SCIENTIFIC WORKING GROUP ON DNA ANALYSIS METHODS[1]
Guidelines for the Use of Probabilistic Genotyping with Autosomal STR Typing Results

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Scope

This document provides guidelines for the use of probabilistic genotyping systems in assisting with the interpretation of short tandem repeat (STR) loci in electrophoresis-based DNA typing results from evidentiary specimens. Information on the application of other methods of analysis and interpretation, including binary methods, is provided in the SWGDAM *Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories.*

[1]The Scientific Working Group on DNA Analysis Methods, better known by its acronym of SWGDAM, is a group of scientists representing federal, state, and local forensic DNA laboratories in the United States and Canada. During meetings, which are held twice a year, subcommittees discuss topics of interest to the forensic DNA community and often develop documents to provide direction and guidance for the community. The use of the term 'shall' or 'must' herein does not transform these guidelines into standards. In the event of a conflict between this document and the *FBI Quality Assurance Standards for Forensic DNA Testing Laboratories* (QAS) or the *SWGDAM Guidance Document for the FBI QAS for Forensic DNA Testing and DNA Databasing Laboratories*, the QAS and its associated *Guidance Document* have precedence. Laboratories should review their standard operating procedures and the underlying validation studies under advisement of these guidelines and update their procedures as needed. Future updates to this document can be anticipated as information and technologies evolve and new methodologies emerge.

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Background:

Probabilistic genotyping refers to the use of biological modeling, statistical theory, computer algorithms, and probability distributions to infer genotypes and/or calculate likelihood ratios (LRs), for the DNA typing results of forensic samples. The use of a probabilistic genotyping system requires that the DNA analyst possess relevant foundational knowledge in DNA mixture interpretation and the calculation of LRs, in addition to understanding the software itself and its underlying models. In accordance with the QAS, in using a probabilistic genotyping system to aid in the interpretation of DNA typing results, the DNA analyst follows laboratory-defined standard operating procedures that are supported by internal validation of the probabilistic genotyping system.

A probabilistic genotyping system is not an expert system but may serve as a valuable tool to assist the DNA analyst in the interpretation of DNA typing data. Probabilistic genotyping is not intended to replace human interpretation but can reduce subjectivity and accomplish complex analyses and calculations. Using a logical, mathematical framework, a probabilistic genotyping system provides a probability of observing the DNA typing results given different proposed

combinations of genotypes. Those genotypes with a better "fit" to the observed results are assigned more weight (deemed more probable) than others. Probabilistic genotyping software calculates a likelihood ratio as a representation of statistical weight associated with the comparison of a reference sample to an evidence sample.

Guidance is provided herein for forensic casework analysis using probabilistic genotyping when analyzing and interpreting autosomal STR typing data and drawing conclusions. This document includes core elements that must be addressed within the laboratory using probabilistic genotyping and provides guidelines for the development of standard operating procedures for probabilistic genotyping of autosomal STR DNA typing results. Details and examples aim to assist the analyst in proper application of the method.

Additional information relating to these guidelines may be found by referring to the following documents:

- QAS, for standards that relate to training (Standard 6), validation (Standard 8), and analytical procedures (Standard 9).
- SWGDAM *Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories*, for definition of terms, information on DNA typing and analysis, verification of DNA typing results, manual procedures for DNA interpretation, and a synopsis on the likelihood ratio statistic.
- SWGDAM *Guidelines for the Validation of Probabilistic Genotyping Systems*, for discussion of principles and information relating to developmental and internal validation of probabilistic genotyping systems and modification of the software. These guidelines include recommended studies and examples with potential outcomes that may be used as a guide to inform and support the laboratory's standard operating procedures.
- *Recommendations of the SWGDAM Ad Hoc Working Group on Genotyping Results Reported as Likelihood Ratios*, for matters relating to the reporting of likelihood ratios.

Core Elements:

Note: The numbers given in parentheses refer to sections within this document that provide additional detail.

The Laboratory must establish and apply standard operating procedures that specify the following:

I. **(1.1)** Appropriate analytical controls.

II. **(1.2; 1.6; 3.2; 3.3)** Criteria for the suitability of DNA typing results for comparisons and/or probabilistic genotyping.

III. **(1.3; 1.4)** Procedures for interpreting the results and using probabilistic genotyping when multiple amplifications of a DNA sample or multiple capillary electrophoresis injections of an amplified sample are obtained.

IV. **(1.5; 2.1)** Procedures for assigning a number of contributors to the DNA typing results if such is required by the probabilistic genotyping software, that do not consider any person of interest (POI). The procedures for assigning a number of contributors may use any reasonably assumed contributor(s), if applicable.

V. **(1.7)** Any manual determination of exclusion in lieu of probabilistic genotyping.

VI. **(2.1; 2.2; 2.3; 2.4)** Procedures for the formulation of propositions used in calculating likelihood ratios and conditioning an interpretation on the genotype of an assumed DNA contributor.

VII. **(2.5)** Procedures complying with *National DNA Index System Procedures* for any CODIS-eligible DNA typing results that are produced using probabilistic genotyping software.

VIII. **(3)** Review of the probabilistic genotyping output relative to the DNA analyst's expectations for those results.

