX	$70	1	$70
PCR tubes, strips	N801-0580	1000/pk	$76	1	$76
PCR tube caps	N801-0535	1000/pk	$60	1	$60
Pipet tips		~$0.10/tip x 550 tips	$55	2	$110
Profiler Plus STR kit	4303326	100 tests/kit	$2,018.94	5	$10,095
COfiler STR kit	4305246	100 tests/kit	$1,816.54	5	$9,083
Syringe, Kloehn 1.0 mL	4304471	each	$82	1	$82
Genetic Analyzer vials, 4 mL	401955	50/pk	$62	1	$62
48-tube sample tray kit	402867	each	$230	1	$230

**following manufacturer's protocols (based on 500 samples total)*

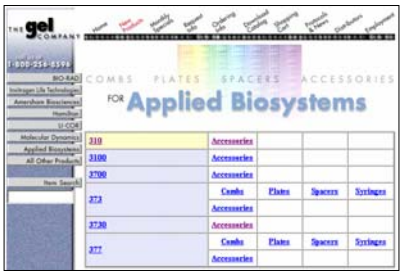
Total per Sample Cost to Obtain Result on 13 CODIS core loci (with Profiler Plus and COfiler STR kits): $43.42

(materials other than STR kits = $5.06)

10 µL PCR (1/5 vol) = $12.73

An Alternative Source for ABI 310 Supplies

The Gel Company **http://www.gelcompany.com**
1-800-256-8596



Capillaries, 47cm, 310, internally uncoated, fused silica (5pack)	**$160**	**ABI = $294**
10X Genetic Analyzer Buffer, 310, w/EDTA, used w/POP-6, 25ml	**$45**	**ABI = $78**

Consumables for ABI 310/3100

What we use at NIST

- A.C.E.™ Sequencing Buffer 10X (Amresco)
  - $155/L = $0.0155/mL 1X buffer (**costs 20 times less!**)
  - http://www.amresco-inc.com
- 3700 POP-6 Polymer (Applied Biosystems)
  - $530 / 200 mL = $2.65/mL (**costs 20 times less!**)

What ABI protocols suggest

- 10X Genetic Analyzer Buffer with EDTA
  - $78/25 mL = $0.312/mL 1X buffer (ABI)
- 3100 POP-4 Polymer
  - $365 / 7 mL = $52/mL

Overall Thoughts on the ABI 310

- Settling on a common instrument platform has been good for the forensic DNA community in terms of data consistency (this is also true with the use of common STR kits)
- I am concerned that the community is very dependent primarily on one company…
- I really like using the instrument and can usually get nice data from it
- Like any instrument, it has its quirks…

Validation Standardization Effort

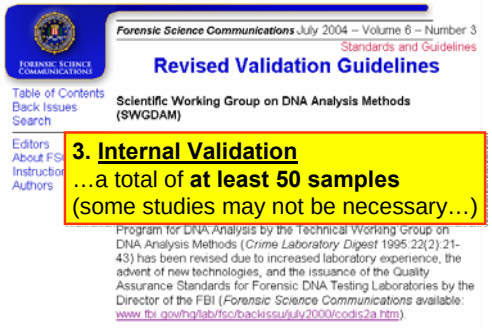
John Butler (NIST), Christine Tomsey (PA State Police), Margaret Kline (NIST)

- **Survey of laboratory practices with questionnaire**
- Literature Review
- Lab notes review/interviews of a few laboratories
- Recommendations for minimum sample numbers
  - *an effort to define the minimum number of samples needed to reliably validate DNA typing procedures*
  - *through a survey of standard practices currently used by practitioners in forensic DNA laboratories*
  - *results will be summarized at the Promega meeting in October 2004 and made available on the NIST STRBase web site.*
- There is a lot of interest from the companies to have guidance in developmental validation and from practitioners for internal validation

Revised SWGDAM Validation Guidelines

(July 2004)

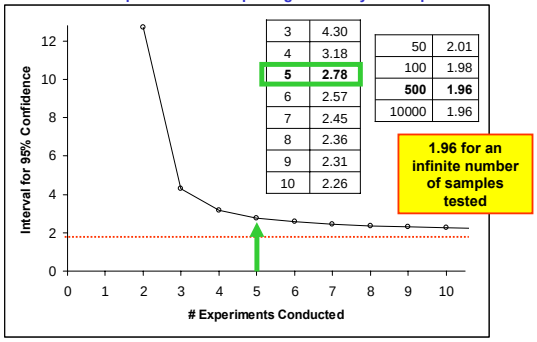
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm



The document provides validation guidelines and definitions approved by SWGDAM July 10, 2003.

