

Inter-laboratory Variation in  
Interpretation of DNA Mixtures  
Study:  
***DNAmix 2021***

Phase 1: Policies & Procedures  
Questionnaire  
***Preliminary Results***

2022 NIJ Symposium

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## Disclaimers

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- This project was supported by Award No. 2020-R2-CX-0049, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice.
- The opinions, findings, and conclusions or recommendations expressed in this program are those of the authors and do not necessarily reflect those of the Department of Justice.
- Manufacturer and brand names are used solely on the basis of actual usage by participants in the study — this does not imply endorsement.
- We would like to thank
  - The participants in the study, and
  - The DNAmix Working Group, a cross-section of experts from across the forensic DNA community who provided input on the study design.
- ***These are preliminary results: Registration is still open!***

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*If two laboratories are given the same mixture (as an electropherogram) and the same person of interest, how consistent are the statistical responses and categorical interpretations?  
And what factors explain any differences in responses?*

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## DNAmix 2021 Overview

- DNAmix 2021: Inter-laboratory Variation in Interpretation of DNA Mixtures Study
- A large-scale independent study conducted
  - to evaluate the extent of consistency and variation among forensic laboratories in interpretations and statistical analyses of electropherograms of DNA mixtures, and
  - to assess the effects of various potential sources of variability.
- Although there have been previous studies of interpretations of DNA mixtures (notably MIX13 and the STRmix Interlaboratory Study), there has not been a large-scale independent study evaluating the extent of variation in interpretation/statistical analysis of DNA mixtures:
  1. With samples selected to be representative of the range of attributes found in actual DNA mixture casework
  2. Including results from current state-of-the-practice probabilistic genotyping software (PGS)
  3. Not restricted to specific products or statistical approaches
- The project was designed to expand on the results and lessons learned from studies conducted to date, and to address issues raised by PCAST and the NIST Scientific Foundation Review.

## Study phases

This study is composed of four phases:

1. *Policies and Procedures (P&P) Questionnaire* — Online questionnaire to assess laboratory policies and procedures relevant to DNA mixture interpretation.
2. *Casework Scenario Questionnaire* — Online questionnaire to assess analysis procedures or decisions that may vary depending upon the case scenario and the nature of mixture casework conducted by the laboratory.
3. *Number of Contributors (NoC) Subtest* — Assessment of suitability and number of contributors for 12 mixture DNA samples, given electropherogram data.
4. *Interpretation, Comparison, and Statistical Analysis (ICSA) Subtest* — Interpretations, comparisons, and statistical analyses for 8 mixture DNA samples, given electropherogram data and reference profiles of assumed and potential contributors.

The use of subtests provides complementary approaches to collect data, to differentiate among the various potential sources of variability and avoid confounding their effects.

## Participation

- Participation is open to all forensic laboratories that conduct DNA mixture interpretation as part of their standard operating procedures (SOPs).
  - Non-U.S. laboratories are welcome to participate if they report the results of their interpretations, comparisons, and statistical analyses in English.
  - Laboratories are permitted to register more than one participant.
- Participants must agree to use the same diligence in performing these analyses as used operationally in casework, and to use their laboratory's SOPs in performing these analyses.
- Study records and test results will be confidential, and reported results will not be aggregated in a way that compromises confidentiality.
- Results will be coded in a way so that after results are published, participants can look up their own anonymized results, if they choose to do so.

## Phase 1: Policies & Procedures (P&P) Questionnaire Preliminary Results

- **These are preliminary results: Registration is still open!**
- These results are limited to participants/forensic laboratories that completed the P&P Questionnaire as of 10 February 2022
- This is a brief summary of a long and detailed questionnaire
- Here we present a subset of the P&P questions that we expect are of most interest

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### Summary of P&P Participation (so far)

- 156 participants from 80 forensic laboratories
  - 22 labs have more than one participant (2 to 14 participants per lab)
- All results in this briefing are reported by laboratory (N=80)
  - For labs with multiple participants, the majority response is reported
  - Labs with no majority response are flagged as "Inconsistent"

Most participating labs are U.S. State or Local labs (66/80)

11 labs are outside the U.S.

