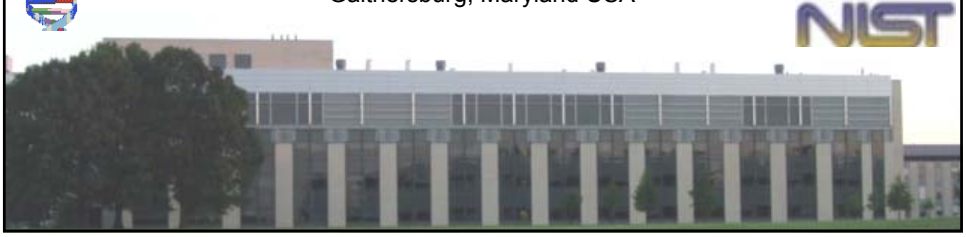
EDNAP and 30th ENFSI DNA WG Meeting
April 22-24, 2009 – Lisbon, Portugal

NIST Update

John M. Butler
and the NIST Human Identity Project Team
National Institute of Standards and Technology
Gaithersburg, Maryland USA



Current Activities at NIST

- **Standard Reference Materials**
  - SRM 2372 (DNA quantitation standard) **(>225 units in use since Oct 2007)**
  - Adding extended ESS loci (D1, D12, D2, D10, D22) to SRM 2391b
- **Technology Evaluation and Development**
  - Rapid multiplex PCR protocols (multiplex STR amplification in <35 min)
  - Low-level DNA studies underway
  - Unusual STR allele characterization
  - New STR loci and assays (STR 26plex, kit concordance, SNP testing)
- **Training Materials**
  - Workshops on mixture interpretation and CE troubleshooting
  - Third edition of *Forensic DNA Typing* textbook (2009 & 2011)

Standard Reference Materials

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

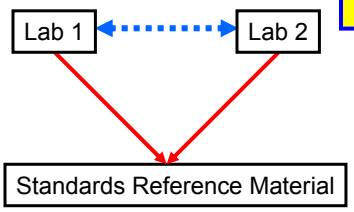
Traceable standards to ensure accurate measurements within DNA laboratories and aid comparison between labs


National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2391b
PCR-based DNA Profiling Standard

Helps meet DAB Std. 9.5 and ISO 17025

SRM 2391b – autosomal STRs
SRM 2392-I – mtDNA
SRM 2395 – Y-STRs
SRM 2372 – DNA quantitation

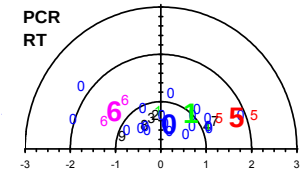
Have updated 2391b with new loci and 2395 with new Y-STRs



Calibration with SRMs enables confidence in comparisons of results between laboratories

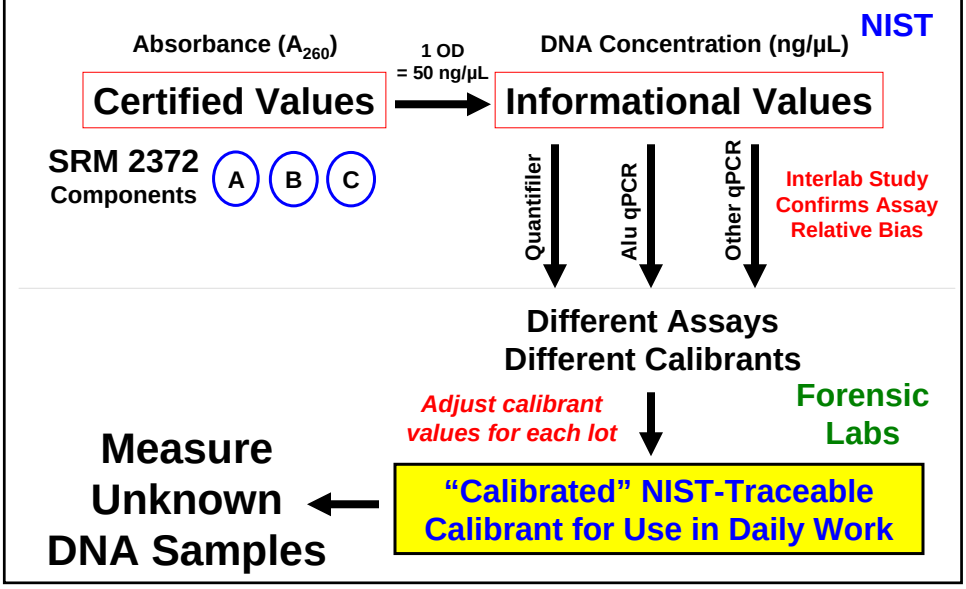
SRM 2372: Human DNA Quantitation Standard

