Using the Scientific Literature
to Become a Lifelong Learner
and NIST Research Update

John M. Butler
National Institute of Standards and Technology

AFDAA winter meeting (Austin, TX)
January 28, 2010

Becoming an Expert Witness – Literature Tracking

Presentation Outline for Literature Portion

- Why bother with the literature?
- What is the literature you should be concerned with reading?
- What are some strategies for reading the literature?
- What resources exist for finding papers?
- What resources exist for storing and retrieving information related to the literature?
- How do I go about writing an article?

Are You an Expert?

- What kind of expert witness will you be?
- Do you know the field as well as you need to?
- **Reading the literature is critical to your ability to be an effective expert!**

Why Discuss the Literature?

- Hopefully you have a desire to continue to learn throughout your career
- The publications of others are key to learning
- How we manage information is critical to success in the information age we live in today

Revised Quality Assurance Standard Requirement for Literature Review

Quality Assurance Standards for Forensic DNA Testing Laboratories
(effective July 1, 2009)

5.1.3.2. The laboratory shall have a program approved by the technical leader for the **annual review of scientific literature** that documents **the analysts' ongoing reading of scientific literature**. **The laboratory shall maintain or have physical or electronic access to a collection of current books, reviewed journals, or other literature applicable to DNA analysis**.

http://www.fbi.gov/hq/lab/fsc/backissu/oct2008/standards/2008_10_standards01b.htm

I am trying to get you "hooked" on literature

- **I want to teach you to fish** rather than just giving you some fish…

Give a man a fish and you feed him for a day. Teach a man to fish and you feed him for a lifetime.
Chinese Proverb

"Give a man a fish; you have fed him for today. Teach a man to use the Net and he won't bother you for weeks."
—Author unknown

Benefits of Literature Scans

- Become familiar with authors and institutions
- Will improve you as a writer and a presenter
- Will improve your lab's performance
- Over time you will be building your knowledge

- Remember: **You don't have to master every paper…**

How many have read any scientific article in the past month?

The Value of a Journal Club

- Some potential approaches
  - Have specific people looking at individual journals
  - Bring relevant articles to attention of everyone

- ***J Forensic Sci*** **and** ***FSI Genetics*** **will cover ~90% of relevant articles in forensic DNA**
  - Scan journal, distill information, distribute to group

- rQAS requires literature to be available

How not to do it based on my experience

- Passing around individual journals with a reader list attached
  - Very inefficient process because journals get stuck on someone's desk
  - It becomes challenging to find a specific issue before it is returned to a central repository
  - Some information may not be as relevant (for research) many months later

Forensic Science Publications


SUPPLEMENT

Progress in Forensic Genetics 13

Proceedings of the 23rd International ISFG Congress
Buenos Aires, Argentina
between 15 and 18 September 2009

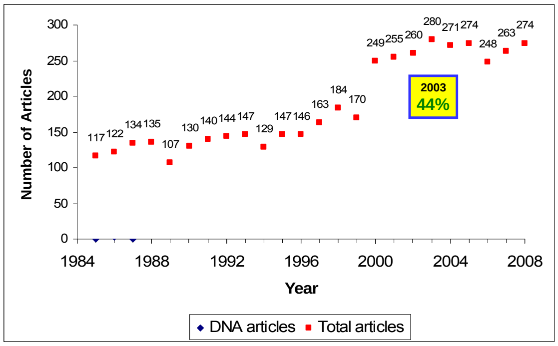
251 articles freely available at http://www.fsigeneticssup.com

Guest Editor

Niels Morling
Section of Forensic Genetics
Department of Forensic Medicine
Faculty of Health Sciences
University of Copenhagen
Denmark

Journal of Forensic Sciences

DNA publications vs total articles



From PubMed searches performed Feb 2009 using http://www.ncbi.nlm.nih.gov/sites/entrez

Forensic Science International: Genetics

http://www.fsigenetics.com/

We need your help as good reviewers and authors

Editor-in-Chief:
Angel Carracedo (Spain)
Associate Editors:
Peter M. Schneider (Germany)
John M. Butler (USA)

FSI: Genetics is a new journal dedicated exclusively to the field of forensic genetics. It has been launched in 2007 by Elsevier Publishers in affiliation with the International Society of Forensic Genetics. **All members of the ISFG receive a free subscription of this journal** (print and online version) as part of their membership benefits.

A Brief Review of Contents
(March 2007-Sept 2009)

- STR population data (40)
- Y-STRs (32)
- Mitochondrial DNA (28)
- X-STRs (13)
- Non-human DNA (11)
- SNPs (10)
- Mixtures (8)
- Low-level DNA (8)
- Degraded DNA/miniSTRs (6)
- Paternity testing (6)
- Rapid screening/portable devices (4)
- Phenotype information (4)
- DNA extraction (4)
- DNA quantitation (3)
- Disaster victim identification (3)
- DNA databases (3)
- Variant & tri-alleles (3)
- Mutation rates (4)
- Forensic pathology (3)
- Statistical issues (2)
- Expert systems (2)
- Review articles (2)
- Other articles not classified (14)

213 articles published in 11 issues

AAFS 2009 Topics Regarding Forensic DNA

From abstracts of presentations at AAFS meeting in Denver, CO (Feb 2009)

- Improved DNA extraction
- Predicting hair color and ancestry with SNPs
- X-chromosome STRs
- **Familial searching**
- Y-STRs and mixtures
- **Low level DNA samples**
- miniSTRs
- DNA screening assays
- Optimizing database labs
- Microfluidic biochip systems
- Use with property crimes
- Recovery from handguns
- DNA from IEDs
- Expert systems
- Automation with robotics
- DNA quantitation – qPCR
- PCR directly from blood
- mtDNA
- RNA
- Non-human DNA (dogs & cows)
- **Mixture interpretation**

Approaches to Maintaining an Awareness of the Literature

- Foster environment where any employee can bring helpful information to their supervisor and team members
- Prepare reference lists on topics of interest to your lab (or team)
  - Examples:
    - **Listing of all articles on mixture interpretation**
    - Gathering 70 articles on low-copy number DNA (pdf files and reference list)

How to Read a Scientific Article

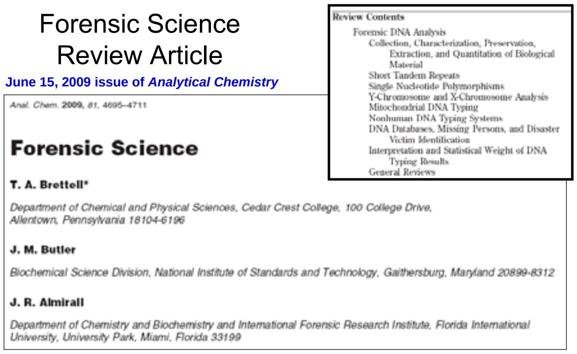
- Skim the article first
  - Start with title and abstract (may consider authors as well)
  - Scan tables, figures and figure captions
- Examine results and conclusions
  - Do the data presented support the statements made?
- Do not worry about trying to comprehend the entire article at first
  - I very rarely read an article from start to finish in its entirety
- Highlight key points and make notes on the paper itself so you can go back to them later to refresh your memory

