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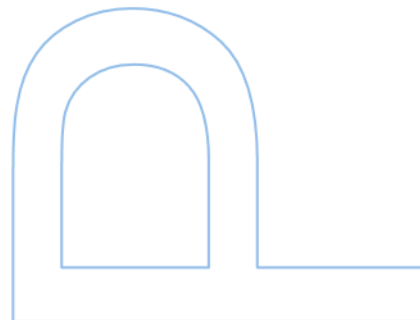
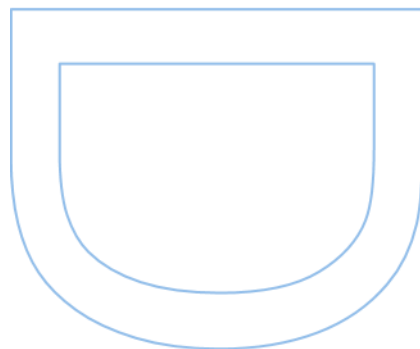
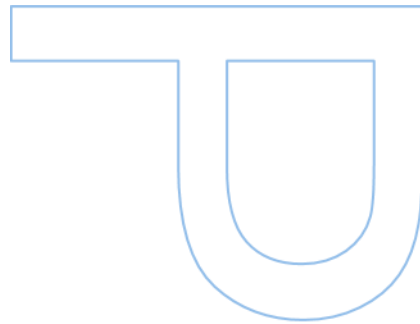
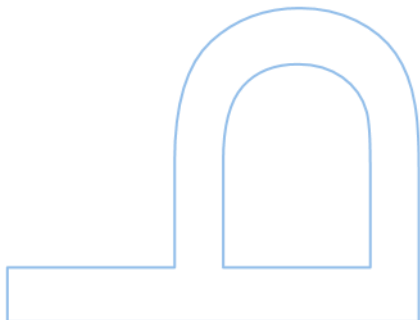
Dismantling Blind-Trusted Black Boxes: Testing the
Limits and Sensitivity of Forensic DNA Software Camila Ferreira da Costa

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Camila Ferreira da Costa
Doctoral Program in Biology
Department of Biology
Faculty of Sciences of the University of Porto
2026



Dismantling Blind-Trusted Black Boxes: Testing the Limits and Sensitivity of Forensic DNA Software

Camila Ferreira da Costa

Thesis carried out as part of the Doctoral Program in Biology
Department of Biology
2026

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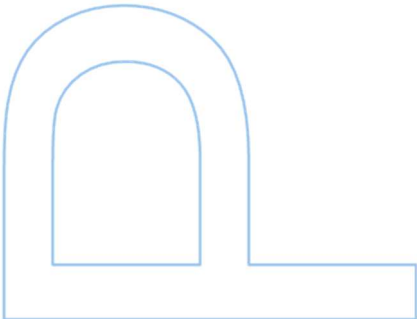
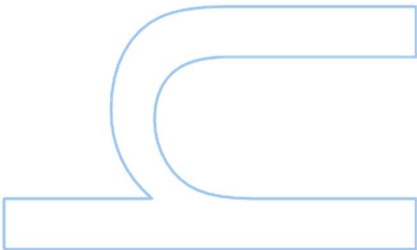
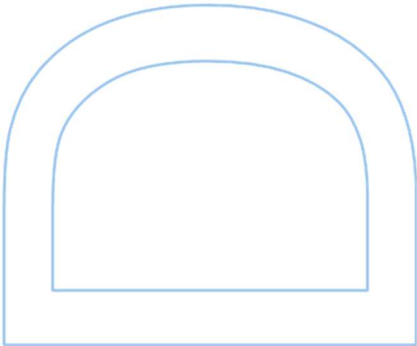


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À minha mãe

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Resumo

A interpretação de misturas complexas de ADN continua a representar um desafio na genética forense, sobretudo em casos que envolvem amostras vestigiais ou degradadas. Nos últimos anos, ferramentas informáticas baseadas em métodos de genotipagem probabilística (PG) têm sido desenvolvidas e implementadas oferecendo enquadramentos estatísticos e computacionais para lidar com este tipo de amostras. Estas ferramentas podem ser baseadas em modelos qualitativos, que consideram apenas os alelos observados no electroferograma da amostra problema, ou quantitativos, que consideram também a altura dos picos, ou seja, a quantidade de ADN. Em ambos os modelos, o valor da prova é quantificado através do cálculo de uma Razão de Verosimilhança (LR, do inglês *Likelihood Ratio*), que consiste na razão entre as probabilidades de observar a evidência assumindo duas hipóteses alternativas. Em problemas de identificação envolvendo misturas e uma amostra de referência de uma pessoa de interesse (Pol), estas hipóteses são tipicamente definidas como: “H1 = A Pol contribuiu para a mistura” e “H2 = A Pol não contribuiu nem está geneticamente relacionada com nenhum contribuidor da mistura”. Neste tipo de cálculo, são incorporados diversos parâmetros que devem ser previamente definidos, incluindo o número de contribuidores da amostra (estimado), o coeficiente de coancestralidade populacional, as frequências alélicas, o limite analítico e outros parâmetros relacionados com o drop-in, dropout e stutters. Até ao desenvolvimento e contemporâneas à presente tese, as análises comparativas publicadas sobre estas ferramentas baseiam-se exclusivamente em amostras simuladas e controladas. Embora importantes, estas amostras não reproduzem a complexidade dos casos reais, dificultando a extrapolação dos resultados para esses cenários práticos.

No contexto da genética forense e dos desafios associados a problemas de identificação, o principal objetivo deste projeto consistiu em avaliar o comportamento de distintos modelos estatísticos e computacionais de interpretação em condições operacionais. Para tal, três ferramentas de PG – uma qualitativa (LRmix Studio) e duas quantitativas (EuroForMix e STRmix™) – foram consideradas em condições harmonizadas, concebidas para manter as suas definições analíticas o mais idênticas possível. Posteriormente, após consolidação desta base operacional, a influência da variação dos supracitados parâmetros no resultado de cada modelo e nas interpretações produzidas foi analisada num crescente nível de escrutínio. Para tal, foi utilizado um conjunto amplo e diversificado de fontes de dados de misturas de ADN: simuladas *in silico*, produzidas em laboratório ou recuperadas de casos reais com

composição desconhecida. Esta tese é pioneira na utilização de amostras reais para avaliar ferramentas estatísticas na área da genética forense, fornecendo perspectivas práticas sobre o seu comportamento nas condições para as quais foram concebidas. Sempre que pertinente, foram também aplicadas simulações e métodos algébricos. Os resultados ilustram discrepâncias relevantes nas respostas dos modelos, enfatizando a necessidade de uma validação empírica robusta entre diferentes enquadramentos de modelação.

Quando foram consideradas condições semelhantes nos diferentes modelos implementados nas ferramentas, as discrepâncias observadas entre ferramentas qualitativas e quantitativas foram maiores do que as observadas entre ferramentas quantitativas, em concordância com os resultados previamente obtidos para amostras simuladas. Contudo, ainda assim foram observadas diferenças entre as ferramentas quantitativas – por vezes suficientemente substanciais para suportar hipóteses opostas –, evidenciando a sensibilidade dos cálculos de LR à modelação estatística subjacente.

Através da variação dos parâmetros considerados em cada modelo, como o número de contribuidores, o coeficiente de coancestralidade populacional, o drop-in (frequência e modelação), a inclusão de picos de stutter da evidência e os limites analíticos, demonstrou-se que tais parâmetros podem influenciar e condicionar o LR calculado. Em alguns casos, observaram-se efeitos contraintuitivos, destacando a complexa interação entre uma parametrização adequada e as características das amostras em análise. Estes resultados realçam a importância crítica dos laboratórios validarem e atualizarem regularmente as suas configurações de parâmetros e versões de software, bem como de assegurarem que os peritos compreendem plenamente os modelos estatísticos subjacentes a cada ferramenta, de modo a aplicá-los corretamente. No conjunto, o estudo fornece uma visão integrada de como as suposições específicas de cada ferramenta, as escolhas de parametrização e as condições das amostras influenciam a quantificação da evidência genética na atividade forense de rotina.

Adicionalmente, através do desenvolvimento e coordenação de uma comissão de trabalho internacional do Grupo de Línguas Espanhola e Portuguesa da *International Society for Forensic Genetics* (GHEP-ISFG), este estudo apresentou informações relevantes tanto para os peritos como para a comunidade científica no que diz respeito à implementação destas ferramentas na prática pericial. Ao promover um diálogo interdisciplinar, este exercício internacional destaca a importância da colaboração entre diferentes áreas, nomeadamente, a academia e a casuística, de forma a reforçar simultaneamente a robustez metodológica e a aplicabilidade prática neste domínio.

Em suma, garantir uma interpretação fiável de ADN forense requer formação contínua e elevada literacia estatística, sendo essencial compreender o funcionamento destas ferramentas. O progresso nesta área dependerá da melhoria da transparência dos modelos, da harmonização da parametrização, da definição de limites de aplicabilidade, da implementação de métricas de qualidade robustas e da manutenção de validações rigorosas. Embora as tecnologias emergentes ofereçam novas capacidades, a sua utilização deve assentar em práticas validadas e normalizadas. Em última análise, o avanço da área depende de esforços coordenados em formação, normalização e validação, de forma a preservar simultaneamente o rigor científico e a confiança operacional.

Palavras-chave: Software de Genotipagem Probabilística, Modelo Qualitativo, Modelo Quantitativo, Casos Reais, Misturas Complexas de ADN, Artefactos, Número de Contribuidores, Drop-in, Dropout, Stutters, Coeficiente de Coancestralidade, Limite Analítico

Abstract

The interpretation of complex DNA mixtures represents a persistent challenge in forensic genetics, particularly in casework involving trace or degraded samples. In recent years, probabilistic genotyping (PG) tools have emerged as a promising solution, offering statistical and computational frameworks for weighing such evidence. These tools can be based on qualitative or quantitative models; the former only consider the alleles observed in the evidence's electropherogram, while the latter also takes into account the peak height, i.e., DNA quantity. In both cases, the weight of the evidence is quantified through a likelihood ratio (LR). This calculation consists of comparing the probabilities of observing the evidence sample assuming two alternative hypotheses, which, in standard identification problems involving mixtures and a reference sample, are "H1 = The person of interest (Pol) is a contributor to the mixture" and "H2 = The Pol is not a contributor nor genetically related to any contributor of the mixture". Several parameters, such as the estimated number of contributors, population coancestry coefficient, allele frequencies, analytical threshold, and others related to drop-in, dropout, and stutters, need to be defined, as they are incorporated in calculations. To date, the comparative analysis published regarding these tools has relied exclusively on controlled mock samples, which, although important, do not reproduce the complexity of real forensic cases, making it difficult to extend the findings to real-world forensic scenarios.

Within the scope of forensic genetics and identification problems, the main objective of this project was to assess the behavior of distinct statistical and computational interpretation models under operational conditions. To this end, three PG tools, one qualitative (LRmix Studio) and two quantitative (EuroForMix and STRmix™), were examined under harmonized conditions designed to maintain their analytical settings as similar as possible. After establishing this aligned operational baseline, the influence of varying different parameters on the outcome of each model and the resulting interpretations was investigated. This evaluation was conducted using a diverse set of DNA mixture data sources, simulated *in silico*, laboratory-mock, and casework DNA samples with unknown composition. This work represents the first in forensic genetics to apply real-world samples in the assessment of statistical software, offering practical insights into these tools' behavior under the conditions for which they were designed. In addition, whenever applicable, simulation and algebraic approaches were employed. The findings highlight key discrepancies in the model responses and interpretation outputs, underscoring the need for robust empirical validation across modeling frameworks.

When similar conditions were considered by the different models implemented in the tools, discrepancies between qualitative and quantitative tools were greater than those observed between quantitative ones, consistent with previous findings from mock samples. Nevertheless, differences among quantitative tools were still observed – sometimes substantial enough to support opposite hypotheses – reflecting the sensitivity of LR computations to the underlying statistical modeling.

Through the variation of parameter settings considered within each model, such as the number of contributors, population coancestry coefficient, drop-in (frequency rate and modeling), inclusion of evidence stutter peaks, and analytical thresholds, it was demonstrated that these parameter conditions can considerably influence the LR assigned. In some cases, counterintuitive effects were observed, emphasizing the complex interplay between proper parameterization and data characteristics. These findings highlight the critical importance for laboratories to validate and regularly update their parameter configurations and software versions, as well as to ensure that practitioners fully understand the statistical models underlying each tool in order to apply them correctly. Overall, the study provides practical insight into how tool-specific modeling assumptions, parameter choices, and sample conditions collectively impact the quantification of genetic evidence in routine forensic casework.

Furthermore, through the establishment and coordination of an international working commission of the Spanish and Portuguese-speaking Working Group of the International Society for Forensic Genetics (GHEP-ISFG), this research also provides valuable perspectives for both forensic practitioners and the scientific community regarding the implementation of such tools in routine casework. By fostering an interdisciplinary dialogue, this international exercise highlights the importance of cross-disciplinary collaboration in strengthening both methodological robustness and practical applicability within the field.

In summary, ensuring reliable forensic DNA interpretation requires continuous training and deep statistical literacy, as understanding the assumptions and limitations of PG is essential. Progress in the field will depend on improving model transparency, harmonizing parameterization, defining limits of applicability, implementing robust quality metrics, and maintaining rigorous validation. Although emerging technologies offer new capabilities, their use must be grounded in validated and standardized practices. Ultimately, advancing the field relies on coordinated efforts in training, standardization, and validation to maintain both scientific rigor and operational trust.

Keywords: Probabilistic Genotyping Software, Qualitative Model, Quantitative Model, Casework samples, Complex DNA Mixtures, Artifacts, Number of Contributors, Drop-in, Dropout, Stutters, Coancestry Coefficient, Analytical Threshold

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General Introduction

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List of Abbreviations

PG	Probabilistic Genotyping
LR	Likelihood Ratio
Pol	Person of Interest
GHEP-ISFG	Spanish and Portuguese-speaking Working Group of the International Society for Forensic Genetics
STR	Short Tandem Repeat
CE	Capillary Electrophoresis
PCR	Polymerase Chain Reaction
MPS	Massively Parallel Sequency
CCD	Charge-coupled Device
EPG	Electropherogram
RFU	Relative Fluorescence Units
AT	Analytical Threshold
MLE	Maximum Likelihood Estimation
MCMC	Markov Chain Monte Carlo
NoC	Number of Contributors
adjLogLik	Adjusted log-likelihood
AIC	Akaike information criterion
MAC	Maximum Allele Count
IBD	Identical-by-descent
NRC II	Second National Research Council
SR	Stutter Ratio
ISFG	International Society for Forensic Genetics

GENERAL INTRODUCTION

General Introduction

1. Forensic Genetics: Evolution and Relevance

In 1900, Karl Landsteiner described the ABO blood grouping system, which allows for classifying individuals based on their blood types: A, B, AB, and O [1] – marking the first step in the emergence of a branch known as Forensic Hemogenetics. Due to advances in molecular biology over time, new techniques and methodologies were developed and applied to genetics. These progresses resulted in the replacement of blood typing with DNA profiling, consequently leading to the evolution of Forensic Hemogenetics into Forensic Genetics [2] – Figure 1.

Forensic Genetics is defined by *Forensic Science International: Genetics* – the second leading journal in the field of Legal Medicine according to the 2025 Journal Citation Reports™ (available at <https://mjl.clarivate.com/> accessed on December 9, 2025) – as “The application of genetics to human and non-human material (in the sense of a science with the purpose of studying inherited characteristics for the analysis of inter- and intra-specific variations in populations) for the resolution of legal conflicts.” [3]. Indeed, the applications of Forensic Genetics are varied, including the analysis of material recovered from a crime scene, paternity and other kinship tests, human remains identification, and even the analysis of non-human DNA [4, 5]. Nevertheless, the core activity of most forensic problems is typically divided into two modes: evaluative and investigative. The evaluative mode relies on the analysis and comparison of evidence and reference samples from the victim(s), suspect(s), or both. In contrast, the investigative mode involves analyzing the evidence without any known suspect [6]. The conclusions drawn in both cases are then summarized in a report to support further investigation.

The strength of the Forensic Genetics field relies on several key elements, including DNA technological advancements, strong and robust theoretical and genetic foundations, rigorous validation protocols, robust statistical approaches, and a substantial body of peer-reviewed literature. Nevertheless, the field’s success and reputation for reliability can sometimes overshadow persistent issues. Thus, despite the abovementioned features that position Forensic Genetics as the gold standard within forensic sciences, it continues to face notable difficulties [7, 8]. An important challenge is the interpretation of complex DNA mixtures, often containing very low levels of template, where subjectivity and inconsistencies between analyses remain a concern and have been documented and discussed in the literature throughout the years [9-11].

Moreover, pitfalls in experts' education and training, particularly in the application of statistical principles that are increasingly complex, can affect the reliability of forensic analyses. The misalignment between theoretical knowledge and the practical challenges of casework among academic researchers and practitioners may further widen these issues, underscoring the importance of closer collaboration and ongoing professional development [12-14].

1900	1920s – 1930s	1953	1975	1977	1980	
Karl Landsteiner described the ABO blood grouping system [1, 15-16]	Protein and enzyme typing for forensic purposes [17]	Watson and Crick discovered the DNA molecule structure [18-19]	Detection of polymorphisms using Southern blotting [20]	Sanger and co-workers developed the Sanger Sequencing method [21]	Wyman and Wilson described a highly polymorphic locus: Variable Number of Tandem Repeats (VNTRs) [22]	
1983	1985	1986	1988	1989	1990s	
Kary Mullis developed the Polymerase Chain Reaction (PCR) [23-26]	Alec Jeffreys developed the "DNA fingerprint" method [27-29]	First DNA-based criminal investigation culminating in the conviction of Colin Pitchfork [30]	Creation of the first forensic DNA laboratories [31]	Characterization of Short Tandem Repeats (STRs) [32]	VNTRs replaced by STRs. PCR using STR technique adopted [33-35]	
1991	1992	1993	1996	1998	1999	2001
First forensic identification using mtDNA from skeletal remains [36-37]	Discovered the first Y-STR and application to forensic [38]	Capillary electrophoresis of STRs first applied [39]	Heid and co-workers described the Real Time quantitative PCR method [40-41]	Launch of NDIS – the national level of CODIS (the first USA DNA database) [42]	Single Nucleotide Polymorphism (SNPs): A new type of marker for forensic purposes [43-48]	Description of X-linked STR and potential forensic application [49]
2007	2010s	2015	2015	2018	2020s	2020s
LoComatioN: one of the first probabilistic genotyping software [50]	Forensic Next Generation Sequencing (NGS) developed [51-52]	Development of a multiplex kit of SNP-STR forensic DNA markers [53]	Emerging field of Forensic Epigenetics [54-55]	"Golden State Killer" case: Successful use of forensic Genetic Genealogy [56-57]	Predictive Forensic DNA Phenotyping [58-59]	Use of Human environmental DNA (eDNA) in Forensic Genetics [60-62]
						Present Day

Figure 1: Key milestones in the History of Forensic Genetics since 1900 [1, 15-62].

2. The Nature of Forensic Genetic Evidence

2.1. Analyzing the Biological Sample: Forensic DNA Profiling

Autosomal Short Tandem Repeats (STRs) remain the standard markers for DNA profiling due to their high variability and discriminatory power [63]. In typical forensic genetics settings, the prevailing method of detection is capillary electrophoresis (CE) [64], which separates and quantifies specific STR fragments amplified by polymerase chain reaction (PCR) [65]. Although Massively Parallel Sequencing (MPS) has emerged as a more advanced and informative technology [66, 67], CE continues to be the method most widely employed in routine forensic casework due to its robustness, efficiency, and established validation protocols.

The CE consists of the migration of PCR products through a narrow glass capillary filled with a polymer solution, allowing their separation based on size. As the DNA fragments pass through a detection window in the capillary, an argon ion laser excites the fluorescent dyes incorporated via labelled PCR primers, causing them to emit fluorescence [68]. The intensity of the fluorescence is detected by a charge-coupled device (CCD) camera, generating an electropherogram (EPG) [69] – a graphical output displaying peaks, each one representing a DNA fragment of a specific length. The peak height, measured in relative fluorescence units (RFU), is proportional to the amount of fluorescence emitted, and thus to the amount of PCR product detected – the more fragments of the same length, the higher the peaks. During this process, an internal lane size standard containing fluorescently labeled DNA fragments of known lengths is analyzed alongside the sample, allowing the sizing of sample fragments [70]. Allelic ladders – containing all previously sequenced alleles for each locus – are also run in parallel and co-electrophoresed with an internal size standard, being crucial for accurate allele calling since it allows sample allele sizes to be directly compared with the known sizes in the allelic ladder [69]. To distinguish true allelic peak signals from background noise, an analytical threshold (AT – detailed in Section 3.2.4) needs to be established. As only signals above the defined AT are considered true allelic peaks, the proper setting of this parameter is essential for accurately interpreting the genotyping. If set too high, it may result in the loss of true alleles, whereas if it is set too low, artifacts and/or noise may be misinterpreted as true alleles [71].

2.2. Reading Between the Strands: Features and Challenges in Forensic DNA Samples

As previously stated, in forensic genetics casework, it is common to encounter biological samples with an unknown composition that may contain genetic material from more than one contributor, resulting in a mixed DNA profile [72]. Several characteristics associated with such samples add different levels of complexity and difficulty to their interpretation. These include allele sharing between contributors, contributions in different proportions, the presence of stochastic effects (heterozygotic peak imbalance, allele drop-in and/or dropout – detailed in Section 2.2.1), or even stutter peaks (also described in Section 2.2.1), which can be easily mistaken for alleles from a minor contributor (or otherwise) [73-75].

Furthermore, forensic samples are frequently exposed to environmental factors, so it is not uncommon for highly degraded DNA profiles to be analyzed in a criminal context. The extent of degradation depends on both the nature of environmental conditions and the duration of exposure. Environmental exposure can lead to various chemical processes that randomly break down DNA molecules into smaller fragments, compromising both the quality and quantity of recoverable DNA. In these cases, the amplification efficiency tends to decrease with increasing fragment size, resulting in a characteristic peak imbalance profile (Figure 2) – larger fragments are less likely to be successfully amplified, and a preferential amplification of smaller ones is observed [76].

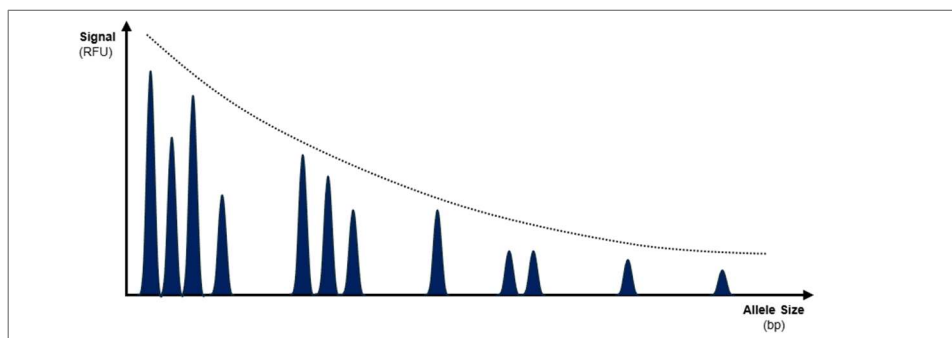


Figure 2: Illustration of the effect of degradation on DNA CE-STR profiles, showing a typical degraded profile with a gradual decrease in product quantity as amplicon size increases.

The frequent presence of low-template DNA samples in the forensic context has motivated the development of molecular techniques to improve the sensitivity of detection, enabling the analysis of increasingly smaller amounts [77-79]. However, this heightened sensitivity also introduces complications that hinder the distinction between true genetic profiles and extraneous material, such as an increased probability of detecting background noise, artifactual signals, and even low-level contamination [64].

2.2.1. Biological and Technical Artifacts in Forensic DNA Data

Both biological and technical artifacts may be generated during different stages of the forensic genetic samples' DNA profiling process, which can subsequently complicate allele interpretation and genotyping.

The most encountered artifact in an EPG of an evidence sample is a stutter peak. This artifact arises from slipped-strand mispairing during PCR amplification, caused by DNA polymerase slippage during the extension phase. The temporary dissociation of the DNA polymerase from the DNA strands allows a loop to form in either the template or the extending strand. If the loop forms in the template strand, a repeat unit is deleted in the newly synthesized strand; but, if it occurs in the extending strand, an additional repeat unit is incorporated [80, 81] (Figure 3). The former results in a back stutter peak, which is more frequently observed and whose height is proportional to 5–10% of the parent allelic peak height; the latter produces a forward stutter peak, which is less common and represents a smaller fraction of the parent allelic peak height (0.5–2%) [32, 81, 82]. This lower frequency of forward stutters compared to back ones may be due to the higher energy required to occur a forward shift or to structural limitations within Taq polymerase [83]. Double back stutters (two repeats' units shorter), and intermediate stutters (e.g., two base-pair shorter in D1S1656 and SE33 [84]) are also observed to a much lesser extent [85]. In each PCR cycle, stutter products are locus- and allele-specific, with longer alleles and certain loci exhibiting higher stutter rates [86]. Although this artifact can be easily identified in single-source samples, it can complicate the DNA profile interpretation in cases with more than one individual, where distinguishing stutters from minor contributor alleles becomes particularly challenging, as previously stated [73-75].

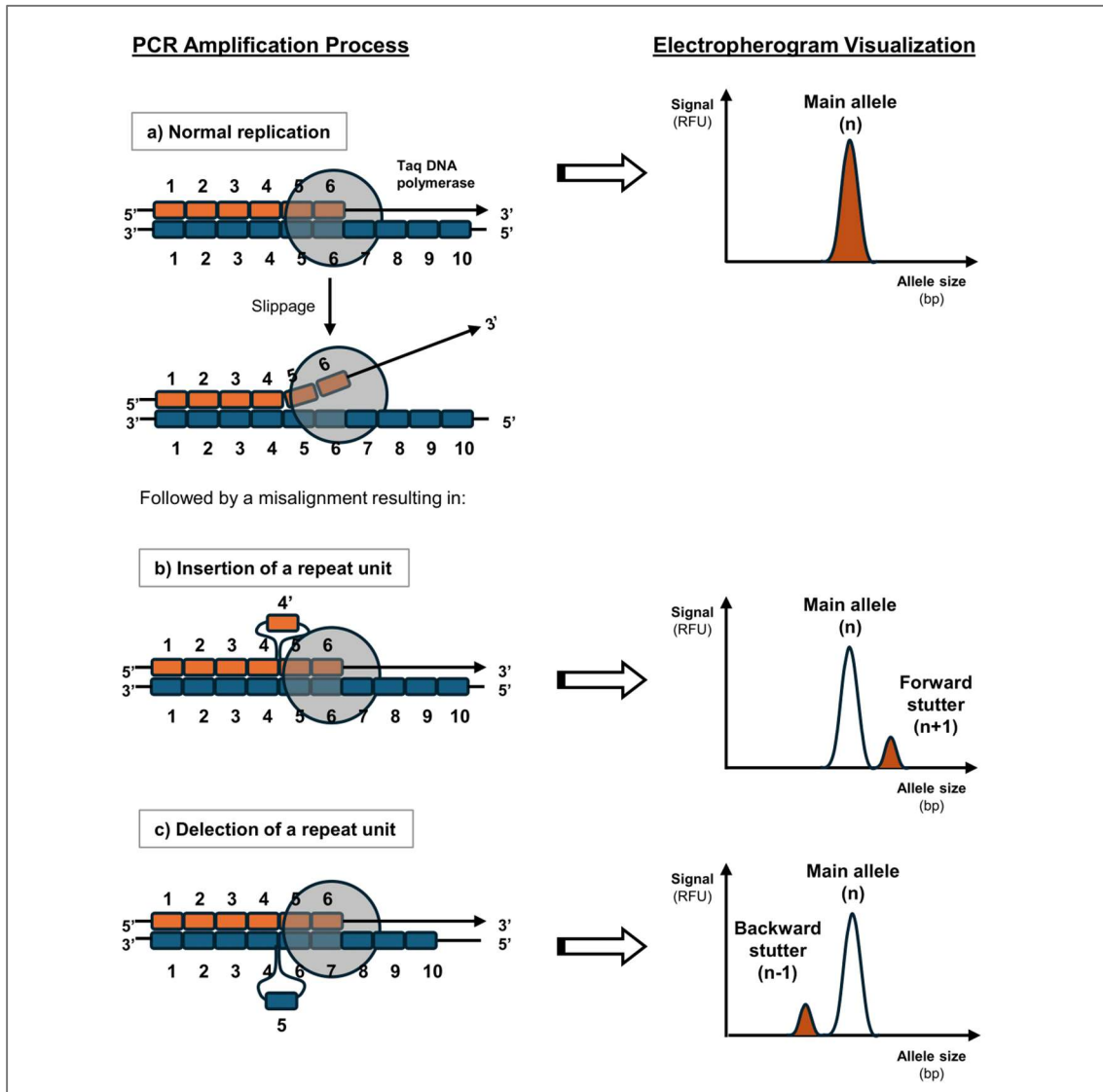


Figure 3: Illustration of the slipped-strand mispairing process that leads to the formation of stutter artifacts during the extension phase of PCR amplification. In (a), normal DNA replication occurs with both strands aligning correctly. In (b), the newly synthesized strand forms a loop due to slipped-strand mispairing, resulting in the insertion of one repeat unit. In contrast, in (c), the loop occurs in the template strand, leading to the deletion of one repeat unit in the newly synthesized strand.

During the amplification process, other artifacts can also be produced, especially when amplifying low amounts of DNA template, as they are more prone to stochastic effects such as heterozygous peak imbalance, allele drop-in, and dropout (Figure 4). The stochastic PCR amplification intensifies heterozygote peak imbalance by preferentially amplifying one of the two alleles at a heterozygotic locus. An extreme form of heterozygote peak imbalance is the allele dropout, where one of the two alleles is not amplified, potentially resulting in false homozygosity [87, 88]. On the other hand, an allele drop-in is a spurious allele that appears sporadically due to contamination or random amplification events whose source is unrelated to the sample, and therefore, is not explained by any contributor [89]. All these features will influence and add a layer of

difficulty to DNA profile interpretation. The peak imbalance makes the deconvolution of the contributors to the mixture more difficult. At the same time, both allele drop-in and dropout will create discordances between the evidence sample and the contributor profile(s), which can result in misinterpretations and, consequently, misleading conclusions [77, 90].

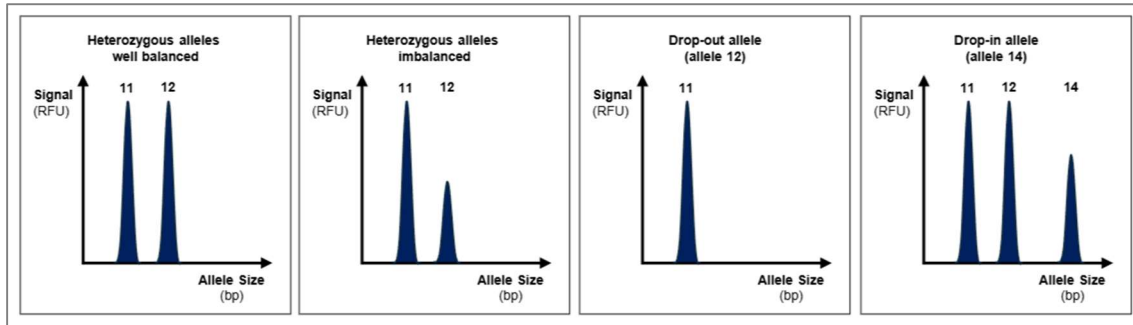


Figure 4: Schematization of the impact of stochastic effects during low-template DNA amplification. The leftmost figure represents a heterozygous genotype without stochastic effects. The remaining figures illustrate the possible stochastic effects: heterozygote imbalance, dropout, and drop-in.

On the other hand, an excessive amount of DNA template can also lead to the formation of artifacts during amplification, namely, split peaks – another PCR product artifact. During PCR amplification, Taq polymerase (DNA polymerase) synthesizes new DNA strands, adding a non-template extra nucleotide (usually an adenine) to the 3' end of the PCR product, making the new strand one base pair longer than the target sequence. This process, known as adenylation, is primer-specific, meaning that different loci may exhibit different adenylation efficiencies due to variations in primer sequences. This results in some PCR products receiving the additional adenine (+A), while others do not (–A), generating split peaks in the EPG (Figure 5). Despite the possibility of interpreting profiles with split peaks, difficulties can occur when the alleles are separated by only a single nucleotide. For example, in marker TH01, a non-adenylated 10 allele (–A) might be mistaken for a fully adenylated 9.3 allele (+A). A high amount of DNA template can also result in incomplete adenylation due to a limited amount of Taq polymerase relative to the number of target molecules, reinforcing the need for DNA quantification before amplification. To avoid this outcome, commonly used approaches include a final extension step and adjustments to reverse primer sequences within the STR protocol [91-93].

Moreover, in the process of STR separation and detection, that is, during the CE, several analytical artifacts can be generated, such as pull-ups, spikes, and dye-blobs [94]. Another consequence of over-amplification is that the CE instrument can become overwhelmed, exceeding its detection range. This saturation prevents the system from

accurately distinguishing between the fluorescent dye colors used to label different alleles. As a result of spectral overlapping, off-scale peaks can cause fluorescence from one dye to bleed into adjacent spectral channels, producing artifacts known as pull-up peaks. These peaks typically appear in the channel immediately above or below the original saturated signal (Figure 5). The occurrence of such artifacts can be minimized through a prior DNA quantification followed by appropriate dilution [95, 96]. An easily identifiable artifact is the spike, as it typically appears at the same data point across all dye sets (Figure 5). This analytical artifact results from voltage fluctuations during electrophoresis caused by air bubbles within the CE capillary, crystal formation in the polymer, or even contamination of the polymer by fluorescent material [94]. Finally, when the fluorescent dye dissociates from the labeled primer, the released residual dye molecules are detected by CE, resulting in an artifact known as dye blobs. To prevent and minimize this issue, PCR products should be filtered to remove residual dye molecules. Nevertheless, due to their predictable location and broad peak shape, dye blobs can be readily identified in the STR profile (Figure 5) [97].

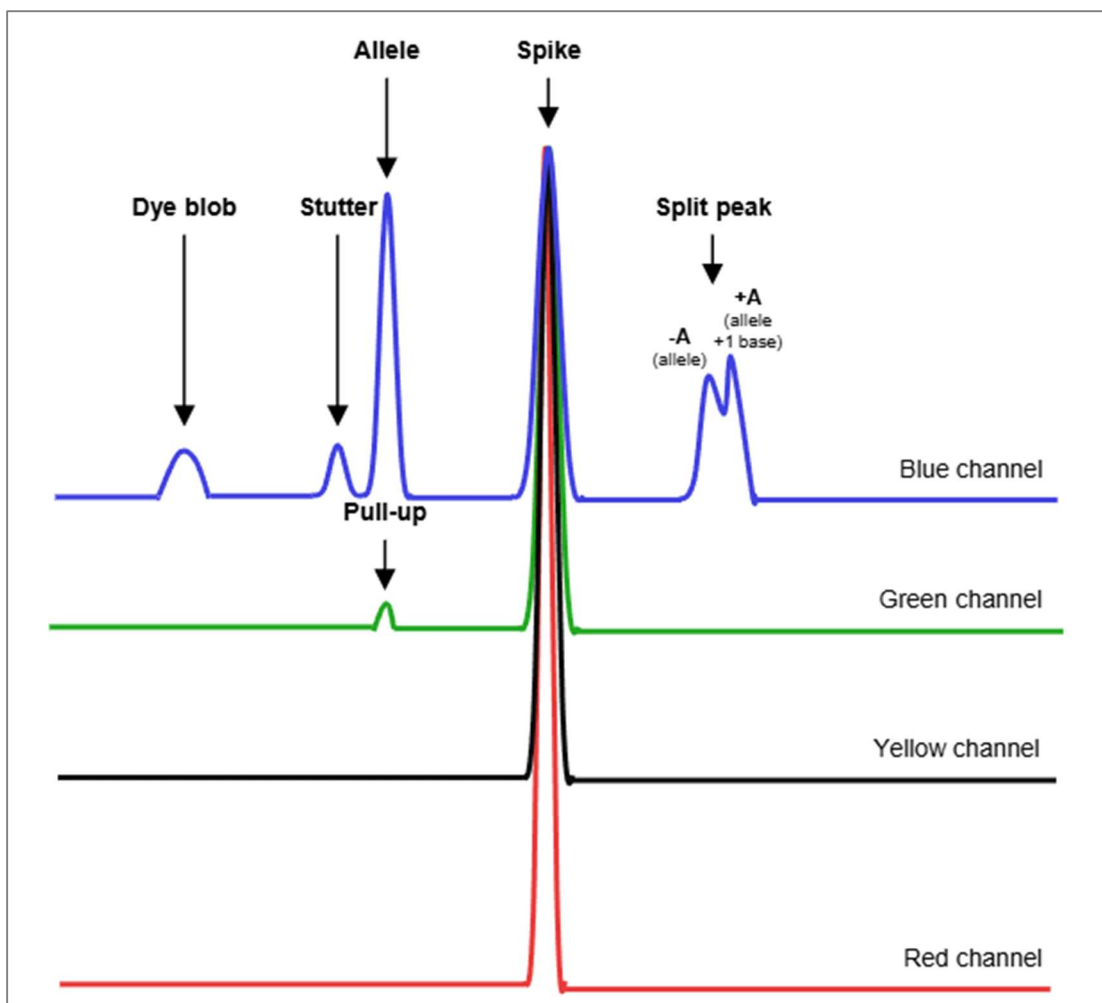


Figure 5: Display of the biological and technical artifacts often encountered in CE-STR profile analysis.

3. Quantifying the Forensic Genetic Evidence

More than four decades ago, the concept of likelihood ratios (LRs) was introduced to forensic science [98] and is currently the recommended method for quantifying the weight of evidence [99]. This approach derives from Bayes' Theorem, in which:

Posterior odds = Likelihood Ratio (LR) × Prior odds

$$\frac{\Pr(H1|E)}{\Pr(H2|E)} = \frac{\Pr(E|H1)}{\Pr(E|H2)} \times \frac{\Pr(H1)}{\Pr(H2)}$$

Assuming equal prior odds for the two hypotheses (H1 and H2), meaning they are considered equally probable before analyzing the DNA evidence (E), the ratio of the probabilities of the DNA evidence given the hypotheses (posterior odds) equals the LR. Consequently, the LR compares the probability of observing the DNA evidence under the two competing hypotheses (H1 and H2):

$$LR = \frac{\Pr(E|H1)}{\Pr(E|H2)}$$

In standard identification problems, where there is typically an evidence sample (E , usually a mixture) and a reference sample of a person of interest (Pol), these hypotheses are formulated as “H1 = The Pol is a contributor of the evidence sample” and, alternatively, “H2 = The Pol is not a contributor nor genetically related to any contributor of the evidence”.

3.1. Statistical Models for Interpreting Forensic DNA Evidence

Different approaches and statistical models can be used to interpret DNA forensic profiles, including binary, semi-continuous (or qualitative), and continuous (or quantitative) models.

The binary method is based on the assumption that a specific genotypic profile of interest is either present or absent in the evidence sample. In practice, this involves assigning a value of zero (for the excluded genotypic configuration) or one (for the included configuration) to the probability that the genotype is present in the questioned sample, given those considered as possible [100]. Nevertheless, this simplistic approach to DNA interpretation cannot account for stochastic effects and artifacts, as well as other mixture's characteristics known to influence statistical interpretation. Combined with the difficulty of applying this framework to evidence samples involving more than two

contributors, this early qualitative model has been gradually replaced by more sophisticated methods such as probabilistic genotyping (PG) systems. These can be based on either a semi-continuous model – which, in addition to considering peak presence, also incorporates probability of allele dropout and drop-in – or a continuous model, in which observed peak heights are modeled by some statistical distributions [101]. Over the past decade, several PG tools have been developed to assist forensic experts in interpreting and assigning LR values to quantify the strength of the evidence [102, 103]. These tools incorporate robust informatics modeling, enabling the weighting of individual genotype contributions through integrated biological and statistical frameworks. While anchored in an LR approach to quantify the evidence, they rely on different probabilistic models and distributional assumptions to assess the data and perform the computations, as outlined in Table 1.

From the currently available tools and respective models, the three PG tools most used worldwide among the forensic genetic community were the focus of this project: one based on a semi-continuous/qualitative model (LRmix Studio) and two based on a continuous/quantitative model (EuroForMix and STRmix™).

Table 1: List of several PG tools developed to quantify the weight of the genetic evidence considering CE-STR profiles, and respective parameters and models. X: Parameter considered by the software; SC: Semi-Continuous; C: Continuous; γ : Gamma; λ : Lambda; MLE: Maximum Likelihood Estimation; MCMC: Markov Chain Monte Carlo; Freq.: Frequency; Distrib.: Distribution; Opt: Optimal; AT: Analytical Threshold; θ : coancestry coefficient; IBD: Identity-by-descent parameters; Deg.: Degradation; NoC: Number of contributors; -?: Information not found.

Software	Commercial Product	Model	Inference approach	AT	Peak Height Distrib.	Population				Allele drop-in		Allele dropout	Deg.	Stutters	NoC	Allow relatedness	Ref
						Allele Freq. Data base	θ	IBD	Mutation and Silent Alleles	Freq	Distrib.						
LoComatioN	Unavailable	SC	Bayesian	-	-	X	X	-	-	X ^b	-	X ^b	-	X	X ^a	No	[50]
LRmix Studio	No	SC	MLE	-	-	X	X ^a	-	-	X ^{ab}	-	X ^{ab}	-	-	X ^a	No	[104]
Lab Retriever	No	SC	MLE	-	-	X	X ^a	X ^a	-	X ^{ab}	-	X ^{ab}	-	-	X ^a	No	[105]
relMix	No	SC	MLE	-	-	X	X ^a	-	X ^{ab}	X ^{ab}	-	X ^{ab}	-	-	-	Yes	[106]
SmartRank	No	SC	MLE	-	-	X	X ^a	-	-	X ^{ab}	-	X ^{ab}	-	-	X ^a	No	[107]
ProbRank	No [*]	C	MLE	X	γ	X	X ^a	-	-	X	λ	X	X	X	X ^a	Yes	[108]
EuroForMix ¹	No	C	Bayesian or MLE	X ^a	γ	X	X ^a	-	-	X ^{ab}	λ^a	X ^b	Opt.	Opt.	X ^{ad}	Yes	[109]
STRmix™	Yes	C	Bayesian using MCMC	X ^a	Log-normal	X	X ^a	-	-	X ^{ab}	γ^a or Uniform	X ^b	X ^b	X ^b	X ^{ad}	Yes	[110]
DNAXs/ DNAstatistX	No ^{**}	C	MLE	X ^a	γ	X	X ^a	-	-	X ^b	λ	X ^b	X ^b	-	X ^{ad}	No	[111]
DNA-VIEW®	Yes	C	-?-	X	γ	X	X	-	-	X	X	X	X ^b	X ^b	X ^a	No	[112]
GenoProof Mixture 3	Yes	C	Bayesian using MCMC	X ^a	Log-normal	X	-	-	-	X ^{ab}	γ^a	X ^b	X ^b	X ^b	X ^a	No	[113]

LikeLTD	No	C	MLE	X ^a	γ	X	X	X ^e	-	X ^b	X ^b	X ^b	X ^b	X ^b	X ^a	Yes	[77]
TrueAllele®	Yes	C	Bayesian using MCMC	-	Truncated normal	X	X ^a	-	-	X	X	X ^b	X ^b	X ^b	X ^a	No	[114]
EFMrep ¹	No	C	Bayesian or MLE	X ^a	γ	X	X	X ^a	-	X ^{ab}	λ ^a	X ^b	Opt.	Opt.	X ^a	Yes	[115]
EFMex ¹	No	C	MLE	X ^a	γ	X	X ^a	-	-	X ^{ab}	λ ^a	X ^b	Opt.	Opt.	X ^a	Yes	[116]
CaseSolver ¹	No	C	MLE	X ^a	γ	X	-	X ^a	-	X ^{ab}	λ ^a	X ^b	Opt.	Opt.	X ^a	No	[117]
DNAmixtures	No ***	C	MLE	X ^a	γ	X	-	-	-	-	-	X ^b	-	X ^b	X ^a	No	[118]
KinMix	No ***	C	MLE	X ^a	γ	X	-	X ^a	-	-	-	X ^b	-	X ^b	X ^a	Yes	[119]
eDNA	No ****	C	Numerical Bayesian, Bayesian using MCMC, and MLE	X	γ	X	X ^a	-	-	X ^{ab}	λ ^a	X ^b	Opt.	Opt.	X ^{ad}	No	[120]
LiRa	Yes	C	Bayesian	X	γ	X	X	X	-	X ^b	γ	X ^b	X	X	X ^a	Yes	[121]
Kongoh	No	C	MCMC	X ^a	γ	X	X	-	-	-	-	X	X ^{ad}	X ^{ad}	X ^{ad}	No	[122]
MaSTR	Yes	C	MCMC	X ^a	-?-	X	X ^a	-	-	X ^{ab}	-?-	X	Opt.	X	X ^a	Yes	[123]
SMART	No *****	C	MCMC	X ^a	Log- normal	X	X ^a	-	-	X	γ ^a or Uniform	X	X	X	X ^a	No	[124]

* Can be accessed via a command-line option in DNASTatistX (available upon request).

** It is necessary to sign a license agreement.

*** The software is free of charge, but to use it, it is necessary to install the commercially licensed software Hugin Expert.

**** Freely available to Bulletproof[®] consortium members.

***** commercial software, but researchers who want to analyze mixed DNA profiles can download it from GitHub (<https://github.com/CGforensic/SMART>) and request permission to use it.

¹ Source code based on EuroForMix.

^a Value introduced by the user.

^b Parameter modeled by the software.

^c Estimates the expected total contribution of drop-in to peak height (in RFU), which is assumed to be distributed across all alleles at the locus in proportion to their population allele fractions.

^d Users can also introduce a range of values for the tool to test the best fit one.

^e Condensed coefficients of identity by descent (Δi) are used to characterize and quantify relationships among mixture contributors.

3.1.1. Semi-Continuous/Qualitative Models

A semi-continuous model is also referred to as a qualitative model, due to the nature of the information considered from the evidence EPG when quantifying the weight of the evidence sample. By relying solely on the qualitative information – i.e., the alleles present/absent in the evidence genotypic profile – this approach has simpler algorithms and computations, making it easier to understand and verify the results empirically, compared to a more complex continuous model. Thus, a critical limitation of this approach is the loss of important information, particularly peak height data, which can contribute to greater accuracy and discriminative power of the analysis in complex DNA mixtures [125, 126].

This approach marked an important step in the development of PG tools. While these systems did not account for peak height information, they do represent an advance over the binary model by accommodating potential allele dropout or drop-in and considering multiple contributors. Although software like LRmix Studio [104], which was based on this model, is no longer updated, it continues to be used in some laboratories. However, as forensic genetic data becomes increasingly complex, experts are gradually shifting from this simpler approach to more advanced systems based on continuous models, which are better suited to handling the challenges of modern casework.

3.1.2. Continuous/Quantitative Models

Like the previous, the continuous model can also be referred to as a quantitative model, based on the information used to quantify the weight of the evidence. The tools based on this model consider not only qualitative but also quantitative information extracted from the evidence EPG – that is, both the detected allele peaks and their corresponding heights [127-129].

These quantitative approaches employ different statistical frameworks to model how allele peaks are expected to appear in DNA profiles. They incorporate various factors that influence profile interpretation, including DNA quantity, degradation, artifacts, and other sources of variability. Unlike qualitative methods, which do not consider peak height information or model artifacts and stochastic effects in the EPG, quantitative models make full use of this data. As a result, these methods generally outperform qualitative ones and are considered more efficient and reliable [74, 130]. For this reason, they are often regarded as the preferred approach for analyzing complex DNA mixtures. The drawback of this approach is that, due to the large number of variables and the

complexity of the models, it can be more difficult to control and verify exactly how the software produces its results [131].

Despite the growing interest in developing tools for analyzing data produced by MPS [132-136], CE-based tools remain the most widely employed in routine forensic casework due to their well-established and comprehensive application in the field [137]. Among these tools, EuroForMix [109] and STRmix™ [128] stand out as the two continuous tools broadly used for interpreting mixed DNA profiles in forensic investigations. Even though both informatics tools consider similar parameters to quantify the weight of the evidence, they differ substantially in their architecture and underlying statistical frameworks (Table 2). EuroForMix (<http://www.euroformix.com/>) is an open-source software implemented in R [138]. This deterministic tool supports both frequentist (via maximum likelihood estimation – MLE) and Bayesian (via integration) approaches to assessing likelihood ratios for DNA mixture interpretation. In contrast, STRmix™ (<http://strmix.esr.cri.nz/>) is a commercial software designed to interpret complex DNA mixtures using a Bayesian framework. It incorporates prior distributions for unknown parameters and employs stochastic Markov Chain Monte Carlo (MCMC) sampling to explore the parameter space. This enables the software to generate weighted genotype possibilities by sampling across potential genotypes of unknown contributors and associated parameter values [128]. While this approach allows for flexible modeling and uncertainty quantification, it can be computationally demanding. If the MCMC chains are not run for enough iterations, or if proper convergence diagnostics are not applied, critical areas of the parameter space may remain insufficiently explored.

Table 2: Software properties, key parameters, and corresponding statistical models applied by EuroForMix and STRmix™ to quantify the weight of the genetic evidence.

		EuroForMix	STRmix™
Properties	<i>Inference approach</i>	Bayesian or Maximum Likelihood Estimation (MLE)	Bayesian
	<i>Peak height distribution</i>	Gamma	Log-normal
	<i>Evidence files with stutter</i>	Optional ^a	Required
Software Parameters	<i>Number of contributors</i>	Input (Fixed value or range ^a)	Input (Fixed value or range ^a)
	<i>Coancestry Coefficient</i>	Input ^b (Fixed value)	Input ^b (Fixed value or beta distribution)
	<i>Dropout Frequency</i>	Directly modeled by the tool	Directly modeled by the tool

	<i>Drop-in Frequency</i>	Input ^b	Input
	<i>Drop-in Distribution Modeling</i>	Exponential ^b (Lambda)	Gamma or Uniform
	<i>Analytical Threshold</i>	Input ^b	Input ^b
	<i>Degradation Modeling</i>	Optional ^a	Directly modeled by the tool

^a The software can test for the best-fit value or condition.

^b Parameter can be set on a per-marker basis.

The increasing interest in implementing these tools in routine forensic practice has been accompanied by a growing awareness of the crucial need for robust comparative studies and careful validation. Particularly, multi-center inter-laboratory studies have been emerging as valuable instruments in this regard, providing insights into variability across laboratories and different tools, and offering guidance for improving the consistency and reliability of forensic interpretations. Studies using mock DNA mixtures, i.e., with known composition – both contributors and corresponding ratios, have highlighted substantial variability in LR results, especially when different software tools or user interpretations are involved [139-153]. Notably, large-scale exercises have reported wide ranges in LR values, often driven not only by different statistical models themselves, but also by analyst-driven factors, such as defining analytical thresholds, allele assessment, or the number of estimated contributors [154-160]. These procedures are essential not only to ensure reliability, reproducibility, and transparency of results but also to deepen understanding of how each tool operates, to identify limitations, and assess potential discrepancies across different settings or protocols.

The purpose of validating PG tools consists of defining their scope, limitations, and reliability by assessing how well models reflect empirical data, even though all models are inherently approximations. Validation involves multiple phases – conceptual, software, and operational – and tests key aspects such as sensitivity, specificity, precision, accuracy, and limitations. Additionally, significant research efforts have been made to better understand trends, characterize different models, and establish best practice guidelines. Several publications from scientific societies [131, 161, 162] and validation studies from various laboratories have contributed to this field [110, 122, 163-170].

A standard procedure for all informatics tools is their continuous updating, including the correction of bugs that may cause the software to produce incorrect or unexpected results, as part of routine maintenance. Consequently, the users should always be informed and aware of these updates.

3.2. Key Software Parameters in DNA Evidence Interpretation

While validation and bug resolution are fundamental to ensuring the reliability of forensic software, a thorough understanding of the parameters built into these tools is equally important. The interpretation of DNA profiles and the quantification of evidential weight depend on a complex interaction between analyst decisions, population data, and modeling assumptions. These parameters not only condition the output but also affect the transparency, reproducibility, and robustness of the conclusions drawn in a forensic context. For this reason, understanding the assumptions and configurable settings within these systems is essential for accurate interpretation and clear communication of the results that should assure their reproducibility.

3.2.1. Estimating the Number of Contributors (NoC)

In a casework context, the true number of individuals who have contributed to the forensic genetic evidence is almost always unknown. Consequently, one of the first and most important steps in the DNA profile interpretation process is for the expert to assign an estimated number of contributors (NoC) [100]. This estimation plays a crucial role in guiding the analysis and statistical modeling of the evidence sample. However, this task is far from straightforward, as factors such as contributors in different proportions, allelic overlap, and stochastic effects and/or artifacts typically associated with such samples (described in Section 2.2.1) complicate both the interpretation process and the further estimation of NoC [77, 90, 171]. As a result, divergent assessments between experts are common, particularly in highly complex or degraded samples [9].

For example, experts may overestimate NoC when analyzing evidence samples with heterozygote imbalance and ambiguous peaks, such as allele drop-in or artifacts, especially augmented stutter peaks. These signals can be easily mistaken for true alleles originating from an additional contributor [172, 173]. Conversely, the NoC can also be underestimated, especially in low-template DNA samples. Such samples often contain alleles from contributors at very low levels, which can be confused with stutter peaks from a major contributor or even dropout, leading to underestimation of the true NoC. In addition, allelic overlap among contributors can further contribute to underestimating NoC, which tends to increase when the number of contributors increases, or when related individuals are involved [174].

Previous studies using mock and controlled mixture samples have shown that misassigning the NoC can have important consequences [175, 176]. Overestimating the

NoC may lead to false positive support with non-contributors ($\log_{10}(\text{LR}) > 0$, but usually low) or to a reduction in the LR values for true contributors; while underestimating it can result in false support for exclusion ($\log_{10}(\text{LR}) \leq 0$) to a true contributor [154, 176-179]. As expected, this will not impact all true contributors evenly, as minor contributors are evidently more susceptible to variations in the NoC compared to major ones. More specifically, their lower peak heights make the alleles of minor contributors more prone to being treated as artefacts to maintain consistency with the NoC, increasing the risk of false exclusion, an outcome far less likely for major contributors [180].

Nowadays, to circumvent this difficulty, some quantitative tools, such as EuroForMix [109] and STRmix™ [128], allow experts to input a contributor's number range, instead of a fixed value. For example, in EuroForMix, the NoC is decided by MLE based on the adjusted log-likelihood (adjLogLik) value, which works as the 'Akaike information criterion' (AIC) [181]. This parameter balances goodness of fit against model complexity to select the most appropriate statistical model for the dataset, favoring simpler models to explain the evidence. STRmix™ treats NoC as a nuisance parameter [182], describing the LR as a weighted average of the individual LRs conditioned on NoC [178, 183]. Whereas other tools only enable the input of a fixed, pre-established NoC, accurately estimating this parameter remains an important issue to address.

Several methodologies have been developed to assist the expert in estimating NoC in forensic DNA mixture profiles, which can be divided into three categories [184]. The first category includes basic methods, which are slow and often suffer from accuracy limitations [100]. The second methodology relies on high-performance computing approaches that offer faster processing but remain highly computing-intensive and still require significant accuracy improvements [185] and have not been widely adopted by the forensic communities. The third category comprises machine learning methods. Even though faster (in seconds), it needs further development as the work published in this area handles this question from different perspectives, lacking transparency regarding the prediction method, classification accuracy, and robustness [186, 187].

The elementary and widely used method is the maximum allele count (MAC) [100, 173]. This approach estimates the lower bound on the NoC based on the locus with the most (observed) alleles divided by two, rounded up to the nearest whole number. That is, it sets the minimum contributors required to explain the set of alleles observed in the sample. However, this tends to underestimate the NoC as the likelihood of all contributors having non-overlapping heterozygous profiles at a given locus – even highly polymorphic ones – decreases as the number of contributors increases. Consequently,

allele sharing becomes more likely, leading to an underestimation of the NoC [188]. Alternatively, other methods have been proposed that rely on various combinations of allele count, allele frequencies, peak heights, stutter ratios, dropout probabilities, and relatedness [177, 189-197]. Recent studies have explored the application of machine learning [198-201] and deep learning [202] to determine the NoC. Although these approaches perform better for mixtures with higher NoC, they pose significant challenges in terms of validation and implementation, mainly due to reproducibility issues.

3.2.2. Accounting for Population Substructure Through Coancestry Coefficient

Population structure develops through the combined influence of geographic, environmental, and behavioral factors that divide a natural population into subgroups or subpopulations [203]. The first genetic metrics for describing and quantifying population substructure were introduced by Wright [204]. Building on Wright's work, Weir and Cockerham later introduced the coancestry coefficient, also known as θ (theta) [205, 206]. Afterward, Balding and Nichols proposed an algebraic framework for incorporating this parameter into forensic DNA match probability calculations, accounting for population structure and coancestry in the population of interest [207]. This approach was further extended by Curran et al. to the interpretation of DNA mixture profiles [208].

The coancestry coefficient represents the probability that a pair of alleles at the same locus, randomly selected from two individuals within the same subpopulation, are identical-by-descent (IBD), i.e., derived from a common ancestor. It serves as an indicator of the background level of genetic relatedness (shared ancestry) within a (sub)population [207]. An increasing coancestry value indicates a higher probability that individuals within the subpopulation share alleles IBD, and thus this probability is further amplified in smaller, highly sub-structured, or geographically and reproductively isolated populations. Such conditions reduce genetic diversity between individuals, leading to greater estimates of genetic relatedness and consequently more conservative match probabilities [209-211]. Conversely, it has been recognized that disregarding population coancestry can lead to an overestimation of the weight of DNA evidence, particularly when the person of interest cannot be excluded as a contributor. This overestimation may result in biased interpretations. While this effect may have limited practical impact in cases with extremely strong statistical support (whether for inclusion or exclusion), its consequences become more concerning in complex, multiple-contributor mixtures or low-template samples, where the reduced genetic variability amplifies the uncertainty of the results [212].

In addition, the population coancestry influences genotype frequency estimates, thereby affecting the quantification of the weight of DNA evidence in forensic identification. Studies using simulated or mock single-source data have demonstrated that different estimators of this parameter can significantly impact the statistical outcome [213-216]. As forensic DNA analysis becomes increasingly common across diverse global populations, incorporating realistic coancestry values into probabilistic models remains essential to ensure the reliability of forensic evidence. As with other parameters considered when weighing DNA evidence [99, 217], the population coancestry coefficient is handled differently across informatics tools. Tools, such as LRmix Studio [104] and EuroForMix [109], require the user to specify a fixed value, whereas others, like STRmix™ [128], can also model it using a probability distribution.

Even though coancestry values are known to be locus-specific, it is standard practice in forensic genetics casework to apply a single estimate across all loci [214]. Given the difficulty of accurately determining this parameter for actual populations, many forensic laboratories rely on previously assigned values from similar populations. In the absence of population-specific data, those recommended by the second National Research Council (NRC II) for STR loci are commonly used, such as $\theta = 0.01$ for urban populations and $\theta = 0.03$ for more isolated populations [218].

3.2.3. Modeling Drop-in and Dropout Events

As discussed in section 2.2.1, stochastic events, such as allele drop-in and dropout, can cause inconsistencies between the evidence sample and the contributors' profiles. The former is due to the presence of an additional peak originating from an unrelated source, while the latter involves the absence of an allele from a known contributor. Both scenarios complicate data interpretation and may influence the conclusions drawn; therefore, considering and appropriately modeling these events is essential to ensure accurate analysis [77, 90, 219, 220].

Existing tools address the drop-in allele phenomenon by incorporating its frequency when quantifying the weight of the evidence, and in the case of quantitative tools that consider the allele's peak height, by also modeling the distribution of drop-in parameters.

The empirical estimation of the drop-in allele frequency is laboratory-specific and based on the number of negative controls on which an additional, unexplained, and unreproducible allele was observed. Negative control consists of an additional tube processed simultaneously with the biological samples under identical conditions, but

without the addition of any biological material [87]. As the drop-in frequency increases, the probability that an observed allele belongs to a contributor of the evidence decreases.

By analyzing drop-in peak height data, quantitative tools can estimate the distribution of drop-in parameters, which may be modeled differently depending on the statistical model implemented in the software [145, 221]. For example, considering the quantitative tools used in this work, EuroForMix models the drop-in through a lambda distribution (λ) [109], while STRmix™ applies either a gamma (γ) or a uniform distribution. For STRmix™ to properly estimate the parameters of the gamma distribution (α and β) and develop a reliable gamma model, a substantial number of drop-in observations is required; otherwise, the use of a uniform distribution is recommended [222].

Dropout events are also accounted for in analyses conducted by the informatics tools, eventually through different statistical approaches as occurs for other parameters [223-225]. When using qualitative tools, a value associated with the probability of dropout alleles must be incorporated into the calculations, which can be either introduced by the user or estimated by the software based on the input data [104]. On the other hand, as quantitative tools consider peak height information, the parameters related to this event are directly modeled through the function applied by the tool to model the peak height information without requiring or allowing user intervention [145]. As previously demonstrated, tools based on a quantitative model may have different built-in statistical methods to handle the same features. For example, in this case, EuroForMix utilizes a gamma distribution (γ) [109], whereas STRmix™ employs a log-normal distribution [222].

3.2.4. Defining and Applying Analytical Threshold

As defined in Section 2.1, an AT in forensic DNA analysis is a benchmark that defines the minimum RFU value set in the peak detection software to differentiate true genetic signals (and artifacts) from baseline noise. Baseline noise refers to non-reproducible peaks that may result from electronic fluctuations, air bubbles, crystals in the CE polymer, or sample contamination. Only signals above the established AT are considered true peaks able to be analyzed and considered in the computations, while those below are assumed to be baseline noise and usually disregarded [71].

ATs aim to minimize false positives and false negatives while promoting consistency and reproducibility across laboratories [226]. Nevertheless, special care is required when setting analytical thresholds, as they inevitably carry the risk of misclassifying peaks affecting the LR values assigned. True alleles with low peak heights may be undetected

if the AT is set too high, resulting in loss of information. Conversely, setting it too low increases the probability that baseline noise will be misinterpreted as true alleles [227].

Thus, the proper establishment of ATs is fundamental to the accurate interpretation of forensic data profiles, particularly in challenging cases involving low-template or mixed DNA samples. As there is no standard method defined for determining these thresholds, each laboratory is responsible for establishing its own through comprehensive internal validation procedures [228]. The selected approach should be adapted to the laboratory's specific needs and may vary depending on several factors, including the type of samples being analyzed. Due to these variables, AT can differ significantly between laboratories, and these differences can influence the analyst's ability to interpret complex DNA profiles correctly. Forensic tools commonly allow users to input RFU thresholds for each color channel, facilitating color-specific thresholds without adding complexity or time to the analysis. Applying a single AT across all channels is not necessarily more efficient and may be less accurate due to significant differences in signal strength between color channels. Therefore, using separate thresholds per color is generally recommended [227].

Recently, efforts have focused on replacing fixed ATs and manual EPG profile reading with a probabilistic approach in which neural networks assign probabilities to EPG signals (allele, stutter, pull-up, etc.), paving the way for automated, high-throughput, and more objective DNA profile interpretation, while reducing subjectivity between laboratories, and enabling the use of low-level information below the traditional threshold [229]. Nevertheless, such complex methods may be impossible to reproduce and may function as a "black box". Thus, to prevent this, the underlying mathematical and statistical frameworks must be clearly established and documented.

3.2.5. Understanding and Managing Stutter Peaks

The well-characterized stutter peak is a PCR product artifact (see section 2.2.1) which arises from a slipped-strand mispairing of DNA polymerase during the PCR extension phase [81]. It is the most prominent artifact observed in the EPG of the evidence genotypic profile. Most commonly, it appears as a peak one repeat unit smaller than the true (parental) allele peak, referred to as a back stutter. Occasionally, a forward stutter – one repeat unit larger than the parent allele – or a peak two repeat units smaller may also be observed. In some loci, such as D1S1656 and SE33 [84], intermediate stutters (e.g., two base pairs shorter) can also occur, though rarely [230].

Like drop-in and dropout events, stutter peaks can also complicate and mislead the interpretation of DNA profiles. However, due to their position and higher frequency, they are often more problematic, especially when involving mixed DNA profiles. Distinguishing stutter peaks from true alleles becomes particularly challenging when a low-level peak, potentially from a minor contributor, occurs at a stutter position. In such cases, alleles coinciding with stutter positions are critical, because misclassifying them as stutter can lead to the erroneous exclusion of potential suspects – an issue that is especially pronounced in mixed profiles where there is a significant imbalance between contributor proportions [73, 74]. Also, the presence of stutter peaks in EPG can contribute to an inaccurate estimate of the number of contributors of the evidence.

The empirical confirmation of the theoretical assumption that an allele's amplification product consists of both true allele copies and their associated stutter peaks [75] reinforces and solidifies the rationale for including stutter peaks into DNA profile evaluation. This approach maximizes the statistical significance of the LR value without erroneously discarding information from true alleles, as might occur when applying a stutter filter during the STR genotyping phase or relying on subjective human judgment to distinguish between stutter and true allele peaks. To address this, the stochastic behavior of the stutter ratio (SR), defined as the height of the stutter peak divided by the height of the parental allele peak, is described and predicted using statistical models [231-234]. A recent study suggests that aging may increase SR, potentially due to increased replication slippage in aging cells [235]. This finding contributes to a better understanding of the stochastic behavior of SR, having implications for improving DNA mixture interpretation in forensic casework and criminal investigations [104].

In the context of PG, several tools have incorporated stutter peak modeling into their frameworks to improve the interpretation accuracy, as is the case of the quantitative tools STRmix™ [128] and EuroForMix [109]. The first requires the inclusion of stutter peaks in the evidence profile, modeling locus-specific amplification efficiencies, and incorporating expected stutter ratios per locus, which are defined using empirical data [233]. In contrast, the latter offers flexibility, allowing the expert to decide whether to model stutter peaks, specify which types of stutters to include (back and/or forward), or let the tool test various stutter model combinations. The extension of the probabilistic model to include multiple types of stutters is achieved by applying a uniform expected stutter rate, rather than using locus-specific amplification efficiencies [145]. Regarding qualitative tools such as LRMix Studio, the inclusion of stutter peaks is not recommended, and a dedicated filter should be applied for tentatively removing them from the evidence sample [104].

OBJECTIVES

Objectives

Technological advances in forensic genetics have enabled the analysis of increasingly complex DNA samples, representing a significant asset in criminal investigations. Nevertheless, this progress has also introduced new challenges, particularly in the analysis of complex mixtures, which often contain low-quality DNA from multiple unknown contributors. Such profiles are susceptible to stochastic effects, including allele dropout, drop-in, and stutter artifacts, making the estimation of the weight of the evidence highly dependent on computational tools. The challenges lie not only in interpreting the results but also in how sensitive quantitative assessments are to the statistical models employed.

There are currently several probabilistic genotyping (PG) tools based on different statistical and mathematical models that allow a quantitative assessment of evidential value through the computation of a likelihood ratio (LR). Each laboratory has its own selection criteria for selecting one (or more) of these tools to apply in its routine. Despite their widespread use, uncertainties remain regarding the extent to which the choice of model, parameter settings, and software tool influences the weight assigned to the evidence. These uncertainties have raised questions about the consistency and robustness of LR values across methodological approaches.

With this in mind, the primary objective of this project was to assess the behavior of widely used computational and statistical models for interpreting complex forensic DNA mixtures. Recognizing that the strength of the quantitative evaluation may not only depend on the statistical models applied, but also on the complexity of the samples analyzed, different approaches were attempted regarding the dataset used. Thus, a wide range of types of sample pairs – including both real casework data from criminal settings (unknown composition) and mock ones (simulated *in silico* and from a public database; known composition) – was considered to reflect different challenges and levels of complexity. Each pair consisted of a DNA mixture (with two or three contributors, estimated number in the case of real samples) and a corresponding single-source reference profile. Where relevant, algebraic approaches were also applied. Under this framework, the specific objectives consisted of:

- a) Assess the impact of considering **statistical models with majorly different architectures**: either incorporating the peak height information or not, or when applying different inference approaches for such information. This was performed by conducting an inter-software analysis, which compared LR

values assigned to the same dataset of pairs of samples using tools based on distinct model architectures under harmonized parameter settings. To evaluate the practical implications in forensic practice, real casework mixture samples, with unknown compositions, were selected for this purpose (**Chapter 1**).

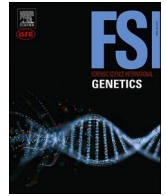
- b) Within each one of the major informatics architectures, perform sensitivity analyses to **evaluate the impact of different parameters** and their associated statistical modeling. This was achieved through an intra-software evaluation through systematically varying one parameter at a time – number of contributors (NoC), drop-in (frequency and modeling), analytical threshold, population coancestry coefficient, and stutter peaks modeling – to assess their influence on LR values and identify conditions under which poor parameter estimation may distort the weight of the evidence. The following analyses were designed to explore critical aspects of parameter sensitivity:
 - As a core parameter, identified as having the greatest impact on results [175, 180, 236], the starting point relied on assessing the sensitivity of LR values when **different NoCs** are considered. Real casework pairs of samples were used to reflect the variability encountered in forensic practice (**Chapter 2**).
 - Following the preceding analysis, the work proceeds by focusing on how varying the assumed **drop-in frequency** affects LR outcomes, considering the same set of real casework data. These preliminary findings highlighted the complexity of modeling artifactual DNA events, reinforcing the need for a broader sensitivity analysis also involving additional key parameters (**Chapter 3**).
 - Extending the previous discussion, the analysis was expanded to a wider set of parameters. Besides deepening the **assessment of the drop-in modeling strategies'** influence on the LR values, the individual impact of **stutter peak treatment and** variation in the **analytical threshold** was also performed. The same set of real casework data was analyzed in this step to reflect practical forensic conditions (**Chapter 4**).

- After addressing analytical and artifactual parameters, the influence of population-genetic-structure modeling also needed to be evaluated. To fulfill this aim, the same set of real casework data was analyzed, varying the **coancestry coefficient value**. Also, to explore the impact of this parameter variation on different genotypic configurations and provide theoretical grounding for the observed LR fluctuations, *in silico* simulated data and corresponding algebraic assessment were analyzed (**Chapter 5**).
 - Finally, a finer-scale analysis was conducted to understand the cumulative effect of some small changes within the same computational architecture, as those verified in software development through its updated versions – in particular, the inclusion of forward stutter modeling and other internal algorithmic improvements. To isolate the impact of these changes, LR values produced by **different versions of the same PG software** were compared under identical input parameter values and conditions. The same set of real casework samples was analyzed to evaluate the practical consequences of these modifications (**Chapter 6**).
- c) Deepen the **understanding of the current awareness, use, and implementation of PG tools in forensic genetics, and compare statistical strategies used across laboratories** for DNA mixtures interpretation. This objective was addressed through the organization and coordination of a collaborative international working commission in which participating laboratories were asked to statistically analyze a set of controlled sample pairs from the public PROVEDIt database (<https://lftdi.camden.rutgers.edu/provedit/>) [237] with varying complexity, gathering information on laboratory practices, software usage, parameter settings, and data handling choices (**Chapter 7**). The aim was to capture real-world diversity in interpretation strategies and evaluate consensus or divergence in methodological choices.

CHAPTER I | Quantification of Forensic Genetic Evidence: Comparison of Results Obtained by Qualitative and Quantitative Software for Real Casework Samples

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As the first and corresponding author, I was deeply involved in all stages of this study. I contributed to the conceptual development, particularly in framing the comparative analysis of qualitative and quantitative probabilistic genotyping software applied to forensic mixture samples. I was responsible for analyzing previously selected real casework data – comprising DNA mixtures with two or three estimated contributors and an associated reference – using probabilistic genotyping tools. Then, I interpreted the resulting likelihood ratios. Additionally, I wrote the first draft of the manuscript and played an active role in the revision process, addressing all comments from the reviewers.



Short communication

Quantification of forensic genetic evidence: Comparison of results obtained by qualitative and quantitative software for real casework samples

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ABSTRACT

To overcome the multifactorial complexity associated with the analysis and interpretation of the capillary electrophoresis results of forensic mixture samples, probabilistic genotyping methods have been developed and implemented as software, based on either qualitative or quantitative models. The former considers the electropherograms' qualitative information (detected alleles), whilst the latter also takes into account the associated quantitative information (height of allele peaks). Both models then quantify the genetic evidence through the computation of a likelihood ratio (LR), comparing the probabilities of the observations given two alternative and mutually exclusive hypotheses.

In this study, the results obtained through the qualitative software LRmix Studio (v.2.1.3), and the quantitative ones: STRmix™ (v.2.7) and EuroForMix (v.3.4.0), were compared considering real casework samples. A set of 156 irreversibly anonymized sample pairs (GeneMapper files), obtained under the scope of former cases of the Portuguese Scientific Police Laboratory, Judiciary Police (LPC-PJ), were independently analyzed using each software. Sample pairs were composed by (i) a mixture profile with either two or three estimated contributors, and (ii) a single contributor profile associated. In most cases, information on 21 short tandem repeat (STR) autosomal markers were considered, and the majority of the single-source samples could not be a priori excluded as belonging to a contributor to the paired mixture sample. This inter-software analysis shows the differences between the probative values obtained through different qualitative and quantitative tools, for the same input samples. LR values computed in this work by quantitative tools showed to be generally higher than those obtained by the qualitative. Although the differences between the LR values computed by both quantitative software showed to be much smaller, STRmix™ generated LRs are generally higher than those from EuroForMix. As expected, mixtures with three estimated contributors showed generally lower LR values than those obtained for mixtures with two estimated contributors.

Different software products are based on different approaches and mathematical or statistical models, which necessarily result in the computation of different LR values. The understanding by the forensic experts of the models and their differences among available software is therefore crucial. The better the expert understands the methodology, the better he/she will be able to support and/or explain the results in court or any other area of scrutiny.

1. Introduction

Whenever biological material is found during a criminal investigation, the standard practice in most countries is to perform DNA analysis

by capillary electrophoresis, after PCR amplification. DNA mixtures, i.e., samples containing contributions from more than one donor (exact number unknown), are typically encountered in this context. An estimation of the number of contributors of the DNA mixture is a parameter

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required by probabilistic genotyping systems to evaluate the weight of the evidence. Although alternative methods have been proposed [1–16], the standard methodology to estimate the number of contributors is the maximum allele count approach [17–20], where the expert establishes the minimum number of contributors based on the number of observed alleles. This estimation may be a challenging procedure, especially when involving high order mixtures (three or more contributors) and/or low levels of DNA, and may not reflect the actual number of contributors.

Beyond the unknown number of contributors, mixture DNA samples may have associated several other characteristics that make it complex and difficult to interpret and analyze, such as allele sharing between contributors, contributions in different proportions, stutters which may be confounded with alleles of a minor contributor (or vice versa), amplification stochastic effects (heterozygotic imbalance, drop-in, and drop-out) due to low-template DNA, or degradation [21–32].

To minimize the impact of the abovementioned conditions and quantify the weight of the forensic genetic evidence under a robust and scientifically supported statistical approach, probabilistic genotyping informatics tools were developed. The standard approach relies on the computation of a Likelihood Ratio (LR) [24], comparing the probabilities of observing the genetic evidence, assuming two alternative and mutually exclusive hypotheses.

Depending on the model on which they are based, existing software can be divided into two major groups: qualitative (e.g., Lab Retriever [33] or LRmix Studio [34]) or quantitative (e.g., EuroForMix [35] or STRmix™ [36]). The qualitative models only consider the qualitative information of the electropherogram (observed alleles in the profile, after the screening of the expert), while the quantitative ones also consider the quantitative information (height of the detected peaks, both alleles and stutters). Therefore, the first approach has simpler algorithms and computations compared to the second, which is more complete and produces complex results that are more sensitive to artifacts and stochastic effects [37].

Several works have been developed to evaluate and compare the performance of the different existing software resorting to controlled, mocked, mixture samples [38–51]. In this case, and in the absence of contamination, the number of contributors and respective proportion of the analyzed mixture samples is, a priori, known.

Under the framework of forensic identification problems, the main goal of this paper is to compare the results obtained by widely used probabilistic genotyping programs, considering real casework samples. This type of samples was chosen due to their unknown composition, uniqueness, and inability to predict and replicate, carrying a much higher level of complexity than mock ones. The software used in this work were based on qualitative – LRmix Studio v.2.1.3 [34] – and quantitative models – EuroForMix v. 3.4.0 [35] and STRmix™ v.2.7 [36]. Both LRmix Studio (<https://github.com/smartrank/lrmixstudio>) and EuroForMix (<http://www.euroformix.com/>) are open-source and free of charge. EuroForMix works in R [52] and assumes either Maximum Likelihood Estimation (MLE) or Bayesian inference approaches. Gamma distribution is assumed for modeling peak heights, and Exponential is used for drop-in occurrence. On other hand, STRmix™ (<http://strmix.esr.cri.nz/>) is a commercial software based on Bayesian approach using Markov Chain Monte Carlo (MCMC). A log-normal distribution is assumed for the peak height and the drop-in is modeled using either a Gamma or Uniform distribution. Indeed, despite both being quantitative software, there are significant differences on the considered approaches to analyze the data, which necessarily results in different evidence quantifications – for a comprehensive review see [50].

2. Methods and materials

A set of 156 irreversibly anonymized mixture/single contributor sample pairs (mixtures with two and three estimated contributors equally distributed) were selected from real casework and analyzed at

Portuguese Scientific Police Laboratory, Judiciary Police (LPC-PJ). For most of the pairs, the single contributor sample could not be excluded from being from one of the respective mixture contributors. The samples were previously processed in the context of the respective casework, under manufacturer protocols. The extraction was carried out in the Automate Express™ Forensic DNA Extraction System (Applied Biosystems™), using the PrepFiler Express BTA™ Forensic DNA Extraction kit (Applied Biosystems™) and QIASymphony SP/AS Extraction System using the QIASymphony DNA Investigator Kit, followed by the quantification in the equipment 7500 Real-Time PCR System (Applied Biosystems™), using the Quantifiler™ Trio DNA Quantification kit (Applied Biosystems™). Then, the amplification was performed in thermal cycler GeneAmp® PCR System 9700 (Applied Biosystems™) using GlobalFiler™ PCR Amplification Kit and GlobalFiler™ Express PCR Amplification Kit (Applied Biosystems™), both 24-locus STR kits; and, finally, the PCR products were detected and separated by capillary electrophoresis in 3500 Genetic Analyzer with GeneMapper™ ID-X (Applied Biosystems™), with an analytical threshold equal to 100 RFU. This work was then developed considering the genetic information obtained (GeneMapper files) for each irreversibly anonymized pair of samples (mixture/single contributor) for the set of 21 autosomal short tandem repeat markers analyzed.

For each mixture/single contributor sample pair, the quantification of the genetic evidence was computed through a global likelihood ratio (LR) assuming the following alternative hypotheses: H_1 : “DNA originated from the person of interest (POI) and N-1 unknown, unrelated individual(s)”, and H_2 : “DNA originated from N unknown, unrelated, individuals”, where N represented the estimated number of contributors. The LR value was computed for the same set of markers using LRmix Studio v.2.1.3 [34], EuroForMix v.3.4.0 [35] and STRmix™ v.2.7 [36]. In all the cases, the single contributor sample was assumed to come from the POI. In EuroForMix, the MLE approach was employed. All LR values were computed using the allelic frequencies of the National Institute of Standards and Technology (NIST) database concerning the Caucasian population [53], available at <https://strbase.nist.gov/NI-STpop.htm>.

To compare the results obtained by the three software, as much as possible similar conditions were maintained for the parameters described in Table 1. A co-ancestry coefficient (F_{ST}) equal to 0.01 was considered in all the tools; this value was used by studies with similar populations [45,54,55] and recommended for a broad geographic group by the second National Research Council report (NRC II) [56]. The drop-in frequency was set to 0.05 in all the programs, a value considered in similar study [54], coinciding with the pre-set value in LRmix Studio. Drop-in peaks, the peak heights are modeled in both quantitative computer tools [37], EuroForMix using a Lambda distribution (λ), where the software's default value of 0.01 was used, and STRmix™ employs either a Gamma (γ) or Uniform distribution, the latter having been considered in this work. A minimum allele frequency of 0.001 (default value in LRmix Studio) was used in both LRmix Studio and EuroForMix, while in STRmix™ this value is locus-specific and a N equal to 0 was considered.

Table 1

Parameter values introduced in LRmix Studio v.2.1.3, EuroForMix v. 3.4.0, and STRmix™ v.2.7, to perform LR computations. N/A: Not Applicable. ^a Dropout is directly estimated through the peak height distribution. ^b Per locus specified by the software considering N = 0.

Parameters	Values		
	LRmix studio	EuroForMix	STRmix™
Co-ancestry Coefficient (F_{ST})	0.010	0.010	0.010
Drop-in frequency	0.05	0.05	0.05
Drop-in parameters' distribution	N/A	λ : 0.01	Uniform
Drop-in cap	N/A	N/A	100
Dropout	0.1	^a	^a
Minimum allele frequency	0.001	0.001	^b
Threshold detection	N/A	100	100

In both EuroForMix and STRmix™, the allele frequencies were normalized. Regarding dropout, in LRmix Studio the pre-set value of 0.1 was used, while in both quantitative programs this parameter is directly estimated through the peak heights distribution [57,58]. A threshold detection equal to 100 RFU was considered in both quantitative software.

For the other parameters, specific to each software implementation, the default values provided by the authors were considered. Since STRmix™ software requires the inclusion of the stutters in the mixture sample files, they were also included in the EuroForMix analysis, both back and forward. LRmix Studio does not consider stutters and therefore its inclusion is not recommended. For LRmix Studio only, the selection of allelic peaks was decided by the same expert for all samples. In the quantitative software the stutter is automatically accommodated by the software, which introduce another source of variation between computed LR values. In the cases where a LR equal to zero was obtained for specific markers, the corresponding markers were removed from the analysis and the corresponding global LR value was denoted as LR_{<21}, as less than 21 markers were analyzed in the three tools.

LR values (log₁₀ scale) for the same mixture/ single contributor sample pair and set of markers were obtained through EuroForMix, LRmix Studio, and STRmix™. Three pairwise differences were then computed for each pair mixture/single contributor sample. Statistical tests (chi-square test and test-t, respectively) were carried out to infer significant trends ($\alpha = 0.05$): (i) a specific software provided greater or smaller LR values than the others, and (ii) LR values obtained for mixtures with two estimated contributors showed to be higher/smaller than those with three.

3. Results and discussion

The global LR values obtained for each of the 156 mixture (with two or three estimated contributors)/ single contributor sample pairs analyzed were computed using the three software – LRmix Studio v.2.1.3 [34], EuroForMix v.3.4.0 [35], and STRmix™ v.2.7 [36]. Values obtained are presented in Fig. 1 and in Tables S1, S2.

STRmix™ returned a LR equal to zero for some mixture/ single contributor sample pairs, namely, in 17% and 9% of the cases with mixtures with two and three estimated contributors, respectively. In these cases, either alleles present in the single contributor sample were absent from the mixture sample, or MCMC sampling failed to identify the true genotype combination, i.e., MCMC algorithms failed to converge, as similarly observed in other studies [44,51,59]. Riman et. al found that this event mainly occurred when minor contributors were compared to mixture profiles with low templates [59]. Nevertheless, in this work, a LR equal to zero was observed indiscriminately for sample pairs whose single contributor profile considered could not be excluded from being either the major or minor contributor of the mixture.

Some approaches have been presented to circumvent this question, which include (i) ignore that locus during deconvolution, (ii) repeat the deconvolution with a random starting seed for the MCMC different than the one that resulted in a LR equal to zero, or (iii) repeat the deconvolution with an increase in number of MCMC parameters values [59,60]. In this case, for simplicity and to maintain the same conditions throughout STRmix™ computations (i.e., MCMC parameters values) these loci were ignored. Inevitably, to guarantee equal circumstances between all tools, these loci were also ignored in both EuroForMix and LRmix Studio software. Thus, as previously mentioned, these pairs were re-analyzed in the three programs, disregarding the marker(s) for which a LR equal to zero was achieved in STRmix™, and so, less than 21

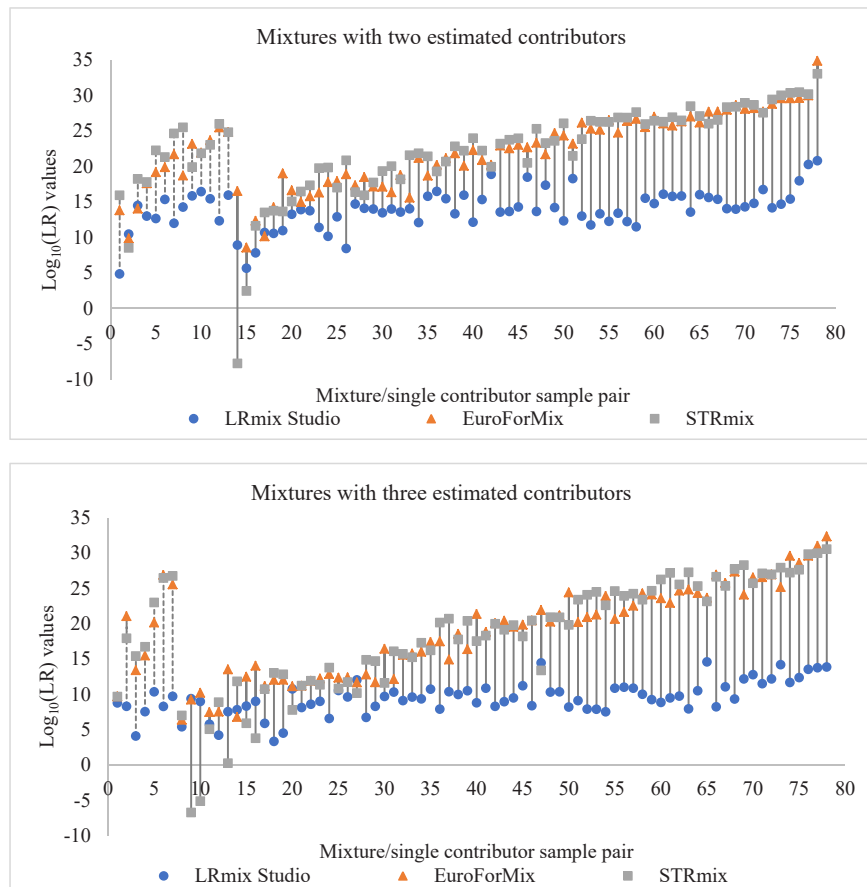


Fig. 1. LR values (log₁₀ scale) computed for the same mixture/ single contributor sample pairs, with two (upper plot) and three estimated contributors (lower plot) in LRmix Studio v.2.1.3 (circle), EuroForMix v.3.4.0 (triangle) and STRmix™ v.2.7 (square). In the first 13 and 7 mixture/single contributor sample pairs illustrated in the upper and lower plot, respectively, less than 21 markers were analyzed (dashed line). Within the pairs highlighted with a dashed line in the upper plot, only 18 markers were considered in the first pair and 20 markers in the other 12 pairs. On the other hand, in the lower plot, in the first two pairs highlighted with a dashed line 19 markers were considered, and 20 markers in the remaining pairs highlighted with a dashed line.

markers (minimum 18) were considered per case. These specific situations are highlighted with a dashed line in Fig. 1.

STRmix™ LR values showed to be higher than those from EuroForMix for cases with mixtures with two estimated contributors ($p = 2.35\text{E-}02$), the same trend not being supported for the cases with mixtures with three estimated contributors ($p = 8.21\text{E-}01$). Also, LR values computed by STRmix™ and EuroForMix showed to be higher than those computed by LRmix Studio, for both cases with mixtures with two ($p = 3.57\text{E-}16$, for both software) and three estimated contributors ($p = 1.09\text{E-}11$ and $p = 3.57\text{E-}16$, resp.).

There are however three cases (2_2, 9_3, and 27_3) for which the LR value computed by LRmix Studio showed to be the highest. For the case 2_2, LR values retrieved by LRmix Studio, EuroForMix, and STRmix™ equated $3.04\text{E}+10$, $7.43\text{E}+09$, and $3.47\text{E}+08$, respectively. For 9_3, LR values computed were $2.45\text{E}+09$, $1.77\text{E}+09$, and $1.89\text{E-}07$, resp. And, finally, for case 27_3, LR values of $1.13\text{E}+12$, $4.77\text{E}+11$, and $1.51\text{E}+10$, resp., were observed. As expected, these exceptions corresponded to mixture/single contributor sample pairs whose single contributor profile could not be excluded from being the minor contributor of the mixture under analysis. Since both EuroForMix and STRmix™ consider the quantitative information related to the height of the peaks, the computed LR values were lower than those computed by LRmix Studio, which does not consider that information.

In concordance with results previously reported [50], LR values obtained for the cases of mixtures with two estimated contributors showed to be higher than those obtained for cases with mixtures with three estimated contributors, for the three software: LRmix Studio ($p = 1.00\text{E-}20$), EuroForMix ($p = 1.19\text{E-}03$) and STRmix™ ($p = 1.45\text{E-}03$). The computation of lower LR values for cases with mixtures with three estimated contributors may be a consequence of the higher complexity associated, which may also be reflected in a greater dispersion of data. In both quantitative software, this trend was verified and corroborated by the standard deviation values computed for the distribution of LR values obtained by each software. The standard deviation values achieved for cases with mixtures with two and three estimated contributors were, respectively – in EuroForMix, 5.311 and 6.428, and in STRmix™, 6.397 and 8.077. On the other hand, for the qualitative software (LRmix Studio) the standard deviation values obtained, for cases with mixtures with two and three contributors showed to be 2.772 and 2.321, respectively, which do not support the trend obtained for quantitative software. The fact that qualitative software uses less information when compared to quantitative ones, may lower its sensitivity to more complex situations.

The observed discrepancies between the LR values ($\log_{10}$ scale) computed by the different software for the same mixture (with two and three estimated contributors)/single contributor sample pairs are summarized in Table 2. After calculating the difference between computed

LR values (represented by D in Table 2), the number of cases with differences within the same range (zero and two units ($\log_{10}$ scale), two and four, and so on) was accounted and divided by N (total number of cases) to calculate the percentage presented. An example clarification regarding the way D was computed is presented made in Table S3.

As expected, the greatest differences were found when the results of the quantitative tool LRmix Studio were compared with those of the qualitative ones: EuroForMix and STRmix™. Indeed, it was noticed that the category with the highest frequency concerns the cases showing differences greater than ten units in a $\log_{10}$ scale – 37% and 46% in cases with mixtures with two, and 46% and 54% in cases with mixtures with three estimated contributors, respectively. Within the cases of mixtures with two estimated contributors, the greatest differences found between LRmix Studio and EuroForMix ($\text{LR} = 3.20\text{E}+11$ vs. $\text{LR} = 5.14\text{E}+26$) was smaller than the one found between LRmix Studio and STRmix™ ($\text{LR} = 8.61\text{E}+08$ vs. $\text{LR} = 1.79\text{E-}08$), the latter also supporting contrasting hypotheses. Similarly, for the cases of mixtures with three estimated contributors, the largest discrepancy observed between LRmix Studio and EuroForMix ($\text{LR} = 1.73\text{E}+08$ vs. $\text{LR} = 8.83\text{E}+26$) was also smaller than the one observed between LRmix Studio and STRmix™ ($\text{LR} = 8.59\text{E}+07$ vs. $\text{LR} = 1.88\text{E}+27$).

In almost all the cases, LR values computed by the quantitative software (EuroForMix and STRmix™) were consistently higher than those computed by qualitative (LRmix Studio). Coincidentally, the single-source profile used as a reference in these cases could not be excluded from being the major contributor of the mixture. The non-consideration of the peak heights information by qualitative tools makes them less sensitive to several aspects, such as contributors in different proportions, which may result in these differences.

A different scenario was observed when comparing LR values obtained through the quantitative tools STRmix™ and EuroForMix. In both cases with mixtures with two and three estimated contributors, most of the cases (73% and 59%, respectively) did not differ by more than two units on a $\log_{10}$ scale. Regarding cases with mixtures with two estimated contributors, only one case showed a difference greater than ten units in a $\log_{10}$ scale between EuroForMix ($3.48\text{E}+16$) and STRmix™ ($1.79\text{E-}08$). It is noteworthy that this was the same sample pair for which LRmix Studio and STRmix™ showed the greatest divergence of results. For the cases with mixtures with three estimated contributors, 5% of the observed LR differences showed to be greater than ten units on a $\log_{10}$ scale. The maximum difference of 15.970 units ($\log_{10}$ scale) was observed in a case for which LR values retrieved by EuroForMix and STRmix™ equated $1.77\text{E}+09$ and $1.89\text{E-}07$, respectively.

For simplicity sake and practical reasons, only cases with differences higher than ten units in a $\log_{10}$ scale between quantitative software were further analyzed, namely, pairs 14_2, 9_3, 10_3, 13_3 and 16_3 – Fig. S1, Tables S4 and S5. None of these pairs contain alleles that have not been

Table 2

Pairwise differences between the LR values ($\log_{10}$ scale) computed by LRmix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7, for cases with mixtures with two and three estimated contributors, and maximum, mean, and median values of these differences.

D = ABS [Log ₁₀ (LR1) – Log ₁₀ (LR2)]	Estimated number of contributors					
	Two			Three		
	EuroForMix and LRmix Studio	STRmix™ and LRmix Studio	EuroForMix and STRmix™	EuroForMix and LRmix Studio	STRmix™ and LRmix Studio	EuroForMix and STRmix™
	N = 78	N = 78	N = 78	N = 78	N = 78	N = 78
0 < D < 2	8%	6%	73%	13%	9%	59%
2 < D < 4	13%	15%	19%	8%	8%	24%
4 < D < 6	19%	13%	4%	8%	9%	9%
6 < D < 8	5%	6%	3%	14%	10%	1%
8 < D < 10	18%	13%	0%	12%	10%	1%
D > 10	37%	46%	1%	46%	54%	5%
Maximum	15.206	16.682	24.288	18.707	19.341	15.970
Mean	8.097	8.823	1.839	9.425	10.252	2.468
Median	8.811	9.687	1.145	9.690	10.445	1.333

previously observed in the used allele frequency database, and therefore differences on the minimum frequencies values cannot justify these discrepancies, as previously shown in other study [51]. For these pairs, the great discrepancies observed in the global LR values resulted from the accumulation of small differences in the analysis of each marker within the specific cases. At this point, it is noteworthy that the differences found and the proportion of cases in these conditions were not greater because, within the same analysis, there were markers where the LR was greater when computed with STRmix™ than when EuroForMix was used, occurring the opposite situation for others. This resulted in smoother global LR differences than those that may have occurred if one of the two software consistently attributed lower (or higher) LR values than the other. Anyway, most of cases with differences greater than ten units ($\log_{10}$ scale) resulted from cases where EuroForMix computed LR values higher than STRmix in most markers.

An extended analysis of the greatest differences found between quantitative software was performed (LRs differing in more than $\log_{10}$ scale units), for both cases with mixtures with two and three estimated contributors.

Starting with the only case of mixture with two estimated contributors (pair 14_2), a difference of 24.288 units ($\log_{10}$ scale) was observed between the two quantitative programs: $LR(\text{EuroForMix}) = 3.48\text{E}+16$ and $LR(\text{STRmix}^{\text{TM}}) = 1.79\text{E}-08$. For this pair, the single contributor profile was considered the minor contributor of the mixture by both EuroForMix and STRmix™ with a mixture proportion of 14.28% and 25.91%, respectively. However, in a per-locus LR analysis, 19 out of the 21 markers favored inclusion ($LR > 1$) in EuroForMix, while in STRmix™ only 9 showed the same trend, as described in Table S4. In Table S6, the profiles of the loci showing the greatest differences, namely, D10S1248 (2.947 units, $\log_{10}$ scale), D8S1179 (2.946), D22S1045 (2.900), and D16S539 (2.068) are described. Neither of the statistic diagnoses given by both software supported a good performance for this analysis. The peak height variability parameter in EuroForMix adjusted to 0.476, a very high value – meaning a poor describing model. A more tolerant peak variance parameter in this software allows a lower false exclusion rate and a higher rate of false support of non-contributors [51]. On the other hand, the STRmix™ equivalent parameter (peak height variance parameter) shows slightly larger values than the mode of the respective prior distribution, which may indicate stochastic effects or incorrect estimation of the number of contributors [60]. Also, the remaining STRmix™ secondary diagnostics – $\log(\text{likelihood})$ and Gelman-Rubin – reinforce the software inability to describe the observed data. A negative $\log(\text{likelihood})$ of -253.95 was observed, which may be due to several reasons such as, profile used as a reference being very low level [60]. In Gelman-Rubin parameter, the acronym NaN (Not a Number) is presented. So, even though the diagnostics of both software pointed out the model bad fitting, the result obtained was very distinct, probably due to different modeling assumptions and parameters settings. STRmix™ models locus specific amplification efficiencies and consider expected stutter ratios defined using empirical data. On the contrary, EuroForMix does not model locus specific amplification efficiencies and has a blanket expected stutter rate. As EuroForMix does not allow a differential amplification between loci, the need for a more tolerant peak height variance parameter is understandable [51].

Within the cases with mixtures with three estimated contributors, the greatest difference occurred in a mixture/single contributor pair (9_3) with a difference of 15.970 units ($\log_{10}$ scale) between EuroForMix ($LR = 1.77\text{E}+09$) and STRmix™ ($LR = 1.89\text{E}-07$). Like the per-marker LR previously analyzed, most markers in EuroForMix (17 out of 21) supported inclusion, while in STRmix™ only 8 out of the 21 showed the same trend – see Table S4. The STR profiles of markers D8S1179, D13S317, and D19S433 are described in Table S7 since the greatest differences were found in those loci – 2.547, 2.092, and 2.087 $\log_{10}$ scale units, respectively. For this case, both EuroForMix and STRmix™ considered the single contributor profile the second contributor of the mixture with a mixture proportion of 15.67% and 22.92%, respectively.

Evaluating the statistic diagnostics of EuroForMix and STRmix™, both tools supported a good performance for this analysis. EuroForMix peak height variability parameter adjusted to a value of 0.195, revealing a good describing model. STRmix™ secondary diagnostics also revealed a good fit of the model – a $\log(\text{likelihood})$ of 28.58 and a Gelman-Rubin value of 1.06 indicating that STRmix™ was able to well describe the data and the model converged [60]. Contrarily to the situation previously analyzed considering two estimated contributors, the diagnostics of both software reflected the model good fitting in this case. The very discrepant results observed are probably due to different modeling assumptions and parameters settings, as stated before.

A more in-depth investigation on these cases where greater differences were reported by different informatics approaches are being performed and soon presented in a dedicated research article.

4. Conclusions

Informatics tools for the analysis and interpretation of forensic samples DNA results, perform a quantification of the genetic evidence through the computation of a probative value using electropherograms' information.

Software based on quantitative models take into account a larger amount of data than the qualitative ones, as these only consider the detected alleles, disregarding the corresponding peak heights. So, while qualitative tools only include statistical models for probability of drop-out and drop-in, quantitative tools model also the peak height information (i.e., peak alleles, peak balance, and stutter peaks). Indeed, the major distinctive feature of existing software is the ability to consider the (inferred) DNA quantity (after amplification).

As expected, the greatest differences between LR were generally found when results retrieved from qualitative and quantitative tools were compared. Notwithstanding, and despite the smaller differences, quantitative software also retrieved different LR results reflecting different mathematical modeling of various parameters. Indeed, even using similar parameter values, each software assigns a different statistical modeling to each parameter leading to the computation of different LR values. Nevertheless, some of the differences observed were unexpected and intriguing large. This prompted us to develop a deeper research to explore the characteristics of the samples that lead to such differences, which results will be presented in a dedicated research article.

The uniqueness, and inability to predict and replicate real casework mixture samples, were the main reasons for the selection of this kind of samples. The drawback is that its composition (donors and proportion) is unknown, contrary to what occurs for mock samples (noting that accidental contaminations may also occur in these cases). Thus, the inference on which software produced more accurate results couldn't be performed. We further anticipate that some differences may be enhanced if the (some of the) contributors are closely related (as is often the case in sexual violence) and or related to the POI.

Software based on probabilistic genotyping methods is essential in the interpretation and quantification of complex DNA mixtures. Different software solutions encode distinct mathematical, statistical and informatics models which lead to the return of different values for the same input samples and approximated parameters. In what seems a paradox, this software dependence for the quantification of the evidence implies a need of improvement of the education and training of the experts, when compared to a previous situation where only a binary exclusion/inclusion interpretation (without statistical calculations) was developed.