Section 1: Initial Interpretation of DNA Typing Results and Manual Comparisons to the Data

Introduction

A qualitative assessment of the data is generally conducted by the DNA analyst prior to using the probabilistic genotyping software. This initial evaluation for both evidence and known DNA profiles involves the verification that the correct results were obtained for controls used in analysis. The qualitative assessment will include characterizing any non-allelic peaks and inferring the number of contributors to a sample, and should be in accordance with the relevant guidelines outlined in the SWGDAM *Interpretation Guidelines for Autosomal STR Typing*, depending upon the probabilistic genotyping system used.

1.1. Analytical controls must be evaluated to determine if the data meet established laboratory criteria. The evaluation of controls can be performed manually or with software in accordance with QAS requirements.

1.2. Laboratories must establish procedures for identifying which DNA typing results will, or will not, be used for comparisons. It is noted that this assessment may be for the DNA profile as a whole, or a part of the profile (e.g., a minor component that is deemed unsuitable for comparisons). These procedures can relate to the manual interpretation of the data prior to probabilistic genotyping or to its output. Such guidelines must be supported by internal validation studies, which may be guided by:

- SWGDAM *Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories* and
- SWGDAM *Guidelines for the Validation of Probabilistic Genotyping Systems*.

1.3. If a single result will be used for probabilistic genotyping analysis, comparisons, or statistical calculations, the laboratory must establish procedures for determining which single result is used when results are generated from multiple amplifications and/or injections of a given sample extract.

1.4. If the software capabilities allow, the laboratory must establish procedures for combining results (i.e., from different amplifications of a given sample extract) into a single probabilistic genotyping analysis.

1.5. Laboratories shall establish procedures for assigning the number of contributors, using any reasonably assumed contributor(s) if applicable. The genotype of any person of interest (POI) shall not be considered in this initial assessment.

1.6. Laboratories shall establish procedures for identifying, documenting, and addressing data that the probabilistic genotyping model cannot accommodate (e.g., presumed tri-allelic locus, excessive number of contributors).

1.7. Laboratories shall establish procedures for any manual determination of exclusion that is based on comparison of a reference sample(s) to the DNA typing results in lieu of probabilistic genotyping.

Section 2: The Likelihood Ratio

Introduction

The likelihood ratio (LR) provides a means to assess the DNA results, given two alternative propositions that are evaluated as a pair. The scientist uses the software to assign the probability of the DNA results given each proposition to calculate the LR. The propositions should reflect two competing explanations for the DNA results. The LR is a value expressing the amount of support of one proposition relative to the other. While the propositions must be mutually exclusive, neither have to necessarily be true, nor do they have to explore all possible proposition pairs. Propositions may change, as necessary, if relevant new information is provided, or previous information is modified. This relevant information may include case circumstances, the associated data, the scientist's assumptions and expert knowledge, and any conditioning information.

Hierarchy of propositions

Presently, DNA testing is conducted to address the question of who may be contributing DNA to the results, and perhaps how much DNA each individual is contributing to a mixed DNA profile. The DNA analyst may be asked about aspects of the evidence that DNA testing does not address, such as the cellular origin of the DNA, the mechanisms or activities that cause the DNA to be found on the evidence, or the offense that is alleged to have caused the DNA transfer. There is a general hierarchy that describes the various levels for which propositions could be assigned further illustrated in Table 1. Each level of the hierarchy requires a separate evaluation of the evidence, which would generally result in a different LR for court.

Table 1. Hierarchy of propositions.

Level	Title	Subject of Propositions
3	Offense	Alleged offense
2	Activity	Alleged activities or DNA transfer mechanisms
1	Source	Cellular source of the DNA (e.g., blood, semen, or saliva)
0	Sub-source	Individual(s) to whom the DNA is attributed
-1	Sub-sub-source	Individual(s) to whom a component(s) (e.g., major/minor) of a DNA mixture is attributed

The propositions utilized by current probabilistic genotyping software are focused on sub-source (individual(s) to whom the DNA is attributed) and/or sub-sub-source (individual(s) to whom a component(s) of a mixture is attributed), without consideration of the cellular source of the DNA (e.g., blood, saliva, or semen), the activity that led to the presence of the DNA on the evidence or the alleged offense.

Likelihood ratios with probabilistic genotyping results

The application of probabilistic genotyping to DNA analysis does not change the steps forensic scientists used for decades to analyze a DNA sample. The difference is that at the end of the process, the data representing the DNA sample is uploaded into probabilistic genotyping software as an aid to interpretation and calculation of statistical weight in the form of an LR.

Assigning propositions

Propositions are assigned by the DNA analyst as simple or compound proposition pairs. Simple proposition pairs evaluate one POI against an unknown contributor(s). Compound proposition pairs evaluate multiple POIs together against any multiple unknown contributor(s)or against a combination of conditioned and unknown contributor(s). For example, given a two-person mixture:

Simple proposition pairs: POI 1 + 1 Unknown / 2 Unknowns and POI 2 + 1 Unknown / 2 Unknowns

Compound proposition pair: POI 1 + POI 2
2 Unknowns

Compound proposition pairs with conditioning: POI 1 + POI 2 and POI 1 + POI 2
POI 1 +1 Unknown POI 2 + 1 Unknown

Within this document, the numerator is referred to as *H*1, and the denominator as *H*2. *H*1 generally includes the POI as a contributor to the DNA profile. *H*2 generally does not include the POI as a contributor to the DNA profile; instead an unknown, unrelated individual is a contributor to the DNA profile in *H*2.