- Released in Oct 2007
  - >225 units in use as of April 2009
- Used by more than 110 forensic laboratories worldwide
- Manuscript describing production is in press with *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

Overview of SRM 2372 Values and Use



SRM 2391b and 2395 Certificate Updates

- **SRM 2391b** (Autosomal STR Loci)
  - **MiniFiler examined** (allele dropout with component 8 and D16S539)
  - **Additional Loci: 26 new miniSTR loci**
  - Demonstrating extended stability (new quantitation data and no significant degradation to existing components)

http://www.cstl.nist.gov/biotech/strbase/srm2391b.htm

- **SRM 2395** (Y-STR and Y-SNP Loci)
  - **Yfiler loci sequenced** (DYS635 now included)
  - **Additional Loci: 20 new Y-STR loci**
  - Demonstrating extended stability (new quantitation data and no significant degradation to existing components)

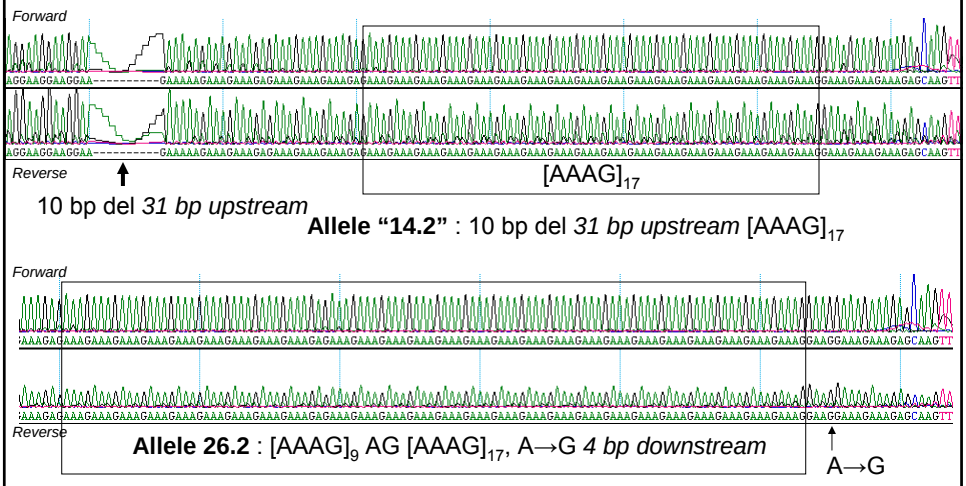
http://www.cstl.nist.gov/biotech/strbase/srm2395.htm

