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DNA Mixture Interpretation Principles: Insights from the NIST Scientific Foundation Review

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Acknowledgment and Disclaimers

I appreciate input on this project from **Rich Cavanagh, Mike Coble** (now at UNTHSC)**, Hari Iyer, John Paul Jones, Willie May** (now at Morgan State University)**, Rich Press, Melissa Taylor, Pete Vallone, and Sheila Willis –** ***and a 13-member DNA Mixtures Resource Group***

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Presentation Topics

- Mixture interpretation challenges and variation
- Importance of defining and understanding principles
- NIST Scientific Foundation Review: goals and progress
- Reflections on some insights learned
- Summary

What is the Biggest Challenge Forensics Laboratories Face Today? (ISHI 28 speakers were asked to share what they thought were the biggest challenges)

Interpretation of complex mixtures and determining relevance

Chantal Frégeau, Royal Canadian Mounted Police

"From a Biology/DNA discipline perspective, the highly sensitive STR kits and capillary electrophoresis-based detection instruments currently used for forensic DNA typing analysis very often generate complex mixtures from "touch DNA" exhibits brought in by the investigators. The biggest challenge remains the interpretation of those complex mixtures and the determination of the relevance of a contributor's DNA profile derived from an exhibit to the crime that has been committed. Probabilistic software can assist with the interpretation of complex mixtures but determining how the genotypes were deposited remains challenging (relevance to the crime)."

Resources, education, and training

Bruce Budowle, UNTHSC

"Resources, education and training. Most of the issues we are facing seem to be related to these needs.."

https://www.ishinews.com/biggest-challenge-forensics-laboratories-face-today/

Sobering Thoughts from a 2014 Article

"There has been very little work published on the **variation of reporting practices of mixtures between laboratories**, but it has been previously demonstrated that **there is little consistency**. **This is because there is no current uniformity of practice, so different laboratories will operate using different rules**. The interpretation of mixtures is not solely a matter of using some software to provide 'an answer.'…"

"We show that **by introducing a structured training [program]**, it is possible to demonstrate, for the first time, that a high degree of standardization, leading to **uniformity of results can be achieved by participating laboratories**."

Prieto et al. (2014) Euroforgen-NoE collaborative exercise on LRmix to demonstrate standardization of the interpretation of complex DNA profiles. *FSI Genetics* 9: 47-54

Butler *et al.* (2018) *FSI Genetics* 37: 81-94

NIST interlaboratory studies involving DNA mixtures (MIX05 and MIX13): Variation observed and lessons learned

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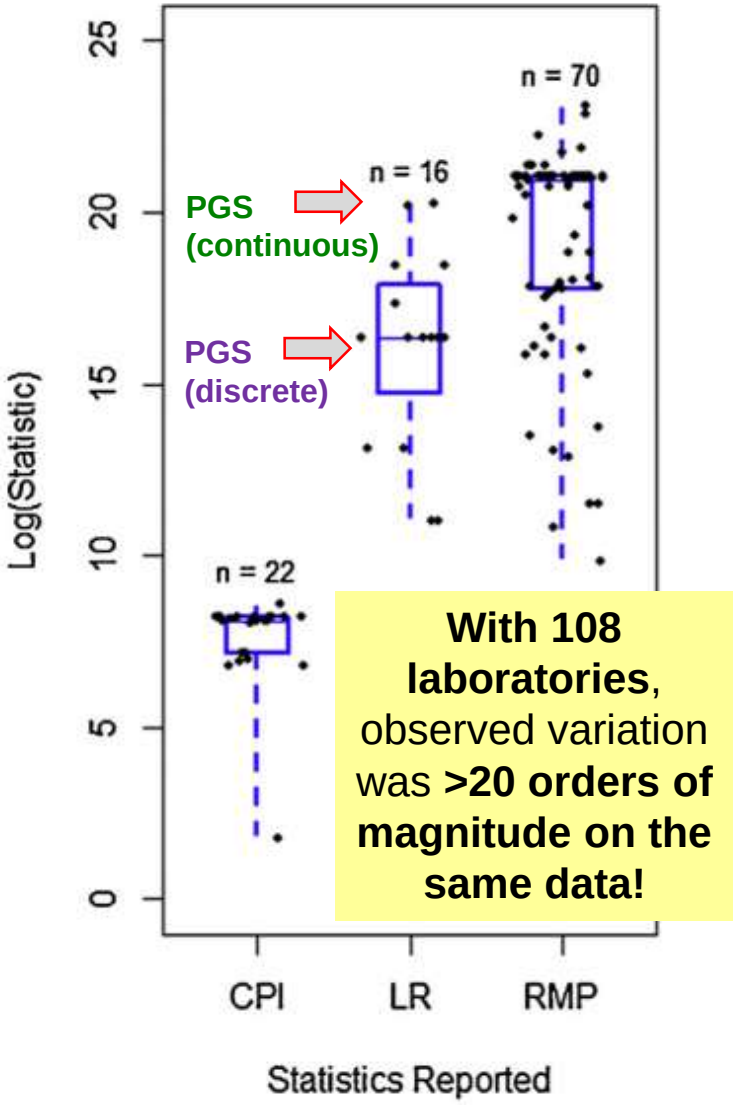
"**All participants correctly included the reference profile "1A" and provided a statistic**. Most of the laboratories inferred the genotype of the unknown contributor and provided either mRMP or LR statistics. However, **a wide range of variation between methods was observed in the statistical values reported**."