Circumstances may warrant consideration of more than one set of propositions (depending on the case information, investigative questions, and results of DNA testing), which may include evaluating simple and compound proposition pairs on the same evidence. Examples of such include varying the number of contributors interpreted to be present, and/or the presence or absence of an assumed contributor within the different proposition pairs.

Number of contributors

The true number of contributors to an evidentiary DNA typing result is unknown. Generally, a higher number of contributors increases the number of potential genotype sets, including the potential for dropout and allele sharing, that can explain the mixture. As a result, adventitious support for a non-contributor may increase, and the support for a true contributor may decrease.

2.1. When the number of contributors is required for probabilistic genotyping analysis, the number of contributors that explain the mixture may be assigned manually by the analyst or with the assistance of software. The use of an assumed contributor may also assist in the assignment of the NOC to a sample.

2.1.1. Given certain DNA typing results, the DNA analyst may not be able to reasonably assign a number of contributors (e.g., limited data or close relatives that share alleles are present in a mixture). Some possible approaches include:

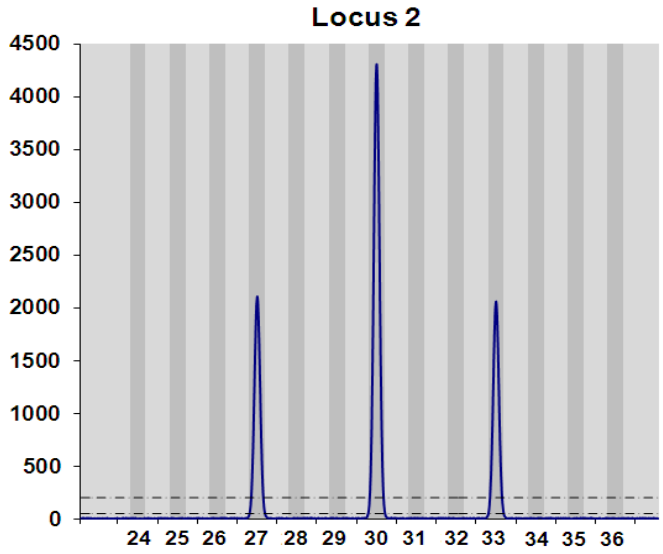
- More than one LR may be calculated using different, yet reasonable, numbers of contributors. For example, if the DNA typing result is interpreted as coming from three or four contributors, LRs could be calculated for both numbers of contributors.
- Multiple numbers of contributors (e.g., three and four) can be used, software permitting, in a single probabilistic genotyping analysis.

2.1.2. Where appropriate, some probabilistic genotyping systems allow for a different number of contributors to be assigned to each proposition within a single analysis. Do not use a number of contributors in *H*2 that is not supported by the data and a number in *H*1 that is supported by the data, as this tends to increase the LR in favor of *H*1.

Conditioning an analysis on a contributor reasonably expected to be present

Probabilistic genotyping software allows for the assumption of an individual's DNA contribution to a sample. The genotype(s) used in such an analysis is referred to as a conditioning profile(s). In such instances, the software considers the assumed contributor's genotype to be present and examines the remainder of the DNA results from the evidence accordingly.

Example: Using the below image of a mixture with two contributors, an unconditioned probabilistic genotyping deconvolution may determine the most likely genotype combinations as either 27,30 and 30,33 or 27,33 and 30,30, with other combinations having much lower probabilities. However, if the software is informed to condition the mixture on an assumed contributor with genotype 27,30, the software may give considerable statistical weight to genotype 30,33 as the second contributor. The software may either outright exclude any other genotype, or give very low weight to any other genotype except 30,33 as the second contributor.



2.2. The laboratory should establish policies for conditioning an interpretation of the DNA typing results based upon the presence of an assumed DNA profile, which is reasonably expected to be present on the evidence. The reason for the conditioning should be clearly documented in the case record. Examples include:

- an individual from whom the evidence sample was taken (e.g., a victim when interpreting evidence from their sexual assault kit)
- an individual who has had contact with the evidence, such as the wearer of clothing, a consensual sexual partner, or a vehicle owner
- a genotype from one fraction (e.g., sperm fraction) of a differential extraction being compared to the other fraction (e.g., non-sperm fraction)

2.2.1. The use of any profile for conditioning must be supported by the DNA results from the evidence.

2.2.2. In some instances, the DNA typing results may be insufficient to support conditioning based solely on a manual evaluation of the data. A laboratory may establish a minimum LR threshold for the conditioning profile as a prerequisite to subsequently conditioning the interpretation of the evidentiary profile.

2.2.2.1. A laboratory may use different minimum LR thresholds for different evidence types, e.g., a lower LR threshold for intimate samples versus non-

intimate samples. Alternatively, a laboratory may define intimate items as not requiring an LR as a prerequisite for conditioning, if 2.2.1 is met.

Multiple POIs

2.3. If DNA from multiple POIs are each associated with the mixture using individual LR calculations, it is necessary, as software allows, to evaluate them together in an LR calculation(s) to address the investigative scenario.

