International Congress Series 1288 (2006) 628–635

www.ics-elsevier.com

Setting standards and developing technology to aid the human identity testing community

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Abstract. Our project team at the U.S. National Institute of Standards and Technology (NIST) is funded by the National Institute of Justice (NIJ) to conduct research that benefits the human identity testing community and to create tools that enable forensic DNA laboratories to be more effective in analyzing DNA. We certify standard reference materials, conduct interlaboratory studies, produce new assays to enable improved recovery of information from degraded DNA, evaluate new loci for potential future use in human identity applications, and generate standard information and training materials that are made available on the NIST STRBase website: http://www.cstl.nist.gov/biotech/strbase/. New genetic markers and assays involving STR and SNP loci are examined in a U.S. reference population data set involving approximately 660 samples that are of Caucasian, Hispanic, and African American origin. Efforts to improve STR and SNP typing resources and assays for the community are described.

Keywords: DNA typing; Y-chromosome; mtDNA; miniSTR; STRBase; STR; SNP; NIST; Interlaboratory studies; Standard reference materials; SRMs

1. Introduction

Since the late 1980s, the U.S. National Institute of Standards and Technology (NIST) has had scientists involved in DNA testing. Over the past two decades NIST has worked closely with the forensic community to develop DNA reference materials and to evaluate new technologies. A human identity (HID) project team was established within the DNA Technologies Group in 2000 and has grown from four to eight research scientists with

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0531-5131/
doi:10.1016/j.ics.2005.09.181

expertise in all aspects of forensic DNA analysis. The National Institute of Justice (NIJ) funds most of the projects conducted by the NIST HID team.

Established by Congress in 1901 under the name National Bureau of Standards, the National Institute of Standards and Technology, as it has been called since 1988, is a non-regulatory federal agency within the U.S. Commerce Department's Technology Administration (see http://www.nist.gov). The mission of NIST is to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life. NIST employs about 3000 scientists, engineers, technicians, and support and administrative personnel primarily in two campuses located in Gaithersburg, Maryland and Boulder, Colorado. NIST supplies over 1300 Standard Reference Materials (SRMs) for industry, academia, and government use in such areas as environmental analysis, health measurements, and industrial materials production and analysis.

To help meet national and international measurement and standards needs, the NIST HID team provides DNA typing SRMs, develops helpful information resources (e.g., STRBase website), conducts interlaboratory studies, and evaluates new technology and genetic loci that may benefit future application within the human identity testing community.

2. Setting standards in reference materials, information, and interlaboratory studies

2.1. Available SRMs

NIST aids quality assurance efforts and helps ensure compatible measurements by generating, certifying, and issuing SRMs. Table 1 lists the various NIST SRMs that have been created or are in development to aid calibration of human identity testing assays. The DNA Advisory Board (DAB) quality assurance standards that govern forensic DNA analysis within the United States require that a laboratory check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST SRM or standard traceable to a NIST standard (DAB standard 9.5). These NIST SRMs also enable laboratories receiving accreditation under ISO 17025 requirements to demonstrate measurement traceability to a national reference material (see ISO 17025 section 5.6).

2.2. STRBase: an information resource

NIST has compiled and maintained a Short Tandem Repeat DNA Internet Database (http://www.cstl.nist.gov/biotech/strbase/) since 1997 commonly referred to as STRBase

Table 1
NIST Standard Reference Materials (SRMs) with human identity testing applications

Number	Release date	Name
SRM 2390	1992	DNA profiling standard (RFLP)
SRM 2391b	1995, 1999, 2002	PCR-based DNA profiling standard (STRs)
SRM 2392-I	2004	Mitochondrial DNA sequencing (human HL-60 DNA)
SRM 2395	2003	Human Y-chromosome DNA profiling standard
SRM 2372	Planned 2006	Human genomic DNA quantitation standard

Table 2
NIST-sponsored interlaboratory studies; see http://www.cstl.nist.gov/biotech/strbase/interlab.htm

