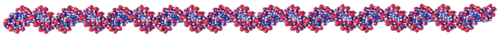
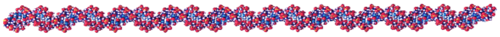


NIST Inter-Laboratory Studies for DNA Mixture Interpretation



Michael D. Coble, PhD

Carolyn R. Hill, MS; David L. Duewer, PhD; and
Margaret C. Kline, MS

National Institute of Standards and Technology

66th Annual AAFS Meeting
February 20, 2014
Seattle, WA

NIST and NIJ Disclaimer

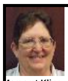
<u>Past and Present Funding</u>: Interagency Agreement between the National Institute of Justice and NIST Office of Law Enforcement Standards

Points of view are mine and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

Interlaboratory Studies

- The method by which multiple laboratories compare results and demonstrate that the methods used in one's own laboratory are reproducible in another laboratory.
- These tests are essential to demonstrate consistency in results from multiple laboratories.

(J.M. Butler, *Forensic DNA Typing*, p. 216)



argaret Kline

NIST DNA Mixture Interlab Studies

Dave Duewe

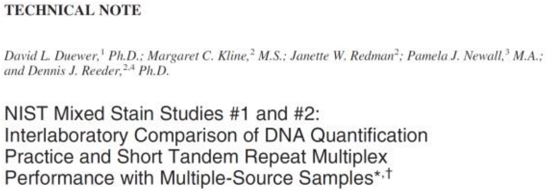
- Mixed Stain Study 1 (MSS1)
- April 1997 – November 1997
- 22/28 labs participated
- 6 reference samples, 4 two-person mixtures, 1 three-person mixture (stains on paper)
- Focus = donor types given a complete set of reference sources.

NIST DNA Mixture Interlab Studies

- Mixed Stain Study 2 (MSS2)
- January 1999 – May 1999
- 45/52 labs participated
- Part A = 4 reference samples, 1 two-person mixture, 1 three-person mixture (stains on paper)
- Part B = 1 mixture at 5 different concentrations
- Focus = donor types given an incomplete set of references; DNA Quantification Study.

MSS1 and MSS2

J Forensic Sci 2001;46(5):1199–1210.



TECHNICAL NOTE

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NIST Mixed Stain Studies #1 and #2: Interlaboratory Comparison of DNA Quantification Practice and Short Tandem Repeat Multiplex Performance with Multiple-Source Samples*,†

Technology in motion

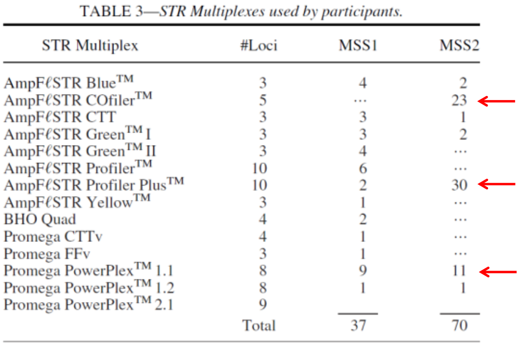


TABLE 3—*STR Multiplexes used by participants.*

STR Multiplex	#Loci	MSS1	MSS2
AmpFℓSTR Blue$^{\text{TM}}$	3	4	2
AmpFℓSTR COfiler$^{\text{TM}}$	5	…	23 ←
AmpFℓSTR CTT	3	3	1
AmpFℓSTR Green$^{\text{TM}}$ I	3	3	2
AmpFℓSTR Green$^{\text{TM}}$ II	3	4	…
AmpFℓSTR Profiler$^{\text{TM}}$	10	6	…
AmpFℓSTR Profiler Plus$^{\text{TM}}$	10	2	30 ←
AmpFℓSTR Yellow$^{\text{TM}}$	3	1	…
BHO Quad	4	2	…
Promega CTTv	4	1	…
Promega FFv	3	1	…
Promega PowerPlex$^{\text{TM}}$ 1.1	8	9	11 ←
Promega PowerPlex$^{\text{TM}}$ 1.2	8	1	1
Promega PowerPlex$^{\text{TM}}$ 2.1	9		
	Total	37	70