For instance, compound propositions with or without alternately conditioning on each POI may be warranted. Compound propositions without conditioning will evaluate the presence of the POIs together in the mixture, while alternately conditioning the analyses on each POI isolates the LR for each individual contributor. Examples of scenarios that require an assessment of whether DNA from both POI1 and POI2 are in the mixture together are:

- POI1 is the victim of a sexual assault at a park, POI2 is the subject, and the evidence is a condom (or a blanket, washcloth, etc.) found at the park
- POI1 is the victim of a homicide, POI2 is the subject, and the evidence is a weapon believed to be related to the crime

2.3.1. If the *H*1 proposition is more ambiguous, all possible proposition pairs may be calculated to cover every scenario. More complex case scenarios should follow this general guidance to select appropriate proposition pairs for LR calculations.

Evaluative versus investigative modes of interpreting DNA typing results

Evaluative assessments are generally made when a person(s) of interest is known and probabilistic genotyping is conducted for reporting LRs. Without a person of interest for a direct comparison to the evidence, the laboratory may utilize probabilistic genotyping software in an investigative mode to be used for database searching in an attempt to identify a person of interest. In investigative instances, deconvolution of the evidence profile may use certain

assumptions and/or conditioning information to create searchable DNA information to develop an investigative lead. Investigative assessments may include:

- Following deconvolution of a mixture by probabilistic genotyping, using assigned genotypes or allele weights to construct a potential multi-locus profile for a contributor to the mixture.
- Using a single source DNA profile from an item as a conditioning profile for another item that is a mixture of two individuals, which includes the single source DNA profile from the first item. This may aid in the isolation of the second profile that is present in the mixed sample.
- Using probabilistic genotyping software to compare DNA profiles derived from multiple items of evidence to evaluate support for the presence of a common contributor across samples. This may aid in the identification of the most discriminating profile for database searching when multiple entries for that contributor are possible or aid the investigator's understanding of the case evidence.

2.4. When working in the investigative mode, the laboratory should establish procedures for conditioning the analysis of a mixed profile on another evidentiary profile or a POI.

2.4.1. The laboratory may require a minimum LR or level of support (e.g., strong support) for the conditioning profile to be used in subsequent analyses.

2.4.2. The use of any profile for conditioning must be supported by the DNA results and documented in the case record.

2.5. The laboratory must establish procedures for the usage of probabilistic genotyping to develop DNA profiles for database searching and must adhere to NDIS requirements, if applicable.

Laboratories that use probabilistic genotyping data to determine profiles for searching should understand the limitations of their procedures on the ability to obtain database matches. A full deconvolution may lack the rarity to be submitted for a database search. However, using less than the full deconvolution (e.g., the full deconvolution includes 4 alleles, but the searched data

includes only 2 alleles) will allow for searching, but risks missing a true contributor in a database search.

Non-contributor testing

Non-contributor testing is an investigative assessment that examines the discrimination power of a DNA result and can help provide context to the LR in the following scenarios:

- evaluating candidate matches from a CODIS search of a forensic mixture
- assessing potential DNA contamination from laboratory staff or evidence handlers
- comparing several individual known profiles to a trace contributor in a mixture
- obtaining an LR below a laboratory defined threshold for a POI in a case

Non-contributor testing is simply a supplement to understanding the result, and does not supplant the reported LR.

For non-contributor testing, a data set of a large number (e.g., ≥10,000) of profiles are tested against the deconvolution, and LRs are calculated for each non-contributor profile. The propositions being evaluated are:

*H*1 = the database individual is a contributor to the mixture

*H*2 = an unrelated, unknown individual is a contributor to the mixture

The resulting LRs are ranked by their magnitude and quantile measurements, resulting in a distribution of LRs for non-contributors. This distribution may be compared against the LR obtained from the comparison to the POI to provide context.

2.6. A laboratory using non-contributor testing in casework must establish standard operating procedures for analysis and reporting.

2.7. Any assessments made should be determined based on the distribution and percentiles for the non-contributor testing, rather than the maximum value obtained, which will be dependent, in part, on the size of the non-contributor data set searched.

Propositions for likelihood ratios when considering biological relatives

Biological relatives tend to have more DNA in common than unrelated individuals, which can impact comparisons made to the results. For example, a non-contributing POI with a close biological relative in a mixture of DNA may produce an LR >1. The impact on the LR value is dependent on the true number of contributors, the number of shared alleles (known or proposed based on the degree of relatedness), the mixture proportions, and the total amount of amplified DNA. More distinct genetic information from the true contributors may allow the exclusion of true non-donors.

2.8. When a close biological relative (e.g., sibling, parent, child) of the person of interest is proposed as a possible contributor to a mixture of DNA, it is best to obtain a sample from the relative for direct comparison. However, if their sample is not available, a likelihood ratio using an alternate proposition for *H*2 which considers that the DNA could have originated from a relative of the person of interest (rather than an unrelated individual) should be calculated if it is within the capabilities of the software.

Section 3: Evaluation of Results and Diagnostic Assessment

The output of each probabilistic genotyping run should be scrutinized to ensure that correct input files were used, the system functioned correctly, and the results are appropriate given the observed data. The following elements are meant as general guidelines, and some specifics may not be applicable to all probabilistic genotyping systems.