Name (when conducted)	# Laboratories participating	Publications
RFLP studies (1992–1999)	20–36	Mudd et al. [3], Duewer et al. [4–7], Stolorow et al. [8]
D1S80 sizing ladders (1994)	23	Kline et al. [9]
CTT evaluation (1996)	34	Kline et al. [10]
Mixed stain study #1 (1997)	45	Duewer et al. [11]
Mixed stain study #2 (1999)	45	Duewer et al. [11]
Mixed stain study #3 (2001)	74	Kline et al. [12], Duewer et al. [13]
DNA quantitation study (2004)	80	Kline et al. [14]
Mixture interpretation study (2005)	71	Forthcoming

[1]. This website is an information resource for the human identity testing community with details on commonly used STR markers and kits, summaries of variant alleles observed by laboratories worldwide, addresses of scientists involved in STR typing, suggestions to aid validation efforts [2], and a fairly comprehensive listing of the literature on STR markers applied to human identity testing. Publications and presentations by the NIST HID team are available as pdf files from http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm.

2.3. Interlaboratory studies

Interlaboratory tests are a means by which multiple laboratories compare results and demonstrate that the methods used in one's own laboratory are reproducible with another laboratory. For over a decade, NIST has been involved in or sponsored interlaboratory studies to assess performance with RFLP and STR typing systems (Table 2).

The most recent NIST sponsored interlaboratory study (MIX05) involved 71 laboratories reviewing the same electropherograms of four different mock case scenarios with mixtures created using five different STR kits. Data for MIX05 are available at http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm.

3. Research efforts and technology development and evaluation

A major focus of recent research efforts by our project team is to understand how potential new genetic loci will perform in relevant U.S. population groups relative to conventional human identity testing assays (e.g., STR kits) already in use. A set of 660 male and 40 female DNA samples have been developed through bulk extraction of purchased blood bank samples from individuals with self-identified ethnicities [15]. Table 3 lists the various assays and loci that have been explored with this U.S. sample set. Thus far, over 100,000 data points have been collected on these samples.

3.1. miniSTRs

Primers designed to bind to STR loci immediately adjacent to the repeat region generate shorter PCR products when compared to commercial STR kits (see http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm). These reduced size STR amplicons have been dubbed "miniSTRs" [25]. Many of the miniSTR primers originated with mass spectrometry efforts for STR typing [26]. MiniSTRs have shown value in recovery of

Table 3
Loci examined with NIST U.S. population samples (http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm)

Assay/Kit	Loci examined	Publication reference
Autosomal STRs—Identifiler kit	13 CODIS STRs, D2S1338, D19S433, amelogenin	Butler et al. [15]
miniSTRs—CODIS loci	12 STRs (all CODIS loci except D3S1358)	Drabek et al. [16]
miniSTRs—new loci	D10S1248, D14S1434, D22S1045, D1, D2, D4	Coble et al. [17]
miniSTRs—new loci	10 new loci	Coble et al. [18]
Y-STRs—NIST 20plex, 11plex	22 Y-STRs including multi-copy locus DYS464	Schoske et al. [19]
Y-STRs—Yfiler kit	17 Y-STRs	*Yfiler database* [20]
Y-STRs—new loci	20 additional Y-STR loci beyond Yfiler	Butler et al. [21]
Y-SNPs	50 Y-SNPs including those in Marligen kits	Vallone et al. [22]
MtDNA typing	Roche LINEAR ARRAYs	Kline et al. [23]
MtDNA sequence	Entire control region was sequenced by AFDIL	Forthcoming
Autosomal SNPs	70 SNPs (see . . ./strbase/SNP.htm)	Vallone et al. [24]

information from degraded DNA samples including many World Trade Center victims [27]. Efforts are underway to characterize new miniSTR loci [17,18]. Three of the new miniSTR loci, D10S1248, D14S1434, and D22S1045 [17], have been recommended by the European Network of Forensic Science Institutes for use in future European DNA databases [28].

3.2. Y-chromosome STRs

Our HID team created the first Y-STR multiplex capable of simultaneous amplifying all of the original European extended haplotype loci [29,30]. Many of these primers were later adopted for use in commercial Y-STR kits such as PowerPlex Y and Yfiler. Core loci [19] and additional loci [21] have been examined in U.S. populations. Y-chromosomal duplications have also been examined [31]. A section of STRBase has been devoted to Y-STR information: http://www.cstl.nist.gov/biotech/strbase/y_strs.htm.