Revised Certificates available since September 5, 2008

D1S1656 and D12S391 Results with SRM 2391b Components

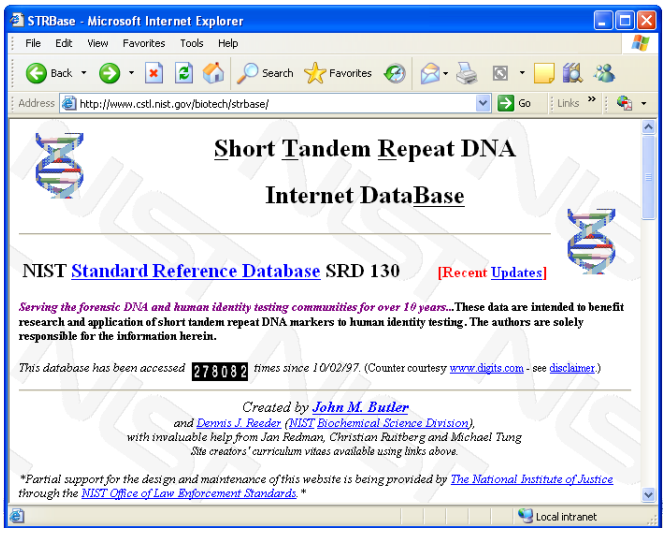
	D1S1656		D12S391	
	Type	Repeat motif	Type	Repeat motif
1	13	[TAGA]13[TG]5	15	[AGAT]8 [AGAC]6 AGAT
	14	[TAGA]13 TAGG [TG]5	18	[AGAT]11 [AGAC]6 AGAT
2	12	[TAGA]12[TG]5	17	[AGAT]10 [AGAC]6 AGAT
	17.3	[TAGA]4 TGA [TAGA]12 TAGG [TG]5	22	[AGAT]13 [AGAC]9
3	14	[TAGA]13 TAGG [TG]5	15	[AGAT]8 [AGAC]6 AGAT
	15	[TAGA]14 TAGG [TG]5	21	[AGAT]12 [AGAC]9
4	15	[TAGA]14 TAGG [TG]5	17	[AGAT]11 [AGAC]5 AGAT
	17.3	[TAGA]4 TGA [TAGA]12 TAGG [TG]5	17	[AGAT]10 [AGAC]6 AGAT
5	11	[TAGA]11[TG]5	18	[AGAT]11 [AGAC]6 AGAT
	16.3	[TAGA]4 TGA [TAGA]11 TAGG [TG]5		
6	11	[TAGA]11[TG]5	21	[AGAT]11 [AGAC]10
	17	[TAGA]16 TAGG [TG]5	22	[AGAT]12 [AGAC]10
7	12	[TAGA]12[TG]5	17	[AGAT]10 [AGAC]6 AGAT
	17.3	[TAGA]4 TGA [TAGA]12 TAGG [TG]5	20	[AGAT]10 [AGAC]9 AGAT
8	14	[TAGA]13 TAGG [TG]5	18	[AGAT]11 [AGAC]6 AGAT
	16.3	[TAGA]4 TGA [TAGA]11 TAGG [TG]5	24	[AGAT]15 [AGAC]9
9	18.3	[TAGA]4 TGA [TAGA]13 TAGG [TG]5	18	[AGAT]11 [AGAC]6 AGAT
			20	[AGAT]12 [AGAC]7 AGAT
10	14	[TAGA]13 TAGG [TG]5	18	[AGAT]11 [AGAC]6 AGAT
	17	[TAGA]16 TAGG [TG]5	24	[AGAT]13 [AGAC]6

SE33 Sequence Data for SRM 2391b Component 3



SE33 Genotype = 14.2, 26.2

STRBase: a Community Resource for Forensic DNA Applications of STRs…



STRBase Info on Extended ESS Loci

- Core Loci Summary Page
- STR Fact Sheets
  - D1S1656, D2S441, D10S1248, D12S391, D22S1045
- Variant allele sequencing capabilities
  - Need to provide ~10 ng of DNA for NIST to sequence sample
  - Submitter will be provided with sequence summary

Variant Allele Sequencing Service (Free)

