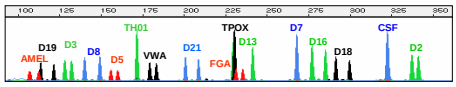


NIST On-Going Projects to Aid the Human Identity Testing Community

John Butler
Margaret Kline, Pete Vallone, Mike Coble
Jan Redman, Amy Decker, Becky Hill, Chris DeAngelis
Dave Duewer (NIST Analytical Chemistry Division)

Visit to Applied Biosystems – July 19, 2005

NIST Human Identity Project Team


John Butler
(Project Leader)


Margaret Kline


Pete Vallone


Mike Coble


Dave Duewer
Anal. Chem. Division


Jan Redman


Amy Decker


Becky Hill


Chris DeAngelis

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

Team Impact on Forensic Community

- **27 publications** since June 2004 (61 since 2000)
- **31 presentations** to the community since June 2004
- All NIST publications and presentations available on STRBase: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
- Training materials: 2 workshops conducted with Bruce McCord
  - NEAFS (Sept 29-30, 2004)
  - Albany DNA Academy (June 13-14, 2005)
- *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*, 2nd Edition (John Butler)

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

Current Areas of NIST Research Effort

- **Resources for "Challenging Samples"** (miniSTRs)
- **Information on New Loci** (SNPs, Y-Chromosome, new STRs)
- **Standard Information Resources** (STRBase website, training materials/review articles, validation standardization)
- **Allele Sequencing and Interlaboratory Studies** (Real-time qPCR, mixture interpretation)


Mike Coble


Becky Hill

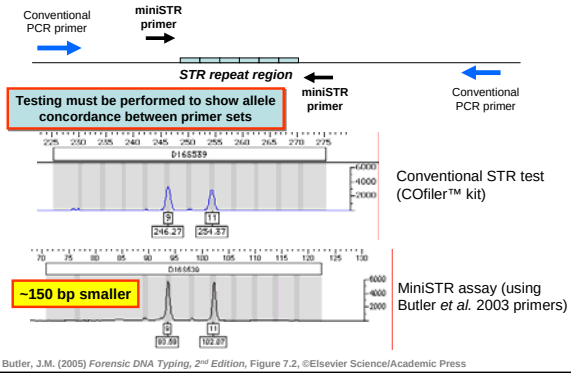

John Butler

miniSTRs for Degraded DNA

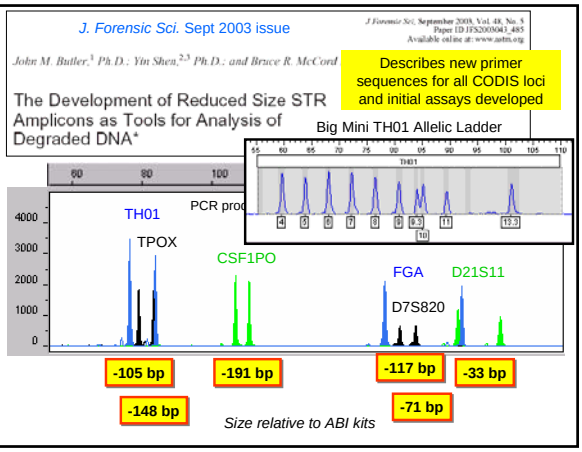
- Original miniSTR paper with CODIS loci, D2, D19, Penta D, Penta E
  - Butler *et al.* (2003) *J. Forensic Sci.* 48: 1054-1064
- Many CODIS loci are too big and make poor miniSTRs
- New miniSTRs and assays: NC01, NC02
  - Coble, M.D. and Butler, J.M. (2005) *J. Forensic Sci.* 50:43-53
- New miniSGM 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

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



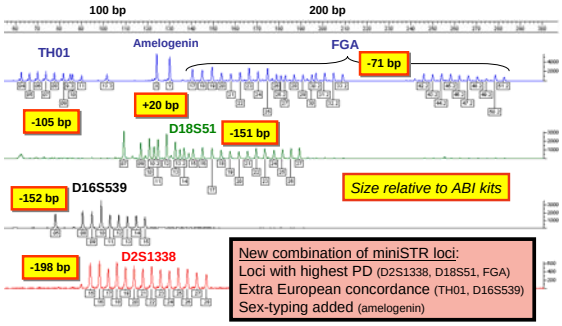
Recent Publications on miniSTRs

- Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5): 1054-1064.
- Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.
- Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. (2004) Concordance study between miniplex STR assays and a commercial STR typing kit, *J. Forensic Sci.* 49(4): 859-860.
- Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA., *J. Forensic Sci.,* 50: 43-53.