Lab Type	Count
US Local	35
US State	31
US Federal	2
US Private	1
non-US State/Provincial	7
non-US National	3
non-US Private	1
<b>Total</b>	<b>80</b>

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  - Labs with no majority response are flagged as “Inconsistent”

# Analysts	Labs
1	1
2 to 10	30
11 to 25	32
26 to 50	10
51+	5
Inconsistent	2

*Most participating labs have 2 to 25 DNA analysts*

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### Summary of Amplification Kits

*Please indicate all autosomal STR amp kit(s) and the number(s) of amp cycles that are validated and used in your lab for DNA mixtures. (check all that apply )*

Amplification Kit	Labs
Applied Biosystems AmpFLSTR Identifiler	1
Applied Biosystems AmpFLSTR Identifiler Plus	7
Applied Biosystems AmpFLSTR Profiler	1
Applied Biosystems AmpFLSTR Profiler Plus	1
Applied Biosystems GlobalFiler	41
Promega Powerplex 16	1
Promega Powerplex 16 HS	1
Promega Powerplex Fusion 5C	7
Promega Powerplex Fusion 6C	24
Qiagen Investigator 24plex	6
Other	4

*About half of the labs use GlobalFiler*

*Over one-quarter use Powerplex Fusion 6C*

*May add to over 80 due to multiple selections. Inconsistent responses not shown.*

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### Summary of Amplification Kits and Cycles

Extensive variability in numbers of cycles used:

- Almost all Fusion 6C labs use 29 cycles
- Much more variation in GlobalFiler labs (only 29/41 use 29 cycles)
- Only 2 other settings had more than 5 labs each

Amplification Kit	Labs	Amplification Cycles						
		25	26	27	28	29	30	>30
Applied Biosystems AmpFLSTR Identifier	1				1			
Applied Biosystems AmpFLSTR Identifier Plus	7			1	7	2	2	
Applied Biosystems AmpFLSTR Profiler	1				1			
Applied Biosystems AmpFLSTR Profiler Plus	1				1			
Applied Biosystems GlobalFiler	41			1	12	29	4	1
Promega Powerplex 16	1							1
Promega Powerplex 16 HS	1							1
Promega Powerplex Fusion 5C	7				1	5	1	
Promega Powerplex Fusion 6C	24	1		1	3	22	1	1
Qiagen Investigator 24plex	6		1		1	2	2	
Other	4							

May add to over 80 due to multiple selections. Inconsistent responses not shown.

### Capillary Electrophoresis (CE) Setup

Please indicate all CE instrument(s), injection voltage(s), and injection time(s) that are validated and used in your lab for DNA mixtures. (check all that apply)

- The vast majority of labs use an ABI 3500 series genetic analyzer at 1.2kV

CE Instrument	Labs	Injection Voltage (kV)					
		1.2	1.3	1.4	1.6	3	>3.0
Applied Biosystems (ABI) 3130	4	1				3	
Applied Biosystems (ABI) 3130xl	4	1				3	1
Applied Biosystems (ABI) 3500	47	45	1			1	
Applied Biosystems (ABI) 3500xl	36	33		1	2	1	1
Other	1						

May add to over 80 due to multiple selections. Inconsistent responses not shown.

### Capillary Electrophoresis (CE) Injection Times

- Notable variability in injection times used in labs
  - ABI 3500: 30 of 47 labs concur on 15 seconds
  - ABI 3500xl: 13 of 36 labs concur on 24 seconds

CE Instrument	Labs	Injection Time (seconds)																					
		<5	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	>24
ABI 3130	4	1	3		1				2														
ABI 3130xl	4	1	3		2			1															
ABI 3500	47	2	6	3	6	2	3	10	2	4	2	2	30	2	2	1	1	1	2	2	1	4	1
ABI 3500xl	36	1		1	2			2		5			3			2		3	1			13	2

May add to over 80 due to multiple selections. Inconsistent responses not shown.

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### Assessing Number of Contributors (NoC)

How do you assess the number of contributors in a mixture?

Method	Labs
Assess number of contributors manually	78
Software or spreadsheet	3

May add to over 80 due to multiple selections. Inconsistent responses not shown.