A Comment on Minimum Numbers of Samples for Validation Studies…

Impact of Number of Experiments on Capturing Variability in a Population of Data



3	4.30
4	3.18
5	**2.78**
6	2.57
7	2.45
8	2.36
9	2.31
10	2.26

50	2.01
100	1.98
500	**1.96**
10000	1.96

1.96 for an infinite number of samples tested

Steps Surrounding "Validation" in a Forensic Lab

Effort to Bring a Procedure "On-Line"

This is what takes the time…

- **Installation** – purchase of equipment, ordering supplies, setting up in lab
- **Learning** – efforts made to understand technique and gain experience troubleshooting; can take place through direct experience in the lab or vicariously through the literature or hearing talks at meetings
- **Validation of Analytical Procedure** – tests conducted in one's lab to verify range of reliability and reproducibility for procedure
- **SOP Development** – creating interpretation guidelines based on lab experience
- **QC of Materials** – performance check of newly received reagents
- **Training** – passing information on to others in the lab
- **Qualifying Test** – demonstrating knowledge of procedure enabling start of casework
- **Proficiency Testing** – verifying that trained analysts are performing procedure properly over time

Issue of "Accuracy" in Forensic DNA Testing

Recent Examples of Lab "Problems"

- Houston Police Department
  - Incompetent or untrained scientists with poor funding
- FBI Laboratory
  - Rogue technician who did not run negative controls
- Washington State Police
  - Accidental sample switch of victim and suspect samples resulted with incorrect association of suspect to crime scene

Josiah Sutton, 21, who was serving a 25-year sentence for rape, was **exonerated by outside retesting of Houston police DNA evidence.**

The New York Times
nytimes.com

March 11, 2003

Review of DNA Clears Man Convicted of Rape
By ADAM LIPTAK

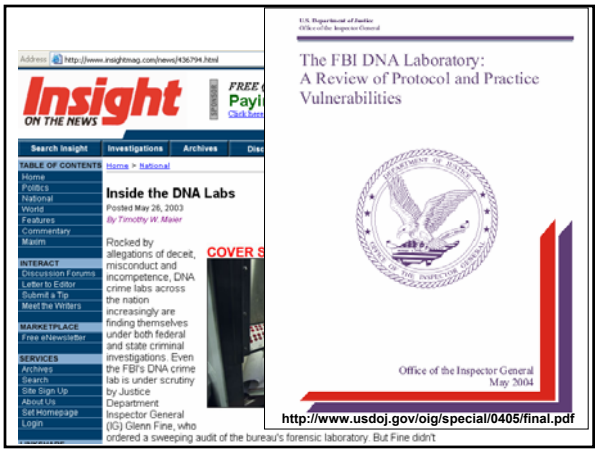
When Josiah Sutton went on trial for rape in 1999, prosecutors in Houston had little to build a case on. The victim was the only eyewitness, and her recollection was faulty. But they did have the rapist's DNA, and **technicians from the Houston police crime laboratory told the jury that it was a solid match**.

That was enough to persuade the jurors to convict Mr. Sutton and send him to prison for 25 years.

But **new testing has conclusively demonstrated that the DNA was not Mr. Sutton's**, the Houston Police Department said yesterday.

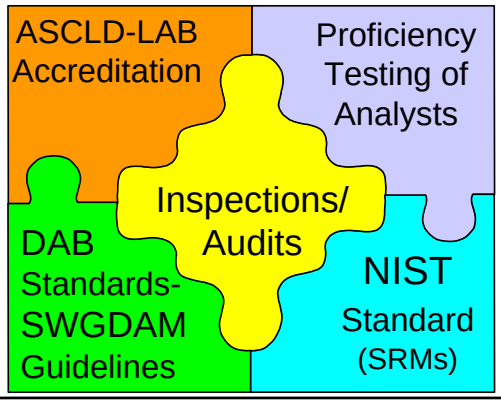
The retesting is part of a review of the laboratory that began after a scathing state audit of its work led to a suspension of genetic testing in January. Mr. Sutton's apparent exoneration is the first to result from the review.