3.1. The laboratory must establish procedures, supported by internal validation, for evaluating the output elements (e.g., LR, mixture proportions, proposed genotypes, weights) obtained from the probabilistic genotyping system and ensure that they conform to qualitative expectations for the sample.

3.2. If diagnostics form part of the output from the probabilistic genotyping system, the laboratory must establish:

- Which diagnostics should be reviewed for determining acceptability of the analysis
- Expected ranges for the relevant diagnostics
- Diagnostic values, individually or collectively, that require further analyst review of the input data (i.e., electropherogram elements), the initial interpretation (e.g., NOC), or the analysis settings (e.g., MCMC accepts)
- Diagnostic values, individually or collectively, that indicate the analysis is unacceptable

3.2.1. The laboratory should have policies that detail possible actions and documentation for when the diagnostics do not meet expectations or established parameters (e.g., the mixture proportions, degradation, genotype weights, or template amounts do not reflect what is expected based on an evaluation of the electropherogram data).

Possible actions may include evaluating the assignment of number of contributors and verifying (and correcting, where needed) the input file to ensure that all non-allelic peaks that are not modeled by the software are removed and that microvariants are resolved. Omitting a discrepant locus from the analysis (e.g., exhibiting unresolved peaks or tri-alleles that cannot be modeled by the software) may be necessary to progress a proper analysis. Additional measures may include

reanalysis after adjusting analysis settings (e.g., degradation, assumptions such as mixture ratio priors, or number of MCMC accepts).

3.3. The laboratory shall establish criteria for determining which DNA typing or probabilistic genotyping results are suitable for comparisons to known DNA profiles.

3.3.1. The laboratory may evaluate the results from software input and/or output information and determine that (a) comparisons may be based on the entirety of the DNA typing results or only the strongest component(s) to a mixture, or (b) the results are of such low quality that the DNA typing results are unsuitable for comparisons.

3.3.2. If DNA typing results (or components thereof) are deemed not suitable for comparisons, the basis for such a determination should be documented in the case record.

3.4. The laboratory should establish procedures for evaluating LR results.

3.4.1. Individual locus LRs should be evaluated to ensure that they conform to qualitative expectations for the sample.

For example, LRs supporting the inclusionary proposition are obtained at all loci except one, where the LR is strongly supporting the exclusionary proposition, and the known sample does not appear to be excluded from the evidentiary profile. This may indicate an incorrect assignment of the number of contributors or an unresolved microvariant allele.

3.4.2. The LR value for a comparison should be evaluated to ensure it aligns with qualitative expectations for the sample.

3.4.3. If compound propositions can be evaluated using a particular probabilistic genotyping system, a compound LR should be calculated when multiple individuals have inclusionary LRs for the same DNA result.

Generally, the LR from the compound proposition pairs with all included individuals should be equal or greater than the product of the individual LRs (or sum of the logLRs) obtained with simple proposition pairs given the same H_2 proposition. For example:

Simple proposition pairs $\frac{\text{POI 1 + 2 unknowns}}{\text{3 unknowns}} = 10^7$

$\frac{\text{POI 2 + 2 unknowns}}{\text{3 unknowns}} = 10^5$

Compound proposition pair $\frac{\text{POI 1 + POI 2 + 1 unknown}}{\text{3 unknowns}}$ = Should be equal or greater than 10^{12} (10^7 x 10^5)

Additivity of the LogLRs is generally expected for true contributors, but exceptions have been reported (Duke *et al.*, 2022). If additivity of the LogLRs is not observed, and the compound proposition LR is greater than 1 (in the above example, this would be any LR between 2 and 10^{11}), this may represent false support for the compound inclusionary proposition or one of the simple inclusionary propositions. As a result, troubleshooting of the analysis may provide additional information for resolving the questions regarding the analysis. Possible approaches may include:

- Reassessing the assigned number of contributors
- Conditioning an analysis on each POI individually (shown below) and performing an LR to the other POI, which isolates the weight of the evidence for the unconditioned POI.

  Conditioned proposition pair 1 of 2 $\frac{\text{POI 1 + POI 2 + 1 unknown}}{\text{POI 2 + 2 unknowns}}$

  Conditioned proposition pair 2 of 2 $\frac{\text{POI 2 + POI 1 + 1 unknown}}{\text{POI 1 + 2 unknowns}}$

  The magnitude of these LRs may be indicative of the amount of support for the association of the unconditioned POI to the data, with conditioned LRs <1 suggesting potentially exclusionary information for the unconditioned POI.
- Where applicable, increasing the number of MCMC accepts in the software may help resolve any false lowering of the LRs by more thoroughly exploring the probability space.

3.5. Laboratories should establish policies that address document retention when multiple probabilistic genotyping analyses are conducted, for example:

- Administrative error (e.g., a wrong or erroneous input file was used)
- Alternative set(s) of propositions (e.g., the wrong propositions were used or multiple proposition sets were required)
- Repeated analysis with the same or updated parameters (e.g., poor diagnostics were obtained from the first analysis)

3.6. Where possible, results from probabilistic genotyping may be evaluated against a quality assurance database to monitor for potential contamination.

Glossary

Analytical threshold: the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles.

Assumed contributor: an individual whose DNA on an item of evidence is reasonably expected based on the relevant case circumstances and supported by the data.

Case record: is the complete documentation, as defined by the laboratory, supporting the final report.