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### Assessing Number of Contributors (NoC)

Do your SOPs limit interpretation and/or comparison based on a maximum total number of contributors?

- 73 laboratories limit their interpretations based on max total NoC
  - The most common threshold for interpretation is a max of 4 contributors (47 labs)
  - The max NoC that any participating lab will interpret/compare is 6 contributors

	Maximum Total NoC	Labs
No		7
Yes	2	1
	3	11
	4	47
	5	12
	6	1
	Inconsistent	1
		73

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### Types of Statistical Values Reported

How do you report the statistical values supporting your interpretations and comparisons of DNA mixtures in casework? (check all that apply)

	Labs		Labs		Labs
Continuous LR	Continuous LR Only	34	Binary or Semi-continuous LR	BLR/SCLR Only	4
	+BLR/SCLR	3		+RMP/mRMP	2
	+RMP/mRMP	7		+CPI/CPE	1
	+BLR/SCLR +RMP/mRMP	3		+RMP/mRMP +CPI/CPE	3
	+RMP/mRMP +CPI/CPE	1			
	+BLR/SCLR +RMP/mRMP +CPI/CPE	6			
	+Inconsistent other stat(s)	3			
		57			10
				Other statistics	
				RMP/mRMP Only	4
				CPI/CPE Only	0
				RMP/mRMP +CPI/CPE	8
					12

One lab indicated that they do not report statistics

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### Types of Statistical Values Reported

- Most labs report continuous LR (57/80)
  - 34 labs report only continuous LRs
  - 23 labs report continuous LRs in addition to other stats

Continuous LR	Labs		Binary or Semi-continuous LR	Labs		Other statistics	Labs	
Continuous LR Only	34		BLR/SCLR Only	4		RMP/mRMP Only	4	
+BLR/SCLR	3		+RMP/mRMP	2		CPI/CPE Only	0	
+RMP/mRMP	7		+CPI/CPE	1		RMP/mRMP +CPI/CPE	8	
+BLR/SCLR +RMP/mRMP	3		+RMP/mRMP +CPI/CPE	3				
+RMP/mRMP +CPI/CPE	1							
+BLR/SCLR +RMP/mRMP +CPI/CPE	6							
+Inconsistent other stat(s)	3							
	57			10			12	

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### Types of Statistical Values Reported

- Most labs report continuous LRs (57/80)
  - 34 labs report only continuous LRs
  - 23 labs report continuous LRs in addition to other stats
- No labs use only CPI/CPE — 19 use it in addition to other stats

Continuous LR	Labs		Binary or Semi-continuous LR	Labs		Other statistics	Labs	
Continuous LR Only	34		BLR/SCLR Only	4		RMP/mRMP Only	4	
+BLR/SCLR	3		+RMP/mRMP	2		CPI/CPE Only	0	
+RMP/mRMP	7		+CPI/CPE	1		RMP/mRMP +CPI/CPE	8	
+BLR/SCLR +RMP/mRMP	3		+RMP/mRMP +CPI/CPE	3				
+RMP/mRMP +CPI/CPE	1							
+BLR/SCLR +RMP/mRMP +CPI/CPE	6							
+Inconsistent other stat(s)	3							
	57			10			12	

One lab indicated that they do not report statistics

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### Types of Statistical Values Reported

- Most labs report continuous LR (57/80)
  - 34 labs report only continuous LRs
  - 23 labs report continuous LRs in addition to other stats
- No labs use only CPI/CPE — 19 use it in addition to other stats
- 4 labs use only RMP/mRMP — 30 use it in addition to other stats

	Continuous LR		Binary or Semi-continuous LR	Other statistics		
		Labs			Labs	
Continuous LR Only		34	BLR/SCLR Only	4	RMP/mRMP Only	4
+BLR/SCLR		3	+RMP/mRMP	2	CPI/CPE Only	0
+RMP/mRMP		7	+CPI/CPE	1	RMP/mRMP +CPI/CPE	8
+BLR/SCLR +RMP/mRMP		3	+RMP/mRMP +CPI/CPE	3		
+RMP/mRMP +CPI/CPE		1				
+BLR/SCLR +RMP/mRMP +CPI/CPE		6				
+Inconsistent other stat(s)		3				
		57		10		12

