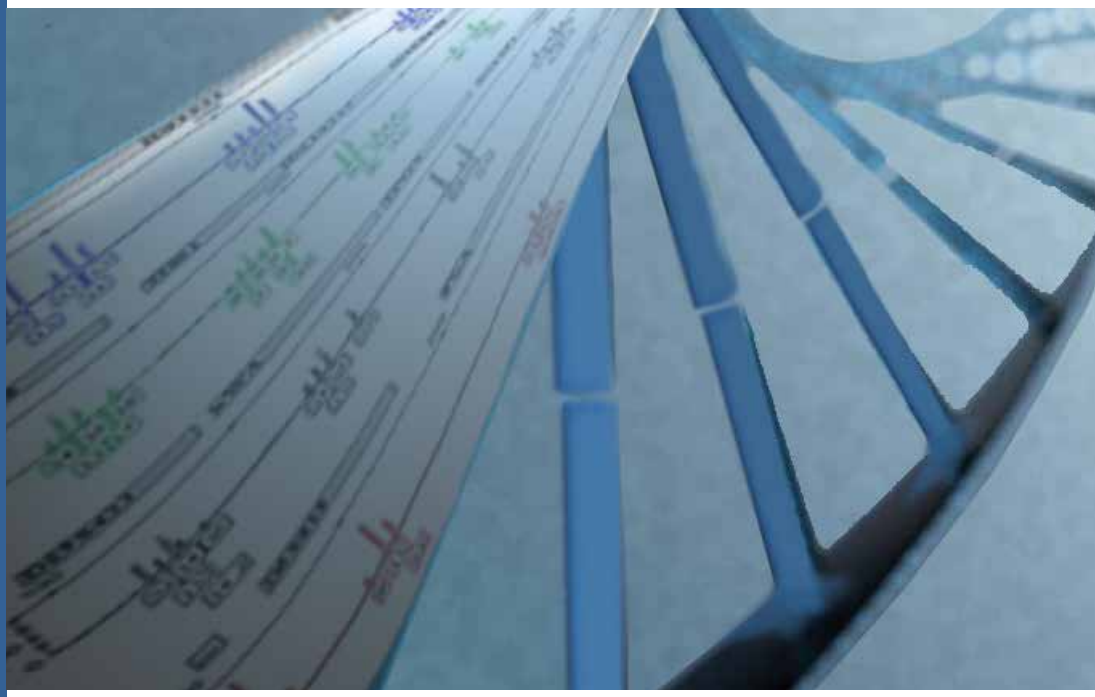
FTCOE
Forensic Technology Center Of Excellence

Landscape Study of **DNA Mixture Interpretation Software**



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Forensic Technology Center of Excellence (FTCoE)

The FTCoE is a collaborative partnership of RTI International and its FEPAC [Forensic Science Education Programs Accreditation Commission]–accredited academic partners: Duquesne University, Virginia Commonwealth University, and the University of North Texas Health Science Center. In addition to supporting the National Institute of Justice's (NIJ's) research and development (R&D) programs, the FTCoE provides testing, and evaluation, and technology assistance to forensic laboratories and practitioners in the criminal justice community. NIJ funds the FTCoE to transition forensic science and technology to practice (Award Number 2011-DN-BX-K564).

The FTCoE is led by RTI International, a global research institute dedicated to improving the human condition by turning knowledge into practice. With a staff of more than 3,700 providing research and technical services to governments and businesses in more than 75 countries, RTI brings a global perspective. The FTCoE builds on RTI's expertise in forensic science, innovation, technology application, economics, DNA analytics, statistics, program evaluation, public health, and information science.

The information shared in this report represents the opinions of the individual practitioners and researchers who participated in this FTCoE project and not the opinions of their agencies or the National Institute of Justice. For more information or questions about this report, visit www.forensiccoe.org, or contact Jeri Ropero-Miller at jerimiller@rti.org or 919-485-5685.

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DISCLAIMER

Information provided herein is intended to be objective and is based on data collected during primary and secondary research efforts available at the time this report was written. Any perceived value judgments may be based on the merits of software features and developer services as they apply to and benefit the law enforcement and forensic communities. The information provided herein is intended to provide a snapshot of current DNA mixture interpretation software developers and a high-level summary of available tools; it is not intended as an exhaustive product summary. Features or capabilities of additional tools or developers identified outside of this landscape may be compared with these tool features and service offerings to aid in the information-gathering or decision-making processes. Experts, stakeholders, and practitioners offered insight related to the use of DNA mixture interpretation software for crime laboratories.

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Commonly used words and phrases

For the purpose of this document the following are defined:

Combined DNA Index System (CODIS): The Federal Bureau of Investigation's program to support criminal justice DNA databases and the software used to run these databases. (https://www.fbi.gov/about-us/lab/biometric-analysis/codis)

Combined Probability of Inclusion (CPI): Assigns equal weight to all genotype possibilities based on the observed alleles and states the fraction of the population that would be included as a possible contributor to a mixture.

Combined Probability of Exclusion (CPE): Calculated as 1 – CPI and states the fraction of the population that would be excluded as a possible contributor to a mixture.

Common Message Format (CMF): Specific file format that includes information about DNA samples to allow for the exchange of data between CODIS and external systems.

Likelihood Ratio (LR): Ratio of probabilities (Pr) for the evidence given two different hypotheses; for example, the prosecution hypothesis (Hp) compared to the defense hypothesis (Hd).

$$LR = \frac{\Pr(E\ H_p)}{\Pr(E\ H_d)}$$

Low Template DNA (LtDNA) or Low Copy Number (LCN): Small amount of total DNA present in the testing process; typically less than 100 pg.

Markov Chain Monte Carlo (MCMC): Mathematical method used to model the data to assess genotypes as more or less probable based on probability distributions.

Mixture: Sample consisting of DNA from more than one individual.

Mixture interpretation software tool: Software that conducts complicated statistical calculations associated with complex mixtures and, in some cases, assists with the deconvolution of complex mixtures.

Modified Random Match Probability (mRMP): Use of the RMP statistic after deconvolution of major and minor components within a mixture.

Probabilistic genotyping: Addresses genotype uncertainty by using probabilistic models to infer genotypes and calculate LRs.

Probabilistic genotyping, Continuous: Uses allele and peak height information while incorporating biological parameters such as peak height ratios, mixture ratios, and stutter percentages.

Probabilistic genotyping, Semi-Continuous: Uses the allele and peak height information without considering biological parameters such as peak height ratios, mixture ratios, and stutter percentages.

Random Match Probability (RMP): Statistic representing the frequency of a DNA profile and the chance of a random match between two DNA profiles.

Scientific Working Group for DNA Analysis Methods (SWGDAM): A forum to discuss, share, and evaluate forensic biology methods, protocols, training, and research to enhance forensic biology services, as well as to provide recommendations to the FBI Director on quality assurance standards for forensic DNA analysis. (http://www.swgdam.org)

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Overview

This effort was directed by the National Institute of Justice's (NIJ's) Forensic Technology Center of Excellence (FTCoE) at RTI International with support from Duquesne University and input from law enforcement, crime laboratories, and practitioners in the criminal justice community.

A landscape study, in concept, is designed to provide a comprehensive list of market participants, their products, and product features to enable better informed decisions by end users. This report provides a "landscape" view of currently available DNA mixture interpretation software tools, and factors impacting their implementation and use. The document is intended to provide forensic laboratory directors, practitioners, and stakeholders with a survey of commercial and open-source software. Specifically, the report provides decision makers and potential end users with the following:

- Exemplary situations that illustrate successful adoption
- Issues to consider related to implementation of DNA mixture interpretation software tools
- Comparison of the capabilities of available DNA mixture interpretation software tools

The report is designed to provide the reader with a basic understanding of DNA mixture interpretation software tools, as well as their use, benefits, and limitations. The document provides a summary of considerations that will impact procurement, training, and validation.

Please Note: This report is a good-faith effort by the FTCoE to accurately represent information available via primary and secondary sources at the time of the analysis. Where appropriate, RTI has sourced the primary research with individual sources, and similarly, key secondary sources are identified. All other information is a composite view developed from literature, trade press, and stakeholder input. This report is funded through a Cooperative Agreement (2011-DN-BX-K564) from NIJ, the research, development, and evaluation agency of the U.S. Department of Justice. The views, policies, and opinions expressed are those of the authors and contributors and do not necessarily reflect those of the U.S. Department of Justice.

DNA Mixture Interpretation Software Tools

DNA mixture interpretation software tools are available to purchase from several vendors or as free open-source downloads. This report explores software features, validation considerations, technical support, and training options to provide a basic overview that will assist crime laboratories in the evaluation process to choose the software tool that best meets their needs.

Purpose

The National Institute of Justice's (NIJ's) Forensic Technology Center of Excellence (FTCoE) at RTI International researched the adoption criteria, use, impact, and availability of DNA mixture interpretation software for crime laboratory and forensic investigation applications. The following factors led the FTCoE to conduct a landscape study of DNA mixture interpretation software:

- A growing number of crime laboratories recognize the benefits of adopting DNA mixture interpretation software that assists with the challenges of complex mixture interpretation and provides statistical analysis.
- An increase in the submission of casework samples has resulted in complex mixtures, which challenge the classic standard operating procedures founded on simple two-person mixture interpretation guidelines.
- Crime laboratories will benefit from an examination of how this technology is chosen, acquired, implemented, and validated.
- Crime laboratories will benefit from a study that reviews current product offerings, features, and capabilities.