3.3. mtDNA

We have collaborated with the Armed Forces DNA Identification Laboratory (AFDIL) to develop a multiplex PCR and SNP typing assay for 11 SNPs from the mtGenome that help separate samples containing the most common HVI/HVII mtDNA sequence in Caucasians [32]. The Roche Linear Array mtDNA HVI/HVII typing system has been evaluated with our U.S. population samples, and the washing and hybridization portions of the assay were automated [23]. In addition, effective strategies for examining variability in the mtDNA coding region have been explored [33]. A focused SNP typing approach [33] has been shown to be more effective than a proposal for selected sequencing of the mtDNA coding region [34].

3.4. SNPs

A number of autosomal SNPs [24], Y-chromosome SNPs [22], mtDNA SNPs [23] have been evaluated in our U.S. population samples. Information on potential forensic SNP loci may be accessed at http://www.cstl.nist.gov/biotech/strbase/SNP.htm. This site has been recommended as a repository for the human identity testing community [35]. A robust 12plex assay has been developed and examined in more than 1000 samples [36].

3.5. qPCR for DNA quantitation

A number of quantitative PCR (qPCR) assays are being evaluated at NIST on the same set of DNA samples. A standard reference material (SRM 2372) is in development based on information gained from the NIST DNA Quantitation Study conducted in 2004 [14]. For more information, see http://www.cstl.nist.gov/biotech/strbase/DNAquant.htm.

3.6. DNA stability studies

Over the past decade, a number of studies have been performed to examine DNA stability on various solid matrices including untreated S&S 903 paper, Whatman, IsoCode, and FTA treated papers [37]. We have been able to successfully recover full STR profiles from bloodstains stored on untreated paper for 15 years at room temperature. For more information, see http://www.cstl.nist.gov/biotech/strbase/NIJprojects.htm.

3.7. Variant allele characterization and sequencing

Sequencing primers have been designed that bind outside of all STR kit primer positions in order to enable characterization of polymorphisms that give rise to allele dropout with a particular primer set. We have performed sequence analysis on a number of variant alleles provided by scientists from around the world. A summary of this information is available at http://www.cstl.nist.gov/biotech/strbase/STRseq.htm.

3.8. Software tools

A number of software tools have been created by our HID project team and are available for download at http://www.cstl.nist.gov/biotech/strbase/software.htm. The **AutoDimer** program, which has been used in all of our multiplex PCR assay development, enables rapid screening of short oligonucleotides for possible primer dimer or hairpin formation [38]. **Multiplex_QA** is an exploratory analysis system using Microsoft Excel macros and is intended to help monitor different performance characteristics of multiplex STR analysis such as instrument resolution and sensitivity over time. The **mixSTR program**, developed in collaboration with the Palm Beach County Sheriff's Office, helps identify reference profiles in sets of mixed source samples particularly cases involving multiple suspects.

3.9. Assay development with collaborators

Over the past few years, we have helped a number of collaborators develop multiplex detection assays [39] including an 11plex SNaPshot assay for mtDNA coding region SNPs (with the Armed Forces DNA Identification Laboratory) [32] and a 12plex assay for cat STRs and gender identification (with the National Cancer Institute's Laboratory of Genomic Diversity) [40]. We welcome future collaborations to develop additional assays to benefit the human identity testing community.

4. Training materials

The NIST HID team will continue to lead research efforts within the human identity testing community and make publications and presentations available on the STRBase

website. In addition, review articles are being written on such topic as the chemistry of capillary electrophoresis [41], validation, and genomics of core STR loci.

Training materials as pdf files and downloadable PowerPoint slides are available at http://www.cstl.nist.gov/biotech/strbase/training.htm. Information is available from workshops presented on topics such as capillary electrophoresis, Y-STRs, and validation. Over 150 slides to aid individuals teaching with *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers* (2nd Edition) [42] are also available at http://www.cstl.nist.gov/biotech/strbase/FDT2e.htm.

Acknowledgments

These projects were supported by the National Institute of Justice Grant Number 1999-IJ-R-A094 and 2003-IJ-R-029, which is an interagency agreement between NIJ and the NIST Office of Law Enforcement Standards, awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice. Points of view in this document 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. SRM 2392-I was created by a different NIST team lead by Dr. Barbara Levin.

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