Technology in motion

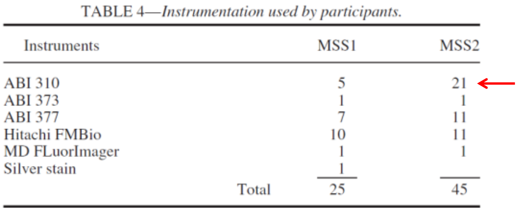


TABLE 4—*Instrumentation used by participants.*

Instruments	MSS1	MSS2
ABI 310	5	21 ←
ABI 373	1	1
ABI 377	7	11
Hitachi FMBio	10	11
MD FLuorImager	1	1
Silver stain	1	
Total	25	45

NIST DNA Mixture Interlab Studies

- Mixed Stain Study 3 (MSS3)
- December 2000 – October 2001
- 74/83 labs participated
- 1 single-source, 5 two-person mixtures, 1 three-person mixture (all extracts)
- Focus = effect of DNA quantitation on STR typing performance.

Anal. Chem. **2004**, *76*, 6928–6934

NIST Mixed Stain Study 3: Sign Balance in Commercial Short Ta Multiplexes

David L. Duewer,* Margaret C. Kline, Janette W. Redman, and J

Chemical Science and Technology Laboratory, National Institute of Standards Gaithersburg, Maryland 20899-8394

Anal. Chem. **2003**, *75*, 2463–2469

NIST Mixed Stain Study 3: DNA Quantitation Accuracy and Its Influence on Short Tandem Repeat Multiplex Signal Intensity

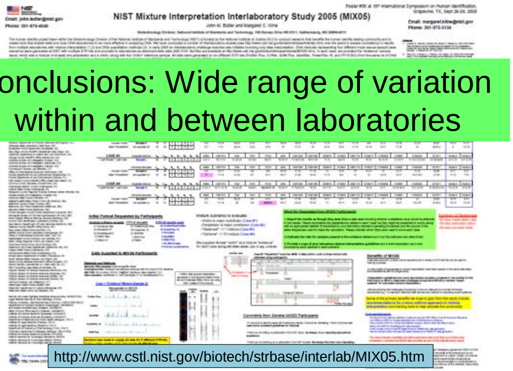
Margaret C. Kline, David L. Duewer,* Janette W. Redman, and John M. Butler

Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899

NIST DNA Mixture Interlab Studies

- Mixture Study 2005 (MIX05)
- January 2005 – August 2005
- 69/94 labs participated
- 4 two-person mixtures (only electronic data)
- Focus = evaluate the "lay of the land" and determine future needs for training and tools for interpretation.

MIX05 Poster Presentation at ISHI



Conclusions: Wide range of variation within and between laboratories

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

How MIX13 differs from MIX05 study

	MIX13 (2013)	MIX05 (2005)
Response	106 labs	69 labs
Number of cases provided	5 cases	4 cases
Case types being mimicked	Sexual assault & touch evidence	Sexual assault evidence
Mixture complexity	2, 3, >3-person (potentially related, low-template, inclusion/exclusion)	all 2-person (all unrelated, male/female; various major/minor ratios)
Scenarios provided	Yes	No

MIX 13 – NIST Interlaboratory Study on Mixture Interpretation - Purpose

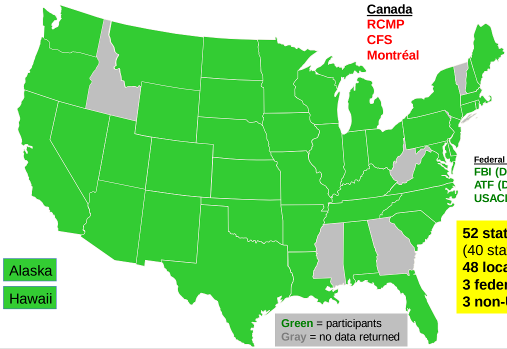
- MIX05 – conducted in 2005. Since then a great deal of effort has been focused on improvements in DNA mixture interpretation.
- 2010 SWGDAM Guidelines approved in January 2010 – many labs have changed their protocols recently.
- MIX13 – Interpretation challenge – no samples to run.