Objectives of the Landscape Study

The objectives of this landscape study are as follows:

- Investigate how DNA mixture interpretation software tools have been used for crime laboratory applications.
- Provide considerations from current users to inform potential technology adopters and assist with implementation planning, where appropriate.
- Provide practical and technical considerations in real-world applications of DNA mixture interpretation software tools to inform crime laboratory practitioners.

Research Methodology

To conduct this landscape study, RTI utilized a process that included the following steps:

- Research secondary sources, including journal and industry literature for information related to need, successful use, developmental validation, and adoption criteria.
- Discuss the state-of-the-art of the technology with subject matter experts, including crime laboratory practitioners, stakeholders, technology developers, academics, and key decision makers.
- Document, summarize, and release key findings to the crime laboratories and the forensic community.

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This study considers the present state of DNA mixture interpretation software and the potential benefits of implementation.

Lessons Learned from Previous User Experiences

This landscape study highlights several laboratories' implementation of DNA mixture interpretation software tools. Each laboratory chose a mixture interpretation software tool, and each is either in the process of validating the tool or has completed implementation. The discussions captured in this study highlight the laboratories' different needs and methods for procurement, validation, and implementation.

The following benefits were observed from successful implementation:

- Improved consistency for complex mixture interpretation results; and
- Use of a likelihood ratio (LR) statistic through probabilistic genotyping, which provides the ability to report on samples previously deemed inconclusive.
- Key considerations for successful implementation include the following:
- Comprehensive training in the use of LRs prior to software implementation;
- Comprehensive training on the software and mathematical model;
- Resources needed for internal validation, including planning, labor, and time; and
- Procurement of the software may require additional funding and/or additional support from the laboratories' information technology (IT) departments.

Current and Future Product Landscape

With the continued increase of complex mixture data, the need for mixture interpretation software tools is of growing importance to the DNA community. Given the availability of open-source and commercial software options, laboratories must assess their needs to find the most suitable software. This report is designed to support the decision process for adoption of this technology.

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Overview Of DNA Mixture Interpretation Software Tools

Introduction

Due to the evolution in the sensitivity of DNA analysis techniques, the need for advanced DNA mixture interpretation methods is a current priority in crime laboratories. Before 2000, the majority of DNA samples submitted to crime laboratories for DNA analysis resulted in single-source DNA profiles. Occasionally, two-person mixtures were observed in sexual assault cases due to imperfect separation of female epithelial cells from male sperm cells during the DNA extraction process. These samples typically contained relatively large quantities of DNA and were often deconvoluted into major and minor contributors using established laboratory processes. Interpretation strategies for these simple mixtures were derived from the rationale outlined by Clayton et al.,[1] which used the number of alleles and their relative proportions as indicators for determining the number of contributors. Sporadically, sexual assault cases produced complex mixtures that could not be deconvoluted, and therefore, the identity of the contributors could not be determined; however, these instances were quite rare (less than 0.3%).[2]

Over the years, DNA detection capabilities have improved dramatically. Currently, profiles can be generated from DNA samples with a quantity less than 100 picograms (referred to as low copy number [LCN] or low template DNA [LtDNA] samples). This increased sensitivity of detection has widened the scope of samples submitted for DNA analysis. These technical advances coincide with a growing national initiative to use DNA analysis to increase prosecution rates for lesser crimes in an effort to prevent more serious crimes.[3] The resulting influx of low-DNA- quantity samples, which often contain DNA from more than one individual, presents a growing analysis challenge for laboratories. Complicating factors, including allele masking, stochastic amplification effects, observable peaks below stochastic and analytical thresholds, and peak height imbalance, necessitate the development of mathematical methods and supporting software capable of advanced mixture interpretation and calculating statistical weight for inclusionary statements.

In 2010, the Scientific Working Group for DNA Analysis Methods (SWGDAM) published updated interpretation guidelines that emphasized the need to apply a statistic for all inclusionary statements that are deemed relevant in the context of the case. The acceptable statistical approaches recommended by SWGDAM were Random Match Probability (RMP), LR, and Random Man Not Excluded (RMNE). These guidelines supported both an allelic and genotypic approach to mixture interpretation and recommended the establishment of stochastic thresholds for the interpretation of data. The statistics used for mixture interpretation are listed in Table 1.

Many laboratories adopted mRMP or RMNE statistical analyses, which provided straightforward statistical weight for two-person mixtures. Laboratories established the required internal thresholds to properly utilize the RMNE statistics, and the use of RMNE continued to be the most frequently used method. This remained true even as mixtures became more complex and various published works,

[1] Clayton, T. M., Whitaker, J. P., Sparkes, R., & Gill, P. (1998). Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Science International 91*, 55-70.

[2] Yolanda, T., Flores, I., Prieto, V., López-Soto, M., José Farfán, M., Carracedo, A., & Sanz, P. (2003). DNA mixtures in forensic casework: a 4-year retrospective study. *Forensic Science International 134*, 180-186.

[3] Office of Justice Programs. (2004). NIJ DNA in minor crimes yields major benefits in public safety. *In Short, Toward Criminal Justice Solutions*. November. Available at: https://www.ncjrs.gov/pdffiles1/nij/207203.pdf.

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Table 1. Statistics for Reporting Mixtures and Properties for Consideration

Allele-Centric Statistics
Random Man Not Excluded (RMNE): Allele-centric approach of which there are two subtypes: Combined Probability of Inclusion and Combined Probability of Exclusion. • Does not adjust for the possibility of drop-out • Does not require an assumption to the number of contributors • Established stochastic threshold; data below the stochastic threshold is not used
Genotype-Centric Statistics
Modified Random Match Probability (mRMP) & Likelihood Ratio (LR): Genotype-centric approaches that compare the probability of observing the mixture data under two alternative hypotheses. • Can adjust for the possibility of drop-out • Requires an assumption to the number of contributors • Established stochastic threshold; data below the stochastic threshold may be used

primarily from the International Society for Forensic Genetics (ISFG), concluded that LR is the more robust and applicable statistical methodology for complex mixture interpretation.[4] Some speculate that the difficulty of explaining LRs in court may have inhibited the adoption of the LR statistical approach.[5]

As laboratories began to evaluate their own processes for complex mixture interpretation, researchers began developing user-friendly statistical software packages to assist crime laboratories with implementing the LR statistical approach and probabilistic genotyping methods. To facilitate the transition from RMNE to LR, many mixture interpretation software developers now provide resources to assist laboratories with internal validation processes, user training, technical support, and expert witness assistance.

What Is A DNA Mixture Interpretation Software Tool?

In some cases, DNA mixture interpretation software tools have the ability to assist with the deconvolution of complex mixtures. Most importantly, these tools conduct statistical calculations associated with complex mixtures, the most prominent of which is LR. The tools can calculate a variety of scenarios within the LR calculation and thereby provide the most likely scenario that supports the data.

Some software is available for purchase from several vendors, and others are available as free, open-source downloads. Some crime laboratories have chosen to develop their own statistical software.

[4] Gill, P., Brenner, C. H., Buckleton, J. S., Carracedo, A., Krawczak, M., Mayr, W. R., Morling, N., Prinz, M., Schneider, P. M., & Weir, B. S. (2006). DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Science International*, 90-101.

[5] Buckleton, J. & Curran, J. (2008). A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Science International: Genetics, 2*(4), 343-348.

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Commercially Available Software

Purchased software tools have the ability to provide extensive customer support and training. However, a laboratory should not assume that services such as training and validation support are part of the purchase price. In addition, laboratories should evaluate the transparency of the developmental validation prior to purchase.

Open-Source Software

Free software tools are fiscally very appealing. Although these tools typically have limited customer support, they are transparent with their developmental validation and resources. These tools often have active user groups for support and additional resources. A laboratory should not assume that training is unavailable for a free tool. Training is often available at a reasonable cost.

Although probabilistic software tools can assist with many of the issues experienced with complex mixtures, it is important for laboratories to understand the features and limitations of the different tools and choose one that best meets their needs.

Benefits Offered by DNA Mixture Interpretation Software Tools

The implementation of DNA mixture interpretation software tools provide the following benefits:

- **Robust:** LR is an accepted statistic for complex mixture interpretation that utilizes more of the data than other statistics.
- **Scientifically sound:** Clearly established methods are supported by peer-reviewed scientific literature.
- **Consistent:** There is improved consistency of mixture interpretation within a laboratory.
- **Compliant:** Laboratory compliance is maintained with SWGDAM guidelines.
- **Supported:** Developer-provided training and resources include the availability of resources for internal validation design.

Statistical Approaches for Mixture Interpretation

The general method for DNA mixture deconvolution that serves as the basis for mixture interpretation software tools was first described by Clayton et al.[6] and expanded upon by Evett et al.,[7,8] Gill et al.,[9] and SWGDAM.

[6] Clayton, T. M., Whitaker, J. P., Sparkes, R., & Gill, P. (1998). Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Science International, 91*, 55-70.

[7] Evett, I. W., Buffery, C., Willott, G., & Stoney, D. (1991). A guide to interpreting single locus profiles of DNA mixtures in forensic cases. *Journal of Forensic Science Society, 31*, 41-47.

