# NIST Research Update

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**Public SWGDAM Meeting – September 25, 2005**
16th International Symposium on Human Identification (Grapevine, TX)

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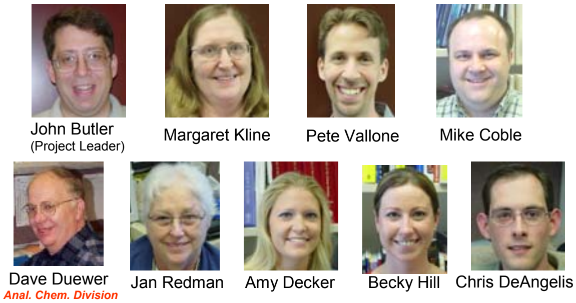
## NIST Human Identity Project Team



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

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## Disclaimers and Collaborations

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

Points of view are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

**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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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

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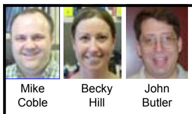

## miniSTRs for Degraded DNA

- 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**

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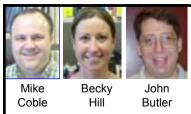
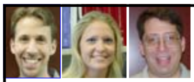


## New Autosomal STR Loci

- 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

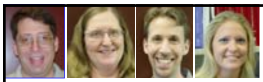
**http://www.cstl.nist.gov/biotech/strbase/newSTRs.htm**



## 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) **Poster P-296 at ISFG**
- Creation of Forensic SNP Information website on STRBase
  - see Gill et al. *Science&Justice* 44(1): 51-53

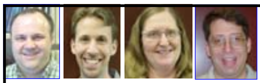
**http://www.cstl.nist.gov/biotech/strbase/SNP.htm**



## 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**

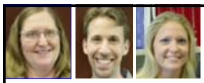
**http://www.cstl.nist.gov/biotech/strbase/y_strs.htm**



## 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

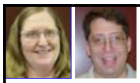
**http://www.cstl.nist.gov/biotech/strbase/mtDNA.htm**



## 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)
- **Talk on Thursday morning here at Promega meeting**

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

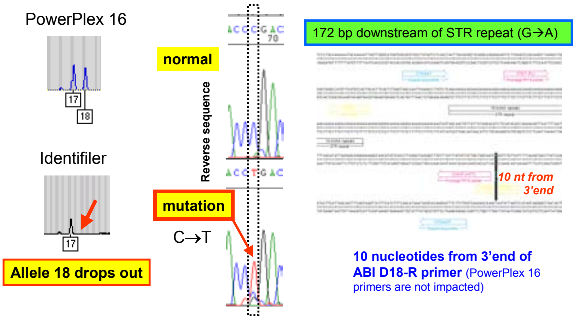


## STR Allele Sequencing and Characterization

- 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)
- **Send us your unusual STR alleles for sequence characterization**

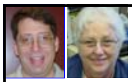
**http://www.cstl.nist.gov/biotech/strbase/STRseq.htm**

## D18S51 Null Allele from Kuwait Samples with ABI Primers



**10 nucleotides from 3’end of ABI D18-R primer** (PowerPlex 16 primers are not impacted)

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 Jan Redman

## 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

## 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 |

Pete Vallone Dave Duewer Chris DeAngelis

## Software Tools

- AutoDimer – multiplex PCR primer screening tool

**http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm**

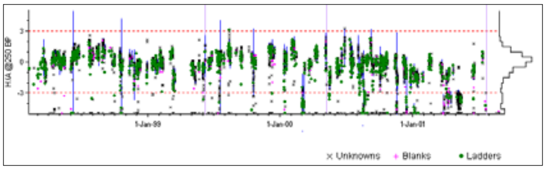
- mixSTR – mixture component resolution tool

- 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)

- 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

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

Margaret Kline Dave Duewer Jan Redman John Butler

## Interlaboratory Studies

- 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)
  - 105 labs signed up (**71** labs returned data)
  - Interpretation requested of provided e-grams for 4 mock sexual assault cases
  - **Come see Poster #56 with MIX05 preliminary results**

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

## NIST Quantitation Study 2004 (QS04)

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

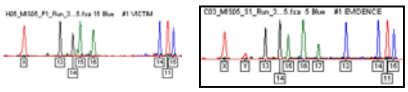
Consisted of:
•8 DNA extracts labeled A – H
•Shipped Dec 2003 –Jan 2004 to 84 laboratories for quantification; data received back by April 2004
•Labs were requested to use multiple methods / multiple analysts

We received data from 80 Labs (95%)
**Total of 287 sets of data**
Participants used 19 different quantification methods (primarily variations on Quantiblot and Real-time PCR)

Information from this interlab study is being used to help construct SRM 2372 (Human DNA Quantitation Standard)

## Mixture Interpretation Interlab Study
(MIX05)

- **Only involves interpretation of data**
- **105 labs enrolled** for participation (20 from overseas)
- **71 labs have returned results**
- Four mock cases supplied with "victim" and "evidence" electropherograms (GeneScan .fsa files – that can be converted for Mac or GeneMapper; gel files made available to FMBIO labs)
- Data available with Profiler Plus, COfiler, SGM Plus, PowerPlex 16, Identifiler, PowerPlex 16 BIO (FMBIO) kits
- Summary of results with involve training materials to illustrate various approaches to solving mixtures

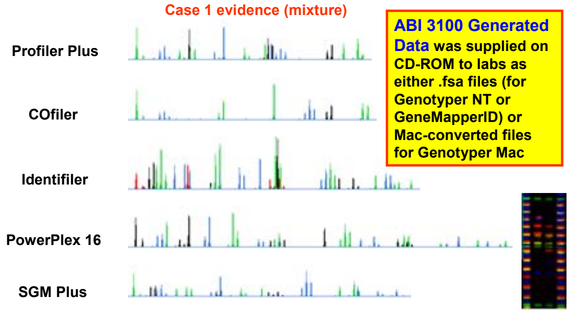


Perpetrator Profile(s) ??

**Along with reasons for making calls and any stats that would be reported**

## MIX05 Results on Multiple Kits

**http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm**



**ABI 3100 Generated Data was supplied on CD-ROM to labs as either .fsa files (for Genotyper NT or GeneMapperID) or Mac-converted files for Genotyper Mac**

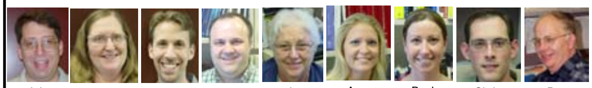
**FMBIO data was also made available upon request**

## Purpose of MIX05 Study

- **Goal is to understand the "lay of the land" regarding mixture analysis across the DNA typing community**
- "If you show 10 colleagues a mixture, you will probably end up with 10 different answers"
  - Peter Gill, Human Identification E-Symposium, April 14, 2005
- One of the primary benefits we hope to gain from this study is **recommendations for a more uniform approach to mixture interpretation** and training tools to help educate the community

## Acknowledgments

**Funding from interagency agreement 2003-IJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards**



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

**MIX05 Credits**

•Margaret Kline (sample prep, running study)
•John Butler (study design and data review)
•Becky Hill (GeneMapper*ID* data review)
•Jan Redman (Access database entry)
•Dave Duewer (*Virtual MixtureMaker* to aid sample selection)
•Chris Tomsey & Frank Krist (FMBIO Mac data)
•Kermit Channel & Mary Robnett (FMBIO NT data)

**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**

## Thank you for your attention…

## Questions?

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

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