NIST DNA Mixture Report (Butler - ISHI 2021)

International Symposium on Human Identification  
September 16, 2021 (Orlando, FL)

NIST Scientific Foundation Review on DNA Mixture Interpretation

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NIST Draft Report Released in June 2021

NISTIR 8351-DRAFT

DNA Mixture Interpretation: A NIST Scientific Foundation Review

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This publication is available free of charge from:  
https://doi.org/10.6028/NISTIR.8351-draft

250 pages  
Executive Summary (9 pages)  
6 chapters and 2 appendices  
528 references cited  
47 terms and acronyms defined  
29 tables  
12 figures  
5 boxes  
16 principles described  
25 key takeaways  
8 future considerations

Released for a 74-day public comment period (June 9 to August 23, 2021)

For a final version of these slides, see https://strbase.nist.gov/NISTpub.htm
Presentation Overview

1. Report Contents and Key Takeaways
   • Why NIST has undertaken this effort
   • Brief summary of our findings

2. Outreach and Public Comments Received
   • Public webinar given on July 21, 2021 (1,000 registrants) – 83 questions/comments
   • Presentations given to FBI SWGDAM (July 14) and NIST/NIJ Human Factors Working Group (July 28)
   • Types of comments received before the August 23 deadline

3. Future Plans
   • A final report will be issued after considering comments received
   • FAQs on a NIST website may also be created in addition to final report

These handouts, which were due to Promega by August 23, do not contain the final slides; for a final version of the presentation, see https://strbase.nist.gov/NISTpub.htm after September 16

Disclaimer & Acknowledgments

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.

Acknowledgments (page i): Members of the DNA Mixture Resource Group (listed in Table 1.2) contributed helpful feedback and assistance in the early stages of drafting this report. Katherine Gettings, Nikola Osborne, and Sarah Riman provided valuable input on the text, including the data summaries used in Chapter 4. Jason Weixelbaum, Susan Ballou, Christina Reed, and Kathy Sharpless assisted with copy editing. Kathryn Miller from the NIST Library helped finalize the document for public release.

Acknowledgments: NIST team members and Resource Group for their insights; all those who provided public comments
Requests for Understanding What Data Exists
Supporting Forensic Science Methods and Practices

NRC Report (2009)
NCFS Recommendation (2016)
PCAST Report (2016)
NIST 8225 (2020)

“demonstrating the validity of forensic methods”
(Recommendation #3)
“technical merit evaluation”
“establishing foundational validity”
Congressional funding uses NCFS language
NIST: a “Scientific Foundation Review”

NIST Scientific Foundation Reviews Underway in 2021

1. DNA Mixture Interpretation (initial pilot study)
   • Began in September 2017
   • AAFS 2019, ISHI 2019, ISHI 2020, AAFS 2021 workshops conducted
   • 250-page report released for 60-day public comment on June 9, 2021,
     with a 3-hour webinar planned for July 21

2. Bitemark Analysis
   • Began in October 2018
   • Workshop held in October 2019

3. Digital Investigation Techniques
   • Began in February 2019
   • Interlaboratory “black box” study conducted from June to November 2020

4. Firearm Examination
   • Began in October 2019
   • Gathering literature and focusing on error rate studies

https://www.nist.gov/topics/forensic-science/interdisciplinary-topics/scientific-foundation-reviews

Reports will be provided with each foundation study and made available for a 60-day public comment period

For a final version of these slides, see https://strbase.nist.gov/NISTpub.htm
DNA Mixture Report Content

In six chapters and two appendices:

• Chapter 1 introduces the topic and challenges of DNA mixture interpretation
• Chapter 2 provides background information on DNA, describes principles and practices underlying mixture measurement and interpretation, and introduces the likelihood ratio (LR) framework and probabilistic genotyping software (PGS)
• Chapter 3 lists data sources used in this study and strategies to locate them
• Chapter 4 and Chapter 5 cover reliability and relevance
• Chapter 6 explores the potential of new technologies to assist mixture interpretation and considerations for implementation
• Appendix 1 reviews the history of how the field has progressed
• Appendix 2 discusses strengthening the field with training & continuing education
• Bibliography includes 528 references cited in the report

