NIST Research Update

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Peter M. Vallone, Michael D. Coble,
Janette W. Redman, Amy E. Decker, Carolyn R. Hill,
Chris DeAngelis, David L. Duewer, John M. Butler

Human Identity Project Team, U.S. National Institute of Standards and Technology

2nd Annual Present & Future Technological Advances in Human Identification (Roanoke,VA) March 27-29, 2006

NIST Human Identity Project Team


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Funding: **Interagency Agreement 2003-IJ-R-029 between National Institute of Justice (NIJ) and NIST Office of Law Enforcement Standards (OLES)**

Disclaimers and Collaborations

Funding: Interagency Agreement 2003-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

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

Past and Present Collaborators (also funded by NIJ):
Mike Hammer and Alan Redd (U. AZ) for Y-chromosome studies
Tom Parsons, Rebecca Just, Jodi Irwin (AFDIL) for mtDNA coding SNP work
Sandy Calloway (Roche) for mtDNA LINEAR ARRAYs
Bruce McCord and students (FL Int. U.) for miniSTR work
Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work
Artie Eisenberg and John Planz (U. North Texas) for miniSTR testing on bones

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Team Impact on Forensic Community

- **28 publications** (published or submitted) since Nov 2004
- **51 presentations** to the community since Nov 2004
- All NIST publications and presentations available on STRBase: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
- Training materials from **2 workshops** **>500 PowerPoint slides**
  - Albany DNA Academy (June 13-14, 2005) with Bruce McCord
  - NFSTC Validation Workshop (August 24-26, 2005) with Robyn Ragsdale
- *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*, 2nd Edition (John Butler)

AAFS 2006 Workshop #6 (John Butler and Bruce McCord)
Advanced Topics in Capillary Electrophoresis and DNA Typing

National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Effort with Forensic DNA

- **Standards**
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

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.*

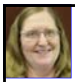
FBI's DNA Advisory Board
Quality Assurance Standards for Forensic DNA Testing Laboratories

(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.

NIST


Margaret Kline


Jan Redman

Standard Reference Materials

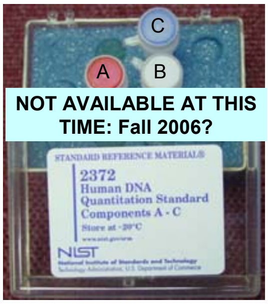
- Relevant Forensic DNA SRMs
  - **SRM 2391b** (DNA profiling – STRs, D1S80, DQA1/PM)
  - SRM 2392-I (mtDNA)
  - SRM 2395 (Y-chromosome)
  - SRM 2372 (Human DNA quantitation); in development
- Provides national/international traceability and compatibility (aids in ISO 17025 compliance)

We are currently working on a manuscript that discusses NIST traceability issues to aid auditors and labs moving to ISO 17025 accreditation

http://www.nist.gov/srm

SRM 2372
Human DNA Quantitation Standard

Anticipated 2006 issue



NOT AVAILABLE AT THIS TIME: Fall 2006?

Component A: Male
Component B: Female
Component C: Mixture

Planned Amounts: Each component 50 µL of Human Genomic DNA with a concentration targeted @ 50 ng/µL. The [DNA] for each component will be list in the materials Certificate of Analysis.

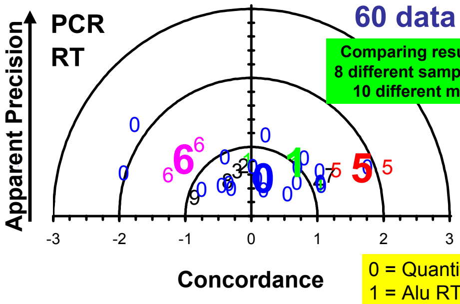
SRM 2372
Human DNA Quantitation Standard

Component A: Male (blood)
Component B: Female (blood)
Component C: Mixture (placenta)

- •Genomic DNA isolated by Salt out procedure
- •Treated with RNAse and re-precipitated
- •UV spectroscopy 340-220 nm on a NIST calibrated spectrophotometer
- •Assume $A^{260} = OD^{260} = 1$ for a 50 µg/mL solution