With 108 laboratories, observed variation was **>20 orders of magnitude on the same data!**

What if probabilistic genotyping had been used?

MIX13 Case 1
2-person
(1:1 ratio)

Buckleton *et al.* (2018) *FSI Genetics* 37: 172-179

NIST interlaboratory studies involving DNA mixtures (MIX13): A modern analysis

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Table 4
PG results for NIST MIX13 case 1.

Software	LR
STRmix™	1.4×10^{20}
EuroForMix v1.10.0	2.7×10^{20}
EuroForMix v1.11.4	1.5×10^{20}
Lab Retriever	4.1×10^{15}
LRmix	3.6×10^{15}
1/RMP	1.9×10^{20}

continuous (~10^{20})

discrete (~10^{15})

Four probabilistic genotyping software (PGS) programs were **run in a single laboratory** on the NIST MIX13 profiles

- **STRmix** and **EuroForMix** use continuous models (allele calls and peak heights)
- **Lab Retriever** and **LRmix** use discrete/semi-continuous models (allele calls only)

The discrete and continuous models are internally consistent, but over four orders of magnitude separate the results. **This is to be expected when different input information is used.**

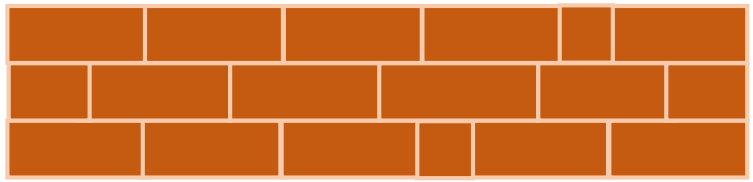
Underlying Principles should be Published (and Understood)

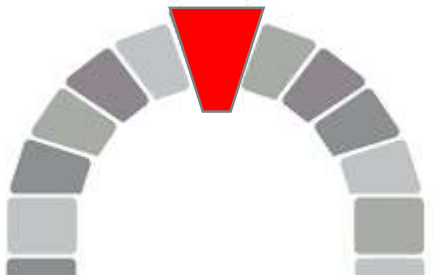
- **FBI QAS (2011, 2019) requires (8.2.2) peer-reviewed publication of underlying scientific principles** of a technology
  - Defined by the QAS as "a rule concerning a natural phenomenon or function that is a part of the basis used to proceed to more detailed scientific functions"

- **Can we define underlying (foundational) principles that govern DNA mixture interpretation** to help us understand "why" something is important and what we should do in specific situations?

What is a “Foundational” Principle?