[8] Evett, I. W., Gill, P. D., & Lambert, J. A. (1998). Taking account of peak areas when interpreting mixed DNA profiles. *Journal of Forensic Sciences, 43* (1) 62-69.

[9] Gill, P., Brenner, C. H., Buckleton, J. S., Carracedo, A., Krawczak, M., Mayr, W. R., Morling, N., Prinz, M., Schneider, P. M., & Weir, B. S. (2006). DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures *Forensic Science International*, 90-101.

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The inherent complexity of DNA mixtures processed by crime laboratories today presents previously unseen challenges for analysis. To successfully deconvolute, analyze, and provide statistical weight to these DNA mixtures, academics, researchers, mathematicians, and statisticians have developed binary and probabilistic models for mixture interpretation:

- **Binary model:** Genotypes are either included (probability = 1) or excluded (probability = 0) by examining the number of alleles per marker and the peak area or peak height ratios.
- **Probabilistic model:** Refers to the use of biological modeling, statistical theory, computer algorithms, and probability distributions to calculate LRs and/or infer genotypes for the DNA typing results of forensic samples (http://www.swgdam.org and SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems). This model assigns a probability between 0 and 1 for each genotype possibility. Probabilistic genotyping can be broken down further into semi-continuous and continuous methods.[10]
  - **Semi-continuous method:** Uses the peak height information and alleles present in a mixture without considering biological parameters such as peak height ratios, mixture ratios, and stutter percentages. This model accounts for the probability of allele drop-out (non-appearance of an allele) and drop-in (appearance of an additional non-reproducible allele). Software programs that use this model generally conduct fast calculations, but the model does not use all of the available data.
  - **Continuous method:** Uses all of the data present, including allele and peak height information, and incorporates biological parameters such as peak height ratios, mixture ratios, and stutter percentages. This method uses the quantitative information from peak heights to calculate the probability of the observed peak heights given all possible genotype combinations. This type of software requires numerous calculations and may use simulations to model the observed data and produce statistics and may have a longer analysis time. The time for such analysis varies by software program.

The final mixture interpretation method consists of seven basic steps:

Step 1: Identify the existence of a mixture.

Step 2: Designate allele peaks.

Step 3: Identify the number of contributors present.

Step 4: Approximate the ratio of each contributor in the mixture.

Step 5: Consider all possible genotype combinations.

Step 6: Compare reference samples.

Step 7: Determine statistical weight-of-evidence if the reference cannot be excluded.

[10] Kelly, H., Bright, J-A., Buckleton, J. S., & Curran, J. M. (2014). A comparison of statistical models for the analysis of complex forensic DNA profiles. Science & Justice, 54(1), 66-70.

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DNA Mixture Interpretation Software Tool Features

The DNA mixture interpretation software tools described in this report have the ability to deconvolute mixtures with two to six unknown contributors. Table 2 outlines the specific capabilities of all of the software tools evaluated in this report. Features of these tools are highlighted below:

Basic Software Applications

Statistical Output: All of the programs perform LR statistics, and some can provide additional statistical calculations.

Input Data Compatibility: This is the ability to directly load .fsa or .hid files into the software or import data tables as text or .csv files.

Number of Contributors: The number of unknown contributors that the software can deconvolute varies from two to six contributors.

Training and Support

Training Availability: An established training program may be available; if not, the developer may offer customized training.

Technical Support: Support varies greatly typically based on the availability of the software.

Testimony Support: Developers may provide expert testimony in admissibility hearings or other court procedures.

Processing Capabilities

Combined DNA Index System (CODIS) Output Compatibility: This indicates the creation of a common message format (CMF) output for CODIS upload.

Database Application: This provides an internal database for comparing samples within and between cases.

Markov Chain Monte Carlo Modeling: This can be used to determine the genotype probabilities.

Relatedness: Some of the software can account for mixtures involving related individuals.

System Updates: The determined number of updates per year is listed in the table; however, some release an update only when needed.

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Table 2. Specific Capabilities of the Software Tools Evaluated in this Report

	ArmedXpert	GeneMarker HID	FST	GenoProof Mixture	Lab Retriever	LikeLTD	LiRa	LRmix Studio	DNAmixtures	DNA•VIEW® Mixture Solution	LiRaHt	STRmix	TrueAllele
Interpretation Model	Binary	Binary	Semi-Continuous	Semi-Continuous	Semi-Continuous	Semi-Continuous	Semi-Continuous	Semi-Continuous	Continuous	Continuous	Continuous	Continuous	Continuous
Availability	Commercial	Commercial	Proprietary	Commercial	Open-source	Open-source	Proprietary	Open-source	Open-source	Commercial	Proprietary	Commercial	Commercial
Developer	USACIL/ NicheVision, Inc.	SoftGenetics	NYC OCME	Qualitype	SCIEG	David Balding	LGC Forensics	Hinda Haned	Therese Graversen	Charles Brenner	LGC Forensics	ESR & FSSA	Cybergenetics
Statistics	RMP, mRMP, CPE/ CPI, LR	LR, CPE/CPI, RMNE	LR	LR, RMNE, probability of identity	LR	LR	LR	LR	LR	LR	LR	LR	LR
Input	ABI GeneMapper or GeneScan Tables OSIRIS .oer projects, Excel, csv, or XML formats	.fsa or hid files	csv	.fsa or hid files, Excel, GeneMapper, GeneScan, and Genotyper export files	csv	csv	csv	Text or csv, GeneMapper IDX	Text, csv, Excel, data. frame	Text or csv	csv	Text file	.fsa or hid files
Max # of unknown contributors	3	2	3	10	4	3	3	4	6	3–4	4	None	5+
Training Available	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No*	Yes	No	Yes	Yes
Technical Support	Extensive	Extensive	None	Extensive	Basic	Basic	None	Basic	Basic	Basic	None	Extensive	Extensive
Testimony Support	No	No	No	No	Yes	No	No	Yes	No*	Yes	No	Yes	Yes
CODIS CMF output	Yes	Yes	No	Yes	No	No	No	No	No	No	No	No	Yes
Database	Yes	Yes	No	Yes	No	No	No	No	No	No	No	Yes	Yes
MCMC	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes
Accounts for Relatedness	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
System Updates	Quarterly	1x per year	Unknown	Yes	As needed	As needed	Unknown	As needed	As needed	For subscribed users	Unknown	1–2x per year	2x per year

* Developer may be available to provide custom support

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Implementation of DNA Mixture Interpretation Software Tools

The implementation of DNA mixture interpretation software by crime laboratories requires a definition of needs, procurement (of software and/or support), training, and validation.

These requirements are summarized in Figure 1.

Figure 1. Implementation of DNA Mixture Interpretation Software Requirements.

Definition of Needs

- Decision making depends on needs and resources of laboratory and capabilities of software tools.
- Collect and review information from technology developers, other laboratories, the literature, and workshops.
- When possible, seek hands-on experience with software (trial period before purchase) to fully evaluate capabilities.
- Evaluation criteria:
  - What is the desired analysis time for output?
  - Is it important to import raw data directly into the software?
  - Is the mathematical model understandable and transparent?
  - What is the defined internal validation plan?
  - Is the developmental validation published or publically available?
  - What are the training requirements?
  - What level of customer support is acceptable?
  - Is there an anticipated need for courtroom testimony support?
  - Is a maintenance agreement required?
  - Are there issues with software updates?

Procurement

- Laboratories must consider all financial commitments:
  - Initial commitments include software costs and computer costs.
  - Intermediate commitments include validation and training.
  - Long-term commitments include maintenance, updates, and training.
- Utilization of grant funds:
  - Purchase must occur during the appropriate grant period
  - Consider software updates and continued licensing as factors when determining the amount of support needed.
- Additional requirements:
  - When possible, include the cost of maintenance and IT support.
  - Purchase of computers and servers may be required prior to implementation.

Training

- Developer-provided training varies from none to extensive.
- It is necessary to determine the number of analysts that initial training will support.
- Desired content:
  - LR training for courtroom presentation.
  - Mathematical model of selected software
  - Correct use of tools
- Laboratory should establish a training plan to benefit from resources beyond the developer:
  - 1-on-1 or small group workshops
  - Supplemental reading with follow-up discussion
  - Advanced training from developer
  - Information sharing from other laboratories, including training plans
  - Collaborative training with nearby laboratories to pool resources and reduce costs
- Supplemental developer support for admissibility hearing and other court proceedings

Validation

- Laboratories should derive a preliminary validation plan prior to purchase.
- The scope of validation must include the mathematical model and the software functionality.
- Validation samples should represent casework samples in complexity.
- Suggested external resources for additional guidance:
  - Academics, researchers, and statisticians
  - SWGDAM guidelines
  - Software developer
- Additional resource considerations:
  - Labor
  - Time
  - Delegation of casework

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Software Validation

As with any new analysis technique, instrumentation, or software, a validation process must be performed to determine the limits of performance and capabilities. Since the use of probabilistic genotyping software is new to the DNA community, SWGDAM has established guidelines to address the necessary testing and information that must be captured in the validation studies for these types of systems. The following information was obtained from the SWGDAM document, *Guidelines for the Validation of Probabilistic Genotyping Systems*,[11] which was posted for public comment on March 16, 2015 and approved on June 15, 2015. The final approved guidelines can be found on the SWGDAM website (http://www.swgdam.org).