Approaches for On-Going Information Searches on Topics of Interest

- Review entire journal listing of articles
  - Pick up journal or view table of contents on-line
- Directed searches on specific topics
  - PubMed
- Sign up for table of contents delivery via email
- **Examine publications cited in review article**

Forensic Science Review Article

June 15, 2009 issue of *Analytical Chemistry*


Anal. Chem. **2009,** *81,* 4695–4711

Forensic Science

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Review Contents

- Forensic DNA Analysis
  - Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material
  - Short Tandem Repeats
  - Single Nucleotide Polymorphisms
  - Y-Chromosome and X-Chromosome Analysis
  - Mitochondrial DNA Typing
  - Nonhuman DNA Typing Systems
  - DNA Databases, Missing Persons, and Disaster Victim Identification
  - Interpretation and Statistical Weight of DNA Typing Results
  - General Reviews

2009 review article covers 160 DNA articles published in 2007-2008

PubMed Searches

Items 1 - 20 of 261 Page 1 of 14 Next

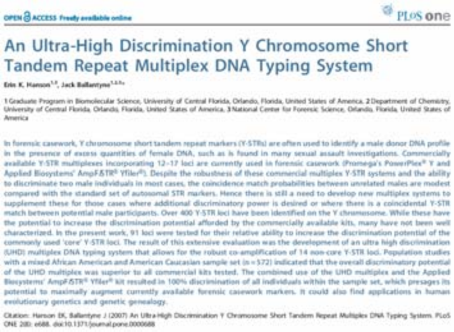
☐ 1: Determination of deleted regions from Yp11.2 of an amelogenin negative male.
Takayama T, Takada N, Suzuki R, Nagaoka S, Watanabe Y, Kumagai R, Aoki Y, Butler JM.
Leg Med (Tokyo). 2009 Mar 5. [Epub ahead of print]
Related Articles

☐ 2: DNA identification of biological traces and interpretation in a sexual assault case.
Apostolov A, Hristov S, Angelova E.
Am J Forensic Med Pathol. 2009 Mar;30(1):57-60.
Related Articles

☐ 3: Y chromosomal STR analysis using Pyrosequencing technology.
Edlund H, Allen M.
Forensic Sci Int Genet. 2009 Mar;3(2):119-24. Epub 2009 Jan 6.
Related Articles

http://www.ncbi.nlm.nih.gov/PubMed

The Public Library of Science (PLoS) offers free on-line access to scientific articles



An Ultra-High Discrimination Y Chromosome Short Tandem Repeat Multiplex DNA Typing System

Erin K. Hanson[1,2], Jack Ballantyne[1,2,3]

1 Graduate Program in Biomolecular Science, University of Central Florida, Orlando, Florida, United States of America, 2 Department of Chemistry, University of Central Florida, Orlando, Florida, United States of America, 3 National Center for Forensic Science, Orlando, Florida, United States of America

In forensic casework, Y chromosome short tandem repeat markers (Y-STRs) are often used to identify a male donor DNA profile in the presence of excess quantities of female DNA, such as is found in many sexual assault investigations. Commercially available Y-STR multiplexes incorporating 12–17 loci are currently used in forensic casework (Promega's PowerPlex® Y and Applied Biosystems' AmpFℓSTR® Yfiler®). Despite the robustness of these commercial multiplex Y-STR systems and the ability to discriminate two male individuals in most cases, the coincidence match probabilities between unrelated males are modest compared with the standard set of autosomal STR markers. Hence there is still a need to develop new multiplex systems to supplement these for those cases where additional discriminatory power is desired or where there is a coincidental Y-STR match between potential male participants. Over 400 Y-STR loci have been identified on the Y chromosome. While these have the potential to increase the discrimination potential afforded by the commercially available kits, many have not been well characterized. In the present work, 91 loci were tested for their relative ability to increase the discrimination potential of the commonly used 'core' Y-STR loci. The result of this extensive evaluation was the development of an ultra high discrimination (UHD) multiplex DNA typing system that allows for the robust co-amplification of 14 non-core Y-STR loci. Population studies with a mixed African American and American Caucasian sample set (n = 572) indicated that the overall discriminatory potential of the UHD multiplex was superior to all commercial kits tested. The combined use of the UHD multiplex and the Applied Biosystems' AmpFℓSTR® Yfiler® kit resulted in 100% discrimination of all individuals within the sample set, which presages its potential to maximally augment currently available forensic casework markers. It could also find applications in human evolutionary genetics and genetic genealogy.

Citation: Hanson EK, Ballantyne J (2007) An Ultra-High Discrimination Y Chromosome Short Tandem Repeat Multiplex DNA Typing System. PLoS ONE 2(8): e688. doi:10.1371/journal.pone.0000688

http://www.plos.org/

Scholar All articles - Recent articles Results 1 - 10 of about 17,800 for forensic DNA typing

Population genetics in forensic DNA typing
RC Lewontin, DL Hartl - Science, 1991 - sciencemag.org
... Population Genetics in Forensic DNA Typing RC LEWONTIN AND DANIEL L. HARTL
Variable number oftandem repeat (VNTR) sequences are ...
Cited by 141 - Related articles - Web Search - Resources @ NIST Library - Get Item at NIST - All 4 versions

[BOOK] Forensic DNA typing
JM Butler - 2001 - Academic Press London
Cited by 96 - Related articles - Web Search - Get Item at NIST

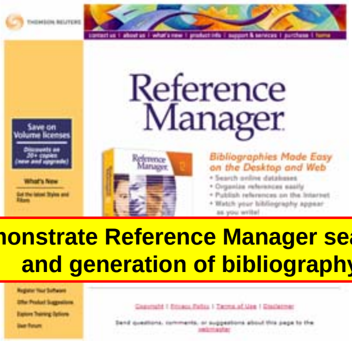
Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material - Get Item at NIST
PS Walsh, DA Metzger, R Higuchi - Biotechniques, 1991 - BioOne
1991 Apr;10(4):506-13. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Walsh PS, Metzger DA, Higuchi R. ...
Cited by 2522 - Related articles - Web Search - Resources @ NIST Library - All 7 versions

[BOOK] Forensic DNA typing: biology, technology, and genetics of STR markers
JM Butler - 2005 - books.google.com
... Page 2. FORENSIC DNA TYPING Page 3. ... These milestones are a tribute to the progress of science and will benefit the field of forensic DNA typing. ...
Cited by 58 - Related articles - Web Search - Get Item at NIST - Library Search - All 3 versions

[BOOK] Forensic DNA typing: biology & technology behind STR markers
JM Butler - 2001 - books.google.com
... In this book, Dr Butler has addressed the biology and technology pertaining to a powerful and relatively new manner of performing forensic DNA typing. ...
Cited by 55 - Related articles - Web Search - Get Item at NIST - Library Search - All 5 versions

http://scholar.google.com

Reference Manager Software



Demonstrate Reference Manager searches and generation of bibliography

http://www.refman.com/

Literature Management

- Used to spend 2+ hours per week in the library
- Now can access articles via NIST Virtual Library from my desk
- Consolidated Reference Manager database