Interlaboratory Comparisons

Laboratory Performances with Real-Time PCR Methods

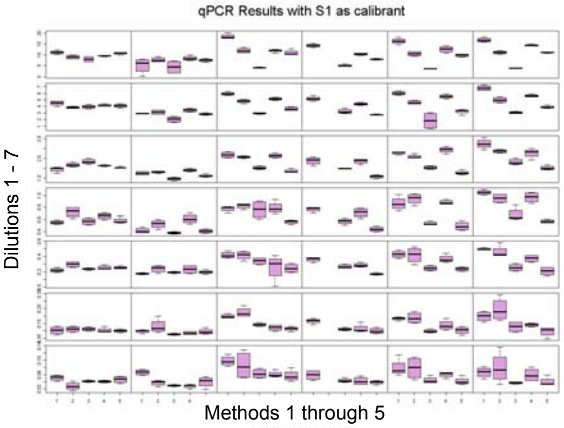


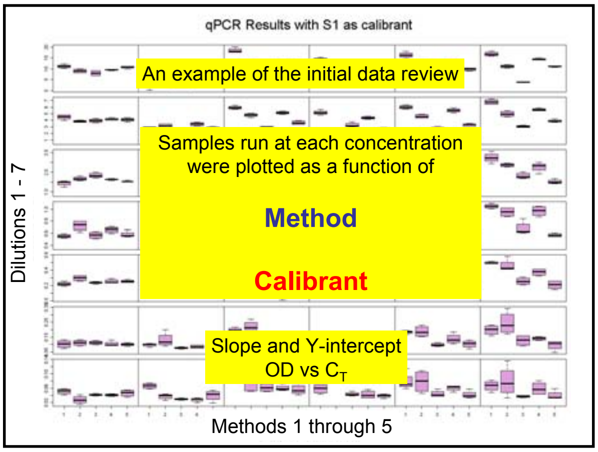
60 data sets

Comparing results from 8 different samples using 10 different methods

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

Kline, *et al.* (2005) *J. Forensic Sci.* 50(3):571-578





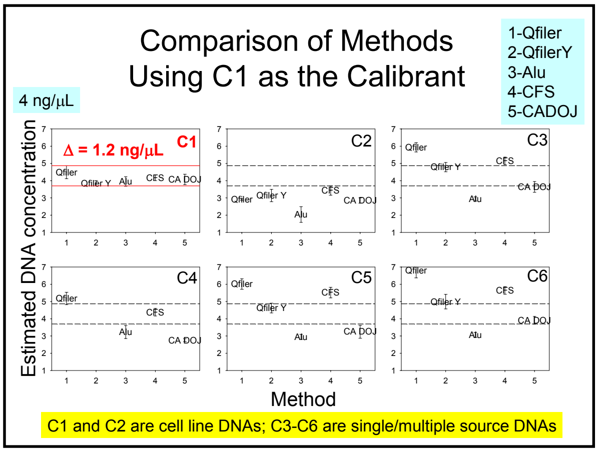
An example of the initial data review

Samples run at each concentration were plotted as a function of

Method

Calibrant

Slope and Y-intercept
OD vs C_T

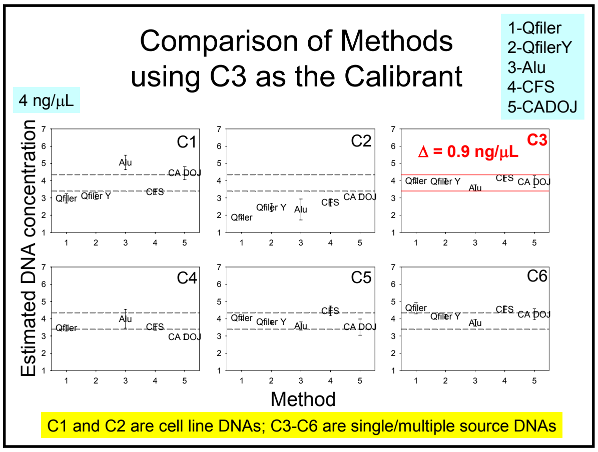


Comparison of Methods Using C1 as the Calibrant

1-Qfiler
2-QfilerY
3-Alu
4-CFS
5-CADOJ

4 ng/µL

C1 and C2 are cell line DNAs; C3-C6 are single/multiple source DNAs

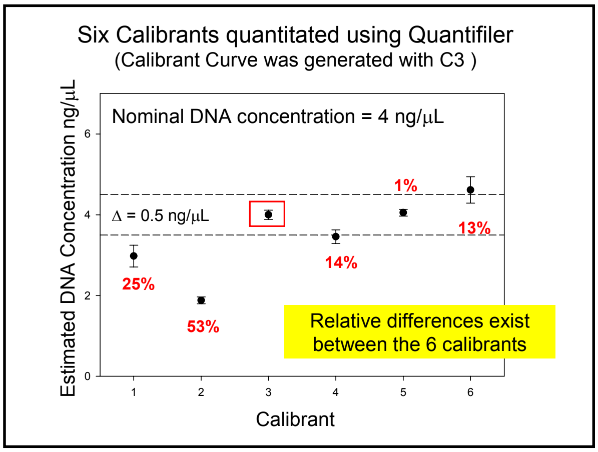


Comparison of Methods using C3 as the Calibrant

1-Qfiler
2-QfilerY
3-Alu
4-CFS
5-CADOJ

4 ng/µL

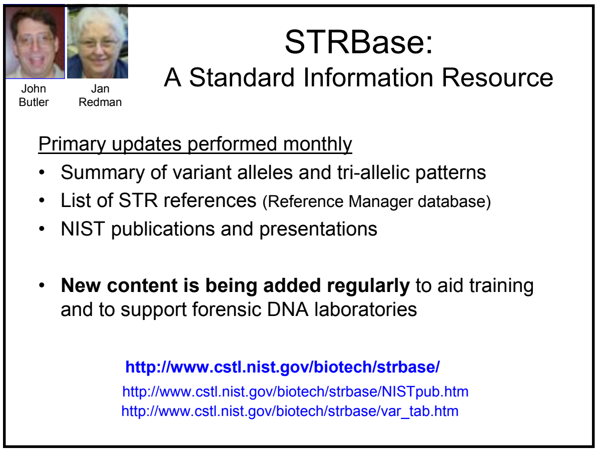
C1 and C2 are cell line DNAs; C3-C6 are single/multiple source DNAs



Six Calibrants quantitated using Quantifiler

(Calibrant Curve was generated with C3)

Relative differences exist between the 6 calibrants



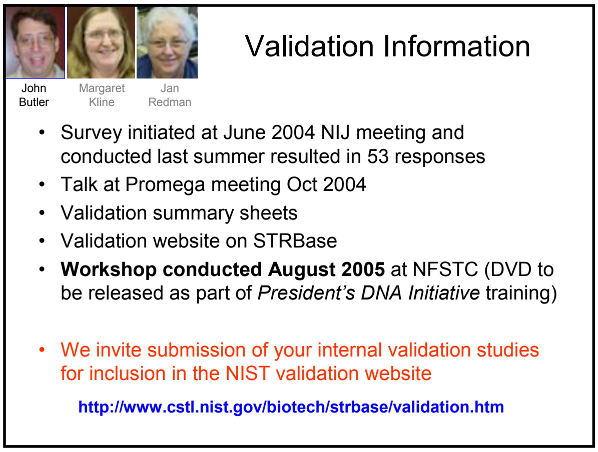
STRBase:
A Standard Information Resource

Primary updates performed monthly

- Summary of variant alleles and tri-allelic patterns
- List of STR references (Reference Manager database)
- NIST publications and presentations
- **New content is being added regularly** to aid training and to support forensic DNA laboratories

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

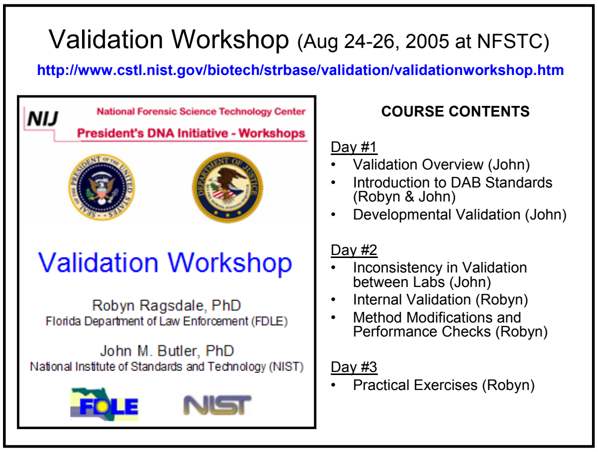
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
http://www.cstl.nist.gov/biotech/strbase/var_tab.htm