Our aim with this short communication is to show how different LR computations can be obtained when considering real casework samples, regardless of whether the informatics tools consider the quantitative information of the electropherogram or not. Quantitative software used (EuroForMix and STRmix™) have built-in diagnostic tools that allow the expert to know if the models are good fits. Nevertheless, for some cases,

despite statistic diagnostics of both tools supported a good fit, discrepant LR results were computed. So, since in the forensic routine the genetic evidence is not usually quantified resorting to more than one informatics tool, there are no confrontations between possibly different results, practitioners in the field assuming the (only) calculated value as steel plated. Thus, we intend to warn experts not to blindly trust the program they use and to be critical of the computed result, paving the way for the routine consideration of more than one tool in routine forensic casuistic. If for a specific case discrepant results are obtained, forensic specialists should investigate the samples at stake, as well as the mathematical modeling corresponding, to further act in conformity and reach a solid, informed, decision.

Indeed, forensic experts play a crucial role in the profile interpretation, from the electropherogram analysis (decisions about the presence of non-allelic peaks and estimation of the number of contributors, for example) to the proper use of the software for LR computations. Furthermore, and considering the different approaches, the expert knowledge should be extended to the understanding of how each software operates and its respective encoded models. Only this way he/she will be able to sustain the conclusions in court or any other scrutiny scenario.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2022.102715.

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Supplementary Material I

The complete supplementary data associated with this article can also be found in the online version at <https://doi.org/10.1016/j.fsigen.2022.102715>

SM 1. LR Values ($\log_{10}$ scale) computed by LRmix Studio v.2.1.3, EuroForMix v.3.4.0 and STRmix™ v.2.7

Table S1: $\log_{10}$ values of the global LR_s computed by LRmix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7, for mixtures with two estimated contributors.

Mixture/single contributor sample pair	Number of Markers analyzed	Log ₁₀ (LR)		
		LRmix Studio	EuroForMix	STRmix™
1_2	18	4.86657204838057	13.8294610937682	15.9646225694356
2_2	20	10.4824447919183	9.87112234580822	8.54071728801072
3_2	20	14.5191885461733	14.0650640266446	18.2511026254141
4_2	20	13.0161345896592	17.6342868867976	17.828124753286
5_2	20	12.6862017574883	19.2064775078269	22.2842276395935
6_2	20	15.3679091541739	19.9042359493822	21.3500346963401
7_2	20	12.0160634463251	21.7196646345689	24.6683579569178
8_2	20	14.2844171971822	18.7390743371681	25.5324103732534
9_2	20	15.8634144687459	23.1965070182103	19.9313103685944
10_2	20	16.4702972714964	21.9688106826449	21.877071729106
11_2	20	15.4572564862835	23.6996274141321	23.0668847431298
12_2	20	12.3455187979382	25.5118385407035	26.0122887398346
13_2	20	15.9692360063515	24.8623072719051	24.8534304968247
14_2	21	8.93505821111056	16.5416457059251	-7.74683167409856
15_2	21	5.68003592267767	8.57215547926539	2.46422119753445
16_2	21	7.84711591107174	12.3498755665475	11.6521012295195
17_2	21	10.7120284396928	10.1490929977319	13.543683213073
18_2	21	10.5818338788459	14.2530952636341	13.7803389182933
19_2	21	10.9851436721674	19.0362121067628	13.6793097669857
20_2	21	13.2869481575052	16.6418330571512	15.089021500795
21_2	21	13.9291869282143	14.9941742874016	16.4936531956769
22_2	21	13.7806035288078	15.8092413562536	17.3637059728547
23_2	21	11.4300441159091	16.3278035152657	19.7905384872419
24_2	21	10.1652924119572	17.7982151390833	19.8640243036114
25_2	21	12.9261767859385	17.937139300617	17.0330214446829
26_2	21	8.45996803173564	18.914403895705	20.874539797071
27_2	21	14.7194026747048	17.3521239586894	16.3947492807005
28_2	21	14.1275991053093	18.5156073166598	15.9632682515262
29_2	21	14.0153634481972	17.1806212152506	17.7559738939978
30_2	21	13.4883064086763	17.1554765889407	19.3744367149817
31_2	21	14.0180087387005	16.3700927419323	20.0617162931599
32_2	21	13.5922157439319	18.7937631104403	18.2062590352093
33_2	21	14.0493449338477	15.6177546649844	21.6083441480519
34_2	21	12.1207094350976	21.2156664775028	21.8719405292931
35_2	21	15.8218745944703	18.716600239649	21.4432160341658
36_2	21	16.5078986579368	20.2270256560828	19.3154875277508
37_2	21	15.4787321833956	21.178958336681	20.7155437506051
38_2	21	13.3407266190049	21.8598735051683	22.8542392221253
39_2	21	15.9778701801983	20.116723170249	22.2338334148844

40_2	21	12.1680559736359	22.3105481887852	24.0022956825549
41_2	21	15.3605939685769	20.8904343299152	22.2517355304378
42_2	21	18.9034756703506	20.238246046583	19.9680577856946
43_2	21	13.5944228408747	22.9605473819747	23.226032503345
44_2	21	13.6793214916022	22.5582577340666	23.781403489496
45_2	21	14.3107943090211	23.0546921324205	23.985192871319
46_2	21	18.5064023048128	22.6998388668291	20.5285696400129
47_2	21	13.6777770544447	23.3603035741593	25.3212047568544
48_2	21	17.3865094132793	21.7147269094052	23.3125158556634
49_2	21	14.2153582531158	24.7142957163447	23.6312915363253
50_2	21	12.3585773310974	24.3725424423411	26.1064628837961
51_2	21	18.3123835333751	23.2190636376932	21.4944607193908
52_2	21	13.0204826438741	26.1840911406115	23.8727038962168
53_2	21	11.7772570164207	25.3235250709434	26.452338543846
54_2	21	13.3426172148295	25.1848227447074	26.3052008452764
55_2	21	12.2903154364377	26.5276286572527	26.278730742747
56_2	21	13.4306364873138	24.7819855304928	26.9135330590988
57_2	21	12.2475950561731	26.4102533727937	26.9013602262447
58_2	21	11.5046960807735	26.7106746065369	27.6763917843913
59_2	21	15.5509190997359	25.5550486031869	25.9958500952539
60_2	21	14.7840828858067	27.0316680424505	26.4513411705978
61_2	21	16.1168749367599	26.0618082844347	26.323540846098
62_2	21	15.7953935212816	25.7668326985701	26.9497509553911
63_2	21	15.852711758157	26.3534414071985	26.5091083925838
64_2	21	13.5960461975942	27.047594236651	28.5203131877761
65_2	21	16.0412664937462	26.1990221488132	27.1232961726453
66_2	21	15.6478066771761	27.7366391207201	26.05468964295
67_2	21	15.3817278774594	27.739055943856	26.577100988801
68_2	21	14.0630081956431	27.9961427909388	28.3834203732457
69_2	21	14.0010204915277	28.6486510167309	28.4236062707212
70_2	21	14.3194425632393	28.1236885660712	28.9799306041087
71_2	21	14.8314732407719	28.2338101009594	28.6836772988187
72_2	21	16.7621263584486	27.7499578326202	27.5917989457305
73_2	21	14.1835738509612	28.8243479586061	29.466363368261
74_2	21	14.699589527138	29.622376677047	30.0417873189718
75_2	21	15.4187244904439	29.6080284373943	30.382341290824
76_2	21	18.0019758924749	29.6430932393099	30.4944607193908
77_2	21	20.2975520979656	30.032149604201	30.225412672866
78_2	21	20.8319403678696	34.9054427835232	33.0772588636929

Table S2: Log₁₀ values of the global LR_s computed by LRmix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7, for mixtures with three estimated contributors.

Mixture/single contributor sample pair	Number of Markers analyzed	Log ₁₀ (LR)		
		LRmix Studio	EuroForMix	STRmix™
1_3	19	8.78722519747517	9.70090122605077	9.68043523556175
2_3	19	8.31208633977482	21.0643998957434	17.9442309057952
3_3	20	4.09917286195281	13.4002091375785	15.4256646196831
4_3	20	7.53920410893427	15.4892608079209	16.7350077292951
5_3	20	10.3600364629684	20.161444477143	23.016029963076
6_3	20	8.28458863040394	26.9004827836319	26.4676968790276
7_3	20	9.71266737604299	25.5541492591491	26.7666135875443
8_3	21	5.40828499539414	6.39179984453314	7.01602996307602
9_3	21	9.38832756782626	9.24700710900774	-6.72312477886845
10_3	21	8.99503653194530	10.2165176818822	-5.12158736630087
11_3	21	5.76563031451247	7.48325144211519	5.07055501583503
12_3	21	4.2131175182878	7.5391325071788	8.89062777647295
13_3	21	7.54463473713168	13.5277082360384	0.255248376961857
14_3	21	7.82619628621419	6.78009519687553	11.8334276227297
15_3	21	8.33066771760188	12.490685762258	5.91034687995707
16_3	21	9.00787442868812	14.0510426116387	3.79051738261732
17_3	21	5.88559331772917	11.157805542533	10.7274192470595
18_3	21	3.34047933685412	12.0427126552787	13.0206512680043
19_3	21	4.51998535926748	12.0638347706296	12.8374621714860
20_3	21	10.7685533983718	11.1376598857465	7.79496181743753
21_3	21	8.13271656124477	11.2151170880555	11.2367639221878
22_3	21	8.5989420096983	11.9196336864301	11.9232336534832
23_3	21	9.00944682976579	12.23243108147	11.3595509167633
24_3	21	6.58745598245138	12.8699188295275	13.7812382205378
25_3	21	10.5439631464745	12.3589900243851	10.829625353358
26_3	21	9.6309600802716	12.3983093636475	11.7379793866908
27_3	21	12.0512640981609	11.6783552013382	10.1796379521578
28_3	21	6.74219010153243	12.8070312886492	14.8851858834841
29_3	21	8.31164546327802	11.6971506770605	14.7182358824283
30_3	21	9.70122925424003	16.4325597847119	11.6212178390672
31_3	21	10.3230760202831	12.1478206629287	16.1022621494943
32_3	21	9.1146034236122	15.595956905226	15.7259197956484
33_3	21	9.6193892917863	15.7599212225258	15.2480959310941
34_3	21	9.34858708191353	16.0186102619306	17.3008345191616
35_3	21	10.7391688922263	17.4071358883201	16.2253609803727
36_3	21	7.90968398630668	17.500078354895	20.16767171623
37_3	21	10.3770915331626	14.9298703160325	20.7140110484215
38_3	21	9.9810718791967	18.5667533562783	17.760723972142
39_3	21	10.5136343450539	16.3855620013867	20.4106085425684
40_3	21	8.8008958483548	21.3696303938414	17.5511571164409
41_3	21	10.8652921341737	18.8397933763735	18.3151303171836
42_3	21	8.30792548445882	20.0618934684376	19.9878494163606

43_3	21	8.95671545425116	20.4219999127221	19.147614498562
44_3	21	9.48728758766046	19.5534006568875	19.8069596466035
45_3	21	11.222270375425	19.8455954472697	18.1947917577219
46_3	21	8.38903098135402	20.4510619818314	20.4486135780856
47_3	21	14.461585601923	21.929927008112	13.3908997971735
48_3	21	10.324735842945	20.2905043029449	20.9200450209477
49_3	21	10.348866036169	21.156322003547	20.9017143543254
50_3	21	8.20797452630088	24.4297405652943	19.8416472359922
51_3	21	9.10614773810907	20.2235758836	23.4090196379315
52_3	21	7.91320712423008	20.9303726321035	24.0957619594775
53_3	21	7.88325516508085	21.2842678374333	24.4999893343342
54_3	21	7.52570948590296	23.9260275198116	22.6119780759939
55_3	21	10.869664868725	20.6592273334067	24.634094196616
56_3	21	11.019296456846	21.6532852190893	23.9310966560155
57_3	21	10.86930593037	22.5366767363943	24.2528287680406
58_3	21	10.004033564776	24.2902045704124	23.4130481693179
59_3	21	9.24554132786121	24.1343336962267	24.6580018516186
60_3	21	8.85013080073263	23.5982182666617	26.2719344938178
61_3	21	9.48951876383595	22.9323347884892	27.1993711613915
62_3	21	9.76015135990851	24.6872102983992	25.557073894366
63_3	21	7.93385129245255	24.838210113684	27.275057846121
64_3	21	10.5176564589578	24.3295616116597	25.3305963821648
65_3	21	14.596162822999	23.6714089408036	23.1626838658779
66_3	21	8.23926455743938	26.9459990616517	26.6380997148397
67_3	21	11.0685931961704	25.7712672888953	25.3445690996885
68_3	21	9.33300499750338	27.3692496957187	27.7557300667124
69_3	21	12.1770035849407	24.1085520663958	28.3081160158656
70_3	21	12.7887344952133	26.5347344686837	25.7242021152189
71_3	21	11.5110842866667	26.5784409177498	27.1288191421945
72_3	21	12.175428861444	26.9744961494731	26.9874249231685
73_3	21	14.199837988177	25.1857881665831	27.9309897602759
74_3	21	11.6850154846904	29.6173400262584	27.2303467224175
75_3	21	12.3676457333853	28.590052930334	27.6374396927037
76_3	21	13.5382594603422	29.6326097433164	29.845284126348
77_3	21	13.762565742915	31.0296978620038	29.993493544769
78_3	21	13.8756561816588	32.3439823515627	30.5543882211507

SM 2. LR values computed by the three software under analysis for the mixture/single contributor sample pair 32_3

Table S3: Illustrative example of how LR differences were computed and included in Table 2. For each mixture/single contributor pair, after calculating $\log_{10}$ for each LR obtained, a pairwise subtraction is performed between $\log_{10}$ (LR) values, namely, $\log_{10}$ (LR LRmix Studio) – $\log_{10}$ (LR EuroForMix); $\log_{10}$ (LR LRmix Studio) – $\log_{10}$ (LR STRmix™); $\log_{10}$ (LR EuroForMix) – $\log_{10}$ (LR STRmix™). Finally, the absolute value of the difference is calculated.

Software	LR	Log ₁₀ (LR)	Dif. = $\log_{10}(\text{LR1}) - \log_{10}(\text{LR2})$			Absolute value (Dif.)		
			LRmix Studio – EuroForMix	LRmix Studio – STRmix™	EuroForMix – STRmix™	LRmix Studio – EuroForMix	LRmix Studio – STRmix™	EuroForMix – STRmix™
LRmix Studio	1.30E+09	9.1146						
EuroForMix	3.94E+15	15.5960	-6.4813	-6.6113	0.1300	6.4813	6.6113	0.1300
STRmix™	5.32E+15	15.7259						

SM 3. Per-marker LR Values for the cases where the difference between the global LR values computed by EuroForMix v. 3.4.0 and STRmix™ v.2.7 showed to be greater than 10 units (log₁₀ scale)

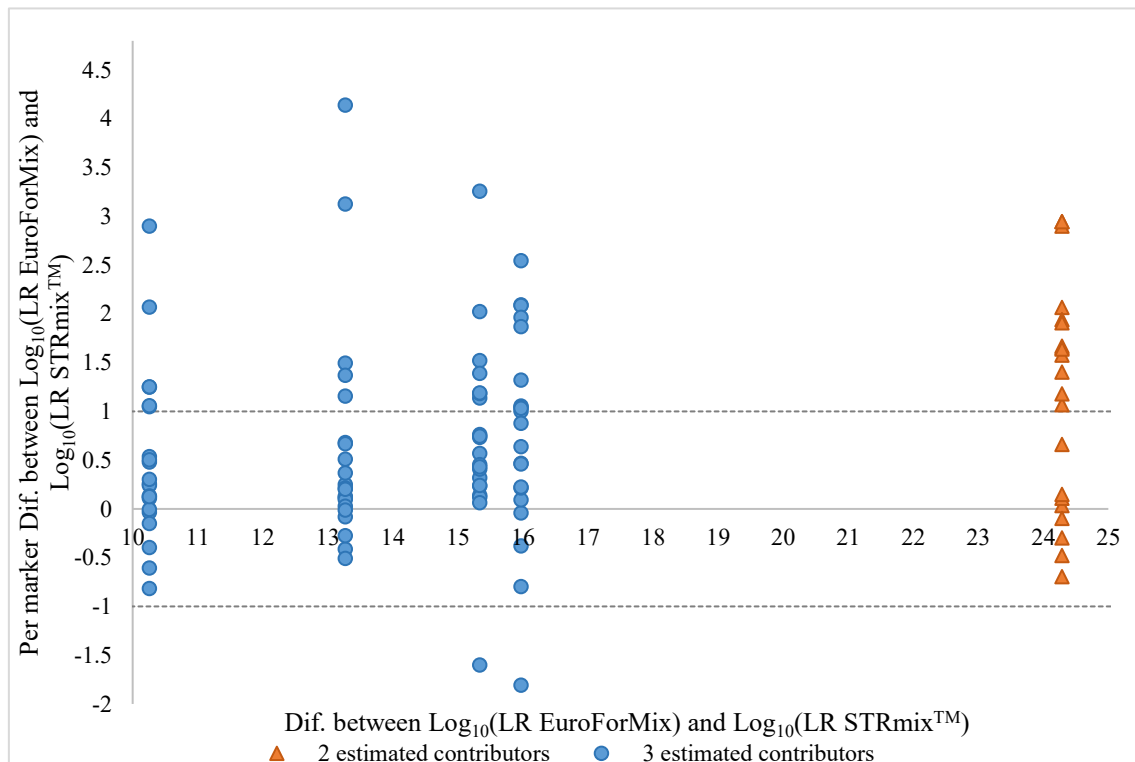


Figure S1: Correlation between the (absolute) higher differences found for global LR values computed by EuroForMix v.3.4.0 and STRmix™ v.2.7 and (log₁₀ scale, x axis) – higher than ten units in a log₁₀ scale – and the corresponding per marker differences (log₁₀ scale, y axis). Each vertical set of points represents a mixture/single contributor sample pair for which global LR values differing in more than ten units (log₁₀ scale) were obtained through EuroForMix v.3.4.0 and STRmix™ v.2.7. Each set is composed by 21 points corresponding to the 21 analyzed markers. Points located above the x-axis represent markers for which the value computed in EuroForMix was higher than the computer by STRmix™. Points located below the x-axis illustrate the reverse situation. The dashed grey line separates markers whose differences do not exceed one unit (log₁₀ scale) from the others.

Table S4: Additional information on the cases where differences greater than ten units (log₁₀ scale) were observed between LR values calculated through EuroForMix (EFM) v.3.4.0 and STRmix™ v.2.7. Highlighted in yellow are i) Greater difference found in both cases with mixtures with two and three estimated contributors; ii) A high number of loci with LR<1; iii) A negative Log(likelihood) value; iv) Gelman-Rubin (GR) values higher than 1.2 or non-value. Within section “Mixture prop.”, all contradictions between both tools about the proportion (and consequent type of contributor) assigned to the single contributor profile are highlighted in orange.

Pair	Final LR		DIF (log ₁₀)	Unobserved alleles in the allele frequency database used			EuroForMix					STRmix 1 st diagnostics				STRmix 2 nd diagnostics				
	EFM	STRmix		Marker	Allele	Ref.	P.H. Variability	Mixture prop.		Locus (LR)		Mixture prop.		Locus (LR)		Log (likelihood)	GR (R<1.2)	Peak Height Variance		
								%	Cont	LR>1	LR<1	%	Cont	LR>1	LR<1			Allele (mode=13.008)	Back Stutter (mode=6.794)	Forward Stutter (mode=5.156)
2 CONTRIBUTORS																				
14_2	3.48E+16	1.79E-08	24.288	-	-	-	0.47601	14.28%	2	19	2	25.91%	2	9	12	-252.95	NaN	22.683	52.148	12.228
3 CONTRIBUTORS																				
16_3	1.12E+14	6.17E+03	10.261	-	-	-	0.3203	44.47%	2	20	1	24.20%	2	15	6	28.27	1.67	16.929	29.428	10.167
13_3	3.37E+13	1.80E+00	13.272	-	-	-	0.30345	47.60%	1	18	3	72.61%	1	13	8	31.86	1.18	13.033	21.325	8.468
10_3	1.65E+10	7.56E-06	15.338	-	-	-	0.26326	52.00%	1	18	3	7.70%	3	12	9	40.33	1.1	15.339	33.309	8.752
9_3	1.77E+09	1.89E-07	15.970	-	-	-	0.19485	15.67%	2	17	4	22.92%	2	8	13	28.58	1.06	9.565	28.03	8.247

Table S5: LR values (log₁₀ scale) computed by EuroForMix (EFM) v.3.4.0 and STRmix™ v.2.7 for each marker and the respective difference (DIF), for all cases where the global LR differed in more than ten units (log₁₀ scale) between tools. All LR values that support the exclusion [Log₁₀(LR) < 0] are highlighted in yellow. For each pair, the biggest differences (DIF > 2) found between markers are highlighted in blue.

Marker	14_2			16_3			13_3			10_3			9_3		
	log (EFM)	log (STRmix)	DIF	log (EFM)	log (STRmix)	DIF	log (EFM)	log (STRmix)	DIF	log (EFM)	log (STRmix)	DIF	log (EFM)	log (STRmix)	DIF
CSF1PO	0.256	0.223	0.033	0.699	0.442	0.257	0.708	1.119	-0.410	0.921	0.156	0.765	-0.096	-0.563	0.467
D10S1248	1.216	-1.730	2.947	0.471	-2.429	2.900	0.494	1.001	-0.507	0.770	-0.752	1.521	0.717	0.079	0.638
D12S391	0.980	-0.671	1.652	0.345	0.235	0.110	0.583	0.073	0.509	0.624	0.305	0.320	0.790	0.829	-0.040
D13S317	-0.496	0.197	-0.693	0.618	0.651	-0.033	0.505	0.374	0.131	0.461	0.226	0.235	0.655	-1.437	2.092
D16S539	1.997	-0.071	2.068	-0.046	-0.582	0.537	0.403	-0.278	0.681	0.479	0.338	0.141	0.642	-0.681	1.323
D18S51	2.410	0.739	1.671	0.556	0.315	0.241	1.441	1.324	0.117	1.306	0.168	1.138	0.225	-0.830	1.055
D19S433	0.361	-1.042	1.403	0.638	-0.611	1.249	1.267	-0.227	1.494	0.610	0.202	0.408	1.757	-0.330	2.087
D1S1656	1.108	-0.471	1.579	0.609	-0.438	1.047	1.458	0.301	1.157	-3.780	-2.179	-1.601	0.604	-1.360	1.964
D21S11	1.883	0.814	1.068	0.663	0.361	0.302	0.773	0.853	-0.080	0.371	0.250	0.121	1.314	1.221	0.093
D22S1045	0.953	-1.947	2.900	0.627	-0.624	1.251	-0.032	-0.700	0.668	0.256	0.192	0.064	-1.175	0.631	-1.807
D2S1338	0.473	-0.188	0.660	1.066	1.070	-0.005	0.898	0.801	0.097	1.055	-0.337	1.392	0.513	0.299	0.214
D2S441	0.441	0.330	0.111	0.194	0.590	-0.396	-0.135	-4.275	4.140	0.952	0.383	0.569	0.355	0.132	0.224
D3S1358	0.973	1.271	-0.298	0.388	1.203	-0.815	0.476	0.751	-0.275	1.476	-1.783	3.259	0.351	-0.651	1.002
D5S818	0.437	-1.504	1.940	0.802	-1.268	2.070	0.785	-2.343	3.128	-0.170	-1.355	1.185	-0.011	-1.881	1.870
D7S820	-0.395	-1.576	1.180	0.379	-0.102	0.481	0.231	-0.021	0.252	0.446	-1.579	2.025	0.457	-0.584	1.041
D8S1179	1.039	-1.907	2.946	1.858	0.800	1.057	0.326	-1.044	1.370	0.306	-0.147	0.453	0.651	-1.896	2.547
FGA	0.769	0.621	0.148	1.256	0.753	0.503	-0.107	-0.322	0.214	1.108	0.374	0.733	0.449	0.829	-0.380
SE33	0.619	-1.019	1.639	1.726	1.592	0.134	1.561	1.191	0.369	1.351	0.603	0.748	0.535	1.330	-0.795
TH01	0.839	-1.069	1.908	0.555	1.160	-0.604	0.991	0.964	0.028	1.070	-0.121	1.190	0.178	-0.701	0.879
TPOX	0.144	0.621	-0.477	0.148	0.298	-0.150	0.296	0.310	-0.013	-0.181	-0.422	0.241	-0.114	-0.576	0.462
VWA	0.535	0.633	-0.098	0.500	0.374	0.126	0.605	0.404	0.200	0.786	0.354	0.432	0.451	-0.583	1.034

Table S6: Profiles of markers D10S1248, D16S539, D22S1045, and D8S1179 of the mixture and single contributor (Ref.) sample of the pair 14_2, since they presented the biggest differences between the quantitative software computations, for the cases with mixtures with two estimated contributors.

14_2															
D10S1248				D16S539				D22S1045				D8S1179			
Mixture			Ref.	Mixture			Ref.	Mixture			Ref.	Mixture			Ref.
Allele	Size	Height	Allele	Allele	Size	Height	Allele	Allele	Size	Height	Allele	Allele	Size	Height	Allele
12	101.75	2464	12	11	251.23	4966		11	97.14	3308	11	13	146.97	11986	
14	109.93	4107	14	12	255.37	5890		14	106.15	2877	14	14	151.08	14561	14
15	113.97	18476		13	259.45	715	13	15	109.14	12052		15	155.12	1513	15
				14	263.44	296	14								

Table S7: Profiles of markers D13S317, D19S433, and D8S1179 of the mixture and single contributor (Ref.) sample of the pair 9_3, the one with the biggest difference between the quantitative software computations, for the cases with mixtures with three estimated contributors.

9_3											
D13S317				D19S433				D8S1179			
Mixture			Ref.	Mixture			Ref.	Mixture			Ref.
Allele	Size	Height	Allele	Allele	Size	Height	Allele	Allele	Size	Height	Allele
8	210.68	2260		12	141.91	625		10	135.16	1217	
10	218.7	1053		13	145.78	525	13	13	147.47	2539	
11	222.81	1070		14	149.65	2494		14	151.6	3173	14
12	226.76	2214	12	14.2	151.68	264	14.2	15	155.65	478	15
13	230.79	497	13	15	153.54	1483		16	159.71	962	

CHAPTER II | The Impact of Considering Different Numbers of Contributors in Identification Problems Involving Real Casework Mixture Samples

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In this work, I served as both the first and corresponding author, being involved from its initial conceptualization to final manuscript preparation. My role embraced designing the study along with other authors, which aimed to assess the effect of varying the number of contributors (NoC) in forensic mixture samples on likelihood ratio (LR) outcomes. I was also involved in developing the methodological framework and was responsible for conducting software-based analysis of the previously selected pairs of real casework data, composed of a mixture (with two and three estimated contributors and an associated reference sample). This included evaluating how different NoC assumptions influenced LR values across qualitative and quantitative tools. I interpreted the results considering real casework complexities and their relevance to forensic practice. I wrote the first draft of the manuscript and actively contributed to the revision process.



The impact of considering different numbers of contributors in identification problems involving real casework mixture samples

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Abstract

Increasingly complex genetic samples are analyzed in forensic genetics routine, including mixtures to which more than one individual contributed. The standard problem relies on identification, aiming to quantify the likelihood of the donor of a reference sample being a contributor to the mixture. This is computed through a likelihood ratio (LR) and requires using devoted probabilistic genotyping software that may consider the quantity of the mixture's DNA (quantitative tools), beyond only the presence/absence of specific alleles (qualitative tools). In any case, the mixture's number of contributors (NoC) is a parameter that the user must introduce. Due to its nature, NoC is unknown for most real casework samples and needs to be estimated, which may be challenging due to poor DNA quality and quantity. This study aims to evaluate the impact of considering different NoC of real mixture samples (both over- and underestimating it after a first assessment of the expert) in identification problems through the pairwise comparison of LR_s, using for the statistical assessment of both qualitative (LRmix Studio) and quantitative tools (EuroForMix and STRmixTM). Different computational models showed different variations of the results, but for all, the impact was greater when considering a smaller NoC than the one initially estimated by the expert. Quantitative tools showed more sensitivity to NoC variation. Taking advantage of using real data, whose possible complexities surpass those of mock ones, this work highlights the impact that the NoC may have on the quantification of the proof, reinforcing the importance of its proper estimation.

Keywords NoC · Estimation · Forensic DNA · Likelihood Ratio · Weight-of-Evidence

Introduction

Forensic DNA samples recovered from the environment and for which more than one individual contributed have several characteristics that make them highly complex and difficult to interpret and analyze. Typically these samples are analyzed in the framework of identification problems, for which probabilistic genotyping methods were developed and implemented as informatic tools allowing the quantification of the weight of the evidence considering both the evidence and corresponding reference profiles [1, 2]. These tools can be based on two different models, either considering only the observed electropherogram's peaks – qualitative model – or also considering their heights – quantitative model [3, 4]. On the other hand, the latter may be based on either maximum likelihood (numeral optimization), as [5], or random sampling (as Markov Chain Monte Carlo – MCMC), as [6], statistical methods. In any case, the weight of the evidence is measured through a Likelihood Ratio (LR)

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computation [7, 8] comparing the probability of observing the genetic evidence assuming two alternative and mutually exclusive hypotheses. This calculation also depends on several parameters regarding population and analytical factors that may need to be introduced by the user as a parameter in the software. This work focuses on the number of contributors (NoC), highlighting and reinforcing the crucial role that the expert continues to have in quantifying the proof as the results provided by the previously mentioned software highly depend on that parameter [9].

By nature, the NoC of most of the evidence samples analyzed in a forensic genetics routine is unknown and needs to be estimated, which can be a challenging task due to a. allele sharing between contributors, b. contributions in different proportions, c. stutter peaks which may be confused with alleles of a minor contributor (or vice-versa), or even d. amplification stochastic effects (heterozygotic imbalance, drop-in, and/or drop-out) due to low quantity and quality of DNA. Indeed, the decision concerning the presence (or not) of non-allelic peaks in the evidence sample [10–12] and the estimation of its NoC relies on the expert's evaluation, which can be subjective and prone to errors [13, 14]. Experts may have different interpretations, especially in the most challenging samples [15]. The standard method for estimating NoC in forensic laboratories is based on the maximum allele count (MAC) observed at the locus with more alleles. A lower bound for NoC is calculated as half of the MAC from the autosomal locus (or loci) with more alleles [7, 13]. This initial estimate should then be re-evaluated by considering the peak imbalance in the electropherogram. In all instances, the subjective nature of MAC as an expert estimation must be acknowledged, as true alleles can be difficult to distinguish from stutters and other artifacts, especially in mixtures. Nevertheless, some informatics tools provide insights into which NoC better fits the information in the evidence electropherogram [16–20], by specifically targeting that variable. In other tools, the NoC is summed out [21].

Some studies have analyzed the impact of an incorrect NoC estimation on the LR value assigned by both qualitative and quantitative tools considering mock profiles [22–25]. The magnitude of the impact showed to be highly dependent on the analyzed samples, as an incorrect estimation can either have no significant effect or significantly decrease the LR value, favoring in some cases the alternative hypothesis of no contribution of the Person of Interest (PoI).

The main goal of this work is to analyze this impact by considering real casework samples with an unknown NoC, instead of measuring it in mock samples where the NoC is known. This methodology allowed to reliably replicate as much as possible the level of complexity of the conditions frequently encountered in the forensic genetics routine, which cannot be completely achieved with mocked samples. For each case, a NoC was estimated by the expert (eNoC)

and then the over ($\text{NoC} = \text{eNoC} + 1$), and underestimation ($\text{NoC} = \text{eNoC} - 1$) of the value were also considered in the computations for comparison purposes. These samples were analyzed in both qualitative (LRmix Studio v.2.1.3 [26]) and quantitative informatics tools (EuroForMix v.3.4.0 [5] and STRmix™ v.2.7 [6]), and the results were compared in an intra-software analysis guaranteeing that the found variations are not due to the different statistical and computational architectures of the tools.

Material and methods

Resorting to evidence material from former cases of the Portuguese Scientific Police Laboratory, Judiciary Police, 152 irreversibly anonymized pairs of samples were selected and analyzed, each one composed of a mixture with either two ($\text{eNoC} = 2$, $N = 75$) or three ($\text{eNoC} = 3$, $N = 77$) estimated contributors and a single-source profile associated – hereafter referred to as “reference sample”. The genetic information obtained for each pair of samples analyzed relies on a set of 21 autosomal short tandem repeat (STR) markers.

For each pair of samples, a statistical evaluation of the evidence was computed through LR_s, under the framework of an identification problem, assuming the alternative hypotheses: “The PoI is a contributor of the mixture” and “The PoI is unrelated to any contributor of the mixture”. The reference sample was assumed to belong to the PoI. The LR values were assigned through both qualitative (LRmix Studio v.2.1.3 [26]) and quantitative (EuroForMix v.3.4.0 [5] and STRmix™ v.2.7 [6]) informatic tools. As the qualitative tool does not allow the inclusion of stutter peaks in the evidence analysis, this artifact was removed from the input file before any LRMix Studio computation, this being the only difference between the input genetic data considered in the tools. For all the calculations, the allele frequencies of the National Institute of Standards and Technology database concerning the Caucasian population were used [27] – see Online Resource 1. The software parameter values considered in the three tools were maintained as similar as possible and are described in Online Resource 2. Nevertheless, it should be remarked that these tools incorporate and apply different models to the input data, and as such, it will impact corresponding results. Regarding the inference approach of each tool, LRMix Studio uses the Maximum Likelihood Estimation (MLE) method, EuroForMix incorporates both MLE and integration (Bayesian) approach, and STRmix™ is based on a Bayesian approach using MCMC. In this work, the MLE approach was employed in EuroForMix as suggested by software developers for model comparison purposes [28]. Also, the parameter optimization is done differently, in both LRMix Studio and EuroForMix, a separate maximum likelihood is established for each of the

abovementioned hypotheses, while in STRmix™, this optimization is done through the MCMC method. Focusing on the quantitative tools, even though both consider the quantitative information of the evidence's EPG (i.e., the height of the peaks), the models implemented on them to analyze this information are quite distinct. EuroForMix applies a gamma function to model peak height distribution, while STRmix™ uses a log-normal one. Also, the artifacts present in the evidence profile as drop-in alleles and stutter peaks are modeled differently. The first is modeled in EuroForMix through an exponential function, while in STRmix™ is capped and can be modeled by either a Gamma (γ) or Uniform distribution, the latter having been considered in this work. Lastly, in EuroForMix the stutter peaks are modeled using a blanket ratio of all alleles, while in STRmix™, allele-specific ratios are applied.

To evaluate the impact in the weight of the evidence of considering different NoCs, cases with mixtures with two initially estimated contributors ($eNoC = 2$) were also analyzed as having three ($NoC = eNoC + 1$), while those with three initially estimated contributors ($eNoC = 3$) were analyzed as having four ($NoC = eNoC + 1$) and two ($NoC = eNoC - 1$) contributors.

For each tool's set of results, an intra-software analysis was performed by comparing the (non-null) LR value calculated under the default ($LR_{NoC=eNoC}$; initial NoC estimation performed by the expert, $eNoC$) and the varied conditions ($(LR_{NoC=eNoC\pm 1})$: either $NoC = eNoC + 1$, or $NoC = eNoC - 1$), through the ratio between the two LR values: $R = LR_{NoC=eNoC} / (LR_{NoC=eNoC\pm 1})$ if $LR_{NoC=eNoC} > (LR_{NoC=eNoC\pm 1})$, or $R = (LR_{NoC=eNoC\pm 1} / LR_{NoC=eNoC})$, otherwise. A single run was performed for each software, pair of samples, and parameter conditions, except when the informatics tool was not able to make compatible the $eNoC$ introduced by the user with marker-specific genotypic data, reporting either a null LR or an error message. For those cases, the marker-specific data that led to that output were withdrawn for comparison purposes, and LRs were recomputed, similar to that carried out in a previous study [9]. Alternatively to removing markers for which a null LR value was retrieved, software developers also suggest the variation of MCMC parameter values to test a possible convergence [29]. Indeed, due to the underlying stochasticity, null LRs could be a result of the lack of convergence of the method on which the software is based, the MCMC algorithms failing to identify a genotype combination compatible with the reference profile, as similarly observed in other studies [29–31]. Nevertheless, to maintain the methodology of a single run for each pair of samples, for all the programs, the first option was selected and a smaller set of markers (varying between 18 and 20) for which a non-null LR was obtained for 17% and 9% of the cases analyzed in STRmix™ with mixtures with two and three estimated contributors, respectively. These pairs

of samples are clearly identified in Online Resource 3 by $LR < 21$ and will also be identified in all the visual representations of the data. Statistical significance of the results was reached considering a significance level $\alpha = 0.05$.

Results and discussion

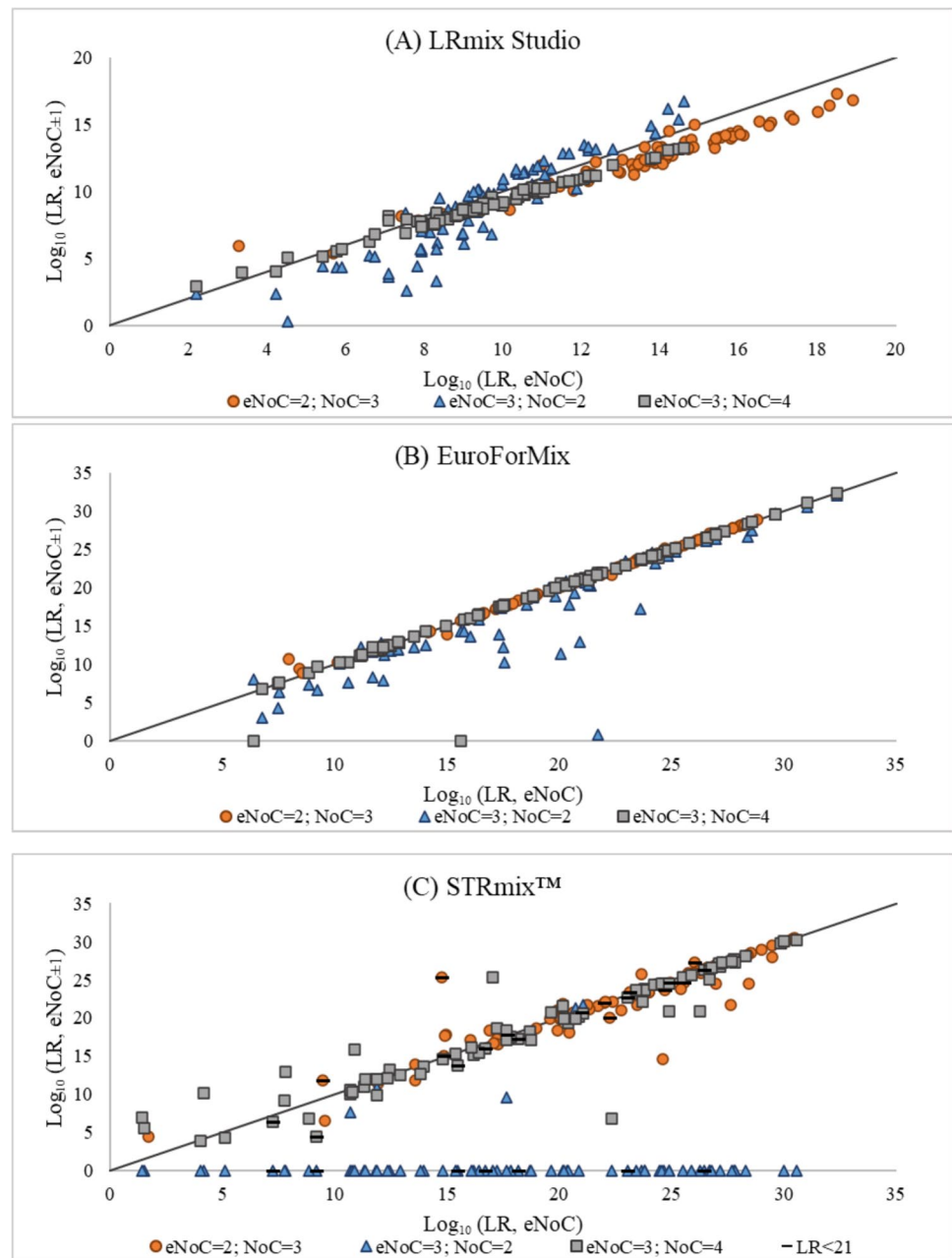
The LR values assigned by LRmix Studio v.2.1.3 [26], EuroForMix v.3.4.0 [5], and STRmix™ v.2.7 [6] assuming the initial estimation of the expert for the number of contributors of the mixture sample ($eNoC$, equal to either two or three) and the varied parameter ($NoC = eNoC + 1$: from two to three and from three to four, and $NoC = eNoC - 1$: from three to two) are displayed in Fig. 1.

Table 1 summarizes the $\log_{10}$ scale of the ratios (R) calculated between LR values computed assuming the initial estimation of the NoC ($eNoC$, equal to either two or three) and the varied parameter ($NoC = eNoC + 1$: from two to three and from three to four, and $NoC = eNoC - 1$: from three to two) for the three informatics tools considered. Broadly speaking these data show how many orders of magnitude the paired values: $LR_{NoC=eNoC}$ and $LR_{NoC=eNoC\pm 1}$, differ between them. Consider as an example the first pair of samples (1_2) for which $eNoC = 2$ and the EuroForMix tool. Assuming both the $eNoC$ value and its overestimation ($NoC = 3$) two LR values were computed: $LR_{NoC=2} = 8.47E + 07$, and $LR_{NoC=3} = 5.13E + 10$. Afterward, a ratio $R > 1$ between the two values was performed and considered for further analyses in a $\log_{10}$ scale: $\log_{10}(R = LR_{NoC=3} / LR_{NoC=2}) = 2.782$. In Table 1, this case is then accounted for the $2 < R < 3$ category, which means that baseline and varied LR values differ in more than two but less than three units on a $\log_{10}$ scale, i.e., the greatest LR is at least 100 times higher than the other but not overpassing 1000 times.

The corresponding trend for the LR to increase or decrease its value when the NoC was varied is also shown in Table 1. See Online Resource 3 for the complete set of LR results assigned by the three software under the tested conditions.

To illustrate how much the values have changed relatively to their size, Fig. 2 presents the cumulative distribution of the differences existing between the baseline ($LR_{NoC=eNoC}$, $\log_{10}$ scale) and the varied values ($LR_{NoC=eNoC\pm 1}$, $\log_{10}$ scale), normalized by their average. For example, for the previously mentioned case 1_2 the computed value equated: $\log_{10}(R = LR_{NoC=3} / LR_{NoC=2}) / ((\log_{10}(LR_{NoC=3}) + \log_{10}(LR_{NoC=2})) / 2) = 2.782 / 9.319 = 0.299$. Assuming this approach, the most striking results were observed for cases with mixtures for which $eNoC = 3$ – see Table 2. From the cases presented in Table 2, special attention should be given to pair 17_3 which was not represented in Fig. 2 due to scale constraints, and that had already been identified in Table 1 as one of those

Fig. 1 Distribution of LR values ($\log_{10}$ scale) assigned through LRmix Studio v.2.1.3 (A), EuroForMix v.3.4.0 (B), and STRmix™ v.2.7 (C), assuming the initial estimation of the expert for the number of contributors of the mixture (x-axis) for both cases with two and three estimated contributors and its variation (y-axis) – over (circles for $eNoC = 2$ and squares for $eNoC = 3$) and underestimation (triangles for $eNoC = 3$). The cases for each less than 21 markers were considered for STRmix™ are highlighted with a ‘-’ over the corresponding point



with the greatest R values. This was the only case for which the variation of NoC led to an LR value smaller than 1, supporting the alternative hypothesis of unrelated individuals. Recognizing the qualitative nature of the LRmix Studio software, inspecting the genotypic data of the pair 17_3 supports its inability to justify via drop-in the high number of peaks present in the evidence sample when underestimating the NoC. Under the same assumptions, quantitative software may have considered other possibilities, such as stutters, to interpret and quantify the genetic evidence. Still, regarding the sample pairs mentioned in Table 2, all the other differences observed for the quantitative tools were due to large partial LR variations in a small (two or three) set of markers,

where the height of the peaks severely impacted the likelihood of the observations assuming the hypotheses, allele frequencies and parameter values.

Differences higher than four log₁₀ units ($R > 10,000$) were obtained for all the software for $eNoC = 2$ and/or $eNoC = 3$. Special attention was devoted to some of these cases trying to disclose the motifs that may have led to such difference, and possible reasons are many fold. Overall, STRmix™ showed the greatest number of cases with $R > 10,000$; however, the stochasticity of the methods on which this tool is based should not be disregarded as these differences may be due to the lack of convergence of MCMC algorithms rather than due to the NoC variation itself. Other

Table 1 Summary of the ratios (R , $\log_{10}$ scale) found between LR values calculated using the computer tools: LRmix Studio v.2.1.3, EuroForMix v.3.4.0 (EFM), and STRmix™ v.2.7, considering as NoC the number of contributors estimated by the expert (eNoC) and + 1 (NoC = eNoC + 1, from two to three and from three to four) and - 1 (NoC = eNoC - 1, from three to two). N/A: “Not Applicable” corresponds to situations where no comparisons were performed, either

because an LR equal to zero was retrieved or an error message was displayed. ^a LR values assigned for less than 21 markers included (17% and 9% of the analyzed cases for eNoC = 2, and eNoC = 3, resp.). *See Online Resource 3 for more data. The statistical significance for the LR to increase or decrease when the NoC was considered as eNoC + 1 and eNoC - 1 is also presented ($\alpha = 0.05$); significant values are underlined

Ratios between LR values (R , $\log_{10}$ scale)	Estimated NoC (eNoC) = 2			Estimated NoC (eNoC) = 3					
	+ 1			+ 1			- 1		
	LR NoC = 2 vs. LR NoC = 3			LR NoC = 3 vs. LR NoC = 4			LR NoC = 3 vs. LR NoC = 2		
	LRmix Studio	EFM	STRmix™	LRmix Studio	EFM	STRmix™	LRmix Studio	EFM	STRmix™
$0 < R < 1$	31%	97%	67% ^a	86%	97%	70% ^a	49%	60%	9%
$1 < R < 2$	61%	1%	16% ^a	14%	0%	14% ^a	27%	21%	1%
$2 < R < 3$	8%	1%	11%	0%	0%	3%	14%	4%	0%
$3 < R < 4$	0%	0%	3%	0%	0%	0%	4%	6%	1%
$R > 4$	0%	0%	4% ^a	0%	0%	13% ^a	5%	9%	1%
N/A	0%	0%	0%	0%	3%	0%	0%	0%	87% ^a
The maximum ratios between LR values (R, $\log_{10}$ scale)									
R	2.713	2.782	10.520	1.333	0.508	15.510	6.356	20.929	8.059
LR NoC = eNoC	1.83E + 03	8.47E + 07	5.58E + 14	2.43E + 14	2.69E + 24	2.10E + 22	2.19E + 03	5.40E + 21	4.45E + 17
LR NoC = eNoC ± 1	9.44E + 05	5.13E + 10	1.85E + 25	7.51E + 13	8.35E + 23	6.52E + 06	9.65E - 04	6.36E + 00	3.88E + 09
Pair*	1_2	1_2	8_2	77_3	49_3	51_3	17_3	5_3	44_3
LR tends to ($\alpha = 0.05$)									
	Decrease	Increase	Decrease	Decrease	Increase	Decrease	-	Decrease	-
p-value	3.47E- 13	6.69E- 06	7.91E- 03	3.61E- 12	4.96E- 02	2.48E- 05	9.09E- 01	2.48E- 05	5.27E- 01

reasons for this and the remaining tools may include the possibility of CE failure to correctly attribute the alleles, errors in experts' analysis of evidence samples (selection of true alleles), highly degraded samples, or even, incorrectly assigning eNoC. In any case, as referred to in Material and Methods section, the same input files were used for the three tools, showing that different software are more sensitive to some variations than the others, depending on samples' characteristics.

a) When considering + 1 contributor than those estimated by the expert

For the three tools, a generally greater impact was observed for cases with mixtures with eNoC = 2 compared to those for which eNoC = 3, which may be due to the smaller complexity of the first (i.e. fewer possibilities of contributors), as observed in previous studies [9, 32]. In any case, it should be noted that the most extreme results, when normalized by the mean values, were obtained for cases with mixtures with eNoC = 3, as shown in Fig. 2 and Table 2.

For the qualitative tool LRmix Studio, most differences were between one and two units ($\log_{10}$ scale): $10 < R < 100$,

for cases with mixtures with eNoC = 2, but did not overstep one unit for most cases with eNoC = 3: $1 < R < 10$.

The smallest differences observed were in fact for the quantitative tool EuroForMix, as in almost all cases, differences lower than one unit were obtained: $1 < R < 10$. Nevertheless, for 3% of cases with mixtures with eNoC = 3, an error message popped up when considering NoC = 4. These cases are represented in Table 1 as N/A and were not considered in the analysis.

On the other hand, only for the other quantitative software STRmix™ differences higher than four units (i.e., $R > 10,000$) were obtained, for both cases eNoC = 2 and eNoC = 3. Those differences resulted indiscriminately from either the decrease or increase of the LR value. The maximum differences achieved in STRmix™ were much higher ($R = 10^{10.520}$ and $R = 10^{15.510}$) than those obtained for LRmix Studio ($R = 10^{2.713}$ and $R = 10^{1.333}$) and EuroForMix ($R = 10^{2.782}$ and $R = 10^{1.262}$), for both cases with eNoC = 2 and eNoC = 3, respectively.

Both LRmix Studio and STRmix™ showed a statistically significant trend for lower LR values when NoC = eNoC + 1, for both types of mixtures with eNoC = 2 and eNoC = 3 (Table 1), similar to previous studies performed with mock samples and known NoC [22, 24]. The decrease in the

Fig. 2 Cumulative distribution of the differences between LR values assigned through LRMix Studio v.2.1.3 (circles), EuroForMix v.3.4.0 (EFM; squares), and STRmix™ v.2.7 (triangles), normalized by their average, for both cases with mixtures with two (eNoC = 2; N = 75; (A)) and three (eNoC = 3; N = 77; (B)) estimated number of contributors, when NoC was either over- (continuous line) or underestimated (dashed line). Data regarding the outlier sample pair 17_3 and the software LRMix Studio was excluded due to scale limitations ($x = 39.10$) – see main text and Table 2 for more details

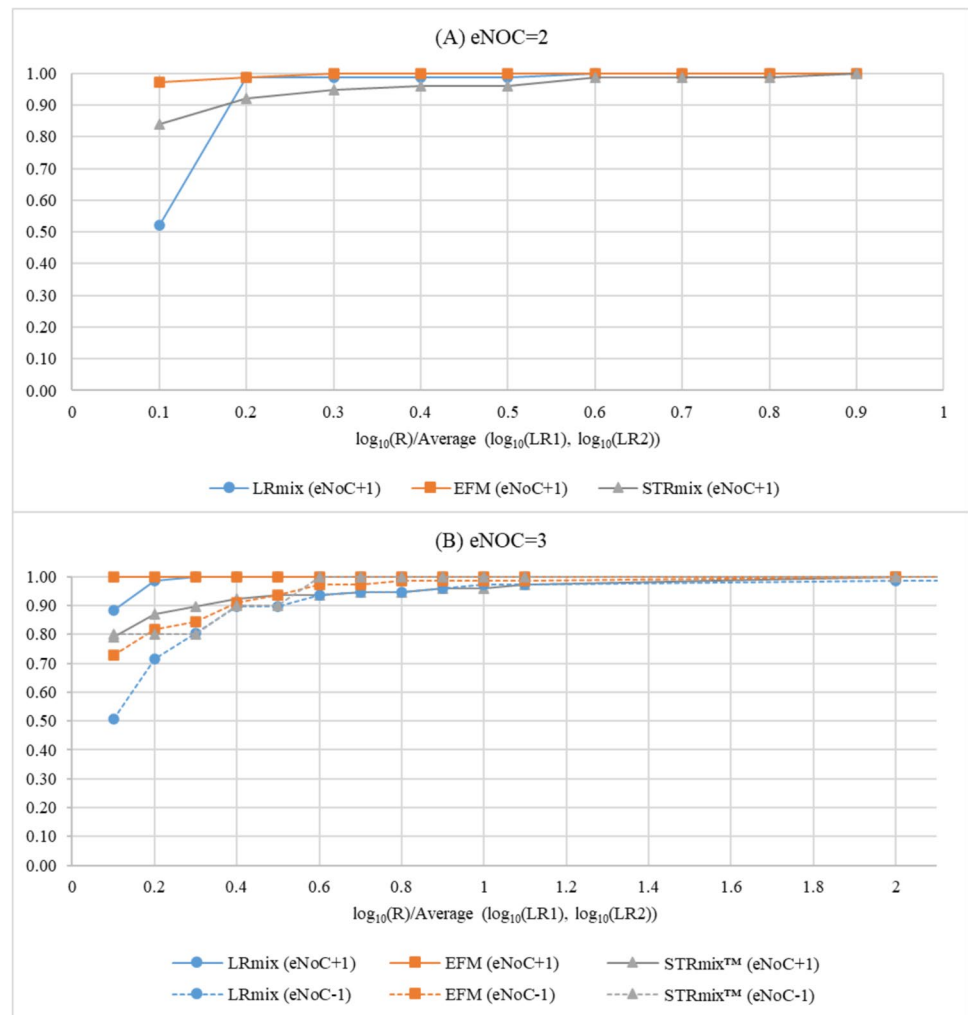


Table 2 Detailed information regarding the most striking results observed (> 1) when the differences ($\log_{10}(R)$) between LR values (assigned assuming both default – eNoC – and varied condition –

eNoC ± 1) were normalized by their average, only observed for cases with mixtures with three initially estimated number of contributors (eNoC = 3; N = 77)

Varied condition	Informatics tool	Pair	LR (default; eNoC = 3)	LR (varied; eNoC ± 1)	$\log_{10}(R)$	Average	Normalization
eNoC + 1	STRmix™	12_3	3.32E + 01	4.45E + 05	4.127	3.585	1.151
		15_3	2.76E + 01	9.75E + 06	5.549	4.215	1.317
		51_3	2.10E + 22	6.52E + 06	15.509	14.568	1.064
eNoC - 1	LRmix Studio	17_3	9.65E - 04	2.19E + 03	6.356	0.163	39.103
		18_3	2.12E + 00	3.31E + 04	4.193	2.424	1.730
	EuroForMix	5_3	5.40E + 21	6.36E + 00	20.929	11.268	1.857

LR value can be associated with the increase in the number of possible combinations of genotypes when assuming one more contributor than initially considered. EuroForMix software showed the opposite trend – i.e., LR values tend to be higher when the NoC = eNoC + 1, being also noteworthy that it was the tool with the greatest proportion of cases differing in less than one order of magnitude (97% of the cases for both eNoC = 2 and eNoC = 3) that supports a modest

practical impact of the NoC variation. In any case, several considerations should be made regarding these results on the increase/decrease of the LR values depending on the NoC. First, it should be borne in mind the unpredictable nature and unknown composition of the analyzed data as, contrarily to what occurs for mocked samples, it is impossible to be sure regarding the true NoC, and even if the profile is considered as a reference truly contributed to the evidence.

Moreover, and in any case, caution should be taken when comparing the LR values obtained by the different tools to evaluate the impact of the different statistical and computational models, not only because STRmix™ is based on a stochastic architecture and a single run was performed for each case, but also because the number of markers analyzed were not always the same, as previously explained and clearly identified in Online Resource 3. For example, for the previously referred pair 1_2, STRmix™ LR values were computed considering 18 out of the 21 markers, as three of them retrieved marker-specific LR values equal to zero under the default condition ($eNoC = 2$), having been removed for further analyses. For the other tools, the complete set of 21 markers was considered as none retrieved an LR equal to zero.

b) When considering – 1 contributor than those estimated by the expert

As abridged in Table 1, the majority of the differences observed were lower than one unit ($\log_{10}$ scale) for all the informatics tools: $1 < R < 10$. Yet, differences higher than four units ($\log_{10}$ scale): $R > 10,000$ were also found. The maximum difference obtained for both LRmix Studio and STRmix™ ($R = 10^{6.356}$ and $R = 10^{8.059}$, respectively) was much lower than the one assigned by EuroForMix ($R = 10^{20.929}$).

Nevertheless, these data must be taken with care, and it should be borne in mind that for most STRmix™ calculations (87%) no comparisons were performed since the software was not able to conciliate the number of $NoC = eNoC - 1$ with the genotypic data – in 74% of cases, the software was unable to assign an LR value presenting an error message, and in 13% it retrieved an LR equal to zero. These cases are represented in Table 1 as N/A and were not considered in the following analysis.

For the remaining STRmix™ results, there was no evidence of a trend for LR value to increase or decrease when the $NoC = eNoC - 1$. An analogous situation was verified for LRmix Studio. Only for EuroForMix results, a statistically significant trend was observed, as the LR decreased when considering one less contributor than initially estimated. This decrease may be a consequence of low-height alleles in the mixture (corresponding to the reference sample) being considered a stutter or even a non-specific peak to enable the tool to deal with the existence of more peaks per marker than would be expected for the estimated NoC . Since EuroForMix has a more tolerant peak variance parameter it allows a higher rate of false support of non-contributors [30]. Therefore, in most cases, the software assigned an LR value but a smaller one. Yet, for some others, it could not justify the presence of so many peaks per marker given the NoC proposed resulting in LR values lower than one. In any case,

and as previously explained, caution should be taken in the generalization and interpretation of these results.

Conclusions

The ability to analyze highly complex DNA forensic data leads to the corresponding statistical evaluation being dependent on the use of informatic tools that consider several parameters of interest, such as the number of contributors (NoC) of the evidence sample. This parameter is usually unknown and needs to be estimated by the forensic expert on a case-by-case basis. This analysis may be subjective particularly when dealing with complex samples with poor quality and quantity DNA, as is the case of some real casework samples as those considered in this work. It is thus fundamental to understand to what extent different values of the NoC may affect the statistical evaluation of the genetic evidence. In this study, 152 pairs of real casework samples composed of a mixture (with the estimated number of contributors – $eNoC$ – equal to either 2 or 3) and a reference sample were analyzed through different tools, two taking into consideration the quantity of DNA (EuroForMix, and STRmix™, quantitative tools) and one disregarding such information (LRmix Studio, qualitative tool). For each pair of samples, the LR value assigned assuming the initial estimation $eNoC$ was compared with the one assigned assuming $+1$ (for both cases with mixtures with $eNoC = 2$ and $eNoC = 3$) and -1 (only for $eNoC = 3$). In the first case: $NoC = eNoC + 1$, a trend for LR decreasing was observed for two tools (LRmix Studio and STRmix™ comparisons), while for EuroForMix the opposite trend was verified. LR discrepancies greater than $10,000 \times$ were only found for one of the quantitative tools: STRmix™, for both $eNoC = 2$ and $eNoC = 3$. On the other hand, when considering $NoC = eNoC - 1$, LR differences greater than $10,000 \times$ were verified for all the analyzed tools, but a statistically significant trend was only found for one of the quantitative tools: EuroForMix, specifically for LR values decreasing. Comparing the impact of considering $eNoC + 1$ and -1 , the latter seems to have a greater impact on all the informatics tools, being more emphasized in quantitative ones (STRmix™ and EuroForMix). Even so, STRmix™ stands out, since for 87% of the complete set of cases no comparisons were performed, showing to be more strict in the analysis of considering the $eNoC$ incompatible with the genotypic data than the other two. When normalizing these values by the corresponding average, the most striking LR differences were obtained for cases involving mixtures with $eNoC = 3$. A limitation of the study is that LRs for non-contributors were not included.

Developing research using real casework data is a straightforward and needed way to better understand and approximate what practitioners encounter in the casuistic

routine as they enclose complexities unable to be predicted or replicated in mocked samples. Recognizing its added value, the nature of the samples analyzed should not be however overlooked and caution should be taken when generalizing and interpreting the obtained results as the challenges are many folds. For example, given the unknown composition of the data, it is impossible to be sure of both the true number of contributors of the evidence samples and if the associated single-source ones contributed to them, which may compromise general conclusions regarding the increase or decrease of LR values depending on the over- or underestimation of the NoC. In fact, this work supports that the impact of varying the NoC parameter in the computed LR is highly variable, depending on both the genotypic profiles of the samples and the computational approach used to accommodate all the parameters.

Even though probabilistic genotyping tools assist the expert in the statistical analyses of complex forensic DNA samples, several parameter values must be introduced in the software before performing any computation, such as the NoC of the evidence samples under analysis. This work emphasizes and demonstrates that different NoC values may have crucial implications in the computation of the weight of the proof, reinforcing the importance of critically analyzing the outcome provided by statistical tools..

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00414-025-03500-7>.

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Data Availability All the statistics that supported the results presented in this study are provided in the Supplementary Material for each one of the analyzed pairs of samples and parameters. The raw genotypic and haplotypic analyzed data concern real forensic casework from the Portuguese Scientific Police Laboratory, Judiciary Police (LPC-PJ), and for that reason, its release is not possible. In any case, specific data from specific analyzed markers (non-haplotypic data) may be available

from the authors upon reasonable request and with the permission of LPC-PJ. The summary statistics of specific runs can also be provided to the readers when solicited. For data sharing issues, contact the corresponding author of the manuscript.

Declarations

Ethics approval Proper ethics approval was obtained from the Committee for Ethical and Responsible Conduct of Research of i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal (5/CECRI/2022) regarding the statistical analysis of both environmental and reference genetic samples (fully and irreversibly anonymized when applied).

Competing interests The authors declare no competing interests.

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Supplementary Material II

The complete supplementary data associated with this article can also be found in the online version at <https://doi.org/10.1007/s00414-025-03500-7>

Table S1: NIST allele frequency database.

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S104	D2S1338	D2S441	D3S1358	D5S818	D7S820	D8S1179	FGA	SE33	TH01	TPOX	vWA
5																			0.0014	0.0014	
6																			0.2355	0.0014	
6.3																					
7														0.0028	0.0277				0.1939		
8	0.00554			0.120499	0.01801									0.0055	0.144	0.01385			0.0956	0.5249	
8.1																0.0014					
9	0.01385			0.077562	0.10665							0.0014		0.0416	0.1676	0.00554			0.1191	0.1274	
9.1												0.0014									
9.3																			0.3449		
10	0.22022			0.047091	0.05679	0.0083	0.00139	0.0028				0.2105		0.0554	0.2562	0.102493			0.0083	0.0499	
10.1																					
10.2																					
10.3																					
11	0.30886	0.001385		0.325485	0.3144	0.0097	0.00554	0.0776		0.13989		0.3435	0.001385	0.356	0.205	0.076177		0.0014	0.0014	0.2521	
11.2																					
11.3												0.0609									
12	0.36011	0.031856		0.268698	0.3144	0.1136	0.07064	0.1163		0.01247		0.0471		0.3878	0.1593	0.16759		0.0069		0.0416	0.0014
12.2								0.00139													
12.3												0.0042									
13	0.08172	0.307479		0.116343	0.16343	0.1233	0.25485	0.0665		0.00693		0.0291	0.001385	0.1427	0.0346	0.32964		0.0166		0.0014	0.0014
13.2								0.00693													
13.3																					
13.4																					
14	0.0097	0.297784		0.042936	0.02632	0.1343	0.3615	0.0789		0.05679		0.241	0.106648	0.0069	0.0042	0.166205		0.0249			0.0928
14.2						0.0014	0.02355											0.0028			
14.3								0.0028													
15		0.196676	0.03186	0.001385		0.1704	0.15651	0.1496		0.32133	0.001385	0.0596	0.272853	0.0014		0.103878		0.0402			0.1053
15.2							0.03601														
15.3								0.0582													
15.4																					
16		0.132964	0.02216			0.1468	0.05679	0.1357		0.38227	0.037396	0.0014	0.238227			0.033241		0.0402			0.2008
16.2						0.0014	0.01524											0.0014			
16.3								0.0609													
17		0.027701	0.12742			0.1385	0.00693	0.0471		0.07479	0.185596		0.210526			0.001385		0.0623			0.2839
17.1																					
17.2							0.00139														

[illegible]

SM2. Parameter values and conditions considered in the three informatics tools: LRmix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7.

Table S2: Software parameter values introduced in the three informatics tools: LRmix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7. ^a Dropout is directly estimated through the peak height distribution. ^b Per locus, specified by the software considering the default conditions (i.e. disregarding the population size).

Parameters \ Values	LRmix Studio	EuroForMix	STRmix™
Coancestry Coefficient	0.01		
Drop-in frequency	0.05		
Drop-in parameters' distribution	N/A	Lambda (λ): 0.01	Gamma (γ): (0.1,0.1)
Drop-in cap	N/A	N/A	100
Dropout	0.1	^a	^a
Minimum allele frequency	0.001	0.001	^b
Threshold detection	N/A	100	100
Stutters	Without	With	With

SM 3: LR values (log10 scale) computed by LRMix Studio v.2.1.3, EuroForMix v. 3.4.0, and STRmixTM v.2.7, for mixtures with two and three estimated contributors.

Table S3_2a: LR values (log10 scale) computed by LRMix Studio v.2.1.3, for mixtures with two estimated contributors.

LRmix Studio				
PAIR	NUMBER OF MARKERS ANALYZED	Log10 of LR values considering .		DIFFERENCE
		Default (2 contributors)	Overestimation (3 contributors)	
1_2	21	3.261555867	5.974774233	2.713218366
2_2	21	7.405694248	8.183648463	0.777954216
3_2	21	11.17615386	10.61477025	-0.561383615
4_2	21	13.87206516	12.14129118	-1.730773974
5_2	21	15.79579188	14.33035012	-1.465441758
6_2	21	12.98362489	11.39911854	-1.584506345
7_2	21	14.03490926	13.33095722	-0.703952047
8_2	21	14.86010405	15.01766806	0.157564013
9_2	21	17.30965826	15.66423087	-1.645427393
10_2	21	14.62467145	13.73672558	-0.887945867
11_2	21	11.1455232	10.49106467	-0.654458533
12_2	21	16.82552327	15.15725003	-1.668273241
13_2	21	8.935058211	8.510897379	-0.424160832
14_2	21	5.680035923	5.396895759	-0.283140163
15_2	21	7.847115911	7.880234589	0.033118678
16_2	21	10.71202844	9.981939207	-0.730089233
17_2	21	10.58183388	10.13279981	-0.449034071
18_2	21	10.98514367	11.95464633	0.969502654
19_2	21	13.28694816	12.07896164	-1.207986513
20_2	21	13.92918693	13.30614363	-0.6230433
21_2	21	13.78060353	12.31588492	-1.464718607
22_2	21	11.43004412	10.41655485	-1.013489269
23_2	21	10.16529241	8.622860869	-1.542431543
24_2	21	12.92617679	11.48721597	-1.438960818
25_2	21	8.459968032	8.287939711	-0.172028321
26_2	21	14.71940267	13.19492087	-1.5244818
27_2	21	14.12759911	12.4258036	-1.701795502
28_2	21	14.01536345	12.67674183	-1.338621616
29_2	21	13.48830641	12.40283316	-1.085473246
30_2	21	14.01800874	12.73340525	-1.284603493
31_2	21	13.59221574	13.33203419	-0.260181556
32_2	21	14.04934493	12.9555363	-1.093808636
33_2	21	12.12070944	11.49897886	-0.621730579
34_2	21	15.82187459	14.1416433	-1.680231298
35_2	21	16.50789866	15.19845822	-1.309440433
36_2	21	15.47873218	14.07790754	-1.400824647
37_2	21	13.34072662	11.68362059	-1.657106024
38_2	21	15.97787018	14.53604402	-1.441826159
39_2	21	12.16805597	10.78307487	-1.384981106
40_2	21	15.36059397	13.64848762	-1.712106353
41_2	21	18.90347567	16.79384235	-2.109633324

42_2	21	13.59442284	11.87634969	-1.718073146
43_2	21	13.67932149	12.36326013	-1.31606136
44_2	21	14.31079431	12.65928702	-1.65150729
45_2	21	18.5064023	17.28580782	-1.220594483
46_2	21	13.67777705	12.27203258	-1.405744477
47_2	21	17.38650941	15.38145253	-2.00505688
48_2	21	14.21535825	14.54509547	0.329737213
49_2	21	12.35857733	12.19346642	-0.165110912
50_2	21	18.31238353	16.41939273	-1.892990803
51_2	21	13.02048264	12.35887589	-0.661606749
52_2	21	11.77725702	10.07452843	-1.702728583
53_2	21	13.34261721	11.28961843	-2.052998785
54_2	21	12.29031544	11.24166608	-1.048649354
55_2	21	13.43063649	12.08810157	-1.342534913
56_2	21	12.24759506	11.36267772	-0.884917334
57_2	21	11.50469608	10.69901937	-0.805676714
58_2	21	15.5509191	14.0101257	-1.540793398
59_2	21	14.78408289	13.85804469	-0.926038198
60_2	21	16.11687494	14.21065247	-1.906222462
61_2	21	15.79539352	13.96000284	-1.835390681
62_2	21	15.85271176	14.14572947	-1.706982293
63_2	21	13.5960462	12.40520474	-1.190841454
64_2	21	16.04126649	14.29478426	-1.746482231
65_2	21	15.64780668	14.23790885	-1.409897824
66_2	21	15.38172788	13.21344108	-2.168286796
67_2	21	14.0630082	12.07002993	-1.992978263
68_2	21	14.00102049	13.04040213	-0.960618357
69_2	21	14.31944256	13.13098245	-1.188460111
70_2	21	14.83147324	13.33811449	-1.493358753
71_2	21	16.76212636	14.89150049	-1.870625865
72_2	21	14.18357385	12.79673814	-1.386835716
73_2	21	14.69958953	13.26400273	-1.435586797
74_2	21	15.41872449	13.95320169	-1.465522805
75_2	21	18.00197589	15.91092306	-2.091052829

Table S3_2b: LR values (log10 scale) computed by EuroForMix v. 3.4.0, for mixtures with two estimated contributors.

EuroForMix				
PAIR	NUMBER OF MARKERS ANALYZED	Log10 of LR values considering .		DIFFERENCE
		Default (2 contributors)	Overestimation (3 contributors)	
1_2	21	7.927961408	10.71001269	2.78205128
2_2	21	8.444753876	9.359112282	0.914358406
3_2	21	14.14865573	14.14724033	-0.001415405
4_2	21	18.9162635	19.03674211	0.120478607
5_2	21	20.55099707	20.55491766	0.003920589
6_2	21	23.43326278	23.55516257	0.121899789
7_2	21	18.15829858	18.24736155	0.089062973
8_2	21	24.34936888	24.41531773	0.065948844
9_2	21	16.58992434	16.59056525	0.000640913
10_2	21	20.71446385	20.71397195	-0.000491901
11_2	21	26.69124692	26.66754094	-0.023705984
12_2	21	26.68903622	27.02565423	0.33661801
13_2	21	16.54164571	16.54516582	0.003520118
14_2	21	8.572155479	8.870027989	0.29787251
15_2	21	12.34987557	12.45913153	0.109255966
16_2	21	10.149093	10.17406766	0.024974665
17_2	21	14.25309526	14.25310259	7.33056E-06
18_2	21	19.03621211	19.09566724	0.059455131
19_2	21	16.64183306	16.6619634	0.020130344
20_2	21	14.99417429	13.89471994	-1.099454345
21_2	21	15.80924136	15.81282816	0.003586804
22_2	21	16.32780352	16.36794733	0.040143815
23_2	21	17.79821514	17.81320647	0.014991331
24_2	21	17.9371393	17.93686749	-0.000271812
25_2	21	18.9144039	18.92756528	0.013161387
26_2	21	17.35212396	17.35305946	0.000935505
27_2	21	18.51560732	18.39495861	-0.12064871
28_2	21	17.18062122	17.18116499	0.000543777
29_2	21	17.15547659	17.16257138	0.007094787
30_2	21	16.37009274	16.36949366	-0.000599082
31_2	21	18.79376311	18.79542684	0.001663728
32_2	21	15.61775466	15.61453125	-0.003223414
33_2	21	21.21566648	21.29200756	0.076341081
34_2	21	18.71660024	18.71660064	3.96191E-07
35_2	21	20.22702566	20.22538001	-0.001645651
36_2	21	21.17895834	21.18374567	0.004787328
37_2	21	21.85987351	21.62523149	-0.234642019
38_2	21	20.11672317	20.117975	0.001251831
39_2	21	22.31054819	21.60247854	-0.708069653
40_2	21	20.89043433	20.8903285	-0.000105834
41_2	21	20.23824605	20.24076628	0.002520231
42_2	21	22.96054738	22.96611669	0.005569312
43_2	21	22.55825773	22.55827938	2.16444E-05
44_2	21	23.05469213	23.05772903	0.003036901
45_2	21	22.69983887	22.69983462	-4.24439E-06

46_2	21	23.36030357	23.36124987	0.0009463
47_2	21	21.71472691	21.71489669	0.000169779
48_2	21	24.71429572	25.17296934	0.458673625
49_2	21	24.37254244	24.37545065	0.002908207
50_2	21	23.21906364	23.21909244	2.88062E-05
51_2	21	26.18409114	26.18392997	-0.000161174
52_2	21	25.32352507	25.32238724	-0.001137831
53_2	21	25.18482274	25.20292611	0.018103369
54_2	21	26.52762866	26.53165376	0.004025105
55_2	21	24.78198553	24.78244991	0.000464377
56_2	21	26.41025337	26.41309338	0.00284001
57_2	21	26.71067461	26.7233639	0.012689289
58_2	21	25.5550486	25.55509942	5.08136E-05
59_2	21	27.03166804	27.105674	0.074005954
60_2	21	26.06180828	26.06684292	0.005034633
61_2	21	25.7668327	25.76000486	-0.006827836
62_2	21	26.35344141	26.3536228	0.000181389
63_2	21	27.04759424	27.05048017	0.002885935
64_2	21	26.19902215	26.20352225	0.004500099
65_2	21	27.73663912	27.73669737	5.82478E-05
66_2	21	27.73905594	27.73921271	0.000156763
67_2	21	27.99614279	27.99709374	0.000950948
68_2	21	28.64865102	28.64869841	4.73964E-05
69_2	21	28.12368857	28.12374837	5.97993E-05
70_2	21	28.2338101	28.23513498	0.001324877
71_2	21	27.74995783	27.75011146	0.000153623
72_2	21	28.82434796	28.82440929	6.13357E-05
73_2	21	29.62237668	29.51668049	-0.105696185
74_2	21	29.60802844	29.60831251	0.000284073
75_2	21	29.64309324	29.64285112	-0.000242121

Table S3_2c: LR values (log10 scale) computed by STRmix™ v.2.7, for mixtures with two estimated contributors.

STRmix				
PAIR	NUMBER OF MARKERS ANALYZED	Log10 of LR values considering .		DIFFERENCE
		Default (2 contributors)	Overestimation (3 contributors)	
1_2	18	14.86573156	15.06621408	0.200482516
2_2	20	9.460010315	11.87255249	2.41254218
3_2	20	17.7093463	17.79027109	0.080924791
4_2	20	22.22287248	20.00715011	-2.215722372
5_2	20	21.01510816	20.77721651	-0.237891649
6_2	20	24.67930977	23.65586805	-1.023441716
7_2	20	25.57216268	24.6740708	-0.898091887
8_2	20	14.74652522	25.2669604	10.52043518
9_2	20	22.00462227	22.0489853	0.044363037
10_2	20	23.15624619	23.43784565	0.281599463
11_2	20	26.0080896	27.25129746	1.243207861
12_2	20	24.9151095	24.65906007	-0.256049433
13_2	21	9.579783597	6.50421252	-3.075571077
14_2	21	1.697438591	4.462847036	2.765408445
15_2	21	11.91505142	11.48512493	-0.429926489
16_2	21	13.55885251	11.90979664	-1.649055869
17_2	21	13.5918323	13.92415001	0.332317717
18_2	21	14.9324484	17.85337572	2.92092732
19_2	21	14.92691324	17.69050618	2.763592939
20_2	21	16.01745073	17.09850528	1.081054554
21_2	21	17.2230804	17.26801602	0.044935623
22_2	21	19.60045012	19.89248974	0.292039621
23_2	21	19.90236737	18.39276728	-1.509600093
24_2	21	17.27443497	16.50543489	-0.769000079
25_2	21	20.44515255	18.08735543	-2.357797121
26_2	21	17.09018749	16.69850941	-0.39167808
27_2	21	16.89389486	18.38569563	1.491800767
28_2	21	17.74497324	17.73934288	-0.005630361
29_2	21	19.93807419	21.0418662	1.103792008
30_2	21	19.99297303	19.95055469	-0.042418335
31_2	21	17.76574304	17.56964307	-0.196099976
32_2	21	20.17897695	21.87990978	1.70093283
33_2	21	21.71560227	21.51141541	-0.204186864
34_2	21	21.34805161	21.17417615	-0.173875466
35_2	21	18.98762605	18.71812785	-0.269498199
36_2	21	20.68670775	20.27538047	-0.411327285
37_2	21	22.74750503	21.06647501	-1.681030014
38_2	21	22.39828731	22.12943207	-0.268855231
39_2	21	23.99390334	23.42941311	-0.564490235
40_2	21	22.05068903	22.16932168	0.118632656
41_2	21	20.04032538	20.00804631	-0.032279073
42_2	21	23.45151012	21.74796307	-1.703547052
43_2	21	23.08346679	22.62174735	-0.461719439
44_2	21	23.99857317	23.37934178	-0.61923139
45_2	21	20.61290036	20.69491217	0.082011808

46_2	21	25.42334419	23.7513484	-1.671995793
47_2	21	23.59149868	23.37476667	-0.216732014
48_2	21	23.66829271	25.70031423	2.032021524
49_2	21	26.08564729	26.08628863	0.000641341
50_2	21	21.23286911	21.77934392	0.546474816
51_2	21	24.59222118	14.66394579	-9.92827539
52_2	21	26.45295118	26.35677136	-0.096179814
53_2	21	26.31272728	26.29221175	-0.02051553
54_2	21	26.2774029	26.27133037	-0.006072533
55_2	21	27.00732095	26.82284124	-0.184479716
56_2	21	26.89873067	26.87155542	-0.027175242
57_2	21	27.6754392	27.62227621	-0.053162994
58_2	21	26.0051376	25.88233666	-0.122800932
59_2	21	26.47846549	25.98521085	-0.49325464
60_2	21	26.32982484	25.9562549	-0.373569948
61_2	21	26.98117507	24.4333057	-2.547869368
62_2	21	26.51160265	26.48498278	-0.026619867
63_2	21	28.52791332	28.53106998	0.003156653
64_2	21	27.14075937	26.74475442	-0.396004951
65_2	21	25.7616347	25.90493183	0.143297124
66_2	21	26.57989787	26.55615771	-0.023740158
67_2	21	28.38530234	28.21423435	-0.171067991
68_2	21	28.4363694	24.5101427	-3.926226705
69_2	21	28.98643581	28.94762485	-0.038810962
70_2	21	29.46737129	27.95587528	-1.511496009
71_2	21	27.61234934	21.7535754	-5.858773946
72_2	21	29.46802223	29.46486198	-0.003160246
73_2	21	30.03726709	29.96628726	-0.070979839
74_2	21	30.38623135	30.39380326	0.007571907
75_2	21	30.48572143	30.52850534	0.042783913

Table S3_3a: LR values (log10 scale) computed by LRMix Studio v.2.1.3, for mixtures with three estimated contributors.

LRmix Studio						
PAIR	NUMBER OF MARKERS ANALYZED	Log10 of LR values considering ...			DIFFERENCE	
		Underestimation (2 contributors)	Default (3 contributors)	Overestimation (4 contributors)		
					Under/Def	Over/Def
1_3	21	7.17940646	8.460106397	8.070640441	-1.280699937	-0.389465956
2_3	21	8.443806045	8.215566604	7.55795616	0.228239442	-0.657610444
3_3	21	2.360575024	2.184035297	2.904577744	0.176539727	0.720542447
4_3	21	3.671278138	7.091176493	8.162096107	-3.419898356	1.070919613
5_3	21	10.20627375	11.8792249	10.86432808	-1.672951153	-1.014896817
6_3	21	3.846707849	7.085112279	7.855885016	-3.23840443	0.770772737
7_3	21	4.436165382	5.408284995	5.171219338	-0.972119613	-0.237065657
8_3	21	10.12463795	9.388327568	8.607014458	0.736310383	-0.78131311
9_3	21	8.347695675	8.995036532	8.367927063	-0.647340857	-0.627109469
10_3	21	4.383799725	5.765630315	5.528199424	-1.38183059	-0.237430891
11_3	21	2.369417752	4.213117518	3.994603579	-1.843699767	-0.218513939
12_3	21	2.585646659	7.544634737	7.939713837	-4.958988078	0.3950791
13_3	21	4.413395709	7.826196286	7.771903613	-3.412800577	-0.054292673
14_3	21	6.184943231	8.330667718	8.068055364	-2.145724486	-0.262612354
15_3	21	6.10620856	9.007874429	8.751240455	-2.901665869	-0.256633974
16_3	21	4.328114548	5.885593318	5.704664597	-1.557478769	-0.180928721
17_3	21	-3.015395463	3.340479337	3.911363798	-6.3558748	0.570884461
18_3	21	0.327101314	4.519985359	5.04647035	-4.192884045	0.526484991
19_3	21	11.64860919	10.7685534	10.00391151	0.880055789	-0.764641884
20_3	21	6.942981228	8.132716561	7.863345437	-1.189735334	-0.269371124
21_3	21	8.613630092	8.59894201	7.959548519	0.014688083	-0.639393491
22_3	21	8.282128443	9.00944683	8.503984882	-0.727318386	-0.505461947
23_3	21	5.233506511	6.587455982	6.280580762	-1.353949472	-0.306875221
24_3	21	11.51947563	10.54396315	9.72423127	0.975512481	-0.819731876
25_3	21	9.902311343	9.63096008	9.270978055	0.271351262	-0.359982025
26_3	21	13.45046172	12.0512641	10.95668456	1.399197622	-1.094579536
27_3	21	5.164569911	6.742190102	6.819685218	-1.577620191	0.077495116
28_3	21	5.713975596	8.311645463	8.187088502	-2.597669868	-0.124556962
29_3	21	6.823073688	9.701229254	9.554888311	-2.878155566	-0.146340943
30_3	21	11.50512713	10.32307602	9.435180007	1.18205111	-0.887896013
31_3	21	7.815472402	9.114603424	8.471609388	-1.299131021	-0.642994036
32_3	21	9.243902147	9.619389292	9.119377519	-0.375487145	-0.500011773
33_3	21	10.15828455	9.348587082	8.523840444	0.80969747	-0.824746638
34_3	21	10.55648327	10.73916889	10.27771806	-0.182685626	-0.461450835
35_3	21	5.579039358	7.909683986	7.672711006	-2.330644628	-0.23697298
36_3	21	11.32931733	10.37709153	9.579138342	0.952225794	-0.797953191
37_3	21	10.51281853	9.981071879	8.990019271	0.531746649	-0.991052608
38_3	21	11.39202022	10.51363435	9.799746634	0.878385872	-0.713887711
39_3	21	8.897266365	8.800895848	8.172681299	0.096370516	-0.628214549
40_3	21	10.13785716	10.86529213	9.932860029	-0.727434976	-0.932432105
41_3	21	3.325760872	8.307925484	8.418961324	-4.982164612	0.11103584
42_3	21	6.826214008	8.956715454	8.597092335	-2.130501447	-0.359623119
43_3	21	7.356553633	9.487287588	9.086469473	-2.130733955	-0.400818115
44_3	21	11.72945484	11.22227038	10.28870544	0.507184468	-0.933564932

45_3	21	9.486779917	8.389030981	7.849929727	1.097748936	-0.539101254
46_3	21	15.4019613	14.4615856	13.13116005	0.940375699	-1.33042555
47_3	21	11.62085758	10.32473584	9.428753718	1.296121741	-0.895982125
48_3	21	9.862912926	10.34886604	9.836746801	-0.48595311	-0.512119235
49_3	21	8.302888833	8.207974526	7.558819263	0.094914307	-0.649155263
50_3	21	9.701606552	9.106147738	8.481179611	0.595458814	-0.624968127
51_3	21	5.684040345	7.913207124	7.529949818	-2.22916678	-0.383257306
52_3	21	5.686343183	7.883255165	7.756489549	-2.196911982	-0.126765616
53_3	21	8.425210955	7.525709486	6.861704676	0.899501469	-0.66400481
54_3	21	9.515658542	10.86966487	10.05887651	-1.354006327	-0.810788358
55_3	21	12.32981919	11.01929646	9.97882863	1.310522735	-1.040467827
56_3	21	11.90761418	10.86930593	10.26521413	1.038308253	-0.604091801
57_3	21	10.9315641	10.00403356	9.185535889	0.927530531	-0.818497676
58_3	21	9.996324644	9.245541328	8.559015401	0.750783316	-0.686525926
59_3	21	8.522510649	8.850130801	8.223001152	-0.327620152	-0.627129649
60_3	21	9.643532832	9.489518764	8.750316148	0.154014068	-0.739202616
61_3	21	9.822704014	9.76015136	8.997584898	0.062552654	-0.762566462
62_3	21	7.060642523	7.933851292	7.373118292	-0.873208769	-0.560733
63_3	21	9.820132146	10.51765646	10.14715468	-0.697524313	-0.37050178
64_3	21	16.73116999	14.59616282	13.26909091	2.135007165	-1.327071916
65_3	21	8.02109238	8.239264557	7.632548141	-0.218172178	-0.606716417
66_3	21	11.27498516	11.0685932	10.20001139	0.206391962	-0.868581807
67_3	21	8.495141352	9.333004998	8.767973899	-0.837863645	-0.565031099
68_3	21	13.10015045	12.17700358	11.12329495	0.923146868	-1.053708638
69_3	21	13.17932977	12.7887345	11.9934052	0.390595276	-0.795329299
70_3	21	12.82742493	11.51108429	10.69142378	1.316340646	-0.819660509
71_3	21	13.32588072	12.17542886	11.22028959	1.150451857	-0.955139274
72_3	21	16.15120397	14.19983799	13.06661025	1.951365984	-1.133227741
73_3	21	12.83670305	11.68501548	10.76859485	1.151687567	-0.916420636
74_3	21	13.16048694	12.36764573	11.15242023	0.792841205	-1.215225507
75_3	21	6.885691289	8.981186252	8.60578569	-2.095494963	-0.375400562
76_3	21	14.93928301	13.76256574	12.45123803	1.176717272	-1.311327711
77_3	21	14.38608388	13.87565618	12.54263761	0.510427694	-1.333018571

Table S3_3b: LR values (log10 scale) computed by EuroForMix v. 3.4.0, for mixtures with three estimated contributors.

EuroForMix						
PAIR	NUMBER OF MARKERS ANALYZED	Log10 of LR values considering ...			DIFFERENCE	
		Underestimation	Default	Overestimation		
		(2 contributors)	(3 contributors)	(4 contributors)	Under/Def	Over/Def
1_3	21	7.334126265	8.872919894	8.858407606	-1.538793629	-0.014512288
2_3	21	13.88157668	17.31926253	17.51528833	-3.437685846	0.196025797
3_3	21	7.566808636	10.59278355	10.27532031	-3.025974915	-0.31746324
4_3	21	10.26831936	17.54233394	17.72071321	-7.274014572	0.178379276
5_3	21	0.80345278	21.73278146	21.93057608	-20.92932868	0.197794617
6_3	21	26.63509842	28.39103356	28.36329945	-1.755935142	-0.027734109
7_3	21	7.94611421	6.391799845	*1	1.554314365	#VALOR!
8_3	21	6.673930102	9.247007109	9.724559297	-2.573077007	0.477552188
9_3	21	10.05890051	10.21651768	10.25949509	-0.157617171	0.042977409
10_3	21	4.211562231	7.483251442	7.465670418	-3.271689211	-0.017581024
11_3	21	6.295991984	7.539132507	7.557124694	-1.243140523	0.017992187
12_3	21	12.19876679	13.52770824	13.61912035	-1.328941447	0.091412112
13_3	21	3.008099609	6.780095197	6.780128411	-3.771995588	3.32145E-05
14_3	21	11.81595632	12.49068576	12.39016549	-0.674729442	-0.100520272
15_3	21	12.41978146	14.05104261	14.31859147	-1.631261152	0.267548855
16_3	21	12.25260085	11.15780554	11.02016854	1.094795308	-0.137637007
17_3	21	12.25131016	12.04271266	11.82286593	0.208597507	-0.219846722
18_3	21	12.70286653	12.06383477	11.88892276	0.639031754	-0.174912006
19_3	21	11.02792346	11.13765989	11.14049001	-0.109736426	0.002830125
20_3	21	11.31817636	11.21511709	11.17765403	0.103059271	-0.037463058
21_3	21	11.83201973	11.91963369	11.89032933	-0.087613957	-0.029304357
22_3	21	11.18943582	12.23243108	12.3543469	-1.042995257	0.121915823
23_3	21	12.69665552	12.86991883	12.71082488	-0.173263309	-0.15909395
24_3	21	12.35645705	12.35899002	12.36124388	-0.002532973	0.002253853
25_3	21	12.39831013	12.39830936	12.40152181	7.69706E-07	0.00321245
26_3	21	11.56155295	11.6783552	11.71778424	-0.116802252	0.039429034
27_3	21	11.90957042	12.80703129	12.87925342	-0.897460874	0.072222132
28_3	21	8.343728843	11.69715068	12.13562242	-3.353421834	0.438471748
29_3	21	15.79971253	16.43255978	16.43869289	-0.632847255	0.006133109
30_3	21	7.862735421	12.14782066	12.14833048	-4.285085242	0.000509821
31_3	21	14.32561948	15.59595691	*1	-1.270337426	#VALOR!
32_3	21	14.23114027	15.75992122	15.84000943	-1.528780957	0.080088205
33_3	21	13.61847075	16.01861026	15.9385798	-2.400139507	-0.080030459
34_3	21	17.34772081	17.40713589	17.43178624	-0.059415079	0.024650352
35_3	21	12.19735174	17.50007835	17.54567625	-5.302726614	0.045597894
36_3	21	14.98886111	14.92987032	14.92978231	0.058990792	-8.801E-05
37_3	21	17.76848305	18.56675336	18.60770696	-0.79827031	0.0409536
38_3	21	16.38224977	16.385562	16.40899979	-0.003312236	0.023437791
39_3	21	20.23823681	21.36963039	21.39719369	-1.131393583	0.027563297
40_3	21	18.66658633	18.83979338	18.86442675	-0.173207049	0.02463337
41_3	21	11.41940953	20.06189347	20.4870402	-8.642483942	0.42514673
42_3	21	17.71777474	20.42199991	20.42115866	-2.704225177	-0.000841253
43_3	21	20.03408307	19.55340066	19.56709107	0.480682411	0.013690417
44_3	21	18.81168444	19.84559545	19.91513433	-1.033911005	0.069538886

45_3	21	20.57379205	20.45106198	20.41577448	0.122730067	-0.035287498
46_3	21	21.96817131	21.92992701	21.92081011	0.038244301	-0.009116898
47_3	21	20.75322997	20.2905043	20.18103409	0.462725667	-0.109470208
48_3	21	20.68503473	21.156322	21.1889126	-0.471287275	0.0325906
49_3	21	24.40427772	24.42974057	23.92182999	-0.025462843	-0.507910575
50_3	21	20.22357656	21.36980017	21.48588826	-1.146223609	0.116088096
51_3	21	12.92131986	20.93037263	21.12040277	-8.009052775	0.190030138
52_3	21	20.39927062	21.28426784	21.0086243	-0.884997222	-0.275643537
53_3	21	23.79622525	23.92602752	23.9136693	-0.12980227	-0.012358219
54_3	21	19.30008528	20.65922733	20.79360689	-1.359142054	0.13437956
55_3	21	21.83123491	21.65328522	21.65383921	0.177949695	0.000553993
56_3	21	22.53936345	22.53667674	22.53394166	0.002686709	-0.002735073
57_3	21	23.23340636	24.29020457	24.29244324	-1.05679821	0.002238668
58_3	21	24.49622066	24.1343337	24.161397	0.361886961	0.027063305
59_3	21	17.13691296	23.59821827	23.6042487	-6.461305303	0.006030431
60_3	21	23.47207194	22.93233479	22.85637633	0.539737154	-0.075958455
61_3	21	24.67980783	24.6872103	24.68759402	-0.007402466	0.000383718
62_3	21	24.20054105	24.83821011	24.83981859	-0.63766906	0.001608473
63_3	21	24.05396192	24.32956161	24.28337908	-0.275599694	-0.046182527
64_3	21	23.63686383	23.67140894	23.67346409	-0.034545114	0.002055154
65_3	21	26.31282118	26.94599906	26.99663392	-0.633177884	0.050634856
66_3	21	25.81132324	25.77126729	25.75340646	0.040055952	-0.017860824
67_3	21	27.37825466	27.3692497	27.35928044	0.009004968	-0.009969251
68_3	21	23.82587815	24.10855207	24.13537095	-0.282673913	0.026818883
69_3	21	26.06412115	26.53473447	26.54037808	-0.470613315	0.005643609
70_3	21	26.63256094	26.57844092	26.55886303	0.054120022	-0.019577887
71_3	21	26.91253858	26.97449615	26.95587071	-0.06195757	-0.018625443
72_3	21	24.69058258	25.18578817	25.18019009	-0.495205583	-0.005598073
73_3	21	29.63349111	29.61734003	29.61577476	0.016151088	-0.001565266
74_3	21	27.44285336	28.59005293	28.60138649	-1.147199573	0.011333557
75_3	21	29.63608598	29.63260974	29.6328832	0.003476235	0.000273455
76_3	21	30.50053813	31.02969786	31.04160171	-0.529159732	0.011903848
77_3	21	32.10518657	32.34398235	32.3594066	-0.238795783	0.015424244

*1 : AN ERROR MESSAGE APPEARED: "Evidence cannot be explained given the parameters you have chosen."

Table S3_3c: LR values (log10 scale) computed by STRmixTM v.2.7, for mixtures with three estimated contribut

STRmix						
PAIR	NUMBER OF MARKERS ANALYZED	Log10 of LR values considering ...			DIFFERENCE	
		Underestimation (2 contributors)	Default (3 contributors)	Overestimation (4 contributors)		
					Under/Def	Over/Def
1_3	19	*1	9.190303678	4.481327948	#VALOR!	-4.70897573
2_3	19	*1	18.18121455	17.20512313	#VALOR!	-0.976091419
3_3	20	*1	15.46993977	13.74874605	#VALOR!	-1.721193728
4_3	20	*1	16.68244293	16.08482642	#VALOR!	-0.597616514
5_3	20	*1	23.02069268	22.755097	#VALOR!	-0.265595678
6_3	20	*1	26.44237001	26.29272114	#VALOR!	-0.149648868
7_3	20	*1	7.249418507	6.399258261	#VALOR!	-0.850160246
8_3	21	*1	7.815112658	12.89305122	#VALOR!	5.077938565
9_3	21	*1	4.179781516	10.23829707	#VALOR!	6.058515552
10_3	21	*1	5.095308861	4.382881167	#VALOR!	-0.712427694
11_3	21	*1	8.857790677	6.810346791	#VALOR!	-2.047443886
12_3	21	*1	1.521138084	5.648067129	#VALOR!	4.126929046
13_3	21	*1	11.8360328	11.96250666	#VALOR!	0.126473859
14_3	21	*1	4.029099567	3.855034317	#VALOR!	-0.17406525
15_3	21	*1	1.440294971	6.989098146	#VALOR!	5.548803175
16_3	21	*2	10.68327224	10.02824582	#VALOR!	-0.65502642
17_3	21	*1	12.46670454	13.23514954	#VALOR!	0.768444992
18_3	21	*1	12.90096762	12.60091847	#VALOR!	-0.300049155
19_3	21	*2	7.773947265	9.210157938	#VALOR!	1.436210673
20_3	21	*2	11.33231724	10.95504762	#VALOR!	-0.377269624
21_3	21	*2	12.36904879	12.0857186	#VALOR!	-0.283330194
22_3	21	*1	11.37604745	12.05146124	#VALOR!	0.675413792
23_3	21	*1	13.97110414	13.68740409	#VALOR!	-0.283700044
24_3	21	7.612381153	10.6792825	10.63833947	-3.066901349	-0.040943027
25_3	21	10.88843786	11.88918961	12.04004824	-1.000751753	0.15085863
26_3	21	*2	10.80632924	10.3339508	#VALOR!	-0.472378433
27_3	21	*1	14.83384168	14.62196519	#VALOR!	-0.211876491
28_3	21	*2	10.86185688	15.82993385	#VALOR!	4.968076971
29_3	21	*1	11.88286592	9.835208459	#VALOR!	-2.047657461
30_3	21	*1	16.17400163	15.23671356	#VALOR!	-0.937288063
31_3	21	*1	15.37417991	15.26528963	#VALOR!	-0.108890284
32_3	21	*1	16.41276285	15.40967957	#VALOR!	-1.003083282
33_3	21	*1	17.22370356	18.66916806	#VALOR!	1.445464497
34_3	21	*1	16.06777736	16.13385813	#VALOR!	0.066080767
35_3	21	*1	20.33663982	19.90120214	#VALOR!	-0.435437681
36_3	21	*1	20.85253489	20.15314386	#VALOR!	-0.69939103
37_3	21	*1	17.83072978	17.59703666	#VALOR!	-0.23369311
38_3	21	*1	20.40401223	19.30241752	#VALOR!	-1.101594707
39_3	21	*1	18.69671451	18.24696611	#VALOR!	-0.449748406
40_3	21	*1	17.62583699	18.43613083	#VALOR!	0.810293837
41_3	21	*1	19.6427612	20.70914271	#VALOR!	1.066381504
42_3	21	*2	18.75797268	17.0728746	#VALOR!	-1.685098084
43_3	21	*2	20.13580028	21.53746648	#VALOR!	1.401666198
44_3	21	9.589044344	17.64837953	17.1059868	-8.059335186	-0.542392734
45_3	21	21.67894609	21.05880549	20.54339747	0.620140603	-0.515408014

46_3	21	*1	13.82714302	12.74649408	#VALOR!	-1.080648938
47_3	21	*1	20.22282048	19.85144181	#VALOR!	-0.371378667
48_3	21	21.33278172	20.74573042	19.97197591	0.587051295	-0.773754516
49_3	21	*1	17.0510367	25.29309726	#VALOR!	8.242060561
50_3	21	*1	23.67368429	23.09229948	#VALOR!	-0.581384813
51_3	21	*1	22.32234336	6.814367476	#VALOR!	-15.50797588
52_3	21	*1	24.48280215	24.37757942	#VALOR!	-0.105222727
53_3	21	*1	23.79169065	23.82992742	#VALOR!	0.038236773
54_3	21	*1	24.65049208	24.5008263	#VALOR!	-0.149665786
55_3	21	*1	23.80110859	23.67006022	#VALOR!	-0.131048374
56_3	21	24.42132418	24.23641129	24.31588642	0.18491289	0.079475128
57_3	21	23.78913632	23.71083636	22.18486171	0.078299959	-1.525974652
58_3	21	*1	24.54501006	24.47944574	#VALOR!	-0.065564325
59_3	21	*1	26.27413475	20.92958239	#VALOR!	-5.344552354
60_3	21	*1	27.16888034	26.66909363	#VALOR!	-0.499786711
61_3	21	*1	25.50821988	25.3437433	#VALOR!	-0.164476584
62_3	21	*1	24.90247068	20.83607081	#VALOR!	-4.066399867
63_3	21	*1	24.90646973	24.43571699	#VALOR!	-0.470752739
64_3	21	*2	23.40599484	23.60176674	#VALOR!	0.195771902
65_3	21	*1	26.69321675	26.11216915	#VALOR!	-0.581047601
66_3	21	*1	26.69321675	24.99758732	#VALOR!	-1.69562943
67_3	21	*1	27.75802575	27.7287838	#VALOR!	-0.02924195
68_3	21	*2	28.28053284	28.1108926	#VALOR!	-0.169640244
69_3	21	*1	25.88939696	25.5774918	#VALOR!	-0.311905157
70_3	21	27.08817154	27.12201917	27.10991586	-0.033847632	-0.012103309
71_3	21	*1	26.80330686	26.61697936	#VALOR!	-0.186327502
72_3	21	*1	27.83932718	27.31454133	#VALOR!	-0.524785852
73_3	21	27.28791365	27.22983537	27.21845673	0.058078281	-0.01137864
74_3	21	*1	27.68372228	27.48398646	#VALOR!	-0.199735818
75_3	21	29.84834309	29.83546861	29.82715595	0.012874482	-0.008312661
76_3	21	*1	29.97573961	29.99080044	#VALOR!	0.015060824
77_3	21	*1	30.56349182	30.1943632	#VALOR!	-0.369128622

*1 : AN ERROR MESSAGE APPEARED: "Evidence cannot be explained given the parameters you have chosen.