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

New miniSGM miniplex assay

Provided to EDNAP/ENFSI group for degraded DNA study (Fall 2004)



Retains same miniSTR primers from Butler et al. (2003) *J. Forensic Sci* 48(5): 1054-1064

Many CODIS Loci Make Poor miniSTRs

- Large allele range (e.g., FGA)
- Large alleles (e.g., D21S11 and FGA)
- Poor flanking regions prohibiting reliable primer annealing immediately adjacent to the repeat region (e.g., D7S820)

Why go beyond CODIS loci

"STRs have proven to be highly successful [for mass disasters] in the past e.g. Waco disaster and various air disasters. However, even if the DNA is high quality there are occasions when there are insufficient family members available to achieve a high level of confidence with an association."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

Why go beyond CODIS loci

"To achieve this purpose, either **new STRs could be developed**, or alternatively, existing STRs could be supplemented with a SNP panel."

"There are also efforts for modifying existing STR panels by decreasing the size amplicons by designing new primers."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

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

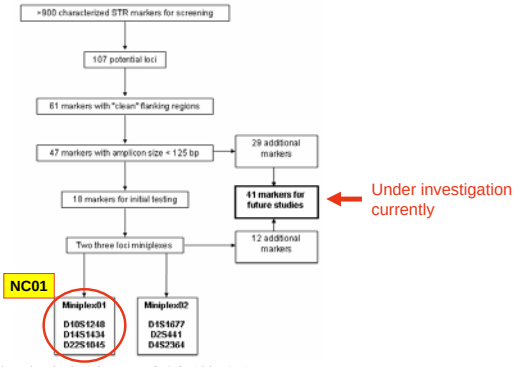
Why go beyond CODIS loci

- Desirable to have markers unlinked from CODIS loci (different chromosomes) for some applications
- Small size ranges to aid amplification from degraded DNA samples
- **New miniSTR loci will benefit missing persons investigations and paternity testing (and perhaps national databases in the future)**

Characterization of New miniSTR Loci

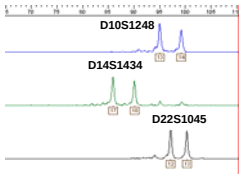
- Candidate STR marker selection
- Chromosomal locations and marker characteristics
- PCR primer design
- Initial testing results
- Population testing
- Allelic ladder construction
- Miniplex assay performance

Initial Testing Results with Potential miniSTR Loci

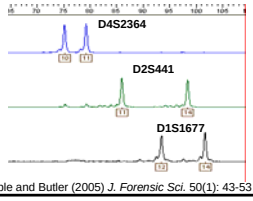


Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

Miniplex NC01



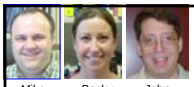
Miniplex NC02



Some Marker Characteristics

Chr.	Marker Name	(Motif)	Ref. Repeat	Amplicon Size	Primer distance from repeat
10	D10S1248	TETRA	13	102	1
	GGAA23C05N	GGAA			0
14	D14S1434	TETRA	10	88	1
	GATA168F06	GATA			0
22	D22S1045	TRI	13	105	3
	ATA37D06	ATA			6
1	D1S1677	TETRA	15	103	0
	GGAA22G10N	GGAA			0
2	D2S441	TETRA	12	92	0
	GATA8F03	GATA			0
4	D4S2364	TETRA	7	78	2
	GAAT1F09	GAAT			1

