# NIST Scientific Foundation Review on DNA Mixture Interpretation

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ISFG 2019
Prague, Czech Republic

International Society for Forensic Genetics

The U.S. National Institute of Standards and Technology (NIST) has been Congressionally-funded to perform scientific foundation reviews of select forensic disciplines. These reviews are intended to establish what is well-known and well-supported empirically in a forensic field and identify gaps that need further study. DNA mixture interpretation was selected as the initial NIST scientific foundation review given the existence of abundant literature and a need expressed by members of the community. Multiple interlaboratory studies conducted by NIST and others have noted variability among accredited laboratories using validated approaches on the same DNA mixture data.

A six-member NIST review team is conducting this review with input from a Resource Group composed of 13 experienced practitioners and researchers. More than 600 articles related to DNA mixture interpretation have been gathered and examined to understand capabilities and limitations as reflected in the scientific literature. An important goal of this project is to identify, consolidate, and share core principles and a list of relevant literature with the community to encourage deeper learning and understanding of DNA mixture interpretation. The output of this review will be a report, entitled *DNA Mixture Interpretation: A NIST Scientific Foundation Review*, to initially be published in draft form so that community feedback can be considered.

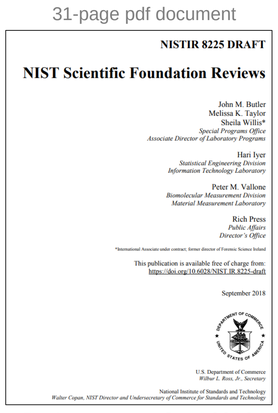
## Purpose of a Scientific Foundation Review

Scientific foundation reviews (initial termed "technical merit evaluations") of forensic disciplines were requested by the National Commission on Forensic Science in June 2016 [1]. In 2018, the United States Congress began funding NIST to conduct these reviews. A draft plan for conducting these reviews was released in September 2018 [2].

**Primary Goals for This Review on DNA Mixture Interpretation**:

1. Develop a bibliography of relevant literature
2. Define underlying principles, characterize capabilities and limitations of methods for DNA mixture interpretation
3. Identify knowledge gaps for future research
4. Inform the forensic community and non-specialists of findings (judges, attorneys, and the general public)
5. Create a framework for future NIST foundational reviews in forensic science (bitemarks, firearms & toolmarks, and digital evidence reviews are planned next)

31-page pdf document


NISTIR 8225 DRAFT

NIST Scientific Foundation Reviews

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This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8225-draft

September 2018

U.S. Department of Commerce
Wilbur L. Ross, Jr., Secretary

National Institute of Standards and Technology
Walter Copan, NIST Director and Undersecretary of Commerce for Standards and Technology

Draft shared in Sept 2018

[1] Views of the National Commission on Forensic Science on Technical Merit Evaluation of Forensic Science Methods and Practices (June 2016): https://www.justice.gov/archives/ncfs/file/881796/download
[2] DRAFT NISTIR 8225: NIST Scientific Foundation Reviews (31 pages): https://www.nist.gov/topics/forensic-science/draft-nistir-8225-nist-scientific-foundation-reviews; 13 public comments received (27 pages): https://www.nist.gov/sites/default/files/documents/2019/02/12/draft_nistir_8225_comments_received.pdf

## Principles and Concepts Involved in DNA Mixture Interpretation

*A principle is a fundamental truth from which others are derived. An understanding of foundational principles can provide the basis for why something is important and can assist in deciding what should be done in specific situations. The following principles and concepts have been distilled out of various publications and aspects of DNA mixture interpretation. These principles and concepts have been grouped by theme and ordered arbitrarily.*

Biology

1. Our DNA generally remains unchanged across time and cell type
2. DNA transfers and persists and can be collected and analyzed
3. A forensic DNA profile comes from analysis of DNA at specific sites in the genome

Genetics

4. DNA passes from parent to offspring according to genetic inheritance patterns
5. Genetic inheritance patterns enable weight-of-evidence statistical calculations
6. DNA profiles from close relatives are more similar than DNA from unrelated people

Analysis

7. With PCR amplification, a copy of a sample's DNA template is attempted, but it may not be exactly representative of the original sample
8. PCR enables sensitive detection but may introduce artifacts (e.g., STR stutter products)
9. When copying low amounts of DNA, the chance of allele drop-out increases
10. Stochastic (random) effects influence repeatability and relative amounts of detected alleles
11. Length-based separations (e.g., capillary electrophoresis) may not fully resolve different STR alleles