Our Project Team Library

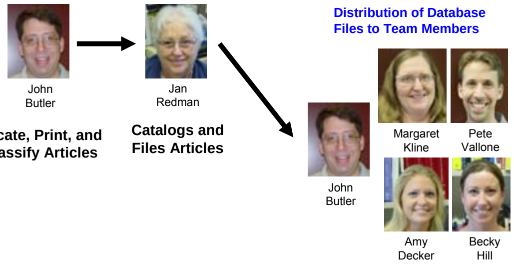
>8,000 references gathered and cataloged in Reference Manager

Comprehensive set of forensic DNA articles

•Started by Christian Ruitberg
•Maintained now by **Jan Redman**
•**Updated Monthly** and provided to NIST Human Identity Project Team

NIST Human Identity Project Team

Strategy for Information Flow



John Butler

Locate, Print, and Classify Articles

Jan Redman

Catalogs and Files Articles

Distribution of Database Files to Team Members

John Butler

Margaret Kline

Pete Vallone

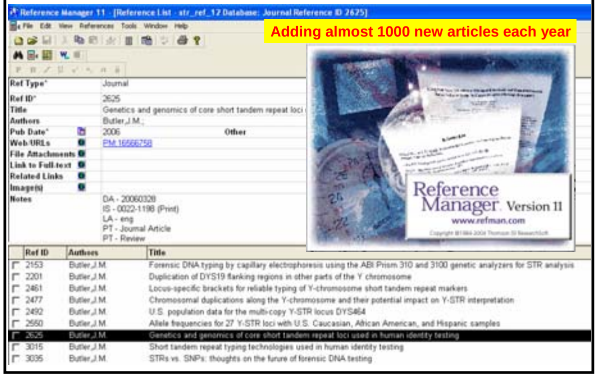
Amy Decker

Becky Hill

Updates supplied once a month on CD

Reference Manager Database

As of Jan 2010: 4887 references in **AllRef** and 3369 references in **STR_Ref**



Reference Manager 11 - [Reference List - str_ref_12 Database: Journal Reference ID 2625]

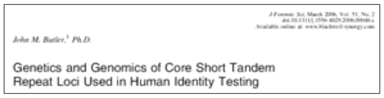
Adding almost 1000 new articles each year

Ref Type*	Journal
Ref ID*	2625
Title	Genetics and genomics of core short tandem repeat loci
Authors	Butler,J.M.;
Pub Date*	2006 Other
Web/URLs	PM:16566758
File Attachments	
Link to Full-text	
Related Links	
Image(s)	
Notes	DA - 20060328 IS - 0022-1198 (Print) LA - eng PT - Journal Article PT - Review

Ref ID	Authors	Title
2153	Butler,J.M.	Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis
2201	Butler,J.M.	Duplication of DYS19 flanking regions in other parts of the Y chromosome
2461	Butler,J.M.	Locus-specific brackets for reliable typing of Y-chromosome short tandem repeat markers
2477	Butler,J.M.	Chromosomal duplications along the Y-chromosome and their potential impact on Y-STR interpretation
2492	Butler,J.M.	U.S. population data for the multi-copy Y-STR locus DYS464
2550	Butler,J.M.	Allele frequencies for 27 Y-STR loci with U.S. Caucasian, African American, and Hispanic samples
2625	Butler,J.M.	Genetics and genomics of core short tandem repeat loci used in human identity testing
3015	Butler,J.M.	Short tandem repeat typing technologies used in human identity testing
3036	Butler,J.M.	STRs vs. SNPs: thoughts on the future of forensic DNA testing

Fruits of a Good Literature Collection

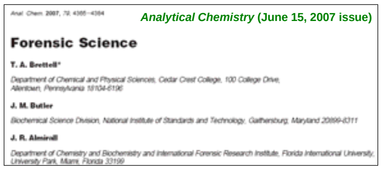
Review Articles



J Forensic Sci, March 2006, Vol. 51, No. 2

John M. Butler,[1] Ph.D.

Genetics and Genomics of Core Short Tandem Repeat Loci Used in Human Identity Testing



Anal. Chem. 2007, 79, 4365–4384

***Analytical Chemistry* (June 15, 2007 issue)**

Forensic Science

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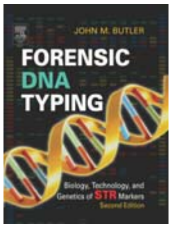
Textbooks



2nd Edition 688 pp.
Feb 2005

And a Useful Reference Website…

STRBase - Microsoft Internet Explorer

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

Short Tandem Repeat DNA

Internet DataBase

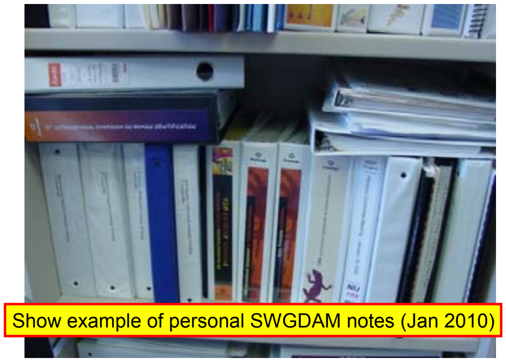
NIST Standard Reference Database SRD 130 [Recent Updates]

Reference Listing regarding STRs and DNA Typing

Complete Set of References (Numerical Order) ***3016 references** as of 11/28/07*

*Created by **John M. Butler***

Collection of Notes from Meetings Attended



Show example of personal SWGDAM notes (Jan 2010)

Why you need to write up your work

- Peer-review usually generates quality information
- Talks are not held to the same standard as a written publication (that has been reviewed)
- A written publication is also accessible to those who did not attend a presentation and is archived for future scientists to read

The Peer-Review Process Based on My Perspective as an Editor

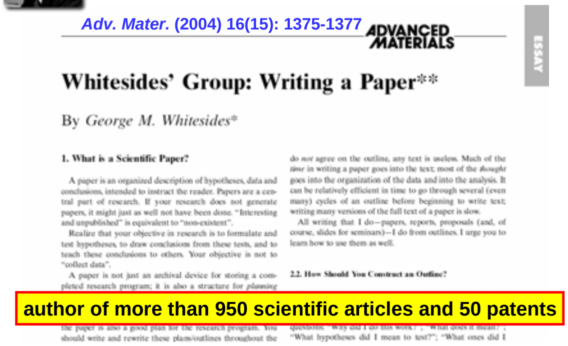
- Authors write article according to journal guidelines (each journal has an "Instructions for Authors")
- Steps during review
  - Article submitted to journal by corresponding author
  - Assigned to an editor
  - Editor asks 2 or more scientists to review the article in a specific timeframe (usually 2-3 weeks)
  - Editor takes reviews into consideration and responds to author with **Accept, Revise, or Reject**; "Revise" is most common
  - Author revises article and resubmits it for another review

Unfortunately, busy scientists often do not complete their review in a timely fashion (requiring the editor to remind them)

How to Write a Scientific Article

- **Outline the ideas first** with a purpose and plan
  - Decide on scope, design experiments, & collect data
- Write Materials and Methods section first
- Prepare all figures & tables
  - captions should be stand-alone
- Write Results and Discussion based on data shown in figures & tables
- Write Introduction to provide context to your work
- Prepare reference list according to journal format
- **Write abstract last**
  - Most critical piece since it will be the most read!