Our Desire with This Report is to Help Move the Field Forward to Improved Practices in DNA Mixture Interpretation

From the Executive Summary (page 1):
“As with any field, the scientific process (research, results, publication, additional research, etc.) continues to lead to advancements and better understanding. Information contained in this report comes from the authors’ technical and scientific perspectives and review of information available to us during the time of our study. Where our findings identify opportunities for additional research and improvements to practices, we encourage researchers and practitioners to take action toward strengthening methods used to move the field forward. The findings described in this report are meant solely to inform future work in the field.”

Public comment received July 19, 2021: “The review is comprehensive, well-considered and well-written. However, [this disclaimer] stands to negate the entire body of work… Please consider whether such a disclaimer is even necessary, and whether it actually contributes to the application of forensic DNA to the fair administration of justice.”
We Recognize That There Are Many Different Perspectives and Lenses on This Report…

This is Why Public Comment is so Important!

Chapter Mapping
25 Key Takeaways (KT) and 8 Future Considerations (FC)

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>Chapter 2</th>
<th>Chapter 3</th>
<th>Chapter 4</th>
<th>Chapter 5</th>
<th>Chapter 6</th>
<th>Appendix 1</th>
<th>Appendix 2</th>
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<tbody>
<tr>
<td>INTRODUCTION</td>
<td>PRINCIPLES</td>
<td>SOURCES</td>
<td>RELIABILITY</td>
<td>RELEVANCE</td>
<td>TECHNOLOGY</td>
<td>HISTORY</td>
<td>TRAINING</td>
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<td>KT #5.1</td>
<td>KT #6.1</td>
<td>KT #A1.1</td>
<td>FC #A2.1</td>
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<td>KT #4.2</td>
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<td>KT #5.4</td>
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<td>16 Principles</td>
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<td>KT #4.8</td>
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<td>FC #A2.8</td>
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</table>

16 Principles
2 Tables
4 Tables
4 Figures
3 Tables
9 Tables
3 Figures
1 Box
5 Tables
3 Figures
3 Tables
5 Figures
3 Tables
4 Boxes

Glossary & Acronyms: 47 terms
Bibliography: 528 references

For a final version of these slides, see https://strbase.nist.gov/NISTpub.htm
**Likelihood Ratios Are Not Measurements**

(p. 42) DNA mixture interpretation is performed in the face of uncertainty. As noted by Ian Evett and Bruce Weir in their 1998 book:

2116 “The origins of crime scene stains are not known with certainty, although these stains may match samples from specific people. The language of probability is designed to allow numerical statements about uncertainty, and we need to recognize that probabilities are assigned by people rather than being inherent physical quantities.”

(Evett & Weir 1998, p. 21, emphasis added).

**KEY TAKEAWAY #2.6:** Likelihood ratios are not measurements. There is no single, correct likelihood ratio (LR). Different individuals and/or PGS systems often assign different LR values when presented with the same evidence because they base their judgment on different kits, protocols, models, assumptions, or computational algorithms. Empirical data for assessing the fitness for purpose of an analyst’s LR are therefore warranted.

