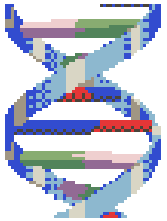
AAFS Jurisprudence Session
Denver, CO
February 23, 2024

DNA Mixture Interpretation: History of the Field and NIST Foundation Study Findings



John M. Butler, Ph.D.
NIST Special Programs Office

NIST NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

Acknowledgments and Disclaimer

Points of view in this presentation are mine and do not necessarily represent the official position or policies of the National Institute of Standards and Technology or of the American Academy of Forensic Sciences.

Certain commercial entities are identified 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 entities identified are necessarily the best available for the purpose.

NIST Forensic Science Program

https://www.nist.gov/spo/forensic-science-program

Special Programs Office

Shyam Sunder

Research at NIST in 8 focus areas:

- Biometrics
- Digital Evidence
- Drugs and Toxicology
- Evidential Statistics
- Firearms and Toolmarks
- Forensic Genetics
- Forensic Science Quality Assurance
- Trace Evidence

Robert Ramotowski

Standards efforts involve administering OSAC

22 forensic disciplines with **>800 participants** from across the community

OSAC REGISTRY

182 forensic science standards
(as of 6 Feb 2024)

OSAC LEXICON

>4,000 terms
organized by forensic discipline

OSAC IMPLEMENTER

>130 implementers
forensic science service providers

John Paul Jones

Foundation Studies


NISTIR 8225

NIST Scientific Foundation Reviews

- DNA Mixture Interpretation
- **Digital Investigation Techniques**
- **Bitemark Analysis**
- Firearm Examination
- Footwear & Tire
- Communicating Findings (LR)

John Butler

Trustworthy Results: A Shared Common Interest

NISTIR 8225 (2020)

NISTIR 8225

NIST Scientific Foundation Reviews

John M. Butler
Melissa K. Taylor
Sheila Willis*
Special Programs Office
Associate Director of Laboratory Programs

Hari Iyer
Statistical Engineering Division
Information Technology Laboratory

Peter M. Vallone
Biomolecular Measurement Division
Material Measurement Laboratory

Rich Press
Public Affairs
Director's Office

*International Associate under contract; retired director of Forensic Science Ireland

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

December 2020

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

NIST: a "Scientific Foundation Review"

Obtaining **reliable (trustworthy, consistently accurate) results is an important goal** for forensic science, which NIST, as part of the forensic science ecosystem, shares in all our activities

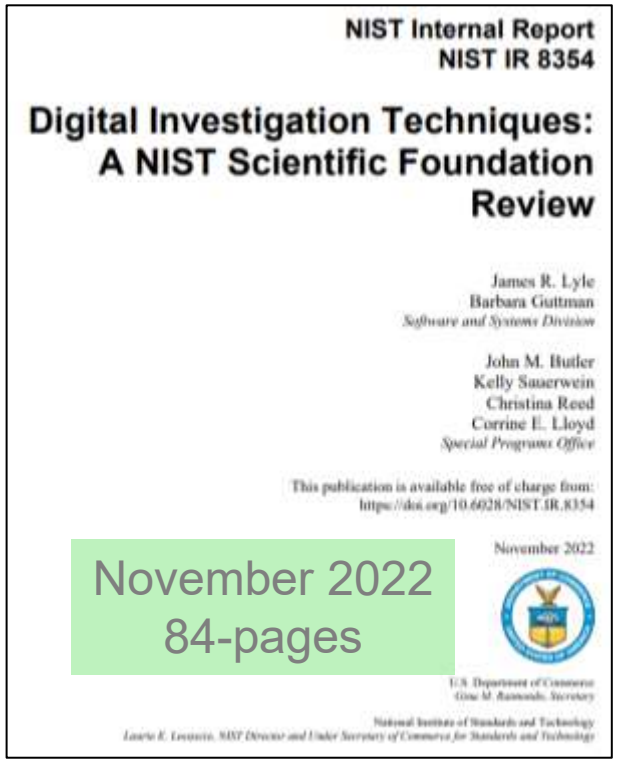
With NIST scientific foundation reviews, we are

1. Documenting the **key scientific principles** that underpin current methods and practices
2. Cataloging **available literature and information** that describe the state of the field
3. Recommending strategies so that the community and its stakeholders **can have confidence in the results obtained** from a particular method or practice

Scientific Foundation Studies

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

Goal: Identify the scientific foundations that support and underpin forensic methods and document and assess empirical evidence for the reliability of these methods using publicly available data and peer-reviewed literature.


NIST Internal Report
NIST IR 8354

Digital Investigation Techniques: A NIST Scientific Foundation Review

James R. Lyle
Barbara Guttman
Software and Systems Division

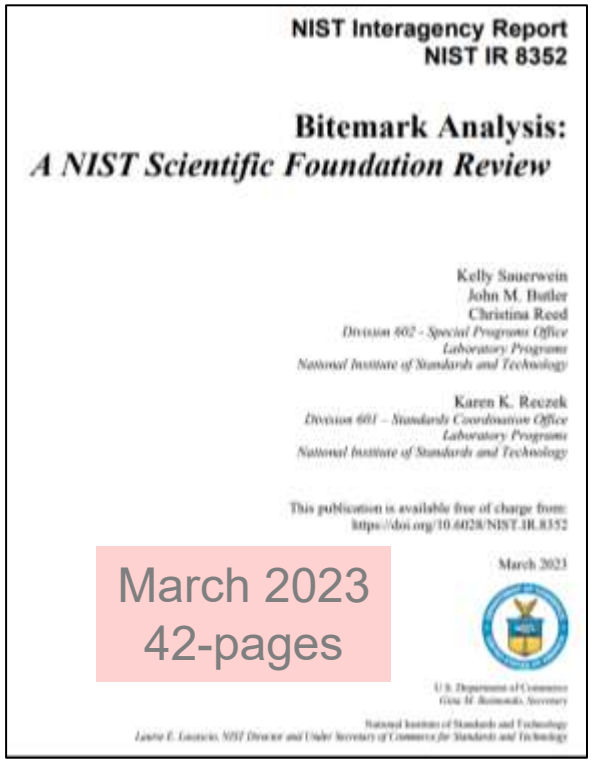
John M. Butler
Kelly Sauerwein
Christina Reed
Corrine E. Lloyd
Special Programs Office

This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8354

November 2022

November 2022
84-pages

Digital evidence examination **rests on a firm foundation based in computer science.** Extensive testing of over 250 widely used digital forensic tools showed that **most tools perform their intended functions with only minor anomalies**.


NIST Interagency Report
NIST IR 8352

Bitemark Analysis: *A NIST Scientific Foundation Review*

Kelly Sauerwein
John M. Butler
Christina Reed
Division 602 - Special Programs Office
Laboratory Programs
National Institute of Standards and Technology

Karen K. Reczek
Division 601 – Standards Coordination Office
Laboratory Programs
National Institute of Standards and Technology

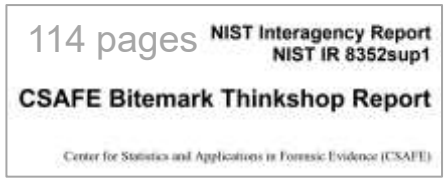
This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8352

March 2023

March 2023
42-pages

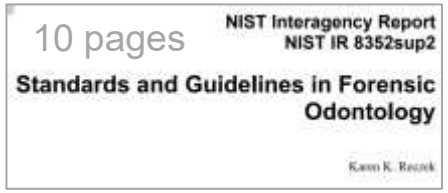
Forensic bitemark analysis **lacks a sufficient scientific foundation** because the three key premises of the field are not supported by the data.