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



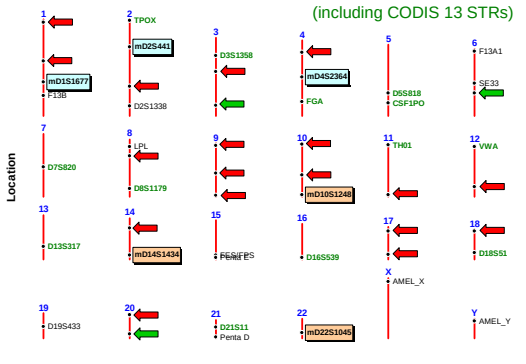
Mike Coble | Becky Hill | John Butler

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
- 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 core loci

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

STR Loci Positions
(including CODIS 13 STRs)



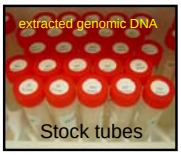
Positions determined along May 2004 Human Genome Reference Sequence (NCBI Build 35)

Standard U.S. Population Dataset

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

260 Caucasians, 260 African Americans, 140 Hispanics, 3 Asians = **663 males**

DNA extracted from whole blood (anonymous; self-identified ethnicities) received from Interstate Blood Bank (Memphis, TN) and Millennium Biotech Inc. (Ft. Lauderdale, FL)

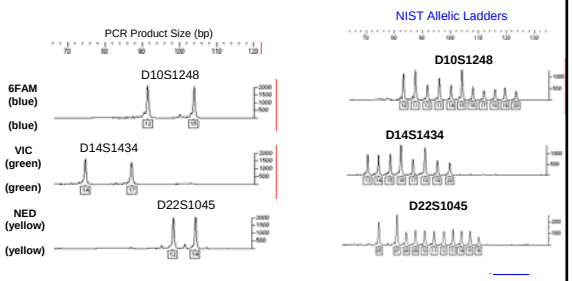


To date: (~95,000 allele calls)

Identifiler (15 autosomal markers + Amelogenin) (10,608)
Roche Linear Arrays (HV1/HV2 10 regions) (6,630)
Y STRs 22 loci—27 amplicons (17,388)
Y STRs 27 new loci (14,535)
Yfiler kit 17 loci (11,237)
Y SNPs 50 markers on sub-set of samples (11,498)
Orchid 70 autosomal SNPs on sub-set (13,230)
miniSTR testing-new loci and CODIS concordance (9,228)
mtDNA full control region sequences by AFDIL

Genotypes with various human identity testing markers

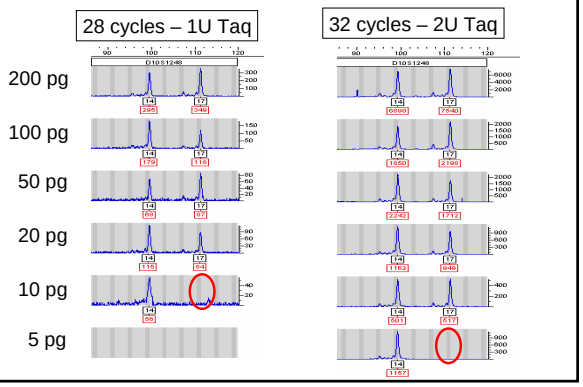
Miniplex "NC01"



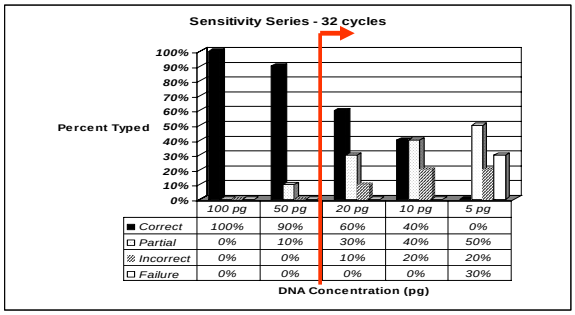
http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm

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

miniSTR Assay Sensitivity (D10S1248)