*2 : Cases where an LR equal to zero was assigned and for that reason, it was not possible to calculate a log10 val

CHAPTER III | Statistical Analysis Tools of Mixture DNA Samples: When the Same Software Provides Different Results

Costa C*, Figueiredo C, Amorim A, Prieto L, Costa S, Ferreira PM, Pinto N: **Statistical analysis tools of mixture DNA samples: When the same software provides different results.** *Forensic Science International: Genetics Supplement Series* 2022, **8**:37-39

This paper resulted from a study selected for oral presentation at the 29th Congress of the International Society for Forensic Genetics (ISFG), held in Washington, DC, USA, 2022. I had the privilege of personally presenting this work, having been awarded one of ten travel bursaries for young scientists offered by ISFG to attend the Congress.

As the first and corresponding author, I was extensively involved in all stages of this study, from the methodological planning to final manuscript preparation. I co-developed the study design, which focused on evaluating the impact of drop-in frequency parameter variation on LR results, using real casework pairs of samples composed of a mixture (with two and three estimated contributors) and an associated reference sample. I was responsible for processing, analyzing, and interpreting the variability observed in LR values across different parameter settings. Finally, I drafted the initial version of the manuscript and subsequently revised and finalized it based on the feedback and suggestions provided by the other co-authors.



Statistical analysis tools of mixture DNA samples: When the same software provides different results

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ABSTRACT

The high complexity of the genetic analysis of crime scene samples is mainly related to the unknown number of contributors, low DNA quantity and quality, and associated stochastic effects. The difficulty and subjectivity of interpreting casework samples was the motto for the development of software to mitigate these conditions and allow the quantification of the genetic evidence. Currently, there are several tools for statistical analysis of mixture samples based on either qualitative or quantitative models. The first considers the electropherograms' qualitative information, while the latter also considers the associated quantitative information. This work's main goal was to evaluate the effect that parameters' settings variation may have on the LR computation, specifically the drop-in frequency parameter. For that, a qualitative – LRmix Studio – and two quantitative software – STRmixTM and EuroForMix – were considered and an intra-software analysis was performed, using as input real casework samples. The drop-in frequency variation showed an impact, leading to differences higher than four units (log₁₀ scale) for some pairs of samples. In addition, for some cases, no comparisons were performed either because the tool computed a null LR value or displayed an error message. Thus, this work reinforces the importance of proper parameters' modeling and estimation in forensic casework evaluation.

1. Introduction

DNA samples recovered from crime scenes may have associated several characteristics that make them difficult and complex for the expert to interpret and analyze. DNA mixtures, i.e., samples containing contributions from more than one donor (exact number unknown), are typically encountered in this context. Beyond the unknown number of contributors (NOC), the high complexity also results from allele sharing between contributors, contributions in different proportions, stutters which may be confounded with alleles of a minor contributor (or vice versa), amplification stochastic effects (heterozygotic imbalance, drop-in, and drop-out) due to low-template DNA, or degradation [1–12].

To deal with the abovementioned conditions and quantify the genetic evidence, several probabilistic genotyping software were

developed. These tools are based on either qualitative or quantitative models. The first only considers the qualitative information of the electropherogram (observed alleles), while the other also considers the quantitative information (height of the detected peaks) [13,14]. Both models quantify the genetic evidence through the computation of a Likelihood Ratio (LR) [4], comparing the probabilities of observing the genetic evidence assuming two alternative and mutually exclusive hypotheses. This calculation can account for several parameters, regarding population and analytical factors, on which the LR depends, and which are introduced by the user, such as NOC, drop-in, co-ancestry coefficient, and threshold detection.

The main goal of this work is to evaluate the impact that the estimation of the drop-in frequency parameter may have on the quantification of the evidence, using casework samples. Both qualitative –

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LRmix Studio v.2.1.3 [15] – and quantitative tools – EuroForMix v. 3.4.0 [16] and STRmix™ v.2.7 [17] were considered. A drop-in allele corresponds to a spurious allele in the problem sample profile, i.e., an allele that shows up in the problem sample profile but cannot be explained by any of the contributors [6]. This stochastic effect generates discordances between the mixture and the contributors' profiles, which can result in misinterpretations and misleading conclusions. So, understanding its impact is extremely important [10,11].

2. Material and methods

Resorting to evidence from *Laboratório de Polícia Científica da Polícia Judiciária* former cases, a set of 156 irreversibly anonymized mixture/single contributor sample pairs were selected. For each case the single contributor sample was either from sampled individuals or single-source problem sample profiles obtained in the same case of the analyzed mixture. It was assumed as belonging to the person of interest (POI) but are not necessarily known. The mixture samples considered were estimated to have either two or three NOC. Casework samples were chosen due to their unknown composition, uniqueness, and inability to predict and replicate, carrying a much higher complexity than mock ones.

For each pair selected, a LR value was computed assuming the alternative hypotheses: “The POI is a contributor of the mixture” and “The POI is unrelated to any contributor of the mixture”. The LR values were computed using both qualitative (LRmix Studio v.2.1.3 [15]) and quantitative (EuroForMix v.3.4.0 [16] and STRmix™ v.2.7 [17]) tools. To understand the impact that drop-in frequency may have on the LR computation, the frequency was varied to lower (0.00) and higher values (0.10) relative to the established default (0.05) [18]. Afterward, an intra-software analysis was carried out through a comparison between the values computed under the default and varied conditions for each pair of samples.

The allelic frequencies of the National Institute of Standards and Technology (NIST) database concerning the Caucasian population were used [19].

3. Results and discussion

In general, the variation of drop-in frequency did not have a great impact on the LR values provided by the same software, as most of the computed differences were within one unit in a log₁₀ scale. For all the qualitative software comparisons (LRmix Studio), no calculated difference exceeded one log₁₀ unit, reflecting its lower sensitivity to this parameter variation compared to quantitative tools.

For both quantitative tools, differences higher than four log₁₀ units were observed - in EuroForMix when the drop-in frequency was considered null (one case with a mixture with two, and one case with a mixture with three estimated NOC), and in STRmix™ when the drop-in frequency was considered either as null or equal to 0.10 (two cases for each comparison; mixtures with three estimated NOC).

The impact of parameter's variation also translates into cases for which no comparison was performed, as the software either computed a null LR value – as STRmix™ – or does not compute a value at all – as both LRMix Studio and EuroForMix. This was observed when a null drop-in frequency was considered. For LRMix Studio and EuroForMix, this occurred in 25% and 27% (respectively) of the cases involving mixtures with two estimated NOC, and in 4% and 5% (respectively) of the cases with mixtures with three estimated. In STRmix™, this occurred in only 1% of the cases for both mixtures with two and three estimated contributors.

4. Conclusion

Before performing any computation, the expert needs to establish and introduce a value for some software parameters, such as NOC, F_{ST}, drop-in, or detection threshold. Thus, it is crucial to understand the

impact those parameters may have on the resulting LR. Focusing on the drop-in frequency parameter, LR values were computed under both the established default and varied values of the drop-in frequency, for each pair analyzed case. The results obtained by LRMix Studio, EuroForMix, and STRmix™ were compared and the impact within software was evaluated.

LRmix Studio showed to be less sensitive to this parameter variation than the quantitative tools. However, in both quantitative differences of more than four units (log₁₀ scale) were observed. Furthermore, for all software, there were cases for which no comparisons were performed due to the computation of a null LR value or an error message display.

This work emphasizes the importance of experts' understanding of the models incorporated in the existing tools and of its proper estimation, specifically for the case of drop-in.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER IV | The Impact of Parameter Variation in the Quantification of Forensic Genetic Evidence

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In this publication, I was also the first and corresponding author. I was involved from the conceptual stage, contributing to the formulation of the research problem and helping shape the methodological approach. I was also responsible for conducting the software-based analysis of the real casework data, which consisted of analyzing the same pairs of samples (composed of a mixture with two or three estimated contributors and an associated reference sample) through three different informatics tools, varying a parameter at a time – drop-in (frequency and modeling), analytical threshold and inclusion or not the stutter peaks in the analysis. Then, I was also responsible for assessing and interpreting the output obtained. I wrote the first draft of the manuscript and the subsequent rounds of editing and peer-review responses during the manuscript revision process.



OPEN The impact of parameter variation in the quantification of forensic genetic evidence

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Technological advancements have allowed the detection of increasingly complex forensic genetics samples, as minimum amounts of DNA can now be detected in crime scenes or other settings of interest. The weight of the evidence depends on several parameters regarding the population and sample-related analytical factors, the latter in a greater number when the DNA amount is considered. This led to the development of probabilistic genotyping software (PGS), able to deal with the associated complexities. This study aims to evaluate the impact on the evidence's weighing, when different analytical threshold values are used, and when different models and/or estimates for analytical artifacts, such as stutters or drop-in parameters, are considered. To reach this goal, three PGS, based on different statistical models, were used to analyze real casework pairs of samples composed of a mixture with either two or three estimated contributors, and a single-source sample associated. The obtained results show that the estimation of these parameters must not be overlooked, as they may considerably impact the outcome. This underlines the importance of proper parametrization in the analysis of forensic genetics identification problems when using complex samples, and the understanding by practitioners of how probabilistic genotyping informatics tools work to use them accurately.

Keywords Forensic DNA, Mixture samples, Likelihood ratio, Drop-in, Analytical threshold, Stutters

With the urge to overcome the difficulty and subjectivity of interpreting complex DNA casework samples, probabilistic genotyping methods were developed and implemented as software. These software tools allow the experts to quantify the weight of the genetic evidence with a robust and scientifically supported statistical approach, considering the short tandem repeat (STR) profiles of evidence and reference(s) samples obtained through the gold standard genotyping methodology of fragment length determination—capillary electrophoresis (CE).

DNA samples recovered from crime scenes may be composed of contributions from more than one donor—DNA mixture—and may have several features that make them complex and difficult to analyze, as described next. The decision on the presence (or not) of non-allelic peaks (artifacts or stochastic effects)^{1–10} is a crucial step for the quantification of the evidence, as well as the estimation of the number of contributors to the mixture, both depending on the evaluation of the expert^{11–16}. Besides the true number of contributors being always unknown and having to be estimated by the expert, other constraints make these samples' interpretation difficult, such as a. allele sharing between contributors, b. contributions in different proportions, c. stutter peaks which may be confused with alleles of a minor contributor (or vice versa), or even d. amplification stochastic effects (heterozygotic imbalance, drop-in, and/or drop-out) due to low-template DNA.

Indeed, despite the important role of computer programs in evaluating the evidence, the expert continues to have a crucial role in the entire course regarding the weight of the evidence¹⁷. It is the expert's responsibility to perform a correct and meticulous analysis from the very beginning of the process, that is, from the evidence collection to the electropherogram (EPG) analysis, so that possible errors are minimized. Also, internal protocols based on international and well-established guidelines should be strictly followed to avoid bias and maintain

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consistency between and within the analysis. A well-collected sample, i.e. maximizing the genetic material subjected to analysis and minimizing the possible sources of contamination, provides a profile easier to interpret, reducing ambiguity and therefore minimizing erroneous conclusions^{18–22}. The STR profile is interpreted by the expert through the EPG analysis after sample processing—that is, after the DNA extraction, purification, quantification, and amplification, followed by STR separation and detection, and finally STR genotyping.

Once the genotypic profiles (of both the unknown and corresponding reference sample) are obtained and pre-processed, the weight of the DNA evidence may be quantified in probabilistic genotyping software^{23,24}. The weight of the evidence is performed through the computation of a Likelihood Ratio (LR) value^{25,26}, which provides a comparison between the probability of observing the genetic evidence assuming two alternative and mutually exclusive hypotheses. In standard identification problems, these hypotheses are “H1 = The person of interest (POI) is a contributor of the mixture” and, alternatively, “H2 = The POI is not a contributor nor genetically related to any contributor of the mixture”. This calculation depends on several parameters regarding population—alleles frequencies and coancestry coefficient—and analytical factors—drop-in, dropout, analytical threshold, and stutters, which will be described next.

The probabilistic genotyping tools are based on different models regarding the EPG information considered to quantify the genetic evidence^{27,28}. If only the qualitative information (observed alleles in the profile) is considered, software are said to be based on a qualitative, or semi-continuous, model—e.g., Lab Retriever²⁹ or LRmix Studio³⁰. On the other hand, if both qualitative (observed alleles) and quantitative information (height of the observed allele peaks) are considered for computations, the software is said to be based on a quantitative, or continuous, model—e.g., EuroForMix³¹ or STRmix³².

It was already shown that different informatics implementations and statistical modeling may lead to important differences in the quantification of the evidence's weight, through both mocked and real casework samples^{33–36}. Specifically in Costa et al.¹⁷ a comparison between LRs assigned by two quantitative (EuroForMix and STRmix[™]) and one qualitative (LRmix Studio) informatics tools, for the same real casework input data, was performed maintaining the values/conditions of the software parameters as similar as possible. In some cases, relevant differences were observed. These results were the motto for the present work where the differences obtained in the statistical LR outputs when considering different parameters' values for the same informatics tools are assessed. A recent study³⁷ has shown that differences due to validated parameters' conditions are small for a particular software and, in most cases, did not produce contradictory interpretations. Nevertheless, it will be interesting to understand the impact of applying different statistical models to the same parameter like the following ones considering different informatics tools.

Drop-in and drop-out

A drop-in allele corresponds to a spurious peak observed in the sample profile, i.e., an allele that shows up in the evidence profile that resulted from a source unassociated with the sample (for example from a crime scene environment or laboratory plasticware) and, therefore, cannot be explained by any contributor. It can be distinguished from gross contamination due to a large amount of peaks detected in the latter^{7,25}. This stochastic effect generates thus discordances between the mixture sample and the contributor profiles, which can lead to misinterpretations and, consequently, wrong conclusions^{8,9}.

The drop-in frequency is laboratory-specific and is usually estimated considering the number of negative controls out of all negative controls in which an additional allelic component, an irreproducible and unexplained peak, appears in the EPG. A negative control is an additional reaction not containing any type of biological material (besides those of the DNA extraction, amplification reaction, and injection) that is analyzed at the same time and under the same conditions as the samples³⁸. The higher the drop-in frequency, the less likely it is that an allele is considered to belong to a mixture contributor. So, the software for statistical analysis of mixture samples may consider the drop-in frequency value (in both quantitative and qualitative models) and the distribution of drop-in parameters (only in quantitative models, those considering peak heights) when computing LR values^{39–41}. The information on the height of the drop-in peaks is used to estimate the distribution of drop-in parameters, which can be modeled by different statistical distributions depending on the software tool^{42–44}. For example, EuroForMix models the drop-in through a lambda distribution (λ), while STRmix[™] employs either a gamma (γ) or a uniform distribution. If limited observations of drop-in have been made or if the dataset is limited in size, the STRmix[™] may not be able to develop a reliable gamma model. So, instead of determining the parameters of the gamma distribution (α and β), the use of a uniform distribution is recommended.

Conversely, allele dropout is a stochastic amplification event that causes an allele present in the contributors' profiles to be absent from the mixture profile to which they contributed due to the low quality and quantity of DNA usually encountered in forensic samples⁷. As for drop-in, discordances between the mixture sample and the contributor profiles may be generated, and the proper modeling of the dropout phenomena should also be considered for an accurate analysis^{8,9}. Parameters related to the dropout probability may have to be estimated and introduced by the user as in qualitative tools³⁰ or could be directly estimated by the software through the height of the peaks in the case of quantitative programs^{26,39–41,44–47}. The latter will model this parameter with the same function used to model the peak height distribution, which can differ between tools. For example, EuroForMix software models peak height using a gamma distribution, while STRmix[™] uses a log-normal distribution³¹. As there has been an increasing use of quantitative tools, under which drop-out estimates are automatically calculated directly by the software with no possible user action, this parameter will not be further analyzed in this study.

Analytical threshold

The analytical threshold is a previously defined value (measured in relative fluorescence units, RFUs) introduced in the peak detection software, for distinguishing true alleles from the baseline noise, considering only as true

alleles peaks higher than the defined threshold. The baseline noise consists of non-reproducible peaks, resulting from electronic fluctuations, air bubbles, and crystals in the polymer in CE capillary, or low-level sample contamination⁵. There is no standard rule for determining the analytical threshold value that should be used, and each laboratory should establish its values based on the corresponding internal validation procedures. However, it should always be taken into account that the estimation of this value encloses a risk-reward decision. Indeed, a value that is too high may result in loss of information due to the discarding of true alleles with smaller heights, substantially affecting the global LR computed value. On the other hand, a value that is too low may result in the incorrect assignment of the peaks, that is, baseline noise peaks being considered true alleles. In any case, it should be reinforced that the determination of this analytical threshold must be part of the internal validation procedure of any laboratory, which may depend on several factors including those related to the type of samples to be analyzed⁴⁸. There are several methods available to assist experts in the estimation of this parameter, as is the case of STR-validator (free and open-source software available at <https://sites.google.com/site/forensicapps/strvalidator>) that can also be used to estimate other parameters⁴⁹.

Quantitative probabilistic genotyping tools incorporate this parameter since they consider alleles' peak height to perform their computations. In contrast, in qualitative tools, any detected peak is considered a true allele.

Stutter artifacts

Stutter peaks are a PCR product artifact that results from a slipped-strand mispairing during the PCR extension phase and constitutes the most encountered artifact in an EPG. When the two strands (template and extending strand) re-anneal, one of them loops out and the other aligns in front of the supposed position. If the loop occurs in the template strand one or more repeat units are deleted in the new strand, but if the loop happens in the extending strand it will result in the addition of repeat(s) unit(s) to the new strand (less common). Considering single-source samples, this artifact can be easily identified by its size and position; however, when analyzing mixed-source samples, it becomes difficult to differentiate it from a minor contributor allele^{1,2}. To maximize the likelihood that no true allele information is lost, various studies advocate the use of software able to model stutters when analyzing the evidence sample instead of either using a stutter filter (during the STR genotyping phase)³ or relying on (subjective) human decisions^{4,5}. Based on these studies, quantitative software such as STRmix™ requires the inclusion of stutters in the evidence sample⁵⁰. On the other hand, other quantitative tools—e.g., EuroForMix—give the user the possibility to decide whether or not to include several types of stutters as an extension of the model³¹. STRmix™ models locus-specific amplification efficiencies and considers expected stutter ratios per locus defined using empirical data. EuroForMix does not model locus-specific amplification efficiencies and uses instead a blanket expected stutter rate⁴⁴. As far as qualitative software are concerned, for tools like LRmix Studio, the inclusion of stutters is not recommended and peak stutters should be removed from mixture samples before analysis³⁰.

Goal of this study

The specific aim of this work is to demonstrate the impact that considering different values and/or conditions for the software parameters related to drop-in, analytical threshold, and stutters may have on the quantification of the evidence. Considering software based in both qualitative—LRmix Studio v.2.1.3³⁰—and quantitative models—EuroForMix v.3.4.0³¹ and STRmix™ v.2.7³², computations were performed using real casework samples and the obtained LR values were compared in an intra-software analysis when parameter values/conditions were varied.

The major objective of this work is to make practitioners aware of the importance of accurate estimation and modeling of the parameters that influence the weight of the evidence, as variations of these may have an important impact in forensic genetics routine analyses.

Material and methods

Resorting to evidence material from former cases of the Portuguese Scientific Police Laboratory, Judiciary Police, 154 irreversibly anonymized DNA mixtures with two and three previously estimated contributors, equally distributed, were selected. In addition, 154 single-source samples, each one related to the forensic cases where the DNA mixtures were detected, were also considered. Some of the single-source samples were real reference samples collected from known individuals (POI, either victim or suspect, e.g.); but some others were single-source evidence profiles detected at the same crime scene where the DNA mixtures were also found (recovered from personal belongings of interest, as a toothbrush or a razor, e.g.). For simplification purposes, both types of single-source samples will be named “reference samples” throughout the paper. For most of the pairs, the donor of the reference sample could not be excluded from being one of the mixture contributors. All methods were carried out in accordance with relevant guidelines and regulations. The samples were previously processed in the context of the respective casework, under manufacturer and internal protocols. Evidence samples were amplified using GlobalFiler™ PCR Amplification Kit and references were amplified by using GlobalFiler™ Express PCR Amplification Kit (Applied Biosystems™), both 24-locus STR kits; and, the PCR products were detected and separated by capillary electrophoresis in 3500 Genetic Analyzer with GeneMapper™ ID-X (Applied Biosystems™), with an analytical threshold equal to 100 RFU for all the dye channels.

To develop this work, the genetic information obtained for each anonymized pair of samples (mixture/reference) analyzed for a set of 21 autosomal STR markers was considered. Real casework samples were selected for the development of this work to mimic as much as possible the conditions that forensic geneticists encountered in routine analyses, as there are levels of complexity that cannot be anticipated in mocked samples. Most of the analyzed genotypic data coincide with those of Costa et al.¹⁷. Proper ethics approval was obtained from the Committee for Ethical and Responsible Conduct of Research of i3S—Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal (5/CECRI/2022), regarding the statistical analysis of both environmental

and reference genetic samples (fully and irreversibly anonymized, when applied). For confidentiality reasons, the EPGs or corresponding data input files of these samples cannot be made publicly available. All the statistics obtained per software and parameter for each pair of samples are available in [Supplementary Tables](#)—please see the section ‘Data Availability’ for more information regarding data sharing.

For each pair of samples, an LR assuming the alternative hypotheses: “The POI is a contributor of the mixture” and “The POI is not a contributor nor genetically related to any contributor of the mixture” was calculated. The reference sample was assumed to belong to the POI. The LR values were computed through both qualitative (LRmix Studio v.2.1.3³⁰) and quantitative (EuroForMix v.3.4.0³¹ and STRmix™ v.2.7³²) software tools. In EuroForMix, the Maximum Likelihood Estimation (MLE) approach was employed as suggested by software developers for model comparison purposes⁵¹. Different software tools were considered to analyze the impact of the variation of each parameter under alternative statistical and computational frameworks and architectures. For all the computations performed the coancestry coefficient of 0.01 was considered, as recommended by the second National Research Council report recommended for general populations⁵²; as well as the allele frequencies of the National Institute of Standards and Technology (NIST) database concerning the Caucasian population⁵³—see Supplementary Table S1.

When computing the LR values using the above-mentioned tools, distinct parameter values were introduced by the user, related to the Drop-in (Frequency and Modeling), Analytical Threshold, and Stutters.

For each one of the parameters, “default” and “varied” values were defined as detailed in Table 1 to quantify the impact the different parameter values may have on LR computations. The parameters’ values set as “default” were either predefined in the software or presented in the literature, while the “varied” values were sub- and/or over-variations of the default ones. The rationale for the different values established is presented in the following subsections. In any case, it should be remarked that most of the parameter values predefined in the different software should be optimized considering each laboratory’s data. The parameter values considered in this work are for comparison purposes.

To verify the effect that each parameter—drop-in, analytical threshold, and stutters—may have on the weight of the evidence, LR values were computed varying only one parameter at a time, as outlined in Supplementary Table S2. As it is the standard practice in forensic routine, in this work is considered a single computation for each software, pair of samples, and set of parameter values. For reseach purposes, exceptions to this procedure are described next. Whenever an LR equal to zero was obtained for a specific marker, under the parameters’ default values, the marker was removed from all the analyses considering that specific pair of samples and software. For these cases, the LR value provided in Supplementary Table S3 is denoted as $LR_{<21}$, as less than 21 markers were considered. For 17% and 9% of the cases with mixtures with two and three estimated contributors, respectively, STRmix™ returned an LR equal to zero. In these cases, either alleles present in the single contributor sample were absent from the mixture sample, or Markov Chain Monte Carlo (MCMC) sampling failed to identify the true genotype combination, i.e., MCMC algorithms failed to converge, as similarly observed in other studies^{36,44,54}. To illustrate the stochasticity of the architecture of the STRmix™ tool and how this may affect the results presented in this study, corresponding computations were also replicated for the pair of samples showing the maximum LR differences after parameter variation. This case is specifically referred to and analyzed in the text. For all the STRmix™ computations, the MCMC-related parameters provided by default in the software were those considered—Burn-in accepts per chain of 10,000 and Post burn-in accepts per chain of 50,000.

Then, for each analyzed parameter, an intra-software analysis was carried out through a comparison of the LR values computed assuming the default (LR1) and varied conditions (LR2) for each pair of samples through the computation of their ratio $R = LR1/LR2$ if $LR1 > LR2$, or $R = LR2/LR1$, otherwise. Thus, for example, $1 < R < 10$ indicates that one of the LRs (either computed assuming the default or varied condition) is at most ten times greater than the other. Afterward, a statistical test was performed to infer a significant increasing or decreasing trend ($\alpha = 0.05$) regarding the LR values that were observed when the parameter value was or not varied in each statistical and informatics framework.

When for certain parameters’ values the software couldn’t compute an LR value different from zero—i.e. either computing an LR equal to zero or presenting an error message—no comparisons were performed for those cases.

Parameters	Values					
	LRmix studio		EuroForMix		STRmix™	
	Default	Variation	Default	Variation	Default	Variation
Drop-in frequency	0.05	0 0.1	0.05	0 0.1	0.05	0 0.1
Drop-in parameters’ distribution	N/A	N/A	λ : 0.01	λ : 0.05	γ : (1.0,1.0)	Uniform
Analytical threshold	N/A	N/A	100	150	100	150
Stutters	Without	N/A	With	Without	With	N/A

Table 1. Default and varied values introduced in LRMix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7 for each analyzed parameter. N/A: not applicable.

Drop-in and drop-out

Remarking that drop-in parameters are laboratory-specific, some values were established due to operational reasons for proceeding with the study. The drop-in frequency was then set to 0.05 as default, a value considered reasonable in similar studies⁵⁵, also coinciding with the pre-set value in LRmix Studio. The variations considered were: (a) 0, meaning that alleles not attributed to a contributor cannot be considered as a drop-in peak; and (b) 0.1, a high probability of unexplained alleles being a drop-in (the double of the determined default value). Also related to drop-in modeling, the impact of considering variations for the distribution of their heights was tested in the quantitative computer tools, which is computed differently in EuroForMix and STRmix™. In EuroForMix the drop-in is modeled using a lambda distribution—the value of the hyperparam λ equal to 0.01 was used as default (which also corresponds to the software's default value) and 0.05 as a varied value; λ cannot be equal to zero. On the other hand, STRmix™ employs either a gamma (γ) or uniform distribution. Therefore, both distributions were tested. The gamma distribution was considered the “default” condition, for which the software default values of parameters α and β were used (1.0 and 1.0, respectively), while uniform distribution was considered the “varied” condition. Regarding Drop-out, and as previously mentioned, it is directly estimated through the peak height distribution of the alleles in the quantitative tools (EuroForMix and STRmix™), while it was maintained the default value equal to 0.1 in the qualitative tool (LRmix Studio).

Analytical threshold

The analytical threshold is a parameter required only in quantitative tools—EuroForMix and STRmix™. This parameter value must be the same as used in the software from which the sample files are exported. In this case, 100 RFU was defined as the default and 150 RFU as the varied value.

Stutter artifacts

The presence/absence of stutters, and corresponding modeling, in mixture sample profiles were also tested. In EuroForMix analysis, modeling of stutters is possible but not required, and so, LR values computed for each pair with (default) and without stutters (varied condition) in mixture samples profiles were compared. In the first condition, EPG data with unfiltered stutters were considered and both backward ($N - 1$) and forward ($N + 1$) stutters were modeled; in the second condition, EPG data with filtered stutters were considered. On the other hand, neither in LRmix Studio nor in STRmix™ was possible to perform this evaluation due to opposite reasons. In LRmix Studio, the developers recommend filtering stutters previously, while in STRmix™ its inclusion is mandatory and its estimation is directly computed by the software.

Results and discussion

The LR values for each one of the 154 pairs of samples, comprising one mixture (with two or three estimated contributors) and a reference sample that in most of the cases cannot be excluded as contributor, were computed using the three software: LRmix Studio v.2.1.3³⁰, EuroForMix v.3.4.0³¹, and STRmix™ v.2.7³², the first considering only the presence/absence of alleles, and the other two also the DNA quantity. LR values were computed under both default and varied conditions for each parameter—see Supplementary Tables S2 and S3.

Drop-in

Frequency

The frequency of drop-in was varied from a null value to the double (0.1) of the considered default value (0.05), as summarily previously presented⁵⁶.

Table 2 shows the differences found between the LR values computed assuming the default and the varied conditions, for both cases with mixtures with two and three estimated contributors. These differences are presented under the format of a ratio between the computed LR values.

Through the analysis of computed LR values, a statistically significant trend ($\alpha = 0.05$) for LR values increase with the decrease of drop-in frequency (both when considering 0.05 to 0, and 0.1 to 0.05) was observed for LRmix Studio and EuroForMix, for both two and three estimated contributors cases. On the other hand, no statistically significant trend towards an increase or decrease in the LR values was observed for STRmix™ (Table 2).

In general, varying the drop-in frequency resulted in LR values diverging less than ten times in most of the cases for which a non-null value was retrieved (74% to 100%). Differences greater than 10,000 times were found for some cases (six, two of them in EuroforMix) with two and/or three estimated contributors when considering quantitative tools. In four of these cases, differences were observed when disregarding drop-in frequency, but in two of them, it occurred when the estimated frequency doubled from 0.05 to 0.1. In these cases showing greater differences ($R > 10,000$) it is noteworthy that when the drop-in frequency was not considered in EuroForMix computation, the LR value followed an opposite trend of the generally showed by the overall results—that is, when the drop-in frequency decreased LR values weakened. On the other hand, it cannot be disregarded the stochasticity of the methods on which STRmix™ is based since it may imply that the observed differences are due to the lack of convergence of MCMC algorithms, rather than due to the parameter variation itself. Indeed, this hypothesis was explored and confirmed for the case showing the greatest differences in the LR values—obtained with default and varied parameters for drop-in modeling, see “Statistical modeling” section).

Besides the differences computed, another factor that demonstrates the impact of the parameter variation is revealed when the software cannot compute an LR value (different from zero), and, therefore, it is not possible to perform a comparison. This situation was observed in all software when comparing LR values computed assuming a null and the default (0.05) drop-in frequencies. The software computed either an LR equal to zero—as STRmix™—or does not compute a value at all—as LRmix Studio and EuroForMix. For the latter, it was observed in both cases with mixtures with two and three estimated contributors, while in STRmix™ it was only verified for

Estimated number of contributors						
2						
Ratio between LR _s (default vs varied, $R > 1$)	LRmix studio		EuroForMix		STRmix™	
	Drop-in frequency		Drop-in frequency		Drop-in frequency	
	0.05 vs. 0	0.05 vs 0.1	0.05 vs. 0	0.05 vs 0.1	0.05 vs. 0	0.05 vs 0.1
$1 < R < 10$	74%	100%	87%	100%	92%	95%
$10 < R < 100$	0%	0%	4%	0%	4%	4%
$100 < R < 1,000$	0%	0%	4%	0%	4%	0%
$1,000 < R < 10,000$	0%	0%	1%	0%	0%	1%
$R > 10,000$	0%	0%	1%	0%	0%	0%
N/A	26%	0%	3%	0%	0%	0%
Maximum ratio between LR values						
R	1.20E+00	8.09E+00	8.50E+10	1.90E+00	8.83E+02	3.05E+03
LR (default)	2.20E+13	1.83E+03	2.71E+23	2.78E+08	3.91E+24	5.58E+14
LR (varied)	2.64E+13	1.48E+04	3.19E+12	5.29E+08	4.43E+21	1.70E+18
Pair*	53_2	1_2	6_2	2_2	51_2	8_2
LR tends to ($\alpha = 0.05$)						
	Increase	Decrease	Increase	Decrease	–	–
p-value	<u>5.62E–08</u>	<u>6.65E–05</u>	<u>1.82E–03</u>	<u>2.48E–05</u>	2.10E–01	1.38E–01
3						
Ratio between LR _s (default vs varied, $R > 1$)	LRmix studio		EuroForMix		STRmix™	
	Drop-in frequency		Drop-in frequency		Drop-in frequency	
	0.05 vs. 0	0.05 vs 0.1	0.05 vs. 0	0.05 vs 0.1	0.05 vs. 0	0.05 vs 0.1
$1 < R < 10$	96%	100%	97%	100%	84%	86%
$10 < R < 100$	0%	0%	1%	0%	9%	8%
$100 < R < 1,000$	0%	0%	0%	0%	3%	3%
$1,000 < R < 10,000$	0%	0%	0%	0%	1%	0%
$R > 10,000$	0%	0%	1%	0%	3%	3%
N/A	4%	0%	1%	0%	0%	1%
Maximum ratio between LR _s						
R	1.18E+00	2.07E+00	1.17E+12	1.81E+00	7.86E+08	4.24E+06
LR (default)	3.31E+04	1.23E+07	4.61E+09	1.77E+09	1.51E+04	1.12E+17
LR (varied)	2.81E+04	2.54E+07	5.40E+21	3.20E+09	1.92E–05	4.75E+23
Pair*	18_3	4_3	5_3	8_3	9_3	49_3
LR tends to ($\alpha = 0.05$)						
	Increase	Decrease	Increase	Decrease	–	–
p-value	<u>1.99E–04</u>	<u>4.11E–04</u>	<u>2.86E–03</u>	<u>9.50E–04</u>	8.19E–01	3.59E–01

Table 2. Ratio R ($R > 1$) between LR values computed considering the varied values of drop-in frequency (0 and 0.1) and the default value (0.05) by LRMix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7, for both cases with mixtures with two and three estimated contributors. N/A: “Not Applicable” corresponds to situations where no comparisons were performed, either because an LR equal to zero was computed, or an error message was displayed. The evidence of a general trend for the LR values either increasing or decreasing when the drop-in frequency was varied is presented along with the corresponding p-value ($\alpha = 0.05$); significant values are underlined. *See Tables S3_1 in Supplementary Table S3 for more data.

cases with mixtures with three. When the drop-in frequency is considered null, the probability of drop-in peaks being treated by software as true peak alleles increases. So, these situations may have resulted from cases where the maximum number of peaks may have been exceeded considering the number of contributors estimated. Even when the number of peaks has not been exceeded, the software can infer, based on the relative height of the peaks, that more alleles are presented than would be expected, considering the number of contributors. Conversely, when the drop-in frequency value was doubled, those situations were only verified in STRmix™ for a case with a mixture with three estimated contributors. Doubling the drop-in frequency value led to an increase in the probability of some peaks being considered drop-in. Therefore, if those peaks belong to the reference sample under analysis, the LR value decreases as observed in STRmix™ computations.

In any case, the most striking results in this regard were found for the qualitative software for 26% and 4% of the cases with two and three estimated contributors, respectively, for which no LR values were retrieved when the drop-in event was disregarded.

Statistical modeling

As previously referred, it should be noted that, even though both software are quantitative, the drop-in modeling is done differently. In EuroForMix the drop-in is modeled using a Lambda distribution (λ) while STRmix™ uses either a Gamma (γ) or a Uniform distribution.

The differences between LR values computed assuming the default condition ($\lambda=0.01$ for EuroForMix and $\gamma=(1.0, 1.0)$ for STRmix™) and the varied condition ($\lambda=0.05$ and Uniform, respectively), for both cases with mixtures with two and three estimated contributors, are summarized in Table 3. A possible trend for LR increase or decrease when this parameter was varied is also presented.

Only EuroForMix showed a statistically significant trend, as for cases with mixtures with three estimated contributors, the LR values tended to increase when the lambda value was varied from 0.01 to 0.05 (Table 3). Nevertheless, all differences observed in EuroForMix were lower than ten times, revealing a lower impact than those observed in STRmix™ when varying the corresponding parameters.

Although most of the computed differences in STRmix™ were also lower than ten times, differences higher than 10,000 times were observed. For those cases, the LR values decreased. In fact, the LR values dropped below one, favoring the alternative hypothesis, and a logical reasoning is that being a consequence of unattributed peaks that could no longer be explained by drop-in. In both cases, in mixtures with two and three estimated contributors, the maximal computed differences were much bigger for STRmix™ values than those from EuroForMix. Nevertheless, and as previously referred, the large differences obtained in STRmix™ analyses raised the hypothesis of these being a consequence of the stochasticity of the model under which the software is based, rather than of the different models themselves. Indeed, STRmix™ assigns a relative weight to the probability of the EPG given each possible genotype configuration using MCMC methods, which implies, by definition, that the results of different runs will not coincide. Nevertheless, small variations in comparison to the LR value itself, with minimal practical impact, were expected. The hypothesis of the lack of convergence was thus further explored considering the genotypic data of the sample pair 13_2, for which the maximum difference R was observed. Under the same conditions, computations were replicated three times for default and varied parameters, and very different results were obtained between trials—see Supplementary Table S4. These results support that the great differences observed may be consequence of a lack of convergence of the model that can be solved by increasing burn-in and post burn-in accepts at the expense of runtime and computer capacity costs.

Analytical threshold

Table 4 shows the impact on the LR value of increasing the analytical threshold from 100 RFU (default value) to 150 RFU (varied value), for both mixtures with two and three estimated contributors in quantitative tools. A possible trend for the LR increasing or decreasing when the analytical threshold was varied is also presented.

No statistical evidence was found regarding a possible general trend to the decrease or increase of the LR values computed by STRmix™. On the other hand, a statistically significant trend for EuroForMix LR values decrease when the analytical threshold value increased was verified for both cases with mixtures with two and three estimated contributors (Table 4).

Ratio between LRs (default vs varied, $R > 1$)	Estimated number of contributors			
	2		3	
	EuroForMix	STRmix™	EuroForMix	STRmix™
	$\lambda = 0.01$ vs 0.05	$\gamma = (1.0, 1.0)$ vs uniform	$\lambda = 0.01$ vs 0.05	$\gamma = (1.0, 1.0)$ vs uniform
$1 < R < 10$	100%	94%	100%	83%
$10 < R < 100$	0%	4%	0%	9%
$100 < R < 1000$	0%	0%	0%	4%
$1000 < R < 10,000$	0%	0%	0%	1%
$R > 10,000$	0%	3%	0%	3%
Maximum ratio between LRs				
R	2.88E+00	2.12E+17	1.94E+00	3.46E+14
LR (default)	1.44E+18	3.80E+09	4.69E+23	6.53E+07
LR (varied)	4.14E+18	1.79E−08	9.10E+23	1.89E−07
Pair*	7_2	13_2#	64_3	8_3
LR tends to ($\alpha=0.05$)				
	–	–	Increase	–
p-value	5.27E−02	4.25E−01	<u>3.04E−02</u>	9.09E−01

Table 3. Ratio R ($R > 1$) between LR values computed in EuroForMix v.3.4.0 under the default and varied drop-in condition ($\lambda=0.01$ and 0.05 , respectively) and in STRmix™ v.2.7 under the default and varied condition ($\gamma=(1.0, 1.0)$ and Uniform, respectively), for cases with mixtures with two and three estimated contributors. The evidence of a general trend for the LR values increasing or decreasing when the drop-in parameter distribution was varied is presented along with the corresponding p-value ($\alpha=0.05$) that supported a trend or the absence of a trend is provided; significant values are underlined. *See Tables S3_2 in Supplementary Table S3. #See the main text and Supplementary Table S4 for more details.

Ratio between LR (100 RFU vs 150 RFU, R > 1)	Estimated number of contributors			
	2		3	
	EuroForMix	STRmix™	EuroForMix	STRmix™
1 < R < 10	81%	92%	87%	87%
10 < R < 100	16%	6%	6%	8%
100 < R < 1000	1%	0%	4%	1%
1000 < R < 10,000	1%	0%	1%	1%
R > 10,000	1%	1%	1%	3%
Maximum ratio between LR				
R	9.44E+04	2.41E+06	3.36E+04	1.37E+11
LR (default)	8.21E+18	3.80E+09	3.46E+07	1.51E+04
LR (varied)	8.70E+13	1.58E+03	1.03E+03	1.10E−07
Pair*	25_2	13_2	11_3	9_3
LR tends to (α = 0.05)				
	Decrease	–	Decrease	–
p-value	<u>2.09E−03</u>	7.32E−01	<u>4.11E−04</u>	7.32E−01

Table 4. Ratio R (R > 1) between LR values computed through EuroForMix v.3.4.0, and STRmix™ v.2.7, considering the analytical threshold equal to 100 RFU (default) and 150 RFU (varied), for mixtures with two and three estimated contributors. The evidence of a general trend for the LR values increasing or decreasing when the analytical threshold was increased is presented along with the corresponding p-value (α = 0.05); significant values are underlined. *See Tables S3_3 in Supplementary Table S3.

For most cases, the difference between the two LR values computed does not overpass ten times. However, differences higher than 10,000 times were found. In almost all cases where differences higher than 10,000 times were reached, a decrease in the LR value occurred. This decrease was determined by alleles that matched the reference sample but were below the analytical threshold—as it happens in EuroForMix—and/or by the change in the allele peak height—as it happens in STRmix™. In only one case, with a difference higher than 10,000 times, the LR value increased when the analytical threshold value was increased. This situation occurred for STRmix™ and resulted from the non-consideration of alleles matching the other possible contributors of the mixture, as they were below the analytical threshold considered. Nevertheless, and as previously referred to and shown, caution should be taken when assuming parameter variation as the main source of different LR values when considering a software based on stochastic methods as is the case of STRmix™ as these can be also due to the lack of convergence of the model.

Stutter artifacts

LR values computed using EuroForMix software were compared, assuming the presence and absence of stutters, for both cases with mixtures with two and three estimated contributors. The differences found between those values are presented in Table 5, as well as an analysis regarding a possible general trend for the LR increase or decrease.

A stutter peak is a PCR product artifact formed during the PCR extension phase, as previously mentioned. So, it can be said that the product from an allele amplification is a combination of correct copies of the target allele and the corresponding stutter peaks. For this reason, the LR value should tend to increase when the stutters’ peaks are included, as confirmed by our data for which the reference sample cannot be excluded as a contributor to problem one.

As demonstrated in Table 5, most cases do not reach differences greater than ten times. Nevertheless, differences of more than 10,000 times were computed, reaching the maximum value of 691,489 times.

Conclusions

The ambition to analyze increasingly complex samples, namely DNA mixture samples, led to the development of software tools to support the expert in the interpretation of the results. These tools apply mathematical models implied in the computation of a probative value to quantify the genetic evidence. This calculation involves the setting of several parameters regarding population genetics and analytical factors, which are estimated either directly by the software or introduced by the user.

Therefore, it is fundamental to demonstrate the impact on statistical evaluation of the genetic evidence when considering different values and/or conditions in the software parameters. So, this work aims to highlight the importance of properly and accurately estimating and modeling parameters such as drop-in, analytical threshold, and stutters, for forensic genetics routine analyses.

LR values were computed under the established default and varied conditions, varying a single parameter at a time, for each one of 154 real casework mixture/single-source sample pairs, the first with two or three estimated contributors. The results obtained through different informatic tools—one qualitative (LRmix Studio v.2.1.3), and two quantitative (EuroForMix v.3.4.0, and STRmix™ v.2.7) for 21 independent and autosomal STRs, were compared and the impact of parameters’ variation within and between software was evaluated.

Ratio between LR (with vs without stutters, $R > 1$)	Estimated number of contributors	
	2	3
$1 < R < 10$	74%	78%
$10 < R < 100$	17%	13%
$100 < R < 1000$	5%	3%
$1000 < R < 10,000$	1%	5%
$R > 10,000$	3%	1%
Maximum ratio between LR		
R	6.91E+05	3.68E+05
LR (default)	1.41E+10	1.65E+10
LR (varied)	9.75E+15	6.07E+15
Pair*	16_2	9_3
LR tends to ($\alpha = 0.05$)		
	Decrease	Decrease
p-value	<u>4.39E-03</u>	<u>9.50E-04</u>

Table 5. Ratio R ($R > 1$) between LR values computed through EuroForMix v.3.4.0 with (default) and without stutters (varied condition), for mixtures with two and three estimated contributors. The evidence of a general trend for the LR values increase or decrease when the stutters were not considered is presented along with the corresponding p-value ($\alpha = 0.05$); significant values are underlined. *See Tables S3_4 in Supplementary Table S3.

Even though the proportion of cases differing more than 10,000 times was low for each varied parameter, the modest dataset size (77 mixtures with two estimated contributors, and 77 with three) and the magnitude of differences reached imply that the impact of these parameters on the weight of the evidence should not be overlooked, especially for cases where considering different values and/or conditions resulted in the support of opposite hypotheses. In this regard, it should however be noted that the stochasticity of the methods grounding one of the informatic tools used: STRmix™, may hamper the corresponding conclusions as the observed differences may be mostly a consequence of a lack of convergence of the MCMC algorithms rather than of the parameter variation itself. Indeed, this was the case for the maximum difference found—LR computed assuming the default condition regarding drop-in modeling in STRmix™ (Gamma distribution $\gamma = (1.0, 1.0)$, $LR = 3.80E+09$) that differed $2.12E+17$ times from the LR computed assuming the varied condition (Uniform distribution, $LR = 1.79E-08$), each one supporting opposite hypotheses. Maintaining the same MCMC parameter values, computations were replicated three more times for default and varied conditions, the variation of the results greatly differing from those obtained in the first trial. The lack of convergence is due to sample-specific factors that affect precision and can be solved by running the problem for longer, i.e. for more MCMC accepts.

Focusing on the Drop-in parameter, in the qualitative software (LRmix Studio) a statistically significant trend for the LR value decrease with increasing drop-in frequency was observed, but none of the calculated differences exceeded 10,000 times. The same trend was observed for EuroForMix. Discrepancies greater than 10,000 times were verified for both quantitative software, varying between 0 and 3% when the frequency value was tested, and also when the distribution model was varied. Nevertheless, and again, the possibility of some of these differences being also a result of the stochastic method on which STRmix™ is based should not be disregarded.

Regarding the analytical threshold parameter, evaluated in quantitative software, only for the LR values computed in EuroForMix was observed a statistically significant trend: when the analytical threshold was increased from 100 to 150 RFU, the retrieved EuroForMix LR values generally decreased. The proportion of cases where the LR differed more than 10,000 times remained at 1% for EuroForMix (for both cases with mixtures with two and three estimated contributors), while for STRmix™ varied between 1 and 3%, respectively. Yet, as previously mentioned, caution should be taken when analyzing STRmix™ LR values, as the source of variation may not be just a result of considering different parameter conditions.

Finally, when considering stutters (only evaluated in EuroForMix), computed LR values were shown to be higher when stutters were considered. Differences greater than 10,000 times were found in both cases with mixtures with two (3%) and three (1%) estimated contributors.

Thus, another step is added to the important role of the experts when they are working with probabilistic genotyping programs. Before performing any computation, the expert must establish and introduce the values determined either empirically following internal protocols or based on values presented in the literature, for each parameter of the software, so that the computation of the LR value is as accurate as possible. The analysis of the unknown sample profile EPG, the evaluation of computed LR value, and the estimation of software parameter values depend on the expert and constitute crucial steps that may influence the interpretation of the results if wrongly or inaccurately performed. Indeed, this work emphasizes the importance of proper modeling and estimation of the parameters that influence the weight of the evidence, along with appropriate training and awareness of the experts on the mathematical/statistical models implemented in the probabilistic genotyping tools that have been introduced in the forensic routine. Special attention should be devoted when computer tools based on stochastic methodologies are used, for which, ideally, different number of runs to testify the

convergence of the algorithms should be performed. Indeed, testing the reproducibility and repeatability of informatics tools is a relevant and important topic that should be further investigated in the future. This is particularly pertinent in the forensic genetics setting where methodologies have been based, at least theoretically, in completely reproducible interpretation results, being the application of stochastic systems as MCMC relatively new in the field.

The parameter settings considered in this work were established to demonstrate the impact that different values may have in the quantification of the evidence using different informatics and statistical approaches. Despite being within a reasonable range for most situations, the considered values cannot be seen, by any means, as suggested values. Each laboratory should estimate and establish their own parameter settings, considering their conditions and specificities, using appropriate, dedicated, and up-to-date methodologies.

In summary, this work reinforces and alerts all practitioners to the importance of considering and accurately estimating the laboratory-specific values/conditions for all the parameters mentioned, underlining the need for proper user training and laboratory-specific validation of any software prior to its use.

Data availability

All the statistics that supported the results presented in this study are provided in Supplementary Material, for each one of the analyzed pairs of samples and parameters. The raw genotypic and haplotypic analyzed data concern real forensic casework from the Portuguese Scientific Police Laboratory, Judiciary Police (LPC-PJ) and for that reason, its release is not possible. In any case, specific data from specific analyzed markers (non-haplotypic data) may be available from the authors upon reasonable request and with the permission of LPC-PJ. For data sharing issues contact the corresponding author of the manuscript.

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Author contributions

Conceptualization, AA, LP, and NP; Methodology: AA, LP, NP, CC, CF, PMF, and SC; Raw data selection: PMF, SC, and CF; Raw data analysis: PMF and CF; Software analysis of raw data: CC and CF; Software results analysis: NP, CC, and CF; Writing—Original Draft Preparation: CC and NP; Writing—Review & Editing, AA, LP, NP, CC, CF, PMF, and SC; Supervision, LP and NP.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Supplementary Material III

The complete supplementary data associated with this article can also be found in the online version at <https://doi.org/10.1038/s41598-024-83841-2>

Table S1: NIST allele frequency database.

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S104	D2S1338	D2S441	D3S1358	D5S818	D7S820	D8S1179	FGA	SE33	TH01	TPOX	vWA
5																			0.0014	0.0014	
6																			0.2355	0.0014	
6.3																					
7														0.0028	0.0277				0.1939		
8	0.00554			0.120499	0.01801									0.0055	0.144	0.01385			0.0956	0.5249	
8.1																0.0014					
9	0.01385			0.077562	0.10665							0.0014		0.0416	0.1676	0.00554			0.1191	0.1274	
9.1												0.0014									
9.3																			0.3449		
10	0.22022			0.047091	0.05679	0.0083	0.00139	0.0028				0.2105		0.0554	0.2562	0.102493			0.0083	0.0499	
10.1																					
10.2																					
10.3																					
11	0.30886	0.001385		0.325485	0.3144	0.0097	0.00554	0.0776		0.13989		0.3435	0.001385	0.356	0.205	0.076177		0.0014	0.0014	0.2521	
11.2																					
11.3												0.0609									
12	0.36011	0.031856		0.268698	0.3144	0.1136	0.07064	0.1163		0.01247		0.0471		0.3878	0.1593	0.16759		0.0069		0.0416	0.0014
12.2								0.00139													
12.3													0.0042								
13	0.08172	0.307479		0.116343	0.16343	0.1233	0.25485	0.0665		0.00693		0.0291	0.001385	0.1427	0.0346	0.32964		0.0166		0.0014	0.0014
13.2								0.00693													
13.3																					
13.4																					
14	0.0097	0.297784		0.042936	0.02632	0.1343	0.3615	0.0789		0.05679		0.241	0.106648	0.0069	0.0042	0.166205		0.0249			0.0928
14.2						0.0014	0.02355											0.0028			
14.3								0.0028													
15		0.196676	0.03186	0.001385		0.1704	0.15651	0.1496		0.32133	0.001385	0.0596	0.272853	0.0014		0.103878		0.0402			0.1053
15.2							0.03601														
15.3								0.0582													
15.4																					
16		0.132964	0.02216			0.1468	0.05679	0.1357		0.38227	0.037396	0.0014	0.238227			0.033241		0.0402			0.2008
16.2						0.0014	0.01524											0.0014			
16.3								0.0609													
17		0.027701	0.12742			0.1385	0.00693	0.0471		0.07479	0.185596		0.210526			0.001385		0.0623			0.2839
17.1																					
17.2							0.00139														

[illegible]

[illegible]

SM2: Software parameter values and conditions introduced in the three informatics tools: LRmix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7.

Table S2: Software parameter values introduced in the three informatics tools: LRmix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7. ^a Dropout is directly estimated through the peak height distribution. ^b Per locus, specified by the software considering the default conditions (i.e. disregarding the population size).

Parameters \ Values	LRmix Studio	EuroForMix	STRmix™
Coancestry Coefficient	0.01		
Drop-in frequency	0.05		
Drop-in parameters' distribution	N/A	Lambda (λ): 0.01	Gamma (γ): (1.0,1.0)
Drop-in cap	N/A	N/A	100
Dropout	0.1	^a	^a
Minimum allele frequency	0.001	0.001	^b
Threshold detection	N/A	100	100
Stutters	Without	With	With

SM 3. LR_s values (log₁₀ scale) computed by LRMix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ 2.7 for mixtures with two and three estimated contributors when varying distinct parameters.

Table S3_1a: Log10 of LR_s values computed by LRMix Studio v.2.1.3 for mixtures with two and three estimated contributors when the drop-in frequency was varied.

Pair of samples	Number of estimated contributors	LRmix Studio v.2.1.3							
		Number of markers analyzed	Log ₁₀ of LR values considering drop-in frequency of ...			Difference between Log ₁₀ (LR) considering drop-in of ...		Absolute value of the difference	
			0	0.05 (Default)	0.1	0.00 - 0.05	0.10 - 0.05	0.00 - 0.05	0.10 - 0.05
1_2	2	21	model couldn't explain the dat	3.261556	4.170292	#VALOR!	0.908736	#VALOR!	0.908736
2_2	2	21	model couldn't explain the dat	7.405694	7.698300	#VALOR!	0.292605	#VALOR!	0.292605
3_2	2	21	model couldn't explain the dat	11.176154	11.437505	#VALOR!	0.261351	#VALOR!	0.261351
4_2	2	21	13.934763	13.872065	13.806626	0.062697	-0.065440	0.062697	0.065440
5_2	2	21	15.826660	15.795792	15.763296	0.030868	-0.032495	0.030868	0.032495
6_2	2	21	model couldn't explain the dat	12.983625	12.928951	#VALOR!	-0.054674	#VALOR!	0.054674
7_2	2	21	14.032951	14.034909	14.037315	-0.001958	0.002406	0.001958	0.002406
8_2	2	21	model couldn't explain the dat	14.860104	15.410303	#VALOR!	0.550199	#VALOR!	0.550199
9_2	2	21	17.349394	17.309658	17.268871	0.039736	-0.040788	0.039736	0.040788
10_2	2	21	14.603467	14.624671	14.645987	-0.021205	0.021316	0.021205	0.021316
11_2	2	21	model couldn't explain the dat	11.145523	11.373782	#VALOR!	0.228259	#VALOR!	0.228259
12_2	2	21	16.856743	16.825523	16.792226	0.031220	-0.033297	0.031220	0.033297
13_2	2	21	8.936974	8.935058	8.933055	0.001916	-0.002004	0.001916	0.002004
14_2	2	21	5.690898	5.680036	5.669822	0.010862	-0.010214	0.010862	0.010214
15_2	2	21	model couldn't explain the dat	7.847116	7.873552	#VALOR!	0.026436	#VALOR!	0.026436
16_2	2	21	10.713255	10.712028	10.710280	0.001226	-0.001748	0.001226	0.001748
17_2	2	21	10.595813	10.581834	10.568101	0.013979	-0.013733	0.013979	0.013733
18_2	2	21	model couldn't explain the dat	10.985144	11.559448	#VALOR!	0.574304	#VALOR!	0.574304
19_2	2	21	13.308353	13.286948	13.264123	0.021405	-0.022825	0.021405	0.022825
20_2	2	21	13.914847	13.929187	13.943696	-0.014340	0.014509	0.014340	0.014509
21_2	2	21	13.818413	13.780604	13.740413	0.037810	-0.040191	0.037810	0.040191
22_2	2	21	11.458858	11.430044	11.400304	0.028814	-0.029740	0.028814	0.029740
23_2	2	21	model couldn't explain the dat	10.165292	9.740988	#VALOR!	-0.424305	#VALOR!	0.424305

24_2	2	21	model couldn't explain the dat	12.926177	13.229691	#VALOR!	0.303514	#VALOR!	0.303514
25_2	2	21	8.446284	8.459968	8.473595	-0.013684	0.013627	0.013684	0.013627
26_2	2	21	14.756850	14.719403	14.679363	0.037448	-0.040040	0.037448	0.040040
27_2	2	21	14.178079	14.127599	14.074425	0.050479	-0.053174	0.050479	0.053174
28_2	2	21	14.032394	14.015363	13.996640	0.017030	-0.018723	0.017030	0.018723
29_2	2	21	13.502078	13.488306	13.473227	0.013772	-0.015080	0.013772	0.015080
30_2	2	21	14.029933	14.018009	14.004085	0.011924	-0.013924	0.011924	0.013924
31_2	2	21	13.554661	13.592216	13.630439	-0.037555	0.038223	0.037555	0.038223
32_2	2	21	14.059699	14.049345	14.037831	0.010354	-0.011514	0.010354	0.011514
33_2	2	21	12.114027	12.120709	12.127041	-0.006683	0.006332	0.006683	0.006332
34_2	2	21	15.860905	15.821875	15.780500	0.039031	-0.041375	0.039031	0.041375
35_2	2	21	model couldn't explain the dat	16.507899	16.729529	#VALOR!	0.221630	#VALOR!	0.221630
36_2	2	21	15.502967	15.478732	15.452774	0.024235	-0.025958	0.024235	0.025958
37_2	2	21	13.385437	13.340727	13.293258	0.044711	-0.047468	0.044711	0.047468
38_2	2	21	15.999814	15.977870	15.953816	0.021944	-0.024054	0.021944	0.024054
39_2	2	21	12.208223	12.168056	12.125631	0.040167	-0.042425	0.040167	0.042425
40_2	2	21	15.392027	15.360594	15.326270	0.031434	-0.034324	0.031434	0.034324
41_2	2	21	model couldn't explain the dat	18.903476	18.867777	#VALOR!	-0.035699	#VALOR!	0.035699
42_2	2	21	13.655234	13.594423	13.530702	0.060811	-0.063721	0.060811	0.063721
43_2	2	21	13.712154	13.679321	13.645274	0.032832	-0.034047	0.032832	0.034047
44_2	2	21	14.352820	14.310794	14.266517	0.042026	-0.044278	0.042026	0.044278
45_2	2	21	model couldn't explain the dat	18.506402	18.517696	#VALOR!	0.011293	#VALOR!	0.011293
46_2	2	21	13.718177	13.677777	13.635157	0.040400	-0.042620	0.040400	0.042620
47_2	2	21	17.422633	17.386509	17.346933	0.036124	-0.039576	0.036124	0.039576
48_2	2	21	model couldn't explain the dat	14.215358	14.510400	#VALOR!	0.295042	#VALOR!	0.295042
49_2	2	21	12.345069	12.358577	12.373252	-0.013509	0.014675	0.013509	0.014675
50_2	2	21	18.338309	18.312384	18.284271	0.025926	-0.028113	0.025926	0.028113
51_2	2	21	13.010674	13.020483	13.030165	-0.009809	0.009682	0.009809	0.009682
52_2	2	21	model couldn't explain the dat	11.777257	11.296641	#VALOR!	-0.480616	#VALOR!	0.480616
53_2	2	21	13.420881	13.342617	13.261092	0.078263	-0.081525	0.078263	0.081525
54_2	2	21	12.317454	12.290315	12.262006	0.027139	-0.028310	0.027139	0.028310
55_2	2	21	13.465730	13.430636	13.393354	0.035093	-0.037282	0.035093	0.037282
56_2	2	21	12.259290	12.247595	12.234735	0.011695	-0.012860	0.011695	0.012860
57_2	2	21	model couldn't explain the dat	11.504696	11.493187	#VALOR!	-0.011509	#VALOR!	0.011509
58_2	2	21	15.581893	15.550919	15.517693	0.030974	-0.033226	0.030974	0.033226
59_2	2	21	model couldn't explain the dat	14.784083	15.023907	#VALOR!	0.239824	#VALOR!	0.239824

60_2	2	21	16.158859	16.116875	16.072380	0.041984	-0.044495	0.041984	0.044495
61_2	2	21	15.839954	15.795394	15.747702	0.044561	-0.047692	0.044561	0.047692
62_2	2	21	15.895763	15.852712	15.806626	0.043051	-0.046086	0.043051	0.046086
63_2	2	21	13.619174	13.596046	13.571749	0.023128	-0.024297	0.023128	0.024297
64_2	2	21	16.082992	16.041266	15.997341	0.041725	-0.043926	0.041725	0.043926
65_2	2	21	15.664507	15.647807	15.629464	0.016700	-0.018342	0.016700	0.018342
66_2	2	21	15.451765	15.381728	15.308021	0.070037	-0.073706	0.070037	0.073706
67_2	2	21	model couldn't explain the dat	14.063008	14.000815	#VALOR!	-0.062193	#VALOR!	0.062193
68_2	2	21	model couldn't explain the dat	14.001020	14.006529	#VALOR!	0.005509	#VALOR!	0.005509
69_2	2	21	14.337984	14.319443	14.299225	0.018541	-0.020217	0.018541	0.020217
70_2	2	21	14.869970	14.831473	14.790721	0.038497	-0.040752	0.038497	0.040752
71_2	2	21	model couldn't explain the dat	16.762126	16.716059	#VALOR!	-0.046067	#VALOR!	0.046067
72_2	2	21	14.223527	14.183574	14.141944	0.039953	-0.041630	0.039953	0.041630
73_2	2	21	14.729056	14.699590	14.668623	0.029467	-0.030966	0.029467	0.030966
74_2	2	21	15.441462	15.418724	15.394237	0.022737	-0.024487	0.022737	0.024487
75_2	2	21	18.043264	18.001976	17.957676	0.041288	-0.044300	0.041288	0.044300
76_2	2	21	20.340904	20.297552	20.250233	0.043352	-0.047319	0.043352	0.047319
77_2	2	21	20.894292	20.831940	20.766932	0.062351	-0.065009	0.062351	0.065009
1_3	3	21	8.453294	8.460106	8.466586	-0.006813	0.006480	0.006813	0.006480
2_3	3	21	8.224604	8.215567	8.205115	0.009038	-0.010451	0.009038	0.010451
3_3	3	21	model couldn't explain the dat	2.184035	2.467896	#VALOR!	0.283861	#VALOR!	0.283861
4_3	3	21	model couldn't explain the dat	7.091176	7.405223	#VALOR!	0.314046	#VALOR!	0.314046
5_3	3	21	11.900918	11.879225	11.856206	0.021693	-0.023019	0.021693	0.023019
6_3	3	21	model couldn't explain the dat	7.085112	7.379937	#VALOR!	0.294825	#VALOR!	0.294825
7_3	3	21	5.405912	5.408285	5.410509	-0.002373	0.002224	0.002373	0.002224
8_3	3	21	9.403418	9.388328	9.371879	0.015091	-0.016448	0.015091	0.016448
9_3	3	21	9.002450	8.995037	8.986918	0.007414	-0.008118	0.007414	0.008118
10_3	3	21	5.754871	5.765630	5.775890	-0.010760	0.010259	0.010760	0.010259
11_3	3	21	4.214404	4.213118	4.211856	0.001287	-0.001262	0.001287	0.001262
12_3	3	21	7.476242	7.544635	7.611846	-0.068392	0.067211	0.068392	0.067211
13_3	3	21	7.821395	7.826196	7.832267	-0.004801	0.006071	0.004801	0.006071
14_3	3	21	8.310309	8.330668	8.349597	-0.020359	0.018929	0.020359	0.018929
15_3	3	21	8.997832	9.007874	9.018013	-0.010043	0.010138	0.010043	0.010138
16_3	3	21	5.870906	5.885593	5.899823	-0.014688	0.014229	0.014688	0.014229
17_3	3	21	3.298357	3.340479	3.385059	-0.042122	0.044580	0.042122	0.044580
18_3	3	21	4.448701	4.519985	4.589544	-0.071285	0.069558	0.071285	0.069558

19_3	3	21	10.781011	10.768553	10.755117	0.012457	-0.013436	0.012457	0.013436
20_3	3	21	8.114551	8.132717	8.149335	-0.018166	0.016619	0.018166	0.016619
21_3	3	21	8.603087	8.598942	8.594098	0.004145	-0.004844	0.004145	0.004844
22_3	3	21	9.025755	9.009447	8.992593	0.016308	-0.016854	0.016308	0.016854
23_3	3	21	6.592395	6.587456	6.582684	0.004939	-0.004772	0.004939	0.004772
24_3	3	21	10.556165	10.543963	10.530575	0.012202	-0.013388	0.012202	0.013388
25_3	3	21	9.632222	9.630960	9.629520	0.001262	-0.001440	0.001262	0.001440
26_3	3	21	12.088050	12.051264	12.013445	0.036786	-0.037820	0.036786	0.037820
27_3	3	21	6.695160	6.742190	6.786633	-0.047031	0.044443	0.047031	0.044443
28_3	3	21	8.288827	8.311645	8.333256	-0.022819	0.021611	0.022819	0.021611
29_3	3	21	9.686281	9.701229	9.716783	-0.014949	0.015554	0.014949	0.015554
30_3	3	21	10.343697	10.323076	10.300820	0.020621	-0.022256	0.020621	0.022256
31_3	3	21	9.116415	9.114603	9.112291	0.001812	-0.002313	0.001812	0.002313
32_3	3	21	9.622857	9.619389	9.615874	0.003468	-0.003515	0.003468	0.003515
33_3	3	21	9.373969	9.348587	9.321836	0.025382	-0.026751	0.025382	0.026751
34_3	3	21	10.727263	10.739169	10.749262	-0.011906	0.010093	0.011906	0.010093
35_3	3	21	7.906761	7.909684	7.912984	-0.002923	0.003300	0.002923	0.003300
36_3	3	21	10.390138	10.377092	10.362684	0.013047	-0.014408	0.013047	0.014408
37_3	3	21	10.005377	9.981072	9.954799	0.024305	-0.026273	0.024305	0.026273
38_3	3	21	10.523065	10.513634	10.503285	0.009431	-0.010349	0.009431	0.010349
39_3	3	21	8.810505	8.800896	8.790098	0.009609	-0.010798	0.009609	0.010798
40_3	3	21	10.891087	10.865292	10.838580	0.025794	-0.026712	0.025794	0.026712
41_3	3	21	8.268921	8.307925	8.346554	-0.039004	0.038628	0.039004	0.038628
42_3	3	21	8.951666	8.956715	8.961673	-0.005049	0.004958	0.005049	0.004958
43_3	3	21	9.488612	9.487288	9.485526	0.001324	-0.001762	0.001324	0.001762
44_3	3	21	11.239083	11.222270	11.203997	0.016812	-0.018274	0.016812	0.018274
45_3	3	21	8.397581	8.389031	8.379557	0.008550	-0.009474	0.008550	0.009474
46_3	3	21	14.482339	14.461586	14.438886	0.020753	-0.022699	0.020753	0.022699
47_3	3	21	10.352377	10.324736	10.296064	0.027641	-0.028672	0.027641	0.028672
48_3	3	21	10.357729	10.348866	10.339265	0.008863	-0.009601	0.008863	0.009601
49_3	3	21	8.227166	8.207975	8.188559	0.019191	-0.019416	0.019191	0.019416
50_3	3	21	9.117699	9.106148	9.092967	0.011552	-0.013181	0.011552	0.013181
51_3	3	21	7.919276	7.913207	7.907881	0.006069	-0.005326	0.006069	0.005326
52_3	3	21	7.847353	7.883255	7.916230	-0.035902	0.032975	0.035902	0.032975
53_3	3	21	7.554269	7.525709	7.496477	0.028560	-0.029232	0.028560	0.029232
54_3	3	21	10.873496	10.869665	10.863750	0.003831	-0.005914	0.003831	0.005914

55_3	3	21	11.050940	11.019296	10.986115	0.031643	-0.033182	0.031643	0.033182
56_3	3	21	10.879518	10.869306	10.858173	0.010212	-0.011133	0.010212	0.011133
57_3	3	21	10.022608	10.004034	9.983672	0.018574	-0.020361	0.018574	0.020361
58_3	3	21	9.263673	9.245541	9.226706	0.018132	-0.018835	0.018132	0.018835
59_3	3	21	8.867949	8.850131	8.831779	0.017819	-0.018352	0.017819	0.018352
60_3	3	21	9.501341	9.489519	9.476637	0.011822	-0.012882	0.011822	0.012882
61_3	3	21	9.778183	9.760151	9.740926	0.018031	-0.019226	0.018031	0.019226
62_3	3	21	7.946366	7.933851	7.920833	0.012515	-0.013018	0.012515	0.013018
63_3	3	21	10.503452	10.517656	10.530472	-0.014204	0.012816	0.014204	0.012816
64_3	3	21	14.616440	14.596163	14.573875	0.020277	-0.022288	0.020277	0.022288
65_3	3	21	8.245121	8.239265	8.230203	0.005857	-0.009061	0.005857	0.009061
66_3	3	21	11.088181	11.068593	11.048055	0.019588	-0.020538	0.019588	0.020538
67_3	3	21	9.351696	9.333005	9.313370	0.018691	-0.019635	0.018691	0.019635
68_3	3	21	12.192123	12.177004	12.159821	0.015119	-0.017182	0.015119	0.017182
69_3	3	21	12.795100	12.788734	12.781562	0.006365	-0.007172	0.006365	0.007172
70_3	3	21	11.524796	11.511084	11.495966	0.013712	-0.015119	0.013712	0.015119
71_3	3	21	12.195305	12.175429	12.154788	0.019876	-0.020641	0.019876	0.020641
72_3	3	21	14.214745	14.199838	14.183400	0.014907	-0.016438	0.014907	0.016438
73_3	3	21	11.700693	11.685015	11.667661	0.015677	-0.017355	0.015677	0.017355
74_3	3	21	12.396706	12.367646	12.336292	0.029060	-0.031353	0.029060	0.031353
75_3	3	21	8.967585	8.981186	8.967585	-0.013602	-0.013602	0.013602	0.013602
76_3	3	21	13.783440	13.762566	13.739443	0.020874	-0.023123	0.020874	0.023123
77_3	3	21	13.907903	13.875656	13.841524	0.032247	-0.034132	0.032247	0.034132

Table S3_1b: Log10 of LR's values computed by EuroForMix v.3.4.0 for mixtures with two and three estimated contributors when the drop-in frequency was varied.

Pair of samples	Number of estimated contributors	EuroForMix v.3.4.0							
		Number of markers analyzed	Log ₁₀ of LR values computed using drop-in frequency equal to ...			Difference between Log ₁₀ (LR) considering drop-in of ...		Absolute value of the difference	
			0	0.05 (Default)	0.1	0.00 - 0.05	0.10 - 0.05	0.00 - 0.05	0.10 - 0.05
1_2	2	21	7.640144	7.927961	8.153761	-0.287818	0.225800	0.287818	0.225800
2_2	2	21	8.055847	8.444754	8.723775	-0.388906	0.279022	0.388906	0.279022
3_2	2	21	14.147007	14.148656	14.147695	-0.001648	-0.000961	0.001648	0.000961
4_2	2	21	17.097286	18.916264	19.187139	-1.818977	0.270875	1.818977	0.270875
5_2	2	21	20.577470	20.550997	20.522266	0.026473	-0.028731	0.026473	0.028731
6_2	2	21	12.504091	23.433263	23.496419	-10.929172	0.063156	10.929172	0.063156
7_2	2	21	model couldn't explain the dat	18.158299	18.366944	#VALOR!	0.208646	#VALOR!	0.208646
8_2	2	21	22.755600	24.349369	24.336790	-1.593768	-0.012579	1.593768	0.012579
9_2	2	21	18.716612	16.589924	16.590606	2.126687	0.000681	2.126687	0.000681
10_2	2	21	20.710632	20.714464	20.713084	-0.003831	-0.001380	0.003831	0.001380
11_2	2	21	26.694244	26.691247	26.688132	0.002997	-0.003115	0.002997	0.003115
12_2	2	21	model couldn't explain the dat	26.689036	26.894840	#VALOR!	0.205804	#VALOR!	0.205804
13_2	2	21	16.549943	16.541646	16.539596	0.008297	-0.002050	0.008297	0.002050
14_2	2	21	8.429284	8.572155	8.674258	-0.142872	0.102102	0.142872	0.102102
15_2	2	21	12.371061	12.349876	12.327395	0.021185	-0.022480	0.021185	0.022480
16_2	2	21	10.270126	10.149093	10.018858	0.121033	-0.130235	0.121033	0.130235
17_2	2	21	14.253114	14.253095	14.253097	0.000019	0.000001	0.000019	0.000001
18_2	2	21	19.038073	19.036212	19.034116	0.001861	-0.002096	0.001861	0.002096
19_2	2	21	13.568001	16.641833	16.706031	-3.073832	0.064198	3.073832	0.064198
20_2	2	21	14.999585	14.994174	14.986872	0.005411	-0.007303	0.005411	0.007303
21_2	2	21	15.810394	15.809241	15.807962	0.001153	-0.001280	0.001153	0.001280
22_2	2	21	16.327945	16.327804	16.326326	0.000142	-0.001477	0.000142	0.001477
23_2	2	21	17.874613	17.798215	17.709227	0.076398	-0.088988	0.076398	0.088988
24_2	2	21	17.936946	17.937139	17.936696	-0.000193	-0.000443	0.000193	0.000443
25_2	2	21	18.968522	18.914404	18.855059	0.054118	-0.059345	0.054118	0.059345

26_2	2	21	17.361423	17.352124	17.342135	0.009299	-0.009989	0.009299	0.009989
27_2	2	21	19.876551	18.515607	18.442893	1.360944	-0.072715	1.360944	0.072715
28_2	2	21	17.180599	17.180621	17.180630	-0.000023	0.000009	0.000023	0.000009
29_2	2	21	17.219863	17.155477	17.085580	0.064387	-0.069897	0.064387	0.069897
30_2	2	21	16.371925	16.370093	16.368078	0.001833	-0.002015	0.001833	0.002015
31_2	2	21	18.792366	18.793763	18.793319	-0.001397	-0.000444	0.001397	0.000444
32_2	2	21	15.607058	15.617755	15.629036	-0.010696	0.011281	0.010696	0.011281
33_2	2	21	21.246490	21.215666	21.176169	0.030824	-0.039498	0.030824	0.039498
34_2	2	21	16.590643	18.716600	18.716588	-2.125957	-0.000013	2.125957	0.000013
35_2	2	21	20.227461	20.227026	20.226798	0.000435	-0.000227	0.000435	0.000227
36_2	2	21	21.161388	21.178958	21.197439	-0.017570	0.018481	0.017570	0.018481
37_2	2	21	21.866755	21.859874	21.851266	0.006882	-0.008608	0.006882	0.008608
38_2	2	21	20.117874	20.116723	20.115197	0.001151	-0.001526	0.001151	0.001526
39_2	2	21	22.329698	22.310548	22.289372	0.019150	-0.021176	0.019150	0.021176
40_2	2	21	20.885450	20.890434	20.895960	-0.004984	0.005526	0.004984	0.005526
41_2	2	21	20.236547	20.238246	20.237444	-0.001699	-0.000802	0.001699	0.000802
42_2	2	21	22.978801	22.960547	22.940666	0.018254	-0.019881	0.018254	0.019881
43_2	2	21	22.558394	22.558258	22.558106	0.000136	-0.000152	0.000136	0.000152
44_2	2	21	23.056843	23.054692	23.057434	0.002151	0.002742	0.002151	0.002742
45_2	2	21	22.699835	22.699839	22.699829	-0.000004	-0.000010	0.000004	0.000010
46_2	2	21	23.368055	23.360304	23.351720	0.007752	-0.008584	0.007752	0.008584
47_2	2	21	21.713493	21.714727	21.715070	-0.001234	0.000343	0.001234	0.000343
48_2	2	21	22.015515	24.714296	24.937850	-2.698780	0.223554	2.698780	0.223554
49_2	2	21	24.390389	24.372542	24.349415	0.017846	-0.023127	0.017846	0.023127
50_2	2	21	23.216960	23.219064	23.217285	-0.002104	-0.001778	0.002104	0.001778
51_2	2	21	26.185001	26.184091	26.179437	0.000910	-0.004654	0.000910	0.004654
52_2	2	21	25.329931	25.323525	25.321498	0.006406	-0.002027	0.006406	0.002027
53_2	2	21	25.185745	25.184823	25.183687	0.000922	-0.001135	0.000922	0.001135
54_2	2	21	26.528507	26.527629	26.526747	0.000878	-0.000882	0.000878	0.000882
55_2	2	21	24.785630	24.781986	24.778578	0.003645	-0.003408	0.003645	0.003408
56_2	2	21	26.411649	26.410253	26.407288	0.001395	-0.002966	0.001395	0.002966
57_2	2	21	26.723119	26.710675	26.696850	0.012444	-0.013825	0.012444	0.013825
58_2	2	21	25.555375	25.555049	25.554691	0.000327	-0.000358	0.000327	0.000358
59_2	2	21	27.057219	27.031668	27.007405	0.025551	-0.024263	0.025551	0.024263
60_2	2	21	26.045607	26.061808	26.093229	-0.016201	0.031421	0.016201	0.031421
61_2	2	21	25.744329	25.766833	25.791111	-0.022503	0.024278	0.022503	0.024278

62_2	2	21	26.355660	26.353441	26.351484	0.002218	-0.001957	0.002218	0.001957
63_2	2	21	27.048792	27.047594	27.046250	0.001198	-0.001345	0.001198	0.001345
64_2	2	21	26.203376	26.199022	26.192513	0.004354	-0.006509	0.004354	0.006509
65_2	2	21	27.737866	27.736639	27.735378	0.001226	-0.001261	0.001226	0.001261
66_2	2	21	27.739609	27.739056	27.738426	0.000553	-0.000630	0.000553	0.000630
67_2	2	21	28.004837	27.996143	27.994078	0.008694	-0.002065	0.008694	0.002065
68_2	2	21	28.648914	28.648651	28.648341	0.000263	-0.000310	0.000263	0.000310
69_2	2	21	28.127717	28.123689	28.120060	0.004029	-0.003628	0.004029	0.003628
70_2	2	21	28.239456	28.233810	28.227763	0.005646	-0.006047	0.005646	0.006047
71_2	2	21	27.750716	27.749958	27.749026	0.000758	-0.000932	0.000758	0.000932
72_2	2	21	28.824629	28.824348	28.824069	0.000281	-0.000279	0.000281	0.000279
73_2	2	21	29.629757	29.622377	29.615232	0.007380	-0.007145	0.007380	0.007145
74_2	2	21	29.613013	29.608028	29.602079	0.004984	-0.005950	0.004984	0.005950
75_2	2	21	29.642473	29.643093	29.644093	-0.000620	0.001000	0.000620	0.001000
76_2	2	21	30.032151	30.032150	30.032149	0.000001	0.000000	0.000001	0.000000
77_2	2	21	34.911653	34.905443	34.896194	0.006210	-0.009249	0.006210	0.009249
1_3	3	21	8.906008	8.872920	8.840773	0.033088	-0.065235	0.033088	0.065235
2_3	3	21	17.311976	17.319263	17.325854	-0.007287	0.013878	0.007287	0.013878
3_3	3	21	10.573041	10.592784	10.459756	-0.019742	-0.113286	0.019742	0.113286
4_3	3	21	model couldn't explain the dat	17.542334	17.680933	#VALOR!	#VALOR!	#VALOR!	#VALOR!
5_3	3	21	9.663887	21.732781	21.914015	-12.068895	12.250128	12.068895	12.250128
6_3	3	21	28.395658	28.391034	28.386549	0.004624	-0.009109	0.004624	0.009109
7_3	3	21	6.360121	6.391800	6.423208	-0.031679	0.063087	0.031679	0.063087
8_3	3	21	8.209871	9.247007	9.504605	-1.037136	1.294734	1.037136	1.294734
9_3	3	21	10.208139	10.216518	10.223391	-0.008379	0.015252	0.008379	0.015252
10_3	3	21	7.499113	7.483251	7.466682	0.015861	-0.032431	0.015861	0.032431
11_3	3	21	7.579659	7.539133	7.495244	0.040526	-0.084415	0.040526	0.084415
12_3	3	21	13.542980	13.527708	13.513649	0.015272	-0.029332	0.015272	0.029332
13_3	3	21	6.779785	6.780095	6.780295	-0.000311	0.000511	0.000311	0.000511
14_3	3	21	12.452248	12.490686	12.518402	-0.038438	0.066153	0.038438	0.066153
15_3	3	21	14.005271	14.051043	14.089479	-0.045772	0.084208	0.045772	0.084208
16_3	3	21	11.237403	11.157806	11.072426	0.079597	-0.164977	0.079597	0.164977
17_3	3	21	12.060246	12.042713	12.025955	0.017533	-0.034291	0.017533	0.034291
18_3	3	21	12.066538	12.063835	12.061502	0.002703	-0.005036	0.002703	0.005036
19_3	3	21	11.119597	11.137660	11.154965	-0.018063	0.035368	0.018063	0.035368
20_3	3	21	11.213458	11.215117	11.214268	-0.001659	0.000810	0.001659	0.000810

21_3	3	21	11.926833	11.919634	11.910464	0.007199	-0.016369	0.007199	0.016369
22_3	3	21	12.234342	12.232431	12.229071	0.001911	-0.005271	0.001911	0.005271
23_3	3	21	12.880917	12.869919	12.855453	0.010998	-0.025464	0.010998	0.025464
24_3	3	21	12.369442	12.358990	12.346786	0.010452	-0.022656	0.010452	0.022656
25_3	3	21	12.393018	12.398309	12.402383	-0.005292	0.009365	0.005292	0.009365
26_3	3	21	11.691428	11.678355	11.663249	0.013073	-0.028179	0.013073	0.028179
27_3	3	21	12.847036	12.807031	12.768361	0.040005	-0.078675	0.040005	0.078675
28_3	3	21	11.657873	11.697151	11.737651	-0.039278	0.079778	0.039278	0.079778
29_3	3	21	16.451829	16.432560	16.413657	0.019269	-0.038172	0.019269	0.038172
30_3	3	21	12.148259	12.147821	12.147342	0.000438	-0.000917	0.000438	0.000917
31_3	3	21	15.639206	15.595957	15.549232	0.043249	-0.089974	0.043249	0.089974
32_3	3	21	15.755660	15.759921	15.762489	-0.004261	0.006829	0.004261	0.006829
33_3	3	21	16.030085	16.018610	16.012063	0.011475	-0.018022	0.011475	0.018022
34_3	3	21	17.407539	17.407136	17.413907	0.000403	0.006368	0.000403	0.006368
35_3	3	21	17.504538	17.500078	17.498518	0.004460	-0.006020	0.004460	0.006020
36_3	3	21	14.930008	14.929870	14.929583	0.000137	-0.000425	0.000137	0.000425
37_3	3	21	18.572415	18.566753	18.561094	0.005662	-0.011322	0.005662	0.011322
38_3	3	21	16.407323	16.385562	16.361703	0.021761	-0.045619	0.021761	0.045619
39_3	3	21	21.391785	21.369630	21.344022	0.022155	-0.047764	0.022155	0.047764
40_3	3	21	18.852737	18.839793	18.829251	0.012944	-0.023487	0.012944	0.023487
41_3	3	21	20.054063	20.061893	20.069320	-0.007831	0.015257	0.007831	0.015257
42_3	3	21	20.425222	20.422000	20.417372	0.003222	-0.007850	0.003222	0.007850
43_3	3	21	19.553435	19.553401	19.553348	0.000035	-0.000088	0.000035	0.000088
44_3	3	21	19.849344	19.845595	19.840466	0.003749	-0.008879	0.003749	0.008879
45_3	3	21	20.458819	20.451062	20.443230	0.007757	-0.015589	0.007757	0.015589
46_3	3	21	21.936169	21.929927	21.926747	0.006242	-0.009422	0.006242	0.009422
47_3	3	21	20.299521	20.290504	20.281279	0.009017	-0.018242	0.009017	0.018242
48_3	3	21	21.157602	21.156322	21.154535	0.001280	-0.003067	0.001280	0.003067
49_3	3	21	24.450225	24.429741	24.285564	0.020485	-0.164661	0.020485	0.164661
50_3	3	21	21.381021	21.369800	21.356542	0.011221	-0.024479	0.011221	0.024479
51_3	3	21	20.833605	20.930373	20.889248	-0.096767	0.055643	0.096767	0.055643
52_3	3	21	21.303990	21.284268	21.266696	0.019722	-0.037295	0.019722	0.037295
53_3	3	21	23.928302	23.926028	23.923620	0.002275	-0.004682	0.002275	0.004682
54_3	3	21	20.658719	20.659227	20.667820	-0.000509	0.009101	0.000509	0.009101
55_3	3	21	21.640903	21.653285	21.665414	-0.012382	0.024510	0.012382	0.024510
56_3	3	21	22.509149	22.536677	22.553632	-0.027528	0.044483	0.027528	0.044483

57_3	3	21	24.288536	24.290205	24.299297	-0.001668	0.010761	0.001668	0.010761
58_3	3	21	24.135546	24.134334	24.134784	0.001212	-0.000762	0.001212	0.000762
59_3	3	21	23.607615	23.598218	23.591266	0.009396	-0.016349	0.009396	0.016349
60_3	3	21	22.940011	22.932335	22.931249	0.007676	-0.008761	0.007676	0.008761
61_3	3	21	24.699511	24.687210	24.675805	0.012301	-0.023705	0.012301	0.023705
62_3	3	21	24.846329	24.838210	24.829596	0.008119	-0.016733	0.008119	0.016733
63_3	3	21	24.337990	24.329562	24.321480	0.008428	-0.016510	0.008428	0.016510
64_3	3	21	23.520227	23.671409	23.800929	-0.151182	0.280703	0.151182	0.280703
65_3	3	21	26.953893	26.945999	26.937779	0.007894	-0.016113	0.007894	0.016113
66_3	3	21	25.769354	25.771267	25.773535	-0.001913	0.004181	0.001913	0.004181
67_3	3	21	27.366486	27.369250	27.363729	-0.002763	-0.002758	0.002763	0.002758
68_3	3	21	24.072902	24.108552	24.143616	-0.035650	0.070714	0.035650	0.070714
69_3	3	21	26.540649	26.534734	26.530000	0.005915	-0.010649	0.005915	0.010649
70_3	3	21	26.570049	26.578441	26.587696	-0.008392	0.017647	0.008392	0.017647
71_3	3	21	27.045689	26.974496	26.897840	0.071193	-0.147849	0.071193	0.147849
72_3	3	21	25.214245	25.185788	25.164830	0.028457	-0.049416	0.028457	0.049416
73_3	3	21	29.618211	29.617340	29.616390	0.000871	-0.001821	0.000871	0.001821
74_3	3	21	28.598348	28.590053	28.580589	0.008295	-0.017759	0.008295	0.017759
75_3	3	21	29.634297	29.632610	29.631454	0.001687	-0.002843	0.001687	0.002843
76_3	3	21	31.034191	31.029698	31.024532	0.004493	-0.009659	0.004493	0.009659
77_3	3	21	32.347759	32.343982	32.340122	0.003777	-0.007637	0.003777	0.007637

Table S3_1c: Log10 of LR values computed by STRmix 2.7 for mixtures with two and three estimated contributors when the drop-in frequency was varied.

Pair of samples	Number of estimated contributors	STRmix v.2.7							
		Number of markers analyzed	Log ₁₀ of LR values computed using drop-in frequency equal to ...			Difference between Log ₁₀ (LR) considering drop-in of ...		Absolute value of the difference	
			0	0.05 (Default)	0.1	0.00 - 0.05	0.10 - 0.05	0.00 - 0.05	0.10 - 0.05
1_2	2	18	15.500525	14.865732	14.540480	0.634793	-0.325252	0.634793	0.325252
2_2	2	20	9.486232	9.460010	9.287242	0.026222	-0.172769	0.026222	0.172769
3_2	2	20	16.914428	17.709346	17.498021	-0.794919	-0.211325	0.794919	0.211325
4_2	2	20	22.216377	22.222872	22.193987	-0.006495	-0.028886	0.006495	0.028886
5_2	2	20	20.658288	21.015108	20.959990	-0.356820	-0.055118	0.356820	0.055118
6_2	2	20	24.625426	24.679310	24.655810	-0.053884	-0.023499	0.053884	0.023499
7_2	2	20	25.506884	25.572163	25.603318	-0.065279	0.031155	0.065279	0.031155
8_2	2	20	14.855053	14.746525	18.230091	0.108527	3.483566	0.108527	3.483566
9_2	2	20	22.052540	22.004622	21.857730	0.047918	-0.146892	0.047918	0.146892
10_2	2	20	22.935487	23.156246	23.069261	-0.220759	-0.086985	0.220759	0.086985
11_2	2	20	25.984301	26.008090	26.020452	-0.023789	0.012363	0.023789	0.012363
12_2	2	20	24.823618	24.915110	24.900367	-0.091492	-0.014742	0.091492	0.014742
13_2	2	21	8.518749	9.579784	8.201151	-1.061034	-1.378632	1.061034	1.378632
14_2	2	21	-0.522156	1.697439	1.061000	-2.219594	-0.636439	2.219594	0.636439
15_2	2	21	11.699213	11.915051	11.854682	-0.215838	-0.060369	0.215838	0.060369
16_2	2	21	13.530699	13.558853	13.480395	-0.028153	-0.078457	0.028153	0.078457
17_2	2	21	11.943742	13.591832	13.809816	-1.648091	0.217983	1.648091	0.217983
18_2	2	21	14.479331	14.932448	15.729966	-0.453118	0.797518	0.453118	0.797518
19_2	2	21	14.969444	14.926913	14.645009	0.042531	-0.281904	0.042531	0.281904
20_2	2	21	16.723086	16.017451	16.376978	0.705635	0.359527	0.705635	0.359527
21_2	2	21	17.332216	17.223080	17.286771	0.109136	0.063690	0.109136	0.063690
22_2	2	21	19.643739	19.600450	19.611139	0.043289	0.010689	0.043289	0.010689
23_2	2	21	19.806085	19.902367	19.756590	-0.096282	-0.145777	0.096282	0.145777
24_2	2	21	17.425860	17.274435	16.682641	0.151425	-0.591794	0.151425	0.591794
25_2	2	21	20.994075	20.445153	20.367319	0.548923	-0.077834	0.548923	0.077834
26_2	2	21	17.263707	17.090187	17.536861	0.173520	0.446674	0.173520	0.446674

27_2	2	21	16.232945	16.893895	16.684540	-0.660950	-0.209355	0.660950	0.209355
28_2	2	21	17.780490	17.744973	17.647579	0.035517	-0.097395	0.035517	0.097395
29_2	2	21	19.197859	19.938074	18.904494	-0.740215	-1.033580	0.740215	1.033580
30_2	2	21	20.109916	19.992973	20.199700	0.116943	0.206727	0.116943	0.206727
31_2	2	21	17.546407	17.765743	17.976790	-0.219336	0.211047	0.219336	0.211047
32_2	2	21	21.779993	20.178977	21.775880	1.601016	1.596903	1.601016	1.596903
33_2	2	21	21.949331	21.715602	21.805827	0.233729	0.090225	0.233729	0.090225
34_2	2	21	21.164531	21.348052	21.054038	-0.183520	-0.294013	0.183520	0.294013
35_2	2	21	18.913332	18.987626	19.172545	-0.074294	0.184919	0.074294	0.184919
36_2	2	21	20.611734	20.686708	20.408562	-0.074974	-0.278146	0.074974	0.278146
37_2	2	21	22.799368	22.747505	23.110354	0.051863	0.362849	0.051863	0.362849
38_2	2	21	22.611352	22.398287	22.485991	0.213064	0.087704	0.213064	0.087704
39_2	2	21	24.023664	23.993903	23.980172	0.029761	-0.013732	0.029761	0.013732
40_2	2	21	22.296292	22.050689	22.165571	0.245603	0.114882	0.245603	0.114882
41_2	2	21	19.844378	20.040325	19.968590	-0.195948	-0.071735	0.195948	0.071735
42_2	2	21	23.960200	23.451510	23.048325	0.508689	-0.403185	0.508689	0.403185
43_2	2	21	23.936257	23.083467	23.642277	0.852791	0.558810	0.852791	0.558810
44_2	2	21	23.399033	23.998573	24.005309	-0.599540	0.006736	0.599540	0.006736
45_2	2	21	20.597201	20.612900	20.483473	-0.015699	-0.129427	0.015699	0.129427
46_2	2	21	25.449725	25.423344	25.332034	0.026381	-0.091310	0.026381	0.091310
47_2	2	21	23.675852	23.591499	23.431589	0.084353	-0.159910	0.084353	0.159910
48_2	2	21	23.737455	23.668293	23.668330	0.069162	0.000037	0.069162	0.000037
49_2	2	21	26.140822	26.085647	26.079254	0.055175	-0.006394	0.055175	0.006394
50_2	2	21	21.474479	21.232869	21.167081	0.241609	-0.065788	0.241609	0.065788
51_2	2	21	21.646217	24.592221	23.627704	-2.946004	-0.964517	2.946004	0.964517
52_2	2	21	26.452293	26.452951	26.459980	-0.000659	0.007029	0.000659	0.007029
53_2	2	21	26.266467	26.312727	26.205448	-0.046260	-0.107279	0.046260	0.107279
54_2	2	21	26.275818	26.277403	26.276301	-0.001585	-0.001102	0.001585	0.001102
55_2	2	21	26.920833	27.007321	27.000087	-0.086488	-0.007234	0.086488	0.007234
56_2	2	21	26.901333	26.898731	26.902732	0.002602	0.004001	0.002602	0.004001
57_2	2	21	27.635856	27.675439	27.698231	-0.039584	0.022792	0.039584	0.022792
58_2	2	21	28.032780	26.005138	25.965606	2.027642	-0.039531	2.027642	0.039531
59_2	2	21	26.156367	26.478465	26.661226	-0.322098	0.182760	0.322098	0.182760
60_2	2	21	26.037665	26.329825	26.677543	-0.292159	0.347718	0.292159	0.347718
61_2	2	21	26.697055	26.981175	26.600505	-0.284120	-0.380670	0.284120	0.380670
62_2	2	21	26.504987	26.511603	26.507923	-0.006616	-0.003679	0.006616	0.003679

63_2	2	21	28.527101	28.527913	28.530712	-0.000812	0.002799	0.000812	0.002799
64_2	2	21	27.129593	27.140759	27.135101	-0.011166	-0.005659	0.011166	0.005659
65_2	2	21	25.985391	25.761635	26.050998	0.223756	0.289363	0.223756	0.289363
66_2	2	21	26.535319	26.579898	26.561233	-0.044578	-0.018665	0.044578	0.018665
67_2	2	21	28.384640	28.385302	28.383923	-0.000662	-0.001379	0.000662	0.001379
68_2	2	21	28.425355	28.436369	28.418732	-0.011015	-0.017637	0.011015	0.017637
69_2	2	21	28.983003	28.986436	28.980222	-0.003432	-0.006214	0.003432	0.006214
70_2	2	21	29.433482	29.467371	29.145383	-0.033889	-0.321988	0.033889	0.321988
71_2	2	21	27.610032	27.612349	27.558900	-0.002318	-0.053449	0.002318	0.053449
72_2	2	21	29.452170	29.468022	29.452339	-0.015852	-0.015684	0.015852	0.015684
73_2	2	21	30.038978	30.037267	30.041748	0.001711	0.004481	0.001711	0.004481
74_2	2	21	30.377233	30.386231	30.388350	-0.008998	0.002119	0.008998	0.002119
75_2	2	21	30.488325	30.485721	30.494057	0.002604	0.008336	0.002604	0.008336
76_2	2	21	30.221049	29.721151	30.241646	0.499898	0.520495	0.499898	0.520495
77_2	2	21	33.100681	33.054115	33.098055	0.046566	0.043940	0.046566	0.043940
1_3	3	19	9.370013	9.190304	9.695385	-0.179710	0.505082	0.179710	0.505082
2_3	3	19	18.310523	18.181215	18.263518	-0.129309	0.082303	0.129309	0.082303
3_3	3	20	15.359650	15.469940	15.450395	0.110290	-0.019544	0.110290	0.019544
4_3	3	20	16.259235	16.682443	17.350050	0.423208	0.667607	0.423208	0.667607
5_3	3	20	23.185174	23.020693	23.101438	-0.164481	0.080745	0.164481	0.080745
6_3	3	20	26.463908	26.442370	26.447034	-0.021538	0.004664	0.021538	0.004664
7_3	3	20	6.970272	7.249419	7.001993	0.279146	-0.247425	0.279146	0.247425
8_3	3	21	7.846368	7.815113	8.150357	-0.031255	0.335245	0.031255	0.335245
9_3	3	21	-4.717809	4.179782	-	8.897590	#VALOR!	8.897590	#VALOR!
10_3	3	21	5.192038	5.095309	4.694746	-0.096729	-0.400563	0.096729	0.400563
11_3	3	21	8.849984	8.857791	8.905180	0.007806	0.047390	0.007806	0.047390
12_3	3	21	1.359266	1.521138	1.618351	0.161872	0.097213	0.161872	0.097213
13_3	3	21	12.141073	11.836033	11.768801	-0.305041	-0.067232	0.305041	0.067232
14_3	3	21	5.842578	4.029100	3.773106	-1.813478	-0.255994	1.813478	0.255994
15_3	3	21	4.026370	1.440295	1.913729	-2.586075	0.473434	2.586075	0.473434
16_3	3	21	11.710803	10.683272	10.733558	-1.027530	0.050286	1.027530	0.050286
17_3	3	21	13.338058	12.466705	13.068520	-0.871353	0.601815	0.871353	0.601815
18_3	3	21	12.706146	12.900968	12.500264	0.194822	-0.400704	0.194822	0.400704
19_3	3	21	7.842297	7.773947	7.639716	-0.068350	-0.134232	0.068350	0.134232
20_3	3	21	11.225361	11.332317	11.445869	0.106956	0.113552	0.106956	0.113552
21_3	3	21	11.927796	12.369049	12.277128	0.441253	-0.091921	0.441253	0.091921

22_3	3	21	11.515463	11.376047	10.485594	-0.139416	-0.890454	0.139416	0.890454
23_3	3	21	13.915748	13.971104	13.638090	0.055356	-0.333014	0.055356	0.333014
24_3	3	21	10.584874	10.679283	10.956207	0.094409	0.276924	0.094409	0.276924
25_3	3	21	11.447654	11.889190	12.168763	0.441536	0.279573	0.441536	0.279573
26_3	3	21	10.389821	10.806329	10.237720	0.416508	-0.568610	0.416508	0.568610
27_3	3	21	14.666602	14.833842	14.651259	0.167239	-0.182583	0.167239	0.182583
28_3	3	21	11.868486	10.861857	15.733189	-1.006629	4.871332	1.006629	4.871332
29_3	3	21	11.713356	11.882866	10.547701	0.169510	-1.335165	0.169510	1.335165
30_3	3	21	16.208871	16.174002	15.981171	-0.034870	-0.192831	0.034870	0.192831
31_3	3	21	15.464802	15.374180	15.228990	-0.090623	-0.145190	0.090623	0.145190
32_3	3	21	15.160318	16.412763	15.467401	1.252444	-0.945362	1.252444	0.945362
33_3	3	21	17.344392	17.223704	17.496459	-0.120689	0.272756	0.120689	0.272756
34_3	3	21	16.066848	16.067777	15.958186	0.000930	-0.109591	0.000930	0.109591
35_3	3	21	20.363838	20.336640	20.489494	-0.027198	0.152855	0.027198	0.152855
36_3	3	21	20.458668	20.852535	20.750724	0.393867	-0.101811	0.393867	0.101811
37_3	3	21	17.821514	17.830730	18.056981	0.009216	0.226251	0.009216	0.226251
38_3	3	21	20.356083	20.404012	20.516324	0.047929	0.112312	0.047929	0.112312
39_3	3	21	19.075255	18.696715	17.222404	-0.378540	-1.474310	0.378540	1.474310
40_3	3	21	18.201097	17.625837	18.202761	-0.575260	0.576924	0.575260	0.576924
41_3	3	21	19.910400	19.642761	19.062920	-0.267639	-0.579841	0.267639	0.579841
42_3	3	21	19.496182	18.757973	18.881567	-0.738209	0.123595	0.738209	0.123595
43_3	3	21	22.205881	20.135800	21.726581	-2.070080	1.590780	2.070080	1.590780
44_3	3	21	17.930251	17.648380	18.587486	-0.281871	0.939107	0.281871	0.939107
45_3	3	21	21.034829	21.058805	20.441192	0.023977	-0.617613	0.023977	0.617613
46_3	3	21	17.350810	13.827143	16.516786	-3.523667	2.689643	3.523667	2.689643
47_3	3	21	19.822305	20.222820	20.413015	0.400515	0.190194	0.400515	0.190194
48_3	3	21	20.418053	20.745730	20.337240	0.327678	-0.408491	0.327678	0.408491
49_3	3	21	25.192651	17.051037	23.676968	-8.141614	6.625931	8.141614	6.625931
50_3	3	21	23.557435	23.673684	23.392873	0.116249	-0.280812	0.116249	0.280812
51_3	3	21	22.721539	22.322343	22.866577	-0.399195	0.544233	0.399195	0.544233
52_3	3	21	24.500950	24.482802	24.508516	-0.018147	0.025714	0.018147	0.025714
53_3	3	21	23.885175	23.791691	22.731073	-0.093484	-1.060618	0.093484	1.060618
54_3	3	21	24.545517	24.650492	24.164442	0.104975	-0.486050	0.104975	0.486050
55_3	3	21	23.963552	23.801109	23.871123	-0.162443	0.070015	0.162443	0.070015
56_3	3	21	24.216219	24.236411	24.281647	0.020193	0.045236	0.020193	0.045236
57_3	3	21	23.879113	23.710836	23.754982	-0.168277	0.044146	0.168277	0.044146

58_3	3	21	24.644350	24.545010	23.469940	-0.099340	-1.075070	0.099340	1.075070
59_3	3	21	24.824315	26.274135	26.239400	1.449820	-0.034735	1.449820	0.034735
60_3	3	21	27.198437	27.168880	27.228760	-0.029557	0.059879	0.029557	0.059879
61_3	3	21	25.534052	25.508220	25.552035	-0.025832	0.043815	0.025832	0.043815
62_3	3	21	26.577400	24.902471	27.263683	-1.674929	2.361213	1.674929	2.361213
63_3	3	21	25.136277	24.906470	25.242740	-0.229807	0.336270	0.229807	0.336270
64_3	3	21	23.199508	23.405995	23.431042	0.206486	0.025047	0.206486	0.025047
65_3	3	21	26.675274	26.693217	26.650103	0.017943	-0.043113	0.017943	0.043113
66_3	3	21	25.364457	26.693217	25.342955	1.328760	-1.350261	1.328760	1.350261
67_3	3	21	27.737542	27.758026	27.750416	0.020483	-0.007610	0.020483	0.007610
68_3	3	21	28.469734	28.280533	28.260596	-0.189201	-0.019937	0.189201	0.019937
69_3	3	21	25.792357	25.889397	25.772351	0.097040	-0.117046	0.097040	0.117046
70_3	3	21	27.119157	27.122019	27.147614	0.002862	0.025595	0.002862	0.025595
71_3	3	21	26.910694	26.803307	26.835240	-0.107387	0.031933	0.107387	0.031933
72_3	3	21	28.065729	27.839327	27.910363	-0.226402	0.071036	0.226402	0.071036
73_3	3	21	27.229810	27.229835	27.225542	0.000026	-0.004293	0.000026	0.004293
74_3	3	21	27.581130	27.683722	27.638509	0.102592	-0.045213	0.102592	0.045213
75_3	3	21	29.838553	29.835469	29.842996	-0.003085	0.007527	0.003085	0.007527
76_3	3	21	29.963325	29.975740	29.984496	0.012415	0.008756	0.012415	0.008756
77_3	3	21	30.489454	30.563492	30.571677	0.074038	0.008185	0.074038	0.008185

Table S3_2a: Log10 of LR's values computed by EuroForMix v.3.4.0 for mixtures with two and three estimated contributors when the drop-in modelation parameter was varied.