Legal experts say the laboratory is the worst in the country, but troubles there are also seen in other crime laboratories. Standards are often lax or nonexistent, technicians are poorly trained and defense lawyers often have no money to hire their own experts. Questions about the work of laboratories and their technicians in Oklahoma City, Montana and Washington State and elsewhere have led to similar reviews. But the possible problems in Houston are much greater. More defendants from Harris County, of which Houston is a part, have been executed than from any other county in the country.



http://www.usdoj.gov/oig/special/0405/final.pdf

Ensuring Accurate Forensic DNA Results

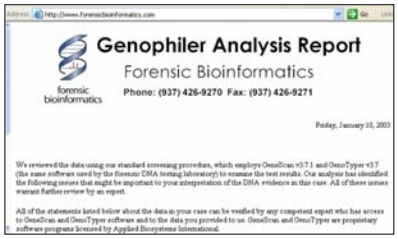


Role and Purpose of a NIST SRM

- DAB Standard 9.5 states …**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.**
- NIST SRM provides certified values that may be used to calibrate a procedure and demonstrate that reliable results may be obtained
  - SRM 2391b is for CODIS loci and other autosomal STRs
  - SRM 2395 is for Y-STR and Y-SNP markers
  - SRM 2392-I is for mtDNA
  - SRM 2372 will be for human DNA quantitation

Forensic Bioinformatics, Inc.

http://www.forensicbioinformatics.com/



forensic bioinformatics

Defense experts that evaluate and often contest STR results…

Forensic Bioinformatics 2850 Presidential Drive Suite 150, Fairborn, OH 45324 **(937) 426 -9270**

Twelve important questions always need to be asked about DNA evidence:

1. Has the prosecution documented the entire **history of the key evidentiary samples** from the time of collection to ultimate disposition, including records of all examinations and tests performed on those samples?

2. Is it possible to determine with certainty **the nature of the biological material from which the DNA originated**? (Particularly in sexual assault cases, it may be important to know whether a sample linked to a suspect originated from semen or some other biological material.)

3. Has the testing laboratory been audited or evaluated by an outside agency? If not, why not? If so, has the prosecution provided copies of the audit documents?

4. Is the testing laboratory accredited? If so, by what agency? If not, why not? (Did the laboratory seek accreditation and fail? If so, has the prosecution provided a copy of the report of the accreditation committee?)

5. Has the laboratory participated in a proficiency testing program? If not, why not? If so, has the prosecution provided documentation of the results?

6. Are there any inconsistencies between the DNA profiles that the lab declared to "match"? Are there any "missing" alleles or "extra" alleles that complicate the interpretation of the test results?

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Twelve important questions always need to be asked about DNA evidence:

7. Did the laboratory run all necessary control samples? **Did the control samples produce the expected results**?

8. Did the laboratory employ "blind" procedures for interpreting the test results? (Failure to use blind procedures can result in "examiner bias" – i.e., the tendency for an analyst to interpret ambiguous data in a manner consistent with the expected or desired outcome and may therefore be an unreliable/incorrect scientific procedure.)

9. How much DNA did the evidentiary samples contain? (Knowing how much DNA was present may help you evaluate whether the results could be explained by contamination or inadvertent DNA transfer.)

10. Do any of the key actors in the case have close relatives who might have been involved? (Labs typically estimate the frequency of DNA profiles among unrelated individuals . The probability of a chance match between DNA profiles is always higher for relatives than for unrelated individuals.)

11. Have the statistical estimates been computed properly in accordance with generally accepted methods? Do they address the right issue? (There continues to be considerable controversy surrounding the proper way to generate statistical estimates for comparisons involving mixed samples and partial or incomplete profiles. Labs often choose methods that are unfairly slanted against the accused.)

12. Is there evidence of unreported additional contributors to any samples? (Labs sometimes overlook or fail to report weak results that may indicate the presence of an additional contributor to evidentiary samples.)

http://www.forensicbioinformatics.com/

Thank you for your attention…

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National Institute of Justice
Funding through NIST Office of Law Enforcement Standards