Supplemental Documents


114 pages
NIST Interagency Report
NIST IR 8352sup1

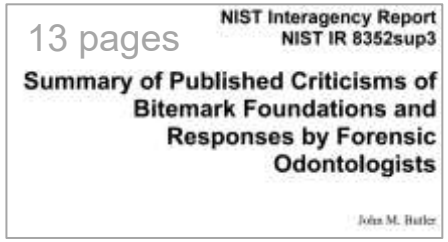
CSAFE Bitemark Thinkshop Report

Center for Statistics and Applications in Forensic Evidence (CSAFE)


10 pages
NIST Interagency Report
NIST IR 8352sup2

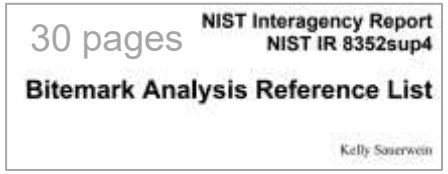
Standards and Guidelines in Forensic Odontology

Karen K. Reczek


13 pages
NIST Interagency Report
NIST IR 8352sup3

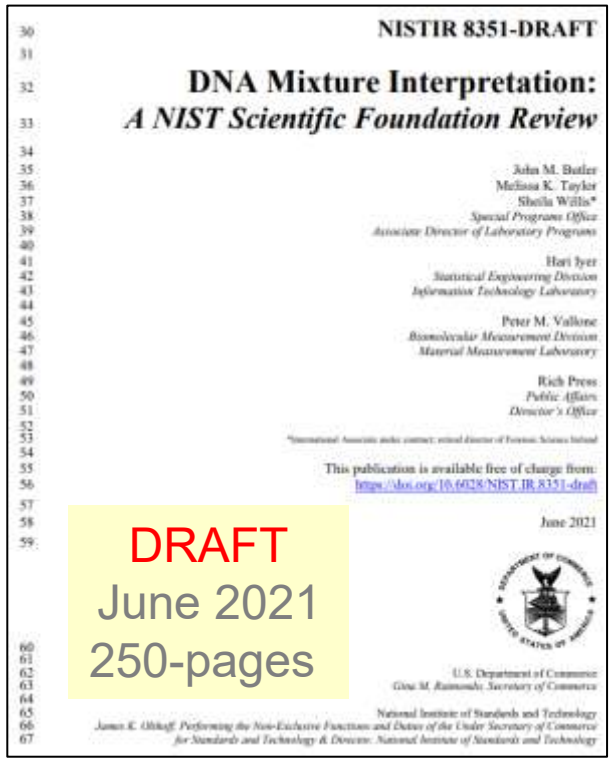
Summary of Published Criticisms of Bitemark Foundations and Responses by Forensic Odontologists

John M. Butler


30 pages
NIST Interagency Report
NIST IR 8352sup4

Bitemark Analysis Reference List

Kelly Sauerwein


NISTIR 8351-DRAFT

DNA Mixture Interpretation: *A NIST Scientific Foundation Review*

John M. Butler
Melissa K. Taylor
Sheila Willis*
Special Programs Office
Associate Director of Laboratory Programs

Hari Iyer
Statistical Engineering Division
Information Technology Laboratory

Peter M. Vallone
Biomolecular Measurement Division
Material Measurement Laboratory

Rich Press
Public Affairs
Director's Office

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

June 2021

DRAFT
June 2021
250-pages

Received extensive public comments (~500 pages) that are being considered along with additional information since June 2021. **We will release a final report when completed**.

Examining History Can Further an Understanding of a Method's Foundations

Some aspects we may want to study regarding the history of a field:

1. When were the earliest documented uses of a forensic method and practice?
2. How has this forensic method and practice progressed over its use?
3. Are there key pivot points or cases that have influenced the field?
4. What are some of the key research studies or publications?
5. Where have capabilities and limitations been documented?
6. What guidance documents have been and are currently available for measurement and interpretation approaches?
7. What documented training materials have been available over time?

History of DNA Mixture Interpretation

Adapted from Appendix 1 of NISTIR 8351-draft
"History of DNA Mixture Interpretation"

To Keep in Mind with DNA Mixtures

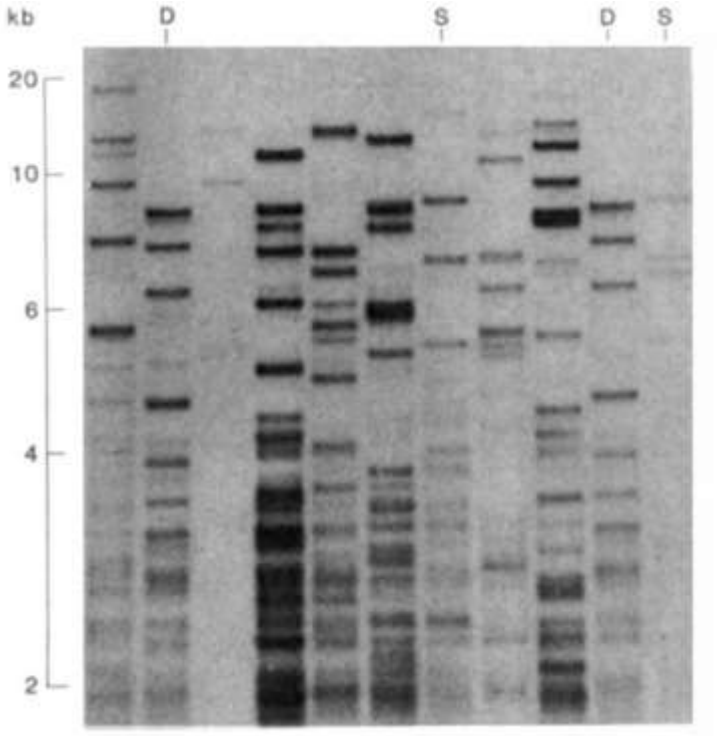
- Mixtures are inherent to many forensic DNA samples
  - For sexual assault samples, **differential extraction**, first described in 1985, can help physically separate (perpetrator) sperm cells from (victim) epithelial cells
  - Mixture **deconvolution using peak height/area information** with two-person mixtures was described in 1998

- Multiple approaches have been used for DNA mixture interpretation
  - **Likelihood ratios (LRs)** have been considered for mixtures since 1991
  - **Combined probability of inclusion (CPI)** was initially described in 1992 but only works when no alleles are missing, which is unlikely when examining low-level DNA samples that come from high-sensitivity systems used today
  - **Probabilistic genotyping software (PGS)** systems have been steadily growing in use by forensic DNA laboratories over the past decade

John M. Butler (2010) *Fundamentals of Forensic DNA Typing*, Figure 3.3

Initial "DNA Fingerprinting" with Multi-Locus Probes **Required Lots of DNA and Were Not Well-Suited for Mixtures**

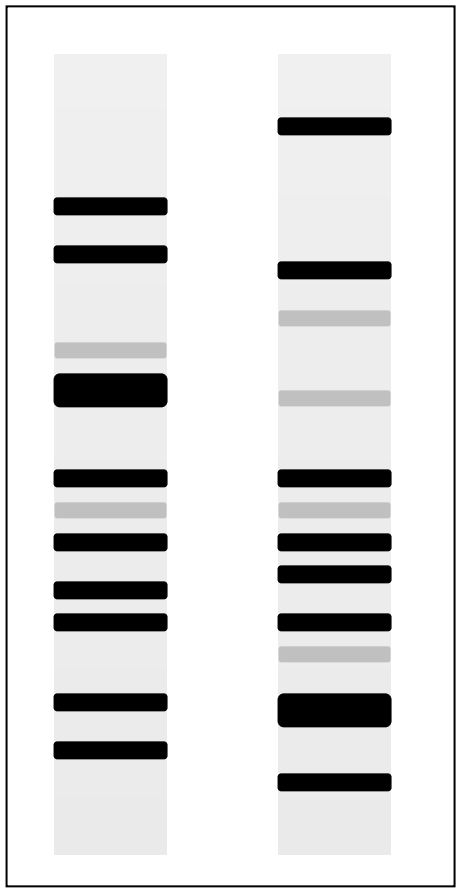
Single-source DNA profiles from 11 individuals including a duplicate (D) and two sisters (S)



Jeffreys AJ, Wilson V, Thein SL (1985) Individual-specific 'fingerprints' of human DNA. *Nature* 316:76-79.