- It is relied upon as being **solid** (i.e., it can be trusted as tried and true)
- It is **established** (i.e., it has been around a while and demonstrated to be trustworthy through repeated studies)
- **The field is built upon it** (i.e., it serves as a center piece – a keystone – to support and underpin other parts of the structure or enterprise)





Retrievable **Respected** **Reliable**

NIST Scientific Foundation Reviews

- Requested and funded by Congress to examine forensic disciplines

- **Initial pilot study on DNA mixture interpretation**
  - Project begun in September 2017

- **6 NIST team members meet weekly with regular input from 13 forensic practitioners/researchers** (our “DNA Mixture Resource Group”)

- **Examining the literature and studying issues…**
  - **>500 articles collected on DNA mixture interpretation**
  - Seeking to compile underlying principles and assess claims

- **Report is being written for release** (as a draft) **later this year**
  - Plan to collect public comment on the report and reactions to its findings
  - Presentation at ISHI 2018 will discuss details, lessons learned, and important principles and challenges faced with DNA mixture interpretation
  - AAFS 2019 workshop planned to discuss the topic and report in detail

Initial Concerns Raised by Some Regarding Our Project

- Everything is fine with DNA – leave it be
- There are standards for DNA interpretation already
  - **FBI QAS 2011 9.6.4** Laboratories analyzing forensic samples shall have and follow a documented procedure for mixture interpretation that addresses major and minor contributors, inclusions and exclusions, and policies for the reporting of results and statistics.
- You need additional experts working on this study
- Available information is being ignored, such as unpublished validation studies

Who Is Involved in the NIST DNA Study?

- **NIST Review Team**
  - Role: conducting review & writing report
  - 6 people who meet weekly (listed as presentation co-authors on title slide)
  - Expertise: research, DNA literature, statistics, human factors, casework management, communications

- **Resource Group**
  - Role: providing input & sounding board
  - 13 practitioners & academics/consultants (Federal, state, local, and international) who provide periodic input & feedback
  - Expertise: DNA casework
  - *Will review draft report but are not being asked to endorse report conclusions or considerations (recommendations)*

Input Provided by a DNA Mixtures Resource Group

Name	Affiliation
Jack Ballantyne	University of Central Florida
Todd Bille	ATFE Laboratory, DNA Technical Leader
Jennifer Breaux	Montgomery County Police Crime Lab
Robin Cotton	Boston University School of Medicine
Roger Frappier	Centre of Forensic Sciences - Toronto
Bruce Heidebrecht	Maryland State Police, DNA Technical Leader
Keith Inman	Cal State East Bay & forensic DNA consultant
Eugene Lien	NYC OCME, DNA Technical Leader
Tamyra Moretti	FBI Laboratory, DNA Support Unit
Lisa Schiermeier-Wood	Virginia Department of Forensic Sciences
Joel Sutton	Defense Forensic Science Center, USACIL
Ray Wickenheiser	NYSP Laboratory Director (ASCLD President)
Charlotte Word	forensic DNA consultant

9 practitioners (3 Federal, 3 state, 2 local, 1 Canadian), **4 academics/consultants**

Where Are We Headed with Our DNA Study?

Primary Goals:

1. **Develop a bibliography of foundational literature**
2. Define underlying principles, characterize capabilities and limitations of methods for mixture analysis
3. Identify knowledge gaps for future research
4. Inform the forensic community and non-specialists of findings (judges, attorneys,& general public)
5. **Create a framework for potential future NIST foundational reviews in forensic science (bitemarks already started)**

Plan to complete a draft report by December 2018 (followed by AAFS workshop in February 2019)

Working on a Comprehensive, Curated Reference List

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>500 articles collected so far

The Current Top Ten Articles*

From 585 references
(8/8/18 version)

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Probabilistic Genotyping

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**** We reserve the right to revise this list with further reading or new publications…***