Pair of samples	Number of estimated contributors	EuroForMix v.3.4.0				
		Number of markers analyzed	Log ₁₀ of LR values computed using drop-in parameters' distribution equal to ...		Difference	Absolute value of the difference
			0.01 (Default)	0.05		
1_2	2	21	7.927961408	8.221510621	0.293549214	0.293549214
2_2	2	21	8.444753876	8.767791691	0.323037816	0.323037816
3_2	2	21	14.14865573	14.14451046	-0.004145276	0.004145276
4_2	2	21	18.9162635	19.28245262	0.366189115	0.366189115
5_2	2	21	20.55099707	20.5771879	0.026190826	0.026190826
6_2	2	21	23.43326278	23.32649837	-0.106764408	0.106764408
7_2	2	21	18.15829858	18.61746065	0.459162068	0.459162068
8_2	2	21	24.34936888	24.33140565	-0.017963231	0.017963231
9_2	2	21	16.58992434	16.59064225	0.000717913	0.000717913
10_2	2	21	20.71446385	20.71453892	7.50746E-05	7.50746E-05
11_2	2	21	26.69124692	26.69346223	0.002215313	0.002215313
12_2	2	21	26.68903622	26.83128327	0.142247048	0.142247048
13_2	2	21	16.54164571	16.5515624	0.009916694	0.009916694
14_2	2	21	8.572155479	8.633034899	0.06087942	0.06087942
15_2	2	21	12.34987557	12.37026065	0.020385087	0.020385087
16_2	2	21	10.149093	10.17128847	0.022195474	0.022195474
17_2	2	21	14.25309526	14.25309604	7.78997E-07	7.78997E-07
18_2	2	21	19.03621211	19.03807323	0.001861126	0.001861126
19_2	2	21	16.64183306	16.51337038	-0.128462679	0.128462679
20_2	2	21	14.99417429	14.99966095	0.005486662	0.005486662
21_2	2	21	15.80924136	15.81040396	0.0011626	0.0011626
22_2	2	21	16.32780352	16.32826867	0.000465159	0.000465159
23_2	2	21	17.79821514	17.81808667	0.019871528	0.019871528
24_2	2	21	17.9371393	17.93694723	-0.000192068	0.000192068
25_2	2	21	18.9144039	18.90531785	-0.009086048	0.009086048
26_2	2	21	17.35212396	17.36131946	0.009195505	0.009195505
27_2	2	21	18.51560732	18.5283742	0.012766883	0.012766883
28_2	2	21	17.18062122	17.18057667	-4.45418E-05	4.45418E-05
29_2	2	21	17.15547659	17.21160314	0.056126549	0.056126549
30_2	2	21	16.37009274	16.37091638	0.000823642	0.000823642
31_2	2	21	18.79376311	18.79418054	0.00041743	0.00041743
32_2	2	21	15.61775466	15.60949504	-0.008259621	0.008259621
33_2	2	21	21.21566648	21.22608388	0.010417406	0.010417406
34_2	2	21	18.71660024	18.71662963	2.93915E-05	2.93915E-05
35_2	2	21	20.22702566	20.22690739	-0.000118266	0.000118266
36_2	2	21	21.17895834	21.16129945	-0.017658883	0.017658883
37_2	2	21	21.85987351	21.84708969	-0.012783812	0.012783812
38_2	2	21	20.11672317	20.11385705	-0.002866121	0.002866121
39_2	2	21	22.31054819	22.30236012	-0.008188069	0.008188069
40_2	2	21	20.89043433	20.88518353	-0.005250801	0.005250801
41_2	2	21	20.23824605	20.23745164	-0.000794408	0.000794408
42_2	2	21	22.96054738	22.96702085	0.006473468	0.006473468
43_2	2	21	22.55825773	22.55839585	0.000138114	0.000138114
44_2	2	21	23.05469213	23.05676218	0.002070052	0.002070052
45_2	2	21	22.69983887	22.69983493	-3.93626E-06	3.93626E-06
46_2	2	21	23.36030357	23.36802434	0.00772077	0.00772077
47_2	2	21	21.71472691	21.7151678	0.000440889	0.000440889
48_2	2	21	24.71429572	24.97481358	0.260517864	0.260517864
49_2	2	21	24.37254244	24.38737008	0.014827635	0.014827635
50_2	2	21	23.21906364	23.21666046	-0.002403182	0.002403182
51_2	2	21	26.18409114	26.18388695	-0.000204192	0.000204192

52_2	2	21	25.32352507	25.32242175	-0.001103325	0.001103325
53_2	2	21	25.18482274	25.18551924	0.000696499	0.000696499
54_2	2	21	26.52762866	26.52850963	0.000880975	0.000880975
55_2	2	21	24.78198553	24.77741033	-0.004575204	0.004575204
56_2	2	21	26.41025337	26.41075482	0.00050145	0.00050145
57_2	2	21	26.71067461	26.70698516	-0.003689446	0.003689446
58_2	2	21	25.5550486	25.55536664	0.000318034	0.000318034
59_2	2	21	27.03166804	27.0508448	0.019176754	0.019176754
60_2	2	21	26.06180828	26.05418457	-0.007623712	0.007623712
61_2	2	21	25.7668327	25.77293165	0.006098956	0.006098956
62_2	2	21	26.35344141	26.35299177	-0.000449637	0.000449637
63_2	2	21	27.04759424	27.04866484	0.001070603	0.001070603
64_2	2	21	26.19902215	26.19132621	-0.007695942	0.007695942
65_2	2	21	27.73663912	27.73785637	0.001217253	0.001217253
66_2	2	21	27.73905594	27.73873031	-0.000325638	0.000325638
67_2	2	21	27.99614279	27.99900807	0.002865275	0.002865275
68_2	2	21	28.64865102	28.64849992	-0.000151092	0.000151092
69_2	2	21	28.12368857	28.12189039	-0.001798179	0.001798179
70_2	2	21	28.2338101	28.23560746	0.001797364	0.001797364
71_2	2	21	27.74995783	27.7493387	-0.00061913	0.00061913
72_2	2	21	28.82434796	28.82422867	-0.000119293	0.000119293
73_2	2	21	29.62237668	29.62327929	0.000902618	0.000902618
74_2	2	21	29.60802844	29.61277995	0.004751513	0.004751513
75_2	2	21	29.64309324	29.64617877	0.003085531	0.003085531
76_2	2	21	30.0321496	30.03215073	1.13045E-06	1.13045E-06
77_2	2	21	34.90544278	34.90131214	-0.004130641	0.004130641
1_3	3	21	8.872919894	8.890394667	0.017474773	0.017474773
2_3	3	21	17.31926253	17.32082638	0.001563845	0.001563845
3_3	3	21	10.59278355	10.83637978	0.243596233	0.243596233
4_3	3	21	17.54233394	17.38736674	-0.154967194	0.154967194
5_3	3	21	21.73278146	22.0090117	0.276230238	0.276230238
6_3	3	21	28.39103356	28.38876865	-0.002264914	0.002264914
7_3	3	21	6.391799845	6.43035685	0.038557006	0.038557006
8_3	3	21	9.247007109	9.125498323	-0.121508786	0.121508786
9_3	3	21	10.21651768	10.21266128	-0.003856404	0.003856404
10_3	3	21	7.483251442	7.500537863	0.017286421	0.017286421
11_3	3	21	7.539132507	7.540787835	0.001655328	0.001655328
12_3	3	21	13.52770824	13.57968988	0.051981645	0.051981645
13_3	3	21	6.780095197	6.780984977	0.00088978	0.00088978
14_3	3	21	12.49068576	12.45431187	-0.036373889	0.036373889
15_3	3	21	14.05104261	14.06781811	0.016775498	0.016775498
16_3	3	21	11.15780554	11.13223798	-0.025567563	0.025567563
17_3	3	21	12.04271266	12.01829239	-0.024420264	0.024420264
18_3	3	21	12.06383477	12.07948282	0.015648048	0.015648048
19_3	3	21	11.13765989	11.16146432	0.023804434	0.023804434
20_3	3	21	11.21511709	11.22835809	0.013241006	0.013241006
21_3	3	21	11.91963369	11.92550675	0.005873067	0.005873067
22_3	3	21	12.23243108	12.23347527	0.001044191	0.001044191
23_3	3	21	12.86991883	12.86110896	-0.008809871	0.008809871
24_3	3	21	12.35899002	12.37652499	0.01753497	0.01753497
25_3	3	21	12.39830936	12.40623473	0.00792537	0.00792537
26_3	3	21	11.6783552	11.6953024	0.016947199	0.016947199
27_3	3	21	12.80703129	12.78988721	-0.017144074	0.017144074
28_3	3	21	11.69715068	11.70451498	0.007364303	0.007364303
29_3	3	21	16.43255978	16.42596981	-0.006589971	0.006589971
30_3	3	21	12.14782066	12.14756937	-0.000251297	0.000251297
31_3	3	21	15.59595691	15.70230549	0.106348583	0.106348583
32_3	3	21	15.75992122	15.76248389	0.002562667	0.002562667
33_3	3	21	16.01861026	16.02808025	0.009469992	0.009469992
34_3	3	21	17.40713589	17.41595655	0.00882066	0.00882066
35_3	3	21	17.50007835	17.50056615	0.000487798	0.000487798

36_3	3	21	14.92987032	14.93001095	0.000140636	0.000140636
37_3	3	21	18.56675336	18.55850188	-0.00825148	0.00825148
38_3	3	21	16.385562	16.4060513	0.020489303	0.020489303
39_3	3	21	21.36963039	21.38203462	0.01240423	0.01240423
40_3	3	21	18.83979338	18.84042213	0.000628753	0.000628753
41_3	3	21	20.06189347	20.06784186	0.005948397	0.005948397
42_3	3	21	20.42199991	20.42657703	0.004577122	0.004577122
43_3	3	21	19.55340066	19.55329122	-0.000109439	0.000109439
44_3	3	21	19.84559545	19.85596489	0.010369446	0.010369446
45_3	3	21	20.45106198	20.45257397	0.001511991	0.001511991
46_3	3	21	21.92992701	21.92160144	-0.008325571	0.008325571
47_3	3	21	20.2905043	20.29292422	0.002419915	0.002419915
48_3	3	21	21.156322	21.15663223	0.000310226	0.000310226
49_3	3	21	24.42974057	24.43495274	0.005212172	0.005212172
50_3	3	21	21.36980017	21.35683077	-0.012969392	0.012969392
51_3	3	21	20.93037263	20.92408502	-0.00628761	0.00628761
52_3	3	21	21.28426784	21.2957534	0.011485566	0.011485566
53_3	3	21	23.92602752	23.92664117	0.000613653	0.000613653
54_3	3	21	20.65922733	20.69421934	0.034992006	0.034992006
55_3	3	21	21.65328522	21.6231699	-0.030115314	0.030115314
56_3	3	21	22.53667674	22.53408632	-0.002590416	0.002590416
57_3	3	21	24.29020457	24.29033626	0.000131691	0.000131691
58_3	3	21	24.1343337	24.1527521	0.018418399	0.018418399
59_3	3	21	23.59821827	23.60761066	0.009392397	0.009392397
60_3	3	21	22.93233479	22.9315641	-0.000770686	0.000770686
61_3	3	21	24.6872103	24.67678382	-0.010426474	0.010426474
62_3	3	21	24.83821011	24.84633099	0.008120879	0.008120879
63_3	3	21	24.32956161	24.32713634	-0.002425274	0.002425274
64_3	3	21	23.67140894	23.95898374	0.287574797	0.287574797
65_3	3	21	26.94599906	26.94567656	-0.000322501	0.000322501
66_3	3	21	25.77126729	25.76310979	-0.0081575	0.0081575
67_3	3	21	27.3692497	27.36459786	-0.00465184	0.00465184
68_3	3	21	24.10855207	24.08155668	-0.026995385	0.026995385
69_3	3	21	26.53473447	26.53694239	0.002207919	0.002207919
70_3	3	21	26.57844092	26.5696621	-0.008778819	0.008778819
71_3	3	21	26.97449615	26.97165552	-0.002840627	0.002840627
72_3	3	21	25.18578817	25.19788009	0.012091922	0.012091922
73_3	3	21	29.61734003	29.6181375	0.000797478	0.000797478
74_3	3	21	28.59005293	28.59738165	0.007328715	0.007328715
75_3	3	21	29.63260974	29.63178345	-0.000826296	0.000826296
76_3	3	21	31.02969786	31.03288969	0.003191826	0.003191826
77_3	3	21	32.34398235	32.34307493	-0.000907425	0.000907425

Table S3_2b: Log10 of LR's values computed by STRmix v.2.7 for mixtures with two and three estimated contributors when the drop-in modelation parameter was varied.

Pair of samples	Number of estimated contributors	STRmix v.2.7				
		Number of markers analyzed	Log ₁₀ of LR values computed using drop-in parameters' distribution equal to ...		Difference	Absolute value of the difference
			(1.0, 1.0) (Default)	Uniform		
1_2	2	18	14.86573156	15.96462257	1.09889101	1.09889101
2_2	2	20	9.460010315	8.540717288	-0.919293027	0.919293027
3_2	2	20	17.7093463	17.82812475	0.118778458	0.118778458
4_2	2	20	22.22287248	22.28422764	0.061355163	0.061355163
5_2	2	20	21.01510816	21.3500347	0.334926536	0.334926536
6_2	2	20	24.67930977	24.66835796	-0.01095181	0.01095181
7_2	2	20	25.57216268	25.53241037	-0.039752309	0.039752309
8_2	2	20	14.74652522	19.93131037	5.184785146	5.184785146
9_2	2	20	22.00462227	21.87707173	-0.127550537	0.127550537
10_2	2	20	23.15624619	23.06688474	-0.089361447	0.089361447
11_2	2	20	26.0080896	26.01228874	0.004199137	0.004199137
12_2	2	20	24.9151095	24.8534305	-0.061679008	0.061679008
13_2	2	21	9.579783597	-7.746831674	-17.32661527	17.32661527
14_2	2	21	1.697438591	2.464221198	0.766782607	0.766782607
15_2	2	21	11.91505142	11.65210123	-0.262950186	0.262950186
16_2	2	21	13.55885251	13.54368321	-0.015169299	0.015169299
17_2	2	21	13.5918323	13.78033892	0.188506623	0.188506623
18_2	2	21	14.9324484	13.67930977	-1.253138629	1.253138629
19_2	2	21	14.92691324	15.0890215	0.16210826	0.16210826
20_2	2	21	16.01745073	16.4936532	0.476202466	0.476202466
21_2	2	21	17.2230804	17.36370597	0.140625575	0.140625575
22_2	2	21	19.60045012	19.79053849	0.190088366	0.190088366
23_2	2	21	19.90236737	19.8640243	-0.038343068	0.038343068
24_2	2	21	17.27443497	17.03302144	-0.241413525	0.241413525
25_2	2	21	20.44515255	20.8745398	0.429387246	0.429387246
26_2	2	21	17.09018749	16.39474928	-0.695438207	0.695438207
27_2	2	21	16.89389486	15.96326825	-0.930626607	0.930626607
28_2	2	21	17.74497324	17.75597389	0.011000658	0.011000658
29_2	2	21	19.93807419	19.37443671	-0.56363748	0.56363748
30_2	2	21	19.99297303	20.06171629	0.068743263	0.068743263
31_2	2	21	17.76574304	18.20625904	0.440515994	0.440515994
32_2	2	21	20.17897695	21.60834415	1.429367201	1.429367201
33_2	2	21	21.71560227	21.87194053	0.156338258	0.156338258
34_2	2	21	21.34805161	21.44321603	0.095164421	0.095164421
35_2	2	21	18.98762605	19.31548753	0.327861477	0.327861477
36_2	2	21	20.68670775	20.71554375	0.028835998	0.028835998
37_2	2	21	22.74750503	22.85423922	0.106734195	0.106734195
38_2	2	21	22.39828731	22.23383341	-0.16445389	0.16445389
39_2	2	21	23.99390334	24.00229568	0.008392341	0.008392341
40_2	2	21	22.05068903	22.25173553	0.201046503	0.201046503
41_2	2	21	20.04032538	19.96805779	-0.072267594	0.072267594
42_2	2	21	23.45151012	23.2260325	-0.225477615	0.225477615
43_2	2	21	23.08346679	23.78140349	0.697936704	0.697936704
44_2	2	21	23.99857317	23.98519287	-0.013380302	0.013380302
45_2	2	21	20.61290036	20.52856964	-0.084330719	0.084330719
46_2	2	21	25.42334419	25.32120476	-0.102139437	0.102139437
47_2	2	21	23.59149868	23.31251586	-0.278982829	0.278982829
48_2	2	21	23.66829271	23.63129154	-0.037001174	0.037001174
49_2	2	21	26.08564729	26.10646288	0.020815595	0.020815595
50_2	2	21	21.23286911	21.49446072	0.261591614	0.261591614
51_2	2	21	24.59222118	23.8727039	-0.719517288	0.719517288

52_2	2	21	26.45295118	26.45233854	-0.000612632	0.000612632
53_2	2	21	26.31272728	26.30520085	-0.007526439	0.007526439
54_2	2	21	26.2774029	26.27873074	0.001327839	0.001327839
55_2	2	21	27.00732095	26.91353306	-0.093787894	0.093787894
56_2	2	21	26.89873067	26.90136023	0.002629561	0.002629561
57_2	2	21	27.6754392	27.67639178	0.000952581	0.000952581
58_2	2	21	26.0051376	25.9958501	-0.009287501	0.009287501
59_2	2	21	26.47846549	26.45134117	-0.027124315	0.027124315
60_2	2	21	26.32982484	26.32354085	-0.006283998	0.006283998
61_2	2	21	26.98117507	26.94975096	-0.031424111	0.031424111
62_2	2	21	26.51160265	26.50910839	-0.002494257	0.002494257
63_2	2	21	28.52791332	28.52031319	-0.007600136	0.007600136
64_2	2	21	27.14075937	27.12329617	-0.017463198	0.017463198
65_2	2	21	25.7616347	26.05468964	0.29305494	0.29305494
66_2	2	21	26.57989787	26.57710099	-0.002796881	0.002796881
67_2	2	21	28.38530234	28.38342037	-0.001881967	0.001881967
68_2	2	21	28.4363694	28.42360627	-0.012763134	0.012763134
69_2	2	21	28.98643581	28.9799306	-0.006505206	0.006505206
70_2	2	21	29.46737129	28.6836773	-0.783693989	0.783693989
71_2	2	21	27.61234934	27.59179895	-0.020550399	0.020550399
72_2	2	21	29.46802223	29.46636337	-0.001658858	0.001658858
73_2	2	21	30.03726709	30.04178732	0.004520224	0.004520224
74_2	2	21	30.38623135	30.38234129	-0.00389006	0.00389006
75_2	2	21	30.48572143	30.49446072	0.008739293	0.008739293
76_2	2	21	29.72115084	30.22541267	0.504261829	0.504261829
77_2	2	21	33.0541149	33.07725886	0.023143963	0.023143963
1_3	3	19	9.190303678	9.680435236	0.490131557	0.490131557
2_3	3	19	18.18121455	17.94423091	-0.236983643	0.236983643
3_3	3	20	15.46993977	15.42566462	-0.044275155	0.044275155
4_3	3	20	16.68244293	16.73500773	0.052564798	0.052564798
5_3	3	20	23.02069268	23.01602996	-0.004662716	0.004662716
6_3	3	20	26.44237001	26.46769688	0.025326873	0.025326873
7_3	3	20	7.249418507	7.016029963	-0.233388544	0.233388544
8_3	3	21	7.815112658	-6.723124779	-14.53823744	14.53823744
9_3	3	21	4.179781516	-5.121587366	-9.301368882	9.301368882
10_3	3	21	5.095308861	5.070555016	-0.024753846	0.024753846
11_3	3	21	8.857790677	8.890627776	0.0328371	0.0328371
12_3	3	21	1.521138084	0.255248377	-1.265889707	1.265889707
13_3	3	21	11.8360328	11.83342762	-0.002605177	0.002605177
14_3	3	21	4.029099567	5.91034688	1.881247313	1.881247313
15_3	3	21	1.440294971	3.790517383	2.350222412	2.350222412
16_3	3	21	10.68327224	10.72741925	0.044147011	0.044147011
17_3	3	21	12.46670454	13.02065127	0.553946724	0.553946724
18_3	3	21	12.90096762	12.83746217	-0.063505452	0.063505452
19_3	3	21	7.773947265	7.794961817	0.021014552	0.021014552
20_3	3	21	11.33231724	11.23676392	-0.095553322	0.095553322
21_3	3	21	12.36904879	11.92323365	-0.445815135	0.445815135
22_3	3	21	11.37604745	11.35955092	-0.016496535	0.016496535
23_3	3	21	13.97110414	13.78123822	-0.189865918	0.189865918
24_3	3	21	10.6792825	10.82962535	0.150342852	0.150342852
25_3	3	21	11.88918961	11.73797939	-0.151210225	0.151210225
26_3	3	21	10.80632924	10.17963795	-0.626691285	0.626691285
27_3	3	21	14.83384168	14.88518588	0.051344201	0.051344201
28_3	3	21	10.86185688	14.71823588	3.856379007	3.856379007
29_3	3	21	11.88286592	11.62121784	-0.261648081	0.261648081
30_3	3	21	16.17400163	16.10226215	-0.071739477	0.071739477
31_3	3	21	15.37417991	15.7259198	0.351739886	0.351739886
32_3	3	21	16.41276285	15.24809593	-1.164666921	1.164666921
33_3	3	21	17.22370356	17.30083452	0.077130956	0.077130956
34_3	3	21	16.06777736	16.22536098	0.157583622	0.157583622
35_3	3	21	20.33663982	20.16767172	-0.168968102	0.168968102

36_3	3	21	20.85253489	20.71401105	-0.138523839	0.138523839
37_3	3	21	17.83072978	17.76072397	-0.070005803	0.070005803
38_3	3	21	20.40401223	20.41060854	0.006596316	0.006596316
39_3	3	21	18.69671451	17.55115712	-1.145557396	1.145557396
40_3	3	21	17.62583699	18.31513032	0.689293325	0.689293325
41_3	3	21	19.6427612	19.98784942	0.345088213	0.345088213
42_3	3	21	18.75797268	19.1476145	0.389641818	0.389641818
43_3	3	21	20.13580028	19.80695965	-0.328840637	0.328840637
44_3	3	21	17.64837953	18.19479176	0.546412228	0.546412228
45_3	3	21	21.05880549	20.44861358	-0.610191909	0.610191909
46_3	3	21	13.82714302	13.3908998	-0.436243222	0.436243222
47_3	3	21	20.22282048	20.92004502	0.69722454	0.69722454
48_3	3	21	20.74573042	20.90171435	0.15598393	0.15598393
49_3	3	21	17.0510367	19.84164724	2.790610541	2.790610541
50_3	3	21	23.67368429	23.40901964	-0.264664652	0.264664652
51_3	3	21	22.32234336	24.09576196	1.773418598	1.773418598
52_3	3	21	24.48280215	24.49998933	0.017187187	0.017187187
53_3	3	21	23.79169065	22.61197808	-1.179712573	1.179712573
54_3	3	21	24.65049208	24.6340942	-0.016397887	0.016397887
55_3	3	21	23.80110859	23.93109666	0.129988065	0.129988065
56_3	3	21	24.23641129	24.25282877	0.01641748	0.01641748
57_3	3	21	23.71083636	23.41304817	-0.297788192	0.297788192
58_3	3	21	24.54501006	24.65800185	0.11299179	0.11299179
59_3	3	21	26.27413475	26.27193449	-0.002200254	0.002200254
60_3	3	21	27.16888034	27.19937116	0.030490819	0.030490819
61_3	3	21	25.50821988	25.55707389	0.048854015	0.048854015
62_3	3	21	24.90247068	27.27505785	2.37258717	2.37258717
63_3	3	21	24.90646973	25.33059638	0.424126655	0.424126655
64_3	3	21	23.40599484	23.16268387	-0.243310975	0.243310975
65_3	3	21	26.69321675	26.63809971	-0.055117034	0.055117034
66_3	3	21	26.69321675	25.3445691	-1.348647649	1.348647649
67_3	3	21	27.75802575	27.75573007	-0.002295688	0.002295688
68_3	3	21	28.28053284	28.30811602	0.027583171	0.027583171
69_3	3	21	25.88939696	25.72420212	-0.165194841	0.165194841
70_3	3	21	27.12201917	27.12881914	0.00679997	0.00679997
71_3	3	21	26.80330686	26.98742492	0.184118061	0.184118061
72_3	3	21	27.83932718	27.93098976	0.091662579	0.091662579
73_3	3	21	27.22983537	27.23034672	0.000511356	0.000511356
74_3	3	21	27.68372228	27.63743969	-0.04628259	0.04628259
75_3	3	21	29.83546861	29.84528413	0.009815514	0.009815514
76_3	3	21	29.97573961	29.99349354	0.017753933	0.017753933
77_3	3	21	30.56349182	30.55438822	-0.009103601	0.009103601

Table S3_3a: Log10 of LR's values computed by EuroForMix v.3.4.0 for mixtures with two and three estimated contributors when the analytical threshold value was varied.

Pair of samples	Number of estimated contributors	EuroForMix v.3.4.0				
		Number of markers analyzed	Log ₁₀ of LR values computed using a threshold of ...		Difference	Absolute value of the difference
			100 (Default)	150		
1_2	2	21	7.927961408	9.333732472	1.40577106	1.405771064
2_2	2	21	8.444753876	7.374772463	-1.0699814	1.069981413
3_2	2	21	14.14865573	14.88141782	0.73276209	0.732762087
4_2	2	21	18.9162635	17.34663741	-1.5696261	1.569626096
5_2	2	21	20.55099707	20.18573061	-0.3652665	0.36526646
6_2	2	21	23.43326278	23.31495139	-0.1183114	0.118311387
7_2	2	21	18.15829858	17.77241002	-0.3858886	0.385888556
8_2	2	21	24.34936888	24.19049519	-0.1588737	0.158873696
9_2	2	21	16.58992434	16.30697388	-0.2829505	0.282950455
10_2	2	21	20.71446385	21.38638848	0.67192463	0.671924631
11_2	2	21	26.69124692	26.67026878	-0.0209781	0.020978145
12_2	2	21	26.68903622	27.03498151	0.34594529	0.34594529
13_2	2	21	16.54164571	16.59228604	0.05064034	0.050640336
14_2	2	21	8.572155479	7.554283285	-1.0178722	1.017872194
15_2	2	21	12.34987557	12.34138942	-0.0084861	0.008486143
16_2	2	21	10.149093	8.33628347	-1.8128095	1.812809528
17_2	2	21	14.25309526	14.26596963	0.01287436	0.012874362
18_2	2	21	19.03621211	19.07633297	0.04012086	0.04012086
19_2	2	21	16.64183306	14.90962722	-1.7322058	1.732205834
20_2	2	21	14.99417429	14.25741877	-0.7367555	0.736755521
21_2	2	21	15.80924136	15.82169973	0.01245837	0.012458373
22_2	2	21	16.32780352	16.01937404	-0.3084295	0.308429479
23_2	2	21	17.79821514	16.15826967	-1.6399455	1.639945469
24_2	2	21	17.9371393	17.8775075	-0.0596318	0.059631804
25_2	2	21	18.9144039	13.9395174	-4.9748865	4.974886497
26_2	2	21	17.35212396	17.26745984	-0.0846641	0.084664119
27_2	2	21	18.51560732	18.13528588	-0.3803214	0.380321437
28_2	2	21	17.18062122	16.88867759	-0.2919436	0.291943626
29_2	2	21	17.15547659	16.50271384	-0.6527627	0.652762744
30_2	2	21	16.37009274	15.81734093	-0.5527518	0.552751809
31_2	2	21	18.79376311	18.31417343	-0.4795897	0.479589681
32_2	2	21	15.61775466	15.48814335	-0.1296113	0.129611318
33_2	2	21	21.21566648	18.19027472	-3.0253918	3.025391754
34_2	2	21	18.71660024	18.41565675	-0.3009435	0.30094349
35_2	2	21	20.22702566	20.23929667	0.01227102	0.012271019
36_2	2	21	21.17895834	21.20080201	0.02184367	0.021843671
37_2	2	21	21.85987351	20.65797102	-1.2019025	1.201902481
38_2	2	21	20.11672317	20.275747	0.15902382	0.159023825
39_2	2	21	22.31054819	20.63097588	-1.6795723	1.679572307
40_2	2	21	20.89043433	20.70424653	-0.1861878	0.186187805
41_2	2	21	20.23824605	20.0711344	-0.1671116	0.167111642
42_2	2	21	22.96054738	21.08551791	-1.8750295	1.875029477
43_2	2	21	22.55825773	20.74099543	-1.8172623	1.817262307
44_2	2	21	23.05469213	21.20717456	-1.8475176	1.847517571
45_2	2	21	22.69983887	22.38551	-0.3143289	0.31432887
46_2	2	21	23.36030357	22.91863291	-0.4416707	0.441670669
47_2	2	21	21.71472691	21.93099784	0.21627093	0.21627093
48_2	2	21	24.71429572	25.06838065	0.35408493	0.354084933
49_2	2	21	24.37254244	23.60761185	-0.7649306	0.76493059
50_2	2	21	23.21906364	22.77754505	-0.4415186	0.441518588
51_2	2	21	26.18409114	26.15378469	-0.0303065	0.030306452
52_2	2	21	25.32352507	25.31082881	-0.0126963	0.012696262
53_2	2	21	25.18482274	24.8921885	-0.2926342	0.292634248
54_2	2	21	26.52762866	26.53855583	0.01092717	0.010927172

55_2	2	21	24.78198553	24.7147584	-0.0672271	0.067227131
56_2	2	21	26.41025337	26.4121473	0.00189392	0.001893924
57_2	2	21	26.71067461	26.06155719	-0.6491174	0.649117413
58_2	2	21	25.5550486	25.466535	-0.0885136	0.088513603
59_2	2	21	27.03166804	27.14810275	0.11643471	0.116434707
60_2	2	21	26.06180828	25.12981144	-0.9319968	0.931996841
61_2	2	21	25.7668327	23.51482191	-2.2520108	2.252010786
62_2	2	21	26.35344141	26.20750899	-0.1459324	0.145932422
63_2	2	21	27.04759424	27.08958752	0.04199328	0.041993284
64_2	2	21	26.19902215	26.07877769	-0.1202445	0.120244457
65_2	2	21	27.73663912	27.84636726	0.10972814	0.109728138
66_2	2	21	27.73905594	27.71435808	-0.0246979	0.024697864
67_2	2	21	27.99614279	28.00228935	0.00614656	0.006146558
68_2	2	21	28.64865102	28.6826002	0.03394918	0.033949183
69_2	2	21	28.12368857	28.12460135	0.00091278	0.000912784
70_2	2	21	28.2338101	27.90608847	-0.3277216	0.327721634
71_2	2	21	27.74995783	27.87864668	0.12868885	0.12868885
72_2	2	21	28.82434796	28.83886194	0.01451398	0.01451398
73_2	2	21	29.62237668	29.60920993	-0.0131667	0.013166749
74_2	2	21	29.60802844	29.53595175	-0.0720767	0.072076686
75_2	2	21	29.64309324	29.66182791	0.01873467	0.018734669
76_2	2	21	30.0321496	30.05168152	0.01953191	0.019531911
77_2	2	21	34.90544278	34.81869174	-0.086751	0.086751043
1_3	3	21	8.872919894	8.681772494	-0.1911474	0.191147399
2_3	3	21	17.31926253	18.11057865	0.79131612	0.79131612
3_3	3	21	10.59278355	10.99339848	0.40061493	0.400614928
4_3	3	21	17.54233394	17.83193683	0.2896029	0.289602897
5_3	3	21	21.73278146	22.28709142	0.55430996	0.554309959
6_3	3	21	28.39103356	28.31081072	-0.0802228	0.080222845
7_3	3	21	6.391799845	6.366180575	-0.0256193	0.02561927
8_3	3	21	9.247007109	8.759079027	-0.4879281	0.487928082
9_3	3	21	10.21651768	12.49265264	2.27613496	2.276134957
10_3	3	21	7.483251442	7.76897477	0.28572333	0.285723328
11_3	3	21	7.539132507	3.013431352	-4.5257012	4.525701155
12_3	3	21	13.52770824	13.68251911	0.15481087	0.154810875
13_3	3	21	6.780095197	7.405517047	0.62542185	0.625421851
14_3	3	21	12.49068576	12.6241789	0.13349313	0.133493134
15_3	3	21	14.05104261	14.15240795	0.10136534	0.10136534
16_3	3	21	11.15780554	7.932892172	-3.2249134	3.224913371
17_3	3	21	12.04271266	11.94608303	-0.0966296	0.096629621
18_3	3	21	12.06383477	12.16052886	0.09669409	0.096694086
19_3	3	21	11.13765989	11.12234692	-0.015313	0.015312964
20_3	3	21	11.21511709	10.25876064	-0.9563564	0.956356447
21_3	3	21	11.91963369	10.50044438	-1.4191893	1.419189304
22_3	3	21	12.23243108	11.50956555	-0.7228655	0.722865529
23_3	3	21	12.86991883	11.68868809	-1.1812307	1.181230736
24_3	3	21	12.35899002	11.85211446	-0.5068756	0.506875561
25_3	3	21	12.39830936	12.04614956	-0.3521598	0.352159803
26_3	3	21	11.6783552	11.14289216	-0.535463	0.535463043
27_3	3	21	12.80703129	11.72725755	-1.0797737	1.079773742
28_3	3	21	11.69715068	12.49591987	0.7987692	0.798769197
29_3	3	21	16.43255978	16.34658671	-0.0859731	0.085973077
30_3	3	21	12.14782066	12.14353651	-0.0042842	0.004284154
31_3	3	21	15.59595691	12.90543893	-2.690518	2.690517979
32_3	3	21	15.75992122	15.01038278	-0.7495384	0.749538438
33_3	3	21	16.01861026	16.02816492	0.00955466	0.009554661
34_3	3	21	17.40713589	17.50225351	0.09511762	0.09511762
35_3	3	21	17.50007835	17.04963923	-0.4504391	0.450439124
36_3	3	21	14.92987032	15.16238089	0.23251058	0.232510576
37_3	3	21	18.56675336	18.6245194	0.05776604	0.05776604
38_3	3	21	16.385562	15.52338658	-0.8621754	0.862175422
39_3	3	21	21.36963039	20.88274462	-0.4868858	0.486885776
40_3	3	21	18.83979338	18.79147568	-0.0483177	0.048317697

41_3	3	21	20.06189347	20.32588885	0.26399538	0.26399538
42_3	3	21	20.42199991	20.12094519	-0.3010547	0.301054722
43_3	3	21	19.55340066	19.44229899	-0.1111017	0.111101668
44_3	3	21	19.84559545	19.74318174	-0.1024137	0.102413705
45_3	3	21	20.45106198	20.25478499	-0.196277	0.196276996
46_3	3	21	21.92992701	21.92747139	-0.0024556	0.002455614
47_3	3	21	20.2905043	19.73891978	-0.5515845	0.551584519
48_3	3	21	21.156322	21.09584379	-0.0604782	0.060478217
49_3	3	21	24.42974057	23.96311576	-0.4666248	0.46662481
50_3	3	21	21.36980017	19.89171537	-1.4780848	1.478084797
51_3	3	21	20.93037263	20.53160767	-0.398765	0.398764959
52_3	3	21	21.28426784	21.20427314	-0.0799947	0.079994693
53_3	3	21	23.92602752	23.81014779	-0.1158797	0.115879727
54_3	3	21	20.65922733	21.14182967	0.48260233	0.482602333
55_3	3	21	21.65328522	20.46952492	-1.1837603	1.1837603
56_3	3	21	22.53667674	22.46824743	-0.0684293	0.068429309
57_3	3	21	24.29020457	23.99676265	-0.2934419	0.293441921
58_3	3	21	24.1343337	24.379163	0.2448293	0.244829303
59_3	3	21	23.59821827	23.78268288	0.18446462	0.184464616
60_3	3	21	22.93233479	22.23334612	-0.6989887	0.698988669
61_3	3	21	24.6872103	24.49053834	-0.196672	0.196671956
62_3	3	21	24.83821011	24.99416136	0.15595125	0.155951247
63_3	3	21	24.32956161	24.30675146	-0.0228102	0.022810155
64_3	3	21	23.67140894	24.01607623	0.34466729	0.344667289
65_3	3	21	26.94599906	26.92804868	-0.0179504	0.017950382
66_3	3	21	25.77126729	25.33371063	-0.4375567	0.437556663
67_3	3	21	27.3692497	27.33729089	-0.0319588	0.031958806
68_3	3	21	24.10855207	23.55555228	-0.5529998	0.552999782
69_3	3	21	26.53473447	26.51646269	-0.0182718	0.018271778
70_3	3	21	26.57844092	26.48252981	-0.0959111	0.095911103
71_3	3	21	26.97449615	24.80719035	-2.1673058	2.167305799
72_3	3	21	25.18578817	25.0707621	-0.1150261	0.115026067
73_3	3	21	29.61734003	29.60997399	-0.007366	0.007366036
74_3	3	21	28.59005293	28.16198567	-0.4280673	0.428067259
75_3	3	21	29.63260974	29.62791415	-0.0046956	0.004695591
76_3	3	21	31.02969786	30.68130313	-0.3483947	0.348394731
77_3	3	21	32.34398235	32.38632201	0.04233966	0.042339663

Table S3_3b: Log10 of LR's values computed by STRmix v.2.7 for mixtures with two and three estimated contributors when the analytical threshold value was varied.

Pair of samples	Number of estimated contributors	STRmix v.2.7				
		Number of markers analyzed	Log ₁₀ of LR values computed using a threshold of ...		Difference	Absolute value of the difference
			100 (Default)	150		
1_2	2	18	14.86573156	15.87767363	1.01194207	1.011942075
2_2	2	20	9.460010315	9.468096135	0.00808582	0.008085821
3_2	2	20	17.7093463	17.72229656	0.01295026	0.01295026
4_2	2	20	22.22287248	22.32506389	0.10219142	0.102191416
5_2	2	20	21.01510816	20.83269409	-0.1824141	0.182414074
6_2	2	20	24.67930977	24.74336079	0.06405103	0.064051026
7_2	2	20	25.57216268	25.22240429	-0.3497584	0.349758392
8_2	2	20	14.74652522	14.57839851	-0.1681267	0.168126717
9_2	2	20	22.00462227	21.7882886	-0.2163337	0.216333667
10_2	2	20	23.15624619	23.07903646	-0.0772097	0.077209733
11_2	2	20	26.0080896	26.02633531	0.0182457	0.018245705
12_2	2	20	24.9151095	24.86593267	-0.0491768	0.049176837
13_2	2	21	9.579783597	3.197501096	-6.3822825	6.3822825
14_2	2	21	1.697438591	1.564890845	-0.1325477	0.132547746
15_2	2	21	11.91505142	12.38521291	0.47016149	0.470161491
16_2	2	21	13.55885251	12.33116401	-1.2276885	1.227688504
17_2	2	21	13.5918323	13.96610417	0.37427188	0.374271875
18_2	2	21	14.9324484	15.65432096	0.72187257	0.721872565
19_2	2	21	14.92691324	15.12804367	0.20113043	0.201130433
20_2	2	21	16.01745073	16.19010749	0.17265676	0.172656759
21_2	2	21	17.2230804	17.3211633	0.0980829	0.098082899
22_2	2	21	19.60045012	19.56099399	-0.0394561	0.039456132
23_2	2	21	19.90236737	19.92155643	0.01918906	0.019189056
24_2	2	21	17.27443497	16.86864444	-0.4057905	0.405790532
25_2	2	21	20.44515255	20.36692701	-0.0782255	0.078225545
26_2	2	21	17.09018749	17.02873388	-0.0614536	0.061453608
27_2	2	21	16.89389486	16.0852192	-0.8086757	0.808675658
28_2	2	21	17.74497324	17.63003103	-0.1149422	0.114942205
29_2	2	21	19.93807419	18.55299655	-1.3850776	1.385077645
30_2	2	21	19.99297303	19.97871906	-0.014254	0.014253975
31_2	2	21	17.76574304	18.04618289	0.28043985	0.280439849
32_2	2	21	20.17897695	21.84511045	1.6661335	1.666133501
33_2	2	21	21.71560227	21.34519723	-0.370405	0.370405039
34_2	2	21	21.34805161	21.40423487	0.05618325	0.056183254
35_2	2	21	18.98762605	18.86479577	-0.1228303	0.12283028
36_2	2	21	20.68670775	19.97044916	-0.7162586	0.716258592
37_2	2	21	22.74750503	22.77102128	0.02351625	0.023516252
38_2	2	21	22.39828731	22.24283947	-0.1554478	0.155447837
39_2	2	21	23.99390334	23.98075762	-0.0131457	0.013145726
40_2	2	21	22.05068903	22.0191994	-0.0314896	0.031489627
41_2	2	21	20.04032538	19.95235648	-0.0879689	0.087968902
42_2	2	21	23.45151012	23.78122385	0.32971373	0.329713728
43_2	2	21	23.08346679	23.58530278	0.50183599	0.50183599
44_2	2	21	23.99857317	23.96596798	-0.0326052	0.032605193
45_2	2	21	20.61290036	20.68532963	0.07242928	0.072429276
46_2	2	21	25.42334419	25.35504903	-0.0682952	0.068295162
47_2	2	21	23.59149868	23.40036527	-0.1911334	0.191133411
48_2	2	21	23.66829271	23.52062762	-0.1476651	0.147665092
49_2	2	21	26.08564729	25.96871171	-0.1169356	0.116935578
50_2	2	21	21.23286911	21.21343806	-0.019431	0.019431043
51_2	2	21	24.59222118	26.43600354	1.84378235	1.843782351
52_2	2	21	26.45295118	26.48174352	0.02879234	0.028792345
53_2	2	21	26.31272728	26.37080906	0.05808177	0.058081773
54_2	2	21	26.2774029	26.28000895	0.00260605	0.002606049

55_2	2	21	27.00732095	26.96240722	-0.0449137	0.044913731
56_2	2	21	26.89873067	26.93266144	0.03393078	0.03393078
57_2	2	21	27.6754392	27.52023454	-0.1552047	0.155204659
58_2	2	21	26.0051376	25.88107066	-0.1240669	0.124066932
59_2	2	21	26.47846549	26.40119368	-0.0772718	0.077271801
60_2	2	21	26.32982484	26.62650461	0.29667976	0.296679764
61_2	2	21	26.98117507	26.94015775	-0.0410173	0.041017322
62_2	2	21	26.51160265	26.57382276	0.06222011	0.062220107
63_2	2	21	28.52791332	28.53770579	0.00979246	0.009792462
64_2	2	21	27.14075937	27.14382639	0.00306702	0.00306702
65_2	2	21	25.7616347	26.0465732	0.28493849	0.284938494
66_2	2	21	26.57989787	26.57083471	-0.0090632	0.009063163
67_2	2	21	28.38530234	28.39885974	0.0135574	0.013557399
68_2	2	21	28.4363694	28.4363376	-3.18E-05	3.18025E-05
69_2	2	21	28.98643581	29.01127433	0.02483852	0.024838519
70_2	2	21	29.46737129	29.36385632	-0.103515	0.103514968
71_2	2	21	27.61234934	27.64237548	0.03002613	0.030026131
72_2	2	21	29.46802223	29.46499598	-0.0030262	0.003026248
73_2	2	21	30.03726709	30.0757294	0.03846231	0.038462305
74_2	2	21	30.38623135	30.37935991	-0.0068714	0.006871436
75_2	2	21	30.48572143	30.58943574	0.10371431	0.103714309
76_2	2	21	29.72115084	30.22133598	0.50018514	0.500185136
77_2	2	21	33.0541149	33.13776542	0.08365052	0.08365052
1_3	3	19	9.190303678	9.233173855	0.04287018	0.042870177
2_3	3	19	18.18121455	18.48559367	0.30437913	0.304379126
3_3	3	20	15.46993977	15.52505777	0.055118	0.055117996
4_3	3	20	16.68244293	16.96904763	0.2866047	0.2866047
5_3	3	20	23.02069268	23.10458953	0.08389685	0.083896848
6_3	3	20	26.44237001	26.29801517	-0.1443548	0.14435484
7_3	3	20	7.249418507	6.342205479	-0.907213	0.907213028
8_3	3	21	7.815112658	6.921655268	-0.8934574	0.893457391
9_3	3	21	4.179781516	-6.95683428	-11.136616	11.1366158
10_3	3	21	5.095308861	5.279826582	0.18451772	0.18451772
11_3	3	21	8.857790677	8.839163683	-0.018627	0.018626994
12_3	3	21	1.521138084	3.489606966	1.96846888	1.968468883
13_3	3	21	11.8360328	11.74519194	-0.0908409	0.090840859
14_3	3	21	4.029099567	3.731757938	-0.2973416	0.297341629
15_3	3	21	1.440294971	1.939199654	0.49890468	0.498904683
16_3	3	21	10.68327224	10.5172223	-0.1660499	0.166049938
17_3	3	21	12.46670454	13.85080547	1.38410092	1.384100924
18_3	3	21	12.90096762	12.84825071	-0.0527169	0.052716909
19_3	3	21	7.773947265	7.324714477	-0.4492328	0.449232788
20_3	3	21	11.33231724	10.99712416	-0.3351931	0.335193089
21_3	3	21	12.36904879	11.61941684	-0.7496319	0.749631946
22_3	3	21	11.37604745	11.45594064	0.07989319	0.079893189
23_3	3	21	13.97110414	13.97799781	0.00689367	0.006893671
24_3	3	21	10.6792825	10.76306843	0.08378593	0.083785927
25_3	3	21	11.88918961	12.37219372	0.48300411	0.483004107
26_3	3	21	10.80632924	10.09558775	-0.7107415	0.71074149
27_3	3	21	14.83384168	14.46188853	-0.3719532	0.371953156
28_3	3	21	10.86185688	13.96444349	3.10258662	3.102586619
29_3	3	21	11.88286592	11.09617978	-0.7866861	0.786686135
30_3	3	21	16.17400163	15.87409316	-0.2999085	0.29990847
31_3	3	21	15.37417991	15.42781057	0.05363066	0.053630663
32_3	3	21	16.41276285	15.60817289	-0.80459	0.80458996
33_3	3	21	17.22370356	17.41493994	0.19123638	0.191236376
34_3	3	21	16.06777736	15.90361083	-0.1641665	0.164166531
35_3	3	21	20.33663982	20.37597437	0.03933455	0.039334548
36_3	3	21	20.85253489	20.31079948	-0.5417354	0.541735403
37_3	3	21	17.83072978	17.68234367	-0.1483861	0.148386107
38_3	3	21	20.40401223	20.14838669	-0.2556255	0.255625539
39_3	3	21	18.69671451	19.57924611	0.8825316	0.882531598
40_3	3	21	17.62583699	18.09289601	0.46705902	0.467059019

41_3	3	21	19.6427612	19.78642487	0.14366367	0.143663666
42_3	3	21	18.75797268	19.00902574	0.25105306	0.251053062
43_3	3	21	20.13580028	21.96517817	1.82937789	1.829377891
44_3	3	21	17.64837953	17.79575492	0.14737539	0.147375393
45_3	3	21	21.05880549	21.21042554	0.15162005	0.151620055
46_3	3	21	13.82714302	12.06235632	-1.7647867	1.764786701
47_3	3	21	20.22282048	19.95199769	-0.2708228	0.270822793
48_3	3	21	20.74573042	20.61369386	-0.1320366	0.132036563
49_3	3	21	17.0510367	26.28618692	9.23515022	9.235150222
50_3	3	21	23.67368429	23.48056743	-0.1931169	0.193116865
51_3	3	21	22.32234336	23.46881977	1.14647641	1.146476414
52_3	3	21	24.48280215	24.53839777	0.05559562	0.055595621
53_3	3	21	23.79169065	22.53030217	-1.2613885	1.261388475
54_3	3	21	24.65049208	24.44053127	-0.2099608	0.209960813
55_3	3	21	23.80110859	23.53239125	-0.2687173	0.268717338
56_3	3	21	24.23641129	24.32580291	0.08939162	0.089391621
57_3	3	21	23.71083636	23.83313436	0.122298	0.122297996
58_3	3	21	24.54501006	24.7554861	0.21047604	0.210476041
59_3	3	21	26.27413475	26.23862311	-0.0355116	0.035511642
60_3	3	21	27.16888034	27.22847735	0.05959701	0.059597012
61_3	3	21	25.50821988	25.65056977	0.14234989	0.14234989
62_3	3	21	24.90247068	27.24711364	2.34464297	2.344642966
63_3	3	21	24.90646973	25.81557775	0.90910802	0.909108021
64_3	3	21	23.40599484	23.69623404	0.29023919	0.290239194
65_3	3	21	26.69321675	26.60337175	-0.089845	0.089845
66_3	3	21	26.69321675	26.60337175	-0.089845	0.089845
67_3	3	21	27.75802575	27.78469573	0.02666997	0.026669975
68_3	3	21	28.28053284	28.20371264	-0.0768202	0.076820204
69_3	3	21	25.88939696	25.94554244	0.05614548	0.056145482
70_3	3	21	27.12201917	27.1735359	0.05151672	0.051516723
71_3	3	21	26.80330686	26.35034495	-0.4529619	0.452961914
72_3	3	21	27.83932718	28.21603388	0.3767067	0.376706697
73_3	3	21	27.22983537	27.22750106	-0.0023343	0.002334302
74_3	3	21	27.68372228	27.45391432	-0.229808	0.229807961
75_3	3	21	29.83546861	29.83405811	-0.0014105	0.001410502
76_3	3	21	29.97573961	29.96850163	-0.007238	0.007237984
77_3	3	21	30.56349182	30.5878146	0.02432277	0.024322775

Table S3_4: Log₁₀ of LR's values computed by EuroForMix v.3.4.0 for mixtures with two and three estimated contributors, when considering and not considering the stutter peaks.

Pair of samples	Number of estimated contributors	Number of markers analyzed	Log ₁₀ of LR values computed ...		Difference	Absolute value of the difference
			With stutters (Default)	Without stutters		
1_2	2	21	7.927961408	6.322077446	-1.605884	1.60588396
2_2	2	21	8.444753876	9.087307762	0.64255389	0.64255389
3_2	2	21	14.14865573	14.8218184	0.67316267	0.67316267
4_2	2	21	18.9162635	20.82527379	1.90901028	1.90901028
5_2	2	21	20.55099707	20.63042737	0.07943029	0.07943029
6_2	2	21	23.43326278	23.58272974	0.14946696	0.14946696
7_2	2	21	18.15829858	23.36978007	5.21148149	5.21148149
8_2	2	21	24.34936888	23.13225627	-1.2171126	1.21711262
9_2	2	21	16.58992434	14.86518782	-1.7247365	1.72473652
10_2	2	21	20.71446385	18.72423323	-1.9902306	1.99023062
11_2	2	21	26.69124692	26.78330997	0.09206305	0.09206305
12_2	2	21	26.68903622	27.16128241	0.4722462	0.4722462
13_2	2	21	16.54164571	16.5100119	-0.0316338	0.0316338
14_2	2	21	8.572155479	9.339852338	0.76769686	0.76769686
15_2	2	21	12.34987557	11.56226056	-0.787615	0.78761501
16_2	2	21	10.149093	15.98891894	5.83982595	5.83982595
17_2	2	21	14.25309526	14.2479281	-0.0051672	0.00516716
18_2	2	21	19.03621211	18.15391085	-0.8823013	0.88230126
19_2	2	21	16.64183306	16.28998548	-0.3518476	0.35184757
20_2	2	21	14.99417429	14.4915568	-0.5026175	0.50261748
21_2	2	21	15.80924136	15.81604827	0.00680691	0.00680691
22_2	2	21	16.32780352	15.98555045	-0.3422531	0.34225306
23_2	2	21	17.79821514	15.96083131	-1.8373838	1.83738383
24_2	2	21	17.9371393	17.43422819	-0.5029111	0.50291111
25_2	2	21	18.9144039	17.33423	-1.5801739	1.5801739
26_2	2	21	17.35212396	17.52859163	0.17646767	0.17646767
27_2	2	21	18.51560732	18.6821553	0.16654798	0.16654798
28_2	2	21	17.18062122	17.04634394	-0.1342773	0.13427728
29_2	2	21	17.15547659	19.60138734	2.44591075	2.44591075
30_2	2	21	16.37009274	15.59077306	-0.7793197	0.77931968
31_2	2	21	18.79376311	18.16491119	-0.6288519	0.62885192
32_2	2	21	15.61775466	15.53606368	-0.081691	0.08169099
33_2	2	21	21.21566648	17.9244209	-3.2912456	3.29124557
34_2	2	21	18.71660024	18.13236952	-0.5842307	0.58423072
35_2	2	21	20.22702566	20.2215497	-0.005476	0.00547595
36_2	2	21	21.17895834	21.30881604	0.1298577	0.1298577
37_2	2	21	21.85987351	21.42447265	-0.4354009	0.43540085
38_2	2	21	20.11672317	19.13960181	-0.9771214	0.97712136
39_2	2	21	22.31054819	23.53705304	1.22650485	1.22650485
40_2	2	21	20.89043433	19.4145607	-1.4758736	1.47587363
41_2	2	21	20.23824605	20.16069167	-0.0775544	0.07755438
42_2	2	21	22.96054738	21.8710045	-1.0895429	1.08954288
43_2	2	21	22.55825773	20.28800779	-2.2702499	2.27024994
44_2	2	21	23.05469213	20.70910791	-2.3455842	2.34558422
45_2	2	21	22.69983887	22.19305551	-0.5067834	0.50678336
46_2	2	21	23.36030357	21.32317758	-2.037126	2.03712599
47_2	2	21	21.71472691	21.02540149	-0.6893254	0.68932542
48_2	2	21	24.71429572	24.10657125	-0.6077245	0.60772447
49_2	2	21	24.37254244	22.68654745	-1.685995	1.685995
50_2	2	21	23.21906364	22.6462288	-0.5728348	0.57283484
51_2	2	21	26.18409114	26.18876018	0.00466904	0.00466904
52_2	2	21	25.32352507	25.40771097	0.0841859	0.0841859
53_2	2	21	25.18482274	24.62873947	-0.5560833	0.55608327
54_2	2	21	26.52762866	26.42619019	-0.1014385	0.10143846
55_2	2	21	24.78198553	24.50790955	-0.274076	0.27407598

56_2	2	21	26.41025337	26.40615697	-0.0040964	0.0040964
57_2	2	21	26.71067461	26.41726614	-0.2934085	0.29340847
58_2	2	21	25.5550486	24.94181905	-0.6132296	0.61322956
59_2	2	21	27.03166804	25.54177316	-1.4898949	1.48989488
60_2	2	21	26.06180828	25.84370083	-0.2181075	0.21810746
61_2	2	21	25.7668327	23.82979056	-1.9370421	1.93704214
62_2	2	21	26.35344141	26.35188634	-0.0015551	0.00155507
63_2	2	21	27.04759424	26.86402689	-0.1835673	0.18356735
64_2	2	21	26.19902215	25.95405768	-0.2449645	0.24496447
65_2	2	21	27.73663912	28.12760393	0.39096481	0.39096481
66_2	2	21	27.73905594	27.84327683	0.10422088	0.10422088
67_2	2	21	27.99614279	28.0017184	0.00557561	0.00557561
68_2	2	21	28.64865102	28.39606589	-0.2525851	0.25258513
69_2	2	21	28.12368857	28.17854932	0.05486075	0.05486075
70_2	2	21	28.2338101	27.29786929	-0.9359408	0.93594081
71_2	2	21	27.74995783	27.49450243	-0.2554554	0.25545541
72_2	2	21	28.82434796	28.81955389	-0.0047941	0.00479407
73_2	2	21	29.62237668	29.63423548	0.01185881	0.01185881
74_2	2	21	29.60802844	28.98901497	-0.6190135	0.61901346
75_2	2	21	29.64309324	29.67067929	0.02758605	0.02758605
76_2	2	21	30.0321496	30.03984958	0.00769998	0.00769998
77_2	2	21	34.90544278	34.99006799	0.0846252	0.0846252
2_3	3	21	17.31926253	20.81424189	3.49497936	3.49497936
3_3	3	21	10.59278355	11.09870117	0.50591762	0.50591762
4_3	3	21	17.54233394	17.54599066	0.00365672	0.00365672
5_3	3	21	21.73278146	25.42419943	3.69141797	3.69141797
6_3	3	21	28.39103356	28.31156123	-0.0794723	0.07947234
7_3	3	21	6.391799845	6.375601478	-0.0161984	0.01619837
8_3	3	21	9.247007109	10.43477653	1.18776942	1.18776942
9_3	3	21	10.21651768	15.78309642	5.56657874	5.56657874
10_3	3	21	7.483251442	7.363014341	-0.1202371	0.1202371
11_3	3	21	7.539132507	7.454044286	-0.0850882	0.08508822
12_3	3	21	13.52770824	13.05153393	-0.4761743	0.47617431
13_3	3	21	6.780095197	7.343883547	0.56378835	0.56378835
14_3	3	21	12.49068576	12.2633082	-0.2273776	0.22737756
15_3	3	21	14.05104261	14.01045132	-0.0405913	0.04059129
16_3	3	21	11.15780554	10.58289284	-0.5749127	0.57491271
17_3	3	21	12.04271266	10.73228634	-1.3104263	1.31042632
18_3	3	21	12.06383477	11.81136153	-0.2524732	0.25247324
19_3	3	21	11.13765989	10.94350838	-0.1941515	0.19415151
20_3	3	21	11.21511709	10.47478922	-0.7403279	0.74032787
21_3	3	21	11.91963369	11.21431539	-0.7053183	0.70531829
22_3	3	21	12.23243108	12.76747496	0.53504388	0.53504388
23_3	3	21	12.86991883	12.80590658	-0.0640123	0.06401225
24_3	3	21	12.35899002	12.17798841	-0.1810016	0.18100162
25_3	3	21	12.39830936	12.2735811	-0.1247283	0.12472826
26_3	3	21	11.6783552	14.60761841	2.92926321	2.92926321
27_3	3	21	12.80703129	12.20068087	-0.6063504	0.60635042
28_3	3	21	11.69715068	12.03935726	0.34220658	0.34220658
29_3	3	21	16.43255978	16.38450828	-0.0480515	0.04805151
30_3	3	21	12.14782066	11.15500484	-0.9928158	0.99281583
31_3	3	21	15.59595691	15.67749064	0.08153373	0.08153373
32_3	3	21	15.75992122	14.66604036	-1.0938809	1.09388087
33_3	3	21	16.01861026	16.0228049	0.00419464	0.00419464
34_3	3	21	17.40713589	17.48071306	0.07357717	0.07357717
35_3	3	21	17.50007835	16.64478724	-0.8552911	0.85529112
36_3	3	21	14.92987032	14.32057176	-0.6092986	0.60929856
37_3	3	21	18.56675336	18.7793254	0.21257205	0.21257205
38_3	3	21	16.385562	14.85706637	-1.5284956	1.52849563
39_3	3	21	21.36963039	20.87523298	-0.4943974	0.49439741
40_3	3	21	18.83979338	18.95244682	0.11265345	0.11265345
41_3	3	21	20.06189347	16.09237061	-3.9695229	3.96952286
42_3	3	21	20.42199991	19.82994491	-0.592055	0.59205501

43_3	3	21	19.55340066	18.56729746	-0.9861032	0.9861032
44_3	3	21	19.84559545	17.69239207	-2.1532034	2.15320338
45_3	3	21	20.45106198	20.28548687	-0.1655751	0.16557511
46_3	3	21	21.92992701	21.83149881	-0.0984282	0.0984282
47_3	3	21	20.2905043	19.83974628	-0.450758	0.45075802
48_3	3	21	21.156322	20.68662337	-0.4696986	0.46969863
49_3	3	21	24.42974057	24.21827576	-0.2114648	0.2114648
50_3	3	21	21.36980017	19.77246743	-1.5973327	1.59733273
51_3	3	21	20.93037263	20.56100378	-0.3693689	0.36936885
52_3	3	21	21.28426784	21.02865795	-0.2556099	0.25560989
53_3	3	21	23.92602752	23.82808124	-0.0979463	0.09794628
54_3	3	21	20.65922733	19.30449848	-1.3547289	1.35472886
55_3	3	21	21.65328522	21.31257762	-0.3407076	0.3407076
56_3	3	21	22.53667674	22.95697726	0.42030052	0.42030052
57_3	3	21	24.29020457	24.03416327	-0.2560413	0.2560413
58_3	3	21	24.1343337	23.41497598	-0.7193577	0.71935772
59_3	3	21	23.59821827	24.0899052	0.49168694	0.49168694
60_3	3	21	22.93233479	22.42384957	-0.5084852	0.50848522
61_3	3	21	24.6872103	24.73100725	0.04379695	0.04379695
62_3	3	21	24.83821011	25.66444561	0.82623549	0.82623549
63_3	3	21	24.32956161	24.3439703	0.01440869	0.01440869
64_3	3	21	23.67140894	25.44917727	1.77776833	1.77776833
65_3	3	21	26.94599906	26.93460719	-0.0113919	0.01139187
66_3	3	21	25.77126729	25.56773237	-0.2035349	0.20353492
67_3	3	21	27.3692497	27.1693174	-0.1999323	0.1999323
68_3	3	21	24.10855207	23.59992361	-0.5086285	0.50862846
69_3	3	21	26.53473447	26.5498013	0.01506683	0.01506683
70_3	3	21	26.57844092	26.32864491	-0.249796	0.24979601
71_3	3	21	26.97449615	25.27525855	-1.6992376	1.6992376
72_3	3	21	25.18578817	25.15004385	-0.0357443	0.03574432
73_3	3	21	29.61734003	29.66376981	0.04642978	0.04642978
74_3	3	21	28.59005293	26.61142192	-1.978631	1.97863101
75_3	3	21	29.63260974	26.53571092	-3.0968988	3.09689883
76_3	3	21	31.02969786	29.82255206	-1.2071458	1.2071458
77_3	3	21	32.34398235	31.85684953	-0.4871328	0.48713282

SM4: LR values (per-marker and overall) computed by STRmix v.2.7 for sample 13_2 when the drop-in modeling parameter was varied

Table S4: LR values (per-marker and overall) computed by STRmix v.2.7 for sample 13_2 when the drop-in modeling parameter was varied - see Table 3 in the main text. Trials 1 to 4 were those obtained in the 1st to 4th attempt, respectively. Trial 1 was the one included and analyzed together with all the other data in the main manuscript. Trials 2 to 4 were only obtained after observing the discrepant result observed in Trial 1, after analyzing the complete set of data. For all the trials: Burn-in accepts per chain of 10,000 and Post burn-in accepts per chain of 50,000 were considered.

	Default condition: $\gamma = (1.0, 1.0)$				Varied condition: Uniform			
Markers	Trial 1	Trial 2	Trial 3	Trial 4	Trial 1	Trial 2	Trial 3	Trial 4
D3S1358	1.15E+01	1.03E+01	3.90E-01	1.26E+01	1.87E+01	1.45E+01	9.64E+00	1.60E+01
vWA	2.82E+00	2.10E+00	1.05E+00	1.29E+00	4.29E+00	1.54E+00	1.26E+00	3.97E+00
D16S539	3.11E+01	9.70E+00	5.74E+01	5.02E+01	8.49E-01	1.43E+01	2.24E+01	4.44E+01
CSF1PO	1.86E+00	2.83E+00	5.81E-01	1.33E+00	1.67E+00	9.43E-01	1.80E+00	1.12E+00
TPOX	2.12E+00	2.05E+00	1.85E+00	1.79E+00	4.18E+00	1.99E+00	1.56E+00	1.71E+00
D8S1179	9.26E-01	4.70E+00	2.45E+00	5.34E-01	1.24E-02	5.93E+00	2.58E+00	2.41E+00
D21S11	6.83E+00	8.65E+00	1.19E+01	6.72E+00	6.52E+00	9.81E+00	5.35E+00	1.11E+01
D18S51	1.67E+02	2.45E+02	2.76E+02	1.35E+02	5.49E+00	1.11E+02	2.01E+02	2.52E+02
D2S441	2.05E+00	8.83E-01	1.17E+00	7.74E-01	2.14E+00	3.11E+00	2.01E+00	2.09E+00
D19S433	1.33E+00	9.90E-01	1.87E+00	1.05E+00	9.07E-02	1.89E+00	1.89E+00	2.14E+00
TH01	7.21E+00	2.87E+00	1.20E+01	4.62E+00	8.53E-02	6.78E+00	6.04E+00	3.90E+00
FGA	1.08E+00	1.26E+00	7.25E-01	5.57E-01	4.18E+00	1.46E+00	8.11E-01	4.32E+00
D22S1045	2.44E+00	1.24E+00	1.59E-01	6.35E+00	1.13E-02	2.79E+00	3.41E+00	1.93E+01
D5S818	1.28E+00	6.73E-01	9.59E-01	4.07E-01	3.14E-02	8.36E-01	1.45E+00	1.75E+00
D13S317	1.73E+00	1.57E+00	2.26E+00	1.73E+00	1.58E+00	1.32E+00	1.58E+00	1.02E+00
D7S820	8.01E-01	5.22E-01	1.51E+00	3.30E-01	2.66E-02	2.21E-01	4.42E-01	2.68E-01
SE33	1.23E+00	1.25E+00	8.11E-01	7.74E-01	9.57E-02	7.79E-01	1.59E+00	5.41E-01
D10S1248	6.06E+00	2.29E+00	2.10E+00	1.30E+00	1.86E-02	9.35E-01	2.74E+00	1.69E+00
D1S1656	5.22E+00	1.26E+01	2.63E+01	4.91E+00	3.38E-01	1.96E+00	5.64E+00	7.09E+00
D12S391	1.93E+00	1.39E+00	6.15E-01	2.08E+00	2.13E-01	2.24E+00	2.80E+00	5.12E+00
D2S1338	1.31E-01	0	2.45E-02	4.11E-01	6.49E-01	4.77E-01	2.50E-01	2.41E-01
Overall	3.80E+09	0.00E+00	1.36E+06	1.23E+07	1.79E-08	2.35E+08	2.34E+09	2.02E+11

CHAPTER V | Navigating Population-level Coancestry in Forensic Mixture Analysis

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As the first and corresponding author of this study, I was involved in the research effort from its early conceptualization through to the final submitted manuscript. I contributed to the development of the research questions and co-designed the methodological framework. My role involved conducting software-based analysis of previously selected real casework data, defining the algebraic formulas used in simulated data development, interpreting the results, and drawing conclusions about the impact of considering or not the coancestry coefficient when quantifying the weight of the evidence. Lastly, I wrote the first version of the manuscript, synthesizing the key findings and aligning them with the broader context of the research objectives. In addition to writing, I collaborated with co-authors during the editing process and addressed comments from reviewers to ensure the manuscript met the standards of the journal.



OPEN Navigating population-level coancestry in forensic mixture analysis

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The genetic similarity of unrelated individuals of the same population can be measured through the coancestry coefficient, which quantifies the probability of a pair of alleles randomly chosen having a common ancestor. It is thus expected that the higher this coefficient, the lower the genetic variation between individuals of a population. This may weaken the evidence's weight in forensic genetics settings, whenever a person of interest cannot be excluded as a contributor to the evidence. Under simple assumptions, the impact of disregarding coancestry when quantifying the likelihood of an individual having contributed to a mixture evidence sample can be evaluated through an algebraic approach. This methodology becomes, however, unfeasible when considering all the information currently obtained from samples in forensic cases, whose analyses are software-dependent. Innovatively, this work assessed the impact of disregarding coancestry when quantifying the weight of the evidence in a modern population under the scope of identification problems involving mixtures. Methodology relied on both an algebraic approach (for simulated data) and an informatics one (for more complex real casework data). The results reinforce the importance of considering the proper population coancestry, whose impact is expected to be even higher for more isolated, substructured, or smaller populations.

Keywords Theta, Algebraic approach, Probabilistic genotyping software, Real casework data

The coancestry coefficient (also referred to as theta— θ) is a parameter that indicates the probability of a pair of alleles, each one randomly chosen from a pair of individuals at the same locus, being identical-by-descent (IBD, i.e., derived from the same common ancestor allele)¹. At a population level and considering a pair of individuals randomly chosen from the population, it can be interpreted as a measure of relatedness (shared ancestry) within a (sub)population. Any level of relatedness between individuals (or samples) must thus be evaluated against the background level of relatedness within the population. The smaller, more substructured, and more isolated the population is, the greater is the probability of the individuals sharing IBD alleles^{2,3}. IBD alleles are, by definition, identical by state, so an increase in the coancestry leads to a decrease in the genetic diversity existing between individuals. Although it has been established that coancestry values are locus-specific, a single estimation of this value for each subpopulation across all loci is generally used in forensic casework⁴. Estimates for the coancestry coefficient have been obtained for worldwide populations, and a value equal to 0.01 for short tandem repeat markers (STRs) and Caucasian (European and West Asian), African, and Hispanic populations has been considered appropriate^{4,5}.

The coancestry influences the population genotypic frequencies, and its proper estimation is crucial, among others, for the accurate quantification of the weight of the evidence in identification problems, such as has been previously shown for simulated or mocked single-source data^{4,6–8}. It is indeed generally accepted that disregarding the possibility of sharing IBD alleles between individuals due to the population's existing background relatedness can lead to an overstatement of the evidence when the person of interest cannot be excluded as a contributor

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to a single-source sample¹. Despite the expectation that a low practical impact would occur when the statistical assessment of evidence is overwhelmingly strong (either high for inclusion or low for exclusion), systematic studies on complex, multi-contributor samples are still absent.

The weight of the forensic DNA evidence is generally computed through a Likelihood Ratio (LR), which compares the probabilities of the genetic observations assuming two mutually exclusive hypotheses. In standard identification problems where an evidence sample and a person of interest (PoI) are identified, these hypotheses are H_1 = “The PoI is a contributor of the evidence sample” and, alternatively, H_2 = “The PoI is neither a contributor nor genetically related to any contributor of the evidence sample”^{9–11}. Algebraic formulations depend on both allele frequencies and coancestry values were established for single-source samples¹ and then extended for problem samples with more than one contributor (the so-called mixtures)².

In addition to coancestry, other parameters must be considered to deal with the high complexity associated with real casework sample mixtures that sometimes are vestigial. These parameters include the number of contributors (an estimated value since the true number is unknown), stutter peaks that may be confused with alleles of a minor contributor (or vice versa), or amplification stochastic effects from low-template DNA, such as heterozygotic imbalance, drop-in, and/or drop-out¹². Given this level of complexity, an algebraic approach is no longer feasible, making computational tools essential for data analysis. Consequently, probabilistic genotyping informatics tools have been developed to address this limitation with several laboratories worldwide currently transitioning to the statistical assessment of the evidence, as shown by a recent GHEP-ISFG working commission¹³. These tools consider different data from the electropherogram when quantifying the genetic evidence's weight^{14,15}. If only the qualitative information (observed alleles in the profile) is taken into account, the software is said to be based on a qualitative, or semi-continuous, model, as is the case of LRmix Studio¹⁶. On the other hand, if also quantitative information (height of the observed allele peaks) is considered, the software is said to be based on a quantitative, or continuous, model, as is the case of EuroForMix¹⁷ or STRmix¹⁸. Even though both consider the DNA quantity, these informatics tools enclose different statistical and computational architectures—the first is based on exact methods for either maximum likelihood estimation or Bayesian frameworks (user option), while the second considers a Bayesian approach based on stochastic Markov Chain Monte Carlo (MCMC) methods.

These tools calculate an LR to weigh the DNA evidence, using a variety of parameters^{9,11}. Specifically, the population coancestry parameter is handled differently by the distinct tools: some, like LRmix Studio¹⁶ and EuroForMix¹⁷, require users to define a specific value, while others, such as STRmix¹⁸, can model it using a probability distribution. The authors recently demonstrated the impact of varying analytical factors—such as drop-in, analytical detection threshold, and the presence/absence of stutters (and corresponding modelling) using the same set of real casework samples considered in this work^{19,20}.

The main goal of this work is to improve the state-of-the-art regarding the impact of the coancestry parameter in the quantification of the weight of evidence in identification problems, extending the analyses for mixtures. This extension is crucial, given that mixtures are now routinely analyzed in forensic settings. To reach this goal, pairs of samples were analyzed using two different approaches. Each pair consisted of a mixture (with two or three contributors, either simulated or estimated) and an associated reference. The first approach, an algebraic framework applied to simulated data, enabled an assessment of the coancestry impact on LR value for different genotypic configurations and allele frequencies, disregarding more complex parameters, such as DNA quantity, uncertainty in the number of contributors, or other biological and technical artifacts. Under the second approach, probabilistic genotyping systems were considered and applied to real casework data to analyze the more complex scenarios that require specialized software. Research on the behavior of real casework data is of utmost importance for forensic genetics practitioners due to their high complexity and unpredictability, which cannot be replicated with simulated or controlled data.

Materials and methods

To measure the impact that the population coancestry coefficient θ may have on the statistical evaluation of identification problems involving mixtures, two values were considered: (a) $\theta = 0$, representing a situation where its effect is ignored, and (b) $\theta = 0.01$, the value recommended by the second National Research Council report for general populations²¹ and also reported in several population studies^{1,2,4,6,12,22,23}. Pairs of samples composed of a mixture and a non-excluded PoI were considered. For this analysis, two different approaches were followed. For the first—see Sections “Algebraic approach with simulated data” and “Algebraic approach”, mixtures with two or three contributors were simulated, one of the contributors was treated as the PoI, and the corresponding allele configurations were analyzed using algebraic formulae. These formulae only consider allele population frequency and the coancestry parameter as variables, allowing for the evaluation of their impact on the quantification of the case-specific evidence, disregarding other confounding factors. In the second approach—see Sections “Informatics approach with real casework data” and “Informatics approach”, were considered real casework data: pairs of samples with estimated two- or three-contributor mixtures and a PoI that cannot be excluded as a contributor. These were analyzed with specialized software, taking into account other complexities, such as DNA quantity and stochastic effects, for which cases an algebraic approach would not be feasible. For both approaches and each mixture/PoI pair of samples, an LR was calculated assuming the hypotheses: “The PoI is a contributor of the mixture” and “The PoI is neither a contributor nor genetically related to any contributor of the mixture”, considering either $\theta = 0$ or $\theta = 0.01$. The LR values obtained for one θ value and another were then compared. Since the experimental design yields paired LRs that are not straightforwardly decomposable, ratios of LRs: $LR(\theta = 0)/LR(\theta = 0.01)$ were used as pragmatic summary statistics, complemented by appropriate statistical tests to ensure interpretability and mitigate potential numerical instability. Given the different magnitudes of the results being compared, and to enable meaningful comparisons within each analysis, different cut-off intervals were used to best accommodate and present the data range. Larger cut-off intervals ($\log_{10}$ scale) were applied

when analyzing data from the complete set of markers, where LR ratios displayed more discrepant values, whereas smaller intervals ($\log_2$ scale) were used for locus-specific analyses due to their narrower value ranges (other cut-off values are presented in Supplementary Material).

For all the computations, the allele frequencies from the National Institute of Standards and Technology database for the Caucasian population were used for 21 autosomal STR markers²⁴—see Table S1.

Algebraic approach with simulated data

Pairs of samples composed of a mixture with either two ($N = 1,000,000$) or three ($N = 1,000,000$) contributors and a profile of one of their contributors were randomly simulated and analyzed using R Studio v.2023.09.1 (available at <https://posit.co/download/rstudio-desktop/>), an integrated development environment for R²⁵, packages dplyr²⁶ and readr²⁷—code publicly available at the https://github.com/napinto/mix_coancestry. Simulations were performed assuming independent markers and marker Hardy–Weinberg equilibrium through the gene dropping strategy to ensure that more frequent alleles are more likely to be involved in the analysis. This resulted in the simulation of the complete set of possible genotypic configurations (six for mixtures with two contributors, and ten for those with three—see Table S2). Noteworthy, the allele frequencies used to simulate profiles correspond to a real, modern population database, which only provides reliable estimates of the mean allele frequencies. As a straightforward approach was adopted (i.e., assuming Hardy–Weinberg equilibrium), the variance associated with population substructure was not accounted for in the simulations. Nevertheless, the main practical effect of this simplification is a slight deviation from the genotype probabilities expected in a natural population, namely, a small excess of heterozygosity. This effect is expected to be minimal due to the very low level of coancestry associated with modern populations.

Algebraic formulae to compute locus-specific LR values were established for each possible genotypic combination between a mixture (with either two or three contributors) and a single-source profile of one of the contributors (either heterozygous or homozygous genotype at the locus in question)—see Table S2. These algebraic formulae depend on both the allele frequencies and the population coancestry coefficient.

For each simulated genotypic configuration of pairs of samples, two LR values were calculated based on the formulas presented in Table S2, considering both $\theta = 0$ and $\theta = 0.01$. Subsequently, the ratio between the two LR values was calculated and analyzed—see Fig. 1 for an example. This methodology was established to show the impact that this parameter may have on the weight of the evidence, resulting in the analysis of $1,000,000 \times 2$ (mixtures with either two or three contributors) $\times 21$ (number of STRs) $\times 2$ (LRs computed considering both $\theta = 0$ and $\theta = 0.01$) = 84,000,000 LRs.

Lastly, to understand whether the differences found per marker would have an impact on the overall LR value, a similar approach was taken combining the marker-specific simulated profiles, which resulted in the analysis of $1,000,000 \times 2$ (mixtures with either two or three contributors) $\times 2$ (LRs computed considering both $\theta = 0$ and $\theta = 0.01$) = 4,000,000 LRs, corresponding to genotypic profiles encompassing 21 STRs.

Summary statistics: mean, median, quartiles, and standard deviation were presented, interpreted, and discussed for the obtained $LR(\theta = 0)/LR(\theta = 0.01)$ results. The coefficient of determination R^2 , the square of the Pearson correlation coefficient, was also computed to assess how well the linear regression model explains the level of variation of the results depending on the heterozygosity (also known as expected heterozygosity, or gene diversity) and the number of alleles of the analyzed markers.

Informatics approach with real casework data

Resorting to evidence material from former cases of the Portuguese Scientific Police Laboratory, Judiciary Police (LPC-PJ), 152 irreversibly anonymized pairs of DNA samples comprising one mixture (with two or three estimated contributors) and a single-source profile were selected. In total, 75 sample pairs with mixtures with two, and 77 with mixtures with three estimated contributors were considered. Each single-source sample is related to the same forensic case of the corresponding DNA mixture, and some of them were also detected at the environmental scene. These single-source samples will be referred to as either “reference samples” or PoI

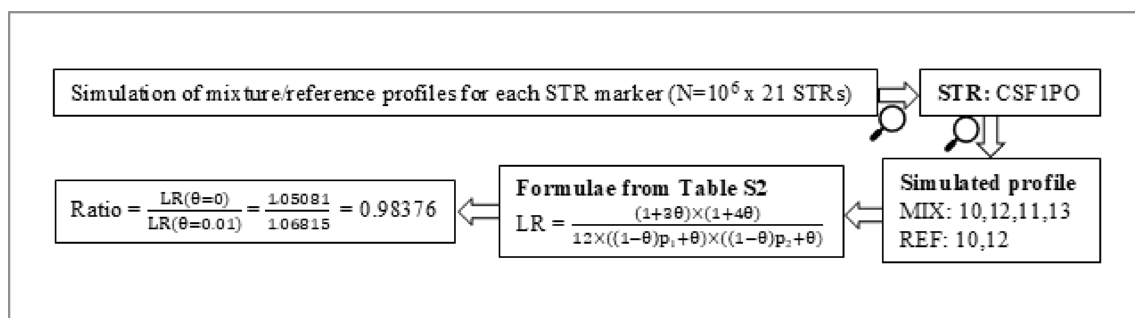


Fig. 1. Illustration of the analyses carried out in R. It represents a case example for the marker CSF1PO, considering a mixture (MIX) with two heterozygous contributors (four distinct alleles) and a heterozygous reference sample of a contributor (REF). The symbol ρ stands for “for example”, LR for “likelihood ratio”, θ for “coancestry coefficient”, and p_i for “frequency of allele i ” – see Table S1 for the database of allele frequencies considered. The theoretical framework for other possible genotypic combinations is presented in Table S2

throughout the paper. The biological samples were previously processed in the context of the respective casework, under manufacturer and internal protocols. All the methods were carried out following relevant guidelines and regulations, and the established genotypic profiles were similarly used as input for the three informatics tools. Proper ethics approval was obtained from the Committee for Ethical and Responsible Conduct of Research of i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal (5/CECRI/2022), regarding the statistical analyses of both environmental and reference genetic samples (fully and irreversibly anonymized, when applied).

Considering the genotypic information obtained for each anonymized pair of samples for a set of 21 STRs, the LR values were computed through both qualitative (LRmix Studio v.2.1.3¹⁶) and quantitative (EuroForMix v.3.4.0¹⁷ and STRmix™ v.2.7¹⁸) software tools. The software parameter values considered in the three tools are described in Table 1. For the STRmix™ results, default software parameters regarding MCMC were considered—Burn-in accepts per chain of 10,000 and Post burn-in accepts per chain of 50,000. In EuroForMix, the maximum likelihood estimation approach was employed as suggested by software developers for model comparison purposes^{28,29}. Except for the specific cases described next, only a single computation was performed for each software and pair of samples, considering ($\theta = 0.01$) and disregarding ($\theta = 0$) the population coancestry, a methodology that may have relevance in the interpretation of the results of STRmix™, which is based on a stochastic model. For comparison purposes, whenever an LR equal to zero was returned by a software for a specific marker, the marker was removed for the analyzed pair of samples and tool, similar to that carried out in a previous study³⁰. This happened for 17% and 9% of the cases with mixtures with two and three estimated contributors, respectively, when analyzed in STRmix™ due to either alleles from the reference sample being absent in the mixture or the MCMC sampling failing to converge and identify the true genotype combination, as observed in other studies^{23,31,32}. Even though these cases could potentially be resolved by increasing the MCMC parameters to allow for better model convergence, consistent settings were maintained across all computations to enable comparison between samples. In any case, a minimum of 18 markers per pair of samples were analyzed.

For each one of the three tools, an analysis was carried out through a comparison between the LR values computed per marker, considering both coancestry values for each pair of samples (in a total of 912 LR values), as performed for simulated data. Also as before, the differences between LRs were analyzed both on a per-marker basis and globally considering the complete set of markers. For visualization purposes, a $\log_{10}$ transformation was used to present the absolute LR results.

Other statistical analyses were applied to measure the paired LR differences; specifically, a Shapiro–Wilk test was used, followed by the analysis through the non-parametric Wilcoxon Signed-Rank test. Additionally, a chi-square goodness-of-fit test was also performed to reinforce the assessment of whether there was a significant difference in the number of cases leading to an increase or decrease of the LR values, assuming equality under the null hypothesis. Confidence intervals for mean, median, and standard deviation were established through bootstrapping (replicates = 10,000). All tests were performed using functions from R software v.4.1.2²⁵, and assuming a level of significance $\alpha = 0.05$.

Results
Algebraic approach with simulated data

A total of 2,000,000 pairs of samples were simulated for 21 STRs, each consisting of a mixture (with either two or three contributors) and a contributor profile. For each one of these genotypic configurations, $LR(\theta = 0) / LR(\theta = 0.01)$ was subsequently analyzed—see Table 2.

For a small proportion of the genotypic configurations simulated, the ratio between the LRs followed the opposite trend of the expected—that is, $LR(\theta = 0) < LR(\theta = 0.01)$. This occurred for both cases with mixtures with two (3.97%) and three contributors (4.77%), with a chi-square test supporting the trend of a higher number of these cases for the latter ($p < 1.00E-05$). Such counterintuitive results were overwhelmingly associated with heterozygous references in both mixture types. Specifically, 100% of these results in two-contributor mixtures involved 3–4 different alleles, while 98% in three-contributor mixtures involved 4–6 different alleles. Noteworthy, 42% of the cases of three contributors’ single allele, mixtures followed this trend, with no similar observations for the analogous genotypic configuration regarding two contributors’ mixtures. In any case, the majority of

Parameters	Values		
	LRmix Studio	EuroForMix	STRmix™
Number of contributors	Estimated		
Drop-in frequency	0.05		
Drop-in parameters’ distribution	N/A	Lambda (λ): 0.01	Gamma (γ): (1.0,1.0)
Drop-in cap	N/A	N/A	100
Dropout	0.1	^a	^a
Minimum allele frequency	0.001	0.001	^b
Threshold detection	N/A	100	100
Stutters	Without	With	With

Table 1. Values introduced in LRMix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7 for each software parameter. N/A: Not Applicable. ^aDropout is directly estimated through the peak height distribution. ^bPer locus, specified by the software, considering the default conditions (i.e., disregarding the population size).

Genotypic configuration	N	$\frac{1/2 \frac{LR(\theta=0)}{LR(\theta=0.01)}}{<1}$		$\frac{1 \frac{LR(\theta=0)}{LR(\theta=0.01)}}{\leq 2}$		$\frac{LR(\theta=0)}{LR(\theta=0.01)}$		> 2	Min	Max	Mean	Median	SD
		N	%	N	%	N	%						
2 Contributors													
Mix: A1,A2,A3,A4/Ref: A1,A2	5,523,787	447,176	8.10	4,766,921	86.30	309,690	5.61	0.965	62.924	1.338	1.103	1.080	
Mix: A1,A2,A3/Ref: A1,A1	1,702,979	0	0.00	1,693,264	99.43	9,715	0.57	1.003	39.022	1.142	1.089	0.214	
Mix: A1,A2,A3/Ref: A1,A2	8,531,734	385,780	4.52	7,930,077	92.95	215,877	2.53	0.990	67.439	1.194	1.080	0.646	
Mix: A1,A2/Ref: A1,A1	2,142,964	0	0.00	2,138,523	99.79	4,441	0.21	1.027	29.190	1.131	1.102	0.135	
Mix: A1,A2/Ref: A1,A2	2,737,917	0	0.00	2,710,734	99.01	27,183	0.99	1.019	26.160	1.140	1.078	0.402	
Mix: A1,A1/Ref: A1,A1	360,619	0	0.00	359,657	99.73	962	0.27	1.080	9.244	1.198	1.172	0.140	
Total	21,000,000	832,956	3.97	19,599,176	93.33	567,868	2.70	-	-	-	-	-	
3 Contributors													
Mix: A1,A2,A3,A4,A5,A6/Ref: A1,A2	1,168,699	56,630	4.85	1,006,411	86.11	105,658	9.04	0.929	60.561	1.518	1.192	1.445	
Mix: A1,A2,A3,A4,A5/Ref: A1,A1	277,785	573	0.21	264,714	95.29	12,498	4.50	0.988	206.982	1.317	1.196	1.431	
Mix: A1,A2,A3,A4,A5/Ref: A1,A2	3,882,190	367,567	9.47	3,324,543	85.64	190,080	4.90	0.957	61.472	1.304	1.092	0.953	
Mix: A1,A2,A3,A4/Ref: A1,A1	1,219,716	0	0.00	1,190,165	97.58	29,551	2.42	1.033	281.078	1.265	1.168	0.959	
Mix: A1,A2,A3,A4/Ref: A1,A2	6,478,476	555,129	8.57	5,751,101	88.77	172,246	2.66	0.982	65.699	1.185	1.067	0.651	
Mix: A1,A2,A3/Ref: A1,A1	1,860,821	0	0.00	1,859,293	99.92	1,528	0.08	1.014	15.630	1.095	1.070	0.103	
Mix: A1,A2,A3/Ref: A1,A2	4,491,700	0	0.00	4,437,409	98.79	54,291	1.21	1.002	60.738	1.124	1.059	0.433	
Mix: A1,A2/Ref: A1,A1	800,334	0	0.00	800,087	99.97	247	0.03	1.040	4.580	1.113	1.090	0.082	
Mix: A1,A2/Ref: A1,A2	770,775	0	0.00	768,121	99.66	2,654	0.34	1.030	11.563	1.109	1.073	0.254	
Mix: A1,A1/Ref: A1,A1	49,504	20,920	42.26	28,584	57.74	0	0.00	0.992	1.638	1.029	1.031	0.042	
Total	21,000,000	1,000,819	4.77	19,430,428	92.53	568,753	2.71	-	-	-	-	-	

Table 2. Genotypic configuration specific ratio between LR values computed disregarding ($\theta=0$) and considering coancestry ($\theta=0.01$), for simulated pairs of samples concerning a mixture (“Mix”) with two ($N=1,000,000$) and three ($N=1,000,000$) contributors and one mixture’s contributor (“Ref”). N = total number of simulations; for all cases, $LR(\theta=0) \neq LR(\theta=0.01)$. See Table S3 for more details, quartiles and intervals of variation.

the observed ratios followed the theoretical expectation of LRs being smaller when considering coancestry: $LR(\theta=0) > LR(\theta=0.01)$ —Table 2. Within this subset of cases and for all genotypic configurations, most of the ratios (57.74–99.97%) were found to be smaller than 2, i.e., considering a null coancestry resulted in a greater LR, but smaller than the double of the one obtained when considering it. Only for cases with mixtures with three contributors, ratios higher than 100 (i.e., $LR(\theta=0) > 100 \times LR(\theta=0.01)$) were observed, and for a relative residual number of cases (Table S3). These results confirm that the effect of coancestry is generally modest on a per-locus basis but varies depending on the genotypic profiles observed and the number of contributors involved.

When focusing on a marker-specific analysis, the counterintuitive, unexpected, trend: $LR(\theta=0) < LR(\theta=0.01)$, was observed for most of the markers (18/21) in varying frequencies (0.35–13.13%), for both mixtures with two or three contributors—see Table 3. As before, within this subset of cases, the ratio showed to be smaller than the double: $LR(\theta=0) < 1/2 \times LR(\theta=0.01)$. Marker D5S818, the one with the second-lowest heterozygosity (0.698), showed the greatest ratio following this trend for both mixtures with two and three contributors. A linear regression was fitted between the number of cases showing this counterintuitive trend: $LR(\theta=0) < LR(\theta=0.01)$ and both heterozygosity and number of alleles. A moderate to strong (negative) correlation exists for heterozygosity ($R^2 = 0.5503$ and $R^2 = 0.7189$), while a weak to moderate one was found for the number of alleles ($R^2 = 0.2206$ and $R^2 = 0.3074$), for mixtures with two and three contributors, respectively.

Such as before for genotypic configurations, for all the markers most ratios $LR(\theta=0)/LR(\theta=0.01)$ showed to be between one and two, with very weak to weak correlation with the analyzed measures regarding allele dispersion: heterozygosity ($R^2 = 0.1991$, and $R^2 = 0.1064$), and number of alleles ($R^2 = 0.0349$, and $R^2 = 0.043$), for mixtures with two and three contributors, respectively.

Among all markers analyzed, SE33 stands out for showing the highest differences— $LR(\theta=0) > 2 \times LR(\theta=0.01)$ was observed for 14.17% and 14.79% of the cases with mixtures with two and three contributors, respectively; a proportion higher than the one observed for other markers (0.07–6.67% and 0.07–5.53%, respectively). Noteworthy, SE33 also has the highest heterozygosity (0.948) and number of alleles (39)—Table 3. For ratios higher than two, i.e., where $LR(\theta=0) > 2 \times LR(\theta=0.01)$, a linear regression indicated a strong to very strong (positive) correlation with the number of alleles per marker, for both cases with mixtures with two ($R^2=0.8674$) and three ($R^2=0.8248$) contributors. In contrast, a weak (positive) correlation was found when marker heterozygosity was used as a predictor ($R^2 = 0.2496$ and 0.2397 , respectively). The maximum differences were also observed for SE33: 67.44 (for two) and 281.08 (for three contributors). Values of $LR(\theta=0) > 100 \times LR(\theta=0.01)$ were found for most markers, but only in mixtures

Markers	Ratio																	Hetero-zygosity	Number of alleles
	$\frac{1/2 \times LR(\theta=0)}{LR(\theta=0.01)} < 1$		$\frac{1 \times LR(\theta=0)}{LR(\theta=0.01)} \leq 2$		$\frac{LR(\theta=0)}{LR(\theta=0.01)} > 2$		Minimum		Maximum		Mean		Median		SD				
	NoC		NoC		NoC		NoC		NoC		NoC		NoC		NoC				
	2 (%)	3 (%)	2 (%)	3 (%)	2 (%)	3 (%)	2	3	2	3	2	3	2	3	2	3			
SE33	0.00	0.00	85.83	85.21	14.17	14.79	1.147	1.104	67.439	281.078	1.846	1.830	1.437	1.408	1.768	1.823	0.948	39	
D1S1656	0.00	0.00	97.69	97.55	2.31	2.45	1.049	1.010	19.754	65.931	1.270	1.264	1.187	1.169	0.462	0.490	0.899	15	
D12S391	0.00	0.00	97.86	97.79	2.14	2.21	1.045	1.006	35.569	57.777	1.291	1.284	1.165	1.157	0.582	0.587	0.891	16	
D12S1338	0.00	0.41	99.69	98.98	0.31	0.62	1.027	0.988	16.432	176.054	1.206	1.200	1.143	1.135	0.412	0.522	0.881	12	
D18S51	0.00	0.35	93.33	94.12	6.67	5.53	1.036	0.997	62.924	157.614	1.270	1.263	1.117	1.107	0.670	0.736	0.876	15	
FGA	0.00	1.41	96.97	95.55	3.03	3.04	1.014	0.976	35.256	142.744	1.249	1.240	1.117	1.111	0.604	0.617	0.859	14	
D21S11	2.43	3.07	93.96	93.18	3.62	3.75	0.995	0.958	63.176	164.421	1.291	1.285	1.105	1.102	0.955	1.017	0.829	16	
D7S820	2.23	4.93	96.65	93.85	1.13	1.23	0.998	0.960	26.033	154.867	1.144	1.135	1.067	1.064	0.456	0.490	0.816	9	
vWA	4.41	4.53	93.37	93.25	2.22	2.22	0.995	0.957	63.440	139.726	1.164	1.156	1.078	1.068	0.704	0.752	0.808	10	
D8S1179	2.16	3.57	96.32	94.90	1.52	1.54	1.000	0.962	21.478	137.396	1.165	1.158	1.091	1.087	0.455	0.468	0.807	10	
D3S1358	6.06	7.35	93.07	91.78	0.87	0.87	0.989	0.952	63.048	132.798	1.145	1.135	1.061	1.050	0.689	0.697	0.790	9	
D13S317	2.22	3.25	97.50	96.46	0.28	0.28	0.979	0.942	18.743	165.564	1.124	1.116	1.078	1.074	0.389	0.457	0.784	8	
D19S433	2.04	5.60	92.90	89.28	5.06	5.12	0.978	0.941	63.871	135.326	1.272	1.260	1.084	1.086	0.929	0.944	0.770	15	
D2S441	5.61	6.88	92.72	91.42	1.66	1.70	0.981	0.944	63.045	133.933	1.186	1.176	1.082	1.084	0.737	0.766	0.770	11	
TH01	3.67	8.02	94.12	89.79	2.21	2.20	0.982	0.945	62.924	132.658	1.125	1.114	1.059	1.050	0.575	0.595	0.765	8	
D16S539	1.89	7.09	98.04	92.84	0.07	0.07	0.975	0.938	4.442	4.078	1.104	1.095	1.065	1.060	0.130	0.127	0.760	7	
D10S1248	5.11	13.13	93.79	85.66	1.10	1.21	0.977	0.940	63.007	128.923	1.145	1.134	1.048	1.045	0.639	0.665	0.759	9	
D22S1045	11.57	6.53	85.88	90.96	2.55	2.50	0.969	0.932	22.178	13.686	1.125	1.115	1.052	1.045	0.281	0.276	0.722	8	
CSF1PO	11.14	12.58	87.17	85.98	1.69	1.43	0.971	0.935	6.375	13.622	1.104	1.094	1.052	1.042	0.253	0.253	0.719	7	
D5S818	12.41	5.48	84.25	91.23	3.33	3.29	0.965	0.929	35.305	128.735	1.147	1.134	1.040	1.036	0.545	0.544	0.698	9	
TPOX	10.36	5.90	88.80	93.27	0.84	0.83	0.970	0.934	64.959	126.444	1.128	1.115	1.043	1.030	0.701	0.686	0.640	8	

Table 3. Marker-specific ratio between LR values computed disregarding ($\theta=0$) and considering coancestry ($\theta=0.01$), for both simulated pairs of samples concerning mixtures with two ($N=1,000,000$) and three ($N=1,000,000$) contributors; for all cases, $LR(\theta=0) \neq LR(\theta=0.01)$. See Table S4 for more details, quartiles, and intervals of variation.

with three contributors and in very low numbers, so they do not appear in the rounded percentage shown in Table S4.

Notably, when the overall LR values were analyzed—that is, when considering the information provided by the complete set of 21 markers for $2 \times 1,000,000$ simulated pairs of samples, no observed ratio presented an opposite trend to the expected: $LR(\theta=0) < LR(\theta=0.01)$ —see Table 4. Most of the observed differences were between 10 and 100, namely, in 70.04% and 63.70% of the cases with mixtures with two and three contributors, respectively. Nevertheless, $LR(\theta=0) > 10,000 \times LR(\theta=0.01)$ were also found for both cases, and the biggest differences observed were $6.91E+05$ and $1.24E+05$ for mixtures with two and three contributors, respectively—Table 4.

Informatics approach with real casework data

The LR values for real casework samples were computed using the three informatics tools: LRmix Studio v.2.1.3¹⁶, EuroForMix v.3.4.0¹⁷, and STRmixTM v.2.7¹⁸—see Table S5. Each pair of samples analyzed comprises one mixture (with either two or three estimated contributors) and an associated reference sample. A set of 21 STRs was considered, and as before, two LR values were computed and compared, considering either $\theta=0.01$ or $\theta=0$.