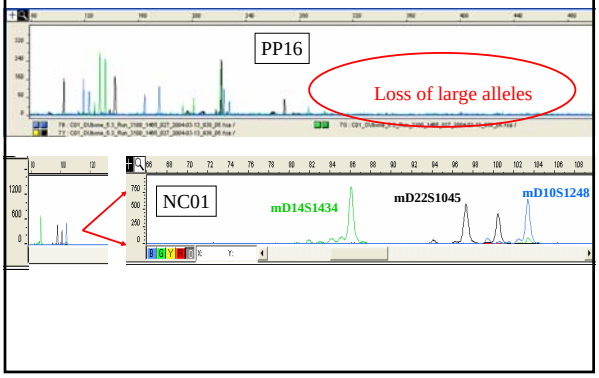
Problems with Obtaining Correct Allele Calls at Low DNA Levels



	100 pg	50 pg	20 pg	10 pg	5 pg
Correct	100%	90%	60%	40%	0%
Partial	0%	10%	30%	40%	50%
Incorrect	0%	0%	10%	20%	20%
Failure	0%	0%	0%	0%	30%

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

Sensitivity - Degraded DNA from an OU Bone Sample



Peter Gill Recommendations to EDNAP and ENFSI (April 2005, Scotland)

- **"miniSTRs are the best way forward for stain work for the foreseeable future…"**
- miniSTRs and 34 cycle PCR seems to be the best option to maximise sensitivity (note importance of minimising cycle number to avoid stochastic effects).
- Recommended to the ENFSI group that miniSTRs are the best way forward.
- Suggested NIST NC01 loci as additional European markers that are being advocated to manufacturers for future STR kits.

Status of Additional STR Loci

- **D10S1248, D14S1434, D22S1045** are chromosomally unlinked to all CODIS STR loci
- Full locus characterization, allelic ladders constructed, population studies completed and published (Coble and Butler JFS Jan 2005)
- Demonstrated success in EDNAP degraded DNA interlab study coordinated by Peter Gill
- EDNAP/ENFSI newly recommended loci to commercial manufacturers for future STR kits
- Being adopted in multiple U.S. paternity testing labs (BRT Labs and Orchid Cellmark East Lansing)

Paper Coming Out Soon…

ARTICLE IN PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT

ELSEVIER

Forensic Science International

Short communication

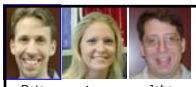
The evolution of DNA databases—Recommendations for new European STR loci

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

…recommended that existing multiplexes are re-engineered to enable small amplicon detection, and that three new mini-STR loci with alleles <130 bp (D10S1248, D14S1434 and D22S1045) are adopted as universal. This will increase the number of European standard Interpol loci from 7 to 10.



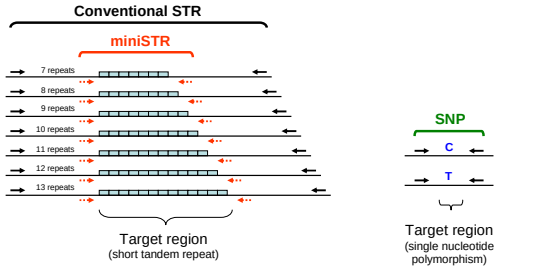
Pete Vallone, Amy Decker, John Butler

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
- 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

Comparison of STRs and SNPs



Larger target region (miniSTR targets same region)
More possible variants than SNPs
Only need a moderate number of STR markers
Range of sizes examined (e.g., 28 bp spread if 4 bp/repeat)

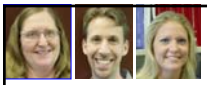
Smaller target region
Fewer possible variants
Need more SNP markers
Constant size examined

John Butler, Margaret Kline, Pete Vallone, Amy Decker

Work with Y-STRs

- Beta-testing of all commercial Y-STR kits
- 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
- Nomenclature defined for new loci
- 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



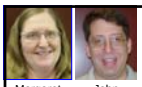
Margaret Kline, Pete Vallone, Amy Decker

Evaluation of qPCR Assays

- Evaluation of published assays on same samples
- Characterization of Quantifiler lot-to-lot performance
- Additional studies under way utilizing qPCR:
  - Examining the challenge of multiplexing qPCR assays
  - Studies to track DNA recovery from various types of tubes
  - Characterizing potential SRM 2372 components (Human DNA Quantitation Standard)