George Whitesides on how to write a scientific article



Adv. Mater. (2004) 16(15): 1375-1377

ADVANCED MATERIALS

ESSAY

Whitesides' Group: Writing a Paper**

By *George M. Whitesides**

1. What is a Scientific Paper?

A paper is an organized description of hypotheses, data and conclusions, intended to instruct the reader. Papers are a central part of research. If your research does not generate papers, it might just as well not have been done. "Interesting and unpublished" is equivalent to "non-existent".

Realize that your objective in research is to formulate and test hypotheses, to draw conclusions from these tests, and to teach these conclusions to others. Your objective is not to "collect data".

A paper is not just an archival device for storing a completed research program; it is also a structure for *planning*

the paper is also a good plan for the research program. You should write and rewrite these plans/outlines throughout the

do *not* agree on the outline, any text is useless. Much of the *time* in writing a paper goes into the text; most of the *thought* goes into the organization of the data and into the analysis. It can be relatively efficient in time to go through several (even many) cycles of an outline before beginning to write text; writing many versions of the full text of a paper is slow.

All writing that I do—papers, reports, proposals (and, of course, slides for seminars)—I do from outlines. I urge you to learn how to use them as well.

2.2. How Should You Construct an Outline?

questions: "Why did I do this work?"; "What does it mean?"; "What hypotheses did I mean to test?"; "What ones did I

author of more than 950 scientific articles and 50 patents

"Source Attribution" and Literature Categories

- **Always cite your sources**
  - Important to know where something came from because you might need to go back to it
  - Not all information is of equal value or importance

Literature can be subdivided into several categories:

- **Peer-reviewed literature** *(containing data)*
- Reports *(evaluating a methodology)*
- Review articles *(commenting on other's data)*
- Non-peer reviewed literature *(representing the authors' opinions only)* – e.g., conference proceedings

Additional Thoughts

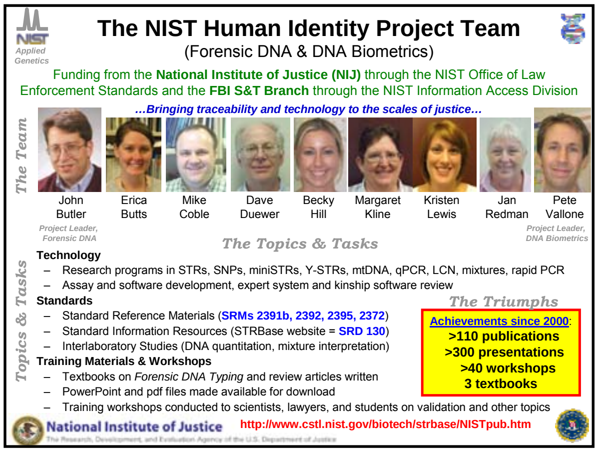
- Make time each week to continue your education
  - read an article once a week during lunch
  - read during your commute (unless you are driving!)
- Take detailed notes with each meeting you attend and then **share what you learned with others** (this will help you understand the information better)

NIST
Applied Genetics

APPLIED GENETICS Group

Major Programs Currently Underway

- **Forensic DNA**
  - New loci and assays (26plex)
  - **STR kit testing**
  - Ancestry SNP assays
  - **Low-template DNA studies**
  - Mixture interpretation
  - STR nomenclature
  - **Variant allele cataloging** and sequencing
  - Expert systems review
  - Training workshops to forensic DNA laboratories
  - Validation information and **software tools**
  - **Textbook** – 3rd ed. (2 vol.)
- **Clinical Genetics**
  - CMV SRM
  - Huntington's SRM
- **Ag Biotech**
  - "universal" GMO detection/ quantitation (35S promoter)
- **DNA Biometrics**
  - **Rapid PCR** methods
  - Efforts to standardize testing of future portable DNA systems
  - Kinship analysis
- **Cell Line Authentication**



The NIST Human Identity Project Team

(Forensic DNA & DNA Biometrics)

Funding from the **National Institute of Justice (NIJ)** through the NIST Office of Law Enforcement Standards and the **FBI S&T Branch** through the NIST Information Access Division

…Bringing traceability and technology to the scales of justice…

John Butler — *Project Leader, Forensic DNA*
Erica Butts
Mike Coble
Dave Duewer
Becky Hill
Margaret Kline
Kristen Lewis
Jan Redman
Pete Vallone — *Project Leader, DNA Biometrics*

The Topics & Tasks

Technology
- Research programs in STRs, SNPs, miniSTRs, Y-STRs, mtDNA, qPCR, LCN, mixtures, rapid PCR
- Assay and software development, expert system and kinship software review

Standards
- Standard Reference Materials (**SRMs 2391b, 2392, 2395, 2372**)
- Standard Information Resources (STRBase website = **SRD 130**)
- Interlaboratory Studies (DNA quantitation, mixture interpretation)

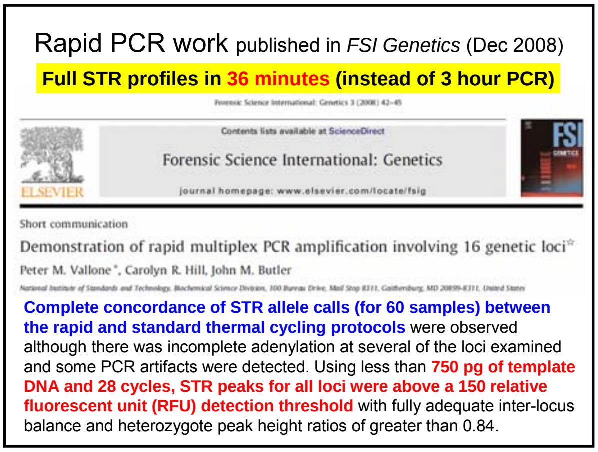
Training Materials & Workshops
- Textbooks on *Forensic DNA Typing* and review articles written
- PowerPoint and pdf files made available for download
- Training workshops conducted to scientists, lawyers, and students on validation and other topics

The Triumphs

Achievements since 2000:
>110 publications
>300 presentations
>40 workshops
3 textbooks

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

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



Rapid PCR work published in *FSI Genetics* (Dec 2008)

Full STR profiles in 36 minutes (instead of 3 hour PCR)

Forensic Science International: Genetics 3 (2008) 42–45

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

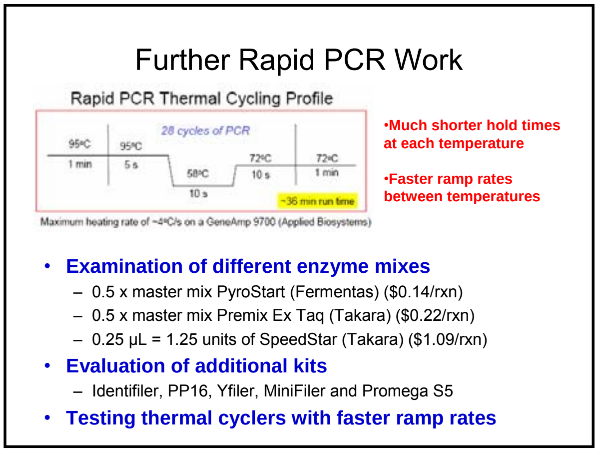
Short communication

Demonstration of rapid multiplex PCR amplification involving 16 genetic loci☆

Peter M. Vallone*, Carolyn R. Hill, John M. Butler

National Institute of Standards and Technology, Biochemical Science Division, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899-8311, United States

Complete concordance of STR allele calls (for 60 samples) between the rapid and standard thermal cycling protocols were observed although there was incomplete adenylation at several of the loci examined and some PCR artifacts were detected. Using less than **750 pg of template DNA and 28 cycles, STR peaks for all loci were above a 150 relative fluorescent unit (RFU) detection threshold** with fully adequate inter-locus balance and heterozygote peak height ratios of greater than 0.84.