Developmental Validation

The first level of validation, which is commonly performed by the software developer, uses test data to verify the accuracy of statistical calculations, establish correct analytical and statistical parameters, determine limitations of the system, and test the overall functionality. SWGDAM offers key components that should be satisfied by software (commercial and open-source) prior to implementation:

- **Sensitivity** tests the ability of the system to determine the presence of a contributor within a mixture and determine the range of LR values expected for contributors over a range of evidentiary samples. Sensitivity testing also examines the potential for Type I errors, which exclude a true contributor.
- **Specificity** tests the ability of the system to exclude non-contributors and determines the range of LR values expected for non-contributors over a range of evidentiary samples. Specificity testing also examines the potential for Type II errors, which supports an inclusion of a non-contributor.
- **Precision** evaluates the variation in LR calculated from repeated analysis of the same input data to establish the acceptable range of LR variation for approaches that do not produce the exact same LR each time.
- **Case-type samples** verify a range of samples representative of those typically encountered by the laboratory. Such samples should include single-source and mixture samples and samples that exhibit features such as stutter, masked/shared alleles, differential and preferential amplification, degradation, and inhibition.
- **Control samples** ensure the correctness of results for control samples.
- **Accuracy** compares software results to manual calculations or an alternate software program (if possible). Also, if the software inputs raw data files, the accuracy of peak calling, sizing, and allele designations must be tested and compared to another validated software system.

[11] Scientific Working Group on DNA Analysis Methods (SWGDAM). (2015). *Guidelines for the Validation of Probabilistic Genotyping Systems*. Available at: http://media.wix.com/ugd/4344b0_22776006b67c4a32a5ffc04fe3b56515.pdf.

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Internal Validation

The second level of validation, which is performed by the laboratory, ensures that the software performs as expected given the established parameters, settings, formulae, algorithms, and functions. Some elements of this internal validation may overlap with the developmental validation if performed by the laboratory. During internal validation, laboratories should conduct a comparison of probabilistic genotyping results to manual methods for general consistency. Be aware that binary and probabilistic genotyping methods are based on different assumptions, thresholds, and formulae, so a direct comparison is not possible.

The following is a list of SWGDAM-recommended parameters that an internal validation plan should address:

- samples with known contributors and case-type samples that can include unknown contributors;
- different hypotheses to assist with policy development;
- variable DNA typing conditions (e.g., variations in amplification or electrophoresis parameters) and allelic peak heights to include off-scale peaks;
- single-source samples;
- allele drop-in;
- forward and reverse stutter;
- intra- and inter-locus peak variance;
- additional challenging samples;
- mixed samples, including the following characteristics:
  - various contributor ratios,
  - varied total DNA template quantities,
  - varied number of contributors in sample and test software by over- and under- estimating the contributors in the sample, and
  - sharing of alleles;
- sensitivity, specificity, and precision testing, as described in the developmental validation; and
- partial profiles, including allele and locus drop-out, DNA degradation, and inhibition.

Table 3 outlines a few laboratories that have shared or published their internal validation plans.

Table 3. Laboratories with Available Validation Plans

Organization	Available Validation Plan
Denver Crime Laboratory	Lab Retriever
Kern County Regional Crime Laboratory	TrueAllele®
New York City Office of Chief Medical Examiner	FST
Phoenix Police Department Crime Laboratory	ArmedXpert™

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Technical Support and Training

The developers of DNA mixture interpretation software tools offer a wide range of support and training. Technical support is offered by many developers and can range from basic technical assistance to more advanced interpretation and aid with validation plan development. Training may be offered on site or virtually and may include multiple courses. Testimony support may range from education and basic resources to full courtroom presentation and testimony.

The range of technical, validation, testimony, and training support options can be divided into three broad categories: none, basic, and extensive. A laboratory should be aware that not all topics are represented at the same categorical level. For example, a developer may have extensive training options, but basic testimony support. These three categories are defined further below:

- **None:** A few of the developers do not offer any type of technical support and training, typically because the software was developed strictly for in-house use or because the software is open-source and there are no resources to offer these additional features.
- **Basic:** Typically, basic support is offered by open-source developers or those in academia. This support may be limited in comparison to other commercial vendors, and may consist of occasional software updates, online resources, some training options, or an informative user network.
- **Extensive:** Developers who provide extensive support are typically commercial vendors who may offer onsite or virtual training. The technical support includes software updates and can range from basic technical assistance with the software to more advanced interpretation assistance. These developers may also offer a wide range of resources, such as FAQ pages, manuals, and how-to videos.

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Subject matter experts shared insights from their product experiences during validation, implementation, and use.

User Profiles

Successful deployment of DNA mixture interpretation software tools in crime laboratories provides insight on implementation. Table 4 and the subsections that follow provide examples of successful implementation of DNA mixture interpretation software tools to illustrate the benefits, potential adoption issues, and examples of how to overcome adoption barriers.

User profiles provide insight into the different ways in which crime laboratories effectively use DNA mixture interpretation software tools to process DNA evidence, enabling a greater degree of confidence in the deconvolution, interpretation, and reporting of mixtures. Special attention is given to the software validation and implementation processes used. In addition, key impacts and lessons learned are highlighted.

Users of DNA mixture interpretation software tools were contacted to determine how these tools have affected forensic DNA analysis procedures and capabilities and to gain insight from lessons learned related to acquisition, validation, and implementation.

Table 4. Profiled Subject Matter Experts for DNA Mixture Interpretation Tools

Organization	Available Validation Plan
California Department of Justice	Software: STRmix™ (currently validating) Contributors: Steven Myers, Senior Criminalist; Gary Sims, Laboratory Director/Technical Leader; and Jeanette Wallin, Acting Assistant Laboratory Director
Denver Police Department Crime Laboratory	Software: Lab Retriever (currently validating) Contributor: Susan Berdine, Technical Leader
Kern County Regional Crime Laboratory	Software: TrueAllele® (validated) Contributors: Kevin Miller, former Technical Leader and Laboratory Director; and Garett Sugimoto, DNA Analyst
New York City Office of Chief Medical Examiner	Software: Forensic Statistical Tool - FST (developed, validated, and implemented) Contributor: Craig O'Connor, DNA Analyst
Phoenix Crime Laboratory	Software: ArmedXpert™ (validated and implemented) Contributors: Jody Wolf, Assistant Crime Laboratory Administrator; and Janel Smith, Technical Leader
U.S. Army Criminal Investigation Laboratory	Software: ArmedXpert™ and STRmix™ (validated and implemented) Contributor: Joel Sutton, DNA Technical Leader
NMS Labs	Software: STRmix™ (currently validating) Contributors: Christian Westring, Director of Criminalistics

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The California Department of Justice is currently validating STRmix™

Contributors

Steven Myers is a Senior Criminalist, Gary Sims is the Laboratory Director/Technical Leader, and Jeanette Wallin is the Acting Assistant Laboratory Director at the California Department of Justice Jan Bashinski DNA Laboratory.

User Profile

The California Department of Justice Jan Bashinski DNA Laboratory currently uses a two-person mixture deconvolution tool that has been developed in-house, which uses threshold and peak height information. However, a growing number of three-person mixture samples has required the laboratory to explore other mixture interpretation tools. These three-person mixtures may be reported as uninterpretable due to their level of complexity. The continuous probabilistic approach, which utilizes peak height data, offered by some mixture interpretation software tools was especially appealing to this laboratory. The laboratory decided to purchase STRmix™ to assist with complicated mixtures.

Key Impacts and Lessons Learned

1. The laboratory's policy is to conduct the analysis twice and report the lower LR, provided that the results are within 1 log unit of each other.
2. To address some of the challenges with deriving the internal validation plan, the laboratory will rely on the SWGDAM probabilistic genotyping guidelines.
3. Future work will include a re-evaluation of laboratory thresholds to assess the effect of providing additional allelic information to the software while increasing the possibility of allelic drop-in and labeled artifacts.

Validation and Implementation

Validation for STRmix™ is currently in progress. Once online, the laboratory expects a full transition to STRmix™ from their current two-person mixture interpretation tool. Currently, the laboratory uses random match probabilities, and LRs for kinship, but the goal is to move to LRs completely. The laboratory's validation process included single-source samples and two- to three-person mixtures composed of different mixture ratios and different total template quantities with assumed and non-assumed contributors, inhibited and degraded samples, and the evaluation of known mixtures to determine false positives and false negatives. Since the current version of STRmix™ cannot account for forward stutter, the laboratory developed an approach to incorporate such ambiguous peaks into a STRmix™ interpretation. Although the version of STRmix™ in validation has been developed to deconvolute four unknown contributors, this laboratory limited the internal validation to three unknown contributors and a "phantom" contributor, which is assumed to account for possible forward stutter peaks. When the laboratory transitions to the newest STRmix™ version with no contributor limits, higher-order mixtures will be evaluated.

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The Denver Police Department Crime Laboratory is validating Lab Retriever

Contributor

Susan Berdine is the DNA Technical Leader at the Denver Police Department Crime Laboratory.