As stated in the Materials and Methods section, a single run was performed by default for each tool and pair of samples, considering ($\theta=0.01$) and disregarding the population coancestry ($\theta=0$). This does not represent any limitation concerning the analyses of the LRmix Studio and EuroForMix results, as they are based on deterministic methods. Nevertheless, special attention needs to be taken regarding STRmix™, as the stochasticity of this tool architecture can affect the results, and the differences observed can be a consequence of MCMC algorithms failing to converge rather than a direct consequence of the parameter variation.

Table 5 provides a summarized example of the marker-specific analysis. These three markers were selected considering their relative heterozygosity—for the complete set of results, see Table S6. For each one of the analyzed markers, cases showing an opposite trend of the expected, i.e. $LR(\theta=0) < LR(\theta=0.01)$, were observed—see Table 5 and Table S6, for the complete set of markers. Contrary to what occurred for simulated data and algebraic formulae shown in the previous section, $LR(\theta=0) < 1/2 \times LR(\theta=0.01)$ were observed for most of the markers (all excepting D13S317 and D1S1656) for at least one of the software considered.

As previously observed for the simulated pairs of samples, the majority of locus-specific ratios: $LR(\theta=0)/LR(\theta=0.01)$, showed to be between one and two for all the markers (63.60–97.40%), indicating

Ratio between LR values	2 Contributors (N = 1,000,000)		3 Contributors (N = 1,000,000)	
	N	%	N	%
$\frac{LR(\theta=0)}{LR(\theta=0.01)} < 1$	0	0.00	0	0.00
$1 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 10^1$	210,298	21.03	290,564	29.06
$10^1 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 10^2$	700,449	70.04	637,005	63.70
$10^2 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 10^3$	85,229	8.52	69,404	6.94
$10^3 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 10^4$	3,918	0.39	2,956	0.30
$\frac{LR(\theta=0)}{LR(\theta=0.01)} > 10^4$	106	0.01	71	0.01
Minimum	2.850		2.136	
Maximum	6.91E+05		1.24E+05	
Mean	51.931		43.295	
Median	17.407		15.019	
Standard deviation	775.349		263.475	
Q1	10.773		9.283	
Q3	36.932		31,538	

Table 4. Ratio between overall LR values computed disregarding ($\theta = 0$) and considering the population coancestry ($\theta = 0.01$), for both cases with mixtures with two ($N = 1,000,000$) and three ($N = 1,000,000$) contributors simulated in R. N = total number of simulations; for all cases, $LR(\theta = 0) \neq LR(\theta = 0.01)$.

that disregarding the coancestry led to an increase in the marker LR value of at most the double. Regarding ratios higher than two: $LR(\theta = 0) > 2 \times LR(\theta = 0.01)$, at least one case was observed for all the markers, at least for one of the tools considered.

Focusing on the cases showing marker-specific striking differences, it is noteworthy that most of the cases with a ratio lower than $1/5$: $LR(\theta = 0) < 1/5 \times LR(\theta = 0.01)$, involved heterozygous references (21 out of 23), most of them exhibiting common alleles (30 out of the 46 observed alleles with frequency higher than 10%), and being STRmix™ the software with higher frequency of accumulated differences (16 out of 23)—see Table S7 for more details. For the cases for which ratios greater than 5 were observed: $LR(\theta = 0) > 5 \times LR(\theta = 0.01)$, also mostly heterozygous references were involved (78 out of 84 cases), but this time most of them showed rare alleles (98 out of 168 observed alleles with frequency lower than 10%), and being LRmix the software with higher frequency of accumulated differences (60 out of 84). Noteworthy, two pairs of samples: 2_70 for CSF1PO and 3_38 for SE33, contributed to both subsets, showing $LR(\theta = 0) > 5 \times LR(\theta = 0.01)$ when analyzed through LRmix and Euroformix, and $LR(\theta = 0) < 1/5 \times LR(\theta = 0.01)$ when using STRmix™—see Table S7. For some markers in the STRmix™ results, such as D2S441, FGA, and SE33, ratios greater than 100 (i.e., $LR(\theta = 0) > 100 \times LR(\theta = 0.01)$) were also observed. These cases all involved references with rare heterozygous genotypes: one allele not reported in the allele frequency database used (with its frequency estimated by the software) and the other with a frequency < 0.079 (see Table S7). To understand if the discrepancies observed per marker are reflected in the overall LR value after considering the information of the complete set of 21 markers, these values were computed for each pair of samples (see Table S5) and then compared. The discrepancies found between LR values are presented in Table 6 and Fig. 2.

Statistically significant differences between paired overall LR results, i.e. considering the joint analysis of 21 markers, were supported for all tools (LRmix Studio, EuroForMix, and STRmix™) for both mixtures with two and three estimated contributors, also supporting the expected general trend: $LR(\theta = 0) > LR(\theta = 0.01)$, (p -value $< 9.78E-12$ and $r < -0.7754$, Wilcoxon Signed-Rank test; p -value $< 1.28E-04$, Shapiro–Wilk Test). Concordantly, the chi-square goodness of fit test also supported that increasing the coancestry value resulted in a decrease in the LR values for a significant number of cases, for mixtures with both two and three contributors estimated, for all the informatics tools used ($p < 1.29E-13$), as demonstrated in Fig. 2.

For the quantitative software STRmix™, there were cases in which the opposite expected trend: $LR(\theta = 0) < LR(\theta = 0.01)$, was observed after analyzing the complete set of markers, namely in 1.33% ($N = 1$) and 7.80% ($N = 3$) of cases with mixtures with two and three estimated contributors, respectively. Within this subset of cases, pair 3_9 stands out as the only one whose $LR(\theta = 0) < 0$ (Fig. 2)—see S8 for more information on this case. Nevertheless, as previously mentioned, the stochasticity of the methods on which STRmix™ is based cannot be disregarded as the main cause of these differences observed in this subset of data and in this particular case. STRmix™ uses MCMC methods to assign a relative weight to the probability of the evidence given each possible genotype configuration, which, by definition, implies that the results of different runs will not be the same. These variations are expected to be minimal compared with the magnitude of the LR value and have an irrelevant practical impact. Nevertheless, in some situations, the MCMC algorithms may fail to converge, and a greater impact can potentially be observed in the LR value.

Markers (and respective heterozygosity)		Ratio												Median (confidence interval)						Standard deviation (confidence interval)					
		$\frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 1/2$				$\frac{1}{2} \leq \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 2$				$\frac{LR(\theta=0)}{LR(\theta=0.01)} > 2$												Maximum		Mean (confidence interval)	
		eNoC		2		3		eNoC		2		3		eNoC		2		3		eNoC		2		3	
		(%)	3	(%)	2	(%)	3	(%)	2	(%)	3	(%)	2	(%)	3	(%)	2	(%)	3	(%)	2	(%)	3	(%)	2
SE33 0.948262 (highest)	Lrmix Studio	1.33	0.00	0.00	1.30	3.90	89.33	90.91	9.33	7.79	0.016	0.802	15.318	12.294	1.713 (1.402; 2.194)	1.770 (1.416; 2.204)	1.343 (1.296; 1.445)	1.413 (1.369; 1.459)	1.789 (0.352; 2.907)	1.730 (0.270; 2.654)					
	EFM	0.00	0.00	1.33	3.90	90.67	88.31	8.00	7.79	0.944	0.579	16.469	11.665	1.768 (1.446; 2.267)	1.622 (1.419; 1.958)	1.369 (1.327; 1.455)	1.403 (1.323; 1.478)	1.896 (0.314; 3.104)	1.239 (0.301; 2.041)						
	STRmix	0.00	5.19	4.00	3.90	77.33	70.13	16.00	16.88	0.596	0.002	16.647	456.748	1.862 (1.506; 2.380)	7.813 (1.494; 20.161)	1.434 (1.323; 1.515)	1.401 (1.325; 1.582)	1.986 (0.55; 3.200)	52.563 (0.751; 89.805)						
D3S1358 0.79003	Lrmix Studio	1.33	0.00	8.00	14.29	86.67	84.42	4.00	1.30	0.315	0.867	10.960	2.106	1.405 (1.046; 1.864)	1.057 (1.033; 1.092)	1.044 (1.023; 1.051)	1.032 (1.019; 1.042)	1.797 (0.110; 2.625)	0.138 (0.036; 0.223)						
	EFM	0.00	0.00	4.00	15.58	92.00	83.12	4.00	1.30	0.983	0.837	11.402	2.289	1.473 (1.105; 1.941)	1.085 (1.051; 1.129)	1.070 (1.061; 1.091)	1.055 (1.030; 1.079)	1.842 (0.118; 2.732)	0.175 (0.075; 0.270)						
	STRmix	1.33	3.90	12.00	18.18	81.33	74.03	5.33	3.90	0.369	0.122	86.131	9.559	3.289 (1.111; 6.538)	1.190 (1.030; 1.445)	1.061 (1.053; 1.079)	1.065 (1.022; 1.102)	12.905 (0.321; 20.258)	1.014 (0.208; 1.693)						
TPOX 0.640449 (lowest)	Lrmix Studio	1.33	0.00	8.00	1.30	82.67	93.51	8.00	5.19	0.012	0.968	15.054	10.166	1.746 (1.254; 3.526)	1.459 (1.146; 1.878)	1.033 (1.022; 1.051)	1.045 (1.032; 1.062)	2.477 (1.156; 3.466)	1.714 (0.748; 2.427)						
	EFM	0.00	0.00	24.00	19.48	68.00	76.62	8.00	3.90	0.936	0.839	45.024	10.755	2.149 (1.248; 3.526)	1.374 (1.054; 1.787)	1.029 (1.015; 1.038)	1.037 (1.017; 1.047)	5.380 (1.173; 8.846)	1.634 (0.074; 2.421)						
	STRmix	0.00	0.00	20.00	23.38	77.33	75.32	2.67	1.30	0.883	0.788	2.862	2.297	1.116 (1.064; 1.184)	1.124 (1.078; 1.176)	1.053 (1.033; 1.066)	1.058 (1.044; 1.094)	0.271 (0.095; 0.416)	0.225 (0.142; 0.306)						

Table 5. Ratio between LR values computed disregarding coancestry ($\theta=0$) and otherwise ($\theta \neq 0$) using Lrmix Studio v.2.1.3, EuroForMix v.3.4.0 (EFM), and STRmix[™] v.2.7, for both cases with mixtures with two (N = 75) and three (N = 77) estimated contributors (eNoC); for all cases, $LR(\theta = 0) \neq LR(\theta \neq 0)$.

Ratio between LR values	2 Contributors (N = 75)			3 Contributors (N = 77)		
	LRmix Studio	EuroForMix	STRmix™	LRmix Studio	EuroForMix	STRmix™
$\frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 1/10^4$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
$1/10^4 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 1/10^3$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
$1/10^3 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
$1/10^2 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 1/10^1$	0.00%	0.00%	0.00%	0.00%	0.00%	2.60%
$1/10^1 < \frac{LR(\theta=0)}{LR(\theta=0.01)} < 1$	0.00%	0.00%	1.33%	0.00%	0.00%	3.90%
$1 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 10^1$	20.00%	16.00%	13.33%	38.96%	23.38%	18.18%
$10^1 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 10^2$	53.33%	50.67%	60.00%	44.16%	57.14%	51.95%
$10^2 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 10^3$	17.33%	21.33%	18.67%	14.29%	15.58%	11.69%
$10^3 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 10^4$	6.67%	8.00%	2.67%	2.60%	2.60%	6.49%
$\frac{LR(\theta=0)}{LR(\theta=0.01)} > 10^4$	2.67%	4.00%	4.00%	0.00%	1.30%	3.90%
Minimum (# pair)	2.197 (2_50)	2.529 (2_14)	0.550 (2_49)	2.021 (3_3)	3.166 (3_28)	1.29E-08 (3_9)
Maximum (# pair)	2.11E+05 (2_43)	3.46E+06 (2_43)	3.05E+06 (2_43)	8,474.550 (3_46)	14,865.107 (3_46)	5.81E+09 (3_49)
Mean (confidence interval)	5,595.653 (180.629; 13,868.917)	51,901.649 (336.297; 1.54E+05)	52,134.414 (170.509; 1.55E+05)	182.576 (41.474; 426.969)	294.627 (55.814; 724.393)	7.55E+07 (254.985; 2.26E+08)
Median (confidence interval)	22.583 (15.656; 41.111)	40.558 (23.603; 63.206)	32.889 (24.813; 46.765)	18.146 (11.438; 24.905)	23.631 (17.358; 34.991)	24.851 (14.717; 46.772)
Standard deviation (confidence interval)	32,443.110 (562.351; 50,778.723)	4.02E+05 (1,243.931; 6.84E+05)	3.63E+05 (524.644; 6.11E+05)	979.102 (75.250; 1,654.495)	1,709.929 (83.752; 2,897.943)	6.62E+08 (845.736; 1.13E+09)

Table 6. Ratio between LR values computed disregarding population coancestry ($\theta=0$) and otherwise ($\theta=0.01$) by LRMix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7, for both cases with mixtures with two (N=75) and three (N=77) estimated contributors, after considering the genotypic information of a set of 21 STRs markers; for all cases, $LR(\theta=0) \neq LR(\theta=0.01)$. Due to the relatively small sets of samples and lack of normality, mean, median, and standard deviation values were reached through bootstrapping (replicates = 10,000).

For the three informatics tools, the largest percentage of overall LR comparisons (21 STRs analyzed) showed results for which $10 < LR(\theta=0)/LR(\theta=0.01) < 100$, for both mixtures with two and three estimated contributors (from 44.16 to 60.0% of the analyzed cases). Nevertheless, cases for which $LR(\theta=0) < 10,000 \times LR(\theta=0.01)$ were also found. Within the cases with mixtures with two estimated contributors, all mixture-reference pairs that showed $LR(\theta=0) > 10,000 \times LR(\theta=0.01)$ in LRMix Studio (2.67%) coincide with the majority of those observed in EuroForMix (4.00%) and STRmix™ (4.00%). In cases with mixtures with three estimated contributors, large differences (either $LR(\theta=0) < 1/10,000 \times LR(\theta=0.01)$ or $LR(\theta=0) > 10,000 \times LR(\theta=0.01)$) were only found when using the quantitative tools—EuroForMix (1.30% for ratios $LR(\theta=0) > 10,000 \times LR(\theta=0.01)$) and STRmix™ (3.90% for ratios $LR(\theta=0) > 10,000 \times LR(\theta=0.01)$ and 1.30% for ratios $LR(\theta=0) < 1/10,000 \times LR(\theta=0.01)$).

For the majority of mixture-reference pairs for which overall $LR(\theta=0) > 10,000 \times LR(\theta=0.01)$, marker-specific LR ratios higher than five were observed. This was also verified when the inverse trend was observed: $LR(\theta=0) < 1/10,000 \times LR(\theta=0.01)$, since marker-specific ratios $LR(\theta=0) < 1/5 \times LR(\theta=0.01)$ were observed. This indicates that large differences in the overall LR are due to large differences in some markers rather than due to the accumulation of small differences in most of them. Interestingly, there are also pairs of samples with marker-specific results pointing to opposite directions $LR(\theta=0) < 1/5 \times LR(\theta=0.01)$ or $LR(\theta=0) > 5 \times LR(\theta=0.01)$ that do not present major differences in the overall LR value (i.e., ratios $LR(\theta=0) < 1/10,000 \times LR(\theta=0.01)$ or $LR(\theta=0) > 10,000 \times LR(\theta=0.01)$, when analyzing the complete set of markers). This indicates that great differences in specific markers can be compensated by the results in other markers showing an opposite trend. Indeed, in these cases, there was a balance between the trend observed for the different markers, that is, some ratios followed the expected trend, and others the opposite, resulting in a modest impact on the overall LR—see examples illustrated in Table 7. For example, comparing the results obtained for pairs 2_21 and 2_43 through LRMix Studio, it was observed that both had a similar number of markers with ratios higher than two (7 and 6, respectively), but the differences in overall LR values resulted in very distinct values (2.49 and 2.11E+05). This occurred due to a balance in the first pair, 2_21, regarding the existence of markers following the opposite trend (11/21) compared with the inexistence of those in pair 2_43.

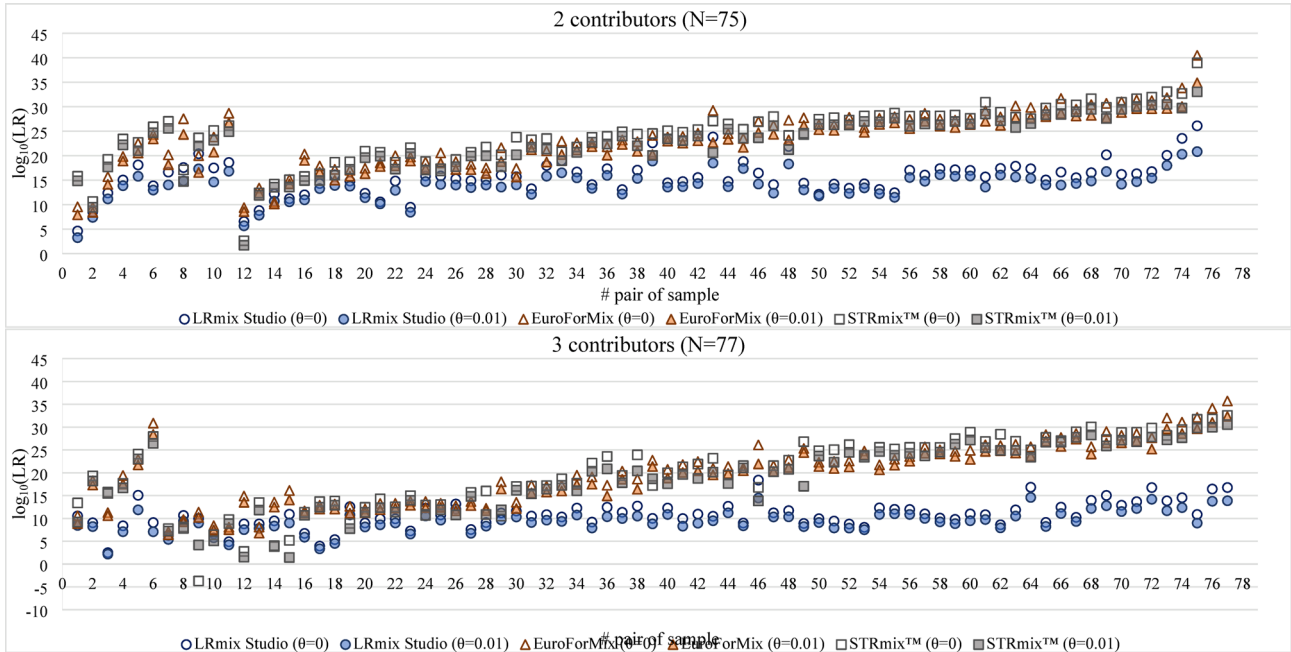


Fig. 2. Distribution of LR values ($\log_{10}$ scale) assigned by the three informatics tools—LRmix Studio v.2.1.3 (circles), EuroForMix v.3.4.0 (triangles), and STRmix™ v.2.7 (squares) for each pair of samples with two (N = 75) and three (N = 77) estimated contributors, disregarding ($\theta = 0$, symbols filled) and considering coancestry ($\theta = 0.01$, symbols unfilled).

Sample pairs		Ratio			
		Overall (21 markers)	Number of Markers for which		
			$\frac{LR(\theta=0)}{LR(\theta=0.01)} < 1$	$1 < \frac{LR(\theta=0)}{LR(\theta=0.01)} \leq 2$	$\frac{LR(\theta=0)}{LR(\theta=0.01)} > 2$
2_21	LRmix	2.49E+00	11	3	7
	EuroForMix	5.21E+00	4	17	0
	STRmix	6.38E+00	6	15	0
2_27	LRmix	2.43E+01	13	2	6
	EuroForMix	1.20E+01	3	17	1
	STRmix	5.48E+00	2	19	0
2_43	LRmix	2.11E+05	0	15	6
	EuroForMix	3.46E+06	1	14	6
	STRmix	3.05E+06	4	13	4
2_70	LRmix	9.87E+01	0	20	1
	EuroForMix	1.56E+02	0	20	1
	STRmix	2.84E+01	9	7	5
2_75	LRmix	1.89E+05	1	15	5
	EuroForMix	3.91E+05	0	16	5
	STRmix	8.21E+05	1	17	3

Table 7. Ratios between LR values (overall—complete set of markers—and by marker) computed disregarding ($\theta = 0$) and considering the population coancestry ($\theta = 0.01$), using LRMix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7, for sample pairs 2_21, 2_27, 2_43, 2_70, and 2_75; for all cases $LR(\theta = 0) \neq LR(\theta = 0.01)$.

Discussion

To evaluate the impact of the population coancestry in the quantification of the weight of the evidence involving a mixture (with either two or three contributors) and an associated reference sample, two different approaches were considered: i. simulated allele data (N = 2 × 1,000,000), analyzed through algebraic expressions, and ii. real casework data (N = 77 + 75, with two and three estimated contributors, respectively), analyzed through devoted software. The first, simplified approach focuses on the interplay between two population parameters: coancestry

level and allele distribution, allowing a controlled examination of their specific effects. Noteworthy, the allele frequencies used to simulate profiles correspond to a real, modern population database that inherently reflects any existing substructure level. The allele frequencies used for profile simulations are based on a real, modern population with unknown substructure, and genotypic data were simulated under the assumptions of linkage equilibrium and Hardy–Weinberg equilibrium. Although the practical impact is expected to be minimal due to the generally low coancestry levels in modern populations, some methodological considerations must be acknowledged in this regard. For example, incorporating deviations from the Hardy–Weinberg equilibrium in the simulations would increase homozygote frequencies, potentially influencing results. Indeed, this work demonstrates that the impact of the coancestry coefficient in the statistical assessment of identification problems is associated with the genotypic configurations of the analyzed individuals (but not limited to). Thus, the potential effects of mismodeling θ in complex population structure and genotype frequency models merit dedicated research, which can potentially be carried out by adapting and expanding the publicly available algorithms and scripts provided with this work.

The second approach adopts a holistic perspective, incorporating several additional parameters and computational and statistical frameworks that necessarily influence the results. These parameters include DNA quantity and quality in each mixture, uncertainty about the number of contributors, drop-in/drop-out events, stutter modeling, and/or detection thresholds, and are accommodated within different computational tools and software architectures. This approach has clear advantages and disadvantages: while reflecting the complexity of real casework, actually faced by forensic geneticists in the field, it precludes cause-and-effect conclusions due to the multitude of interacting variables.

If the reference sample cannot be excluded as a contributor to the mixture, it is expected an inverse correlation between coancestry and LR, i.e., as population coancestry increases, LR values are expected to decrease: $LR(\theta = 0) > LR(\theta = 0.01)$. This is due to an increasing population background level of relatedness implying a higher likelihood of alleles' sharing between unrelated individuals, as previously stated^{2,33}. In fact, for most cases analyzed, in both simulated and real casework data, the obtained results were congruent with these expectations for both mixtures with two and three contributors, but cases showing the opposite trend were also observed. Notably, when focusing on the different simulated genotypic configurations, 42.26% of the cases of the three-contributor configuration "Mix: A1, A1 / Ref: A1, A1", followed this counter intuitive trend: $LR(\theta = 0) < LR(\theta = 0.01)$. Most of the other cases following this trend, were observed in pairs of mixtures involving heterozygous contributors and mixtures with greater allele diversity. Interestingly, these are also the genotypic configurations showing higher differences in the opposite (and expected) direction, which supports the case-sensitive nature of the problem. On the other hand, when focusing on marker-specific data, both simulated and real, markers with lower heterozygosity showed a general trend for having a greater proportion of cases following the counterintuitive trend. However, only in the informatics approach were observed cases where $LR(\theta = 0) < 1/2 \times LR(\theta = 0.01)$: 13.38% of the total marker-specific cases analyzed through the three tools. Regarding the overall LR values obtained after the analysis of 21 markers, this counterintuitive increase of the LR value when considering coancestry was only observed for the informatic approach (4.61%, all for STRmix™ results), reaching a minimum value equal to 1.29E-08 (i.e., LR considering coancestry showed to be 10E+08 times greater than when coancestry was disregarded) for one case with a mixture with three contributors. Nevertheless, and again, the possibility of some of these STRmix™ differences being a result of the stochasticity of the method on which this tool is based should not be overlooked.

For most locus-specific cases, for both simulated and real data, the disregard of population coancestry led to an increase, no higher than the double of the LR values. On the other hand, considering overall LR values, after the analysis of the complete set of markers, the majority of cases presented $10 < LR(\theta = 0) / LR(\theta = 0.01) < 100$. This occurred for 70.04% and 63.70% of the simulated and 50.67–60.00% and 44.16–57.14% of the real data, for mixtures with two and three contributors, respectively. Although the differences observed are generally not of high magnitude, as shown in Fig. 2, special attention should be taken when dealing with lower LR values, as even small variations can shift an LR across interpretative thresholds—for instance, turning a result from inconclusive to conclusive—thereby carrying greater interpretative weight. Despite having a vestigial percentual expression in simulated simplified simulated data or having a low percentage in the set of real data, marker-specific cases for which $LR(\theta = 0) > 100 \times LR(\theta = 0.01)$ were observed. For overall computations, gathering information from the complete set of 21 markers, cases for which $LR(\theta = 0) > 10,000 \times LR(\theta = 0.01)$ were observed in both algebraic and informatics approaches. As observed in the marker-specific analysis, the percentage of these extreme results were lower for the simulated data (0.01% for both cases with mixtures with two and three contributors) than for real data analyses (2.67–4.00% and 0.00–3.90%, respectively). Also, for all the analyzed overall LRs, the greatest difference was observed for a pair of real casework samples composed of a mixture with three estimated contributors, again when analyzed with STRmix™ (5.81E+09; the maximum difference observed for simulated data equated to 6.91E+05 for a mixture with two contributors).

In any case, when rare alleles are involved (usually in markers with greater heterozygosity), it sustains and gives more weight to the possibility of IBD, accentuating these LR differences. Conversely, in an identical situation but involving a common allele, the impact of IBD is expected to be lower. This generally supports that, despite the differences encountered when locus-specific analyses are performed, those observed when considering the overall LR value, after analyzing the complete set of markers, might be less impacted than could be anticipated. Indeed, the overall LR value benefits from compensation between markers, as the observed differences often balance, preventing the much larger impact that would result if all marker differences followed a consistent trend for either increasing or decreasing.

While a θ value of 0.01 is commonly used for general outbred populations, populations with greater isolation and substructure often exhibit higher θ values, leading to more pronounced genetic similarity among individuals. Consequently, further accentuated LR differences for forensic mixture analyses are expected, especially when

rare alleles or complex profiles are involved. This highlights the need for accurate, population-specific estimation and incorporation of θ in probabilistic genotyping models.

Conclusions

The coancestry coefficient (θ) quantifies the relatedness or shared ancestry within a (sub)population, corresponding higher θ values to decreased genetic diversity between individuals. Due to this, properly estimating θ is crucial for accurately determining profile frequencies and, consequently, assessing the strength of DNA evidence in identification cases. This study extends previous analyses of coancestry impact in identification cases, from single-source samples to complex mixtures, by combining an algebraic framework applied to simulated data with an informatics approach analyzing real casework data. This dual strategy provides a comprehensive understanding of coancestry's effect on LR computations in challenging multi-contributor samples, which are increasingly common in forensic practice.

Results show that disregarding coancestry—even measured through a modest value such as $\theta = 0.01$, commonly applied to outbred modern populations—can lead to substantial differences in both locus-specific and overall LR values. Specifically, the number of cases for which the unexpected trend: $LR(\theta = 0) < LR(\theta = 0.01)$, was observed is far from negligible. Although modest effects at single loci are the most common, larger LR differences emerge in some markers and mixture complexities, demonstrating nonuniform coancestry impacts. These differences are expected to be even more pronounced in isolated, substructured, or smaller populations characterized by higher coancestry. Moreover, this work highlights the case-sensitive nature of coancestry effects, where genotype composition, allele frequency, mixture complexity, and number of contributors influence LR variability. When rare alleles are involved, coancestry adjustments amplify differences in LR estimates, whereas common alleles often mitigate these effects. Given these findings, forensic practitioners should carefully consider including coancestry adjustments in probabilistic genotyping, since ignoring this parameter risks over- or understating the strength of DNA evidence, potentially impacting judicial outcomes.

Data availability

All the statistics that supported the results presented in this study are provided in the Supplementary Material. Regarding the simulated data, the code developed to simulate and analyze pairwise genotypic profiles of mixtures with two and three contributors, and one of those contributors is available in https://github.com/napinto/mix_coancestry. On the other hand, concerning real casework data, the raw genotypic and haplotypic information analyzed are from former cases of the Portuguese Scientific Police Laboratory, Judiciary Police (LPC-PJ), and, for confidentiality reasons, their public release is not possible. In any case, specific data from specific analyzed markers (non-haplotypic data) may be available from the authors upon reasonable request and with the permission of LPC-PJ. For data sharing issues contact the corresponding author of the manuscript.

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Author contributions

Conceptualization, CC and NP; Methodology: CC, ECS, CF, SC, PMF, AA, LP, and NP; Software: CC, ECS, and NP; Validation: CC, ECS, CF, and NP; Formal analysis: CC, ECS, and CF; Investigation: CC, CF, SC, and PMF; Resources: SC, PMF, and AA; Data Curation: CC, ECS, CF, and NP; Writing—Original Draft: CC and NP; Writing—Review & Editing: CC, ECS, CF, SC, PMF, AA, LP, and NP; Supervision: AA, LP, and NP; Project administration: NP; Visualization: CC; Funding acquisition: CC, AA, and NP.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Supplementary Material IV

The complete supplementary data associated with this article can also be found in the online version at <https://doi.org/10.1038/s41598-025-33132-1>

SM1. NIST allele frequency database.

Table S1: NIST allele frequency database.

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S104	D2S1338	D2S441	D3S1358	D5S818	D7S820	D8S1179	FGA	SE33	TH01	TPOX	vWA
5																			0.0014	0.0014	
6																			0.2355	0.0014	
6.3																					
7														0.0028	0.0277				0.1939		
8	0.00554			0.120499	0.01801									0.0055	0.144	0.01385			0.0956	0.5249	
8.1															0.0014						
9	0.01385			0.077562	0.10665							0.0014		0.0416	0.1676	0.00554			0.1191	0.1274	
9.1												0.0014									
9.3																			0.3449		
10	0.22022			0.047091	0.05679	0.0083	0.00139	0.0028				0.2105		0.0554	0.2562	0.102493			0.0083	0.0499	
10.1																					
10.2																					
10.3																					
11	0.30886	0.001385		0.325485	0.3144	0.0097	0.00554	0.0776		0.13989		0.3435	0.001385	0.356	0.205	0.076177		0.0014	0.0014	0.2521	
11.2																					
11.3												0.0609									
12	0.36011	0.031856		0.268698	0.3144	0.1136	0.07064	0.1163		0.01247		0.0471		0.3878	0.1593	0.16759		0.0069		0.0416	0.0014
12.2							0.00139														
12.3												0.0042									
13	0.08172	0.307479		0.116343	0.16343	0.1233	0.25485	0.0665		0.00693		0.0291	0.001385	0.1427	0.0346	0.32964		0.0166		0.0014	0.0014
13.2							0.00693														
13.3																					
13.4																					
14	0.0097	0.297784		0.042936	0.02632	0.1343	0.3615	0.0789		0.05679		0.241	0.106648	0.0069	0.0042	0.166205		0.0249			0.0928
14.2						0.0014	0.02355											0.0028			
14.3								0.0028													
15		0.196676	0.03186	0.001385		0.1704	0.15651	0.1496		0.32133	0.001385	0.0596	0.272853	0.0014		0.103878		0.0402			0.1053
15.2							0.03601														
15.3								0.0582													
15.4																					
16		0.132964	0.02216			0.1468	0.05679	0.1357		0.38227	0.037396	0.0014	0.238227			0.033241		0.0402			0.2008
16.2						0.0014	0.01524											0.0014			
16.3								0.0609													
17		0.027701	0.12742			0.1385	0.00693	0.0471		0.07479	0.185596		0.210526			0.001385		0.0623			0.2839
17.1																					
17.2							0.00139														
17.3			0.02078					0.133										0.0014			

18	0.001385	0.17175	0.0776	0.0055	0.00554	0.073407	0.15097	0.0249	0.072	0.2022
18.1										
18.2			0.00139							
18.3		0.02493		0.0499					0.0014	
19	0.00277	0.12465	0.0402			0.120499	0.01662	0.0499	0.072	0.1039
19.1										
19.2									0.0042	
19.3		0.00416		0.0152						
19.4										
20		0.1108	0.018			0.15651	0.001385	0.1233	0.0582	0.0069
20.1										
20.2									0.0097	
20.3										
21		0.12881	0.0097			0.037396		0.1787	0.0249	0.0014
21.2								0.0055	0.0235	
21.3										
22		0.09557	0.0069			0.034626		0.205	0.0139	
22.2								0.0125	0.0374	
22.3										
23		0.06925				0.105263		0.1524	0.0028	
23.2								0.0028	0.036	
23.3										
24		0.04709				0.114958		0.1343	0.0014	
24.2								0.0014	0.0222	
24.3										
25		0.01662				0.102493		0.0789		
25.2				0.0014					0.0416	
26		0.00277				0.030471		0.0263		
26.2									0.0416	
27		0.00139		0.0222				0.0042		
27.2									0.0942	
27.3										
28				0.1593						
28.2									0.0762	
28.3									0.0014	
29				0.2022						
29.2				0.0028					0.0554	
29.3										
30				0.2825						
30.2				0.0291					0.0568	
30.3										
31				0.072					0.0028	

[illegible]

SM 2: LR algebraic formulas for all cases composed of a mixture profile and a reference profile that cannot be excluded from belonging to one of the mixture contributors.

Table S2: LR algebraic formulas for all cases composed of a mixture profile (with two or three contributors) and a reference profile (homo or heterozygotic) that cannot be excluded from belonging to one of the mixture contributors. The following nomenclature must be considered: **NOC** = Number of contributors; **MIX** = mixture profile; **REF** = Reference profile; θ = co-ancestry coefficient; p_i = frequency of allele A_i ; $a(k, \theta) = 1 + k \times \theta$; $S(i, \dots, n, z, \theta) = (1 - \theta) \times (\sum_{i=1}^n j p_i) + z \times \theta$; **Sjz** is a simplified notation of the values of the function $S(i, \dots, n, z, \theta)$ described in detail in Table S2A.

NOC	MIX	REF	LR
2	A1,A2, A3,A4	A1, A2	$\frac{a(3, \theta) \times a(4, \theta)}{12 \times (S41,1 \times S42,1)}$
		A1, A2	$\frac{a(3) \times a(4) \times S310,5}{12 \times (S31,1 \times S32,1 \times S37,5)}$
	A1,A2, A3	A1, A1	$\frac{a(3, \theta) \times a(4, \theta)}{6 \times (S31,2 \times S37,5)}$
		A1, A2	$\frac{a(3, \theta) \times a(4, \theta) \times (S21,1 \times S23,4 + S22,1 \times S22,2)}{2 \times S21,1 \times S22,1 \times (S21,2 \times S25,12 + 2 \times S22,2 \times S22,3)}$
	A1,A2	A1, A1	$\frac{a(3, \theta) \times a(4, \theta) \times S24,5}{2 \times S21,1 \times (S21,3 \times S25,11 + 2 \times S22,1 \times S22,2)}$
		A1, A1	$\frac{a(3, \theta) \times a(4, \theta)}{S11,4 \times S11,5}$
3	A1,A2,A3, A4,A5,A6	A1, A2	$\frac{a(5, \theta) \times a(6, \theta)}{30 \times S61,1 \times S62,1}$
		A1, A2	$\frac{a(5, \theta) \times a(6, \theta) \times S54,7}{30 \times S51,1 \times S52,1 \times S53,7}$
	A1,A2,A3, A4,A5	A1, A1	$\frac{a(5, \theta) \times a(6, \theta)}{30 \times S51,1 \times S51,2 \times S53,7}$
		A1, A2	$\frac{a(5, \theta) \times a(6, \theta) \times (6 \times S41,1 \times S410,6 + 3 \times S42,1 \times S47,6 + S43,1 \times S45,7 + 2 \times S44,1 \times S44,2)}{30 \times S41,1 \times 42,1 \times (S41,2 \times S411,18 + S42,2 \times S411,12 + S43,1 \times S45,7 + 2 \times S44,1 \times S44,2)}$
	A1,A2, A3,A4	A1, A1	$\frac{a(5, \theta) \times a(6, \theta) \times 2 \times S16,3}{5 \times S41,1 \times S41,2 \times (S41,3 \times S11,17 + S42,1 \times S48,10 + S43,1 \times S45,7 + S41,1 \times S44,2)}$
		A1, A2	$\frac{a(5, \theta) \times a(6, \theta) \times (S31,1 \times (S31,2 \times 2 \times S311,15 + 32,1 \times 12 \times S34,3 + 4 \times S33,1 \times S33,2) + S32,1 \times (S32,2 \times 2 \times S36,9 + 4 \times S33,1 \times S33,2) + S33,1 \times S33,2 \times S33,3)}{30 \times S31,1 \times S32,1 \times (S31,2 \times (S31,3 \times S39,10 + S32,2 \times S36,9 + 2 \times S33,1 \times S33,2) + S32,2 \times (S32,3 \times S35,6 + 2 \times S33,1 \times S33,2) + S33,1 \times S33,2 \times S33,3)}$
	A1,A2, A3	A1, A1	$\frac{a(5, \theta) \times a(6, \theta) \times (S31,2 \times 6 \times S37,5 + S32,1 \times S36,7 + 2 \times S33,1 \times S33,2)}{15 \times S31,2 \times (S31,3 \times (S31,4 \times S39,9 + S32,1 \times S36,7 + 2 \times S33,1 \times S33,2) + S32,1 \times (S32,2 \times S35,5 + 2 \times S33,1 \times S33,2) + S33,1 \times S33,2 \times S33,3)}$
		A1, A2	$\frac{a(5, \theta) \times a(6, \theta) \times (S21,1 \times (S22,1 \times (S21,2 \times 2 \times S25,12 + 4 \times S22,2 \times S22,3) + S21,2 \times S21,3 \times S21,4) + S22,1 \times S22,2 \times S22,3 \times S22,4)}{S21,1 \times S22,1 \times (S21,2 \times S21,3 \times (S22,2 \times 5 \times S26,24 + 6 \times S21,4 \times S21,5) + S22,2 \times S22,3 \times S22,4 \times 3 \times S28,20)}$
	A1,A2	A1, A1	$\frac{a(5, \theta) \times a(6, \theta) \times (S21,2 \times (S21,3 \times 2 \times S25,11 + 4 \times S22,1 \times S22,2) + S22,1 \times S22,2 \times S22,3)}{S21,1 \times (S21,3 \times (S21,4 \times S21,5 \times S29,51 + S22,1 \times S22,2 \times 5 \times S27,25) + 6 \times S22,1 \times S22,2 \times S22,3 \times S22,4)}$
		A1, A1	$\frac{a(5, \theta) \times a(6, \theta)}{S11,6}$

Table S2a: Nomenclature for Sjz considered in the mixture calculations. ‘N’ is the number of different alleles present in the mixture considered.

N	S _{iz}			
1	S11,z = S (1,z)			
2	S21,z = S (1,0,z)	S24,z = S (2,1,z)	S27,z = S (4,3,z)	
	S22,z = S (0,1,z)	S25,z = S (2,3,z)	S28,z = S (5,2,z)	
	S23,z = S (1,2,z)	S26,z = S (3,4,z)	S29,z = S (6,15,z)	
3	S31,z = S (1,0,0,z)	S34,z = S (0,1,1,z)	S37,z = S (1,1,1,z)	S310,z = S (2,2,1,z)
	S32,z = S (0,1,0,z)	S35,z = S (0,1,2,z)	S38,z = S (1,0,2,z)	S311,z = S (2,6,3,z)
	S33,z = S (0,0,1,z)	S36,z = S (0,2,3,z)	S39,z = S (1,2,2,z)	
4	S41,z = S (1,0,0,0,z)	S44,z = S (0,0,0,1,z)	S47,z = S (0,2,1,1,z)	S410,z = S (1,2,1,1,z)
	S42,z = S (0,1,0,0,z)	S45,z = S (0,0,2,3,z)	S48,z = S (0,2,3,3,z)	S411,z = S (2,3,3,3,z)
	S43,z = S (0,0,1,0,z)	S46,z = S (0,1,1,1,z)	S49,z = S (1,1,1,1,z)	
5	S51,z = S (1,0,0,0,0,z)	S52,z = S (0,1,0,0,0,z)	S53,z = S (1,1,1,1,1,z)	S54,z = S (2,2,1,1,1,z)
6	S61,z = S (1,0,0,0,0,0,z)	S62,z = S (0,1,0,0,0,0,z)		

SM 3: Ratio between LR values computed per genotypic configuration, not considering the population coancestry (0; LR1) and considering (0.01, LR2), for one million cases with mixtures with two and three contributors simulated in R

Table S3: Ratio between LR values computed per genotypic configuration not considering the population coancestry (0; LR1) and considering (0.01, LR2), for one million cases with mixtures with two and three contributors simulated in R Studio. Ratio=LR1/LR2; ‘2’: cases with mixtures with two contributors; ‘3’: cases with mixtures with three contributors. Highlighted in bold ratios with an insufficient number of cases to have a percentage expression. N=1,000,000 for each marker.

Genotypic configuration	N	1/2<R<1		1<R<2		2<R<5		5<R<10		10<R<100	
		N	%	N	%	N	%	N	%	N	%
2 CONTRIBUTORS											
Mix:A1,A2,A3,A4 / Ref: A1,A2	5,523,787	447,176	8.10%	4,766,921	86.30%	231,856	4.20%	69,833	1.26%	8,001	0.14%
Mix:A1,A2,A3 / Ref: A1,A1	1,702,979	0	0.00%	1,693,264	99.43%	9,340	0.55%	291	0.02%	84	0.00%
Mix:A1,A2,A3 / Ref: A1,A2	8,531,734	385,780	4.52%	7,930,077	92.95%	165,535	1.94%	47,342	0.55%	3,000	0.04%
Mix:A1,A2 / Ref: A1,A1	2,142,964	0	0.00%	2,138,523	99.79%	4,350	0.00%	66	0.00%	25	0.00%
Mix:A1,A2 / Ref: A1,A2	2,737,917	0	0.00%	2,710,734	99.01%	20,940	0.76%	5,970	0.22%	273	0.01%
Mix:A1,A1 / Ref: A1,A1	360,619	0	0.00%	359,657	99.73%	950	0.26%	12	0.00%	0	0.00%
TOTAL	21,000,000	832,956	3.97%	19,599,176	93.33%	432,971	2.06%	123,514	0.59%	11,383	0.05%
3 CONTRIBUTORS											
Mix: A1,A2,A3,A4,A5,A6 / Ref: A1,A2	1,168,699	56,630	4.85%	1,006,411	86.11%	77,822	6.66%	23,402	2.00%	4,434	0.38%
Mix: A1,A2,A3,A4,A5 / Ref: A1,A1	277,785	573	0.21%	264,714	95.29%	11,841	4.26%	457	0.16%	178	0.06%
Mix: A1,A2,A3,A4,A5 / Ref: A1,A2	3,882,190	367,567	9.47%	3,324,543	85.64%	142,274	3.66%	44,474	1.15%	3,332	0.09%
Mix: A1,A2,A3,A4 / Ref: A1,A1	1,219,716	0	0.00%	1,190,165	97.58%	28,454	2.33%	733	0.06%	326	0.03%
Mix:A1,A2,A3,A4 / Ref: A1,A2	6,478,476	555,129	8.57%	5,751,101	88.77%	129,989	2.01%	40,886	0.63%	1,371	0.02%
Mix:A1,A2,A3 / Ref: A1,A1	1,860,821	0	0.00%	1,859,293	99.92%	1,485	0.08%	30	0.00%	13	0.00%
Mix:A1,A2,A3 / Ref: A1,A2	4,491,700	0	0.00%	4,437,409	98.79%	41,170	0.92%	12,996	0.29%	125	0.00%
Mix:A1,A2 / Ref: A1,A1	800,334	0	0.00%	800,087	99.97%	247	0.03%	0	0.00%	0	0.00%
Mix:A1,A2 / Ref: A1,A2	770,775	0	0.00%	768,121	99.66%	1,943	0.25%	706	0.09%	5	0.00%
Mix:A1,A1 / Ref: A1,A1	49,504	20,920	42.26%	28,584	57.74%	0	0.00%	0	0.00%	0	0.00%
TOTAL	21,000,000	1,000,819	4.77%	19,430,428	92.53%	435,225	2.07%	123,684	0.59%	9,784	0.05%

R>100		Minimum	Maximum	Mean	Median	Q1	Q3	Standard Deviation
N	%							
0	0.00%	0.9654	62.9239	1.3381	1.1029	1.0442	1.2733	1.0802
0	0.00%	1.0032	39.0222	1.1419	1.0890	1.0502	1.1787	0.2141
0	0.00%	0.9898	67.4390	1.1940	1.0804	1.0310	1.1515	0.6463
0	0.00%	1.0271	29.1900	1.1308	1.1018	1.0650	1.1465	0.1354
0	0.00%	1.0186	26.1598	1.1402	1.0776	1.0430	1.1470	0.4021
0	0.00%	1.0802	9.2442	1.1984	1.1718	1.1418	1.2153	0.1401
0	0.00%	-	-	-	-	-	-	-
0	0.00%	0.9292	60.5607	1.5176	1.1923	1.0614	1.4109	1.4448
22	0.01%	0.9883	206.9821	1.3168	1.1962	1.0969	1.2934	1.4310
0	0.00%	0.9568	61.4723	1.3044	1.0916	1.0401	1.2565	0.9527
38	0.00%	1.0329	281.0782	1.2650	1.1684	1.1115	1.3203	0.9594
0	0.00%	0.9821	65.6990	1.1848	1.0673	1.0215	1.1367	0.6511
0	0.00%	1.0138	15.6301	1.0948	1.0700	1.0430	1.1134	0.1031
0	0.00%	1.0022	60.7384	1.1240	1.0593	1.0257	1.1167	0.4335
0	0.00%	1.0405	4.5796	1.1131	1.0899	1.0592	1.1455	0.0818
0	0.00%	1.0295	11.5626	1.1094	1.0732	1.0452	1.1229	0.2537
0	0.00%	0.9922	1.6380	1.0288	1.0305	0.9922	1.0464	0.0417
60	0.00%	-	-	-	-	-	-	-

SM 4: Ratio between LR values computed per marker not considering the population coancestry (0; LR1) and considering (0.01, LR2), for one million cases with mixtures with two and three contributors simulated in R

Table S4: Ratio between LR values computed per marker not considering the population coancestry (0; LR1) and considering (0.01, LR2), for one million cases with mixtures with two and three contributors simulated in R Studio. Ratio=LR1/LR2; ‘2’: cases with mixtures with two contributors; ‘3’ cases with mixtures with three contributors. Highlighted in bold ratios with an insufficient number of cases to have a percentage expression. N=1,000,000 for each marker.

Ratio	Markers															
	CSF1PO		D10S1248		D12S391		D13S317		D16S539		D18S51		D19S433		D1S1656	
	2	3	2	3	2	3	2	3	2	3	2	3	2	3	2	3
1/2 < R < 1	11.14%	12.58%	5.11%	13.13%	0.00%	0.00%	2.22%	3.25%	1.89%	7.09%	0.00%	0.35%	2.04%	5.60%	0.00%	0.00%
1 < R < 2	87.17%	85.98%	93.79%	85.66%	97.86%	97.79%	97.50%	96.46%	98.04%	92.84%	93.33%	94.12%	92.90%	89.28%	97.69%	97.55%
2 < R < 5	1.68%	1.42%	0.51%	0.63%	1.71%	1.82%	0.01%	0.01%	0.07%	0.07%	6.12%	4.94%	3.93%	3.99%	2.03%	2.31%
5 < R < 10	0.01%	0.02%	0.57%	0.57%	0.39%	0.35%	0.27%	0.28%	0.00%	0.00%	0.52%	0.55%	1.06%	1.06%	0.27%	0.13%
10 < R < 100	0.00%	0.00%	0.02%	0.01%	0.03%	0.03%	0.00%	0.00%	0.00%	0.00%	0.04%	0.03%	0.07%	0.07%	0.01%	0.01%
R > 100	0.00%	0.00%	0.00%	0.00% (N=4)	0.00%	0.00%	0.00%	0.00% (N=3)	0.00%	0.00%	0.00%	0.00% (N=5)	0.00%	0.00% (N=5)	0.00%	0.00%
Minimum	0.971	0.935	0.977	0.940	1.045	1.006	0.977	0.942	1.045	0.938	0.979	0.997	0.978	0.941	1.049	1.010
Maximum	6.375	13.622	63.007	128.923	35.569	57.777	63.007	165.564	35.569	4.078	18.743	157.614	63.871	135.326	19.754	65.931
Mean	1.104	1.094	1.145	1.134	1.291	1.284	1.145	1.116	1.291	1.095	1.124	1.263	1.272	1.260	1.270	1.264
Median	1.052	1.042	1.048	1.045	1.165	1.157	1.048	1.074	1.165	1.060	1.078	1.107	1.084	1.086	1.187	1.169
Q1	1.008	1.010	1.018	1.018	1.098	1.089	1.018	1.037	1.098	1.028	1.046	1.066	1.041	1.037	1.124	1.113
Q3	1.085	1.069	1.099	1.090	1.312	1.335	1.099	1.139	1.312	1.110	1.152	1.227	1.173	1.166	1.253	1.243
Standard Deviation	0.253	0.253	0.639	0.665	0.582	0.587	0.639	0.457	0.582	0.127	0.389	0.736	0.929	0.944	0.462	0.490
Heterozygosity	0.719431		0.758627		0.89085		0.783726		0.759973		0.875834		0.769446		0.899237	
Number of alleles	7		9		16		8		7		15		15		15	

D21S11		D22S1045		D12S1338		D2S441		D3S1358		D5S818		D7S820		D8S1179		FGA	
2	3	2	3	2	3	2	3	2	3	2	3	2	3	2	3	2	3
2.43%	3.07%	11.57%	6.53%	0.00%	0.41%	5.61%	6.88%	6.06%	7.35%	12.41%	5.48%	2.23%	4.93%	2.16%	3.57%	0.00%	1.41%
93.96%	93.18%	85.88%	90.96%	99.69%	98.98%	92.72%	91.42%	93.07%	91.78%	84.25%	91.23%	96.65%	93.85%	96.32%	94.90%	96.97%	95.55%
2.44%	2.56%	2.54%	2.49%	0.03%	0.34%	0.84%	0.88%	0.03%	0.03%	2.98%	2.98%	0.84%	0.95%	1.23%	1.25%	2.63%	2.69%
1.07%	1.10%	0.01%	0.01%	0.26%	0.27%	0.79%	0.81%	0.82%	0.83%	0.34%	0.30%	0.27%	0.27%	0.28%	0.28%	0.37%	0.33%
0.11%	0.09%	0.00%	0.00%	0.02%	0.00%	0.03%	0.01%	0.02%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.03%	0.03%
0.00%	0.00% (N=7)	0.00%	0.00%	0.00%	0.00% (N=5)	0.00%	0.00% (N=4)	0.00%	0.00% (N=2)	0.00%	0.00% (N=1)	0.00%	0.00% (N=2)	0.00%	0.00% (N=1)	0.00%	0.00% (N=1)
0.995	0.958	0.969	0.932	1.027	0.988	0.981	0.944	0.989	0.952	0.965	0.929	0.998	0.960	1.000	0.962	1.014	0.976
63.176	164.421	22.178	13.686	16.432	176.054	63.045	133.933	63.048	132.798	35.305	128.735	26.033	154.867	21.478	137.396	35.256	142.744
1.291	1.285	1.125	1.115	1.206	1.200	1.186	1.176	1.145	1.135	1.147	1.134	1.144	1.135	1.165	1.158	1.249	1.240
1.105	1.102	1.052	1.045	1.143	1.135	1.082	1.084	1.061	1.050	1.040	1.036	1.067	1.064	1.091	1.087	1.117	1.111
1.065	1.059	1.025	1.019	1.093	1.082	1.024	1.024	1.025	1.019	1.019	1.018	1.041	1.027	1.054	1.053	1.067	1.057
1.195	1.197	1.114	1.094	1.264	1.262	1.153	1.139	1.098	1.095	1.134	1.102	1.132	1.138	1.137	1.131	1.207	1.220
0.955	1.017	0.281	0.276	0.412	0.522	0.737	0.766	0.689	0.697	0.545	0.544	0.456	0.490	0.455	0.468	0.604	0.617
0.82885		0.721994		0.881424		0.769266		0.79003		0.697662		0.816135		0.807199		0.859355	
16		8		12		11		9		9		9		10		14	

SE33		TH01		TPOX		vWA	
2	3	2	3	2	3	2	3
0.00%	0.00%	3.67%	8.02%	10.36%	5.90%	4.41%	4.53%
85.83%	85.21%	94.12%	89.79%	88.80%	93.27%	93.37%	93.25%
10.46%	11.16%	1.65%	1.64%	0.00%	0.00%	1.39%	1.39%
2.98%	3.01%	0.55%	0.55%	0.83%	0.83%	0.81%	0.82%
0.72%	0.62%	0.01%	0.01%	0.00%	0.00%	0.01%	0.01%
0.00%	0.00% (N=12)	0.00%	0.00% (N=3)	0.00%	0.00% (N=1)	0.00%	0.00% (N=4)
1.147	1.104	0.982	0.945	0.970	0.934	0.995	0.957
67.439	281.078	62.924	132.658	64.959	126.444	63.440	139.726
1.846	1.830	1.125	1.114	1.128	1.115	1.164	1.156
1.437	1.408	1.059	1.050	1.043	1.030	1.078	1.068
1.297	1.275	1.026	1.025	1.019	1.014	1.043	1.039
1.666	1.668	1.090	1.082	1.080	1.072	1.110	1.108
1.768	1.823	0.575	0.595	0.701	0.686	0.704	0.752
0.948262		0.764627		0.640449		0.807621	
39		8		8		10	

SM 5: LR's values (overall and per-marker) computed by the three tools for both cases with mixtures with two and three estimated contributors, considering an FST value of 0 and 0.01.

S5 2a: LR's values (overall and per-marker) computed by LRMix Studio v.2.1.3 for mixtures with two estimated contributors, considering an FST value of 0 and 0.01. The ratio between the two LR values was calculated.

PAIR		NUMBER OF MARKERS ANALYZED	LR																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
			OVERALL		CSFIPO												D10S1248				D12S391				D13S317				D16S539				D18S51				D19S433				D1S1656				D21S11				D22S1045				D2S1338				D2S441																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
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					0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01		0.00	0.01	

D3S1358			D5S818			D7S820			D8S1179			FGA			SE33			TH01			TPOX			vWA																															
RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO																									
1.056	0.871	0.890	0.979	1.097	1.109	0.989	2.526	2.472	1.021	4.670	4.391	1.064	1.374	1.264	1.087	31.652	24.430	1.296	1.833	1.813	1.011	186.288	23.835	7.816	1.780	1.756	1.014	1.066	2.229	2.187	1.019	4.161	3.748	1.110	14.059	11.253	1.249	5.532	4.875	1.098	12.790	11.115	1.151	0.001	0.001	1.016	5.308	4.933	1.076	2.048	2.015	1.016	5.958	5.503	1.083
1.046	3.911	3.720	1.051	6.919	6.044	1.145	4.724	4.440	1.064	1.977	1.758	1.124	13.262	11.162	1.188	35.693	26.581	1.343	1.811	1.793	1.010	8.797	7.591	1.159	2.053	2.032	1.010	1.066	2.607	2.549	1.023	1.141	1.150	0.993	5.306	4.977	1.066	22.345	17.032	1.312	13.291	11.183	1.188	0.062	0.057	1.095	17.545	14.755	1.189	12.316	10.094	1.220	3.864	3.670	1.053
1.013	4.880	4.568	1.068	2.790	2.678	1.042	2.523	2.470	1.021	0.849	0.809	1.049	9.476	7.717	1.228	59.751	38.662	1.545	1.394	1.280	1.089	2.048	2.015	1.016	4.393	4.148	1.059	1.039	2.441	2.387	1.023	1.401	1.355	1.034	2.625	2.559	1.026	4.030	3.784	1.065	15.713	12.992	1.209	88.083	54.092	1.628	2.903	2.806	1.035	2.112	2.054	1.028	7.490	6.810	1.100
1.278	2.607	2.549	1.023	1.947	1.840	1.059	2.636	2.568	1.026	3.976	3.738	1.064	7.833	7.094	1.104	0.666	0.563	1.183	4.472	4.199	1.065	15.793	11.138	1.418	2.747	2.646	1.038	1.126	687.185	81.777	8.403	0.535	0.525	1.019	5.306	4.977	1.066	10.232	9.016	1.135	2.255	2.222	1.015	19.603	16.024	1.223	4.012	3.761	1.067	3.808	3.604	1.057	87.744	36.044	2.434
1.395	1.436	1.443	0.995	1.947	1.840	1.059	20.326	15.192	1.038	2.632	2.433	1.082	247.723	70.782	3.500	28.520	21.859	1.305	7.399	6.179	1.197	1.826	1.737	1.051	10.612	9.400	1.129	1.395	1.436	1.443	0.995	1.947	1.840	1.059	20.326	15.192	1.038	2.632	2.433	1.082	247.723	70.782	3.500	28.520	21.859	1.305	7.399	6.179	1.197	1.826	1.737	1.051	10.612	9.400	1.129
1.148	1.439	1.446	0.995	1.401	1.355	1.034	4.724	4.440	1.064	8.107	7.267	1.116	3.664	3.507	1.045	22.056	17.876	1.234	4.422	4.155	1.064	0.391	0.389	1.005	0.152	0.147	1.037	1.077	3.015	2.901	1.039	4.969	4.530	1.097	17.291	13.539	1.277	4.322	4.114	1.051	22.715	16.596	1.369	15.086	12.688	1.189	5.901	5.436	1.086	3.054	2.881	1.060	3.129	2.978	1.050
1.278	1.051	0.985	1.066	1.097	1.109	0.989	3.312	3.184	1.040	2.936	2.853	1.029	0.786	0.705	1.116	7.193	5.253	1.370	3.057	2.952	1.035	0.305	0.296	1.029	11.745	10.300	1.140	1.149	1.272	1.285	0.990	0.535	0.525	1.019	3.569	3.416	1.045	1.993	1.922	1.037	6.472	5.949	1.088	0.601	0.515	1.166	1.747	1.707	1.007	0.105	0.105	0.996	5.989	5.531	1.083
1.056	3.533	3.392	1.041	4.969	4.530	1.097	4.687	4.126	1.136	136.447	47.725	2.859	5.202	4.855	1.072	18.029	14.977	1.204	0.232	0.225	1.034	0.125	0.125	1.005	5.885	5.442	1.081	1.000	3.240	2.955	1.096	1.097	1.109	0.989	4.241	4.030	1.052	0.804	0.769	1.045	5.897	5.404	1.091	11.500	10.022	1.147	7.148	6.520	1.096	1.280	1.240	1.033	7.807	7.038	1.109
1.019	2.607	2.419	1.078	0.493	0.485	1.015	0.096	0.093	1.033	4.811	4.512	1.066	6.023	5.592	1.077	24.900	19.704	1.264	6.710	6.569	1.186	1.826	1.737	1.051	11.470	10.098	1.136	1.020	3.911	3.720	1.051	1.489	1.436	1.037	6.850	6.275	1.092	14.493	12.403	1.169	26.495	19.127	1.385	81.961	51.085	1.604	1.238	1.249	0.992	1.199	1.203	0.997	7.807	7.038	1.109
1.036	5.175	4.812	1.076	5.674	5.119	1.108	2.037	2.012	1.012	5.438	4.723	1.151	13.817	11.514	1.200	6103.396	398.427	15.319	11.478	9.035	1.270	2.313	2.232	1.036	2.043	2.023	1.010	1.028	5.431	4.893	1.110	1.982	1.941	1.021	4.724	4.314	1.095	4.116	3.742	1.100	5.372	4.859	1.106	65.132	32.437	2.008	3.512	3.299	1.064	2.048	2.000	1.024	2.659	2.376	1.119
1.285	1.643	1.643	1.000	1.401	1.355	1.034	3.900	3.077	1.102	28.491	17.040	1.672	5.813	5.393	1.078	16.848	14.134	1.192	4.319	4.089	1.056	2.048	2.015	1.016	4.948	4.587	1.079	0.720	3.633	3.730	0.974	1.097	2.279	0.481	19.364	3.193	6.805	1.065	4.886	4.252	1.376	36.886	27.576	1.265	0.180	0.210	0.085	3.601	2.028	1.776	11.753	14.43	8.145		
1.046	3.911	3.720	1.051	1.870	1.663	1.125	4.724	4.440	1.064	9.534	8.433	1.131	0.075	0.075	1.008	19.059	15.620	1.220	4.319	4.089	1.056	0.388	0.386	1.005	77.310	70.386	10.944	1.039	1.185	1.103	1.075	0.614	0.635	0.967	4.724	4.440	1.064	2.531	2.464	1.027	13.494	11.521	1.171	0.446	0.395	1.129	9.976	8.811	1.132	0.042	0.043	0.987	1.450	1.456	0.996
1.133	3.738	6.739	1.095	5.674	5.119	1.108	20.368	15.308	1.331	8.209	7.416	1.107	2.923	2.484	1.177	31.893	23.883	1.335	9.631	7.808	1.233	0.405	0.350	1.158	3.864	3.670	1.053	1.010	3.911	3.720	1.051	1.982	1.954	1.014	6.850	6.275	1.092	9.957	8.755	1.137	2.659	2.595	1.025	20.691	16.778	1.233	2.502	1.960	1.022	5.467	4.599	1.145	7.807	7.038	1.109
1.143	2.498	2.437	1.025	1.401	1.355	1.034	4.724	4.440	1.064	4.166	3.898	1.066	6.104	5.657	1.079	102.995	60.555	1.701	1.024	1.041	0.983	1.080	1.057	1.022	4.393	4.148	1.059	0.585	1.185	3.762	0.315	0.614	1.109	0.554	4.724	2.460	1.920	2.531	2.649	0.956	13.494	70.745	0.191	0.446	27.318	0.016	9.976	0.847	11.783	0.042	3.604	0.012	1.450	4.418	0.350
1.046	2.119	2.089	1.014	4.519	4.246	1.064	4.895	4.602	1.064	2.531	2.464	1.027	6.600	6.079	1.086	125.787	65.754	1.913	3.192	2.913	1.096	1.080	1.057	1.022	4.393	4.148	1.059	1.056	3.911	3.720	1.051	3.837	3.463	1.108	5.306	4.977	1.066	723.379	85.397	8.471	8.036	6.970	1.153	47.701	32.410	1.472	2.198	2.153	1.021	0.464	0.456	1.016	3.903	3.704	1.054
1.039	4.920	4.613	1.067	1.401	1.355	1.034	3.102	2.997	1.035	2.636	2.556	1.031	12.026	10.411	1.155	275.322	121.507	2.266	3.057	2.952	1.035	1.080	1.057	1.022	5.916	5.469	1.082	1.050	1.991	1.967	1.013	0.686	0.662	1.036	7.161	6.536	1.096	2.198	1.935	1.136	20.410	15.168	1.346	51.032	35.663	1.431	112.857	49.849	2.264	0.637	0.656	1.071	2.659	2.462	1.080
1.013	2.273	2.234	1.018	4.519	4.246	1.064	2.840	2.745	1.035	7.032	6.379	1.102	3.022	2.925	1.033	82.024	51.115	1.605	9.673	8.606	1.124	2.048	2.015	1.016	1.641	1.487	1.103	1.096	3.240	2.955	1.096	2.363	2.305	1.025	2.656	2.586	1.027	8.925	7.966	1.120	3092.988	223.200	13.857	27.424	21.177	1.295	1.818	1.809	1.005	3.808	3.604	1.057	7.807	7.038	1.109
1.060	1.643	1.643	1.000	1.982	1.954	1.014	4.724	4.440	1.064	8.052	7.226	1.114	7.766	6.473	1.200	47.034	33.657	1.397	5.354	5.001	1.071	9.433	8.139	1.159	8.744	7.790	1.123	1.046	3.911	3.720	1.051	1.097	1.109	0.989	4.724	4.440	1.064	4.251	2.339	1.035	11.505	10.094	1.140	66.579	45.793	1.454	4.012	3.761	1.067	2.048	2.015	1.016	2.400	2.351	1.021
1.375	4.732	4.616	1.136	4.519	4.246	1.064	4.426	4.185	1.057	12.543	10.897	1.151	4.838	4.549	1.063	47.338	34.035	1.391	2.972	2.716	1.094	2.048	2.015	1.016	6.978	6.334	1.102	1.020	5.020	4.684	1.072	1.097	1.109	0.989	3.569	3.416	1.045	6.413	5.842	1.098	15.258	11.518	1.325	37.122	25.696	1.445	3.097	2.987	1.037	0.464	0.456	1.016	4.390	4.122	1.065
1.060	2.344	2.301	1.019	1.947	1.840	1.059	5.317	4.972	1.069	4.322	4.114	1.051	4.869	4.454	1.064	2301.778	311.076	7.399	3.811	3.628	1.050	2.048	2.015	1.016	4.393	4.148	1.059	13.628	3.533	3.392	1.041	128.543	46.117	2.787	5.902	5.461	1.081	21.942	16.777	1.308	3.774	3.605	1.047	535.344	164.446	3.255	5.354	5.001	1.071	2.375	2.286	1.039	1.676	1.654	1.014
1.139	2.119	2.089	1.014	21.635	16.957	1.276	5.902	5.461	1.081	2.675	2.590	1.033	10.932	9.549	1.149	13.445	8.436	1.594	1.811	1.793	1.010	7.709	6.544	1.178	3.942	3.739	1.054	1.034	1.436	1.443	0.995	1.097	1.109	0.989	17.291	13.539	1.277	4.670	4.391	1.064	8.707	7.752	1.127	3.62	10.280	9.125	1.127	2.048	2.015	1.016	1.439	1.319	1.091		
1.144	2.																																																						

S5_2b: LR's values (overall and per-marker) computed by EuroForMix for mixtures with two estimated contributors, considering an F_{ST} value of 0 and 0.01. The ratio between the two LR values was calculated.

For Mix

PAIR	NUMBER OF MARKERS ANALYZED	LR																																				MARKET									
		OVERALL		CSFPO				D10S1248				D12S391				D13S317				D16S539				D18S51				D19S433				D1S1656				D21S11				D22S1045				D2S13KFF			
				0.00	0.01	RATIO		0.00	0.01	RATIO		0.00	0.01	RATIO		0.00	0.01	RATIO		0.00	0.01	RATIO		0.00	0.01	RATIO		0.00	0.01	RATIO		0.00	0.01	RATIO		0.00	0.01	RATIO		0.00	0.01	RATIO					
2.1	21	3.55E+09	8.47E+07	4.19E+01	1.0230	1.0245	0.9985	39.7145	29.9573	1.3257	0.5492	0.5236	1.0489	0.2446	0.2451	0.9977	1.3237	1.2450	1.0632	0.1260	0.1187	1.0611	0.13153	12.1396	1.0721	0.0004	0.0004	0.9732	6.8911	6.3583	1.0838	5.3059	4.8228	1.1002	7.4075	6.5085											
2.2	21	2.83E+09	2.78E+08	1.01E+01	2.0350	2.0077	1.0136	8.1167	7.3691	1.1014	0.1993	0.2038	0.9778	2.0916	2.0278	1.0315	1.5599	1.5253	1.0095	5.8823	5.3858	1.0242	1.9249	1.8007	1.0690	7.8379	6.8729	1.1404	163.7851	46.6266	3.5127	2.2203	2.2061	1.0064	0.4222	0.4177											
2.3	21	5.13E+15	1.41E+14	3.64E+01	9.7824	8.1223	1.2044	3.3592	3.1148	1.0745	7.3875	6.9179	1.0679	4.4269	4.1783	1.0595	3.0268	2.9274	1.0340	0.0018	0.0015	1.2904	151.9984	56.9343	2.6697	16.9895	12.4120	1.3688	4.1254	3.7901	1.0885	2.4372	2.5231	0.9659	33.7127	21.7295											
2.4	21	6.84E+19	8.25E+18	8.30E+01	1.8137	1.7844	1.0164	16.0244	13.3760	1.1980	77.6211	69.2357	1.1211	12.0743	11.5407	1.0462	2.4260	2.2720	1.0678	40.4381	35.1953	1.1490	3.0111	3.0267	0.9948	39.2215	28.1158	1.3950	97.5674	86.7602	1.1246	1.3743	1.4298	0.9612	0.4811	0.5106											
2.5	21	3.61E+22	3.56E+20	1.02E+02	40.6787	22.7092	1.7913	7.0225	6.5968	1.0645	54.3012	40.0439	1.3494	13.3033	11.8664	1.1211	1.1645	1.1500	1.0126	8.9189	8.2914	1.0757	1543.9068	194.8481	7.9236	231.0025	167.0180	1.3831	6.0963	5.6556	1.0749	33.8011	28.9381	1.1680	7.4933	6.7290											
2.6	21	2.09E+24	2.71E+23	7.72E+01	16.5533	13.1118	1.2625	3.4739	3.6883	0.9419	19.5209	17.2220	1.1335	3.2431	3.1511	1.0292	6.0746	5.7579	1.0550	38.2612	35.0641	1.0917	4.5429	4.5112	1.0070	63.3688	55.4075	1.1437	18.5231	16.9397	1.1163	33.4476	29.1404	1.1478	53.8748	48.8970											
2.7	21	1.50E+20	1.44E+18	1.04E+02	916.6399	86.7628	10.5649	7.7277	7.2183	1.0706	1113.8483	596.5620	1.8671	1.6016	1.5914	1.0064	2.4335	2.3833	1.0210	10.7466	10.5466	1.1591	39.3688	24.1676	1.0620	40.2069	30.6575	1.3115	8.1709	7.4859	1.1163	14.5261	12.6763	1.1459	5.5561	5.2576											
2.8	21	3.56E+27	2.24E+24	1.59E+03	1232.7897	272.5665	4.4414	19.5370	14.8692	1.3139	33.3008	25.7665	1.2924	2.1197	1.9035	1.1136	8.3928	7.2690	1.1546	42.0079	26.3094	1.5967	3.4796	3.4603	1.0056	18.1577	14.5764	1.2457	23.0407	20.6371	1.1455	17.4577	15.3494	1.1374	41.1647	33.3236											
2.9	21	1.05E+20	3.89E+16	2.71E+03	8617.0277	307.5858	28.4782	9.1710	7.2490	1.2651	4.7215	4.7202	1.1057	7.0992	6.7286	1.0551	4.9541	4.5176	1.0966	38.4531	29.6661	1.2962	1.4181	1.4073	1.0077	17.1099	14.1750	1.2071	3.0649	2.9402	1.0220	0.0000	0.0000	52.6364	6.7691												
2.10	21	6.66E+23	5.18E+20	1.28E+03	1.8357	1.7812	1.0306	996.4299	130.7664	7.6202	58.0119	32.2674	1.7978	21.61628	158.0846	1.3702	19.9596	17.1388	1.1646	8.7519	7.6133	1.1496	1684.1782	208.2275	8.0882	77.8530	69.2623	1.1240	220.0484	160.5900	1.3709	3.2521	2.9604	1.0985	39.9202	27.2269											
2.11	21	4.47E+28	4.89E+26	9.69E+01	1684.8984	350.2812	4.8101	2.6431	3.0357	0.8707	13.04072	101.3468	1.3277	6.5978	6.3137	1.0450	13.9257	12.2651	1.1354	25.4627	18.8998	1.3472	33.1090	26.2729	1.0602	52.5150	44.9944	1.1671	17.4427	15.5088	1.1247	0.9034	9.0104	1.0097	76.1338	55.2768											
2.12	21	2.44E+09	3.73E+08	6.55E+00	0.5130	0.5076	1.0105	4.0885	4.4568	0.9174	3.3231	2.7847	1.1933	2.7763	2.5172	1.1029	0.9218	0.8845	1.0422	5.9408	5.4290	1.0943	3.3840	3.1282	1.0818	1.1898	0.9562	1.2443	6.4051	4.9522	1.2934	0.5349	0.6020	0.8885	3.8672	3.1199											
2.13	21	2.39E+13	2.24E+12	1.07E+01	8.7596	7.1840	1.2193	6.4037	5.1261	1.2492	52.8644	37.5255	1.4088	15.0367	13.3094	1.1298	3.3578	3.1081	1.0303	24.0482	20.8995	1.1200	0.3456	0.4871	0.9968	50.9504	33.1579	1.5366	2.6547	2.6589	0.9984	5.5134	4.9305	1.1812	0.5923	0.5332											
2.14	21	3.56E+10	1.41E+10	2.53E+00	1.7803	1.7493	1.0177	1.0298	1.0339	0.9961	2.5140	2.4985	1.0062	1.7284	1.7137	1.0086	2.0574	2.0882	0.9853	0.8293	0.8398	0.9875	8.1810	7.5766	1.0798	42.3811	37.0139	1.1450	1.0473	1.0489	0.9985	1.5340	1.5516	0.9887	73.9662	56.0025											
2.15	21	1.46E+15	1.74E+14	8.14E+00	9.7225	8.0850	1.2025	4.8610	4.5927	1.0584	5.9234	4.9244	1.2029	1.9901	1.9626	1.0140	19.5657	15.9763	1.2247	6.1897	5.8115	1.0651	6.4981	5.4914	1.1833	16.4964	14.1320	1.1673	2.1733	2.1804	0.9968	6.5171	5.6957	1.1442	3.3931	3.2453											
2.16	21	2.15E+20	1.09E+19	1.98E+01	5.4951	4.9283	1.1150	0.6819	0.6444	1.0582	28.3815	25.6266	1.1075	21.1940	17.6549	1.2005	49.9220	42.5580	1.1730	20.5789	16.8162	1.2238	44.3551	31.2806	1.4180	14.0296	12.2181	1.1483	14.8919	11.1513	1.3354	4.6833	4.5882	1.0207	26.2090	22.6239											
2.17	21	7.84E+17	4.38E+16	1.79E+01	0.6849	0.6777	1.0105	1.5438	1.3512	1.1426	28.1979	21.2856	1.3247	10.9575	10.0211	1.0934	1.4007	1.3640	1.0269	4.9402	4.8590	1.0167	2.0314	1.8230	1.1143	15.1872	13.3101	1.1410	8.3166	7.0822	1.1743	18.2623	14.6968	1.2426	6.4389	5.2241											
2.18	21	1.06E+17	9.87E+14	1.68E+02	2.8013	2.6740	1.0476	5.5308	5.8359	0.9477	5.0078	4.5565	1.0990	1.5061	1.2247	1.2298	1.1919	8.4458	1.4113	1.4931	4.3146	1.1549	3.7283	4.0913	0.9113	23.9298	20.0384	1.1624	2.0235	1.8084	1.1189	3.1020	3.1071	0.9984	36.8660	24.2073											
2.19	21	1.05E+17	6.45E+15	1.63E+01	3.1242	3.0391	1.0280	26.6399	20.7948	1.2837	116.5056	69.2028	1.6835	2.5647	2.4395	1.0513	22.9422	17.0494	1.3546	12.4848	10.4655	1.1934	2.1100	1.9093	1.1051	10.7427	9.0676	1.1847	1.3193	1.3064	1.0099	2.7404	2.6387	1.0249	4.2277	3.8479											
2.20	21	2.73E+17	2.13E+16	1.28E+01	2.5627	2.3796	1.0769	3.6967	3.2919	1.1229	13.3745	11.6258	1.1504	3.9371	3.5027	1.1240	4.5495	4.5013	1.2129	5.5828	4.5438	1.2286	9.8682	9.3310	1.0576	21.3158	19.7351	1.0801	4.4798	4.3756	1.0238	4.4647	4.4367	1.0063	3.4855	3.1625											
2.21	21	3.27E+18	6.28E+17	5.21E+00	0.3086	0.3122	0.9885	5.8786	5.8366	1.0072	56.0588	48.0492	1.1667	8.5910	7.6973	1.1161	2.2242	2.2368	0.9944	31.0819	29.2228	1.0636	8.0931	7.5530	1.0715	17.2698	15.3231	1.1270	2.2051	2.4643	1.0152	2.9311	2.7455	1.0910	30.7520	28.2265											
2.22	21	9.67E+19	8.65E+17	1.12E+02	1.5511	1.4587	1.0633	0.5572	0.5677	0.9814	9.9355	9.3704	1.0603	18.3312	15.5372	1.1798	10.5456	9.1456	1.1531	25.9446	21.0199	1.2343	0.9131	0.8747	1.0440	69.9774	56.4463	1.2397	7.9118	7.2231	1.0953	3.3513	3.1625	1.0597	55.9135	38.9558											
2.23	21	5.46E+19	8.21E+18	6.64E+00	3.2812	3.1226	1.0508	7.0667	7.1053	0.9946	16.3982	15.0934	1.0864	13.9722	12.9358	1.0805	22.8175	21.2352	1.0745	1.5966	1.0756	1.0573	11.4466	10.9733	1.0431	50.0854	41.1454	1.1731	29.8415	26.4179	1.1296	92.6296	40.0894	2.3106	12.6960	11.9230											
2.24	21	4.94E+18	2.25E+17	2.19E+01	4.1773	3.9982	1.0448	4.8986	8.6529	0.9822	13.1574	11.9566	1.1004	1.0643	0.9978	1.0666	1.1303	1.1760	0.9612	17.7986	14.7791	1.2043	7.7820	7.7566	1.0033	13.4708	11.8847	1.1335	36.8291	25.2172	1.4605	17.0204	14.9300	1.1400	1.9883	1.7063											
2.25	21	3.82E+20	3.28E+18	1.17E+02	346.9642	33.0141	10.5096	1.1995	1.1563	1.3074	3.6220	3.4815	1.0404	6.7309	5.9411	1.1329	3.0627	3.0013	1.0204	25.5568	23.4436	1.0901	0.8479	0.8503	0.9972	38.4819	29.8643	1.2886	8.9907	7.5690	1.1878	18.2428	14.3576	1.2706	42.7931	28.0766											
2.26	21	6.15E+18	1.52E+17	4.06E+01	2.7406	2.6857	1.0204	4.3114	4.0912	1.0538	88.9473	54.7913	1.6234	6.7798	5.7309	1.1830	4.0148	3.8386	1.0459	14.7143	12.0561	1.2205	91.4831	54.2440	1.6865	7.8886	6.9988	1.1271	2.8131	2.6460	1.0632	2.9061	2.7776	1.0462	84.3872	36.0414											
2.27	21	1.72E+18	1.43E+17	1.20E+01	1.7951	1.8047	0.9947	17.8325	16.3268	1.0922	12.3639	10.7422	1.1165	3.1437	3.0447	1.0325	0.8509	0.8496	1.0104	5.6233	5.2357	1.0740	4.1484	4.1307	1.0043	21.9966	18.6574	1.1790	4.6730	4.4585	1.0481	0.6253	0.6897	0.9066	70.9920	58.8257											
2.28	21	3.23E+17	2.34E+16	1.38E+01	1.1295	1.0805	1.0453	16.1783	13.6698	1.1835	98.3502	62.0258	1.5856	11.9746	9.9481	1.2037	10.4517	8.9728	1.1648	7.8819	6.7670	1.1647	4.8983	4.5923	1.0666	30.0817	23.7869	1.2646	5.1431	4.8753	1.0549	7.1058	7.2468	0.9805	5.1463	4.6428											
2.29	21	4.43E+21	6.22E+18	6.99E+02	41.8339	15.4409	2.70																																								

Σ	D2S441						D3S1358						D5S818						D7S820						D8S1179						FGA						SE33						TH01						TPOX						vWA																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO</

SS_2c: LRs values (overall and per-marker) computed by STRmix™ v2.7 for mixtures with two estimated contributors, considering an FST value of 0 and 0.01. The ratio between the two LR values was calculated.

PAIR		NUMBER OF MARKERS ANALYZED	OVERALL		CSF1PO		D10S1248		D12S391		D13S317		D16S539		D18S51		D19S433		D1S1656		D21S11		D22S1045		D2S1338											
			0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO										
2_1	18	6.321E+15	7.3406E+14	8.61012724	1.51E+00	1.52E+00	0.9927	8.77E+01	6.03E+01	1.4540	-	#VALOR!	7.01E-02	1.04E-01	0.6736	4.33E+00	3.90E+00	1.1100	-	#VALOR!	1.31E+01	1.15E+01	1.1386	-	#VALOR!	4.90E+01	3.55E+01	1.3816	8.81E+00	8.03E+00	1.0973	1.25E+01	1.09E+01			
2_2	20	4.22E+10	2.88E+09	1.46E+01	1.75E+00	1.71E+00	1.0229	1.09E+00	1.05E+00	1.0403	5.44E-02	9.01E-02	0.6035	1.02E+00	1.06E+00	0.9673	4.76E+00	4.57E+00	1.0418	5.09E+00	4.52E+00	1.1269	3.99E+01	4.04E+01	0.9871	1.76E+01	1.55E+01	1.1380	1.95E+03	1.97E+02	9.8597	9.70E+00	8.29E+00	1.1703	2.08E+01	2.31E-01
2_3	20	1.75E+19	5.12E+17	3.42E+01	8.39E+00	7.00E+00	1.1984	2.05E+00	1.90E+00	1.0792	9.91E+00	8.40E+00	1.1800	6.75E+00	6.13E+00	1.1026	5.29E+00	4.84E+00	1.0914	-	#VALOR!	1.08E+02	6.66E+01	1.6269	4.51E+01	2.89E+01	1.5594	9.80E+00	8.48E+00	1.1560	3.74E+00	3.63E+00	1.0302	2.42E+01	1.65E+01	
2_4	20	2.59E+23	1.67E+22	1.55E+01	2.40E+00	2.36E+00	1.0179	6.85E+01	5.22E+01	1.3113	3.01E+02	2.03E+02	1.4821	2.75E+01	2.49E+01	1.1051	1.47E+01	1.26E+01	1.1708	2.49E+01	2.36E+01	1.0538	5.03E+00	4.76E+00	1.0565	2.14E+01	1.60E+01	1.3389	1.76E+02	1.31E+02	1.3435	5.20E+00	5.00E+00	1.0405	-	-
2_5	20	5.08E+22	1.04E+21	4.91E+01	1.46E+02	8.44E+01	1.7302	1.69E+01	1.57E+01	1.0757	9.85E+01	5.30E+01	1.8577	3.77E+01	3.14E+01	1.2034	2.04E+00	1.66E+00	1.2294	1.69E+01	1.51E+01	1.1225	3.33E+02	2.08E+02	1.5999	1.77E+02	1.36E+02	1.3064	1.04E+01	1.00E+01	1.0429	1.23E+01	1.21E+01	1.0157	1.70E+01	1.55E+01
2_6	20	7.90E+25	4.78E+24	1.65E+01	6.45E+01	4.04E+01	1.5969	1.57E+01	1.47E+01	1.0694	2.20E+01	1.91E+01	1.1524	6.96E+00	6.86E+00	1.0137	9.37E+00	8.83E+00	1.0613	3.69E+01	3.42E+01	1.0815	6.42E+00	6.29E+00	1.0219	6.84E+01	5.83E+01	1.1728	1.54E+02	1.04E+02	1.4854	2.29E+01	1.94E+01	1.1830	-	-
2_7	20	1.05E+27	3.73E+25	2.80E+01	9.09E+01	6.67E+01	1.3624	1.08E+01	1.01E+01	1.0669	7.80E+02	3.94E+02	1.9800	5.52E+00	5.25E+00	1.0509	6.37E+00	5.93E+00	1.0742	3.23E+01	2.74E+01	1.1764	2.54E+02	1.58E+02	1.6074	3.29E+01	2.69E+01	1.2216	1.60E+02	1.15E+02	1.3942	1.07E+01	1.03E+01	1.0344	3.36E+01	3.09E+01
2_8	20	1.33E+17	5.58E+14	2.38E+02	3.63E+01	1.45E+01	2.5042	2.04E+00	1.18E+00	1.7302	4.37E+01	3.30E+01	1.3244	3.96E+00	3.68E+00	1.0754	6.59E+00	5.88E+00	1.1219	1.08E+02	7.60E+01	1.4211	9.26E-01	1.1380	-	#VALOR!	3.17E+01	2.72E+01	1.1667	1.31E+00	1.20E+00	1.1002	3.77E+01	3.49E+01		
2_9	20	4.39E+23	1.01E+22	4.35E+01	1.43E+02	7.32E+01	1.9580	1.15E+01	1.10E+01	1.0528	9.10E+00	7.86E+00	1.1581	2.61E+00	2.39E+00	1.0883	3.91E+00	3.49E+00	1.1189	3.12E+01	2.65E+01	1.1777	2.12E+00	2.10E+00	1.0123	2.22E+01	1.87E+01	1.1906	3.23E+01	2.68E+01	1.2024	-	#VALOR!	4.74E+00	4.44E+00	
2_10	20	1.40E+25	1.43E+23	9.78E+01	7.60E-01	8.16E-01	0.9314	1.33E+02	7.56E-01	1.7594	9.13E+01	3.17E+01	2.8801	2.01E+02	1.51E+02	1.3274	1.53E+01	1.40E+01	1.0937	2.53E+01	2.14E+01	1.1800	2.30E+02	1.50E+02	1.5353	3.67E+01	3.39E+01	1.0826	2.69E+02	1.78E+02	1.5108	4.72E+00	4.56E+00	1.0354	6.96E+01	4.74E+01
2_11	20	1.44E+26	8.22E+24	1.75E+01	2.84E+02	1.48E+02	1.9208	1.58E+01	1.48E+01	1.0726	7.25E+01	6.20E+01	1.1695	5.58E+00	5.54E+00	1.0072	9.72E+00	8.87E+00	1.0960	1.95E+01	1.69E+01	1.1521	2.44E+01	2.07E+01	1.1817	3.60E+01	3.31E+01	1.0862	5.56E+01	4.42E+01	1.2601	1.23E+01	1.21E+01	1.0157	-	-
2_12	21	4.27E+02	4.98E+01	8.56E+00	3.82E-01	3.61E-01	1.0590	8.39E+00	8.22E+00	1.0197	1.60E+00	1.17E+00	1.3583	9.60E-01	8.55E-01	1.1238	9.12E-01	8.79E-01	1.0375	6.47E+00	5.72E+00	1.1307	2.19E+00	2.05E+00	1.0639	3.45E-02	3.59E-02	0.9606	1.60E+00	1.33E+00	1.2070	1.23E-02	9.08E-03	1.3588	2.56E+00	1.88E+00
2_13	21	2.45E+12	8.22E+11	2.98E+00	9.69E+00	8.93E+00	1.0856	8.54E+00	7.99E+00	1.0677	2.29E+01	2.00E+01	1.1440	4.87E+00	4.39E+00	0.8910	4.65E+00	4.27E+00	1.0879	1.31E+01	1.39E+01	0.9412	1.38E+00	1.35E+00	1.0223	5.12E+01	3.14E+01	1.6311	1.35E+00	1.68E+00	0.8045	1.13E+01	9.39E+00	1.2022	2.11E+01	1.95E+01
2_14	21	1.54E+14	3.62E+13	4.24E+00	2.32E+00	2.30E+00	1.0107	3.01E+00	2.79E+00	1.0790	4.20E+00	3.82E+00	1.1002	2.09E+00	2.05E+00	1.0193	4.96E+00	5.34E+00	0.9285	1.49E+00	1.42E+00	1.0486	6.67E+00	6.78E+00	0.9838	1.59E+02	1.22E+02	1.3079	1.41E+00	1.34E+00	1.0516	3.06E+00	3.02E+00	1.0126	4.73E+01	4.25E+01
2_15	21	1.20E+15	3.91E+13	3.06E+01	1.33E+01	6.09E+00	2.1867	6.50E+00	6.37E+00	1.0216	5.62E+00	3.70E+00	1.5183	1.85E+00	1.88E+00	0.9822	8.36E+00	7.62E+00	1.0965	5.46E+00	5.33E+00	1.0245	6.29E+00	5.75E+00	1.0930	1.20E+01	1.11E+01	1.0768	2.11E+00	2.36E+00	0.8957	1.13E+01	7.63E+00	1.4803	3.53E+00	2.51E+00
2_16	21	5.73E+15	8.56E+14	6.69E+00	8.09E+00	7.24E+00	1.1171	1.26E+00	1.05E+00	1.1981	1.09E+01	9.90E+00	1.1004	2.05E+01	1.65E+01	1.2397	7.37E+00	7.01E+00	1.0518	1.46E+00	3.04E+00	0.4812	1.30E+01	1.09E+01	1.1883	1.52E+01	1.27E+01	1.1908	1.46E+01	1.10E+01	1.3301	1.19E+00	1.16E+00	1.0253	2.29E+01	2.02E+01
2_17	21	2.14E+16	8.45E+14	2.54E+01	1.15E+00	9.69E-01	1.1909	1.07E+01	9.30E+00	1.1507	9.01E+00	7.58E+00	1.1883	6.22E+00	5.57E+00	1.1162	6.93E-01	5.44E-01	1.2733	1.97E+00	2.42E+00	0.8162	7.78E+00	6.48E+00	1.1993	6.83E+00	5.84E+00	1.1706	7.01E+00	6.13E+00	1.1424	1.09E+01	1.01E+01	1.0698	6.91E+00	4.87E+00
2_18	21	4.51E+18	1.04E+16	4.33E+02	5.04E+00	4.71E+00	1.0704	5.60E+00	5.64E+00	0.9944	5.77E+00	5.59E+00	1.0337	1.49E-01	6.92E-02	2.1534	5.51E+00	4.22E+00	1.3069	7.81E+00	6.61E+00	1.1813	5.84E+00	5.73E+00	1.0194	5.81E+01	4.77E+01	1.2171	4.24E+00	3.02E+00	1.4027	9.03E+00	8.57E+00	1.0535	4.82E+01	3.30E+01
2_19	21	5.50E+18	1.67E+17	3.29E+01	3.64E+00	3.48E+00	1.0457	1.74E+01	1.55E+01	1.1186	2.26E+02	1.03E+02	2.1914	2.62E+00	2.45E+00	1.0662	2.54E+01	1.76E+01	1.4472	1.12E+01	9.40E+00	1.1902	2.25E+00	1.93E+00	1.1634	2.00E+01	1.52E+01	1.3295	6.56E+00	5.58E+00	1.1770	3.95E+00	3.82E+00	1.0345	6.93E+00	6.53E+00
2_20	21	8.12E+20	3.99E+19	2.04E+01	3.34E+00	3.07E+00	1.0888	3.14E+00	2.70E+00	1.1640	1.20E+01	1.03E+01	1.1582	4.17E+00	3.81E+00	1.0965	7.40E+00	5.55E+00	1.3340	1.24E+01	1.11E+01	1.1149	1.69E+01	1.55E+01	1.0869	2.69E+01	2.46E+01	1.0976	1.72E+01	1.42E+01	1.2081	5.10E+00	5.06E+00	1.0082	1.16E+01	1.11E+01

R																														
	D2S441			D3S1358			D5S818			D7S820			D8S1179			FGA			SE33			TH01			TPOX			vWA		
RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO
1.1510	3.93E+00	3.49E+00	1.1285	3.22E+00	3.24E+00	0.9933	3.52E+00	3.42E+00	1.0309	1.19E+01	1.03E+01	1.1526	1.11E+01	9.98E+00	1.1146	7.51E+00	6.51E+00	1.1534	1.27E+02	8.24E+01	1.5455	3.65E+00	3.52E+00	1.0373	1.81E+01	1.34E+01	1.3519	5.94E+00	5.49E+00	1.0821
0.9013	9.29E+00	8.47E+00	1.0962	4.70E+00	4.43E+00	1.0613	1.60E+00	1.63E+00	0.9780	1.08E+01	8.77E+00	1.2363	4.92E+00	4.58E+00	1.0744	9.63E+00	8.12E+00	1.1850	-	-	#VALOR!	4.34E+00	4.07E+00	1.0660	2.73E+00	2.74E+00	0.9970	1.41E+00	1.56E+00	0.9029
1.4669	5.23E+00	4.97E+00	1.0517	4.24E+00	4.04E+00	1.0497	5.62E+00	5.27E+00	1.0703	5.42E+00	4.98E+00	1.0870	7.95E+00	6.72E+00	1.1831	1.81E+01	1.38E+01	1.3061	3.96E+01	2.74E+01	1.4426	5.03E+00	4.56E+00	1.1027	7.12E+00	5.73E+00	1.2410	4.79E+00	4.47E+00	1.0174
#VALOR!	2.76E+00	2.49E+00	1.1121	7.59E+00	7.26E+00	1.0460	1.20E+00	1.18E+00	1.0119	1.43E+01	1.28E+01	1.1174	4.32E+01	3.59E+01	1.2023	9.57E+01	7.97E+01	1.2016	4.78E+01	4.69E+01	1.0184	2.32E+01	1.98E+01	1.1698	3.39E+01	2.87E+01	1.1845	9.47E+00	8.74E+00	1.0638
1.0930	6.27E+00	6.21E+00	1.0095	2.01E+01	1.84E+01	1.0904	-	-	#VALOR!	1.58E+01	1.54E+01	1.0255	1.38E+01	1.27E+01	1.0926	1.81E+01	1.32E+01	1.3639	2.90E+02	1.95E+02	1.4877	1.52E+02	1.90E+02	0.8004	1.96E+00	1.85E+00	1.0611	2.92E+01	1.76E+01	1.1685
#VALOR!	5.33E+00	5.00E+00	1.0649	6.53E+00	6.14E+00	1.0635	1.84E+00	1.67E+00	1.0999	6.85E+00	6.65E+00	1.0302	1.36E+01	1.23E+01	1.1017	5.10E+01	4.22E+01	1.2097	6.67E+02	3.87E+02	1.7224	1.52E+01	1.49E+01	1.0196	6.56E+00	6.38E+00	1.0282	4.72E+01	4.42E+01	1.1091
1.0871	6.91E+01	5.49E+01	1.2583	3.26E+00	3.28E+00	0.9942	6.93E+00	6.42E+00	1.0807	1.79E+00	1.30E+00	1.3765	1.33E+01	1.30E+01	1.0197	1.89E+01	1.79E+01	1.0557	-	-	#VALOR!	7.99E+00	8.26E+00	0.9679	3.28E+01	2.73E+01	1.1986	1.37E+01	1.33E+01	1.0256
1.0792	3.31E-02	4.18E-02	0.7914	2.28E+01	4.28E+00	5.3425	5.69E-01	5.41E-01	1.0518	1.29E+01	1.18E+01	1.0911	2.96E+00	2.52E+00	1.1733	9.23E+00	9.15E+00	1.0084	4.09E+01	3.63E+01	1.1276	5.80E+00	5.43E+00	1.0689	7.60E+00	7.00E+00	1.0854	3.68E+01	1.80E+01	2.0431
1.0659	3.89E+01	2.83E+01	1.3759	4.26E+00	4.40E+00	0.9683	5.98E+00	5.52E+00	1.0846	8.77E+01	5.57E+01	1.5750	1.18E+01	1.02E+01	1.1574	1.45E+02	9.36E+01	1.5485	5.89E+01	4.68E+01	1.2584	2.27E+01	1.75E+01	1.2954	7.07E+00	6.57E+00	1.0769	9.05E+00	7.89E+00	1.1473
1.4678	5.80E+01	4.89E+01	1.1861	1.11E+00	8.27E-01	1.3476	3.12E+00	2.99E+00	1.0436	3.64E+00	3.50E+00	1.0406	2.66E+01	2.31E+01	1.1521	1.63E+01	1.51E+01	1.0786	5.95E+01	4.59E+01	1.2950	1.36E+00	1.21E+00	1.1282	1.02E+00	1.02E+00	1.0057	-	-	#VALOR!
#VALOR!	2.42E+01	2.09E+01	1.1555	8.71E+00	8.45E+00	1.0308	8.03E+00	6.89E+00	1.1657	6.82E+01	5.07E+01	1.3446	1.51E+01	1.36E+01	1.1123	1.75E+01	1.47E+01	1.1905	2.43E+01	2.22E+01	1.0942	1.44E+01	1.28E+01	1.1235	4.60E+00	4.47E+00	1.1017	1.43E+01	1.38E+01	1.0383
1.3600	8.54E-01	7.54E-01	1.1322	2.30E+01	1.97E+01	1.1709	4.44E+00	4.45E+00	0.9973	2.79E+00	2.50E+00	1.1194	8.04E+00	7.99E+00	1.0072	5.79E+01	5.25E+01	1.1025	5.64E+00	4.69E+00	1.1020	1.98E+00	1.91E+00	1.0329	1.01E+00	1.01E+00	1.0052	3.01E+01	3.18E+01	0.9471
1.0840	5.77E+00	4.41E+00	1.3086	6.81E-01	7.37E-01	0.9248	3.73E+00	3.62E+00	1.0305	3.22E+00	3.27E+00	0.9829	6.12E+00	5.73E+00	1.0677	4.04E+00	4.18E+00	0.9649	1.04E+00	1.04E+00	1.2074	2.49E+00	2.32E+00	1.0745	4.33E+01	4.90E+01	0.8835	8.50E+00	7.29E+00	1.1658
1.1128	1.82E+00	1.75E+00	1.0427	2.46E+00	2.30E+00	1.0659	5.72E+00	5.05E+00	1.1339	1.19E+01	9.89E+00	1.2055	6.37E+00	4.95E+00	1.2870	3.75E+00	3.63E+00	1.0326	9.75E+00	9.23E+00	1.0562	1.07E+00	1.05E+00	1.0278	9.88E+01	9.63E+01	1.0269	2.07E+01	1.99E+01	1.0380
1.4077	1.71E+00	1.94E+00	0.8839	1.23E+01	1.02E+01	1.2113	1.38E+00	1.41E+00	0.9743	4.53E+00	4.46E+00	1.0161	2.81E+00	2.67E+00	1.0517	6.34E+00	6.66E+00	0.9531	2.28E+01	1.09E+01	2.1010	3.35E+00	2.87E+00	1.1684	2.40E+00	2.05E+00	1.1706	1.07E+01	8.75E+00	1.2192
1.1352	1.19E+00	1.22E+00	0.9727	7.67E-01	7.78E-01	0.9864	1.31E+00	1.21E+00	1.0771	6.62E+01	5.04E+01	1.3146	6.92E+00	6.13E+00	1.1297	1.12E+01	1.03E+01	1.0861	4.94E+01	3.81E+01	1.2993	9.85E+00	8.44E+00	1.1675	3.83E+00	3.66E+00	1.0471	2.64E+01	2.12E+01	1.2484
1.4197	5.69E+00	5.27E+00	1.0783	5.44E+00	5.13E+00	1.0603	4.70E+00	4.13E+00	1.1376	1.12E+01	1.02E+01	1.0959	4.19E+01	4.57E-01	0.9158	6.50E+01	3.19E+01	2.0368	1.91E+02	1.10E+02	1.3107	3.55E+00	3.71E+00	0.9554	2.16E+00	1.96E+00	1.1001	1.26E+01	1.11E+01	1.1360
1.4583	7.81E+00	7.65E+00	1.0212	1.76E+01	1.60E+01	1.1011	1.00E+01	8.39E+00	1.1974	2.68E+01	2.92E+01	0.9153	3.81E+00	3.00E+00	1.2700	2.53E+01	2.10E+01	1.2068	8.43E+03	5.06E+02	16.6471	1.59E+01	1.26E+01	1.2668	2.00E+00	1.82E+00	1.1020	7.17E+01	7.11E+01	1.0094
1.0616	6.02E+00	5.77E+00	1.0426	1.41E+01	1.25E+01	1.1320	1.98E+00	1.90E+00	1.0435	4.89E+00	4.57E+00	0.9666	7.89E+00	7.11E+00	1.0936	5.41E+01	2.63E+01	2.0574	2.74E+00	2.75E+00	0.9956	1.92E+00	1.92E+00	1.90E+00	7.41E+00	6.21E+00	1.1948	6.21E+00	1.1948	1.1948
1.0405	7.60E+01	6.02E+01	1.2630	8.46E+00	8.18E+00	1.0349	6.80E+00	6.09E+00	1.1151	2.12E+00	1.95E+00	1.0880	5.17E+01	2.79E+01	1.8559	1.27E+01	1.09E+01	1.1718	1.87E+01	1.41E+01	1.3234	1.08E+01	1.01E+01	1.0748	2.56E+00	2.43E+00	1.0532	1.15E+01	1.06E+01	1.0942
1.0517	6.32E+00	6.53E+00	0.9686	3.45E+00	3.53E+00	0.9770	3.43E+00	3.51E+00	0.9785	2.31E+01	1.61E+01	1.4378	7.29E+01	6.16E+01	1.1836	1.65E+01	1.45E+01	1.1380	3.87E+02	2.58E+02	1.5015	1.98E+01	1.63E+01	1.2144	6.09E+00	6.00E+00	1.0145	3.87E+01	3.57E+01	1.0846
1.2402	5.16E+00	4.82E+00	1.0710	4.17E+00	3.99E+00	1.0451	1.00E+01	8.32E+00	1.2070	5.38E+00	5.03E+00	1.0684	2.11E+01	1.97E+01	1.0720	2.41E+00	2.86E+00	0.8428	4.20E+01	3.45E+01	1.2170	5.85E+00	5.53E+00	1.0562	7.12E+01	7.21E+01	0.9879	8.91E+02	1.23E+02	7.2714
1.0935	5.36E+00	5.13E+00	1.0443	9.31E+00	8.47E+00	1.0983	4.49E+00	4.62E+00	0.9715	4.79E+00	4.53E+00	1.0594	9.93E+00	9.33E+00	1.0648	1.48E+01	1.31E+01	1.1313	5.72E+00	5.17E+00	1.1069	1.09E+01	1.00E+01	1.0880	1.22E+00	1.12E+00	1.0866	1.06E+01	1.05E+01	1.0041
1.2470	4.54E+01	3.90E+01	1.1629	5.43E+01	4.43E+01	1.2271	1.00E+01	8.39E+00	1.1974	4.88E+00	3.37E+00	1.4486	1.51E+01	1.33E+01	1.1310	6.99E+01	6.45E+01	1.0849	3.45E+01	2.61E+01	1.3180	2.16E+01	1.65E+01	1.3054	1.99E+01	1.70E+01	1.1698	6.65E+00	3.46E+00	1.1928
1.6240	3.11E+00	5.36E+00	0.9955	5.53E+00	5.13E+00	1.0446	2.36E+00	2.31E+00	1.0220	1.17E+01	1.05E+01	1.1145	3.59E+01	2.54E+01	1.2146	3.58E+00	4.23E+00	0.8450	9.84E+00	1.29E+01	0.7602	4.								