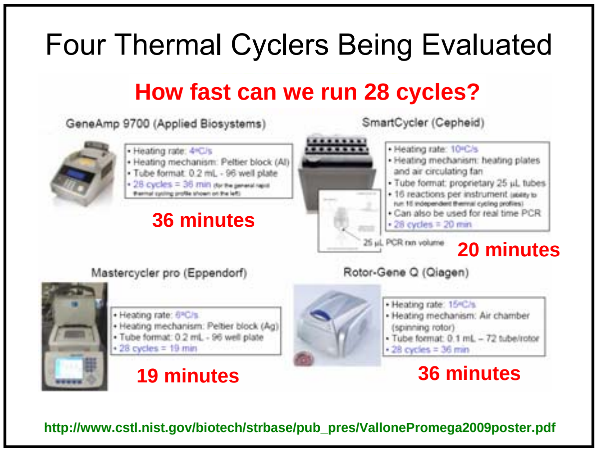
Further Rapid PCR Work

Rapid PCR Thermal Cycling Profile

Maximum heating rate of ~4°C/s on a GeneAmp 9700 (Applied Biosystems)

- **Much shorter hold times at each temperature**
- **Faster ramp rates between temperatures**

- **Examination of different enzyme mixes**
  - 0.5 x master mix PyroStart (Fermentas) ($0.14/rxn)
  - 0.5 x master mix Premix Ex Taq (Takara) ($0.22/rxn)
  - 0.25 μL = 1.25 units of SpeedStar (Takara) ($1.09/rxn)
- **Evaluation of additional kits**
  - Identifiler, PP16, Yfiler, MiniFiler and Promega S5
- **Testing thermal cyclers with faster ramp rates**



Four Thermal Cyclers Being Evaluated

How fast can we run 28 cycles?

GeneAmp 9700 (Applied Biosystems)
- Heating rate: 4°C/s
- Heating mechanism: Peltier block (Al)
- Tube format: 0.2 mL - 96 well plate
- 28 cycles = 36 min (for the general rapid thermal cycling profile shown on the left)

36 minutes

SmartCycler (Cepheid)
- Heating rate: 10°C/s
- Heating mechanism: heating plates and air circulating fan
- Tube format: proprietary 25 μL tubes
- 16 reactions per instrument (ability to run 16 independent thermal cycling profiles)
- Can also be used for real time PCR
- 28 cycles = 20 min

20 minutes

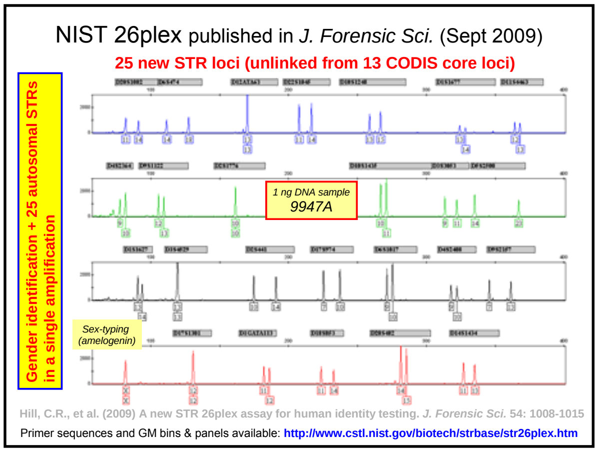
Mastercycler pro (Eppendorf)
- Heating rate: 6°C/s
- Heating mechanism: Peltier block (Ag)
- Tube format: 0.2 mL - 96 well plate
- 28 cycles = 19 min

19 minutes

Rotor-Gene Q (Qiagen)
- Heating rate: 15°C/s
- Heating mechanism: Air chamber (spinning rotor)
- Tube format: 0.1 mL – 72 tube/rotor
- 28 cycles = 36 min

36 minutes

http://www.cstl.nist.gov/biotech/strbase/pub_pres/VallonePromega2009poster.pdf



NIST 26plex published in *J. Forensic Sci.* (Sept 2009)

25 new STR loci (unlinked from 13 CODIS core loci)

Gender identification + 25 autosomal STRs in a single amplification

1 ng DNA sample
9947A

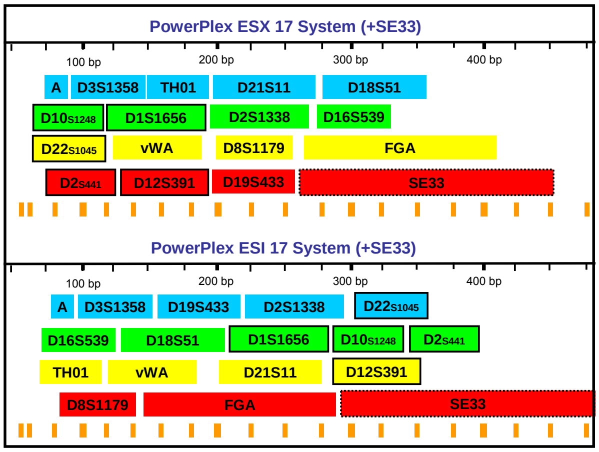
Sex-typing (amelogenin)

Hill, C.R., et al. (2009) A new STR 26plex assay for human identity testing. *J. Forensic Sci.* 54: 1008-1015

Primer sequences and GM bins & panels available: http://www.cstl.nist.gov/biotech/strbase/str26plex.htm

NIST Studies with New STR Kits

- Over the past year, our NIST team has worked with Promega to evaluate their new STR kits
  - PowerPlex ESX 17 & ESI 17 - with new European loci
  - PowerPlex 16 HS – with new enzyme & buffer system
- **Concordance studies**, population data analysis, and additional validation information
- Plan to do similar studies with Applied Biosystems on their NGM and other new kits



New NIST Software Tools

Developed by Dave Duewer (NIST)

From NIST STRBase Website:


Lab Resources and Tools

- Addresses for scientists working with STRs
- Training Materials
- STR Allele Sequencing
- Population data
- Data from NIST U.S. Population Samples
- NIST-Developed Software including AutoDimer, mixSTR, and Multiplex_QA
- NIST Standard Reference Material for PCR-Based Testing
- New STR Markers under Development at NIST
- Chromosomal Locations
- DNA Advisory Board Quality Assurance Standards
- Interlaboratory Studies
- NIST Mixture 2005 Interlab Study MIX05 Data
- Validation information
- DNA Quantitation - SRM 2372 (available as of October 5, 2007)
- Technology for resolving STR alleles