Statistics

12. Ability to differentiate DNA profiles generally increases as more DNA sites are tested
13. Probability is used to account for uncertainties when interpreting complex DNA data
14. Validation and calibration with known samples can be used to assess reliability of probability assignments
15. Different statistical approaches (e.g., LR, RMP, CPI) can produce different results
16. Weight-of-evidence assessments require at least two (mutually exclusive or different) points of view

Mixture Interpretation

17. Mathematical models can digitally deconvolute mixture components that cannot be physically separated
18. Continuous models use more information than discrete or binary approaches
19. Ability to deconvolute mixture components depends on the degree of allele sharing and stochastic effects, which can limit reliable pairing of alleles into potential contributor genotypes
20. Probabilistic genotyping software (PGS) can assist in complex DNA mixture interpretation but should not replace the judgment of DNA analysts

Propositions

21. Use of the hierarchy of propositions helps address different questions (e.g., who? vs. how?)
22. Information from DNA transfer and persistence studies should inform activity level evaluations
23. Sub-source likelihood ratios, if taken in isolation, can potentially be misleading

## Some Common Challenges with DNA Mixture Interpretation

- Differentiating stutter products from true alleles of another contributor
- Determining the number of possible contributors in a complex mixture
- Presenting multiple scenarios and accompanying LR values in reports and testimony
- Placing limits on the degree of complexity to examine or a lower limit on the amount of DNA to attempt amplifying and interpreting
- Obtaining sufficient training to understand probabilistic genotyping systems
- Understanding the possibilities of DNA transfer and their potential impact on a case
- Conducting validation experiments to cover the range of samples seen in casework
- Conveying to report users the meaning of results with low LR values
- Obtaining consistent results across analysts and laboratories

**Acknowledgements and Disclaimer:** Congressional funding for this work was administered through the NIST Special Programs Office. 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.

*Poster available for download from STRBase:*
***http://strbase.nist.gov/pub_pres/ButlerISFG2019poster.pdf***

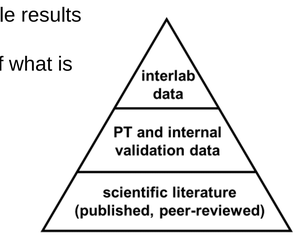
## Study Input and Data Sources

**Data Resources Sought for Examination in Our Review**

- **Interlaboratory data** reveal the degree of reproducibility with a method across multiple laboratories
- **Proficiency test (PT) and internal validation data** demonstrate the ability to obtain reliable results under specific laboratory conditions in a single laboratory
- **Published articles** in peer-reviewed scientific journals typically establish the broad base of what is possible

**NIST Review Team**: six (6) researchers with expertise in forensic DNA literature, statistics, human factors, casework management, and communications have met weekly over the past two years to conduct this review and write a report

**Resource Group:** thirteen (13) practitioners & academics/consultants with expertise in DNA casework from U.S. federal, state, local, and Canadian forensic laboratories provided periodic input and feedback across 12 meetings with the NIST team; they reviewed the initial draft report (in June 2019) but were not asked to endorse the report or its findings




An illustration of general relationships for information in support of a method and its use

## Outputs

Workshops have been conducted and others are planned to present our findings. A plain-language DNA explainer was released in April 2019 and a draft report is being finalized.

### Topics Covered in Draft Report Being Finalized

- Executive Summary, Acknowledgments & Disclaimer
- Introduction to the Review
- DNA Mixture Approaches, Principles, and History
- Data Sources and Study Input
- Reliability (validation and LR discrimination & calibration)
- Relevance (DNA transfer & activity)
- New Technologies (potential & limitations)
- Training and Continuing Education
- Key Takeaways Summarized
- Relevant Literature
- DNA Basics & Glossary
- Comments on PCAST Requirements for Scientific Validity

**AAFS 2019 Workshop Speakers**
(*left-to-right in picture below, NIST team bolded*):

Joel Sutton (Defense Forensic Science Center)
Jack Ballantyne (University of Central Florida)
Keith Inman (California State University - East Bay)
**John Butler** (NIST Special Programs Office)
Lisa Schiermeier-Wood (Virginia Department of Forensic Sciences)
**Peter Vallone** (NIST Applied Genetics Group)
**Melissa Taylor** (NIST Special Programs Office)
Ray Wickenheiser (New York State Police)
Robin Cotton (Boston University)
Bruce Heidebrecht (Maryland State Police)
**Hari Iyer** (NIST Statistical Engineering Division)
Eugene Lien (NYC Office of Chief Medical Examiner)
**Sheila Willis** (NIST guest researcher)
Jennifer Breaux (Montgomery County, Maryland)
Charlotte Word (consultant)
Roger Frappier (Center for Forensic Science-Toronto)
**Rich Press** (NIST Special Programs Office)