Send us any unusual variant or null alleles and we will sequence them…

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

Variant allele characterization

Locus	Variant Allele	Sample Source	Comments
TPOX	10.3	Maryland State Police	Deletion of a "G" that is 157 bp from the repeat region under PowerPlex 1.1 and Identifiler primers does not affect primer binding or allele sizing . However, PowerPlex 2.1 and PowerPlex 16 products are 1 bp smaller because they are further away from the repeat and encompass the deletion.
FGA	46.2	Denver Crime Laboratory	Checked with Identifiler allelic ladder
D18S51	null allele 18	FSS and Kuwait government lab	Base change was a C-to-T transition 172 bp downstream of the repeat region which impacts the ABI D18S51 reverse primer but not the PowerPlex 16 D18S51 reverse primer that is internal to this mutation
D18S51	40	Nebraska State Crime Lab	DNA sequence analysis showed 40 GAAA repeats
D18S51	"5.3"	DNA Solutions	DNA sequence analysis revealed a 9 bp deletion beyond the end of the 8th repeat unit to produce a "5.3" allele
Penta D	[illegible]	University	$[AAAGA]_{11}$ repeat
Penta D	6	Peter de Knijff's lab at Leiden University	DNA sequence analysis confirmed 6 repeats

Send 10-20 ng of DNA (or 2-3 FTA bloodstain punches)
Contact margaret.kline@nist.gov or john.butler@nist.gov
Information will be posted on **STRBase …/STRseq.htm**
Sequence details provided back to sender

Summary of Variant Alleles Sequenced *(only 15 shown)*

Locus	Allele	Repeat Motif
D2S1338	12	$[TGCC]_4[TTCC]_8$
D2S1338	31	$[TGCC]_7[TTCC]_6$**TTAC** $[TTCC]_{14}$ **GTCC** $[TTCC]_2$
D3S1358	16.2	TCTA $[TCTG]_3$ **TC** $[TCTA]_{12}$
D3S1358	20	TCTA $[TCTG]_3$ $[TCTA]_{16}$
D3S1358	23	TCTA $[TCTG]_3$ $[TCTA]_{19}$
D5S818	10.1	**A**$[AGAT]_{10}$
D5S818	"29"	$[AGAT]_{12}$ **+68 bp**
D7S820	8.3	$[GATA]_9$ del **A** 22 bp DS
D16S539	11	$[GATA]_{11}$ (U83**T**→**C**) results in an anomalous migration 11/10.3
D18S51	15.2	$[AGAA]_7$ **AA** $[AGAA]_8$
D21S11	"24.3"	$[TCTA]_5$ $[TCTG]_6$ $[TCTA]_3$ TA $[TCTA]_3$ TCA $[TCTA]_2$ TCCATA $[TCTA]_9$ del 13 bp, 11 bp DS (28 allele -13 bp)
D21S11	28.1	$[TCTA]_5$ $[TCTG]_6$ $[TCTA]_3$ TA $[TCTA]_3$ TCA $[TCTA]_2$ TCCATA $[TCTA]_9$ **+T**
FGA	50	$TTTC]_4$ TTTT $[TTCT]_6$ TTTT $[CTTT]_{12}$ CTGT $[CTTT]_{14}$ $[CTTC]_3$ $[CTTT]_3$ CTCC $[TTCC]_4$
Penta E	27	$[AAAGA]_{27}$
DYS389II	29.1	$[TCTG]_4$ $[TCTA]_{13}$ N_{49} $[TCTG]_3$ $[TCTA]_9$ or $[TCTG]_4$ $[TCTA]_{14}$ N_{45} $[TCTG]_3$ $[TCTA]_9$ (D3Tins = **+T** 3bp downstream)

Variant Alleles Cataloged in STRBase

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

Off-Ladder Alleles

466 total variants reported **as of 10/06/2008**

[click on loci listed below for det

Currently 483 at 13/13 CODIS loci + F13A01, FES/FPS, Penta D, Penta E, D2S1338, D19S433

Core STR Loci

- CSF1PO (19)
- FGA (102)
- TH01 (17)
- TPOX (17)
- VWA (11)
- D3S1358 (28)
- D5S818 (12)
- D7S820 (25)
- D8S1179 (19)
- D13S317 (17)
- D16S539 (19)
- D18S51 (40)
- D21S11 (30)