Multi-Locus Probe

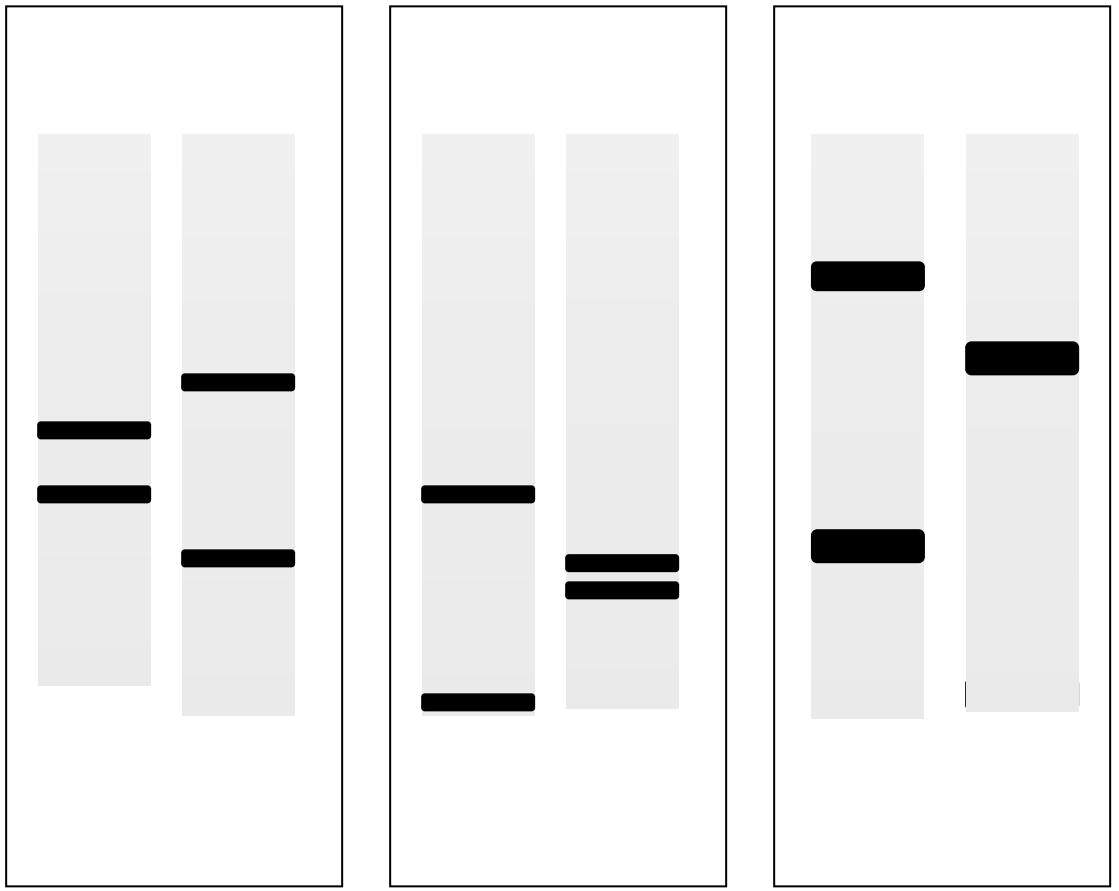
Originally developed by Alec Jeffreys



Probe 33.6

Complex patterns

Single-Locus Probe



D1S7 **D2S44** **D4S139**

Better for forensic samples containing mixtures

A Brief History of DNA Mixture Interpretation

Measurement

- RFLP-VNTR (single-locus probes)
- Early PCR (dot-blot PM+DQA1)
- **Fluorescently-labeled STRs** (20+ years)

Interpretation

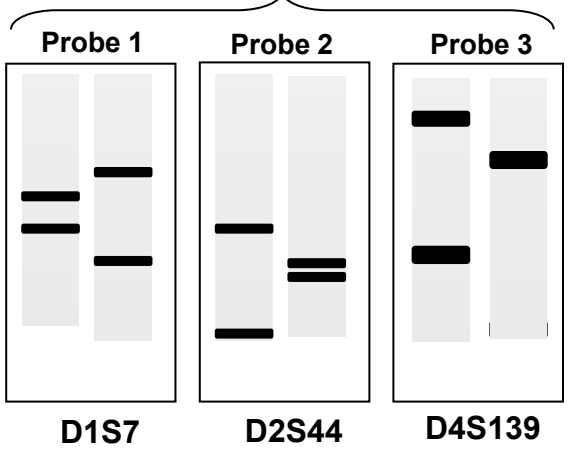
- **CPI** (common U.S. approach)
- RMP (deconvoluted major)
- LR (binary with simple mixtures)
- **PGS** (discrete & continuous models)

Key Cases

- O.J. Simpson (1995)
- Omagh bombing (2007)
- Amanda Knox (2007, 2011)
- *US v. Gissantaner* (2019)

Probabilistic Genotyping Software (PGS): theory described by Gill et al. 2000

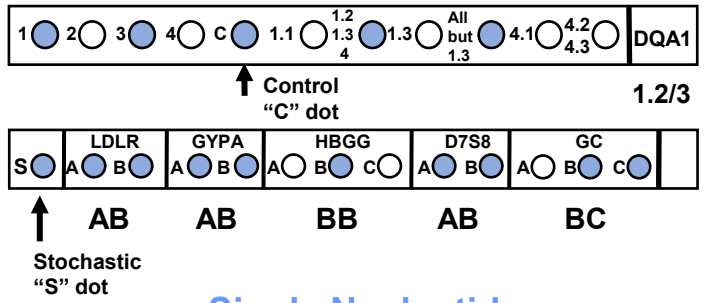
Restriction Fragment Length Polymorphisms (RFLP) Single-Locus Probes



Variable Number of Tandem Repeats (VNTRs)

Poor sensitivity (no PCR), many alleles, few loci

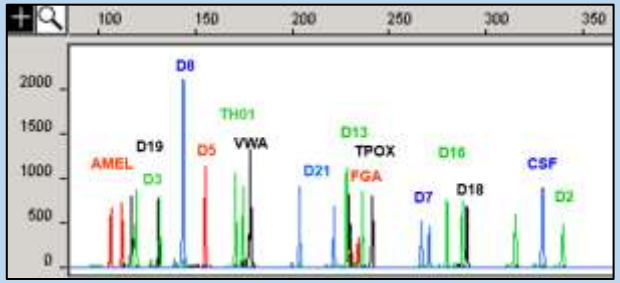
AmpliType PM + DQA1 (PolyMarker)



Single Nucleotide Polymorphisms (SNPs)

Improved sensitivity (PCR), few alleles, few loci

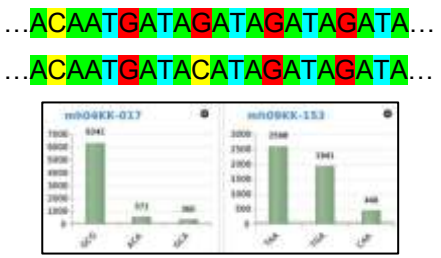
Fluorescently-Labeled, Short Tandem Repeats (STRs) with Capillary Electrophoresis (CE)



Short Tandem Repeats (STRs)