User Profile

To stay compliant with SWGDAM guidelines, the Denver Crime Laboratory needed to look at new methods for applying statistical weight to the interpretation of mixtures and wanted to move forward with a probabilistic approach. The laboratory chose to validate Lab Retriever because it was scientifically sound with peer-reviewed resources. The design and mathematical model were transparent, and because it was free, there were no significant budget concerns. The laboratory is using Lab Retriever as a statistical tool, not as a mixture interpretation tool; therefore, mixture deconvolution is still done manually by the analysts. The speed of analysis in Lab Retriever has been less than 1 second for all calculations performed.

Key Impacts and Lessons Learned

1. New versions and updates to the software may become potential barriers depending on the level of effort and resources required to address the changes.
2. To address some of the challenges with incorporating LRs for mixture interpretation, the standard operating procedures will contain specific language for reports.
3. The main challenge to completing the validation has been limited resources, with the competing interests of casework, grant goals, and staff training complicating the process.

Validation and Implementation

The laboratory looked to the published work of the New York City Office of Chief Medical Examiner for initial guidance during development of the validation plan, and also discussed validation design with the developers of Lab Retriever. The validation is broken down into several steps. The laboratory has completed the first few validation steps, including the probability of drop-out curve, but is still in the process of completing the validation. The Lab Retriever website has a list of references, including, in many cases, full text access to journal articles, which is a valuable resource for both the validation process and training. Although the laboratory had been using LRs for paternity casework, the application of an LR that incorporates a probability of drop-out, as well as applying it to mixture interpretation, still needed to be addressed. The training plan consisted of reading literature, onsite training conducted by Lab Retriever developers, familiarization with the validation, and a competency test.

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The Kern County Regional Crime Laboratory has validated TrueAllele® to assist with the analysis of complex mixtures

Contributors

Garett Sugimoto is a DNA analyst, and Kevin Miller was the former Laboratory Director/Technical Leader at the Kern County Regional Crime Laboratory.

User Profile

Kern County Regional Crime Laboratory validated TrueAllele® to assist with the analysis of complex mixtures. Prior to the implementation of a software tool, mixture interpretation was primarily limited to straightforward two-person mixtures. The laboratory-established–stochastic threshold in combination with the updated SWGDAM guidelines resulted in analysts' reluctance to interpret more complex mixtures. The laboratory purchased TrueAllele® to assist with the analysis of complex mixtures. The appealing features were the ability to utilize as much of the data as possible and perform the appropriate math to produce correct statistical results. The tool has greatly improved the ability and confidence in reporting complex mixtures.

Key Impacts and Lessons Learned

1. As a result of implementation, LR is the most commonly used statistic for mixture samples because it provides a standard statistic the court intuitively understands.
2. Grant funding enabled the purchase of the software, maintenance agreement, and training for three people.
3. The laboratory has observed a significant increase in caseload due to the success of addressing complex mixtures and is now processing samples it would have previously rejected.

Validation and Implementation

Based on validation, the software can deconvolute a mixture of up to five contributors. The laboratory's validation was published in the *Journal of Forensic Sciences*.[12] The laboratory worked directly with Dr. Mark Perlin to develop the validation plan, which was extensive and took over 1 year to complete. This plan included an inter-laboratory comparison that demonstrated 100% concordance of the validation data, as well as guidance for reporting the statistic. Based on the validation, multiple runs are conducted and the result with two concordant runs is reported. In addition, the laboratory is using the database function within TrueAllele® to track within- and between-case matching.

[12] Perlin, M. W., Hornyak, J. M., Sugimoto, G., & Miller, K. W. P. (2015). TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *Journal of Forensic Sciences, 60*, 857-868.

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The Office of Chief Medical Examiner in New York City has developed and implemented the Forensic Statistical Tool (FST)

Contributor

Craig O'Connor is a DNA analyst at the New York City Office of Chief Medical Examiner (NYC OCME).

Key Impacts and Lessons Learned

1. An extensive training plan that consisted of a range of information and resources was implemented to address the challenges associated with effectively teaching the concept of LR.
2. Frequent exposure was necessary for trainees to develop a deep understanding and familiarity with the software and statistics.

User Profile

The Office of Chief Medical Examiner in New York City has developed and implemented the Forensic Statistical Tool (FST) to enable the calculation of LR for mixtures based on SWGDAM guidelines. FST is a probabilistic genotyping software that provides a quantitative LR statistic to support the qualitative assessment of DNA mixture interpretation. This copyrighted software tool, which took approximately 3 years to develop, has been shared with other labs within the system and may eventually be publicly released. FST was designed because, at the time, other mixture interpretation software tools did not meet the requirements of the NYC OCME laboratory, including working with LCN samples. Stochastic effects, such as peak height imbalance, drop-in, drop-out, and elevated stutter, are prevalent in LCN. This software has the advantage of incorporating drop-in and drop-out as part of the peak height ratio; therefore, more of the data can be used.

Validation and Implementation

The FST validation was published in 2012 and could be used as a guide to derive other internal validations for similar software types. The validation covered the software and the mathematical model and incorporated drop-in and drop-out rates. In addition, the laboratory hired a biostatistician for validation support.

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The Phoenix Police Department Crime Laboratory has validated and implemented ArmedXpert™

Contributors

Jody Wolf is the Assistant Crime Laboratory Administrator, and Janel Smith is the Technical Leader at the Phoenix Police Department Crime Laboratory.

Key Impacts and Lessons Learned

1. The training for the software was more extensive than what has previously been done for training on new technologies.
2. Grant funding was utilized to purchase the software.

User Profile

The Phoenix Police Department Crime Laboratory purchased and validated ArmedXpert™. At the time of purchase, there were limited DNA mixture interpretation software tools from which to choose. Although TrueAllele® was available, this laboratory felt that the analysis time was too long to meet its needs, and there was a lack of training for user-based knowledge to thoroughly understand the software. ArmedXpert™ had the features in which the laboratory was interested, such as fast turnaround time, the ability to import data directly from the instrument analysis software GeneMapper® ID-X, the ability to directly export results to CODIS and other local databases, and the use of the modified RMP as a mixture statistic and the use of formulae directly from the SWGDAM guidelines for ease of user understanding. This laboratory conducts a manual deconvolution of the mixture where the number of contributors is determined with the aid of a worksheet developed in house. ArmedXpert™ is used as a statistical analysis tool, where the likelihood of alternative scenarios is determined through the generation of an LR statistic. The analysts then report the most likely scenario as the result. This laboratory felt that the mathematical models used by ArmedXpert™ are easily understood and explained in court.

Validation and Implementation

This laboratory is very open to sharing its internal validation of ArmedXpert™ and suggested that NicheVision, Inc. may also be a resource for assistance with the internal validation process for other laboratories. Training in an expanded use of RMP for mixtures was a component of the validation, as the laboratory had never used RMP for mixtures or the use of modified RMP formulae such as "allele any" or "allele obligate." Internal training consisted of assigned readings, completion of written questions, one-on-one training covering the software by members of the validation team, and a final competency exam. Overall, the laboratory is pleased with the extent of customer support that has been available beyond the initial purchase, and the vendor was very supportive during the implementation process.

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The U.S. Army Criminal Investigation Laboratory (USACIL) utilizes the functionality of ArmedXpert™ in combination with the statistical output of STRmix™

Contributor

Joel Sutton is the Technical Leader of the U.S. Army Criminal Investigation Laboratory (USACIL) DNA Casework Branch within the Defense Forensic Science Center (DFSC).

Key Impacts and Lessons Learned

1. Analysts are comfortable reporting LRs and have improved consistency and confidence in the interpretation of mixtures.
2. Computer security requirements and processing speeds were initial barriers.
3. An effective training strategy is to divide training into lectures and interactive exercises.

User Profile

In 2007, USACIL began a concerted effort to develop an Excel program to better assist with casework mixture interpretation. This included functionality to better organize data and make comparisons, as well as to interpret mixtures and report statistics. In 2011, in collaboration with NicheVision, Inc., this program evolved into what is now ArmedXpert™. This software tool can deconvolute up to three contributors by applying certain binary rules and thresholds to evaluate likely contributor genotypes and account for allelic drop-out. Even with this tool, the laboratory identified a need to explore probabilistic genotyping to better evaluate complicated mixtures. The laboratory purchased STRmix™ to supplement interpretation of complex mixture samples. Several cases using STRmix™ have now been presented at court martial proceedings with no admissibility or legal challenges. The laboratory does not use either software as a mixture interpretation tool. The analysts perform the initial interpretation, followed by utilization of both ArmedXpert™ (final allele calls, control checks, initial comparisons, and CODIS uploads) and STRmix™ (mixture deconvolution and for reporting LR statistics).

Validation and Implementation

The developmental validation for ArmedXpert™ included hand-verifying the accuracy of the math using known single-source and mixture samples. The statistical calculations were verified with Popstats where applicable. Once the commercial version was released, it was back-checked with the laboratory-developed software, and any new statistical calculations implemented by NicheVision were verified. The STRmix™ validation was comprehensive and included verification of the mathematical model and the functionality of the software. A subset of the analysts were sent for a 2-week train-the-trainer session with the STRmix™ developers, and then all analysts at USACIL were trained (over 5 full days) at the DFSC.

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NMS Labs is validating STRmix™

Contributor

Christian Westring is the Director of Criminalistics at NMS Labs.