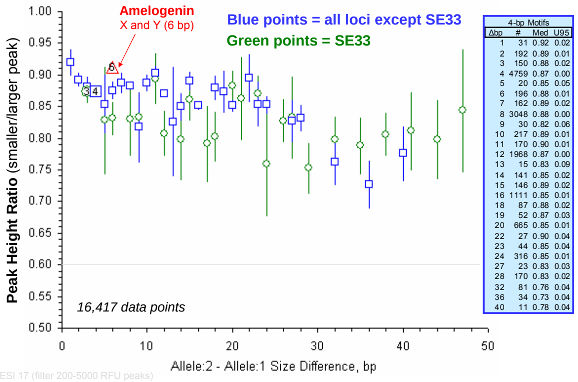
http://www.cstl.nist.gov/biotech/strbase/tools/STR_MatchSamples.xls

STR_MatchSamples
- An Excel-based tool developed to aid comparison of STR genotypes from two or more data sets.

Tools under development
(to aid validation studies)
- Peak height ratio
- Inter-locus balance
- Stutter percentages
- Allele frequency

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

Peak Height Ratio as a Function of Allele Spread



4-bp Motifs

Δbp	#	Med	U95
1	31	0.92	0.02
2	192	0.89	0.01
3	150	0.88	0.02
4	4759	0.87	0.00
5	20	0.85	0.05
6	196	0.88	0.01
7	162	0.89	0.02
8	3048	0.88	0.00
9	30	0.82	0.06
10	217	0.89	0.01
11	170	0.90	0.01
12	1968	0.87	0.00
13	15	0.83	0.09
14	141	0.85	0.02
15	146	0.89	0.02
16	1111	0.85	0.01
18	87	0.88	0.02
19	52	0.87	0.03
20	665	0.85	0.01
22	27	0.90	0.04
23	44	0.85	0.04
24	316	0.85	0.01
27	23	0.83	0.03
28	170	0.83	0.02
32	81	0.76	0.04
36	34	0.73	0.04
40	11	0.78	0.04

Trinucleotide **D22S1045**

Allele-Specific Stutter Percentages

ESX 17

Allele	Size	#	Stutter Median
10	84.5	21	1.8
11	87.4	134	3.0
12	90.4	37	4.2
14	96.4	51	7.2
15	99.4	165	8.9
16	102.4	120	10.5
17	105.5	105	14.7
	Avg	633	7.2
	SD		4.6

633 data points

Avg + 3SD **21.0%**

ESI 17

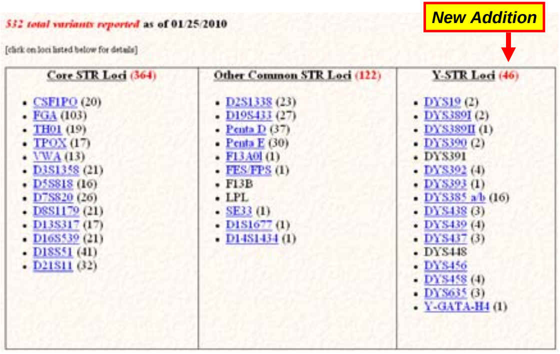
Allele	Size	#	Stutter Median
10	308.7	22	1.9
11	311.8	98	2.8
12	314.8	32	4.5
14	321.0	36	6.1
15	324.0	150	9.9
16	327.1	94	9.8
17	330.1	95	14.2
	Avg	527	7.0
	SD		4.4

527 data points

Avg + 3SD **20.2%**

532 Variant Alleles on STRBase

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

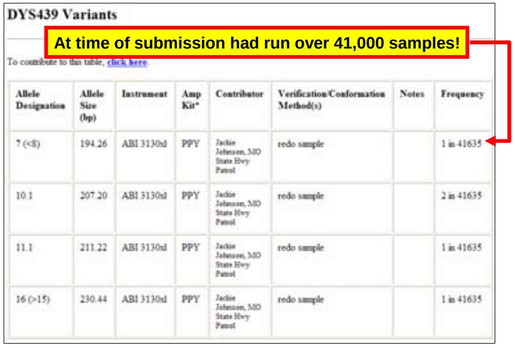

532 total variants reported **as of 01/25/2010**

New Addition

[click on loci listed below for details]

Core STR Loci (364)	Other Common STR Loci (122)	Y-STR Loci (46)
CSF1PO (20) FGA (103) TH01 (19) TPOX (17) VWA (13) D3S1358 (21) D5S818 (16) D7S820 (26) D8S1179 (21) D13S317 (17) D16S539 (21) D18S51 (41) D21S11 (32)	D2S1338 (23) D19S433 (27) Penta D (37) Penta E (30) F13A01 (1) FES/FPS (1) F13B LPL SE33 (1) D1S1677 (1) D14S1434 (1)	DYS19 (2) DYS389I (2) DYS389II (1) DYS390 (2) DYS391 DYS392 (4) DYS393 (1) DYS385 a/b (16) DYS438 (3) DYS439 (4) DYS437 (3) DYS448 DYS456 DYS458 (4) DYS635 (3) Y-GATA-H4 (1)

Many New Y-STR Variants Being Reported by Missouri State Highway Patrol


DYS439 Variants

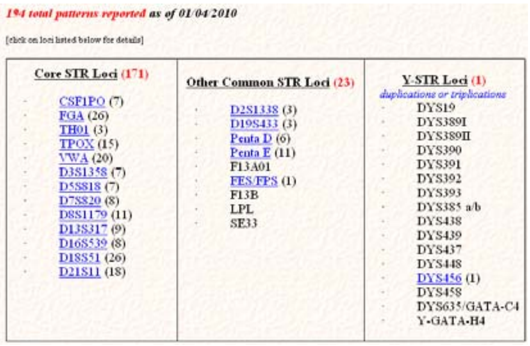
At time of submission had run over 41,000 samples!

To contribute to this table, click here.