High sensitivity (PCR), many alleles, many loci

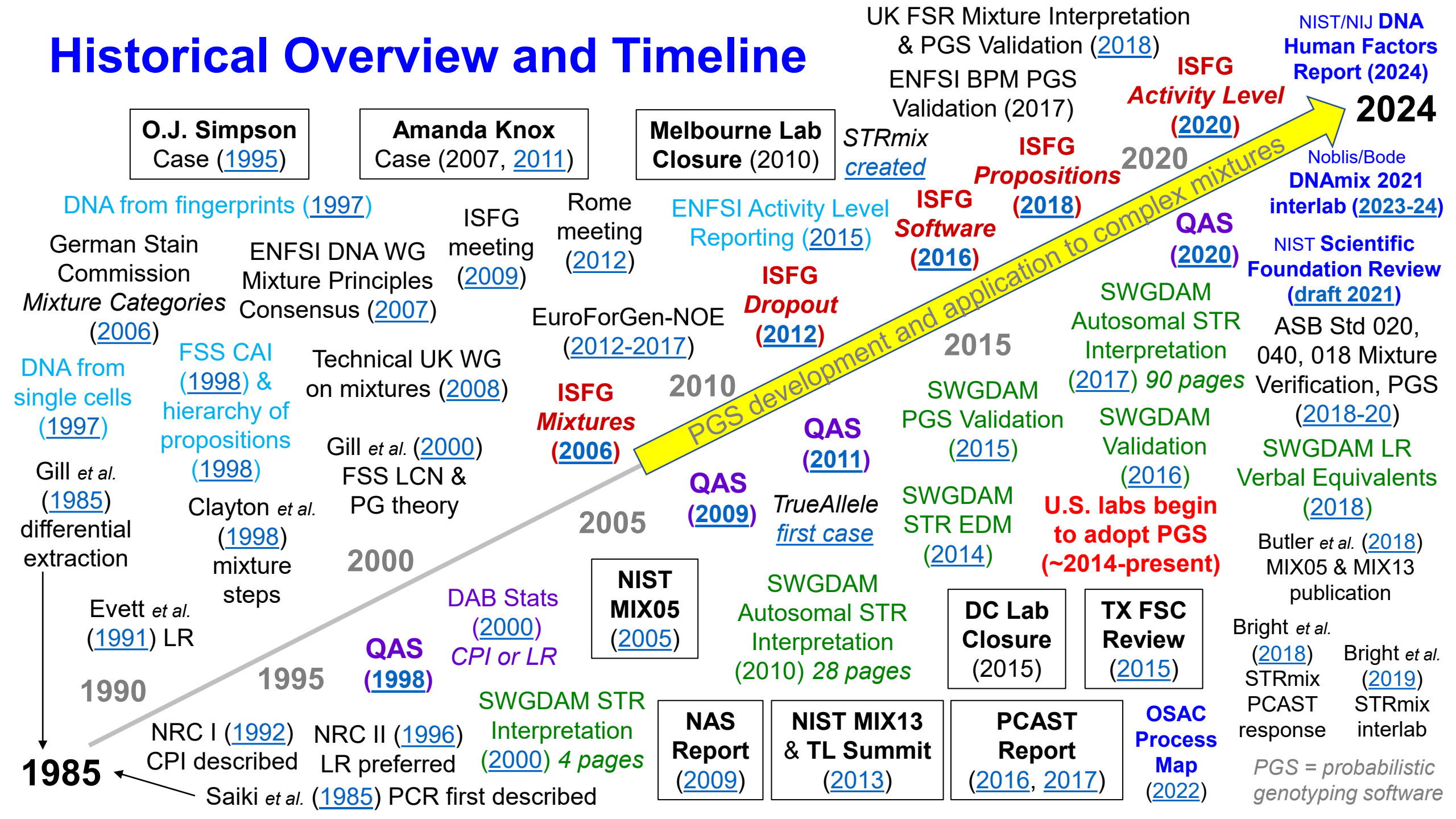
Next-Generation Sequencing (NGS); Massively Parallel Sequencing (MPS)



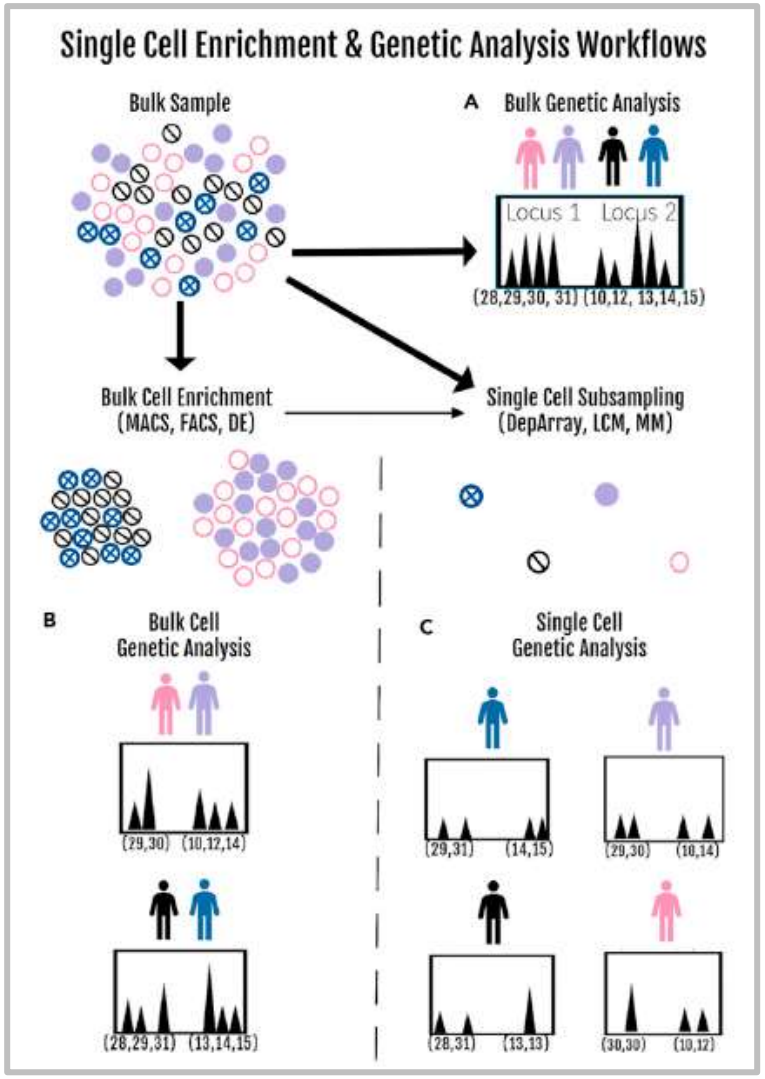
STRs, SNPs, Microhaplotypes

High sensitivity (PCR), more alleles, more loci

Historical Overview and Timeline



Looking to the Future: Single Cell Analysis in the Past Year…



Huffman & Ballantyne (2023)
https://doi.org/10.1016/j.isci.2023.107961

Single cell separation and mixture work by Grgicak et al. and Huffman & Ballantyne