Resource Group members not pictured:
Todd Bille (ATFE Laboratory)
Tamyra Moretti (FBI Laboratory)

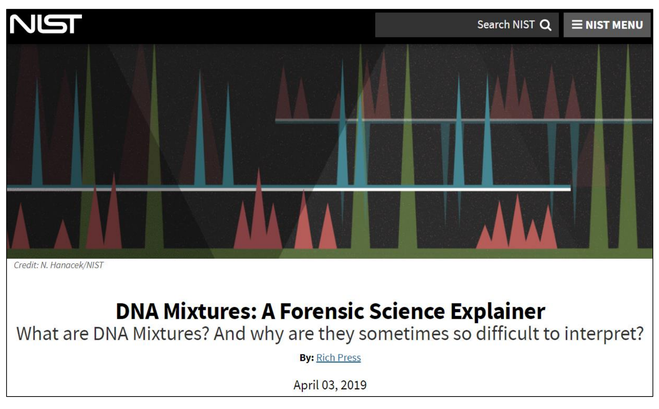
### American Academy of Forensic Sciences Workshop (February 2019)

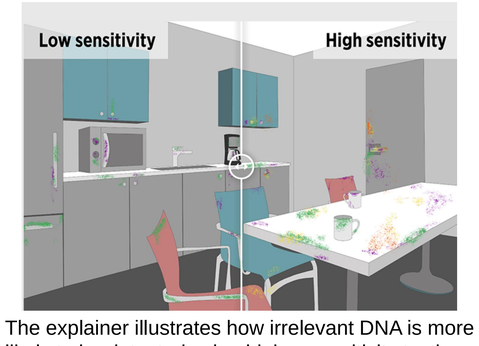
**DNA Mixture Interpretation Principles: Observations from a NIST Scientific Foundation Review**

**8 hours, 17 presenters, 19 talks, 406 slides**
Available at
**https://strbase.nist.gov/AAFS2019-W10.htm**

### DNA Mixtures Explainer (April 2019)

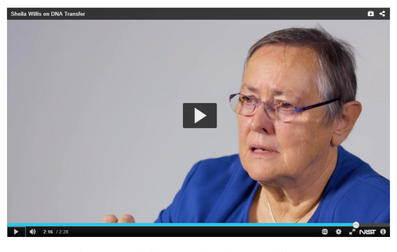
Available at https://www.nist.gov/featured-stories/dna-mixtures-forensic-science-explainer



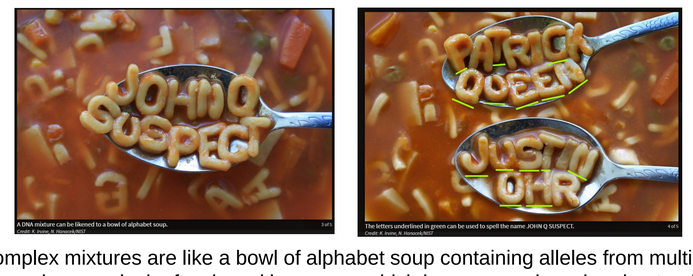




The explainer illustrates how irrelevant DNA is more likely to be detected using higher sensitivity testing

- **Why have DNA mixtures and trace DNA become so prevalent?**
- **Are all DNA mixtures difficult to interpret?**
- **Why are complex DNA mixtures difficult to interpret?**
  - **Uncertainty #1: When is a peak a peak?**
  - **Uncertainty #2: Whose peak is it anyway?**
- **What is probabilistic genotyping software, and how does it help?**
- **How confident can one be that the DNA is related to the crime?**
- **Should labs just stop analyzing complex DNA mixtures altogether?**




In a 2-minute video, Sheila Willis, a guest researcher at NIST and the former director general of Forensic Science Ireland, discusses the risks associated with DNA transfer when the evidence in a criminal case contains very small amounts of DNA. These risks can be mitigated, she says, by considering the evidence in the larger context of the case.




Complex mixtures are like a bowl of alphabet soup containing alleles from multiple contributors. One of the early realizations in our review was lack of a shared language, which hampers a shared understanding. A plain-language DNA mixtures explainer was prepared to assist in understanding why mixtures can be difficult to interpret.