Allele Designation	Allele Size (bp)	Instrument	Amp Kit*	Contributor	Verification/Conformation Method(s)	Notes	Frequency
7 (<8)	194.26	ABI 3130xl	PPY	Jackie Johnson, MO State Hwy Patrol	redo sample		1 in 41635
10.1	207.20	ABI 3130xl	PPY	Jackie Johnson, MO State Hwy Patrol	redo sample		2 in 41635
11.1	211.22	ABI 3130xl	PPY	Jackie Johnson, MO State Hwy Patrol	redo sample		1 in 41635
16 (>15)	230.44	ABI 3130xl	PPY	Jackie Johnson, MO State Hwy Patrol	redo sample		1 in 41635

194 Tri-Allelic Patterns on STRBase

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



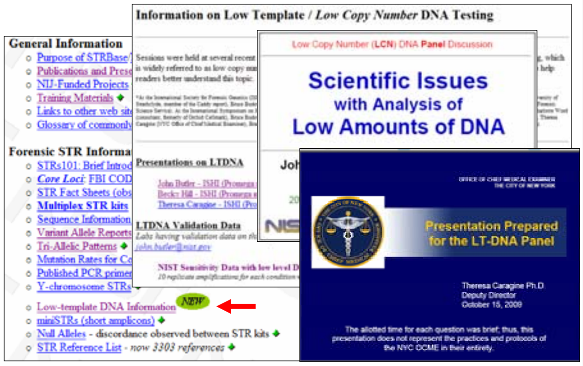
194 total patterns reported as of 01/04/2010

[click on loci listed below for details]

Core STR Loci (171)	**Other Common STR Loci (23)**	**Y-STR Loci (1)** *duplications or triplications*
CSF1PO (7) FGA (26) TH01 (3) TPOX (15) VWA (20) D3S1358 (7) D5S818 (7) D7S820 (8) D8S1179 (11) D13S317 (9) D16S539 (8) D18S51 (26) D21S11 (18)	D2S1338 (3) D19S433 (3) Penta D (6) Penta E (11) F13A01 FES/FPS (1) F13B LPL SE33	DYS19 DYS389I DYS389II DYS390 DYS391 DYS392 DYS393 DYS385 a/b DYS438 DYS439 DYS437 DYS448 DYS456 (1) DYS458 DYS635/GATA-C4 Y-GATA-H4

New STRBase Website on LT-DNA (LCN)

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



Information on Low Template / *Low Copy Number* DNA Testing

General Information

- Purpose of STRBase
- Publications and Prese
- NIJ-Funded Projects
- Training Materials
- Links to other web sit
- Glossary of commonly

Forensic STR Informa

- STRs101: Brief Introd
- *Core Loci*: FBI COD
- STR Fact Sheets (obs
- **Multiplex STR kits**
- Sequence Information
- Variant Allele Reports
- Tri-Allelic Patterns
- Mutation Rates for Co
- Published PCR primer
- Y-chromosome STRs
- Low-template DNA Information NEW
- miniSTRs (short amplicons)
- Null Alleles - discordance observed between STR kits
- STR Reference List - *now 3303 references*

Sessions were held at several recent … is widely referred to as low copy nu… readers better understand this topic.

Presentations on LTDNA

John Butler - ISHI (Promega
Becky Hill - ISHI (Promega
Theresa Caragine - ISHI (Pro

LTDNA Validation Data

Labs having validation data on th
john.butler@nist.gov

NIST Sensitivity Data with low level D
10 replicate amplifications for each condition

Low Copy Number (**LCN**) DNA **Panel** Discussion

Scientific Issues
with Analysis of
Low Amounts of DNA

Presentation Prepared for the LT-DNA Panel

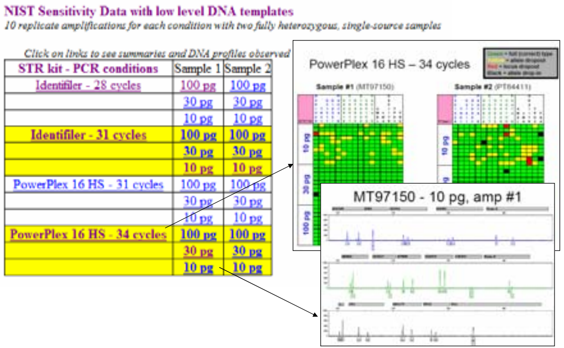
OFFICE OF CHIEF MEDICAL EXAMINER
THE CITY OF NEW YORK

Theresa Caragine Ph.D
Deputy Director
October 15, 2009

The allotted time for each question was brief; thus, this presentation does not represent the practices and protocols of the NYC OCME in their entirety.

Complete Set of NIST Sensitivity Data Available on New LT-DNA Website

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



NIST Sensitivity Data with low level DNA templates
10 replicate amplifications for each condition with two fully heterozygous, single-source samples

Click on links to see summaries and DNA profiles observed

STR kit - PCR conditions	Sample 1	Sample 2
Identifiler - 28 cycles	100 pg	100 pg
	30 pg	30 pg
	10 pg	10 pg
Identifiler - 31 cycles	100 pg	100 pg
	30 pg	30 pg
	10 pg	10 pg
PowerPlex 16 HS - 31 cycles	100 pg	100 pg
	30 pg	30 pg
	10 pg	10 pg
PowerPlex 16 HS - 34 cycles	100 pg	100 pg
	30 pg	30 pg
	10 pg	10 pg

Comparison of Approaches

What "LCN Labs" Are Doing

Replicate Amplification with Consensus Profile

Low amount of DNA examined

Stochastic effects

Amplification #1
Amplification #2
Amplification #3

Consensus Profile Developed
(from repeated alleles observed)

Interpretation Rules Applied
(based on validation experience)
e.g., specific loci may dropout more

Result can be and usually is Reliable & Reproducible

Single Amplification

Low amount of DNA examined

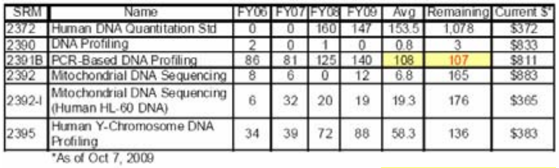
Stochastic effects

Amplification #1
(only a single test)

Result can be Unreliable

Individual results may vary but a consensus profile is reproducible
(based on our experience with sensitivity studies and replicate amplifications)

NIST SRM 2391b will need to be replaced within the next year



SRM	Name	FY06	FY07	FY08	FY09	Avg	Remaining	Current $*
2372	Human DNA Quantitation Std	0	0	160	147	153.5	1,078	$372
2390	DNA Profiling	2	0	1	0	0.8	3	$833
2391B	PCR-Based DNA Profiling	86	81	125	140	108	107	$811
2392	Mitochondrial DNA Sequencing	8	6	0	12	6.8	165	$883
2392-I	Mitochondrial DNA Sequencing (Human HL-60 DNA)	6	32	20	19	19.3	176	$365
2395	Human Y-Chromosome DNA Profiling	34	39	72	88	58.3	136	$383

*As of Oct 7, 2009

PCR-based DNA Profiling Standard

*coverage for all commercially available kit STR loci at the time of release

Considering preparing DNA in different forms:
3 liquid samples
2 swabs
1 paper punch

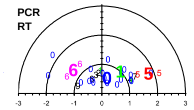
Plan to certify both autosomal and Y-STRs
on SRM 2391c components

http://www.cstl.nist.gov/biotech/strbase/pub_pres/Promega2009poster_SRM2391c.pdf

SRM 2372:

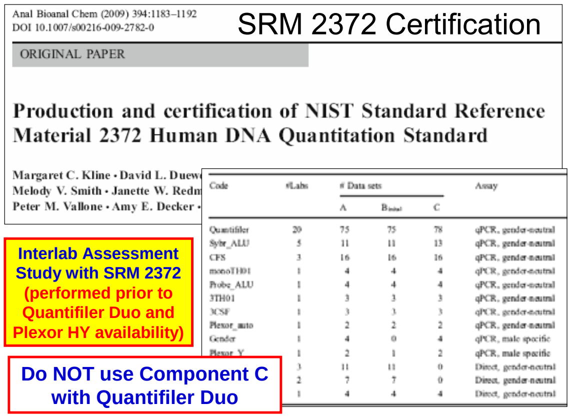
Human DNA Quantitation Standard

- Released in Oct 2007
  - >300 units in use as of October 2009
- **Used by more than 110 forensic laboratories worldwide**
- Manuscript describing production published in *Anal. Bioanal. Chem.*
- **Serves to adjust qPCR calibrants supplied by manufacturers and adjust for assay-specific bias**