Validation Information

- Survey initiated at June 2004 NIJ meeting and conducted last summer resulted in 53 responses
- Talk at Promega meeting Oct 2004
- Validation summary sheets
- Validation website on STRBase
- **Workshop conducted August 2005** at NFSTC (DVD to be released as part of *President's DNA Initiative* training)
- We invite submission of your internal validation studies for inclusion in the NIST validation website

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



Validation Workshop (Aug 24-26, 2005 at NFSTC)

http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

NIJ
National Forensic Science Technology Center
President's DNA Initiative - Workshops

Validation Workshop

Robyn Ragsdale, PhD
Florida Department of Law Enforcement (FDLE)

John M. Butler, PhD
National Institute of Standards and Technology (NIST)

COURSE CONTENTS

Day #1
- Validation Overview (John)
- Introduction to DAB Standards (Robyn & John)
- Developmental Validation (John)

Day #2
- Inconsistency in Validation between Labs (John)
- Internal Validation (Robyn)
- Method Modifications and Performance Checks (Robyn)

Day #3
- Practical Exercises (Robyn)

Laboratory Internal Validation Summaries

Address http://www.cstl.nist.gov/biotech/strbase/validation.htm

We invite updates to this table. Please contact John Butler <john.butler@nist.gov> if you would like to add a summary of your laboratory's validation studies with a particular forensic DNA test, instrument, or software program. Please submit information in a standard format summarizing the studies conducted, a description of samples run, and the number of samples examined using this downloadable Excel file [click here].

Summaries of Validation Studies Conducted in Individual Laboratories (not published in the literature)

Kit, Assay or Instrument	Laboratory	Submitter
PowerPlex 16 Kit with ABI 310	Pennsylvania State Police	Christine Tomsey
Quantifiler with ABI 7000	Alabama Department of Forensic Sciences	Angelo Della Manna

Helpful Information with STR Kit Validation (with SWGDAM revised validation guideline sections indicated)

Information will be included here to help with individual lab validation studies and is based on a community-wide effort either through literature extraction or direct contribution. If you have something that you feel would be helpful to others regarding a particular aspect of validation, please contact John Butler <john.butler@nist.gov>. Information will be posted along with the source and contact information if someone wants to learn more.

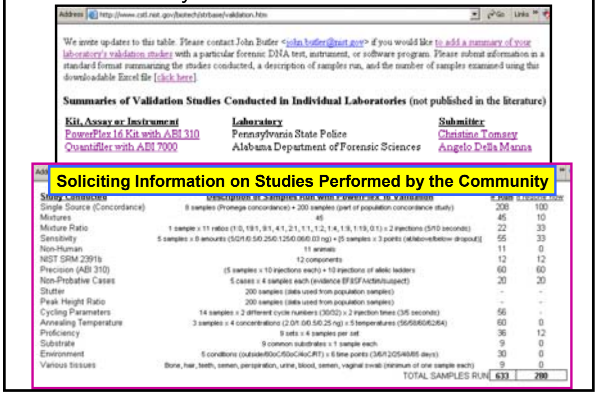
The information below is organized following the SWGDAM Revised Validation Guidelines with the numbers in parentheses referring to the various sections of the document.

Developmental Validation

Characterization of Genetics Markers (2.1): STRBase STR Fact Sheets, Forensic SNP information

Inheritance (2.1.1):

Laboratory Internal Validation Summaries



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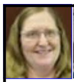
Soliciting Information on Studies Performed by the Community

Study Conducted	Description of Samples Run with PowerPlex 16 Validation	# Run	[illegible]
Single Source (Concordance)	8 samples (Promega concordance) + 200 samples (part of population concordance study)	208	100
Mixtures	45	45	10
Mixture Ratio	1 sample x 11 ratios (1:0, 19:1, 9:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:9, 1:19, 0:1) x 2 injections (5/10 seconds)	22	33
Sensitivity	5 samples x 8 amounts (5/2/1/0.5/0.25/0.125/0.06/0.03 ng) + [5 samples x 3 points (at/above/below dropout)]	55	33
Non-Human	11 animals	11	0
NIST SRM 2391b	12 components	12	12
Precision (ABI 310)	(5 samples x 10 injections each) + 10 injections of allelic ladders	60	60
Non-Probative Cases	5 cases x 4 samples each (evidence EF/SF/victim/suspect)	20	20
Stutter	200 samples (data used from population samples)	-	-
Peak Height Ratio	200 samples (data used from population samples)	-	-
Cycling Parameters	14 samples x 2 different cycle numbers (30/32) x 2 injection times (3/5 seconds)	56	-
Annealing Temperature	3 samples x 4 concentrations (2.0/1.0/0.5/0.25 ng) x 5 temperatures (56/58/60/62/64)	60	0
Proficiency	9 sets x 4 samples per set	36	12
Substrate	9 common substrates x 1 sample each	9	0
Environment	5 conditions (outside/60oC/50oC/4oC/RT) x 6 time points (3/6/12/24/48/65 days)	30	0
Various tissues	Bone, hair, teeth, semen, perspiration, urine, blood, semen, vaginal swab (minimum of one sample each)	9	0
	TOTAL SAMPLES RUN	633	280

Interlaboratory Studies



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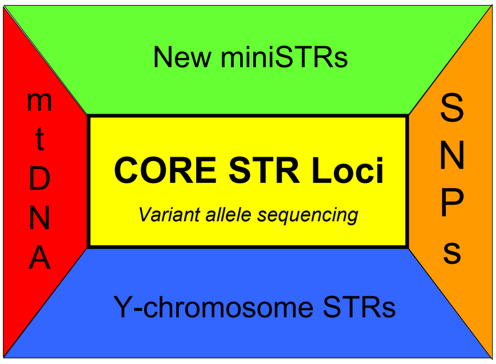
- DNA Quantitation Study (QS04)
  - 8 DNA samples supplied
  - 84 laboratories signed up (80 labs returned results)
  - 287 data sets using 19 different methods
  - 60 data sets with real-time qPCR (37 Quantifiler data sets)
  - Publication in May 2005: *J. Forensic Sci.* 50(3): 571-578
- Mixture Interpretation Study (MIX05)
  - 94 labs signed up (**69** labs returned data)
  - Interpretation requested of provided e-grams for 4 mock sexual assault cases
  - Data analysis is still on-going…