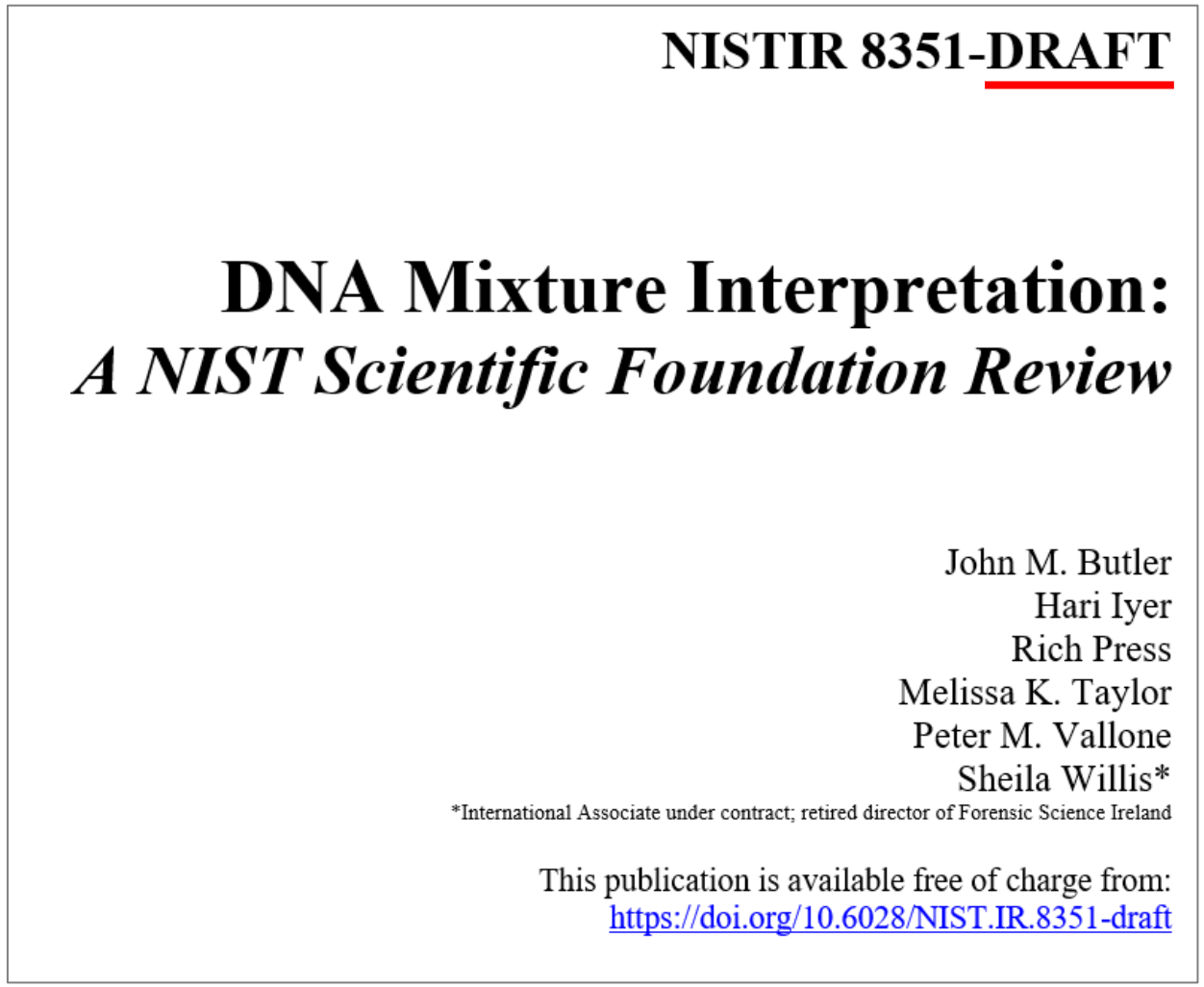
- Grgicak, C. M., Bhembe, Q., Slooten, K., Sheth, N. C., Duffy, K. R., & Lun, D. S. (2023). Single-cell investigative genetics: Single-cell data produces genotype distributions concentrated at the true genotype across all mixture complexities. *Forensic science international. Genetics*, *69*, 103000. Advance online publication. https://doi.org/10.1016/j.fsigen.2023.103000
- Duffy, K. R., Lun, D. S., Mulcahy, M. M., O'Donnell, L., Sheth, N., & Grgicak, C. M. (2023). Evidentiary evaluation of single cells renders highly informative forensic comparisons across multifarious admixtures. *Forensic science international. Genetics*, *64*, 102852. https://doi.org/10.1016/j.fsigen.2023.102852
- **Huffman, K., & Ballantyne, J. (2023). Single cell genomics applications in forensic science: Current state and future directions. *iScience*, *26*(11), 107961. https://doi.org/10.1016/j.isci.2023.107961**
- Huffman, K., Kruijver, M., Ballantyne, J., & Taylor, D. (2023). Carrying out common DNA donor analysis using DBLR™ on two or five-cell mini-mixture subsamples for improved discrimination power in complex DNA mixtures. *Forensic science international. Genetics*, *66*, 102908. https://doi.org/10.1016/j.fsigen.2023.102908
- Huffman, K., & Ballantyne, J. (2023). Validation of Probabilistic Genotyping Software for Single Cell STR Analysis. *Genes*, *14*(3), 674. https://doi.org/10.3390/genes14030674

See Chapter 6 of NISTIR 8351 for other methods (e.g., sequencing and microhaplotypes)

Foundation Study

DNA Mixture Interpretation

NIST Draft Report Released in June 2021



NISTIR 8351-DRAFT

DNA Mixture Interpretation:
A NIST Scientific Foundation Review

John M. Butler
Hari Iyer
Rich Press
Melissa K. Taylor
Peter M. Vallone
Sheila Willis*
*International Associate under contract; retired director of Forensic Science Ireland

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

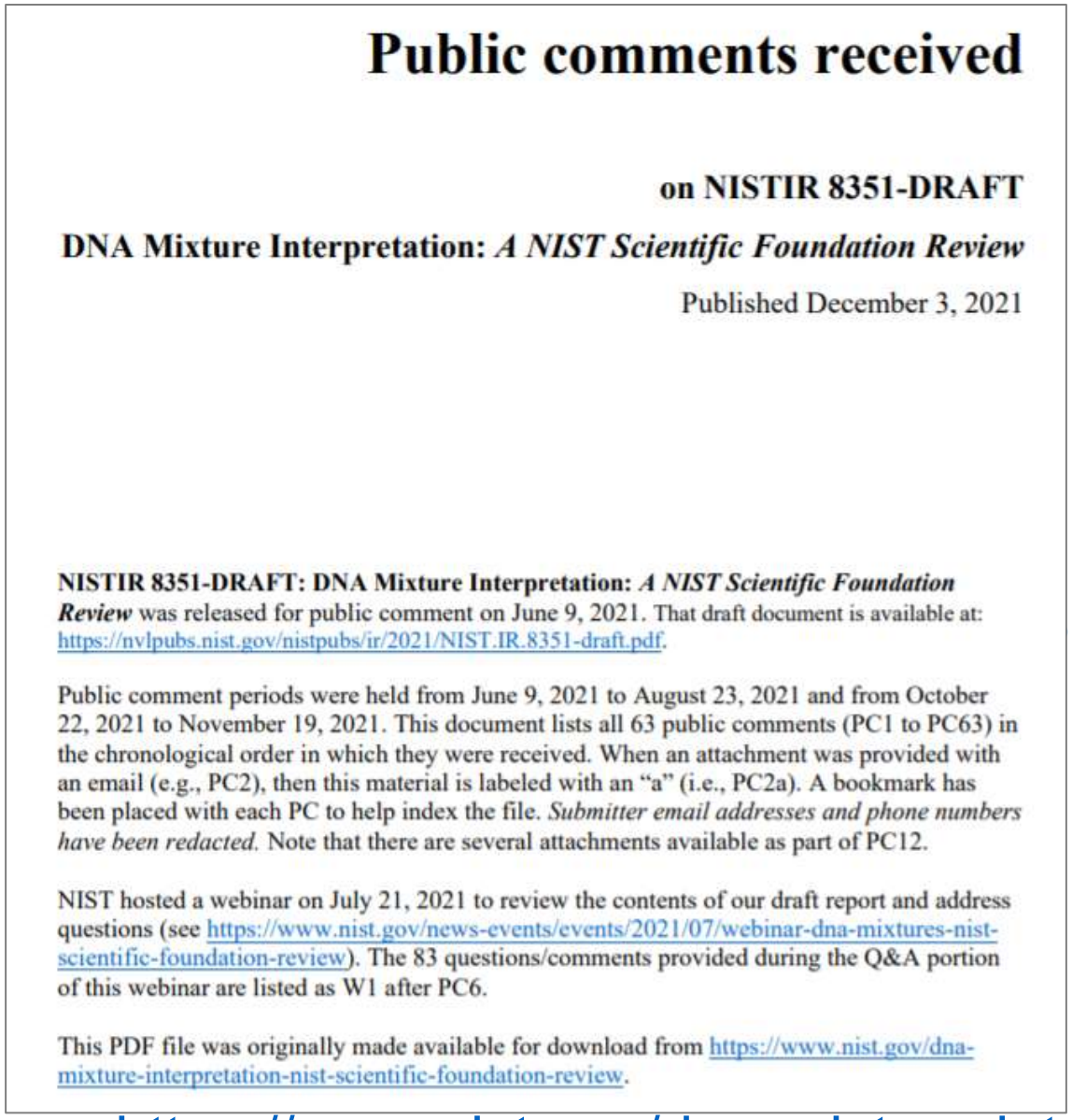
16 principles described

25 key takeaways

8 future considerations

Collected public comments on this draft report (June to November 2021)

Public Comments Received on Our Draft DNA Report



Public comments received

on NISTIR 8351-DRAFT

DNA Mixture Interpretation: *A NIST Scientific Foundation Review*

Published December 3, 2021

NISTIR 8351-DRAFT: DNA Mixture Interpretation: *A NIST Scientific Foundation Review* was released for public comment on June 9, 2021. That draft document is available at: https://nvlpubs.nist.gov/nistpubs/ir/2021/NIST.IR.8351-draft.pdf.