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

SRM 2372 Certification


Anal Bioanal Chem (2009) 394:1183–1192
DOI 10.1007/s00216-009-2782-0

ORIGINAL PAPER

Production and certification of NIST Standard Reference Material 2372 Human DNA Quantitation Standard

Margaret C. Kline • David L. Duew
Melody V. Smith • Janette W. Redm
Peter M. Vallone • Amy E. Decker •

Code	#Labs	# Data sets			Assay
		A	$B_{initial}$	C	
Quantifiler	20	75	75	78	qPCR, gender-neutral
Sybr_ALU	5	11	11	13	qPCR, gender-neutral
CFS	3	16	16	16	qPCR, gender-neutral
monoTH01	1	4	4	4	qPCR, gender-neutral
Probe_ALU	1	4	4	4	qPCR, gender-neutral
3TH01	1	3	3	3	qPCR, gender-neutral
3CSF	1	3	3	3	qPCR, gender-neutral
Plexor_auto	1	2	2	2	qPCR, gender-neutral
Gender	1	4	0	4	qPCR, male specific
Plexor_Y	1	2	1	2	qPCR, male specific
	3	11	11	0	Direct, gender-neutral
	2	7	7	0	Direct, gender-neutral
	1	4	4	4	Direct, gender-neutral

Interlab Assessment Study with SRM 2372 (performed prior to Quantifiler Duo and Plexor HY availability)

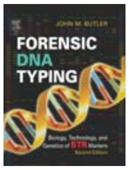
Do NOT use Component C with Quantifiler Duo

The Expansion of *Forensic DNA Typing*

1st Edition

Jan 2001
335 pp.
17 chapters

2nd Edition

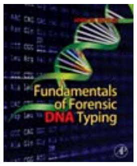


Feb 2005
688 pp.
24 chapters

Chinese Translation (2007) Y. Hou, translator

Japanese Translation (2009) Y. Fukuma, translator

3rd Edition



Sept 2009

Fundamentals
18 chapters (504 pp.)

Advanced Topics
25 chapters (~600 pp.)

Planned for Spring 2011

Improved Reference Format

***Forensic DNA Typing* (2nd Edition)**

Full list of authors but no article title


Lazaruk, K., Walsh, P.S., Oaks, F., Gilbert, D., Rosenblum, B.B., Menchen, S., Scheibler, D., Wenz, H.M., Holt, C. and Wallin, J. (1998) *Electrophoresis*, 19, 86–93.

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Mansfield, E.S., Vainer, M., Enad, S., Barker, D.L., Harris, D., Rappaport, E. and Fortina, P. (1996) *Genome Research*, 6, 893–903.

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McCord, B.R. (2003) Troubleshooting capillary electrophoresis systems. *Profiles in DNA*, 6 (2); Available at: http://www.promega.com/profiles/602/ProfilesinDNA_602_10.pdf.

Madabhushi, R.S. (1998) *Electrophoresis*, 19, 224–230.

***Fundamentals* (3rd Edition)**

Subdivided by subject with article title provided


Instrument Platforms

ABI 373 and 377

Frazier, R. R. E., et al. (1996). Validation of the Applied Biosystems Prism™ 377 automated sequencer for forensic short tandem repeat analysis. *Electrophoresis, 17*, 1550–1552.

Fregeau, C. J., et al. (1999). Validation of highly polymorphic fluorescent multiplex short tandem repeat systems using two generations of DNA sequencers. *Journal of Forensic Sciences, 44*, 133–166.

FMBIO Gel Imager

Greenspoon, S. A., et al. (2004). Validation and implementation of the PowerPlex 16 BIO System STR multiplex for forensic casework. *Journal of Forensic Sciences, 49*, 71–80.

ABI Prism 310 Genetic Analyzer

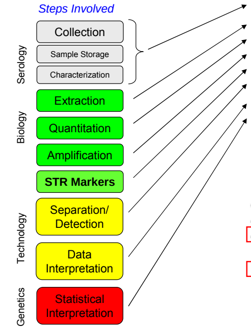
Buel, E., et al. (1998). Capillary electrophoresis STR analysis: Comparison to gel-based systems. *Journal of Forensic Sciences, 43*, 164–170.

Butler, J. M., et al. (2004). Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. *Electrophoresis, 25*, 1397–1402.

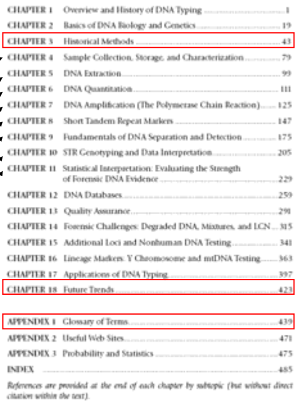
Lazaruk, K., et al. (1998). Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. *Electrophoresis, 19*, 86–93.

>1500 references total
(>600 new since 2nd edition)

Chapters Re-ordered to Reflect DNA Testing Process



***Fundamentals of Forensic DNA Typing* (2009)**


CHAPTER 1 Overview and History of DNA Typing 1
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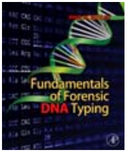
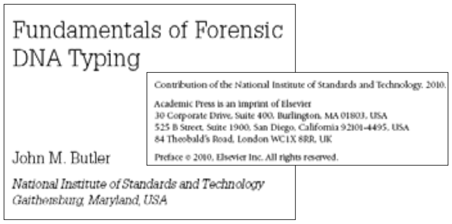
References are provided at the end of each chapter by subtopic (but without direct citation within the text).



Written as Part of My Job at NIST
(no royalties to be received)


Fundamentals of Forensic DNA Typing

John M. Butler

National Institute of Standards and Technology
Gaithersburg, Maryland, USA

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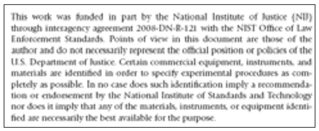
This work was funded in part by the National Institute of Justice (NIJ) through interagency agreement 2008-DN-R-121 with the NIST Office of Law Enforcement Standards. Points of view in this document are those of the author and do not necessarily represent the official position or policies of the U.S. 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.

Entire book was subjected to NIST review process for approval prior to publication

Thank you for your attention!

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Margaret Kline
STR allele sequencing


Jan Redman
Variant allele cataloging


Becky Hill
Kit analysis, miniSTRs, LCN, and 26plex work


Kristen Lewis
Kinship analysis


Pete Vallone
Rapid PCR, SNPs, & DNA Biometrics


Erica Butts
DNA Biometrics

Thanks to numerous collaborators and variant allele submitters

http://www.cstl.nist.gov/biotech/strbase
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Dave Duewer
(data analysis tools)