Conditioning Profile: the profile of an individual whose DNA is included as a contributor to the profile in both propositions of the likelihood ratio. A conditioning profile may be DNA from an assumed contributor or it may be that of a POI when attempting to isolate the LR for another POI.

Deconvolution: separation of contributors to a mixed DNA profile based on quantitative peak height information and any underlying assumptions.

Dropout: failure to detect one or more alleles within a sample (i.e., above the analytical threshold) or failure to amplify an allele during PCR.

Evidence sample: biological sample recovered from a crime scene or collected from persons or objects associated with a crime; also known as a questioned sample or a forensic sample.

Exclusion: a conclusion that eliminates an individual as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other).

Genotype: results of autosomal STR analysis of an individual at one or more genetic loci.

Guidelines: a set of general principles used to provide directions and parameters for decision making.

Intimate sample: a biological sample from an evidence item that is obtained directly from an individual's body; it is not unexpected to detect that individual's allele(s) in the DNA typing results.

Known sample: biological material for which the identity of the donor or DNA type is established (also referred to as a reference sample).

Likelihood ratio (LR): the ratio of two probabilities of the same event under different and mutually exclusive hypotheses (or propositions); typically the numerator is the inclusionary proposition (i.e., $H1$) and the denominator is the exclusionary proposition (i.e., $H2$).

Locus: the specific physical location of a genetic marker. In forensic DNA analysis, it refers to the specific sites being tested (e.g., D3S1358, vWA or D5S818).

Mixture: a DNA typing result originating from two or more individuals.

Mixture proportion: the relative proportion of the DNA contributions of multiple individuals to a mixed DNA typing result; variously expressed as proportion, ratio or percentage.

Person of interest (POI): an individual whose DNA profile is the subject of the evaluation.

Probabilistic genotyping: the use of biological modeling, statistical theory, computer algorithms, and probability distributions to calculate likelihood ratios (LRs) and/or infer genotypes for the DNA typing results of forensic samples.

Proposition: a theory or hypothesis proposed as one possible explanation for the evidence observed.

Threshold: the level or point at which the interpretation of the data changes.

Weight: a value assigned to the probability of observing the profile given the proposed genotype combination.

References and Suggested Readings

General interpretation guidelines

T. Bille, S. Weitz, J.S. Buckleton, and J.-A. Bright. *Interpreting a major component from a mixed DNA profile with an unknown number of minor contributors*. Forensic Sci. Int. Genet. 40 (2019) 150-159.

J.M. Curran, C.M. Triggs, J. Buckleton, and B.S. Weir. *Interpreting DNA mixtures in structured populations*. J. Forensic Sci. 44 (1999) 987-995.

Forensic Science Regulator Guidance – DNA Mixture Interpretation. FSR-G-222 Consultation, (2018) www.gov.uk/government/collections/dna-guidance

P. Gill, C.H. Brenner, J.S. Buckleton, A. Carrecedo, M. Krawczak, W.R. Mayr, N. Morling, M. Prinz, P.M. Schneider, B.S. Weir, *DNA Commission of the International Society of Forensic Genetics: recommendations on the interpretation of mixtures.* Forensic Sci. Int. 160 (2006) 90-101.

P. Gill, L. Gusmao, H. Haned, W.R. Mayr, N. Morling, W. Parson, L. Prieto, M. Pronz, H. Schneider, P.M. Schneider, and B.S. Weir. *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 Sci. Int. Genet. 6 (2012) 679-688.

P. Gill, J. Whitaker, C. Flaxman, N. Brown, J. Buckleton. *An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA*. Forensic Sci. Int. 112 (2000) 17-40.

B. Mallinder, S. Pope, J. Thomson, L.-A. Beck, A. McDonald, D. Ramsbottom, D.S. Court, D. Vanhinsbergh, M. Barber, I. Evett, K. Sullivan, and J. Whitaker. *Interpretation and reporting of mixed DNA profiles by seven forensic laboratories in the UK and Ireland.* Forensic Sci. Int. Genet. 58 (2022) 102674.

Scientific Working Group on DNA Analysis Methods (SWGDAM) Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories. Approved January 12, 2017, www.swgdam.org

PG modeling

E. Alladio, M. Omedei, S. Cisana, G. D'Amico, D. Caneparo, M. Vincenti, and P. Garofano. *DNA mixtures interpretation – A proof of concept multi-software comparison highlighting different probabilistic genotyping methods' performances on challenging samples*. Forensic Sci. Int. Genet. 37 (2018) 143-150.

O. Bleka, C.C.G. Benschop, G. Storvik, and P. Gill. *A comparative study of qualitative and quantitative models used to interpret complex STR DNA profiles*. Forensic Sci. Int. Genet. 25 (2016) 85-96.

J.S. Buckleton, K. Lohmueller, K. Inman, K. Cheng, J.M. Curran, S. Pugh, J.-A. Bright, and D. Taylor. *Testing whether stutter and low level peaks are additive*. Forensic Sci. Int. Genet. 43 (2019) 102166.

R.G. Cowell, S.L. Lauritzen, and J. Mortera. *Probabilistic modelling for DNA mixture analysis.* Forensic Sci. Int. Genet. Suppl. Ser. 1(1) (2008) 640-642.