User Profile

NMS Labs initially chose LRmix because it was available at no cost, its functionality was transparent, it could perform multiple statistical analyses, and it was in alignment with discussions from the ISFG pertaining to mixture interpretation. However, the extensive analysis time for complex mixtures was not a good fit for the laboratory. The laboratory then implemented Lab Retriever, which uses a semi-continuous model and has faster analysis speeds. During the internal validation process, the laboratory decided this tool did not quite meet the options it was seeking. The laboratory then purchased STRmix™. The appealing features were the excellent customer support, the transparency of the developmental validation, and the accuracy and transparency of the mathematical model. As STRmix™ is a fully continuous model, the laboratory felt this would allow for the most use of the data.

Validation and Implementation

NMS Labs developed an internal validation by evaluating published guidelines from SWGDAM and ISFG. External academic resources on statistical analysis and molecular biology were also used to define reproducibility, precision, and accuracy. The laboratory is currently working through their internal validation, which is progressing well and so far meeting expectations.

Key Impacts and Lessons Learned

NMS Labs experienced several unexpected circumstances over the course of implementing three different DNA mixture interpretation software tools and have provided some example considerations:

1. Effective LR training requires commitment that extends beyond a single training event. Training should leverage available courses, webinars, and resources. Sufficient funds should be set aside for analysts to participate in these sessions. Extensive training may be available from developers.
2. Those seeking to implement mixture interpretation software should contact other laboratories to discuss implementation and training processes and to request additional resources from software developers.
3. Additional resources were required for training the analysts beyond the functionality of the software. The additional training included the mathematical aspects of the software and better prepared the analysts for expert testimony.

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Developers shared information and key features about their products to assist with evaluation, validation, implementation, and use.

Developer Profiles

The developers of DNA mixture interpretation software tools were contacted to discuss key features, developmental validation procedures, and technical support offerings that are important to end users. The consensus of information gathered was used to build the profiles and summaries that follow.

This section provides a brief summary of the DNA mixture interpretation software tools currently available. The creators, distributors, and basic details are provided in text, and specific software features are outlined in Table 2. In total, six detailed developer profiles are included in this report along with summaries of seven additional DNA mixture interpretation software tools. The detailed profiles were selected based primarily on their availability and use in the United States in addition to the extent of technical support and training offered. Most of the software tools outlined in the detailed profiles require payment and are distributed by commercial entities.

ArmedXpert™

http://www.armedxpert.com/

http://www.nichevision.com/index.php/forensics/armedxpert

ArmedXpert™ was originally developed by USACIL in Atlanta, Georgia, and made commercially available through NicheVision, Inc. ArmedXpert™ utilizes binary modeling, but does allow for drop-out using probabilities calculated by a Markov Chain Monte Carlo (MCMC) method. The software can deconvolute up to three contributors in a mixture and perform full statistics, including RMP (modified, restricted, and unrestricted), CPE/CPI (RMNE), LR (restricted and unrestricted), and single-source with relatedness. ArmedXpert™ is sold per license, which includes installation, a 1-year maintenance agreement, and virtual training. The price per license is on a declining scale as the number of purchased copies increases. Additional cost considerations include onsite training and yearly maintenance fees.

Additional features include the following:

- DNA sizing and quality allele calls with OSIRIS plugin;
- QA/QC checks (ladders, controls, staff profiles, etc.);
- matching (within case, among cases, etc.);
- full documentation of interpretation (every screen is printable); and
- profile preparation for CODIS entry.

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DNA•VIEW® Mixture Solution™

http://dna-view.com/

The new DNA•VIEW® Mixture Solution™ module uses a continuous interpretation method that assesses peak heights and models artifacts, including drop-out, stutter, and drop-in. The program, created by Charles Brenner, can automatically evaluate all possible hypotheses for prosecution and defense by testing all combinations of the references, with up to four unknown contributors for each combination, and by considering all mixture proportions.

Additional features include the following:

- visual aids (e.g., depiction of mixture fit to hypothesis);
- fast operation (usually seconds per hypothesis);
- large and user-extendable collection of reference population data;
- validation of input data;
- kinship mixture hypotheses;
- racial estimation for unknown contributors;
- automatic evaluation of calibration data; and
- LIMS integration available through customization.

GeneMarker® HID

http://www.softgenetics.com/GeneMarkerHID.html

GeneMarker® HID, developed by SoftGenetics® in collaboration with Dr. Mitchell Holland, is based on the recommendations of the DNA Commission of ISFG. The software has a mixture analysis application that identifies the number of possible contributors; ranks the most likely genotype combinations (with or without the use of reference samples) using the binary interpretation method; searches the database; and performs statistical analysis, including LR, CPI, CPE, and RMNE. Cost is affected by the setup of the software license as local, network, or site, but discounts apply as the volume of licenses increases. The initial purchase price includes online training and technical support. Additional costs include onsite visits and yearly technical support/software update fees after the initial purchase.

Additional features include the following:

- audit trail;
- case comparison tool;
- profile search capability within the missing persons application and kinship analysis;
- replicate comparison tool;
- CODIS-compatible output (National DNA Index System–approved expert system);
- paternity testing (trio and single parent, AABB calculations); and
- automated contamination check for lane-to-lane and sample to staff contamination database.

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Lab Retriever

http://scieg.org/lab_retriever.html

Lab Retriever was developed by the Scientific Collaboration, Innovation and Education Group (SCIEG) whose mission is to develop a knowledge base and provide tools to improve the accuracy and reliability of forensic DNA typing. Lab Retriever modified R-code originally developed by David Balding to calculate LRs incorporating a probability of allelic drop-out and developed an easy-to-use GUI for data input and output. A separate universal calculator following Tvedebrink et al.[13] is provided to assist in empirically estimating the probability of drop-out. Lab Retriever is an open-source probabilistic genotyping system that can calculate statistics for up to four unknown contributor mixtures. Training and expert testimony services are available at a fee. A formal manual is available for download, and the developers are available for direct support as time allows. Additionally, an extensive user group exists for the open exchange of ideas, technical information, and validation. The following input information is required: probability drop-out, probability drop-in, theta (FST), evidence profile, any assumed contributor profiles (victim, consensual partner, masking stutter, etc.), suspect profile, and the hypotheses for H1 and H2.

Additional features include the following:

- fee-based training and expert testimony services available;
- extensive user group for support;
- peak heights and masking stutter information is manually modeled; and
- control over the entire analysis process.

STRmix™

http://strmix.esr.cri.nz/

STRmix™ was developed by the Institute of Environmental Science and Research Limited (ESR), a New Zealand crown research institute, with Forensic Science South Australia (FSSA). The software is commercially available in the United States through NicheVision, Inc. The statistical models are outlined in Bright et al.[14,15] and Taylor et al.[16] STRmix™ uses a continuous interpretation model with MCMC modelling to calculate LR statistics. There is no restriction on the number of contributors STRmix™ can deconvolute, and it includes database capabilities, which provides the ability to search a deconvoluted DNA profile as well as the database for related individuals. STRmix™ is sold per license, with optional support and an annual upgrade. Training is mandatory and is offered on site or in external multilaboratory training sessions.

Additional features include the following:

- batch multiple deconvolutions;
- combining multiple electropherograms (EPGs) into a single analysis;
- familial searching capabilities; and
- kinship hypothesis testing.

[13] Tvedebrink, T. Erikson, P. S., Morgensen, H. S., & Morling, N. (2009). Estimating the probability of allelic drop-out of STR alleles in forensic genetics. *Forensic Science International: Genetics, 3*, 222-226.

[14] Bright, J.-A., Taylor, D., Curran, J. M., & Buckleton, J. (2013). Developing allelic and stutter peak height models for a continuous method of DNA interpretation. *Forensic Science International: Genetics, 7*(2), 296-304.

[15] Bright, J.-A., Taylor, D., Curran, J. M., & Buckleton, J. (2013). Degradation of forensic DNA profiles. *Australian Journal of Forensic Sciences, 45*(4), 445-449.

[16] Taylor, D., Bright, J.-A., & Buckleton, J. (2013). The interpretation of single source and mixed DNA profiles. *Forensic Science International: Genetics, 7*(5), 516-528.

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TrueAllele® Casework

http://www.cybgen.com/systems/casework.shtml

TrueAllele® Casework is a DNA interpretation software tool developed by Cybergenetics and outlined in Perlin et al.[17,18] and Perlin & Sinelnikov.[19] The continuous interpretation method utilizes a server for MCMC modelling and statistical analysis for resolving mixtures with five or more contributors.[20] The software has been validated to resolve mixtures involving five unknown contributors, but the software is capable of resolving more than five total contributors. The basic package includes software, servers, 1-year support, maintenance, and virtual training. The software is also functional utilizing cloud servers, which can limit expenses and allow laboratories to utilize more or less servers as needed. Additional costs include annual maintenance, advanced training for more analysts, and expert testimony fees.

Additional features include the following:

- kinship relations, familial search, and match capabilities;
- evidence-to-evidence search and match capability;
- a database that enables matching evidence across serial crimes; and
- an ability to interface with LIMS system.

Additional Software Profiles

The following additional software tools may be unavailable in the United States or are open-source with limited support. The developers/laboratories may be contacted directly for further information.