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

NIST Initiated Interlaboratory Studies

Studies involving STRs	# Labs	Publications
Evaluation of CSF1PO, TPOX, and TH01	34	Kline MC, Duewer DL, Newall P, Redman JW, Reeder DJ, Richard M. (1997) Interlaboratory evaluation of STR triplex CTT. *J. Forensic Sci.* 42: 897-906
Mixed Stain Studies #1 and #2 (Apr–Nov 1997 and Jan–May 1999)	45	Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. (2001) NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples. *J. Forensic Sci.* 46: 1199-1210
MSS3 Mixed Stain Study #3 (Oct 2000-May 2001)	74	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. Duewer, D.L., Kline, M.C., Redman, J.W., Butler, J.M. (2004) NIST Mixed Stain Study #3: signal intensity balance in commercial short tandem repeat multiplexes, *Anal. Chem.* 76: 6928-6934.
DNA Quantitation Study (Jan-Mar 2004) QS04	80	Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2005) Results from the NIST 2004 DNA Quantitation Study, *J. Forensic Sci.* 50(3):571-578
MIX05 Mixture Interpretation Study (Jan-Mar 2005)	**69**	***Data analysis currently on-going …*** Some information presented at NIJ Grantees (June 2005), ISFG (Sept 2005), Promega (Sept 2005)

Genetic Markers Under Examination



New miniSTRs

mtDNA

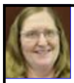
CORE STR Loci

Variant allele sequencing

SNPs

Y-chromosome STRs

STR Allele Sequencing and Characterization



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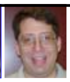
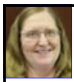


John Butler

- Variant characterization
  - TPOX 10.3 (Maryland State Police)
  - **D18S51 null alleles** (FSS and Kuwait govt)
  - D18S51 allele 40 (Nebraska State Crime Lab)
  - D18S51 allele “5.3” (DNA Solutions)
  - VWA allele “15.1” (Peter de Knijff)
  - DYS19 allele 9 (Italy)
  - DYS392 allele “10.2” (AFDIL)
  - DYS439 allele 7 (Italy)

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



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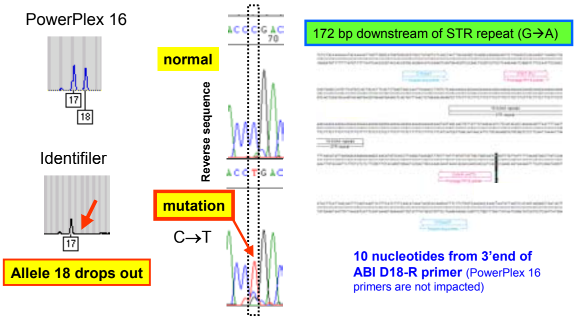
STR Allele Sequencing and Characterization

- Locus duplication or deletion
  - DYS390 deletion (CFS Toronto)
  - DYS19 duplication (Portugal)
  - DYS392 deletion (MN BCA) - H4 duplication (Portugal)
  - DYS385 triplication (Italy)
  - DYS389II duplication (Italy)
  - DYS439 duplication (Italy)
- **Forensic labs are sending us unusual STR alleles for sequence characterization**

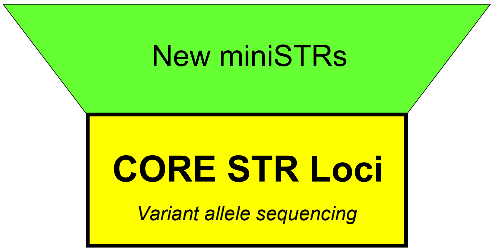
http://www.cstl.nist.gov/biotech/strbase/STRseq.htm

D18S51 Null Allele from Kuwait Samples with ABI Primers

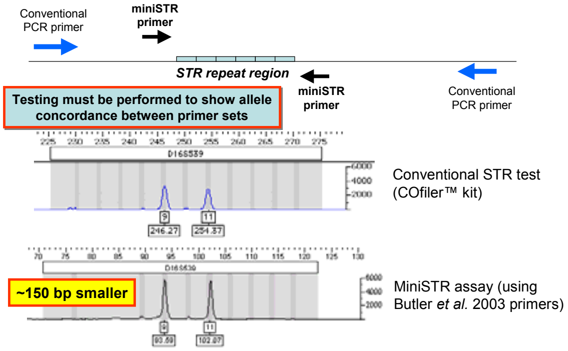


Clayton et al. (2004) **Primer binding site mutations affecting the typing of STR loci contained within the AMPFISTR SGM Plus kit.** *Forensic Sci Int.* 139(2-3): 255-259

Genetic Markers Under Examination



A miniSTR is a reduced size STR amplicon that enables higher recovery of information from degraded DNA samples



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition,* Figure 7.2, ©Elsevier Science/Academic Press

Mike
Coble

Becky
Hill

John
Butler

miniSTRs for Degraded DNA

- Original miniSTR paper with CODIS loci, D2, D19, Penta D, Penta E
  - Butler *et al.* (2003) *J. Forensic Sci.* 48: 1054-1064
- Many CODIS loci are too big and make poor miniSTRs
- New miniSTRs and assays: NC01, NC02
  - Coble, M.D. and Butler, J.M. (2005) *J. Forensic Sci.* 50:43-53
- New miniSGM miniplex: AMEL, TH01, FGA, D18, D16, D2
- EDNAP/ENFSI degraded DNA study coordinated by Peter Gill
- Creation of miniSTR information on STRBase

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

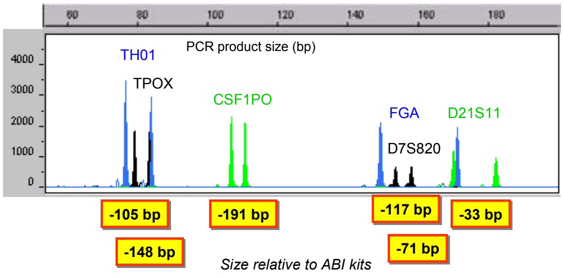
J. Forensic Sci. Sept 2003 issue

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

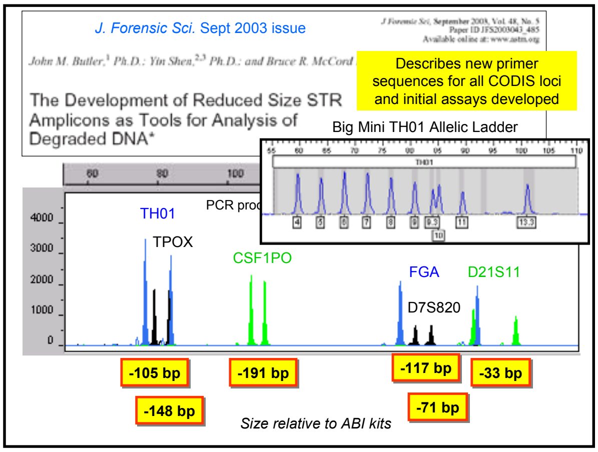
John M. Butler,[1] Ph.D.; Yin Shen,[2,3] Ph.D.; and Bruce R. McCord