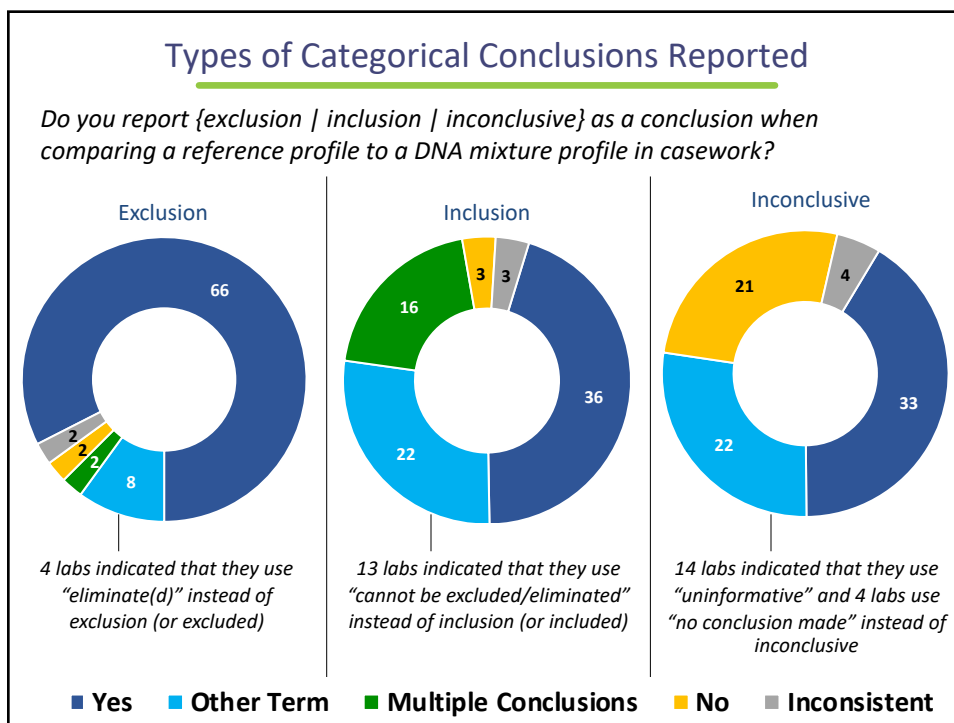
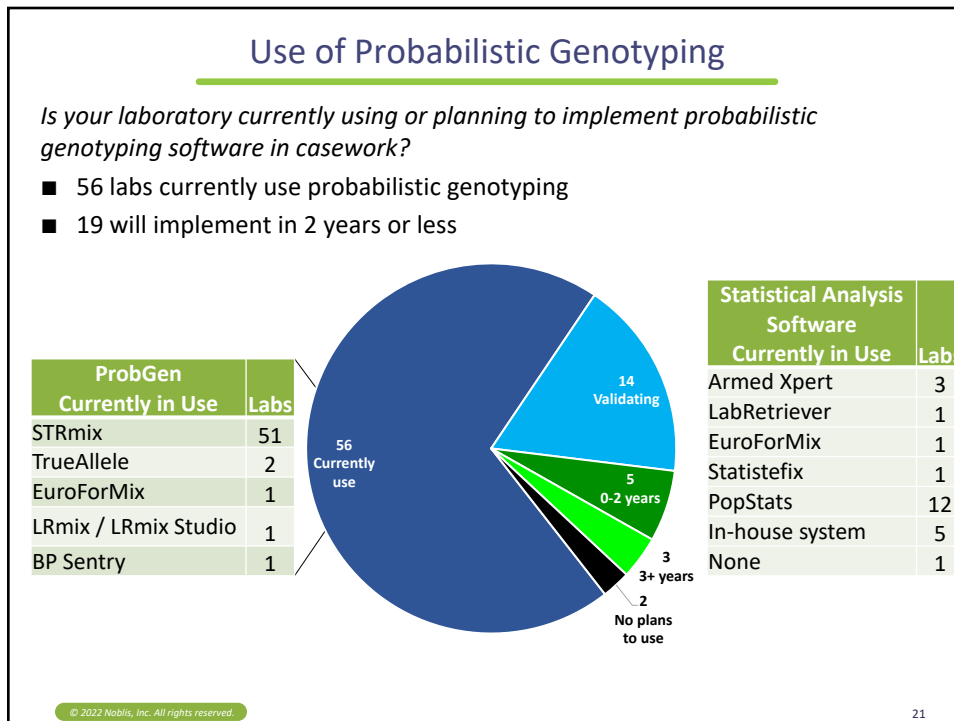
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### Types of Statistical Values Reported