MIX 13 – NIST Interlaboratory Study on Mixture Interpretation - Goals

- (1) To evaluate the current “lay of the land” regarding STR mixture interpretation across the community.
- (2) To measure consistency in mixture interpretation across the U.S. after the publication of the 2010 SWGDAM guidelines.
- (3) To learn where future training and research could help improve mixture interpretation and reporting.

MIX13 Participants from **106 Laboratories**

45 states had at least one lab participate



Canada

RCMP

CFS

Montréal

Federal Labs

FBI (DOJ)

ATF (DOJ)

USACIL (DOD)

52 state labs

(40 states)

48 local labs

3 federal

3 non-U.S.

Due to the number of laboratories responding and the federal, state, and local coverage obtained, this MIX13 interlaboratory study can be assumed to provide **a reasonable representation of current U.S. forensic DNA lab procedures across the community**

MIX13 was also used an intra-lab study

Comments from TL of a MIX13 Lab

- Thank you for the opportunity to participate in this exercise! Some of these were very challenging and provoked a lot of conversation.
- I had a majority of the analysts in our Forensic Biology Unit interpret these profiles independently in an effort to identify inconsistencies and areas where we need to improve. **It was very interesting how much the results varied!** I've included two spreadsheets that demonstrate this – "MIX13 summary of allele calls" and "MIX13 summary of stats and conclusions."

16 different analysts examined the data in this particular lab

Purpose of MIX13 Cases

	Challenge provided to study responses
Case 1	~1:1 mixture (2-person)
Case 2	**Low template** profile with potential dropout (2-person)
Case 3	Potential **relative** involved (3-person)
Case 4	Minor component (2-person)
Case 5	Complex mixture (>3-person) with **# of contributors**; inclusion/exclusion issues

According to German Stain Commission (2009) mixture types: 1 = A, 2 = C, 3 = ?, 4 = B, 5 = ?

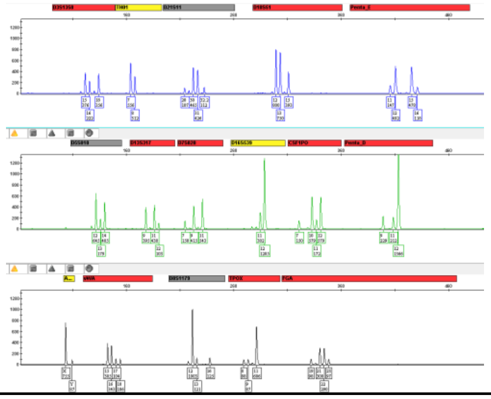
Case 04 – Bite Mark Evidence

Minor component (2-person)
“German Type B”

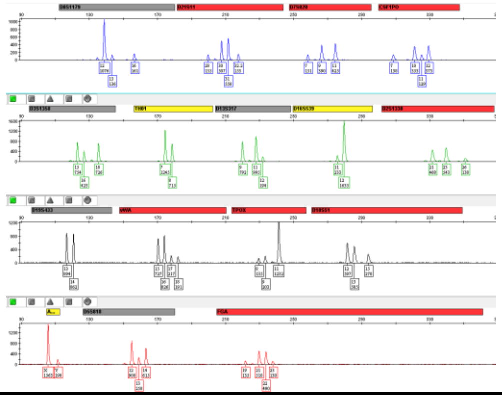
Scenario

- Evidence: saliva swab from a bite mark on the victim.
- A female waiting at a bus stop in the late evening is attacked from behind and pushed to the ground. A motorist driving by witnesses the attack, pulls his car over, and runs to her aid. As the Good Samaritan comes upon the scene, the perpetrator bites the victim on the back of her neck before running away.
- The motorist is able to give a good description of the perpetrator and a few days later, the police arrest a suspect. He is positively identified in a police lineup by the witness.