Public comment periods were held from June 9, 2021 to August 23, 2021 and from October 22, 2021 to November 19, 2021. This document lists all 63 public comments (PC1 to PC63) in the chronological order in which they were received. When an attachment was provided with an email (e.g., PC2), then this material is labeled with an "a" (i.e., PC2a). A bookmark has been placed with each PC to help index the file. *Submitter email addresses and phone numbers have been redacted.* Note that there are several attachments available as part of PC12.

NIST hosted a webinar on July 21, 2021 to review the contents of our draft report and address questions (see https://www.nist.gov/news-events/events/2021/07/webinar-dna-mixtures-nist-scientific-foundation-review). The 83 questions/comments provided during the Q&A portion of this webinar are listed as W1 after PC6.

This PDF file was originally made available for download from https://www.nist.gov/dna-mixture-interpretation-nist-scientific-foundation-review.

- We are extremely grateful for the detailed feedback provided during our public comment periods and acknowledge the significant time and effort of those who carefully read and provided valuable written feedback on our draft report
- **We are carefully considering each comment as part of the NIST process to finalize this report and working to clarify language regarding data**
- A final report will be issued when we have completed this process

https://www.nist.gov/dna-mixture-interpretation-nist-scientific-foundation-review

Some High-Level Revisions Planned in Our Final Report

1. **Additions to preface** thanking public commenters and providing overview of primary changes
2. **Rewording of most key takeaways** to improve clarity, removing several, and **adding several new key takeaways**
3. **Restructuring (reordering information) in Chapters 4 and 5**
4. **Addition of new information** either from public comment or publications since the draft version was released in June 2021
5. **Separating the two appendices** into a single supplemental document on history of DNA mixture interpretation and removing the training/continuing education information

Two Appendices in Draft Report → One Supplemental Document

DRAFT 8351 – Appendix 1

NIST Interagency Report
NIST IR 8351sup1

History of DNA Mixture Interpretation

John M. Butler

This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8351sup1

3 Key Takeaways

NIST NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY U.S. DEPARTMENT OF COMMERCE

DRAFT 8351 – Appendix 2

NIST Interagency Report
NIST IR 8351sup2

Training and Continuing Education for DNA Mixture Interpretation

John M. Butler

This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8351sup2

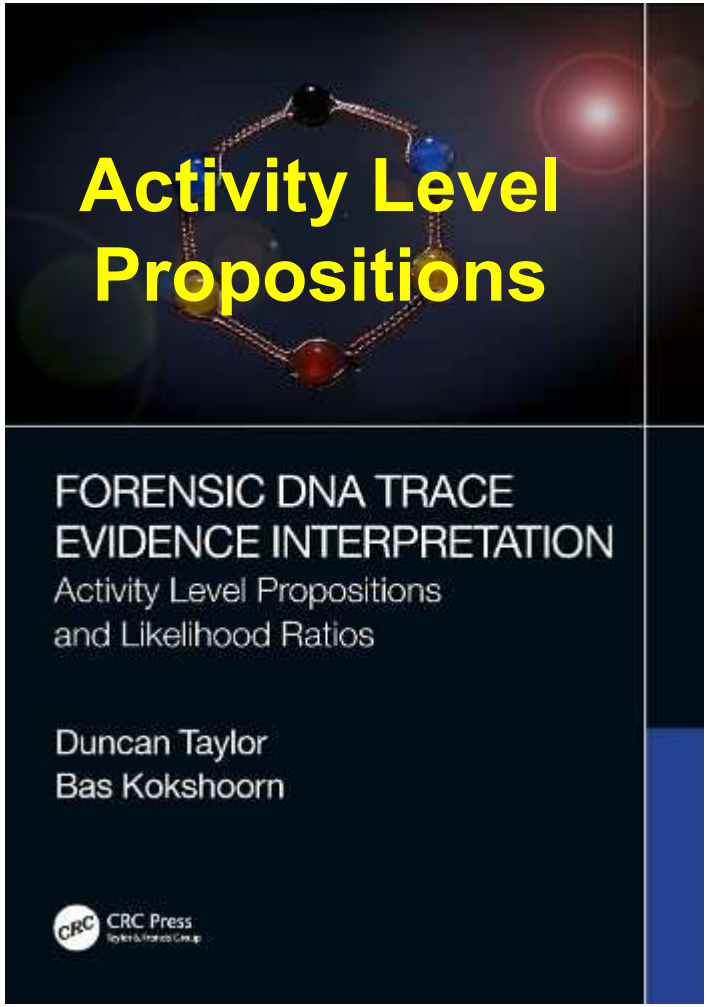
8 Future Considerations

Well-aligned with Chapter 9 in the forthcoming DNA Human Factors report "Education, Training, and Professional Credentialing"

NIST NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY U.S. DEPARTMENT OF COMMERCE

Why is Chapter 5 in Our Report?

566-page book



CRC Press (May 2023)

- When considering reliability of the entire DNA mixture interpretation process (including judgments from the analyst prior to using any software), it is important to understand and consider the assumptions around **DNA transfer, persistence, prevalence, and recovery (TPPR)**
- If this report had focused solely on likelihood ratios with sub-source level propositions assigned by probabilistic genotyping software systems, without considering questions of TPPR, then the resulting assessment would have been incomplete
- Chapter 5 provides a discussion of DNA TPPR

This topic is also addressed in Chapter 7 of the forthcoming DNA Human Factors report

Publicly Accessible Data for Independent Review

Foundational scientific data should be *publicly accessible for independent review* **so that interested parties can judge for themselves** the efficacy of the underpinning information.

- Several forensic laboratories invited us in their public comments to privately review their validation studies. We do not believe that this would be a useful exercise in the context of our efforts. **NIST evaluation of validation information would only move the needle from "trust the laboratories" to "trust the NIST authors" (who have looked at the laboratory data) rather than allowing the available and accessible data to be independently reviewed by other parties.**

New Journal Requirements for Open-Access Data

Forensic Science International: Genetics 65 (2023) 102877

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Editorial

Editorial considerations for publication in *Forensic Science International: Genetics*

Kayser et al. (2023) *FSI Genetics* 65: 102877

Published in April 2023

https://doi.org/10.1016/j.fsigen.2023.102877

"As in any field of science, **scientific publication** in forensic genetics and genomics **is an important corner stone in the advancement of forensic science**, technology and their practical applications in forensic casework…"

Check list for editors and peer-reviewers to follow

1) Is the work substantive and does it show high quality and impact as well as a significant amount of novelty to be published in the Journal? Does the work represent a step change to be published in the Journal? Or is it incremental, recycling existing ideas with minor benefit, which is not in line with the Journal's aim?
2) Incremental papers, which build upon existing concepts and simply update details or explain differences in approach, are not typically suitable for publication by the Journal, at least not as an original research article. Such manuscripts may be appropriate as Short Communication or Correspondence papers as long as they advance the field significantly enough; otherwise they are not line with the Journal's aim.
3) Are the conclusions supported by a comprehensive set of open-access data and/or open-source software and/or other documentation to ensure that the work is accessible and useful to the broader community to be published in the Journal?
4) Are the research findings presented in a clear and organized manner without using excessive technical jargon allowing other researchers and practitioners to easily understand the content including all used methods?