DNAmixtures

http://dnamixtures.r-forge.r-project.org/

DNAmixtures is an open-source R statistical package created by Therese Graversen based on the model proposed by Cowell et al.[21] with the computational aspects further described by Graversen & Lauritzen.[22] DNAmixtures is a statistical tool intended for use in casework and in research and development. It provides a flexible statistical toolbox that enables the user to explore various aspects of the model. In particular, it offers a set of diagnostic tools for investigating whether the model explains the data well. A variety of user-specified extensions and modifications to the model are easily accommodated. Deconvolution of the DNA mixture utilizes the continuous probabilistic genotyping model. On a single computer, it will be computationally feasible to handle up to six unknown contributors.

[17] Perlin, M. W., Legler, M. M., Spencer, C. E., Smith, J. L., Allan, W. P., Belrose, J. L., Duceman, B. W. (2011). Validating TrueAllele® DNA Mixture Interpretation. *Journal of Forensic Sciences, 56*(6), 1430-1447.

[18] Perlin, M. W., Belrose, J. L., & Duceman, B. W. (2013). New York State TrueAllele® Casework Validation Study. *Journal of Forensic Sciences, 58*(6), 1458-1466.

[19] Perlin, M. W. & Sinelnikov, A. (2009). An Information gap in DNA evidence interpretation. *PLoS ONE, 4*(12), e8327.

[20] Perlin, M. W., Hornyak, J. M., Sugimoto, G., & Miller, K. W. P. (2015). TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *Journal of Forensic Sciences, 60*, 857-868.

[21] Cowell, R.G., Graversen, T., Lauritzen, S., & Mortera, J. (2015). Analysis of forensic DNA mixtures with artefacts. *Journal of the Royal Statistical Society: Series C (Applied Statistics), 64*(1), 1-48.

[22] Graversen, T., & Lauritzen, S. (2015). Computational aspects of DNA mixture analysis. *Statistics and Computing, 25*(3), 527-541.

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Forensic Statistical Tool (FST)

FST is a program created in house at the NYC OCME and described in Mitchell et al.[23] FST utilizes a semi-continuous approach to LR calculations that incorporates drop-out and drop-in for single-source samples and 2- and 3-person mixtures.

GenoProof Mixture

http://www.genoproofmixture.com/en

GenoProof Mixture, created by Qualitype GmbH, provides comprehensive calculation options for evaluation of reference samples and mixtures. This software is marketed worldwide and follows the ISFG 2012 recommendations.

LikeLTD

http://cran.r-project.org/web/packages/likeLTD/index.html

https://sites.google.com/site/baldingstatisticalgenetics/

LikeLTD is an open-source R package initially created by David Balding and developed by colleagues at University College London, using a semi-continuous interpretation model that evolved from Balding & Buckleton[24] for up to three unknown contributor mixtures in addition to known contributors. Limited training is available from Cellmark (UK), and some technical support is provided from Dr. Balding in the academic sector.

LiRa

LiRa is used by LGC Forensics based on methods by Puch-Solis & Clayton[25] for the deconvolution of up to three-person mixtures with drop-in and drop-out. It is not currently available in the United States. The continuous version of the software (LiRaHt) is currently being validated by LGC Forensics.

[23] Mitchell, A.A., Tamariz, J., O'Connell, K., Ducasse, N., Budimlija, Z., Prinz, M., & Caragine, T. (2012). Validation of a DNA mixture statistics tool incorporating allelic drop-out and drop-in. *Forensic Science International: Genetics, 6*(6), 749-761.

[24] Balding, D. J. & Buckleton, J. (2009). Interpreting low template DNA profiles. *Forensic Science International: Genetics, 4*(1), 1-10.

[25] Puch-Solis, R., Rodgers, L., Mazumder, A., Pope, S., Evett, I., Curran, J., & Balding, D. (2013). Evaluating forensic DNA profiles using peak heights, allowing for multiple donors, allelic dropout and stutters. *Forensic Science International: Genetics 7*(5), 555-63.

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LRmix & LRmix Studio

https://sites.google.com/site/forensicdnastatistics/PCR-simulation/lrmix
http://lrmixstudio.org/

LRmix & LRmix Studio, created by Hinda Haned, are open-source programs using a semi-continuous interpretation model for LR calculations, which incorporate the probability of drop-out and drop-in based on recommendations by Gill et al.[26,27] and Gill & Haned.[28] LRmix Studio is the successor to LRmix, offering an updated interface and faster analysis.

NOC*It*

http://www.bu.edu/dnamixtures/pages/help/downloads/

NOC*It* is a publically available software tool created through a collaboration between Boston University, Rutgers University, and the Massachusetts Institute of Technology, that determines the a posteriori probability on the number of contributors in a mixture. These results can then be used by MatchIt, a companion software system currently under development, which calculates (1) the LR; (2) the LR's distribution, conditioned on the defense hypothesis; and (3) an associated *p*-value. Laboratories can reach out to the academic developers for more information. This software is currently being modified and expanded with additional properties and is not yet available for casework.

[26] Gill, P., Brenner, C. H., Buckleton, J. S., Carracedo, A., Krawczak, M., Mayr, W. R., Morling, N., Prinz, M., Schneider, P. M., & Weir, B. S. (2006). DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures *Forensic Science International*, 90-101.

[27] Gill, P., Gusmão, L., Haned, H., Mayr, W. R., Morling, N., Parson, W., Prieto, L., Prinz, M., Schneider, H., Schneider, P.M., & Weir, B.S. (2012). DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. *Forensic Science International: Genetics, 6*(6), 679-688.

[28] Gill, P. & Haned, H. (2013). A new methodological framework to interpret complex DNA profiles using likelihood ratios. *Forensic Science International: Genetics, 7*(2), 251-263.

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To gain the benefits that DNA mixture interpretation software tools offer, agencies must understand the factors necessary for a successful implementation.

Summary

The implementation of DNA mixture interpretation software tools provides results that are:

- Robust: LR is an accepted statistic that can be applied to complex mixtures.
- Scientifically sound: Clearly established methods are supported by peer-reviewed scientific literature.
- Consistent: There is improved consistency of mixture interpretation within a laboratory.
- Compliant: Laboratory compliance is maintained with SWGDAM guidelines.
- Supported: Developer-provided training and resources include the availability of resources for internal validation design.

Implementation of DNA mixture interpretation software will require the following:

- Definition of needs: An assessment of laboratory needs and resources must be conducted to properly evaluate DNA mixture interpretation software tools. Finding a software tool that fits the laboratory's need is critical. Potential areas to evaluate include time of analysis, type of interpretation model the software performs, and type of training and support offered.
- Procurement (for software and/or support): Laboratories must look beyond the initial cost of the tool and consider intermediate and long-term financial commitments. Even if a laboratory chooses one of the free tools, substantial resources are needed for labor associated with the validation and analyst training. Additional cost considerations include maintenance agreements.
- User training: Training should cover three major areas: the application of the LR, the mathematical model the tool uses, and the correct use of the tool, all of which need to be explained fluently in court.
- Validation: The internal validation process for implementing a DNA mixture interpretation software tool will be challenging, as the scope of the validation must incorporate both the mathematical and the software component of the chosen system across a variety of samples that mimic the complex mixtures observed in casework. Therefore, the laboratory should be cognizant of additional resources in labor, time, and finances to support the scope of the validation, as it will be a more extensive process than that of previous validation studies.

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ADDITIONAL RESOURCES

To learn more about DNA mixture interpretation software technology, consider these additional resources.

DNA Analyst Training on Mixture Interpretation:

- NIST webinar available online: http://www.nist.gov/oles/forensics/dna-analyst-training-on-mixture-interpretation-webcast.cfm
- NIST DNA Analyst Webinar Series: Probabilistic Genotyping and Software Programs (Part 1): http://www.nist.gov/forensics/nist-dna-analyst-webinar-series-pt1.cfm and http://www.nist.gov/forensics/nist-dna-analyst-webinar-archive.cfm.
- NIST DNA Analyst Webinar Series: Probabilistic Genotyping and Software Programs (Part 2): http://www.nist.gov/forensics/nist-dna-analyst-webinar-series-part-2.cfm and http://www.nist.gov/forensics/dna-analyst-webinar-probabilistic-genotyping-software-programs.cfm.