- Penta E (27)
- F13A01 (1)
- FES/FPS (1)
- F13B
- LPL
- SE33 (1)
- D1S1677 (1)
- D14S1434 (1)

Tri-Allelic Patterns

176 total patterns reported **as of 08/07/2008**

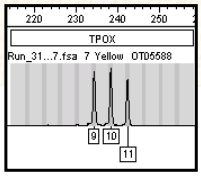
[click on loci listed below for details]

Currently 178 at 13/13 CODIS loci + FES/FPS, Penta D, Penta E, D2S1338, D19S433

Core STR Loci

- CSF1PO (7)
- FGA (22)
- TH01 (3)
- TPOX (15)
- VWA (19)
- D3S1358 (7)
- D5S818 (6)
- D7S820 (7)
- D8S1179 (11)
- D13S317 (8)
- D16S539 (7)
- D18S51 (23)
- D21S11 (19)

- F13A01
- FES/FPS (1)
- F13B
- LPL
- SE33



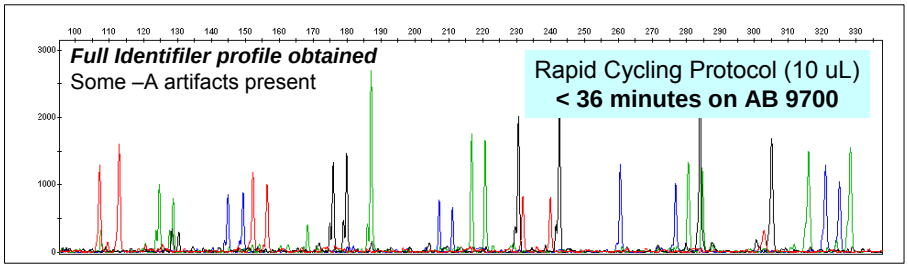
Apparent Null Alleles Observed During Concordance Studies

New Section of STRBase (launched to track MiniFiler discordance and allele dropout frequency):
http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm

Locus	STR Kits/Assays Compared	Results	Frequency of Primer Binding Site Mutation	Source
CSF1PO	MiniFiler vs ID vs PP16	MF: 11,11 and ID: 11,11.1 One base insertion in Identifiler amplicon outside of MiniFiler and PP16 primers	1/1308	Hill *et al.* (2007)
CSF1PO	PP16 vs COfiler	Loss of allele 14 with COfiler; fine with PP16	2/1537	Budowle *et al.* (2001)
FGA	SGM vs SGM Plus	Loss of allele 26 with SGM Plus; weak amp of same allele with SGM		Cotton *et al.* (2000)
FGA	PP16 vs ProPlus	Loss of allele 22 with ProPlus; fine with PP16		Budowle and Sprecher (2001)
TH01	PP16 vs COfiler	Loss of allele 9 with COfiler; fine with PP16	1/1537	Budowle *et al.* (2001)
TH01	SGM vs SGM Plus	Loss of allele 6 with SGM Plus; fine with SGM	1/4245	Clayton *et al.* (2004)
VWA	PP1.1 vs ProPlus	Loss of allele 19 with ProPlus; fine with PP1.1	2/1483	Kline *et al.* (1998) and Walsh (1998)
VWA	PP16 vs ProPlus	Loss of alleles 15 and 17 with ProPlus; fine with PP16	2/1537	Budowle *et al.* (2001)
VWA	ID vs miniplexes	Loss of alleles 12, 13, and 14 with miniplex assay; fine with ID	9/532	Drabek *et al.* (2004)

Rapid Multiplex PCR Protocols Under Development

Utilizing AB 9700 cycler and 'fast' commercial enzymes



Identifiler STR kit
28 cycles, 1ng template DNA

Initial results presented by at 60th Annual Meeting of the American Academy of Forensic Sciences (Washington, DC), February 23, 2008, "Developing Rapid PCR Multiplex Assays with miniSTR Loci"

More recent results on a different thermal cycler in as little as ~15 minutes…

What is "Rapid PCR"?