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



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

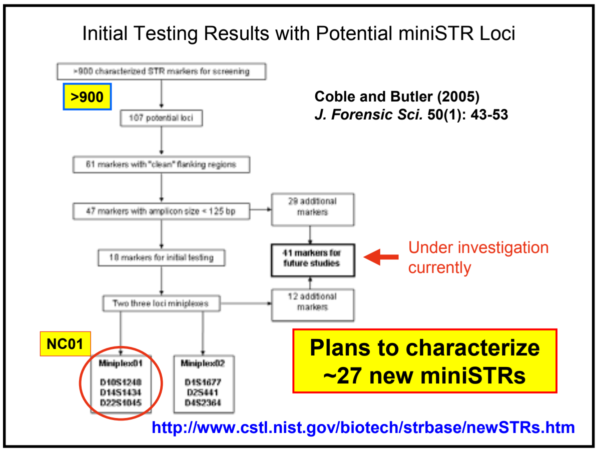
The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA*

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

Big Mini TH01 Allelic Ladder

-105 bp
-191 bp
-117 bp
-33 bp
-148 bp
-71 bp

Size relative to ABI kits



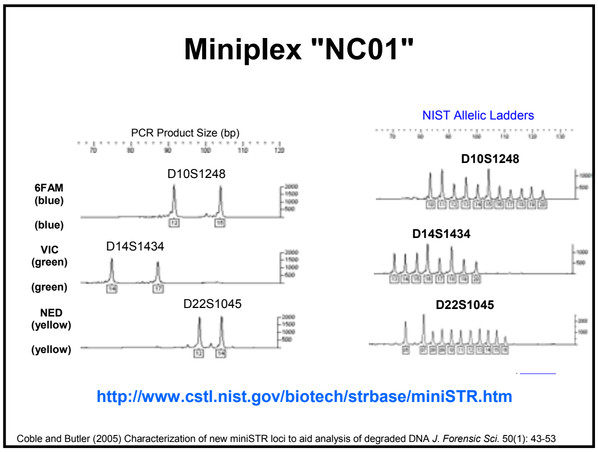
Initial Testing Results with Potential miniSTR Loci

Coble and Butler (2005)
***J. Forensic Sci.* 50(1): 43-53**

Under investigation currently

Plans to characterize ~27 new miniSTRs

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



Miniplex "NC01"

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

Coble and Butler (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA *J. Forensic Sci.* 50(1): 43-53

Forthcoming Article Advocating miniSTRs

DTD 5

ARTICLE IN PRESS

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE @ DIRECT®

Forensic Science International xxx (2005) xxx–xxx

Forensic Science International
www.elsevier.com/locate/forsciint

Short communication

The evolution of DNA databases—Recommendations for new European STR loci

Peter Gill[a,*], Lyn Fereday[b], Niels Morling[c], Peter M. Schneider[d]

[a] Forensic Science Service, Birmingham, UK
[b] Forensic Science Service, London, UK
[c] Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark
[d] Institute of Legal Medicine, University of Cologne, Germany

Received 25 May 2005; accepted 26 May 2005

Forthcoming Article Advocating miniSTRs

They recommend that miniSTRs "be adopted as the way forward to increase both the robustness and sensitivity of analysis."

ELSEVIER

Forensic Science International xxx (2005) xxx–xxx

Science International
www.elsevier.com/locate/forsciint

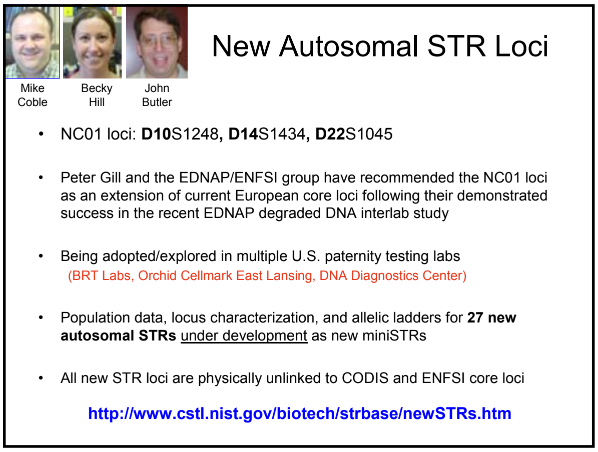
Short communication

The evolution of DNA databases—Recommendations for new European STR loci

Peter Gill[a,*], Lyn Fereday[b], Niels Morling[c], Peter M. Schneider[d]

[a] Forensic Science Service, Birmingham, UK
[b] Forensic Science Service, London, UK
[c] Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark

They recommend that European laboratories adopt three new mini-STR loci, namely: D10S1248, D14S1434 and D22S1045.