Principles Contained in These Top Ten Articles

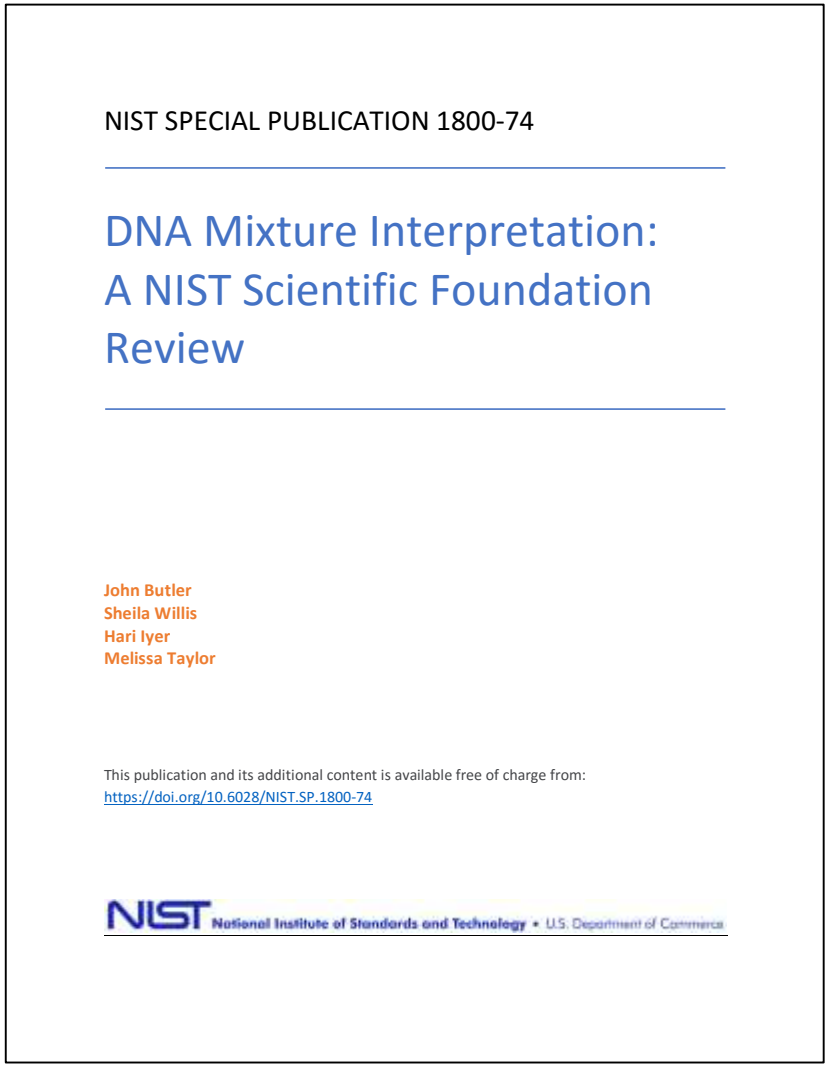
10. Are we **addressing the right question(s)** with our results?
9. Are we aware of possible **stochastic effects**?
8. Are we able to **deconvolute the mixture into component genotypes**?
7. Are we recognizing **peaks in stutter positions as potential minor alleles**?
6. Are we **aware of variation** in how others may approach a mixture?

Probabilistic Genotyping

5. Are we performing **validation studies to estimate drop-out and drop-in probabilities** with known samples?
4. Are we assessing **performance with potential non-contributors**?
3. Are we reporting results with **clear propositions and limited significant figures**?
2. Are we **disclosing assumptions made** and contextual information used?
1. Are we **thinking carefully about the case data and context** and not just feeding information into a computer program?

Overall Project Goal: Communicating Findings

Report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DNA Mixture Interpretation:
A NIST Scientific Foundation Review

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This publication and its additional content is available free of charge from:
https://doi.org/10.6028/NIST.SP.1800-74

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Supplemental Documents:

- Plain Language Summary
- Key Takeaways
- FAQs about this Report
- Why this is Important
- Considerations

- DNA Mixtures Explainer

- Public Documents
  - October 2017 press release
  - Report press release
  - PowerPoint presentations
    - SWGDAM
    - AAFS
    - ISHI
  - …

Website

A Quick Primer on DNA Mixtures and Touch DNA

A Brief History

What is a DNA Profile?

DNA in Context: Transfer & Persistence

Why Complex Mixtures are Difficult to Interpret

Probabilistic Genotyping

Validation and Identifying Limits

What is NIST Doing to Help?