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

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



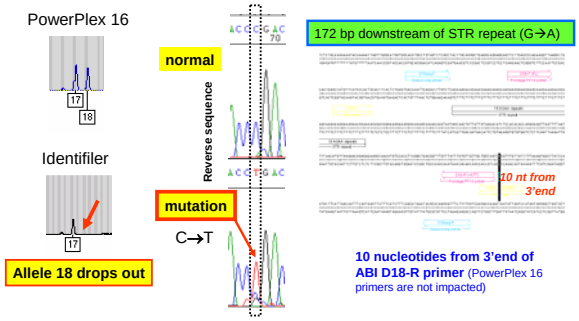
Margaret Kline John Butler

STR Allele Sequencing and Characterization

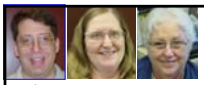
- Variant characterization
  - TPOX 10.3 (Maryland State Police)
  - D18S51 null alleles (FSS and Kuwait govt)
  - D18S51 allele 40 (Nebraska State Crime Lab)
  - D18S51 allele 5.3 (DNA Solutions)
  - FGA allele 46.2 (Denver Crime Lab)
  - DYS392 allele "10.3" (AFDIL)
- Locus duplication or deletion
  - DYS390 (CFS Toronto)
  - DYS392 (MN BCA)
- **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



John Butler Margaret Kline Jan Redman

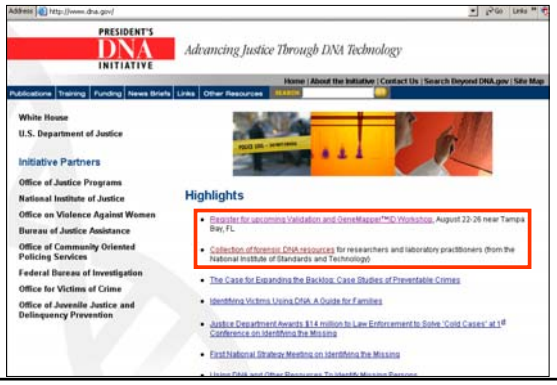
Validation Standardization

- 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

- We invite submission of your internal validation studies for inclusion in the NIST validation website

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

www.dna.gov website



John Butler Jan Redman

STRBase Updates

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

Content of STRBase Website

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

- …/str_fact.htm STR Fact Sheets on Core Loci
- …/multiplx.htm Multiplex STR Kit Information
- …/y_strs.htm Y-Chromosome Information
- …/var_tab.htm Variant Alleles Reported
- …/mutation.htm Mutation Rates for Common STRs
- …/str_ref.htm Reference List with ~2,300 Papers
- …/training.htm Downloadable PowerPoints for Training
- …/validation.htm Validation Information
- …/miniSTR.htm miniSTR Information
- …/address.htm Addresses for Scientists
- …/NISTpub.htm Publications & Presentations from NIST

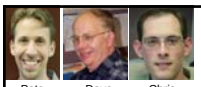
Training Materials and Review Articles


John Butler

- 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)
- PowerPoint slides from *Forensic DNA Typing*, 2nd Edition
- Review articles
  - ABI 310 and 3100 chemistry – Electrophoresis 2004, 25, 1397-1412
  - Forensic DNA analysis – Anal. Chem. 2005, 77, 3839-3860
  - STR core loci – J. Forensic Sci., *in press* (Nov 2005)

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

Software Tools


Pete Vallone Dave Duewer Chris DeAngelis

- 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

Interlaboratory Studies


Margaret Kline Dave Duewer Jan Redman John Butler

- 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)
  - 91 labs signed up (64 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

Acknowledgments

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John Butler Margaret Kline Pete Vallone Mike Coble Jan Redman Amy Decker Becky Hill Chris DeAngelis Dave Duewer

Past and Present Collaborators (also funded by NIJ):
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Artie Eisenberg and John Planz (U. North Texas)

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