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**Chapter 4: Reliability of DNA Mixture Measurements and Interpretation**

(4.1.1) System Reliability vs Component Reliability
(4.1.2) Definitions of Measurement, Uncertainty, Assessment, and Interpretation
(4.1.3) Empirical Assessments of Reliability
(4.1.4) Factor Space and Factor Space Coverage
(4.1.5) Provider-User Responsibilities and Examples
(4.2) Data Sources Used to Examine Reliability
(4.3) Review of Publicly Available Data and Factor Space Coverage
(4.4) Discussion
(4.5) Thoughts on a Path Forward

**KEY TAKEAWAY #4.1:** The degree of reliability of a component or a system can be assessed using empirical data (when available) obtained through validation studies, interlaboratory studies, and proficiency tests.
Table 4.3, Factor space coverage for published PGS validation data from peer-reviewed literature. Studies are grouped by PGS system and publication date. Studies listed on rows #6, #7, #9, #10, #11, #12, #13, #14, and #49 were part of the PCAST 2016 review. Nikola Osbourne and Sarah Riman (NIST Associates) assisted with early versions of these summaries. NoC = number of contributors; N.E.S. = not explicitly stated in the referenced publication; N/A = not applicable. Comparison of multiple PGS systems is discussed in Table 4.4. Inclusion of ranges is not meant to imply that all combinations of DNA quantities and mixture ratios were covered. A 31-laboratory compilation (Bright et al. 2018) contained data from eight different STR kits: GlobalFiler, Identifiler Plus, NGM Select, PowerPlex Fusion 5C, PowerPlex Fusion 6C, PowerPlex ESI17 Pro, PowerPlex ESI17 Fast, and PowerPlex 16 HS.

Table 4.4 (pp. 66-69)

Factor Space Coverage for Published PGS Validation Studies

Table 4.3

<table>
<thead>
<tr>
<th>#</th>
<th>Reference</th>
<th>PGS System</th>
<th>STR Kit</th>
<th>NoC Range</th>
<th># samples by NoC</th>
<th>Total DNA Quantity Range (pg)</th>
<th>Mixture Ratio Range</th>
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<tr>
<td>1</td>
<td>Perlin &amp; Snelikov 2009</td>
<td>TrueAllele</td>
<td>PowerPlex 16</td>
<td>2</td>
<td>40</td>
<td>125 to 1000</td>
<td>1:1 to 9:1</td>
</tr>
<tr>
<td>2</td>
<td>Perlin et al. 2011</td>
<td>TrueAllele</td>
<td>Pro+CoFliger</td>
<td>2</td>
<td>16</td>
<td>N.E.S.</td>
<td>N.E.S.</td>
</tr>
<tr>
<td>3</td>
<td>Perlin et al. 2013</td>
<td>TrueAllele</td>
<td>Pro+CoFliger</td>
<td>2/3</td>
<td>73/14</td>
<td>N.E.S.</td>
<td>N.E.S.</td>
</tr>
<tr>
<td>4</td>
<td>Ballantyne et al. 2015</td>
<td>TrueAllele</td>
<td>Identifier</td>
<td>2</td>
<td>2</td>
<td>N.E.S.</td>
<td>1:1</td>
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<tr>
<td>5</td>
<td>Perlin et al. 2014</td>
<td>TrueAllele</td>
<td>PowerPlex 16</td>
<td>2/3/4</td>
<td>40/65/8</td>
<td>N.E.S.</td>
<td>N.E.S.</td>
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<tr>
<td>6</td>
<td>Perlin et al. 2015</td>
<td>TrueAllele</td>
<td>Identifiler Plus</td>
<td>2/3/4/5</td>
<td>10/10/10/10</td>
<td>200, 1000</td>
<td>1:1 to 32:160/5.2</td>
</tr>
<tr>
<td>7</td>
<td>Greenspoon et al. 2015</td>
<td>TrueAllele</td>
<td>PowerPlex 16</td>
<td>1/2/3</td>
<td>11/18/15/7</td>
<td>10 to 1000</td>
<td>1:1 to 17:1:1</td>
</tr>
<tr>
<td>59</td>
<td>You &amp; Bolding 2019</td>
<td>multiple</td>
<td>N.GM Select</td>
<td>1/2/3</td>
<td>36/24/12</td>
<td>4 to 328</td>
<td>1:1 to 16:1/1:1:1</td>
</tr>
<tr>
<td>60</td>
<td>Riman et al. 2021</td>
<td>multiple</td>
<td>GlobalFiler</td>
<td>2/3/4</td>
<td>154/147/127</td>
<td>30 to 750</td>
<td>1:1 to 1:9/2:1</td>
</tr>
</tbody>
</table>