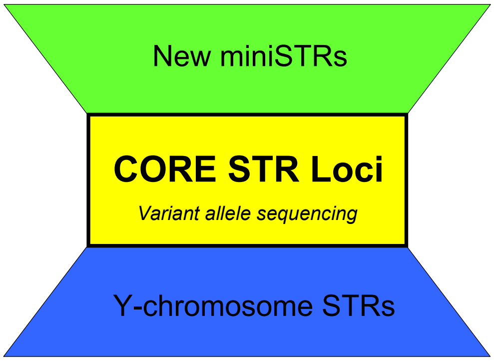
Mike Coble
Becky Hill
John Butler

New Autosomal STR Loci

- NC01 loci: **D10**S1248, **D14**S1434, **D22**S1045
- Peter Gill and the EDNAP/ENFSI group have recommended the NC01 loci as an extension of current European core loci following their demonstrated success in the recent EDNAP degraded DNA interlab study
- Being adopted/explored in multiple U.S. paternity testing labs (BRT Labs, Orchid Cellmark East Lansing, DNA Diagnostics Center)
- Population data, locus characterization, and allelic ladders for **27 new autosomal STRs** under development as new miniSTRs
- All new STR loci are physically unlinked to CODIS and ENFSI core loci

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

Genetic Markers Under Examination



New miniSTRs

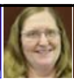
CORE STR Loci

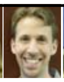
Variant allele sequencing

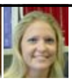
Y-chromosome STRs

Work with Y-STRs


John Butler


Margaret Kline


Pete Vallone

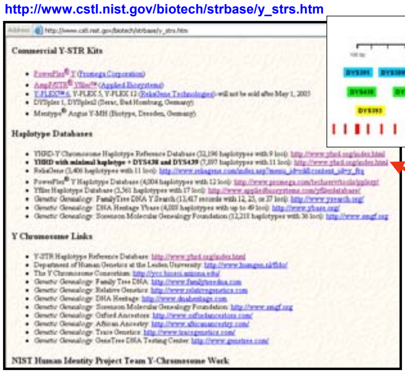

Amy Decker

- Beta-testing of all commercial Y-STR kits
- U.S. population data supplied to Yfiler haplotype database
- **49 Y-STR loci evaluated with ~650 U.S. samples**
- New Y-chromosome information on STRBase linking to all available haplotype databases
- Human Y-Chromosome DNA Profiling Standard Reference Material (SRM 2395) – updates with DYS635 for Yfiler
- **Separation of two brothers with 47 Y-STRs**

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

New Y-Chromosome Information Resources on STRBase

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



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

Commercial Y-STR Kits

- PowerPlex® Y (Promega Corporation)
- AmpFlSTR® Yfiler™ (Applied Biosystems)
- Y-PLEX™6, Y-PLEX 5, Y-PLEX 12 (ReliaGene Technologies)–will not be sold after May 1, 2005
- DYSplex 1, DYSplex2 (Serac, Bad Homburg, Germany)
- Mentype® Argus Y-MH (Biotype, Dresden, Germany)

Haplotype Databases

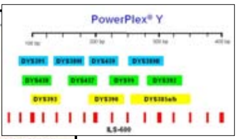
- YHRD-Y Chromosome Haplotype Reference Database (32,196 haplotypes with 9 loci): http://www.yhrd.org/index.html
- **YHRD with minimal haplotype + DYS438 and DYS439** (7,897 haplotypes with 11 loci): http://www.yhrd.org/index.html
- ReliaGene (3,406 haplotypes with 11 loci): http://www.reliagene.com/index.asp?menu_id=[illegible]content_id=Y_fq
- PowerPlex® Y Haplotype Database (4,004 haplotypes with 12 loci): http://www.promega.com/techserv/tools/pplexy/
- Yfiler Haplotype Database (3,561 haplotypes with 17 loci): http://www.appliedbiosystems.com/yfilerdatabase/
- Genetic Genealogy: FamilyTree DNA Y Search (13,417 records with 12, 25, or 37 loci): http://www.ysearch.org/
- Genetic Genealogy: DNA Heritage Ybase (4,088 haplotypes with up to 49 loci): http://www.ybase.org/
- Genetic Genealogy: Sorenson Molecular Genealogy Foundation (12,218 haplotypes with 36 loci): http://www.smgf.org

Y Chromosome Links

- Y-STR Haplotype Reference Database: http://www.yhrd.org/index.html
- Department of Human Genetics at the Leiden University: http://www.humgen.nl/lfdo/
- The Y Chromosome Consortium: http://ycc.biosci.arizona.edu/
- Genetic Genealogy: Family Tree DNA: http://www.familytreedna.com
- Genetic Genealogy: Relative Genetics: http://www.relativegenetics.com
- Genetic Genealogy: DNA Heritage: http://www.dnaheritage.com
- Genetic Genealogy: Sorenson Molecular Genealogy Foundation: http://www.smgf.org
- Genetic Genealogy: Oxford Ancestors: http://www.oxfordancestors.com/
- Genetic Genealogy: African Ancestry: http://www.africanancestry.com/
- Genetic Genealogy: Trace Genetics: http://www.tracegenetics.com/
- Genetic Genealogy: GeneTree DNA Testing Center: http://www.genetree.com/

NIST Human Identity Project Team Y-Chromosome Work

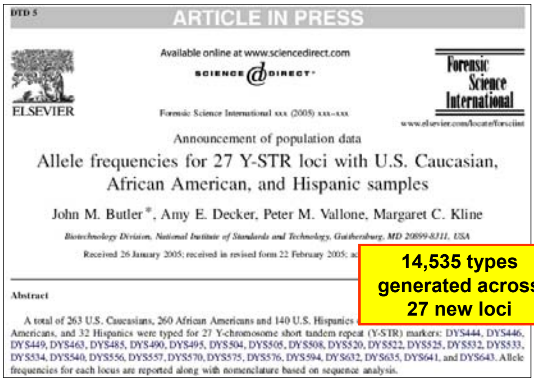
Locus boxes are hyperlinked to STR Fact Sheets



Largest Y-STR Database

YHRD has **9,634 haplotypes** (from 61 populations) with SWGDAM recommended loci

NIST Work with New Y-STR Loci



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Forensic Science International xxx (2005) xxx–xxx

Forensic Science International

www.elsevier.com/locate/forsciint

Announcement of population data

Allele frequencies for 27 Y-STR loci with U.S. Caucasian, African American, and Hispanic samples

John M. Butler*, Amy E. Decker, Peter M. Vallone, Margaret C. Kline

Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899-8311, USA

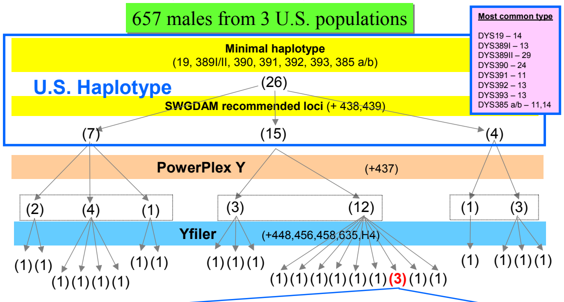
Received 26 January 2005; received in revised form 22 February 2005; ac

Abstract

A total of 263 U.S. Caucasians, 260 African Americans and 140 U.S. Hispanics … Americans, and 32 Hispanics were typed for 27 Y-chromosome short tandem repeat (Y-STR) markers: DYS444, DYS446, DYS449, DYS463, DYS485, DYS490, DYS495, DYS504, DYS505, DYS508, DYS520, DYS522, DYS525, DYS532, DYS533, DYS534, DYS540, DYS556, DYS557, DYS570, DYS575, DYS576, DYS594, DYS632, DYS635, DYS641, and DYS643. Allele frequencies for each locus are reported along with nomenclature based on sequence analysis.