New DNA Material Available to Assist with Open-Access Data

Forensic DNA Research Grade Test Material: RGTM 10235

Samples were collected and prepared by the NIST Applied Genetics Group
under explicit informed consent allowing for public sharing of genetic data

Sample	Format	Sex	Carrier**	Concentration*** (ng/µL)
Sample 1	Single source A	M	Y	5.1 ± 0.4
Sample 2	Single source A - degraded*	M	Y	3.6 ± 0.3
Sample 3	Single source B	F	N	4.9 ± 0.4
Sample 4	Single source B - degraded*	F	N	3.0 ± 0.2
Sample 5	Single source C	M	Y	5.1 ± 0.4
Sample 6	2p mixture D+E	F:M - 90:10	Y	4.5 ± 0.4
Sample 7	3p mixture D+F+G	F:M:M - 20:20:60	Y	4.3 ± 0.3
Sample 8	3p mixture H+G+E	F:M:M - 10:30:60	Y	4.3 ± 0.4

*Degraded with UV light for 5 minutes

**Samples with carrier contain 50 ng/µL of yeast tRNA to improve nucleic acid stability

***As measured by digital PCR (dPCR) **DOI:**10.6028/NIST.SP.260-189
https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-189.pdf

A collaborative effort with the community to examine fit-for-purpose methods for developing future SRMs

- **Will assist in addressing public sharing/consent issues**
- **Contains 2p and 3p mixture samples**
- **Submitters are anonymous and data are not being used to assess mixture issues**

Contact: ForensicRGTM@nist.gov

NIST APPLIED GENETICS

Erica Romsos
Pete Vallone

https://strbase.nist.gov/Information/RGTM_10235

Steps in DNA Analysis and Interpretation

stain

swab

Gathering the Data

Understanding Results Obtained

Collection/Storage/ Characterization → Extraction/ Quantitation → Amplification/ Marker Sets → Separation/ Detection → Data → Stats → Report → **result**

Interpretation

Probabilistic Genotyping Software (PGS)

https://cts-forensics.com/program-1.php

Publicly Accessible Proficiency Test Data

Start with stains → CTS **Forensic Biology** (6 data sets per year) — usually 2p mixtures

Start with stains → CTS **DNA Semen** (2 data sets per year) — usually 2p mixtures

Start with stains → CTS **DNA Mixture** (2 data sets per year) — usually 2p mixtures

Each data set includes two evidence items (#3,#4) with two reference items (#1,#2)

Graded by appropriate inclusion or exclusion of Item #1 or Item #2 with Item #3 and Item #4

Start with data files; analyst inputs & approaches can vary → CTS **DNA Interpretation** (2 data sets per year) — **Begun in 2013** — 2p, 3p mixtures

Samples are prepared by **mixing human whole blood and/or semen** in specific ratios and spotting on cloth or paper

Start with stains → CTS **Probabilistic Genotyping** (2 data sets per year) — **Begun in 2022** — 2p, 3p mixtures

New CTS Probabilistic Genotyping Proficiency Tests

https://cts-forensics.com/reports/22-5904.5_Web.pdf

	Victim (Item 1)		Suspect (Item 2)	
	Item 3	Item 4	Item 3	Item 4
Yes	**20**	**19**	**20**	**0**
No	**0**	**1** False negative	**0**	**20**
Inc	**0**	**0**	**0**	**0**
No Interpretation	**0**	**0**	**0**	**0**
No Response	**0**	**0**	**0**	**0**

20 participants

https://cts-forensics.com/reports/23-5901.2_Web.pdf

	Victim (Item 1)		Suspect (Item 2)	
	Item 3	Item 4	Item 3	Item 4
Yes	**48**	**46**	**0**	**48**
No	**0**	**2** False negatives	**48**	**0**
Inc	**0**	**0**	**0**	**0**
No Interpretation	**0**	**0**	**0**	**0**
No Response	**0**	**0**	**0**	**0**

48 participants

https://cts-forensics.com/reports/23-5904.5_Web.pdf

	Victim (Item 1)		Suspect (Item 2)	
	Item 3	Item 4	Item 3	Item 4
Yes	**72**	**72**	**0**	**72**
No	**0**	**0**	**72**	**0**
Inc	**0**	**0**	**0**	**0**
No Interpretation	**0**	**0**	**0**	**0**
No Response	**0**	**0**	**0**	**0**

72 participants

- Tests include a three-person mixture with a 3:2:1 mixture ratio and a two-person mixture with 1:1, 2:1, or 3:2 mixture ratios
- **A range of LR values were reported with a variety of formats and details** (perhaps offering an opportunity to improve reporting formats and standardize information shared?)

New CTS Probabilistic Genotyping Proficiency Tests

While proficiency test success is graded on proper inclusion or exclusion of the items compared, reported LR values are provided and can be assessed

CTS PGS Proficiency Test	Number of Participants	NOC and Mixture Ratio	Range of Reported LRs (summarized from Tables 7 & 8) ban = an order of magnitude
22-5904/5	20	Item 3: **3p** (2:3:1) Item 4: 2p (2:1)	LR (S) Item 3: 10^{7} to 10^{42} = **35 ban** Item 4: *(suspect not included)*
23-5901/2	48	Item 3: 2p (2:1) Item 4: **3p** (1:3:2)	LR (V) Item 3: 10^{10} to 10^{33} = **23 ban** LR (S) Item 4: 10^{9} to 10^{36} = **27 ban**
23-5904/5	72	Item 3: 2p (1:1) Item 4: 2p (3:2)	LR (V) Item 3: 10^{7} to 10^{22} = **15 ban** LR (S) Item 4: 10^{9} to 10^{29} = **20 ban**

https://cts-forensics.com/reports/22-5904.5_Web.pdf
https://cts-forensics.com/reports/23-5901.2_Web.pdf
https://cts-forensics.com/reports/23-5904.5_Web.pdf

2p = 2-person
3p = 3-person

High quantity and quality samples with little-to-no allele drop-out

Variation may arise from different STR kits, propositions, PGS models, pop. databases (allele frequencies), or reporting policies

https://cts-forensics.com/reports/22-5882_Web.pdf

A Recent DNA Interpretation Proficiency Test

CTS DNA Interpretation Test 22-5882

(posted online: January 30, 2023)

Item 4

	Item 1		Item 2
Included	**0**	correct →	**35**
Excluded	**35**		**0**
Inconclusive	**0**		**0**
No Response	**0**		**0**
Total	**35**		**35**

- **2-person mixture (3:1)** EPG assessed
- Item 1 was not included
- **Item 2 was included** (as the minor contributor)
- These 35 participants provide **correct responses in terms of exclusions and inclusions** and report no inconclusives
- But the **LR values** assigned for the same data **varied** significantly…

25 of 35 (other 10 did not report an LR value)