K. Duke, D. Cuenca, S. Myers, and J. Wallin. *Compound and Conditioned Likelihood Ratio Behavior within a Probabilistic genotyping Context*. Genes (Basel) 2022 Dec 29;14(1):102. doi: 10.3390/genes14010102. PMID: 36672842

K. Duke and S. Myers. *Systematic evaluation of STRmix performance on degraded DNA profile data*. Forensic Sci Int Genet. 44 (2020):102174.

P. Gill and H. Haned. *A new methodological framework to interpret complex DNA profiles using likelihood ratios*. Forensic Sci. Int. Genet. 7 (2013) 251-263.

K. Inman, N. Rudin, K. Cheng, C. Robinson, A. Kirschner, L. Inman-Semerau, et al. *Lab retriever: a software tool for calculating likelihood ratios incorporating a probability of drop-out for forensic DNA profiles.* BMC Bioinformatics 16 (2015) 298.

M. Kruijver and J.-A. Bright. *A comparison of likelihood ratios with and without assuming relatedness for DNA mixtures interpreted using a continuous model.* FSI Gen 62 (2023) 102800.

M.W. Perlin. *Efficient construction of match strength distributions for uncertain multi-locus genotypes*. Heliyon 4 (2018) e00824.

D. Taylor, J-A Bright, and J. Buckleton. *The interpretation of single source and mixed DNA profiles.* Forensic Sci. Int. Genet. 7(5) (2013) 516-528.

D. Taylor, J-A Bright, H. Kelly, M-H Lin, and J. Buckleton. A *fully continuous system of DNA profile evidence evaluation that can utilise STR profile data produced under different conditions within a single analysis*. Forensic Sci. Int. Genet. 31 (2017) 149-154.

Validation strategies

D.J. Balding and J. Buckleton. *Interpreting low template DNA profiles*. Forensic Sci. Int. Genet. 4 (2009) 1-10.

D.W. Bauer, N. Butt, J.M. Hornyak, and M.W. Perlin. *Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors*. J Forensic Sci (2020) 65(2):380-398.

J.-A. Bright, D. Taylor, C. McGovern, S. Cooper, L. Russel, D. Abarno, and J. Buckleton. *Developmental validation of STRmix, expert software for the interpretation of forensic DNA profile.* Forensic Sci Intl: Genet. 23 (2016) 226-239.

J.-A. Bright, J.M. Curran, and J.S. Buckleton. *The effect of the uncertainty in the number of contributors to mixed DNA profiles on profile interpretation*. Forensic Sci Intl. Genet 12 (2014) 208-214.

J.-A. Bright, K.E. Stevenson, J.M. Curran, and J.S. Buckleton. *The variability in likelihood ratios due to different mechanisms.* Forensic Sci Intl: Genet. 14 (2015) 187-190.

J.-A. Bright, et al. *Internal validation of STRmix – a multi laboratory response to PCAST.* Forensic Sci Intl: Genet. 34 (2018) 11-24.

J. Buckleton, H. Kelly, J-A Bright, D. Taylor, T. Tvedibrink, and J. Curran. *Utilising allelic drop-out probabilities estimated by logistic regression in casework*. Forensic Sci. Int. Gent. 9 (2014) 9-11.

M.D. Coble, J. Buckleton, J.M. Butler, T. Egeland, R. Fimmers, and P. Gill. *DNA commission of the International Society for Forensic Genetics: recommendations on the validation of software programs performing bio statistical calculations for forensic genetics applications*. Forensic Sci. Int. Genet. 25 (2016) 191-197.

S.A. Greenspoon, L. Schiermeier-Wood, and B.C. Jenkins. (2015) *Establishing the limits of TrueAllele® Casework: a validation study*. J Forensic Sci 60(5):1263-1276.

H. Haned, T. Egeland, D. Pontier, L. Pene, and P. Gill. *Estimating drop-out probabilities in forensic DNA samples: a simulation approach to evaluate different models.* Forensic Sci. Int. Genet. 5 (2011) 525-531.

T. Moretti, R. Just, S. Kehl, L. Willis, J. Buckleton, J.-A. Bright, D. Taylor, and A. Onorato. *Internal validation of STRmix for the interpretation of single source and mixed DNA profiles*. Forensic Sci Intl: Genet. 29 (2017) 126-144.

S. Noel, J. Noel, D. Granger, J-F Lefebvre, and D. Seguin. *STRmix™ put to the test: 300 000 non-contributor profiles compared to four-contributor DNA mixtures and the impact of replicates*. Forensic Sci. Int. Genet. 41 (2019) 24-31.

M.W. Perlin, M.M. Legler, C.E. Spencer, J.L. Smith, W.P. Allan, J.L. Belrose, et al. *Validating TrueAllele® DNA mixture Interpretation*. J. Forensic Sci. 56(6) (2011) 1430-1447.

R. Puch-Solis and T. Clayton. *Evidential evaluation of DNA profiles using a discrete statistical model implemented in the DNA LiRa software*. Forensic Sci. Int. Genet. 11 (2014) 220-228.

Scientific Working Group on DNA Analysis Methods (SWGDAM) Guidelines for the Validation of Probabilistic Genotyping Systems. Approved June 15, 2015, www.swgdam.org

D. Taylor, J.-A. Bright, J. Buckleton, and J. Curran. *An illustration of the effect of various sources of uncertainty on DNA likelihood ratio calculations*. Forensic Sci. Int. Gent. 11 (2014) 56-63.