PAIR	NUMBER OF MARKERS ANALYZED	L																														
		OVERALL		CSFIPO			D10S1248			D12S391			D13S317			D16S539			D18S51			D19S433			DIS1656			D21S11				
		0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	
		0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	
3_1	21	3.46E+10	2.88E+08	1.20E+02	0.270325711	2.57E-01	1.0538	2.197329488	2.14E+00	1.0275	3.843136613	3.52E+00	1.0923	1.61594627	1.58E+00	1.0239	1.480844593	1.45E+00	1.0193	4.931834737	4.46E+00	1.1063	5.480996822	4.21E+00	1.3007	5.660619098	4.99E+00	1.1340	2.612272405	2.47E+00	1.0584	0.545253415
3_2	21	1.11E+09	1.64E+08	6.76E+00	3.0051	4.1792	0.7191	0.5965	0.5686	1.0489	17.5571	17.5566	1.0000	1.3118	1.3146	0.9978	4.9690	6.0980	0.8149	4.1406	4.6792	0.8849	11.2515	8.8736	1.2680	2.1595	2.1176	1.0198	5.5954	4.5877	1.2197	0.5453
3_3	21	3.09E+02	1.53E+02	2.02E+00	2.4180	2.2919	1.0550	1.4385	1.4199	1.0131	1.5182	1.5075	1.0071	1.4565	1.4398	1.0116	1.3974	1.3793	1.0131	3.1443	2.9635	1.0610	0.3751	0.3976	0.9433	1.6441	1.6258	1.0112	6.1483	4.8297	1.2730	0.4728
3_4	21	2.39E+08	1.23E+07	1.94E+01	1.2324	1.1812	1.0433	0.8606	0.8780	0.9802	2.3627	2.0154	1.1723	3.6922	3.4623	1.0664	0.9706	0.9826	0.9878	0.6878	0.6240	1.1023	7.2692	5.4723	1.3284	3.8422	3.5244	1.0902	1.9142	1.7575	1.0891	1.1926
3_5	21	1.18E+15	7.57E+11	1.55E+03	1.1828	1.0957	1.0795	0.9859	0.9906	0.9953	2.0250	1.9630	1.0316	2.9581	2.8008	1.0562	9.2291	7.0662	1.3061	2.7521	2.6150	1.0525	44.7180	16.8533	2.6534	2.0828	1.8111	1.1500	2.9824	2.8140	1.0598	331.1969
3_6	21	1.24E+09	1.22E+07	1.02E+02	0.9874	0.9981	0.9893	0.5965	0.6172	0.9664	0.9667	0.8453	1.1436	3.0581	2.7181	1.1251	4.9690	4.3792	1.1347	1.9760	1.7292	1.1427	36.1681	15.6146	2.3163	11.2720	9.0885	1.2402	6.0863	5.3499	1.1377	2.3764
3_7	21	1.90E+06	2.56E+05	7.42E+00	0.6998	0.6209	1.1271	0.5742	0.5639	1.0183	2.3535	2.2483	1.0468	1.7429	1.6102	1.0824	1.6111	1.5665	1.0285	0.8763	0.7547	1.1611	1.0661	1.0612	1.0046	11.1854	9.1189	1.2266	7.2890	6.3128	1.1546	3.6095
3_8	21	4.44E+10	2.45E+09	1.81E+01	0.7827	0.7976	0.9813	3.8826	3.1438	1.2350	17.7630	13.1526	1.3505	1.5165	1.4938	1.0152	1.9721	1.9076	1.0338	3.1858	2.9963	1.0633	7.2075	5.4319	1.3269	6.1910	4.8720	1.2707	12.3996	8.8797	1.3964	1.1926
3_9	21	9.75E+09	9.89E+08	9.86E+00	2.4180	2.2919	1.0550	2.1973	2.1385	1.0275	3.4519	3.1932	1.0810	1.6159	1.5782	1.0239	4.0604	3.7151	1.0930	47.7668	20.0510	2.3823	2.5031	2.3791	1.0521	3.2026	2.9905	1.0709	3.7747	3.4704	1.0877	1.9808
3_10	21	4.55E+06	5.83E+05	7.81E+00	1.2007	1.1981	1.0022	1.2074	1.2102	0.9977	0.0753	0.0726	1.0368	1.5033	1.4822	1.0142	1.3974	1.3793	1.0131	3.3857	3.1598	1.0715	0.6189	0.6374	0.9711	7.2516	6.2175	1.1663	1.1467	1.1483	0.9986	1.4061
3_11	21	7.70E+04	1.63E+04	4.71E+00	1.5539	1.5396	1.0093	2.4759	2.3713	1.0441	3.7801	3.4512	1.0953	3.6056	3.2012	1.1263	0.0611	0.0616	0.9928	0.3621	0.3314	1.0926	0.8262	0.7899	1.0459	3.3219	3.0684	1.0826	8.8827	6.9366	1.2806	0.7967
3_12	21	6.33E+08	3.50E+07	1.81E+01	3.6014	3.3022	1.0906	1.1436	1.1021	1.0376	3.2523	3.0330	1.0723	1.6159	1.5782	1.0239	2.5672	2.3552	1.0900	50.1582	28.9583	1.7321	8.0157	5.9359	1.3504	15.8741	10.1465	1.5645	3.5772	3.2129	1.1134	0.7612
3_13	21	6.26E+08	6.70E+07	9.35E+00	1.2007	1.1981	1.0022	1.1252	1.0472	1.0745	17.0519	1.0723	1.6253	1.5789	1.0294	1.0723	1.6253	1.5789	1.0294	1.8860	1.8434	1.0231	0.9659	0.9719	0.9938	6.0335	5.3086	1.1365	2.2430	2.1574	1.0397	1.8425
3_14	21	2.88E+09	2.14E+08	1.35E+01	1.8227	1.7639	1.0334	1.4385	1.4200	1.0131	5.4771	4.9114	1.1152	1.6159	1.5782	1.0239	2.4163	2.3265	1.0386	2.9371	2.7647	1.0623	0.6393	0.6205	1.0303	3.3338	3.0783	1.0830	3.7747	3.4704	1.0877	2.6121
3_15	21	8.16E+10	1.02E+09	8.01E+01	3.6014	3.3022	1.0906	1.1436	1.1021	1.0376	2.5680	2.1494	1.1947	1.2315	1.1778	1.0456	0.9706	0.9826	0.9878	2.5410	2.4156	1.0519	0.6393	0.6205	1.0303	4.6927	4.1138	1.1407	3.7747	3.4704	1.0877	2.6121
3_16	21	5.12E+06	7.68E+05	6.66E+00	1.5539	1.5396	1.0093	6.2366	5.0345	1.2388	2.3120	2.2095	1.0464	3.7434	3.4366	1.0893	2.8775	2.7190	1.0583	5.5043	4.9268	1.1172	0.9749	0.9800	0.9947	4.5541	4.1012	1.1104	20.5279	14.0612	1.4599	3.3858
3_17	21	8.64E+03	2.19E+03	3.94E+00	0.0658	0.0666	0.9887	1.2314	1.2337	0.9982	4.6450	4.2092	1.1035	0.2257	0.2158	1.0456	0.6412	0.6241	1.0274	1.4008	3.6565	1.0944	1.0731	1.0674	1.0053	6.0453	5.2909	1.1426	1.0976	1.0233	1.0726	0.4793
3_18	21	2.25E+05	3.31E+04	6.80E+00	0.1881	0.1834	1.0255	0.2482	0.2470	1.0047	13.3306	9.5700	1.3930	1.2315	1.1778	1.0456	0.5802	0.5994	0.9680	5.0216	4.4464	1.1294	0.2278	0.2276	1.0011	26.9512	16.4416	1.6392	2.2540	2.1666	1.0403	0.5542
3_19	21	3.61E+12	5.87E+10	6.15E+01	1.5539	1.5396	1.0093	9.4637	7.4837	1.2646	7.4813	6.3707	1.1743	3.5157	3.2287	1.0889	1.0177	1.0147	1.0030	48.7917	20.2218	2.4128	1.0653	1.0293	1.0349	0.4045	0.3635	1.1128	2.0732	2.0151	1.0288	86.1897
3_20	21	9.79E+08	1.36E+08	7.21E+01	1.5539	1.5396	1.0093	1.2109	1.2102	1.0006	0.6997	0.5955	1.1750	2.8744	2.7286	1.0534	1.4808	1.4528	1.0193	5.5254	4.9186	1.1234	1.9243	1.7547	1.0966	5.8366	5.1209	1.1398	3.3823	3.1506	1.0736	1.2519
3_21	21	8.05E+09	3.97E+08	2.03E+01	1.4497	1.4378	1.0083	0.8384	0.8561	0.9794	25.1302	17.0680	1.4724	1.6159	1.5782	1.0239	2.8775	2.7190	1.0583	5.9026	5.2310	1.1284	52.5111	29.5246	1.7664	12.5066	9.9841	1.2527	1.4892	1.4617	1.0188	0.9572
3_22	21	8.30E+09	1.02E+09	8.12E+00	0.9874	0.9981	0.9893	0.5742	0.5639	1.0183	13.0482	9.9895	1.3062	2.8744	2.7286	1.0534	3.0578	2.7689	1.1044	7.8759	6.4826	1.2149	2.1114	1.9809	1.0658	3.6509	3.3759	1.0815	3.5772	3.2129	1.1134	1.0179
3_23	21	1.79E+07	3.87E+06	4.63E+00	1.5539	1.5396	1.0093	4.5451	3.7380	1.2159	13.5872	9.8852	1.3745	0.6007	0.5872	1.0229	1.9721	1.9076	1.0338	7.8784	3.4737	1.0762	0.9317	0.9408	0.9904	2.8542	2.6858	1.0627	2.8657	2.6856	1.0671	3.0736
3_24	21	1.78E+12	3.50E+10	5.10E+01	2.1391	2.0553	1.0408	4.8693	4.3843	1.1106	10.6564	8.8082	1.2098	2.9581	2.8008	1.0562	2.5403	2.4052	1.0562	7.8159												

2mix Studio																																							
LR																																							
MARKER																																							
D22S1045		D2S1338		D2S441		D3S1358		D5S818		D7S820		D8S1179		FGA		SE33		TH01		TPOX		vWA																	
0.01	RATIO	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00																	
5.37E-01	1.0146	3.0579677	2.88E+00	1.0604	0.664105652	6.90E-01	0.9631	2.983853265	2.72E+00	1.0973	1.935063376	1.89E+00	1.0244	5.54752233	4.74E+00	1.1703	1.048580885	8.76E-01	1.1968	9.233106494	7.08E+00	1.3045	932.4956192	7.59E+01	12.2939	16.649054	8.30E+00	2.0056	1.34320065	1.30E+00	1.0320	2.215050333	2.13E+00	1.0399					
0.0482	11.3177	1.5591	1.5541	1.0032	1.1491	1.1645	0.9868	1.9955	2.3005	0.8674	1.8702	2.3049	0.8114	2.9028	3.5424	0.8194	4.5317	6.1543	0.7363	3.4119	3.5935	0.9494	24.4977	21.4998	1.1394	2.0885	2.3952	0.8720	0.8448	0.7621	1.1085	1.3595	1.4487	0.9384					
0.4924	0.9602	0.0122	0.0122	1.0069	1.0247	1.0301	0.9948	1.1812	1.1835	0.9981	1.8702	1.8259	1.0242	1.8969	1.8476	1.0267	0.9753	0.9825	0.9927	1.9321	1.8760	1.0299	15.0987	11.5422	1.3081	1.1678	1.1686	0.9993	1.7877	1.7383	1.0284	1.6322	1.5925	1.0249					
1.1865	1.0051	7.3461	6.0652	1.2112	6.2411	5.4149	1.1526	1.2872	1.2851	1.0016	4.1955	3.6093	1.1624	3.6445	3.3745	1.0800	2.7453	2.2407	1.2252	19.2249	13.6435	1.4091	0.8231	0.3564	2.3094	1.0307	1.0363	0.9946	2.8848	2.5699	1.1225	5.2015	4.6343	1.1224					
31.0291	10.6738	12.9533	10.1529	1.2758	5.9534	5.0670	1.1749	1.2872	1.2851	1.0016	1.3393	1.3279	1.0086	0.9148	0.8662	1.0561	5.8308	5.2086	1.1195	1.4362	1.4192	1.0120	59.2689	33.0514	1.7932	4.4327	4.0853	1.0850	119.7584	15.7403	7.6084	2.2512	2.1571	1.0436					
2.2304	1.0655	0.0632	0.0632	0.9999	1.4265	1.4175	1.0064	1.8649	1.8147	1.0277	0.9052	0.8871	1.0204	2.3187	2.2190	1.0449	2.4171	2.2966	1.0525	2.0960	1.8208	1.1511	16.3339	12.4094	1.3163	1.4998	1.4891	1.0072	164.4599	16.1776	10.1659	1.7032	1.6763	1.0161					
3.3219	1.0866	2.8494	2.5210	1.1303	4.2063	3.7995	1.1070	2.1334	2.0672	1.0320	3.0529	2.7601	1.1061	2.8359	2.6751	1.0601	3.2643	3.0771	1.0608	0.3145	0.2667	1.1793	1.6510	1.2330	1.3389	3.7490	3.2035	1.1703	1.3432	1.3015	1.0320	1.3774	1.3649	1.0091					
1.1865	1.0051	6.6180	5.2367	1.2638	4.5002	3.8825	1.1591	1.9437	1.8930	1.0268	0.4361	0.4548	0.9588	1.4417	1.4275	1.0100	2.5770	2.4613	1.0470	8.0859	6.9678	1.1605	59.1476	34.9526	1.6922	4.4131	3.6631	1.2047	0.4461	0.3903	1.1430	1.4806	1.4544	1.0180					
1.9244	1.0293	3.6307	3.3822	1.0735	3.6462	3.2578	1.1192	2.5762	2.4713	1.0424	0.4972	0.4928	1.0090	1.2269	1.2268	1.0000	1.4576	1.4271	1.0214	4.0628	3.7428	1.0855	40.4120	26.5186	1.5239	4.0471	3.7339	1.0839	0.3982	0.3977	1.0014	3.5269	3.2659	1.0799					
1.3888	1.0124	4.8710	4.3438	1.1214	3.8131	3.3693	1.1317	1.1812	1.1835	0.9981	1.4038	1.3909	1.0092	2.8033	2.6423	1.0609	77.5124	27.8254	2.7857	6.8201	5.8328	1.1693	8.3250	7.0185	1.1862	0.5366	0.5287	1.0150	0.7136	0.7065	1.0101	5.5738	5.0087	1.1128					
0.8068	0.9875	5.2500	4.6829	1.1211	1.6636	1.6321	1.0193	3.9891	3.6866	1.0821	1.6465	1.5815	1.0411	2.7563	2.6280	1.0488	3.5383	3.3026	1.0714	6.2329	5.4662	1.1403	0.9604	0.7891	1.2171	0.3014	0.2823	1.0676	1.3432	1.3015	1.0320	2.5194	2.3870	1.0555					
0.7743	0.9831	4.8411	4.3606	1.1102	0.8464	0.8073	1.0485	2.0984	2.0134	1.0422	0.7407	0.6686	1.1078	1.1599	1.0719	1.0821	0.2690	0.2657	1.0124	4.0307	0.4965	1.0688	58.9721	31.0863	1.8970	2.8345	2.6860	1.0553	1.7633	1.7222	1.0239	2.1109	2.0433	1.0331					
1.7556	1.0495	8.4401	6.7418	1.2519	3.6462	3.2578	1.1192	2.5762	2.4713	1.0424	0.4972	0.4928	1.0090	1.2269	1.2268	1.0000	1.4576	1.4271	1.0214	4.0628	3.7428	1.0855	40.4120	26.5186	1.5239	4.0471	3.7339	1.0839	0.3982	0.3977	1.0014	3.5269	3.2659	1.0799					
2.4483	1.0669	6.8096	5.9195	1.1504	0.5789	0.5671	1.0207	3.4471	3.2066	1.0750	121.9949	45.3807	2.6883	1.4649	1.4477	1.0119	1.5156	1.4775	1.0258	4.4653	4.0585	1.1002	34.7359	19.3104	1.7988	2.8345	2.6860	1.0553	1.3432	1.3015	1.0320	0.3834	0.3691	1.0388					
2.4483	1.0669	7.0359	6.1009	1.1532	1.0458	1.0491	0.9969	2.0984	2.0134	1.0422	2.5091	1.7445	1.4383	1.8969	1.8476	1.0267	259.7344	32.4713	7.9989	12.5173	9.3382	1.3404	47.6079	25.3669	1.8768	4.7638	4.3480	1.0956	1.3432	1.3015	1.0320	1.1797	1.1876	0.9933					
3.1161	1.0865	0.0996	0.0937	1.0634	1.8412	1.7084	1.0778	1.6015	1.5023	1.0660	1.9351	1.8889	1.0244	0.0940	0.0921	1.0211	2.2855	2.1689	1.0538	3.6857	3.4228	1.0768	16.8539	12.9488	1.3016	0.4805	0.4336	1.1081	1.7613	1.6883	1.0432	1.9426	1.8797	1.0335					
0.4756	1.0079	6.5301	5.3882	1.2119	1.0655	1.0305	1.0340	0.6610	0.6416	1.0286	1.5026	1.4043	1.0700	0.7796	0.7480	1.0422	2.4968	2.3630	1.0566	4.8615	4.3550	1.1163	15.6515	12.1457	1.2886	3.0987	2.8833	1.0747	1.7633	1.7222	1.0239	2.0797	2.0152	1.0320					
0.5439	1.0189	2.5097	2.3913	1.0495	2.3245	2.1478	1.0823	0.9891	0.9305	1.0630	0.9052	0.8871	1.0204	2.3187	2.2190	1.0449	1.5639	1.5276	1.0237	9.4710	7.8130	1.2122	18.6281	13.0885	1.4232	2.4686	2.3800	1.0372	1.7877	1.7383	1.0284	0.8918	0.9076	0.9826					
30.6324	2.8140	5.6763	5.0554	1.1228	11.5130	8.8090	1.3070	2.0984	2.0134	1.0422	1.1687	1.1603	1.0072	2.1549	1.9637	1.0974	1.4804	1.4551	1.0173	2.1262	2.0448	1.0398	96.4534	45.1119	2.1381	3.2582	3.0369	1.0729	3.0518	2.8167	1.0834	2.3387	2.2273	1.0500					
0.9856	1.2701	10.8032	8.4145	1.2839	1.9680	1.9239	1.0229	2.0157	1.9533	1.0319	0.4670	0.4642	1.0060	2.1794	2.1129	1.0315	3.3223	3.0803	1.0785	5.5101	4.9167	1.1207	16.2711	12.6063	1.2907	3.5851	3.3629	1.0661	1.7633	1.7222	1.0239	8.0924	6.5565	1.2342					
0.9644	0.9926	1.1164	0.9316	1.1984	1.9680	1.9239	1.0229	10.4425	4.9582	2.1061	1.3488	1.3364	1.0092	1.6831	1.6522	1.0187	2.4171	2.2966	1.0525	3.2196	3.0049	1.0714	24.3032	17.5298	1.3864	1.3122	1.2456	1.0535	1.3432	1.3015	1.0320	0.5951	0.5267	1.1299					
0.9899	1.0283	2.4194	2.3241	1.0410	4.1937	3.8179	1.0984	1.5778	1.5490	1.0186	1.3488	1.3364	1.0092	7.4295	5.8064	1.2795	3.3245	3.0934	1.0747	3.2144	2.9976	1.0723	12.3668	8.7487	1.4136	2.2459	2.0404	1.1007	5.2168	4.4613	1.1694	2.3256	2.2157	1.0496					
2.7822	1.1047	2.7602	2.4511	1.1261	1.0630	1.0645	0.9986	1.6490	1.6204	1.0154	1.9351	1.8889	1.0244	1.8969	1.8476	1.0267	0.9498	0.9602	0.9892	1.7399	1.7058	1.0200	31.9489	21.8389	1.4629	2.2400	2.1284	1.0524	1.2047	1.1960	1.0073	1.4629	0.7115	0.6873	1.0353				
2.7525	1.0706	2.3174	1.8262	1.2690	4.1937	3.8179	1.0984	3.3.																															

S5_3b: LRs values (overall and per-marker) computed by EuroForMix for mixtures with three estimated contributors, considering an FST value of 0 and 0.01. The ratio between the two LR values was calculated.

EuroForMix			LR																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
PAIR	NUMBER OF MARKERS ANALYZED	MARKER																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
		OVERALL												CSFIPO												D10S1248				D12S391				D13S317				D16S539				D18S51				D19S433				D1S1656				D21S11				D22S1045				D2S1338																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
		0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
3_1	21	3.84E+10	7.46E+08	51.38887	1.81482	1.78473	1.01686	3.19488	3.17270	1.00699	38.61388	35.27312	1.09471	3.20214	3.20046	1.00053	5.46620	5.29635	1.03207	101.23177	87.68642	1.15447	4.72673	4.76228	0.99253	285.07831	57.32996	4.97259	33.25416	30.21453	1.10060	33.03869	18.56460	1.77966	110.39373	85.93580	1.28461																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				

D2S411			D3S1358			D5S818			D7S820			D8S1179			FGA			SE33			TH01			TPOX			YVA		
0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO
11.71102	10.76881	1.08749	9.99699	9.94194	1.00571	47.67038	38.06616	1.25230	7.26197	6.92173	1.04915	10.24058	9.81452	1.04341	10.05372	9.59921	1.04735	154.09011	127.83502	1.20538	7.24031	7.10913	1.01845	3.34650	3.33213	1.00431	50.39614	40.83448	1.23416
4.70828	4.56063	1.04488	5.24783	5.15963	1.01709	6.33939	6.13263	1.03371	9.35605	8.62421	1.08486	6.89667	6.32847	1.08979	16.03143	15.04500	1.06557	253.32612	166.05427	1.52556	11.28024	10.55070	1.06915	4.44144	4.48902	0.98940	5.84722	5.58904	1.04619
1.54546	1.50235	0.98920	1.92689	1.86999	1.03043	5.82422	5.32148	1.09447	1.65683	1.60927	1.03046	1.60652	3.61203	0.99447	4.91424	4.65564	1.05555	87.25534	60.06109	1.45278	4.07760	4.02852	1.01218	3.30792	3.15926	1.04705	2.98026	2.77375	1.07445
23.49464	18.24103	1.28801	2.08570	2.03579	1.02452	1.33498	1.33910	0.99693	12.45637	10.21119	1.21988	1.52810	1.57926	0.96761	3.87251	3.83248	1.01044	58.05773	41.36797	1.40345	3.36057	3.31542	1.07869	2.54066	2.50671	1.01354	9.49421	8.54998	1.11044
7.89738	6.52235	1.21082	1.29837	1.51776	0.85545	12.70164	10.63147	1.19472	3.92801	3.60959	1.08822	12.76966	21.94343	1.23817	6.47040	5.90895	1.09502	40.64637	35.50257	1.14489	27.97081	23.63991	1.18325	9.15023	9.69568	0.94374	5.19092	4.80512	1.08129
9.59742	9.17285	1.04629	7.68620	7.48180	1.02732	8.22289	7.66078	1.07338	9.42603	8.90749	1.05821	9.43744	9.00196	1.04938	53.92686	41.57889	1.29698	213.28402	162.40291	1.31330	10.65956	10.47548	1.01757	1886.24051	175.37632	10.75539	12.81565	12.24665	1.04646
3.05115	2.76075	1.10519	2.87730	2.68200	1.07282	3.28707	2.87632	1.14280	1.77696	1.71915	1.03635	5.51609	5.05138	1.09919	5.58649	6.06979	0.96180	2.75073	2.25672	1.21842	4.93913	4.02848	1.22605	0.72304	0.71146	1.01627	1.05174	1.00927	
2.48786	2.26650	1.09767	2.21583	2.24161	0.98850	1.00121	0.97594	1.02589	2.80735	2.86664	0.97932	4.33006	4.47317	0.96801	2.90718	2.81264	1.03361	3.14724	3.24227	0.91910	1.62111	1.50784	1.07512	0.90368	0.76837	1.17610	1.27667	2.82384	0.93080
11.18832	8.50762	1.24999	31.86141	29.92040	1.06487	0.70795	0.67561	1.04787	2.81907	2.79516	1.00855	2.03127	2.02416	1.00352	14.35555	12.81139	1.12053	32.57521	22.42685	1.45251	13.85081	11.74207	1.17959	0.66774	0.65865	1.01380	6.55653	6.11505	1.07025
9.29288	7.84882	1.17588	1.54081	1.49531	1.03043	1.96329	1.94852	1.00758	2.84079	2.64363	1.07458	53.00570	19.30046	2.74634	12.23524	10.57890	1.15657	16.60215	14.94432	1.11093	0.36366	0.36347	0.98962	0.93167	0.91591	1.01720	7.16688	6.00473	1.17546
1.96884	1.99515	0.98681	2.11334	2.05167	1.03006	1.59567	1.54026	1.03597	2.56345	2.44788	1.04721	3.84813	4.09456	0.93982	4.44420	4.01870	1.10588	2.66124	2.30013	1.15700	0.87193	0.85613	1.01846	1.00492	1.00171	1.00321	5.37103	5.00068	1.07406
0.79110	0.73349	1.07853	3.10796	2.99514	1.03767	6.95787	6.06969	1.14120	1.86661	1.70118	1.09724	2.28271	2.11733	1.07811	0.82699	0.78088	1.05904	72.02252	36.36791	1.98011	11.15056	9.79803	1.13804	2.01274	1.97912	1.01699	4.27348	4.02550	1.06160
0.90418	0.61661	1.36765	1.11921	1.03744	1.07882	1.22127	1.20738	1.01151	1.05583	1.04582	1.03330	2.98034	2.76069	1.05977	10.49803	7.54707	1.39101	32.94830	16.90282	1.94882	0.00071	0.00080	1.88054	3.87089	3.81739	1.01402	5.47583	4.14569	1.31729
0.68676	0.64317	1.03979	2.83171	2.68696	1.05387	11.44205	64.60066	1.72509	1.27726	1.23552	1.03379	7.52847	6.62611	1.13618	5.90427	5.38350	1.09673	39.75313	21.44718	1.85354	10.56050	9.22370	1.14493	1.53567	1.47470	1.04134	1.94087	1.78674	1.08626
1.57320	1.56146	1.00752	2.62324	2.44350	1.07356	9.19064	6.34057	1.44950	2.48470	2.39156	1.03895	618.20659	72.04585	8.58074	23.78619	18.03151	1.31915	105.06647	53.24612	1.97322	4.14380	3.59154	1.53777	1.44476	1.40509	1.02823	3.28856	3.16144	1.04021
4.81231	4.46460	1.07788	4.42221	4.48011	1.08384	2.48402	2.48058	1.00139	0.51023	0.52141	0.97856	2.15302	1.99406	1.07931	3.27311	3.18708	1.02699	47.94728	38.19518	1.25532	1.22908	1.23300	0.99682	0.80814	0.91767	0.96673	6.02308	5.78721	1.04059
3.64686	3.34901	1.08893	10.15745	8.89953	1.14135	12.09748	11.46012	1.05562	9.59442	8.64539	1.11035	3.87807	3.50543	1.06360	4.84627	6.15087	1.05460	34.42949	25.91592	1.32851	4.71844	4.54847	1.03737	2.00076	1.98001	1.01044	7.76631	7.03731	1.10359
8.64670	7.72849	1.11881	9.54193	8.17220	1.16761	2.69102	2.46735	1.09065	6.68370	6.15058	1.08668	3.80279	3.75294	1.01328	19.77348	16.07297	1.23023	32.73903	22.85763	1.43230	4.04551	3.83253	1.05557	1.47547	1.44532	1.02086	0.72123	0.73925	0.97563
25.40683	20.45974	1.24180	1.92749	1.84090	1.04703	1.82453	1.81944	1.00280	2.01805	2.02743	0.99966	1.36438	1.30996	1.04154	2.12182	2.06848	1.02579	189.15791	93.82835	2.01600	2.08410	2.01842	1.03254	1.78066	1.69251	1.05028	2.20021	2.09429	0.98927
3.18238	3.51552	0.90575	1.58291	1.82937	0.86528	1.86906	2.08322	0.89720	2.65607	2.38290	1.11463	3.95204	3.47939	1.13584	7.65618	6.15771	1.24335	18.03715	14.76317	1.22177	1.85606	1.79674	1.03301	2.07276	1.99937	1.01395	4.22901	3.87187	1.09224
3.99618	4.27576	0.93461	105.18589	45.94376	2.28945	2.66896	2.82088	0.94614	1.96086	1.77655	1.04311	3.51166	3.16583	1.09214	4.13243	3.53234	1.16989	27.20685	20.55822	1.32339	0.72217	0.71273	1.01325	2.02716	2.00104	0.99673	5.52096	0.54111	0.98126
6.18090	5.60460	1.10282	2.03747	2.12123	0.96051	1.48676	1.46491	1.01492	0.06082	0.06338	0.95949	4.95855	4.54700	1.09051	5.47411	5.23654	1.04537	25.18205	15.26635	1.64951	12.07797	9.63515	1.25353	6.85144	5.68068	1.06110	4.27213	4.27104	1.00045
4.31575	4.26020	1.01304	2.40339	2.44350	1.04935	7.59655	6.91983	0.99779	4.39551	4.18878	1.04975	1.52813	1.51831	1.00646	3.64354	3.46660	1.05104	39.96826	29.12391	1.37235	1.82912	1.73403	1.05484	1.73987	1.75469	0.99155	1.19426	1.14896	1.03943
0.05639	3.89954	1.04022	2.76276	5.08555	1.13316	3.52198	3.23829	1.08761	3.88749	3.67377	1.05817	10.42398	9.40030	1.10890	0.46146	0.46980	0.98224	42.39238	18.05613	2.34781	4.47914	3.97654	1.12639	5.99231	5.41250	1.10713	1.58287	1.50516	1.05163
0.40820	0.80708	0.94198	8.36533	3.42893	1.11887	3.72982	3.42616	1.08863	7.74913	6.91010	1.08714	23.76387	18.81487	1.26304	0.176420	14.68967	14.91491	1.21096	70.87499	46.0889	2.33366	2.17527	1.07282	0.85670	0.81749	1.04796	2.30457	2.18208	1.05161
6.71090	5.74445	1.16824	4.31391	4.21898	1.02250	0.95253	0.93344	1.02045	4.12384	3.86284	1.06757	4.54048	3.81488	1.19020	4.17246	3.79304	1.10003	26.15043	19.78373	1.32182	3.11967	3.09756	1.00691	1.52677	1.46792	1.04009	7.75546	6.81135	1.13861
2.81191	2.51871	1.11641	6.21111	5.80817	1.06937	1.54604	1.55863	0.99307	3.15209	3.05760	1.03093	7.55035	7.23361	1.04174	89.17913	49.93635	1.07831	26.31487	20.02248	1.31427	3.30866	3.04499	1.08560	2.14769	2.08921	1.02799	13.69455	12.41287	1.06901
0.50418	0.50272	1.00289	79.50847	66.12767	1.20235	3.04579	2.89462	1.05222	57.72443	49.6771	1.16233	8.10998	6.97430	1.16284	10.58652	9.74724	1.08610	3.09437	2.99368	1.03363	3.82174	3.64187	1.10395	1.95153	1.85297	1.03355	1.54964	1.58834	0.99441
11.62558	9.29454	1.25080	1.97347	1.94499	1.01464	2.10858	2.05742	1.04004	12.96109	10.61133	1.23895	2.45397	2.36877	1.03597	3.85547	3.69714	1.04283	72.13366	48.68731	1.48157	4.18667	3.62449	1.15511	0.42509	0.43243	0.98303	4.09368	3.73855	1.09499
6.79926	5.99855	1.13348	1.27252	1.29869	0.97985	1.37561	1.38224	0.99521	1.91348	1.85672	1.03057	1.44094	1.35349	1.06461	12.51237	10.59450	1.18103	103.44004	55.10666	1.87698	0.99891	0.99799	1.00092	0.77780	0.79365	0.98003	45.57295	18.94856	2.40509
1.37914	1.28143	1.07625	17.87984	14.65080	1.22040	1.93485	1.84241	1.05017	7.94389	6.80922	1.16664	5.34022	5.07110	1.05307	10.58290	2.96369	1.03815	25.96153	19.52688	1.32955	0.21010	0.21687	0.96082	3.29019	2.96173	1.11090	7.47925	6.65488	1.07292
14.86561	12.35998	1.20272	3.84701	3.70217	1.03912	3.56639	3.54252	1.00674	30.92112	24.84356	1.26293	1.47799	1.36878	1.07978	13.80119	12.76130	1.08149	11.58085	7.169224	1.55639	4.46138	3.87141	1.15239	1.66699	1.60942	1.03577	2.97811	2.89119	1.03006
23.49464	18.24103	1.28801	2.08570	2.03579	1.02452	1.33498	1.33910	0.99693	12.45637	10.21119	1.21988	1.52810	1.57926	0.96761	3.87251	3.83248													

S5_3c: LR's values (overall and per-marker) computed by STRmix™ v.2.7 for mixtures with three estimated contributors, considering an FST value of 0 and 0.01. The ratio between the two LR values was calculated.

PAIR		NUMBER OF MARKERS ANALYZED	OVERALL		LR																														
					CSF1PO			D10S1248			D12S391			D13S317			D16S539			D18S51			D19S433			D1S1656			D21S11			D22S1045			
			0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO	0.00	0.01	RATIO			
3.1	19	2.5E+13	1.5E+09	1.6E+04	0.967	0.975	0.992	16.797	15.289	1.099	3.619	3.470	1.043	1.947	1.932	1.008	1.026	0.956	1.073	7.180	5.936	1.210	2.396	1.244	1.925	8.912	6.993	1.274	0.263	0.268	0.984	-	-	#VALOR!	
3.2	20	2.1E+19	1.5E+18	1.4E+01	5.414	4.741	1.142	-	-	-	148.810	115.480	1.289	-	-	#VALOR!	8.636	7.681	1.124	23.390	20.719	1.129	35.117	25.886	1.357	36.208	31.723	1.141	31.447	17.094	1.840	6.153	5.743	1.072	
3.3	20	5.8E+15	3.0E+15	2.0E+00	11.556	9.690	1.193	1.259	1.338	0.941	18.204	18.162	1.002	16.336	14.980	1.091	6.107	5.746	1.063	4.592	4.748	0.967	6.523	6.648	0.981	20.033	19.631	1.020	78.515	64.685	1.214	1.090	1.193	0.913	
3.4	20	4.8E+17	4.8E+16	1.0E+01	6.508	6.226	1.045	1.894	2.013	0.941	3.094	3.066	1.009	12.673	11.093	1.142	3.290	3.837	0.857	13.361	11.974	1.116	20.259	16.056	1.262	9.946	8.747	1.137	2.011	2.038	0.987	4.481	4.289	1.045	
3.5	20	1.3E+24	1.0E+23	1.2E+01	7.412	6.598	1.123	5.182	5.244	0.988	35.373	32.819	1.078	7.085	6.033	1.174	162.860	116.100	1.403	11.555	10.898	1.060	50.447	38.026	1.327	24.959	20.942	1.192	150.170	119.540	1.256	229.670	150.540	1.526	
3.6	20	8.8E+27	2.8E+26	3.2E+01	6.116	5.941	1.029	6.102	6.248	0.977	43.946	36.627	1.200	28.831	24.509	1.176	18.005	15.871	1.134	45.237	36.219	1.249	133.230	104.260	1.278	333.480	217.630	1.532	68.023	41.294	1.647	11.882	11.917	0.997	
3.7	20	5.8E+07	1.8E+07	3.2E+00	1.315	1.274	1.033	1.474	1.332	1.107	1.604	1.641	0.978	1.033	1.026	1.429	1.402	1.019	1.414	1.444	0.979	1.059	1.102	0.961	24.273	16.192	1.499	20.228	16.757	1.207	2.741	2.530	1.084		
3.9	21	1.4E+08	6.5E+07	2.1E+00	2.031	2.020	1.005	11.507	8.474	1.358	5.925	4.967	1.193	3.716	3.616	1.028	3.283	3.472	0.946	5.253	4.934	1.065	22.123	19.685	1.124	2.472	2.450	1.009	5.809	5.139	1.130	0.001	0.001	0.830	
3.10	21	2.5E+06	1.2E+05	2.0E+01	1.638	4.617	0.355	0.154	1.218	0.126	2.386	1.242	1.922	1.729	1.158	1.493	2.316	6.964	0.333	2.051	39.814	0.052	1.700	2.134	0.797	0.011	0.001	11.894	1.849	6.893	0.268	1.725	0.836	2.064	
3.11	21	5.5E+09	7.2E+08	7.6E+00	1.188	1.248	0.952	3.548	3.574	0.993	7.886	5.751	1.371	2.673	2.527	1.058	0.287	0.288	0.997	0.656	0.660	0.963	2.494	2.245	1.111	9.714	10.109	0.961	29.558	15.520	1.905	2.338	2.518	0.929	
3.12	21	5.8E+02	3.3E+01	1.8E+01	15.238	13.301	1.146	11.405	10.003	1.140	1.229	1.162	1.058	2.352	2.395	0.982	0.491	0.562	0.875	27.522	21.398	1.286	0.661	0.567	1.167	3.824	2.330	1.641	8.895	7.010	1.269	0.172	0.215	0.802	
3.13	21	3.0E+13	6.9E+11	4.4E+01	2.309	2.164	1.067	2.769	2.268	1.221	23.294	16.929	1.376	8.747	7.126	1.227	2.807	2.538	1.106	0.909	0.875	1.038	1.808	1.776	1.018	31.488	19.651	1.602	3.210	3.299	0.973	2.696	2.433	1.108	
3.14	21	6.7E+03	1.1E+04	6.3E+01	3.167	2.855	1.109	5.885	5.868	1.003	3.262	3.401	0.959	1.828	1.946	0.939	0.750	0.821	0.914	2.584	2.931	0.881	0.272	0.202	1.348	0.247	0.225	1.096	2.124	2.375	0.894	0.215	0.188	1.144	
3.15	21	1.5E+05	2.8E+01	5.5E+03	3.099	2.336	1.327	0.006	0.001	3.931	2.177	1.476	1.475	4.779	4.355	1.097	0.214	0.173	1.237	2.170	1.940	1.119	0.257	0.181	1.422	0.327	0.242	1.349	2.421	2.018	1.200	0.228	0.257	0.886	
3.16	21	2.2E+11	4.8E+10	4.6E+00	0.988	0.964	1.025	6.140	5.435	1.130	11.999	11.130	1.078	4.164	3.561	1.169	2.060	2.044	1.008	6.669	6.316	1.056	1.189	1.065	1.116	27.362	23.121	1.183	20.370	17.872	1.140	1.790	1.676	1.068	
3.17	21	4.9E+13	2.9E+12	1.7E+01	0.025	0.016	1.560	6.874	7.044	0.976	11.726	10.657	1.100	0.281	0.172	1.638	6.599	7.017	0.940	13.814	12.453	1.109	5.491	5.482	1.001	80.433	64.002	1.257	12.679	11.761	1.078	0.006	0.003	2.241	
3.18	21	6.5E+13	8.0E+12	8.2E+00	0.154	0.171	0.904	2.344	2.051	1.143	7.975	6.711	1.188	2.205	1.972	1.144	2.070	2.325	0.891	8.097	6.240	1.298	3.001	2.988	1.015	86.846	52.803	1.645	3.202	2.831	1.131	11.227	9.622	1.167	
3.19	21	7.8E+08	5.9E+07	1.3E+01	1.380	1.402	0.984	1.355	1.476	0.918	5.855	5.019	1.167	4.940	4.807	1.028	1.334	1.312	1.016	64.783	29.904	2.166	1.394	1.300	1.073	0.320	0.329	0.970	1.555	1.968	0.790	0.133	0.096	1.384	
3.20	21	2.7E+12	2.1E+11	1.3E+01	2.435	2.390	1.019	3.247	3.388	0.958	1.202	0.942	1.276	2.810	2.851	0.986	2.763	2.674	1.033	8.752	9.004	0.972	2.129	2.280	0.934	2.287	1.542	1.483	7.941	7.544	1.053	104.860	47.251	2.219	
3.21	21	1.9E+14	2.3E+12	8.2E+01	1.568	1.575	0.995	1.759	1.737	1.013	90.161	45.713	1.972	2.251	2.260	0.996	2.241	2.117	1.059	4.968	3.901	1.273	134.890	64.960	2.077	37.282	25.566	1.458	3.044	2.775	1.097	3.628	2.845	1.275	
3.22	21	3.5E+12	2.4E+11	1.5E+01	1.450	1.433	1.012	2.628	2.440	1.077	21.529	17.508	1.230	2.795	2.511	1.113	6.554	5.449	1.203	9.058	7.942	1.140	5.163	4.504	1.146	4.041	2.396	1.686	6.432	4.968	1.295	2.053	1.959	1.048	
3.23	21	9.5E+14	9.4E+13	1.0E+01	2.766	2.681	1.032	8.322	7.916	1.051	102.540	53.956	1.900	1.537	1.440	1.067	1.348	1.334	1.011	4.920	4.523	1.088	3.136	2.886	1.087	9.520	8.636	1.102	35.265	28.385	1.242	16.677	13.613	1.225	
3.24	21	8.2E+12	4.8E+10	1.7E+02	2.299	2.100	1.095	12.591	11.862	1.061	13.191	12.006	1.099	1.992	1.852	1.076	2.044	1.623	1.260	13.611	11.354	1.199	1.578	1.399	1.128	209.490	71.056	2.948	4.251	3.576	1.189	2.132	1.925	1.108	
3.25	21	1.1E+13	7.7E+11	1.4E+01	4.000	3.827	1.045	11.097	9.112	1.218	8.009	6.893	1.162	4.937	4.507	1.095	7.889	7.118	1.108	18.802	15.759	1.193	0.920	0.940	0.979	10.058	8.525	1.180	9.175	8.206	1.118	0.775	0.727	1.067	
3.26	21	5.6E+10	6.4E+10	8.8E+01	2.930	3.017	0.971	0.001	0.010	0.123	10.761	7.109	1.514	4.484	4.399	1.019	4.752	4.382	1.085	5.429	6.052	0.897	12.639	10.386	1.041	1.217	5.564	4.901	1.135	9.486	7.405	1.281	4.151	3.666	1.132
3.27	21	4.9E+15	6.8E+14	7.2E+00	1.505	1.509	0.998	13.502	12.190	1.108	6.135	5.343	1.148	2.021	2.178	0.928	1.495	1.430	1.045	6.237	5.801	1.075	8.213	7.288	1.127	14.846	14.043	1.057	2.113	2.048	1.032	0.394	0.537	0.734	
3.28	21	1.0E+16	7.3E+10	1.4E+05	4.310	3.545	1.230	2.102	1.532	1.372	5.230	1.039	5.034	1.918	0.188	10.223	1.751	1.491	1.174	11.397	7.310	1.559	3.094	1.546	2.001	13.898	6.818	2.038	9.435	6.175	1.528	12.984	14.343		
3.29	21	3.1E+10	7.6E+11	4.1E+02	27.344	7.899	3.462	9.045	5.457	1.657	1.978	8.411	0.235	3.220	1.449	2.222	6.302	12.810	0.492	12.632	6.689	1.888	2.259	2.338	0.966	17.429	7.234	2.409	13.183	9.089	1.450	2.390	1.546	1.546	
3.30	21	1.0E+17	1.5E+16	6.8E+00	2.123	2.631	0.807	0.633	0.747	0.847	31.748	27.259	1.165	8.576	7.306	1.174	26.129	18.969	1.377	4.692	3.694	1.270	6.509	6.349	1.025	16.894	16.384	1.031	3.856	3.363	1.147	1.797	1.722	1.043	
3.31	21	1.2E+17	2.4E+15	5.0E+01	1.371	1.367	1.003	2.439	2.019	1.208	98.283	50.930	1.930	9.443	8.126	1.162	25.845	17.787	1.453	11.512	10.580	1.088	65.807	42.325	1.555	52.288	33.330	1.569	14.162	12.424	1.140	0.833	0.653	1.276	
3.32	21	1.6E+17	2.6E+16	1.6E+00	1.346	1.358	0.992	0.862	0.671	1.285	11.044	8.714	0.973	1.064	1.093	0.673	1.930	1.668	1.157	104.850	64.625	1.622	2.151	2.023	1.063	47.751	41.409	1.153	50.904	38.401	1.326	3.515	3.698	1.034	
3.33	21	4.9E+18	1.7E+17	2.9E+01	1.821	1.892	0.963	16.609	12.180	1.364	143.840	73.685	1.952	5.070	4.421	1.147	5.324	5.235	1.017	7.287	7.374	0.988	7.681	6.854	1.121	13.900	11.881	1.170	25.319	15.820	1.600	4.156	4.034	1.030	
3.34	21	5.3E+17	1.2E+16	4.6E+01	15.557	12.075	1.288	0.784	0.806	0.973	6.237	5.740	1.087	6.365	5.782	1.107	13.578	8.448	1.607	5.527	5.079	1.088	10.721	9.137	1.173	11.044	10.781	1.024	14.613	11.770	1.242	3.332	3.041	1.063	
3.35	21	1.3E+22	2.2E																																

[illegible]

SM 6: Ratio between LR values computed by marker not considering population coancestry (0; LR1) and considering (0.010, LR2) by the three tools

Table S6: Ratio between LR values computed by marker not considering population coancestry (0; LR1) and considering (0.010, LR2) by LRmix Studio v.2.1.3, EuroForMix v.3.4.0, and STRmix™ v.2.7, for both cases with mixtures with two and three estimated contributors. R=LR1/LR2. N/A: “Not Applicable” corresponds to situations where an LR equal to zero was assigned when an coancestry of 0.01 was considered. Due to the relatively small sets of samples and lack of normality, mean, median, and standard deviation values were reached through bootstrapping (number of replicates: 10,000).

Marker	Ratio	2 contributors (N=75)			3 contributors (N=77)		
		LRmix	EuroForMix	STRmix™	LRmix	EuroForMix	STRmix™
CSF1PO	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
CSF1PO	$1/10^2 < R < 1/10$	1.30%	0.00%	0.00%	0.00%	0.00%	0.00%
CSF1PO	$1/10 < R < 1/5$	0.00%	0.00%	1.30%	0.00%	0.00%	0.00%
CSF1PO	$1/5 < R < 1/2$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
CSF1PO	$1/2 < R < 1$	8.00%	9.30%	16.00%	13.00%	22.10%	22.10%
CSF1PO	$1 < R < 2$	76.00%	78.70%	78.70%	80.50%	70.10%	74.00%
CSF1PO	$2 < R < 5$	8.00%	5.30%	4.00%	3.90%	5.20%	2.60%
CSF1PO	$5 < R < 10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
CSF1PO	$10 < R < 10^2$	6.70%	6.70%	0.00%	2.60%	2.60%	0.00%
CSF1PO	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
CSF1PO	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
CSF1PO	Minimum	1.72E-02	9.80E-01	1.10E-01	7.19E-01	6.51E-01	3.55E-01
CSF1PO	Maximum	2.80E+01	2.85E+01	2.50E+00	1.05E+01	1.09E+01	4.24E+00
CSF1PO	Mean	2.08E+00	2.10E+00	1.16E+00	1.35E+00	1.37E+00	1.19E+00
CSF1PO	Median	1.05E+00	1.06E+00	1.08E+00	1.04E+00	1.02E+00	1.05E+00
CSF1PO	Standard Deviation	3.73E+00	3.80E+00	3.37E-01	1.53E+00	1.61E+00	4.91E-01
CSF1PO	Mean_ci_low	1.37E+00	1.37E+00	1.08E+00	1.07E+00	1.07E+00	1.09E+00
CSF1PO	Mean_ci_high	3.04E+00	3.04E+00	1.24E+00	1.75E+00	1.78E+00	1.31E+00
CSF1PO	Median_ci_low	1.04E+00	1.04E+00	1.05E+00	1.02E+00	1.02E+00	1.03E+00
CSF1PO	Median_ci_high	1.08E+00	1.09E+00	1.09E+00	1.06E+00	1.05E+00	1.09E+00
CSF1PO	Standard Deviation_ci_low	1.32E+00	1.31E+00	2.21E-01	2.72E-01	2.81E-01	1.88E-01
CSF1PO	Standard Deviation_ci_high	5.75E+00	5.91E+00	4.32E-01	2.34E+00	2.46E+00	7.38E-01
D10S1248	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D10S1248	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D10S1248	$1/10 < R < 1/5$	1.30%	0.00%	0.00%	0.00%	0.00%	2.60%
D10S1248	$1/5 < R < 1/2$	0.00%	0.00%	0.00%	0.00%	1.30%	0.00%
D10S1248	$1/2 < R < 1$	16.00%	22.70%	13.30%	28.60%	26.00%	27.30%
D10S1248	$1 < R < 2$	76.00%	72.00%	84.00%	70.10%	71.40%	63.60%
D10S1248	$2 < R < 5$	1.30%	0.00%	0.00%	0.00%	0.00%	3.90%
D10S1248	$5 < R < 10$	5.30%	4.00%	2.70%	1.30%	1.30%	1.30%
D10S1248	$10 < R < 10^2$	0.00%	1.30%	0.00%	0.00%	0.00%	0.00%
D10S1248	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D10S1248	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D10S1248	Minimum	1.19E-01	7.13E-01	7.39E-01	8.12E-01	2.84E-01	1.23E-01
D10S1248	Maximum	9.79E+00	1.02E+01	7.75E+00	7.99E+00	8.48E+00	7.75E+00
D10S1248	Mean	1.45E+00	1.49E+00	1.28E+00	1.16E+00	1.17E+00	1.21E+00
D10S1248	Median	1.05E+00	1.06E+00	1.07E+00	1.02E+00	1.05E+00	1.06E+00

D10S1248	Standard Deviation	1.60E+00	1.71E+00	1.03E+00	7.97E-01	8.62E-01	8.77E-01
D10S1248	Mean_ci_low	1.14E+00	1.17E+00	1.10E+00	1.05E+00	1.05E+00	1.05E+00
D10S1248	Mean_ci_high	1.85E+00	1.91E+00	1.55E+00	1.35E+00	1.39E+00	1.44E+00
D10S1248	Median_ci_low	1.03E+00	1.05E+00	1.05E+00	1.01E+00	1.03E+00	1.02E+00
D10S1248	Median_ci_high	1.06E+00	1.09E+00	1.10E+00	1.03E+00	1.07E+00	1.09E+00
D10S1248	Standard Deviation_ci_low	6.63E-01	7.66E-01	1.45E-01	9.48E-02	1.34E-01	2.40E-01
D10S1248	Standard Deviation_ci_high	2.27E+00	2.42E+00	1.60E+00	1.36E+00	1.46E+00	1.40E+00
D12S391	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D12S391	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D12S391	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D12S391	$1/5 < R < 1/2$	0.00%	0.00%	1.30%	0.00%	0.00%	2.60%
D12S391	$1/2 < R < 1$	1.30%	2.70%	5.30%	1.30%	3.90%	5.20%
D12S391	$1 < R < 2$	96.00%	96.00%	82.70%	96.10%	92.20%	87.00%
D12S391	$2 < R < 5$	2.70%	1.30%	8.00%	2.60%	3.90%	3.90%
D12S391	$5 < R < 10$	0.00%	0.00%	1.30%	0.00%	0.00%	1.30%
D12S391	$10 < R < 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D12S391	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D12S391	N/A	0.00%	0.00%	1.30%	0.00%	0.00%	0.00%
D12S391	Minimum	7.57E-01	8.87E-01	4.04E-01	6.78E-01	6.65E-01	2.35E-01
D12S391	Maximum	4.54E+00	4.70E+00	8.56E+00	4.36E+00	4.60E+00	5.03E+00
D12S391	Mean	1.27E+00	1.28E+00	1.40E+00	1.26E+00	1.32E+00	1.36E+00
D12S391	Median	1.13E+00	1.15E+00	1.16E+00	1.15E+00	1.15E+00	1.19E+00
D12S391	Standard Deviation	4.61E-01	4.58E-01	9.53E-01	4.63E-01	5.39E-01	6.21E-01
D12S391	Mean_ci_low	1.18E+00	1.20E+00	1.23E+00	1.18E+00	1.21E+00	1.23E+00
D12S391	Mean_ci_high	1.38E+00	1.40E+00	1.65E+00	1.38E+00	1.45E+00	1.51E+00
D12S391	Median_ci_low	1.11E+00	1.13E+00	1.14E+00	1.12E+00	1.13E+00	1.16E+00
D12S391	Median_ci_high	1.16E+00	1.19E+00	1.21E+00	1.18E+00	1.20E+00	1.27E+00
D12S391	Standard Deviation_ci_low	1.74E-01	1.87E-01	3.46E-01	1.48E-01	2.06E-01	3.27E-01
D12S391	Standard Deviation_ci_high	7.19E-01	7.22E-01	1.52E+00	7.16E-01	7.96E-01	8.98E-01
D13S317	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D13S317	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D13S317	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D13S317	$1/5 < R < 1/2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D13S317	$1/2 < R < 1$	2.70%	5.30%	17.30%	2.60%	9.10%	19.50%
D13S317	$1 < R < 2$	94.70%	94.70%	81.30%	97.40%	89.60%	75.30%
D13S317	$2 < R < 5$	1.30%	0.00%	1.30%	0.00%	1.30%	1.30%
D13S317	$5 < R < 10$	1.30%	0.00%	0.00%	0.00%	0.00%	1.30%
D13S317	$10 < R < 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D13S317	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D13S317	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D13S317	Minimum	9.80E-01	8.06E-01	6.74E-01	9.63E-01	9.07E-01	5.28E-01
D13S317	Maximum	5.30E+00	1.44E+00	2.15E+00	1.25E+00	3.32E+00	1.02E+01
D13S317	Mean	1.17E+00	1.10E+00	1.11E+00	1.06E+00	1.12E+00	1.29E+00
D13S317	Median	1.07E+00	1.07E+00	1.08E+00	1.05E+00	1.07E+00	1.09E+00
D13S317	Standard Deviation	5.19E-01	1.04E-01	2.09E-01	5.35E-02	2.77E-01	1.16E+00
D13S317	Mean_ci_low	1.08E+00	1.08E+00	1.07E+00	1.05E+00	1.07E+00	1.10E+00
D13S317	Mean_ci_high	1.31E+00	1.13E+00	1.16E+00	1.08E+00	1.19E+00	1.60E+00
D13S317	Median_ci_low	1.05E+00	1.06E+00	1.05E+00	1.03E+00	1.05E+00	1.05E+00
D13S317	Median_ci_high	1.10E+00	1.10E+00	1.12E+00	1.06E+00	1.09E+00	1.11E+00
D13S317	Standard Deviation_ci_low	7.03E-02	7.68E-02	1.32E-01	4.31E-02	7.30E-02	1.86E-01
D13S317	Standard Deviation_ci_high	8.61E-01	1.27E-01	2.81E-01	6.28E-02	4.53E-01	1.90E+00

D16S539	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D16S539	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D16S539	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D16S539	$1/5 < R < 1/2$	0.00%	0.00%	1.30%	0.00%	0.00%	2.60%
D16S539	$1/2 < R < 1$	6.70%	6.70%	10.70%	11.70%	14.30%	20.80%
D16S539	$1 < R < 2$	92.00%	93.30%	88.00%	88.30%	85.70%	74.00%
D16S539	$2 < R < 5$	0.00%	0.00%	0.00%	0.00%	0.00%	2.60%
D16S539	$5 < R < 10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D16S539	$10 < R < 10^2$	1.30%	0.00%	0.00%	0.00%	0.00%	0.00%
D16S539	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D16S539	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D16S539	Minimum	6.04E-01	9.61E-01	4.13E-01	8.15E-01	8.85E-01	3.33E-01
D16S539	Maximum	1.37E+01	1.55E+00	1.75E+00	1.63E+00	1.55E+00	2.47E+00
D16S539	Mean	1.25E+00	1.11E+00	1.13E+00	1.07E+00	1.10E+00	1.10E+00
D16S539	Median	1.07E+00	1.08E+00	1.09E+00	1.03E+00	1.06E+00	1.05E+00
D16S539	Standard Deviation	1.46E+00	1.15E-01	1.75E-01	1.24E-01	1.37E-01	2.64E-01
D16S539	Mean_ci_low	1.07E+00	1.09E+00	1.09E+00	1.05E+00	1.07E+00	1.05E+00
D16S539	Mean_ci_high	1.61E+00	1.14E+00	1.17E+00	1.10E+00	1.13E+00	1.16E+00
D16S539	Median_ci_low	1.06E+00	1.06E+00	1.07E+00	1.03E+00	1.04E+00	1.02E+00
D16S539	Median_ci_high	1.09E+00	1.10E+00	1.12E+00	1.05E+00	1.08E+00	1.09E+00
D16S539	Standard Deviation_ci_low	7.27E-02	8.41E-02	1.25E-01	7.40E-02	9.86E-02	1.55E-01
D16S539	Standard Deviation_ci_high	2.50E+00	1.42E-01	2.23E-01	1.64E-01	1.67E-01	3.59E-01
D18S51	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D18S51	$1/10^2 < R < 1/10$	1.30%	0.00%	0.00%	0.00%	0.00%	1.30%
D18S51	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D18S51	$1/5 < R < 1/2$	1.30%	0.00%	1.30%	0.00%	1.30%	0.00%
D18S51	$1/2 < R < 1$	0.00%	1.30%	5.30%	2.60%	6.50%	16.90%
D18S51	$1 < R < 2$	89.30%	90.70%	84.00%	90.90%	84.40%	77.90%
D18S51	$2 < R < 5$	8.00%	8.00%	6.70%	6.50%	7.80%	3.90%
D18S51	$5 < R < 10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D18S51	$10 < R < 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D18S51	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D18S51	N/A	0.00%	0.00%	2.70%	0.00%	0.00%	0.00%
D18S51	Minimum	2.95E-02	9.87E-01	4.81E-01	8.85E-01	2.65E-01	5.15E-02
D18S51	Maximum	2.43E+00	2.45E+00	3.89E+00	2.48E+00	2.49E+00	2.40E+00
D18S51	Mean	1.21E+00	1.26E+00	1.28E+00	1.23E+00	1.21E+00	1.21E+00
D18S51	Median	1.12E+00	1.15E+00	1.15E+00	1.12E+00	1.11E+00	1.14E+00
D18S51	Standard Deviation	3.76E-01	3.26E-01	5.21E-01	3.19E-01	3.46E-01	3.27E-01
D18S51	Mean_ci_low	1.13E+00	1.19E+00	1.17E+00	1.16E+00	1.14E+00	1.14E+00
D18S51	Mean_ci_high	1.30E+00	1.34E+00	1.40E+00	1.30E+00	1.29E+00	1.28E+00
D18S51	Median_ci_low	1.10E+00	1.12E+00	1.12E+00	1.11E+00	1.10E+00	1.11E+00
D18S51	Median_ci_high	1.14E+00	1.17E+00	1.18E+00	1.14E+00	1.15E+00	1.19E+00
D18S51	Standard Deviation_ci_low	2.58E-01	2.07E-01	2.49E-01	2.01E-01	2.25E-01	2.24E-01
D18S51	Standard Deviation_ci_high	4.69E-01	4.16E-01	7.26E-01	4.10E-01	4.41E-01	4.13E-01
D19S433	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D19S433	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D19S433	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D19S433	$1/5 < R < 1/2$	1.30%	0.00%	0.00%	0.00%	0.00%	2.60%
D19S433	$1/2 < R < 1$	8.00%	10.70%	8.00%	7.80%	16.90%	14.30%
D19S433	$1 < R < 2$	77.30%	77.30%	86.70%	84.40%	76.60%	77.90%
D19S433	$2 < R < 5$	9.30%	8.00%	4.00%	7.80%	6.50%	3.90%

D19S433	5 < R < 10	4.00%	4.00%	0.00%	0.00%	0.00%	1.30%
D19S433	10 < R < 10 ²	0.00%	0.00%	1.30%	0.00%	0.00%	0.00%
D19S433	R > 10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D19S433	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D19S433	Minimum	4.10E-01	9.11E-01	7.75E-01	9.43E-01	7.95E-01	4.11E-01
D19S433	Maximum	8.49E+00	8.63E+00	2.65E+01	2.66E+00	2.81E+00	6.44E+00
D19S433	Mean	1.52E+00	1.54E+00	1.61E+00	1.24E+00	1.25E+00	1.29E+00
D19S433	Median	1.07E+00	1.10E+00	1.12E+00	1.08E+00	1.07E+00	1.13E+00
D19S433	Standard Deviation	1.48E+00	1.44E+00	2.98E+00	3.89E-01	4.16E-01	7.53E-01
D19S433	Mean_ci_low	1.23E+00	1.26E+00	1.18E+00	1.16E+00	1.16E+00	1.15E+00
D19S433	Mean_ci_high	1.88E+00	1.91E+00	2.38E+00	1.33E+00	1.35E+00	1.48E+00
D19S433	Median_ci_low	1.05E+00	1.07E+00	1.09E+00	1.05E+00	1.06E+00	1.11E+00
D19S433	Median_ci_high	1.11E+00	1.15E+00	1.16E+00	1.12E+00	1.11E+00	1.16E+00
D19S433	Standard Deviation_ci_low	4.79E-01	4.46E-01	3.47E-01	2.58E-01	2.88E-01	2.39E-01
D19S433	Standard Deviation_ci_high	2.13E+00	2.06E+00	5.01E+00	4.94E-01	5.24E-01	1.18E+00
D1S1656	R < 1/10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D1S1656	1/10 ² < R < 1/10	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D1S1656	1/10 < R < 1/5	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D1S1656	1/5 < R < 1/2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D1S1656	1/2 < R < 1	1.30%	1.30%	4.00%	1.30%	5.20%	7.80%
D1S1656	1 < R < 2	94.70%	97.30%	92.00%	96.10%	90.90%	81.80%
D1S1656	2 < R < 5	2.70%	0.00%	1.30%	2.60%	3.90%	9.10%
D1S1656	5 < R < 10	1.30%	1.30%	0.00%	0.00%	0.00%	0.00%
D1S1656	10 < R < 10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D1S1656	R > 10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D1S1656	N/A	0.00%	0.00%	2.70%	0.00%	0.00%	0.00%
D1S1656	Minimum	9.80E-01	9.73E-01	8.39E-01	9.18E-01	8.94E-01	5.35E-01
D1S1656	Maximum	5.11E+00	5.18E+00	3.85E+00	4.67E+00	4.97E+00	1.19E+01
D1S1656	Mean	1.25E+00	1.26E+00	1.25E+00	1.24E+00	1.34E+00	1.51E+00
D1S1656	Median	1.16E+00	1.19E+00	1.19E+00	1.14E+00	1.19E+00	1.25E+00
D1S1656	Standard Deviation	4.85E-01	4.70E-01	3.37E-01	4.70E-01	6.50E-01	1.29E+00
D1S1656	Mean_ci_low	1.17E+00	1.19E+00	1.19E+00	1.15E+00	1.21E+00	1.29E+00
D1S1656	Mean_ci_high	1.38E+00	1.38E+00	1.34E+00	1.36E+00	1.50E+00	1.85E+00
D1S1656	Median_ci_low	1.15E+00	1.16E+00	1.17E+00	1.13E+00	1.15E+00	1.18E+00
D1S1656	Median_ci_high	1.19E+00	1.22E+00	1.24E+00	1.17E+00	1.25E+00	1.28E+00
D1S1656	Standard Deviation_ci_low	6.51E-02	8.09E-02	1.10E-01	1.08E-01	1.86E-01	3.49E-01
D1S1656	Standard Deviation_ci_high	7.99E-01	7.93E-01	5.48E-01	7.51E-01	9.64E-01	2.12E+00
D21S11	R < 1/10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D21S11	1/10 ² < R < 1/10	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D21S11	1/10 < R < 1/5	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D21S11	1/5 < R < 1/2	0.00%	0.00%	0.00%	0.00%	0.00%	2.60%
D21S11	1/2 < R < 1	5.30%	6.70%	5.30%	6.50%	9.10%	13.00%
D21S11	1 < R < 2	90.70%	89.30%	85.30%	89.60%	84.40%	75.30%
D21S11	2 < R < 5	4.00%	4.00%	8.00%	1.30%	2.60%	7.80%
D21S11	5 < R < 10	0.00%	0.00%	1.30%	0.00%	0.00%	0.00%
D21S11	10 < R < 10 ²	0.00%	0.00%	0.00%	2.60%	3.90%	1.30%
D21S11	R > 10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D21S11	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D21S11	Minimum	8.60E-01	8.95E-01	8.04E-01	9.30E-01	8.09E-01	2.68E-01
D21S11	Maximum	3.30E+00	3.55E+00	9.86E+00	1.06E+01	1.10E+01	2.04E+01
D21S11	Mean	1.23E+00	1.25E+00	1.47E+00	1.38E+00	1.56E+00	1.55E+00

D21S11	Median	1.10E+00	1.12E+00	1.26E+00	1.09E+00	1.12E+00	1.21E+00
D21S11	Standard Deviation	3.94E-01	4.53E-01	1.07E+00	1.49E+00	1.87E+00	2.23E+00
D21S11	Mean_ci_low	1.15E+00	1.16E+00	1.29E+00	1.12E+00	1.19E+00	1.22E+00
D21S11	Mean_ci_high	1.33E+00	1.37E+00	1.75E+00	1.75E+00	2.03E+00	2.10E+00
D21S11	Median_ci_low	1.08E+00	1.09E+00	1.20E+00	1.07E+00	1.09E+00	1.15E+00
D21S11	Median_ci_high	1.12E+00	1.13E+00	1.32E+00	1.11E+00	1.17E+00	1.26E+00
D21S11	Standard Deviation_ci_low	1.65E-01	1.66E-01	2.92E-01	1.08E-01	2.61E-01	3.63E-01
D21S11	Standard Deviation_ci_high	5.53E-01	6.50E-01	1.75E+00	2.31E+00	2.71E+00	3.76E+00
D22S1045	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D22S1045	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D22S1045	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D22S1045	$1/5 < R < 1/2$	1.30%	0.00%	1.30%	0.00%	0.00%	0.00%
D22S1045	$1/2 < R < 1$	9.30%	14.70%	6.70%	10.40%	24.70%	20.80%
D22S1045	$1 < R < 2$	86.70%	82.70%	89.30%	81.80%	70.10%	71.40%
D22S1045	$2 < R < 5$	1.30%	2.70%	1.30%	3.90%	3.90%	6.50%
D22S1045	$5 < R < 10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D22S1045	$10 < R < 10^2$	1.30%	0.00%	0.00%	3.90%	1.30%	0.00%
D22S1045	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D22S1045	N/A	0.00%	0.00%	1.30%	0.00%	0.00%	1.30%
D22S1045	Minimum	4.36E-01	7.45E-01	4.02E-01	9.22E-01	7.25E-01	6.81E-01
D22S1045	Maximum	1.70E+01	2.32E+00	2.74E+00	1.13E+01	1.07E+01	2.24E+00
D22S1045	Mean	1.32E+00	1.15E+00	1.12E+00	1.51E+00	1.28E+00	1.17E+00
D22S1045	Median	1.06E+00	1.09E+00	1.07E+00	1.05E+00	1.06E+00	1.08E+00
D22S1045	Standard Deviation	1.84E+00	2.61E-01	2.38E-01	1.91E+00	1.15E+00	3.30E-01
D22S1045	Mean_ci_low	1.07E+00	1.09E+00	1.07E+00	1.14E+00	1.10E+00	1.10E+00
D22S1045	Mean_ci_high	1.78E+00	1.21E+00	1.18E+00	1.98E+00	1.58E+00	1.25E+00
D22S1045	Median_ci_low	1.04E+00	1.06E+00	1.05E+00	1.03E+00	1.03E+00	1.06E+00
D22S1045	Median_ci_high	1.09E+00	1.12E+00	1.10E+00	1.07E+00	1.10E+00	1.11E+00
D22S1045	Standard Deviation_ci_low	1.29E-01	1.47E-01	1.07E-01	3.51E-01	2.34E-01	2.35E-01
D22S1045	Standard Deviation_ci_high	3.14E+00	3.51E-01	3.65E-01	2.79E+00	1.91E+00	4.02E-01
D2S1338	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D2S1338	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D2S1338	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D2S1338	$1/5 < R < 1/2$	1.30%	0.00%	0.00%	0.00%	0.00%	1.30%
D2S1338	$1/2 < R < 1$	1.30%	2.70%	5.30%	2.60%	5.20%	13.00%
D2S1338	$1 < R < 2$	96.00%	94.70%	89.30%	97.40%	89.60%	77.90%
D2S1338	$2 < R < 5$	0.00%	1.30%	0.00%	0.00%	3.90%	2.60%
D2S1338	$5 < R < 10$	1.30%	1.30%	1.30%	0.00%	1.30%	0.00%
D2S1338	$10 < R < 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D2S1338	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D2S1338	N/A	0.00%	0.00%	4.00%	0.00%	0.00%	2.60%
D2S1338	Minimum	3.16E-01	8.02E-01	5.66E-01	8.09E-01	7.26E-01	6.63E-02
D2S1338	Maximum	7.91E+00	7.52E+00	7.56E+00	1.67E+00	6.32E+00	9.28E+01
D2S1338	Mean	1.28E+00	1.33E+00	1.31E+00	1.17E+00	1.28E+00	2.44E+00
D2S1338	Median	1.14E+00	1.19E+00	1.18E+00	1.13E+00	1.14E+00	1.17E+00
D2S1338	Standard Deviation	7.99E-01	7.58E-01	7.79E-01	1.49E-01	6.53E-01	1.06E+01
D2S1338	Mean_ci_low	1.15E+00	1.21E+00	1.19E+00	1.14E+00	1.17E+00	1.16E+00
D2S1338	Mean_ci_high	1.48E+00	1.53E+00	1.52E+00	1.20E+00	1.45E+00	4.93E+00
D2S1338	Median_ci_low	1.13E+00	1.16E+00	1.14E+00	1.11E+00	1.12E+00	1.13E+00
D2S1338	Median_ci_high	1.23E+00	1.27E+00	1.26E+00	1.15E+00	1.20E+00	1.22E+00
D2S1338	Standard Deviation_ci_low	1.37E-01	1.65E-01	1.85E-01	1.12E-01	1.92E-01	2.54E-01

D2S1338	Standard Deviation_ci_high	1.35E+00	1.26E+00	1.30E+00	1.81E-01	1.05E+00	1.81E+01
D2S441	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D2S441	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D2S441	$1/10 < R < 1/5$	0.00%	0.00%	1.30%	0.00%	0.00%	1.30%
D2S441	$1/5 < R < 1/2$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D2S441	$1/2 < R < 1$	8.00%	9.30%	14.70%	11.70%	14.30%	19.50%
D2S441	$1 < R < 2$	90.70%	88.00%	82.70%	87.00%	84.40%	72.70%
D2S441	$2 < R < 5$	0.00%	1.30%	0.00%	0.00%	0.00%	2.60%
D2S441	$5 < R < 10$	0.00%	0.00%	0.00%	1.30%	1.30%	2.60%
D2S441	$10 < R < 10^2$	1.30%	1.30%	0.00%	0.00%	0.00%	0.00%
D2S441	$R > 10^2$	0.00%	0.00%	1.30%	0.00%	0.00%	0.00%
D2S441	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D2S441	Minimum	5.85E-01	9.66E-01	1.84E-01	8.68E-01	5.35E-01	1.85E-01
D2S441	Maximum	1.36E+01	1.32E+01	1.41E+02	7.86E+00	8.33E+00	9.07E+00
D2S441	Mean	1.24E+00	1.26E+00	2.95E+00	1.17E+00	1.19E+00	1.29E+00
D2S441	Median	1.06E+00	1.07E+00	1.06E+00	1.07E+00	1.09E+00	1.07E+00
D2S441	Standard Deviation	1.45E+00	1.40E+00	1.62E+01	7.77E-01	8.38E-01	1.08E+00
D2S441	Mean_ci_low	1.05E+00	1.08E+00	1.05E+00	1.07E+00	1.07E+00	1.10E+00
D2S441	Mean_ci_high	1.59E+00	1.60E+00	6.71E+00	1.36E+00	1.40E+00	1.58E+00
D2S441	Median_ci_low	1.04E+00	1.04E+00	1.05E+00	1.05E+00	1.05E+00	1.04E+00
D2S441	Median_ci_high	1.07E+00	1.10E+00	1.10E+00	1.10E+00	1.12E+00	1.14E+00
D2S441	Standard Deviation_ci_low	8.34E-02	7.35E-02	9.80E-02	7.10E-02	1.12E-01	2.61E-01
D2S441	Standard Deviation_ci_high	2.48E+00	2.38E+00	2.77E+01	1.32E+00	1.42E+00	1.72E+00
D3S1358	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D3S1358	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D3S1358	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	2.60%
D3S1358	$1/5 < R < 1/2$	1.30%	0.00%	1.30%	0.00%	0.00%	1.30%
D3S1358	$1/2 < R < 1$	8.00%	4.00%	12.00%	14.30%	15.60%	18.20%
D3S1358	$1 < R < 2$	86.70%	92.00%	81.30%	84.40%	83.10%	74.00%
D3S1358	$2 < R < 5$	0.00%	0.00%	1.30%	1.30%	1.30%	2.60%
D3S1358	$5 < R < 10$	1.30%	1.30%	1.30%	0.00%	0.00%	1.30%
D3S1358	$10 < R < 10^2$	2.70%	2.70%	2.70%	0.00%	0.00%	0.00%
D3S1358	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D3S1358	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D3S1358	Minimum	3.15E-01	9.83E-01	3.69E-01	8.67E-01	8.37E-01	1.22E-01
D3S1358	Maximum	1.10E+01	1.14E+01	8.61E+01	2.11E+00	2.29E+00	9.56E+00
D3S1358	Mean	1.40E+00	1.47E+00	3.29E+00	1.06E+00	1.08E+00	1.19E+00
D3S1358	Median	1.04E+00	1.07E+00	1.06E+00	1.03E+00	1.06E+00	1.06E+00
D3S1358	Standard Deviation	1.80E+00	1.84E+00	1.29E+01	1.38E-01	1.75E-01	1.01E+00
D3S1358	Mean_ci_low	1.05E+00	1.10E+00	1.11E+00	1.03E+00	1.05E+00	1.03E+00
D3S1358	Mean_ci_high	1.86E+00	1.94E+00	6.54E+00	1.09E+00	1.13E+00	1.44E+00
D3S1358	Median_ci_low	1.02E+00	1.06E+00	1.05E+00	1.02E+00	1.03E+00	1.02E+00
D3S1358	Median_ci_high	1.05E+00	1.09E+00	1.08E+00	1.04E+00	1.08E+00	1.10E+00
D3S1358	Standard Deviation_ci_low	1.10E-01	1.18E-01	3.21E-01	3.59E-02	7.47E-02	2.08E-01
D3S1358	Standard Deviation_ci_high	2.62E+00	2.73E+00	2.03E+01	2.23E-01	2.70E-01	1.69E+00
D5S818	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D5S818	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D5S818	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D5S818	$1/5 < R < 1/2$	1.30%	0.00%	0.00%	0.00%	1.30%	0.00%
D5S818	$1/2 < R < 1$	12.00%	8.00%	12.00%	15.60%	18.20%	27.30%
D5S818	$1 < R < 2$	74.70%	81.30%	82.70%	77.90%	76.60%	67.50%

D5S818	2 < R < 5	10.70%	9.30%	1.30%	6.50%	3.90%	2.60%
D5S818	5 < R < 10	1.30%	1.30%	1.30%	0.00%	0.00%	0.00%
D5S818	10 < R < 10 ²	0.00%	0.00%	1.30%	0.00%	0.00%	1.30%
D5S818	R > 10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D5S818	N/A	0.00%	0.00%	1.30%	0.00%	0.00%	0.00%
D5S818	Minimum	4.81E-01	9.70E-01	8.92E-01	8.11E-01	2.73E-01	5.42E-02
D5S818	Maximum	7.61E+00	7.81E+00	9.53E+01	3.06E+00	2.79E+00	1.12E+01
D5S818	Mean	1.29E+00	1.32E+00	2.50E+00	1.16E+00	1.14E+00	1.25E+00
D5S818	Median	1.04E+00	1.07E+00	1.07E+00	1.02E+00	1.05E+00	1.05E+00
D5S818	Standard Deviation	8.91E-01	8.90E-01	1.10E+01	4.52E-01	3.49E-01	1.24E+00
D5S818	Mean_ci_low	1.13E+00	1.15E+00	1.12E+00	1.07E+00	1.06E+00	1.06E+00
D5S818	Mean_ci_high	1.52E+00	1.55E+00	5.16E+00	1.27E+00	1.22E+00	1.58E+00
D5S818	Median_ci_low	1.03E+00	1.05E+00	1.04E+00	1.02E+00	1.03E+00	1.03E+00
D5S818	Median_ci_high	1.06E+00	1.10E+00	1.10E+00	1.03E+00	1.06E+00	1.08E+00
D5S818	Standard Deviation_ci_low	3.83E-01	3.37E-01	1.89E-01	2.40E-01	1.74E-01	1.95E-01
D5S818	Standard Deviation_ci_high	1.38E+00	1.39E+00	1.87E+01	6.08E-01	4.80E-01	2.05E+00
D7S820	R < 1/10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D7S820	1/10 ² < R < 1/10	0.00%	0.00%	1.30%	0.00%	0.00%	1.30%
D7S820	1/10 < R < 1/5	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D7S820	1/5 < R < 1/2	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D7S820	1/2 < R < 1	1.30%	4.00%	12.00%	5.20%	9.10%	23.40%
D7S820	1 < R < 2	97.30%	96.00%	86.70%	94.80%	90.90%	71.40%
D7S820	2 < R < 5	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D7S820	5 < R < 10	1.30%	0.00%	0.00%	0.00%	0.00%	1.30%
D7S820	10 < R < 10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D7S820	R > 10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D7S820	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D7S820	Minimum	9.99E-01	8.83E-01	6.22E-02	8.19E-01	9.33E-01	1.92E-02
D7S820	Maximum	6.07E+00	1.38E+00	1.58E+00	1.29E+00	1.73E+00	9.83E+00
D7S820	Mean	1.17E+00	1.11E+00	1.11E+00	1.06E+00	1.10E+00	1.22E+00
D7S820	Median	1.07E+00	1.08E+00	1.09E+00	1.04E+00	1.07E+00	1.06E+00
D7S820	Standard Deviation	5.86E-01	1.01E-01	1.94E-01	7.22E-02	1.12E-01	1.05E+00
D7S820	Mean_ci_low	1.09E+00	1.09E+00	1.07E+00	1.04E+00	1.07E+00	1.06E+00
D7S820	Mean_ci_high	1.32E+00	1.13E+00	1.16E+00	1.08E+00	1.12E+00	1.49E+00
D7S820	Median_ci_low	1.06E+00	1.07E+00	1.07E+00	1.03E+00	1.06E+00	1.04E+00
D7S820	Median_ci_high	1.08E+00	1.10E+00	1.12E+00	1.06E+00	1.08E+00	1.10E+00
D7S820	Standard Deviation_ci_low	7.44E-02	7.86E-02	1.27E-01	5.07E-02	7.14E-02	1.88E-01
D7S820	Standard Deviation_ci_high	9.89E-01	1.19E-01	2.67E-01	8.99E-02	1.55E-01	1.75E+00
D8S1179	R < 1/10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D8S1179	1/10 ² < R < 1/10	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D8S1179	1/10 < R < 1/5	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D8S1179	1/5 < R < 1/2	0.00%	0.00%	0.00%	0.00%	0.00%	2.60%
D8S1179	1/2 < R < 1	1.30%	1.30%	9.30%	9.10%	11.70%	15.60%
D8S1179	1 < R < 2	92.00%	94.70%	86.70%	83.10%	80.50%	71.40%
D8S1179	2 < R < 5	4.00%	2.70%	2.70%	5.20%	5.20%	9.10%
D8S1179	5 < R < 10	2.70%	1.30%	1.30%	2.60%	2.60%	0.00%
D8S1179	10 < R < 10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D8S1179	R > 10 ²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
D8S1179	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
D8S1179	Minimum	9.56E-01	9.73E-01	8.31E-01	7.36E-01	5.86E-01	2.97E-01
D8S1179	Maximum	9.59E+00	9.22E+00	7.34E+00	8.00E+00	8.92E+00	3.97E+00

D8S1179	Mean	1.41E+00	1.32E+00	1.27E+00	1.36E+00	1.39E+00	1.36E+00
D8S1179	Median	1.10E+00	1.10E+00	1.11E+00	1.06E+00	1.09E+00	1.11E+00
D8S1179	Standard Deviation	1.36E+00	1.04E+00	8.20E-01	1.16E+00	1.27E+00	7.44E-01
D8S1179	Mean_ci_low	1.15E+00	1.14E+00	1.12E+00	1.14E+00	1.15E+00	1.20E+00
D8S1179	Mean_ci_high	1.75E+00	1.60E+00	1.48E+00	1.65E+00	1.71E+00	1.53E+00
D8S1179	Median_ci_low	1.06E+00	1.08E+00	1.09E+00	1.05E+00	1.07E+00	1.06E+00
D8S1179	Median_ci_high	1.11E+00	1.13E+00	1.13E+00	1.08E+00	1.12E+00	1.16E+00
D8S1179	Standard Deviation_ci_low	2.99E-01	1.37E-01	1.36E-01	3.36E-01	2.94E-01	4.89E-01
D8S1179	Standard Deviation_ci_high	2.06E+00	1.70E+00	1.32E+00	1.73E+00	1.92E+00	9.37E-01
FGA	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
FGA	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
FGA	$1/10 < R < 1/5$	1.30%	0.00%	0.00%	0.00%	0.00%	0.00%
FGA	$1/5 < R < 1/2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
FGA	$1/2 < R < 1$	0.00%	0.00%	9.30%	2.60%	6.50%	16.90%
FGA	$1 < R < 2$	90.70%	89.30%	81.30%	88.30%	84.40%	74.00%
FGA	$2 < R < 5$	2.70%	5.30%	6.70%	6.50%	6.50%	5.20%
FGA	$5 < R < 10$	1.30%	1.30%	0.00%	1.30%	0.00%	2.60%
FGA	$10 < R < 10^2$	4.00%	4.00%	0.00%	1.30%	2.60%	0.00%
FGA	$R > 10^2$	0.00%	0.00%	2.70%	0.00%	0.00%	0.00%
FGA	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
FGA	Minimum	1.91E-01	1.01E+00	6.33E-01	7.80E-01	7.32E-01	9.96E-02
FGA	Maximum	1.39E+01	1.41E+01	2.08E+02	1.15E+01	3.06E+01	8.92E+00
FGA	Mean	1.75E+00	1.85E+00	6.54E+00	1.51E+00	1.81E+00	1.37E+00
FGA	Median	1.12E+00	1.12E+00	1.14E+00	1.10E+00	1.10E+00	1.12E+00
FGA	Standard Deviation	2.39E+00	2.47E+00	3.22E+01	1.53E+00	3.59E+00	1.11E+00
FGA	Mean_ci_low	1.28E+00	1.36E+00	1.20E+00	1.22E+00	1.23E+00	1.16E+00
FGA	Mean_ci_high	2.35E+00	2.46E+00	1.46E+01	1.90E+00	2.76E+00	1.66E+00
FGA	Median_ci_low	1.09E+00	1.10E+00	1.11E+00	1.07E+00	1.07E+00	1.07E+00
FGA	Median_ci_high	1.15E+00	1.14E+00	1.18E+00	1.13E+00	1.14E+00	1.16E+00
FGA	Standard Deviation_ci_low	1.01E+00	1.10E+00	3.15E-01	5.36E-01	5.38E-01	4.10E-01
FGA	Standard Deviation_ci_high	3.37E+00	3.42E+00	5.03E+01	2.34E+00	5.92E+00	1.70E+00
SE33	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
SE33	$1/10^2 < R < 1/10$	1.30%	0.00%	0.00%	0.00%	0.00%	0.00%
SE33	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
SE33	$1/5 < R < 1/2$	0.00%	0.00%	0.00%	0.00%	0.00%	2.60%
SE33	$1/2 < R < 1$	0.00%	1.30%	4.00%	1.30%	3.90%	3.90%
SE33	$1 < R < 2$	89.30%	90.70%	77.30%	90.90%	88.30%	70.10%
SE33	$2 < R < 5$	6.70%	4.00%	13.30%	5.20%	6.50%	13.00%
SE33	$5 < R < 10$	1.30%	2.70%	1.30%	0.00%	0.00%	2.60%
SE33	$10 < R < 10^2$	1.30%	1.30%	1.30%	2.60%	1.30%	1.30%
SE33	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
SE33	N/A	0.00%	0.00%	2.70%	0.00%	0.00%	2.60%
SE33	Minimum	1.63E-02	9.44E-01	5.96E-01	8.02E-01	5.79E-01	2.31E-03
SE33	Maximum	1.53E+01	1.65E+01	1.66E+01	1.23E+01	1.17E+01	4.57E+02
SE33	Mean	1.71E+00	1.77E+00	1.86E+00	1.77E+00	1.62E+00	7.81E+00
SE33	Median	1.34E+00	1.37E+00	1.43E+00	1.41E+00	1.40E+00	1.40E+00
SE33	Standard Deviation	1.79E+00	1.90E+00	1.99E+00	1.73E+00	1.24E+00	5.26E+01
SE33	Mean_ci_low	1.40E+00	1.45E+00	1.51E+00	1.46E+00	1.42E+00	1.49E+00
SE33	Mean_ci_high	2.19E+00	2.27E+00	2.38E+00	2.20E+00	1.96E+00	2.02E+01
SE33	Median_ci_low	1.30E+00	1.33E+00	1.32E+00	1.37E+00	1.32E+00	1.32E+00
SE33	Median_ci_high	1.44E+00	1.45E+00	1.52E+00	1.46E+00	1.48E+00	1.58E+00

SE33	Standard Deviation_ci_low	3.52E-01	3.14E-01	5.50E-01	2.70E-01	3.01E-01	7.51E-01
SE33	Standard Deviation_ci_high	2.91E+00	3.10E+00	3.20E+00	2.65E+00	2.04E+00	8.98E+01
TH01	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TH01	$1/10^2 < R < 1/10$	1.30%	0.00%	0.00%	0.00%	0.00%	0.00%
TH01	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TH01	$1/5 < R < 1/2$	0.00%	0.00%	0.00%	0.00%	1.30%	2.60%
TH01	$1/2 < R < 1$	5.30%	9.30%	13.30%	10.40%	19.50%	22.10%
TH01	$1 < R < 2$	86.70%	85.30%	81.30%	88.30%	79.20%	70.10%
TH01	$2 < R < 5$	5.30%	5.30%	5.30%	1.30%	0.00%	5.20%
TH01	$5 < R < 10$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TH01	$10 < R < 10^2$	1.30%	0.00%	0.00%	0.00%	0.00%	0.00%
TH01	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TH01	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TH01	Minimum	8.51E-02	9.13E-01	8.00E-01	8.39E-01	2.32E-01	4.85E-01
TH01	Maximum	1.18E+01	2.67E+00	2.66E+00	2.01E+00	1.61E+00	3.54E+00
TH01	Mean	1.26E+00	1.16E+00	1.18E+00	1.06E+00	1.06E+00	1.13E+00
TH01	Median	1.06E+00	1.07E+00	1.07E+00	1.05E+00	1.05E+00	1.05E+00
TH01	Standard Deviation	1.27E+00	3.14E-01	3.73E-01	1.21E-01	1.41E-01	4.17E-01
TH01	Mean_ci_low	1.07E+00	1.10E+00	1.10E+00	1.04E+00	1.02E+00	1.05E+00
TH01	Mean_ci_high	1.59E+00	1.24E+00	1.27E+00	1.09E+00	1.09E+00	1.23E+00
TH01	Median_ci_low	1.06E+00	1.06E+00	1.05E+00	1.04E+00	1.02E+00	1.03E+00
TH01	Median_ci_high	1.08E+00	1.10E+00	1.08E+00	1.06E+00	1.07E+00	1.06E+00
TH01	Standard Deviation_ci_low	1.97E-01	1.61E-01	2.11E-01	4.00E-02	7.09E-02	1.98E-01
TH01	Standard Deviation_ci_high	2.13E+00	4.31E-01	4.96E-01	1.96E-01	2.02E-01	5.95E-01
TPOX	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TPOX	$1/10^2 < R < 1/10$	1.30%	0.00%	0.00%	0.00%	0.00%	0.00%
TPOX	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TPOX	$1/5 < R < 1/2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TPOX	$1/2 < R < 1$	8.00%	24.00%	20.00%	1.30%	19.50%	23.40%
TPOX	$1 < R < 2$	82.70%	68.00%	77.30%	93.50%	76.60%	75.30%
TPOX	$2 < R < 5$	0.00%	0.00%	2.70%	0.00%	0.00%	1.30%
TPOX	$5 < R < 10$	5.30%	5.30%	0.00%	3.90%	2.60%	0.00%
TPOX	$10 < R < 10^2$	2.70%	2.70%	0.00%	1.30%	1.30%	0.00%
TPOX	$R > 10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TPOX	N/A	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
TPOX	Minimum	1.18E-02	9.36E-01	8.83E-01	9.68E-01	8.39E-01	7.88E-01
TPOX	Maximum	1.51E+01	4.50E+01	2.86E+00	1.02E+01	1.08E+01	2.30E+00
TPOX	Mean	1.75E+00	2.15E+00	1.12E+00	1.46E+00	1.37E+00	1.12E+00
TPOX	Median	1.03E+00	1.03E+00	1.05E+00	1.05E+00	1.04E+00	1.06E+00
TPOX	Standard Deviation	2.48E+00	5.38E+00	2.71E-01	1.71E+00	1.63E+00	2.25E-01
TPOX	Mean_ci_low	1.25E+00	1.25E+00	1.06E+00	1.15E+00	1.05E+00	1.08E+00
TPOX	Mean_ci_high	2.37E+00	3.53E+00	1.18E+00	1.88E+00	1.79E+00	1.18E+00
TPOX	Median_ci_low	1.02E+00	1.01E+00	1.03E+00	1.03E+00	1.02E+00	1.04E+00
TPOX	Median_ci_high	1.05E+00	1.04E+00	1.07E+00	1.06E+00	1.05E+00	1.09E+00
TPOX	Standard Deviation_ci_low	1.16E+00	1.17E+00	9.50E-02	7.48E-01	7.40E-02	1.42E-01
TPOX	Standard Deviation_ci_high	3.47E+00	8.85E+00	4.16E-01	2.43E+00	2.42E+00	3.06E-01
vWA	$R < 1/10^2$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
vWA	$1/10^2 < R < 1/10$	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%
vWA	$1/10 < R < 1/5$	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
vWA	$1/5 < R < 1/2$	1.30%	0.00%	1.30%	0.00%	1.30%	1.30%
vWA	$1/2 < R < 1$	1.30%	1.30%	5.30%	5.20%	11.70%	16.90%

vWA	1 < R < 2	85.30%	89.30%	84.00%	89.60%	81.80%	79.20%
vWA	2 < R < 5	6.70%	5.30%	4.00%	5.20%	5.20%	1.30%
vWA	5 < R < 10	4.00%	1.30%	2.70%	0.00%	0.00%	0.00%
vWA	10 < R < 10²	1.30%	2.70%	1.30%	0.00%	0.00%	0.00%
vWA	R > 10²	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
vWA	N/A	0.00%	0.00%	1.30%	0.00%	0.00%	0.00%
vWA	Minimum	3.50E-01	6.84E-01	4.50E-01	9.38E-01	4.52E-01	8.19E-02
vWA	Maximum	1.09E+01	1.25E+01	8.13E+01	2.42E+00	2.91E+00	2.12E+00
vWA	Mean	1.57E+00	1.56E+00	2.43E+00	1.13E+00	1.15E+00	1.14E+00
vWA	Median	1.08E+00	1.11E+00	1.09E+00	1.06E+00	1.07E+00	1.07E+00
vWA	Standard Deviation	1.82E+00	1.92E+00	9.36E+00	2.97E-01	3.72E-01	3.02E-01
vWA	Mean_ci_low	1.21E+00	1.18E+00	1.18E+00	1.07E+00	1.08E+00	1.07E+00
vWA	Mean_ci_high	2.02E+00	2.05E+00	4.77E+00	1.20E+00	1.24E+00	1.21E+00
vWA	Median_ci_low	1.06E+00	1.09E+00	1.08E+00	1.04E+00	1.05E+00	1.05E+00
vWA	Median_ci_high	1.09E+00	1.14E+00	1.13E+00	1.07E+00	1.09E+00	1.10E+00
vWA	Standard Deviation_ci_low	8.72E-01	3.26E-01	3.09E-01	1.53E-01	1.69E-01	2.17E-01
vWA	Standard Deviation_ci_high	2.57E+00	2.84E+00	1.59E+01	4.09E-01	5.14E-01	3.74E-01

SM 7: Genetic profiles of pairs of samples for markers in which ratios $< 1/5$ and/or > 5 were observed in at least one of the computer tools considered

MARKER	NR.	CONTR.	PAIR	RATIO		RATIO LRMIX	RATIO EFM	RATIO STRMIX	REF	REF	REF	REF	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX		
				< 1/5	> 5				A1	FREQ A1	A2	FREQ A2	A1	HA1	FREQ A1	A2	HA2	FREQ A2	A3	HA3	FREQ A3	A4	HA4	FREQ A4	A5	HA5	FREQ A5	A6	HA6
CSF1P0	2	2_21	1	#N/D	0.017	0.988	0.917	10	0.2202	11	0.3089	10	1269	0.2202	12	166	0.3601	13	1458	0.0817									
	2	2_7	#N/D	1	10.737	10.565	1.362	7	-	10	0.2202	7	205	-	10	473	0.2202	11	3777	0.3089									
	2	2_9	#N/D	1	27.995	28.478	1.958	7	-	8	0.0055	7	762	-	8	602	0.0055	11	827	0.3089	13	513	0.0817						
	2	2_25	#N/D	1	10.550	10.510	1.311	7	-	12	0.3601	7	344	-	11	329	0.3089	12	359	0.3601									
	2	2_69	#N/D	1	10.283	10.877	1.353	7	-	11	0.3089	7	698	-	9	2658	0.0140	11	1388	0.3089	12	2561	0.3601						
	2	2_70	1	1	10.235	10.570	0.110	7	-	11	0.3089	7	2519	-	10	758	0.2202	11	2086	0.3089	12	600	0.3601						
	3	3_69	#N/D	1	10.524	10.935	1.400	7	-	11	0.3089	7	3565	-	11	5628	0.3089	12	1560	0.3601									
	3	3_75	#N/D	1	10.292	10.922	1.413	7	-	11	0.3089	7	1874	-	10	130	0.2202	11	2494	0.3089									
D10S1248	2	2_10	#N/D	1	7.991	7.620	1.759	11	0.0014	14	0.2978	10	118	-	11	2526	0.0014	13	555	0.3075	14	7319	0.2978	15	3889	0.1967			
	2	2_27	1	#N/D	0.119	1.092	1.362	13	0.3075	17	0.0277	12	348	0.0319	13	5926	0.3075	15	313	0.1967	16	3375	0.1330	17	538	0.0277			
	2	2_32	#N/D	1	7.696	7.839	6.922	13	0.3075	18	0.0014	12	1625	0.0319	13	1954	0.3075	14	1239	0.2978	17	151	0.0277	18	1444	0.0014			
	2	2_69	#N/D	1	6.552	8.545	7.754	16	0.1330	18	0.0014	12	191	0.0319	13	3468	0.3075	14	4484	0.2978	15	146	0.1967	16	1491	0.1330	18	1122	0.0014
	2	2_75	#N/D	1	9.793	10.221	1.893	11	0.0014	12	0.0319	11	1532	0.0014	12	1727	0.0319	13	457	0.3075	15	729	0.1967						
	3	3_9	1	#N/D	1.028	1.072	0.126	15	0.1967	16	0.1330	13	331	0.3075	14	1656	0.2978	15	1110	0.1967	16	1316	0.1330						
	3	3_26	1	#N/D	1.285	1.016	0.123	16	0.1330	17	0.0277	12	118	0.0319	13	821	0.3075	14	474	0.2978	15	326	0.1967	16	607	0.1330			
	3	3_36	#N/D	1	7.987	8.481	7.745	16	0.1330	18	0.0014	12	243	0.0319	13	3536	0.3075	15	8216	0.1967	16	4221	0.1330	18	4565	0.0014			
D12S339	2	2_30	#N/D	1	4.538	4.700	8.557	21	0.1288	26	0.0028	17.3	421	0.0208	18	500	0.1717	21	736	0.1288	26	581							
	3	3_28	#N/D	1	1.118	1.022	5.034	18	0.1717	21	0.1288	18	1169	0.1717	20	119	0.1108	21	1038	0.1288	22	269	0.0956						
D13S317	2	2_27	#N/D	1	5.300	1.032	1.149	12	0.2687	12	0.2687	8	3728	0.1205	11	257	0.3255	12	4649	0.2687									
	3	3_28	#N/D	1	1.031	1.004	10.223	8	0.1205	12	0.2687	8	2385	0.1205	11	603	0.3255	12	1146	0.2687	13	167	0.1163						
	3	3_49	#N/D	1	1.012	1.218	5.160	8	0.1205	12	0.2687	8	5791	0.1205	9	2407	0.0776	11	3169	0.3255	12	4865	0.2687	13	412	0.1163			
D16S539	2	2_27	#N/D	1	13.707	1.001	0.413	12	0.3144	12	0.3144	11	110	0.3144	12	505	0.3144	13	3267	0.1634									
	2	2_27	1	#N/D	0.030	1.074	1.124	15	0.1704	16	0.1468	15	1067	0.1704	16	6385	0.1468												