https://cts-forensics.com/reports/22-5882_Web.pdf

Participant ID (WebCode)	Item 4: Item 1	Item 4: Item 2	LR Value (3:1)	NOC	AT Used (RFUs)	ST Used (RFUs)	STR Kit	PGS	Database
BLHE2K	Excluded	Included	>1000000 (1.00E+6) *actual LR upon request*	2	--	--	GlobalFiler, Investigator 24plex, Fusion 5C, Fusion 6C, Identifiler Plus	LabRetriever 2.2.1	NIST 1036
YCQWHU	Excluded	Included	1.00E+09	2	--	--	GlobalFiler, Investigator 24plex, Fusion 5C, Fusion 6C, Identifiler Plus	STRmix	GlobalFiler Caucasian
KT4P4C	Excluded	Included	1.00E+11	2	75	100	GlobalFiler	STRmix	FBI Caucasian
X2DFBX	Excluded	Included	3.90E+12	2	75	75	Fusion 5C	--	NIST
6RNWXQ	Excluded	Included	4.56E+12	2	50	100	GlobalFiler, Fusion 6C	--	NIST 1036
BQFTU2	Excluded	Included	1.06E+14	2	100	600	Investigator 24plex	--	PopStats
4DQJ7A	Excluded	Included	1.39E+14	3	50	560	GlobalFiler	LRmix Studio	STRidER
9J36YN	Excluded	Included	3.80E+16	2	100	100	GlobalFiler	LRmix Studio	NIST 1036
W2KX3H	Excluded	Included	1.40E+17	2	100	300	GlobalFiler	--	[Local]
9DEFL6	Excluded	Included	1.98E+17	2	75	100	GlobalFiler	--	[Local]
66QMEN	Excluded	Included	7.10E+17	2	--	--	Fusion 5C	LR Mix 2.1.3	STRidER
7XAACJ	Excluded	Included	1.20E+18	2	--	600	GlobalFiler	--	STRidER
EP8REK	Excluded	Included	1.90E+18	2	75	100	GlobalFiler	LRmix Studio	STRidER
6EEGQL	Excluded	Included	3.10E+18	2	185	500	--	LRmix Studio	ABI Pop
4LKNYB	Excluded	Included	3.80E+21	2	120	360	GlobalFiler	--	[Local]
DBAEM4	Excluded	Included	3.80E+21	2	120	360	GlobalFiler	--	[Local]
VEQULK	Excluded	Included	3.80E+21	2	120	360	GlobalFiler	--	[Local]
8LAYM9	Excluded	Included	4.50E+25	2	75	100	--	--	[Local]
FBLVU2	Excluded	Included	4.90E+25	2	150	300	GlobalFiler, Investigator 24plex, Fusion 5C, Fusion 6C, Identifiler Plus	--	[Local]
PPUVJN	Excluded	Included	3.30E+26	2	75	100	--	STRmix	FBI Afr Am
4RABNN	Excluded	Included	4.60E+28	2	--	--	GlobalFiler	BP Sentry	NIST General
GQTFPB	Excluded	Included	4.60E+28	2	50	n/a for PGS	GlobalFiler	BP Sentry	NIST General
ETP62T	Excluded	Included	4.98E+29	3	75	230	Fusion 6C	STRmix	*none reported*
JFPX9T	Excluded	Included	8.50E+32	2	125 to 345	430 to 820	Fusion 6C	STRmix	[Local]
EKWWF3	Excluded	Included	1.00E+53	2	--	--	GlobalFiler, Investigator 24plex, Fusion 5C, Fusion 6C, Identifiler Plus	--	[Local]

All correctly included Item 2 (suspect)

However, the reported LR values ranged over 47-orders of magnitude! 10^6 to 10^{53}

https://cts-forensics.com/reports/22-5882_Web.pdf

With a Different Item in the Same Proficiency Test

CTS DNA Interpretation Test 22-5882

(posted online: January 30, 2023)

	Item 3		
	Item 1		Item 2
Included	**0**	correct	**11**
Excluded	**30**		**13**
Inconclusive	**5**		**11**
No Response	**0**		**0**
Total	**35**		**35**

- **3-person mixture (3:1:1)** EPG assessed
- Item 1 was not included
- **Item 2 was included** (as a trace minor contributor)
- **Consensus mixture profile was missing 17 alleles** due to allele drop-out → *more like low-level DNA casework!*
- These same 35 participants provide **a correct response for Item 2 only 11/35 (31%)**

Steps in DNA Analysis and Interpretation

stain

swab

Gathering the Data

Understanding Results Obtained

Collection/Storage/ Characterization → Extraction/ Quantitation → Amplification/ Marker Sets → Separation/ Detection → Data → Stats → Report → **result**

Interpretation

Probabilistic Genotyping Software (PGS)

Inter-laboratory and Intra-laboratory Studies

Started with stains — NIST Mixed Stain Study #1 (1997) and #2 (1999) — 1p, 2p, 3p mixtures

Started with DNA extracts — NIST Mixed Stain Study #3 (2001) — 1p, 2p, 3p mixtures

Started with data files; analyst inputs & approaches varied — NIST MIX05 (2005) — 2p mixtures

NIST MIX13 (2013) — 2p, 3p, 4p mixtures

Other interlab studies: GHEP-ISFG (2010, 2011, 2012, 2014, 2015), EuroForGen (2013), DFSC (2014-15), NFI (2016), UK Regulator (2014), AFSP (2018), STRmix (2014)

Started with common PGS, fixed inputs (same AT) — STRmix (2018) — 3p, 4p mixtures

Started with data files; analyst inputs & approaches varied — DNAmix (2021/2022) — 2p, 3p, 4p, 5p, 6p mixtures

Ideally, **we would like to characterize uncertainty for the entire system** (swab-to-result), but most **interlaboratory studies only provide a partial picture of the variability** because of the difficulty of generating and providing consistent mixture samples

DNAmix 2021 study

(1) independent assessment
(2) collaborative design
(3) range of factors explored

A Comparison of These Two NIST DNA Reports

Scientific Foundation

- DNA Mixture Interpretation
- NIST Effort
  - Technical merit evaluations requested and funded by Congress
- Written by NIST authors with early input from a Resource Group
- Draft version released for public comment before finalizing
- ~180 pages + 2 supplementals (history, summary of public info)
- Issues actionable proposals to strengthen the field

Accessible Data Summarized

Human Factors

- DNA Interpretation
- Joint NIJ/NIST Effort
  - Builds on previous human factors with fingerprints and handwriting analysis
- Written by diverse committee with later input from external reviewers
- To be released as a final version when complete
- ~350 pages (12 chapters covering wide range of issues)
- Issues actionable proposals to strengthen the field

Process Map Created

Future Plans (FY2024 and beyond)

- ✓ Finalize NISTIR 8354 (**Digital Investigation Techniques**) using comments received
- ✓ Finalize NISTIR 8352 (**Bitemark Analysis**) using comments received
- Finalize NISTIR 8351 (**DNA Mixture Interpretation**) using comments received
- Complete NISTIR 8353-draft report (**Firearm Examination**) and hold public comment

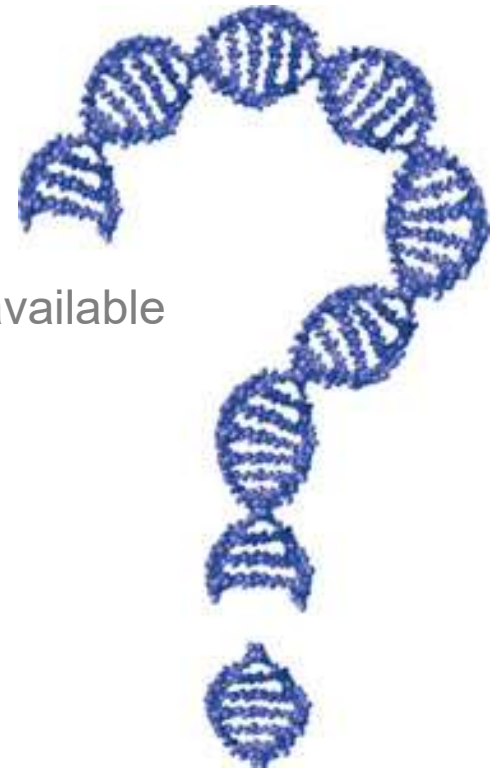
- Conduct **Footwear & Tire** foundation study (held IAI workshop: August 25, 2023)
- Begin a cross-cutting study on **Communicating Findings with Likelihood Ratios** (planning workshop for June 2024)
- Assist with NIST validation and data efforts

We welcome your input on future foundation studies

→ contact John Butler, Sandra Koch, or Kelly Sauerwein

Thank you for your attention!

Acknowledgments:
- Congressional funding to NIST for technical merit evaluations
- Report co-authors: Hari Iyer, Rich Press, Melissa Taylor, Pete Vallone, Sheila Willis
- Input from others at NIST including Sarah Riman and Kathy Sharpless
- CTS (Cassandra Swart & Cathy Barden) for making proficiency test information publicly available



John Butler
john.butler@nist.gov

https://www.nist.gov/spo/forensic-science-program

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

Questions?

Feel free to email me anytime