Current STR kits were optimized by manufacturers for slower PCR (~3 hours)

- Use of new commercial DNA polymerases

  - **Replace the current standard polymerase** (AmpliTaq Gold) and buffer but **keep commercial STR kit primer mixes**
    - rapid hot start (save ~10min)
    - 'faster' nucleotide incorporation (processivity >100 bases/sec)

- Use with common thermal cycler (GeneAmp 9700)

  - **Utilize maximum ramp rate of 4 °C/sec with 9700**
  - Shorten cycling hold times (to 1-5 sec vs 1 min)
  - Eliminate 60 °C adenylation soak (to save ~30-60 min)

- **Explore possibilities with faster thermal cyclers** (e.g., 10 °C/sec ramp) and possibly new primer mixes

Goal: to obtain full STR profiles in as little time as possible (<30 min?)

Rapid PCR Article

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.

Comparison of Thermal Cycling Times

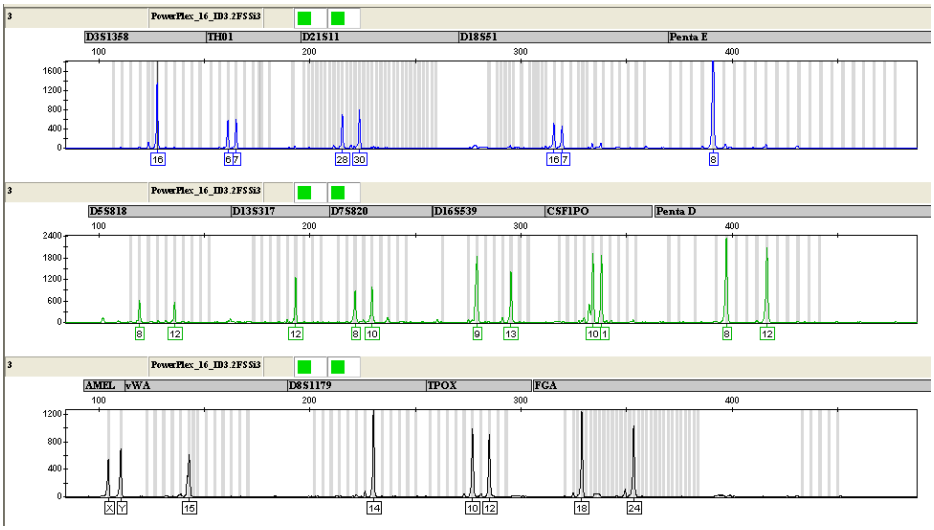
Parameter	Unit	Trad	Rapid	Difference (min)	%
Hot Start	Min	10	1	9.0	6.3
Hold	Sec	60	5/10	72.3	50.6
Soak	Min	60	1	59.0	41.2
Ramp rate	(deg/sec)	1	4	22.4	15.7
				Saving 2 hours, 23 minutes	
Cycles		28	28		
Time		2:58:41	0:35:38	2:23:03	

Overall time reduction on GeneAmp 9700 from 3 hours to 35 minutes

Parameter	Purpose
Hot Start	Primer Dimer, non-specific amplification
Hold	Denature, annealing, elongation, Inter and intra locus balance
Soak	Full adenylation of PCR products

Rapid DNA Polymerases used: PyroStart (Fermentas, Glen Burnie, MD) and SpeedSTAR (Takara Bio USA, Madison, WI)