Report Chapters Planned

- Front Material: Acknowledgments and disclaimer
- **Chapter 1**: Scientific foundation review and purpose of study
- **Chapter 2**: DNA mixture background and historical timeline
- **Chapter 3**: Review process and input (materials and methods) – literature examined, criteria and terminology used
- **Chapter 4**: Relevance: case context including DNA transfer issues
- **Chapter 5**: Reliability: measurement and validation
- **Chapter 6**: Additional issues to consider: new technologies and forces at play
- **Chapter 7**: Considerations and summary
- Appendix: Reference list (with annotation)

Literature Searches Conducted for Chapter 5 Information

- Published validation studies examined
  - Prior to probabilistic genotyping, >65 developmental and internal validation studies were published
  - **Almost all contain only 2-person mixtures** with around five ratios (usually something like 9:1, 4:1, 1:1, 1:4, 1:9)

- Some observations
  - Most forensic DNA literature is methods focused and describes new markers or population data (i.e., it does not assess reliability of interpretation approaches)
  - Theoretical papers often describe a particular model and may perform some simulations with relatively little data presented
  - Often broad claims are made in validation studies without explicit support for these claims; **some claims are simply that SWGDAM validation guidelines were followed**

Published Validation Summaries for Chapter 5 Information

Reference	Type of Validation	Instru-ment	Kit/Assay	Method	Mixtures Examined	Mixture Ratios Explored	# Contributors Tested	DNA tested
Jäger 2017 FSIG 28:52-70	Developmental	MiSeq FGx	ForenSeq (Illumina)	NGS	NA12877/NA18507 (male/male); NA12878/NA19238 (female/female); NA12878/NA18507 (female/male)	**9 ratios** for MM and FF - 99.9:0.1 (999:1), 99:1, 95:5, 93.75:6.25, 90.9:9.1, 90:10, 87.5:12.5, 75:25, 50:50 (1:1); 4 ratios for FM - 95:5, 90:10, 75:25, 50:50	**2person** (male/male, female/female)	not clear
Du 2017 IJLM 131:605-620	Developmental	ABI 3500xl	HG19+14Y System (AGCU, China)	CE	9947A/9948 (1:1, 1:4, 1:9, 1:19); 9948/2800M (1:1, 1:4, 1:9, 1:19)	**4 ratios** - 19:1, 9:1, 4:1, 1:1	**2person** (male/female, male/male)	1 ng total
Cisana 2017 CMJ 58:26-33	Evaluation study	ABI 3500	PowerPlex Fusion 6C (Promega)	CE	1:1, 1:5, 1:10 (91pg & 909pg), 10:1, 5:1	**5 ratios** - 10:1, 5:1, 1:1, 1:5, 1:10	**2person** (male/female)	1 ng total
Kraemer 2017 FSIG 29:9-20	Developmental	ABI 3500	Investigator 24plex QS (Qiagen)	CE	1:15 (31 pg & 469 pg)	**9 ratios** - 15:1, 10:1, 7:1, 3:1, 1:1, 1:3, 1:7, 1:10, 1:15	**2person** (male/female)	500 pg total
Li 2017 FSIG 27:67-73	Developmental	ABI 3130	Microreader 23sp ID (Suzhou, China)	CE	9947A/9948 (19:1, 9:1, 4:1, 2:1, 1:1)	**5 ratios** - 19:1, 9:1, 4:1, 2:1, 1:1	**2person** (male/female)	1 ng total

Claims for most of these articles is that they conducted enough experiments to meet the SWGDAM validation guideline requirements

Ideas going into Chapter 5 regarding validation

- Validation of a method is not binary or universal
- Verification of interpretation protocol performance is important
- Limitations can be best established with performance-based analyses