Case 04 – PP16HS



Case 04 – IDPlus

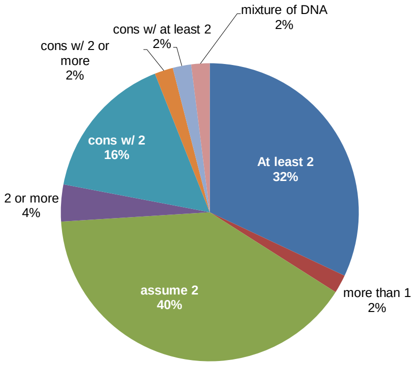


Case 04 – One Suspect

Individual	Inclusion?	Ratio
Victim	Included	~3.5
Suspect 4A	Included	1

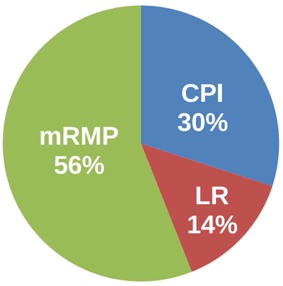
Primary Goals

- Primary purpose – will labs choose to deconvolve this mixture since the mixture ratio is close to the limit of deconvolution for many labs?
- All labs have included the suspect in the mixture.



# of Contributors

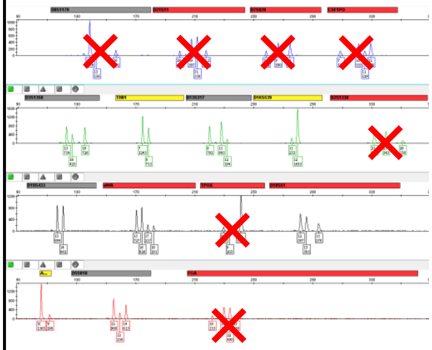
Statistical Interpretation



CPI Analysis

- Stats ranged from 1 in 3,070 to 1 in 862,000 with a median of 1 in 14,380.

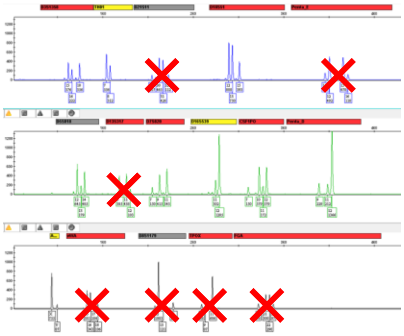
Alleles below ST (ID+)



7 Loci
D8
D21
D7
CSF
D2
TPOX
FGA

1 of 12 ID+ labs included all but D7

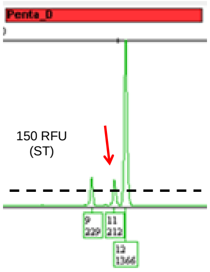
Alleles below ST (PP16HS)



7 Loci
D21
Penta E
D13
vWA
D8
TPOX
FGA

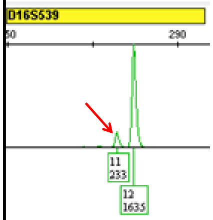
All PP16 labs excluded these markers

One PP16HS Lab (CPI)



Excluded Penta D from the CPI stats because the 11 allele may fall below ST due to stutter from the 12 allele

A similar situation with ID+



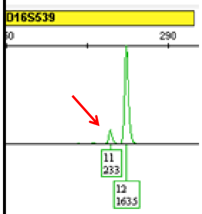
If 10% stutter from the 12 allele (163 RFU) is part of the 11 allele, then the remaining peak (70 RFU) is below the ST

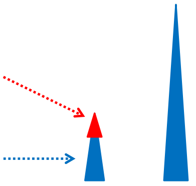
No CPI labs excluded D16 from the stat

mRMP/LR Analysis

- Stats ranged from 1 in 358,000 to 1 in 412 Quintillion with a median of 1 in 2.58 Quadrillion