PowerPlex 16 Rapid Cycling

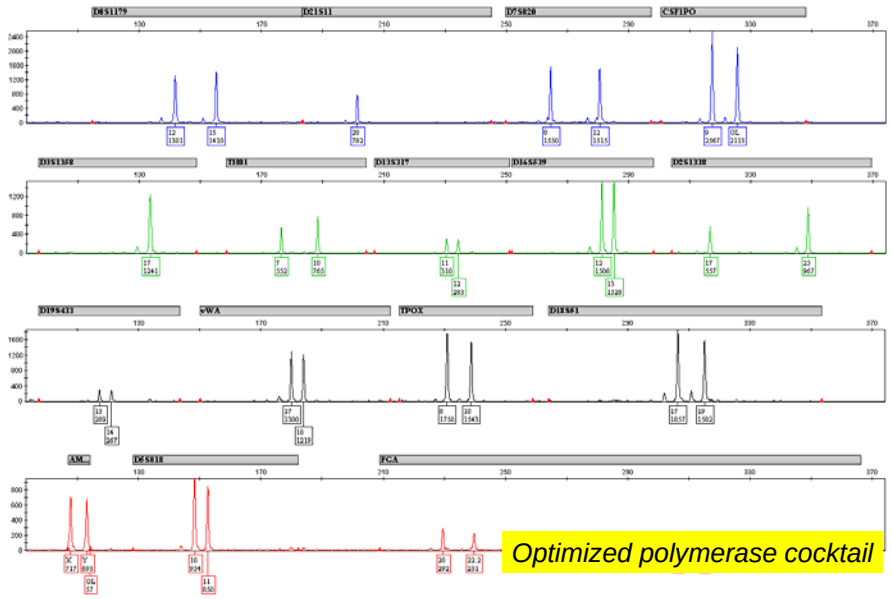


Rapid DNA Polymerases used: PyroStart (Fermentas, Glen Burnie, MD) and SpeedSTAR (Takara Bio USA, Madison, WI)

< 36 minutes on AB 9700

Identifiler Profile from 17 minute PCR

Further details will be given at the ISFG meeting in Buenos Aires



Optimized polymerase cocktail

Potential Applications with Rapid PCR Capabilities

- **Improve overall laboratory throughput**
  - Multiplex PCR amplification is already in many situations the longest part of the DNA analysis process (depending on DNA extraction and DNA quantitation methods)
  - With increased use of robotic sample preparation and expert system data analysis, bottleneck for sample processing will shift to time for PCR amplification…
- **Enable new potential DNA biometric applications** (because the overall DNA analysis process is faster)
  - Permit analysis of individuals at a point of interest such as an embassy, an airport, or a country border

Mixture Interpretation Workshop

http://www.cstl.nist.gov/biotech/strbase/training/AAFS2008_MixtureWorkshop.htm

AAFS (February 19, 2008)

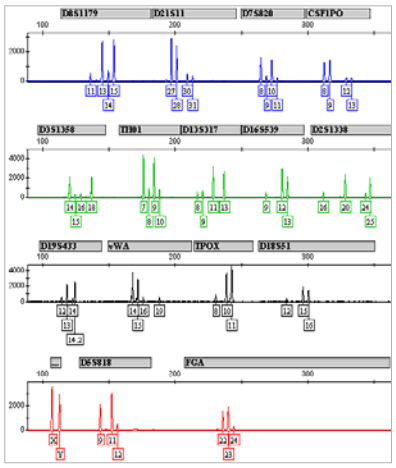
DNA Mixture Interpretation: Principles and Practice in Component Deconvolution and Statistical Analysis

196 page handout

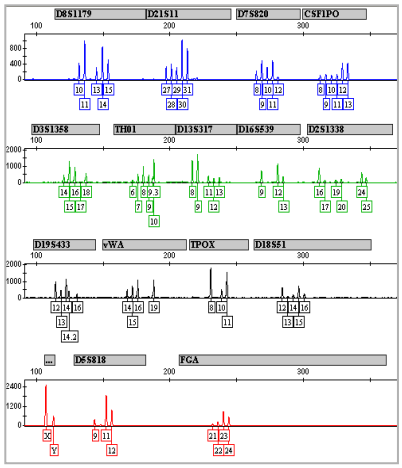
- **John Butler (NIST)**
- Ann Gross (MN)
- George Carmody (Carleton U.)
- Gary Shutler (WA)
- Joanne Sgueglia (MA)
- Angela Dolph (Marshall U./NIST)
- Tim Kalafut (USACIL)