D1:	3	3_9	1	#N/D	2.382	2.465	0.052	13	0.1232	22	0.0069	13	682	0.1232	16	189	0.1468	17	647	0.1385	19	657	0.0402	22	574	0.0069
	2	2_5	#N/D	1	8.488	7.924	1.600	12	0.0706	12.2	0.0014	12	1074	0.0706	12.2	453	0.0014	13	3365	0.2548	14	2747	0.3615			
	2	2_10	#N/D	1	8.184	8.088	1.535	12.2	0.0014	15	0.1565	14	2034	0.3615	16	1280	0.0568	18	134	-	19	1326	-			
	2	2_70	#N/D	1	1.120	1.123	26.455	13	0.2548	16	0.0568	12	246	0.0706	13	2899	0.2548	14	100	0.3615	15	1451	0.1565	16	3341	0.0568
	2	2_74	#N/D	1	8.430	8.633	3.784	10	0.0014	15	0.1565	10	4372	0.0014	11	2441	0.0055	15	4988	0.1565				17	791	0.0069
D19S433	3	3_49	#N/D	1	1.233	1.314	6.441	14	0.3615	15.2	0.0360	13	2207	0.2548	14	5584	0.3615	15.2	4223	0.036						
	2	2_47	#REF!	#REF!	5.106	5.184	3.845	14.3	0.0028	17	0.0471	11	127	0.0776	12	2068	0.1163	14.3	413	0.0028	16.3	115	0.061	17	566	0.0471
	3	3_9	#N/D	1	1.071	1.095	11.894	11	0.0776	17.3	0.1330	10	112	0.0028	11	1712	0.0776	12	763	0.1163	13	111	0.0665	14	251	0.0789
DIS165	2	2_47	#REF!	#REF!	5.106	5.184	3.845	14.3	0.0028	17	0.0471	11	127	0.0776	12	2068	0.1163	14.3	413	0.0028	16.3	115	0.061	17	566	0.0471
	3	3_9	#N/D	1	1.071	1.095	11.894	11	0.0776	17.3	0.1330	10	112	0.0028	11	1712	0.0776	12	763	0.1163	13	111	0.0665	14	251	0.0789
D21S11	2	2_2	#N/D	1	3.295	3.513	9.860	28	0.1593	34.2	0.0042	28	791	0.1593	30	188	0.2825	31.2	455	0.0983	34.2	292	0.0042			
	3	3_46	#N/D	1	10.609	11.014	20.430	30	0.2825	33.1	-	28	7844	0.1593	30	15872	0.2825	31	160	0.072	33.1	7151	-			
	3	3_51	#N/D	1	1.347	10.958	1.403	32.2	0.0900	33.2	0.0263	28	4812	0.1593	29	142	0.2022	30	201	0.2825	30.2	4720	0.0291	32.2	2337	0.09
	3	3_76	#N/D	1	10.151	10.096	3.083	33.2	0.0263	35	0.0014	28	1399	0.1593	29	1480	0.2022	32.2	288	0.09	33.2	3186	0.0263	34	114	-
D22S1045	2	2_27	#N/D	1	16.969	0.907	1.037	15	0.3213	16	0.3823	15	666	0.3213	16	5623	0.3823	17	204	0.0748						
	3	3_2	#N/D	1	11.318	1.018	1.072	15	0.3213	15	0.3213	11	324	0.1399	14	290	0.0568	15	2151	0.3213	16	2715	0.3823	17	2664	0.0748
	3	3_5	#N/D	1	10.674	2.782	1.526	10	-	11	0.1399	10	1291	-	11	823	0.1399	15	799	0.3213	16	7837	0.3823	17	5010	0.0748
	3	3_46	#N/D	1	10.345	10.741	1.518	10	-	15	0.3213	10	18311	-	15	27091	0.3213	16	2120	0.3823	17	11876	0.0748			
D2S1338	2	2_39	#N/D	1	7.909	7.522	7.564	15	0.0014	17	0.1856	14	120	-	15	1985	0.0014	16	139	0.0374	17	2569	0.1856	21	1463	0.0374
	3	3_9	1	#N/D	1.073	1.115	0.066	24	0.1150	25	0.1025	17	157	0.1856	19	414	0.1205	20	375	0.1565	24	512	0.115	25	913	0.1025
	3	3_49	#N/D	1	1.038	1.320	92.821	19	0.1205	24	0.1150	17	2582	0.1856	19	2726	0.1205	20	230	0.1565	23	2081	0.1053	24	3454	0.115
	3	3_51	#N/D	1	1.018	6.324	1.299	17	0.1856	23	0.1053	17	2582	0.1856	19	2726	0.1205	20	230	0.1565	23	2081	0.1053	24	3454	0.115
D2S441	2	2_39	#N/D	1	13.628	13.155	141.448	10.1	-	13	0.0291	9.1	124	0.0014	10	139	0.2105	10.1	1728	-	11	2897	0.3435	13	1394	0.0291
	2	2_70	1	#N/D	1.136	1.148	0.184	14	0.2410	15	0.0596	10	221	0.2105	11	3338	0.3435	13	164	0.0291	14	3825	0.241	15	4225	0.0596
	3	3_40	#N/D	1	1.327	1.220	5.647	10	0.2105	13	0.0291	10	1246	0.2105	13	1174	0.0291	14	2701	0.241						
	3	3_42	#N/D	1	7.865	8.331	9.075	9	0.0014	10	0.2105	9	493	0.0014	10	1215	0.2105	11	1059	0.3435	11.3	145	-			
	3	3_46	1	#N/D	1.157	1.140	0.185	11	0.3435	12	0.0609	11	9826	0.3435	12	9666	0.0609	13	6283	0.0291	14	6548	0.241			
D3S1358	2	2_8	#N/D	1	8.403	8.584	5.342	13	0.0014	14	0.1066	12	204	-	13	2869	0.0014	14	3158	0.1066	15	227	0.2729	16	3241	0.2382
	2	2_46	#N/D	1	10.808	11.015	74.772	9	-	17	0.2105	9	552	-	15	210	0.2729	16	2128	0.2382	17	1702	0.2105			
	2	2_75	#N/D	1	10.960	11.402	86.131	9	-	18	0.1510	9	1050	-	15	690	0.2729	18	911	0.151						
	3	3_9	1	#N/D	1.042	1.065	0.122	14	0.1066	18	0.1510	14	1371	0.1066	15	355	0.2729	16	210	0.2382	17	407	0.2105	18	838	0.151
	3	3_29	1	#N/D	1.002	1.015	0.124	15	0.2729	17	0.2105	15	10288	0.2729	16	13052	0.2382	17	9717	0.2105	18	9438	0.151			
D5S818	3	3_21	#N/D	1	2.106	2.289	9.559	19	0.0166	19	0.0166	15	167	0.2729	16	387	0.2382	18	437	0.151	19	920	0.0166			
	2	2_70	#N/D	1	1.008	1.003	95.299	11	0.3560	13	0.1427	10	242	0.0554	11	4861	0.356	12	2097	0.3879	13	4721	0.1427	15	1381	0.0014
	2	2_61	#N/D	1	7.605	7.809	7.127	12	0.3879	15	0.0014	10	242	0.0554	11	4861	0.356	12	2097	0.3879	13	4721	0.1427	15	1381	0.0014
	3	3_9	1	#N/D	1.009	1.048	0.054	11	0.3560	11	0.3560	9	145	0.0416	10	120	0.0554	11	1564	0.356	12	888	0.3879	13	661	0.1427
	3	3_15	#N/D	1	1.438	1.449	11.247	9	0.0416	9	0.0416	7	130	0.0028	9	2715	0.0416	11	101	0.356	12	421	0.3879	13	2487	0.1427
820	2	2_21	#N/D	1	6.065	1.217	1.438	9	0.1676	13	0.0346	9	1886	0.1676	10	2256	0.2562	13	117	0.0346						
	2	2_70	1	#N/D	1.330	1.335	0.062	7	0.0277	8	0.1444	7	5258	0.0277	8	4001	0.1444	10	674	0.2562	11	1159	0.205			

D7	3	3_9	1	#N/D	1.000	1.009	0.019	10	0.2562	12	0.1593	7	104	0.0277	8	239	0.1444	10	932	0.2562	11	698	0.205	12	615	0.1593			
	3	3_49	#N/D	1	1.039	1.154	9.830	8	0.1444	9	0.1676	8	3243	0.1444	9	6306	0.1676	10	2273	0.2562	11	247	0.205						
D8S1179	2	2_21	#N/D	1	9.587	1.230	1.184	12	0.1676	16	0.0332	12	535	0.1676	13	3151	0.3297	14	2597	0.1662	16	408	0.0332						
	2	2_29	#N/D	1	8.471	9.216	3.573	15	0.1039	17	0.0014	14	1544	0.1662	15	3458	0.1039	17	996	0.0014									
	2	2_73	#N/D	1	4.475	4.732	7.344	8	0.0139	9	0.0055	8	2324	0.0139	9	2400	0.0055												
	3	3_15	#N/D	1	7.999	8.581	2.849	10	0.1025	17	0.0014	9	112	0.0055	10	2097	0.1025	12	394	0.1676	13	3433	0.3297	14	220	0.1662	17	914	0.0014
	3	3_74	#N/D	1	7.784	8.924	3.259	14	0.1662	17	0.0014	12	162	0.1676	13	582	0.3297	14	6005	0.1662	15	280	0.1039	16	3657	0.0332	17	2216	0.0014
FGA	2	2_27	1	#N/D	0.191	3.818	1.532	25	0.0790	27	0.0042	21	110	0.1787	22	1925	0.205	23	1602	0.1524	25	234	0.079	27	278	0.0042			
	2	2_33	#N/D	1	13.857	14.075	2.747	26	0.0263	28	-	21	3702	0.1787	25	3556	0.079	26	2306	0.0263	28	2898	-						
	2	2_43	#N/D	1	11.294	9.538	191.737	23.3	-	25	0.0790	20	1328	0.1233	21	1142	0.1787	23.3	1021	-	25	1014	0.079						
	2	2_66	#N/D	1	9.044	11.704	2.823	25	0.0790	28	-	20	1401	0.1233	21	919	0.1787	25	1959	0.079	27	180	0.0042	28	2093	-			
	2	2_75	#N/D	1	11.672	11.824	207.757	25	0.0790	41.2	-	20	228	0.1233	25	722	0.079	41.2	347	-									
	3	3_29	1	#N/D	1.067	1.043	0.100	20	0.1233	22	0.2050	20	3468	0.1233	21	4725	0.1787	22	34447	0.205	27	2748	0.0042						
	3	3_51	#N/D	1	8.033	30.553	8.923	23	0.1524	24.2	0.0014	22	3650	0.205	23	1637	0.1524	24	2558	0.1343	24.2	1461	0.0014						
	3	3_60	#N/D	1	4.680	4.786	5.065	20	0.1233	23.2	0.0028	19	357	0.0499	20	134	0.1233	21	1305	0.1787	25.2	282	-	28.2	969	-			
	3	3_77	#N/D	1	11.503	11.692	2.903	19	0.0499	28	-	19	1163	0.0499	21	157	0.1787	22	143	0.205	23	324	0.1524	25	385	0.079	28	1114	-
SE33	2	2_18	#N/D	1	15.319	16.469	16.647	17.3	0.0014	20.2	0.0097	17.3	464	0.0014	19	516	0.072	20.2	960	0.0097	23.2	355	0.036						
	2	2_27	1	#N/D	0.016	1.311	1.412	15	0.0402	29.2	0.0554	15	329	0.0402	18	198	0.072	19	2099	0.072	29.2	572	0.0554	30.2	2760	0.0568			
	2	2_38	#N/D	1	7.399	6.561	7.017	22	0.0139	23	0.0028	17	347	0.0623	21	143	0.025	22	1046	0.0139	23	690	0.0028						
	2	2_43	#N/D	1	3.561	5.327	4.184	18	0.0720	33.2	0.0042	11	137	0.0014	12	2453	0.0069	17	132	0.0623	18	1618	0.072	23.2	150	0.036	24.2	1983	0.0222
	3	3_1	#N/D	1	12.294	1.205	456.748	20	0.0582	29	-	20	526	0.0582	28.2	131	0.0762	29	323	-									
	3	3_4	#N/D	1	2.309	1.403	-	32.2	0.0125	32.2	0.0125	13	149	0.0166	16	1113	0.0402	19	306	0.072	22.2	1190	0.0374	25.2	1631	0.0416	32.2	6366	0.0125
	3	3_5	#N/D	1	1.793	1.145	-	21	0.0250	24.2	0.0222	17	289	0.0623	17.2	111	-	18	3380	0.072	21	479	0.025	24.2	561	0.0222	25.2	2484	0.0416
	3	3_9	1	#N/D	1.524	1.453	0.186	22.2	0.0374	26.2	0.0416	19	994	0.072	22.2	570	0.0374	26.2	354	0.0416									
	3	3_24	#N/D	1	2.668	2.348	6.791	12	0.0069	19	0.0720	12	566	0.0069	19	149	0.072	20	1545	0.0582	21.2	541	0.0235						
	3	3_28	#N/D	1	1.332	1.034	12.407	17	0.0623	18	0.0720	17	990	0.0623	18	1468	0.072	31.2	159	0.0235									
	3	3_38	1	1	11.817	11.665	0.002	19	0.0720	35	-	15	368	0.0402	19	302	0.072	26.2	131	0.0416	35	269	-						
	3	3_42	#N/D	1	3.655	3.454	5.895	27.2	0.0942	33.2	0.0042	17	109	0.0623	18	1142	0.072	27.2	362	0.0942	33.2	331	0.0042						
TH01	2	2_27	#N/D	1	11.783	1.013	1.020	6	0.2355	6	0.2355	6	277	0.2355	7	1967	0.1939	9.3	2189	0.3449									
	2	2_21	1	#N/D	0.085	1.042	1.214	9	0.1191	10	0.0083	7	1829	0.1939	9	425	0.1191	9.3	2044	0.3449									
TPOX	2	2_27	1	#N/D	0.012	0.962	1.053	8	0.5250	9	0.1274	8	1190	0.525	9	1425	0.1274												
	2	2_1	#N/D	1	7.816	7.917	1.352	6	0.0014	8	0.5250	6	424	0.0014	8	843	0.525	11	299	0.2521									
	2	2_43	#N/D	1	15.054	45.024	2.862	6	0.0014	6	0.0014	6	1587	0.0014	8	801	0.525	9	702	0.1274									
	2	2_48	#N/D	1	10.940	11.035	2.322	7	-	9	0.1274	7	816	-	8	883	0.525	9	410	0.1274									
	2	2_64	#N/D	1	8.176	8.278	1.256	6	0.0014	9	0.1274	6	407	0.0014	8	874	0.525	9	350	0.1274									
	2	2_74	#N/D	1	7.908	7.736	1.364	6	0.0014	8	0.5250	6	1838	0.0014	8	3750	0.525	10	848	0.0499									
	2	2_75	#N/D	1	8.176	8.443	1.328	6	0.0014	9	0.1274	6	511	0.0014	8	101	0.525	9	368	0.1274									

vWA	3	3_5	#N/D	1	7.608	0.944	1.207	6	0.0014	11	0.2521	6	501	0.0014	7	4558	-	8	4558	0.525	9	1260	0.1274	11	366	0.2521			
	3	3_6	#N/D	1	10.166	10.755	2.297	7	-	11	0.2521	7	952	-	8	484	0.525	9	225	0.1274	11	1095	0.2521	12	122	0.0416			
	3	3_72	#N/D	1	8.017	7.738	1.376	6	0.0014	9	0.1274	6	854	0.0014	8	2543	0.525	9	4443	0.1274									
	3	3_73	#N/D	1	8.876	9.520	1.409	6	0.0014	10	0.0499	6	1176	0.0014	9	274	0.1274	10	907	0.0499	11	129	0.2521						
	2	2_21	#N/D	1	8.145	1.069	1.085	14	0.0928	15	0.1053	14	174	0.0928	15	445	0.1053	16	3733	0.2008									
	2	2_22	#N/D	1	10.944	11.000	7.271	11	-	15	0.1053	11	842	-	15	588	0.1053	16	1575	0.2008	18	976	0.2022						
	2	2_43	#N/D	1	8.556	12.521	81.347	18	0.2022	22	-	14	121	0.0928	15	1559	0.1053	16	137	0.2008	17	1789	0.2839	18	1713	0.2022	22	1217	-
	2	2_48	#N/D	1	8.297	8.340	7.806	14	0.0928	21	0.0014	14	1362	0.0928	15	947	0.1053	18	887	0.2022	21	1038	0.0014						
	3	3_29	1	#N/D	1.035	1.095	0.082	17	0.2839	17	0.2839	14	9535	0.0928	16	8893	0.2008	17	12525	0.2839	18	176	0.2022						

SM 8: Pair 3_9 STRmix results and genotypic data

S8a: STRmix results computed for pair 3_9 disregarding ($\theta=0$) and considering coancestry ($\theta=0.01$) and respective ratio.

 $\theta = 0$

LOCUS	Pr(E Hp)	Pr(E Hd)	LR
D3S1358	4.58E-06	1.36E-04	3.36E-02
vWA	5.90E-04	2.67E-04	2.21E+00
D16S539	8.50E-04	3.67E-04	2.32E+00
CSF1PO	4.59E-04	2.80E-04	1.64E+00
TPOX	6.36E-05	1.51E-04	4.21E-01
D8S1179	8.06E-05	1.24E-04	6.48E-01
D21S11	1.66E-04	9.00E-05	1.85E+00
D18S51	1.57E-06	7.66E-07	2.05E+00
D2S441	1.12E-03	4.30E-04	2.61E+00
D19S433	2.83E-03	1.67E-03	1.70E+00
TH01	5.42E-04	5.92E-04	9.15E-01
FGA	2.69E-04	1.05E-04	2.56E+00
D22S1045	1.25E-03	7.23E-04	1.73E+00
D5S818	1.49E-05	3.59E-04	4.14E-02
D13S317	1.16E-03	6.73E-04	1.73E+00
D7S820	1.81E-06	4.01E-05	4.51E-02
SE33	5.84E-06	1.11E-06	5.26E+00
D10S1248	5.73E-05	3.72E-04	1.54E-01
D1S1656	3.40E-08	3.08E-06	1.10E-02
D12S391	8.82E-05	3.70E-05	2.39E+00
D2S1338	1.00E-05	1.84E-05	5.45E-01
SUB-SUB LR			1.96E-04

 $\theta = 0.01$

LOCUS	Pr(E Hp)	Pr(E Hd)	LR	Ratio
D3S1358	4.12E-05	1.49E-04	2.76E-01	1.22E-01
vWA	3.88E-04	3.17E-04	1.22E+00	1.81E+00
D16S539	3.20E-03	4.60E-04	6.96E+00	3.33E-01
CSF1PO	1.44E-03	3.11E-04	4.62E+00	3.55E-01
TPOX	7.85E-05	1.47E-04	5.35E-01	7.88E-01
D8S1179	4.85E-05	1.19E-04	4.09E-01	1.58E+00
D21S11	7.51E-04	1.09E-04	6.89E+00	2.68E-01
D18S51	6.83E-05	1.71E-06	3.98E+01	5.15E-02
D2S441	1.48E-03	5.71E-04	2.59E+00	1.01E+00
D19S433	3.93E-03	1.84E-03	2.13E+00	7.97E-01
TH01	1.17E-03	6.22E-04	1.89E+00	4.85E-01
FGA	5.16E-04	1.31E-04	3.93E+00	6.52E-01
D22S1045	6.39E-04	7.64E-04	8.36E-01	2.06E+00
D5S818	2.61E-04	3.42E-04	7.64E-01	5.42E-02
D13S317	8.03E-04	6.93E-04	1.16E+00	1.49E+00
D7S820	8.66E-05	3.69E-05	2.35E+00	1.92E-02
SE33	5.67E-05	2.01E-06	2.83E+01	1.86E-01
D10S1248	4.63E-04	3.80E-04	1.22E+00	1.26E-01
D1S1656	3.45E-09	3.72E-06	9.27E-04	1.19E+01
D12S391	4.79E-05	3.86E-05	1.24E+00	1.92E+00
D2S1338	1.61E-04	1.95E-05	8.22E+00	6.63E-02
SUB-SUB LR			1.51E+04	1.29E-08

S8b: Evidence genotypic profile consider in pair 3_9.

EVIDENCE PROFILE

Marker	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Height 1	Height 2	Height 3	Height 4	Height 5
D3S1358	14	15	16	17	18	1371	355	210	407	838
vWA	15	17	18			1036	1214	473		
D16S539	12	13				1606	450			
CSF1PO	10	11	12	13		402	777	275	690	
TPOX	8	10	11	12		728	138	207	405	
Yindel	2					2546				
AMEL	X	Y				3182	3296			
D8S1179	11	12	13	14	15	554	132	1094	1365	675
D21S11	29	30				2509	622			
D18S51	13	16	17	19	22	682	189	647	657	574
DYS391	10					1866				
D2S441	10	14				538	1929			
D19S433	13	14				618	1348			
TH01	6	7	9			201	1305	565		
FGA	21	22	24			796	286	697		
D22S1045	11	15	16			1107	1209	820		
D5S818	9	11	12	13		145	1564	888	661	
D13S317	8	11	12			793	1477	1186		
D7S820	7	8	10	11	12	104	239	932	698	615
SE33	19	22.2	26.2			994	570	354		
D10S1248	13	14	15	16		331	1656	1110	1316	
D1S1656	11	12	13	14	16	1712	763	111	251	157
D12S391	15	17	18	19		440	588	974	536	
D2S1338	17	19	20	24	25	157	414	375	512	913

S8c: Reference genotypic profile considered in pair 3_9.

REFERENCE PROFILE

Marker	Allele 1	Allele 2
D3S1358	14	18
vWA	15	17
D16S539	12	13
CSF1PO	11	13
TPOX	8	
Yindel	2	
AMEL	X	Y
D8S1179	13	15
D21S11	29	30
D18S51	13	22
DYS391	10	
D2S441	14	
D19S433	13	14
TH01	7	9
FGA	21	24
D22S1045	11	16
D5S818	11	
D13S317	11	12
D7S820	10	12
SE33	22.2	26.2
D10S1248	15	16
D1S1656	11	17.3
D12S391	18	19
D2S1338	24	25

CHAPTER VI | Stutter Modeling in Probabilistic Genotyping for Forensic DNA Analysis: A Casework-Driven Assessment

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As the first author, I contributed significantly to the conceptual and methodological foundations of the work. My specific responsibilities included processing, analyzing, and interpreting the outputs generated by different versions of EuroForMix when applied to the same real casework data, under varying stutter modeling conditions – either modeling both forward and back stutter, or only back stutter. I wrote the initial draft of the manuscript, and I was deeply involved in the revision process.

Article

Stutter Modeling in Probabilistic Genotyping for Forensic DNA Analysis: A Casework-Driven Assessment

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Abstract

Background: Probabilistic genotyping software has become an essential tool in forensic genetics, particularly for interpreting complex DNA mixtures. Previous studies measured the impact of considering widely divergent statistical approaches in quantifying evidence, both inter- and intra-software. At a much smaller scale, this data-driven study shows how different models implemented on distinct versions of the same tool may affect the results. Among the available tools, EuroForMix stands out as a quantitative, open-source software that models various aspects of the DNA profile, including artefacts like stutter peaks. Its freeware nature allowed the use of both versions 1.9.3. and 3.4.0, between which several updates were made, including the possibility to model both back and forward stutter, compared to only modeling back stutters inputted by the expert in the earlier version. **Methods:** A total of 156 real casework sample pairs (comprising mixtures with two or three estimated contributors and associated reference) from the Portuguese Scientific Police Laboratory were analyzed using both software versions. The same input data, containing alleles and artefactual peaks, were used to reflect operational conditions. Statistical measurements were compared and further investigated. **Results:** Most Likelihood Ratio values differed in less than one order of magnitude across versions. However, exceptions were found in more complex samples, such as those with more contributors, unbalanced contributions, or greater degradation. **Conclusions:** This work emphasizes the relevance of model selection in forensic evidence quantification, even when considering different versions of the same tool. The impact of different models in statistical evaluation depends on several factors, such as sample technical conditions, genotypic profiles, and population distribution.

Keywords: probabilistic genotyping software; EuroForMix; forward stutter; back stutter; DNA mixtures; weight-of-evidence



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1. Introduction

Technological advances in forensic genetics have made it possible to obtain genetic profiles from increasingly smaller quantities of DNA. However, this progress has also led to greater complexity of the samples, creating a growing need for sophisticated informatics tools to overcome the associated difficulties and weigh the genetic evidence [1–3]. These tools can be based on two different models, qualitative or quantitative, depending on the extent of information used to quantify the genetic evidence [4,5]. In the first ones, only the qualitative information is taken into account (i.e., presence or absence of alleles), while in the second ones, the quantitative information (i.e., the height of the observed alleles) is also considered.

Nevertheless, both models quantify the weight of evidence through the computation of a Likelihood Ratio (LR) value [6,7], which compares the probability of observing the evidence assuming two alternative and mutually exclusive hypotheses. In standard identification cases with DNA mixtures, these hypotheses are “H1 = The person of interest (PoI) is a contributor to the mixture” and, alternatively, “H2 = The PoI is not a contributor nor genetically related to any contributor of the mixture”. To perform this calculation, several parameters regarding population—allele frequencies and coancestry coefficient—and analytical factors—drop-in, drop-out, analytical threshold, and modeling of stutters—need to be considered.

Informatics tools based on a quantitative model, like STRmix™ [8] or EuroForMix [9], have become the preferred approach over the more simplistic qualitative one. They account for a large amount of data and information, allowing the modeling of artefacts, such as stutter peaks, which may be present on the electropherogram (EPG) of the evidence profile. The key advantages of these tools rely on the ability to deconvolve complex DNA mixtures, estimating genotype combinations and corresponding likelihoods [5].

A stutter peak is an artefact originating during the PCR extension phase that results from a slipped-strand mispairing. During the re-annealing of the strands (template and extending strand), one may loop and be aligned in a position different from the supposed one. The more common is for the loop to occur in the template strand, resulting in the deletion of one (or more) repeat units in the new strand—the so-called back stutter, whose height corresponds to a considerable proportion of the allelic peak height (5–10%). Even though it is less common, the loop can also occur in the new strand, leading to the addition of repeat unit(s) in the new strand—forward stutter, resulting in a peak that accounts for a smaller fraction of the parent allelic height (0.5–2%) [10–12].

This artefact is one of the most observed in EPG and may be challenging to distinguish from alleles from minor donors, especially in mixed-source samples, and can lead to an inaccurate estimation of the number of contributors. Also, different studies emphasize that, to maximize the statistical significance of the LR value, stutter peaks should be included in the analysis and appropriately modeled, rather than applying a stutter filter (during the STR genotyping phase) or relying on (subjective) human decisions in what is a stutter or a true allele peak. These studies further reinforce that the amplification product of an allele is a combination of both true allele copies and their associated stutter peaks, which should not be disregarded [13].

For these reasons, some quantitative tools such as STRmix™ [8] require the inclusion of stutter peaks in the analysis [13] and model them by considering expected stutter ratios per locus derived from empirical data. Others, like EuroForMix [9], let the user decide whether to model stutters, applying a general expected stutter rate as an extension of the probabilistic model [14]. The effort and dedication to include and analyze this parameter have been evident over time. For example, in early versions of EuroForMix, as version 1.9.3, it was only possible for the expert to choose model back stutters. The more recent versions, as v. 3.4.0,

also support the modeling of forward stutters (available at: <https://www.euroformix.com/>, accessed on 3 April 2023). Despite this major improvement, it is noteworthy that between these versions, EuroForMix underwent other algorithmic improvements, especially related to optimization strategies. Previous research has compared widely differing statistical architectures and models used in various software tools, and even within the same tool. It has already been shown that distinct statistical frameworks—including those for DNA quantity and parameter estimations—can lead to substantially different results for the same input data [15–18]. However, a finer-scale comparison of modeling differences between versions of the same tool, specifically regarding the stutter model, has never been attempted.

This work aims to demonstrate the implications of analyzing the same input data under different stutter modeling options: modeling either back or both back and forward stutters by using different versions of the same software. This methodology was selected to replicate real-world forensic workflows where entire software versions are replaced rather than individual features switched. To accomplish this, pairs of real casework samples composed of a mixture (with two or three estimated contributors) and an associated reference were analyzed using the two EuroForMix versions abovementioned (1.9.3 and 3.4.0), as the older version only enables the expert to input whether to model back stutter, while the newer enables modeling both back and forward stutters.

2. Materials and Methods

In this study, real casework samples were used, rather than mocked ones, to more authentically replicate the complex and unique conditions that forensic experts encounter during routine genetic analyses. A total of 156 irreversibly anonymized DNA pairs of mixtures (78 with two and 78 with three contributors previously estimated by independent analysts) and an associated single-source profile were selected from former cases of the Portuguese Scientific Police Laboratory of the Judiciary Police. All samples were previously processed in the context of the respective casework, under manufacturer's and internal protocols, and all methods were carried out following relevant guidelines and regulations. The genetic information of each selected pair analyzed in this work was amplified using the GlobalFiler™ PCR Amplification Kit (Applied Biosystems™, Thermo Fisher Scientific Inc., Waltham, MA, USA) and GlobalFiler™ Express PCR Amplification Kit (Applied Biosystems™, Thermo Fisher Scientific Inc., Waltham, MA, USA), both 24-locus STR kits with an analytical threshold equal to 100 RFU. A set of 21 autosomal STR markers was considered, coinciding with the data analyzed in [15,17]. Proper ethics approval was obtained from the Committee for Ethical and Responsible Conduct of Research of i3S—Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal (5/CECRI/2022) concerning all the statistical analyses.

To conduct this study, two versions of the quantitative software EuroForMix [9] were used: v.1.9.3 and 3.4.0, as they incorporate different approaches to stutter modeling—the first allows only the modeling of back stutters, while the updated version allows the modeling of both back and forward stutters. These were the features correspondingly selected for the analysis. As is the standard methodology for comparative analyses, the same input profiles—containing alleles and artefactual peaks, including back and forward stutters—were used in both tools. This also reflects a typical operational scenario, where casework profiles may contain artefacts—indistinguishable from true alleles, unmodeled by the software used.

Through the comparison of the results obtained by each software version, it is possible to evaluate the impact of software modeling updates when quantifying the same input data. All the remaining parameter settings were held constant across versions, as detailed in Supplementary Table S1. For each version and selected pair of samples, LR values

were calculated assuming the alternative hypotheses: H1—“The PoI is a contributor to the mixture” versus H2—“The PoI is not a contributor and is not genetically related to any contributor of the mixture”. The reference sample used was assumed to belong to the PoI. Following recommendations from the software developers [9], the Maximum Likelihood Estimation (MLE) was employed in both versions for model comparison. To perform the calculations, the allele frequencies of the National Institute of Standards and Technology (NIST) database concerning the Caucasian population were used [19]—see Supplementary Table S2.

After completing all calculations, a comparison of the LR values assigned by both versions was performed for each pair of samples using their ratio: $R = LR1/LR2$ if $LR1 > LR2$, or $R = LR2/LR1$, otherwise. Thus, for example, a value of $1 < R < 10$ indicates that one LR (either obtained using only back stutter modeling or both back and forward stutters) is at most ten times greater than the other. The level of complexity of the mixture samples analyzed was assessed based on two parameters estimated under H1—the contributors’ mixture proportion and the degradation slope. Through the first one, it is possible to understand how imbalanced the contributions are; while the second reflects the level of degradation, typically ranging between 0 and 1, with 1 corresponding to no degradation and values below 0.60 to very degraded samples [20].

Diagnostic metrics estimated under H1 and associated with the LR value, such as Loglik and Peak Height Variability, were also assessed. Both values allow the expert to understand if the model fits the observed data: the higher the Loglik value, the better the model fit; on the other hand, a lower Peak Height Variability means a better description of the model. Statistical tests were performed to assess the paired differences in the results obtained from the different software versions. The Shapiro–Wilk test was used to assess data normality. Subsequently, if normality could be assumed, the differences between the corresponding means were evaluated using a Paired T-test; otherwise, the Wilcoxon Signed-Rank test was applied. All analyses were performed using functions from the R software v.4.1.2. Additionally, a chi-square goodness-of-fit test was conducted to infer whether there was a significant increasing or decreasing trend, assuming an equal number of decreasing and increasing LRs as the expected distribution. A significant level of $\alpha = 0.05$ was used for all the statistical analyses.

3. Results and Discussion

LR values obtained assuming only back (EuroForMix v.1.9.3) and both back and forward stutter modeling (v.3.4.0) were compared for mixtures with both two and three estimated contributors—see Supplementary Table S3 for the complete set of LR results and corresponding diagnostic values and Figure 1 for their distribution. The differences observed between LR values are presented in Table 1.

In Supplementary Table S3, other estimations performed under H1 are present as well for all the analyses and samples. These include contributors’ mixture proportions, expected peak height, peak height variability, and degradation slope. Notably, the complexity of the mixture samples analyzed, for both mixtures with two and three estimated contributors, becomes evident in these parameters. Indeed, using v.1.9.3, PoI was considered the minor contributor in 32 of the 78 analyzed cases with two estimated contributors (under H1, estimated proportion of contribution: 0.0937 to 0.4694, median = 0.3238, and a mean = 0.3112), and in 3 out of the 78 cases with three estimated contributors (under H1, estimated proportion of contribution: 0.1025 to 0.3161, median = 0.2365, and a mean = 0.2137). On the other hand, the estimated degradation slope provides insight into the degree of sample degradation. For both two and three estimated mixture contributors, the degradation slope medians were similar (values estimated under H1, 0.7510 and 0.7434, respectively), and the

means were also close (0.7389 and 0.7252, respectively). Notably, 25% of the samples in both groups had estimated slopes of 0.6498 (two contributors) and 0.6367 (three contributors), values close to the 0.60 threshold considered typical for highly degraded samples. For version v.3.4.0, similar parameter values were observed for both cases with mixtures with two and three estimated contributors.

Table 1. Ratio R ($R > 1$) between LR values computed through EuroForMix v.1.9.3 (only back stutter modeled) and v.3.4.0 (back and forward stutters modeled) for mixtures with two and three estimated contributors; the mean, median, and maximum LR values are also described. The evidence of a general trend for the LR values to increase or decrease when using the more recent version is presented. * See Supplementary Table S3 for all LR values assigned with both software versions and respective diagnostic values.

Ratio Between LRs (v.1.9.3 vs. v.3.4.0, $R > 1$)	Estimated Number of Contributors	
	2	3
$1 < R < 10$	99%	95%
$10 < R < 100$	1%	3%
$100 < R < 1000$	0%	1%
$1000 < R < 10,000$	0%	0%
$R > 10,000$	0%	1%
Mean	0.1244	0.2794
Median	0.0400	0.1034
Maximum Ratio Between LRs		
R	3.70×10^{01}	3.34×10^{05}
LR (v.1.9.3)	1.70×10^{16}	7.93×10^{21}
LR (v.3.4.0)	6.28×10^{17}	2.65×10^{27}
Pair *	24_2	7_3

As illustrated in Table 1, for most cases with mixtures with two and three estimated contributors (99% and 95%, respectively), the LR values assigned by both software versions were within the same order of magnitude—that is, for these cases the LR differences modeling both back and forward stutters or only modeling back did not exceed a factor of ten. Despite no statistically significant differences between median LR difference results being found for both mixtures with two (p -value = 3.89×10^{-01} , Wilcoxon Signed-Rank test; p -value = 2.53×10^{-12} , Shapiro–Wilk Test) and three estimated contributors (p -value = 4.40×10^{-01} , Wilcoxon Signed-Rank test; p -value = 2.67×10^{-15} , Shapiro–Wilk Test), some cases exhibited large differences, particularly for mixtures with three estimated contributors. This is expected, as mixtures with three contributors may have more complexity associated and exhibit more pronounced effects from artefacts such as forward stutters, especially if not explicitly modeled. Indeed, from the 78 pairs of samples analyzed with mixtures with two estimated contributors, only one showed LR differences greater than ten times ($R = 3.70 \times 10^{01}$), raising this number to four in the case of mixtures with three estimated contributors ($R = 1.32 \times 10^{01}$, $R = 1.39 \times 10^{01}$, $R = 1.64 \times 10^{02}$, and $R = 3.34 \times 10^{05}$).

Unsurprisingly, these cases correspond to particularly complex mixtures and sensitive situations that stand out not only due to the presence of unbalanced contributors and degraded samples but also because the genotypic configurations (often involving rare alleles) are difficult to explain under the stringency of the stutter model. For illustrative purposes, special attention was devoted to the case with the most striking difference observed ($R = 3.34 \times 10^{05}$). Through a thorough per-marker analysis of this case with a mixture of three estimated contributors, most showed modest differences between software versions, with LR ratios ranging from 0.36 to 1.73, and consistently supported the same hypoth-

esis across versions. In contrast, two markers (D21S11 and D18S51) stood out not only because different versions supported different hypotheses—the older version supported H2, while the recent version supported H1—but also due to the substantially higher ratios obtained (501.53 and 228.79, respectively)—see Supplementary Material Table S4. Thus, these two loci were mainly responsible for the largest discrepancies observed. At D18S51, the mixture showed alleles 13 (232 RFU), 14 (160 RFU), 15 (2101 RFU), 16 (139 RFU), and 17 (1820 RFU), while the reference was homozygous 15,15. The pronounced imbalance and presence of several minor peaks made interpretation difficult. In version 1.9.3, the extra peaks and their respective heights were difficult to justify due to modeling limitations in explaining the genotypic observations, given the 8:1:1 mixture ratio with the PoI as the major contributor. These difficulties lowered the likelihood under Hp and resulted in a low LR value (0.088). In version 3.4.0, the model incorporated these peaks more efficiently—some, such as peak 16, could have been modeled as forward stutters—along with other algorithmic improvements. These changes allowed the reference genotype to better explain the evidence profile, substantially increasing the LR (20.20). This demonstrates the locus's sensitivity to interpretative assumptions in mixtures. At D21S11, the effect was even more pronounced. The evidence showed alleles 27 (293 RFU), 28 (2367 RFU), and 29 (2356 RFU). Given the 8:1:1 mixture ratio with the PoI as the major contributor, the strong 29 signal is fully consistent with the reference profile (29, 29), and 28 can be readily attributed to a minor contributor. The key interpretative challenge is the smaller allele 27, which complicates the profile despite its lower height. In the earlier version, despite already modeling back stutter, allele 27 was treated as a true allele from an unknown contributor, most likely because its relative peak height did not match well with expected stutter proportions. Given its low frequency (0.02), this assignment heavily penalized the probability of Hp, resulting in a very low LR (0.028). The updated version was able to accommodate allele 27 more consistently as a back stutter from 28, avoiding the need to assign it as a genuine allele of a minor contributor. This improvement raised the LR to 14.18 and shifted the balance of evidence in favor of Hp. Together, these loci illustrate how features like allelic imbalance, additional low peaks, but also rare alleles in the population can make results highly sensitive.

On the other hand, when analyzing the diagnostic values associated—Loglik and Peak Height Variability—statistical evidence seems to support better modeling of observed data by the latest version. Statistically significant differences between median of results were found for both mixtures with two (p -value = 7.00×10^{-09} and 1.54×10^{-08} , Wilcoxon Signed-Rank test, respectively; p -value = 0 and 3.16×10^{-12} , Shapiro–Wilk Test, respectively) and three estimated contributors (p -value = 5.26×10^{-12} and 7.98×10^{-07} , Wilcoxon Signed-Rank test, respectively; p -value = 1.06×10^{-13} and 1.96×10^{-10} , Shapiro–Wilk Test, respectively). The Loglik tended to increase when both back and forward stutters were modeled (p -value = 1.90×10^{-07} and 2.22×10^{-12} , respectively, chi-square test), and the Peak Height Variability tended to decrease (p -value = 5.92×10^{-06} and 6.29×10^{-07} , resp., chi-square test) for both cases with two and three estimated contributors. Nevertheless, caution should be taken when generalizing these results for several reasons, from both theoretical and practical points of view. Regarding the first, it is noteworthy that increasing the number of parameters in a statistical model inevitably increases the degrees of freedom, which necessarily impacts the diagnostic values and corresponding model fit. From a practical, methodological point of view, it is essential to note that the same input files were used in both software versions, thereby replicating the standard approach employed in both comparative analyses and laboratory procedures when software updates are installed. In the scope of this work, depending on the case under analysis, this may have important consequences for the diagnostic values of the earlier version, which only considers back-

ward stutters. This scenario is easy to explain—if the longer peak observed in the data is, in fact, a forward stutter (likely with a small DNA quantity), then models that do not account for forward stutters might incorrectly consider it as an allele. This misclassification could lead to skewed diagnostic values, where the low peak height influences the model's interpretation, potentially compromising the accuracy of the results.

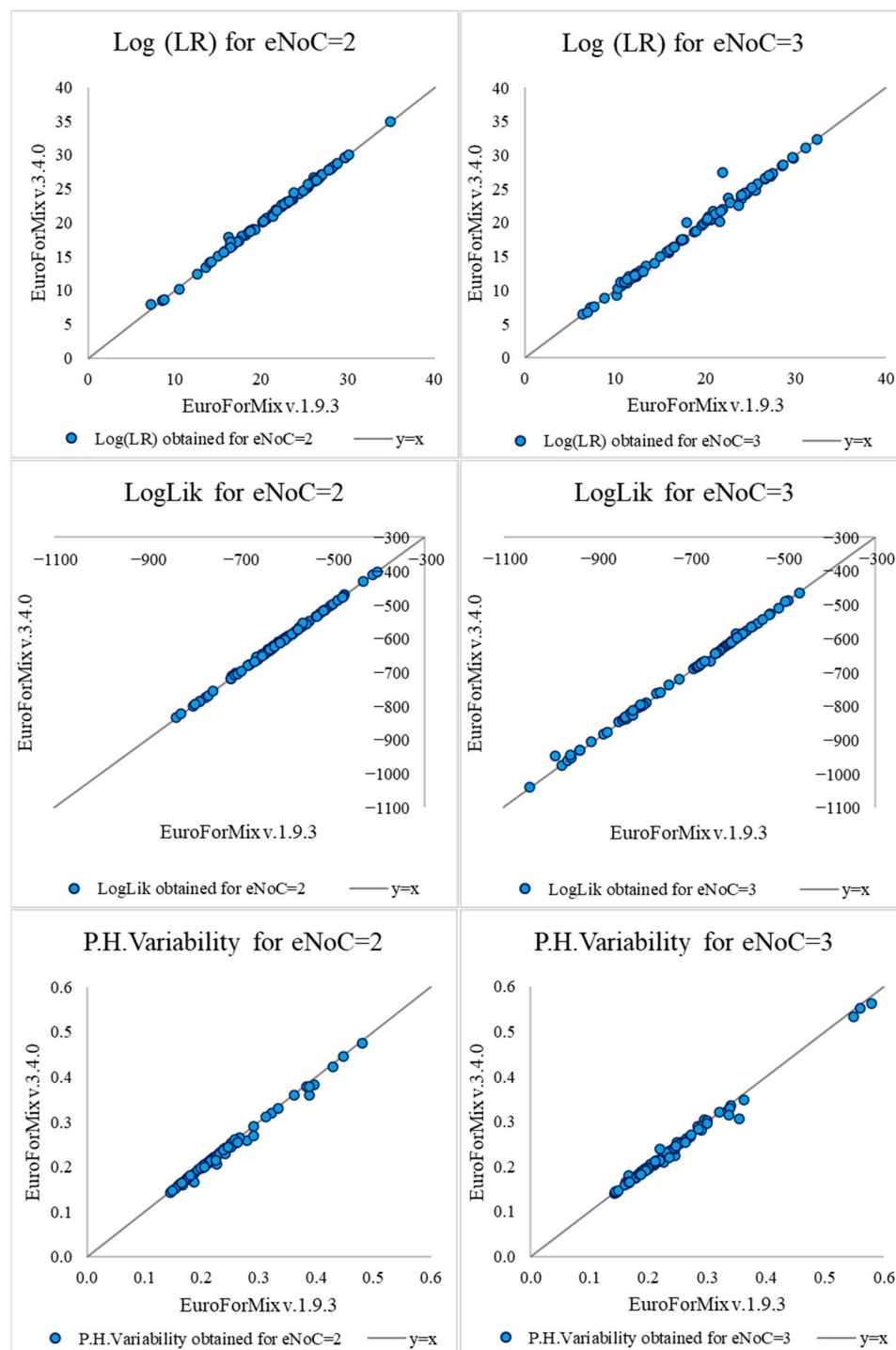


Figure 1. Distribution of LRs and associated diagnostic values, LogLik and Peak Height Variability (estimated under H1) obtained for both EuroForMix versions (1.9.3 in x-axis and 3.4.0 in y-axis), for both cases with mixtures with two (left plots) and three (right plots) estimated contributors. The order of pairs of samples is the same in each plot and corresponds to the same data displayed in Supplementary Material Table S3. For example, the first dot in each plot corresponds to pair 1_eNoC, in which eNoC (estimated number of contributors) is either 2 or 3 if considering the left or right plots, respectively.

4. Conclusions

The implementation and use of probabilistic genotyping tools in forensic genetics has increased over the past few years, enabling experts to quantify the weight of highly complex genetic samples encountered in casework. Software based on quantitative models has become the preferred approach over the more simplistic qualitative one, as it accounts for a large amount of data and information, also modeling artefacts, such as stutter peaks. Nevertheless, despite their advantages, these tools are still often perceived as black boxes by forensic geneticists, as the mathematical and statistical models incorporated are beyond the typical user's expertise. This complexity (and consequent understanding) increases as tools evolve to handle more detailed information from increasingly complex samples. Thus, using a data-driven approach, the main goal of this paper is to demonstrate the impact that applying different modeling approaches to the same input data may have, even using the same software. As software versions advance and new modeling capabilities are introduced, such as the ability to include forward stutters, it becomes crucial to understand how these developments may influence the interpretation of forensic evidence.

Staying true to the complexity of DNA samples encountered in real forensic casework, it is impossible to mimic with mocked ones; exclusively, real casework data were analyzed in this study. Each data pair consisted of a DNA mixture (with either two or three estimated contributors) and an associated reference profile. The selected samples were processed using two different versions of the same quantitative informatics tool (EuroForMix v.1.9.3 and 3.4.0). The main difference between the two versions lies in the modeling of stutter peaks—the first is only able to model back stutters, while the latter models both back and forward stutters.

By comparing LR results obtained for each pair of samples, along with the associated diagnostic values (Loglik and Peak Height Variability), it was possible to observe that most LR values remained within the same order of magnitude between the two software versions. Diagnostic values tended to indicate improved model fit when both back and forward stutters were included, as evidenced by generally higher Loglik values and lower Peak Height Variability values. However, it is important to interpret these findings cautiously. The inclusion of forward stutters modeling introduces additional parameters, thereby increasing the model's degrees of freedom, which naturally tends to improve fit diagnostics. Consequently, the better diagnostic performance of the latest software version should not be taken as definitive proof of superior modeling, but rather as an indication that more complex models may capture additional data features. Furthermore, the consistent use of identical input files across software versions ensures comparability but also highlights potential misclassifications in the earlier model that only considers back stutters, leading to forward stutter peaks being mistakenly classified as alleles, especially in low DNA quantity samples, potentially biasing diagnostic values. Thus, while the results suggest advantages in modeling both stutter peaks, the interpretation must be balanced with an understanding of the underlying model complexity and the biological and technical context of each case.

This work provides a practical example of how changes in modeling strategies—implemented even within the same tool—can influence the statistical results regarding the quantification of the observations in the forensic genetics setting. The use of more comprehensive models allows the software to better address the inherent complexity of the real casework data, as illustrated in this study. It also reinforces the importance of laboratories regularly updating the software used in their workflow, as ongoing developments contribute to improving accuracy, transparency, and reliability in forensic DNA interpretation. Therefore, it is strongly recommended that forensic laboratories not only validate updated software versions prior to implementation but also remain aware of the interpretive implications such changes may bring, particularly in complex or borderline cases.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/genes16091053/s1>, Table S1: Software parameters values and conditions considered in both EuroForMix versions used in this work: 1.9.3 and 3.4.0. Table S2: Allele frequencies of the National Institute of Standards and Technology (NIST) database considered. Table S3: LR: diagnostic values, mixture proportions, and degradation slope (all estimated under H1) obtained from both EuroForMix versions (1.9.3 and 3.4.0), for both cases with mixtures with three estimated contributors. Table S4: LR values (overall and per-marker) and diagnostic values obtained from both EuroForMix versions (1.9.3 and 3.4.0), for the pair 7_3, the one with the biggest discrepancy between versions. The genotypic data of both the evidence and reference profiles are also provided.

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Data Availability Statement: All the statistical analyses supporting the findings of this study are included in the Supplementary Material. The raw genotypic and haplotypic data are derived from real forensic casework from the Portuguese Scientific Police Laboratory, Judiciary Police (LPC-PJ), and therefore cannot be publicly released. However, non-haplotypic data related to specific analyzed markers may be available upon reasonable request and with prior permission of LPC-PJ. For inquiries regarding data access, contact the corresponding author of the manuscript.

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Supplementary Material V

The complete supplementary data associated with this article can also be found in the online version at <https://doi.org/10.3390/genes16091053>

SM1. Parameter values considered in both EuroForMix versions

Table S1: Software parameter values introduced in both versions (1.9.3 and 3.4.0) of the quantitative tool EuroForMix.

Parameters	Values
Drop-in frequency	0.05
Drop-in parameters' distribution (λ)	0.01
Analytical Threshold	100
Coancestry coefficient	0.01

Table S2: NIST allele frequency database.

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S104	D2S1338	D2S441	D3S1358	D5S818	D7S820	D8S1179	FGA	SE33	TH01	TPOX	vWA
5																			0.0014	0.0014	
6																			0.2355	0.0014	
6.3																					
7														0.0028	0.0277				0.1939		
8	0.00554			0.120499	0.01801									0.0055	0.144	0.01385			0.0956	0.5249	
8.1																0.0014					
9	0.01385			0.077562	0.10665							0.0014		0.0416	0.1676	0.00554			0.1191	0.1274	
9.1												0.0014									
9.3																			0.3449		
10	0.22022			0.047091	0.05679	0.0083	0.00139	0.0028				0.2105		0.0554	0.2562	0.102493			0.0083	0.0499	
10.1																					
10.2																					
10.3																					
11	0.30886	0.001385		0.325485	0.3144	0.0097	0.00554	0.0776		0.13989		0.3435	0.001385	0.356	0.205	0.076177		0.0014	0.0014	0.2521	
11.2																					
11.3												0.0609									
12	0.36011	0.031856		0.268698	0.3144	0.1136	0.07064	0.1163		0.01247		0.0471		0.3878	0.1593	0.16759		0.0069		0.0416	0.0014
12.2								0.00139													
12.3												0.0042									
13	0.08172	0.307479		0.116343	0.16343	0.1233	0.25485	0.0665		0.00693		0.0291	0.001385	0.1427	0.0346	0.32964		0.0166		0.0014	0.0014
13.2								0.00693													
13.3																					
13.4																					
14	0.0097	0.297784		0.042936	0.02632	0.1343	0.3615	0.0789		0.05679		0.241	0.106648	0.0069	0.0042	0.166205		0.0249			0.0928
14.2						0.0014	0.02355											0.0028			
14.3								0.0028													
15		0.196676	0.03186	0.001385		0.1704	0.15651	0.1496		0.32133	0.001385	0.0596	0.272853	0.0014		0.103878		0.0402			0.1053
15.2							0.03601														
15.3								0.0582													
15.4																					
16		0.132964	0.02216			0.1468	0.05679	0.1357		0.38227	0.037396	0.0014	0.238227			0.033241		0.0402			0.2008
16.2						0.0014	0.01524											0.0014			
16.3								0.0609													
17		0.027701	0.12742			0.1385	0.00693	0.0471		0.07479	0.185596		0.210526			0.001385		0.0623			0.2839
17.1																					
17.2							0.00139														

[illegible]

SM3. LR_s, logLik and Peak Height Variability values computed by EuroForMix v. 1.9.3 and 3.4.0

Table S3a: LR_s, logLik and Peak Height Variability values computed by EuroForMix v. 1.9.3 and 3.4.0, for mixtures with two estimated contributors.

2 contr.	v.1.9.3					v.3.4.0					Differences					Estimated under Hp																
	LR	logLik (Hp)	P.H. variability (Hp)			LR	logLik (Hp)	P.H. variability (Hp)			LR	logLik (Hp)	P.H. variability (Hp)			v.1.9.3								v.3.4.0								
			Mix_C1 MLE	Mix_C 1_SE				Mix_C2 MLE	Mix_C2 SE				E	PHE_ML PHE_SE		PHV_ML PHV_SE	Degrad MLE	Degrad SE	Mix_C1 MLE	Mix_C1 SE	Mix_C2 MLE	Mix_C2 SE	E	PHE_ML PHE_SE	PHV_ML PHV_SE	Degrad MLE	Degrad SE					
1_2	1.62E+07	-616.7	0.32113		8.47E+07	-608.76	0.32017		7.17E-01	7.940	0.000960		0.600	0.037	0.400	0.037	2182.000	168.000	0.321	0.036	0.718	0.052	0.589	0.038	0.411	0.038	2227.800	170.700	0.320	0.035	0.708	0.051
2_2	3.74E+08	-517.7	0.38847		2.78E+08	-517.28	0.35895		1.28E-01	0.420	0.029520		0.504	0.054	0.496	0.054	1163.000	106.400	0.388	0.045	0.626	0.055	0.480	0.059	0.520	0.059	1181.600	101.600	0.359	0.049	0.618	0.052
3_2	3.46E+13	-503.8	0.25081		2.56E+13	-503.49	0.25174		1.31E-01	0.310	0.000930		0.456	0.045	0.544	0.045	4438.500	273.200	0.251	0.027	0.403	0.032	0.458	0.045	0.542	0.045	4443.900	274.500	0.252	0.027	0.402	0.032
4_2	1.17E+14	-611	0.27825		1.41E+14	-608.3	0.25996		7.94E-02	2.700	0.018290		0.571	0.034	0.429	0.034	2875.200	187.800	0.278	0.030	0.724	0.044	0.568	0.032	0.432	0.032	2910.000	177.000	0.260	0.028	0.717	0.041
5_2	1.19E+19	-661	0.22172		8.25E+18	-661.03	0.2218		1.58E-01	0.030	0.000080		0.250	0.024	0.750	0.024	3678.500	200.900	0.222	0.019	0.553	0.033	0.250	0.024	0.750	0.024	3679.000	201.000	0.222	0.019	0.553	0.033
6_2	4.07E+20	-773.8	0.26605		3.56E+20	-773.79	0.26605		5.82E-02	0.010	0.000000		0.177	0.021	0.823	0.021	6493.300	399.900	0.266	0.021	0.748	0.041	0.177	0.021	0.823	0.021	6493.000	400.000	0.266	0.021	0.748	0.041
7_2	4.42E+23	-623.6	0.22048		2.71E+23	-622.33	0.22073		2.12E-01	1.270	0.000250		0.209	0.018	0.791	0.018	5647.800	292.900	0.220	0.019	0.852	0.038	0.208	0.018	0.792	0.018	5648.800	293.300	0.221	0.019	0.852	0.038
8_2	1.40E+18	-718.8	0.22154		1.44E+18	-719.11	0.2215		1.35E-02	0.310	0.000040		0.107	0.014	0.893	0.014	3940.000	203.000	0.222	0.017	0.846	0.037	0.107	0.014	0.893	0.014	3940.000	203.000	0.222	0.017	0.846	0.037
9_2	2.27E+24	-767.9	0.20631		2.24E+24	-768.23	0.20632		7.35E-03	0.330	0.000010		0.604	0.023	0.396	0.023	4841.300	237.100	0.206	0.018	0.750	0.034	0.604	0.023	0.396	0.023	4841.300	237.200	0.206	0.018	0.750	0.034
10_2	3.98E+16	-662.2	0.22043		3.89E+16	-662.25	0.221		1.00E-02	0.050	0.000570		0.421	0.022	0.579	0.022	2696.300	137.100	0.220	0.019	0.828	0.036	0.421	0.022	0.579	0.022	2698.700	137.400	0.221	0.018	0.828	0.036
11_2	4.20E+20	-715.9	0.21087		5.18E+20	-711.53	0.20868		9.10E-02	4.370	0.002190		0.322	0.022	0.678	0.022	4139.300	210.100	0.211	0.017	0.714	0.035	0.319	0.022	0.681	0.022	4153.500	208.900	0.209	0.017	0.713	0.035
12_2	5.04E+26	-631.1	0.20529		4.91E+26	-630.83	0.2037		1.13E-02	0.270	0.001590		0.835	0.019	0.165	0.019	2684.900	133.200	0.205	0.019	0.718	0.034	0.834	0.020	0.166	0.020	2692.700	132.900	0.204	0.019	0.717	0.034
13_2	4.66E+26	-568.5	0.16277		4.89E+26	-563.92	0.15986		2.04E-02	4.580	0.002910		0.720	0.019	0.280	0.019	2358.460	92.840	0.163	0.017	0.650	0.026	0.721	0.019	0.279	0.019	2375.510	92.150	0.160	0.016	0.650	0.026
14_2	2.89E+16	-645	0.48039		3.48E+16	-644.86	0.47601		8.03E-02	0.140	0.004380		0.141	0.027	0.859	0.027	12495.000	1337.000	0.480	0.052	0.918	0.079	0.143	0.027	0.857	0.027	12681.000	1334.000	0.476	0.051	0.903	0.075
15_2	6.26E+08	-473.5	0.39619		3.73E+08	-469.89	0.38225		2.24E-01	3.610	0.013940		0.587	0.049	0.413	0.049	942.930	90.040	0.396	0.042	0.526	0.053	0.575	0.056	0.425	0.056	970.480	90.090	0.382	0.041	0.515	0.051
16_2	4.35E+12	-563.1	0.21698		2.24E+12	-558.23	0.21193		2.88E-01	4.870	0.005050		0.422	0.028	0.578	0.028	1966.800	101.700	0.217	0.021	0.543	0.030	0.424	0.028	0.576	0.028	2001.900	101.600	0.212	0.020	0.539	0.029
17_2	3.33E+10	-526	0.18588		1.41E+10	-525.97	0.18516		3.74E-01	0.030	0.000720		0.094	0.014	0.906	0.014	1785.080	76.760	0.186	0.016	0.828	0.031	0.093	0.014	0.907	0.014	1787.100	77.790	0.185	0.017	0.828	0.031
18_2	1.83E+14	-614.4	0.21035		1.79E+14	-611.13	0.20828		8.88E-03	3.270	0.002070		0.535	0.022	0.465	0.022	7897.000	382.000	0.210	0.020	0.914	0.037	0.534	0.022	0.466	0.022	7924.000	379.800	0.208	0.019	0.912	0.036
19_2	1.08E+19	-611.7	0.18265		1.09E+19	-611.25	0.18137		1.67E-03	0.450	0.001280		0.415	0.021	0.585	0.021	2898.000	125.000	0.183	0.016	0.744	0.030	0.413	0.021	0.587	0.021	2905.500	124.700	0.181	0.016	0.746	0.030
20_2	4.74E+16	-586.1	0.20243		4.38E+16	-586.36	0.200030		3.42E-02	0.260	0.000030		0.402	0.028	0.598	0.028	2278.000	110.700	0.202	0.018	0.615	0.030	0.402	0.028	0.598	0.028	2278.100	110.800	0.202	0.018	0.615	0.030
21_2	1.13E+15	-629.8	0.25733		9.87E+14	-625.66	0.25348		5.82E-02	4.140	0.003850		0.346	0.035	0.654	0.035	3196.000	201.700	0.257	0.024	0.513	0.038	0.343	0.035	0.657	0.035	3214.400	200.200	0.253	0.024	0.511	0.037
22_2	6.48E+15	-517.7	0.23828		6.45E+15	-517.63	0.23899		2.42E-03	0.070	0.000710		0.548	0.031	0.452	0.031	1938.900	111.100	0.238	0.026	0.711	0.039	0.549	0.032	0.451	0.032	1939.800	111.600	0.239	0.026	0.711	0.039
23_2	2.06E+16	-595.1	0.21151		2.13E+16	-595.13	0.21129		1.43E-02	0.030	0.000220		0.582	0.030	0.418	0.030	2824.400	141.900	0.212	0.019	0.678	0.033	0.585	0.030	0.415	0.030	2840.500	142.800	0.211	0.019	0.676	0.033
24_2	1.70E+16	-659.3	0.20203		6.28E+17	-654.89	0.19879		1.57E+00	4.410	0.003240		0.144	0.016	0.856	0.016	2812.800	133.700	0.202	0.016	0.813	0.034	0.143	0.015	0.857	0.015	2837.000	133.000	0.199	0.016	0.811	0.033
25_2	6.06E+17	-601.5	0.2146		8.65E+17	-598.31	0.21258		1.55E-01	3.190	0.002020		0.411	0.023	0.589	0.023	2823.300	142.300	0.215	0.019	0.742	0.035	0.412	0.023	0.588	0.023	2841.400	142.100	0.213	0.019	0.741	0.034
26_2	1.74E+19	-565.2	0.23978		8.21E+18	-565.81	0.23902		3.25E-01	0.610	0.000760		0.248	0.021	0.752	0.021	1500.090	82.330	0.240	0.021	0.754	0.037	0.248	0.021	0.752	0.021	1501.500	82.920	0.239	0.022	0.754	0.037
27_2	2.69E+17	-626.3	0.25965		2.25E+17	-622.2	0.25763		7.76E-02	4.100	0.002020		0.575	0.043	0.425	0.043	3196.300	203.900	0.260	0.027	0.515	0.038	0.577	0.044	0.423	0.044	3217.200	204.200	0.258	0.026	0.511	0.038
28_2	3.14E+18	-580.2	0.21631		3.28E+18	-580.07	0.21668		1.80E-02	0.130	0.000370		0.609	0.037	0.391	0.037	2277.400	116.300	0.216	0.022	0.616	0.033	0.610	0.038	0.390	0.038	2277.700	118.500	0.217	0.022	0.617	0.033
29_2	1.57E+17	-513.7	0.23045		1.52E+17	-513.72	0.23065		1.50E-02	0.020	0.000200		0.469	0.030	0.531	0.030	1930.100	108.500	0.230	0.023	0.716	0.037	0.470	0.030	0.530	0.030	1930.300	106.600	0.231	0.023	0.716	0.037
30_2	2.89E+16	-647.8																														

54_2	1.86E+25	-662.3	0.16718	1.53E+25	-654	0.161	8.47E-02	8.300	0.006180	0.670	0.020	0.330	0.020	2595.400	101.600	0.167	0.015	0.760	0.027	0.669	0.019	0.331	0.019	2638.580	99.880	0.161	0.014	0.757	0.026
55_2	4.01E+26	-634.2	0.18603	3.37E+26	-630.92	0.16592	7.55E-02	3.280	0.020110	0.803	0.019	0.197	0.019	2827.900	123.500	0.186	0.019	0.750	0.030	0.792	0.018	0.208	0.018	2858.500	111.900	0.166	0.017	0.746	0.027
56_2	6.19E+24	-556.2	0.3615	6.05E+24	-556.2	0.36002	9.64E-03	0.000	0.001480	0.796	0.035	0.204	0.035	1708.000	142.000	0.362	0.035	0.906	0.062	0.796	0.034	0.204	0.034	1712.000	142.000	0.360	0.035	0.903	0.062
57_2	2.69E+26	-625.1	0.25686	2.57E+26	-624.47	0.26067	1.95E-02	0.630	0.003810	0.906	0.017	0.094	0.017	3522.100	209.600	0.257	0.023	0.865	0.044	0.914	0.019	0.086	0.019	3527.000	213.000	0.261	0.023	0.866	0.045
58_2	1.11E+26	-562.8	0.24154	5.14E+26	-552.67	0.22956	6.66E-01	10.130	0.011980	0.803	0.024	0.197	0.024	1843.200	107.700	0.242	0.022	0.550	0.035	0.812	0.026	0.188	0.026	1876.300	104.800	0.230	0.021	0.548	0.033
59_2	3.13E+25	-707.4	0.19961	3.59E+25	-703.1	0.19796	6.00E-02	4.300	0.001650	0.674	0.024	0.326	0.024	4129.300	198.300	0.200	0.018	0.715	0.033	0.675	0.024	0.325	0.024	4143.500	197.700	0.198	0.017	0.714	0.033
60_2	9.22E+26	-535.4	0.1538	1.08E+27	-535.03	0.1529	6.68E-02	0.370	0.000900	0.680	0.020	0.320	0.020	2696.600	102.300	0.154	0.015	0.492	0.022	0.682	0.021	0.318	0.021	2708.100	103.300	0.153	0.015	0.492	0.022
61_2	1.10E+26	-402	0.33387	1.15E+26	-401.94	0.33011	2.16E-02	0.060	0.003760	0.797	0.038	0.203	0.038	717.340	56.820	0.334	0.036	0.845	0.057	0.801	0.038	0.199	0.038	725.290	57.520	0.330	0.035	0.841	0.056
62_2	2.45E+25	-488.4	0.42861	5.85E+25	-487.33	0.4236	3.78E-01	1.070	0.005010	0.848	0.039	0.152	0.039	1238.400	123.300	0.429	0.041	0.770	0.069	0.869	0.042	0.131	0.042	1250.500	123.700	0.424	0.040	0.768	0.068
63_2	1.83E+26	-476.1	0.38779	2.26E+26	-476.05	0.37843	9.04E-02	0.050	0.009360	0.919	0.024	0.081	0.024	1223.400	103.300	0.388	0.038	1.043	0.066	0.927	0.025	0.073	0.025	1277.530	74.990	0.378	0.035	1.000	0.000
64_2	1.14E+27	-799.7	0.1751	1.12E+27	-800.03	0.1751	9.01E-03	0.330	0.000000	0.245	0.014	0.755	0.014	6088.200	252.200	0.175	0.013	0.858	0.031	0.245	0.014	0.755	0.014	6088.100	252.200	0.175	0.013	0.858	0.031
65_2	2.36E+26	-710.1	0.22532	1.58E+26	-708.7	0.2067	1.74E-01	1.400	0.018620	0.811	0.033	0.189	0.033	6518.500	344.100	0.225	0.024	0.808	0.038	0.772	0.036	0.228	0.036	6531.600	316.800	0.207	0.021	0.807	0.035
66_2	5.51E+27	-756.5	0.29069	5.45E+27	-756.38	0.29023	4.26E-03	0.120	0.000460	0.840	0.026	0.160	0.026	6519.000	439.400	0.291	0.026	0.745	0.045	0.840	0.026	0.160	0.026	6533.300	439.800	0.290	0.025	0.743	0.045
67_2	5.56E+27	-703.5	0.17659	5.48E+27	-703.5	0.17676	5.70E-03	0.000	0.000170	0.809	0.018	0.191	0.018	5946.200	249.200	0.177	0.016	0.560	0.025	0.809	0.018	0.191	0.018	5946.800	249.500	0.177	0.016	0.560	0.025
68_2	1.01E+28	-613	0.2373	9.91E+27	-611.1	0.23899	7.77E-03	1.900	0.001690	0.801	0.023	0.199	0.023	5633.200	315.300	0.237	0.024	0.854	0.041	0.803	0.022	0.197	0.022	5651.600	318.400	0.239	0.023	0.851	0.042
69_2	4.37E+28	-784.7	0.2038	4.45E+28	-785.05	0.2038	8.06E-03	0.350	0.000000	0.714	0.018	0.287	0.018	4494.100	208.600	0.204	0.017	0.956	0.036	0.714	0.018	0.287	0.018	4494.100	208.700	0.204	0.017	0.956	0.036
70_2	1.36E+28	-696.6	0.24579	1.33E+28	-697.26	0.2452	9.24E-03	0.660	0.000590	0.908	0.017	0.092	0.017	3925.100	224.500	0.246	0.021	0.851	0.042	0.907	0.017	0.093	0.017	3933.600	224.500	0.245	0.021	0.849	0.041
71_2	1.76E+28	-573.9	0.17744	1.71E+28	-573.75	0.17775	1.24E-02	0.150	0.000310	0.681	0.023	0.319	0.023	2465.500	104.200	0.177	0.016	0.657	0.027	0.681	0.023	0.319	0.023	2466.000	104.400	0.178	0.017	0.657	0.027
72_2	5.51E+27	-785.5	0.19764	5.62E+27	-785.82	0.19764	8.84E-03	0.320	0.000000	0.279	0.015	0.721	0.015	4494.800	201.700	0.198	0.015	0.956	0.035	0.279	0.015	0.721	0.015	4494.700	201.700	0.198	0.015	0.956	0.035
73_2	6.57E+28	-795.9	0.1803	6.67E+28	-795.84	0.1803	6.60E-03	0.060	0.000000	0.758	0.016	0.242	0.016	6086.400	259.600	0.180	0.014	0.858	0.032	0.758	0.016	0.242	0.016	6086.400	259.600	0.180	0.014	0.858	0.032
74_2	4.22E+29	-650.8	0.16458	4.19E+29	-650.61	0.16423	2.48E-03	0.190	0.000350	0.902	0.012	0.098	0.012	3729.600	147.600	0.165	0.014	0.671	0.026	0.902	0.012	0.098	0.012	3732.300	147.300	0.164	0.014	0.670	0.026
75_2	4.29E+29	-668.2	0.22471	4.06E+29	-667.07	0.21575	2.43E-02	1.130	0.008960	0.776	0.022	0.224	0.022	2728.700	141.700	0.225	0.021	0.882	0.039	0.771	0.021	0.229	0.021	2745.800	137.500	0.216	0.020	0.878	0.038
76_2	4.38E+29	-478.8	0.20462	4.40E+29	-477.87	0.20087	1.22E-03	0.930	0.003750	0.937	0.015	0.063	0.015	1789.660	85.010	0.205	0.019	0.826	0.034	0.938	0.015	0.062	0.015	1801.220	85.020	0.201	0.019	0.824	0.033
77_2	1.17E+30	-533.7	0.1488	1.08E+30	-532.8	0.14865	3.55E-02	0.900	0.000150	0.642	0.019	0.358	0.019	7048.100	247.600	0.149	0.016	0.847	0.026	0.641	0.019	0.359	0.019	7048.400	247.400	0.149	0.016	0.847	0.026
78_2	7.57E+34	-519.2	0.26174	8.04E+34	-518.34	0.25509	2.64E-02	0.860	0.006650	0.778	0.024	0.222	0.024	1498.600	90.100	0.262	0.025	0.752	0.040	0.778	0.024	0.222	0.024	1513.190	89.310	0.255	0.025	0.750	0.039

Table S3b: LR, logLik and Peak Height Variability values computed by EuroForMix v. 1.9.3 and 3.4.0, for mixtures with three estimated contributors.

3 contr.	v.1.9.3			v.3.4.0			Differences			Estimated under Hp																													
	LR	logLik (Hp)	P.H.V. (Hp)	LR	logLik (Hp)	P.H.V. (Hp)	LR	logLik (Hp)	P.H.V. (Hp)	v.1.9.3										v.3.4.0																			
										Mix_					PHV					Degra					Mix_					PHV					Degra				
										C1_	Mix_C1	Mix_C2_	Mix_C2_S	Mix_C3_	Mix_C3	PHE_ML	PHE	SE	E	ML	SE	E	ML	SE	E	ML	SE	E	ML	SE	E	ML	SE	E	ML	SE	E	ML	SE
1_3	5.96E+08	-911.3	0.54782	7.46E+08	-905.09	0.5326	9.77E-02	6.21	0.01526	0.43	0.09	0.28	0.08	0.28	0.06	9021.00	1117.00	0.55	0.05	0.47	0.06	0.45	0.11	0.28	0.10	0.28	0.07	9154.00	1104.00	0.53	0.05	0.46	0.05						
2_3	2.00E+17	-851.9	0.16783	2.09E+17	-846.59	0.1680	1.86E-02	5.31	0.00015	0.22	0.02	0.07	0.01	0.71	0.02	3766.90	149.90	0.17	0.01	0.77	0.03	0.21	0.03	0.72	0.01	0.07	0.02	3789.80	151.10	0.17	0.01	0.77	0.03						
3_3	5.18E+10	-936.5	0.22532	3.92E+10	-929.11	0.2097	1.22E-01	7.39	0.01564	0.53	0.07	0.17	0.05	0.30	0.04	5350.80	289.80	0.23	0.02	0.58	0.03	0.50	0.08	0.25	0.08	0.25	0.04	5379.40	271.40	0.21	0.02	0.57	0.03						
4_3	3.68E+17	-831.6	0.18164	3.49E+17	-831.56	0.1821	2.33E-02	0.04	0.00048	0.49	0.02	0.08	0.01	0.44	0.02	8606.60	371.40	0.18	0.02	0.78	0.03	0.49	0.04	0.44	0.01	0.08	0.03	8615.00	373.00	0.18	0.02	0.78	0.03						
5_3	7.13E+20	-1045	0.141626	5.40E+21	-1038.75	0.1407	8.80E-01	6.25	0.000925	0.10	0.02	0.45	0.10	0.45	0.08	8500.70	285.30	0.14	0.01	0.85	0.03	0.10	0.03	0.45	0.14	0.45	0.12	8521.30	284.40	0.14	0.01	0.85	0.02						
6_3	2.93E+28	-823.2	0.16574	2.46E+28	-817.72	0.1644	7.61E-02	5.48	0.00135	0.70	0.03	0.08	0.01	0.22	0.02	3761.80	148.20	0.17	0.02	0.77	0.03	0.70	0.05	0.22	0.02	0.08	0.03	3781.90	147.90	0.16	0.02	0.77	0.03						
7_3	7.93E+21	-600.7	0.35418	2.65E+27	-586.86	0.3064	5.52E+00	13.84	0.04782	0.80	0.06	0.10	0.03	0.10	0.04	2466.70	201.80	0.35	0.03	0.84	0.06	0.86	0.06	0.07	0.03	0.07	0.03	2463.00	175.60	0.31	0.03	0.84	0.05						
8_3	2.35E+06	-487.1	0.28298	2.46E+06	-486.99	0.2907	2.00E-02	0.11	0.00769	0.35	0.08	0.45	0.07	0.19	0.04	964.18	67.12	0.28	0.04	0.50	0.04	0.35	0.07	0.20	0.07	0.44	0.05	976.51	69.47	0.29	0.04	0.49	0.04						
9_3	1.45E+10	-845	0.20074	1.77E+09	-841.62	0.1949	9.14E-01	3.38	0.00589	0.17	0.04	0.56	0.04	0.27	0.03	3862.50	185.60	0.20	0.03	0.70	0.03	0.16	0.03	0.57	0.03	0.27	0.03	3891.80	182.10	0.19	0.02	0.69	0.03						
10_3	1.93E+10	-645.8	0.265	1.65E+10	-645.46	0.2633	6.91E-02	0.34	0.00174	0.52	0.08	0.39	0.03	0.09	0.05	1643.70	103.10	0.27	0.03	0.82	0.05	0.52	0.08	0.39	0.03	0.09	0.05	1648.30	102.90	0.26	0.03	0.82	0.04						
11_3	1.55E+07	-771.2	0.2141	3.04E+07	-761.02	0.2098	2.93E-01	10.18	0.00433	0.25	0.03	0.38	0.03	0.38	0.03	2538.00	131.10	0.21	0.02	0.73	0.04	0.24	0.03	0.38	0.03	0.38	0.03	2585.60	131.30	0.21	0.02	0.73	0.04						
12_3	3.92E+07	-588.4	0.25478	3.46E+07	-588.43	0.2534	5.36E-02	0.03	0.00135	0.19	0.03	0.15	0.03	0.65	0.03	1255.24	76.29	0.25	0.03	0.73	0.04	0.19	0.03	0.65	0.03	0.16	0.03	1261.14	76.34	0.25	0.03	0.73	0.04						
13_3	2.67E+13	-837.5	0.29506	3.37E+13	-831.65	0.3035	1.02E-01	5.85	0.00839	0.47	0.08	0.43	0.03	0.11	0.06	4427.50	315.20	0.30	0.03	0.53	0.04	0.48	0.09	0.42	0.04	0.11	0.07	4442.20	324.70	0.30	0.03	0.53	0.04						
14_3	7.06E+06	-975	0.27131	6.03E+06	-973.72	0.2656	6.86E-02	1.28	0.00571	0.32	0.06	0.34	0.05	0.34	0.05	8340.80	530.40	0.27	0.03	0.85	0.05	0.31	0.05	0.35	0.05	0.35	0.05	8384.50	521.40	0.27	0.02	0.84	0.04						
15_3	1.79E+12	-840.6	0.28956	3.10E+12	-834.74	0.2901	2.39E-01	5.86	0.00051	0.44	0.08	0.47	0.02	0.09	0.06	4460.90	313.60	0.29	0.03	0.52	0.04	0.45	0.10	0.47	0.03	0.08	0.07	4496.90	316.60	0.29	0.03	0.52	0.04						
16_3	2.03E+14	-833.8	0.29955	1.12E+14	-828.91	0.3031	2.56E-01	4.89	0.0035	0.46	0.04	0.10	0.03	0.44	0.05	4438.50	322.30	0.30	0.03	0.53	0.04	0.46	0.10	0.43	0.03	0.10	0.07	4459.00	327.00	0.30	0.03	0.53	0.04						
17_3	3.96E+10	-629.4	0.2987	1.44E+11	-627.12	0.2962	5.60E-01	2.28	0.00249	0.12	0.02	0.04	0.02	0.84	0.02	2450.20	169.30	0.30	0.03	0.84	0.05	0.12	0.15	0.87	0.02	0.01	0.13	2460.30	169.20	0.30	0.03	0.84	0.05						
18_3	3.23E+11	-687.5	0.32025	1.10E+12	-685.38	0.3221	5.33E-01	2.12	0.00187	0.53	0.07	0.24	0.05	0.24	0.04	2135.40	161.90	0.32	0.03	0.58	0.04	0.55	0.08	0.22	0.06	0.23	0.05	2154.80	164.50	0.32	0.03	0.58	0.05						
19_3	2.14E+12	-722.9	0.24522	1.16E+12	-719.7	0.2243	2.66E-01	3.2	0.02088	0.48	0.06	0.18	0.08	0.34	0.05	1862.90	111.80	0.25	0.04	0.64	0.04	0.47	0.07	0.37	0.04	0.17	0.04	1884.90	103.80	0.22	0.03	0.64	0.04						
20_3	1.88E+11	-587.6	0.3399	1.37E+11	-586.07	0.3349	1.36E-01	1.53	0.005	0.29	0.09	0.62	0.04	0.10	0.06	1192.63	97.12	0.34	0.04	0.81	0.06	0.29	0.11	0.64	0.04	0.07	0.07	1203.44	96.67	0.33	0.04	0.81	0.06						
21_3	1.02E+11	-566.6	0.33531	1.64E+11	-565.14	0.3254	2.05E-01	1.46	0.00993	0.46	0.12	0.45	0.04	0.09	0.08	1463.00	120.70	0.34	0.04	0.56	0.05	0.47	0.15	0.46	0.04	0.07	0.11	1488.90	120.30	0.33	0.04	0.56	0.05						
22_3	9.98E+11	-563	0.36125	8.31E+11	-562.29	0.3492	7.93E-02	0.71	0.01207	0.49	0.07	0.10	0.06	0.41	0.06	1469.40	130.70	0.36	0.05	0.56	0.05	0.48	0.15	0.42	0.06	0.10	0.10	1493.20	129.40	0.35	0.05	0.55	0.05						
23_3	1.93E+12	-641.6	0.22936	1.71E+12	-641.23	0.2274	5.21E-02	0.37	0.00192	0.39	0.04	0.08	0.02	0.53	0.03	1636.31	88.07	0.23	0.02	0.82	0.04	0.39	0.06	0.53	0.02	0.08	0.04	1640.52	88.33	0.23	0.02	0.82	0.04						
24_3	5.17E+12	-671.9	0.24238	7.41E+12	-670.57	0.2398	1.56E-01	1.33	0.00263	0.48	0.06	0.35	0.03	0.18	0.04	1562.85	89.01	0.24	0.03	0.68	0.04	0.48	0.07	0.34	0.04	0.18	0.04	1586.91	90.14	0.24	0.03	0.68	0.04						
25_3	2.24E+12	-527.7	0.57801	2.29E+12	-527.85	0.5633	9.64E-03	0.15	0.01467	0.54	509.25	0.46	0.00	0.00	509.20	1167.80	150.90	0.58	0.07	0.88	0.09	0.53	326.45	0.47	0.00	0.00	326.50	1186.40	150.60	0.56	0.07	0.87	0.09						
26_3	2.66E+12	-528.7	0.55901	2.50E+12	-528.93	0.5516	2.65E-02	0.23	0.00741	0.66	0.06	0.00	0.00	0.34	0.06	1156.90	144.70	0.56	0.06	0.89	0.09	0.65	360.52	0.35	0.00	0.00	360.50	1170.40	145.10	0.55	0.06	0.88	0.09						
27_3	4.51E+11	-553.9	0.33739	4.77E+11	-553.47	0.3290	2.43E-02	0.43	0.00837	0.44	0.06	0.08	0.04	0.48	0.05	973.98	76.53	0.34	0.04	0.88	0.06	0.44	0.06	0.07	0.04	0.49	0.05	983.61	76.44	0.33	0.04	0.88	0.06						
28_3	1.18E+13	-676.3	0.16217	6.41E+12	-673.39	0.1652	2.66E-01	2.91	0.00303	0.38	0.02	0.09	0.02	0.53	0.02	3199.30	130.80	0.16	0.01	0.38	0.02	0.38	0.06	0.54	0.02	0.08	0.04	3202.10	133.20	0.17	0.01	0.38	0.02						
29_3	2.02E+11	-623.1	0.2101	4.98E+11	-621.36	0.2039	3.91E-01	1.74	0.00617	0.32	0.08	0.58	0.02	0.10	0.07	2252.60	114.70	0.21	0.02	0.74	0.04	0.32	0.07	0.60	0.02	0.09	0.06	2274.40	112.90	0.20	0.02	0.73	0.03						
30_3	2.46E+16	-828.1	0.19671	2.71E+16	-821.72																																		

54_3	8.10E+23	-634.9	0.14438	8.43E+23	-634.66	0.1453	1.75E-02	0.24	0.00093	0.65	0.04	0.18	0.02	0.18	0.03	1978.12	68.25	0.14	0.02	0.80	0.02	0.65	0.04	0.18	0.03	0.18	0.03	1979.09	68.67	0.15	0.02	0.80	0.02
55_3	1.49E+20	-691.4	0.27069	4.56E+20	-690.19	0.2669	4.87E-01	1.21	0.00377	0.28	0.07	0.67	0.02	0.05	0.05	1667.00	100.70	0.27	0.02	0.96	0.05	0.28	0.08	0.68	0.02	0.04	0.06	1672.10	102.30	0.27	0.02	0.96	0.05
56_3	4.66E+21	-610.2	0.24271	4.50E+21	-609.55	0.2421	1.47E-02	0.65	0.00066	0.64	0.12	0.27	0.05	0.08	0.08	2153.60	124.10	0.24	0.02	0.56	0.03	0.64	0.13	0.27	0.05	0.09	0.07	2160.80	124.20	0.24	0.03	0.56	0.03
57_3	3.38E+22	-826.1	0.2357	4.69E+23	-821.28	0.2199	1.14E+00	4.82	0.01582	0.75	0.03	0.01	0.01	0.24	0.03	8207.40	457.20	0.24	0.02	0.72	0.04	0.76	1.12	0.24	0.01	0.00	1.11	8217.90	462.10	0.24	0.02	0.72	0.04
58_3	2.81E+24	-586.2	0.14873	1.95E+24	-585.4	0.1468	1.58E-01	0.8	0.00196	0.62	0.04	0.31	0.01	0.07	0.03	1674.52	59.56	0.15	0.01	0.81	0.03	0.62	0.05	0.31	0.02	0.07	0.04	1682.00	64.69	0.15	0.01	0.81	0.03
59_3	8.83E+23	-684.8	0.17861	1.36E+24	-684.02	0.1765	1.88E-01	0.78	0.00214	0.21	0.05	0.75	0.01	0.04	0.04	2242.22	90.11	0.18	0.01	0.95	0.03	0.21	0.06	0.76	0.01	0.03	0.05	2251.44	89.79	0.18	0.01	0.95	0.03
60_3	9.37E+23	-803.5	0.19379	3.96E+23	-798.67	0.1951	3.73E-01	4.83	0.00129	0.30	0.04	0.65	0.01	0.05	0.03	9713.10	445.50	0.19	0.02	0.82	0.03	0.30	0.05	0.65	0.01	0.05	0.04	9742.90	450.40	0.20	0.02	0.81	0.03
61_3	4.80E+22	-683.6	0.3363	8.56E+22	-682.98	0.3155	2.51E-01	0.62	0.02083	0.67	0.11	0.10	0.10	0.23	0.04	1669.00	126.00	0.34	0.05	0.96	0.06	0.69	0.19	0.27	0.04	0.04	0.15	1679.30	117.50	0.32	0.03	0.96	0.05
62_3	5.68E+24	-680.3	0.28466	4.87E+24	-680.05	0.2842	6.69E-02	0.25	0.00044	0.72	0.05	0.03	0.03	0.25	0.03	2072.50	136.80	0.28	0.03	0.96	0.05	0.72	0.19	0.26	0.02	0.02	0.17	2080.70	135.30	0.28	0.03	0.96	0.05
63_3	3.51E+25	-799.9	0.21041	6.89E+24	-795.68	0.2118	7.07E-01	4.22	0.00137	0.66	0.03	0.05	0.01	0.29	0.02	9703.40	482.60	0.21	0.02	0.82	0.04	0.66	0.07	0.29	0.01	0.05	0.05	9741.10	488.50	0.21	0.02	0.81	0.04
64_3	2.33E+24	-681	0.19113	2.14E+24	-680.38	0.1920	3.72E-02	0.62	0.00083	0.67	0.06	0.30	0.01	0.03	0.06	8343.20	390.40	0.19	0.02	0.70	0.03	0.67	0.07	0.30	0.01	0.03	0.06	8357.30	393.10	0.19	0.02	0.70	0.03
65_3	4.54E+23	-821.4	0.21996	3.44E+22	-825.94	0.2383	1.12E+00	4.54	0.01833	0.24	0.02	0.01	0.01	0.76	0.02	8207.50	425.80	0.22	0.02	0.72	0.04	0.24	0.42	0.76	0.01	0.00	0.41	8213.00	426.00	0.22	0.02	0.72	0.04
66_3	1.68E+27	-936	0.19096	8.83E+26	-929.8	0.1903	2.78E-01	6.2	0.00068	0.68	0.03	0.07	0.02	0.25	0.02	5265.30	232.40	0.19	0.02	0.73	0.03	0.68	0.06	0.25	0.02	0.08	0.04	5279.50	232.50	0.19	0.02	0.73	0.03
67_3	5.74E+25	-543.4	0.251	5.91E+25	-543.28	0.2489	1.27E-02	0.12	0.00214	0.65	0.10	0.18	0.07	0.18	0.04	1258.03	75.77	0.25	0.02	0.73	0.04	0.65	0.10	0.18	0.07	0.18	0.04	1265.80	75.72	0.25	0.02	0.72	0.04
68_3	2.33E+27	-676.2	0.1946	2.34E+27	-675.89	0.1917	2.65E-03	0.31	0.00291	0.74	0.06	0.21	0.02	0.05	0.04	2239.57	98.31	0.19	0.02	0.95	0.03	0.75	0.06	0.21	0.02	0.05	0.05	2246.40	97.60	0.19	0.02	0.95	0.03
69_3	1.01E+24	-821.3	0.18386	1.28E+24	-811.51	0.1819	1.05E-01	9.79	0.002	0.17	0.01	0.02	0.01	0.81	0.02	4555.00	197.00	0.18	0.01	0.75	0.03	0.17	0.11	0.82	0.01	0.01	0.11	4572.00	195.90	0.18	0.01	0.75	0.03
70_3	3.30E+26	-641.6	0.27151	3.43E+26	-641.98	0.2704	1.62E-02	0.38	0.00112	0.71	0.03	0.01	0.00	0.28	0.03	8363.20	520.60	0.27	0.03	0.94	0.05	0.71	0.13	0.28	0.00	0.01	0.12	8377.40	519.50	0.27	0.03	0.94	0.05
71_3	3.79E+26	-597.4	0.18703	3.79E+26	-596.55	0.1833	6.07E-04	0.85	0.00375	0.84	0.05	0.05	0.03	0.11	0.03	3393.80	155.30	0.19	0.02	0.52	0.03	0.84	0.08	0.11	0.03	0.04	0.06	3406.10	153.20	0.18	0.02	0.52	0.03
72_3	9.92E+26	-494	0.26205	9.43E+26	-490.84	0.2548	2.21E-02	3.16	0.00727	0.74	0.07	0.13	0.04	0.13	0.04	1592.90	102.80	0.26	0.03	0.43	0.03	0.76	0.10	0.12	0.06	0.12	0.05	1614.50	101.90	0.25	0.02	0.43	0.03
73_3	1.39E+25	-644.1	0.24674	1.53E+25	-644.68	0.2462	4.42E-02	0.58	0.00058	0.30	0.02	0.01	0.00	0.69	0.02	8319.40	468.90	0.25	0.02	0.95	0.04	0.30	0.11	0.69	0.00	0.01	0.11	8335.60	467.50	0.25	0.02	0.95	0.04
74_3	4.56E+29	-806.8	0.21843	4.14E+29	-796.84	0.2147	4.12E-02	9.96	0.0037	0.81	0.07	0.06	0.05	0.13	0.03	4553.50	234.30	0.22	0.02	0.75	0.04	0.84	0.17	0.14	0.02	0.02	0.15	4580.70	231.80	0.21	0.02	0.75	0.03
75_3	3.50E+28	-885.4	0.16055	3.89E+28	-883.25	0.1603	4.55E-02	2.15	0.0003	0.32	0.02	0.03	0.01	0.65	0.02	6223.70	239.40	0.16	0.01	0.66	0.03	0.32	0.07	0.65	0.01	0.03	0.06	6233.40	239.40	0.16	0.01	0.66	0.03
76_3	4.20E+29	-508.5	0.21145	4.29E+29	-509.24	0.2113	9.18E-03	0.74	0.00012	0.99	0.07	0.01	0.04	0.01	0.04	3356.80	162.10	0.21	0.02	0.94	0.04	0.99	0.44	0.01	0.09	0.00	0.34	3357.40	164.30	0.21	0.02	0.94	0.04
77_3	1.07E+31	-878.1	0.16649	1.07E+31	-876.11	0.1657	4.05E-04	1.99	0.00076	0.65	0.02	0.03	0.01	0.32	0.02	6227.00	249.00	0.17	0.01	0.66	0.03	0.65	0.07	0.32	0.01	0.03	0.06	6236.90	248.40	0.17	0.01	0.66	0.03
78_3	2.20E+32	-667.5	0.1682	2.21E+32	-666.41	0.1648	2.25E-03	1.09	0.00336	0.72	0.07	0.22	0.02	0.06	0.05	3498.10	141.20	0.17	0.02	0.52	0.02	0.72	0.07	0.23	0.02	0.05	0.06	3513.20	139.80	0.16	0.02	0.52	0.02

SM4. LR with the biggest difference between EuroForMix versions.

Table S4: LR per marker assigned using EuroForMix version 1.9.3 and 3.4.0 for pair 7_3, and respective ratio alongside with each marker heterozygosity

Markers	v.1.9.3	v.3.4.0	ratio	heterozygosity
SE33	44.31107	74.53319	1.682044	0.948262
D1S1656	92.54164	128.0234	1.383414	0.899237
D12S391	286.8032	496.5013	1.731157	0.89085
D2S1338	58.22835	88.31658	1.516728	0.881424
D18S51	0.088292	20.20076	228.7944	0.875834
FGA	9.9549	15.26879	1.533796	0.859355
D21S11	0.028279	14.18278	501.5269	0.82885
D7S820	68.40416	89.38092	1.306659	0.816135
VWA	33.10798	40.67218	1.228471	0.807621
D8S1179	8.004167	9.252359	1.155943	0.807199
D3S1358	9.222859	12.15385	1.317796	0.79003
D13S317	16.87226	13.67655	0.810594	0.783726
D19S433	37.16447	19.91454	0.535849	0.769446
D2S441	17.9363	21.295	1.187257	0.769266
TH01	113.7115	40.83018	0.359068	0.764627
D16S539	5.02544	5.078238	1.010506	0.759973
D10S1248	4.173581	5.36602	1.285711	0.758627
D22S1045	2.808284	4.104967	1.461735	0.721994
CSF1PO	4.558679	6.238593	1.368509	0.719431
D5S818	9.495495	5.974298	0.629172	0.697662
TPOX	8.541714	3.5835	0.419529	0.640449

Table S4a: Gentotypic data of the evidence sample of pair 7_3

Marker	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6
D3S1358	14	15	16	17				353	3791	109	294		
vWA	15	16	18	19				1186	180	308	1590		
D16S539	9	11	12					156	1087	1315			
CSF1PO	10	11	12					1741	108	1099			
TPOX	8	11						636	684				
Yindel	2							3266					
AMEL	X	Y						3271	2547				
D8S1179	10	11	12	13	14			408	242	183	2925	2511	
D21S11	27	28	29					293	2367	2356			
D18S51	13	14	15	16	17			232	160	2101	139	1820	
DYS391	10							1113					
D2S441	10	11	15					422	1676	1556			
D19S433	13	14	15	16				219	1109	213	827		
TH01	6	9						335	1627				
FGA	21	22	23					205	1401	1485			
D22S1045	14	15	16	17				191	2369	2361	194		
D5S818	11	12	13					974	5724	397			
D13S317	8	12	13					3321	3483	146			
D7S820	7	8	9	10				2520	2964	153	202		
SE33	20	26.2	27	27.2	29.2			120	372	119	3606	111	
D10S1248	12	13	14	15				467	3175	2696	105		
D1S1656	11	12	13	14	16	17	17.3	237	133	1819	217	165	1545
D12S391	18	18.3	19.3	21	22	23		327	128	1919	150	158	1357
D2S1338	16	18	19	23	24			2250	351	1557	153	261	

Table S4b: Gentotypic data of the reference sample of pair 7_3

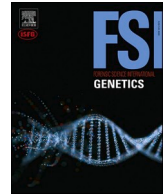
Marker	Allele 1	Allele 2
D3S1358		15
vWA		15
D16S539		11
CSF1PO		10
TPOX		8
Yindel		2
AMEL	X	X
D8S1179		13
D21S11		29
D18S51		15
DYS391		10
D2S441		11
D19S433		14
TH01		9
FGA		22
D22S1045		15
D5S818		12
D13S317		8
D7S820		7
SE33	27.2	27.2
D10S1248		13
D1S1656		13
D12S391	19.3	23
D2S1338		16

CHAPTER VII | Statistical Interpretation of Cases Involving Mixtures: A Spanish and Portuguese-Speaking Working Group (GHEP-ISFG) Collaborative Exercise

Costa C*, Álvarez JC, Angeletti S, Biagini S, Caputo M, Carnevali E, Castillo MI, Cordoba S, Cortes S, de la Puente M, Dorigón A, García O, Ginart S, Guinudinik A, Merino C, Minervino A, Miozzo C, Mosquera-Miguel A, Nicolotti ME, Onofri M, Palencia-Madrid L, Ramella MI, Rena V, Sala A, Trindade B, Vinueza-Espinosa DC, Wirz LN, Prieto L, Pinto N: **Statistical Interpretation of Cases Involving Mixtures: A Spanish and Portuguese-Speaking Working Group (GHEP-ISFG) Collaborative Exercise**. *Forensic Science International: Genetics* 2025, 103383.

This paper marks a significant milestone in my PhD work plan, as it involved the creation of an international working commission that I co-led alongside my supervisors, and with the support of the Spanish and Portuguese-speaking Working Group of the International Society for Forensic Genetics (GHEP-ISFG). As both first and corresponding author, I was responsible for shaping the conceptual direction of the initiative and articulating its academic and practical objectives. I contributed to methodological planning, data preparation, and results analysis and interpretation. I was responsible for drafting the

manuscript and ensuring it reflected the collective work and vision of the commission. Throughout the revision process, I coordinated input from multiple contributors and ensured the final version was well-aligned with the objectives of this working commission. This paper not only reflects my academic and analytical contributions but also my organizational role in developing and maintaining a collaborative, international research effort that is central to my present and future journey.



Statistical interpretation of cases involving mixtures: A Spanish and Portuguese-Speaking Working Group (GHEP-ISFG) collaborative exercise

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ABSTRACT

The increasing complexity of forensic genetic samples has driven the development and implementation of probabilistic genotyping software (PGS) to assist experts in quantifying the weight of evidence. A common challenge lies in quantifying the likelihood that a person of interest (PoI) is a contributor to a DNA mixture, especially in samples with low quantity and quality, stochastic effects (drop-in allele, dropout, and/or heterozygotic peaks imbalance), shared alleles, and stutter peaks. PGS can be based on either qualitative models (allele presence/absence) or quantitative (including allele peak heights) to assess the likelihood ratio, which consists of comparing the probability of observing the evidence given two hypotheses: “H1 = The PoI is a contributor to the evidence” and “H2 = The PoI is not a contributor nor genetically related to any contributor”. Key parameters included in these calculations regarding population – allele frequency distributions, and co-ancestry coefficient –, analytical factors – drop-in, dropout, analytical threshold –, and stutter presence, can influence the outcomes depending on how they are set. This collaborative exercise, organized by the Spanish and Portuguese-speaking

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Working Group of the International Society for Forensic Genetics, aimed to assess the current state of knowledge, use, and implementation of PGS among forensic laboratories. The goal was to evaluate how non-binary informatics tools are applied in practice and to understand the methodologies used to statistically interpret complex DNA mixtures. Participants were asked to analyze 30 pairs of samples composed of DNA mixtures (with varying the number of contributors, mixture ratios, and degradation levels) and corresponding reference profiles, selected from the PROVEDit database. Laboratories employed different tools and approaches to quantify the evidence's weight, including decisions regarding the number of contributors (NoC), population, laboratory, and case-specific parameters, such as coancestry coefficient, minimum allele frequency, dropout frequency, drop-in (frequency and modeling), analytical threshold, and the modeling of stutter peaks and degradation. Even though all laboratories received the same genotypic and frequency data, methodological differences led to different LR results, particularly for more complex samples with low-template and degraded DNA. The greatest differences were observed in the interplay between analytical thresholds and NoC estimation, with discrepancies amplified when alleles from minor contributors overlapped with expected stutter positions. This exercise highlights the importance of expert training and underscores the need for a comprehensive understanding of the statistical models underlying PGS. Ensuring accurate and consistent interpretation of complex DNA evidence requires not only technical proficiency but also an integrated approach to parameter selection and genotypic data evaluation.

1. Introduction

Facing the increasing complexity of analyzed samples, forensic genetic laboratories worldwide have adopted probabilistic genotyping software (PGS) tools to address identification challenges commonly encountered in routine casework [1]. A typical identification problem involves quantifying the likelihood that the donor of a reference sample (also referred to as the person of interest – PoI, typically a suspect or victim) is a contributor to the evidence sample under analysis. The development and implementation of PG tools in the routine casework of the laboratories aim to handle the growing complexity of genetic profiles derived from crime scene samples, which are frequently DNA mixtures. Beyond the number of contributors (NoC) usually unknown [2], these profiles are likely to have several other characteristics that make them extremely challenging to interpret, such as allele sharing between contributors, contributions in different proportions, and stutter peaks that may be confounded with alleles from minor contributors (or vice versa) [3]. Further complicating the analysis are stochastic effects (heterozygotic imbalance, drop-in, and/or drop-out), typically resulting from low-template DNA [4,5], as well as potential degradation of the DNA [6,7].

Depending on the modeling framework implemented, PGS may follow either a qualitative or quantitative approach, considering different types of information from the evidence's electropherogram (EPG) to fit the respective model. Qualitative models consider only the presence or absence of peaks in the genotypic profiles, whereas quantitative models also incorporate peak height information (including both allelic and stutter peaks) observed in the EPG [1]. Regardless of the model, both approaches quantify the weight of the evidence through the recommended and widely accepted Likelihood Ratio (LR), which compares the probability of observing the evidence assuming two alternative and mutually exclusive hypotheses. In standard identification scenarios, these hypotheses are typically formulated as “H1 = The PoI is a contributor of the evidence” and, alternatively, “H2 = The PoI is not a contributor nor genetically related to any contributor of the evidence” [8].

To perform this calculation, several parameters' values and conditions need to be considered regarding the population, including allele frequency distributions and coancestry coefficients, as well as analytical factors related to drop-in, dropout, analytical threshold, and the existence of stutters in the evidence file. Therefore, before performing any computation, the expert must specify the values of these parameters – either by setting them explicitly (e.g., through empirical laboratory estimates, or using values from the literature) or, when applicable, by allowing the PGS to infer maximum likelihood estimates based on the data. This is an important step in quantifying the evidence, as it has been demonstrated that different software parameter values and conditions can have a relevant practical impact on the assigned LR value for both

mocked [2,9,10] and real casework samples [11–13]. Moreover, experts should be aware that when analyzing the same input data while considering the same or very similar parameter conditions in different tools, distinct LR values will be obtained, as the parameters are modeled differently [14–17].

With this growing interest of forensic genetics laboratories to implement PG tools in their workflow, the Spanish and Portuguese-speaking Working Group of the International Society for Forensic Genetics (GHEP-ISFG) organized a collaborative exercise whose main results are herein presented and discussed. The main goal of this initiative is to understand the state-of-the-art regarding the knowledge, use, and implementation of non-binary software among participating laboratories. To this end, a series of mock DNA mixtures was distributed, varying the number of contributors (NoC), sample treatments, and levels of degradation. It also aims to evaluate the different approaches and methodologies currently used by the participants for the statistical interpretation of cases involving DNA mixtures.