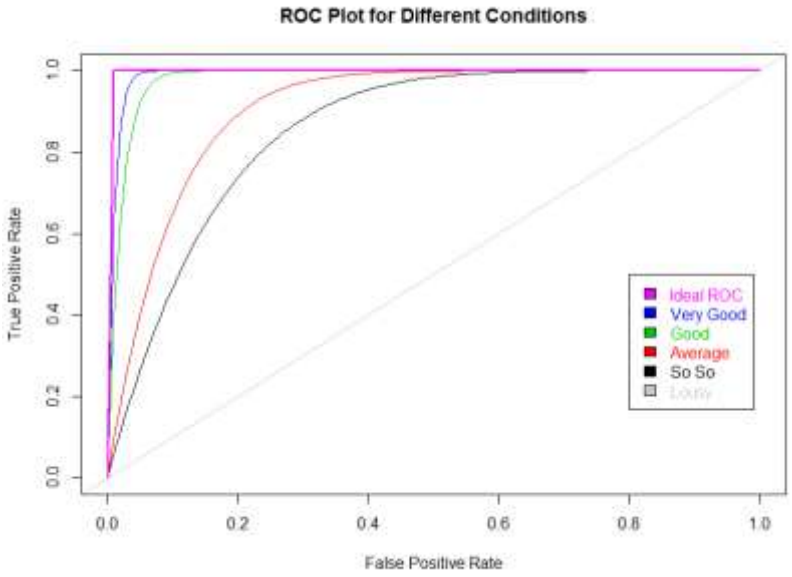
Task-driven

SWGDAM Validation Guidelines for DNA Analysis Methods (2016)

- ✓ 4.1 Known and nonprobative evidence samples
- ✓ 4.2 Sensitivity and stochastic studies
- ✓ 4.3 Precision and accuracy: repeatability
- ✓ 4.3 Precision and accuracy: reproducibility
- ✓ 4.4 Mixture studies
- ✓ 4.5 Contamination assessment

✓ *4.4 Mixed DNA samples that are representative of those typically encountered by the testing laboratory should be evaluated*

Performance-based



Summary

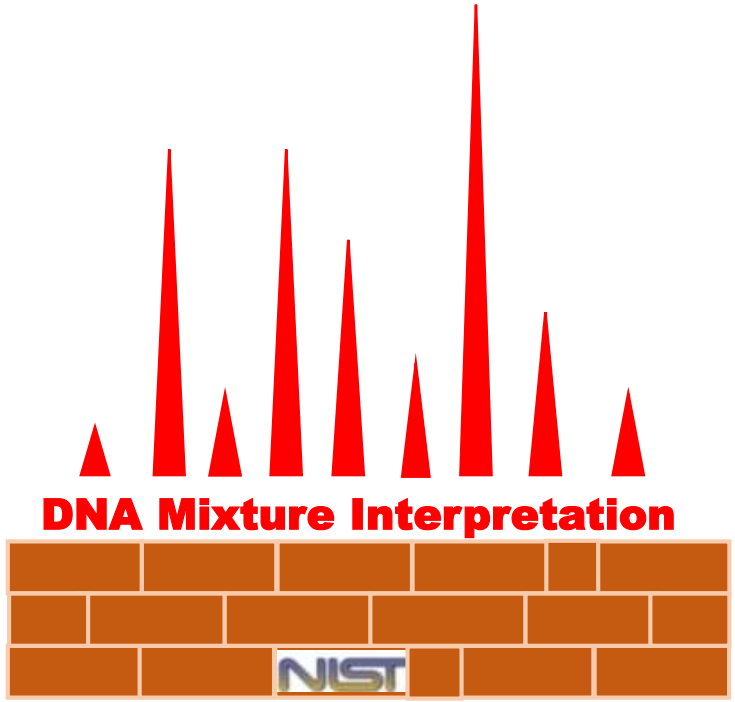
- Input from our Resource Group has been extremely helpful in examining DNA mixture interpretation issues and challenges

- We should approach validation of DNA mixture interpretation methods from a performance basis rather than a list of tasks and tests to conduct
  - Future interlaboratory studies can assist

- There is value in identifying and spelling out foundational principles and why they matter

- It is important to communicate and work together as a community to improve performance with DNA mixture interpretation

Thank you for your attention!



Scientific Foundation Review

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Upcoming Meetings

Forensics@NIST
November 7-8, 2018

Research Innovation to Implementation (RI2I)
June 19-20, 2019