Creating Known Mixtures for Testing Software Tools

NIST 2-person mixture
(Identifiler data, 1ng DNA, **1:5**)



NIST 3-person mixture
(Identifiler data, 1ng DNA, **5:2:1**)



Mixtures were created for research purposes and are synthetic mixtures of extracted DNA created in a controlled environment without PCR inhibitors or an unknown amount of degraded DNA as may be found in forensic casework.

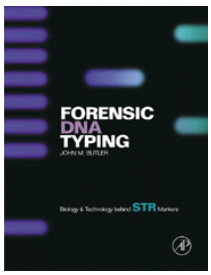
Some Training Workshops Planned

- ISFG Meeting (Sept 2009, Buenos Aires, Argentina)
  - **CE Fundamentals and Troubleshooting**
  - **Validation**

- Int. Symposium on Human Identification (Promega) Meeting (October 2009, Las Vegas, NV)
  - **Validation & Threshold Determination**

Forensic DNA Typing Textbook

1st Edition



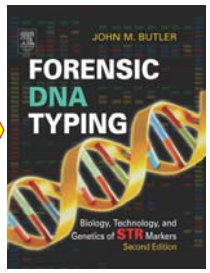
Jan 2001

335 pp.

17 chapters

With Y. Fukuma
(Japanese translator)

2nd Edition



Feb 2005

688 pp.

24 chapters

Now available in **Chinese**
(Yiping Hou)

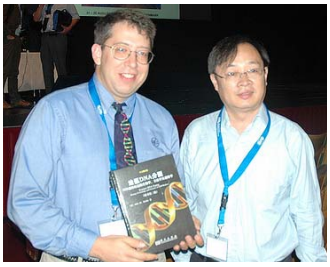
Japanese in preparation
(Yoshiya Fukuma)

3rd Edition

Fundamentals
Chapters 1-18
Sept 2009

Advanced Topics
Chapters 1-25
Feb 2011

Planned for 2009 & 2011
~1000 pages



With Y. Hou (Chinese translator)

Going Beyond the Core Competencies of Forensic DNA Testing

Core Competency

Standard STR Typing (DNA Profile)

Direct Matching (or Parentage)

Sufficient DNA quantity (ng)

Challenging kinship search questions

Familial Searching Attempts **(fishing for brothers or other relatives)**

Solution: Additional Markers (Y-chromosome, more STRs) and Multiple Reference Samples

Lower amounts of DNA being tested

Touch DNA Attempts
(poor quality, mixtures, low-level stochastic effects)

Solution: Replicate Testing

Be very cautious when outside the box…

Figure 18.3 in forthcoming Butler (2009) *Fundamentals of Forensic DNA Typing*

The Future of Forensic DNA

is Similar to the Olympic Motto of

"Swifter, Higher, Stronger"

Resources Training Action

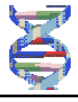
A new group was formed in Oct 2008 with an expanded mission

NIST *Applied Genetics*

Applied Genetics Group
Mission Statement

Advancing technology and traceability through quality genetic measurements to aid work in

- **forensic DNA testing,**
- **clinical genetics,**
- **agricultural biotechnology,** and
- **DNA biometrics.**



Thank you for your attention!

Funding from the **National Institute of Justice (NIJ)**
through NIST Office of Law Enforcement Standards

Margaret Kline

STR allele sequencing

Jan Redman

Variant allele cataloging

Becky Hill

miniSTRs, LCN, and 26plex work

Amy Decker

Mixtures & Y-STRs

Pete Vallone

SNPs & Rapid PCR

Dave Duewer

Data analysis tools

http://www.cstl.nist.gov/biotech/strbase
john.butler@nist.gov
301-975-4049