D. Taylor, J. Buckleton, and I. Evett. *Testing likelihood ratios produced complex DNA profiles*. Forensic Sci. Int. Genet. 16 (2015) 165-171.

D. Taylor, J. Buckleton, and J.-A. Bright. *Factors affecting peak height variability for short tandem repeat data*. Forensic Sci. Int. Genet. 21 (2016) 126-133.

T. Tvedibrink, P.S. Eriksen, H.S. Mogensen, and N. Morling. *Estimating the probability of allelic drop-out of STR alleles in forensic genetics*. Forensic Sci. Int. Genet. 3 (2009) 222-226.

T. Tvedibrink, P.S. Eriksen, M. Asplund, H.S. Morgensen, and N. Morling. *Allelic drop-out probabilities estimated by logistic regression – further considerations and practical implementation.* Forensic Sci. Int. Genet. 6 (2012) 263-267.

LR framework and reporting strategies with PG

J.-A. Bright, J.M. Curran, and J.S. Buckleton. *The effect of uncertainty in the number of contributors to mixed DNA profiles on profile interpretation*. Forensic Sci. Int. Genet. 12 (2014) 208-214.

T. Hicks, J. Buckleton, V. Castella, I. Evett, and G. Jackson. *A Logical Framework for Forensic DNA Interpretation*. Genes 2022 13, 957. https://doi.org/10.3390/genes13060957

R. Cook, I.W. Evett, G. Jackson, P.J. Jones, and J.A. Lambert. *A hierarchy of propositions: deciding which level to address in casework.* Sci. Justice 38(4) (1998) 231-240.

P. Gill, T. Hicks, J.M. Butler, E. Connolly, L. Gusmao, B. Kakshoorn, N. Morling, R.A.H. van Oorschot, W. Parson, M. Prinz, P.M. Schneider, T. Sijen, and D. Taylor. *DNA commission of the International Society for Forensic Genetics: Assessing the value of forensic biological evidence – Guidelines highlighting the importance of propositions Part 1: evaluation of DNA profiling comparisons given (sub-) source propositions*. Forensic Sci. Int. Genet. 36 (2018) 189-202.

S. Gittelson, T. Kalafut, S. Myers, D. Taylor, T. Hicks, F. Taroni, et al. *A practical guide for the formulation of propositions in the Bayesian approach to DNA evidence interpretation in an adversarial environment*. J. Forensic Sci. 61(1) (2016) 186-195.

H. Haned, G. Dorum, T. Egeland, and P. Gill. *On the meaning of the likelihood ratio: Is a large number always an indication of the strength of evidence?* Forensic Sci. Int. Genet. Supplemental Series 4 (2013) e176-e177.

H. Haned, C.C.G. Benschop, P.D. Gill, and T. Sijen. *Complex DNA mixture analysis in a forensic context: evaluating the probative value using a likelihood ratio model*. Forensic Sci. Int. Genet. 16 (2015)17-25.

B. Robertson, G.A. Vignaux, and C.E. Berger. *Interpreting Evidence, Evaluating Forensic Science in the Courtroom.* Second Edition (2016) Wiley and Sons, Ltd.

Scientific Working Group on DNA Analysis Methods (SWGDAM) Recommendations on Genotyping Results Reported as Likelihood Ratios. Approved July 12, 2018, www.swgdam.org

D. Taylor. *Using continuous DNA interpretation methods to revisit likelihood ratio behavior*. Forensic Sci. Int. Genet. 11 (2014) 144-153.

D. Taylor, J-A Bright, and J. Buckleton. *Considering relatives when assessing the evidential strength of mixed DNA profiles*. Forensic Sci. Int. Genet. 13 (2014) 269-280.

G.A. Williams and P.D. Maskell. *Embracing likelihood ratios and highlighting the principles of forensic interpretation.* Forensic Sci. Int.: Reports 3 (2021) 10029.

Investigative mode/database searching

J.-A. Bright, D. Taylor, J. Curran, and J. Buckleton. *Searching mixed DNA profiles directly against profile databases*. Forensic Sci Int Genet 9 (2014) 102-110.

M. Kruijver, J-A Bright, H. Kelly, and J. Buckleton. *Exploring the probative value of mixed DNA profiles*. Forensic Sci. Int. Genet. 41 (2019) 1-10.

S. Myers. *Searching CODIS with binary conversions of STRmix interpretations*. Forensic Sci Int Genet. 2021 Nov;55:102569.

C. Schuerman, T. Kalafut, C. Buchanan, J.Sutton, J-A Bright. *Using the Nondonor Distribution to Improve Communication and Inform Decision Making*. J. Forensic Sci. 2020 doi: 10.1111/1556-4029.14306.

K. Slooten. *Identifying common donors in DNA mixtures, with applications to database searches*. Forensic Sci Int Genet 26 (2017) 40-47.

D. Taylor and D. Abarno. *Using big data from probabilistic genotyping to solve crime*. Forensic Sci. Int. Genet. 57 (2022) 102631.

D. Taylor and M. Kruijver. *Combining evidence across multiple mixed DNA profiles for improved resolution of a donor when a common contributor can be assumed.* Forensic Sci. Int. Genet. 49 (2020) 102375.