2. Materials and methods

A working commission was created to carry out the present study after being previously presented and approved at the general assembly during the XXIX Meeting of GHEP-ISFG held in Salta, Argentina, in June 2024.

2.1. Participants

This collaborative exercise was open to all GHEP-ISFG members, and representatives from 47 worldwide laboratories registered once the call was launched. From these, only 16 laboratories from seven countries have concretized their participation, sending results: Argentina (7), Spain (4), Colombia (2), Brazil (1), Italy (1), and Peru (1). Most participating laboratories belong to public institutions: Justice Administration (5), Police (5), University/Research Institute or Unit (4), and State Agency (1). Only one participating laboratory represents a private party.

2.2. Exercise structure and files provided to the participants

Each participating laboratory was provided with 30 different mock cases, each consisting of a pair of evidence/reference STR profiles to be analyzed. The aim was to quantify the weight of the evidence, considering the possibility of the reference sample belonging to one contributor of the mixture (i.e., evidence) sample, while the alternative was not being a contributor nor related to any of the contributors. In addition to reporting the LR results for the 30 cases (including both total and per-locus LRs) and respective conclusions, participants were asked to complete a questionnaire. This questionnaire collected information about

both laboratory and software-specific settings, as well as the criteria used to analyze the data provided and report the results.

2.2.1. Questionnaire

Along with all the genetic information of the sample pairs, a questionnaire was provided for all participants to collect information regarding the informatics framework(s) used not only in the exercise but also in their routine casework. The latter allowed obtaining insights regarding the level of experience of the participants in the use of such tools in real-world problems. Regarding the exercise, each laboratory was asked to specify which software was used, if it is the same software they use in their casework, and the corresponding stage of the implementation process. A section to describe all software parameters' values considered in the present exercise and corresponding justification was included in the questionnaire. Focusing on case-specific data, besides the LR results (total and per-marker) and the respective conclusion, information concerning the estimated NoC, the evidence file used (with or without stutters), the stutters and/or degradation modeling, and any other relevant information was also solicited – see [Supplementary Material S1](#).

2.2.2. Samples

All the samples provided were selected from the publicly available Rutgers University *PROVEDIt* Initiative (Project Research Openness for Validation with Experimental Data) database (<https://lftdi.camden.rutgers.edu/provedit/>) [18]. A set of 60 Excel files with the STR profiles corresponding to pairs of evidence/reference samples was made available to all participating laboratories, the first corresponding to mixture profiles with allelic heights. As informed by *PROVEDIt*'s representatives, the selected mixtures were amplified with the GlobalFiler™ kit (29 cycles) and genotyped on the 3500 Genetic Analyzer instrument using injection times of 5, 15, or 25 s. These samples comprised either two ($N = 15$) or three ($N = 15$) known contributors at differing ratios (1:1, 1:2, 1:4, 1:9, 1:2:1, 1:9:1, 1:2:2, and 1:9:9). Some mixtures shared the same composition and contributors' ratios but differed either the treatment applied afterward (none, 3, 6, 12, and 24 mU of DNase I Degradation enzyme) or in DNA template quantity (range between 0.03 ng and 0.75 ng), mimicking different degradation levels. All samples' profiles were analyzed using GeneMapper® ID-X with Local Southern sizing, applying an analytical threshold of 1 Relative Fluorescent Unit (RFU). For each case, two versions of the evidence file were provided: one with artifacts (e.g., stutter peaks) and one without. Having access to the contributor profiles for the selected mixtures enabled the creation of artifact-free files containing only alleles corresponding to those profiles. Laboratories were free to choose which version to analyze. A description of the complete set of the selected mixtures and their respective reference samples is detailed in [Supplementary Material S2](#). While contextual information may assist experts in actual casework assessments, participants in this commission received only the genotype profiles of the evidence and reference samples, and no further information.

All participants used the same allele frequency database corresponding to the National Institute of Standards and Technology (NIST) database for the Caucasian population [19] – see [Supplementary Material S3](#).

2.3. Assessment of participating laboratories' results

Each participating laboratory provided both the filled questionnaire and the reports directly exported from the software for each pair of samples analyzed. Through the questionnaire, it was possible to assess A: how many of the participating laboratories have implemented and validated the tools used in their routine casework, B: whether they use more than one tool, the type of model on which the tool(s) is (are) based, and if this (these) coincide with the one used to develop the exercise; and, C: the method(s) used both to define and establish the values of the

parameters used in computations.

The statistical evaluation performed by each participating laboratory for each case was evaluated to assess how the laboratories analyze and interpret the same genotypic data. Also, as some of the pairs shared identical contributor composition but different treatments, the exercise also allowed for an assessment of how DNA quality and quantity can affect the results obtained.

Although the questionnaire requested participants to report the results and software parameters used for each case, all such information was ultimately extracted from the case-specific reports directly exported from the software by the laboratories.

3. Results and discussion

As stated in the Materials and Methods section, only 16 of the 47 initially registered laboratories (34 %) submitted results for this working commission. The high number of initial registrations reflects strong community interest in the subject, supporting its relevance and the need for continued working commissions and training on the topic. Even after a one-month deadline extension (from December 1st to December 31st, 2024), some laboratories justified the absence of results with a high casework workload as the reason for being unable to participate, reinforcing the importance and need of laboratory managers to foresee dedicated time for experts' training and learning.

Some laboratories did not report LR values for the complete set of samples (4 laboratories, 21 LRs in total), and other LR results were not further considered because they could not be confirmed with the software's reports (2 laboratories, 3 cases in total). Within the first set of laboratories, one explained that LRs were not calculated due to a large number of alleles falling below their specified analytical threshold (e.g.: $AT=122$ RFU; pairs: #1, #3, #6, and #8). In addition, the results presented by one laboratory were excluded from the analysis as procedures showed systematic major errors in half of the cases analyzed, making it unreasonable to compare them with the remaining. This laboratory stated that neither had a PG tool implemented in their routine casework nor in an implementation process. It is also worth noting that two laboratories submitted two complete sets of results, obtained with different tools, that were both included in the subsequent analyses.

3.1. Software

The level of experience of the 15 laboratories in using PGS in their routine casework varied widely: 10 have at least one tool validated and implemented (8 of which are qualitative). Experience with more than one tool was also reported – see [Supplementary Material S4](#).

The PGS used for this exercise may or may not coincide with the one used in the laboratory. All 5 laboratories without any tool implemented in their routine casework have used the quantitative tool EuroForMix [20] to perform the exercise, under different implementation stages. [Table 1](#) illustrates all the tools (and respective versions) used for this exercise by participants, alongside their implementation status in their laboratories. Regarding the two laboratories that submitted two complete sets of results using two different software, one used a qualitative (LRmix Studio [21], implemented and validated) and a quantitative tool (EuroForMix, in the implementation process); while the other used two quantitative software (EuroForMix and DNASTatX [22], whose source code is largely based on EuroForMix, both planned to be implemented in the near future).

3.1.1. Software parameters

Before performing any computations, each participating laboratory needed to make decisions regarding the establishment of values and conditions for the various software parameters, which can be divided into two major groups: laboratory-specific (which also includes population-related parameters) and case-specific.

Table 1

List of all the PGS (and respective versions) used to perform this work, along with the implementation stage (Stg 1–4) they were at by the time the results presented were produced.

Stage of implementation in routine casework	Qualitative		Quantitative				Total
	LRmix Studio		EuroForMix			DNASTatistX	
	v.2.1.5	v.2.1.3	v.4.1.0	v.4.0.9	v.4.0.8	v.2.3.2	
Stg 1: Implemented	1	2	-	-	2	-	5
Stg 2: In the implementation process	-	-	3	3	-	-	6
Stg 3: Planned to be implemented in the near future	-	-	2	1	1	1	5
Stg 4: Neither implemented nor planned to be implemented in the near future	-	-	-	1	-	-	1
Total	3		13			1	17

3.1.1.1. Software and laboratory-specific parameter values and conditions. Alongside case-specific parameter values and conditions, probabilistic genotyping tools also require accounting for several software, population, and laboratory-specific settings to assign an LR: coancestry coefficient, minimum allele frequency, drop-in, dropout, and, for the quantitative ones, the analytical threshold. In Table 2, all values and conditions considered by the laboratories for each of the above-mentioned parameters are provided along with the respective methodology applied to determine each one.

As shown in Table 2, various methods were applied to the same input data. Some parameter values and conditions were predefined – either based on published literature or lab-specific validation studies – and then manually entered into the software. In other cases, tool-specific defaults or estimation features played a more significant role.

a) Coancestry Coefficient

The coancestry coefficient (θ) indicates the probability of a pair of alleles, each one randomly chosen from a pair of individuals from the same population, being identical-by-descent (IBD, i.e., derived from the same common ancestor allele) [24]. For modern human populations, as those from participating laboratories, published studies tend to support low (0.01) but non-null values of θ [24,25].

As summarized in Table 2, most participating laboratories (10 out of 17) did not incorporate a coancestry coefficient in their calculations, effectively considering it to be null. Of these, the majority used a specific estimated value based on their working population, with only two reporting that their choice was based on a literature review.

For the remaining laboratories (7 out of 17), most ($N = 5$) used $\theta = 0.01$, which aligns with EuroForMix's default value. However, only one laboratory that utilized both EuroForMix and DNASTatistX explicitly stated using this value because it was the software's default; the other three supported their choice based on literature references. Finally, the laboratory employing both LRmix Studio and EuroForMix used different co-ancestry values for each: 0.01 and 0.02 (case-dependent) for LRmix Studio, and only 0.02 for EuroForMix. This $\theta = 0.02$ was reported as specifically estimated by their population.

b) Minimum Allele Frequency

The participating laboratories either opted to consider the lowest allele frequency observed in the database (0.001385, $N = 7$) or estimated a value ($N = 10$), as described in Table 2. Within the latter group, the majority ($N = 7$) applied the formula: $5/(2N)$, $N = 1036$, in which 'N' represents the size of the allele frequency database. This is the formula incorporated in the EuroForMix tool to determine this parameter, which was also the reason declared by two of these laboratories for considering this value. Also considering the size of the allele frequency database ($N = 1036$), one laboratory estimated a minimum allele frequency of 0.0005 through the application of this formula: $1/(2N + 1)$. Another laboratory determined a marker-specific minimum allele frequency using Chakraborty's formula [23]. Lastly, one laboratory considered 0.002258 as the minimum allele frequency with no further justification.

c) Drop-in Frequency

Most laboratories (10 out of 17) applied the EuroForMix default value (0.05) as the drop-in frequency, and two of them reported the use

of this value based on a literature review. One laboratory using LRmix Studio also considered this value, as it was a laboratory-specific estimation. It is noteworthy that one laboratory used EuroForMix and its default value, also considering a different drop-in frequency (0.0005) in a single case, without further justification.

The laboratories using other values opted either to resort to literature ($N = 2$) or to estimate a laboratory-specific frequency ($N = 4$). The values are shown in Table 2.

d) Drop-in Modeling

Drop-in modeling is only possible with quantitative tools, since they consider the allele peak heights in their computations. Among the 14 laboratories using such tools, most ($N = 12$) used the EuroForMix default value ($\lambda = 0.01$), with one of them justifying it through published literature, as illustrated in Table 2. The remaining set of results ($N = 2$) came from a laboratory using both EuroForMix and DNASTatistX, which applied $\lambda = 0.00882$ to both tools, with no further justification.

e) Dropout Frequency

In contrast to drop-in modeling, dropout frequency is a parameter that qualitative tools require experts to input, as quantitative tools directly model it from peak height distributions. Although the three laboratories using a qualitative tool (LRmix Studio) have this PGS implemented, dropout frequency estimations varied significantly between laboratories for the same evidence sample. These variations, reported as ranges of 0–0.10, 0–0.20, and 0–0.53, likely resulted from laboratories considering different individuals during their simulations – see Table 2. Laboratories also differed in whether they applied a uniform dropout frequency across all individuals considered in the Likelihood Ratio (LR) calculation (i.e., the reference profile in H1 and the unknown individuals in H1 and H2). The laboratory reporting the smallest dropout frequency range (0–0.10) consistently applied the same frequency for all individuals across all analyzed cases. The other two laboratories followed this same approach in most cases (15 and 20 cases, respectively). For the remaining cases, the laboratory with the largest range (0–0.53) applied the same dropout frequency to the unknown individuals in H1 and H2, but a different one for the reference profile. A similar strategy was adopted by the laboratory with the intermediate range (0–0.20) in most cases (13 out of 15), but differed in two cases, where the dropout frequency was the same for the reference profile and the unknown individuals in H2, while considering a different frequency for the unknown individuals in H1.

f) Analytical Threshold

As shown in Table 2, an analytical threshold is explicitly required for quantitative tools ($N = 14$), whereas qualitative ones do not rely on this parameter, as they do not use peak height information. The majority of the participants ($N = 6$) used an analytical threshold of 50 RFU, either by resorting to literature (2), by directly estimating it (1), or even accepting the software default (3). Also, other laboratories ($N = 2$) considered an analytical threshold of 50 RFU, but not consistently for all cases.

Among the remaining participants, most considered a fixed analytical threshold for the complete set of pairs and markers: 1 RFU (1), 35 RFU (1), 40 RFU (2), and 85 RFU (1). All these values were reported as determined empirically by the laboratories, except for the one using an analytical threshold of 35 RFU, which indicated that this value was

Table 2

Software-, population-, and laboratory-specific parameter values and conditions used by participating laboratories. The methodology applied to define each parameter is also provided. Laboratories are grouped according to the tool implementation stage at the time this exercise was performed: Stg1 – Implemented; Stg2 – In the implementation process; Stg3 – Planned to be implemented in the near future; and Stg4 – Neither implemented nor planned to be implemented in the near future. NA: Not applicable.

Software Parameter	Value/Condition	N	Software	Methodology applied considering the Tool Implementation Stage (Stg)			
				Inputted by the user		Probabilistic Genotyping Tool	
				From literature	Laboratory-specific	Default	Estimated
Coancestry coefficient	0	10	Qualitative: 2 Quantitative: 8	- Stg2: 2	Stg1: 2 Stg1: 1 Stg2: 3 Stg3: 1 Stg4: 1	- -	NA NA
	0.01	5	Quantitative: 5	Stg1: 1 Stg2: 1 Stg3: 1	-	Stg3: 2*	NA
Minimum allele frequency	0.02	1	Quantitative: 1	-	Stg3: 1	-	NA
	0.01 and 0.02 ^z	1	Qualitative: 1	-	Stg1: 1	-	NA
	Marker-specific (Chakraborty's formula [23])	1	Qualitative: 1	-	Stg1: 1	-	NA
	0.0005	1	Quantitative: 1	-	Stg2: 1	NA	-
	(1/(2 N + 1); N = 1036)						
	0.001385 (lowest allele frequency from the database used)	7	Quantitative: 7	-	Stg1: 1 Stg2: 2 Stg3: 4*	NA	-
	0.002258	1	Qualitative: 1	-	Stg1: 1	-	NA
Drop-in Frequency	0.002413 (5/(2 N); N = 1036)	7	Qualitative: 1 Quantitative: 6	- -	Stg1: 1 Stg1: 1 Stg1: 1 Stg2: 2 Stg3: 1 Stg4: 1	- - NA	NA NA Stg2: 1
	0	1	Qualitative: 1	-	Stg1: 1	-	NA
	0 and 0.01 ^z	1	Qualitative: 1	Stg1: 1	-	-	NA
	0.01	1	Quantitative: 1	Stg3: 1	-	-	NA
	0.00165	2	Quantitative: 2	-	Stg3: 2*	-	NA
	0.03	1	Quantitative: 1	-	Stg2: 1	-	NA
	0.05	10	Qualitative: 1 Quantitative: 9	- Stg2: 2	Stg1: 1 -	- Stg1: 2 Stg2: 3 Stg3: 1 Stg4: 1	NA NA
	0.05 and 0.0005 ^y	1	Quantitative: 1	-	Stg3: 1	-	NA
	0.01	12	Quantitative: 12	Stg3: 1	-	Stg1: 2 Stg2: 6 Stg3: 2 Stg4: 1	-
	0.00882	2	Quantitative: 2	-	Stg3: 2*	-	-
Dropout Frequency ^b	0–0.10	1	Qualitative: 1	-	-	-	Stg1: 1
	0–0.20	1	Qualitative: 1	-	-	-	Stg1: 1
	0–0.53	1	Qualitative: 1	-	-	-	Stg1: 1
Analytical Threshold (RFU) ^a	1	1	Quantitative: 1	-	Stg1: 1 ^c	-	NA
	35	1	Quantitative: 1	Stg3: 1	-	-	NA
	40	2	Quantitative: 2	-	Stg3: 2 *	-	NA
	50	6	Quantitative: 6	Stg1: 1 Stg2: 1	Stg1: 1	Stg3: 1 Stg2: 1 Stg4: 1	NA
	85	1	Quantitative: 1	-	Stg2: 1	-	NA
	1 and 50 ^z	1	Quantitative: 1	Stg2: 1	-	-	NA
	50 and Marker-specific ^y	1	Quantitative: 1	-	Stg3: 1	-	NA
	Marker-specific (2 different sets) ^z	1	Quantitative: 1	-	Stg2: 1	-	NA

* The laboratory that used two quantitative tools considered the default settings of one of the tools to perform the calculations in both.

^a Applied only to quantitative tools;

^b This parameter is directly modeled by quantitative tools, thus, only data regarding qualitative ones is provided;

^c Considered the same value used in the genotyping analysis tool;

^y The alternative parameter value presented was considered in only one case;

^z The alternative parameter value reported was considered in 18 %-38 % of the analyzed cases.

sourced from literature. Nevertheless, and in contrast to all others, one laboratory reported using two sets of marker-specific analytical thresholds; that is, different threshold values were defined for each marker individually, rather than applying a fixed value across all markers.

3.1.1.2. Case-specific parameter values and conditions. This set of parameters includes NoC, degradation, and back and forward stutter modeling, which are addressed differently depending on the software used. As previously stated in the Materials and Methods section, two versions of each evidence file were provided to the participants: with

and without artefacts, such as stutter peaks and drop-in alleles. The type of evidence files selected by each laboratory and the different approaches applied to determine the values and conditions of the case-specific parameters are described in [Table 3](#).

Almost all participants (15 out of 17) selected the genotypic files without artefacts. Only two laboratories, both using EuroForMix, analyzed the genotypic files with artefacts. Finally, one laboratory, which assigned LR values with both EuroForMix and DNASTatX, considered all files without artefacts except for pair #28.

As qualitative tools like the one used in the exercise (LRmix Studio) do not consider the height of the peaks, they are not able to model the stutter peaks or degradation. Consequently, all laboratories that utilized this tool ($N = 3$, stg1) used files without artefacts, and no modeling was applied to stutters or degradation.

EuroForMix was the predominant quantitative software used in this exercise ($N = 13$). Its recent versions, like those employed here, enable experts to either provide pre-defined values and conditions for computations or let the software test various combinations regarding the NoC and whether degradation and stutter (back and/or forward) models should be applied. In the latter case, once the user defines which conditions the tool should test, an informative table showing the outcome of each model combination is generated. The optimal model – i.e., the best fit for the genotypic input data – is then automatically selected based on the adjusted log-likelihood (adjLogLik) value. This value, which incorporates a penalty for the number of effective parameters, is equivalent to using the Akaike Information Criterion (AIC), a metric that balances goodness of fit with model complexity to select the best statistical model for a given dataset. Thus, the model with the highest adjLogLik value is considered the best fit [20]. [Table 3](#) outlines the diverse methods employed by each laboratory using EuroForMix. For both NoC and degradation modeling parameters, all laboratories using a quantitative tool adopted the second procedure for at least a subset of cases; most of them also adopted the same approach for stutter modeling. A more detailed analysis of these parameters is provided in sections a), b), and c), respectively.

a) Number of Contributors (NoC)

The number of contributors (NoC) of a DNA mixture is generally unknown in real forensic casework contexts and has been reported as the parameter with the greatest impact in the quantification of the weight of the evidence, especially its underestimation [2,10,11,26]. Its estimation can be handled in two ways: either the user inputs a value into the software, which is then assumed to be known in computations (the sole option for qualitative tools), or quantitative tools can estimate it based on genotypic observations. In the latter case, the expert provides the software with a range of possible NoCs, and the software selects the value that best fits the data. All the participants who input a NoC value declared having estimated it empirically. Half of the participating laboratories using quantitative tools mixed both approaches depending on the case under analysis – see [Table 3](#).

The NoC estimated by each of the participating laboratories for each of the 30 pairs of samples analyzed is presented in [Table 4](#) along with the corresponding methodology. For laboratory-specific data, see [Table S5](#).

The value of the evidence sample's NoC was correctly estimated by all the laboratories in 8 out of the 30 cases analyzed. As expected, these correspond to sample pairs with a lower level of complexity and/or without degradation.

For 19 out of 30 pairs, even though most laboratories correctly estimated the NoC, some over- and underestimations were observed ($eNoC = NoC + 1$ and $NoC - 1$, respectively).

Focusing on laboratories that allowed the software to test different values rather than inputting a fixed estimation, overestimation occurred in only one laboratory and for two pairs of samples: #19 and #29. Both cases involved high peak imbalance – contributors' proportions equal to 1:9:9. This laboratory was one of the two that considered evidence files

with artifacts and, as far as it could be inferred, this wrong estimation have primarily resulted from an application of a low analytical threshold ($T = 50$ RFU, for all the markers), leading that stutter peaks of major contributor(s) were attributed to possible minor contributors. Noteworthy, the other laboratory that also used evidence files with artifacts correctly estimated the NoC's value in consequence of having considered higher marker-specific analytical thresholds (ranging from $T = 81$ – 161 RFU), which led to fewer alleles being identified in most of the markers (in 13 out of the 21 analyzed in the case of sample #19, for example).

The NoC's underestimation was more frequent and was largely due to the interplay between the analytical threshold considered and/or a low template amount that led to the loss of allelic peaks. For example, for pair #1 ($NoC = 2$), 22 out of the 52 alleles observed were lower than 50 RFU, the analytical threshold considered by the 5 laboratories that reported $eNoC = 1$ as being the best fit for the input data according to the software.

Finally, the remaining three cases (samples #6, #7, and #8), all with an actual NoC of 3, were particularly notable as most participating laboratories underestimated this value. For sample pair #6, in fact, every laboratory underestimated the NoC, reporting either 1 or 2. This may have resulted from an accumulation of different complicating factors, including low template amounts (for all the cases), mixture profiles being degraded (for pairs #6 and #7), or a highly imbalanced donors' proportions (1:9:1, for case #7). Noteworthy, the two laboratories that, after analysis of the PGS, provided the correct NoC for both cases #7 and #8 used low analytical thresholds: $T = 1$ RFU, and $T = 35$ RFU – in fact, the two lowest values used by the participating laboratories ([Table 2](#)).

Even when laboratories allow the tool to test different NoCs, accurate estimation of this parameter might still be compromised. This is not solely due to decisions about the NoC itself, such as providing an inappropriate range, but also due to choices concerning other parameters, primarily the analytical threshold.

b) Stutter peaks and degradation modeling

Other case-specific parameters – namely, the modeling of stutter peaks and degradation – are features exclusive to quantitative tools. As previously stated, evidence files with and without artifacts, such as stutter peaks and drop-in alleles, were provided to participating laboratories. This allowed users of quantitative tools, EuroForMix (13) and DNASTatX (1), to choose which type of evidence file to analyze, as both tools can model degradation and both back and forward stutters. All EuroForMix versions used in this exercise allow experts two approaches: either to allow or disallow the modeling of both stutters (only back or both back and forward) and degradation prior to analysis or let the tool test different combinations for the specific case and then select the one that best fits the genotypic input data. The methodology applied to both stutter peaks and degradation by each laboratory is detailed in [Tables S6 and S7](#), respectively.

It is important to note that the mocked samples in this exercise, denominated “without artifacts”, are artifact-free mixtures because, as their contributors were known, it was possible to remove unwanted peaks that did not belong to any of the mixture's contributors. This is a significant departure from routine real-world analyses, where samples often contain unknown contributors. In real samples, deciding if a peak is an artifact or a true allele is complex, especially with degraded and/or low-template DNA. Consequently, some laboratories likely mimicked routine casework procedures by allowing stutter modeling, even when analyzing these artifact-free samples.

[Table 5](#) compiles the number of laboratories using quantitative tools, the respective evidence files (with and without artefacts) analyzed, and whether they modeled stutter peaks for each pair of samples' computation. Information regarding the method followed to determine whether to model or not the stutter peaks is also provided – either input the information or let the software test the different combinations to

Table 3

Approaches applied by the participant laboratories to determine the number of contributors (NoC), degradation, and back and/or forward stutter modeling. The establishment of values and conditions of these case-specific parameters can be input directly into the tool (Input), or the expert can insert a range of conditions and select the best fit once the tool tests all condition combinations (PGS). Category 'Both' refers to laboratories that do not apply the same method to all cases, alternating between manual input and software-driven inference. A detailed Table regarding the case-specific parameters used by each laboratory for each pair of samples and the method applied can be found in [Supplementary Material S5](#). In both Tables, laboratories were divided into groups based on the software implementation stage at the time this exercise was performed: Stg 1 – Implemented; Stg 2 – In the implementation process; Stg 3 – Planned to be implemented in the near future; and Stg 4 – Neither implemented nor planned to be implemented. Information regarding the use of evidence files with (W) or without (WO) artefacts is also indicated. NA: Not applicable.

Stage (Stg)	Laboratory	Software	version	NoC			Degradation			Back stutter			Forward stutter			Evidence File		
				Input	PGS	Both	Input	PGS	Both	Input	PGS	Both	Input	PGS	Both	W	WO	Both
1	Lab1	LRmix Studio	2.1.5	X	NA		NA			NA			NA			-	X	-
1	Lab2a	LRmix Studio	2.1.3	X												-	X	-
1	Lab3	LRmix Studio	2.1.3	X												-	X	-
1	Lab4	EuroForMix	4.0.8	-	-	X	-	-	X	-	-	X	-	-	X	-	X	-
1	Lab5	EuroForMix	4.0.8	-	-	X	-	-	X	X ^a	-	-	X ^a	-	-	-	X	-
2	Lab6	EuroForMix	4.1.0	-	X	-	-	X	-	-	X	-	-	X	-	-	X	-
2	Lab7	EuroForMix	4.1.0	-	-	X	-	-	X	-	-	X	X ^a	-	-	-	X	-
2	Lab8	EuroForMix	4.1.0	-	X	-	-	X	-	-	X	-	-	X	-	X	-	-
2	Lab9	EuroForMix	4.0.9	-	-	X	-	-	X	-	-	X	-	-	X	-	X	-
2	Lab10	EuroForMix	4.0.9	-	X	-	-	X	-	-	X	-	-	X	-	-	X	-
2	Lab11	EuroForMix	4.0.9	-	-	X	-	-	X	-	-	X	X ^a	-	-	-	X	-
3	Lab12	EuroForMix	4.1.0	-	X	-	-	X	-	X ^a	-	-	X ^a	-	-	-	X	-
3	Lab13	EuroForMix	4.1.0	-	X	-	-	X	-	-	X	-	-	X	-	-	X	-
3	Lab14a	EuroForMix	4.0.9	-	X	-	-	-	X	-	-	X	-	-	X	-	-	X ^b
3	Lab2b	EuroForMix	4.0.8	-	X	-	-	-	X	-	X	-	-	X	-	X	-	-
3	Lab14b	DNAStatistX	2.3.2	-	*	-	-	-	*	-	-	*	-	-	*	-	-	*
4	Lab15	EuroForMix	4.0.9	-	-	X	-	X	-	X ^a	-	-	X ^a	-	-	-	X	-

* The conditions considered were transposed from those used and estimated using EuroForMix by this laboratory (Lab14);

^a For all the cases, the information inputted by the user was not used to model the parameter;

^b Only for pair #28 was considered the evidence file with stutters.

Table 4

Estimated number of contributors (eNoC) and true NoC for each pair of samples analyzed. The methodology applied by each laboratory to estimate this parameter is detailed in [Table S5](#) – Estimations were performed empirically (input directly into the tool, Input) or determined by the PGS through automated model selection. The pairs of samples were grouped by taking into account the donors to the mixture. For example, pairs #1 and #21 were grouped since the contributors were the same: donor of profiles #31 (contributor #1) and #32 (contributor #2). The difference between the pairs relies on the treatment applied to each of the mixtures – see [Supplementary Material S2](#). ‘Total’ indicates the number of laboratories that assigned an LR value for the pair.

Profile ID		Pair ID	True NoC	Total	Estimated NoC (eNoC)											
					1			2			3			4		
Mixture	Ref.				N	Input	PGS	N	Input	PGS	N	Input	PGS	N	Input	PGS
31_32	31	1	2	14	6	1	5	8	3	5	-	-	-	-	-	-
		21	2	17	-	-	-	17	6	11	-	-	-	-	-	-
33_34	33	2	2	17	-	-	-	17	5	12	-	-	-	-	-	-
		34	3	15	2	1	1	13	3	10	-	-	-	-	-	-
35_50		22	2	17	-	-	-	17	6	11	-	-	-	-	-	-
		35	12	2	16	-	-	-	15	3	12	1	1	-	-	-
		50	5	2	17	1	-	1	16	4	12	-	-	-	-	-
39_40		24	2	17	-	-	-	17	5	12	-	-	-	-	-	-
		39	13	2	16	-	-	-	16	4	12	-	-	-	-	-
		40	15	2	16	1	-	1	15	6	9	-	-	-	-	-
48_49		25	2	17	-	-	-	17	5	12	-	-	-	-	-	-
		48	4	2	16	-	-	-	16	3	13	-	-	-	-	-
		14	2	17	1	-	1	16	4	12	-	-	-	-	-	-
36_37_38		49	11	2	17	1	-	1	16	5	11	-	-	-	-	-
		23	2	17	-	-	-	16	4	12	1	1	-	-	-	-
		36	6	3	13	4	2	2	9	3	6	-	-	-	-	-
41_42_43		30	3	14	-	-	-	1	-	1	13	3	10	-	-	-
		37	10	3	17	-	-	-	-	-	-	17	5	12	-	-
		26	3	17	-	-	-	1	-	1	16	5	11	-	-	-
44_45_46		38	17	3	17	-	-	-	1	-	1	16	5	11	-	-
		41	7	3	16	-	-	-	13	3	10	3	1	2	-	-
		42	18	3	17	-	-	-	2	1	1	15	4	11	-	-
47_48_49		43	27	3	17	-	-	-	1	-	1	16	5	11	-	-
		44	8	3	15	1	-	1	11	2	9	3	1	2	-	-
		28	3	16	-	-	-	1	-	1	15	4	11	-	-	
		45	16	3	17	-	-	-	1	-	1	16	5	11	-	-
		46	20	3	17	-	-	-	2	1	1	15	4	11	-	-
		47	9	3	16	-	-	-	1	-	1	15	4	11	-	-
		19	3	16	-	-	-	-	-	-	15	6	9	1	-	1
		29	3	15	-	-	-	1	-	1	13	2	11	1	-	1

then select the better fit.

As illustrated in [Table 3](#), the majority of the participants using quantitative tools analyzed the evidence files without artefacts (12 out of 14). Although most laboratories did not model either back or forward stutter peaks in the reported LR values, their methodologies in this regard differed. Indeed, some laboratories consistently chose not to model stutters, providing this prior assumption to the software (3); others consistently allowed the tool to test the different combinations to then select the best fit (3), and the remaining used either approach depending on the pair of samples under analysis.

Within the set of cases using artifact-free evidence files, stutter peaks were modeled by PGS as the best fit only for the pairs #4 (N = 1) and #7 (N = 4). In pair #4, the laboratory considered a high analytical threshold (T = 85 RFU), which, combined with the high imbalance of the mixture's contributors (1:4), resulted in peaks able to be explained as stutter peaks, due to their sizes and positions. In pair #7, although the true mixture involved three contributors, it was analyzed as a two-person mixture; in this context, stutter modeling helped account for the multiple unassigned peaks observed.

On the other hand, only two laboratories consistently used evidence files including artifacts for all the pairs analyzed, both letting the software test the different conditions and then selecting the best fit.

Regarding degradation, only six laboratories consistently allowed the tool to quantify the weight of genetic evidence both with and without modeling degradation, subsequently choosing the better-fitting condition for all pairs analyzed ([Table 3](#)). The remaining participants applied this method selectively, pre-establishing whether degradation should be considered for a subset of cases. [Table 6](#) summarizes the case-specific degradation state and the modeling approach used by laboratories employing quantitative tools; laboratory-specific details can be

found in [Table S7](#).

Within the set of mixture samples subjected to any degree of degradation treatment, all the reported LR values have modeled degradation, except for one case (pair #6, N = 1). As the mixture profile of this pair combines both low DNA template amount and degradation, the majority of its peaks are lower than the maker-specific analytical threshold considered by the laboratory (ranging from T = 81–161 RFU; 20/21 loci with no detectable alleles). Thus, when allowing the software to quantify the weight of the evidence with and without modeling the degradation, the best fit to the genotypic data was obtained without modeling degradation.

For the 13 cases to which no treatment was applied, the majority of the reported LR values did not model degradation (ranging from N = 9/12 to N = 13/14). In most cases, this outcome followed the software's evaluation of different modeling conditions to identify the best (ranging from N = 6/9 to N = 12/13). Those for whom the best fit relied on modeling degradation despite no treatment have been applied: pairs #5 (N = 1), #15 (N = 1), and #30 (N = 3), involved situations where no modeling degradation was the second best fit for the input data and the differences between the LR values assigned were very slight, maintaining in the same order of magnitude in all cases. For example, for pair #5, the adjLogLik and log₁₀(LR) for the best fit (modeling degradation: -444.48 and 33.85, resp.) and the second best fit (no modeling degradation: -444.5 and 33.81, resp.) were very similar (in both cases, not modeling both back and forward stutter).

3.2. LR values assigned, and respective conclusions reported

Once all parameter values and conditions had been established, participating laboratories assigned an LR value for each pair of samples.

Summary of the number of participants using a quantitative tool (14 out of 17), the mixture profile chosen to be analyzed (without or with artefacts), and the information regarding stutter peaks modeling. The method followed to determine whether to model or not the stutter peaks is also described – either modeled by choice of the user (Input) or letting the software test the different combinations and then selecting the best fit for the genotypic input data (PGS). As before, the pairs of samples were grouped based on the contributor profiles that comprise each mixture.

Profiles ID		Pair ID	Total	Evidence file without (and with) artefacts	Back stutter modeled															
					No						Yes									
Mixture	Ref.				N	Input	PGS	N	Input						PG					
									Forward stutter modeled						Forward stutter modeled					
									No			Yes			No			Yes		
									N	Input	PGS	N	Input	PGS	N	Input	PGS	N	Input	PGS
31_32	31	1	13	11	11 (1)	6	5	-	-	-	-	-	-	-	-	-	-	-	-	-
		21	14	(2)	12	8	(1)	(1)	-	-	-	-	-	-	(1)	(1)	(1)	-	-	-
33_34	33	2	14	12	12	7	5	(2)	-	-	-	-	-	-	(2)	(2)	(2)	-	-	-
	34	3	14	(2)	12 (2)	8	(2)	-	-	-	-	-	-	-	(2)	(2)	(2)	-	-	-
		22	14	12	12	8	4	(2)	-	-	-	-	-	-	(2)	(1)	(1)	-(1)	-	-
35_50	35	12	14	(2)	12	8	4	(2)	-	-	-	-	-	-	(2)	(2)	(2)	-	-	-
	50	5	14	12	12	6	6	(2)	-	-	-	-	-	-	(2)	(1)	(1)	-(1)	-	-
		24	14	(2)	12	8	4	(2)	-	-	-	-	-	-	(2)	-	(2)	-	-	(2)
39_40	39	13	14	12	11 (1)	7	(1)	1	1	-	-	-	1	1	-	(1)	(1)	(1)	-	-
	40	15	13	(2)	10 (1)	6	(1)	1	1	-	-	-	1	1	-	(1)	-	-	-(1)	-
		25	14	12	12	7	5	(2)	-	-	-	-	-	-	(2)	-	(2)	-	-	(1)
48_49	48	4	14	(2)	11 ^a (2)	7	(2)	1	-	-	-	-	-	-	1	(2)	(2)	1	-	1
		14	14	12	11	7	4	(2)	1	1	-	-	-	1	1	-	(2)	(1)	(1)	-(1)
	49	11	14	(2)	12	8	4	(2)	-	-	-	-	-	-	(2)	-	-	-(2)	-	(1)
		23	14	12	12	8	4	(2)	-	-	-	-	-	-	(2)	-	-	-(1)	-	(2)
36_37_38	36	6	12	10	10 (2)	8	(2)	-	-	-	-	-	-	-	-	-	-	-	-	-
		30	12	(2)	10	7	3	(2)	-	-	-	-	-	-	(2)	(2)	(2)	-	-	-
	37	10	14	12	12 (1)	8	(1)	-	-	-	-	-	-	-	(1)	(1)	(1)	-	-	-
		26	14	(2)	12	7	5	(2)	-	-	-	-	-	-	(2)	(2)	(2)	-	-	-
	38	17	14	12	12	8	4	(2)	-	-	-	-	-	-	(2)	(2)	(2)	-	-	-
41_42_43	41	7	14	12	6 (1)	5	(1)	6	2	1	1	-	1	1	-	4	1	1	3	3
	42	18	14	(2)	11	7	4	(2)	1	1	1	-	-	-	(2)	-	-	-(2)	-	(2)

(continued on next page)

Table 5 (continued)

Profiles ID	Pair ID	Total	Evidence file without (and with) artefacts	Back stutter modeled				Forward stutter modeled				Forward stutter modeled			
				No		Yes		No		Yes		No		Yes	
				N	Input	PGS	N	N	Input	PGS	N	N	Input	PGS	N
Mixture	Ref.														
43	27	14	12	12	8	4	-	-	-	-	-	-	-	-	-
44	8	14	(2)	12 (2)	8	4	(2)	-	-	-	-	(1)	-	(1)	-
45	16	14	(2)	10 (1)*	6	4 (1)*	-	-	-	-	-	(2)	-	(2)	-
46	20	14	(4*)	12	8	4	(3)	-	-	-	-	-	-	-	-
47	9	14	(2)	11 (2)	7	4	(2)	1	1	-	1	-	-	-	-
48	19	14	(2)	12	8	4	-	-	-	-	-	(2)	-	(2)	-
49	29	13	(2)	11 (1)	7	4	(1)	1	1	-	1	-	-	(1)	-
				11	7	4	(2)	-	-	-	-	-	-	(2)	-

*One of the two laboratories that used two tools to perform the exercise applied different models in each tool for pair #28.

^a For this pair, one laboratory inputted the decision of modelling only the forward stutters.

Fig. 1 provides an overview of the distribution of those LR values, with laboratories according to the implementation stage of the tool used to perform the exercise.

The LR distribution offers insights into how the sample complexity affects the results. As expected, more complex samples – those combining highly imbalanced contributor ratios, multiple contributors, DNase I degradation, and low DNA input – yielded lower and more dispersed LR values (see Table 7 for some statistics). Indeed, these conditions create complex mixtures that are more difficult to interpret and more susceptible to stochastic effects, resulting in greater variability across laboratory analyses. This was the case for the top five cases #8, #9, #14, #18, and #19, with greater variability and less consistent results across laboratories. The most critical factor underlying this trend was the combination of a high number of contributors and imbalanced ratios – see Table 8. The highly imbalanced mixture ratios are more prone to stochastic effects, especially for the minor contributor, since increased variability in allele detection leads to higher dispersion of results that are especially amplified when coupled with DNA degradation – see cases #14 and #19 in Table 8.

When comparing pairs that shared the same mixture and reference profile with similar low template amounts (0.03 ng and 0.062 ng), but differed in degradation levels, the pair with greater degradation consistently yielded lower and more dispersed LR values – see, for example, cases #1 and #21 in Table 8. When the same mixture, reference profile, and degradation treatment were considered, but differed in the total template amount, the pair with the least template showed lower and more dispersed LR values – see, for example, cases #4 and #14 in Table 8. Finally, a comparison of pairs sharing the same mixture and reference profiles but differing in template amount and degradation treatment (absent/present), revealed that the non-degraded pair with a lower template amount showed lower and slightly more clustered LR values – see, for example, cases #15 and #25 in Table 8. This observation may support the hypothesis that the amount of template had a greater impact on the LR of the analyzed cases than degradation.

Regarding the range of LR values presented, it is important to stress that laboratories were only provided with the mixture profiles, the associated reference profiles, and the allele frequency database. These represent the only consistent data across all participants. As detailed in Section 3.1, several factors varied in the statistical assessment performed by the different laboratories. These included the PG tool used, the type of evidence files considered (with or without artefacts), estimates for population and laboratory-specific parameters and conditions, and whether combinations of factors like the number of contributors, stutters, and/or degradation were tested or pre-established. These differences inevitably affect the LR values reported.

For example, considering the same PG tool and letting it test the best-fit conditions for the same input data, but applying different analytical thresholds will lead the PGS to consider different conditions as the best fit. This situation was observed, for example, for pair #4, where three laboratories let EuroForMix test all possible combinations regarding NoC, stutters, and degradation modeling, but applied different analytical thresholds ($T = 1, 50$, and 85 RFU). This resulted in a discrepancy between the number of peaks considered by each one, which led to the tool assuming different best-fit conditions for the same input data and, consequently, assigning different LR values ($1.22\text{E}+24$, $1.69\text{E}+17$, and $5.96\text{E}+07$, respectively).

Even when the same analytical threshold is applied (50 RFU), very discrepant LR values may be obtained either due to considering a different NoC or the decision to model (or not) stutters and/or degradation. As observed for pair #29, one laboratory allowed the software to test all combinations of conditions. The resulting LR value varied from $1.23\text{E}+11$ (best-fit; back stutter modeled) to $2.14\text{E}+06$ (considering the same conditions as the best-fit, except in this case the back stutter was not modeled).

Thus, a comprehensive, integrated analysis is necessary to understand the differences observed instead of a parameter-by-parameter

Table 6

Number of laboratories using a quantitative tool (14 out of 17) that modeled the degradation or not for each pair of samples. The method followed is also described – either model by choice (Input) or letting the software test the different combinations and then selecting the best fit for the genotypic input data (PGS). As before, the pairs of samples were grouped based on the contributor profiles. The level of degradation was simulated by adding DNase I at different concentrations: A= 6 mU, B= 12 mU, and C= 24 mU. ‘Total’ represents the number of laboratories using a quantitative tool that provided an LR value for the given pair.

Profiles ID	Ref.	Pair ID	Degradation Treatment	Total	Modeled Degradation					
					No			Yes		
Mixture					N	Input	PGS	N	Input	PGS
31_32	31	1	A	13	-	-	-	13	5	8
		21	None	14	12	2	10	2	2	-
33_34	33	2	None	14	13	3	10	1	1	-
		34	A	14	-	-	-	14	4	10
35_50	35	22	None	14	12	1	11	2	2	-
		12	B	14	-	-	-	14	3	11
		5	None	14	12	1	11	2	1	1
		24	A	14	-	-	-	14	3	11
39_40	39	13	None	14	12	1	11	2	2	-
		15	None	13	10	2	8	3	2	1
		25	A	14	-	-	-	14	2	12
48_49	48	4	A	14	-	-	-	14	2	12
		14	A	14	-	-	-	14	3	11
		11	C	14	-	-	-	14	5	9
		23	None	14	13	1	12	1	1	-
36_37_38	36	6	A	12	1	-	1	11	3	8
		30	None	12	9	3	6	3	-	3
		10	None	14	11	2	9	3	3	-
		26	None	14	13	2	11	1	1	-
41_42_43	41	17	B	14	-	-	-	14	3	11
		7	A	14	-	-	-	14	3	11
		18	B	14	-	-	-	14	3	11
		27	None	14	13	3	10	1	1	-
44_45_46	44	8	None	14	13	3	10	1	1	-
		28	A	14	-	-	-	14	3	11
		16	A	14	-	-	-	14	3	11
		20	A	14	-	-	-	14	4	10
47_48_49	47	9	None	14	13	4	9	1	1	-
		19	A	14	-	-	-	14	6	8
		29	A	13	-	-	-	13	2	11

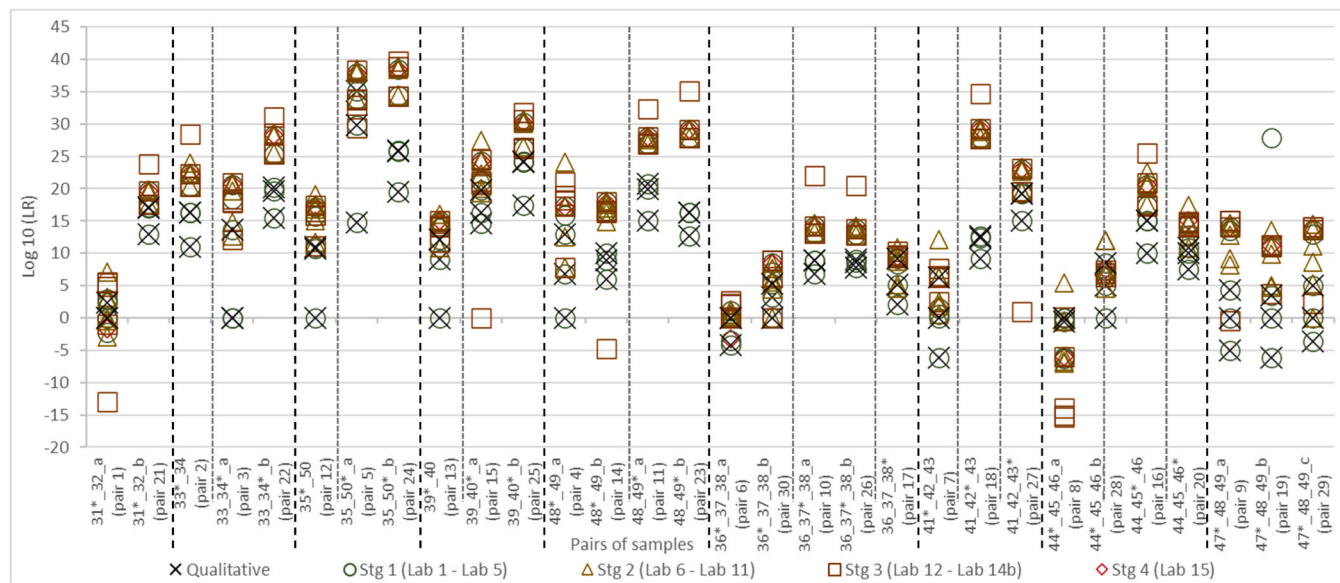


Fig. 1. Distribution of LR values ($\log_{10}$ scale) assigned by the participating laboratories for each pair of samples. The results reported were grouped according to the implementation stage of the tool at the time this exercise was performed: Stg1 (circles) – Implemented; Stg2 (triangles) – In the implementation process; Stg3 (squares) – Planned to be implemented in the near future; and Stg4 (diamonds) – Neither implemented nor planned to be implemented in the near future. Results obtained using qualitative tools are highlighted with an ‘X’. The pairs of samples, whose ID is in parentheses, were organized based on the mixture sample profiles considered; the reference sample included in the pair is highlighted with an ‘*’, and, for pairs considering the same mixture and reference pair, the different treatments to which they were subjected are highlighted alphabetically (a, b, c, ...). For example, in pairs #1 and #21, although the mixture composition (31_32) and reference sample (31*_32) are the same, different treatments (a and b) were applied: ‘31*_32_a’ for pair #1, and ‘31*_32_b’ for pair #21.

Table 7

Conclusions reported by the participating laboratories for each pair of samples analyzed regarding the potential contribution of the reference sample to the evidence mixture: “The reference cannot be excluded”, “Non-conclusive/Non-informative”, “The reference can be excluded”, and “Not answer”. The pairs of samples were grouped based on the mixture and reference sample (Ref.) combinations. Within each conclusion category, N’ denotes the number of laboratories reporting the same conclusion, ‘Ø’ indicates the ones that did not assign an LR value for the respective pair, while ‘LR < 1’ and ‘LR > 1’ are the number of laboratories that reported an LR lower and higher than 1, respectively; the minimum and maximum LR values obtained are also provided, along with the mean LR and its standard deviation.

Profiles ID		Pair ID	LR values Mean	Standard Deviation (stdv)	Does the reference belong to one of the contributors to the evidence sample?																					
Mixture	Ref.				The reference cannot be excluded						Non-conclusive / non-informative						The reference can be excluded						Not answered			
					N	Ø	LR < 1	LR > 1	Minimum	Maximum	N	Ø	LR < 1	LR > 1	Minimum	Maximum	N	Ø	LR < 1	LR > 1	Minimum	Maximum		N		
31_32	31	1	8.91E+ 00	4.944	1	-	-	1	-	3.86E+ 05	11	1	3	7	1.04E-13	1.34E+ 07	4	1	3	-	1.34E-03	2.19E-02	1			
		21	3.85E+ 18	2.172	17	-	-	17	7.87E+ 12	7.24E+ 23	-	-	-	-	-	-	-	-	-	-	-	-	-			
33_34	33	2	6.42E+ 20	3.606	17	-	-	17	1.02E+ 11	3.07E+ 28	-	-	-	-	-	-	-	-	-	-	-	-	-			
		34	1.03E+ 18	3.084	13	-	-	13	4.45E+ 12	8.22E+ 20	3	1	-	2	1.20E+ 12	4.25E+ 13	1	1	-	-	-	-	-	-		
		22	7.40E+ 25	3.888	17	-	-	17	2.65E+ 15	1.21E+ 31	-	-	-	-	-	-	-	-	-	-	-	-	-			
		35_50	35	12	2.36E+ 15	2.627	16	-	-	16	4.66E+ 10	1.27E+ 19	-	-	-	-	-	1	1	-	-	-	-	-	-	
		50	5	1.68E+ 34	5.651	17	-	-	17	6.21E+ 14	2.78E+ 38	-	-	-	-	-	-	-	-	-	-	-	-	-		
		24	6.49E+ 34	5.632	17	-	-	17	3.55E+ 19	5.52E+ 39	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
39_40	39	13	3.65E+ 13	1.914	16	-	-	16	1.29E+ 09	1.16E+ 16	-	-	-	-	-	-	1	1	-	-	-	-	-	-		
		40	15	4.07E+ 21	3.192	16	-	-	16	3.91E+ 14	2.94E+ 27	-	-	-	-	-	-	-	-	-	-	-	-	1		
		25	6.16E+ 27	3.557	17	-	-	17	2.33E+ 17	6.74E+ 31	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		48_49	48	4	3.88E+ 15	4.695	15	-	-	15	5.96E+ 07	1.22E+ 24	1	-	-	1	-	6.16E+ 06	1	1	-	-	-	-	-	
		14	1.72E+ 14	5.884	16	-	-	16	1.11E+ 06	1.21E+ 18	1	-	1	-	-	1.85E-05	-	-	-	-	-	-	-	-		
		49	11	1.86E+ 26	3.890	17	-	-	17	1.27E+ 15	2.14E+ 32	-	-	-	-	-	-	-	-	-	-	-	-	-		
		23	4.21E+ 26	5.634	17	-	-	17	4.55E+ 12	1.14E+ 35	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		36_37_38	36	6	1.20E+ 00	2.155	-	-	-	-	-	10	1	-	9	1.25E+ 00	4.09E+ 02	6	2	3	1	5.68E-05	3.87E+ 00	1		
		30	9.65E+ 06	1.800	11	-	-	11	1.49E+ 03	8.17E+ 08	3	-	-	3	1.89E+ 05	3.03E+ 08	2	2	-	-	-	-	-	1		
		37	10	1.99E+ 13	3.116	17	-	-	17	6.00E+ 06	1.06E+ 22	-	-	-	-	-	-	-	-	-	-	-	-	-		
		26	1.05E+ 13	2.695	17	-	-	17	7.20E+ 07	3.12E+ 20	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		38	17	1.71E+ 08	2.377	15	-	-	15	1.13E+ 02	8.02E+ 10	2	-	-	2	1.23E+ 05	2.08E+ 09	-	-	-	-	-	-	-		
41_42_43	41	7	8.36E+ 03	4.044	6	-	-	6	1.83E+ 06	1.50E+ 12	9	-	-	9	3.13E+ 00	1.83E+ 06	2	1	1	-	-	-	6.33E-07	-		
		42	18	1.01E+ 26	6.961	17	-	-	17	1.56E+ 09	5.40E+ 34	-	-	-	-	-	-	-	-	-	-	-	-	-		
		43	27	3.42E+ 19	5.137	16	-	-	16	9.69E+ 14	1.61E+ 23	1	-	-	1	-	9.31E+ 00	-	-	-	-	-	-	-		
		44_45_46	44	8	2.40E-06	5.714	-	-	-	-	-	5	1	2	2	2.94E-01	2.71E+ 05	12	1	11	-	4.86E-16	2.40E-01	-		
		28	2.66E+ 07	1.945	14	-	-	14	4.15E+ 04	9.74E+ 11	2	-	-	2	5.03E+ 06	2.37E+ 07	1	1	-	-	-	-	-	-		
		45	16	6.91E+ 18	3.409	17	-	-	17	1.20E+ 10	2.89E+ 25	-	-	-	-	-	-	-	-	-	-	-	-	-		
		46	20	2.66E+ 13	2.327	17	-	-	17	3.11E+ 07	2.55E+ 17	-	-	-	-	-	-	-	-	-	-	-	-	-		
		47_48_49	47	9	5.93E+ 10	5.918	13	-	-	13	1.50E+ 08	1.06E+ 15	2	-	1	1	4.23E-01	1.84E+ 04	2	1	1	-	-	-	8.49E-06	
		19	2.68E+ 09	6.767	11	-	-	11	5.06E+ 04	6.40E+ 27	4	-	-	4	2.60E+ 03	9.25E+ 10	2	1	1	-	-	-	-	6.30E-07		
		29	16	1.60E+ 10	5.329	10	-	-	10	4.65E+ 08	2.74E+ 14	4	-	-	4	1.83E+ 02	4.54E+ 13	2	1	1	-	-	-	-	2.53E-04	

Table 8

Descriptive summary of relevant pairs of samples: the top five pairs for which the most discrepant results were obtained between laboratories; and pairs used in the main text as examples to illustrate the impact of some conditions associated with the problem samples may have on the result.

	Pair ID	Profiles ID		Mixture Proportion	Degradation Treatment (mU DNase I)	DNA in PCR (ng)	LR values Mean	Standard Deviation (stdv)	Notable Features regarding the Mixture Profile
		Mixture	Ref.						
Higher Standard Deviation	8	44_45_46	44	1:2:2	Untreated	0.075	2.40E-06	5.714	3 contributors with a moderate contributor ratio, and a low DNA amount
	9	47_48_49	47	1:9:9	Untreated	0.500	5.93E+ 10	5.918	3 contributors with a highly imbalanced contributor ratio
	14	48_49	48	1:4	6	0.075	1.72E+ 14	5.884	2 contributors, degraded, with a highly imbalanced contributor ratio and low DNA amount
	18	41_42_43	42	1:9:1	12	0.693	1.01E+ 26	6.961	3 contributors, highly degraded, with a highly imbalanced contributor ratio
	19	47_48_49	47	1:9:9	6	0.750	2.68E+ 09	6.767	3 contributors, degraded, with a highly imbalanced contributor ratio
Other examples	1	31_32	31	1:1	6	0.030	8.91E+ 00	4.944	Both with 2 contributors with a balanced contributor ratio and a low DNA amount, but only one was subject to degradation treatment
	21				Untreated	0.062	3.85E+ 18	2.172	
	4	48_49	48	1:4	6	0.155	3.88E+ 15	4.695	Both with 2 contributors, degraded, and a highly imbalanced contributor ratio, but different DNA amounts
	14					0.075	1.72E+ 14	5.884	
	15	39_40	40	1:2	Untreated	0.045	4.07E+ 21	3.192	Both with two contributors and a moderate contributor ratio, but with different DNA amounts, and only one was subject to degradation treatment
	25				6	0.189	6.16E+ 27	3.557	

analysis. Also, the analysis should always be combined with information regarding the genotypic configuration of the mixture profile, as the number of allele peaks in stutter positions, for example, is one of the main factors that influence the results.

For all these reasons, the LR values were examined alongside each laboratory's conclusions for each sample pair. This allowed a more comprehensive understanding of the results, considering the specific methods and conditions applied by each participant.

Based on their analyses, laboratories were asked to conclude whether the reference could be considered a contributor to the mixture. Three response options were provided: (1) "The reference cannot be excluded from belonging to one of the contributors to the evidence sample", (2) "non-conclusive/non-informative", and (3) "The reference can be excluded from belonging to one of the contributors to the evidence sample". A summary of all the conclusions reported for each analyzed case can be found in Table 7.

Table 7 also shows that laboratories apply different criteria when interpreting LR values. Considering pair #1 as an example, the only laboratory concluding that "The reference cannot be excluded from belonging to one of the contributors to the evidence sample" assigned an LR value of 3.86E+ 05, which falls within the broad LR range (1.04E-13 – 1.34E+07) reported by laboratories that classified the result as "non-conclusive/non-informative". On the other hand, all the laboratories that concluded that "The reference can be excluded from belonging to one of the contributors to the evidence sample" assigned an LR value (1.34E-03 – 2.19E-02) higher than some that reported a "non-conclusive/non-informative result".

As observed in Table 3, pairs #1, #6, #7, and #8 stood out once again from the others. The DNA mixture in these pairs was treated with DNase, and/or the DNA template amount was very low, and/or the reference profile belonged to a minor contributor of the mixture. Out of the 42 alleles for each reference sample (21 markers analyzed, each with 2 alleles), 6, 8, 3, and 7 reference alleles, respectively, were missing from the corresponding mixture profiles.

Noteworthy, it was previously reported that reference profile #41 (included in pairs #7, #18 and #27) carries a possible primer binding site mutation at locus D1S1656 [27], which causes heterozygote imbalance between reference's allele 14 and 15. Although this mutation could influence the statistical interpretation, its impact is reflected differently in the LR results of each pair. Both pairs #7 and #18 were treated with DNase, resulting in more dispersed LR values overall.

However, in pair #18, where the reference profile was the major contributor (ratio 1:9:1), LR results tended to cluster into two distinct groups: one resulted by quantitative tools computations, which yielded higher LR values due to considering peak heights in their calculations, and another by qualitative ones, which produce lower LR values. In contrast, even though the reference profile of pair #27 was a minor contributor, the evidence sample was untreated and as such the impact was lower.

4. Conclusions

This collaborative exercise, with the Spanish and Portuguese-speaking Working Group of the International Society for Forensic Genetics (GHEP-ISFG), was driven by the growing willingness of forensic genetics laboratories worldwide to implement probabilistic genotypic tools in their modern interpretation protocols. GHEP-ISFG collaborative exercises have long demonstrated their significant value, improving participating laboratories' knowledge and practice quality while contributing to the wider scientific community – see, e.g., the more recent ones [28–30]. Despite significant initial interest, only one-third of those who initially registered in this exercise completed their participation, with high workload often cited as a barrier. This highlights the broader challenges faced by many forensic laboratories in dedicating time and resources to continuing professional development, including participation in inter-laboratory exercises that can contribute to advanced interpretative capabilities within forensic casework. Aiming to have a general overview of the knowledge and use of non-binary software, both in the routine casework and preliminary stages of implementation, the participating laboratories were asked to evaluate the weight of evidence of 30 mock contributor/mixture samples. These samples, selected from the PROVEDIt Initiative, encompassed a variety of NoCs, treatments, and levels of degradation. The corresponding genotypic profiles were provided to all participants. Through this collaborative exercise, it was possible to assess the different approaches and methodologies used to statistically interpret cases involving DNA mixtures, including parametrization choices and tool configuration.

Even though all participating laboratories have received the same genotypic profiles and allelic frequency data, different results were reported for each pair of samples due to the varied methodologies applied. Despite using distinct tools (qualitative or quantitative) at different implementation stages, laboratories also employed diverse approaches

to establish the NoC, as well as other population, laboratory, and case-specific parameter values and conditions. These included the coancestry coefficient, minimum allele frequency, dropout frequency, drop-in (frequency and modeling), analytical threshold, and the modeling of stutter peaks and degradation.

While such methodological variability might have a limited practical impact for some parameters and simpler cases, it inevitably led to different LR values. As expected, the more striking differences have been reached for more complex samples characterized by a higher level of degradation and low DNA template. As far as it could be determined, the most influential factors in these discrepancies were the analytical threshold and the estimated NoC, both of which directly affect the number of alleles included in the interpretation. Additionally, LR discrepancies may have been amplified when reference samples belonged to a minor contributor, especially when alleles of the minor contributor coincided with stutter peak positions. Therefore, to fully understand all the factors that influence and condition an LR result, it is crucial to perform an integrated analysis combining both parameters' values and condition variations with the genotypic profile of the evidence and reference samples under analysis.

No direct correlation between implementation stage and interpretation accuracy was observed in this study. This may be due to the limited number of participating laboratories ($N = 17$) and the uneven distribution between stages 1–2 ($N = 11$) and 3–4 ($N = 6$). Moreover, experience with PGS was not necessarily linked to the implementation stage of the software used in the exercise, as some participants laboratories routinely use different tool(s). Thus, a laboratory using a tool in implementation stage 3 or 4 may still have substantial expertise with similar ones.

Nevertheless, proficiency in complex DNA interpretation requires not only experience but also continuous and specialized training. The evolving nature of PGS and interpretation guidelines requires ongoing education to maintain high standards and reduce errors even for experienced practitioners.

Thus, for forensic genetics experts, a truly deep understanding of PGS is essential. This means not only user proficiency but also comprehending the statistical models and population genetics principles that underpin these tools, how to accurately set their parameters, and the downstream impact on results. Accordingly, continuous and specialized training in forensic genetics is paramount, sustaining professional development as a fundamental component of quality assurance in forensic genetics and enabling practitioners to correctly interpret and report complex DNA profile analyses.

CRedit authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.fsigen.2025.103383](https://doi.org/10.1016/j.fsigen.2025.103383).

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Supplementary Material VI

The complete supplementary data associated with this article can also be found in the online version at <https://doi.org/10.1016/j.fsigen.2025.103383>

SM 1: Questionnaire provided to participating laboratories

S1a: Participant_ID

PARTICIPANT IDENTIFICATION			
Acronym for the data*			
#1			
Name and Surname			
E-mail			
#2 (if any)			
Name and Surname			
E-mail			
LABORATORY IDENTIFICATION			
Name			
Address			
City			
Country			
Type of institution	Please select ...	If 'other', please specify	

*Example: Corresponding participant: Nadia Pinto from Instituto de Investigação e Inovação em Saúde da Universidade do Porto (i3S), Porto, Portugal: NPinto_i3S_Porto_PT

S1b: Software_Info

INFORMATICS TOOL

(A) INFORMATICS TOOL USED TO PERFORM THE COMPUTATIONS
--

Name	Please select ...
If 'other', please specify	
Version	
It is implemented in the laboratory?	Please select ...

(B) INFORMATICS TOOL VALIDATED AND IN USE IN YOUR LABORATORY TO PERFORM MIXTURE SAMPLE ANALYSES
--

Name	Please select ...
If 'other', please specify	
Version	

(C) ADDITIONAL INFORMATICS TOOL USED IN YOUR LABORATORY (if any)

Name	Please select ...
If 'other', please specify	
Version	

OTHER REVELANT INFORMATIONS

--

DATA ANALYSIS CONDITIONS USED IN THIS GHEP-ISFG WORKING COMMISSION

Did you use any type of tool to calculate the number of contributors?		
Please select ...	If 'other', please	

Do you use the GlobalFiler ® kit in your laboratory?	Please select ...
Do you have the GlobalFiler ® kit validated under your laboratory conditions?	Please select ...

PARAMETERS:	Values*:	Justification for the values used:	If 'other', please specify	Other relevant information, including references or formulae applied
Drop-out frequency		Please select ...		
Drop-in frequency		Please select ...		
Drop-in modelling (e.g. $\lambda=0.01$ in EuroForMix)		Please select ...		
Coancestry coefficient (FST, θ)		Please select ...		
Threshold detection		Please select ...		
Minimum allele frequency		Please select ...		

* If different parameters' values were considered for the different markers, please specify them in the table below.

MARKERS	PARAMETERS VALUES			
	Drop-in frequency	Drop-in modelling	Coancestry coefficient (FST, θ)	Threshold detection
D3S1358				
vWA				
D16S539				
CSF1PO				
TPOX				
D8S1179				
D21S11				
D18S51				
D2S441				
D19S433				
TH01				
FGA				
D22S1045				
D5S818				
D13S317				
D7S820				
SE33				
D10S1248				
D1S1656				
D12S391				
D2S1338				

OTHER REVELANT INFORMATIONS

S1d: Report_Results

PAIR OF SAMPLES	NUMBER OF CONTRIBUTORS	PROBLEM SAMPLE FILE USED	STUTTERS MODELLING If other, please specify	DEGRADATION MODELLING
1	Please select ...	Please select ...	Please select ...	Please select ...
2	Please select ...	Please select ...	Please select ...	Please select ...
3	Please select ...	Please select ...	Please select ...	Please select ...
4	Please select ...	Please select ...	Please select ...	Please select ...
5	Please select ...	Please select ...	Please select ...	Please select ...
6	Please select ...	Please select ...	Please select ...	Please select ...
7	Please select ...	Please select ...	Please select ...	Please select ...
8	Please select ...	Please select ...	Please select ...	Please select ...
9	Please select ...	Please select ...	Please select ...	Please select ...
10	Please select ...	Please select ...	Please select ...	Please select ...
11	Please select ...	Please select ...	Please select ...	Please select ...
12	Please select ...	Please select ...	Please select ...	Please select ...
13	Please select ...	Please select ...	Please select ...	Please select ...
14	Please select ...	Please select ...	Please select ...	Please select ...
15	Please select ...	Please select ...	Please select ...	Please select ...
16	Please select ...	Please select ...	Please select ...	Please select ...
17	Please select ...	Please select ...	Please select ...	Please select ...
18	Please select ...	Please select ...	Please select ...	Please select ...
19	Please select ...	Please select ...	Please select ...	Please select ...
20	Please select ...	Please select ...	Please select ...	Please select ...
21	Please select ...	Please select ...	Please select ...	Please select ...
22	Please select ...	Please select ...	Please select ...	Please select ...
23	Please select ...	Please select ...	Please select ...	Please select ...
24	Please select ...	Please select ...	Please select ...	Please select ...
25	Please select ...	Please select ...	Please select ...	Please select ...
26	Please select ...	Please select ...	Please select ...	Please select ...
27	Please select ...	Please select ...	Please select ...	Please select ...
28	Please select ...	Please select ...	Please select ...	Please select ...
29	Please select ...	Please select ...	Please select ...	Please select ...
30	Please select ...	Please select ...	Please select ...	Please select ...

LR VALUES																	How would you report the LR result obtained?	OTHER RELEVANT INFORMATIONS								
PAIR OF SAMPLES	D3S1358	vWA	D16S539	CSF1PO	TPOX	D8S1179	D21S11	D18S51	D2S441	D19S433	TH01	FGA	D22S1045	D5S818	D13S317	D7S820			SE33	D10S1248	D1S1656	D12S391	D2S1338	GLOBAL LR VALUE		
1																								Please select ...		
2																									Please select ...	
3																									Please select ...	
4																									Please select ...	
5																									Please select ...	
6																									Please select ...	
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28																									Please select ...	
29																									Please select ...	
30																									Please select ...	

STATISTICAL INTERPRETATION IN IDENTIFICATION CASES WITH MIXTURES

Information regarding data provided:

We are providing 30 pairs of mixture/reference profiles to be analyzed to quantify the weight of the evidence, given a possible contributor to the mixture sample. Mixture and reference profiles of the i -th pair are named `problem_i` and `ref_i`, respectively, and the complete set of pairs to be analyzed is provided in the files 'problem_i' and 'reference_i', respectively.

The allele frequency database to be used in the computations is provided in the Excel file 'NIST allele frequency database', corresponding to [1]. In case of need to consider minimum frequency allele please consider $N = 1,036$, as the total number of analyzed subjects

All the samples provided were obtained from the *PROVEDIt* database [2]: <https://lftdi.camden.rutgers.edu/provedit/>, considering filtered data for the markers analyzed with the GlobalFiler® kit. As indicated by the authors, the data from *PROVEDIt* were exported from GeneMapper® ID-X using Local Southern sizing at an analytical threshold (AT) of one Relative Fluorescent Unit (RFU). The file corresponding to each problem sample contains information on the allele, size, and height for all the peaks. Problem sample profile files with and without stutters will be provided. For the reference samples only the alleles are provided. Although the samples were analyzed by using the GlobalFiler® kit with an $AT=1$, participants can use other thresholds that they consider more suitable (the ones from their laboratory's internal validation or others).

A likelihood ratio (LR) consists of a comparison between the probability of observing the evidence (E) assuming two opposite, alternative, and mutually exclusive hypotheses, $H1$: "DNA originated from the person of interest and $N-1$ unknown, unrelated individual(s)", and $H2$: "DNA originated from N unknown, unrelated, individuals", where N represented the estimated number of contributors:

$$LR = \frac{P(E|H1)}{P(E|H2)}$$

Markers analyzed are considered as both unlinked and in linkage equilibrium in the population and therefore for each case the global LR can be obtained by multiplying the partial LRs obtained for the different markers.

Information regarding data required:

- Global LR values, as well as marker-specific ones, should be provided under the format required.
- Information on the informatics tool used (and corresponding version), number of estimated contributors, and parameters' values used should be carefully and accurately provided to allow the re-computation of the results.
- All the data should be provided by filling the Excel file 'REPORT_RESULTS', without modifying its structure or format. Participants proceeding with the latter will be kindly asked to resend their data under the requested format.
- Cells with open answers should be carefully filled, providing objective and accurate information to allow its global analysis with those from the other participants.

information to allow its global analysis with those from the other participants.

- Beyond the Excel file, reports exported from the software for the complete set of cases should be provided to the coordinators at the time of sending the results.

These reports should be named as follows: ParticipantAcronym_pair_i, for the case involving samples problem_i / ref_i.

- Results considering another allele frequency database than the one provided will not be considered.

Deadlines:

- Deadline for sending results: December 1, 2024

(send to camilac@i3s.up.pt; Cc: npinto@i3s.up.pt; lourditasmt@gmail.com)

References:

[1] C.R. Hill, D.L. Duewer, M.C. Kline, M.D. Coble, J.M. Butler, US population data for 29 autosomal STR loci, *Forensic Sci. Int. Genet.* 7 (3) (2013) e82–e83.

[2] Alfonse, L.E., Garrett, A.D., Lun, D.S., Duffy, K.R. & Grgicak, C.M. A large-scale dataset of single and mixed-source short tandem repeat profiles to inform human identification strategies: PROVEDIt. *Forensic Sci. Int. Genetics* 32, 62-70. DOI:10.1016/j.fsigen.2017.10.006

SM 2: List of all pairs of samples provided to the participant laboratories.

Table S2: List of all pairs of samples provided to the participant laboratories to analyze in the context of this working commission, along with their composition and respective treatment.

Pair ID	Ref. ID	MIXTURE CONDITIONS								PROVEDIt sample reference
		Contributors ID	Proportion	Number of dilutions	Treatment	ng included in PCR	DNA extract quantified	Capilar number	Injection Time	
1	31	31_32	1;1	2	6 mU DNase I Degradation	0.03	2	1	5	A02_RD14-0003-31_32-1;1-M2c-0.03GF-Q2.0_01.5sec.hid
21	31	31_32	1;1	2	Untreated	0.062	1.2	4	25	D01_RD14-0003-31_32-1;1-M2a-0.062GF-Q1.2_04.25sec.hid
2	33	33_34	1;2	2	Untreated	0.375	0.7	1	5	A03_RD14-0003-33_34-1;2-M2a-0.375GF-Q0.7_01.5sec
3	34	33_34	1;2	3	6 mU DNase I Degradation	0.045	1	1	5	A04_RD14-0003-33_34-1;2-M3c-0.045GF-Q1.0_01.5sec.hid
22	34	33_34	1;2	3	Untreated	0.093	0.8	4	25	D03_RD14-0003-33_34-1;2-M3a-0.093GF-Q0.8_04.25sec.hid
12	35	35_50	1;9	3	12 mU DNase I Degradation	0.9	0.9	1	15	A09_RD14-0003-35_50-1;9-M3d-0.15GF-Q0.9_01.15sec.hid
5	50	35_50	1;9	3	Untreated	0.8	0.8	1	5	A08_RD14-0003-35_50-1;9-M3a-0.15GF-Q0.8_01.5sec.hid
24	50	35_50	1;9	2	6 mU DNase I Degradation	0.7	0.7	4	25	D08_RD14-0003-35_50-1;9-M2c-0.31GF-Q0.7_04.25sec.hid
13	39	39_40	1;2	3	Untreated	0.8	0.8	1	15	A10_RD14-0003-39_40-1;2-M3a-0.093GF-Q0.8_01.15sec.hid
15	40	39_40	1;2	3	Untreated	0.045	0.8	2	15	B10_RD14-0003-39_40-1;2-M3a-0.045GF-Q0.8_02.15sec.hid
25	40	39_40	1;2	2	6 mU DNase I Degradation	0.189	1	4	25	D10_RD14-0003-39_40-1;2-M2c-0.189GF-Q1.0_04.25sec.hid
4	48	48_49	1;4	3	6 mU DNase I Degradation	0.155	0.8	1	5	A06_RD14-0003-48_49-1;4-M3c-0.155GF-Q0.8_01.5sec.hid
14	48	48_49	1;4	3	6 mU DNase I Degradation	0.075	0.8	2	15	B06_RD14-0003-48_49-1;4-M3c-0.075GF-Q0.8_02.15sec.hid
11	49	48_49	1;4	3	24 mU DNase I Degradation	0.155	1	1	15	A07_RD14-0003-48_49-1;4-M3e-0.155GF-Q1.0_01.15sec.hid
23	49	48_49	1;4	3	Untreated	0.315	0.6	4	25	D05_RD14-0003-48_49-1;4-M3a-0.315GF-Q0.6_04.25sec.hid
6	36	36_37_38	1;2;1	4	12 mU DNase I Degradation	0.06	1.1	1	5	A02_RD14-0003-36_37_38-1;2;1-M4c-0.06GF-Q1.1_01.5sec.hid
30	36	36_37_38	1;2;1	3	Untreated	0.06	0.7	5	25	E01_RD14-0003-36_37_38-1;2;1-M3a-0.06GF-Q0.7_05.25sec.hid
10	37	36_37_38	1;2;1	2	Untreated	0.5	0.6	2	5	B01_RD14-0003-36_37_38-1;2;1-M2a-0.5GF-Q0.6_02.5sec.hid
26	37	36_37_38	1;2;1	3	Untreated	0.124	0.7	4	25	D01_RD14-0003-36_37_38-1;2;1-M3a-0.124GF-Q0.7_04.25sec.hid
17	38	36_37_38	1;2;1	2	12 mU DNase I Degradation	0.252	0.9	3	15	C02_RD14-0003-36_37_38-1;2;1-M2d-0.252GF-Q0.9_03.15sec.hid
7	41	41_42_43	1;9;1	2	6 mU DNase I Degradation	0.341	1.1	1	5	A04_RD14-0003-41_42_43-1;9;1-M2c-0.341GF-Q1.1_01.5sec.hid
18	42	41_42_43	1;9;1	1	12 mU DNase I Degradation	0.693	1	3	15	C04_RD14-0003-41_42_43-1;9;1-M1d-0.693GF-Q1.0_03.15sec.hid
27	43	41_42_43	1;9;1	2	Untreated	0.5	0.6	4	25	D03_RD14-0003-41_42_43-1;9;1-M2a-0.5GF-Q0.6_04.25sec.hid
8	44	44_45_46	1;2;2	3	Untreated	0.075	0.6	1	5	A06_RD14-0003-44_45_46-1;2;2-M3a-0.075GF-Q0.6_01.5sec.hid
28	44	44_45_46	1;2;2	3	6 mU DNase I Degradation	0.155	0.9	4	25	D06_RD14-0003-44_45_46-1;2;2-M3c-0.155GF-Q0.9_04.25sec.hid
16	45	44_45_46	1;2;2	2	6 mU DNase I Degradation	0.625	0.8	2	15	B06_RD14-0003-44_45_46-1;2;2-M2c-0.625GF-Q0.8_02.15sec.hid
20	46	44_45_46	1;2;2	3	6 mU DNase I Degradation	0.155	0.9	4	15	D06_RD14-0003-44_45_46-1;2;2-M3c-0.155GF-Q0.9_04.15sec.hid
9	47	47_48_49	1;9;9	2	Untreated	0.5	0.7	1	5	A08_RD14-0003-47_48_49-1;9;9-M2a-0.5GF-Q0.7_01.5sec.hid
19	47	47_48_49	1;9;9	2	6 mU DNase I Degradation	0.75	1.1	3	15	C08_RD14-0003-47_48_49-1;9;9-M2c-0.75GF-Q1.1_03.15sec.hid
	47	47_48_49	1;9;9	2	6 mU DNase I Degradation	0.589	1.1	4	25	D08_RD14-0003-47_48_49-1;9;9-M2c-0.589GF-Q1.1_04.25sec.hid

SM 3. NIST allele frequency database.

Table S3: NIST allele frequency database.

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S165	D21S11	D22S104	D2S1338	D2S441	D3S1358	D5S818	D7S820	D8S1179	FGA	SE33	TH01	TPOX	vWA
5																			0.0014	0.0014	
6																			0.2355	0.0014	
6.3																					
7														0.0028	0.0277					0.1939	
8	0.00554			0.120499	0.01801									0.0055	0.144	0.01385			0.0956	0.5249	
8.1															0.0014						
9	0.01385			0.077562	0.10665							0.0014		0.0416	0.1676	0.00554			0.1191	0.1274	
9.1												0.0014									
9.3																			0.3449		
10	0.22022			0.047091	0.05679	0.0083	0.00139	0.0028				0.2105		0.0554	0.2562	0.10249			0.0083	0.0499	
10.1																					
10.2																					
10.3																					
11	0.30886	0.001385		0.325485	0.3144	0.0097	0.00554	0.0776		0.13989		0.3435	0.001385	0.356	0.205	0.07618		0.0014	0.0014	0.2521	
11.2																					
11.3												0.0609									
12	0.36011	0.031856		0.268698	0.3144	0.1136	0.07064	0.1163		0.01247		0.0471		0.3878	0.1593	0.16759		0.0069		0.0416	0.0014
12.2							0.00139														
12.3														0.0042							
13	0.08172	0.307479		0.116343	0.16343	0.1233	0.25485	0.0665		0.00693		0.0291	0.001385	0.1427	0.0346	0.32964		0.0166		0.0014	0.0014
13.2							0.00693														
13.3																					
13.4																					
14	0.0097	0.297784		0.042936	0.02632	0.1343	0.3615	0.0789		0.05679		0.241	0.106648	0.0069	0.0042	0.1662		0.0249			0.0928
14.2						0.0014	0.02355											0.0028			
14.3								0.0028													
15		0.196676	0.03186	0.001385		0.1704	0.15651	0.1496		0.32133	0.001385	0.0596	0.272853	0.0014		0.10388		0.0402			0.1053
15.2							0.03601														
15.3								0.0582													
15.4																					
16		0.132964	0.02216			0.1468	0.05679	0.1357		0.38227	0.037396	0.0014	0.238227			0.03324		0.0402			0.2008
16.2						0.0014	0.01524											0.0014			
16.3								0.0609													
17		0.027701	0.12742			0.1385	0.00693	0.0471		0.07479	0.185596		0.210526			0.00139		0.0623			0.2839
17.1																					
17.2							0.00139														
17.3			0.02078					0.133										0.0014			

18	0.001385	0.17175	0.0776	0.0055	0.00554	0.073407	0.15097	0.0249	0.072	0.2022
18.1										
18.2			0.00139							
18.3		0.02493		0.0499					0.0014	
19	0.00277	0.12465	0.0402			0.120499	0.01662	0.0499	0.072	0.1039
19.1										
19.2									0.0042	
19.3		0.00416		0.0152						
19.4										
20		0.1108	0.018			0.15651	0.001385	0.1233	0.0582	0.0069
20.1										
20.2									0.0097	
20.3										
21		0.12881	0.0097			0.037396		0.1787	0.0249	0.0014
21.2								0.0055	0.0235	
21.3										
22		0.09557	0.0069			0.034626		0.205	0.0139	
22.2								0.0125	0.0374	
22.3										
23		0.06925				0.105263		0.1524	0.0028	
23.2								0.0028	0.036	
23.3										
24		0.04709				0.114958		0.1343	0.0014	
24.2								0.0014	0.0222	
24.3										
25		0.01662				0.102493		0.0789		
25.2				0.0014					0.0416	
26		0.00277				0.030471		0.0263		
26.2									0.0416	
27		0.00139						0.0042		
27.2				0.0222					0.0942	
27.3										
28				0.1593						
28.2									0.0762	
28.3									0.0014	
29				0.2022						
29.2				0.0028					0.0554	
29.3										
30				0.2825						
30.2				0.0291					0.0568	
30.3										
31				0.072					0.0028	

31.2	0.0983	0.0235
32	0.0055	0.0014
32.2	0.09	0.0125
33	0.0014	0.0014
33.1		
33.2	0.0263	0.0042
34		0.0083
34.2	0.0042	0.0014
35	0.0014	
36	0.0014	

HETE1	0.71943	0.758627	0.89085	0.783726	0.75997	0.8758	0.76945	0.8992	0.8288	0.72199	0.881424	0.7693	0.79003	0.6977	0.8161	0.8072	0.8594	0.9483	0.7646	0.6404	0.8076
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SM 5: Method followed by each laboratory to determine the NoC of each pair of samples analyzed

Table S5: Method followed by each laboratory to determine the NoC of each pair of samples analyzed – either model by choice (‘Input’) or letting the software test the different combinations and then selecting the best fit for the genotypic input data (‘PG’). The pairs of samples are grouped based on their composition. ‘N/A’ represents the number of laboratories that did not assign an LR value for the pair and, therefore, did not estimate the NoC. * The conditions considered in this set were transposed from the ones used and estimated for the set of data produced using EuroForMix by this laboratory (Lab13).

					Profiles	Mix Ref	31_32 31						33			33_34 34					
					Pair		#1			#21			#2			#3			#22		
Stage	Lab	Software	version	Method	#Pairs analyzed	Corrected eNoC	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method
1	Lab1	LRmix Studio	2.1.5	Input for all	26	24	2	N/A	-	2	2	Input	2	2	Input	2	N/A	-	2	2	Input
1	Lab2a	LRmix Studio	2.1.3	Input for all	30	28	2	2	Input	2	2	Input	2	2	Input	2	2	Input	2	2	Input
1	Lab3	LRmix Studio	2.1.3	Input for all	17	17	2	N/A	-	2	2	Input	2	2	Input	2	N/A	-	2	2	Input
1	Lab4	EuroForMix	4.0.8	Mixed	30	26	2	1	PG	2	2	Input	2	2	PG	2	2	Input	2	2	Input
1	Lab5	EuroForMix	4.0.8	Mixed	30	27	2	2	Input	2	2	PG	2	2	PG	2	2	PG	2	2	PG
2	Lab6	EuroForMix	4.1.0	PG for all	30	27	2	2	PG	2	2	PG	2	2	PG	2	2	PG	2	2	PG
2	Lab7	EuroForMix	4.1.0	Mixed	28	25	2	2	Input	2	2	Input	2	2	Input	2	2	Input	2	2	Input
2	Lab8	EuroForMix	4.1.0	PG for all	30	24	2	1	PG	2	2	PG	2	2	PG	2	2	PG	2	2	PG
2	Lab9	EuroForMix	4.0.9	Mixed	30	23	2	1	Input	2	2	Input	2	2	PG	2	1	Input	2	2	Input
2	Lab10	EuroForMix	4.0.9	PG for all	28	26	2	N/A	-	2	2	PG	2	2	PG	2	2	PG	2	2	PG
2	Lab11	EuroForMix	4.0.9	Mixed	30	29	2	2	PG	2	2	PG	2	2	Input	2	2	PG	2	2	PG
3	Lab12	EuroForMix	4.1.0	PG for all	30	29	2	2	PG	2	2	PG	2	2	PG	2	2	PG	2	2	PG
3	Lab13	EuroForMix	4.1.0	PG for all	28	25	2	1	PG	2	2	PG	2	2	PG	2	2	PG	2	2	PG
3	Lab14a	EuroForMix	4.0.9	PG for all	29	26	2	2	PG	2	2	PG	2	2	PG	2	2	PG	2	2	PG
3	Lab2b	EuroForMix	4.0.8	PG for all	30	11	2	1	PG	2	2	PG	2	2	PG	2	1	PG	2	2	PG
3	Lab14b	DNAstatistX	2.3.2	*	30	26	2	2	*	2	2	*	2	2	*	2	2	*	2	2	*
4	Lab15	EuroForMix	4.0.9	Mixed	30	26	2	1	PG	2	2	PG	2	2	PG	2	2	PG	2	2	PG

[illegible]

49				36_37_38												41_42_43										
				36						37						38			41			42			43	
#23				#6			#30			#10			#26			#17			#7			#18				
Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	
Input	2	2	Input	3	N/A	-	3	3	Input	3	3	Input	3	3	Input	3	3	Input	3	2	Input	3	3	Input	3	
Input	2	3	Input	3	2	Input	3	3	Input	3	3	Input	3	3	Input	3	3	Input	3	3	Input	3	3	Input	3	
Input	2	2	Input	3	N/A	-	3	N/A	-	3	3	Input	3	3	Input	3	3	Input	3	N/A	-	3	3	Input	3	
Input	2	2	Input	3	1	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	2	PG	3	3	PG	3	
PG	2	2	PG	3	2	Input	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	2	PG	3	3	PG	3	
PG	2	2	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	2	PG	3	3	PG	3	
Input	2	2	PG	3	2	Input	3	N/A	-	3	3	Input	3	3	Input	3	3	Input	3	2	Input	3	3	Input	3	
PG	2	2	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	2	PG	3	3	PG	3	
Input	2	2	Input	3	1	Input	3	3	Input	3	3	Input	3	3	Input	3	3	Input	3	2	Input	3	2	Input	3	
PG	2	2	PG	3	N/A	-	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	2	PG	3	3	PG	3	
PG	2	2	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	
PG	2	2	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	
PG	2	2	PG	3	N/A	-	3	N/A	-	3	3	PG	3	3	PG	3	3	PG	3	2	PG	3	3	PG	3	
PG	2	2	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	2	PG	3	3	PG	3	
PG	2	2	PG	3	1	PG	3	2	PG	3	3	PG	3	2	PG	3	2	PG	3	2	PG	3	2	PG	3	
*	2	2	*	3	2	*	3	3	*	3	3	*	3	3	*	3	3	*	3	2	*	3	3	*	3	
PG	2	2	PG	3	1	Input	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	2	PG	3	3	PG	3	

43		44_45_46									47_48_49								
		44			45			46			47								
#27		#8			#28			#16			#20			#9			#19		
eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method	NoC	eNoc	Method
3	Input	3	N/A	-	3	3	Input	3	3	Input	3	2	Input	3	3	Input	3	3	Input
3	Input	3	3	Input	3	3	Input	3	3	Input	3	3	Input	3	3	Input	3	3	Input
3	Input	3	N/A	-	3	N/A	-	3	3	Input	3	3	Input	3	N/A	-	3	N/A	-
3	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	Input	3	3	PG
3	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	Input	3	3	PG
3	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG
3	Input	3	2	Input	3	3	Input	3	3	Input	3	3	Input	3	3	Input	3	N/A	-
3	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	4	PG
3	Input	3	2	Input	3	3	Input	3	3	Input	3	3	Input	3	3	Input	3	3	PG
3	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG
3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG
3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG
3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG
3	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG
3	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG
2	PG	3	1	PG	3	2	PG	3	2	PG	3	2	PG	3	2	PG	3	3	PG
3	*	3	2	*	3	3	*	3	3	*	3	3	*	3	3	*	3	3	*
3	PG	3	2	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG	3	3	PG

SM 6: Method followed by each laboratory to determine whether to model stutter peaks for each pair of samples analyzed

Table S6: Method followed by each laboratory to determine whether to model stutter peaks for each pair of samples analyzed – either model by choice ('Input') or letting the software test the different combinations and then selecting the best fit for the genotypic input data ('PG'). The pairs of samples are grouped based on their composition. 'X' represents the number of laboratories that did not assign an LR value for the pair. The evidence file analyzed by each laboratory for each pair of samples is also described: 'WO' evidence file analyzed without artefacts and 'W' evidence file analyzed with artefacts.

			Profiles	Mix Ref	31_32 31										33					33_34 34											
			Pair				#1				#21				#2					#3					#22						
							Ev. File	No	Yes Forward		No	Yes	Ev. File	No	Yes Forward		No	Yes	Ev. File	No	Yes Forward		No	Yes	Ev. File	No	Yes Forward		No		
Stage	Lab	Software version	Ev. File	No				Ev. File	No				Ev. File	No				Ev. File	No				Ev. File	No			Ev. File	No			
1	Lab4	EuroForMix 4.0.8	WO	PG	-	PG	-	WO	Input	-	Input	-	WO	PG	-	PG	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input		
1	Lab5	EuroForMix 4.0.8	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input		
2	Lab6	EuroForMix 4.1.0	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG		
2	Lab7	EuroForMix 4.1.0	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input		
2	Lab8	EuroForMix 4.1.0	W	PG	-	PG	-	W	-	PG	PG	-	W	-	PG	PG	-	W	PG	-	PG	-	W	-	PG	-	PG	W	-		
2	Lab9	EuroForMix 4.0.9	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	PG	-	PG	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input		
2	Lab10	EuroForMix 4.0.9	WO	X	-	X	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG		
2	Lab11	EuroForMix 4.0.9	WO	PG	-	Input	-	WO	PG	-	Input	-	WO	Input	-	Input	-	WO	PG	-	Input	-	WO	PG	-	Input	-	WO	PG		
3	Lab12	EuroForMix 4.1.0	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input		
3	Lab13	EuroForMix 4.1.0	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG		
3	Lab14a	EuroForMix 4.0.9	WO	PG	-	PG	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input		
3	Lab2b	EuroForMix 4.0.8	W	-	PG	PG	-	W	-	PG	PG	-	W	-	PG	PG	-	W	PG	-	PG	-	W	-	PG	PG	-	W	-		
3	Lab14b	DNAStatistX 2.3.2	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input		
4	Lab15	EuroForMix 4.0.9	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input		

35_50												39_40																	
35		50						39						40															
#12		#5				#24				#13				#15				#25				#4							
Yes Forward No Yes		Ev. File	No	Yes Forward No Yes		Ev. File	No	Yes Forward No Yes		Ev. File	No	Yes Forward No Yes		Ev. File	No	Yes Forward No Yes		Ev. File	No	Yes Forward No Yes		Ev. File	No	Yes Forward No					
-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input			
-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input			
-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG			
-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input			
PG	PG	-	W	-	PG	PG	-	W	-	PG	-	PG	-	W	PG	-	PG	-	W	-	PG	PG	-	W	PG	-	PG		
-	Input	-	WO	PG	-	PG	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	PG	-	PG	-	WO	Input	-	Input			
-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	-	PG	-			
-	Input	-	WO	PG	-	Input	-	WO	PG	-	Input	-	WO	PG	-	Input	-	WO	PG	-	Input	-	WO	PG	-	Input			
-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input	-	WO	Input	-	Input			
-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG	-	WO	PG	-	PG			
-	Input	-	WO	PG	-	PG	-	WO	Input	-	Input	-	WO	Input	-	X	-	WO	Input	-	Input	-	WO	PG	-	PG			
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SM 7: Method followed by each laboratory to determine whether to model degradation('Yes') or not ('No') for each pair of samples analyzed

Table S7: Method followed by each laboratory to determine whether to model degradation('Yes') or not ('No') for each pair of samples analyzed – either model by choice ('Input') or letting the software test the different combinations and then selecting the best fit for the genotypic input data ('PG'). The pairs of samples are grouped based on their composition. 'N/A' represents the number of laboratories that did not assign an LR value for the pair.

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GENERAL CONCLUSIONS

General Conclusions

The interpretation of complex DNA mixtures remains one of the most challenging tasks in forensic genetics casework. As the field moves towards adopting probabilistic genotyping (PG) tools in routine workflows to address the difficulties associated with such samples, research has emerged to assess their performance, accuracy, and reliability. To reach this goal, studies (both prior and contemporary to this thesis) have used mock or laboratory-generated samples produced under controlled conditions, essential for establishing baseline performance and validating the statistical models incorporated into the PG software [139-153]. Nevertheless, and even though unexplored until now, it is equally important to perform a similar evaluation of these tools using real casework samples with unknown composition. Only through testing under the complex, unique, unpredictable, and non-replicable conditions of real forensic genetics evidence can the true capabilities and limitations of these systems be fully understood.

Focusing on identification problems involving an evidence sample (commonly a mixture) and an associated reference sample, this work primarily aimed to examine how different statistical and computational interpretation models behave under practical operational conditions. Three widely used informatics tools – one based on a qualitative model (LRmix Studio [104]), which considers only the presence or absence of alleles, and two based on a quantitative model (EuroForMix [109] and STRmix™ [128]), which take into account both the presence or absence of alleles and their respective heights – were explored. These tools were applied to different types of sample pairs to reflect a spectrum of challenges and complexity: simulated *in silico*, laboratory-mock, and real casework data with unknown composition. In that sense, this work represents a significant step forward in forensic genetics, not only presenting the first published studies to assess how PG tool models behave and perform on real casework samples – the very samples these systems were designed to interpret – but also providing an integrated analysis of the obtained results.

The comparison of the distinct statistical models' behavior, applied to real casework data, and under conditions maintained as similar as possible across the three tools, was performed. As expected, the qualitative tool's values differed more from the quantitative tools than the quantitative tools differed among themselves. Nevertheless, although in general terms smaller, differences between the results produced by the quantitative tools were still present and should not be overlooked. These differences arise from the fact that each tool applies different statistical modeling to the various

parameters they both consider in the quantification of genetic evidence. Unexpectedly, some of these differences were strikingly large, to the point that one tool supported the hypothesis of inclusion, while the other supported the opposite hypothesis, assigning LR values close to zero or even numerically underflowing to zero, as described in Chapter I [238]. Recognizing that, in some cases, such discrepancies may result from the stochastic nature of methods implemented in tools such as STRmix™, where the lack of convergence of MCMC algorithms cannot be ruled out, these outcomes highlight that obtaining reliable results requires a deep understanding of the underlying statistical models and their assumptions, rather than uncritical reliance on software outputs. These findings therefore reinforce the importance of expert scrutiny when interpreting likelihood ratios generated by complex probabilistic tools.

On a more fine-grained analytical approach, within each one of the major informatics architectures considered, the impact of varying parameter values and model conditions on the quantification of genetic evidence was evaluated. A set of values and/or conditions was defined as the default for parameters such as the estimated number of contributors (NoC) to the evidence, drop-in (frequency and modeling), population coancestry coefficient, analytical threshold, and inclusion of evidence stutter peaks. For each tool, the results obtained considering the default conditions were compared with those computed after varying a parameter at a time. Although a more accentuated general impact was observed for the NoC in Chapter II [239], especially when underestimated in the quantitative tools analyses, the results demonstrated substantial discrepancies in some cases for all the varied parameters [240-242], as shown in Chapters III to V. Noteworthy, in some analyses, there were situations where the observed differences followed a counter-intuitive and opposite trend to the one expected and/or demonstrated for mock samples. For example, it is expected that increasing the population coancestry decreases the LR values due to an increased probability of allele sharing by chance. However, in some marker-specific cases, when the coancestry value was increased, the LR value also increased. Additionally, and similarly to what was observed in the comparison of the distinct statistical models, in some cases, considering different parameter values and/or conditions within computational architectures resulted in LR values supporting opposite hypotheses, showing that the impact of these parameters should not be overlooked. Thus, demonstrating the operational impact of varying parameter values and conditions on the behavior of models implemented in currently worldwide used informatics tools is an effective way to draw attention to the importance of each laboratory accurately estimating and defining its own parameter settings based on its specific conditions and requirements.

Furthermore, at an even more detailed level, the practical impact of small modifications within the same computational architecture throughout software updates on tools' outputs was assessed. The results obtained by two EuroForMix versions (1.9.3 and 3.4.0), differing particularly in the inclusion of forward stutter modeling and other internal algorithmic improvements, were compared under identical input conditions in Chapter VI [243]. This comparison highlighted the need for laboratories to validate software versions before implementation and maintain version-controlled tracking of results. It also emphasized the importance of regularly updating the software used in workflows, as continuous improvements enhance the accuracy and reliability of DNA interpretation.

The differences observed throughout this work – both between statistical models with markedly different architectures and within the same architecture framework under varying parameter values or conditions – highlight the strong influence of case-specific factors on the resulting outcomes. Factors such as low DNA quantity and quality, degradation, a high number of contributors, the composition of evidence and reference genotypic profiles, allele frequency, heterozygote imbalance, varying contributor ratios, and the proportion of DNA contributed by the reference individual (i.e., whether the person is a major or minor contributor), as well as the presence of common forensic artifacts (such as drop-in, dropout, and stutter peaks) are a great source of divergence in interpretation and weight of genetic evidence. Collectively, these characteristics can significantly affect the performance of PG tools when quantifying the weight of the evidence, leading to highly variable outcomes across different case conditions.

Recognizing these complexities and the need for accurate parameter estimation, an international working group supported by the Spanish and Portuguese-speaking Working Group of the International Society for Forensic Genetics (GHEP-ISFG) was launched, as displayed in Chapter VII, involving multiple international forensic laboratories [244]. The aim was to assess the extent to which forensic laboratories are familiar with and have PG software implemented in their workflows, as well as the methodologies applied to statistically interpret complex DNA mixtures. Over 45 laboratories, with varying levels of experience with PG tools, registered to participate in this working group, reflecting a strong interest within the forensic community. Nevertheless, only one-third of these concretized their participation, citing heavy workloads as a constraint. This illustrates how difficult it can be for forensic laboratories to invest in professional development, such as inter-laboratory exercises that enhance their interpretative skills. Although the participants analyzed the same data, the results reported varied due to differences in the tools, parameter settings, and interpretation

strategies used. While these methodological discrepancies had a limited effect in simpler cases, they led to significant variation in LR for complex or degraded samples. The study emphasizes the importance of a comprehensive understanding of the available tools, as well as the influence of parameter values and conditions on outputs. Regardless of the level of expertise, the findings reaffirm the fundamental need for continuous and specialized training. Furthermore, international collaborations such as this not only provide insight into the current state of PG tools implementation in routine practice but also help harmonize methodologies, ensure reliable results, and strengthen the scientific robustness of forensic DNA interpretation. Indeed, enhancing collaboration between researchers and practitioners, coupled with targeted continuous training, is crucial to align theoretical advances with the practical demands of forensic casework.

Beyond bridging the gap between theoretical development and operational practice in forensic DNA interpretation, this work also highlights the urgent need for continuous training and deep statistical literacy among practitioners. As forensic science increasingly depends on complex computational models, it is essential that experts not only operate these tools but also understand the statistical frameworks and parameter assumptions underpinning them. Looking ahead, further progress will depend on addressing a set of unresolved challenges that continue to shape the field. A main priority is improving the transparency of models, not only by clarifying their statistical foundations but also by ensuring that experts and courts can truly understand the assumptions and limitations of these systems. Closely linked to this is the need to harmonize parameterization practices, as variations in modeling dropout, drop-in, stutter, and other artefacts can lead to inconsistencies across laboratories and software packages. Thus, consistent guidelines for parameter selection would enhance both reproducibility and interpretability. Equally important is the clarification of the limits of applicability of these tools. Although probabilistic systems have expanded the range of interpretable profiles, it remains unclear where the boundary lies in situations involving extremely low template DNA, highly degraded material, or complex mixtures with a high number of contributors or in highly unbalanced contributions. Developing universal quality metrics to evaluate data suitability and model robustness is essential to define these limits. Another area requiring continued attention is software validation. As PG tools evolve, laboratories must adopt validation frameworks that not only assess implementation but also ensure comparability and reliability across successive software updates. Although emerging technologies such as Massively Parallel Sequencing (MPS) and machine and deep learning approaches hold the potential to further expand the capabilities of PG, moving to these tools before fundamental issues of validation, standardization, and interpretation

are fully addressed could introduce additional complexity and uncertainty. The premature introduction of such models without a solid foundation of consistent practices and universal quality metrics risks increasing noise in the results and the difficulty of understanding them, both for experts and for the courts, undermining the operational trust the field is still striving to establish. Together, these future directions highlight that the continued evolution of PG depends not only on technical innovation but also, and foremost, on coordinated efforts in standardization, validation, communication, and professional training. Only by addressing these interconnected challenges can the field progress in a way that is both scientifically rigorous and operationally trustworthy.

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