Uncertainty with D16





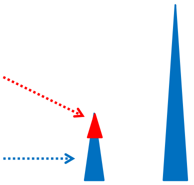
70 RFU
(POI)

163 RFU
(Stutter)

11
233

12
1633

Uncertainty with D16
Observed Approaches



70 RFU
(POI)

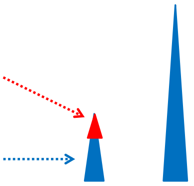
163 RFU
(Stutter)

11
233

12
1633

Drop the locus
(Below ST)

Uncertainty with D16
Observed Approaches



70 RFU
(POI)

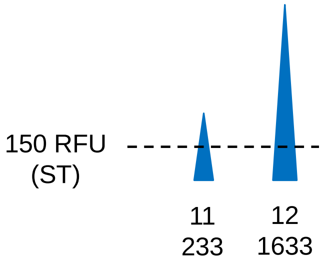
163 RFU
(Stutter)

11
233

12
1633

Use 2p
(Below ST)

Uncertainty with D16 Observed Approaches

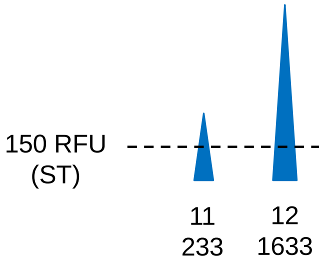


Use 2pq or p^2

(to infer genotypes)

11,12 or 11,11

Uncertainty with D16 Observed Approaches



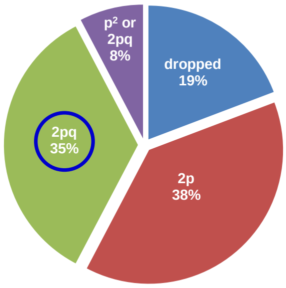
Use 2pq

(to infer genotype)

11,12

(matches POI)
“Certainty”

Statistics with D16 (mRMP/LR)



4.2. For calculating the CPE or RMP, any DNA typing results used for statistical analysis ***must be*** derived from **evidentiary items** and not known samples.

Probabilistic approaches to interpretation
can be useful to reduce uncertainty and subjectivity

Summary

- Most labs have validated and implemented AT and STs since MIX05. However, there is still a great deal of variation in interpretation across the US.
- An Idea – if everyone uses the same AT/ST, then one would expect to see similar results.
- Reality – the results were all over the place, Some of this was to be expected since each lab's protocol is different (e.g. dropping a locus vs, 2p).
- Probabilistic approaches will also deconvolve the mixture (without dropping loci), and can do so without bias.

NIST/NRC Postdoc Program

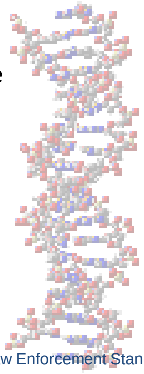
Working in the Applied Genetics Group at NIST

- Current stipend (2014) is **$66,256 per year**
  - Currently a limit of 120 slots per year
  - Congressionally-mandated program for NIST
  - Maximum 2-year appointments
- Awardees **must be U.S. citizens**
- Awardees are chosen through a **national competition** administered by the National Research Council of the National Academy of Sciences.
- Two competitions per year
  - **deadlines of February 1 and August 1**
- **Contact either Dr. Peter Vallone (peter.vallone@nist.gov) or or Dr. Michael Coble (michael.coble@nist.gov)**

Selected Topics
Rapid DNA Typing
DNA Mixture Analysis
Forensic Applications of Next-Gen Sequencing
DNA Extraction efficiency
Forensic SNPs
Y-STRs
Open to suggested topics/projects

http://www.nist.gov/iaao/postdoc.cfm
http://nrc58.nas.edu/RAPLab10/Opportunity/Program.aspx?LabCode=50

Thank You!



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Outside funding agencies:
NIJ – Interagency Agreement with the Office of Law Enforcement Standards