- Most labs report continuous LR (57/80)
  - 34 labs report only continuous LRs
  - 23 labs report continuous LRs in addition to other stats
- No labs use only CPI/CPE — 19 use it in addition to other stats
- 4 labs use only RMP/mRMP — 30 use it in addition to other stats
- 4 labs use only Binary or Semi-continuous LRs — 18 in addition to other stats

	Continuous LR		Binary or Semi-continuous LR	Other statistics		
		Labs			Labs	
Continuous LR Only		34	BLR/SCLR Only	4	RMP/mRMP Only	4
+BLR/SCLR		3	+RMP/mRMP	2	CPI/CPE Only	0
+RMP/mRMP		7	+CPI/CPE	1	RMP/mRMP +CPI/CPE	8
+BLR/SCLR +RMP/mRMP		3	+RMP/mRMP +CPI/CPE	3		
+RMP/mRMP +CPI/CPE		1				
+BLR/SCLR +RMP/mRMP +CPI/CPE		6				
+Inconsistent other stat(s)		3				
		57		10		12

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### Use of Verbal Equivalents for Likelihood Ratios

Do you employ verbal equivalents in reporting your LR results?

	Labs
Report LR and verbal equivalents	36
Only report the LR	29
<i>Inconsistent</i>	1
<i>N/A (do not report LRs)</i>	14

Which verbal equivalent scale(s) do you use in reporting LR results?

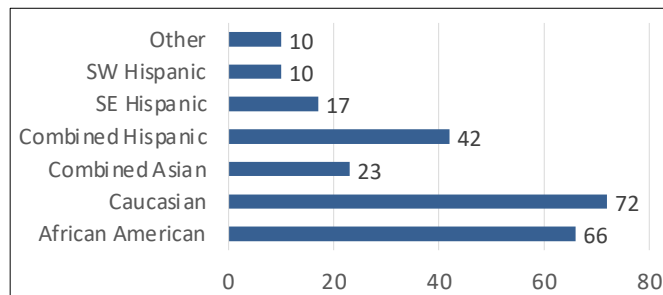
	Labs
SWGDM	28
Internally developed scale	4
ENFSI	1
ISFG	1
<i>Inconsistent</i>	3
<i>N/A (do not use LR Verbal Equivalents)</i>	43

### Population Databases Used

What is your source of the population databases that are routinely used in your casework? (check all that apply)

Population Database Source	Labs
NIST	50
FBI	23
Internal/In-house	6
National peer reviewed publication	1
STRider	2

Which population databases are ROUTINELY used for computing and reporting the results of statistical analyses for DNA mixtures? (check all that apply)



## Status & Schedule

	Start	End
Participant registration	28 Jun 2021	6 Mar 2022
Phase 1: P&P Questionnaire	14 Jul 2021	10 Mar 2022
Phase 2: Casework Scenario Questionnaire	3 Sep 2021	13 Mar 2022
Phase 3: NoC Subtest	22 Nov 2021	27 Mar 2022
Phase 4: ICSA Subtest	19 Jan 2022	11 May 2022

*Note: although we closed registration on Jan 31, we received multiple requests to allow additional participants, so we re-opened registration and P&P, and have extended the deadlines for the remaining phases by 3 weeks.*

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For eligible laboratories to register to participate:

**<https://dnamix.edgeaws.noblis.org>**

**by 6 March 2022**

To be added to the interested parties list  
and be informed of future presentations/publications, email

**DNAmix@noblis.org**

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