8 PGS studies were available and cited in the 2016 PCAST report. We examined and summarized 60 published PGS studies.

Published PGS Comparison Studies

11 + 1 NIST study (conducted during our review)

Table 4.4 (pp. 69-72)

<table>
<thead>
<tr>
<th>PGS Systems Compared Reference</th>
<th>Samples Tested</th>
<th>Observations Made</th>
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<tbody>
<tr>
<td>Available at <a href="https://www.biorxiv.org/content/10.1101/2021.05.26.445891v1">https://www.biorxiv.org/content/10.1101/2021.05.26.445891v1</a></td>
<td>Provided LR values for 1279 Hp true tests (Supplemental Table 4) and 1279 Hd true tests (Supplemental Table 5) for each software: explored LR distributions observed and used ROC plots, scatter plots, histograms with distribution of differences; evaluated apparent discrepancies between PGS models, adventitious exclusionary and inclusionary support, and verbal equivalent discordance; the authors reported: “in certain cases differences in numerical LR values from both software resulted in differences in one or more than one verbal categories (Table 8). These differences were substantially more with low template minor contributors and higher [number of contributors].”</td>
<td></td>
</tr>
<tr>
<td>EuroForMix (v2.1.0) STTRmix (v2.6)</td>
<td>Examined 154 two-person, 147 three-person, and 127 four-person mixtures from the PROVEDIt dataset, see Supplemental Table 4 in their article</td>
<td></td>
</tr>
<tr>
<td>Riman et al. 2021</td>
<td>*Supplementary Tables 4 and 5 contain all LR values and provide an example</td>
<td></td>
</tr>
</tbody>
</table>

For a final version of these slides, see https://strbase.nist.gov/NISTpub.htm
**Table 4.5** (pp. 73-75)

N.E.S. = Not Explicitly Stated in the referenced public source

The data most likely exist within the laboratory but are not currently available in the publicly accessible validation summary.

### Chapter 5: Context and Relevance Related to DNA Mixture Interpretation

**High Sensitivity Methods Impact Scientific Relevance**

**KEY TAKEAWAY #5.3:** Highly sensitive methods increase the likelihood of detecting contaminating DNA that might affect an investigation. Contamination avoidance procedures should be robust both at the crime scene and in the laboratory.

**Case Context is Important to Scientific Relevance**

**KEY TAKEAWAY #5.5:** The fact that DNA transfers easily between objects does not negate the value of DNA evidence. However, the value of DNA evidence depends on the circumstances of the case.

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Public Comments Received

Next Steps (DRAFT Report to FINAL Report)

• The public comment session closed on August 23, 2021
  • Public comments are being examined and considered
  • THANK YOU for taking time to read our draft report and providing your valuable input!

• A final report will be issued when our review process is complete
  • Are there sections that need to be clarified or changed? Is additional research needed?
  • In the end, the draft report will be superseded by the final version
  • We plan to provide a summary of changes made between the draft and final version

• An FAQ (frequently asked questions) webpage may also be created to help address why requested changes were made or not made
AAFS 2019 Workshop with NIST DNA Team and Resource Group Members

Thank you to our Resource Group members and their agencies who permitted them to assist us in this study.

Email received after our last meeting: John and NIST colleagues; thank-you very much for the invitation to participate in this illustrious group. I gained a great deal from our robust discussion and enjoyed it thoroughly. Many viewpoints always makes the product stronger.

Thank you for your attention!

John Butler
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https://www.nist.gov/topics/forensic-science

RESEARCH. STANDARDS. FOUNDATIONS.

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