ArmedXpert™ Resources:

- http://www.armedxpert.com/
- http://www.nichevision.com/index.php/forensics/armedxpert

DNAmixtures Resources:

- Cowell, R. G., Graversen, T., Lauritzen, S. L., & Mortera, J. (2015). Analysis of forensic DNA mixtures with artefacts. *Journal of the Royal Statistical Society: Series C (Applied Statistics), 64*(1), 1-48.
- Graversen, T., & Lauritzen, S. (2014). Computational aspects of DNA mixture analysis. *Statistics and Computing, 25*(3), 527-541.
- Steele, C. D., & Balding, D. J. (2014). Statistical Evaluation of forensic DNA profile evidence. *Annual Review of Statistics and Its Application, 1*(1), 361-384.
- http://dnamixtures.r-forge.r-project.org/

DNA•View® Mixture Solution™ References/Resources:

- Puch-Solis, R., Rodgers, L., Mazumder, A., Pope, S., Evett, I., Curran, J., & Balding, D. (2013). Evaluating forensic DNA profiles using peak heights, allowing for multiple donors, allelic dropout and stutters. *Forensic Science International: Genetics 7*(5), 555-63.
- http://www.bodetech.com/technologies/dnaview-dna-statistics-calculations/
- http://dna-view.com/

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FST Resources:

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GeneMarker® HID Resources:

- Clayton, T. M., Whitaker, J. P., Sparkes, R., & Gill, P. (1998). Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Science International*, *91*, 55-70.
- Gill, P., Brenner, C. H., Buckleton, J. S., Carracedo, A., Krawczak, M., Mayr, W. R., Morling, N., Prinz, M., Schneider, P. M., & Weir, B. S. (2006). DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures *Forensic Science International*, 90-101.
- Gill, P., Sparkes, R., Pinchin, R., Clayton, T., Whitaker, J., & Buckleton, J. (1998). Interpreting simple STR mixtures using allele peak areas. *Forensic Science International, 91*(1), 41-53.
- http://www.softgenetics.com/GeneMarkerHID.html

GenoProof Mixture Resources:

- Curran, J. M., Triggs, C. M., Buckleton, J., & Weir, B. S. (1999). Interpreting DNA mixtures in structured populations. *Journal of Forensic Sciences, 44*, 987-995.
- Gill, P., Gusmão, L., Haned, H., Mayr, W. R., Morling, N., Parson, W., Prieto, L., Prinz, M., Schneider, H., Schneider, P. M., & Weir, B. S. (2012). DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. *Forensic Science International: Genetics, 6*(6): 679-688.
- http://www.genoproofmixture.com/en

Lab Retriever Resources:

- Lohmueller, K. E., Rudin, N., & Inman, K. (2014). Analysis of allelic drop-out using the Identifiler® and PowerPlex® 16 forensic STR typing systems. *Forensic Science International: Genetics, 12*(0), 1-11.
- Lohmueller, K. E. & Rudin, N. (2013). Calculating the weight of evidence in low-template forensic DNA casework. *Journal of Forensic Sciences, 58*, S243-S249.
- http://scieg.org/lab_retriever.html

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likeLTD Resources:

- Balding, D. J. (2013). Evaluation of mixed-source, low-template DNA profiles in forensic science. *Proceedings of the National Academy of Sciences, 110*(30), 12241-12246.
- Balding, D. J. & Buckleton, J. (2009). Interpreting low template DNA profiles. *Forensic Science International: Genetics, 4*(1): 1-10.
- Steele, C. D. & Balding, D. J. (2014). Statistical evaluation of forensic DNA profile evidence. *Annual Review of Statistics and Its Application, 1*(1), 361-384.
- Steele, C. D. & Balding, D. J. (2014). Choice of population database for forensic DNA profile analysis. *Science and Justice, 54*(6), 487-493.
- Steele, C. D., Greenhalgh, M., & Balding, D .J. (2014). Verifying likelihoods for low template DNA profiles using multiple replicates. *Forensic Science International: Genetics, 13*(0), 82-89.
- http://cran.r-project.org/web/packages/likeLTD/index.html
- https://sites.google.com/site/baldingstatisticalgenetics/

LiRa Resources:

- Puch-Solis, R. & Clayton, T. (2013). Evidential evaluation of DNA profiles using a discrete statistical model implemented in the DNA LiRa software. *Forensic Science International: Genetics, 11*, 220-228.
- Puch-Solis, R., Rodgers, L., Mazumder, A., Pope, S., Evett, I., Curran, J., & Balding, D. (2013). Evaluating forensic DNA profiles using peak heights, allowing for multiple donors, allelic dropout and stutters. *Forensic Science International: Genetics, 7*(5), 555-563.
- Puch-Solis, R., Kirkham, A. J., Gill, P., Read, J., Watson, S., & Drew, D. (2011). Practical determination of the low template DNA threshold. *Forensic Science International: Genetics, 5*(5), 422-427.

LRmix Resources:

- Haned, H., Benscho, C., Gill, P. D., & Sijen, T. (2015). Complex DNA mixture analysis in a forensic context: Evaluating the probative value using a likelihood ratio model. *Forensic Science International: Genetics, 16*, 17-25.
- Haned, H., Dorum, G., & Gill, P. (2013). On the meaning of the likelihood ratio: Is a large number always an indication of strength of evidence? *Forensic Science International: Genetics Supplement Series, 4*(1), e176-e177.
- Haned, H., Slooten, K., & Gill, P. (2012). Exploratory data analysis for the interpretation of low template DNA mixtures. *Forensic Science International: Genetics, 6*(6), 762-774.
- Haned, H. (2011). Forensim: An open-source initiative for the evaluation of statistical methods in forensic genetics. *Forensic Science International: Genetics, 5*(4), 265-268.
- Haned, H., Egeland, T., Pontier, D., Pène, L., & Gill, P. (2011). Estimating drop-out probabilities in forensic DNA samples: a simulation approach to evaluate different models. *Forensic Science International: Genetics, 5*(5), 525-531.

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- Haned, H. & Gill, P. (2011). Analysis of complex DNA mixtures using the Forensim package. *Forensic Science International: Genetics Supplement Series, 3*(1), e79-e80.
- Gill, P. & Haned, H. (2013). A new methodological framework to interpret complex DNA profiles using likelihood ratios. *Forensic Science International: Genetics, 7*(2): 251-263.
- Gill, P., Gusmão, L., Haned, H., Mayr, W. R., Morling, N., Parson, W., Prieto, L., Prinz, M., Schneider, H., Schneider, P. M., & Weir, B. S. (2012). DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. *Forensic Science International: Genetics, 6*(6): 679-688.
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- http://lrmixstudio.org/
- https://sites.google.com/site/forensicdnastatistics/PCR-simulation/lrmix

NOC*It* Resources:

- Swaminathan, H., Grgicak, C. M., Medard, M., & Lun, D. S. (2014). NOCIt: A computational method to infer the number of contributors to DNA samples analyzed by STR genotyping. *Forensic Science International: Genetics, 16*, 172-180.
- http://www.bu.edu/dnamixtures/pages/help/downloads/

STRmix™ Resources:

- Bright, J.-A., Evett, I.A., Taylor, D., Curran, J. M., & Buckleton, J. (2015). A series of recommended tests when validating probabilistic DNA profile interpretation software. *Forensic Science International: Genetics, 14*, 125-131.
- Bright, J.-A., Taylor, D., Curran, J. M., & Buckleton, J. (2014). Searching mixed DNA profiles directly against profile databases. *Forensic Science International: Genetics, 9*(0), 102-110.
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- Bright, J.-A., McManusa, K., Harbison, S-A., Gill, P. & Buckleton, J. (2012). A comparison of stochastic variation in mixed and unmixed casework and synthetic samples. *Forensic Science International: Genetics, 6*(2), 180-184.
- Buckleton, J., Kelly, H., Bright, J.-A., Taylor, D., Tvedebrink, T., & Curran, J. M. (2014). Utilising allelic dropout probabilities estimated by logistic regression in casework. *Forensic Science International: Genetics, 9*(0), 9-11.

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- Taylor, D., & Buckleton, J. (2015). Do low template DNA profiles have useful quantitative data? *Forensic Science International: Genetics, 16*, 13-16.
- Taylor, D., Buckleton, J., & Evett, I. (2015). Testing likelihood ratios produced from complex DNA profiles. *Forensic Science International: Genetics, 16*, 165-171.
- Taylor, D., Bright, J.-A., & Buckleton, J. (2013). The interpretation of single source and mixed DNA profiles. *Forensic Science International: Genetics, 7*(5), 516-528.
- http://strmix.esr.cri.nz/

TrueAllele® Resources:

- Perlin, M. W., Hornyak, J. M., Sugimoto, G. and Miller, K. W.P. (2015), TrueAllele® Genotype Identification on DNA Mixtures Containing up to Five Unknown Contributors. *Journal of Forensic Sciences, 60*: 857–868.
- Perlin, M. W., Dormer, K., Hornyak, J., Schiermeier-Wood, L., & Greenspoon, S. (2014). TrueAllele® Casework on Virginia DNA mixture evidence: Computer and manual interpretation in 72 reported criminal cases. *PLoS ONE, 9*(3), e92837.
- Perlin, M. W., Belrose, J. L., & Duceman, B. W. (2013). New York State TrueAllele® Casework Validation Study. *Journal of Forensic Sciences, 58*(6), 1458-1466.
- Perlin, M. W., (2012). When good DNA goes bad. *Journal of Forensic Research, 04*(01).
- Perlin, M. W., Legler, M. M., Spencer, C. E., Smith, J. L., Allan, W. P., Belrose, J. L., Duceman, B.W. (2011). Validating TrueAllele® DNA Mixture Interpretation. *Journal of Forensic Sciences, 56*(6), 1430-1447.
- Perlin, M. W. & Sinelnikov, A. (2009). An information gap in DNA evidence interpretation. *PLoS ONE, 4*(12), e8327.
- Perlin, M. W. & Szabady, B. (2001). Linear mixture analysis: a mathematical approach to resolving mixed DNA samples. *Journal of Forensic Sciences, 46*(6), 1372-1378.
- http://www.cybgen.com/systems/casework.shtml
- https://www.youtube.com/user/TrueAllele

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