14,535 types generated across 27 new loci

Subdividing Common Types with More Loci



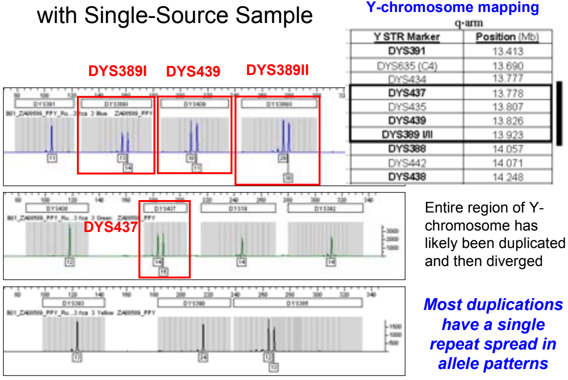
Most common type

DYS19 – 14
DYS389I – 13
DYS389II – 29
DYS390 – 24
DYS391 – 11
DYS392 – 13
DYS393 – 13
DYS385 a/b – 11,14

Identical: DYS…444,446,485,495,505,508,534,540,556

Subdivide into two groups (2)(1): DYS…449,463,520,532,533,557,570,594,643
Subdivide into three groups (1)(1)(1): **DYS522, DYS576**

Duplication at Multiple Loci with Single-Source Sample



Y-chromosome mapping

q-arm

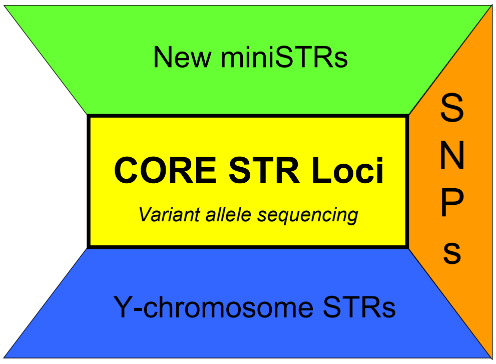
Y STR Marker	Position (Mb)
DYS391	13.413
DYS635 (C4)	13.690
DYS434	13.777
DYS437	13.778
DYS435	13.807
DYS439	13.826
DYS389 I/II	13.923
DYS388	14.057
DYS442	14.071
DYS438	14.248

Entire region of Y-chromosome has likely been duplicated and then diverged

Most duplications have a single repeat spread in allele patterns

Butler *et al.* (2005) *J. Forensic Sci. 50(4):853-859*

Genetic Markers Under Examination



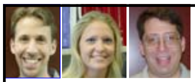
New miniSTRs

CORE STR Loci

Variant allele sequencing

SNPs

Y-chromosome STRs



Pete Vallone · Amy Decker · John Butler

Work with SNP Loci

- U.S. population frequencies with 70 autosomal SNPs
  - Vallone *et al.* (2005) *Forensic Sci. Int.* 149: 279-286
- U.S. population information with 50 Y-SNPs
  - Vallone *et al.* (2004) *J. Forensic Sci.* 49: 723-732
- Construction of 12plex autosomal SNP assay
  - Vallone *et al.* (2005) *Progress in Forensic Genetics 11*

>1,000 samples examined from 10 populations

- Creation of Forensic SNP Information website on STRBase
  - see Gill et al. *Science&Justice* 44(1): 51-53

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

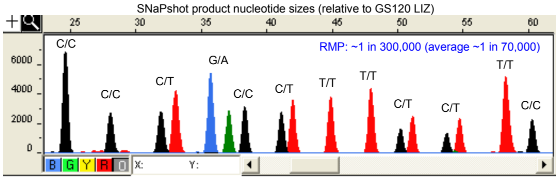
Vallone, *et al.* (2005) *Progress in Forensic Genetics 11, in press*

Autosomal 12plex SNP Assay

12plex PCR and 12plex ASPE from 0.5 ng DNA template

all PCR products <90 bp

SNaPshot product nucleotide sizes (relative to GS120 LIZ)



RMP: ~1 in 300,000 (average ~1 in 70,000)

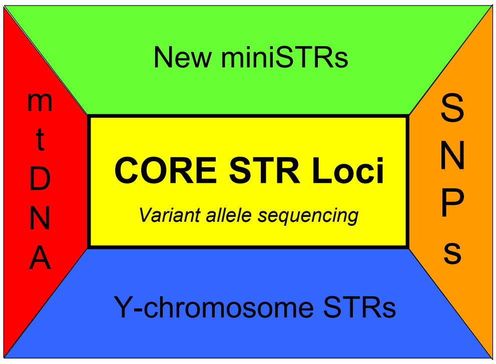
locus:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
chr:	5	15	10	1	17	13	17	1	6	11	20	11

“Best” 12 SNPs selected from 70 originally tested;
>0.45 heterozygosity in U.S. Caucasians, Hispanics, and African Americans

Vallone, P.M., Decker, A.E., Butler, J.M. (2005) Allele frequencies for 70 autosomal SNP loci with U.S. Caucasian, African American, and Hispanic Samples. *Forensic Sci. Int.* 149:279-286.

Population data has been collected on >1,000 samples (662 U.S. and 375 world samples) **from 10 different population groups**

Genetic Markers Under Examination



New miniSTRs

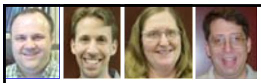
mtDNA

CORE STR Loci

Variant allele sequencing

SNPs

Y-chromosome STRs



Mike Coble · Pete Vallone · Margaret Kline · John Butler

Work with mtDNA

- **Collaboration with Armed Forces DNA Identification Laboratory** to develop multiplex mtDNA SNP assays for coding region polymorphisms
- Beta-testing and automation of Roche LINEAR ARRAY HVI/HVII probes
- Population study performed with LINEAR ARRAY HVI/HVII probes
- Exploration of effective strategies for forensic analysis in the mitochondrial DNA coding region

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

Typing frequencies for 666 NIST population samples

# in Group	Freq	% Types	% People
1	185	65.6	27.8
2	46	16.3	13.8
3	18	6.4	8.1
4	4	1.4	2.4
5	3	1.1	2.3
6	4	1.4	3.6
7	1	0.4	1.1
8	9	3.2	10.8
9	2	0.7	2.7
10	4	1.4	6.0
11	1	0.4	1.7
12	1	0.4	1.8
18	1	0.4	2.7
23	1	0.4	3.5
28	1	0.4	4.2
51	1	0.4	7.7

Summary of Our Population Typing with Roche mtDNA LINEAR ARRAYS

LINEAR ARRAY summary
- 282 different types
- 185 were unique (occurred only once)
- 51 samples had “Most Common Type”

HV1/HV2 sequencing summary
- 518 different types
- 454 were unique (occurred only once)
- 17 samples had “Most Common Type”

“Most Common Type” evaluated further with mtDNA coding region SNP assay

Kline *et al.* (2005) *J. Forensic Sci.* 50(2): 377-385


Pete Vallone


Dave Duewer

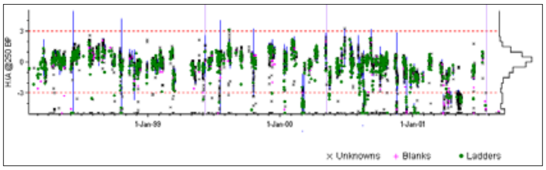

Chris DeAngelis

Software Tools

- AutoDimer – multiplex PCR primer screening tool
  http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm
- mixSTR – mixture component resolution tool
- **Multiplex_QA** – quality assessment tool for monitoring instrument performance over time
- NIST U.S. population database (internal Access database)

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

Multiplex_QA Overview



- **Research tool** that provides quality metrics to review instrument performance over time (e.g., examines resolution on internal size standard peaks)
- Runs with Microsoft Excel macros. Requires STR data to be converted with NCBI's BatchExtract program into numerical form.

Available for download from STRBase:
http://www.cstl.nist.gov/biotech/strbase/software.htm


John Butler

Training Materials and Review Articles

- Workshops on STRs and CE (ABI 310/3100)
  - Taught with Bruce McCord (Florida Int. Univ.)
  - NEAFS (Sept 29-30, 2004)
  - U. Albany DNA Academy (June 13-14, 2005)
  - **AAFS Feb 2006 Workshop #6 (February 20, 2006)**
- Validation Workshop
  - Taught with Robyn Ragsdale (FDLE) at NFSTC (August 24-26, 2005)
- PowerPoint slides from *Forensic DNA Typing*, 2nd Edition
  - >150 slides available now (~1,000 planned) for download
  - **http://www.cstl.nist.gov/biotech/strbase/FDT2e.htm**
- Review articles
  - ABI 310 and 3100 chemistry – Electrophoresis 2004, 25, 1397-1412
  - **Core STR Loci** – *J. Forensic Sci.* (May 2006)

http://www.cstl.nist.gov/biotech/strbase/training.htm
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

AAFS Workshop #6 (Feb 2006, Seattle)
Advanced Topics in STR DNA Analysis
Instructors: John Butler and Bruce McCord

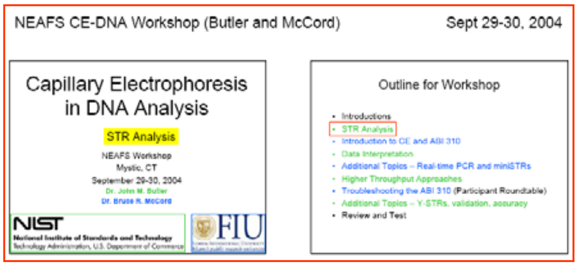
For DNA analysts using the ABI 310 or ABI 3100 who would like to better understand the underlying issues and science involved with STR DNA typing

- **STR Biology, Markers, and Methods**
- **Capillary Electrophoresis Instrumentation: Theory and Application**
- **Validation Aspects to Consider in Bringing a New STR Kit "On-line"**
- **CE Troubleshooting**
- **STR Mixture Interpretation**
- **DNA Quantitation with Real-Time qPCR**
- **Low-copy Number Issues**
- **Y-STRs and mtDNA**

Workshop Slide Handouts

Handouts available as downloadable pdf files from
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm#NEAFSworkshop

2 day workshop with **>500 slides** describing STRs and CE (ABI 310 and ABI 3100)


NEAFS CE-DNA Workshop (Butler and McCord) Sept 29-30, 2004

Capillary Electrophoresis in DNA Analysis

STR Analysis

NEAFS Workshop
Mystic, CT
September 29-30, 2004
Dr. John M. Butler
Dr. Bruce R. McCord

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

Review Article on Core STR Loci

- J.M. Butler **"Genetics and Genomics of Core STR Loci Used in Human Identity Testing"**
- ***Journal of Forensic Sciences, in press* (March 2006)**
- Reviews STR kits, genomic locations, mutation rates, potential genetic linkage, and known variant alleles for autosomal STR and Y-STR loci
- **Covers characteristics of 18 autosomal loci (13 core CODIS loci, D2, D19, Penta D, Penta E, SE33) and 11 SWGDAM-recommended Y-STR loci**

Acknowledgments

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John Butler


Margaret Kline


Pete Vallone


Mike Coble


Jan Redman


Amy Decker


Becky Hill


Chris DeAngelis


Dave Duewer

The many forensic scientists and their supervisors who took time out of their busy schedules to examine the MIX05 data provided as part of this interlaboratory study

Past and Present Collaborators (also funded by NIJ):
Mike Hammer and Alan Redd (U. AZ) for Y-chromosome studies
Tom Parsons, Rebecca Just, Jodi Irwin (AFDIL) for mtDNA coding SNP work
Sandy Calloway (Roche) for mtDNA LINEAR ARRAYs
Bruce McCord and students (FL Int. U.) for miniSTR work
Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work
Artie Eisenberg and John Planz (U. North Texas) for miniSTR testing on bones
Murray Brilliant (U. AZ) for phenotype markers
Ken Kidd (Yale U.) for SNP typing population samples

Thank you for your attention…

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

http://www.cstl.nist.gov/biotech/strbase
margaret.kline@nist.gov